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# Convergence dans l'évolution de la spécialisation d'hôte chez des tiques : modèle tiques-oiseaux de mer à distribution mondiale

Marlène Dupraz

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# THÈSE

Pour obtenir le grade de  
**Docteur**

Délivré par  
**Université de Montpellier**

Préparée au sein de l'école doctorale GAIA  
Et de l'unité de recherche MIVEGEC - IRD

Spécialité : **Biologie des interactions**

Présentée par  
**Marlène Dupraz**

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spécialisation d'hôte chez des tiques :  
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distribution mondiale**

Soutenue le jeudi 15 décembre 2016 devant le jury composé de

Elena Gómez-Díaz, CR, EBD-CSIC, Séville, Espagne

Vincent Debat, MC, MNHN, Paris, France

Johan Michaux, Professeur, Université de Liège, Belgique

Laurence Vial, CR, CIRAD, Montferrier-le-lez, France

Dale Clayton, Professeur, Université de l'Utah, USA

Karen McCoy, DR2, CNRS, Montpellier, France

Rapportrice

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Examinatrice

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## Résumé

Convergence dans l'évolution de la spécialisation d'hôte chez des tiques : modèle tiques-oiseaux de mers à distribution mondiale

Les interactions intimes et répétées entre hôtes et parasites peuvent engendrer la spécialisation d'un parasite à son hôte, grâce à des adaptations comportementales, morphologiques et/ou génétiques, combinées avec un flux de gènes limité. C'est un processus clef car il participe à l'évolution de la biodiversité parasitaire et peut ainsi permettre de mieux comprendre l'émergence d'organismes pathogènes. Encore peu étudié, une spécialisation d'hôte a néanmoins été démontrée lors de précédentes études chez deux espèces de tiques nidicoles : chez *Ixodes uriae* une tique dure, parasite des oiseaux marins coloniaux en zone arctique, et dans un complexe de tiques molles *Ornithodoros capensis* sensu lato, parasitant aussi de nombreuses espèces d'oiseaux marins, mais cette fois-ci en zones tempérées et tropicales. Ces espèces sont vectrices d'une grande diversité d'agents pathogènes incluant des virus, des bactéries et des protozoaires. Cependant, les facteurs impliqués dans le phénomène de spécialisation d'hôte restent inconnus. Dans ce cadre, le but de ma thèse était donc de déterminer 1) si l'évolution des divergences en fonction des hôtes est toujours accompagnée par les mêmes changements phénotypiques et 2) si ces changements pourraient permettre d'identifier les facteurs de sélection sous-jacents. Dans ce contexte, des campagnes d'échantillonnage de tiques ont été menées durant la période de reproduction des hôtes oiseaux dans les différentes zones de leur répartition et nous avons réalisé des analyses morphométriques, basées sur l'utilisation de landmarks et de contours sur chaque individu tique et des analyses phylogénétiques et génétiques des populations sur les mêmes individus. L'ensemble de ces résultats suggère la présence de convergences morphologiques au sein de ces systèmes et souligne un rôle de la sélection dans ce processus de divergence. En effet, les caractéristiques écologiques des hôtes mais aussi le micro-habitat exercent des pressions sélectives importantes dans ces deux systèmes pouvant être à l'origine de la divergence observée entre les populations. De plus, les caractéristiques biologiques de chaque espèce de tiques, telle que la capacité de dispersion, entrent également en jeu et peuvent fortement modifier l'épidémiologie des agents infectieux dont elles sont vectrices.

Mots clés : Argasidae, écologie de la transmission, évolution convergente, interactions hôte-parasite, Ixodidae, oiseaux marins.

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## Abstract

Convergence in the evolution of host specialization of ticks: insights from two worldwide tick-seabird model systems

Intimate and repeated interactions between hosts and parasites can lead to parasite specialization to a given host via behavioral, morphological and/or genetic adaptations that act in combination with restricted gene flow. Specialization is a key process leading to the generation of parasite biodiversity and can help us understand the emergence of pathogenic organisms. Although little studied, host specialization has already been demonstrated to occur in previous studies of two nidicolous tick species: *Ixodes uriae* a hard tick parasitizing colonial seabirds in polar regions, and soft ticks of the complex *Ornithodoros capensis* sensu lato, that also exploit colonial seabirds, but this time in temperate and tropical zones. These species act as vector to a wide variety of pathogenic organisms, including viruses, bacteria and protozoa. However, the factors involved in host specialization remain unknown. In this context, the aim of my thesis was to determine 1) whether the evolution of host specialization is always accompanied by the same phenotypic changes and 2) whether these changes could help to identify the selective factors that influence this phenomenon. In this context, tick collections were conducted during the breeding period of the host birds in different areas of their distribution and morphometric analyses, based on landmark and contour methods, were performed on each individual tick. Phylogenetic and population genetic analyses were also carried out using the same individuals. Overall, the results demonstrate that morphological convergence occurs within these systems, highlighting the role of selection in the divergence process. Indeed, the ecological characteristics of the hosts, but also their micro-habitat, may exert significant selective pressures on ticks and may cause the observed divergence among populations. Likewise, the biological characteristics of each tick species, particularly in relation to dispersal capacity, may also come into play and will greatly modify the epidemiology of associated infectious agents.

Keywords: Argasidae, convergent evolution, host-parasite interactions, Ixodidae, transmission ecology, seabirds.



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# Sommaire

▪ <b>Chapitre 1 – Introduction</b>	<b>1</b>
♦ Evolution de la diversité parasitaire	3
♦ Convergences dans la formation des races d’hôtes	5
♦ Systèmes d’étude	9
× <i>Ixodes uriae</i> , la tique des pôles	
× <i>Ornithodoros capensis</i> sensu lato, un complexe d’espèces complexe	
♦ Objectifs de la thèse	14
▪ <b>Chapitre 2 – Identification et discrimination des espèces</b>	<b>17</b>
♦ 2.1 Description morphologique et tiques	19
♦ 2.2 La morphométrie géométrique	21
<i>Article 1</i>	23
<b>Estrada-Peña et al.</b> The results of a blind test of ixodid tick identification by European experts. Soumis.	
<i>Article 2</i>	47
<b>Dujardin et al, 2014.</b> Outline based morphometrics, an overlooked method in arthropod studies? <i>Infection, Genetics and Evolution</i> . 28: 704-714.	
▪ <b>Chapitre 3 – Convergences dans l’évolution des patrons de diversification</b>	<b>61</b>
<i>Article 3</i>	65
<b>Dupraz et al, 2016.</b> Linking morphometric and genetic divergence with host use in the tick complex <i>Ornithodoros capensis</i> s.l. <i>Infection, Genetics and Evolution</i> . <i>Sous presse</i> .	
<i>Article 4</i>	121
<b>Dupraz et al.</b> Morphological convergence in <i>Ixodes uriae</i> host races. En préparation.	
▪ <b>Chapitre 4 – Rôle de l’isolement local dans la divergence</b>	<b>141</b>
<i>Article 5</i>	145
<b>Dupraz et al.</b> Circulation of vector-borne infectious agents at local scales: structure of the soft tick <i>Ornithodoros maritimus</i> within a colony of its seabird host <i>Larus michahellis</i> . En préparation.	
▪ <b>Chapitre 5 – Discussion générale et perspectives</b>	<b>173</b>
▪ <b>Références bibliographiques</b>	<b>187</b>
▪ <b>Annexes</b>	<b>201</b>
<b>Duron et al, 2015.</b> The Recent Evolution of a Maternally-Inherited Endosymbiont of Ticks Led to the Emergence of the Q Fever Pathogen, <i>Coxiella burnetii</i> .	
<b>Boulinier et al, 2016.</b> Migration, Prospecting, Dispersal? What Host Movement Matters for Infectious Agent Circulation?	





# Chapitre 1 - Introduction

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*Evolution de la diversité parasitaire*

La spéciation est un processus clef dans la diversification du vivant conduisant, à partir d'une seule espèce, à l'apparition de deux ou plusieurs nouvelles espèces distinctes. Étudier le phénomène de spéciation revient à étudier les mécanismes sous-jacents qui conduisent à la divergence des populations. Cette divergence peut survenir grâce à deux modes principaux de spéciation, encore sujets à débats (Bird et al., 2012). La spéciation allopatrique survient lorsque des populations sont spatialement (ou temporellement) séparées l'une de l'autre, par exemple par des barrières géographiques (montagne, océan), par des changements environnementaux (changements climatiques) ou par la disponibilité des ressources. Ces populations ne peuvent donc plus se reproduire ensemble si bien que le flux de gènes entre elles est réduit ou devient inexistant (Wiens, 2004). Elles seront alors soumises à des pressions de sélection différentes et vont évoluer au gré des mutations qui peuvent se fixer dans chacune des populations. En revanche, le second mode de spéciation ne découle pas d'un isolement géographique mais peut survenir lorsque la divergence des populations se fait malgré un flux de gènes, c'est la spéciation sympatrique (Rice, 1987). Pour qu'il y ait formation de deux sous-populations se différenciant en sympatrie, il est nécessaire que la sélection disruptive mène à deux optimums distincts. Cette sélection divergente peut survenir dans un environnement spatialement hétérogène présentant des habitats ou niches différentes (Levene, 1953). L'adaptation à un habitat donné, ou une niche écologique<sup>1</sup>, entraînera en principe une perte de valeur sélective dans les autres habitats (Levins and MacArthur, 1966). Le choix du partenaire, se faisant préférentiellement avec les individus présents dans un type d'habitat donné, renforcera la divergence des sous-populations (Ravigné et al., 2010). La nature des variations environnementales, l'histoire des populations et les forces en jeu telles que la dérive<sup>2</sup> ou le flux génique donneront lieu à des adaptations évolutives graduelles, le long d'un continuum, allant d'un polymorphisme phénotypique au sein d'une population jusqu'à la spéciation complète, en passant éventuellement par un stade intermédiaire, dont la formation des races (Magalhães et al., 2007).

Les parasites constituent un modèle biologique idéal pour étudier les processus évolutifs de la spéciation de par leur grande diversité et les nombreuses transitions depuis un mode de vie libre vers le parasitisme (de Meeûs and Renaud, 2002; Poulin and Morand, 2000). De par leur cycle de vie, deux types de pression de sélection sont exercés sur les parasites : l'environnement biotique, donc l'hôte et l'environnement abiotique dans lequel vit l'hôte et/ou que le parasite doit subir lors de son cycle de vie.

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<sup>1</sup> Ensemble des conditions environnementales telles qu'une espèce donnée peut former des populations viables

<sup>2</sup> Modification aléatoire de la diversité des allèles au sein d'une population d'une génération à l'autre

Ces variations interviennent tant sur le plan temporel que spatial. Les facteurs abiotiques peuvent inclure la température, l'humidité, le rythme circadien ou encore la structure physique et chimique du milieu. Les facteurs biotiques sont déterminés par la présence et la disponibilité d'hôtes compétents, la présence de compétiteurs ou encore de prédateurs, ceci pouvant également être modifié par les facteurs abiotiques (Meyers and Bull, 2002). L'ensemble de ces facteurs peut être cyclique ou périodique, étendu sur de courtes ou longues périodes et s'opérer sur de larges échelles spatiales ou localement. L'environnement et les changements climatiques jouent donc un rôle à une échelle globale dans la distribution et la diversification des parasites car ils produisent des environnements hétérogènes impactant l'abondance des populations, la composition des communautés et la physiologie des organismes (Hoberg and Brooks, 2015; Tylianakis et al., 2008). Selon ces conditions locales, les interactions antagonistes entre hôte et parasite peuvent également être plus ou moins intenses, induisant des pressions de sélection différentielles sur les mécanismes de défense réciproques (Hochberg and Baalen, 1998). En conséquence, l'assemblage de ces facteurs crée une mosaïque géographique des interactions interspécifiques au niveau local qui modifiera les trajectoires co-évolutives des espèces (Thompson, 1997).

Parmi les facteurs qui agissent sur l'évolution des parasites, les plus fortes pressions de sélection doivent venir de l'hôte car il représente la majeure partie des besoins écologiques du parasite (habitat, ressource alimentaire, lieu de reproduction). L'hôte représente également un environnement spatialement (individus, populations) et temporellement (phénologie) hétérogène. Ainsi, pour assurer sa survie le parasite n'a d'autre choix que de s'adapter à l'hôte (De Meeûs et al., 1998). En retour, l'hôte développe des mécanismes de défense (immunité, physiologie, comportement) pour lutter contre le parasite modifiant ainsi l'histoire évolutive propre à chacun des systèmes hôte-parasite (Van Valen, 1977). Dans un monde idéal, il convient donc à un parasite généraliste, c'est-à-dire un parasite capable d'utiliser différents types d'habitats ou de ressources, d'investir dans des adaptations à large spectre afin d'exploiter différents types d'hôtes et faire face aux diverses réponses immunitaires et mécanismes de défenses. Cependant, les ressources en hôtes sont souvent inégales, et les contraintes adaptatives peuvent être fortes. Ainsi, pour optimiser sa fitness, un parasite peut devenir spécialiste d'un seul type d'hôte et parviendra à contourner la gamme des défenses de cet hôte (Wikel, 1984, Combes, 2001). Cette adaptation a néanmoins un coût potentiel sur la capacité du parasite à exploiter efficacement d'autres types d'hôte; comme dans le cas de tout type de spécialisation écologique, on parle alors d'un compromis entre la performance et le spectre des ressources exploitables (Futuyma and Moreno, 1988).

Discerner le lien entre diversité-préférences-performances des parasites par rapport à leurs hôtes ainsi que déterminer les facteurs qui affectent ou maintiennent leurs interactions est alors nécessaire pour prédire la dynamique des populations de parasites et leur trajectoire évolutive. Dans le cas des parasites qui sont aussi vecteurs d'agents pathogènes, la capacité à exploiter différents hôtes aura une influence majeure sur la dynamique des maladies associées (Ostfeld et al., 2005) et ces informations seront alors indispensables pour comprendre et prédire l'épidémiologie (Cleaveland et al., 2001).

### *Convergences dans la formation des races d'hôtes*

Nous savons que des interactions intimes et répétées entre un parasite et son hôte peuvent entraîner la spécialisation du parasite et, combinées à un flux de gène limité, mener à la formation de races d'hôtes (encadré 1). Il s'agit de populations isolées reproductivement, qui diffèrent par des caractéristiques biologiques et des variations morphologiques subtiles, elles ne sont donc pas considérées comme des espèces distinctes (Jaenike, 1981). Les étapes de la formation de races d'hôtes sont détaillées dans l'encadré 1 (Bush and Butlin, 2004). Ce phénomène est une étape le long du continuum menant à la spéciation et il est considéré comme un mécanisme clé pour la spéciation sympatrique (Mopper and Strauss, 2013). Cependant, les critères pour établir si une race d'hôte est devenue une espèce à part entière sont relativement difficiles à établir car la détection du flux de gènes entre races se réduisant au cours du temps est problématique et la notion d'espèce n'est pas encore communément admise.

#### **Encadré 1 - Etapes de la formation de races d'hôtes (d'après Bush et Butlin, 2004)**

1. Des mutations ou recombinaisons génèrent dans une population de parasites, des individus capables d'utiliser un nouvel hôte.
2. Un accouplement non aléatoire se produit entre les parasites présents sur le nouvel hôte ce qui réduit le flux de gènes avec la population d'origine et permet le maintien des gènes d'adaptation dans la population.
3. Au cours de l'adaptation au nouvel hôte, une race d'hôte génétiquement distincte évolue de par les différences de fitness et la fidélité à l'hôte augmente avec le temps.
4. Pour les loci qui ne sont pas impliqués dans l'adaptation au nouvel hôte, des similarités génétiques avec la population d'origine sont maintenues par un faible flux de gènes, en particulier durant les premières étapes de la formation des races d'hôte.
5. La spéciation est complétée par renforcement ou parce que la divergence continue de s'opérer, réduisant le flux de gènes à zéro.

Les insectes phytophages ont largement été utilisés dans les études concernant la spéciation sympatrique et la formation de races d'hôtes de par leur grande diversité, l'association étroite existant

entre les préférences alimentaires et la reproduction ainsi que leur facilité d'utilisation dans des expériences en conditions contrôlées (Drès and Mallet, 2002). Citons comme exemple la galéruque du nénuphar *Galerucella nymphaeae*, un insecte coléoptère de la famille des Chrysomelidae, montrant des races associées à deux familles d'hôtes (Nymphaeaceae et Polygonaceae) dont les différences de performance résultent de traits morphologiques distincts et de préférences alimentaires (Pappers et al., 2002). Dans le cas de la mouche du pommier, *Rhagoletis pomonella*, présentant deux races d'hôtes spécialisées sur l'aubépine et sur le pommier, il a été montré que les différences de choix d'hôte, fondées sur la discrimination des odeurs de fruits et la phénologie de l'hôte entraîneraient un accouplement non aléatoire et donc un isolement reproductif facilitant la spéciation (Feder et al., 1994; Dambroski et al., 2005).

Les acariens présentent également des prédispositions à la formation de races d'hôtes du fait de leurs interactions rapprochées avec leurs hôtes, de leur philopatrie et de leur faible capacité de dispersion. Cependant relativement peu d'études se sont portées sur ce groupe et la plupart se focalisent sur *Tetranychus urticae*, un parasite généraliste de plantes (Magalhães et al., 2007) (voir encadré 2 ci-dessous).

#### Encadré 2 - Exemple de races d'hôte: le cas d'un acarien *Tetranychus urticae*



*T. urticae* est un parasite généraliste de plantes de la famille des Tetranychidae, considéré comme un ravageur important des cultures mais aussi des plantes sauvages. La formation de toiles, qui affectent les plantes hôtes, lui permet de maintenir l'humidité et lui assure une protection. Son cycle biologique s'effectue en plusieurs stases : œuf, larve, deux stases de nymphe, une stase adulte, dont la durée est variable en fonction de la température (20 jours au maximum dans les conditions optimales). La femelle peut vivre de 2 à 4 semaines et pondre plusieurs centaines d'œufs dans ce laps de temps (Van de Vrie et al., 1972). De par son large spectre d'hôte, la facilité à le maintenir en élevage et à le manipuler, cet acarien présente des caractéristiques idéales pour étudier la spécialisation d'hôte. Les races d'hôte identifiées sont associées par exemple à la tomate, le coton ou le concombre et sont morphologiquement semblables. Des études expérimentales ont montré que la formation de ces races d'hôtes dans ce système est basée sur des différences de préférences et de performances (oviposition, survie, développement) selon l'hôte utilisé qui découlent principalement de phénologies différentes des plantes hôtes (Agrawal, 2000; Egas and Sabelis, 2001) mais reposent aussi sur la production de composés volatiles permettant de lutter contre les parasites (Gotoh et al., 1993; Takabayashi et al., 2000).

Malgré ces exemples classiques, l'identification des cas de spécificité d'hôte d'une manière générale et la compréhension de son origine pourraient souvent être freinés par des ressemblances phénotypiques. Avec le développement d'outils de génétique et génomique, nous avons aujourd'hui accès à de plus amples informations sur la spécificité d'hôte et son évolution. Combiné avec des études phénotypiques détaillées, ces informations peuvent permettre d'identifier les convergences quant à la réponse évolutive d'espèces soumises aux mêmes contraintes écologiques. La convergence phénotypique, ou l'homoplasie, correspond à la présence de caractères analogues chez deux espèces appartenant à des lignées distinctes, découlant d'une même adaptation face à une pression de sélection naturelle ou sexuelle. Ces convergences peuvent être comportementales (Blackledge and Gillespie, 2004), morphologiques (Kocher et al., 1993) ou fonctionnelles (Ribeiro, 1995), reposant sur des mécanismes génétiques et/ou moléculaires (Stern, 2013). Les convergences phénotypiques liées au phénomène de spécialisation d'hôte peuvent donc être appréhendées par des mesures de variations phénotypiques au sein des différentes sous-populations ou races d'hôtes qui, grâce aux données génétiques, sont connues pour avoir des origines indépendantes. J'ai utilisé cette approche pour étudier l'évolution de la spécialisation d'hôte chez un groupe d'arthropodes d'importance médicale et vétérinaire, les tiques (Ordre Ixodida).

Les tiques sont des arthropodes hématophages présents dans toutes les régions du monde. Avec 900 espèces décrites actuellement, dont environ 700 espèces de tiques « dures » (famille Ixodidae) et 200 espèces de tiques « molles » (famille Argasidae), un grand nombre de facteurs (disponibilité et prédictibilité d'hôte) et de pressions exercées par l'hôte (comportement, immunité) ou l'environnement (température et humidité du micro-habitat) peuvent conduire à la spécialisation des tiques. Les relations physiologiques et immunologiques qu'elles entretiennent avec une large gamme d'hôtes incluant des oiseaux, des reptiles, de petits et grands mammifères mais également l'Homme sont très étroites (Jongejan and Uilenberg, 2004). Elles ont un impact direct sur le succès reproducteur et les dynamiques de populations d'hôtes, en particulier quand le taux d'infestation est élevé (Boulinier and Danchin, 1996; Duffy, 1983; Feare, 1976; Jongejan and Uilenberg, 2004; Lehmann, 1993). Il existe donc une pression sélective importante pour l'hôte à lutter contre l'infestation. De plus, les tiques constituent un groupe de vecteurs des plus importants au monde en termes de diversité d'agents infectieux transmissibles (Parola and Raoult, 2001), incluant des bactéries, des virus mais aussi des parasites. Les enjeux économiques en santé humaine et animale sont alors considérables (Sonenshine and Roe, 2014). Il est donc particulièrement pertinent d'étudier ce groupe d'arthropodes pour comprendre les mécanismes d'évolution associés à l'hôte et leurs conséquences.



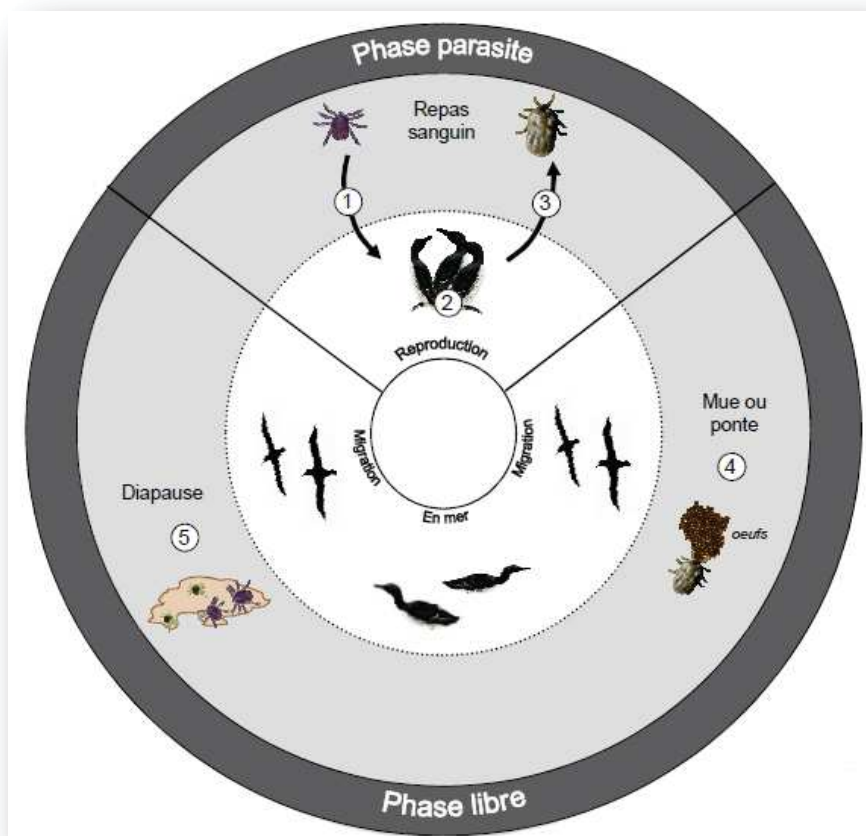
Les associations tiques-hôtes sont diverses, certaines espèces étant très spécialistes d'un type d'hôte et d'autres plutôt considérées comme généralistes. Cependant, les degrés de spécificité restent encore difficiles à établir du fait d'observations opportunistes, d'effort d'échantillonnage et/ou d'erreurs d'identification (McCoy et al., 2013). Le temps passé sur l'hôte pour le repas de sang est relativement court par rapport au temps passé dans l'environnement externe, il a donc été suggéré que la distribution des tiques n'est pas limitée par l'utilisation d'hôte mais plutôt par les conditions environnementales (climat, végétation)(Estrada-Peña, 2001; Klompen et al., 1996). En effet, en étudiant 229 espèces de tiques africaines, Cumming (1999) observa que la moitié présentaient une distribution plus limitée que celle de leurs hôtes (les informations concernant l'autre moitié n'étant pas assez solides pour en tirer des conclusions). Cette étude ne prenait en compte ni l'abondance des tiques sur chaque type d'hôte, ni l'abondance des hôtes, facteurs pouvant modifier la distribution des espèces de tiques (McCoy et al., 2013). De même, Nava et Guglielmono (2013) ont conclu que les interactions tiques-hôtes dans la zone néotropicale étaient principalement dues aux similarités écologiques d'habitats des hôtes plutôt qu'aux hôtes eux-mêmes. Contrastant avec ces résultats, des analyses de génétique des populations ont révélé plusieurs cas de divergence en fonction des hôtes (McCoy et al., 2013) et suggèrent que ce type de divergence peut être fréquent dans ces systèmes. Pour mieux évaluer cette hypothèse et pour comprendre les facteurs à son origine, je me suis focalisée spécifiquement sur deux systèmes-tiques complémentaires. Le premier implique la tique dure *Ixodes uriae* qui exploite de nombreuses espèces d'oiseaux marins coloniaux dans les régions polaires du globe. Dans ce système, plusieurs groupes génétiquement distincts en relation avec l'utilisation d'hôte ont été détectés à plusieurs reprises et indépendamment dans différentes zones de sa distribution grâce à des outils de génétique des populations (McCoy et al., 2005). Il reste cependant à identifier l'origine de ces divergences. Le deuxième système est celui des tiques molles du complexe *Ornithodoros capensis* sensu lato. Ces tiques exploitent également des oiseaux marins coloniaux, mais cette fois-ci en zones tropicales et tempérées. Un premier indice d'une diversification en fonction de l'hôte a été révélé dans une étude initiale de ce système (Gómez-Díaz et al., 2012), mais ces patrons à plus grande échelle et leur origine restent aussi à élucider. La biologie de ces systèmes est détaillée ci-dessous.

## Systèmes d'étude

*Ixodes uriae*, la tique des pôles

**Figure 1** : Photos représentant la tique *Ixodes uriae*. De gauche à droite : une femelle non gorgée; des individus en gorgement autour de l'œil d'un Albatros ; des individus gorgés rassemblés en agrégats sous une pierre. Crédits photos : M. Dupraz, C. McKenzie et P. Bize.

*Ixodes uriae* (White, 1852) est un ectoparasite appartenant à la famille des Ixodidae, que l'on nomme « tique dure » du fait de la présence d'une partie chitinisée en face dorsale. Cette espèce est largement répartie en zones tempérées froides et polaires : Atlantique Nord, Pacifique Nord, Océan Arctique, îles subantarctiques et Antarctique (Dietrich et al., 2011). Elle infeste une soixantaine d'espèces d'oiseaux marins coloniaux, incluant entre autres des oiseaux des genres Laridae, Alcidae, Procellariidae et Sulidae (Arthur, 1962; Zumpt, 1952) qui se reproduisent à terre durant quelques mois puis partent pour passer l'hiver en mer (Coulson, 2001). *I. uriae* est une espèce à cycle triphasique (larve-nymphé-adulte) avec un seul stade nymphal et un repas long par stase dont la durée est variable selon la stase (de 3 à 12 jours), excepté pour les mâles qui ne se gorgent pas (Eveleigh and Threlfall, 1974). Comme pour toutes tiques dures, le repas de sang implique un dialogue moléculaire très étroit entre le système immunitaire de l'hôte et la production de molécules anticoagulantes et immunosuppressives par la tique pour contourner cette réponse (Brossard and Wikel, 2004). Après le repas de sang, les tiques se retirent dans un endroit protégé où elles muent et passent l'hiver ou, pour la femelle, pond une centaine d'œufs et meurt (Eveleigh and Threlfall, 1974) (Figure 2).



**Figure 2** : Cycle biologique d'*Ixodes uriae* (cercle gris) déterminé par la présence de ses hôtes (cercle blanc)(tiré du rapport de thèse de M. Dietrich, 2011). 1 : les tiques non gorgées (larves, nymphes et femelles) retrouvent l'hôte lors de son retour dans la colonie ; 2 : La durée du repas de sang sur l'hôte dépend de la stase ; 3 : A la fin du gorgement, les tiques se détachent de l'hôte et repassent dans le substrat environnant ; 4 : Les tiques se mettent à l'abri sous des pierres ou dans la roche pour pondre ou muer ; 5 : Elles passent l'hiver en diapause en attendant le retour des hôtes.

C'est une espèce nidicole, c'est-à-dire que son habitat se restreint au nid de ses hôtes ou aux alentours. Hors de la période de nidification des hôtes oiseaux, elle se réfugie en agrégats dans le sol, les crevasses rocheuses ou au fond des nids des oiseaux (Barton et al., 1995) pour y passer la majeure partie de son cycle dans des conditions climatiques stables jusqu'au retour des hôtes. Son cycle de vie dépend donc très fortement de la présence de ses hôtes et est généralement achevé en 4 à 5 ans (Eveleigh and Threlfall, 1974). L'exploitation de ses hôtes est facilitée par certaines caractéristiques des oiseaux marins coloniaux qui peuvent par ailleurs favoriser la spécialisation : ils se reproduisent à la même période et au même endroit chaque année (espèces philopatrides), ils se regroupent en colonie monospécifique (une seule espèce) ou hétérospécifique (plusieurs espèces) très denses et sont spatialement et temporellement prévisibles, et ils sont longévifs, pouvant vivre 15-20 ans (Coulson, 2001). Dans le cas de fortes infestations, *I. uriae* peut avoir un impact direct sur les populations d'hôtes par la spoliation sanguine en diminuant le succès reproducteur (Boulinier and Danchin, 1996; Mangin et al., 2003). De plus, elles transmettent des agents pathogènes, tels que les bactéries du complexe *Borrelia burgdorferi*

sensu lato, responsables de la maladie de Lyme chez l'Homme ; les effets de ces agents infectieux sur les hôtes sont encore mal connus (mais voir Chambert et al., 2012).

Cependant, de par sa phase libre relativement longue, la survie d'*I. uriae* dépend aussi des conditions de température et d'humidité qu'elle rencontre dans le micro-habitat de l'hôte et qui peuvent contraindre sa distribution. La dispersion de cette tique est réalisée par ses hôtes, et notamment à l'échelle inter-colonie. Pendant la période de reproduction, les mouvements des oiseaux se résument à des allers-retours entre les zones de nourrissage en mer et le nid, il est donc peu probable que la tique puisse disperser grâce aux adultes reproducteurs. Néanmoins, les individus en échec et les jeunes immatures, en quête d'un nouveau site de nidification ont été proposés comme moteur de dispersion de ces parasites (Boulinier et al., 2016, 2008; Danchin, 1992).

Sa large répartition géographique et sa capacité à exploiter de nombreuses espèces d'hôtes ont participé à la classification d'*I. uriae* parmi les espèces de parasites généralistes. Cependant, grâce à des échantillonnages dans des colonies mono- et hétérospécifiques et l'utilisation d'outils de génétique des populations, les études précédentes ont démontré que cette espèce avait évolué en races d'hôtes et ceci indépendamment dans différentes zones de sa répartition, les populations des deux hémisphères étant génétiquement isolées l'une de l'autre (McCoy et al., 2001, 2012, 2005). Dans l'Atlantique Nord, cette évolution serait relativement récente (Kempf et al., 2009), mais ces divergences ne semblent pas aller au delà des races d'hôtes dans l'ensemble de la distribution (Dietrich et al., 2014a). De plus, une expérience de transplantation a mis en lumière des différences de performance sur un hôte alternatif (Dietrich et al., 2014b) et des analyses de morphométrie ont révélé que les divergences génétiques étaient supportées par des divergences morphologiques entre races (Dietrich et al., 2013). Néanmoins, ces deux études ont été réalisées à l'échelle d'une seule colonie sympatrique, reflétant un processus d'adaptation au niveau local. Dans le cadre de ma thèse, je me suis donc attachée à tester s'il existe une convergence morphologique des patrons d'association d'*I. uriae* à ces hôtes à une échelle plus globale, incluant différentes populations de l'Atlantique Nord. A partir de ces patrons, nous pouvons ensuite entreprendre l'identification des traits impliqués dans ces divergences.

*Ornithodoros capensis* sensu lato, un complexe d'espèces complexe

**Figure 3** : Photos représentant différentes espèces du complexe *Ornithodoros capensis* sensu lato.

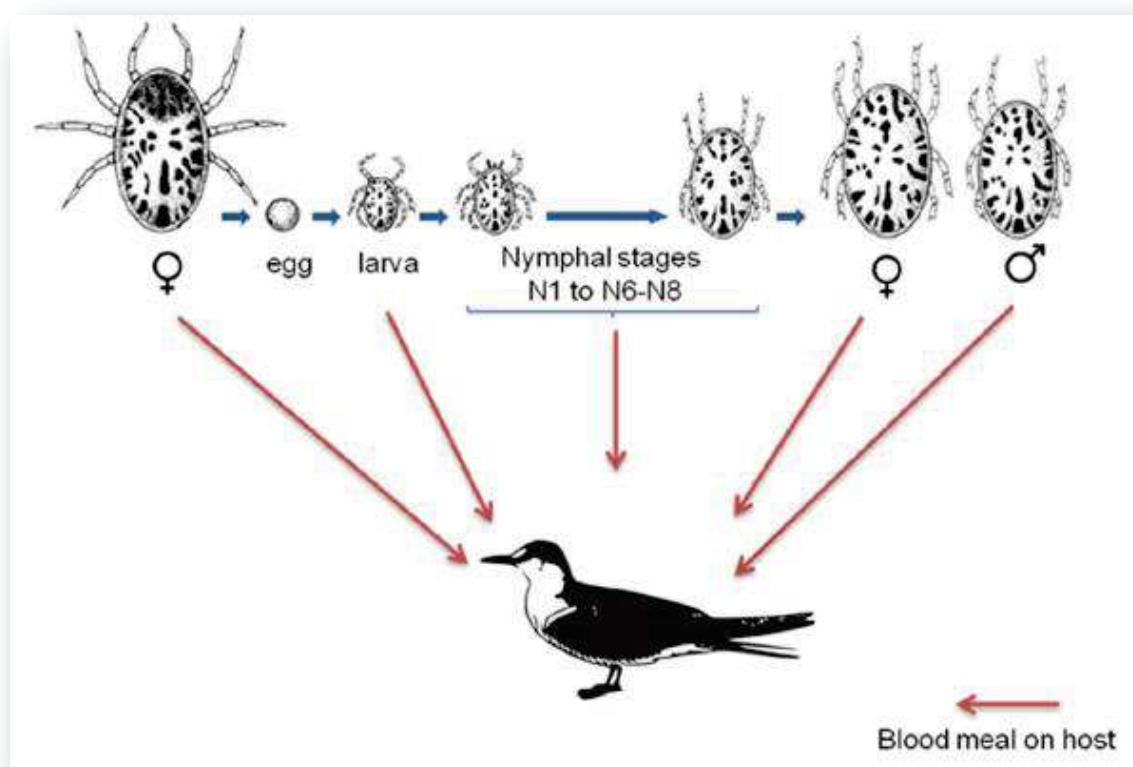
*Ornithodoros (Carios) capensis* sensu lato est un complexe de huit espèces de la famille des Argasidae, que l'on nomme « tiques molles » car elles possèdent un tégument souple mammelonné, sans partie chitinisée sur la face dorsale. A la différence des tiques dures, le capitulum (portant les pièces buccales) est positionné en face ventrale aux stases nymphales et adultes (Sonenshine, 1993). Ce groupe cryptique<sup>3</sup> est réparti largement en zones tropicales et subtropicales et les différentes espèces ont été décrites à partir d'individus récoltés de manière opportuniste et en se basant sur des critères morphologiques définis sur les stases larvaires :

- *O. capensis sensu stricto* (Neumann, 1901)
- *O. maritimus* (Vermeil and Marguet, 1967)
- *O. amblus* (Chamberlin, 1920)
- *O. denmarki* (Kohls, Sonenshine et Clifford 1965)
- *O. muesebecki* (Hoogstraal, 1969)
- *O. sawaii* (Kitaoka and Suzuki, 1973)
- *O. spheniscus* (Hoogstraal, Wassef, Hays et Keirans, 1985)
- *O. yunkerii* (Keirans, Clifford et Hoogstraal, 1984)

Plus récemment, Vermeil et al (1997) ont décrit une espèce proche d'*O. capensis* s.s. en stase larvaire mais proche d'*O. maritimus* à la stase femelle, collectée dans des nids de sternes en Mauritanie. Cependant, aucune information concernant son statut taxonomique ou ses affinités d'hôtes ne sont disponibles depuis cette publication et son inclusion dans le complexe reste encore à vérifier.

<sup>3</sup> Des espèces cryptiques ne sont pas morphologiquement différenciables mais sont isolées reproductivement et/ou ont une différenciation génétique importante.

Ce complexe d'espèces nidicoles exploite un grand nombre d'oiseaux marins coloniaux incluant, entre autres, des Alcidae, des Laridae, des Procellariidae, des Sulidae et des Spheniscidae, nichant sur ou près du sol, généralement sur des côtes arides (Hoogstraal, 1985). Leur cycle est polyphasique impliquant un repas de sang pour la stase larvaire et un à plusieurs repas pour chacun des quatre à huit stades nymphaux et la stase adulte. Comparativement aux tiques dures, la durée des repas est très courte, de quelques heures pour la stase larvaire à quelques minutes pour les stases nymphales et adultes, les mâles ne se gorgeant généralement pas ou peu. En conséquence, on collecte souvent les nymphes et les adultes dans l'environnement et les larves sur les hôtes. Les femelles sont capables de se nourrir et de pondre quelques centaines d'œufs à plusieurs reprises (Pérez-Eid, 2007). Le cycle d'*O. capensis* s.s. est décrit dans la figure 4, extraite de McCoy et al (2016).



**Figure 4:** Cycle biologique d'*O. capensis* s.s. La durée du repas de sang dépend de la stase considérée. Plusieurs repas courts sont pris sur l'hôte par chacun des stades nymphaux et par la stase adulte.

Malgré l'effort de différentes écoles pour clarifier la position systématique de ce groupe dans la classification (Camicas and Morel, 1977; Hoogstraal, 1985; Klompen and Oliver, 1993; Pospelova-Shtrom, 1969) par des études morphologiques et cladistiques, leur position reste encore inexacte. (Guglielmone et al., 2010). Voilà pourquoi l'on se réfère aussi au complexe comme *Carios capensis* s.l. (Horak et al., 2002). Cependant, si nous adoptons le genre *Carios*, le genre *Ornithodoros* devient



paraphylétique. Nous avons alors décidé de rester conservatifs dans l'utilisation du nom du genre, mais un travail important sur la systématique de ces argasidés est nécessaire (Estrada-Peña et al., 2010).

De la même manière, la distribution géographique et les relations de parenté entre les membres du complexe *O. capensis* s.l. restent à clarifier. En effet, une première étude génétique sur ces tiques dans les îles du Cap-Vert a remis en question la description du complexe (Gómez-Díaz et al., 2012). Ceci s'explique par le fait que l'on récolte majoritairement des individus adultes souvent sporadiquement, pour lesquels la ressemblance morphologique avec des critères classiques est très marquée, en comparaison des larves, qui peuvent être différenciées plus facilement. De plus, aucune étude génétique regroupant les différentes espèces du complexe n'a été menée pour l'instant et peu de données sont encore disponibles concernant l'écologie et l'évolution de ce complexe.

En revanche, on connaît la capacité de ces espèces à transmettre de nombreux agents pathogènes : des bactéries telles que les *Borrelia* responsables de la fièvre récurrente (Takano et al., 2009) et de nombreux virus, tel que le virus Soldado, qui peut induire une forte mortalité dans les populations d'oiseaux, la désertion des zones de reproduction (Converse et al., 1975) et des infections prurigineuses chez l'Homme (Camicas, 1980; Feare, 1976) ou encore des Flavivirus comme le virus Meaban et le virus Kémérovo (Chastel et al., 1985).

De par les nombreuses espèces d'oiseaux marins qu'elles exploitent, les tiques de ce complexe sont également considérées comme généralistes. Cependant, l'étude de Gómez-Díaz et al (2012) a soulevé des interrogations par rapport à cette considération. A partir d'échantillons provenant des îles du Cap-Vert et grâce à l'utilisation de marqueurs mitochondriaux (ARNr 16S et 18S), Gómez-Díaz et al (2012) ont montré que différentes lignées de tiques, provenant de zones distantes du globe, coexistent sur ces îles. Au sein de cette diversité génétique, attribuée aux mouvements des hôtes, les auteurs ont pu observer des associations tiques-oiseaux au sein des différentes îles. Néanmoins, les espèces en présence n'ont pas été identifiées. Plus généralement, peu d'informations sur la biologie et l'utilisation d'hôte des espèces du complexe *O. capensis* s.l. sont actuellement disponibles. Mon travail a donc consisté à identifier et à clarifier la distribution et les interactions des différentes espèces du complexe avec leurs hôtes à une échelle plus globale.

### *Objectifs de la thèse*

Dans le cadre de ma thèse, je me suis intéressée **aux patrons de spécialisation d'hôte et aux facteurs à l'origine de cette spécialisation dans les deux systèmes tiques-oiseaux de mer** présentés précédemment. Les objectifs étaient de déterminer si l'évolution de la spécialisation d'hôte est toujours

accompagnée des mêmes changements phénotypiques ainsi que de dégager les facteurs agissant sur la sélection. J'ai donc utilisé des méthodes de morphométrie afin de détecter les variations phénotypiques fines entre les différentes populations de tiques en relation avec l'utilisation de l'hôte, couplées à des outils de phylogénie et de génétique des populations. J'ai ensuite utilisé une approche à échelle locale, basée sur une étude *in situ*, pour tenter de dégager le rôle de l'isolement dans ces divergences.

Pour commencer, le chapitre 2 traite des questions techniques liées à l'efficacité des outils de morphologie et de morphométrie pour caractériser les espèces et les populations de tiques. L'article 1 évalue la fiabilité des descriptions classiques pour l'identification des tiques. L'article 2 se focalise ensuite sur l'analyse plus fine des populations par la morphométrie géométrique, une méthode qui m'a permis de comparer différentes populations de tiques dans le chapitre 3.

Le chapitre 3 porte sur l'étude des deux systèmes-tiques pour examiner les relations entre morphométrie et utilisation d'hôte avec comme objectif d'identifier les phénomènes de convergence attestant de la spécialisation de ces tiques à leurs hôtes. L'article 3 présente alors les patrons de divergence génétique et morphologique dans le complexe *Ornithodoros capensis* sensu lato au niveau mondial. L'article 4 descend à une échelle biologique plus fine pour tester explicitement les convergences morphologiques entre races d'hôtes d'*Ixodes uriae* afin d'identifier les facteurs à l'origine des divergences répétées dans ce système.

Le chapitre 4 est dédié aux facteurs de divergence impactant l'isolement des populations et notamment la capacité de dispersion locale des tiques. J'ai abordé cette question par un design expérimental sur le terrain pour déterminer la dynamique des tiques au sein des nids, le degré des mouvements au sein de la colonie (Article 5) et comment ceux-ci pouvaient impacter la circulation des agents pathogènes.

Finalement, je conclus la thèse avec une discussion générale (chapitre 5) sur les différents aspects de la spécialisation d'hôtes dans les deux systèmes-tiques, incluant les perspectives à poursuivre pour mieux comprendre leur histoire évolutive. J'inclus ces résultats dans un cadre plus large pour évaluer à quel points ils peuvent être applicables à d'autres systèmes tiques et d'autres systèmes 'vecteurs' plus généralement.



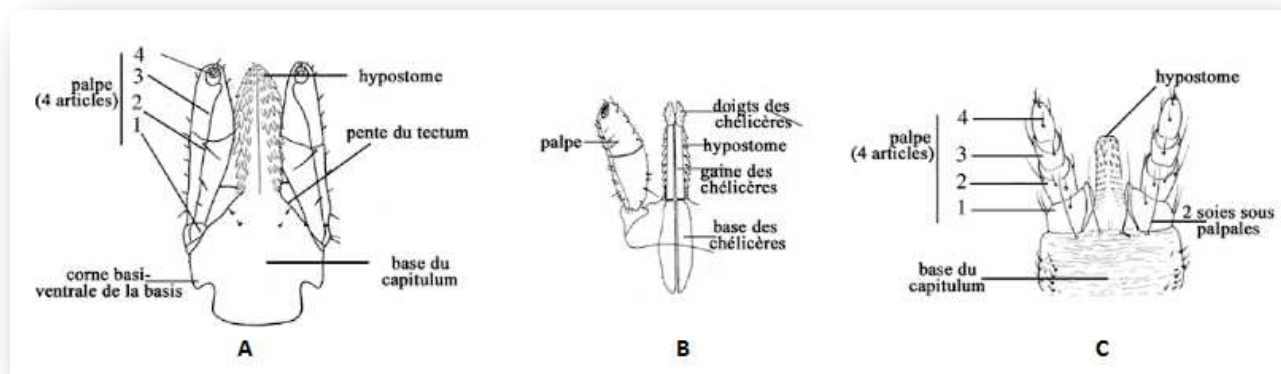


## **Chapitre 2 – Identification et discrimination des espèces**

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## 2.1 Description morphologique et tiques

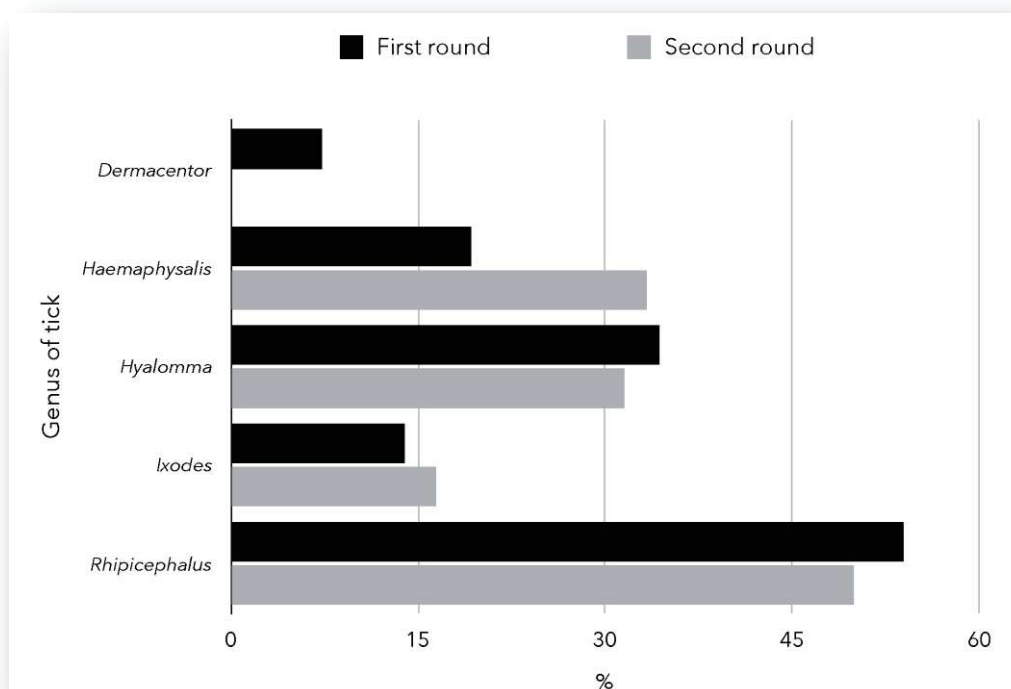


**Figure 5** : Morphologie schématisée du capitulum des tiques utilisée comme critère pour les identifications. A et B : Ixodina, C : Aragasina (d'après Pérez-Eid, 2007).

La description morphologique, basée sur la diversité des structures, est la première étape de l'identification et de la classification des espèces (Adams et al., 2004). Une première vision fixiste définissant une espèce comme « un ensemble d'individus qui se ressemblent », a laissée la place à une vision évolutionniste insufflée par Charles Darwin au XIXe siècle. Ce courant considère une espèce comme « un groupe d'individus interféconds présentant des caractères communs » (Thomas et al., 2010), laissant apparaître la notion de variabilité au sein d'une espèce. Les taxonomistes se sont donc attachés à définir ou redéfinir les espèces sur la base de caractères partagés, entreprenant un travail de grande ampleur notamment en ce qui concerne les arthropodes qui représentent la biodiversité la plus étendue avec 1,5 millions d'espèces décrites actuellement (Lecointre et Guyader, 2001). Les espèces cryptiques, qui ne peuvent être distinguées l'une de l'autre par un examen morphologique, posent un challenge conséquent (Bickford et al., 2007) et contribuent aujourd'hui à la description de nouvelles espèces notamment grâce aux outils moléculaires, comme dans le cas des Anophèles (Collins and Paskewitz, 1996) ou des acariens (Navia et al., 2013).

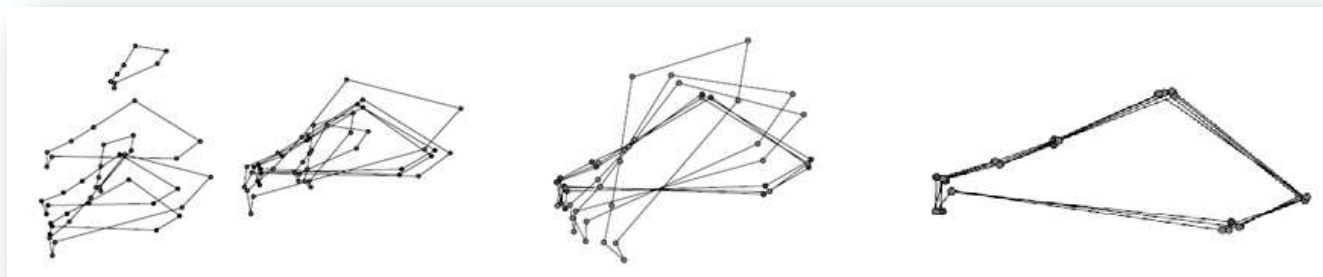
Chez les tiques, l'identification des espèces est traditionnellement basée sur des critères morphologiques tels que la position du capitulum, la taille du rostre, la position du sillon anal ou encore la longueur des articles des palpes (Bonnet et al., 2015)(Figure 5). A l'heure actuelle, plusieurs classifications, basées sur des clefs morphologiques ou sur l'utilisation de marqueurs génétiques, sont disponibles, illustrant la difficulté des chercheurs à trouver un consensus sur la systématique de ce groupe d'arthropodes, notamment concernant les tiques molles (Estrada-Pena et al., 2010). Trois familles de tiques ont été définies : les *Nuttalliellidae*, représentées par une seule espèce *Nuttammiella namaqua*, les Argasidae ou tiques molles contenant 193 potentielles espèces appartenant aux genres

*Antricola*, *Argas*, *Nothoaspis*, *Ornithodoros* et *Otobius*, et les Ixodidae ou tiques dures contenant 702 espèces de 14 genres différents, par exemple *Ixodes*, *Amblyomma*, *Hyalomma*, *Rhipicephalus* et *Dermacentor* (Guglielmo et al., 2010). Au sein de ces familles, des différences morphologiques fines peuvent donner lieu à des erreurs d'identification des espèces et donc de classification notamment en raison de la disponibilité ou de l'indisponibilité de différentes clefs d'identification. Le développement des techniques d'identification moléculaire permet à l'heure actuelle une meilleure conception de la systématique des tiques et conduit régulièrement à des changements de nomenclatures (Plantard et al., 2015). La capacité d'une communauté de spécialistes à identifier différentes espèces de tiques des genres *Dermacentor*, *Ixodes*, *Haemaphysalis*, *Hyalomma* et *Rhipicephalus*, a été testée au cours d'un « blind test » (Article 1). Deux rounds permettant d'identifier les tiques grâce à l'identification morphologique ou des analyses génétiques lors du premier et à seulement des clefs morphologiques, sans illustrations, spécialement produites pour le test lors du second round. Les résultats montrent des taux d'identification variables, de 0 à 54% selon le genre (Figure 6) et révèlent un manque d'assurance des spécialistes à identifier morphologiquement les tiques. Il apparaît donc important de pouvoir s'appuyer sur des descriptions morphologiques et des outils moléculaires correctement définis pour différencier les espèces.



**Figure 6:** Taux d'erreur d'identification des différents genres de tiques lors des deux rounds.

## 2.2 La morphométrie géométrique



**Figure 7** : Représentation des différentes étapes nécessaires à l'analyse Procrustéenne basée sur l'agencement de landmarks (translation, rotation, mise à l'échelle).

Le concept évolutionniste de l'espèce admet qu'il peut exister une grande variabilité morphologique intra-spécifique, par exemple entre juvéniles et adultes, entre mâles et femelles ou même au sein d'une classe d'âge. Cette variabilité morphologique ou plasticité phénotypique peut être quantifiée grâce à des méthodes de morphométrie basées sur l'étude de la forme d'un objet (taille + conformation) et de ses variations. Contrairement à la morphologie, la morphométrie n'a pas pour but de décrire les espèces mais de les comparer (Thompson, 1945). La morphométrie classique repose sur une série de mesures linéaires, de surfaces ou de volumes comme illustré par le modèle de Raup pour décrire les coquilles de gastéropodes (Raup, 1966). Cependant, elle présente des inconvénients concernant la configuration d'un objet (forme), étant donné que les mesures de distances linéaires sont fortement corrélées à la taille des individus et doivent donc être corrigées ou que deux objets de formes différentes peuvent présenter des mesures linéaires identiques. Dans les années 80, les travaux de Bookstein ont révolutionné la discipline en instaurant le concept de morphométrie géométrique basée sur la superposition de points homologues ou landmarks, afin de comparer des formes et leurs variabilités grâce aux coordonnées X et Y de chaque point (Bookstein, 1997). La première phase de cette méthode repose sur le positionnement des landmarks sur des structures anatomiques présentes chez chacun des organismes étudiés, en nombre égal et suffisant pour obtenir une bonne couverture de la forme. Ces landmarks peuvent être collectés sur des images photographiques ou radiographiques ou en 3D à l'aide de lasers ou de bras tactiles. La seconde phase consiste en la superposition des formes géométriques générées par les landmarks. Ceci peut être réalisée par différentes approches, par exemple par une analyse Procrustéenne ou GPA (generalized Procrustes analysis), basée sur la détermination du centroid, correspondant à la moyenne des coordonnées de chaque landmark et nécessitant différentes étapes de mise à l'échelle (homothétie), translation et rotation (Dujardin, 2011). Ces étapes permettent de définir

de nouvelles coordonnées pour décrire le positionnement des landmarks et comparer la forme sans interférence de la taille (Figure 7). L'allométrie, représentant la relation entre la taille et la forme, pourra ensuite être calculée grâce à des techniques de régression linéaire. L'étape finale permet d'analyser statistiquement le positionnement des landmarks et de générer une représentation graphique de la variabilité. Ceci peut être réalisé par différents outils : analyse en composantes principales (PCA), méthode des plaques minces (thin-plate spline), analyse discriminante (DA). La morphométrie géométrique permet de mesurer les caractères continus qui sont affectés par les changements micro-évolutifs, tels que les traits morphologiques associés à différentes activités biologiques, par exemple l'alimentation, la reproduction ou la locomotion et elle constitue donc un bon outil pour étudier les changements phénotypiques. La morphométrie géométrique a largement été utilisée pour distinguer des espèces proches (Barluenga et al., 2006; Michaux et al., 2007; Patterson et al., 2001), pour discriminer des sous-populations (Dietrich et al., 2013; Kaba et al., 2012; Ledevin et al., 2010) ou des cas de ré-infestations après traitement aux acaricides (Dujardin, 2011; Dujardin et al., 1997). Elle peut également permettre de relier les différences de taille et de forme à des variables environnementales et donc d'appréhender les mécanismes à l'origine de la divergence (Dujardin, 2008). Rapide, peu coûteuse et non destructive, cette méthode peut compléter des analyses génétiques mais aussi les remplacer dans le cas de spécimens de collection (Garros and Dujardin, 2013).

De plus, la morphométrie géométrique permet d'obtenir des informations sur des structures non articulées, ne permettant pas le positionnement de landmarks, en utilisant la capture de contours. Par exemple, l'analyse de Fourier utilise les coordonnées de chaque landmarks formant un contour afin de comparer les formes relatives de chaque individu (Lawing et Polly, 2010). Cette méthode a été évaluée en comparaison avec des analyses basées sur l'utilisation de landmarks, en utilisant des espèces proches ou conspécifiques incluant des punaises, des anophèles, des glossines et des tiques molles du complexe *O. maritimus*. Les deux types d'analyses ont révélé des scores similaires dans la discrimination des populations. La méthode des contours est donc particulièrement adaptée pour les tiques molles dont la cuticule ne permet pas le positionnement de landmarks. (Article 2).

## Article 1

A. Estrada-Peña, G. D'Amico, A. M. Palomar, M. Dupraz, M. Fonville, D. Heylen, M.A. Habela, S. Hornok, L. Lempereur, M. Madder, M. S. Nuncio, D. Otranto, M. Pfaffle, O. Plantard, M. M. Santos-Silva, H. Sprong, Z. Vatansever, L. Vial, A.D. Mihalca. *Submitted to Ticks and Tick-borne Diseases*. The results of a blind test of ixodid tick identification 1 by European experts.





The results of a blind test of ixodid tick identification by European experts.

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## Abstract.

This study reports the results of a blind test of identification of ticks occurring in Western Europe and Northern Africa. A total of 14 laboratories were voluntarily enrolled in the test. Each participant received between 22 and 25 specimens of adult and nymphal ticks of the species *Dermacentor marginatus*, *D. reticulatus*, *Haemaphysalis punctata*, *Hyalomma lusitanicum*, *Hy. marginatum*, *Ixodes ricinus*, *I. hexagonus*, *Rhipicephalus annulatus*, *R. bursa*, *R. rossicus*, and/or *R. sanguineus* s.l. Ticks were morphologically identified by three of the co-authors and the identification confirmed by a fourth co-author who used molecular methods based on several genes. Then ticks were randomly selected and blindly distributed among participants, together with a questionnaire. Only specimens collected while questing and, if possible, in the same survey, were circulated. Because of the random nature of the test, a participant could receive several specimens of the same species. Species in the different genera had variable misidentification rates (MR) of 7% (*Dermacentor*), 14% (*Ixodes*), 19% (*Haemaphysalis*), 36% (*Hyalomma*), and 54% (*Rhipicephalus*). Within genera, the MR was also variable ranging from 5.4% for *I. ricinus* or 7.4% for *D. marginatus* or *D. reticulatus* to 100% for *R. rossicus*. The test provided a total misidentification rate of 29.6% of the species of ticks. There are no significant differences in MR according to the sex of the tick. Participants were requested to perform a second round of identifications on the same set of ticks, using only purposely prepared keys (without illustrations), circulated to the enrolled participants, including 2 species of the genus *Dermacentor*, 8 of *Haemaphysalis*, 10 of *Hyalomma*, 23 of *Ixodes*, and 6 of *Rhipicephalus*. The average MR in the second round was 28%: 0% (*Dermacentor*), 33% (*Haemaphysalis*), 30% (*Hyalomma*) 18% (*Ixodes*), and 50% (*Rhipicephalus*). Species which are not reported in the countries of a participating laboratory had always highest MR, i.e. purely Mediterranean species had highest MR by laboratories in Central and Northern Europe. Participants expressed their concerns about a correct identification for almost 50% of the ticks of the genera *Hyalomma* and *Rhipicephalus*. The results revealed the lack of total confidence identifying the most prominent species of ticks in the Western Palearctic, and underpin the need of adequate reference libraries for specialists involved in this task. Results also showed that an adequate combination of several genes may adequately identify the target species of ticks.

Keywords: blind test; identification; morphology; molecular; Ixodid ticks; Western Palearctic

## Background

Ticks are known to transmit a large variety of pathogens of medical and veterinary concern and are among the most important disease-transmitting arthropods (Estrada-Peña and de la Fuente, 2014). Most of the field studies on ticks are based on a correct identification of the specimens collected, as a prerequisite for further research. For example, in a recent literature review on the occurrence of ticks and tick-borne pathogens in Europe and the Mediterranean Basin (Maioli et al., 2012), only studies that used morphological keys for tick identification were considered. More than 60 species of the family Ixodidae can be found in Europe, and its morphological identification is not trivial, as some species form complexes with cryptic or sibling species, i.e. *Rhipicephalus sanguineus* group (Dantas-Torres et al., 2013) or have relatively complex morphological features for an accurate determination, which may require a deep training, like the genus *Hyalomma*. Even in a thoroughly surveyed area like Europe, new species have been recently described (i.e. *Ixodes inopinatus* Estrada-Peña, Nava & Petney, or *I. ariadnae* Hornok). Interspecific hybridization between two species of *Ixodes* has also been reported, a biological event that may pose additional concerns for specific identification, even using molecular markers (Kovalev et al. 2015). Moreover, morphological keys for ticks commonly cover only the species with medical interest, not every stages are included (Arthur, 1963; Nosek and Sixl, 1972; Cordas et al., 1993; Hillyard, 1996; Filippova, 1997; Manilla, 1998; Estrada-Peña et al., 2004; Cringoli et al., 2005; Pérez-Eid, 2007) and some of them may be unreliable because they do not include the most recent concepts about the species identity. Therefore, recent studies on comparative morphology of ticks in Europe are rare (Heylen et al., 2014). The availability of different methods has provided insights in the use of cuticular hydrocarbons composition (Estrada-Peña et al., 1996) or MALDI-TOF (Yssouf et al., 2013) for the identification of ticks, while most reports focused on the use of adequate genetic markers, like 16S rRNA, 12S rRNA, or *cytochrome c oxidase I (coxI)*. Although these technologies are useful, they will always be based on the use of reference specimens for which morphological species-specific identification need to be correctly conducted (Nava et al., 2009; Araya-Ancheta et al., 2015). “Garbage sequences” obtained from unreliably identified specimens that accumulate in databanks like GenBank are a source of misidentification. They may introduce a background noise when included in the context of a phylogenetic reconstruction of species of ticks (Zhang and Zhang, 2014) or produce an incorrect identification of separate specimens.

Further concerns on the topic include the possible spread of some tick species beyond their historical ranges because natural factors such as weather (Jaenson et al., 2012; Medlock et al., 2013; Jore et al., 2014), the peculiarities of the landscape (Li et al., 2012; Vanwambeke et al., 2016), or an increase of the density of adequate hosts (Li et al., 2014). If some species are prone to spread and colonize areas where they were previously unreported, there is a pressure for a correct identification to promote the adequate measures of control and evaluate the potential impact on either human or domestic animals.

Blind tests are often applied for the unbiased determination of events in different facets of the science, including very different events like cut marks versus carnivore tooth marks on bone surfaces (Blumenschine et al., 1996), plant or animal residues in archeological research (Leach, 1998; Lombard and Wadley, 2007) or the degree of anonymity of the blind or double-blind review process for scholarly research articles (Hill and Provost, 2003). Biological sciences have benefited of blind tests for the identification of organisms, the procedure being generally applied to compare the degree of similarity between the opinion of several specialists about the classification of organisms. In other cases, the classification obtained by both morphological and molecular methods has been compared (i.e. Kim et al., 1999; Coupe et al., 2005; Druzhinina et al., 2005). Most of the non-morphological methods used for the identification of some parasitic arthropods always used the blind approach, comparing the results of a candidate method against the background of morphological identification by experts (i.e. Dieme et al., 2014; Yssouf et al., 2013). Examples of a blind test approach to ticks so far cover the use of DNA-barcoding for the detection of blood remnants on specimens collected while feeding (Garipey et al., 2012). Nevertheless, the morphological identification of ticks is more routinely used in laboratories around the world than molecular methods, mainly in large sample sets. However, a comparative test on the opinion of experts about the specific identity on batches of ticks in a large region has never been done. In summary, there is no objective measure about the comparative reliability of experts in the field to recognize widely distributed and common species of ticks in a large territory.

We aimed to candidly and openly test the reliability of a blind test of morphological identification of ticks reported as established in Europe and Northern Africa, by several teams of experts, based on specimens which were previously selected to fit some requirements, carefully identified by their morphology, confirmed by molecular tools,

and circulated to the participants. We derived statistics of the rates of correct determinations compared with the genus and the sex/stage of each tick. Special attention was paid to the reliability of the identification according to the distribution of the ticks (e.g. species existing in the country of residence of the participant, and therefore familiar to him/her) and the approach used by participants in the identification of the specimens (i.e. using dedicated books, reprints, voucher specimens, etc.). A secondary aim was to estimate the rate of confidence of the participants with their identifications as compared with the rate of mismatches, a ratio that express how efficiently the experts can manage the threat derived by ticks. Finally, our last aim was to evaluate the reliability and usability of a comprehensive key for all the species of ticks reported in the Western Palearctic as a synthetic tool specifically aimed to improve the task.

#### Material and Methods

For an adequate representation of the most common species reported in the Western Palearctic (including Northern Africa), the following species have been included in the test: *Dermacentor marginatus* (Panzer) (14♀, 13♂), *Dermacentor reticulatus* (Fabricius) (14♀, 14♂), *Haemaphysalis punctata* Canestrini & Fanzago (14♀, 12♂), *Hyalomma lusitanicum* Koch (7♀, 11♂), *Hyalomma marginatum* Koch (14♀, 12♂), *Ixodes hexagonus* Leach (14♀, 13♂, and 15 nymphs), *Ixodes ricinus* (Linnaeus) (14♀, 11♂, and 12 nymphs), *Rhipicephalus annulatus* (Say) (14♀, 11♂), *Rhipicephalus bursa* Canestrini & Fanzago (12♀, 12♂), *Rhipicephalus rossicus* Yakimov and Kohl-Yakimova (12♀, 15♂), and *Rhipicephalus sanguineus* s.l. (9♀, 17♂). The test was explicitly focused on the tick fauna from the two large biogeographical regions of the target territory: the countries bordering the Mediterranean basin, which also includes species from Northern Africa, and countries in Central and Northern Europe. We did not include species of ticks which are monoxenous (like *Ha. hispanica* Gil-Collado *R. pusillus* Gil-Collado or *I. ventalloi* Gil-Collado, restricted to the Mediterranean wild rabbit, or *I. lividus* Koch, restricted to the European sand martin) or species inadequately described or scarcely reported (i.e. *I. eldaricus* Dzhaparidze, *I. festai* Tonelli-Rondelli, *I. kaiseri* Arthur). The species chosen for the study have special significance in both human and animal health, and they are well known vectors of pathogens to humans or livestock (Jongejan and Uilenberg, 2004).

The standardization of the batches of ticks circulated to the participants was a point of special concern. This interest prevented the inclusion of some species of potential importance in the test, like *I. frontalis* Panzer, *I persulcatus* Schulze, or *I acuminatus* Neumann, because the available specimens were in a variable degree of repletion, or obtained from a wide variety of sources, far from the standards adhered in the protocol. Only unengorged ticks were used. Specimens used for the test were always collected while questing, never feeding, to avoid the distortion of the morphological proportions. In the case of *R. annulatus*, which is a one host species, specimens were collected as engorged nymphs on cattle, and allowed to molt in the laboratory to have flat adults. All the specimens of the same species were collected in the same place and, when possible, they were collected in the same survey, site and date, to have the most homogeneous sample set. Every specimen which did not conform to the description of the species, or with morphological abnormalities was removed from the test. Specimens of *R. sanguineus* s.l. were collected on the walls of a kennel in Mediterranean Spain. In this case, we included in the test only specimens conforming to the classic description by Filippova (1997). This description overlaps with the morphology of the “type II” specimens reported by Dantas-Torres et al. (2013) and it intends to represent the Mediterranean populations of this complex of species. All specimens were determined by one of three co-authors (none of these participated in the blind test) and confirmed by the two others. A fourth author enrolled in the blind test, performed a molecular determination of every species and results were 100% in agreement with the morphological determination of the three authors mentioned above. Tick species were confirmed by PCR targeting the tick mitochondrial 16S ribosomal RNA (16S rRNA) (Black and Piesman, 1994). For some specimens, e.g. tick species in which this fragment gene had not been characterized, or was not specific enough, PCR assays for the mitochondrial 12S rRNA gene and the nuclear 5.8S-28S rRNA intergenic transcribed spacer 2 (ITS2) were also performed (Beati and Keirans, 2001; Labruna et al., 2002). Again, this is of special interest in the case of *R. sanguineus* s.l. for which molecular identity of the specimens included in the test was confirmed to belong to the so-called temperate clade. The genes targeted and the primers used for molecular identification are included in Table 1. The Table 2 includes the maximum identities of sequences obtained in this study for the molecular identification of the ticks.

The assignation of samples to each participant was blind and random. Once each individual tick was placed separately in a small vial containing 70% ethanol, a unique



Table 1. PCR primer pairs and conditions used for the genetic identification of tick, together with the original references.

Target gene	Primer sequence (5'→3')	Melting temperature (°C)	Fragment size (base pairs)	Reference
16S rRNA	16S+1: CTGCTCAATGATTTTTTAAATTGCTGT GG	48 54	456	Black and Piesman, 1994
	16S-1: CCGGTCTGAACTCAGATCAAGT			
12S rRNA	T1B: AAAC TAGGATTAGATACCT	51	Approx.. 360	Beati and Keirans, 2001
	T2A: AATGAGAGCGACGGGCGATGT	53		
ITS2	RIB-4F: CCATCGATGTGAAYTGCAGGACA	55	variable, 800-1,100	Zahler et al. 1995; McLain et al. 1995 (from Labruna et al., 2002)
	RIB-R: GTGAATTCTATGCTTAAATTCAGGGG GT			

Table 2. Maximum identities of sequences obtained in this study for the genetic characterization of tick species.

Tick species	Gene sequences; % identity & GenBank no (no of sequences)		
	16S rRNA	12S rRNA	ITS2
<i>Dermacentor marginatus</i>	99.5 JX051098 (1)	-	-
	99.5 JX051097 (1)	100 AF031848 (1)	99.6 FN296278 (1)
<i>Dermacentor reticulatus</i>	99.8 JF928493 (1)	-	-
<i>Haemaphysalis punctata</i>	99.5 KR870978 (3)	100 AF483218 <sup>1</sup> (3)	-
<i>Hyalomma lusitanicum</i>	99.7-100 Z97881 (3)	-	-
<i>Hyalomma marginatum</i>	99.5 L34307 (1)	-	-
<i>Ixodes hexagonus</i>	100 JF928502 (1)	99.6 AF081828 (1)	100 GQ924083 (1)
<i>Ixodes ricinus</i>	100 GU074616 (1)	100 AF150029 (1)	-
	100 GU074606 (1)	100 KF197118 (1)	-
	99.8 GU074590 (1)	100 JN248424 (1)	-
	100 GU074589 (1)	-	-
<i>Rhipicephalus annulatus</i>	99.8 L34311 (2)	-	-
<i>Rhipicephalus bursa</i>	100 KU664351 (4)	100 KC243833 (2)	-
<i>Rhipicephalus rossicus</i>	100 KP866202 <sup>2</sup> ; 99.6 KU848178 (1)	100 AF150021 (1)	99.3 AF271282 <sup>3</sup> (1)
<i>Rhipicephalus sanguineus</i> s.l.	100 KT382469 (2)	-	-

<sup>1</sup> Identified as *Haemaphysalis* sp.; <sup>2</sup> *R. sanguineus* sequence, only a short sequence from *R. rossicus* available in GenBank (KU848178); <sup>3</sup> Sequence identified as *Rhipicephalus pumilio*. No ITS2 sequence from *R. rossicus* available in GenBank.

number was randomly allocated to each specimen, which was sent to one of the participants. Participating laboratories also were identified by a random number. Each participant received 22 to 25 specimens. Because of the random nature of the test, a participant could receive several specimens of the same species, without a balance of gender or stage of the ticks in the samples. Participants were informed of this characteristic of the test, and instructed that even if a species was already identified in the set of material, the same species could appear again, or not, in the received material.

Participants enrolled voluntarily in the study. All of them have a longstanding experience in the identification of ticks, and have a background of peer-reviewed publications on the topic. Only three of the authors (the ones which did the primary identification of ticks) knew the names of the other participants, to avoid the exchange of information among enrolled experts. In any case, the random identification numbers for the ticks were unique to avoid any attempt of information exchange among participants. Only one of the authors knew the complete correspondence between species of ticks, identification numbers for ticks, and details of the enrolled laboratories. Sixteen experts, from 14 laboratories (one of them involved in the molecular information of the specific identity of the specimens), were finally enrolled in the test.

The samples were circulated together with a questionnaire to be filled individually for each identified specimen. These forms were labelled with the number of the tick, and included questions about the identity of the specimen, its gender or stage. We specifically aimed to collect details about the process of identification, involving the procedures adhered by the participant regarding the use of keys/reprints/monographs and how confident he/she felt about his/her identification. The complete questionnaire is included in the supplementary material. After the first round of identification, participants received a key tailored for every stage and species of Ixodidae found in the Palearctic region, without illustrations. The keys included the adults of 2 species of the genus *Dermacentor*, 8 species of *Haemaphysalis*, 10 of *Hyalomma*, 23 of *Ixodes*, and 6 of *Rhipicephalus*, as well as keys for nymphs of 23 species of *Ixodes*. Participants were requested to perform a second round of identifications on the same set of ticks, using only the purposely prepared keys, and submit again the results. We received the response from the 14 laboratories in the first round but only 11 sets of responses in the

second round. This resulted in 306 ticks identified by morphological methods in the first round and 259 in the second.

Every participant was confidentially informed of his/her rates of success in the determination of the batches of ticks in both rounds. We did not consider that some species could be more difficult to identify than others, and therefore the rate of success is a crude, unweighted percentage. The compared performance of each laboratory is not included in this study because obvious rules of ethics and confidentiality. We calculated total rates of correct identifications, in both the first and the second round, as well as the same figures split by genera, to evaluate if some genera had poorer identification rates than others, and by sex, to check if males or females had different identification rates. We further compared if the rates of wrong identifications are higher for species that do not exist in the geographical zone of each participating laboratory. In other words, we tested if participating laboratories are able to identify potentially invasive ticks. We also compiled the list of references used to identify each species.

## Results

All the species of ticks classified on morphological grounds and circulated to the participants hit a 100% of correct identification rates for every species when determined by molecular methods (see Table 2). Regarding the identifications made on morphological grounds, every genera of ticks were adequately identified but for one specimen, and misclassification rates (MR) were at the level of species or stage. The total MR in the first round was of 29.6%, which decreased to 28.5% in the second round. The MR of stages was 1.6% (5 out of 306) and 0.8% (2 out of 259) in the first and second rounds, respectively. Two males of *D. marginatus* were initially considered as females during the first round of identification while there were in fact females; because numerous morphological characters allow to distinguish males and females in ixodid ticks, this mistake illustrates the existence of errors of inattention (possibly during the filling of the individual form for each tick specimen) among the misidentifications, which are not possible to address in the statistical assessment. Figure 1 shows the MR split by genera, in both the first and second rounds. It must be noted that the number of responses by participants was lower in the second round than in the first: 306 vs. 259 ticks were identified in each round. The species in the genera *Dermacentor* and *Ixodes* obtained the lowest misidentification rates, 7.27% and 13.92% in the first round, respectively. However, every specimen of *Dermacentor* was correctly identified in the second round but the misidentification rate of *Ixodes* increased to 18.03%. Within

genera, the misidentification rate varied from 5.4% for *I. ricinus* or 7.4% for *D. marginatus* or *D. reticulatus* (the three most studied tick species in Europe). Species belonging to the genus *Haemaphysalis* had misclassification rates of 19.23% and 33.33% in the first and second rounds, respectively. The species in *Hyalomma* and *Rhipicephalus* were those that had highest rates of misidentifications in both rounds, with similar figures, around 36% in *Hyalomma* and 54% in *Rhipicephalus*. The MR by species are included in Figure 2. The species was adequately identified if the specimen was a male in 71% and 72% of cases (first and second round), or in 68% in the case of females (both rounds). The misidentification rate was 100% for *R. rossicus* in the first round, a species rarely included in most morphological keys used by european tick taxonomy experts.

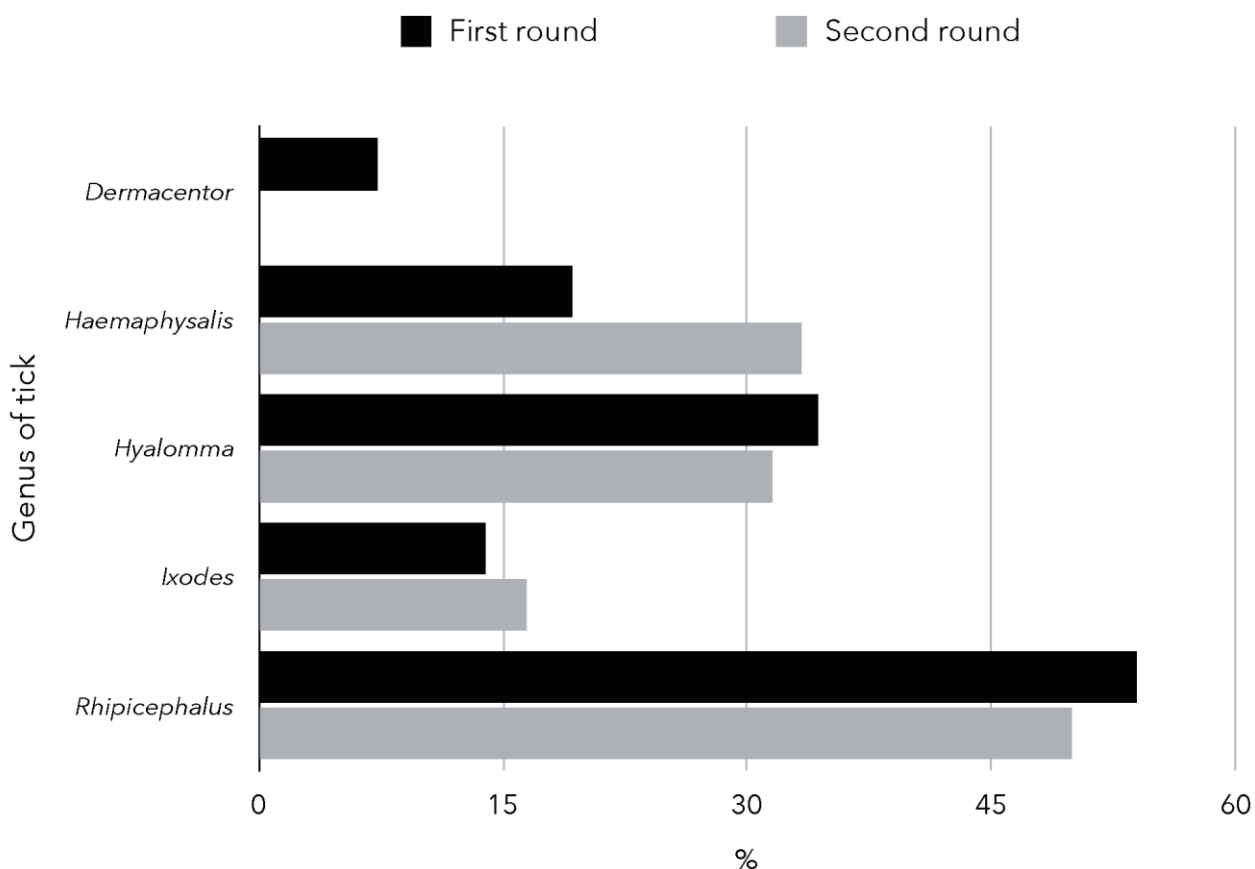


Figure 1: The misclassifications rates of ticks, split by genera (in %) in both the first and second round of morphological identification.

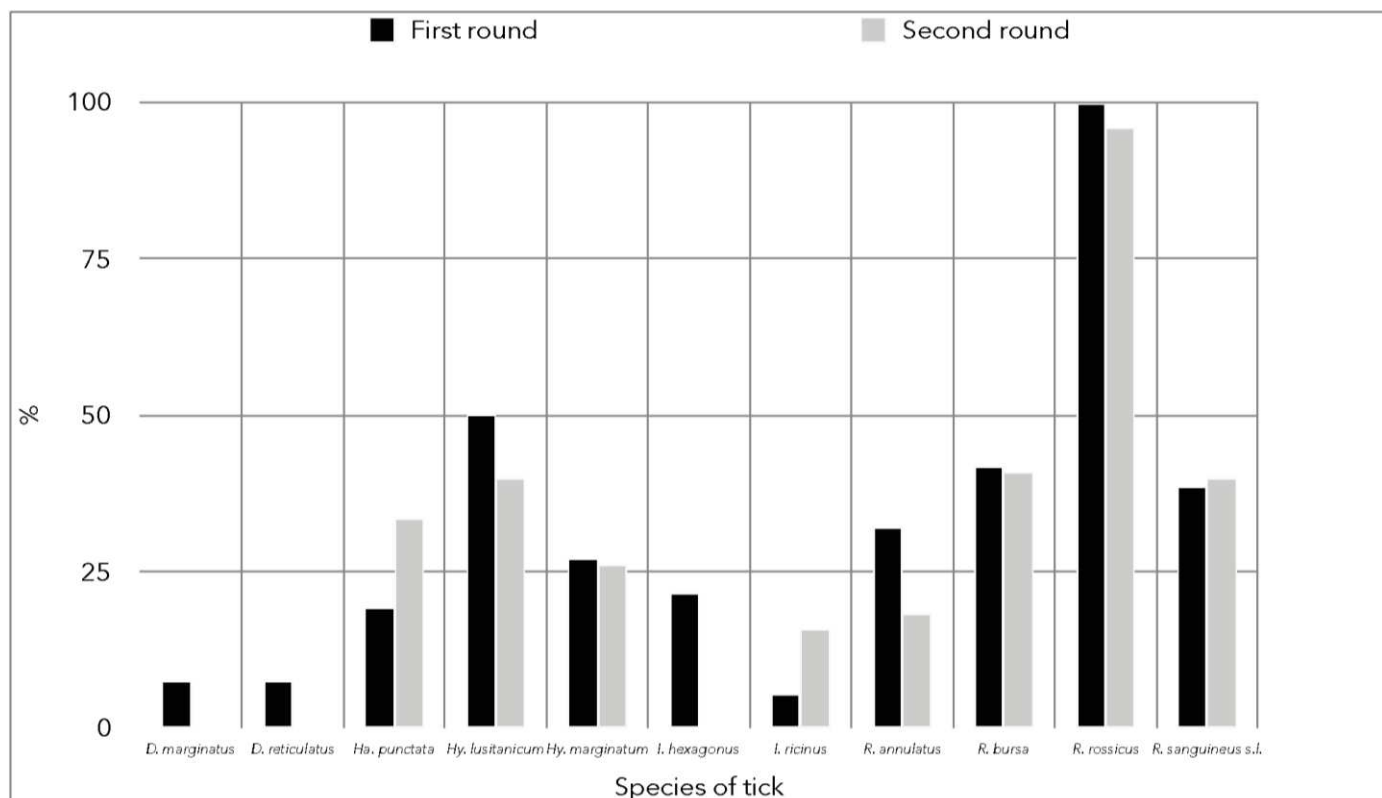


Figure 2: The misclassification rates of species of ticks (in %) in both the first and second round.

We compared the performance rate of identifications with the presence/absence of the tick in the national territory of each participating laboratory. We refer to these specimens as “endemic” or “non-endemic” if they have been reported or not in the national territory of the participating researcher. Only 8.49% of “endemic” ticks were misidentified in the first round, a value that increased to 10.45% in the second round of identifications. However, the misidentification rates of “non-endemic” ticks were 21.1% and 13.6%, in the first and second rounds, respectively. Regarding the use of bibliographical resources, 21% of specimens were identified without the help of further references, because participants were familiar with the tick, 49% used reprints for the identification (listed separately in the supplementary material), 5% used generalist book(s) that compile(s) data on species from a large territory, and 24% used both reprints and books. The self-perception of the participants about the reliability of identifications was very variable. The participants had the idea that 27% of specimens were reliably identified after a first look because they were familiar with the tick, and that 49% of specimens were identified after checking the bibliographical references. The participants had “serious concerns about the reliability of the morphological identification” of 23% of the specimens (see Figure 2). Comparing these figures about self-perception with the MR, 2% of specimens were erroneously identified in the first category, 7% in the second, and 15% in the third. However, these rough figures were

category, 7% in the second, and 15% in the third. However, these rough figures were highly variable when split by tick genera (see Figures 3 and 4). Most participants felt that identifications of *Dermacentor* and *Ixodes* were reliable. The degree of self-perception of identification reliability decreased in the species of *Haemaphysalis*. Most participants expressed “serious concerns” about the reliability of the identification of species in both *Hyalomma* and *Rhipicephalus*. The comparison of these crude figures

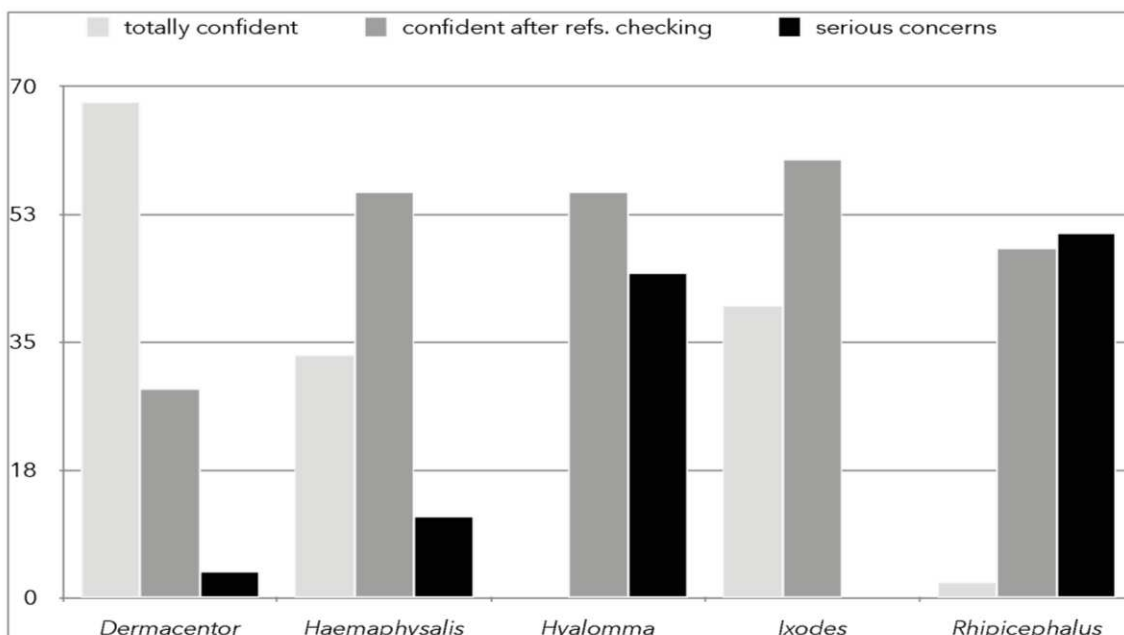


Figure 3: The degree of self-perception by participants about the reliability of the identification of ticks, split by the genus of the tick (in %).

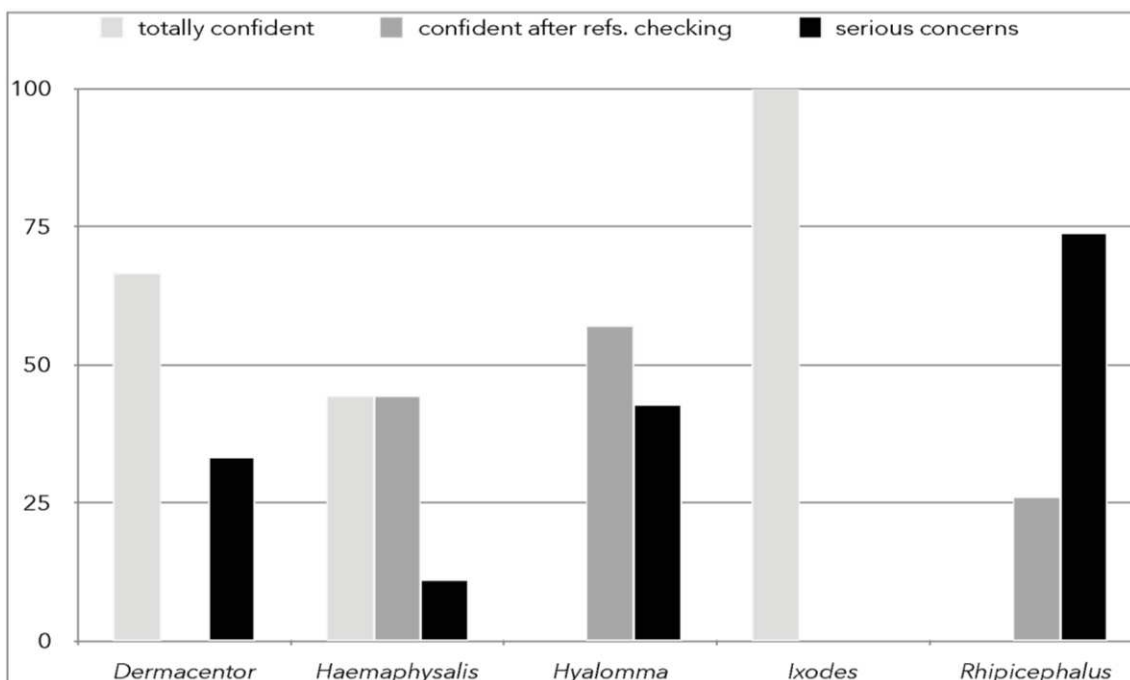


Figure 4: The percent of erroneously identified ticks (grouped by genera) according to the self-perception by participants about the reliability of the identification of ticks.

with the accuracy of the identifications provide some significant findings: participants were aware that a high percentage of the ticks in the genera *Rhipicephalus* and *Hyalomma* were probably wrongly determined.

#### Discussion

This paper reports the results of a blind test of identification of ticks carried out by 14 voluntarily enrolled laboratories in Europe. The study was intended to evaluate our capacity to identify both the well-established species of ticks which are common in the country of the participants, or to quantify the competences of the European groups to cope with potentially invasive species. The test included species of the genera *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* and *Rhipicephalus*, distributed in Europe and Northern Africa. The study was intended to focus on the most prominent species of ixodid ticks of the target region to clearly delineate the potential of the experts in the management of tick species with the highest impact on human or animal health. Results showed that species in the genera *Ixodes* and *Dermacentor* had the lowest misidentification rates, while species of *Hyalomma* and *Rhipicephalus* had the highest rates of unreliable identifications. Crude results showed about 30% of misidentifications, which decreased merely to 28% when a purposely prepared key without illustrations was circulated among participants in a second round. Claims about the possibility of spread of ticks currently reported in the Mediterranean to northern latitudes (Jaenson et al., 2012) are thus a concern after these results, since 21% of these “non-endemic” species were unreliably identified.

There are several factors that could bias the results reported here. The most obvious is the “gold standard” established for the identity of each specimen. This was done by three participants in the study, something which could arguable lead to different diagnostic criteria when the specimens were examined by other participants. The three co-authors initiating the study, identifying, and circulating the ticks were not involved in any other step of the study. Additionally, these three authors were aware also about the exact geographical origin of the ticks. However, the morphological identification of the batches of ticks matched at 100% when identified by molecular methods. For every other step, we adhered to the randomness of both batches of ticks and participants, therefore eliminating potential biases. Even at the stage of manuscript editing and final agreement of the submitted paper, the co-authors were unaware of the rates obtained by the other participants.

It is interesting to notice that the genera *Dermacentor*, *Ixodes* and *Haemaphysalis* were well known by almost every participant, with low rates of misidentifications. However, the genera *Rhipicephalus* and *Hyalomma* accumulated the highest rates of mistakes. In other words, the purely Mediterranean species were inadequately identified by most of the participating laboratories. It is however important to mention that only 6 laboratories in Mediterranean countries were involved in the test, of a total of 14. In the case of *Rhipicephalus*, the errors in classification were mainly due to the lack of a harmonized definition of the *R. sanguineus* s.l. group, which needs a re-description and the designation of a neotype of *R. sanguineus* s.s. (Nava et al., 2015). We circulated adults of both *R. rossicus*, from large batches obtained in Romania (Sándor et al., 2014) and *R. sanguineus* s.l., collected in a heavily infested kennel in Spain, which closely overlapped with the classic description of the “species” (Filippova, 1997), had highest similarity with the molecular signature reported for the Mediterranean populations (Nava et al., 2015) and closely adhered to the specimens mentioned as clade II by Dantas-Torres et al. (2013). As a further proof of the inherent difficulties of the genus *Rhipicephalus*, nobody was able to identify correctly the adults of the neglected *R. rossicus* (rarely included in most of the books used by European experts) in the first round, and only 1 laboratory managed a correct identification after a key containing the species was circulated among the participants.

Of particular interest is the fact that the circulation of the key without illustrations did not improve significantly the rate of reliable identifications, and, in some cases, introduced even higher rates of misidentifications. The second round produced poorer rates by some participants who already identified reliably a specimen in the first round. The obvious translation is that: (i) the inclusion of more species of ticks in the key produced a background noise that confused the participants, (ii) the lack of illustrations is a serious issue when only the crude text is used for the identification. The key included 2 species of the genus *Dermacentor*, 8 species of *Haemaphysalis*, 10 of *Hyalomma*, 23 of *Ixodes*, and 6 of *Rhipicephalus*. Whereas most mistakes using the keys were observed for the genera *Ixodes*, *Hyalomma*, and *Rhipicephalus*, we conclude that the “noise” generated by the new information regarding species of ticks which are strange to the participants, produced additional confusion. Being unfamiliar with these “new” species, researchers tended to identify ticks by close similarity rather than by complete identity. This explanation is further confirmed by the misclassification rates of “endemic” versus “non-endemic” species. The keys could probably help the participants



to identify the ticks with which they were not familiar, but introduced a further complication when dealing with species which they already knew, because several species (reported in the target territory) were included in the keys adding a “background noise” to the efforts of researcher.

It is necessary to stress that participants were aware in most cases of their unreliable identifications. Highest rates of confidence were obtained for the genera *Dermacentor* and *Ixodes*, meaning that participants were confident about the reliability of their identifications. Highest rates of concern about the validity of identification were obtained for species of *Hyalomma* and *Rhipicephalus*. Again, there is a high agreement between the rates of concern about the validity of classifications and the actual unreliable identifications for species of these genera.

Results from this blind test show the importance of an adequate source of information for the experts involved in the identification of ticks, both to cope with a reliable determination of the risk by a pathogen, and to adequately address further studies devoted to the epidemiology of tick-borne pathogens. Studies in multiple fields would benefit from adequate identifications of ticks. We believe that the best approach would be a curated library of specimens for reference, including every species and stages in the target territory. Such a reference collection does not exist and the forecasted difficulties in assembling such large number of species and stages render the procedure unpractical. Molecular approaches for the identification of ticks were reliable in this study, if performed with adequate primers and a careful selection of reference sequences (either produced in house or obtained from GenBank). The 5' region of the mitochondrial DNA gene *cytochrome c oxidase subunit I (coxI)* is the standard marker for DNA barcoding (Hebert, 2004). Nevertheless, 16S rRNA is a reliable marker for the tick identification at the species level (Lv et al., 2014a,b), and sequences of this fragment gene are the most available on GenBank. Moreover, other markers such as 12S rRNA or internal transcribed spacers (ITS) can be complementary for the tick classification (Lv et al., 2014a,b).

Undoubtedly, the success of DNA barcoding for any parasite species identification highly relies on the accurate morphological identification of reference specimens. Indeed, barcoding of ticks using alone the molecular approach could lead to inconsistent results (Lv et al., 2014a) and a combination of three DNA markers (*coxI*, 16S rDNA, and 18S rDNA) could efficiently separate several species of ticks (Lv et al., 2014a, b). The specific identification of ticks by molecular methods should not be considered as

definitive since it requires personal experience and adequate libraries, leading to the need of deposition of voucher specimens (i.e. Beati et al., 2013; Nava et al., 2014). The procedures used in this study show that an adequate combination of several genes and of the portions that produce the highest phylogenetic information is adequate for identification of ticks.

While the awareness about ticks and tick-borne pathogens increases worldwide, there is a lack of adequate knowledge about the identity of the most prominent species colonizing a large territory in the Western Palearctic. As far as we know, a similar blind test has never been performed in other parts of the world. It thus remains of interest to check how specialists of other regions can address the issue, or how other less known species are identified by the experts. Although other species of ticks may lack interest in circulating pathogens, they must be considered potentially confusing entities when compared with the focal species, introducing noise in reporting. We wanted to candidly show these results while claiming about the need of adequate training of experts involved in the identification of ticks, a step necessary either to address epidemiological studies or to cope with the risk by invasive species.

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## References

- Araya-Anchetta, A., Busch, J.D., Scoles, G.A., Wagner, D.M., 2015. Thirty years of tick population genetics: A comprehensive review. *Inf. Gen. Evol.* 29, 164-179.
- Arthur, D.R., 1963. *British Ticks*, London, Butterworth & Co. (Publishers) Ltd. 218 pp.
- Beati, L., Keirans, J.E., 2001. Analysis of the systematic relationships among ticks of the genera *Rhipicephalus* and *Boophilus* (Acari: Ixodidae) based on mitochondrial 12S ribosomal DNA gene sequences and morphological characters. *J. Parasitol.* 87, 32-48.
- Beati, L., Nava, S., Burkman, E. J., Barros-Battesti, D.M., Labruna, M.B., Guglielmone, A. A., Faccini, J. L., 2013. *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae), the Cayenne tick: phylogeography and evidence for allopatric speciation. *BMC Evol. Biol.* 13, 1.
- Black, W.C., Piesman, J., 1994. Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. *Proc. Nat. Acad. Sci. USA* 91, 10034-10038.
- Blumenschine, R.J., Marean, C.W., Capaldo, S.D., 1996. Blind tests of inter-analyst correspondence and accuracy in the identification of cut marks, percussion marks, and carnivore tooth marks on bone surfaces. *J. Archaeol. Sci.* 23, 493-507.
- Cordas, T., Aeschlimann, A., Morel, P.C., 1993. Étude morphologique des ixodidae s. str. (Schulze, 1937) de Suisse au microscope électronique à balayage. *Acarologia.* 34, 21-46.
- Coupe, S., Sarfati, C., Hamane, S., Derouin, F., 2005. Detection of *Cryptosporidium* and identification to the species level by nested PCR and restriction fragment length polymorphism. *J. Clin. Microbiol.* 43, 1017-1023.
- Cringoli, G., Lori, A., Rinaldi, L., Veneziano, V., Genchi, C., 2005. *Mappe parassitologiche - Zecche*. Series Edit. Giuseppe Cringoli, Napoli.
- Dantas-Torres, F., Latrofa, M. S., Annoscia, G., Giannelli, A., Parisi, A., Otranto, D., 2013. Morphological and genetic diversity of *Rhipicephalus sanguineus* sensu lato from the New and Old Worlds. *Parasit Vectors.* 6, 213.
- Dieme, C., Yssouf, A., Vega-Rúa, A., Berenger, J. M., Failloux, A. B., Raoult, D., Almeras, L. ,2014. Accurate identification of Culicidae at aquatic developmental stages by MALDI-TOF MS profiling. *Parasit Vectors.* 7, 544.
- Druzhinina, I.S., Kopchinskiy, A.G., Komoń, M., Bissett, J., Szakacs, G., Kubicek, C.P., 2005. An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea*. *Fungal Gen. Biol.* 42, 813-828.

- Estrada-Peña, A., de la Fuente, J., 2014. The ecology of ticks and epidemiology of tick-borne viral diseases. *Antiviral Res.* 108, 104-128.
- Estrada-Peña, A., Gray, J.S., Kahl, O., 1996. Variability in cuticular hydrocarbons and phenotypic discrimination of *Ixodes ricinus* populations (Acarina: Ixodidae) from Europe. *Exp. Appl. Acarol.* 20, 457-466.
- Estrada-Peña, A., Bouattour, A., Camicas, J.-L., Walker, A.R., 2004. Ticks of domestic animals in the Mediterranean region. University of Zaragoza.
- Filippova, N.A., 1997. Fauna of Russia and neighbouring countries. Arachnoidea, Ixodid ticks of subfamily Amblyomminae, Vol IV (5), Russian Academy of Sciences, Zoological Institute, New Series 145, Nauka Publishing House, St Petersburg.
- Gariepy, T.D., Lindsay, R., Ogden, N., Gregory, T.R., 2012. Identifying the last supper: utility of the DNA barcode library for bloodmeal identification in ticks. *Mol. Ecol. Res.* 12, 646-652.
- Hebert, P.D., Stoeckle, M.Y., Zemlak, T.S., Francis, C.M., 2004. Identification of Birds through DNA Barcodes. *PLoS Biol.* 2:e312.
- Heylen, D., De Coninck, E., Jansen, F., Madder, M., 2014. Differential diagnosis of three common *Ixodes* spp. ticks infesting songbirds of Western Europe: *Ixodes arboricola*, *I. frontalis* and *I. ricinus*. *Ticks Tick-borne Dis.* 5, 693-700.
- Hill, S., Provost, F., 2003. The myth of the double-blind review?: author identification using only citations. *ACM SIGKDD Explorations Newsl.* 5, 179-184.
- Hillyard, P.D., 1996. Ticks of North-West Europe. Field Studies Council, UK.
- Jaenson, T.G., Jaenson, D.G., Eisen, L., Petersson, E., Lindgren, E., 2012. Changes in the geographical distribution and abundance of the tick *Ixodes ricinus* during the past 30 years in Sweden. *Parasit. Vectors.* 5, 1
- Jongejan, F., Uilenberg, G., 2004. The global importance of ticks. *Parasitol.* 129(S1), S3-S14.
- Jore, S., Vanwambeke, S.O., Viljugrein, H., Isaksen, K., Kristoffersen, A.B., Woldehiwet, Z., Larsen, I. L., 2014. Climate and environmental change drives *Ixodes ricinus* geographical expansion at the northern range margin. *Parasit Vectors.* 7(1), 1-14.
- Kim, B.J., Lee, S.H., Lyu, M.A., Kim, S.J., Bai, G.H., Kim, S.J., Kook, Y.H., 1999. Identification of mycobacterial species by comparative sequence analysis of the RNA polymerase gene (*rpoB*). *J. Clin. Microbiol.* 37, 1714-1720.

- Kovalev, S.Y., Mikhaylishcheva, M.S., Mukhacheva, T.A., 2015. Natural hybridization of the ticks *Ixodes persulcatus* and *Ixodes pavlovskyi* in their sympatric populations in Western Siberia. *Infection. Gen. Evol.* 32, 388-395.
- Labruna, M.B., Marrelli, M.T., Heinemann, J.M., Fava, A.B., Cortez, A., Soares, R.M., Sakamoto, S.M., Richtzenhain, L.J., Marinotti, O., Schumaker, T.T., 2002. Taxonomic status of *Ixodes didelphidis* (Acari:Ixodidae). *J. Med. Entomol.* 39, 135-142.
- Leach, J.D., 1998. A brief comment on the immunological identification of plant residues on prehistoric stone tools and ceramics: results of a blind test. *J. Archaeol. Sci.* 25, 171-175.
- Li, S., Heyman, P., Cochez, C., Simons, L., Vanwambeke, S.O., 2012. A multi-level analysis of the relationship between environmental factors and questing *Ixodes ricinus* dynamics in Belgium. *Parasit Vectors* 5, 149.
- Li, S., Vanwambeke, S.O., Licoppe, A.M., Speybroeck, N., 2014. Impacts of deer management practices on the spatial dynamics of the tick *Ixodes ricinus*: A scenario analysis. *Ecol. Modell.* 276, 1-13.
- Lombard, M., Wadley, L., 2007. The morphological identification of micro-residues on stone tools using light microscopy: progress and difficulties based on blind tests *J. Archaeol. Sci.* 34, 155-165.
- Lv, J., Wu, S., Zhang, Y., Chen, Y., Feng, C., Yuan, X., Mei, L., 2014a. Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari: Ixodida). *Parasit Vectors.* 7, 93.
- Lv, J., Wu, S., Zhang, Y., Zhang, T., Feng, C., Jia, G., Lin, X., 2014b. Development of a DNA barcoding system for the Ixodida (Acari: Ixodida). *Mitochondrial DNA.* 25, 142-149.
- Maioli, M., Galletti, G., Calzolari, M., Biasi, G., Galletti, E., Dottori, M., 2012. Systematic literature review on the occurrence of ticks and tick-borne pathogens in the EU and Mediterranean Basin, EFSA Supporting Publications 9.
- Manilla, G., 1998. *Fauna d'Italia* (Vol. 36). Edizioni Calderini.
- Mclain, D.K., Wesson, D.M., Oliver, J.H., Collins, F.H., 1995. Variation in ribosomal DNA internal transcribed spacers 1 among eastern populations of *Ixodes scapularis* (Acari: Ixodidae). *J. Med. Entomol.* 32, 353-360.

- Medlock, J. M., Hansford, K. M., Bormane, A., Derdakova, M., Estrada-Peña, A., George, J. C., Kazimirova, M., 2013. Driving forces for changes in geographical distribution of *Ixodes ricinus* ticks in Europe. *Parasit Vectors*. 6, 1-11.
- Nava, S., Beati, L., Labruna, M.B., Cáceres, A.G., Mangold, A.J., Guglielmone, A.A., 2014. Reassessment of the taxonomic status of *Amblyomma cajennense* (L) with the description of three new species, *Amblyomma tonelliae* n. sp., *Amblyomma interandinum* n. sp. and *Amblyomma patinoi* n. sp., and reinstatement of *Amblyomma mixtum*, and *Amblyomma sculptum* (Ixodida: Ixodidae). *Ticks Tick-borne Dis*. 5, 252-276.
- Nava, S., Estrada-Peña, A., Petney, T., Beati, L., Labruna, M. B., Szabó, M. P., Guglielmone, A. A., 2015. The taxonomic status of *Rhipicephalus sanguineus* (Latreille, 1806). *Vet. Parasitol*. 208, 2-8.
- Nava, S., Guglielmone, A.A., Mangold, A.J., 2009. An overview of systematics and evolution of ticks. *Frontiers Biosci*. 14, 2857-2877.
- Nosek, J., Sixl, W., 1972. Central European ticks (Ixodoidea). Key for determination. *Jahrbuch der naturwissenschaftlichen Abteilung Joanneum* 1, 61-92.
- Pérez-Eid, C., 2007. Les tiques: identification, biologie, importance médicale et vétérinaire. Lavoisier Eds. 314pp.
- Sándor, A.D., Dumitrache, M.O., D'amico, G., Kiss, B.J., Mihalca, A.D., 2014. *Rhipicephalus rossicus* and not *R. sanguineus* is the dominant tick species of dogs in the wetlands of the Danube Delta, Romania. *Vet. Parasitol*. 204, 430-432.
- Vanwambeke, S.O., Artois, J., Davidson, R.K., Meyfroidt, P., Jore, S., 2016. Forest classes and tree cover gradient: tick habitat in encroached areas of southern Norway. *Exp. Appl. Acarol*. 68, 375-385.
- Yssouf, A., Flaudrops, C., Drali, R., Kernif, T., Socolovschi, C., Berenger, J. M., Parola, P., 2013. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for rapid identification of tick vectors. *J. Clin. Microbiol*. 51, 522-528.
- Zahler. M., Gothe, R., Rinder, H., 1995. Genetic evidence against a morphologically suggestive conspecificity of *Dermacentor reticulatus* and *D. marginatus* (Acari: Ixodidae). *Int. J. Parasitol*. 25, 1413-1419.
- Zhang, R.L., Zhang, B., 2014. Prospects of using DNA barcoding for species identification and evaluation of the accuracy of sequence databases for ticks (Acari: Ixodida). *Ticks Tick-borne Dis*. 5, 352-358.



Article 2

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## Outline-based morphometrics, an overlooked method in arthropod studies?



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### ABSTRACT

Modern methods allow a geometric representation of forms, separating size and shape. In entomology, as well as in many other fields involving arthropod studies, shape variation has proved useful for species identification and population characterization. In medical entomology, it has been applied to very specific questions such as population structure, reinfestation of insecticide-treated areas and cryptic species recognition. For shape comparisons, great importance is given to the quality of landmarks in terms of comparability. Two conceptually and statistically separate approaches are: (i) landmark-based morphometrics, based on the relative position of a few anatomical “true” or “traditional” landmarks, and (ii) outline-based morphometrics, which captures the contour of forms through a sequence of close “pseudo-landmarks”.

Most of the studies on insects of medical, veterinary or economic importance make use of the landmark approach. The present survey makes a case for the outline method, here based on elliptic Fourier analysis. The collection of pseudo-landmarks may require the manual digitization of many points and, for this reason, might appear less attractive. It, however, has the ability to compare homologous organs or structures having no landmarks at all. This strength offers the possibility to study a wider range of anatomical structures and thus, a larger range of arthropods.

We present a few examples highlighting its interest for separating close or cryptic species, or characterizing conspecific geographic populations, in a series of different vector organisms. In this simple application, i.e. the recognition of close or cryptic forms, the outline approach provided similar scores as those obtained by the landmark-based approach.

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### 1. Introduction

Contrary to classical morphology, the main objective of morphometrics is not to describe organisms, but to compare them. Because of the geometric constraints of modern morphometrics, data are generally collected on a non-articulated part, often a single organ (but see David et al. (1996) and Adams (1999)). A few characters are enough, provided that they are homologous.

Insect species studies generally made use of the wings because these structures are almost bidimensional and relatively rigid, reducing digitizing error; also, importantly, because the wings of many groups of insects provide a large number of landmarks. Not only are they many, they also are of good quality, called “true”

(or “anatomical”, or “traditional”) landmarks. True landmarks are considered homologous. Homology here refers to the positional equivalence of a small biological structure, as small as a point at the requisite scale. The level of homology of a landmark is governed by the precision with which it can be localized from one organism to another. Thus, true, anatomical landmarks are homologous in the sense that they are relocatable points, and according to this criterion various levels of homology have been recognized (see type I, II and III landmarks, Bookstein (1991)). A special development of type III landmarks, called “semi-landmarks” (also “sliding semi-landmarks”) allows the description of curved lines between two classical landmarks (Bookstein, 1997).

Anatomical, true landmarks are opposed to “pseudo-landmarks” used in the outline-based approach. Pseudo-landmarks describing contours or boundary outlines do not depend on the presence of true anatomical landmarks, they can exist with no

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anatomical landmark at all, or can include one or more of them. Obviously, pseudo-landmarks are of another nature than true landmarks: comparability is not expected from them separately, but from the structure they describe. Thus, if carefully chosen, like the mandible of a beetle, the genital paramere of a sandfly or an internal cell of the wing, a contour represents an homologous structure allowing interindividual and/or intergroup comparisons.

It is quite possible to develop both approaches on the same organ (Baylac and Frieß, 2005). One can see the wings of an insect as a configuration of anatomical landmarks, but the wing can be seen also as an assemblage of closed contours, i.e. the internal cells designated by the intersecting veins (Francoy et al., 2012). Moreover, other anatomical parts, because they are generally deficient in true landmarks, are only amenable to an outline analysis. For instance, many insect species, including very close species, are separated by the shape of some genital pieces. Such parts often lack anatomical landmarks, like the paramere of sandflies, the pronotum of Coleoptera (Faille et al., 2007), the genital leaflet of *Culex neavei* (Boussès et al., 2013; Garros and Dujardin, 2013) or the mandible parts of beetles (Tatsuta et al., 2009). Eggs and juvenile instars which also provide important taxonomic characters generally lack acceptable configurations of true landmarks: they can be considered through outline analyzes. Also good or natural candidates for the outline approach are the many wingless forms of insects, as for instance fleas, lice, ants, many of them of great veterinary, medical or agronomic importance. Finally, arthropods other than insects may present poorly defined landmarks but a particular anatomy which can be submitted to outline analyzes, as for instance the rounded body of ticks. In sum, the outline approach offers the possibility to study a wider range of organs, along with a wider range of arthropods.

The question is not limited however to technical considerations about presence or absence of landmarks (Rohlf, 1986), it also concerns the biological relevance of outlines. For entomologists, the primary needs are to distinguish species, especially cryptic species, and to detect them where they are not expected. Conspecific, but geographically or ecologically separated populations, are of interest too, in order to design relevant control strategies (Kaba et al., 2012) or to detect the origin of reinfestation after treatment (Felicangeli et al., 2007; Hernández et al., 2013; Gaspé et al., 2013). Does the outline approach distinguish species and populations as well as the landmark approach? We present here a few examples showing no significant difference between the two methods when it is about discriminating between close species or between conspecific populations.

## 2. Materials and methods

### 2.1. Material

#### 2.1.1. *Rhodnius prolixus* and *Rhodnius robustus*

*R. prolixus* Stal, 1859 and *R. robustus* Larrouse, 1927 are morphologically very close species with a long history of controversy among taxonomists (Bargues et al., 2010). *R. prolixus* is a major vector of Chagas disease in the northern part of Latin America, recently eradicated from Central America (Hashimoto and Schofield, 2012) but still active in Venezuela and Colombia (Gorla et al., 2010). The two species generally are found in different ecological conditions: in domestic and peridomestic structures for *R. prolixus*, in the crown of palm trees for *R. robustus*. Because of its silvatic habit preferences, *R. robustus* is not considered as an important vector of Chagas disease, but it is involved in some local transmission (Felicangeli et al., 2002). The overall morphology of the two species is very similar, although striking size differences are often observed, with *R. robustus* being generally the larger species. In

the field, there is frequently some size overlap, so specimens collected from trees are assumed to be *R. robustus*, and those collected from houses are assumed to be *R. prolixus*. Our material contains three groups previously identified by molecular tools (Monteiro et al., 2003): one is *R. prolixus* from houses in the Pampanito village (State of Trujillo, Venezuela), the second one is *R. prolixus* from palm trees collected in another locality, San José Tiznados (State of Guárico, Venezuela), and the last one is *R. robustus* from two localities of Venezuela: Pampanito and Candelaria (State of Trujillo).

We performed analyzes both combining the two *R. prolixus* habitats, as well as the two *R. robustus* origins, into single samples and considering them as separated groups. A total of 7 landmarks could be used, thus excluding the eighth used in Felicangeli et al. (2007) because of the inconsistency of the clavum position on the pictures. The outline submitted to the elliptical Fourier analysis (EFA, see Section 2.2.2) contained 5 of them, as well as the external boundary of the membranous part of the wing (Fig. 1).

#### 2.1.2. *Glossina palpalis palpalis* and *Glossina palpalis gambiensis*

*G. p. palpalis* (Robineau-Desvoidy), 1830 and *G. p. gambiensis* Vanderplank 1949 (Diptera: Glossinidae) are important vectors of sleeping sickness in West Africa. Both species hybridize readily in the laboratory but hybridized females produce fewer offspring and hybrid males are sterile (Gooding, 1988). These two subspecies are difficult to separate on morphological ground. Although males show consistent differences in the terminal dilatation of inferior claspers of their genitalia (Machado, 1954), morphological differentiation of female flies is not conclusive. The material we used here is from Ivory Coast and was collected in 2007. Male and female specimens of *G. p. palpalis* were collected in Aniassee, South of Ivory Coast. The *G. p. gambiensis* specimens came from Ganse, North of Ivory Coast. The subspecies identity of all the female and male specimens of the present study had no morphological nor molecular confirmation.

Ten landmarks were used as in Kaba et al. (2012). The EFA method was applied to the comparison of the central cell of the wing which includes five landmarks (Fig. 2). This cell is considered as having an important taxonomic significance for tsetse flies (De la Rocque et al., 2002).

#### 2.1.3. *Anopheles strodei* and *Anopheles oswaldoi*

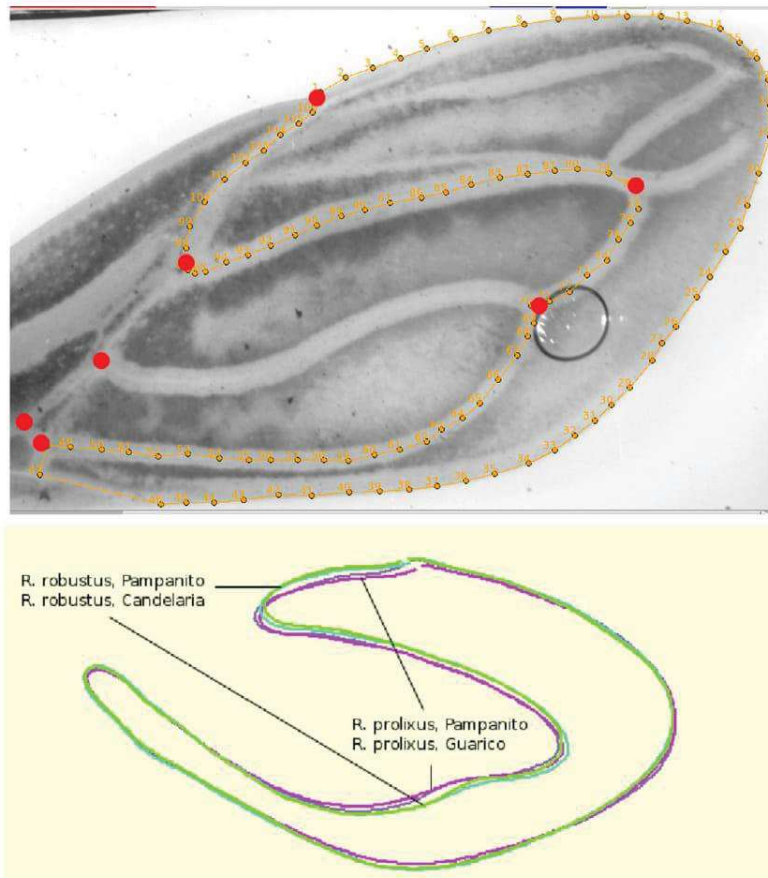
*A. oswaldoi* (Peryassú) has an unclear taxonomic and vectorial status in South America (Ruiz-Lopez et al., 2013), and *A. strodei* Root shows a high levels of morphological polymorphism (Bourke et al., 2013). Both species are difficult to distinguish based on existing dichotomous keys (Faran, 1980; Faran and Linthicum, 1981; Gonzales and Carrejo, 2007). Females were collected from the municipality of Puerto Asis, department of Putumayo, Colombia. They were induced to oviposit in the laboratory, where the male genitalia and associated immature stages of offspring were used for taxonomic identification. *A. oswaldoi* specimens were confirmed by PCR-RFLP of the rDNA marker ITS-2 (Ruiz et al., 2005). From *A. oswaldoi*, 30 adult F1 females and from *A. strodei*, 32 adult F1 females were randomly chosen for the morphometric studies.

Eleven anatomical landmarks were selected on the crossing of wing veins, while the EFA method used the contour of a small cell at the internal base of the wing, a contour which did not include any of the eleven landmarks (Fig. 3).

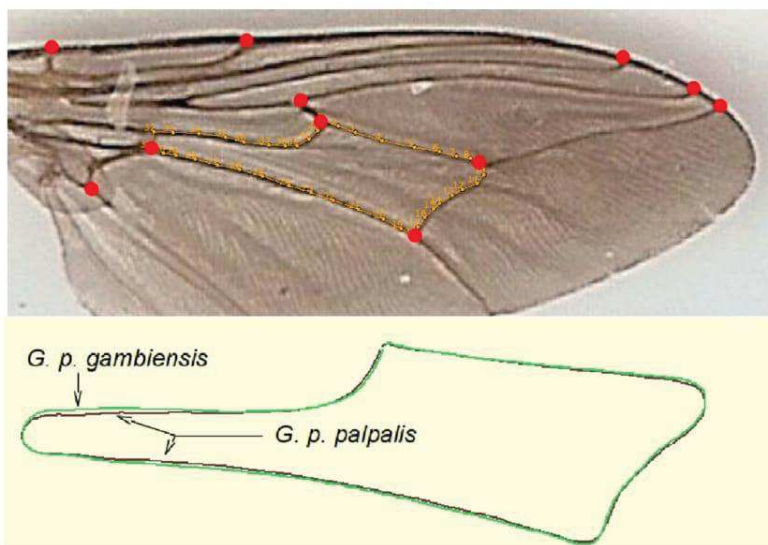
#### 2.1.4. *Ornithodoros maritimus*

*O. maritimus* is a soft tick (Family Argasidae) and part of the widespread species complex, *Ornithodoros capensis* sensu lato, exploiting colonial seabirds in tropical and sub-tropical areas of the world (Dietrich et al., 2011). The different members of this complex are known vectors of several viruses and bacteria,





**Fig. 1.** Top: Outline as digitized for a *Rhodnius* sp. wing, and seven landmarks (red dots). Bottom: Shape differences between outlines of *R. prolixus* and *R. robustus*. It can be seen that the selected outline remains stable within each species. The two samples of *R. prolixus* are almost identical in spite of having different sizes (see Fig. 5), different geographic origins (Pampanito, Guarico) and habitats (domestic, silvatic). A similar observation can be made for the two samples of *R. robustus*.



**Fig. 2.** Top: Outline as digitized for a *Glossina* sp. wing, and 10 landmarks (red dots). Bottom: Shape differences between outlines of female *Glossina palpalis palpalis* and *G. p. gambiensis*. The contours are very similar, with the elongated part of the cell thinner for *G. p. palpalis*.

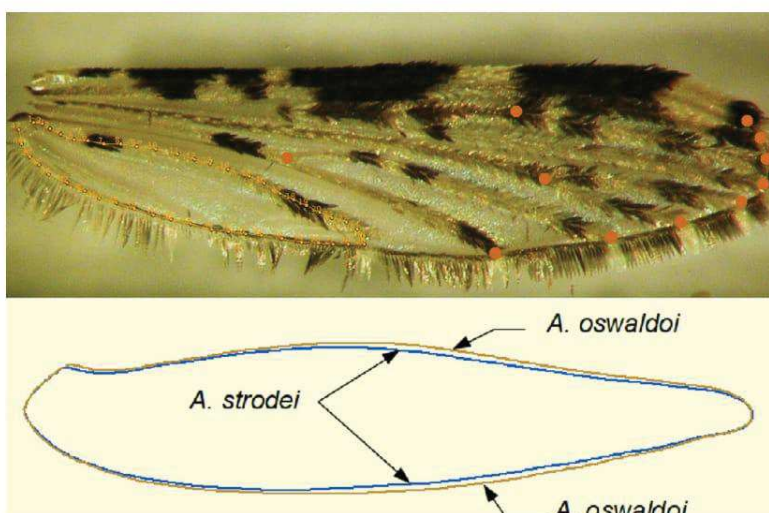


Fig. 3. Top: Outline as digitized for an *Anopheles* sp. wing, and 11 landmarks (red dots). Bottom: Shape differences between outlines of *A. strodei* and *A. oswaldoi*.

including the agents responsible for tick-borne relapsing fever and African tick bite fever (Takano et al., 2009; Dietrich et al., 2011; Dietrich et al., 2014). Although the *O. capensis* complex currently includes eight species, the actual number of species and their geographical distribution have been recently called into question (Gómez-Díaz et al., 2012). Given the lack of reliable morphological characters for identifying soft tick species in the adult life stage (Estrada-Pena et al., 2010), alternative methods are greatly needed. In the present study, two distant French populations were analyzed, both within the described distribution of *O. maritimus*: one from Rouzic, one of the islands of the Sept-Iles archipelago off the northern coast of Brittany and one from Riou, a Mediterranean island lying near the city of Marseilles. In Rouzic, 18 adult ticks were collected from around the nests of the Northern gannet (*Morus bassanus*), whereas in Riou, the 20 adult ticks analyzed came from nests of the Yellow-legged gull (*Larus michahellis*).

Fifteen landmarks were used on the ventral face of the ticks. The EFA method used the contour of the external boundary of the dorsal face (Fig. 4).

## 2.2. Statistical approaches

### 2.2.1. Landmark-based analyzes

Shape variables were obtained through the generalized Procrustes analysis (GPA) superimposition algorithm and subsequent projection of the Procrustes residuals into an euclidean space (Rohlf, 1990b). Both non-uniform and uniform components were used as shape variables. The shape variables were produced separately for males and females. These variables – also called “partial warps” (Slice et al., 1996) – actually describe the differences in shape as deviations from an average configuration of landmarks, and their principal components or a subset of them (see Section 2.2.3) were used as input for the discriminant analysis.

### 2.2.2. Outline-based analyzes

For each organism, except for the ticks, a previous study was necessary to select the outline to be used in the comparisons. Various outlines are possible on the same wing, and their discriminating power can be different (Francoy et al., 2012). We tried to select outlines containing the fewest possible landmarks, since the justification for using the outline-based approach is just that: no landmarks, or not enough of them. In each comparison, we used the elliptic Fourier analysis (EFA) (Kuhl and Giardina,

1982). Briefly, the observed contour is decomposed in terms of sine and cosine curves of successive frequencies called harmonics, and each harmonic is described by four coefficients. With this method (Kuhl and Giardina, 1982), the first harmonic ellipse parameters are used to standardize the Fourier coefficients so that they are invariant to size, rotation and the starting position of the outline trace. By doing this, the three first coefficients become constant (1, 0 and 0) and are not used in the remaining analyses. The fourth coefficient, the one related to the width-on-length ratio of the outline (Lestrel, 1989), has been used in our study.

As for the conventional Fourier analysis, the maximum number of harmonics is half the number of sample points (pseudo-landmarks). The optimal number of harmonics can be selected in various ways: by appraising the goodness-of fit using the sum of squared distances between the original data and reconstructed outline, by examining the spectrum of harmonic Fourier power, or simply by visualizing the reconstructed outlines (Lestrel, 1989, 1997; Claude, 2008). Ideally, more harmonics should be able to capture more shape parameters and produce a higher level of discrimination. However, more harmonics also inflate the digitization error and an optimal number should be selected (Crampton, 1995; Renaud et al., 1999; Firmat et al., 2010). In our study, the threshold regarding the number of harmonics was governed by the problem of high-dimensionality in the resulting data set (see next section).

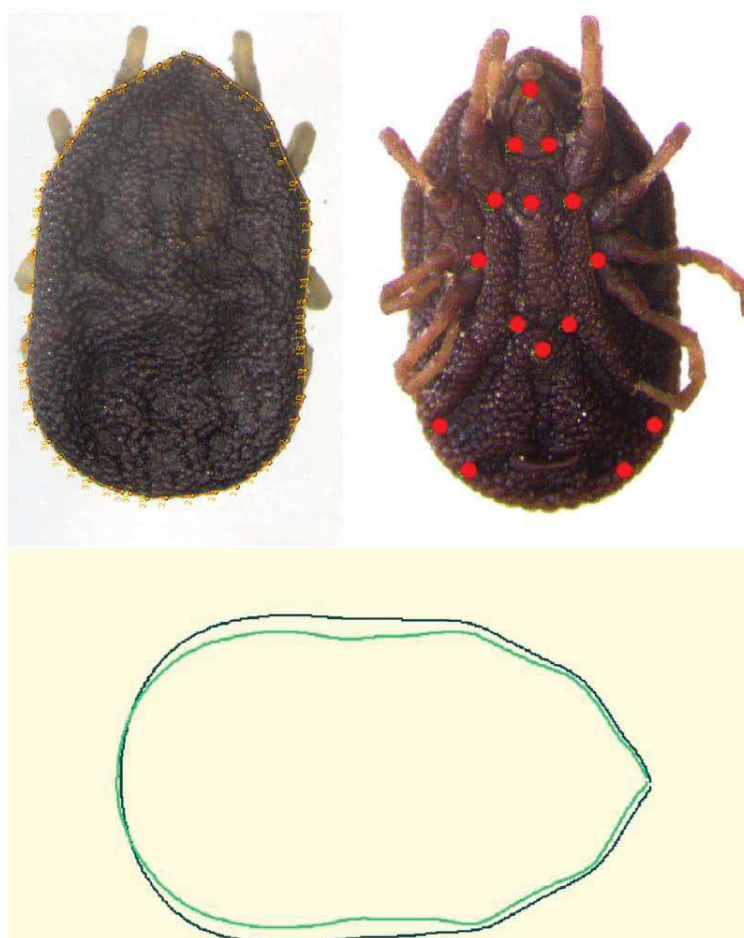
The superiority of the elliptic Fourier algorithm over other methods (Rohlf, 1990a) is now commonly accepted: the algorithm does not require the points to be equidistant (as in eigenshape analysis (Lohmann, 1983)), and the use of the normalized coefficients “saves the investigator from having to worry about aligning the images in a standard fashion” (Rohlf and Archie, 1984).

### 2.2.3. Assigning individuals to their corresponding groups

Each individual was classified after a discriminant analysis of shape variables according to a validated classification procedure, also called the jackknife classification (Manly, 2004), where each individual is allocated to its closest group without being used to help determine a group center.

Because of statistical constraints (Sheets et al., 2006), the discriminant analysis did not use the original shape variables as input. The number of shape variables produced by a two-dimensional landmark approach is two times the number of landmarks less four (Rohlf, 1996), and can be larger than the number of individuals. To circumvent the problem of high-dimensionality in the data set, a





**Fig. 4.** Top, left: Outline as digitized for *Ornithodoros maritimus*. Top, right: 15 landmarks on the ventral face (red dots). Bottom: Riou and Rouzic localities have different outlines for *Ornithodoros maritimus*.

few first principal components (PC) of shape variables were used as input for the discriminant analysis and subsequent validated classification. The size of this subset of PC was simply the number of specimens of the smallest group less one. There are other, more sophisticated ways to select for an adequate morphological subspace, but most rules of selection are heuristic or ad hoc methods (Jolliffe, 1986; Krzanowski, 1987). One of the least subjective is to select the number of retained components in each analysis that minimize the total cross-validated misclassification percentages (Baylac et al., 2003; Baylac and Frieß, 2005; Sheets et al., 2006). We used here a much simpler approach by placing the two morphometric techniques in similar statistical conditions, even if these conditions were not optimal for obtaining the best scores.

The use of the outline method also poses difficulties for the discriminant analysis. To accurately represent a curve many harmonics may be needed, each with four coefficients, so that the analyzes would require the measurement of numerous samples (Iwata, 2011). In each of our examples, the PC of the (normalized) Fourier coefficients were not derived from the total number of coefficients because it was frequently larger than the total sample. Thus, only a part of the Fourier coefficients was submitted to the PCA, and only a part of the first PC was used as input for the DA, where the same rule as above (“the smallest group less one”) was adopted.

#### 2.2.4. Size estimation and its contribution to shape discrimination

Whichever way shape is computed, the estimate of size in modern morphometrics is restricted to the geometric area

examined and is contained in a single variable: the root-squared area of a contour (instead of its perimeter (Lestrel, 1997)), or the “centroid size” for a configuration of landmarks (Bookstein, 1991). The centroid size is defined as the square root of the sum of the squared distances between the center of the configuration of landmarks and each separate landmark (Bookstein, 1991). The root-squared area of a contour was actually the one of the starting ellipse in the Fourier decomposition (Rohlf, 1990a).

The estimate of size was then used to evaluate its contribution to species discrimination based on shape: in each specific study, the coefficient of determination was computed after regressing the first (and generally unique) discriminant function on size.

#### 2.2.5. Measurement error

The precision of both techniques was estimated using the repeatability index. For each sample, we used an ANOVA design on repeated measurement performed on the totality the sample. The repeatability was measured using different variance components: the among-measurement ( $V_a$ ) and the within-measurement ( $V_w$ ) components. It was expressed in percentage, and computed as the ratio of  $V_a/(V_a + V_w)$  (Arnqvist and Mårtensson, 1998), which is the reverse of the measurement error as suggested by Yezerinac et al. (1992). The input variables for the ANOVA were the four first principal components of shape. We only report the average and standard deviation of the repeatability across species and comparisons for each morphometric approach.

2.3. Software

All analyzes and graphical output were obtained through the various modules of the CLIC package (Dujardin and Slice, 2006; Dujardin et al., 2010): the COO module to digitize either landmarks or curves, the MOG and PAD modules for the landmark-based approach, the FOG module for EFA analyzes and subsequent validated classifications, and the VAR module for estimating the repeatability of size and shape.

3. Results

The quality of group discrimination was generally satisfactory in both methods (Table 1, see the “%” columns) (86% of correct assignation on average versus 78% for landmarks). On average, the repeatability was slightly higher for outlines (85 ± 11%) than for landmarks (79 ± 17%). The contribution of size to the discrimination appears in Table 1 as a percentage in the “r2%” column. On average, it was lower in the landmark-based approach (23 ± 18% versus 33 ± 23%).

3.1. Group distinction

3.1.1. *R. prolixus* and *R. robustus*

The landmark approach produced relatively satisfactory results: 15 (out of 17) *R. robustus* were correctly assigned, while 2 (out of 24) *R. prolixus* were wrongly attributed to *R. robustus*. More impressive results were obtained from outlines, where no *R. prolixus* was assigned to the *R. robustus* species, and only one *R. robustus* (out of 18) was confounded with *R. prolixus*. The *R. robustus* outline was not affected by the different geographic areas (Fig. 1) and the different sizes (Fig. 5). Similarly, the two samples of *R. prolixus*

showed a very close outline in spite of different habitats (Fig. 1) and very different sizes (Fig. 5).

3.1.2. *G. p. palpalis* and *G. p. gambiensis*

There was a notable difference in the morphometric discrimination of subspecies according to the sex. Both morphometric techniques produced comparable and unsatisfactory results for male specimens (67–70% versus 66–77%, for landmarks and outlines, respectively). For females, only the outline comparisons produced satisfactory results (80–85%, versus 70–73% for landmark comparisons). Thus, outlines could only discriminate the two forms for females.

3.1.3. *A. strodei* and *A. oswaldoi*

In this simple pairwise comparison of female mosquitoes, both landmarks and outlines performed satisfactorily (more than 83% were correctly classified). The scores obtained using landmarks (84%–86%) were slightly inferior to the ones obtained using the contour of a single internal cell of the wing (87%–94%).

3.1.4. *O. maritimus*

The geographic origin of the 39 specimens of *O. maritimus* was almost perfectly recognized by the body contour (94%–100%), while imperfectly – but still consistently – by the landmark approach (80%–83%).

4. Discussion

For analyzing curves containing anatomical landmarks, as is the case for most of the material used in this study, and in spite of some unresolved drawbacks (Sheets et al., 2004), the sliding semi-landmark approach becomes more frequently adopted in

Table 1

Validated classification based on shape. “n”, sample sizes; “%”, percentages of correct assignation of individuals to relevant species or group. “LM”, the number of true landmarks used; “H”, the number of harmonics corresponding to the number of normalized Fourier coefficients used as input for the principal component analysis; “r2%”, percent contribution of size to the first discriminant factor based on shape. The analyzes comparing *R. robustus* and *R. prolixus* were repeated with two different sampling distribution: either 14, 10 (both *R. prolixus*) and 18 *R. robustus* (mixing their geographic origins, i.e. Candelaria and Pampanito), or 14, 10, 8 *R. robustus* from Candelaria and 10 from Pampanito. 23/22, 30/22, 17/18 or 7/8 mean a different sample according to the approach: the first number refers to the sample size analyzed with landmarks, the second number to the one analyzed with outlines; F, females; M, males.

Species and groups	n	Validated classifications					
		Landmarks			Outlines		
		LM	%	r2%	H	%	r2%
Tsetse flies, females		10		1	11		0
<i>G. p. palpalis</i>	26		73			80	
<i>G. p. gambiensis</i>	20		70			85	
Tsetse flies, males		10		2	9		2
<i>G. p. palpalis</i>	15		67			66	
<i>G. p. gambiensis</i>	23/22		70			77	
Mosquitoes		11		27	13		59
<i>A. strodei</i>	32		87			87	
<i>A. oswaldoi</i>	30/22		93			94	
Kissing bugs, 2 groups		7		45	10		41
<i>R. prolixus</i> (2 habitats)	24		83			100	
<i>R. robustus</i>	17/18		94			94	
Kissing bugs, 3 groups		7		39	10		40
<i>R. prolixus</i> (domestic)	14		86			92	
<i>R. prolixus</i> (silvatic)	10		70			80	
<i>R. robustus</i>	17/18		88			94	
Kissing bugs, 4 groups		7		11	10		52
<i>R. prolixus</i> (domestic)	14		86			92	
<i>R. prolixus</i> (silvatic)	10		70			80	
<i>R. robustus</i> (Candelaria)	7/8		100			87	
<i>R. robustus</i> (Pampanito)	10		77			100	
Soft ticks		15		34	9		40
<i>O. maritimus</i> Rouzic	18		80			94	
<i>O. maritimus</i> Riou	20		83			100	



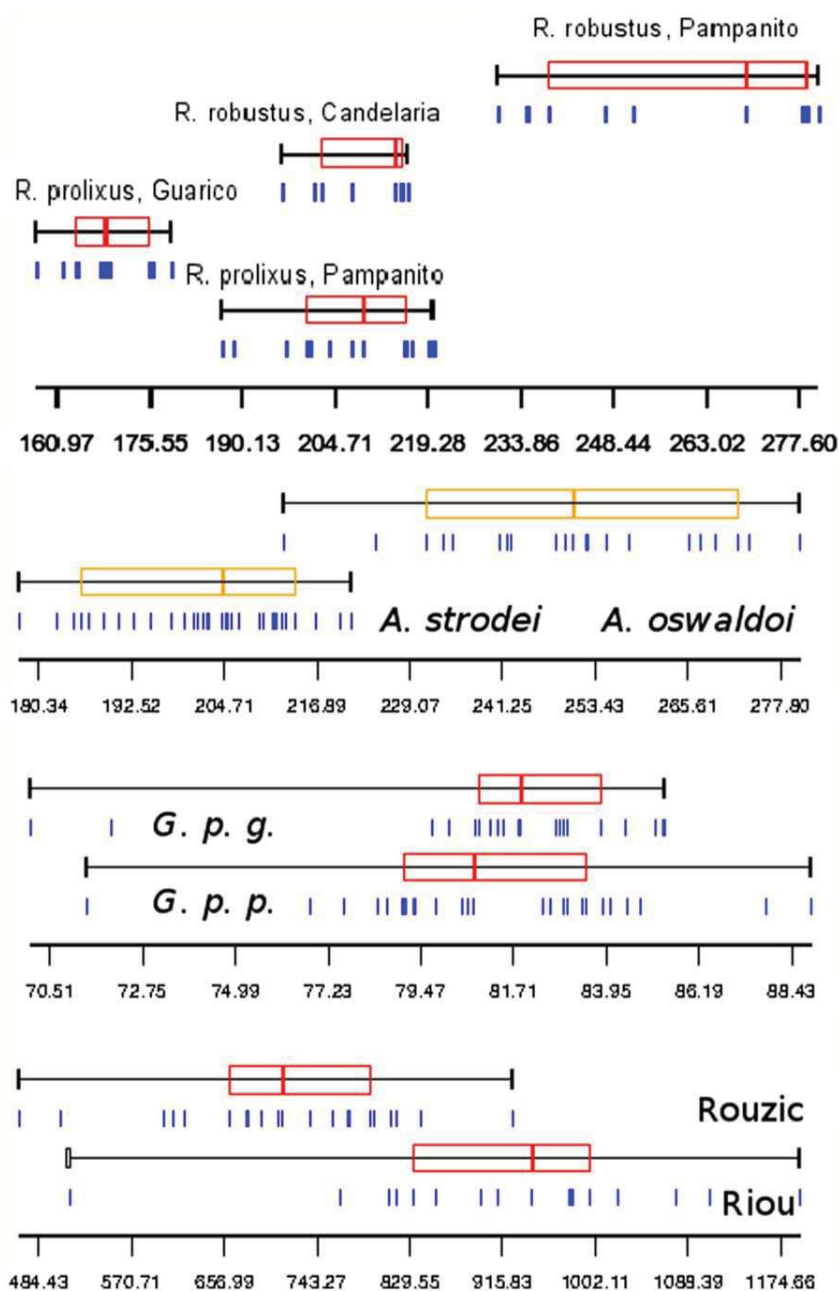


Fig. 5. Size variation as estimated by the square root of outline areas. From top to bottom: kissing bugs (*Rhodnius robustus* and *R. prolixus*), mosquitoes (*Anopheles strodei* and *A. oswaldoi*), tsetse flies (*G. p. p.* = *Glossina palpalis palpalis*, and *G. p. g.* = *Glossina p. gambiensis*) and ticks (*Ornithodoros maritimus* from two localities, Riou and Rouzic).

arthropods (Yee et al., 2011; Palestrini et al., 2012; Stephens and Juliano, 2012). The main benefit is that both landmarks and semi-landmarks can be arranged in one data set submitted to a single analysis. The discriminating power has been shown to be similar to the elliptic Fourier analysis (EFA) and some other outline methods (Sheets et al., 2006). The semi-landmark approach considers both curves and landmarks. As long as it requires true landmarks in order to describe the curves, it is a landmark-dependent method. The outline-based approach is completely different, as outlines may or may not include landmarks; it is a landmark-free method. Thus, for corresponding structures having very few landmarks or simply lacking landmarks, it is advisable to turn to the classical outline analysis based on EFA. We showed here that this option does not mean lower discriminating power. The EFA applies to closed contours only. Other statistical techniques can be applied

to curves, closed or not (Lohmann, 1983), as reviewed by Rohlf (1990a) and, more recently, by Sheets et al. (2006). The attractive feature of the EFA algorithm, which is used here, is that points need not to be equidistant nor equal in number.

#### 4.1. Data collection

In both approaches, we used manual data collection: a manual collection of landmarks, and a manual digitization of contours. For the landmark approach, it is not advisable to mix data from different users because of the “user effect”: small but persistent differences may arise between different users in pointing to the exact location of some landmarks, with effects on the final output (Dujardin et al., 2010). Because of the high comparability of each separate landmark, it is mandatory to collect each one very



carefully. Since the comparability of points (pseudolandmarks) is not a problem in outline digitization, it might be expected that the user effect is lower. However, it has been shown that different users, hence different ways to capture the contour, may have a more (Firmat et al., 2010) or less (Qing-Bin and Xin-Li, 2012) significant effect on the final output. We did not test for the compared magnitude of the user effect between landmark and outline techniques, but we show that, for the same images, both techniques suffered from a more or less similar amount of measurement error.

For both landmark and outline approaches, the automated capture of data could appear as the solution, but no such solution has been widely adopted so far. Automated identification of landmarks was recently proposed for Dipteran wings (Houle et al., 2003; Palaniswamy et al., 2010), but it may be a very difficult task when applied to wings covered with scales (the *Anopheles* in this study) or when crosses between veins are wide zones instead of points (kissing bugs). Automated curve tracing is already part of many imagery software and might look easier to generalize. It is a possible task when the contour is the external boundary of the body, as can be the case for rounded bodies as for instance in mussels (Ferson et al., 1985; Claude, 2008). The problem becomes more difficult when the perimeter to be digitized is a substructure of the main image, an internal part of the body. These and other obstacles in performing automatic captures of outlines have led some authors to rely on the more flexible manual collection (Sheets et al., 2006; Firmat et al., 2010).

Even if adopting the same starting point is not mandatory for the EFA method we used, we digitized the contour trying to start from the same point. This strategy, when possible, allows other methods to be applied to the same set of pseudolandmarks, as for instance the Procrustes superposition (after equalizing the number of points) used for our graphical output (Figs. 1–4).

#### 4.2. Statistical analyzes

The reclassification procedure used in the present study was based on the discriminant analysis. This analysis requires that there be more specimens than variables in the smallest group. As we were unable to increase the sample sizes, we used a principal component analysis (PCA) to reduce the dimensionality of the data: the scores of a limited number of PC was used instead of the original data. The criterion to determine the number of PC selected was simply the number of specimens in the smallest group less one. Thus, in our study, the selected number of PC can be deduced from Table 1 by looking at the sample sizes in each comparison (see the “n” column). More sophisticated procedures exist (Baylac and Frieß, 2005), some of them not based on the PCA method (Sheets et al., 2006).

Because of the PCA-based dimension reduction method, only a part of shape variation, although generally a consistent part, was used to classify individuals, which probably lowered the power of the analyzes. There are two more reasons to think that low sample sizes probably represented a heavier handicap for the outline approach than for the landmark one. First, according to Baylac and Frieß (2005), the first PCA axes of Fourier coefficients rarely account for a significant proportion of the between-group differences. Second, we were not even able to use the maximum possible number of harmonics because the total sample was too low for the PCA to include them all. Table 1 indicates the number of harmonics used for the PCA (see the column “H”; which can also be deduced by computing the total sample of each group less 1, and divided by 4). Between close or cryptic species, and *a fortiori* between conspecific populations, we expect to have discrete, localized differences, if any. Such differences are captured by the higher level harmonics, while the first few ones generally describe more global differences in shape (Lestrel, 1989).

Thus, poor sample sizes could have reduced the discriminating power of outlines relative to landmarks. In spite of that, the outline classification showed satisfactory results.

#### 4.3. Compared scores

The morphometric methods used here to build shape representation remove the isometric change of size, but were not designed to remove the allometric effect of size on shape variation. Thus, shape variables, be they partial warps or normalized Fourier coefficients, contain an allometric residue. Here, the effect of this residue on shape discrimination was estimated by regressing the shape-derived discriminant factor on size variation; which is reported in the column “r2%” of Table 1. On average, it was slightly less present in the landmark approach, which suggests the landmark-based approach might provide a slightly greater independence between size and shape.

##### 4.3.1. *R. prolixus* and *R. robustus*

Previous morphometric studies based on landmarks could distinguish the two species to some extent, but in the favorable situation of allopatry (samples coming from distinct geographic areas) (Matias et al., 2001; Villegas et al., 2002; Márquez et al., 2011). In our material, we compared the two species from samples collected in the same village (Pampanito, Venezuela).

Morphological differences between the two taxa were frequently thought to be related to their separate environment, domestic for *prolixus* and silvatic for *robustus*. In our material, we could compare a sample of domestic *R. prolixus* (Pampanito) with another one collected in silvatic conditions in another locality (Guárico). In spite of coming from different habitats and localities, the two *R. prolixus* samples were similar, and remained distinct from *R. robustus*.

Both landmarks and outlines did a relatively good job, with again better results obtained from outlines. Actually, the outline-based distinction was almost perfect, since only one *R. robustus* out of 18 was confounded with *R. prolixus*, and no *R. prolixus* were confounded with *R. robustus*. Moreover, these outline-scores were not affected by subdividing the *R. robustus* sample according to its geographic origin. Within both species, the stability of the outline was remarkable: it was constant whatever the size (Fig. 5), the habitat and the geographic origin (Table 1).

As for previous studies on these two close species, the shape differences could not be clearly separated from the size variation (see the column “r2%” of Table 1). Feliciangeli et al. (2007) showed that in silvatic conditions size variance of *R. prolixus* may be significantly smaller. Size is generally considered more evolutionarily labile than shape, but it seems to have played a major role in the evolutionary divergence between *R. prolixus* and *R. robustus* (Fig. 5).

##### 4.3.2. *Tsetse flies*

Males of *G. p. palpalis* and *G. p. gambiensis* were unsatisfactorily discriminated by both landmarks and outlines. Since each individual of this study was not identified by a more accurate criterion than its geographic origin, we cannot certify that the two groups were completely homogeneous. However, in the case of these two subspecies, for which the taxonomic status is still discussed, the problem concerns particularly the females. Males indeed can be recognized by observable variations of the genitalia (terminal dilatations of inferior clusters). Fortunately, it appears that, for females, modern morphometrics can contribute to their identification, especially by using the boundary of the central cell (80%–85%).



### 4.3.3. Mosquitoes

Mosquitoes represent a group of insects where very few outline-based morphometric analyses have been published (Garros and Dujardin, 2013). Yet the first application of EFA to the shape of living organisms was the quantitative analysis of the boundary of mosquito wings (Rohlf and Archie, 1984). The material of this seminal study was a set of drawings coming from an entomological book (plates 1 to 127 in Carpenter and LaCasse (1955)). As far as we know, no other outline studies have been performed on mosquitoes, except a study of the genital leaflet of *C. neavei* (Boussès et al., 2013; Garros and Dujardin, 2013), and the use of sliding-landmarks combining the posterior edge of the wing with a set of true landmarks in *Aedes aegypti* and *Aedes albopictus* (Stephens and Juliano, 2012). The outline used in our mosquito sample was this posterior edge united to the anal vein of the wing (Fig. 3). None of the landmarks used for the landmark-based approach was included in this contour, but it could still discriminate the two close species.

### 4.3.4. Soft ticks

Fifteen landmarks of the ventral face were used, all of them however of type II, i.e. landmarks providing less homology (Bookstein, 1991). In spite using low quality landmarks, good scores were obtained, but the contour of the dorsal face of the tick provided even better scores. Whatever the approach, significant shape divergence was disclosed between geographic populations of the same species. These differences could reflect adaptation to different hosts and/or geographic isolation (two remote islands). From these initial results, we expect this approach may perform well in species discrimination within the *O. capensis* group.

## 5. Conclusion

Our four case-studies do not (and cannot) claim superiority of one technique over another, but do suggest that one should not overlook the outline approach for discriminating close forms. It produced high scores of correct group allocation, it suffered from a relatively low measurement error and could be applied to various organs of the body, including or not relocatable landmarks. Our study suggests that if the main objective is to distinguish morphologically close entities, and even if a landmark-based approach is possible, one should consider also to test the outline technique.

In some organisms other than arthropods where such comparisons have been performed, either the two methods were considered as equivalent (Jensen et al., 2002) or the outlines were said to perform better (Loy et al., 2000; Pavlinov, 2000; Baylac and Frieß, 2005; Albutra et al., 2012). A recent study on Hymenoptera wings showed that landmarks performed better than (internal wing cells) outlines, and that their combination provided the best results (Francoy et al., 2012). In our various examples involving arthropods, similar or even better discrimination scores were attained using the outline-based approach. Although our sample sizes were generally low, similar performances of outlines in four comparisons involving quite different organisms (ticks, tsetse flies, mosquitoes and kissing bugs) is a significant signal. It suggests that for discriminating close forms, the choice of an outline-based analysis can be as relevant as the choice of a landmark-based one. Ideally, the outline should be selected in areas of the body where landmarks are lacking. This is not to say that, in other applications than simple discrimination, the outline approach would be less informative. In general, there was a powerful biological signal in the variation of outlines for arthropods (Foster and Kaesler, 1988; Monti et al., 2001; Baltanas et al., 2002; Faille et al., 2007; Tatsuta et al., 2009), as well as for other organisms like fishes (Loy et al., 2000), birds (Sheets et al., 2006), mammals (Renaud

et al., 1996, 1999; Pavlinov, 2000; Renaud and Michaux, 2003; Friess and Baylac, 2003) and plants (Yamanaka et al., 2001; Jensen et al., 2002; Iwata et al., 2010; Iwata, 2011).

The objective of this study was to illustrate the interest of a relatively neglected method in modern morphometrics as applied to arthropods. We show that outline analyses can be applied in complement or as an alternative to the landmark-based approach.

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## References

- Adams, D.C., 1999. Methods for shape analysis of landmark data from articulated structures. *Evol. Ecol. Res.* 1 (8), 959–970.
- Albutra, Q.B., Torres, M.A.J., Demayo, C.G., 2012. Outline and landmark based geometric morphometric analysis in describing sexual dimorphism in wings of the white stem borer (*Schirpophaga innotata* walker). *ABAH* 4 (1), 5–14.
- Arngqvist, G., Mårtensson, T., 1998. Measurement error in geometric morphometrics: empirical strategies to assess and reduce its impact on measure of shape. *Acta Zool. Acad. Sci. Hung.* 44 (1–2), 73–96.
- Baltanas, A., Alcorlo, P., Danielopol, D.L., 2002. Morphological disparity in populations with and without sexual reproduction: a case study in *Eucypris virens* (Crustacea: Ostracoda). *Biol. J. Linn. Soc.* 75 (1), 9–19.
- Bargues, M.D., Schofield, C., Dujardin, J.P., 2010. The phylogeny and classification of the triatominae. In: Telleria, J., Tibayrenc, M. (Eds.), *American Trypanosomiasis: Chagas disease, One hundred years of research*.
- Baylac, M., Frieß, M., 2005. Fourier descriptors, Procrustes superimposition, and data dimensionality: an example of cranial shape analysis in modern human populations. In: *Modern Morphometrics in Physical Anthropology*, Slice ed. p. 145–165 (Chapter 6).
- Baylac, M., Villemant, C., Simbolotti, G., 2003. Combining geometric morphometrics with pattern recognition for the investigation of species complexes. *Biol. J. Linn. Soc.* 80 (1), 89–98.
- Bookstein, F.L., 1991. *Morphometric Tools for Landmark Data. Geometry and Biology*. Cambridge University Press, NY.
- Bookstein, F.L., 1997. Landmark methods for forms without landmarks: localizing group differences in outline shape. *Med. Image Anal.* 1, 225–243.
- Bourke, B.P., Oliveira, T.P., Suesdek, L., Bergamo, E.S., Sallum, M.A., 2013. A multi-locus approach to barcoding in the *Anopheles strodei* subgroup (Diptera: Culicidae). *Parasites Vectors* 6. <http://dx.doi.org/10.1186/1756-3305-6-111>.
- Boussès, P., Dehecq, J.S., Brengues, C., Fontenille, D., 2013. Updated inventory of mosquitoes (Diptera: Culicidae) of the island of La Réunion, Indian ocean. *Bull. Soc. Pathol. Exot.* 106, 113–125.
- Carpenter, S.J., LaCasse, W.J., 1955. *Mosquitoes of North America (north of Mexico)*. Univ. California Press, Los Angeles.
- Claude, J., 2008. *Morphometrics with R*. Springer Science+Business Media, LLC, ISBN 978-0-387-77789-4, p. 330.
- Crampton, J.S., 1995. Elliptic Fourier shape analysis of fossil bivalves: some practical considerations. *Lethaia* 28, 179–186.
- David, B., Dommergues, J.L., Magniez-Jannin, F., 1996. Exploration d'un espace morphologique les carabes (Coleoptera) du nord-est de la France. *C. R. Acad. Sci., Life Sci.* 319, 835–842.
- De la Rocque, S., Geoffroy, B., Michel, J.F., Borne, F., Solano, P., Meunier, J.Y., Cuisance, D., 2002. Tsetse flies wings, an identity card of the insect? *Parasite* 9 (3), 275–281.
- Dietrich, M., Gómez-Daz, E., McCoy, K.D., 2011. Worldwide distribution and diversity of seabird ticks: implications for the ecology and epidemiology of tick-borne pathogens. *Vector-Borne Zoonotic Dis.* 11 (5), 447–453.
- Dietrich, M., Lebarbenchon, C., Jaeger, A., Le Rouzic, C., Bastien, M., Lagadec, E., McCoy, K.D., Pascalis, H., Le Corre, M., Dellagi, K., Tortosa, P., 2014. *Rickettsia* spp. in Seabird Ticks from Western Indian Ocean Islands, 2011–2012. *Emerg. Infect. Dis.* 20 (5), 838–842.
- Dujardin, J.P., Kaba, D., Henry, A.B., 2010. The exchangeability of shape. *BMC Res. Notes* 3, 266. <http://dx.doi.org/10.1186/1756-0500-3-266>.
- Dujardin, J.-P., Slice, D.E., 2006. *Contributions of Morphometrics to Medical Entomology*. John Wiley & Sons, Inc. <<http://dx.doi.org/10.1002/9780470114209.ch25>>, p. 435–447.
- Estrada-Pena, A., Mangold, A.J., Nava, S., Venzal, J.M., Labruna, M., Guglielmo, A.A., 2010. A review of the systematics of the tick family Argasidae (Ixodida). *Acarologia* 50, 317–334.
- Faille, A., Déliot, P., Quéinnec, E., 2007. A new cryptic species of Aphaenops (Coleoptera: Carabidae: Trechinae) from a french Pyrenean cave: Congruence between morphometrical and geographical data confirm species isolation. *Ann. soc. entomol. Fr.* 43 (3), 363–370.



- Faran, M.E., 1980. Mosquito studies (Diptera: Culicidae) XXXIV a revision of the *Albimanus* section of the subgenus *Nyssorhynchus* of *Anopheles*. *Contrib. Am. Entomol. Inst.* 15, 1–214.
- Faran, M.E., Linthicum, K.J., 1981. A handbook of the species of *Anopheles* (*Nyssorhynchus*) (Diptera: Culicidae). *Mosq. Syst.* 13 (1), 1–81.
- Feliciangeli, M.D., Dujardin, J.P., Bastrenta, B., Mazzarri, M., Villegas, J., Flores, M., Muñoz, M., 2002. Is *Rhodnius robustus* (Hemiptera: Reduviidae) responsible for Chagas disease transmission in Western Venezuela? *Trop. Med. Int. Health* 7 (3), 280–287.
- Feliciangeli, M.D., Sanchez-Martin, M., Marrero, R., Davies, C., Dujardin, J.P., 2007. Morphometric evidence for a possible role of *Rhodnius prolixus* from palm trees in house re-infestation in the State of Barinas (Venezuela). *Acta Trop.* 101, 169–177.
- Ferson, S., Rohlf, F.J., Koehn, R.K., 1985. Measuring shape variation of two-dimensional outlines. *Syst. Zool.* 34 (1), 59–68.
- Firmat, C., Gomes-Rodriguez, H., Renaud, S., Claude, J., Hutterer, R., Garcia-Talavera, F., Michaux, J., 2010. Mandible morphology, dental microwear, and diet of the extinct giant rats *Canariomys* (Rodentia: Murinae) of the Canary Islands (Spain). *Biol. J. Linn. Soc.* 101, 28–40.
- Foster, D.W., Kaesler, R.L., 1988. Shape analysis, ideas from the Ostracoda. In: McKinney (Ed.), *Heterochrony in Evolution: A Multidisciplinary Approach*, N.Y. Topics in Geobiology.
- Francoy, T.M., Faria Franco, F., Roubik, D.W., 2012. Integrated landmark and outline-based morphometric methods efficiently distinguish species of *Euglossa* (Hymenoptera, Apidae, Euglossini). *Apidologie* 43, 609–617.
- Friess, M., Baylac, M., 2003. Exploring artificial cranial deformation using elliptic fourier analysis of procrustes aligned outlines. *Am. J. Phys. Anthropol.* 122 (1), 11–22.
- Garros, C., Dujardin, J.P., 2013. Genetic and phenetic approaches to *Anopheles* systematics. In: *Anopheles mosquitoes – New insights into malaria vectors*. <<http://www.intechopen.com/books/export/citation/BibTex/anopheles-mosquitoes-new-insights-into-malaria-vectors/genetic-and-phenetic-approaches-to-anopheles-systematics>>.
- Gaspe, M.S., Gurevitz, J.M., Gürtler, R.E., Dujardin, J.P., 2013. Origins of house reinfestation with *Triatoma infestans* after insecticide spraying in the Argentine Chaco using wing geometric morphometry. *Infect Genet Evol.*, p. 93–100.
- Gómez-Díaz, E., Morris-Pocock, J.A., Gonzalez-Solis, J., McCoy, K.D., 2012. Trans-oceanic host dispersal explains high seabird tick diversity on cape verde islands. *Biol. Lett.* 8, 616–619.
- Gonzales, R., Carrejo, N., 2007. Introducción al estudio taxonómico de *Anopheles* de Colombia. Claves y notas de distribución. Cali: Editorial Litocencia, p. 237.
- Gooding, R.H., 1988. Preliminary analysis of genetics of hybrid sterility in crosses of *Glossina palpalis palpalis* (robineau-desvoidy) and *Glossina palpalis gambienseis vanderplanck*. *The Can. Entomol.* 120 (11), 997–1001.
- Gorla, D., Ponce, C., Dujardin, J.P., Schofield, C.J., 2010. Control strategies against triatominae. In: Telleria, J., Tibayrenc, M. (Eds.), *American Trypanosomiasis Chagas Disease. One Hundred Years of Research*. Elsevier, pp. 233–245 [Chapter 10].
- Hashimoto, K., Schofield, C., 2012. Elimination of *Rhodnius prolixus* in Central America. *Parasites Vectors* 5 (1), 45 <<http://www.parasitesandvectors.com/content/5/1/45>>.
- Hernández, M.L., Dujardin, J.P., Gorla, D.E., Catalá, S.S., 2013. Potential sources of *Triatoma infestans* reinfesting peridomestic identified by morphological characterization in los Llanos, La Rioja, Argentina. *Mem. Inst. Oswaldo Cruz*, 91–97.
- Houle, D., Mezey, J., Galpern, P., Carter, A., 2003. Automated measurement of *Drosophila* wings. *BMC Evol. Biol.* 3, 25.
- Iwata, H., 2011. Quantitative genetic analyses of crop organ shape based on principal components of elliptic fourier descriptors (p. 50–66). In: Lestrel, P. (Ed.), *Biological Shape Analysis, Proceedings of the 1st International Symposium, Tsukuba Japan 3–6 June 2009*, p. 305.
- Iwata, H., Ebana, K., Uga, Y., Hayashi, T., Jannink, J.L., 2010. Genome-wide association study of grain shape variation among *Oryza sativa* 1 germplasms based on elliptic fourier analysis. *Mol. Breed.* 25, 203–215.
- Jensen, R.J., Ciofani, K.M., Miramontes, L.C., 2002. Lines, outlines, and landmarks: morphometric analyses of leaves of *Acer rubrum*, *Acer saccharinum* (aceraceae) and their hybrid. *Taxon* 51 (3), 475–492.
- Jolliffe, I.T., 1986. *Principal Component Analysis. Series in Statistics*. Springer, New York.
- Kaba, D., Ravel, S., Acapovi-Yao, G., Solano, P., Allou, K., Bosson-Vanga, H., Gardes, L., N'Goran, E.K., Schofield, C.J., Koné, M., Dujardin, J., 2012. Phenetic and genetic structure of tsetse fly populations (*Glossina palpalis palpalis*) in southern Ivory Coast. *Parasites Vectors* 5, 153.
- Krzanowski, W.J., 1987. Cross-validation in principal component analysis. *Biometrics* 43, 575–584.
- Kuhl, F.P., Giardina, C.R., 1982. Elliptic fourier features of a closed contour. *Comput. Graphics Image Process.* 18, 236–258.
- Lestrel, P.E., 1989. Methods for analyzing complex two-dimensional forms: elliptic fourier functions. *Am. J. Hum. Biol.* 1, 149–164.
- Lestrel, P.E., 1997. Introduction and overview of Fourier descriptors. In: *Fourier Descriptors and their Applications in Biology*. Cambridge University Press, Cambridge, pp. 22–44 [Chapter 2].
- Lohmann, G.P., 1983. Eigenshape analysis of microfossils: a general morphometric procedure for describing changes in shape. *Math. Geol.* 15, 659–672.
- Loy, A., Busilacchi, S., Costa, C., Ferlin, L., Cataudella, S., 2000. Comparing geometric morphometrics and outline fitting methods to monitor fish shape variability of *Diplodus puntazzo* (Teleostea: Sparidae). *Aquacult. Eng.* 21 (4), 271–283.
- Machado, A., 1954. Révision systématique des glossines du groupe *palpalis* (Diptera). *Publ. cult. Co. Diam. Angola* 22, 189.
- Manly, B.F.J., 2004. *Multivariate Statistical Methods: A primer*, third ed. Chapman & Hall Statistics Texts.
- Márquez, E.J., Jaramillo-O, N., Gómez-Palacio, A., Dujardin, J.P., 2011. Morphometric and molecular differentiation of a *Rhodnius robustus*-like form from *R. robustus* Lrousse, 1927 and *R. prolixus* Stal, 1859 (Hemiptera, Reduviidae). *Acta Trop.* 120: 103–120: 109.
- Matias, A., De la Riva, J.X., Torrez, M., Dujardin, J.P., 2001. *Rhodnius robustus* in Bolivia identified by its wings. *Mem. Inst. Oswaldo Cruz* 96 (7), 947–950.
- Monteiro, F.A., Barrett, T.V., Fitzpatrick, S., Cordon-Rosales, C., Feliciangeli, D., Beard, C.B., 2003. Molecular phylogeography of the Amazonian Chagas disease vectors *Rhodnius prolixus* and *R. robustus*. *Mol. Ecol.* 12 (4), 997–1006.
- Monti, L., Baylac, M., Lalanne Cassou, B., 2001. Elliptic Fourier analysis of the form of genitalia in two *Spodoptera* species and their hybrids (Lepidoptera: Noctuidae). *Biol. J. Linn. Soc.* 72 (3), 391–400.
- Palaniswamy, S., Thacker, N.A., Klingenberg, C.P., 2010. Automatic identification of morphometric landmarks in digital images. *IET Comput. Vis.* 4 (4), 247–260 <<http://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.105.7188>>.
- Palestrini, C., Roggero, A., Lindsay Karen Hernández, N., Giachino, M.P., Rolando, A., 2012. On the evolution of shape and size divergence in *Nebria* (Nebriola) ground beetles (Coleoptera,Carabidae). *Systematics and Biodiversity*, 147–157 <<http://dx.doi.org/10.1080/14772000.2012.685775>>.
- Pavlinov, I.Y., 2000. Supplementary data on application of geometric morphometrics in analyzing variation of molar shape in voles (Rodentia, Alticola). *Zoologicheskyy Zhurnal* 79 (1), 104–107.
- Qing-Bin, Z., Xin-Li, W., 2012. Elliptic fourier analysis of the wing outline shape of five species of antlion (Neuroptera: Myrmeleontidae: Myrmeleontini). *Zool. Stud.* 51 (3), 399–405.
- Renaud, S., Michaux, J., Jaeger, J.-J., Aufray, C., 1996. Fourier analysis applied to *Stephanomys* (rodentia: Muridae) molars: non-progressive evolutionary patterns in a gradual lineage. *Paleobiology* 22, 251–261.
- Renaud, S., Michaux, J., Mein, P., Aguilar, J.-P., Aufray, C., 1999. Patterns of size and shape differentiation during the evolutionary radiation of the european miocene murine rodents. *Lethaia* 32, 61–71.
- Renaud, S., Michaux, J.R., 2003. Adaptive latitudinal trends in the mandible shape of *Apodemus* wood mice. *J. Biogeog.* 30 (10), 1617–1628.
- Rohlf, F.J., 1986. Relationships among eigenshape analysis, Fourier analysis and analysis of coordinates. *Math. Geol.* 18 (8), 845–854.
- Rohlf, F.J., 1990a. Fitting curves to outlines. In: Rohlf, F.J., Bookstein, F.L. (Eds.), *Proceedings of the Michigan Morphometrics Workshop*. University of Michigan Museums, Ann Arbor, pp. 168–177, ISBN 0-9628499-01.
- Rohlf, F.J., 1990b. Rotational fit (Procrustes) methods. In: Rohlf, F., Bookstein, F. (Eds.), *Proceedings of the Michigan morphometrics workshop*, Special Publication No. 2. University of Michigan Museums, Ann Arbor, pp. 227–236, The University of Michigan Museum of Zoology, Ann Arbor, MI, p. 380.
- Rohlf, F.J., 1996. Morphometric spaces, shape components and the effects of linear transformations. In: Marcus, L.F., Corti, M., Loy, A., Naylor, G., Slice, D. (Eds.), *Advances in Morphometrics. Proceedings of the 1993 NATO-ASI on Morphometrics*, NATO ASI, Ser. A, Life Sciences. Plenum Publ., New York, pp. 117–129.
- Rohlf, F.J., Archie, J.W., 1984. A comparison of Fourier methods for the description of wing shape in mosquitoes (Diptera: Culicidae). *Syst. Zool.* 33, 302–317.
- Ruiz, F., Quiñones, M.L., Erazo, H.F., Calle, D.A., Alzate, J.F., Linton, Y.M., 2005. Molecular differentiation of *Anopheles* (*Nyssorhynchus*) *benarrochi* and *A* (N.) *oswaldoi* from Southern Colombia. *Mem. Inst. Oswaldo Cruz* 100, 155–160.
- Ruiz-Lopez, F., Wilkerson, R.C., Ponsonby, D.J., Herrera, M., Sallum, M.A.M., Dario Velez, I., Quiñones, M.L., Flores-Mendoza, C., Chadee, D.D., Alarcon-Ormasa, J., Linton, Y.-M., 2013. Systematics of the Oswaldo complex (*Anopheles*, *Nyssorhynchus*) in South America. *Parasites Vectors* 6, 324.
- Sheets, D.H., Kim, K., Mitchell, C.E., 2004. A combined landmark and outline-based approach to ontogenetic shape change in the Ordovician trilobite *Triarthrus becki*. In: *Morphometrics. Applications in Biology and Paleontology*. Springer-Verlag, Berlin, Heidelberg, pp. 67–82 [Chapter 6].
- Sheets, H.D., Covino, K.M., Panasiewicz, J.M., Morris, S.M., 2006. Comparison of geometric morphometric outline methods in the discrimination of age-related differences in feather shape. *Front. Zool.* 3, 15.
- Slice, D.E., Bookstein, F.L., Marcus, L.F., Rohlf, F.J., 1996. A glossary for morphometrics. pp 531–551. In: Naylor, G.J.P., Slice, D. (Eds.), *Advances in Morphometrics. Proceedings of the 1993 NATO-ASI on Morphometrics*, NATO ASI, ser. A, Life Sciences. Plenum Publ., New York, pp. 387–398.
- Stephens, C.R., Juliano, S.A., 2012. Wing shape as an indicator of larval rearing conditions for *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). *J. Med. Entomol.* 49 (4), 927–938.
- Takano, A., Muto, M., Sakata, A., Ogasawara, Y., Ando, S., Hanaoka, N., Tsurumi, M., Sato, F., Nakamura, N., Fujita, H., Watanabe, H., Kawabata, H., 2009. Relapsing fever spirochete in seabird tick, Japan. *Emerg. Infect. Dis.* 15, 1528–1530.
- Tatsuta, H., Iwata, H., Goka, K., 2009. Morphometric studies of male Lucanid beetle mandibles: shape variation between hybrid subspecies. In: Lestrel, P.E. (Ed.), *Biological Shape Analysis*. World Scientific Publishing Co. Pte. Ltd., London, ISBN 10-981-4355-23-2.



- Villegas, J., Feliciangeli, M.D., Dujardin, J.P., 2002. Wing shape divergence between *Rhodnius prolixus* from Cojedes (Venezuela) and *R. robustus* from Mérida (Venezuela). *Infect. Genet. Evol.* 2, 121–128.
- Yamanaka, N., Ninomiya, S., Hoshi, M., Tsukura, Y., Yano, M., Nagamura, Y., Sasaki, T., Harada, K., 2001. An informative linkage map of soybean reveals qtls for flowering time, leaflet morphology and regions of segregating distortion. *DNA Res.* 8, 61–72.
- Yee, W.L., Sheets, H.D., Chapman, P.S., 2011. Analysis of surstylus and aculeus shape and size using geometric morphometrics to discriminate *Rhagoletis pomonella* and *Rhagoletis zephyria* (Diptera: Tephritidae). *Ann. Entomol. Soc. Am.* 104 (2), 105–114. <http://dx.doi.org/10.1603/AN10029>.
- Yezerinac, S.M., Loogheed, S.C., Handford, P., 1992. Measurement error and morphometric studies: statistical power and observer experience. *Syst. Biol.* 41, 471–482.



## **Chapitre 3 - Convergence dans l'évolution des patrons de diversification**

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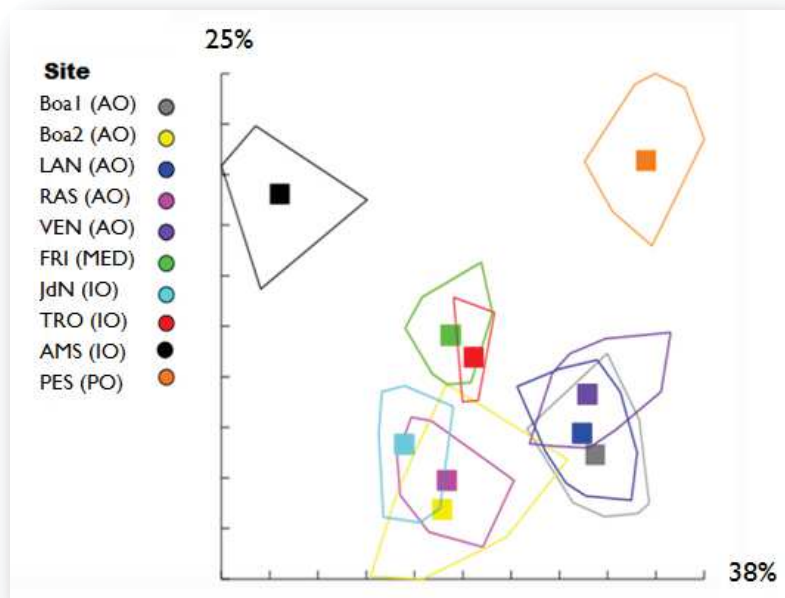


De fortes pressions de sélection sexuelle et/ou de sélection divergente dans différents environnements peuvent engendrer rapidement la divergence écologique et morphologique de populations sympatriques et conduire à la spéciation (Rundell and Price, 2009). La sélection divergente peut provenir des facteurs abiotiques et/ou biotiques tels que la nature des ressources alimentaires, les conditions climatiques de l'habitat ou les relations interspécifiques comme la compétition ou la prédation (Schluter, 2009). Des modèles de divergence adaptative tels que les pinsons de Darwin (*Geospiza fortis*) dans les îles Galápagos ou les lézards du genre *Anolis* dans les îles Caraïbes ont montré la coexistence à différentes localités de plusieurs espèces présentant des différences de taille et de micro-habitat (Grant and Grant, 2008; Losos, 1990). De même, la grande diversité des poissons Cichlidés observée dans les lacs d'Afrique de l'est résulte d'un phénomène d'adaptation à des ressources et des habitats spécifiques engendrant des convergences morphologiques en sympatrie (Muschick et al., 2012). Certaines de ces convergences morphologiques sont directement liées à des mécanismes génétiques, comme par exemple, l'apparition des plaques de protection chez les épineches (Colosimo et al., 2005).

Les systèmes hôte-parasite s'apparentent aux systèmes insulaires dans lesquels les espèces d'hôtes forment des « îles isolées » et pourraient présenter des cas fréquents de divergence adaptative. Par exemple, Johnson et al (2012) se sont intéressés à la morphologie de différentes espèces de poux fortement corrélée au comportement adopté par celles-ci pour échapper à la toilette effectuée par différents hôtes-oiseaux. Ils ont montré que la spécialisation de poux à différentes parties du corps des hôtes-oiseaux (tête, aile, corps) était due à un processus de divergence adaptative répété au sein d'un groupe d'hôtes, engendrant des différenciations morphologiques suivant la partie du corps utilisée. Ils ont mis en évidence un phénomène de convergence morphologique entre les différents groupes d'hôtes, reflétant des pressions de sélection identiques subies par les différentes espèces de poux en fonction de la partie du corps des hôtes utilisée.

Au sein de nos deux systèmes tiques-oiseaux de mers, j'ai donc utilisé deux approches de morphométrie géométrique combinée à des analyses génétiques pour étudier la variabilité phénotypique des différentes espèces ou races d'hôtes afin de dégager des patrons de spécialisation d'hôte et pour tenter d'identifier les facteurs sous-jacents. Dans l'article 3, l'utilisation combinée d'outils de génétique des populations et de morphométrie géométrique au sein du complexe *O. capensis* s.l. a montré que les variations de taille et de forme sont étroitement corrélées à la structure génétique, révélant une forte structuration des tiques en fonction de l'utilisation de l'hôte. Ce résultat se répète également dans les populations retrouvées en sympatrie. Des populations distantes de tiques, collectées dans les colonies de Sulidae, présentent des variations phénotypiques semblables (Figure 8). Nous discutons l'implication de pressions de sélection associées à l'utilisation d'hôtes.





**Figure 8:** Résultat d'une analyse discriminante basée sur la méthode des contours et montrant des regroupements de populations en fonction de l'utilisation d'hôte dans le complexe *O. capensis* sensu lato.

L'article 4 porte sur l'utilisation de la morphométrie géométrique (landmarks et contours) pour discriminer les races d'hôtes d'*I. uriae* collectées sur différents hôtes-oiseaux au Canada, en Islande et en Norvège. Des signes d'un phénomène de convergence morphologique ont été mis en évidence mais ce signal est faible entre les différents sites échantillonnés. Nous discutons les patrons de variations phénotypiques observées en fonction des pressions de sélection potentiellement exercées par les hôtes et/ou le micro-habitat.

Article 3

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Linking morphometric and genetic divergence with host use in the tick complex, *Ornithodoros capensis* sensu lato.

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**Abstract**

Host specific adaptations in parasites can lead to isolation and divergence of conspecific populations. However, divergence can be often difficult to measure because morphological changes may not be expressed or because obvious changes may simply reflect phenotypic plasticity. Combining both genetic and phenotypic information can enable a better understanding of the divergence process and help identify the underlying selective forces, particularly in closely-related species groups. Here, we link genetic and phenotypic data to understand divergence patterns within the *Ornithodoros (Carios) capensis* complex, a group of soft ticks (Argasidae) exploiting colonial seabirds across the globe. Species designations in this complex were historically based on larval morphology and geographic location. However, recent work has suggested that divergence within the group may be at least partially linked to host specificity. Using genetic and geometric morphometrics (landmark and outline analyses), we test whether host use in the complex is always accompanied by the same phenotypic changes. Genetic and morphometric analyses revealed strong structure in relation to host use, both when populations were sympatric and widely allopatric. Tick size varied among populations, along with relative body width after correcting for size. Shape and size variation was closely linked to genetic structure, but only weakly with geography. These results support the hypothesis that speciation in this tick group has been more strongly shaped by host use than by geographic barriers per se. The revealed phenetic patterns now require detailed investigation to link them with host-specific selective forces.

Keywords: adaptation, host specificity, population genetic structure, morphometry, ectoparasite, tropical seabirds

## 1 Introduction

Being a food resource and principal habitat, the host often represents the majority of a parasite's ecological needs. The optimal exploitation of one host type may require specific adaptations that can lead to the divergence of conspecific populations (Ravigné et al. 2009). However, host-associated divergence is sometimes difficult to measure in natural populations, as adaptation may not require morphological changes (Bickford et al, 2007) or because these changes may simply represent phenotypic plasticity (Agrawal, 2001). Molecular techniques, such as DNA barcoding (Hajibabaei et al, 2007) and genome-wide typing (Manske et al, 2012), have become increasingly popular and accessible allowing us to measure levels of genetic isolation, but identifying the drivers of this divergence remains difficult, particularly in non-model organisms. One tool that can be used to help understand the link between genetic divergence and host specific adaptations is morphometry, the quantitative analysis of form. Morphometric methods, although developed in the 1980's, have gained in popularity over the last few years (Adams et al, 2004) because they represent a cheap, rapid and biologically significant way of measuring phenotypic changes (Lawing and Polly, 2010). As these methods are also non-destructive, they can be used in cases where genetic analyses are impossible, for example with certain collection specimens. Morphometric tools not only complement more traditional morphological species descriptions, but are also able to quantify subtle population-level variation (eg. Barluenga et al, 2006) and can help identify the factors underlying divergence. Although rarely employed for parasitic organisms, coherent results have been obtained for several taxa, including blood-feeding arthropods (Dujardin, 2008, Dietrich et al. 2013) and ectoparasitic trematodes (Huyse and Volckaert, 2002). For example, morphometric analyses of different sympatric host-associated populations of the hard tick *Ixodes uriae* showed that the overall body shape of ticks differed with host use (Dietrich et al. 2013) and correlated with the genetic divergence among tick subpopulations and the relative parasite fitness on alternative host types (McCoy et al. 2005, Dietrich et al, 2012, Dietrich et al, 2014). However, the morphometrical analysis in this case was limited to patterns within a single location and it remains unknown whether the

same morphological changes occur on the same host types in other areas of the distribution. The ability to make inferences on host traits associated with these changes is therefore limited.

The *Ornithodoros (Carios) capensis* complex is a soft tick (Argasidae) taxon that includes eight described species (Hoogstraal et al, 1976). These species are considered to be host generalists parasitizing a range of tropical and temperate colonial seabird species, including at least 35 species of 12 avian families from across the Pacific, Atlantic and Indian Oceans. Although poorly studied to date, these ectoparasites are considered to have direct effects on host reproductive success when occurring in high density (e.g., Duffy, 1983) and are known to harbour numerous pathogenic agents for humans including Soldado and West Nile viruses and relapsing fever bacteria (Dietrich et al, 2011). Like other soft ticks, their endophilic life style should restrict encounters with alternative host species (Vial, 2009), which may favor the evolution of local adaptation and host specialization (Whitlock 1996), processes already reported for other tick species (McCoy et al, 2013).

The members of the *O. capensis* complex share a high degree of morphological similarity. Species descriptions in this taxon have been based on a combination of larval characteristics and geography (Colas-Belcour and Rageau, 1960, Hoogstraal et al, 1976, Clifford et al, 1980). However, recent work on the Cape Verde Islands suggested that both these descriptions and the generalist nature of these species may be erroneous; surprisingly high genetic diversity was found within islands, with several putative species occurring on different sympatric seabird species (Gómez-Díaz et al, 2012). The authors suggest that this diversity is due to a combination of long-distance colonization events via seabird movement and adaptations to specific host genera. Here, we examine this hypothesis at a global scale, by combining genetic and morphometric data to compare the diversity and structure of *O. capensis* ticks associated with different hosts in the Cape-Verde and Canary Islands with ticks collected from across the distribution of the complex. We expected that genetic results would reveal isolation and divergence among tick populations in relation to the host genus and that this factor would better explain genetic distance than geography. We also expected that patterns of host-associated divergence would match with differences in tick size and shape, potentially revealing adaptive variation. We discuss our results in light of the current

systematics of this species group and the relative importance of different evolutionary forces in driving parasite divergence.

## 2 Material and methods

### 2.1 Biological system

The species of the *O. capensis* complex, their geographic ranges, and host associations have been described somewhat opportunistically by different authors over the past 100 years. Eight species are currently recognized within the complex (Hoogstraal et al. 1985), with a possible ninth species that remains poorly studied as yet (Vermeil et al, 1997): *O. capensis sensu stricto* Neumann 1901 has been recorded on albatrosses, boobies, cormorants, gulls, penguins and terns, and is widely distributed within tropical, subtropical and southern temperate islands and along the coasts of the Atlantic, Pacific and Indian Oceans, the Caribbean and Corral seas and lakes of the East African Rift Valley (Hoogstraal et al, 1985); *O. amblus* Chamberlin 1920 is associated with boobies, cormorants and pelicans and found on islands off the coasts of Peru (Clifford et al, 1980); *O. denmarki* Kohls, Sonenshine & Clifford 1965 was described from boobies and terns in Florida, California, Hawaii and Mexico (Kohls et al, 1965, Amerson, 1968); *O. muesebecki* Hoogstraal 1969 is associated with boobies, cormorants and terns in the Arabian gulf; *O. maritimus* Vermeil and Marguet 1966 is found on numerous species of cormorants, gulls, alcids and terns in Great Britain, Ireland, France, Italy, Tunisia (Hoogstraal et al, 1976); *O. sawaii* Kitaoka and Suzuki 1973 was initially collected from Streaked Shearwaters (*Calonectris leucomelas*) on the Amani-Oshima Islands in Japan, but has since also been found on Swinhoe's Storm Petrels (*Oceanodroma monorhis*) (Kawabata et al, 2006; Takano et al, 2009); *O. yunkeri* Keirans, Clifford & Hoogstraal 1984 is associated with albatrosses, boobies, cormorants and penguins of the Galapagos Islands and *O. spheniscus* Hoogstraal, Wassef, Hays & Keirans 1985 was found to be associated with the Humboldt Penguin in different localities in Peru and has been more recently described on these hosts in Chile (González-Acuña et al, 2008). *O. cheikhi*, a species morphologically close to *O. capensis s.s* and *O.*

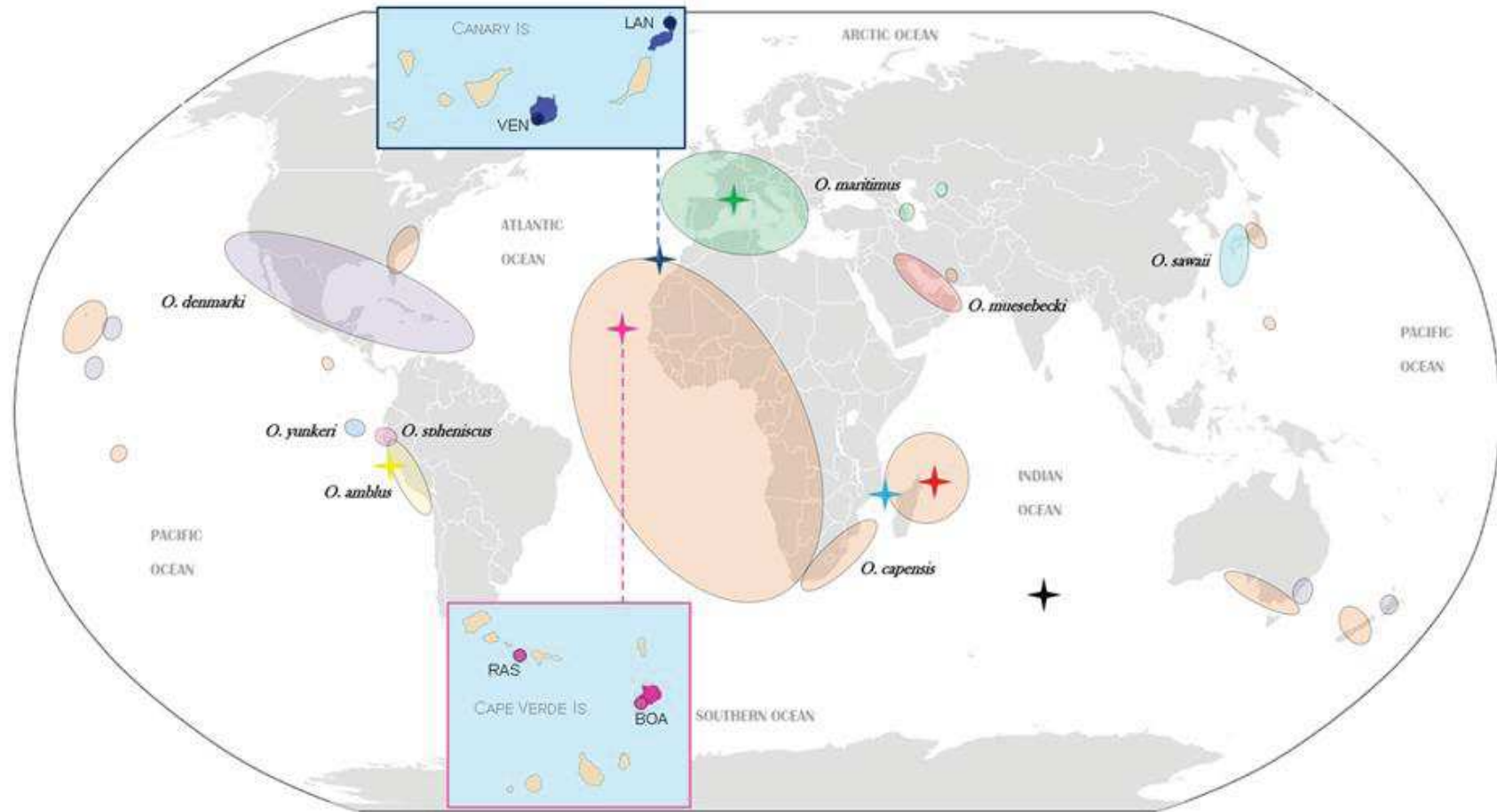


*maritimus* was described from tern nests in Mauritania (Vermeil et al, 1997), but no additional data concerning its taxonomic status or host affinities have since been reported.

Like other argasid ticks, species of the *O. capensis* complex have three developmental stages: larva, nymph and adult, with a variable number of nymphal instars (Pérez-Eid, 2007). Although obligate parasites, these species only associate with the host for the time of their relatively short blood meal, taking from a few hours to engorge for larval ticks to several minutes for nymphal and adult stages. The greatest part of the life cycle is therefore confined to the microhabitat of the host nesting area (within nests, burrows or under nearby rocks and vegetation) (Hoogstraal, 1985) and we generally refer to these species as being endophilic. Feeding occurs multiple times for nymphal and adult stages, typically at night, and depends on the presence of the host at the nest site (Duffy & Daturi, 1987). Colonial seabirds are particular as hosts since many species are only on land for a few months each year to breed. However, as they tend to show high natal and breeding fidelity to their colony and nest site, their presence is highly predictable for their nest parasites (McCoy et al, 2016).

## 2.2 Tick sampling

Ticks were collected on different seabird species or from host nests from a series of islands (see Fig.1 and Table.1 for details) and were preserved in 70% ethanol. They were initially assigned to the species level based on historical records of geographic distribution (Fig.1). For genetic analyses, 206 collected ticks were sequenced at three genes (16S, CO1, 18S) and 12 additional 16S rDNA sequences were added from GenBank (Table 1). The same 206 ticks (94 females and 112 males) used for sequencing were also analyzed for morphometry, with a mean of 20 ticks per population (Table 1). All analyzed ticks were in the adult stage and non-engorged to avoid body distortion from the blood meal in the morphometrical analyses. For tick populations in which larvae were collected, species identifications were verified based on classical morphological traits using available keys: Hoogstraal et al, 1976; Hoogstraal, 1969; Hoogstraal et al, 1985; Keirans et al, 1984; Kitaoka and Suzuki, 1973; Kohls et al, 1965; Clifford et al, 1980.



**Fig.1:** Sampled sites in seabird colonies across the range of the *Ornithodoros capensis sensu lato* complex. The distribution of the eight currently described species is indicated by colored ellipses. Purple: *O. denmarki*; Blue: *O. yunkeri*; Pink: *O. spheniscus*; Yellow: *O. amblus*; Orange: *O. capensis*; Green: *O. maritimus*; Red: *O. muesebecki*; Light blue: *O. sawaii*. The sampled sites are indicated by stars. Yellow: Pescadore Island (PES) on *S. variegata*; Dark blue (inset map): Canary Islands on *C. borealis* (LAN and VEN); Pink (inset map): Cape-Verde Islands on *C. edwardsii* (Boa1) and *S. leucogaster* (Boa2 and RAS); Green: Frioul Island (FRI) on *L. michahellis*; Light blue: Juan de Nova Island (JdN) on *O. fuscatus*; Red: Tromelin Island (TRO) on *S. sula*; Black: Amsterdam Island (AMS) on *E. chrysocome*. Distributional data from Dietrich et al, 2011. See Table 1 for more detail on sampling locations.

**Table.1:** Tick populations used in this study, with collection localities, geographic coordinates, population abbreviations, presumed tick species based on geographic distributions, host species and the number of ticks used in morphometric and genetic (16S, COI and 18S genes) analyses. F: female; M: male. Accession numbers for 16S gene sequences are also provided (See SM for accession numbers for 18S and COI gene sequences).

Origin	Sample location (Lat. Lon.)	Pop	Presumed tick species	Host	N° ticks morphometry	N° ticks 16S	16S Accession number	Reference study	N° ticks COI	N° ticks 18S
Atlantic Ocean - Cape-Verde - Boavista1	15.993700, -22.790108	Boa1	<i>O. capensis</i>	Cape Verde shearwater <i>Calonectris edwardsii</i>	12F - 12M	22	KX825947-50	present study	9	16
Atlantic Ocean - Cape-Verde - Boavista1	15.993700, -22.790108	Boa1	<i>O. capensis</i>	Cape Verde shearwater <i>Calonectris edwardsii</i>	-	1	JQ824298.1	Gómez-Díaz et al, 2012	-	-
Atlantic Ocean - Cape-Verde - Boavista2	15.993700, -22.790108	Boa2	<i>O. capensis</i>	Brown booby <i>Sula leucogaster</i>	13F - 12M	9	KX825953	present study	8	14
Atlantic Ocean - Cape-Verde - Boavista2	15.993700, -22.790108	Boa2	<i>O. capensis</i>	Brown booby <i>Sula leucogaster</i>	-	2	JQ824315.1	Gómez-Díaz et al, 2012	-	-
Atlantic Ocean - Cape-Verde - Raso	16.616844, -24.586501	RAS	<i>O. capensis</i>	Brown booby <i>Sula leucogaster</i>	8F - 16M	21	KX825954-56	present study	21	11
Atlantic Ocean - Cape-Verde - Raso	16.616844, -24.586501	RAS	<i>O. capensis</i>	Brown booby <i>Sula leucogaster</i>	-	2	JQ824308.1	Gómez-Díaz et al, 2012	-	-
Atlantic Ocean - Canary - Lanzarote	29.297243, -13.534957	LAN	<i>O. capensis</i>	Cory's shearwater <i>Calonectris borealis</i>	7F - 20M	24	KX825951,52,58	present study	10	10
Atlantic Ocean - Canary - Lanzarote	29.297243, -13.534957	LAN	<i>O. capensis</i>	gull <i>Larus michahellis</i>	-	3	KX825970	present study	-	-

Atlantic Ocean - Canary - Veneguera	27.905011, -15.730907	VEN	<i>O. capensis</i>	Cory's shearwater <i>Calonectris borealis</i>	14F - 10M	13	KX825957	present study	17	22
Atlantic Ocean – Canary - Tenerife	28.372326, -16.76808	TEN	<i>O. capensis</i>	Cory's shearwater <i>Calonectris borealis</i>	-	3	JQ824316.1	Gómez-Díaz et al, 2012	-	-
Mediterranean Sea - France - Frioul	43.274548, 5.302565	FRI	<i>O. maritimus</i>	Yellow-legged gull <i>Larus michahellis</i>	6F - 16M	20	KX825959	present study	6	20
Indian Ocean - TAAF - Juan de Nova	-17.055907, 42.724928	JdN	<i>O. capensis</i>	Sooty tern <i>Onychoprion fuscatus</i>	16F - 5M	18	KX825960,61	present study	10	9
Indian Ocean - TAAF - Amsterdam	-37.834645, 77.557291	AMS	?	Rockhopper penguin <i>Eudyptes chrysocome</i>	9F - 5M	15	KX825965-69	present study	5	12
Indian Ocean - TAAF - Tromelin	-15.891818, 54.523823	TRO	<i>O. capensis</i>	Red-footed booby <i>Sula sula</i>	8M	8	KX825962,63	present study -	6	2
Pacific Ocean - Peru - Pescadores	-11.756390, -77.284593	PES	<i>O. amblus</i>	Peruvian booby <i>Sula variegata</i>	9F - 8M	20	KX825964	present study	14	9
Pacific Ocean - Peru - Islas Chincha	-13.642826, -76.399884	Peru	<i>O. amblus</i>	Peruvian booby <i>Sula variegata</i>	-	2	KX825971	present study	-	-
Pacific Ocean - Mexico - Isabel Island	21.848529, -105.884897	Mex	<i>O. denmarki</i>	Blue-footed booby <i>Sula nebouxii</i>	-	2	KX825972	present study	-	-
Pacific Ocean - Japan - Hanmya Island	28.046214, 129.187829	HanmyaA	<i>O. capensis</i>	Streaked shearwater <i>Calonectris leucomelas</i>	-	1	AB057538	Kawabata et al, 2006	-	-

Pacific Ocean - Japan - Ishikawa	?	Ishikawa1	<i>O. sawaii</i>	Streaked shearwater <i>Calonectris leucomelas</i>	-	1	AB242427	Kawabata et al, 2006	-	-
Pacific Ocean – Hawaii – Laysan Island	25.768278, -171.732304	Hawaii1	<i>O. capensis</i>	Laysan albatross <i>Diomedea immutabilis</i>	-	1	AB057538	Ushijima et al, 2003	-	-
Atlantic Ocean – Texas – Longman reef Island	?	TexasA	<i>O. capensis</i>	Reddish egret <i>Egretta rufescens</i>	-	1	AB076081	Ushijima et al, 2003	-	-

### 2.3 Genetic analyses

*DNA isolation and PCR amplification:* After taking standardized images, the 206 newly collected ticks were washed in two water baths, placed individually in a sterile tube and crushed with a pestle. DNA extractions were performed with the DNeasy Tissue kit (Qiagen, Valencia, CA, USA) following manufacturer's instructions. Mitochondrial 16S and COI, and nuclear rDNA 18S gene fragments were PCR-amplified (Black and Piesman, 1994; Simon et al, 1994; Black et al, 1997) for each individual tick and sequenced using standard methods (see A1 in supplementary materials for detailed amplification protocols and sequencing methods).

*Genetic and statistical analyses:* DNA sequences were aligned with the MEGA software (version 6.0.5; Tamura et al, 2013) and used in DNAsp (version 5.10.01) (Librado and Rozas, 2009) to calculate haplotype ( $H_d$ ) and nucleotide ( $\pi$ ) diversities (Nei 1973) (see A2 in supplementary material for accession numbers of used DNA sequences).

In order to test for genetic differentiation among populations, we calculated KST\*, a distance index based on the number of nucleotide differences between sequences (Hudson et al, 1992). Both an overall estimate of KST\* (KST\*<sub>Tot</sub>) including all populations and pairwise population values were calculated for each gene fragment. Permutation tests, with 1000 repetitions, were used to assess the significance of KST\* estimates.

To visualize genetic differences among populations, cluster analyses were performed for each gene fragment and for concatenated sequences of the 16S and COI genes (800bp) using the UPGMA method with Splitstree (version 4.13.1; Huson and Bryant, 2006). Five 16S gene sequences from the study of Gómez-Díaz et al. (2012) and 4 additional sequences available from Genbank (See Table 1) were used to construct phylogenetic trees with MEGA (version

6.0.5) using maximum likelihood with sequences from the hard tick *Ixodes uriae* (Ixodidae) as an outgroup.

In order to attribute genetic structure to different sources (host versus geography), we carried out an analysis of molecular variance (AMOVA) on haplotypic data using ARLEQUIN (version 3.5.1.2; Excoffier and Lischer, 2010). For this analysis, we considered only those host groups containing at least two tick populations (i.e. shearwater/booby/gull). Consequently, we excluded the AMS, JdN, Hawaii1 and TexasA populations.

## 2.4 Morphometric analyses

We used both landmark and outline-based analyses to describe tick morphology. The first type enables us to obtain precise measures of morphological traits associated with different biological activities (i.e., feeding, reproduction, locomotion) as landmarks are positioned on genetically determined anatomical structures. The outline analysis, in contrast, provides an overall measure of differences in body shape and size because it can be used on body parts lacking clear anatomical structure, and is thus especially relevant for the soft tick body form (Dujardin et al, 2014). For both analyses, we used a standardized image of each tick taken using a binocular microscope (Leica M80). All morphometrical analyses were performed using the different modules of the CLIC package (Dujardin & Slice, 2006, Dujardin et al., 2010), freely available at <http://mome-clic.com/>.

For the landmark analysis, a set of 15 type II (Bookstein, 1997) landmarks was established on the ventral face of the tick (Fig.2a). Shape variables (uniform and non-uniform components) were obtained by computing a generalized Procrustes analysis (GPA). Briefly, after removing the size factor through the Procrustes superimposition on the consensus shape, the residual coordinates were converted into “partial warps” (PW) to be used by standard multivariate

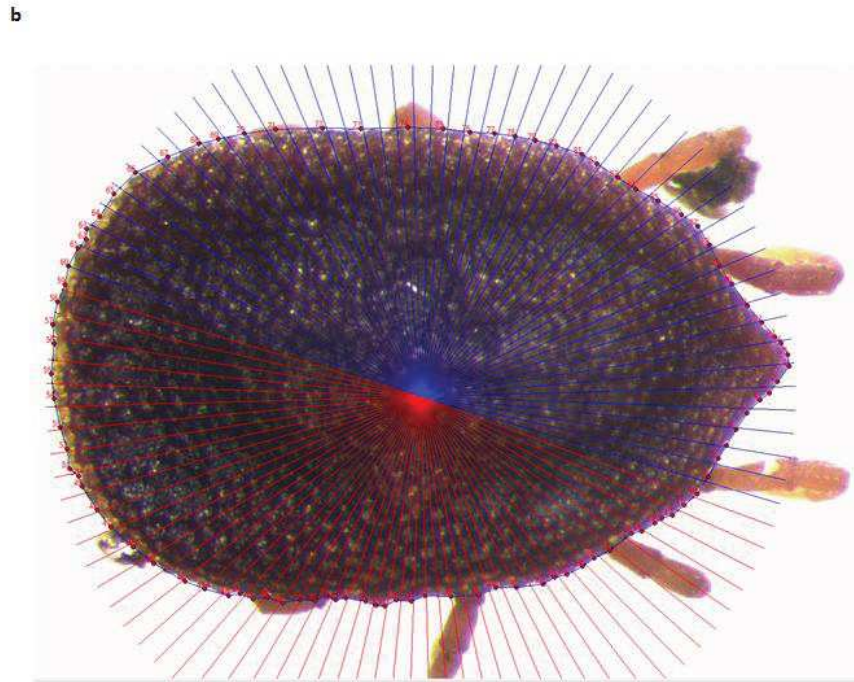
analyses (principal component and discriminant analyses). The connected residual coordinates of the 15 landmarks provided polygons allowing visual comparisons of mean shape among tick populations. A discriminant analysis was then performed using the principal components of the PW (i.e., the relative warps, or RW) as input.

For the outline-based analysis, we used the elliptic Fourier analysis (EFA) method (Kuhl & Giardina, 1982) to compute the contour of the dorsal face of each tick (Fig.2b) with the aim of comparing overall tick shape. The outline was decomposed into elliptic Fourier coefficients which were normalized (NEF) in order to be compared without the influence of size, orientation or starting point. A variance-covariance matrix was then constructed with these coefficients to summarize variation and was used as input for multivariate analyses.

a







**Fig.2 (a)** Image representing the 15 true landmarks used on the ventral face of each tick (here shown on a female tick from Juan de Nova). Each point represents one landmark. Landmarks N°1-2: width between landmarks 1 and 2 on extremities of the transverse post-anal groove; N°3-4: width between landmarks 1 and 2 on extremities of the transverse post-anal groove; N°5: anus; N°8-9: width between landmarks 8 and 9 at coxae III/IV; N°10-12: width between landmarks 10 and 12 at coxae I/II; N°11: genital pore; N°13-14: width between landmarks 13 and 14 on the lower extremity of the basis capitulum; N°15: capitulum extremity. **(b)**: Image representing the outline method used on the dorsal face of ticks (an image of a female from Juan de Nova). The central point represents the centroid. The presence of radii is a decorative feature of the software; pseudo-landmarks were collected manually.

For size analyses, we computed the centroid size (CS) for the landmark analysis, defined as the square root of the sum of the squared distances between the center of the landmark configuration and each individual landmark (Bookstein, 1997). For outline analyses, we used the perimeter (P) as an estimate of body size. Size means and variances were analyzed using permutation tests due to low sample sizes in some populations. At each permutation cycle, individuals were randomly exchanged among populations, and the mean and variance was recalculated (1000 permutations in total). Then, we used a Bonferroni correction to determine the significance of size differences between populations. The permutation tests were also used to test size and shape differences between females and males for each tick population.

Shape differences among populations were quantified based on Mahalanobis distances (Dujardin et al, 2010) that were used to construct dendrograms in the R software (version 3.0.2) using the UPGMA method. Size variation among populations for landmarks and outlines was visualized using boxplots, showing values for each individual tick, the median value per population, and the 25<sup>th</sup> and 75<sup>th</sup> percentiles. If boxes overlap, landmark arrangements or outlines were considered similar among populations. A random validated classification method (CCC, for “cross checked classification”), based on the jackknife procedure, was used to test the affiliation of each individual tick to each population.

The effect of size (either CS or P) on the discrimination of shape (either RW or principal components of NEF) was estimated by linear regression, using the correlation ( $r$ ) and determination ( $r^2$ ) coefficients.

The morphometrical Mahalanobis distance matrix was compared statistically against the genetic distance matrix (KST\*) using Mantel tests to evaluate their relationship. All Mantel tests were performed using XLSTAT software (version 2015), with significance assigned based on 1000 matrix permutations and a critical level of  $\alpha = 0.05$ .

## **3 Results**

### **3.1 Population genetic analyses**

Gene fragments were successfully sequenced for the three genes (16S 351bp, COI 400bp and 18S 312bp), although some amplification problems occurred for the 18S gene. As generally expected, the COI gene fragment showed the highest polymorphism with 146 variable sites, followed by the mitochondrial 16S gene with 90 polymorphic sites and the 18S nuclear gene fragment, with 33 polymorphic sites (Table 2 and Table A1).

Haplotypic and nucleotidic diversities were relatively low within populations but high among tick populations (Table 2 and SM Table A1). This was supported by measures of population differentiation;

**Table.2:** Genetic indexes of population diversity for the 16S gene fragment. Seq: number of sequences; NS: number of variable sites; K: mean number of nucleotide differences; H: number of haplotypes; Hd  $\pm$  SD: Haplotype diversity and standard deviation;  $\pi$   $\pm$  SD: Nucleotide diversity and standard deviation.

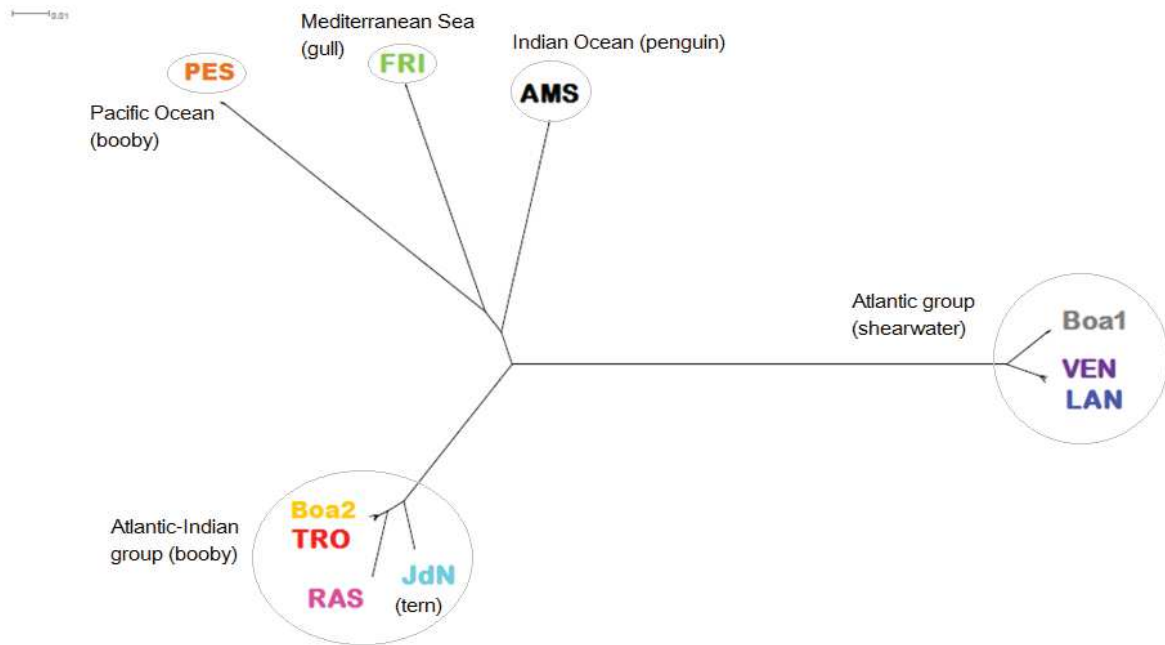
Population	rDNA 16S – 351 bp					
	Seq	NS	K	H	Hd $\pm$ SD	$\pi$ $\pm$ SD
<b>Boavista 1</b>	22	67	6.255	4	0.333 $\pm$ 0.124	0.0183 $\pm$ 0.01557
<b>Boavista 2</b>	9	0	0.000	1	0 $\pm$ 0	0 $\pm$ 0
<b>Lanzarote</b>	24	2	0.373	3	0.359 $\pm$ 0.110	0.00109 $\pm$ 0.00036
<b>Raso</b>	21	1	0.267	3	0.267 $\pm$ 0.120	0.00077 $\pm$ 0.00035
<b>Veneguera</b>	13	0	0.000	1	0 $\pm$ 0	0 $\pm$ 0
<b>Frioul</b>	20	0	0.000	1	0 $\pm$ 0	0 $\pm$ 0
<b>Juan de Nova</b>	18	6	0.667	2	0.111 $\pm$ 0.096	0.00193 $\pm$ 0.00167
<b>Amsterdam</b>	15	4	1.829	7	0.857 $\pm$ 0.065	0.00530 $\pm$ 0.00082
<b>Pescadores</b>	20	1	0.100	2	0.100 $\pm$ 0.088	0.00029 $\pm$ 0.00026
<b>Tromelin</b>	8	1	0.429	2	0.429 $\pm$ 0.169	0.00124 $\pm$ 0.00049
<b>Total</b>	<b>170</b>	<b>90</b>	<b>35.392</b>	<b>20</b>	<b>0.888 <math>\pm</math> 0.009</b>	<b>0.1278 <math>\pm</math> 0.00304</b>

KST\* coefficients indicated strong genetic differentiation among all populations (Table 3). The high number of variable sites (67) observed for 16S mitochondrial gene fragments in the Boal tick population originated from two sequences that more closely matched sequences from the FRI tick population; interestingly, this was not the case for the other gene fragments, nor for morphometrical analyses suggesting a historical introgression event.

**Table.3:** Pairwise KST\* indexes between populations for the 16S gene fragment. Significance is indicated as follow: NS = not significant; \*0.01<P<0.05; \*\*0.001<P<0.01; \*\*\*P<0.001. Green and purple boxes represent respectively results within and between genetic identified groups.

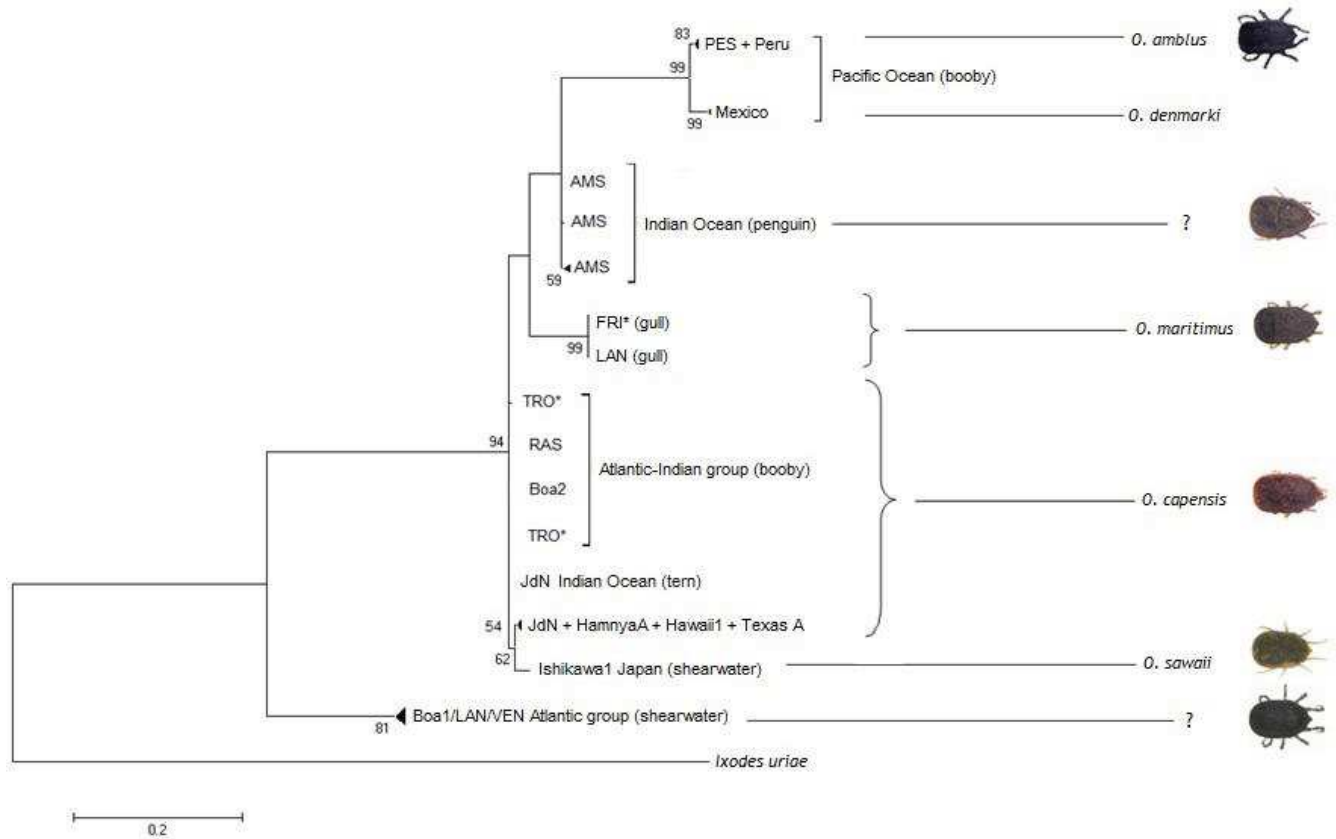
16S KST*		Boa 1	Boa 2	Lan	Ras	Ven	Fri	JdN	Ams	Pes
Cape Verde	Boavista 2	<b>0.79655</b> ***								
Canarias	Lanzarote	<b>0.69279</b> ***	<b>0.8932</b> 9 ***							
Cape Verde	Raso	<b>0.83639</b> ***	- 0.0213 2 NS	<b>0.9003</b> 6 ***						
Canary	Veneguera	<b>0.71604</b> ***	<b>1</b> ***	3 NS	<b>0.9438</b> 9 ***					
Mediterranean Sea	Frioul	<b>0.86840</b> ***	<b>1</b> ***	<b>7</b> ***	<b>8</b> ***	<b>1</b> ***				
Indian Ocean	Juan de Nova	<b>0.82440</b> ***	<b>0.8404</b> 1 ***	<b>0.8924</b> 1 ***	<b>0.8096</b> 7 ***	<b>0.9407</b> 8 ***	<b>0.9413</b> 1 ***			
Indian Ocean	Amsterdamm	<b>0.70254</b> ***	<b>0.7338</b> 9 ***	<b>0.7735</b> 8 ***	<b>0.7058</b> 6 ***	<b>0.7884</b> 9 ***	<b>0.7887</b> 6 ***	<b>0.7005</b> 8 ***		
Peru	Pescadores	<b>0.86147</b> ***	<b>0.9694</b> 6 ***	<b>0.9244</b> 2 ***	<b>0.9328</b> 4 ***	<b>0.9798</b> 8 ***	<b>0.9817</b> 2 ***	<b>0.9282</b> 1 ***	<b>0.7785</b> 7 ***	
Indian Ocean	Tromelin	<b>0.75013</b> ***	<b>0.5924</b> 1 **	<b>0.8514</b> 0 ***	<b>0.4108</b> 1 ***	<b>0.9500</b> 1 ***	<b>0.9474</b> 4 ***	<b>0.7608</b> 7 ***	<b>0.5943</b> 7 ***	<b>0.9208</b> 1 ***
KST* Tot 16S			<b>0.9226</b> 9	***						

Cluster analyses showed the presence of two main population groups, one group of tick populations associated with shearwater hosts [Boa1, VEN, LAN] and a second with mainly booby hosts [Boa2, RAS, TRO, JdN]. The tick populations of AMS, FRI and PES (collected on Peruvian Boobies) fell outside of these groups (Fig.3). The shearwater group was the most genetically differentiated at all genes (See SM Fig A1.1, A1.2, and A1.3). It should be noted that the 18S gene fragments were not included in the concatenated tree due to missing sequences in some populations.



**Fig.3:** Cluster analysis of the concatenated sequences of the 16S and COI mitochondrial gene fragments. Populations are identified by abbreviations as outlined in Table 1.

Phylogenetic analyses of the three genes (including additional sequences for 16S gene) were well-resolved and concordant with the cluster analyses (Fig.4 and See SM Fig.A2.1 and A2.2). The shearwater group was the most isolated, with the Atlantic/Indian ocean booby group and the other populations showing more recent divergence. The PES tick population branched with *O. amblus* samples collected from Peru and were closely related to *O. denmarki* samples collected from Mexico, constituting a Pacific booby group. The FRI tick population branched with ticks collected on Lanzarote Island from gull nests identified as *O. capensis*. The AMS tick population was isolated from all other typed populations and no known species could be attributed to these ticks. The JdN tick population branched with *O. capensis* samples from Texas, Hawaii and Japan collected on different seabird species (Reddish Egret, Laysan Albatross and Streaked Shearwater) and grouped with *O. capensis s.s.* This large group tended to be related to *O. sawaii* collected from Streaked Shearwaters in Japan.



**Fig.4:** Phylogenetic tree based on sequences of the 16S mitochondrial marker. *Ixodes uriae* represents the outgroup. To the right, we have provided the expected species names based on historical descriptions and an associated image, when available. The population names with a star indicate that species identifications have been verified using classical morphological traits of larval ticks.

The AMOVA results showed that genetic differentiation was highly dependent on host (54.07% of the variation due to among host-group differences), but that geography also played a substantial role with 43% of the variation attributed to among population differences within groups (Table 4). Much of the variation within host groups can be attributed to differences among booby tick populations in the Atlantic and Pacific Oceans (see above).

**Table.4:** Analysis of molecular variance (AMOVA) using haplotypic data from populations of three host groups (Shearwater, Booby, Gull) (see text for details).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P-value
Among host groups	2	1507.631	12.20141	54.07	0.00782
Among populations within groups	12	1013.299	9.64217	42.73	0.00000
Within populations	140	101.348	0.72391	3.21	0.00000
Total	154	2622.277	22.56750		

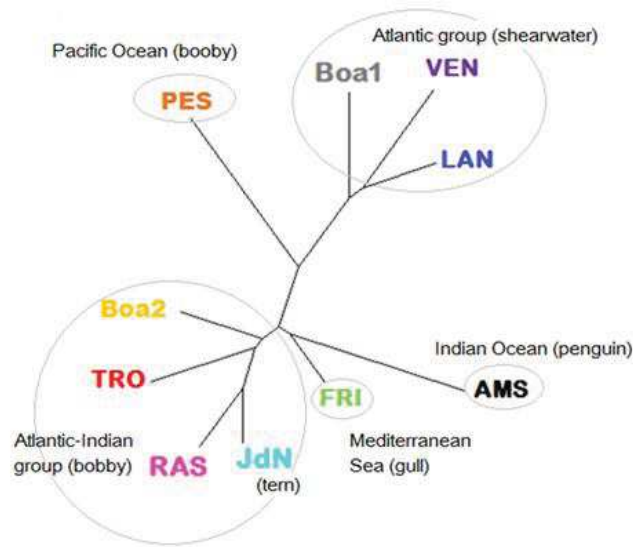
### 3.2 Morphometrical analyses

Similar results were obtained for males and females for both types of morphometrical analyses; we therefore combined the sexes for population level comparisons (pairwise differences among males and females of each population  $p > 0.0120$ : starting value for the Bonferroni test: 0.000292).

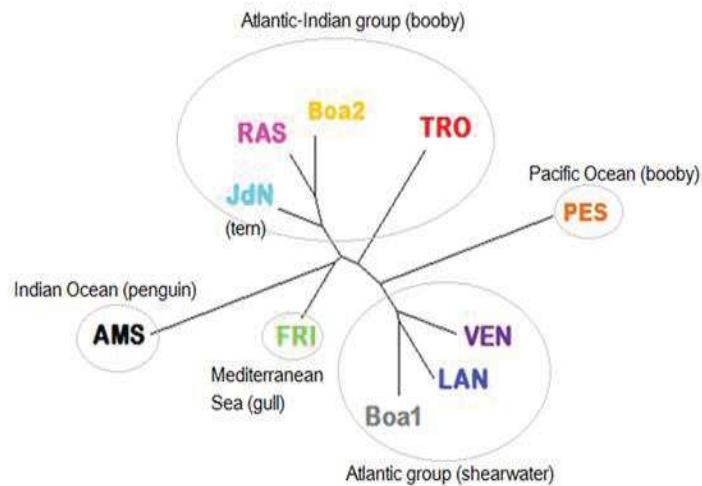
The UPGMA trees, based on Mahalanobis distances indicated the presence of two main population groups (Fig.5): one composed of shearwater tick populations [Boa1, LAN and VEN], and another formed by four populations [Boa2, RAS, TRO, JdN] with slightly more diverse host relationships (i.e. boobies and terns). The other three populations – AMS, FRI and PES (collected respectively on penguins, gulls and Peruvian boobies) - were largely isolated from these main groups and from each other. The same results were obtained for the discriminant analyses (See SM Fig.A3).



a



b



**Fig.5:** UPGMA tree based on Mahalanobis distances from the landmark **(a)** and outline **(b)** analyses of tick populations. See Table 1 for population abbreviations.

Boa1 and LAN showed the largest size variation and overall centroid size, whereas Boa2 and RAS were significantly smaller than the other populations (See SM Fig.A4 and A5 and Table.A4). The cross-check classification provided strong support for the distinctness of populations with a minimum of 50% for TRO based on the landmark analysis (similar to JdN and Boa2) and 48% for Boa2 with the outline analysis (similar to RAS). Maximal scores were obtained for PES at 88% (strongly conserved landmark configuration) and 95% for FRI

(strongly conserved outline configuration) (See SM Fig.A4 ad A5). AMS and PES showed the most distant inter-population morphologies based on both analyses (See Fig.5 and SM Fig.A6 and A7).

For the landmark analysis, the size effect on shape represented 18.3% of the overall variation in tick morphology (DF1 regression:  $r=-0.260$ ;  $r^2=6.8\%$ ;  $df=204$ ;  $t=3.85$ ,  $p=8.2 \times 10^{-5}$ ; DF2 regression:  $r=-0.339$ ;  $r^2=11.5\%$ ;  $df=204$ ;  $t=5.15$ ,  $p=3.1 \times 10^{-7}$ ), whereas for outline analyses, the size effect represented 22.5% of the overall shape variation (DF1 regression:  $r=0.444$ ;  $r^2=19.7\%$ ;  $df=204$ ;  $t=7.08$   $p=1.17 \times 10^{-11}$ ; DF2 regression  $r=0.167$ ;  $r^2=2.8\%$ ;  $df=204$ ;  $t=2.42$   $p=0.008$ ).

Landmark arrangement variation among populations seemed to be due to changes in the relative position of the genital aperture and the anus (See SM Fig.A6). The position of the anus, and thus the distance between the anus and the genital aperture, of shearwater tick populations [Boa1, LAN and VEN] and the PES population seemed to differ from the other populations. The distance between the posterior ends of the preanal groove also varied among tick populations, principally due to changes in tick body width (See SM Fig.A6). The majority of shape variation in the outline analyses originated from the form of anterior margin of the body and the relative width of the body contour (See SM Fig A7).

### **3.3 Correlation between genetic and morphological analyses**

Significant correlations were found between morphological and genetic distances among populations (See Table 5). However, the results from the Mantel tests of the 16S and CO1 suggest that genetic distance only explains a small part of the morphometric variation among tick populations; this relationship was stronger for the 18S gene fragment ( $r^2=65.6\%$

$p \leq 0.0001$  for the landmarks; See Table 5) and may be linked to ventral traits on the tick body.

**Table.5:** Mantel test results between morphometric (Mahalanobis distances) and genetic (KST\* distances) matrices. The corresponding graphs for each analysis for the landmark (LM) distances are shown in SM Table.A.3.

	16S			COI			18S		
	r-value	r <sup>2</sup> (%)	p-value	r-value	r <sup>2</sup> (%)	p-value	r-value	r <sup>2</sup> (%)	p-value
<b>Landmarks</b>	0.378	14.3	0.025	0.532	28.3	0.001	0.810	65.6	<0.0001
<b>Outline</b>	0.316	10.0	0.059	0.449	20.2	0.005	0.603	36.4	0.0003

## 4 Discussion

The *O. capensis sensu lato* complex is a diverse group of ticks found in seabird colonies across the globe. Previous descriptions of this complex were based on both classical morphology and geographic location, but included largely fragmented and opportunistic samples. As a consequence, host relationships are vague and most species have been considered to be seabird generalists, exploiting any locally available seabird (Dietrich et al, 2011). Here, we examined genetic structure among populations of this complex from across the globe to determine the relative role of host versus geography in driving divergence. We compared these patterns with detailed analyses of tick morphometry to test congruence and infer the potential phenotypic basis of divergence.

### 4.1 Genetic and associated morphometric structure

Our genetic analyses, based on three genes, were able to discriminate tick populations in relation to host use and/or locality and were in strong agreement with morphometric analyses, confirming the presence of at least five different species among the sampled ticks (Fig.4 and 5). Distant populations using the same or closely-related host species shared haplotypes across

large spatial scales and were morphologically similar. Moreover, differences among host-associated populations remained even when ticks were sampled from different hosts in complete sympatry (ex., Boa1 and Boa2). However, despite these global patterns, significant differences in haplotype frequencies were evident among populations. Indeed, pairwise population genetic analyses showed that almost all populations are genetically distinct. Therefore, although host-associated groups are evident at large spatial scales, gene flow is not frequent among populations within groups (Table 2 and 3). This is not surprising given the life history traits of soft ticks. Indeed, although birds can migrate over large distances, soft ticks only exploit their hosts for a short period of time (several minutes) and typically at night when most birds do not travel. Therefore, dispersal events should be rare. These events may occur during bird immigration from distant colonies (Thibault & Bretagnolle, 1998) or with the movements of young birds that prospect different colonies prior to breeding, a period that can last 2 to 6 years in these seabird species (Votier et al. 2011; Peron & Gremillet 2013, Boulinier et al, 2016).

Despite the presence of clear host-associated groups, some evidence of mixing among host groups was still apparent in the data. In the Boa1 population (shearwater hosts), haplotypes typically associated with the FRI Mediterranean population (gull hosts) were found at the 16S gene in two sampled ticks, but were not present at the other two genes. These two individuals were not morphological outliers, suggesting a signal of a past introgression events among ticks of these two species. Some degree of hybridization among distinct tick lineages may not be surprising given that different tick lineages are known to co-occur sympatrically on some islands (Gómez-Díaz et al, 2012). Indeed, gull ticks collected on Lanzarote Island matched with the FRI tick population from the same host and co-occurred with the shearwater group on this island (LAN). However, given the relative distinctness of the different host-associated populations we found, hybridization events must be very rare and may depend on the

occurrence of specific ecological conditions (e.g., low tick population size, unavailability of typical host, etc). *O. spheniscus*, for example, is specialized on the Humboldt penguin, *Spheniscus humboldti* breeding in dense colonies, and has never been found to occur on other seabird species that breed sympatrically with these penguin hosts (Hoogstraal et al, 1985).

#### **4.2 Host-association and the geographic distribution of species within the *O. capensis* complex**

Based on genetic and morphometrical evidence, we suggest that at least five different species of the *O. capensis* complex have been sampled in our study. At least two tick species were sympatric on the Cape Verde Islands. One of these species, associated with booby hosts, is also found within the Indian Ocean. Given this large distribution and historical descriptions, these populations should be classified as *O. capensis* s.s. Indeed, larval ticks from the TRO tick population were identified as *O. capensis* s.s. using traditional morphological criteria (data not shown). The JdN tick population, collected on terns also grouped with *O. capensis* s.s., although some genetic divergence was evident between these ticks and those of booby hosts. This may suggest a recent shift of ticks between booby and tern hosts within this region of the world. The ticks from terns may also represent a distinct species: *O. cheikhi*, a species described on terns in Mauritania was suggested to be morphologically related to *O. capensis* (Vermeil et al, 1997), but no further work has been done on this species. The JdN tick population was genetically similar to the Ishikawa1 sample representing *O. sawaii*, a tick species found in Japanese seabird colonies and again reported to be closely related to *O. capensis* (Kitaoka and Suzuki, 1973). A more complete sampling of tern colonies within the Indian Ocean and Japan, with representative samples of larval ticks, is now called for to clarify the specific status of these populations.

The second tick population present on the Cape-Verde Islands was strictly associated with shearwater hosts and was also found in the Canary Islands. This tick could not be identified to any described species using traditional morphology on larval ticks (data not shown), and likely represents a new species. The strong morphological and genetic distances between this group and the other ticks sampled suggest that this species is not part of the *O. capensis* complex at all, and may represent an independent shift to seabird hosts.

Like the shearwater tick group, ticks from AMS did not match any previously described species of the *O. capensis* complex, nor did sequence alignments match with available *O. sawaii* sequences (Kawabata et al, 2006), or *O. denmarki*, described from coastal regions of Northern and Central America (Fig.1). However, unlike the ticks associated with shearwaters, ticks from penguins on AMS seem to belong to the *O. capensis* complex, their clade being well-nested within the complex. Ticks from PES could be attributed to *O. amblyus*, a species distributed along the west coast of Peru and known to exploit different host species (Clifford et al, 1980). The morphology and genetic differences of this species with respect to the Atlantic-Indian booby group is likely related to geographic isolation of the hosts. Ticks from FRI correspond to *O. maritimus* which occurs throughout gull colonies of the Mediterranean Sea and along the Atlantic coast of Europe and Africa (Hoogstraal et al, 1976; Guiguen et al, 1987; Vermeil et al, 1997). Larval ticks from this population were identified as *O. maritimus* using traditional morphological criteria, confirming this identification (data not shown). These ticks grouped with those collected on gulls on Lanzarote Island, indicating the presence of *O. maritimus* on the Canary Islands. As host affinities are subtle in the *O. capensis* complex and our samples reflect only part of its diversity, additional sampling and official descriptions, notably of larval stages, will now be necessary to match existing descriptions with these new observations.

### 4.3 Drivers of diversification: host versus geography

Both landmark and outline analyses showed that ticks from boobies are typically smaller than the other tick populations. Following Harrison's Rule (Harrison, 1915), parasite size is thought to be directly correlated with host size, i.e. parasite size increases with increasing host size. Malenke et al. (2009), for example, showed a proportional relationship between parasite size and host size in *Columbicola* feather lice. However, Johnson et al (2005) found no such relationship in body lice. This rule does not seem to apply to the present tick populations, as boobies are larger overall (>70cm total length) than terns, gulls, penguins and shearwaters (<50cm total length). Reduced tick size could be due to several alternative factors. For example, Amin and Sonenshine (1969) demonstrated that size variation is directly linked to the differential engorgement success of immature stages in the American dog tick, *Dermacentor variabilis*. Engorgement success can depend on host traits associated with physiology and immunity that alter the quantity of blood imbibed. Tick size reduction could also come from differences in tick digestive capacities, reflecting intrinsic differences in the ability of ticks to exploit different host resources (Willadsen et al, 1984; Dietrich et al, 2013). However, in both of these cases, we would expect that adaptation to the host should enable a release from such constraints allowing for increased tick size. The smaller size of booby ticks could also be linked to a physical constraint imposed by the host, and more specifically the ability to remove ticks during preening (Clayton et al, 2010). Indeed, beak shape and size variation have been shown to play a major role in determining the size and load of ectoparasites (Clayton et al, 1999, Clayton et al, 2005). In our study, beak shape strongly differs shearwaters and boobies, with shearwaters having hooked beaks and boobies pointed; these differences may alter the ability of hosts to preen off ticks. High CCC scores based on Mahalanobis distances indicated that size is relatively constant within booby tick populations (Fig.A4 and A5), suggesting strong host-imposed size selection on this trait. Indeed, ticks



exploiting boobies in the Pacific (PES population) were also among the smallest-sized populations, despite being only distantly related to the other booby tick lineage. These elements suggest preening rather than variation due to the quantity or quality of the bloodmeal may explain at least some of the size patterns in our data.

Tick shape was largely independent of body size variation (only 18-22.5% of shape variation was due to size effects). The dorsal boundary outline of the tick body showed strong population structure in relation to host use. Contrary to size, which can be influenced by environmental conditions, shape is thought to be more stable (Dujardin et al, 2003; Perrard et al, 2012), and can modify the ability of ticks to move within the feather environment and escape preening (Bush et al, 2011). As for size, variance in shape was lower for ticks on booby hosts compared to the other populations suggesting strong selective pressures on tick body shape. Comparative studies of bill morphology will now be required to test the role of preening in determining tick size and shape.

Aside from selective pressures directly related to feeding and survival on the host, geographic differences in climate, landscape or ecological perturbations may alter tick shape and size and play a role in their diversification. Indeed, ticks spend the major part of their life-cycle in the off-host environment and require specific conditions of temperature and humidity to survive (Gray et al, 2014). Such factors could present an alternative explanation for morphological differences observed among AMS, FRI and PES tick populations, but are unlikely to explain divergences observed within islands among hosts or populations located in similar climatic zones (ex. Cape-Verde and Canary archipelagos). It is possible that the micro-habitats associated with the use of different sympatric hosts (e.g., burrows versus rocky nests) exert off-host selection pressures on tick populations and can explain some of this variation. However, soft ticks seem to be able to readily move at the within colony scale, if

environmental conditions are suitable. *O. savignyi*, for example, is able to cross more than twenty meters to find a host (Mans and Neitz, 2004).

Although genetic and morphometric analyses revealed tick population structure linked to host use and geographic location, correlations between morphometric and genetic distances were variable in strength. The relationship between the two distance measures was stronger for the 18S nuclear gene than for the mitochondrial genes. This stronger relationship may be logical if morphological structures have a genetic basis and represent gene flow of both tick sexes, rather than only female gene flow as is the case for the two mitochondrial genes. Likewise, Mahalanobis distances based on landmark measures of morphology were more strongly correlated with genetic distances than outline measures. These results suggest that the ventral traits measured by the landmark analysis are more genetically conserved than overall body shape.

## 5 Conclusions

Combining genetic and morphometric analyses enabled us to test specific predictions on the role of host specificity in the diversification of the *O. capensis* complex. Genetic-based analyses were able to discriminate the presence of different species and were supported by morphometric results, illustrating the need for more complete studies of the *O. capensis* complex in relation to host use if we are to fully understand their systematic and global diversity. Although not mutually exclusive from climate or distance-based isolation, divergence associated with host specific adaptations remains the most parsimonious explanation for the morphometric and genetic structures that we observed among tick populations. Therefore, our results also highlight the utility of combining genetics and morphometrics for studying cryptic parasite biodiversity and the potential selective forces

acting on this diversity. Finally, our results support previous hypotheses suggesting that ticks are rarely true host generalists and that host-associated adaptations likely play a major role in the diversification of these parasite vectors and in the circulation of tick-borne pathogens (McCoy et al, 2013).

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## References

- Adams, D.C., Rohlf, F.J., Slice, D.E., 2004. Geometric morphometrics: Ten years of progress following the “revolution.” *Italian Journal of Zoology* 71, 5–16. doi:10.1080/11250000409356545
- Agrawal, A.A., 2001. Phenotypic Plasticity in the Interactions and Evolution of Species. *Science* 294, 321–326. doi:10.1126/science.1060701
- Amerson, A.B., Jr, 1968. Tick distribution in the central Pacific as influenced by sea bird movement. *J. Med. Entomol.* 5, 332–339.
- Amin, O.M., Sonenshine, D.E., 1969. Development of the American Dog Tick, *Dermacentor variabilis*, Following Partial Feeding by Immatures. *Annals of the Entomological Society of America* 63, 128–133.
- Barluenga, M., Stölting, K.N., Salzburger, W., Muschick, M., Meyer, A., 2006. Sympatric speciation in Nicaraguan crater lake cichlid fish. *Nature* 439, 719–723. doi:10.1038/nature04325
- Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K., Das, I., 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution* 22, 148–155. doi:10.1016/j.tree.2006.11.004
- Black IV, W.C., Klompen, J.S.H., Keirans, J.E., 1997. Phylogenetic Relationships among Tick Subfamilies (Ixodida: Ixodidae: Argasidae) Based on the 18S Nuclear rDNA Gene. *Molecular Phylogenetics and Evolution* 7, 129–144. doi:10.1006/mpev.1996.0382
- Black, W.C., Piesman, J., 1994. Phylogeny of hard-and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. *Proceedings of the National Academy of Sciences* 91, 10034–10038.
- Bookstein, F.L., 1997. *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge University Press.
- Boulinier, T., Kada, S., Ponchon, A., Dupraz, M., Dietrich, M., Gamble, A., Bourret, V., Duriez, O., Bazire, R., Tornos, J., Tveraa, T., Chambert, T., Garnier, R., McCoy, K.D., 2016. Migration, Prospecting, Dispersal? What Host Movement Matters for Infectious Agent Circulation? *Integr. Comp. Biol.* icw015. doi:10.1093/icb/icw015
- Bush, S.E., Villa, S.M., Boves, T.J., Brewer, D., Belthoff, J.R., 2011. Influence of Bill and Foot Morphology on the Ectoparasites of Barn Owls. *Journal of Parasitology* 98, 256–261. doi:10.1645/GE-2888.1
- Clayton, D.H., Koop, J.A., Harbison, C.W., Moyer, B.R., Bush, S.E., 2010. How birds combat ectoparasites. *Open Ornithol J* 3, 41–71.
- Clayton, D.H., Lee, P.L.M., Tompkins, III, E.D.B., 1999. Reciprocal Natural Selection on Host-Parasite Phenotypes. *The American Naturalist* 154, 261–270. doi:10.1086/303237
- Clayton, D.H., Moyer, B.R., Bush, S.E., Jones, T.G., Gardiner, D.W., Rhodes, B.B., Goller, F., 2005. Adaptive significance of avian beak morphology for ectoparasite control.

- Proceedings of the Royal Society of London B: Biological Sciences 272, 811–817. doi:10.1098/rspb.2004.3036
- Clifford, C.M., Hoogstraal, H., Radovsky, F.J., Stiller, D., Keirans, J.E., 1980. *Ornithodoros (Alectorobius) amblus* (Acarina: Ixodoidea: Argasidae): Identity, Marine Bird and Human Hosts, Virus Infections, and Distribution in Peru. *The Journal of Parasitology* 66, 312–323. doi:10.2307/3280825
- Colas-Belcour, J., Rageau, J., 1960. Biologie et élevage d'*Ornithodoros capensis* Neumann (acarien Argasidae). *Bulletin de la Société de Pathologie Exotique* 53, 723–727.
- Dietrich, M., Beati, L., Elguero, E., Boulinier, T., McCoy, K.D., 2013. Body size and shape evolution in host races of the tick *Ixodes uriae*. *Biological Journal of the Linnean Society* 108, 323–334. doi:10.1111/j.1095-8312.2012.02021.x
- Dietrich, M., Gómez-Díaz, E., McCoy, K.D., 2011. Worldwide Distribution and Diversity of Seabird Ticks: Implications for the Ecology and Epidemiology of Tick-Borne Pathogens. *Vector-Borne and Zoonotic Diseases* 11, 453–470. doi:10.1089/vbz.2010.0009
- Dietrich, M., Kempf, F., Gómez-Díaz, E., Kitaysky, A.S., Hipfner, J.M., Boulinier, T., McCoy, K.D., 2012. Inter-oceanic variation in patterns of host-associated divergence in a seabird ectoparasite. *Journal of Biogeography* 39, 545–555. doi:10.1111/j.1365-2699.2011.02620.x
- Dietrich, M., Lobato, E., Boulinier, T., McCoy, K.D., 2014. An experimental test of host specialization in a ubiquitous polar ectoparasite: a role for adaptation? *J Anim Ecol* 83, 576–587. doi:10.1111/1365-2656.12170
- Duffy, D.C., 1983. The Ecology of Tick Parasitism on Densely Nesting Peruvian Seabirds. *Ecology* 64, 110. doi:10.2307/1937334
- Duffy, D.C., Daturi, A., 1987. Diel rhythms of tick parasitism on incubating African penguins. *Medical and Veterinary Entomology* 1, 103–106. doi:10.1111/j.1365-2915.1987.tb00329.x
- Dujardin, J.-P., 2008. Morphometrics applied to medical entomology. *Infection, Genetics and Evolution* 8, 875–890. doi:10.1016/j.meegid.2008.07.011
- Dujardin, J.-P.A., Kaba, D., Henry, A.B., 2010. The exchangeability of shape. *BMC Research Notes* 3, 266. doi:10.1186/1756-0500-3-266
- Dujardin, J.-P., Kaba, D., Solano, P., Dupraz, M., McCoy, K.D., Jaramillo-O, N., 2014. Outline-based morphometrics, an overlooked method in arthropod studies? *Infection, Genetics and Evolution* 28, 704–714. doi:10.1016/j.meegid.2014.07.035
- Dujardin, J.-P., Pont, F.L., Baylac, M., 2003. Geographical versus interspecific differentiation of sand flies (Diptera: Psychodidae): a landmark data analysis. *Bulletin of Entomological Research* 93, 87–90. doi:10.1079/BER2002206
- Dujardin, J.-P., Slice, D.E., 2006. Contributions of morphometrics to medical entomology. *Encyclopedia of infectious diseases: modern methodologies* 435–447.

- Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10, 564–567. doi:10.1111/j.1755-0998.2010.02847.x
- Gómez-Díaz, E., Morris-Pocock, J.A., González-Solís, J., McCoy, K.D., 2012. Trans-oceanic host dispersal explains high seabird tick diversity on Cape Verde islands. *Biol. Lett.* 8, 616–619. doi:10.1098/rsbl.2012.0179
- González-Acuña, D., Moreno, L., Guglielmone, A.A., 2008. First report of *Ornithodoros spheniscus* (Acari: Ixodoidea: Argasidae) from the Humboldt Penguin in Chile. *Systematic and Applied Acarology* 13, 120–122. doi:10.11158/saa.13.2.4
- Guiguen, C., Monnat, J., Launay, H., Beaucournu, J., 1987. Ectoparasites des oiseaux de Bretagne, III. Ixodoidea. *Cahiers Orstom, Ser. Ent. Méd. Parasitol.* 73–81.
- Hajibabaei, M., Singer, G.A.C., Hebert, P.D.N., Hickey, D.A., 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics* 23, 167–172. doi:10.1016/j.tig.2007.02.001
- Harrison, L., 1915. Mallophaga from Apteryx, and their Significance; with a Note on the Genus *Rallicola*. *Parasitology* 8, 88–100. doi:10.1017/S0031182000010428
- Hoogstraal, H., 1985. Argasid and Nuttalliellid Ticks as Parasites and Vectors, in: J.R. Baker and R. Muller (Ed.), *Advances in Parasitology*. Academic Press, pp. 135–238.
- Hoogstraal, H., 1969. *Ornithodoros (Alectorobius) muesebecki* n. sp., a parasite of the blue-faced booby (*Sula dactylatra melanops*) on Hasikiya Island, Arabian Sea. *Proceedings of the Entomological Society of Washington* 71, 368–374.
- Hoogstraal, H., Clifford, C.M., Keirans, J.E., Kaiser, M.N., Evans, D.E., 1976. The *Ornithodoros (Alectorobius) Capensis* Group (Acarina: Ixodoidea: Argasidae) of the Palearctic and Oriental Regions. O. (A.) *Maritimus*: Identity, Marine Bird Hosts, Virus Infections, and Distribution in Western Europe and Northwestern Africa. *The Journal of Parasitology* 62, 799. doi:10.2307/3278964
- Hoogstraal, H., Wassef, H.Y., Hays, C., Keirans, J.E., 1985. *Ornithodoros (Alectorobius) spheniscus* n. sp. [Acarina: Ixodoidea: Argasidae: *Ornithodoros (Alectorobius) capensis* group], a tick parasite of the Humboldt penguin in Peru. *J. Parasitol.* 71, 635–644.
- Hudson, R.R., Boos, D.D., Kaplan, N.L., 1992. A statistical test for detecting geographic subdivision. *Mol Biol Evol* 9, 138–151.
- Huson, D.H., Bryant, D., 2006. Application of Phylogenetic Networks in Evolutionary Studies. *Mol Biol Evol* 23, 254–267. doi:10.1093/molbev/msj030
- Huyse, T., Volckaert, F.A., 2002. Identification of a host-associated species complex using molecular and morphometric analyses, with the description of *Gyrodactylus rugiensoides* n. sp. (Gyrodactylidae, Monogenea). *International Journal for Parasitology* 32, 907–919. doi:10.1016/S0020-7519(02)00026-7

- Johnson, K.P., Bush, S.E., Clayton, D.H., 2005. Correlated Evolution of Host and Parasite Body Size: Tests of Harrioso's Rule Using Birds and Lice. *Evolution* 59, 1744–1753. doi:10.1111/j.0014-3820.2005.tb01823.x
- Kawabata, H., Ando, S., Kishimoto, T., Kurane, I., Takano, A., Nogami, S., Fujita, H., Tsurumi, M., Nakamura, N., Sato, F., Takahashi, M., Ushijima, Y., Fukunaga, M., Watanabe, H., 2006. First Detection of Rickettsia in Soft-Bodied Ticks Associated with Seabirds, Japan. *Microbiology and Immunology* 50, 403–406. doi:10.1111/j.1348-0421.2006.tb03807.x
- Keirans, J.E., Clifford, C.M., Hoogstraal, H., 1984. *Ornithodoros (Alectorobius) yunkeri*, new species (Acari: Ixodoidea: Argasidae), from seabirds and nesting sites in the Galapagos Islands. *Journal of Medical Entomology* 21, 344–350.
- Kitaoka, S., Suzuki, H., 1973. *Ornithodoros (Alectorobius) sawaii* sp. n. (Ixodoidea, Argasidae) associated with the streaked shear-water, *Colonectris leucomelas*, from the Amami-oshima Islands, Japan. *Natl Inst Anim Health Q (Tokyo)* 13, 142–148.
- Kohls, G.M., Sonenshine, D.E., Clifford, C.M., 1965. The systematics of the subfamily Ornithodorinae (Acarina: Argasidae). II. Identification of the larvae of the Western Hemisphere and descriptions of three new species. *Ann. Entomol. Soc. Am.* 58, 331–364.
- Kuhl, F.P., Giardina, C.R., 1982. Elliptic Fourier features of a closed contour. *Computer Graphics and Image Processing* 18, 236–258. doi:10.1016/0146-664X(82)90034-X
- Lawing, A.M., Polly, P.D., 2010. Geometric morphometrics: recent applications to the study of evolution and development. *Journal of Zoology* 280, 1–7. doi:10.1111/j.1469-7998.2009.00620.x
- Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25, 1451–1452. doi:10.1093/bioinformatics/btp187
- Malenke, J.R., Johnson, K.P., Clayton, D.H., 2009. Host Specialization Differentiates Cryptic Species of Feather-Feeding Lice. *Evolution* 63, 1427–1438. doi:10.1111/j.1558-5646.2009.00642.x
- Mans, B.J., Neitz, A.W.H., 2004. The sand tampan, *Ornithodoros savignyi*, as a model for tick-host interactions. *South African journal of science* 100, 283–288.
- Manske, M., Miotto, O., Campino, S., Auburn, S., Almagro-Garcia, J., Maslen, G., O'Brien, J., Djimde, A., Doumbo, O., Zongo, I., Ouedraogo, J.-B., Michon, P., Mueller, I., Siba, P., Nzila, A., Borrmann, S., Kiara, S.M., Marsh, K., Jiang, H., Su, X.-Z., Amaratunga, C., Fairhurst, R., Socheat, D., Nosten, F., Imwong, M., White, N.J., Sanders, M., Anastasi, E., Alcock, D., Drury, E., Oyola, S., Quail, M.A., Turner, D.J., Ruano-Rubio, V., Jyothi, D., Amenga-Etego, L., Hubbard, C., Jeffreys, A., Rowlands, K., Sutherland, C., Roper, C., Mangano, V., Modiano, D., Tan, J.C., Ferdig, M.T., Amambua-Ngwa, A., Conway, D.J., Takala-Harrison, S., Plowe, C.V., Rayner, J.C., Rockett, K.A., Clark, T.G., Newbold, C.I., Berriman, M., MacInnis, B., Kwiatkowski, D.P., 2012. Analysis of *Plasmodium falciparum* diversity in natural infections by deep sequencing. *Nature* 487, 375–379. doi:10.1038/nature11174



- McCoy, K.D., Chapuis, E., Tirard, C., Boulinier, T., Michalakis, Y., Bohec, C.L., Maho, Y.L., Gauthier-Clerc, M., 2005. Recurrent evolution of host-specialized races in a globally distributed parasite. *Proc. R. Soc. B* 272, 2389–2395. doi:10.1098/rspb.2005.3230
- McCoy, K.D., Dietrich, M., Jaeger, A., Wilkinson, D.A., Bastien, M., Lagadec, E., Boulinier, T., Pascalis, H., Tortosa, P., Le Corre, M., Dellagi, K., Lebarbenchon, C., 2016. The role of seabirds of the Iles Eparses as reservoirs and disseminators of parasites and pathogens. *Acta Oecologica* 72, 98–109. doi:10.1016/j.actao.2015.12.013
- McCoy, K.D., Leger, E., Dietrich, M., 2013. Host specialization in ticks and transmission of tick-borne diseases: a review. *Front Cell Infect Microbiol* 3. doi:10.3389/fcimb.2013.00057
- Nei, M., 1973. Analysis of Gene Diversity in Subdivided Populations. *PNAS* 70, 3321–3323.
- Pérez-Eid, C., 2007. Les tiques : identification, biologie, importance médicale et vétérinaire. Lavoisier.
- Perrard, A., Villemant, C., Carpenter, J.M., Baylac, M., 2012. Differences in caste dimorphism among three hornet species (Hymenoptera: Vespidae): forewing size, shape and allometry. *Journal of Evolutionary Biology* 25, 1389–1398. doi:10.1111/j.1420-9101.2012.02527.x
- Ravigné, V., Dieckmann, U., Olivieri, I., 2009. Live where you thrive: joint evolution of habitat choice and local adaptation facilitates specialization and promotes diversity. *Am. Nat.* 174, E141–169. doi:10.1086/605369
- Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, Weighting, and Phylogenetic Utility of Mitochondrial Gene Sequences and a Compilation of Conserved Polymerase Chain Reaction Primers. *Annals of the Entomological Society of America* 87, 651–701. doi:10.1093/aesa/87.6.651
- Takano, A., Muto, M., Sakata, A., Ogasawara, Y., Ando, S., Hanaoka, N., Tsurumi, M., Sato, F., Nakamura, N., Fujita, H., Watanabe, H., Kawabata, H., 2009. Relapsing Fever Spirochete in Seabird Tick, Japan. *Emerg Infect Dis* 15, 1528–1530. doi:10.3201/eid1509.090459
- Tamura, K., Stecher, G., Peterson, D., Filipowski, A., Kumar, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30, 2725–2729. doi:10.1093/molbev/mst197
- Thibault, J.-C., Bretagnolle, V., 1998. A Mediterranean breeding colony of Cory's Shearwater *Calonectris diomedea* in which individuals show behavioural and biometric characters of the Atlantic subspecies. *Ibis* 140, 523–528. doi:10.1111/j.1474-919X.1998.tb04615.x
- Ushijima, Y., Oliver, J.H., Keirans, J.E., Tsurumi, M., Kawabata, H., Watanabe, H., Fukunaga, M., 2003. Mitochondrial Sequence Variation in *Carios capensis* (Neumann), a Parasite of Seabirds, Collected on Torishima Island in Japan. *Journal of Parasitology* 89, 196–198. doi:10.1645/0022-3395(2003)089[0196:MSVICC]2.0.CO;2
- Vermeil, C., Marjolet, M., Vermeil, F., 1997. *Ornithodoros* (*Alectorobius*) *cheikhi* n.sp. (Acarina, Ixodoidea, Argasidae, groupe *Ornithodoros* (*Alectorobius*) *capensis*); Tique

parasite de sternes au banc d'Arguin - Mauritanie. Bulletin de la Société des sciences naturelles de l'Ouest de la France 19, 66–76.

Vial, L., 2009. Biological and ecological characteristics of soft ticks (Ixodida: Argasidae) and their impact for predicting tick and associated disease distribution. Parasite 16, 191–202.

Votier, S.C., Grecian, W.J., Patrick, S., Newton, J., 2010. Inter-colony movements, at-sea behaviour and foraging in an immature seabird: results from GPS-PPT tracking, radio-tracking and stable isotope analysis. Mar Biol 158, 355–362. doi:10.1007/s00227-010-1563-9

Willadsen, P., Kemp, D.H., McKenna, R.V., 1984. Bloodmeal ingestion and utilization as a component of host specificity in the tick, *Boophilus microplus*. Z. Parasitenkd. 70, 415–420. doi:10.1007/BF00927829

## Supplementary material

**A1:** PCR amplification conditions and information for mitochondrial 16S and COI and nuclear 18S gene fragments:

DNA extractions were performed with the DNeasy Tissue kit (Qiagen, Valencia, CA, USA) following manufacturer's instructions. Each fragment was amplified in a 25µl reaction mixture containing 2.5 µl of 10X reaction buffer (Qiagen, Courtaboeuf, France), 2.5mM of MgCl<sub>2</sub>, 200µM of each deoxynucleotide triphosphate (Eurogentec, France), 1 unit of Taq DNA polymerase (Cat. No 201205 Qiagen, Courtaboeuf, France), and 10 pmol of each forward and reverse primers.

For the amplification of the 16S gene fragment, we used the primers developed by Black and Piesman (1994): forward 5'-CTGCTCAATGATTTTTTAAATTGC-3', reverse 5'-CCGGTCTGAACTCAGATCATGTA-3'. For the COI gene fragment, we used the primers from Simon et al. (1994): forward 5'-GGAGGATTTGGAAATTGATTAGTTCC-3' and reverse 5'-ACTGTAAATATATGATGAGCTCA-3'. For the 18S gene fragment, we used the primers from Black et al. (1997): forward 5'-GCAAGTCTGGTGCCAGCAGCC-3', reverse 5'-CTCCGTCAATTCCTTTAAG-3'.

The PCR conditions included an initial denaturation step at 94°C for 2min, followed by 35 cycles at 94°C for 45s, 50°C for 45s and 72°C for 45s, with a final extension step at 72°C for 10min. Amplification products were sequenced in both directions using Sanger method by Eurofins Genomics (Courtaboeuf, France).

## References:

Black IV, W.C., Klompen, J.S.H., and Keirans, J.E. (1997). Phylogenetic Relationships among Tick Subfamilies (Ixodida: Ixodidae: Argasidae) Based on the 18S Nuclear rDNA Gene.

*Molecular Phylogenetics and Evolution* 7, 129–144.

Black IV, W.C., and Piesman, J. (1994). Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. *Proceedings of the National Academy of Sciences, USA* 91, 10034–10038.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., and Flook, P. (1994). Evolution, Weighting, and Phylogenetic Utility of Mitochondrial Gene Sequences and a Compilation of Conserved Polymerase Chain Reaction Primers. *Annals of the Entomological Society of America* 87, 651–701.

**A2:** Accession numbers of the DNA sequences used in this study. Each number corresponds to distinct haplotypes seen in each population.

	<i>COI</i>	<i>16S</i>	<i>18S</i>
<b>Boa1</b>	KX826007	KX825947	KX825974
	KX826008	KX825948	
		KX825949	
		KX825950	
<b>Boa2</b>	KX826003	KX825953	KX825977
	KX826004		
	KX826005		
<b>LAN</b>	KX825994	KX825951	KX825975
	KX825995	KX825952	KX825986
	KX825996	KX825958	KX825987
		KX825970	
<b>VEN</b>	KX825991	KX825957	KX825973
	KX825992		
	KX825993		
<b>RAS</b>	KX825997	KX825954	KX825976
	KX825998	KX825955	
	KX825999	KX825956	
	KX826000		
	KX826001		
	KX826002		

	<i>COI</i>	<i>16S</i>	<i>18S</i>
<b>TRO</b>	KX826006	KX825962	KX825985
		KX825963	
<b>JdN</b>	KX826013	KX825960	KX825979
	KX826014	KX825961	KX825984
<b>AMS</b>	KX826015	KX825965	KX825988
		KX825966	KX825989
		KX825967	KX825990
		KX825968	
		KX825969	
<b>PES</b>	KX826009	KX825964	KX825983
	KX826010		
	KX826011		
	KX826012		
<b>FRI</b>	KX826016	KX825959	KX825978
	KX826017		KX825980
			KX825981
			KX825982
<b>Per</b>		KX825971	
<b>Mex</b>		KX825972	

**Table A1:** Genetic indexes of population diversity for COI and 18S gene fragments. Seq: number of sequences; NS: number of variable sites; K: mean number of nucleotide differences; H: number of haplotypes; Hd  $\pm$  SD: Haplotype diversity and standard deviation;  $\pi$   $\pm$  SD: Nucleotide diversity and standard deviation.

COI – 400 bp						
Population	Seq	NS	K	H	Hd $\pm$ SD	$\pi$ $\pm$ SD
Boavista 1	9	1	0.222	2	0.222 $\pm$ 0.166	0.00056 $\pm$ 0.00042
Boavista 2	8	2	1.071	3	0.750 $\pm$ 0.096	0.00311 $\pm$ 0.00048
Lanzarote	10	2	0.400	3	0.378 $\pm$ 0.181	0.00101 $\pm$ 0.00052
Raso	21	21	2.248	6	0.552 $\pm$ 0.122	0.00565 $\pm$ 0.00316
Veneguera	17	5	0.588	3	0.228 $\pm$ 0.129	0.00148 $\pm$ 0.00103
Frioul	6	2	0.867	3	0.733 $\pm$ 0.155	0.00218 $\pm$ 0.00062
Juan de Nova	10	1	0.200	2	0.200 $\pm$ 0.154	0.00050 $\pm$ 0.00039
Amsterdam	5	0	0.000	1	0 $\pm$ 0	0 $\pm$ 0
Pescadores	14	4	1.033	4	0.648 $\pm$ 0.116	0.00299 $\pm$ 0.00082
Tromelin	6	0	0.000	1	0 $\pm$ 0	0 $\pm$ 0
<b>Total</b>	<b>106</b>	<b>146</b>	<b>51.704</b>	<b>24</b>	<b>0.888 <math>\pm</math> 0.017</b>	<b>0.15030 <math>\pm</math> 0.00532</b>
rDNA 18S – 312 bp						
Population	Seq	NS	K	H	Hd $\pm$ SD	$\pi$ $\pm$ SD
Boavista 1	16	0	0.000	1	0 $\pm$ 0	0 $\pm$ 0
Boavista 2	14	10	1.429	2	0.143 $\pm$ 0.119	0.00468 $\pm$ 0.00390
Lanzarote	10	2	0.822	3	0.511 $\pm$ 0.164	0.00263 $\pm$ 0.00086
Raso	11	0	0.000	1	0 $\pm$ 0	0 $\pm$ 0
Veneguera	22	0	0.000	1	0 $\pm$ 0	0 $\pm$ 0
Frioul	20	11	1.384	4	0.284 $\pm$ 0.128	0.00452 $\pm$ 0.00290
Juan de Nova	9	2	0.444	2	0.222 $\pm$ 0.166	0.00144 $\pm$ 0.00108
Amsterdam	12	2	0.697	3	0.591 $\pm$ 0.108	0.00228 $\pm$ 0.00055
Pescadores	9	0	0.000	1	0 $\pm$ 0	0 $\pm$ 0
Tromelin	2	2	2.000	2	1 $\pm$ 0.5	0.00662 $\pm$ 0.00331
<b>Total</b>	<b>125</b>	<b>33</b>	<b>6.804</b>	<b>13</b>	<b>0.7001 <math>\pm</math> 0.026</b>	<b>0.02246 <math>\pm</math> 0.00111</b>

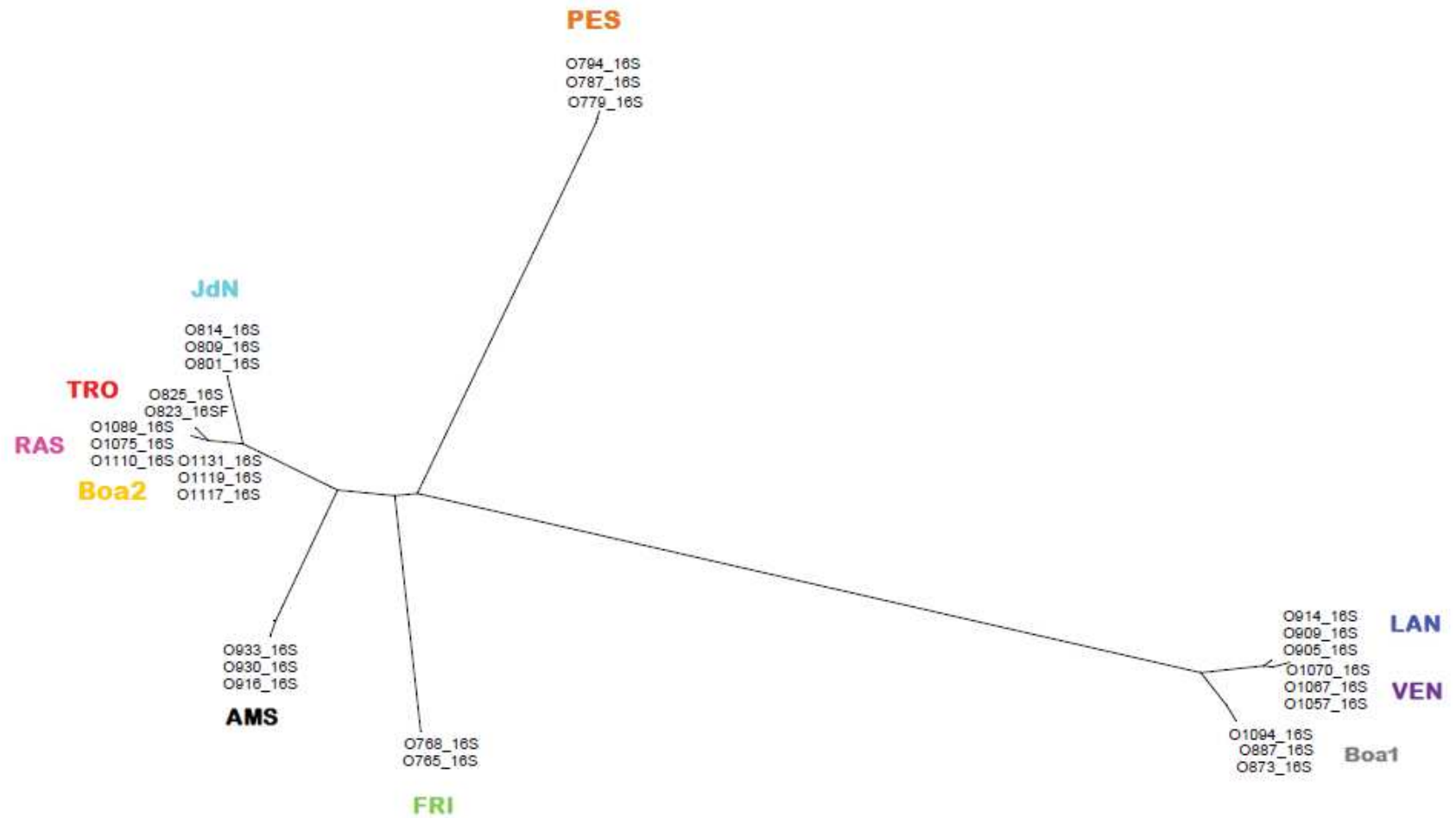
**Table A2:** KST\* indexes for the COI and 18S gene fragments. Significance is indicated as follow: NS = not significant; \*0.01<P<0.05; \*\*0.001<P<0.01; \*\*\*P<0.001

	<b>COI KST*</b>	Boavista 1	Boavista 2	Lanzarote	Raso	Veneguera	Frioul	Juan de Nova	Amsterdam	Pescadores
		<b>0.84520-</b> ***								
Cape Verde	Boavista 2									
		<b>0.84710 -</b> ***								
Canary	Lanzarote		<b>0.82358 - ***</b>							
		<b>0.76706 -</b> ***								
Cape Verde	Raso		<b>0.09014 - **</b>	<b>0.76297 - ***</b>						
		<b>0.36976 -</b> ***		(-0.00754 - NS)	<b>0.80310 - ***</b>					
Canary	Veneguera		<b>0.83180 - ***</b>							
Mediterranean		<b>0.87886 -</b> ***				<b>0.83751 -</b> ***				
Sea	Frioul		<b>0.78129 - ***</b>	<b>0.85076 - ***</b>	<b>0.66309- ***</b>					
		<b>0.94017 -</b> ***				<b>0.90223 -</b> ***				
Indian Ocean	Juan de Nova		<b>0.70214 - ***</b>	<b>0.91596 - ***</b>	<b>0.62142 - ***</b>		<b>0.87763 - ***</b>			
		<b>0.95378 -</b> ***				<b>0.87913 -</b> ***				
Indian Ocean	Amsterdam		<b>0.81681 - **</b>	<b>0.91416 - ***</b>	<b>0.67154 - ***</b>		<b>0.86800 - **</b>	<b>0.95249 - **</b>		
		<b>0.82330 -</b> ***				<b>0.83738-</b> ***		<b>0.82518 -</b> ***	<b>0.77029 -</b> ***	
Peru	Pescadores		<b>0.74313 - ***</b>	<b>0.81254 - ***</b>	<b>0.73578- ***</b>		<b>0.74885- ***</b>	***	***	
		<b>0.95818 -</b> ***			(-0.03912 - NS)	<b>0.89025 -</b> ***		<b>0.92350 -</b> ***		<b>0.79485 -</b> ***
Indian Ocean	Tromelin		<b>0.23322 - ***</b>	<b>0.92168 - ***</b>			<b>0.88734 - ***</b>		<b>1 - **</b>	
		<b>KST* Tot COI</b>	<b>0.88398</b>	***						

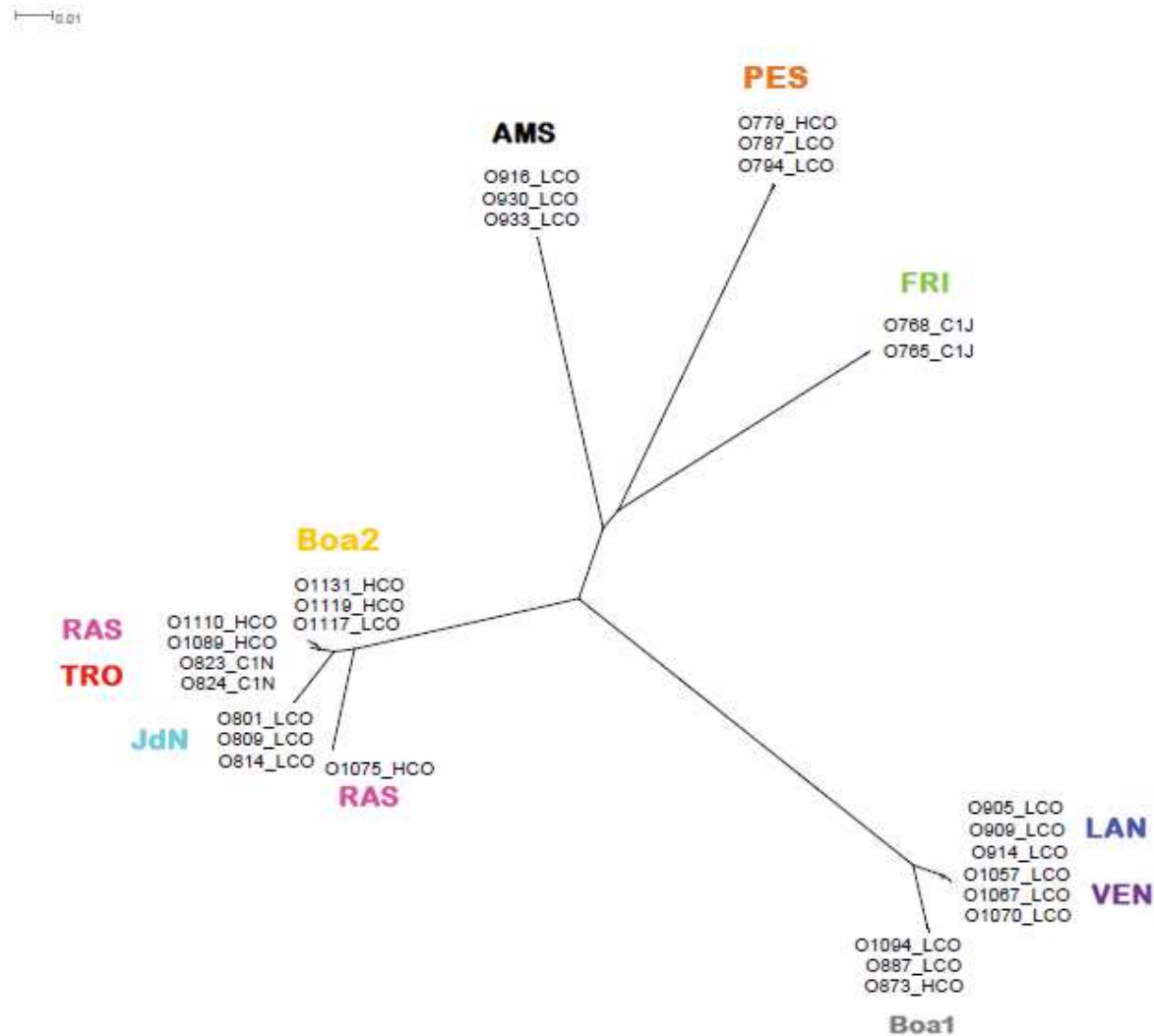


<b>18S KST*</b>		Boavista 1	Boavista 2	Lanzarote	Raso	Veneguera	Frioul	Juan de Nova	Amsterdam	Pescadores
		<b>0.87022 -</b> ***								
Cape Verde	Boavista 2									
Canary	Lanzarote	<b>0.16338 - *</b>	<b>0.70563 - ***</b> (- 0.02041 - NS)	<b>0.83630 - ***</b>						
Cape Verde	Raso	<b>1 - ***</b>								
Canary	Veneguera	-	<b>0.88712 - ***</b>	<b>0.20098 - **</b>	<b>1 - ***</b>					
Mediterranean Sea	Frioul	<b>0.82183 -</b> ***	(-0.01062 - NS)	<b>0.67492 - ***</b>	(-0.02216 - NS)	<b>0.84688 -</b> ***				
Indian Ocean	Juan de Nova	<b>0.93157 -</b> ***	(-0.01976 - NS)	<b>0.74844 - ***</b>	(0.02778 - NS)	<b>0.93960 -</b> ***	(-0.02119 - NS)			
Indian Ocean	Amsterdam	<b>0.86875 -</b> ***	<b>0.60894 - ***</b>	<b>0.71304 - ***</b>	<b>0.73467 - ***</b>	<b>0.88494 -</b> ***	<b>0.55466 - ***</b>	<b>0.62344 -</b> ***		
Peru	Pescadores	<b>1 - ***</b>	<b>0.83213 - ***</b>	<b>0.84303 - ***</b>	<b>1 - **</b>	<b>1 - ***</b>	<b>0.74909 - ***</b>	<b>0.90517 -</b> ***	<b>0.81519 -</b> ***	
Indian Ocean	Tromelin	<b>1 - *</b>	(0.20206 - NS)	<b>0.56640 - **</b>	(1 - NS)	<b>1 - **</b>	(0.10675 - NS)	(0.37209 - NS)	<b>0.42243 - **</b>	<b>1 - *</b>
	<b>KST* Tot</b>									
	<b>18S</b>		<b>0.86822</b>	***						

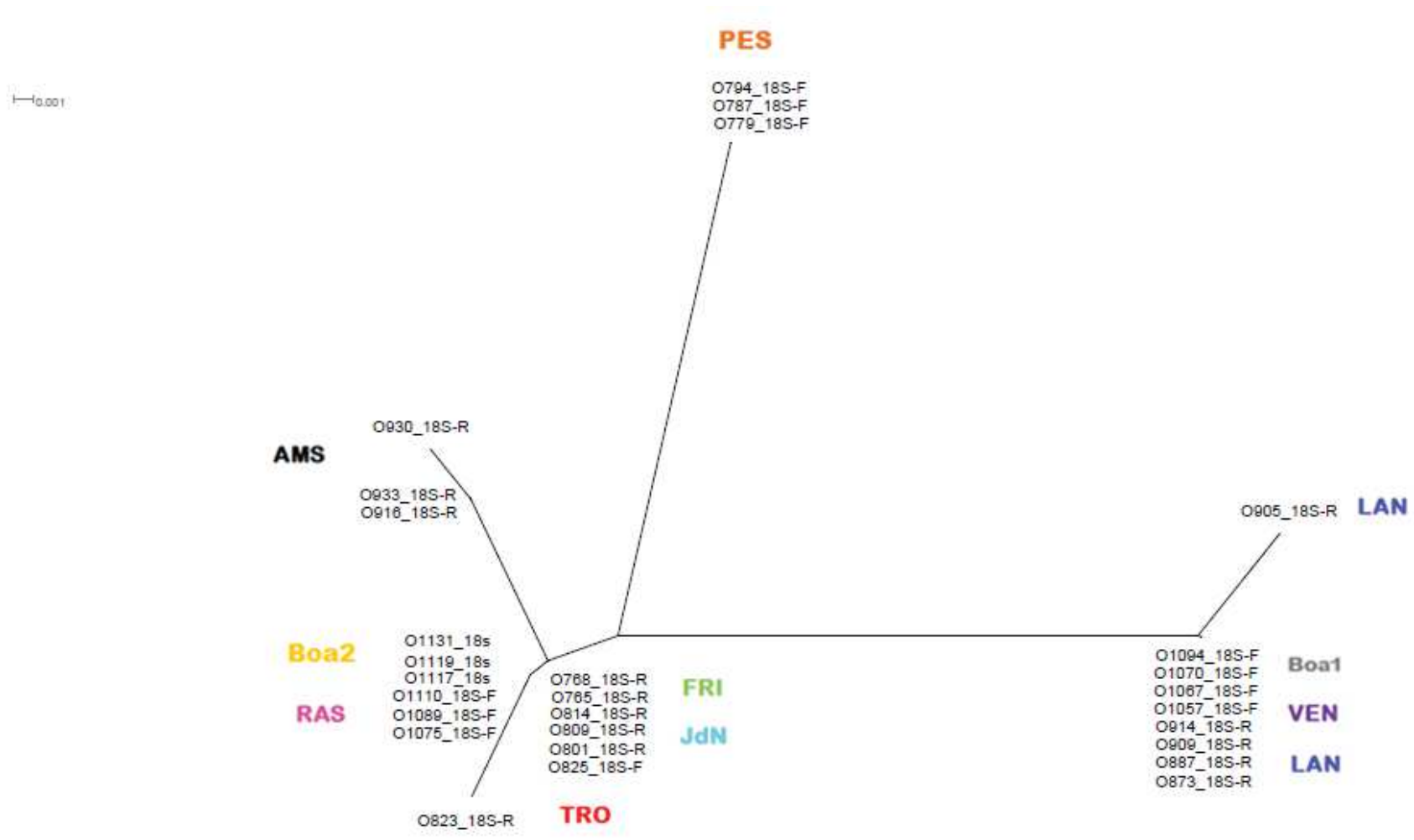
0.01



**Fig.A1.1:** Cluster analysis based on sequences of 16S mitochondrial marker. Labels under population abbreviations represent sequence numbers.



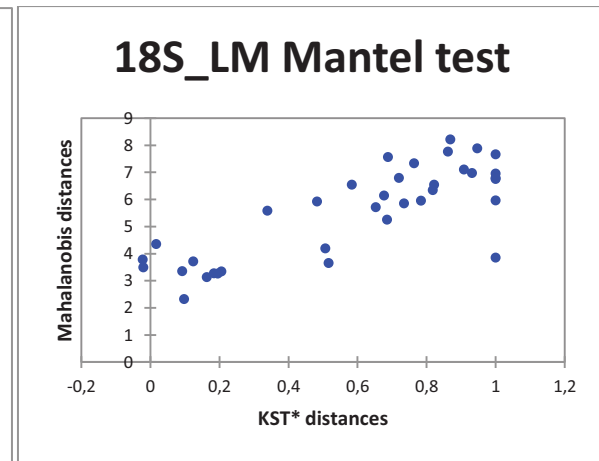
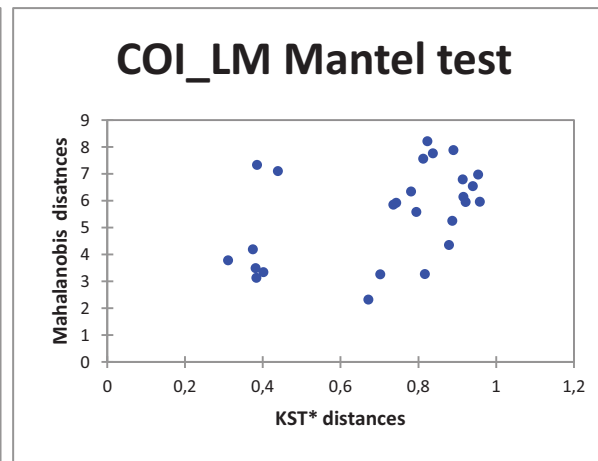
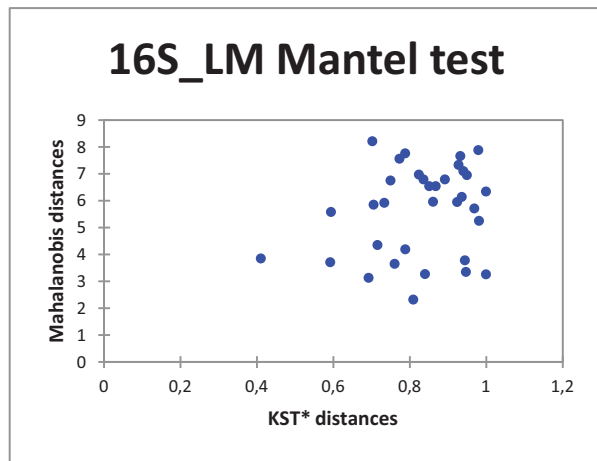
**Fig.A1.2:** Cluster analysis based on sequences of COI mitochondrial marker. Labels under population abbreviations represent sequence numbers.

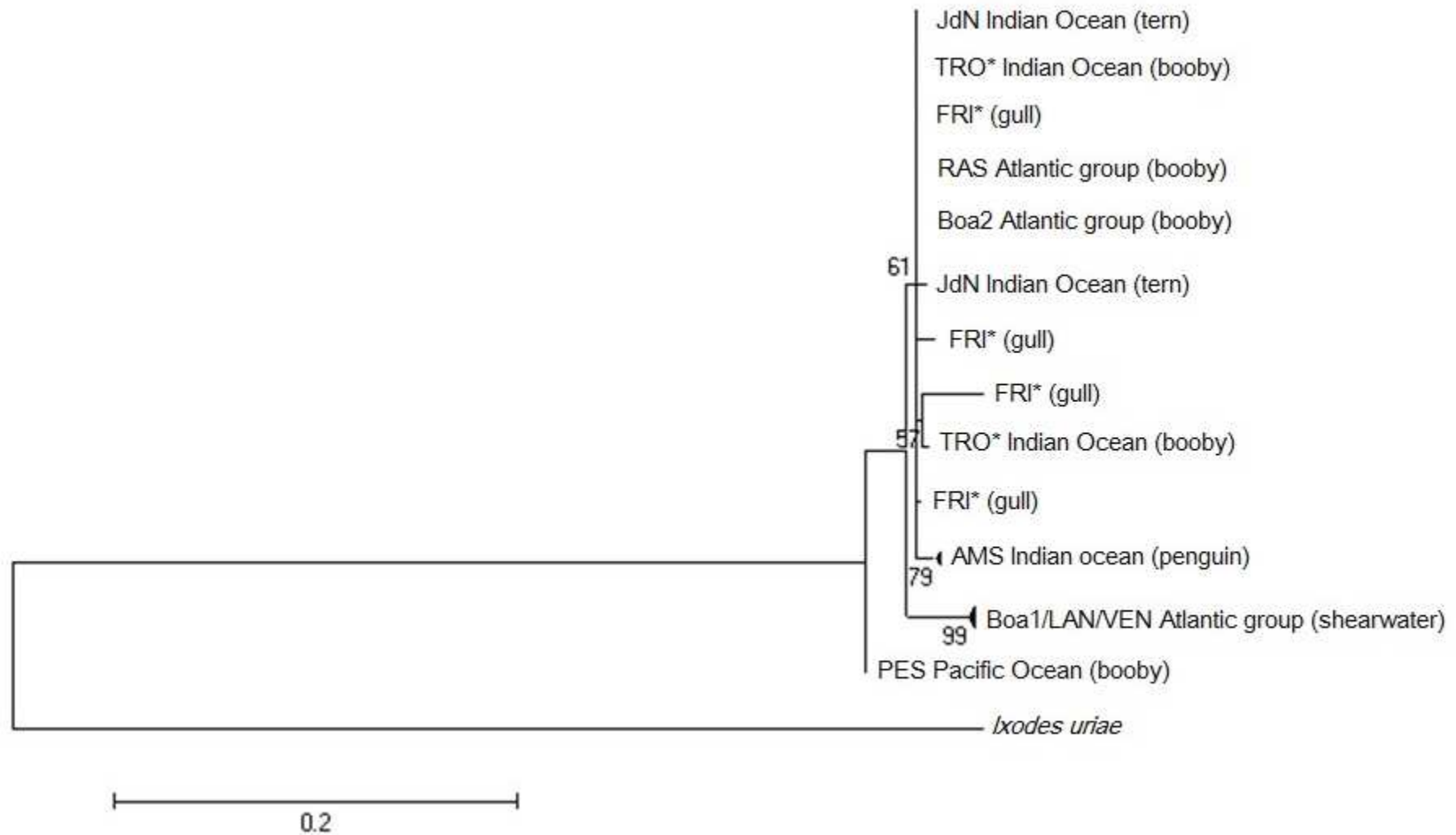


**Fig.A1.3:** Cluster analysis based on sequences of 18S nuclear marker.

**Table A3:** Mantel test results between morphometric (Mahalanobis distances) and genetic (KST\* distances) matrices. The corresponding graphs for each analysis for the landmark (LM) distances are shown below.

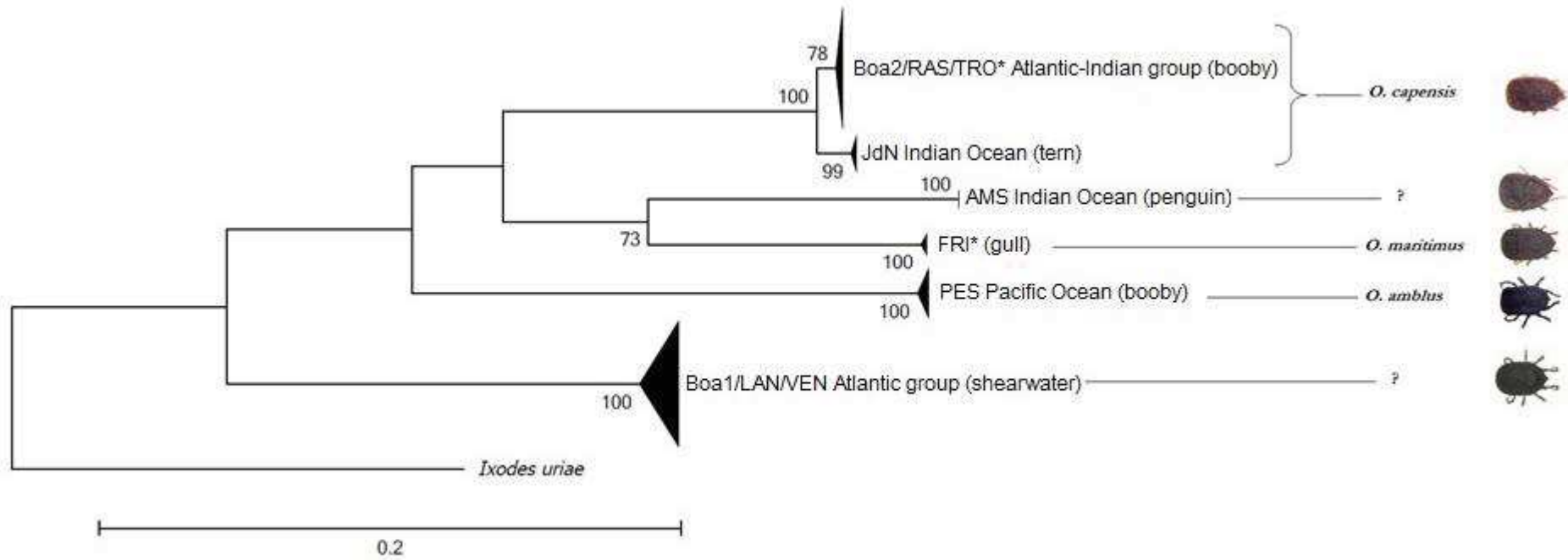
	<b>16S</b>			<b>COI</b>			<b>18S</b>		
	r-value	r <sup>2</sup> (%)	p-value	r-value	r <sup>2</sup> (%)	p-value	r-value	r <sup>2</sup> (%)	p-value
<b>Landmarks</b>	0.378	14.3	0.025	0.532	28.3	0.001	0.810	65.6	<0.0001
<b>Outline</b>	0.316	10.0	0.059	0.449	20.2	0.005	0.603	36.4	0.0003





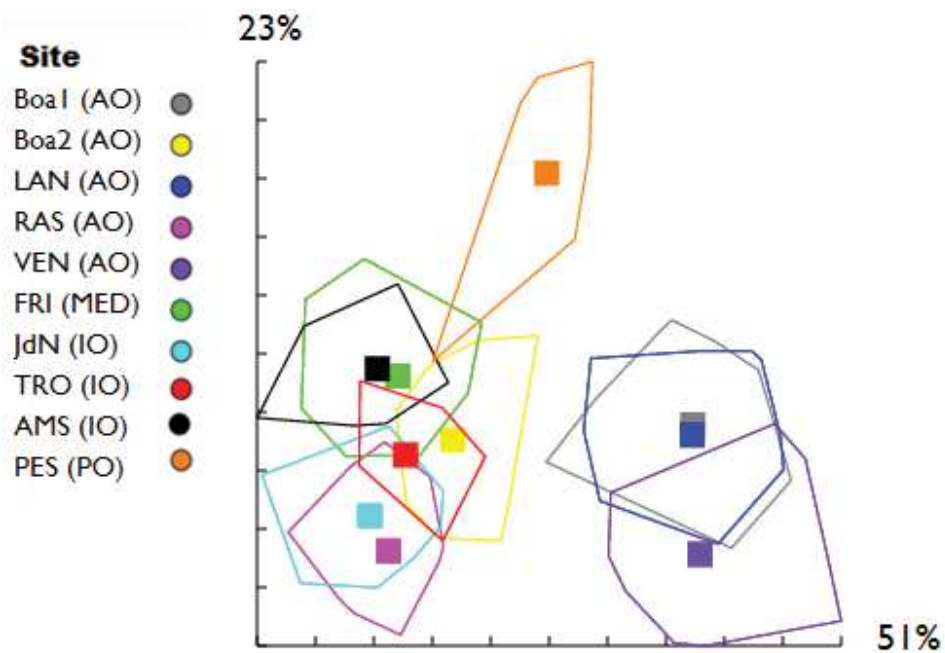
**Fig.A2.1:** Phylogenetic tree based on sequences of 18S nuclear marker. *Ixodes uriae* represents the outgroup. Population names with star have been verified based on classical morphological traits of larval ticks.



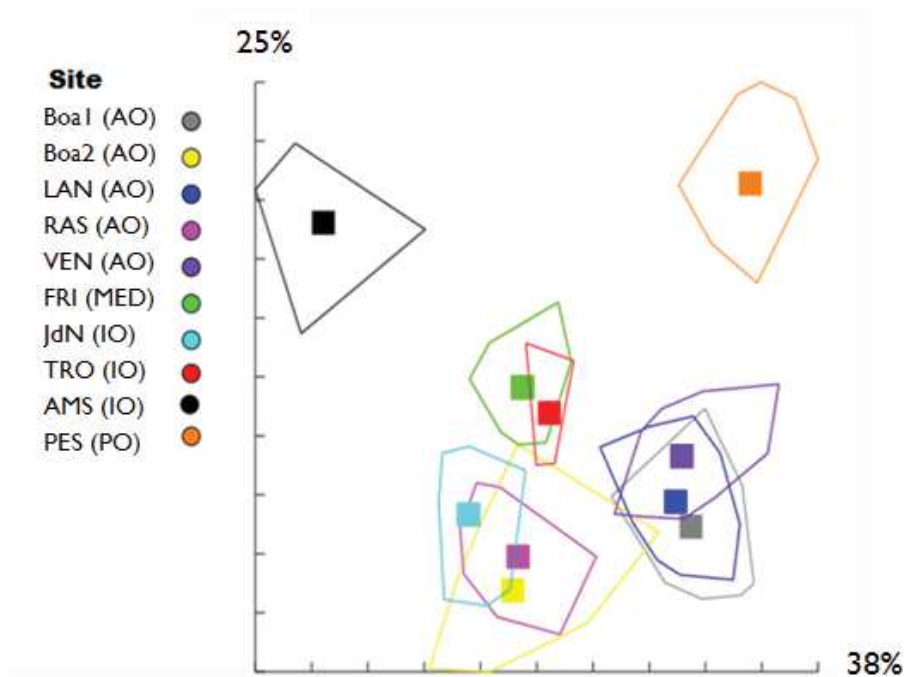


**Fig.A2.2:** Phylogenetic tree based on sequences of COI mitochondrial marker. *Ixodes uriae* represents the outgroup. Population names with star have been verified based on classical morphological traits of larval ticks.

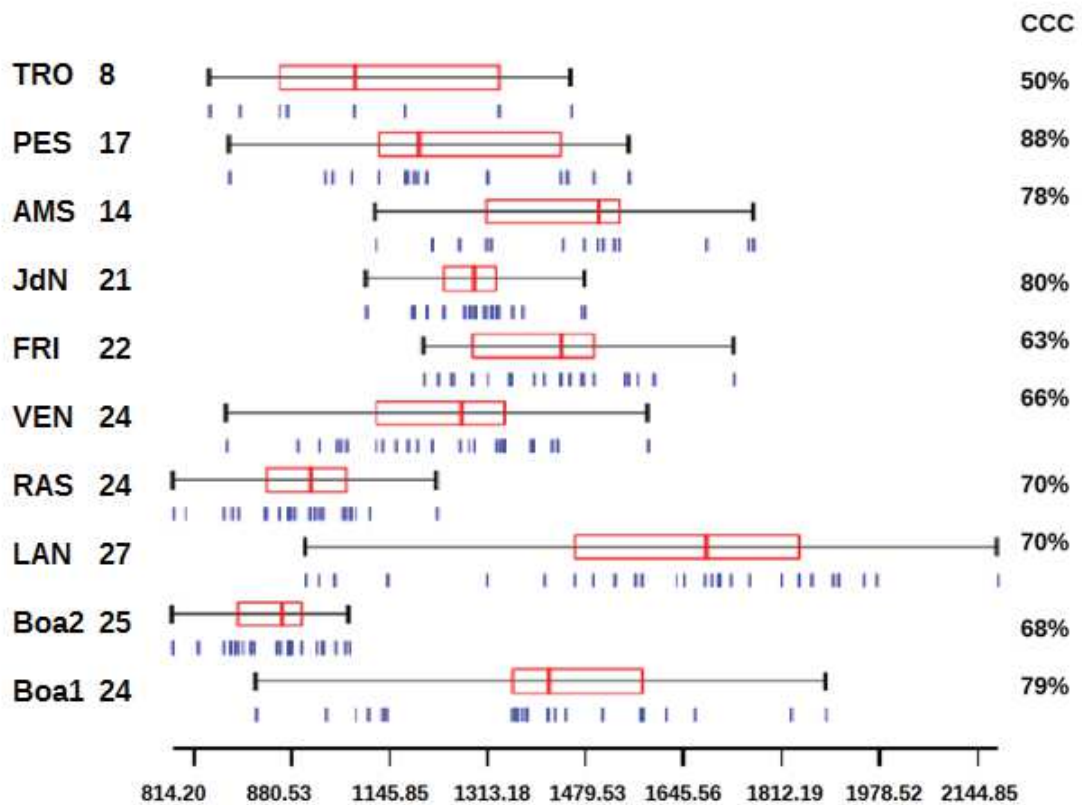
a



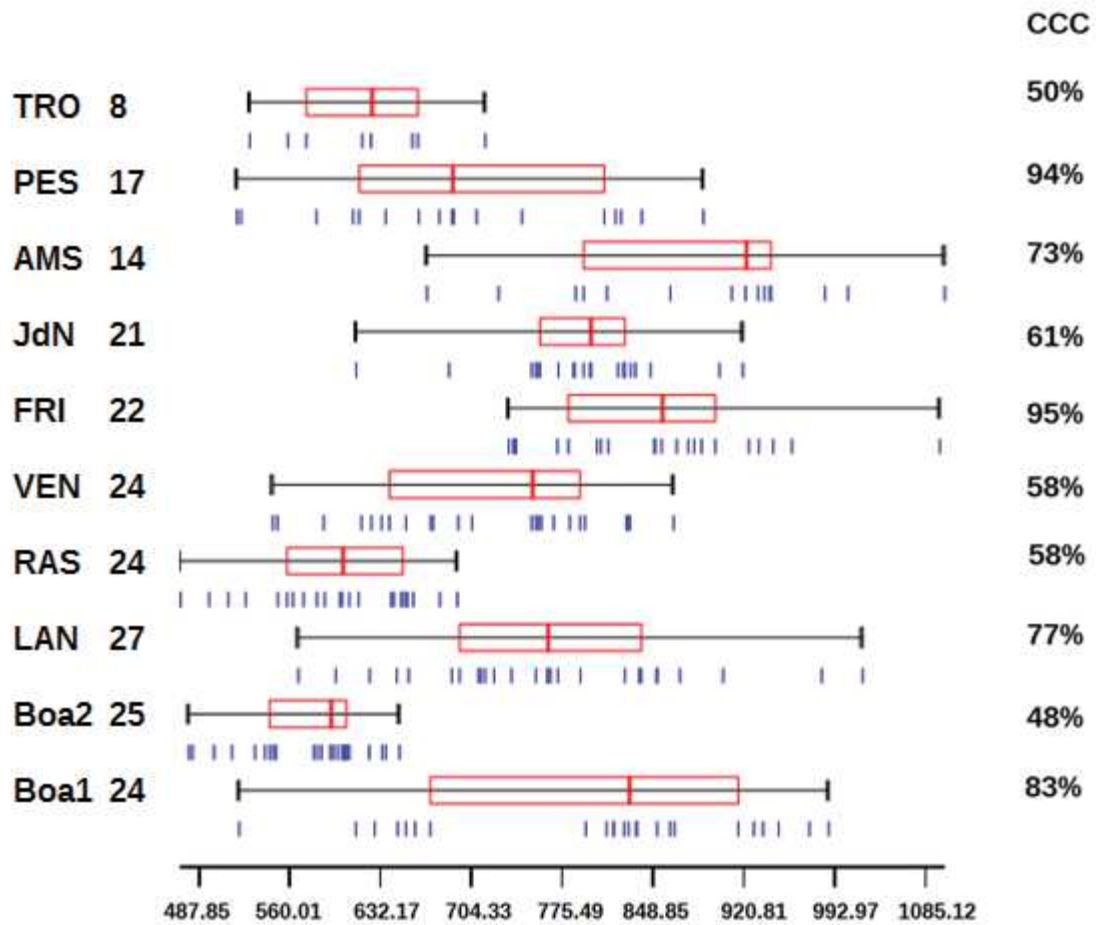
b



**Fig.A3:** First two functions of a discriminant analysis performed on **(a)**: a set of first RW based on the landmark arrangement and **(b)**: on normalized Fourier coefficients based on the outline analysis and showing the proportion of total variance explained. The polygons represent the maximal parameter space of each population and the squares the population mean. The site locations are: AO: Atlantic Ocean. MED: Mediterranean Sea. IO: Indian Ocean. PO: Pacific Ocean.



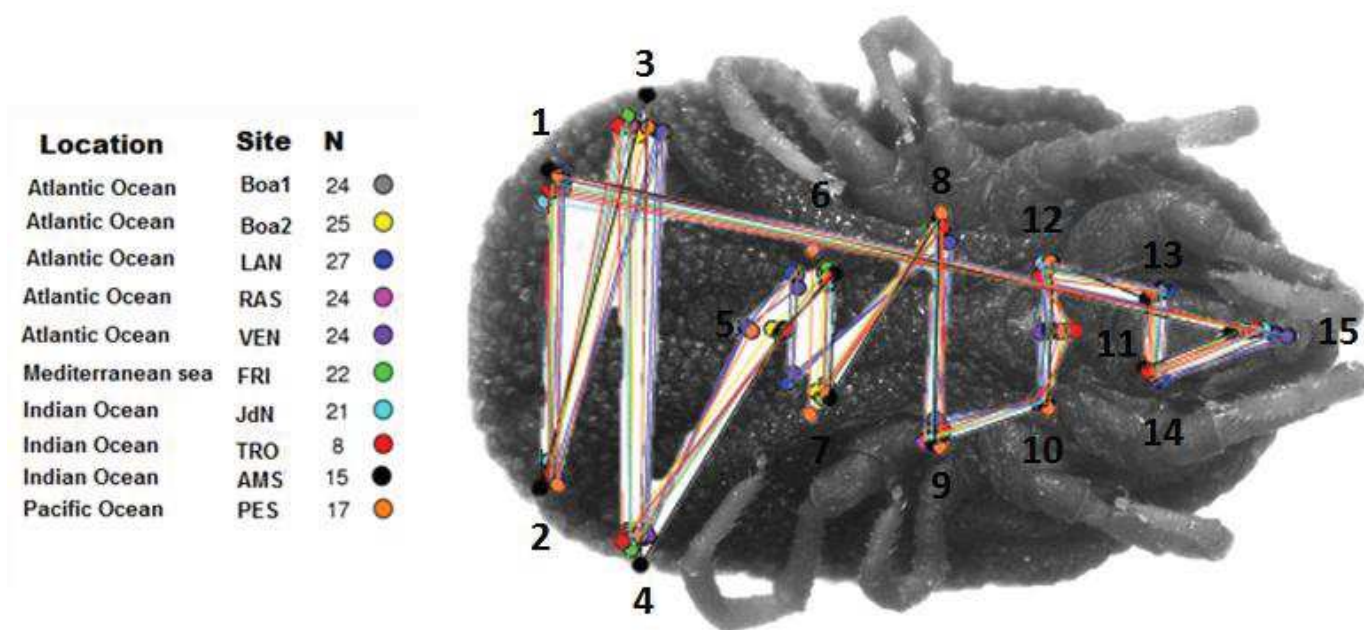
**Fig.A4:** Size variation (in pixels) presented as quantile plots for tick populations based on landmark arrangements. The box shows the median as a line across the middle and the quartiles (25<sup>th</sup> and 75<sup>th</sup> percentiles) as its ends. Extremities represent the minimal and maximal values. The right column represents the validated percent classification (CCC) result based on shape, used to test the affiliation of each individual tick to each population. The population abbreviations (Table 1) and the sample size for each population are indicated on the left.



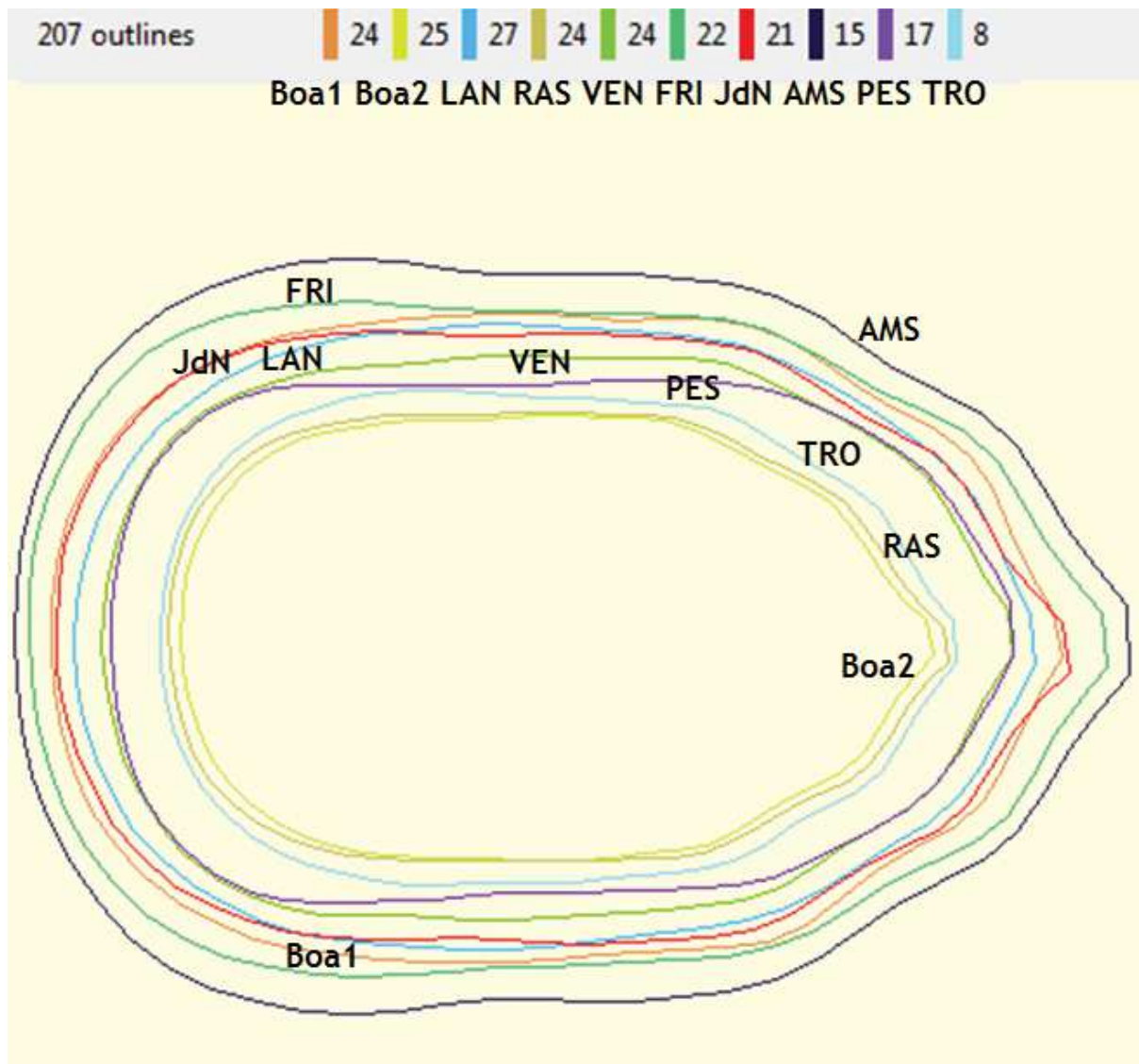
**Fig.A5:** Size variation (in pixels) presented as quantile plots for tick populations based on the outlines analysis. The box shows the median as a line across the middle and the quartiles (25<sup>th</sup> and 75<sup>th</sup> percentiles) as its ends. Extremities represent the minimal and maximal values. The right column represents the validated percent classification (CCC) result based on shape, used to test the affiliation of each individual tick to each population. The population abbreviations (Table 1) and the sample size for each population are indicated on the left.

**Table A4:** P-value tables of the pairwise mean comparisons among populations after 1000 permutations, for the landmarks and outline analyses (starting value for the Bonferroni test: 0.00111111111111111111). Significant values are in bold.

Landmarks	Boa1	Boa2	LAN	RAS	VEN	FRI	JdN	AMS	PES
Boa2	<b>0.0000</b>								
LAN	0.0070	<b>0.0000</b>							
RAS	<b>0.0000</b>	0.1220	<b>0.0000</b>						
VEN	0.0040	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>					
FRI	0.8650	<b>0.0000</b>	0.0070	<b>0.0000</b>	<b>0.0000</b>				
JdN	0.0550	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	0.1490	<b>0.0000</b>			
AMS	0.4040	<b>0.0000</b>	0.0930	<b>0.0000</b>	<b>0.0000</b>	0.3820	0.0020		
PES	0.0290	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	0.8100	<b>0.0010</b>	0.3250	0.0050	
TRO	0.0040	0.0080	<b>0.0000</b>	0.0840	0.0690	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	0.0700
Outlines	Boa1	Boa2	LAN	RAS	VEN	FRI	JdN	AMS	PES
Boa2	<b>0.0000</b>								
LAN	0.1620	<b>0.0000</b>							
RAS	<b>0.0000</b>	0.3170	<b>0.0000</b>						
VEN	0.0090	<b>0.0000</b>	0.0590	<b>0.0000</b>					
FRI	0.3480	<b>0.0000</b>	0.0150	<b>0.0000</b>	<b>0.0000</b>				
JdN	0.2450	<b>0.0000</b>	0.7100	<b>0.0000</b>	0.0140	0.0030			
AMS	0.0400	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	0.0870	<b>0.0000</b>		
PES	0.0090	<b>0.0000</b>	0.0320	<b>0.0000</b>	0.5020	<b>0.0000</b>	0.0050	<b>0.0000</b>	
TRO	<b>0.0000</b>	0.0240	<b>0.0010</b>	0.2100	0.0110	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	0.0780



**Fig.A6:** Mean landmark arrangements for each population as shown on a female tick from the JdN population. Each point represents one landmark. Landmark N°1-2: width between landmarks 1 and 2 on extremities of the transverse post-anal groove; N°3-4: width between landmarks 3 and 4 each located on extremities of the pre-anal groove; N°5: anus; N°8-9: width between landmarks 8 and 9 at coxae III/IV; N°10-12: width between landmarks 10 and 12 at coxae I/II; N°11: genital pore; N°13-14: width between landmarks 13 and 14 on the lower extremity of the basis capitulum; N°15: capitulum extremity.



**Fig.A7:** Mean contours for each population. Each colored bar represents one population: Orange: Boavista 1; Yellow: Boavista 2; Blue: Lanzarote; Light brown: Raso; Light green: Veneguera; Green: Frioul; Red: Juan de Nova; Black: Amsterdam; Purple: Pescadores; Light blue: Tromelin. Numbers beside colored bars indicate the number of used ticks in each population.





Article 4

Dupraz M, Toty C, Boulinier T, Dujardin J-P, McCoy K-D.  
Morphological convergence in *I. uriae* host races. En préparation.



Morphological convergence in *Ixodes uriae* host races.

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## Abstract

Parasite diversification occurs within a particular ecological context resulting from the dynamic equilibrium between extinction, colonization and divergence in different microhabitats. For ectoparasites, the host often represents the greatest part of the parasite needs, i.e. alimentation, habitat and reproduction, and host-associated traits may exert strong selective pressures on the parasite to specialize. Among the parasite traits under pressure, morphology can be of primary importance because physical defenses used by the host can be very efficient at eliminating parasites, particularly ectoparasites. Previous studies in the widely-distributed seabird tick, *Ixodes uriae*, demonstrated the occurrence of genetically distinct host races within multi-specific colonies and showed that a given host race could evolve independently in different isolated areas of the tick distribution. Here, we use geometric morphometrics to test whether host-associated selection pressure has also resulted in phenotypic congruence in two different tick host races associated with Atlantic puffins (*Fratercula arctica*) and common guillemots (*Uria aalge*). Using both landmark and outline analyses, we assessed the degree of morphological congruence among ticks using the same host species but in three distinct colony locations. We found that adult females showed some degree of congruence when exploiting puffins, but that female ticks on guillemots tended to be morphologically distinct in each colony location. These patterns were much less apparent in male ticks, which may not be surprising as this life stage does not feed on the host. These results suggest that some phenotypic divergence in relation to host use does occur, but that either selection is not strong enough to find the same distinct traits associated with each host type and/or the populations that we have analyzed are not yet at equilibrium. We discuss the potential host factors that may drive the observed patterns and the future directions required to evaluate our alternative hypotheses.

## 1 Introduction

Parasite diversification occurs within a particular ecological context resulting from a dynamic equilibrium between extinction, colonization and divergence in the different microhabitats occupied by the parasite (Poulin, 2006). For parasites, the host represents the greatest part of its biological needs, i.e. food, habitat and site of reproduction. Because of this, we generally consider that high predictability in parasite-host encounters should favor the evolution of specialist parasites, parasites that avoid the cost of investing or maintaining multiple host-specific adaptations, for example to different immunological systems (Combes, 1995). For ectoparasites, like ticks, contact with the host can be more temporary, largely restricted to feeding (De Meeûs et al., 1998). However, although these contacts are short, the molecular dialogue can be intense at the host interface and may lead to the emergence of host specialization (Magalhães et al., 2007; Mans and Neitz, 2004; McCoy et al., 2013). For those parasites that spend a good part of their life cycle off-host, environmental constraints such as temperature and humidity can also greatly impact their fitness and may select for specific parasite traits related to parasite physiology, morphology or behavior (Clayton et al., 2010; Kuclu et al., 2011; Prudhomme et al., 2012). Depending on the interplay between selection in the on-host and off-host environments and its effects on assortative mating, parasite divergence among host types can either be helped or hindered.

The involvement of the different host and environmental factors in population divergence are often complicated to distinguish, particularly when trait changes are not highly visible. Morphometrical analyses is one method that can be used to differentiate cryptic population divergence as it can detect and quantify subtle population-level variation and help identify factors implicated in the divergence process (Barluenga et al., 2006; Dujardin et al., 2014; Klimov et al., 2006).

*Ixodes uriae* is a hard tick (family Ixodidae) widely distributed across the circumpolar regions of both hemispheres and parasitizing a great diversity of seabird species breeding in dense and predictable colonies (Dietrich et al., 2011). This species is nidicolous, spending the major part of its life cycle off-host, in the nest or under rocks or crevices and interacting with the seabird host for a brief period of 5-10 days each year during the seabird breeding period (Sonenshine and Roe, 2014). Based on their large host spectra and wide distribution, this tick interacts within a diverse array of ecological conditions. Indeed, parasitized hosts have different life history traits and behaviors which can impose different selection pressures on infesting ticks. These hosts may also use different microhabitats within colonies, again imposing different abiotic conditions for tick survival and persistence. Previous studies in this model system demonstrated the existence of genetically distinct host races occurring within multi-host colonies (Dietrich et al., 2012; Kempf et al., 2009; McCoy et al., 2005; McCoy et al., 2001) and that these

races evolve recurrently, even for the same host species (Dietrich et al., 2014a; McCoy et al., 2013). Adaptive specialization of these races for different host species was tested by a field-cross-infestation experiment and revealed performance differences on alternative hosts, indicating that the genetic divergence observed was at least partially link to host specialization (Dietrich et al., 2014b). An initial morphometric study of these tick races further revealed variation in tick size and shape in relation to host use (Dietrich et al., 2013). However, this study was limited to different tick life stages in a single multi-host colony. It is unknown as yet whether the specific morphological differences observed among the sympatric races reflect direct selection pressure from the host. If this is the case, we would expect the same morphological traits to occur for each race, and particularly so when host race formation has occurred independently.

Here, we test this hypothesis using two tick races of *Ixodes uriae* sampled from three multi-host colonies where sympatric host races were previously demonstrated to occur. We use two geometric morphometric techniques, landmarks and outlines, to determine whether morphometrical convergence has occurred in different locations in relation to host use. As understanding the pattern of morphological changes in specialized parasites can help us to identify the factors underlying the diversification process, we try to relate observed differences with selection factors coming from the host and the abiotic environment.

## 2 Material and methods

### 2.1 Tick sampling and predictions

During the 2016 breeding season, non engorged adult *Ixodes uriae* ticks were collected on seabirds or from the host nesting area in three multi-host seabird colonies on island in the North Atlantic and preserved in 70% ethanol: Hornoya in northern Norway (HOR), Grimsey (GRI) in northern Iceland and Gull Island (GUL) in Newfoundland, Canada. On each island, ticks were sampled from three sympatric seabird species: the black-legged kittiwake (KT) *Rissa tridactyla*, the common guillemot (CG) *Uria aalge* and the Atlantic puffin (PF) *Fratercula arctica* (Table 1).



**Table 1:** Sampled sites of *Ixodes uriae* in North Atlantic seabird colonies.

Origin	Lat. Long.	Pop	Host species	N° ticks
Iceland - Grimsey	66.549744, -17.992711	GRI CG	<i>Uria aalge</i>	10F – 10M
Iceland - Grimsey	66.549744, -17.992711	GRI PF	<i>Fratercula arctica</i>	10F – 10M
Canada - Gull	47.952609, -53.040384	GUL CG	<i>Uria aalge</i>	10F – 10M
Canada - Gull	47.952609, -53.040384	GUL PF	<i>Fratercula arctica</i>	4F – 10M
Norway - Hornøya	70.389348, 31.154289	HOR CG	<i>Uria aalge</i>	10F - 10M
Norway – Hornøya	70.389348, 31.154289	HOR PF	<i>Fratercula arctica</i>	10F - 10M

Previous studies suggested that the evolution of host race formation in the western North Atlantic was largely independent of this process in the eastern North Atlantic, with some exchange occurring between certain colonies in Iceland and Norway (McCoy et al., 2013). This is particularly the case for the PF tick race which tends to show more spatially extensive gene flow than the other tick races (McCoy et al., 2003). In 2016, KT populations were in failure or not synchronous with our passage in the colonies, so we were not able to collect enough ticks to include in our analyses. Consequently, we used only CG and PF populations to test for large-scale convergence in morphological traits in relation to host use. Based on previous genetic analyses and the initial morphometric study carried out on HOR (Dietrich et al., 2013), we expected that PF ticks should be larger overall and relatively wider than CG ticks in all three colony locations. Some direct gene flow may occur between the colonies of HOR and GRI which could account for similar phenotypic patterns (McCoy et al., 2013). However, this should not be the case for GUL where host race evolution has been independent. The occurrence of similar between-race patterns in this colony would provide evidence for morphological convergence related to host selection pressures. As male adult ticks do not feed on the host, we also expected that patterns of host-associated divergence would be stronger in female ticks than in male ticks.

## 2.2 Morphometric analyses

Standardized images of 10 females and 10 males were taken using a binocular microscope (Leica M80) except for GUL PF female population containing only 4 ticks. Morphometrical analyses were performed using the CLIC package (Dujardin et al., 2010; Dujardin and Slice, 2006), freely available at <http://mome-clic.com/>. Landmark analyses were used to acquire measures of morphological features involved in biological activities (feeding, reproduction, locomotion) and outline analyses to obtain an overall measure of shape and size of each tick population. Females and males were treated separately.

*Landmark-based analyses:* We used a set of 10 landmarks for females and 12 for males on the ventral face (Fig. 1). Shape variables (uniform and non-uniform components) were obtained by computing a

generalized Procrustes analysis (GPA) with a superimposition algorithm using the landmark arrangement and removing the size factor. The connected residual coordinates of the landmarks were converted into “partial warps” (PW) to be used by standard multivariate analyses (principal component and discriminant analyses) allowing visual comparisons of mean shape between tick populations. The principal components of the PW (i.e., the relative warps, or RW) were computed to show the morphospace, represented by polygons, for each population separately. In each morphospace, the horizontal axis is the first relative warp (RW1), the vertical axis is the second one (RW2). Overlapping polygons share individuals for which outlines or landmark configurations are similar, while non-overlapping polygons are constituted by individuals sharing a particular conformation, typical of the population.

*Outline-based analysis:* The elliptic Fourier analysis (EFA) method (Kuhl and Giardina, 1982) was used to compute the contour of the dorsal face of each tick. The outline was decomposed into Fourier coefficients which were normalized (NEF) in order to be compared without the influence of size, orientation or starting point. A variance-covariance matrix was then constructed with these coefficients to summarize variation and principal components of NEF were used as input for multivariate analyses.





**Fig.1** : Images representing the 10 and 12 landmarks used on the ventral face of female and male ticks. Each point represents one landmark. Landmarks N°1-2: width between landmarks 1 and 2 on the lower extremity of the basis capitulum; N°3-4: width between landmarks 10 and 12 at coxae I; N°5: genital pore; N°6: anus; For female: N°7-8: width between landmarks 7 and 8 extremities of the genual groove; N°9-10: width between landmarks 9 and 10 on the spiracular plate; For male: N°7-8, 8-9, 9-10: width between landmarks of the festoons; N°11-12: width between landmarks 11 and 12 on the spiracular plate.

We computed the centroid size (CS) for each landmark configuration in size analyses, defined as the square root of the sum of the squared distances between the center of the landmark configuration and each individual landmark (Bookstein, 1997). We used the perimeter (P) as an estimate of body size in outlines analyses. Using the VAR module in CLIC software, we analyzed size means and variances among populations using permutation tests. At each permutation cycle, individuals were randomly exchanged among populations, and the mean and variance was recalculated (1000 permutations in total). Then, we used a Bonferroni correction to determine the significance of size differences between populations.

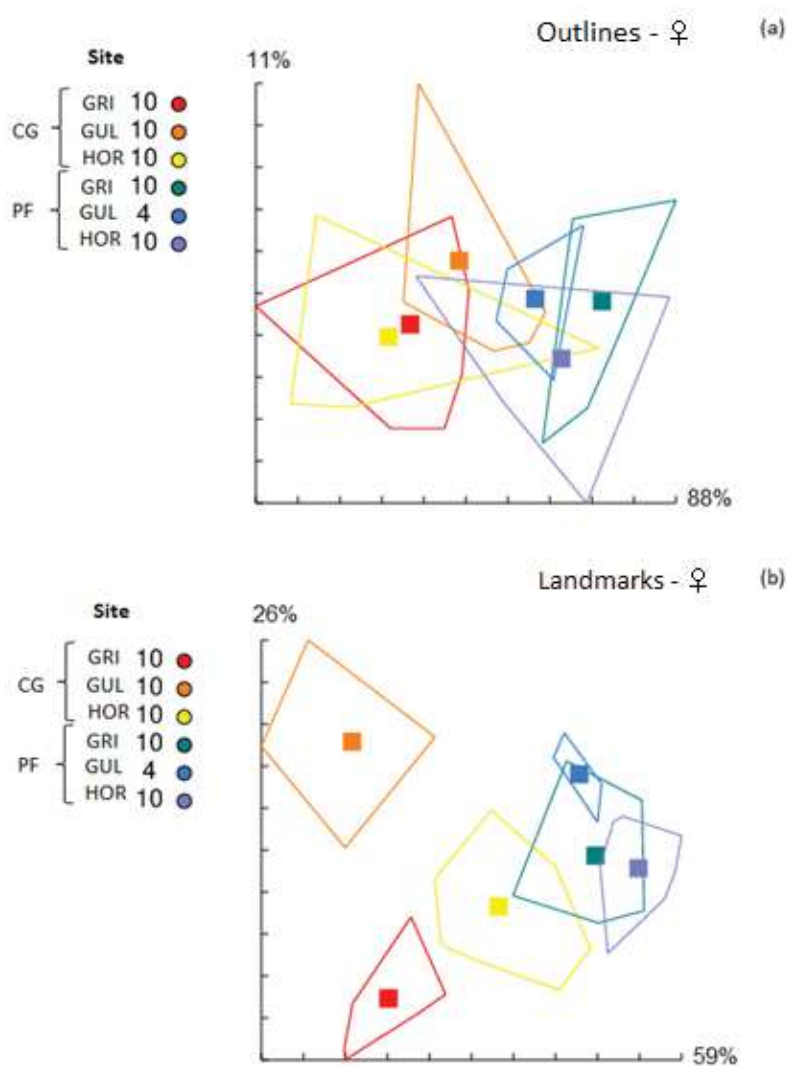
A random validated cross-check classification method (CCC), based on the jackknife method, was used to test the affiliations of each individual to each population.

The size effect (CS or P) on body shape (RW or principal component of NEF), was estimated by a linear regression using the PAD module in CLIC software.

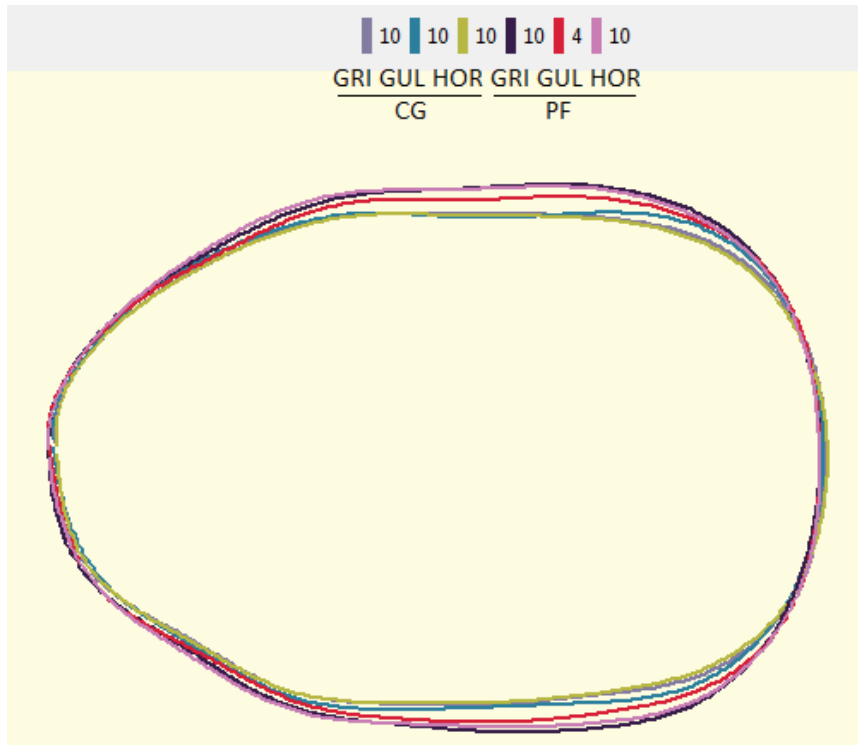
### 3 Results

#### Females

Outline analyses revealed that all tick populations were overlapping, sharing an overall body shape, with a slight tendency for CG and PF populations to be grouped together (Fig.2a). PF ticks showed significant size differences (Table 2) and tended to be larger than CG ticks (Fig.3). The size effect on shape represented 16.9% of the overall shape variation (DF1 regression:  $r=0.288$ ;  $r^2=8.3\%$ ;  $df=52$ ;  $t=2.17$ ,  $p=0.01737$ ; DF2 regression:  $r=-0.293$ ;  $r^2=8.6\%$ ;  $df=52$ ;  $t=2.21$ ,  $p=0.0007$ ). The cross-check classification showed that populations were not well-defined based on size alone, ranging from 10% correctly-assigned individuals for GRI CG to 60% for GRI PF (Fig.4a).



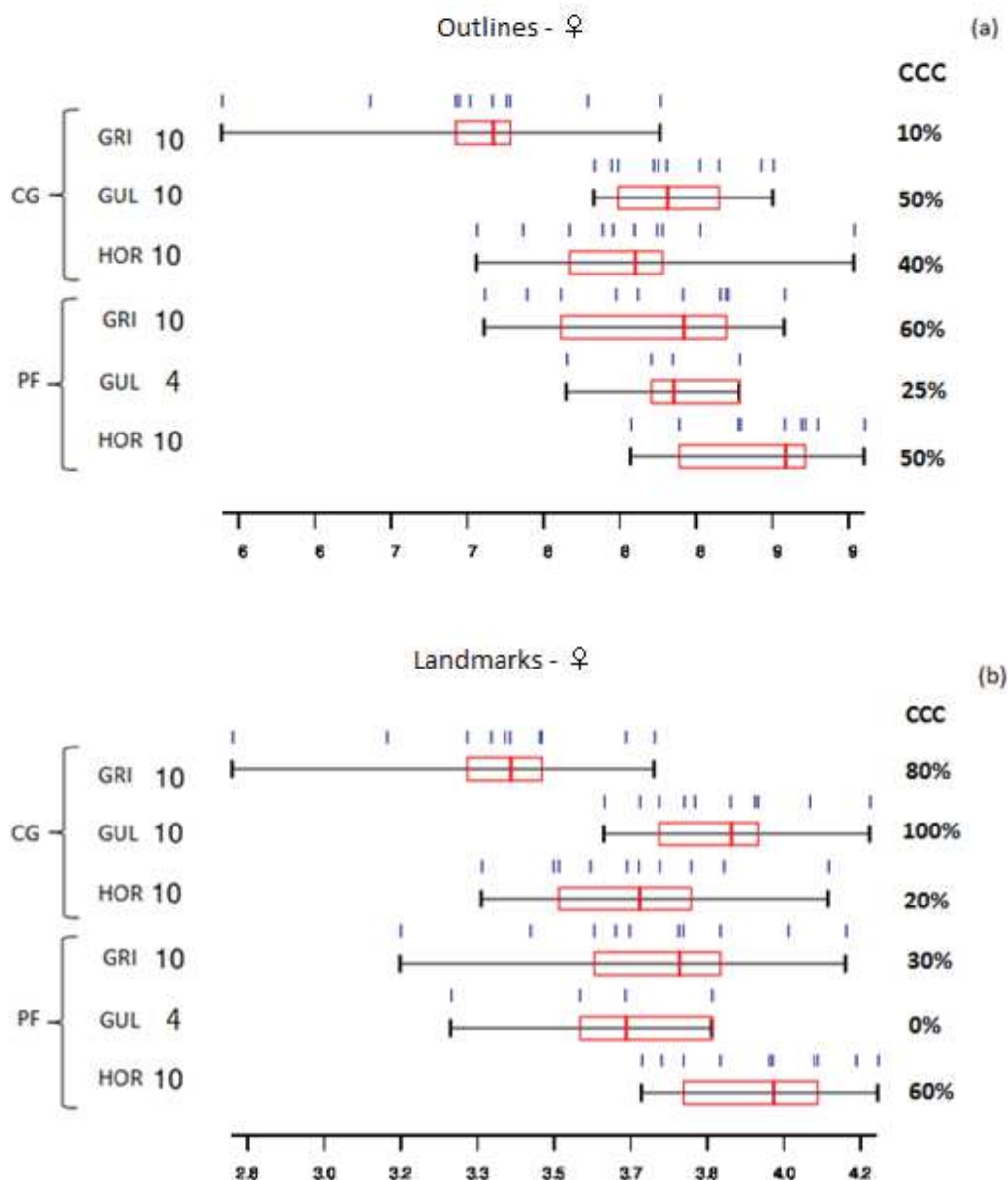
**Fig.2:** First two functions of a discriminant analysis performed on (a): normalized Fourier coefficients based on the outline analysis and (b): a set of first RW based on the landmark arrangement of females and showing the proportion of total variance explained. The polygons represent the maximal parameter space of each population and the squares the population mean. The site locations are: GRI: Grimsey; GUL: Gull; HOR: Hornoeya.



**Fig.3:** Mean contours for each female tick population. Each colored bar represents one population: grey: GRI CG; cyan: GUL CG; olive green: HOR CG; black: GRI PF; red: GUL PF; pink: HOR PF. Numbers beside colored bars indicate the number of used ticks for each population.

**Table 2:** Results of permutation tests of size differences in female tick outline contours (upper matrix) and landmark (lower matrix) configurations. The threshold value for significance using a Bonferroni correction is 0.0033. Significant values are in red. Blue cells indicate comparisons between sympatrically occurring host races.

	GRI CG	GUL CG	HOR CG	GRI PF	GUL PF
GUL CG	0.0000				
HOR CG	0.0040	0.1850			
GRI PF	0.0010	0.3360	0.7180		
GUL PF	0.0060	0.6180	0.5880	0.7850	
HOR PF	0.0000	0.0430	0.0060	0.0190	0.0760
	GRI CG	GUL CG	HOR CG	GRI PF	GUL PF
GUL CG	0.0010				
HOR CG	0.0090	0.0440			
GRI PF	0.0100	0.1360	0.8110		
GUL PF	0.1750	0.0370	0.4880	0.4470	
HOR PF	0.0000	0.2460	0.0030	0.0250	0.0120



**Fig.4:** Size variation (in pixels) of females presented as quantile plots for tick populations based on (a) outlines and (b) landmark arrangements. The box shows the median as a line across the middle and the quartiles (25<sup>th</sup> and 75<sup>th</sup> percentiles) as its ends. Extremities represent the minimal and maximal values. The right column represents the validated classification (CCC) result based on shape in percentage, used to test the affiliation of each individual tick to each population. The site locations are: GRI: Grimsey; GUL: Gull; HOR: Hornoeya.

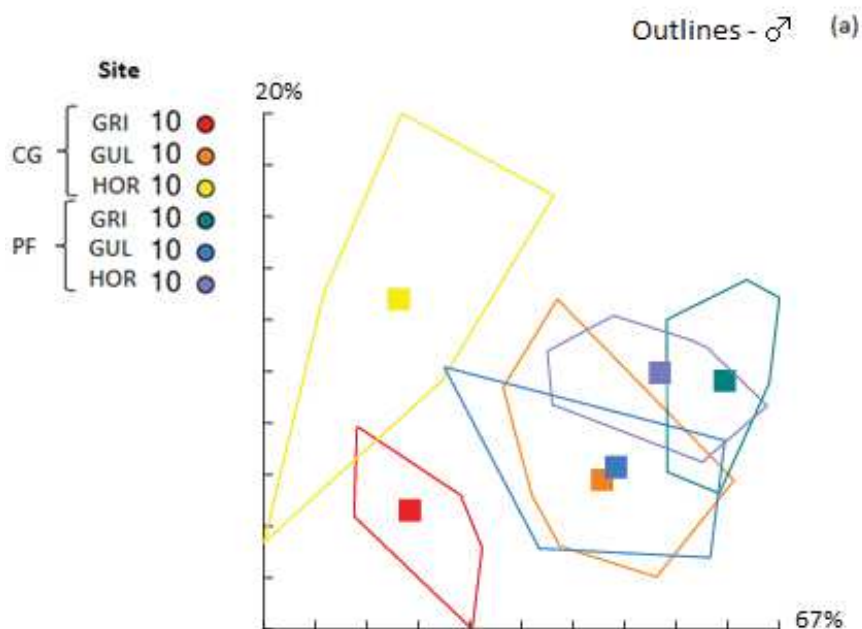
Landmark analyses showed greater discrimination among tick populations and tended to group PF ticks more closely than CG ticks, which were each highly distinct (Fig.2b). As for the outline analysis, size was significantly different between CG and PF ticks, except on GUL where this pattern seemed to be reversed (Fig.4a). The size effect on shape represented 10.7% of the overall variation in tick morphology (DF1 regression:  $r=0.101$ ;  $r^2=10\%$ ;  $df=52$ ;  $t=0.73$ ,  $p=0.23308$ ; DF2 regression:  $r=-0.083$ ;  $r^2=0.7\%$ ;  $df=52$ ;  $t=0.59$ ,  $p=0.0001$ ). The cross check classification showed that populations were differently supported,



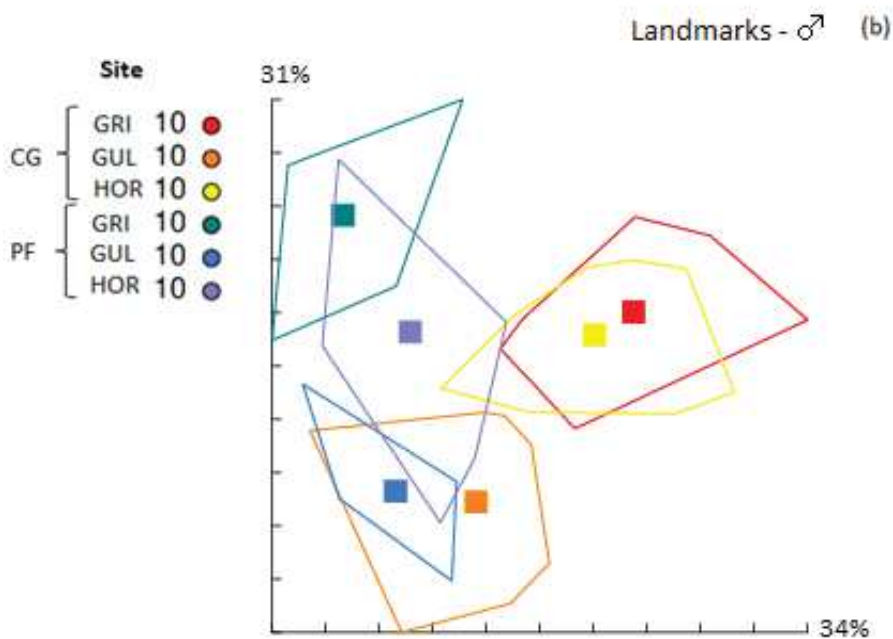
ranging from 0% well-assigned individuals for GUL PF (but only 4 female ticks constituted this population) to 100% for GUL CG (Fig.4b).

*Males*

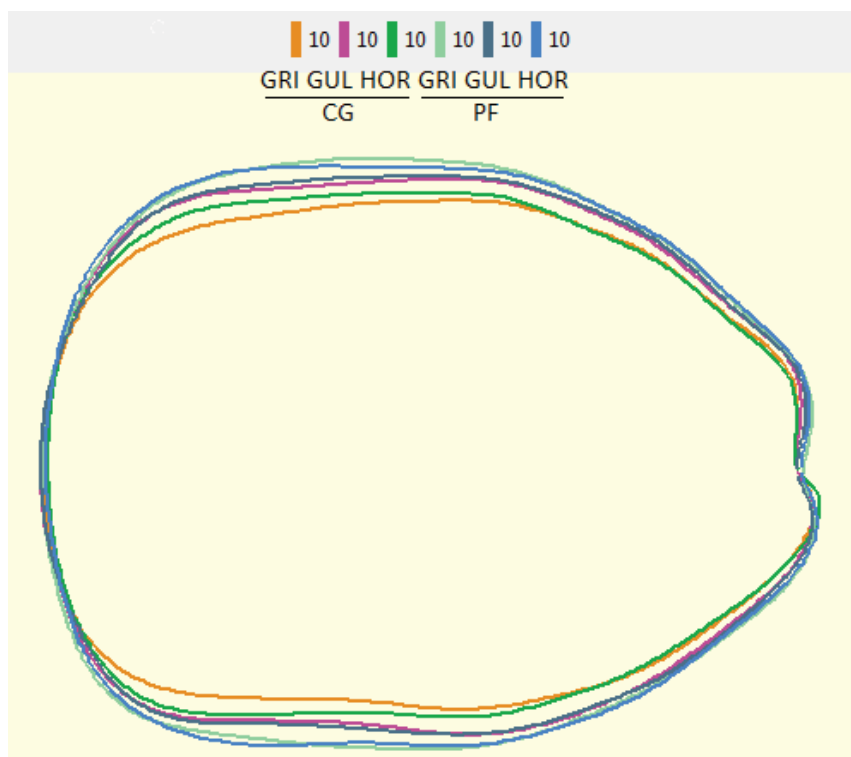
Male morphogroups were not as well discriminated as female morphogroups. The overall body shape of CG males differed between tick races in the HOR and GRI colonies with the populations of each race grouping together for the two PF populations, but this was not the case for the GUL population where ticks from CG and PF largely overlapped for both outline (Fig.5a) and landmark (Fig.5b) analyses. As for females, outlines showed that PF ticks tended to be wider than CG ticks (Fig.6). The GRI CG tick size was the smallest and was significantly different from all other populations (Fig.6, 7 and Table 3). The size effect on shape represented 36% of the overall shape variation for the outline analysis (DF1 regression:  $r=0.470$ ;  $r^2=22.1\%$ ;  $df=58$ ;  $t=4.06$ ,  $p=7.65 \times 10^{-5}$ ; DF2 regression:  $r=-0.373$ ;  $r^2=13.9\%$ ;  $df=58$ ;  $t=3.06$ ,  $p=0.0127$ ) and 12.3% of the overall variation in tick morphology for landmark analysis (DF1 regression:  $r=0.320$ ;  $r^2=10.2\%$ ;  $df=58$ ;  $t=2.57$ ,  $p=0.0064$ ; DF2 regression:  $r=-0.144$ ;  $r^2=2.1\%$ ;  $df=58$ ;  $t=1.11$ ,  $p=0.142$ ). The cross check classification showed that populations are differently supported with the GUL PF population showing the least support in both analyses and GRI PF population showing the most (Fig.7).



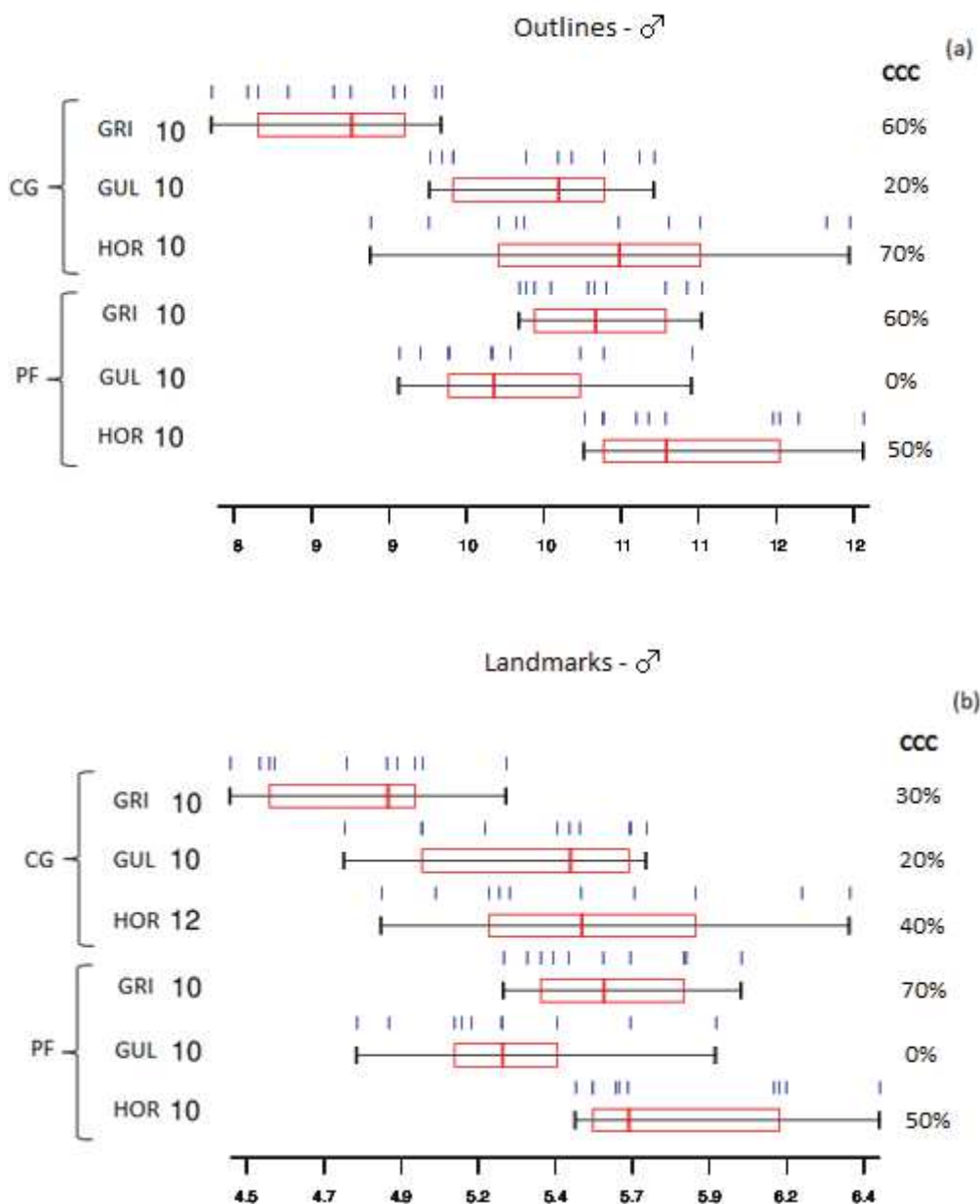




**Fig.5:** First two functions of a discriminant analysis performed on (a): normalized Fourier coefficients based on the outline analysis and (b): a set of first RW based on the landmark arrangement of males and showing the proportion of total variance explained. The polygons represent the maximal parameter space of each population and the squares the population mean. The site locations are: GRI: Grimsey; GUL: Gull; HOR: Hornøya.



**Fig.6:** Mean contours for each male tick population. Each colored bar represents one population: orange: GRI CG; pink: GUL CG; dark green: HOR CG; light green: GRI PF; grey: GUL PF; blue: HOR PF. Numbers beside colored bars indicate the number of used ticks for each population.



**Fig.7:** Size variation (in pixels) of males presented as quantile plots for tick populations based on (a) outlines and (b) landmark arrangements. The box shows the median as a line across the middle and the quartiles (25<sup>th</sup> and 75<sup>th</sup> percentiles) as its ends. Extremities represent the minimal and maximal values. The right column represents the validated classification (CCC) result based on shape in percentage, used to test the affiliation of each individual tick to each population. The site locations are: GRI: Grimsey; GUL: Gull; HOR: Hornoeya.

**Table 3** Results of permutation tests of size differences in male tick outline contours (upper matrix) and landmark (lower matrix) configurations. The threshold value for significance using a Bonferroni correction is 0.0033. Significant values are in red. Blue cells indicate comparisons between sympatrically occurring host races.

	GRI CG	GUL CG	HOR CG	GRI PF	GUL PF
GUL CG	0.0000				
HOR CG	0.0000	0.2280			
GRI PF	0.0000	0.1120	0.7550		
GUL PF	0.0010	0.4740	0.0970	0.0200	
HOR PF	0.0000	0.0010	0.2030	0.0280	0.0010
	GRI CG	GUL CG	HOR CG	GRI PF	GUL PF
GUL CG	0.0000				
HOR CG	0.0000	0.3250			
GRI PF	0.0000	0.0980	0.7850		
GUL PF	0.0020	0.6130	0.1800	0.0310	
HOR PF	0.0000	0.0080	0.1320	0.0670	0.0000

## 4 Discussion

Host races were phenotypically distinct in each colony location in relation to both size and shape in two of the three colonies examined here. Congruent patterns in size and shape for each tick race were found for the HOR and GRI populations, but the morphological divergence of ticks from the GUL population was not clear. PF ticks tended to be wider than CG ticks, as observed in a previous study (Dietrich et al., 2013) but this pattern was less clear for male ticks of the GUL population. These results suggest that no one simple host-associated factor is responsible for the phenotypic divergence observed among co-occurring tick races, but rather that processes other than host-associated selection need to be considered.

Previous genetic analyses have suggested that CG and PF tick races on HOR and GRI population share a common origin (McCoy et al., 2013). We cannot therefore rule out that the observed patterns of phenotypic congruence in these colonies are due to selection *per se*. The similarity that we find may be largely associated with historical constraints and/or contemporary patterns of gene flow. However,

although the morphology of CG ticks was largely variable among colonies (with the same tendencies in HOR and GRI), this was not the case for PF ticks where morphology was relatively coherent across colonies. This may suggest that within-colony divergence comes from asymmetric selection on ticks exploiting puffins to evolve a specific body form in order to escape host preening. Indeed, Clayton et al (1999) showed that efficient preening (grooming and scratching) select for small body size in lice so they can better hide between feather barbs. In the *I. uriae*-seabird system, we observed that ticks on puffins tended to be wider than ticks on common guillemots. This does not correspond to host body size (28 to 35 cm in length for puffins and 40 to 46 cm in length for guillemots (Dif et al., 1982); suggesting that Harrison's rule (Harrison, 1915) does not apply to this system. Tick size differences could thus come from other sources, such as beak size and shape. Indeed, puffin beaks are large and slightly hooked measuring 23 to 32mm in length while common guillemot beaks are thin and pointed measuring 37 to 49mm in length. These differences could favor wider size in PF ticks and thinner size in CG ticks. Observations of seabird behavior while grooming and scratching and morphometrical analyses of the different seabird beaks could provide more support to this hypothesis, in particular by measuring gap widths between the mandibles.

Given the nidicolous lifestyle of *I. uriae*, size and shape differences among host races and colonies could also come from different selection pressures from the environment. Indeed, as organisms physically interact with their environment, micro-habitat conditions might induce morphological changes. The seabird species involved in this study use very different nesting substrates with potential differences in temperature and humidity conditions, known to affect tick distributions (Estrada-Peña, 2001). Common guillemots breed close to each other directly on rocks, whereas puffins breed in individual burrows which can be up to 5 meters deep. These different conditions may select for optimal tick size and shape. For example, thinner guillemot ticks may better fit into the rocky crevices of guillemot breeding ledges than the wider puffin ticks. More information concerning the abiotic conditions of each seabird nesting area with for example the use of temperature and humidity data loggers placed in the tick sheltered areas, are now needed to investigate this hypothesis.

Other factors may also explain the weak divergence patterns we observed in this study. For example, the pattern we observed for the GUL population could come from weak selection, the fitness consequences of tick size and shape are not strong enough to lead to population divergence. Alternatively, the patterns found in the GUL population may come from extinction-recolonization dynamics. Indeed, twenty years ago, infestation levels on kittiwake hosts were extremely high on GUL and the number of nesting guillemots was very low (McCoy et al., 1999). In 2016 almost no nestling or adult kittiwakes carried ticks in this colony and guillemots were found breeding throughout the island.

This suggests a potential shift in the local levels of infestation and host use. Such dynamics may mean that the ticks currently infesting the birds of this colony have shifted over time and may not yet have reached equilibrium. This can be tested by analyzing the sampled ticks in each colony at neutral genetic markers and determining whether the host-associated populations are still genetically distinct and whether there has been a recent host shift. These population dynamic events could also lead to host “mistakes” that add error to our measures of morphological divergence; genetic data can also allow us to estimate this error rate. If genetically-distinct host races are still present in each colony, we can use assignment tests to evaluate the degree to which host misuse has blurred patterns of phenotypic divergence (see Dietrich et al., 2013). Finally, our results are based on relatively low sample sizes. Resampling efforts should therefore be made to improve the power of our analyses and make strong inferences concerning the divergence process of these *I. uriae* populations.

In conclusion, although our morphometrical analyses partially support congruent phenotypic divergence in the *I. uriae*-seabird system across the North Atlantic in relation to host use, this divergence is not homogeneous in all examined populations. Additional samples and supplementary analyses are now required to confirm this pattern and to distinguish among the different possible factors shaping divergence process.

## 5 Acknowledgements

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## References

- Barluenga, M., Stölting, K.N., Salzburger, W., Muschick, M., Meyer, A., 2006. Sympatric speciation in Nicaraguan crater lake cichlid fish. *Nature* 439, 719–723. doi:10.1038/nature04325
- Bookstein, F.L., 1997. *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge University Press.
- Clayton, D.H., Koop, J.A., Harbison, C.W., Moyer, B.R., Bush, S.E., 2010. How birds combat ectoparasites. *Open Ornithol J* 3, 41–71.
- Clayton, D.H., Lee, P.L.M., Tompkins, III, E.D.B., 1999. Reciprocal Natural Selection on Host-Parasite Phenotypes. *Am. Nat.* 154, 261–270. doi:10.1086/an.1999.154.issue-3
- Combes, C., 1995. *Interactions durables: écologie et évolution du parasitisme*. Masson.
- De Meeûs, T., Michalakis, Y., Renaud, F., 1998. Santa Rosalia Revisited: or Why Are There So Many Kinds of Parasites in 'The Garden of Earthly Delights'? *Parasitol. Today* 14, 10–13. doi:10.1016/S0169-4758(97)01163-0
- Dietrich, M., Beati, L., Elguero, E., Boulinier, T., McCoy, K.D., 2013. Body size and shape evolution in host races of the tick *Ixodes uriae*. *Biol. J. Linn. Soc.* 108, 323–334. doi:10.1111/j.1095-8312.2012.02021.x
- Dietrich, M., Gómez-Díaz, E., McCoy, K.D., 2011. Worldwide Distribution and Diversity of Seabird Ticks: Implications for the Ecology and Epidemiology of Tick-Borne Pathogens. *Vector-Borne Zoonotic Dis.* 11, 453–470. doi:10.1089/vbz.2010.0009
- Dietrich, M., Kempf, F., Boulinier, T., McCoy, K.D., 2014a. Tracing the colonization and diversification of the worldwide seabird ectoparasite *Ixodes uriae*. *Mol. Ecol.* 23, 3292–3305. doi:10.1111/mec.12815
- Dietrich, M., Kempf, F., Gómez-Díaz, E., Kitaysky, A.S., Hipfner, J.M., Boulinier, T., McCoy, K.D., 2012. Inter-oceanic variation in patterns of host-associated divergence in a seabird ectoparasite. *J. Biogeogr.* 39, 545–555. doi:10.1111/j.1365-2699.2011.02620.x
- Dietrich, M., Lobato, E., Boulinier, T., McCoy, K.D., 2014b. An experimental test of host specialization in a ubiquitous polar ectoparasite: a role for adaptation? *J. Anim. Ecol.* 83, 576–587. doi:10.1111/1365-2656.12170
- Dif, G., Hainard, R., Lamouche, T., Dorst, J., 1982. *Les oiseaux de mer d'Europe*. Arthaud, Paris.
- Dujardin, J.-P.A., Kaba, D., Henry, A.B., 2010. The exchangeability of shape. *BMC Res. Notes* 3, 266. doi:10.1186/1756-0500-3-266
- Dujardin, J.-P., Kaba, D., Solano, P., Dupraz, M., McCoy, K.D., Jaramillo-O, N., 2014. Outline-based morphometrics, an overlooked method in arthropod studies? *Infect. Genet. Evol.* 28, 704–714. doi:10.1016/j.meegid.2014.07.035
- Dujardin, J.-P., Slice, D.E., 2006. Contributions of morphometrics to medical entomology. *Encycl. Infect. Dis. Mod. Methodol.* 435–447.
- Estrada-Peña, A., 2001. Forecasting habitat suitability for ticks and prevention of tick-borne diseases. *Vet. Parasitol., Promotion Advancement, Preserving Tradition* 98, 111–132. doi:10.1016/S0304-4017(01)00426-5

- Harrison, L., 1915. Mallophaga from Apteryx, and their Significance; with a Note on the Genus Rallicola. *Parasitology* 8, 88–100. doi:10.1017/S0031182000010428
- Kempf, F., Boulonier, T., De Meeûs, T., Arnathau, C., McCOY, K.D., 2009. Recent evolution of host-associated divergence in the seabird tick *Ixodes uriae*. *Mol. Ecol.* 18, 4450–4462. doi:10.1111/j.1365-294X.2009.04356.x
- Klimov, P.B., Bochkov, A.V., Oconnor, B.M., 2006. Host specificity and multivariate diagnostics of cryptic species in predacious cheyletid mites of the genus *Cheletophyes* (Acari: Cheyletidae) associated with large carpenter bees. *Biol. J. Linn. Soc.* 87, 45–58. doi:10.1111/j.1095-8312.2006.00554.x
- Kuclu, O., Aldemir, A., Demirci, B., 2011. Altitudinal variation in the morphometric characteristics of *Aedes vexans* Meigen from northeastern Turkey. *J. Vector Ecol. J. Soc. Vector Ecol.* 36, 30–41. doi:10.1111/j.1948-7134.2011.00138.x
- Kuhl, F.P., Giardina, C.R., 1982. Elliptic Fourier features of a closed contour. *Comput. Graph. Image Process.* 18, 236–258. doi:10.1016/0146-664X(82)90034-X
- Magalhães, S., Forbes, M.R., Skoracka, A., Osakabe, M., Chevillon, C., McCoy, K.D., 2007. Host race formation in the Acari. *Exp. Appl. Acarol.* 42, 225–238. doi:10.1007/s10493-007-9091-0
- Mans, B.J., Neitz, A.W.H., 2004. Adaptation of ticks to a blood-feeding environment: evolution from a functional perspective. *Insect Biochem. Mol. Biol.* 34, 1–17. doi:10.1016/j.ibmb.2003.09.002
- McCoy, K.D., Boulonier, T., Chardine, J.W., Danchin, E., Michalakis, Y., 1999. Dispersal and Distribution of the Tick *Ixodes uriae* within and among Seabird Host Populations: The Need for a Population Genetic Approach. *J. Parasitol.* 85, 196. doi:10.2307/3285619
- McCoy, K.D., Boulonier, T., Tirard, C., Michalakis, Y., 2003. Host-Dependent Genetic Structure of Parasite Populations: Differential Dispersal of Seabird Tick Host Races. *Evolution* 57, 288–296.
- Mccoy, K.D., Boulonier, T., Tirard, C., Michalakis, Y., 2001. Host specificity of a generalist parasite: genetic evidence of sympatric host races in the seabird tick *Ixodes uriae*. *J. Evol. Biol.* 14, 395–405. doi:10.1046/j.1420-9101.2001.00290.x
- McCoy, K.D., Chapuis, E., Tirard, C., Boulonier, T., Michalakis, Y., Bohec, C.L., Maho, Y.L., Gauthier-Clerc, M., 2005. Recurrent evolution of host-specialized races in a globally distributed parasite. *Proc. R. Soc. B Biol. Sci.* 272, 2389–2395. doi:10.1098/rspb.2005.3230
- McCoy, K.D., Léger, E., Dietrich, M., 2013. Host specialization in ticks and transmission of tick-borne diseases: a review. *Front. Cell. Infect. Microbiol.* 3. doi:10.3389/fcimb.2013.00057
- Poulin, R., 2006. *Evolutionary Ecology of Parasites*., 2nd edition, New Second edition. ed. Princeton University Press, Princeton, N.J.
- Prudhomme, J., Gunay, F., Rahola, N., Ouanaimi, F., Guernaoui, S., Boumezzough, A., Bañuls, A.-L., Sereno, D., Alten, B., 2012. Wing size and shape variation of *Phlebotomus papatasi* (Diptera: Phlebotomidae) populations from the south and north slopes of the Atlas Mountains in Morocco. *J. Vector Ecol.* 37, 137–147. doi:10.1111/j.1948-7134.2012.00210.x
- Sonenshine, D.E., Roe, R.M., 2014. *Biology of Ticks Vol.2*, Second edition. ed. Oxford University Press.



## **Chapitre 4 – Facteurs de divergence associés à l’hôte et rôle de l’isolement local**

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La dispersion est un sujet central en écologie et en évolution puisqu'elle conditionne le flux génique et donc la structure génétique des populations. La distribution des parasites n'est pas spatialement uniforme et leur adaptation locale dépend fortement de leur capacité de dispersion (Gandon, 2002). Dans le cas d'*I. uriae*, il a été mis en évidence la présence de quatre grands groupes génétiquement différenciés au sein de son aire de répartition mondiale, liés aux contraintes locales des populations d'hôtes (Dietrich et al., 2014a). Boulinier et al. (2016, voir annexe) soulignent l'importance des différents mouvements des oiseaux marins (migration, dispersion, prospection) dans la dispersion de la tique *Ixodes uriae*, dont les taux d'infestation des nids varient entre falaises et entre colonies (Gasparini et al., 2001; McCoy et al., 1999). L'utilisation d'outils de génétique des populations sur les populations de tiques montre, par exemple, que la structuration des populations ne s'opère qu'à partir d'une distance de 200 km autour de la colonie d'origine pour certaines races d'*I. uriae* (McCoy et al., 2003). En effet, l'utilisation de GPS posés sur des oiseaux bagués montre que ceux-ci peuvent prospecter jusqu'à des centaines de kilomètres depuis leur colonie d'origine, permettant ainsi de disperser des tiques et de potentiels agents infectieux (Ponchon et al., 2014). Ces mouvements de prospection peuvent jouer un rôle majeur dans la dispersion des tiques dures car leur repas de sang nécessite plusieurs jours à toutes les stases. De plus dans ce système, une étude a révélé l'importance d'évaluer la structure des populations de tiques pour comprendre leur implication dans les cycles épidémiologiques. En effet, il a été montré que les différentes races d'hôtes d'*I. uriae* portaient des prévalences et des intensités d'infection variables par des bactéries du complexe *Borrelia burgdorferi* s.l. et que ceci avait un impact sur les dynamiques locales d'infection. De plus, les différentes races d'hôtes des tiques ne partageaient pas les mêmes souches de la bactérie au sein d'une colonie, soulignant l'effet en cascade de la divergence des vecteurs sur l'épidémiologie des agents infectieux (Gómez-Díaz et al., 2010).

Dans le cas des tiques molles, on peut prédire que les mouvements des hôtes impacteront la dispersion des tiques en fonction de la stase considérée. Ceci serait dû au fait que les adultes et les nymphes se nourrissent rapidement, généralement la nuit quand les oiseaux ne bougent pas, alors que les larves peuvent rester attachées à l'hôte plusieurs jours (Kada et al, in prep). Ceci suggère donc que la structuration génétique des tiques molles à échelle locale devrait être plus élevée que celle des tiques dures, réduisant en conséquence la circulation d'agents pathogènes.

Pour tester cette hypothèse, j'ai donc réalisé une étude expérimentale *in situ* pour tester l'isolement local de tiques molles à l'échelle d'une colonie d'hôte-oiseaux en combinaison avec l'utilisation d'une puce Fluigdim pour la détection des agents pathogènes. Les résultats ont révélé une faible structuration spatiale des tiques et des micro-organismes entre nids et suggèrent une circulation régulière des tiques au sein de la colonie. Ceci indique que les caractéristiques propres à chaque espèce, telle que la capacité

de dispersion doivent être prises en compte si l'on s'attache à comprendre la dynamique des populations de tiques et la circulation des agents infectieux.

Article 5

Dupraz M, Toty C, Devillers E, Blanchon T, Elguero E, Vittecoq M, Moutailler S, McCoy K-D. Circulation of vector-borne infectious agents at local scales: structure of the soft tick *Ornithodoros maritimus* within a colony of its seabird host *Larus michahellis*. En preparation.



Circulation of vector-borne infectious agents at local scales: structure of the soft tick *Ornithodoros maritimus* within a colony of its seabird host *Larus michahellis*.

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## Abstract

The epidemiology of vector-borne zoonoses depends on the movements of both hosts and vectors at different spatial scales, with the role of vectors being potentially constrained at local spatial scales. However, very little information is currently available on local scale movements of vector organisms, and particularly so for ticks, the vector group transmitting the greatest diversity of infectious agents of medical and veterinary importance. Here, we investigated the dynamics and structure of the soft tick *Ornithodoros maritimus* over the course of a breeding season at the scale of a single seabird host colony to understand the degree to which ticks disperse among nests and are responsible for local scale dissemination of tick-borne infectious agents. As the host species, the Yellow-legged gull *Larus michahellis*, is highly territorial, we expected ticks to be the main motor of local pathogen dissemination. Ticks were sampled within a series of nests across the colony at regular intervals over the course of the season; in half of the nests, all ticks were removed (destructive sampling) and in the other half they were counted and returned to the nest. At the last sampling event, all ticks were removed and a sample of all collected ticks was screened for a wide array of known infectious agents (bacteria, viruses, parasites) using a high throughput real-time PCR system. We then quantified tick dynamics over the course of the season in relation to the sampling treatment and tested for spatial auto-correlation in the presence of ticks and tick-borne agents among nests. The results show a temporal dynamic in the presence of the different tick stages over the season. Adult females and males were present throughout the reproductive season, increasing linearly until the second last passage. The appearance of nymphs and larvae coincided with chick hatching suggesting a potential adaptation to host phenology. No tick spatial structure was detected and the effect of destructive sampling reduced tick abundance in the nest near the end of the sampling period, suggesting that ticks move frequently to find shelter and/or an available host within the colony. The spatial distribution of bacteria carried by the ticks supported this view. Three symbiotic bacteria, four potentially pathogenic bacteria, one parasite and one virus were found in the sampled ticks, but none showed evidence of spatial structure within the colony. These results highlight the importance of considering vector movement patterns to predict and control pathogen circulation.

Keywords: Argasidae, within-nest dynamics, dispersal, epidemiology

## 1 Introduction

Vector-borne zoonoses are often maintained in complex transmission cycles including different vector and reservoir host species (Sonenshine and Mather, 1994; Brisson et al, 2008; Keesing et al, 2006). Locally, vector spatial dynamics condition pathogen population structure among hosts, the outcome of host-parasite co-evolutionary interactions (Gandon and Michalakis, 2002) and ultimately the dynamics of disease emergence (Ostfeld et al, 2005). Understanding the spatial and temporal dynamics of vectors is thus of major importance to prevent or control potential outbreaks, especially as global change favors the redistribution of biodiversity (Tylianakis et al, 2008; Ogden et al, 2013). Ticks are among the most important vectors of diseases worldwide and are able to transmit bacteria, viruses and parasites (Jongejan and Uilenberg, 2004) to a wide variety of hosts including birds, reptiles and mammals (Sonenshine and Roe, 2014). However, very little information is currently available on local scale movements of this vector organism. Indeed, few studies have focused on vector dispersal. Abrahan et al. (2011) showed that different Triatominae species use active movement to disperse locally, rather than passive transportation with more wide ranging vertebrate hosts. McCoy et al. 2003b found that cliff topography and nest site organization are of key importance for predicting the local population structure of the seabird tick *Ixodes uriae*. Likewise, soft ticks of the *Ornithodoros turicata* species showed low interburrow movement and specific depth preferences for tortoise burrows (Adeyeye and Butler, 1989). These few studies highlight the variety of different biotic and abiotic factors that may come into play when trying to understand local scale vector structure.

*Ornithodoros maritimus* Vermeil and Marguet 1966 is a member of the soft tick (Argasidae) complex *Ornithodoros capensis sensu lato* containing 8 described species exploiting colonial seabirds in tropical and sub-tropical areas of the globe (Perez-Eid, 2007). *O. maritimus* has been recorded in association with a wide range of seabird host species including cormorants, gulls, alcids and terns in Great Britain, Ireland, France, Italy, Spain and Tunisia (Hoogstraal et al, 1976). It is also known to vector numerous viruses such as Meaban and West Nile viruses (Arnal et al, 2014; Dietrich et al, 2011), and Soldado virus which can induce high mortality rates in bird populations and pruritus in humans (Converse et al, 1975; Feare et al, 1976). More recently, these ticks have also been found to harbor endosymbiotic bacteria that, in addition to playing a potentially important role in tick biology, may favor the emergence of medically important pathogens, such as the Q fever agent *Coxiella burnetii* (Duron et al, 2015). *O. maritimus* is largely nidicolous, feeding on the host rapidly at night in the nymphal and adult life stages. This limited contact with the host should result in low tick dispersal among colonies, and should have a cascading effect on pathogen spread (Kada et al, submitted).

Here, we investigate the dynamics of the different tick stages in bird-host nests over the course of a seabird breeding season and test for spatial structuring among nests at the scale of an island. To examine the degree to which ticks move among nests, we destructively sampled ticks in half of our study nests to determine whether we could alter within-nest population dynamics by removing ticks. As the study location is situated in a biodiversity hotspot, we used a high throughput real-time PCR system to test for the presence of the most common tick-borne infectious agents including bacteria, parasites and viruses potentially harbored by the ticks and examined their spatial structure within the colony.

## 2 Material and methods

### 2.1 Biological system

Like other soft ticks, the life cycle of *O. maritimus* is polyphasic, composed of three active stages: a single larval stage, several nymphal instars and a sexual adult stage. As larvae, the ticks attach and feed for several hours. In nymphal and adult stages, the ticks feed to repletion in a few minutes during periods when the host is resting – typically at night. Females oviposit after each blood meal, depositing few hundred eggs each time (Vial, 2009). While not feeding, the ticks of all life stages are found in the nest or surrounding area (nidicolous) to benefit from stable temperature and humidity conditions (Vial, 2009). Ticks can be found in nests during the host breeding season, but spend the rest of the year in diapause in shelters or deeper in the ground waiting for the return of their host the following season.

The breeding range of the yellow-legged gull *Larus michahellis* is centered on the Mediterranean Sea. These gulls breed in dense colonies (pairs usually nest several meters apart) laying eggs in nests built with a sparse mound of vegetation on the ground or on cliff ledges (Olsen, 2004). These birds are very territorial, with relatively limited movements during the breeding season, mainly from feeding areas to the nest area. Outside the breeding season, the species remains gregarious, congregating around ports, harbours and dumps (Olsen, 2004).

### 2.2 Tick sampling and study location

Ticks were collected in the Camargue area of southern France from March to May 2015 on Carteau, a flat island in the Gulf of Fos of 210 meters long and 65 meters wide, entirely occupied by a breeding colony of yellow-legged gulls *L. michahellis*. In 2015, 385 breeding pairs occupied the island over the reproduction season (February to June). We selected, marked and took the gps coordinates of 30 nests across the island (Fig.1). At each visit, a nest was searched for 3 minutes by two people; one person examined the upper nest materials in a white tray and, the other searched directly inside each nest and

all ticks found were removed. For 15 of the 30 focal nests, ticks were returned to the nest once all life stages were identified and counted (non-engorged females, engorged females, males, non-engorged nymphs, engorged nymphs and larvae); we refer to these nests as “count nests”. In the other 15 nests, all ticks were collected and kept alive in the laboratory; we refer to these as “collected nests” (Fig.1). The sampling protocol was repeated once per week over six weeks on the same nests. Only during the last visit were all ticks were collected from all nests. After collection, ticks were identified and used for DNA/RNA extractions. At each passage, the number of gull eggs, hatching eggs or chicks in each nest was noted. In 2015, eggs started hatching during the fourth passage (16.04.15).

### **2.3 RNA and genomic DNA extraction**

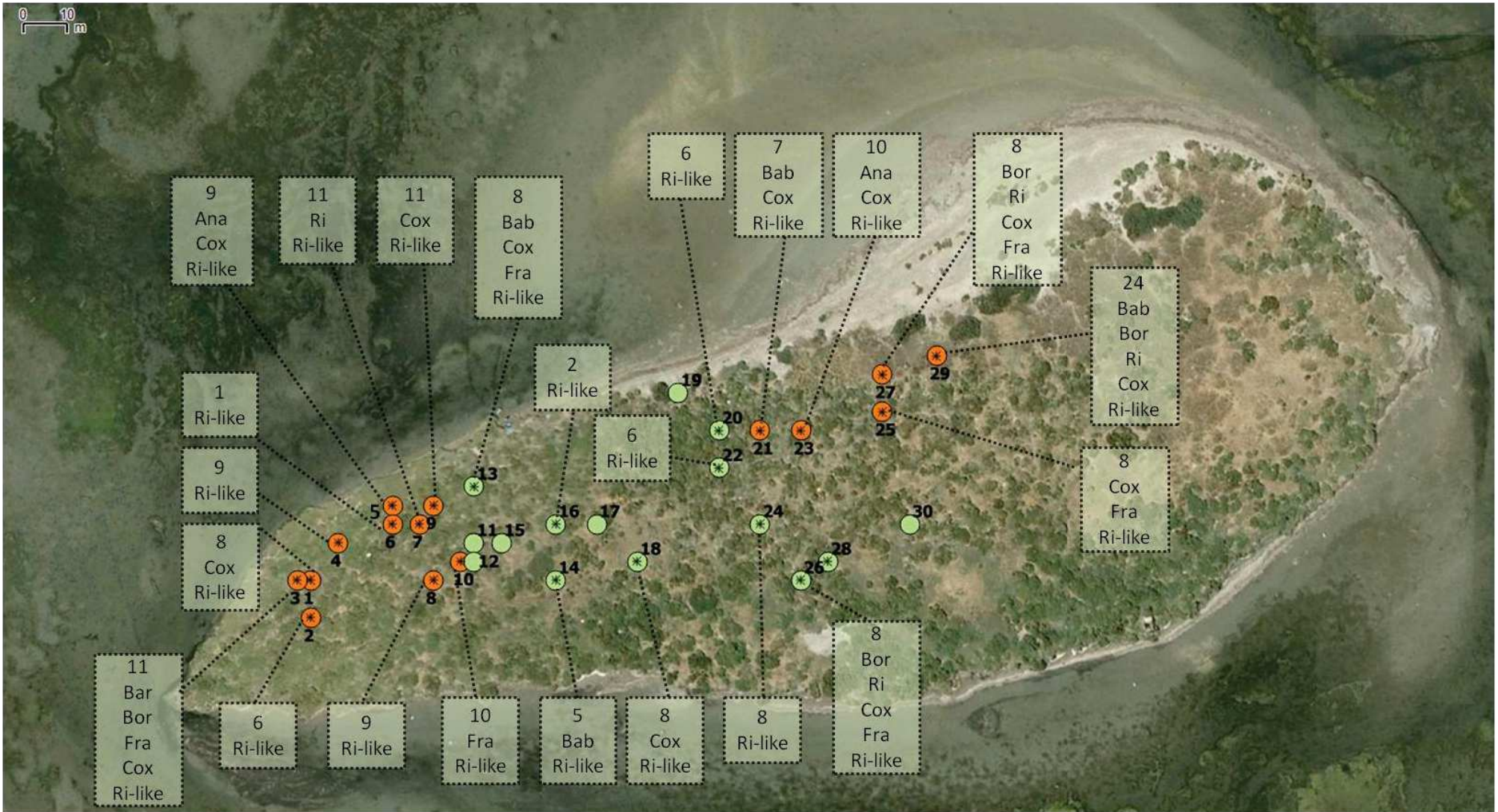
Extraction protocols followed those outlined in Moutailler et al. (2016) and Michelet et al. (2014). 201 adult ticks (162 females and 39 males) collected at the second, fourth, fifth and sixth passages were selected from 24 study nests to examine the presence of infectious agents (~7ticks/nest). All ticks were washed during 5 min in an ethanol bath, 10 min in two successive water baths and placed individually in sterile tubes and crushed in 300  $\mu$ L of DMEM with 10% fetal calf serum using the Precellys<sup>®</sup>24 Dual homogenizer (Bertin, France). The supernatant was divided into 3 fractions: 100  $\mu$ L for the DNA extraction, 100  $\mu$ L for the RNA extraction and the rest was used as back-up and conserved at -80°C. Genomic tick DNA was then extracted using the Wizard genomic DNA purification kit (Promega, France) according to the manufacturer’s instructions. RNA was extracted using the Nucleospin RNA II kit (Macherey Nagel, Germany) according to the manufacturer’s instructions. Purified DNA was eluted into 50  $\mu$ L elution buffer and stored at -20°C, whereas RNA was eluted into 50  $\mu$ L of RNase-free water and conserved at -80°C. Tick DNA and RNA quality was assessed, via the amplification of the ITS2 and COI respectively (Michelet et al, 2014; Moutailler et al, 2016).

### **2.4 High throughput real-time PCR system**

Ticks were analyzed for the most common tick-borne infectious agents using the BioMark real-time PCR system (Fluidigm, USA) for high-throughput microfluidic real-time PCR amplification. The DNA primer chip developed by Michelet et al. (2014) includes primers for detecting 28 bacterial species, 12 parasite species and 25 viruses (SM Table A1).

All RNAs were reverse transcribed into cDNAs using random primers and oligos (dT). The remaining methods followed those of Michelet et al, 2014 and Moutailler et al, 2016.





**Fig.1:** Map showing the position of the 30 tracked nests on Carteau Island, Camargue, France. Orange points represent the 15 nests in which ticks were counted and released. The green points are those nests where all ticks were counted and collected. Stars within the points represent the nests in which ticks were used for pathogen screening. Boxes indicate the number of ticks screened and the detected infectious agents: Ana: *Anaplasma* spp; Bab: *Babesia* spp; Bar: *Bartonella* spp; Bor: *Borrelia* spp; Cox: *Coxiella*-like symbiont; Fra: *Francisella*-like symbiont; Ri: *Rickettsia helvetica*; Ri-like: *Rickettsia*-like symbiont.

**Table 1:** List of pathogens detected using the high-throughput microfluidic real-time PCR amplification. The target gene refers to the gene used for the initial detection of the infectious agent and the confirmation method, the gene and procedure used for verifying the correction identification of the organism. NP: Not performed.

Pathogens	Species	Target Gene (HT)	Positive ticks - Prevalence % (n=201)	Confirmation method (gene targeted)	Reference confirmation technique	Confirmation	Sequence reference
Bacterium	<i>Anaplasma</i> spp.	msp2	3 – 1.5	PCR (16S)	Hornok et al, 2008	<i>Anaplasma</i> spp.	Sequence obtained without known species match
Bacterium	<i>Bartonella henselae</i>	pap31	1 – 0.5	PCR (gltA)	Norman et al, 1995	none	No sequence obtained
Bacteria	<i>Borrelia</i> spp.	23s	3 – 1.5	Nested PCR (IGS and p66)	Bunikis et al, 2004	none	No sequence obtained
Bacterium	<i>Coxiella</i> spp.	icd	25 – 12.4	NP	-	<i>Coxiella</i> -like symbiont	-
Bacterium	<i>Francisella</i> spp	tul4	3 – 1.5	NP	-	<i>Francisella</i> like symbiont	-
Bacterium	<i>Rickettsia helvetica</i>	ITS	6 - 3	NP	-	<i>R. helvetica</i>	-
Bacteria	<i>Rickettsia endosymbiont</i>	gltA	164 – 81.6	PCR (gltA)	Regnery et al, 1991	<i>R. lusitaniae</i>	gb JQ771933.1 (Milhano et al, 2014)
Parasite	<i>Babesia</i> spp.	hsp70	6 - 3	PCR (18S)	Bonnet et al, 2007	<i>Babesia</i> spp.	Sequence obtained without known species match
Virus	West Nile (WNV)	polyprotein	6 - 3	RT-PCR (NS5)	Scaramozzino et al, 2001	none	No sequence obtained

DNA and cDNA pre-amplifications were performed using the TaqMan PreAmp Master Mix (Applied Biosystems, France) according to the manufacturer's instructions. Primers for bacteria or viruses were pooled by combining equal volume of primers (200nM final each). This step was performed in a final volume of 5  $\mu$ L containing 2.5  $\mu$ L TaqMan PreAmp Master Mix (2X), 1.2  $\mu$ L pooled primer mix (0.2X) and 1.3  $\mu$ L DNA. Thermal cycling conditions were as follows: one cycle at 95°C for 10min, 14 cycles at 95°C for 15s and 4min at 60°C (Michelet et al., 2014).

The qPCR reactions were then performed using 6-carboxyfluorescein (FAM) and black hole quencher (BHQ1)-labeled TaqMan probes (Michelet et al, 2014) with TaqMan Gene expression Master Mix, in accordance to the manufacturer's instructions (Applied Biosystem, France). PCR cycling comprised 5min at 95°C, 45 cycles at 95°C for 10s, 15s at 60°C and 10s at 40°C.

Data were acquired on the BioMark Real-Time PCR system and analyzed using the Fluidigm Real-Time PCR Analysis software to obtain crossing point (CP) values. The assays were performed in duplicate using two negative water controls per chip and *Escherichia coli* strain EDL933 was added in each run to control for internal inhibition (Michelet et al, 2014).

## 2.5 Validation of detected infectious agents

Conventional and nested PCRs using different primers than those of the BioMark® system were used to confirm the presence of the detected infectious agents in the samples (Table 1). Amplicons were sequenced by Eurofins MWG Operon (Germany) and assembled using the BioEdit software (Ibis Biosciences, Carlsbad). An online BLAST (National Center for Biotechnology Information) was used to identify the sequenced organisms.

## 2.6 Statistical analyses

To evaluate the impact of destructive sampling on tick dynamics, we fit Poisson generalized linear mixed models to the tick numbers over time. The explanatory variables were i) nest treatment, ie whether ticks were collected or counted and ii) an exponential time trend. The effect of collecting on tick dynamics was assessed via the interaction between these two variables. Note that exponential trends are standard in Poisson models, as what is modeled is actually the logarithm of the response. To complete the models, a random effect nest was introduced to take into account unobserved differences between nests. Females, males and nymphs were studied separately. Calculations were performed with the R software (R-core team, 2015).

To test for spatial structure in tick numbers among nests, we calculated Moran's I index using the *correlogram* function in pgirmess package for R software using total tick numbers and female tick

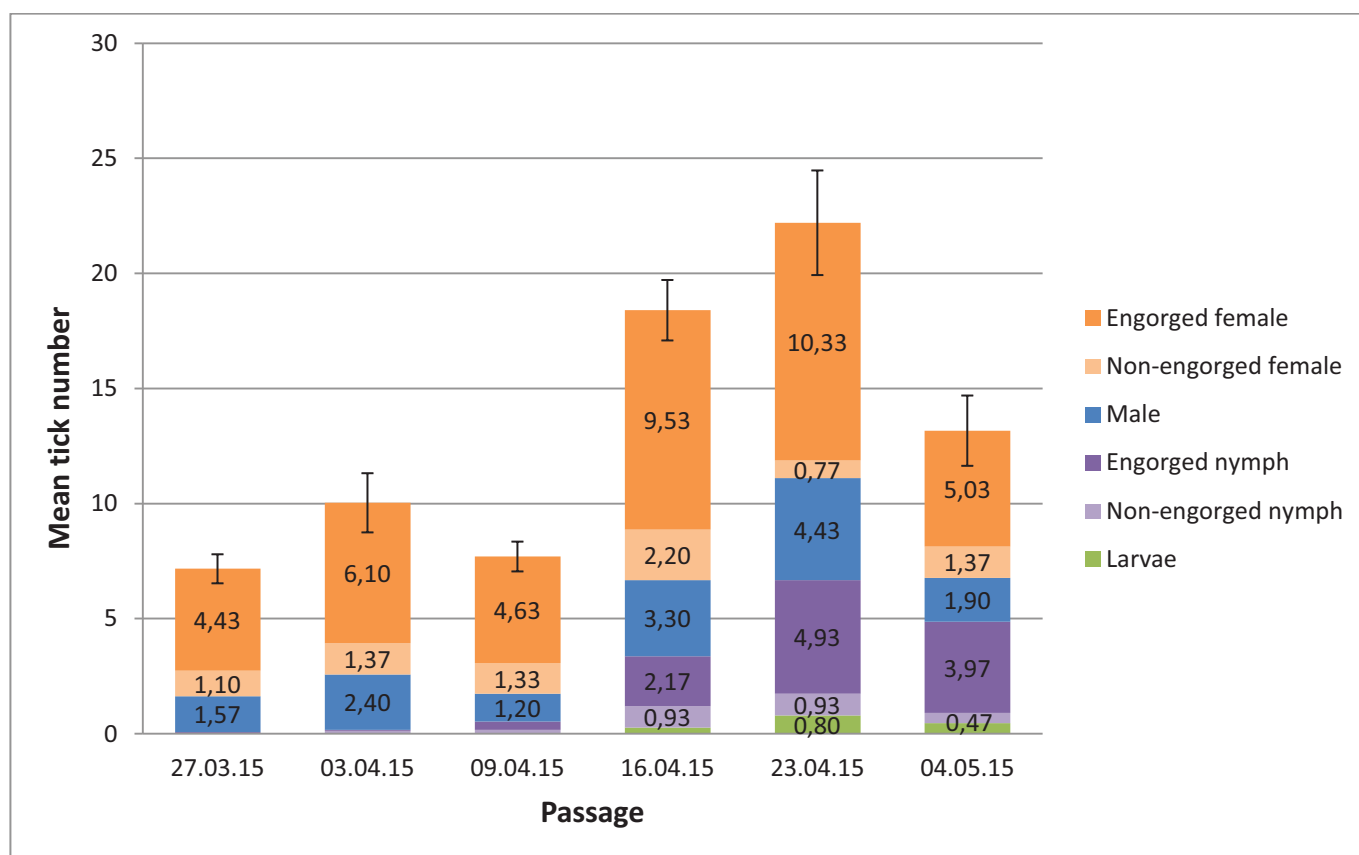


numbers only; this was not done for the other life stage due to low numbers. Ten distance classes among nests were defined at the first passage to test for step-by-step tick dispersal. Three distance classes were then defined for counted nests at each subsequent passage to test for short, medium and large-scale patterns of tick movement. The presence of structure in the distribution of the detected micro-organisms was also tested using infected nests and ten distance classes.

### 3 Results

#### 3.1 Within season tick dynamics

Ticks were found in all nests, but not at each passage; the prevalence of infested nests per passage varied from 86 to 100%, with all nests infested during passages 4 and 5. The total number of ticks per nest increased over time up to passage 5 and then started to decline (Fig. 2). During the first three passages, we observed predominantly adult ticks (Fig. 3a and 3b). From the third passage onward, we started to observe engorged and non-engorged nymphs (Fig. 3c) and larvae. The most stable tick life stage was engorged females (mean abundance over time:  $6.68 \pm 8.89$  or see Fig. 2). At the fifth passage, one week after egg hatching, tick abundance was maximal and all life stages were found.

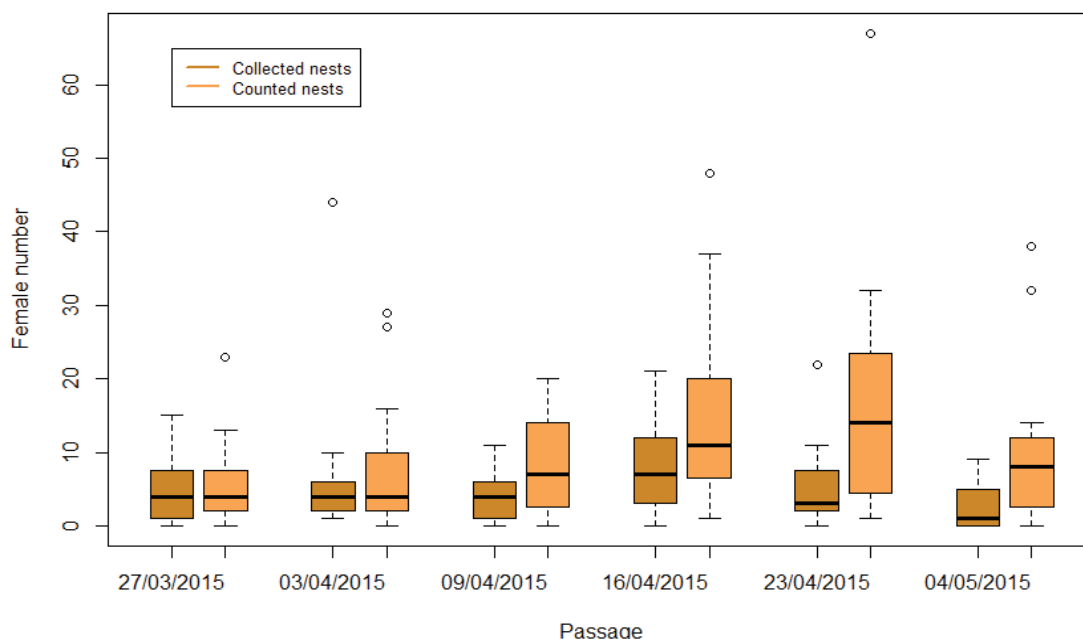


**Fig.2:** Histogram presenting the mean number of ticks observed in all nests over time. Bars represent mean standard errors of the total number of ticks.

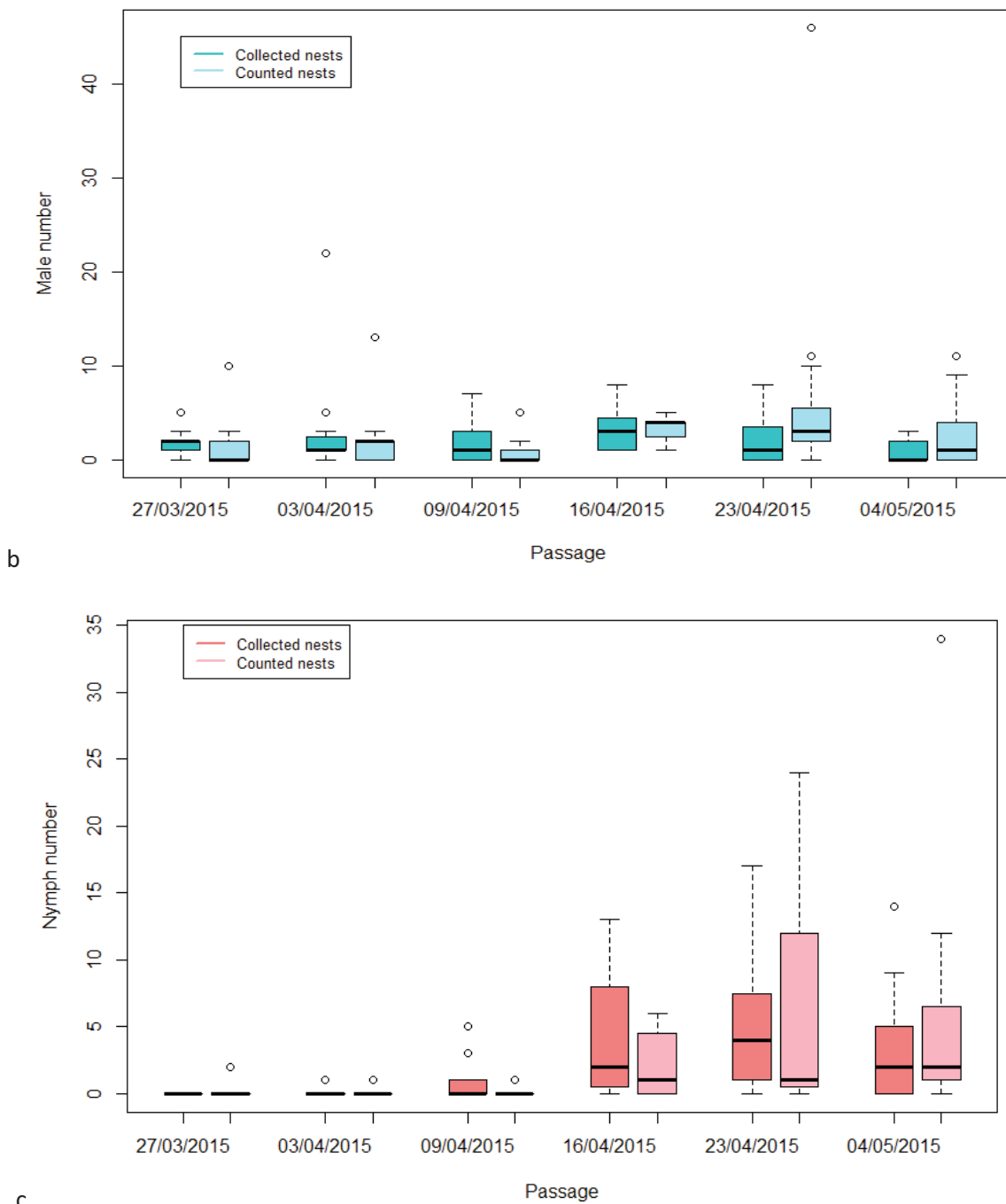
Table 2 shows the results of the generalized linear models. All slopes were significant, except for the male population of the collected nests. For females, males and nymphs, collecting had a significant effect on the population size (ie, significant interaction). There was also an interesting trend in nymphal ticks, where abundance seemed to be higher in the collected nests prior to passage 4 and, after this time, this trend reversed itself, suggesting a marked reduction in the presence of this life stage with destructive sampling (Fig. 3).

**Table 2:** Generalized linear model results. As tick number was modeled by an exponential trend, a time slope above 1 indicates an increase in the population size, whereas a time slope less than 1 indicates a decrease compared to expectations. A significant interaction effect denotes a difference in the population size between the two sampling treatments.

Nest type	Time slope	95% confidence interval	P-value	Interaction p-value
<b>Females</b>				
Counted	1.15	1.11 – 1.20	<1.0E-7	
Collected	0.94	0.89 – 0.99	0.023	<1.0E-7
<b>Males</b>				
Counted	1.30	1.20 – 1.40	<1.0E-7	
Collected	0.94	0.86 – 1.02	0.11	<1.0E-7
<b>Nymphs</b>				
Counted	2.04	1.82 – 2.28	<1.0E-7	
Collected	1.67	1.51 – 1.84	<1.0E-7	0.0089



a.



**Fig.3:** Boxplot representations of tick numbers in counted and collected nests over time: a, females only; b, males only; c, nymphs. The box shows the median as a line across the middle and the quartiles (25<sup>th</sup> and 75<sup>th</sup> percentiles) at either end. Extremities represent the minimal and maximal values and circles represent outliers.

### 3.2 Infectious agents in ticks

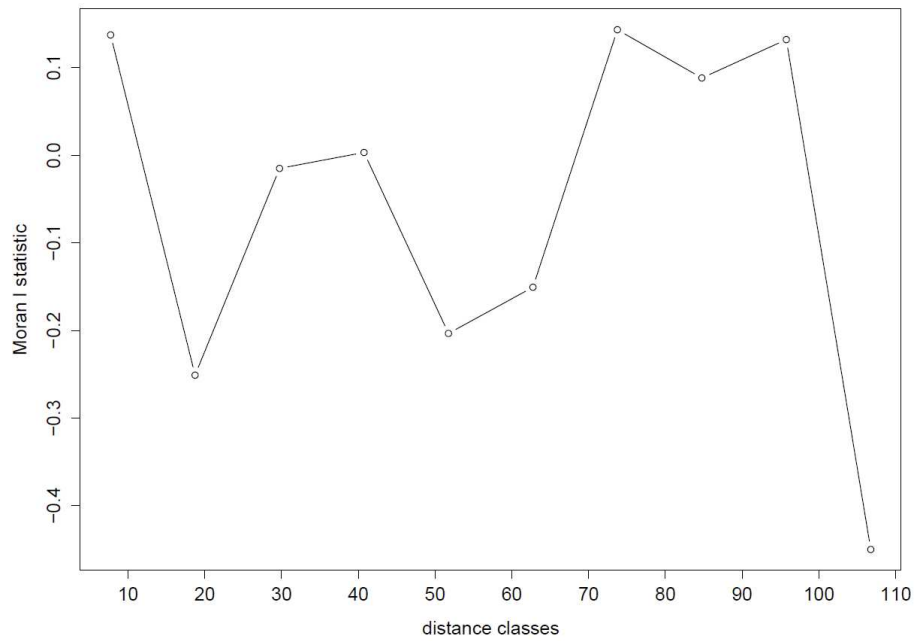
Among the 24 nests and 201 tick individuals analyzed by real-time PCR, 177 (88%) harbored at least one micro-organism including 7 bacteria, 1 parasite and 1 virus (Table 1). After subsequent amplification and sequencing, 159 ticks (79%) were found to harbor only symbiotic bacteria, 4 (2%) were infected by known pathogenic agents, 14 (7%) ticks were co-infected by both symbiotic bacteria and pathogens and 24 (12%) were found uninfected. Three presumably symbiotic bacteria were detected: the *Rickettsia*-like

bacterium was detected in all nests in high prevalence (50-100% of sampled ticks) and matched sequences of *Rickettsia lusitaniae* (Milhano et al, 2014) (99% Blast identity), one *Coxiella*-like bacterium was found in 10 nests with variable prevalence among ticks (9-50%), and one *Francisella*-like bacterium was detected in 3 nests in low prevalence (10-12.5%). Four potentially pathogenic bacteria were detected in low prevalence. *Anaplasma* spp was found in three nests (2-11%), but the reamplified sequence did not match on GenBank. *Bartonella henselae* was detected in a single nest with species-specific primers (9%), but could not be reamplified for confirmation. *Borrelia* spp. was also detected in three nests at low prevalence (4-12.5%), but could not be reamplified for confirmation; we do not yet know what bacterium species was initially amplified. Finally, *Rickettsia helvetica* was detected and confirmed to occur in five nests (4-25%). Only one parasite was detected, *Babesia* spp. and occurred in five nests (8-20%). However, the reamplified sequences of this *Babesia* spp. did not match any known sequences on GenBank and may represent a new species. Two tick co-infections were observed for potential pathogens: *B. henselae* and *R. helvetica* in one female tick (nest 3) and *Borrelia* spp and *Rickettsia helvetica* in a different female tick (nest 26). In addition, co-infections of presumed pathogens also occurred at the nest level: *Anaplasma* spp., *B. henselae* and *R. helvetica* (nest 3); *R. helvetica* and *Borrelia* spp. in nests 26 and 27; *Babesia* spp., *R. helvetica* and *Borrelia* spp. (nest 29).

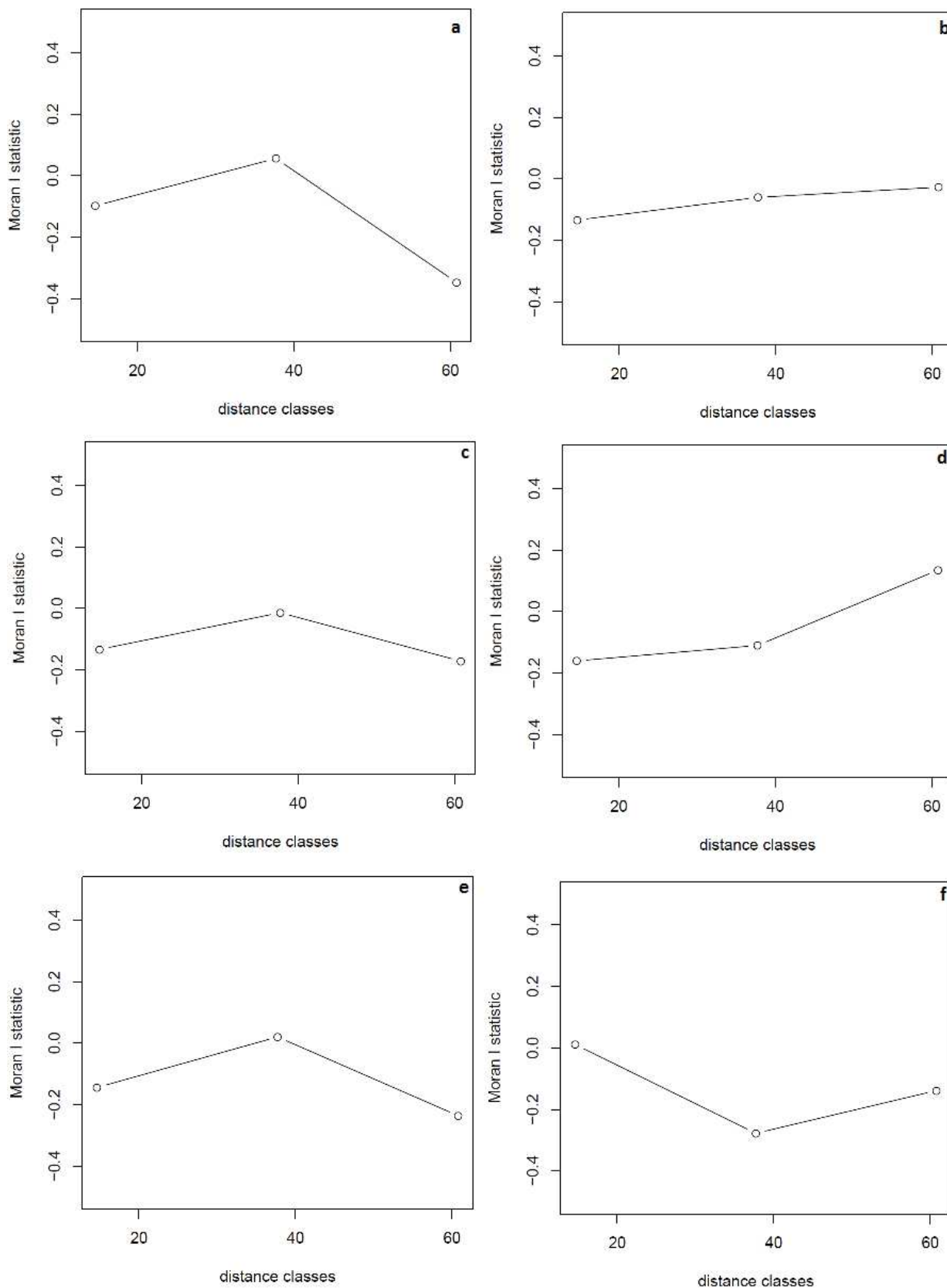
Among the 25 tested viruses, only one, the West Nile virus (WNV), occurred on Carteau in 6 individual ticks from 5 different nests. Two individual ticks infected by WNV were also infected by *Babesia* spp. (nest 13) and by *R. helvetica* (nest 29). Nevertheless, we were not able to obtain a sequence of this virus with RT-PCR or nested PCRs and the presence of this particular virus remains to be confirmed.

### 3.3 Spatial and temporal structuring of ticks and their infectious agents

The tests for spatial auto-correlation in tick abundance at the initial passage were non-significant (Fig. 4) and no clear spatial patterns in tick presence could be found for counted nests at each passage (Fig. 5). These patterns were similar when we tested for within-colony structure in the occurrence of infectious agents in the ticks (data not shown).



**Fig.4:** Spatial autocorrelation in total tick number estimated by Moran's I (Sokal and Oden, 1978). Data are from the first passage in the colony and include nests of both treatments. Ten distance classes have been defined in metres. No index value was significantly different from zero. The same results were obtained using female count data only (results not shown).



**Fig.5:** Spatial autocorrelation in total tick number and distance between counted nests at each passage with three distance classes based on Moran's I: a, 1<sup>st</sup> passage; b, 2<sup>nd</sup> passage; c, 3<sup>rd</sup> passage; d, 4<sup>th</sup> passage; e, 5<sup>th</sup> passage; f, 6<sup>th</sup> passage. Circles indicate the autocorrelation coefficients. The same results were obtained with female count numbers.

## 4 Discussion

To understand the degree to which ticks disperse among nests and are responsible for pathogen dissemination at local scales, we investigated the dynamics and structure of a soft tick *Ornithodoros maritimus* over the course of a host breeding season at the scale of a single island colony. We found a temporal dynamic in the presence of the different tick stages over the season, with the simultaneous appearance juvenile stages and hatched chicks. Destructive sampling reduced tick abundance in the nest from the fourth passage onward, suggesting the presence of some localized populations, but no significant spatial structure within the colony was found for either tick abundance or pathogen presence.

### 4.1 Within-nest tick dynamics and impact of destructive sampling

In this study, one of the goals was to understand and describe the dynamics of *O. maritimus* ticks within host nests during the breeding season. What we observed is that adult ticks were present from the start of the season, with females feeding on brooding adult birds. As engorged females continued to occur and increased in number over time after chick hatching, it is likely that this life stage also feeds on chicks (Fig. 2 and Fig.3a). The proportion of males increased in line with females, potentially to maximize copulation opportunities (Fig. 3b). Nymphs and larvae only started to appear at the third and fourth passages respectively. However, observations of nymphs and larvae in the nest are extremely difficult due to their small size, particularly when unengorged: 1 to 5mm for nymphs and 0.5 to 1 mm for larvae (Hoogstraal et al, 1976). The abundance data associated with these life stages should therefore be considered with caution as the relative quantities of these stages are likely greatly underestimated compared to the larger and more conspicuous adult ticks. The number of juvenile ticks was maximal at the fifth passage (Fig. 3c) which may represent an adaptation to host phenology due to their intimate and repeated interactions (Magalhães et al, 2007). In effect, larvae were found feeding on chicks during the fourth passage. These larvae may have been produced from eggs laid a few weeks prior by females that starting feeding at the start of incubation. Due to their longer blood meal (several hours), larvae that feed on stationary chicks may have a higher probability of finding suitable off-host habitat post-bloodmeal than larvae that feed on the mobile adult birds. This kind of adaptation to host phenology is already known in other parasite-host systems, and notably in phytophagous insects (Filchack et al, 2000; Rouault et al, 2004). To further test the hypothesis that life stage appearance in ticks has evolved to



match breeding phenology, detailed sampling in other colonies that show different timing in breeding will be necessary.

The decrease in all tick stages at the sixth passage was surprising, but may be explained by the fact that chicks are no longer restricted to the nest site. Indeed, at this point, the young birds start to use the entire territory and notably sheltered areas in and around the vegetation. This behavior may favor the return of ticks to overwintering microhabitats where temperature and humidity conditions may be more stable (Hoogstraal, 1985).

At the scale of the seabird colony, we tested whether the repeated collection of ticks from nests over six weeks would affect within-nest dynamics of the tick population. On the whole, tick dynamics were relatively homogenous between collected and counted nests despite destructive sampling (Fig.3). Nevertheless, tick number (female, male and nymph) was significantly reduced in collected nests (Table 2). Although the number of ticks observed in collected nests was lower than that of counted nests, collected nests were still recolonized week after week by all tick stages suggesting that tick dispersal at the within colony level is frequent. Even though ticks were surely missed during sampling, it is difficult to imagine that the number of uncollected ticks in a nest was the sole contributor to ticks retrieved at the next passage (ie, some movement into the nest must be occurring).

#### 4.2 Diversity and structure of symbionts and infectious agents

Micro-organisms present in ticks of Carteau Island included both presumed endosymbionts and pathogens. The presence of *Coxiella* symbionts in *O. maritimus* was expected based on previous results obtained from this island colony (Duron et al, 2015) and has been recently reported in members of the *O. capensis* complex from across the globe (Al Deeb et al, 2015; Duron et al, 2014; Reeves et al, 2006). The *Rickettsia*-like symbiont was initially isolated from a soft tick parasitizing birds in Portugal, *O. erraticus* (Milhano et al, 2014) and from cell lines of *O. capensis* collected from coastal Georgia, USA (Mattila et al, 2007). Likewise, the *Francisella*-like symbiont has been previously described in both soft ticks, *O. moubata* (Noda et al, 1997) and *Argas persicus* (Suitor et Weiss, 1961), and hard ticks (Niebylski et al, 1997; Sun et al, 2000, Michelet et al, 2013). These different endosymbiotic bacteria are suggested to perform essential functions required for the completion of the tick life cycle, but details on their physiological impacts are scarce for now and we cannot yet explain the high diversity of these organisms within our population (Duron et al, 2015). Although they likely have no impact on the bird host, recent work has suggested that mutations in endosymbiotic bacteria can directly lead to the emergence of novel pathogenic organisms (Duron et al, 2015).

The prevalence of suspected pathogenic agents was much lower within the tick population, as would be expected. Different pathogenic species of *Anaplasma* are transmitted by hard ticks to mammals (including humans) (Dantas-Torres et al, 2012) and by a soft tick *Argas reflexus* to pigeons (Jongejan and Uilenberg, 2004). The *Anaplasma* species detected in our study did not match any known species available on public databases and may represent a new species. Although the msp2 target gene shows high intra and interspecies polymorphism, it is typically used to identify the species of this genus (Rymaszewska, 2010). We also detected a bacterium that matches with *Bartonella henselae*, the known agent of cat-scratch fever. The first identification of a *Bartonella* species, related to *B. henselae*, in a tick was in the soft tick *O. sonrai* in Senegal but the principal vectors of *B. henselae* are typically lice of wild felids (Mediannikov et al, 2013). The primers used in our real-time PCR assay were specific to this species, but we were not able to reamplify this bacterium using an alternative gene. This may have been due to low DNA quantity or to the fact that this bacterium may represent a genetic variant of the species. These issues require further investigation. *Rickettsia helvetica*, an infectious agent causing meningitis in humans, is well-known to be transmitted by hard ticks (Parola et al, 2005) and notably to wild birds by *I. ventalloi* in Portugal (Santos-Silva et al, 2006). Our study represents the first confirmed report of this bacterium in association with soft ticks to our knowledge. We also found an unknown species of *Babesia* in our *O. maritimus* colony. These apicomplexan parasites cause different forms of hemolytic disease, or babesiosis, in mammals. Most species of *Babesia* are vectored by hard ticks. Only one soft tick species has so far been recorded to vector these parasites; *O. erraticus* transmitting *Babesia meri* to sand rats in Africa (Yabsley and Shock, 2013). The *Babesia* spp. we found in *O. maritimus* could not be identified using data available on Genbank. Further work will therefore be required to determine the relationship of this species to other described species of *Babesia*. The presence of *Borrelia* spp. and a type of West Nile Virus (a Flavivirus) were also detected in our ticks, but could not be confirmed. Both agents are known to be transmitted by different species of soft ticks (Cutler, 2010; Lawrie et al, 2004) and WNV has been previously reported in the Camargue region (Balança et al, 2009; Pradier et al, 2014; Vittecoq et al 2013). However, a previous study of Yellow-legged gulls on Carteau did not find evidence for the presence of Flavivirus antibodies in eggs, suggesting that WNV and other related viruses did not circulate in gulls (Arnal et al, 2014). These results therefore also require further study.

Compared to results obtained using the same molecular detection assay and 267 females of the hard tick *Ixodes ricinus* from nine sites in France (Moutailler et al, 2016), we found much fewer pathogens and in lower prevalence (Table 1) in our soft ticks. This may be a direct consequence of the soft tick lifestyle; soft ticks feed on the host for only a short time compared to hard ticks and therefore may be more isolated because they have fewer opportunities for host-associated dispersal (Kada et al.

submitted). Alternatively, ticks or host-birds may be more efficient at clearing infections compared to hard ticks and their hosts (Brown and O'Brien, 2011). It should also be noted that the detection of pathogen DNA in the ticks does not mean that these pathogens were still alive and transmissible to a new host. Laboratory tests are therefore required now to determine true vector competence. Likewise, data from different colonies across the Mediterranean are needed to evaluate the role of *O. maritimus* in the circulation of these different infectious agents at larger spatial scales.

### 4.3 Vector movement at the within-colony scale

Removal sampling and the analysis of spatial structure reveal little to no structuring for ticks or their infectious agents in the gull colony of Carteau Island, even among nests separated by several meters. Topography is known to be an important factor impacting the isolation of hard tick populations and may modify pathogen circulation at small spatial scales (McCoy et al, 2003b). However, no apparent spatio-temporal structuring was observed for the Meaban virus transmitted by *O. maritimus* in Spain (Arnal et al, 2014) suggesting that it could be a less important factor in soft ticks especially on a flat and densely occupied island as Carteau. Hosts are considered to be the major driver of tick dispersal and may transport them over long distances (e.g., Smith et al, 1996; McCoy et al, 2003a). In the case of soft ticks however, feeding times are so short that the role of host movements in structuring tick populations and their infectious agents may be minimal. Indeed, Arnal et al. (2014) found that the seroprevalence of the Meaban virus was highly stable at the colony level, but variable at the nest level. This suggests little intercolony dispersal of either birds or ticks, but rather shifts in either nest use by birds and/or movement of infected ticks within the colony. In relation to our results, we suggest that it is the latter mechanism at play and that ticks are disseminating from one nest to another relatively easily. Moreover, on Carteau Island, we observed that exact nest sites are not necessarily reused by hosts from one year to the next. Ticks must therefore move at least over the area of the territory each season in order to find a suitable host. Indeed, the within-nest patterns of infection that we found suggest frequent within-colony movements; surprisingly few ticks from a same nest were infected with the same infectious agents, which would have been expected if all ticks in a nest repeatedly fed on the same individual bird. Analyses of tick genetic structure are now required to determine the degree to which ticks from different nests represent single, panmictic population. Further analyses on the presence of infectious agents or agent-specific antibodies in birds (Arnal et al, 2014) would also provide additional information on exposure rates to ticks at the within-colony level. As soft ticks differ substantially in life history traits from hard ticks, these results highlight the need to understand the spatial and temporal dynamics of this

vector at local scales in order to better to predict and control pathogen circulation at larger spatial scales.

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## References

- Abrahan, L.B., Gorla, D.E., Catalá, S.S., 2011. Dispersal of *Triatoma infestans* and other Triatominae species in the arid Chaco of Argentina: Flying, walking or passive carriage? The importance of walking females. *Memórias do Instituto Oswaldo Cruz* 106, 232–239. doi:10.1590/S0074-02762011000200019
- Adeyeye, O.A., Butler, J.F., 1989. Population structure and seasonal intra-burrow movement of *Ornithodoros turicata* (Acari: Argasidae) in gopher tortoise burrows. *Journal of medical entomology* 26, 279–283.
- Al-Deeb, M.A., Frangoulidis, D., Walter, M.C., Kömpf, D., Fischer, S.F., Petney, T., Muzaffar, S.B., n.d. Coxiella-like endosymbiont in argasid ticks (*Ornithodoros muesebecki*) from a Socotra Cormorant colony in Umm Al Quwain, United Arab Emirates. *Ticks and Tick-borne Diseases*. doi:10.1016/j.ttbdis.2015.10.012
- Arnal, A., Gómez-Díaz, E., Cerdà-Cuéllar, M., Lecollinet, S., Pearce-Duvel, J., Busquets, N., García-Bocanegra, I., Pagès, N., Vittecoq, M., Hammouda, A., Samraoui, B., Garnier, R., Ramos, R., Selmi, S., González-Solís, J., Jourdain, E., Boulinier, T., 2014. Circulation of a Meaban-Like Virus in Yellow-Legged Gulls and Seabird Ticks in the Western Mediterranean Basin. *PLoS ONE* 9, e89601. doi:10.1371/journal.pone.0089601
- Balança, G., Gaidet, N., Savini, G., Vollot, B., Foucart, A., Reiter, P., Boutonnier, A., Lelli, R., Monicat, F., 2009. Low West Nile Virus Circulation in Wild Birds in an Area of Recurring Outbreaks in Southern France. *Vector-Borne and Zoonotic Diseases* 9, 737–741. doi:10.1089/vbz.2008.0147
- Bonnet, S., Jouglin, M., Malandrin, L., Becker, C., Agoulon, A., Hostis, M., Chauvin, A., 2007. Transstadial and transovarial persistence of *Babesia divergens* DNA in *Ixodes ricinus* ticks fed on infected blood in a new skin-feeding technique. *Parasitology* 134, 197–207. doi:10.1017/S0031182006001545
- Brisson, D., Dykhuizen, D.E., Ostfeld, R.S., 2008. Conspicuous impacts of inconspicuous hosts on the Lyme disease epidemic. *Proceedings of the Royal Society of London B: Biological Sciences* 275, 227–235. doi:10.1098/rspb.2007.1208
- Brown, C., O'Brien, V., 2011. Are Wild Birds Important in the Transport of Arthropod-Borne Viruses?, in: *Ornithological Monographs No. 71, Ornithological Monographs*. American Ornithologists' Union, pp. 1–64.
- Bunikis, J., Garpmo, U., Tsao, J., Berglund, J., Fish, D., Barbour, A.G., 2004. Sequence typing reveals extensive strain diversity of the Lyme borreliosis agents *Borrelia burgdorferi* in North America and *Borrelia afzelii* in Europe. *Microbiology* 150, 1741–1755. doi:10.1099/mic.0.26944-0
- Converse, J.D., Hoogstraal, H., Moussa, M.I., Feare, C.J., Kaiser, M.N., 1975. Soldado virus (Hughes group) from *Ornithodoros (Alectorobius) capensis* (Ixodoidea: Argasidae) infesting Sooty Tern colonies in the Seychelles, Indian Ocean. *Am J Trop Med Hyg* 24, 1010–1018.
- Cutler, S. j., 2010. Relapsing fever – a forgotten disease revealed. *Journal of Applied Microbiology* 108, 1115–1122. doi:10.1111/j.1365-2672.2009.04598.x
- Dantas-Torres, F., Chomel, B.B., Otranto, D., 2012. Ticks and tick-borne diseases: a One Health perspective. *Trends in Parasitology* 28, 437–446. doi:10.1016/j.pt.2012.07.003

- Dietrich, M., Gómez-Díaz, E., McCoy, K.D., 2011. Worldwide Distribution and Diversity of Seabird Ticks: Implications for the Ecology and Epidemiology of Tick-Borne Pathogens. *Vector-Borne and Zoonotic Diseases* 11, 453–470. doi:10.1089/vbz.2010.0009
- Duron, O., Jourdain, E., McCoy, K.D., 2014. Diversity and global distribution of the *Coxiella* intracellular bacterium in seabird ticks. *Ticks and Tick-borne Diseases* 5, 557–563. doi:10.1016/j.ttbdis.2014.04.003
- Duron, O., Noël, V., McCoy, K.D., Bonazzi, M., Sidi-Boumedine, K., Morel, O., Vavre, F., Zenner, L., Jourdain, E., Durand, P., Arnathau, C., Renaud, F., Trape, J.-F., Biguezoton, A.S., Cremaschi, J., Dietrich, M., Léger, E., Appelgren, A., Dupraz, M., Gómez-Díaz, E., Diatta, G., Dayo, G.-K., Adakal, H., Zoungrana, S., Vial, L., Chevillon, C., 2015. The Recent Evolution of a Maternally-Inherited Endosymbiont of Ticks Led to the Emergence of the Q Fever Pathogen, *Coxiella burnetii*. *PLoS Pathog* 11, e1004892. doi:10.1371/journal.ppat.1004892
- Feare, C.J., 1976. Desertion and Abnormal Development in a Colony of Sooty Terns *Sterna Fuscata* Infested by Virus-Infected Ticks. *Ibis* 118, 112–115. doi:10.1111/j.1474-919X.1976.tb02015.x
- Filchak, K.E., Roethele, J.B., Feder, J.L., 2000. Natural selection and sympatric divergence in the apple maggot *Rhagoletis pomonella*. *Nature* 407, 739–742. doi:10.1038/35037578.
- Gandon, S., Michalakis, Y., 2002. Local adaptation, evolutionary potential and host–parasite coevolution: interactions between migration, mutation, population size and generation time. *Journal of Evolutionary Biology* 15, 451–462. doi:10.1046/j.1420-9101.2002.00402.x
- Hoogstraal, H., 1985. Argasid and Nuttalliellid Ticks as Parasites and Vectors, in: J.R. Baker and R. Muller (Ed.), *Advances in Parasitology*. Academic Press, pp. 135–238.
- Hoogstraal, H., Clifford, C.M., Keirans, J.E., Kaiser, M.N., Evans, D.E., 1976. The *Ornithodoros* (*Alectorobius*) *Capensis* Group (Acarina: Ixodoidea: Argasidae) of the Palearctic and Oriental Regions. *O. (A.) Maritimus*: Identity, Marine Bird Hosts, Virus Infections, and Distribution in Western Europe and Northwestern Africa. *The Journal of Parasitology* 62, 799. doi:10.2307/3278964
- Hornok, S., Földvári, G., Elek, V., Naranjo, V., Farkas, R., de la Fuente, J., 2008. Molecular identification of *Anaplasma marginale* and rickettsial endosymbionts in blood-sucking flies (Diptera: Tabanidae, Muscidae) and hard ticks (Acari: Ixodidae). *Veterinary Parasitology* 154, 354–359. doi:10.1016/j.vetpar.2008.03.019
- Jongejan, F., Uilenberg, G., 2004. The global importance of ticks. *Parasitology* 129, S3–S14. doi:10.1017/S0031182004005967
- Lawrie, C.H., Uzcátegui, N.Y., Gould, E.A., Nuttall, P.A., 2004. Ixodid and argasid tick species and West Nile virus. *Emerg Infect Dis* 10, 653–657.
- Keesing, F., Holt, R.D., Ostfeld, R.S., 2006. Effects of species diversity on disease risk. *Ecology Letters* 9, 485–498. doi:10.1111/j.1461-0248.2006.00885.x
- Magalhães, S., Forbes, M.R., Skoracka, A., Osakabe, M., Chevillon, C., McCoy, K.D., 2007. Host race formation in the Acari. *Exp Appl Acarol* 42, 225–238. doi:10.1007/s10493-007-9091-0



- Mattila, J.T., Burkhardt, N.Y., Hutcheson, H.J., Munderloh, U.G., Kurtti, T.J., 2007. Isolation of Cell Lines and a Rickettsial Endosymbiont from the Soft Tick *Carios capensis* (Acari: Argasidae: Ornithodorinae). *Journal of Medical Entomology* 44, 1091–1101. doi:10.1093/jmedent/44.6.1091
- McCoy, K.D., Boulinier, T., Tirard, C., Michalakis, Y., 2003a. Host-Dependent Genetic Structure of Parasite Populations: Differential Dispersal of Seabird Tick Host Races. *Evolution* 57, 288–296.
- McCoy, K.D., Tirard, C., Michalakis, Y., 2003b. Spatial genetic structure of the ectoparasite *Ixodes uriae* within breeding cliffs of its colonial seabird host. *Heredity* 91, 422–429. doi:10.1038/sj.hdy.6800339
- Mediannikov, O., Diatta, G., Kasongo, K., Raoult, D., 2013. Identification of Bartonellae in the Soft Tick Species *Ornithodoros sonrai* in Senegal. *Vector-Borne and Zoonotic Diseases* 14, 26–32. doi:10.1089/vbz.2013.1326
- Michelet, L., Bonnet, S., Madani, N., Moutailler, S., 2013. Discriminating *Francisella tularensis* and *Francisella*-like endosymbionts in *Dermacentor reticulatus* ticks: Evaluation of current molecular techniques. *Veterinary Microbiology* 163, 399–403. doi:10.1016/j.vetmic.2013.01.014
- Michelet, L., Delannoy, S., Devillers, E., Umhang, G., Aspan, A., Juremalm, M., Chirico, J., van der Wal, F.J., Sprong, H., Boye Pihl, T.P., Klitgaard, K., BÅdker, R., Fach, P., Moutailler, S., 2014. High-throughput screening of tick-borne pathogens in Europe. *Frontiers in Cellular and Infection Microbiology* 4. doi:10.3389/fcimb.2014.00103
- Milhano, N., Palma, M., Marcili, A., Nuncio, M.S., de Carvalho, I.L., de Sousa, R., 2014. *Rickettsia lusitaniae* sp. nov. isolated from the soft tick *Ornithodoros erraticus* (Acarina: Argasidae). *Comparative Immunology, Microbiology and Infectious Diseases* 37, 189–193. doi:10.1016/j.cimid.2014.01.006
- Moutailler, S., Moro, C.V., Vaumourin, E., Michelet, L., Tran, F.H., Devillers, E., Cosson, J.-F., Gasqui, P., Van, V.T., Mavingui, P., Vourc'h, G., Vayssier-Taussat, M., 2016. Co-infection of Ticks: The Rule Rather Than the Exception. *PLOS Negl Trop Dis* 10, e0004539. doi:10.1371/journal.pntd.0004539
- Niebylski, M.L., Peacock, M.G., Fischer, E.R., Porcella, S.F., Schwan, T.G., 1997. Characterization of an endosymbiont infecting wood ticks, *Dermacentor andersoni*, as a member of the genus *Francisella*. *Appl. Environ. Microbiol.* 63, 3933–3940.
- Noda, H., Munderloh, U.G., Kurtti, T.J., 1997. Endosymbionts of ticks and their relationship to *Wolbachia* spp. and tick-borne pathogens of humans and animals. *Appl. Environ. Microbiol.* 63, 3926–3932.
- Norman, A.F., Regnery, R., Jameson, P., Greene, C., Krause, D.C., 1995. Differentiation of Bartonella-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. *J. Clin. Microbiol.* 33, 1797–1803.
- Ogden, N., 2013. Changing geographic ranges of ticks and tick-borne pathogens: drivers, mechanisms and consequences for pathogen diversity. *Front. Cell. Infect. Microbiol* 3, 46. doi:10.3389/fcimb.2013.00046
- Olsen, K.M., 2004. Gulls of Europe, Asia and North America. Christopher Helm Publishers Ltd, London.



- Ostfeld, R.S., Glass, G.E., Keesing, F., 2005. Spatial epidemiology: an emerging (or re-emerging) discipline. *Trends in Ecology & Evolution*, Special Issue: Bumper Book Review 20, 328–336. doi:10.1016/j.tree.2005.03.009
- Parola, P., Paddock, C.D., Raoult, D., 2005. Tick-Borne Rickettsioses around the World: Emerging Diseases Challenging Old Concepts. *Clin. Microbiol. Rev.* 18, 719–756. doi:10.1128/CMR.18.4.719-756.2005
- Perez-Eid, C., 2007. Les tiques : identification, biologie, importance médicale et vétérinaire. Lavoisier.
- Pradier, S., Sandoz, A., Paul, M.C., Lefebvre, G., Tran, A., Maingault, J., Lecollinet, S., Leblond, A., 2014. Importance of Wetlands Management for West Nile Virus Circulation Risk, Camargue, Southern France. *International Journal of Environmental Research and Public Health* 11, 7740–7754. doi:10.3390/ijerph110807740
- R Core Team, 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Reeves, W.K., Loftis, A.D., Sanders, F., Spinks, M.D., Wills, W., Denison, A.M., Dasch, G.A., 2006. *Borrelia*, *Coxiella*, and *Rickettsia* in *Carios capensis* (Acari: Argasidae) from a brown pelican (*Pelecanus occidentalis*) rookery in South Carolina, USA. *Exp Appl Acarol* 39, 321–329. doi:10.1007/s10493-006-9012-7
- Regnery, R.L., Spruill, C.L., Plikaytis, B.D., 1991. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. *J. Bacteriol.* 173, 1576–1589.
- Rouault, G., Turgeon, J., Candau, J.-N., Roques, A., Aderkas, P. von, 2004. Oviposition strategies of conifer seed chalcids in relation to host phenology. *Naturwissenschaften* 91, 472–480. doi:10.1007/s00114-004-0554-4
- Rymaszewska, A., 2010. Variability within the *msp2* gene in populations of *Anaplasma phagocytophilum*. *Folia Biol. (Praha)* 56, 269–275.
- Santos-Silva, M.M., Sousa, R., Santos, A.S., Melo, P., Encarnação, V., Bacellar, F., 2006. Ticks Parasitizing Wild Birds in Portugal: Detection of *Rickettsia aeschlimannii*, *R. helvetica* and *R. massiliae*. *Exp Appl Acarol* 39, 331. doi:10.1007/s10493-006-9008-3
- Scaramozzino, N., Crance, J.-M., Jouan, A., DeBriel, D.A., Stoll, F., Garin, D., 2001. Comparison of Flavivirus Universal Primer Pairs and Development of a Rapid, Highly Sensitive Heminested Reverse Transcription-PCR Assay for Detection of Flaviviruses Targeted to a Conserved Region of the NS5 Gene Sequences. *J. Clin. Microbiol.* 39, 1922–1927. doi:10.1128/JCM.39.5.1922-1927.2001
- Smith, R.P., Rand, P.W., Lacombe, E.H., Morris, S.R., Holmes, D.W., Caporale, D.A., 1996. Role of Bird Migration in the Long-Distance Dispersal of *Ixodes dammini*, the Vector of Lyme Disease. *J Infect Dis.* 174, 221–224. doi:10.1093/infdis/174.1.221
- Sokal, R.R., Oden, N.L., 1978. Spatial autocorrelation in biology: 1. Methodology. *Biological Journal of the Linnean Society* 10, 199–228. doi:10.1111/j.1095-8312.1978.tb00013.x
- Sonenshine, D.E., Mather, T.N., 1994. *Ecological Dynamics of Tick-Borne Zoonoses*. Oxford University Press.

- Sonenshine, D.E., Roe, R.M., 2014. *Biology of Ticks Vol.2*, Second edition. ed. Oxford University Press.
- Sutor, E.C., Weiss, E., 1961. Isolation of a Rickettsialike Microorganism (*Wolbachia Persica*, N. SP.) From *Argas Persicus* (Oken). *J Infect Dis.* 108, 95–106. doi:10.1093/infdis/108.1.95
- Sun, L.V., Scoles, G.A., Fish, D., O'Neill, S.L., 2000. Francisella-like Endosymbionts of Ticks. *Journal of Invertebrate Pathology* 76, 301–303. doi:10.1006/jipa.2000.4983
- Tylianakis, J.M., Didham, R.K., Bascompte, J., Wardle, D.A., 2008. Global change and species interactions in terrestrial ecosystems. *Ecology Letters* 11, 1351–1363. doi:10.1111/j.1461-0248.2008.01250.x
- Vial, L., 2009. Biological and ecological characteristics of soft ticks (Ixodida: Argasidae) and their impact for predicting tick and associated disease distribution. *Parasite* 16, 191–202.
- Vittecoq, M., Lecollinet, S., Jourdain, E., Thomas, F., Blanchon, T., Arnal, A., Lowenski, S., Gauthier-Clerc, M., 2013. Recent Circulation of West Nile Virus and Potentially Other Closely Related Flaviviruses in Southern France. *Vector-Borne and Zoonotic Diseases* 13, 610–613. doi:10.1089/vbz.2012.1166
- Yabsley, M.J., Shock, B.C., 2013. Natural history of Zoonotic Babesia: Role of wildlife reservoirs. *International Journal for Parasitology: Parasites and Wildlife* 2, 18–31. doi:10.1016/j.ijppaw.2012.11.003

## Supplementary material

Table A1: List of the screened but non-detected pathogens.

Pathogen	Species	Target gene	Pathogen	Species	Target gene	Pathogen	Species
Bacterium	<i>A. marginale</i>	msp1	Parasite	<i>Babesia divergens</i>	hsp70	Virus	Tick Borne Encephalitis (TBEV)
Bacterium	<i>A. platys</i>	groEL	Parasite	<i>B. microti</i>	CCTeta	Virus	Langat (LGTV)
Bacterium	<i>A. ovis</i>	msp4	Parasite	<i>B. venatorum EU1</i>	18s	Virus	Louping ill (LIV)
Bacterium	<i>A. centrale</i>	groEL	Parasite	<i>B. canis</i>	18s	Virus	Deer tick virus
Bacterium	<i>B. quintana</i>	btq	Parasite	<i>B. bovis</i>	CCTeta	Virus	Powassan virus (POW)
Bacterium	<i>Borrelia burgdorferi ss</i>	rpoB	Parasite	<i>B. caballi</i>	rap1	Virus	Meaban (MEAV)
Bacterium	<i>B. garinii</i>	rpoB	Parasite	<i>B. bigemina</i>	18s	Virus	Kyasanur Forest disease (KFDV)
Bacterium	<i>B. afzelii</i>	fla	Parasite	<i>B. major</i>	CCTeta	Virus	Omsk hemorrhagic fever (OHFV)
Bacterium	<i>B. valaisiana</i>	ospA	Parasite	<i>B. ovis</i>	18s	Virus	African swine fever (AFSV)
Bacterium	<i>B. lusitaniae</i>	rpoB	Parasite	<i>Theileria equi</i>	ema1 R2	Virus	Thogoto virus
Bacterium	<i>B. spielmanii</i>	fla	Parasite	<i>T. annulata</i>	18s	Virus	Bourbon (BOUV)
Bacterium	<i>B. bissetii</i>	rpoB				Virus	Dhori (DHOV)
Bacterium	<i>B. miyamotoi</i>	glpQ				Virus	Heartland (HRTV)
Bacterium	<i>Coxiella burnetii</i>	IS1111				Virus	Kemerovo (KEMV)
Bacterium	<i>Francisella tularensis</i>	fopA				Virus	Colorado (CTF)
Bacterium	<i>Ehrlichia chaffeensis</i>	dsb				Virus	Eyach virus
Bacterium	<i>E. ruminantium</i>	dsb				Virus	Crimean Congo haemorrhagic fever (CCHF)
Bacterium	<i>E. canis</i>	dsb				Virus	Dugbe virus
Bacterium	<i>Neoehrlichia mikurensis</i>	groEL				Virus	Nairobi (NSD)
Bacterium	<i>Rickettsia conorii</i>	ITS				Virus	Uukuniemi (UUKV)
Bacterium	<i>R. slovaca</i>	ITS				Virus	Schmallenberg (SBV)
Bacterium	<i>R. massiliae</i>	ITS				Virus	Severe fever thrombocytopenia syndrome (SFTSV)



## **Chapitre 5- Discussion générale et perspectives**

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La spécialisation d'hôte est un processus particulièrement intéressant à étudier car il est générateur de biodiversité. Néanmoins, ce processus est parfois difficile à mettre en évidence, notamment dans les systèmes naturels dans lesquels l'expérimentation est lourde voire impossible. Il convient donc de trouver des méthodes d'analyses efficaces pour détecter les variations phénotypiques fines qui découlent de la différenciation des populations et qui pourraient être associées à des variations de valeur sélective des individus. L'étude du phénomène de convergence évolutive permet d'apporter des preuves pour soutenir la divergence entre populations en liaison avec des facteurs de sélection spécifiques. En effet, retrouver les mêmes patrons de divergence entre des populations n'échangeant pas ou peu d'information génétique, atteste de l'action similaire de pressions évolutives. Le but de cette thèse était donc de savoir si la spécialisation d'hôte dans des systèmes tiques-oiseaux de mer est toujours accompagnée des mêmes changements phénotypiques et de dégager les facteurs qui peuvent influencer ce phénomène de convergence.

L'ensemble des résultats de cette thèse montre que des signes de convergence phénotypique existent dans les systèmes étudiés et que diverses sources de pressions de sélection provenant de l'hôte et/ou de l'environnement, ainsi que les caractéristiques biologiques propres à chaque système participent à la structuration des populations.

### **Les pressions de sélection au sein des systèmes étudiés**

La divergence des races d'hôtes d'*I. uriae* est le résultat de différents facteurs propres à chaque population (dynamique des interactions hôtes-parasites, traits d'histoire de vie) en combinaison avec des pressions de sélection exercées par les hôtes et/ou l'environnement et/ou des mécanismes évolutifs tels que la dérive, qui modifient la réponse adaptative des parasites. Par exemple, la reconnaissance des signaux chimiques de l'hôte (kairomones)(Benoit et al., 2007), les différences de phénologie des hôtes (Barrett and Erikstad, 2011) et les mécanismes de résistance des hôtes vis-à-vis des parasites (comportement, immunité, micro-habitat) constituent des facteurs importants impliqués dans le phénomène de divergence des races d'hôtes chez *I. uriae*. Dans ce système, des patrons de variations morphologiques, en ce qui concerne la taille et la forme sont retrouvées dans différentes zones de sa répartition en fonction de l'utilisation d'hôte (Article 4) et sont en accord avec les résultats obtenus précédemment (Dietrich et al., 2013). Des différences de performance des races d'hôtes sur un hôte alternatif, découlant potentiellement de la capacité différentielle des tiques à faire face aux systèmes immunitaires et à digérer le sang de leurs hôtes ont été démontrées (Dietrich et al., 2014b). La réponse immunologique des hôtes pourrait donc potentiellement impacter la taille des tiques mais certainement



pas leur forme, suggérant que d'autres facteurs peuvent être impliqués dans la formation des patrons de variations morphologiques observées chez *I. uriae*. Par exemple, les défenses physiques des hôtes pourraient constituer un facteur important dans l'évolution des parasites. En effet, le bec des oiseaux, dont il est admis que la forme est fonction de l'alimentation, est aussi utilisé pour le nettoyage des plumes et peut être complétée par l'action des griffes dans le cas de becs longs, moins faciles à utiliser (Clayton et al., 2010; Clayton and Cotgreave, 1994). Les différences de taille et de forme des becs (en particulier la correspondance des mandibules inférieure et supérieure et la présence d'un crochet à l'extrémité du bec) peuvent influencer la taille et la charge des ectoparasites (Clayton et al., 2005, 1999). Chez les hôtes d'*I. uriae* examinés lors de notre étude, la forme des becs est variable. En effet, les macareux moines ont un bec court, large et légèrement crochu, les mouettes tridactyles ont un bec fin et légèrement crochu de longueur moyenne et les guillemots de Troïl ont un bec long, fin et pointu (Dif et al., 1982)(Figure 9).



**Figure 9:** Photographies illustrant les différences de morphologie des becs des hôtes d'*Ixodes uriae*. De gauche à droite : Le macareux moine (*Fratercula arctica*), la mouette tridactyle (*Rissa tridactyla*) et le guillemot de Troïl (*Uria aalge*). Crédits photos : M. Dupraz.

Ces différences de taille et de forme pourraient donc être à l'origine des différentes morphologies détectées chez *I. uriae*. De plus, le plumage de ces différents hôtes-oiseaux, agissant comme une barrière mécanique et chimique contre les parasites (Clayton et al., 2010) mais leur permettant aussi de réguler leur température en leur procurant une couche imperméable (Grémillet et al., 2005), est différent selon l'espèce considérée. Ces caractéristiques, telle que la densité des plumes, pourraient également avoir un impact sur la morphologie des tiques, en sélectionnant une forme et/ou une taille idéale pour se déplacer facilement dans le plumage et atteindre les zones affectionnées pour le repas de sang. Sur la base d'études comme celle réalisée par Yang et al (2006), montrant des différences de densité de plumes entre des oiseaux terricoles, nageurs et plongeurs, les différences de densité entre les plumages des différents hôtes d'*I. uriae* pourraient être investiguées en relation avec les données

morphométriques des tiques pour évaluer leur implication potentielle dans la divergence des races d'hôtes.

En outre, dans ce système impliquant des tiques endophiles, le micro-habitat utilisé par les hôtes pendant la période de reproduction peut jouer également un rôle non négligeable dans le phénomène de divergence. Chez les tiques l'adaptation à un nouvel environnement dépend de leur capacité à survivre aux conditions climatiques (Estrada-Peña, 2001). En effet, la perméabilité de leur cuticule, dépendante de la production d'hydrocarbures cuticulaires, peut constituer un facteur limitant. Dans le système *I. uriae*, les hôtes-oiseaux exploitent différentes zones de nidification : les macareux moines utilisent des terriers individuels qui peuvent être profonds jusqu'à 5 mètres ; les mouettes tridactyles nichent dans des nids individuels faits de divers débris végétaux dans des falaises abruptes et les guillemots de Troil nichent côte à côte à même les rochers. L'exposition aux variations de température et d'humidité endurée par les tiques et la possibilité de trouver un refuge dans lequel les conditions sont favorables à leur survie, sont donc variables suivant l'hôte exploité. De plus, la reproduction d'*I. uriae*, impliquant des phéromones sexuelles de contact (Sonenshine, 1985), se produit dans le micro-habitat (Eveleigh and Threlfall, 1974), ce qui peut entraîner une reproduction non-aléatoire réduisant le flux de gènes entre races d'hôtes et favoriser la spécialisation. Pour comprendre l'influence différentielle des micro-habitats, des capteurs de température et d'humidité pourraient être placés au sein des zones de nidification des hôtes afin d'appréhender les variations journalières ou saisonnières. Grâce à l'acquisition de ces données, des tests de résistance des différentes races d'hôtes en conditions contrôlées, mimant les conditions de température et d'humidité des différents micro-habitats, pourraient être mis en place en laboratoire pour tester l'influence du micro-habitat dans la divergence des races d'hôtes.

En ce qui concerne le complexe de tiques molles *Ornithodoros capensis* sensu lato, parasitant une grande diversité d'oiseaux marins, des groupes génétiquement et morphologiquement différents ont été décrit en fonction de l'utilisation d'hôte. Bien que nos échantillonnages des différentes espèces de tiques et d'hôtes aient été incomplets et que les marqueurs génétiques employés pour l'instant soient assez conservatifs en terme de capacité de détection des événements récents de divergence, des préférences d'hôte au sein de ce complexe sont apparentes. Deux espèces de tiques, clairement distinguées par les deux types d'analyses employées, sont présentes en sympatrie dans les îles du Cap-Vert. La première est associée aux populations de fous du genre *Sula* et appartient à l'espèce *O. capensis* sensu stricto, parasitant de nombreux oiseaux marins et largement distribuée dans les océans Atlantique et Indien (Dietrich et al., 2011). Cependant, la distance génétique que nous avons observée entre des tiques de cette espèce, parasitant des fous et des sternes dans l'Océan Indien (Article 3), ainsi

que la description d'*O. cheikhi*, une espèce proche parasitant des sternes en Mauritanie (Vermeil et al., 1997), semblent suggérer qu'il existe potentiellement d'autres divergences associées à l'hôte dans ce système. La seconde espèce de tique détectée mais encore non identifiée, est présente dans les populations de puffins du genre *Calonectris* et ne semble pas faire partie du complexe *O. capensis* sensu lato. Pour l'instant, on ne connaît pas l'origine évolutive de cette deuxième espèce, mais il est clair qu'une attention particulière est nécessaire quant à l'identification des tiques molles échantillonnées dans ce système. En effet, les résultats du blind-test (Article 1), soulignent la nécessité d'un examen minutieux des caractères morphologiques variables pour identifier convenablement les espèces concernées. L'utilisation combinée d'outils de génétique et des descriptions morphologiques adéquates permet d'identifier correctement de nouvelles espèces ou de différencier des espèces morphologiquement très proches comme les tiques molles exploitant les oiseaux de mer. Cependant, une tentative de développement de marqueurs microsatellites s'est révélée infructueuse sur les espèces du complexe *O. capensis*. Pour mieux identifier et discriminer la présence des différentes espèces, nous allons donc entreprendre la recherche de marqueurs de type SNPs (Single-Nucleotide Polymorphism) dans les zones exprimées du génome.

Comme dans le système impliquant *I. uriae*, différents paramètres peuvent exercer des pressions sélectives dans ce système. Par exemple, les différences entre les puffins et les fous qui forment des colonies hétérospécifiques dans lesquelles sont retrouvées deux espèces de tiques au Cap-Vert. La forme de leur bec, respectivement crochus et pointus et les habitats constitués respectivement par des terriers et des nids rocaillieux, peuvent être à l'origine de l'isolement associé à l'hôte observé entre les populations de tiques. Néanmoins, les préférences d'hôtes mises en lumière par nos analyses ne représentant qu'une partie de la biologie des tiques, des échantillonnages supplémentaires, en combinaison avec des analyses morphologiques et génétiques plus poussées, devront être réalisés pour décrire plus précisément la distribution des espèces et leur utilisation d'hôte.

En revanche, la comparaison des deux systèmes tiques-oiseaux de mer nous apporte déjà une indication sur l'impact potentiel de la sélection par la forme et la taille des becs des hôtes-oiseaux. En effet, dans le système *I. uriae*, les becs longs et pointus des guillemots de Troil sélectionnent des tiques dont la forme du corps est plus longue et fine par rapport aux individus parasitant d'autres hôtes (Article 4). Ce résultat est retrouvé dans le système *O. capensis* s.l. dans lequel les becs longs et pointus des fous semblent aussi sélectionner des tiques plus petites et plus fines (Article 3). Cette hypothèse pourrait être testée en se basant sur des photos de becs d'individus capturés en zone naturelle ou disponibles dans les musées, sur lesquelles des analyses morphométriques pourraient être conduites et mises en

parallèle avec les différences morphométriques observées entre les races d'hôtes de tiques (voir Foster et al., 2008 pour les mesures de la morphométrie des becs).

Les patrons observés, en termes de divergence des populations de tiques en fonction de leurs hôtes, vont être largement conditionnés par le flux de gènes à différentes échelles spatiales. Même s'il est admis que la dispersion des parasites est assurée par l'hôte à grande échelle spatiale, ce n'est pas toujours le cas à des échelles plus fines. La dispersion locale des tiques repose sur les caractéristiques de chaque espèce ainsi que de leur niche écologique. Par exemple, Adeyeye et Butler (1989) ont étudié durant 14 mois les mouvements d'*Ornithodoros turicata* à l'intérieur de terriers de tortues gaufrées (*Gopherus polyphemus*), grâce à des marquages fluorescents de différentes couleurs. Cette tique parasite des amphibiens, des reptiles et des mammifères (Adéyeyè and Phillips, 1996) mais n'est pas connue pour se nourrir sur les tortues gaufrées, profitant seulement des conditions favorables de température et d'humidité de leur terrier, dont la profondeur peut atteindre cinq mètres (Young and Goff, 1939). Ils ont observé que la concentration des tiques à différentes profondeurs était variable en fonction de la saison mais n'ont pas constaté de mouvements inter-terriers. D'autres tiques molles telles que *O. savignyi* (Andouin, 1826), connues pour leurs toxines causant des paralysies et des toxicoses (Mans et al., 2002), vivent dans les zones semi-arides et se réfugient dans le sable, sous des arbres ou aux abords des zones d'élevage, à l'abri des fortes chaleurs en attendant l'arrivée d'un hôte potentiel (Basu et al., 2015). De par ses habitudes alimentaires impliquant des repas courts aux stases nymphales et adultes, la dispersion de cette tique est considérée comme limitée. Néanmoins, Mans et Neitz (2004b) précisent que cette espèce est capable de se déplacer sur une distance de plus de vingt mètres pour accéder à un hôte. Cette distance, relativement longue pour une tique, pourrait être facilitée chez cette espèce par la présence d'une paire d'yeux (Sonenshine and Roe, 2014). Nos travaux ont montré une faible structuration locale de la tique molle *O. maritimus* entre les nids de ses hôtes suggérant des mouvements réguliers des tiques au sein de la colonie (Article 5). Nous avons observé que les hôtes n'utilisent pas obligatoirement les mêmes nids d'une année sur l'autre, obligeant les tiques à se déplacer au moins au sein d'un territoire pour trouver un nouvel hôte. De plus, l'île d'étude est peu élevée et soumise aux intempéries durant l'hiver et les nids peuvent être détruits, il est donc nécessaire que les tiques trouvent des refuges adéquats pour survivre. Tout ceci suggère que la dispersion de cette espèce peut s'effectuer par ses propres moyens. De surcroît, trois bactéries symbiotiques, quatre bactéries potentiellement pathogènes, un parasite protozoaire et un virus ont été détectés dans les tiques échantillonnées, mais aucune structuration spatiale n'a été mise en évidence, suggérant de même des mouvements fréquents des tiques au sein de la colonie. Ces résultats pourront être appuyés par une étude de génétique des populations pour déterminer le degré d'apparement des tiques

entre nids. Une méthode de marquage telle que celle utilisée par Adeyeye et Butler pourrait aussi être employée pour déterminer l'amplitude des mouvements des différentes stases d'*O. maritimus* entre les nids. Ces informations seront cruciales dans la compréhension de l'adaptation de cette tique à ses hôtes et de son rôle dans la dissémination d'agents pathogènes. En effet, observer une structuration spatiale des tiques au sein d'une colonie pourrait donc indiquer une stabilité dans l'utilisation d'un territoire qui pourrait conduire à l'adaptation spécifique des tiques à un couple d'oiseaux.

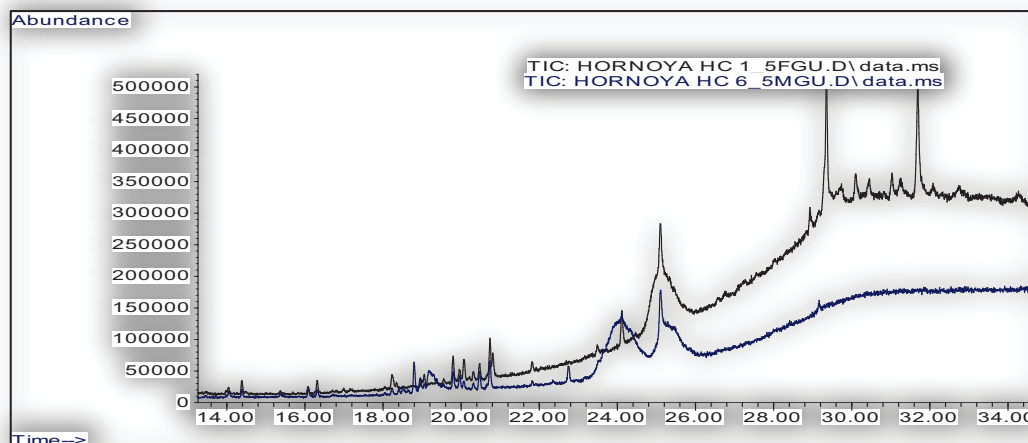
Un autre facteur susceptible de modifier les dynamiques de populations d'arthropodes et d'impacter le rôle des facteurs de sélection et des flux de gènes, est la présence d'endosymbiontes. En effet, en plus de compléter leurs hôtes en vitamines et d'être capable de modifier leur succès reproducteur, il a été montré que les endosymbiontes pouvaient participer à une adaptation rapide de l'hôte arthropode à une nouvelle ressource (Hosokawa et al., 2007). Les insectes infectés peuvent aussi montrer de plus amples performances sous différentes conditions de température (Dunbar et al., 2007; Russell and Moran, 2006). Chez les tiques, l'implication des endosymbiontes est encore peu connue mais, dans le cas des bactéries du genre *Coxiella*, une grande diversité a été détectée dans différentes espèces de tiques prélevées sur le terrain, et notamment sur notre île d'étude d'*Ornithodoros maritimus*, révélant que ce groupe pouvait être à l'origine de l'émergence de nouveaux agents pathogènes, notamment l'agent de la fièvre Q, *C. burnetii* (Duron et al., 2015; voir Annexe). Les endosymbiontes semblent donc un élément important à prendre en compte dans la description de la distribution des tiques, dans leur utilisation des hôtes et dans la transmission d'agents pathogènes.

### Utilisation des analyses phénotypiques pour mettre en lumière la divergence

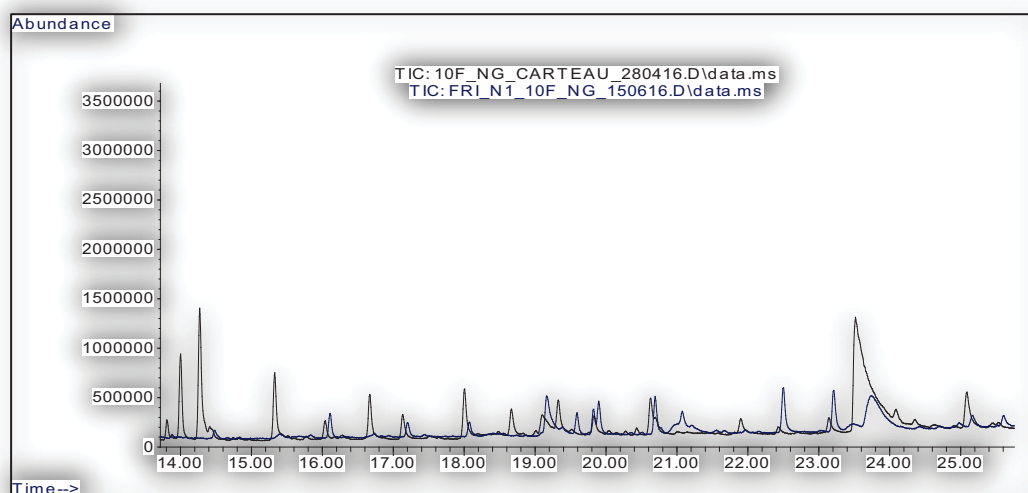
Pour être pertinentes lors d'études d'écologie évolutive, les analyses phénotypiques employées doivent pouvoir quantifier la variabilité intra et inter-espèces. La morphométrie géométrique s'est révélée être un outil de choix pour discriminer les espèces du complexe *O. capensis* et les races d'hôtes d'*I. uriae*. L'utilisation des contours semble être appropriée lorsque l'utilisation des landmarks est compliquée ou impossible comme dans le cas de la cuticule des tiques molles (Articles 2 et 3) et est tout autant discriminante que les landmarks comme dans le cas des races d'hôtes d'*I. uriae* (Article 4). Peu coûteuse et relativement rapide, la morphométrie géométrique peut constituer une méthode de substitution pour détecter rapidement les espèces en présence et pour tester une structuration potentielle entre populations. De plus, cette méthode permet de mesurer l'asymétrie fluctuante (AF), souvent subtile, entre les individus appartenant à différentes populations. L'AF est généralement mesurée comme la déviation d'un trait par rapport à une symétrie parfaite, c'est à dire la variance d'un trait entre les côtés

droit et gauche (Debat and Peronnet, 2013). Il a été proposé que l'AF puisse augmenter lorsqu'un organisme est soumis à un stress environnemental. Ce stress engendrerait des différences de forme entre les individus, pouvant être détectées grâce à des analyses basées sur l'utilisation de landmarks.

D'autres analyses phénotypiques peuvent être utilisées pour investiguer les pressions de sélection à l'origine des différences entre populations d'arthropodes. Par exemple, l'analyse des hydrocarbures cuticulaires (HC), chaînes de carbone et d'hydrogène volatiles qui constituent la couche lipidique de la cuticule chez les arthropodes (Lockey, 1988), repose sur l'extraction des HC dans un solvant, leur séparation par chromatographie en phase liquide à haute performance (HPLC) et leur analyse par chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC-MS)(Gołębiowski et al., 2010). Ces HC forment une véritable signature chimique variant qualitativement entre les espèces et quantitativement au sein d'une espèce. Ils peuvent participer à la rétention d'eau, à l'agrégation, à la communication et/ou à la reproduction avec les individus apparentés (Blomquist and Bagnères, 2010; van Zweden and d'Ettoire, 2010). La signature chimique est sous contrôle génétique mais leur production est liée aux variations environnementales (Estrada-Peña, 1993; Gibbs et al., 1991). Dans les systèmes tiques-oiseaux de mer, la production d'HC peut donc être potentiellement liée à l'utilisation d'hôte et la capacité à survivre dans les conditions de température et d'humidité des différents microhabitats. Afin de tester cette hypothèse, nous avons réalisé des extractions d'HC de tiques dures et de tiques molles, individuellement à partir de larves, nymphes et adultes vivants. Les premiers résultats ont montré des concentrations en HC trop basses avec beaucoup de bruit de fond, empêchant l'identification des composés en présence. Les extractions suivantes ont été réalisées par pool de 5 puis de 10 tiques, mais les concentrations, encore très faibles, particulièrement pour les mâles (Fig. 10a), n'ont pas permis ni d'identifier correctement les composés extraits ni d'effectuer des comparaisons entre populations (Figure 10b).



a.



b.

**Figure 10:** Courbes d'identification des hydrocarbures extraits : **(a)** de pools de 5 tiques femelles (courbe noire) et 5 tiques mâles (courbe bleue) d'*I. uriae* collectées dans une colonie de guillemots de Troil sur Hornøya en Norvège ; **(b)** de pools de 10 femelles non gorgées d'*O. maritimus* collectées dans la colonie de Carreau en Camargue (courbe noire) et sur l'île du Frioul au large de Marseille (courbe bleue).

La méthode employée a pourtant fait ses preuves chez d'autres organismes comme les fourmis ou les araignées (Lenoir et al., 2001; van Zweden et al., 2009) et les HC ont également permis de discriminer des sous-populations d'arthropodes (Bagneres et al., 1991; Jallon and David, 1987; Simmons et al., 2014) y compris des tiques molles et dures (Estrada-Peña et al., 1996; Estrada-Peña and Dusbabek, 1993), montrant même une composition en HC variable selon l'hôte utilisé chez des tiques du genre *Rhipicephalus* telles que *R. bursa* ou *R. sanguineus* (Shimshoni et al., 2013). *I. uriae* et *O. capensis* étant des espèces nidicoles et fréquentant de ce fait des milieux compartimentés dans lesquels les conditions sont relativement stables, en particulier durant les mois d'hiver, il est possible que la production d'HC soit inutile et métaboliquement coûteuse comme le soulignent Sonenshine et Roe (2014b) et serait donc réduite par rapport à des tiques chassant à l'affût. Cette hypothèse est soutenue par les concentrations très basses que nous avons obtenues pour les extractions réalisées sur les tiques mâles, qui ne font pas



de repas de sang et n'ont donc pas à endurer les conditions du milieu extérieur (Fig.10a). De plus, ce mode de vie endophile pourrait inciter ces espèces à utiliser d'autres modes de communication entre apparentés. Par exemple, dans une colonie de manchot Adélie *Pygoscelis adeliae* en Antarctique, il a été montré que toutes les stases d'*I. uriae* utilisent un mélange de guanine et autres composés présents dans leurs excréta comme phéromone d'assemblage marquant les micro-habitats favorables (Benoit et al., 2007). Les phéromones d'assemblage sont typiquement peu volatiles, nécessitant un contact physique pour être perçues et elles persistent durablement dans l'environnement (Sonenshine, 1985). De plus, Eveleigh et Threlfall (1974) soulignent que l'accouplement ne s'initie qu'après un contact physique, sans lequel la femelle n'est pas attirée par le mâle. De telles phéromones (guanine, xanthine et hypoxanthine), présentes dans les excréta et impliquées dans la reproduction, ont également été mises en évidence chez les tiques molles (Dusbábek et al., 1991). D'autre part, *I. uriae*, notamment les stases non gorgées, utilise le guano (mélange d'acide urique et autres composés) comme kairomone<sup>4</sup> pour repérer ses hôtes (Benoit et al., 2007). Sur la base de ces publications, des tests d'attraction entre excréta des différentes races d'hôtes d'*I. uriae* ou des guanos produits par ses hôtes pourraient apporter de plus amples informations sur le rôle de ces produits dans l'isolement reproducteur des tiques associées à des hôtes différents et donc la mise en place de la spécificité d'hôte chez cette espèce. Ce type d'expérience pourrait également être conduit sur les tiques molles, collectées sur différents hôtes.

Difficiles à réaliser en conditions naturelles, des expériences d'attraction et d'assemblage peuvent être plus facilement mises en place en laboratoire mais nécessitent le maintien de populations de tiques en élevage. Une unique étude, impliquant des gorgements sur des poulets, a démontré les limites du maintien en laboratoire d'*I. uriae* (Eveleigh et Threlfall, 1974), dues vraisemblablement à la spécificité de cette tique aux oiseaux marins. Néanmoins, l'élevage des tiques du complexe *O. capensis* s'est révélée plus facile à réaliser sur des jeunes poulets ou des pigeons (Colas-Belcour et Rageau, 1960). Suite à de multiples échantillonnages, nous avons donc entrepris l'élevage de différentes populations de tiques molles appartenant au complexe *O. capensis*. Ces tiques sont maintenues à l'intérieur d'une étuve dont les conditions de température (24°C) et d'humidité (90%) sont stables. Un cycle de 12h de jour et 12h de nuit est également programmé pour mimer les conditions naturelles. Le gorgement des tiques s'effectue grâce à l'utilisation de membranes artificielles comme décrit dans Boulanger et al. (2015). Les tiques sont disposées sur la membrane sous laquelle est chauffé du sang de vache à la température du sang des oiseaux, approximativement 41°C. Le gorgement s'effectue dans le noir durant une nuit. Les femelles gorgées sont ensuite isolées individuellement et pondent leurs œufs en une dizaine de jours.

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<sup>4</sup> Kairomone : composé chimique émis par un hôte attirant les tiques.

Ce type d'élevage nous permettra à terme de tester le degré de spécificité d'hôte et de déterminer les facteurs sous-jacents dans ce système.

### **La base génétique des variations phénotypiques**

Mettre en avant des variations phénotypiques ne suffit pas pour comprendre l'évolution de la divergence. Un volet important dans la détermination des facteurs impliqués dans la diversification des tiques sera aussi de déterminer les bases génétiques des différences phénotypiques observées. En effet, l'évolution d'un trait est le produit de l'intensité de la sélection sur ce trait et de son héritabilité (Falconer, 1960). Un trait donné doit donc être suffisamment sélectionné et transmis à la descendance pour être maintenu. D'autres facteurs génétiques auront également un impact dans l'évolution des populations tels que le nombre de gènes impliqués influençant un trait et leur interaction avec d'autres traits (pléiotropie, épistasie), le type d'accouplement (assortatif ou aléatoire), la dispersion entre populations ou encore le temps de génération entre les espèces (Clayton et al., 2015). Ainsi, pour confirmer les patrons de variations morphologiques observées chez *I. uriae* dans l'Atlantique Nord (Article 4), les analyses morphométriques doivent être couplées à des analyses de génétique des populations pour déterminer si les variations observées ont une base génétique. Des scans de génome entier avec des méthodes de type RADseq (Wang et al., 2012) pourraient fournir des informations intéressantes pour trouver ces zones de sélection.

En ce qui concerne le complexe *O. capensis* sensu lato, les études génétiques ne reposent pour l'instant que sur l'utilisation de marqueurs génétiques conservés (Article 3) car le développement de marqueurs plus variables, de types microsatellites, s'est révélé impossible dû à la complexité du génome des tiques (large génome contenant de nombreuses séquences répétées) (Nene, 2009). D'autres méthodes, comme le RNAseq reposant sur le séquençage du transcriptome (Wang et al., 2009), seront donc utilisées pour identifier de nouveaux marqueurs neutres et potentiellement sous sélection, afin de discriminer les différentes espèces de tiques de ce complexe et d'identifier les traits liés au processus de spéciation.

### **Devenir des divergences associées à l'hôte**

L'évolution de la spécialisation d'*I. uriae* à ses hôtes au cours du temps pourra être investiguée sur la base d'individus récoltés à plusieurs années d'intervalle provenant de différentes zones de sa répartition. Ces analyses pourront soutenir l'idée évoquée que les races d'hôtes sont stables au cours du

temps et qu'elles n'évoluent pas vers la spéciation complète (Dietrich et al., 2014a). Les analyses morphométriques réalisées dans différentes zones de sa répartition vont dans ce sens et suggèrent que les populations évoluent différemment selon la zone considérée (Article 4). En revanche, dans le complexe *O. capensis* s.l., les divergences ont pu se produire plus rapidement et donner lieu à la diversité des espèces que l'on observe actuellement. Pour comprendre plus précisément ces patrons, il convient donc de prendre en compte les facteurs de dispersion qui impactent les dynamiques d'extinction et/ou de recolonisation et de déterminer si la formation d'une nouvelle espèce ou d'une race d'hôtes est localisée ou si elle résulte de la dispersion d'une autre colonie.

### Conclusions générales

De nombreux facteurs sont donc potentiellement impliqués dans le phénomène de spécialisation des tiques et peuvent impacter leur divergence au niveau local. Les pressions d'hôtes (immunité, comportement, micro-habitat), les caractéristiques biologiques des tiques (mode de vie, durée du repas, dispersion), mais aussi le macro-environnement constituent des paramètres déterminant leur capacité d'adaptation et leur distribution. L'ensemble de ces facteurs et l'utilisation d'analyses phénotypiques et génétiques peuvent donc nous aider à comprendre le phénomène de spécialisation d'hôtes dans ces systèmes et à définir les risques épidémiologiques locaux qui leur sont associés. Nos résultats indiquent des convergences morphologiques entre les populations de tiques qui semblent résulter des pressions de sélection exercées par le comportement des hôtes. Ces facteurs ainsi que les caractéristiques propres à chaque espèce telles que le mode de vie ou les capacités de dispersion ainsi que l'utilisation des micro-habitats entrent également en jeu dans la structuration des populations de tiques et peuvent impacter la dissémination des agents pathogènes qu'elles transmettent. Comme souligné ci-dessus, il reste beaucoup de perspectives scientifiques associées à ce travail de thèse. Dans un premier temps, il semble essentiel d'investiguer plus en détail les pressions de sélection qui s'exercent dans les différentes populations de tiques et de définir les bases génétiques des variations phénotypiques observées pour mieux comprendre les phénomènes de spécialisation d'hôtes dans ces deux systèmes modèles tiques-oiseaux de mer.



## Références bibliographiques

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- A. Lenoir, P. D’Ettorre, Errard, and C., Hefetz, A., 2001. Chemical Ecology and Social Parasitism in Ants. *Annu. Rev. Entomol.* 46, 573–599. doi:10.1146/annurev.ento.46.1.573
- Adams, D.C., Rohlf, F.J., Slice, D.E., 2004. Geometric morphometrics: Ten years of progress following the “revolution.” *Ital. J. Zool.* 71, 5–16. doi:10.1080/11250000409356545
- Adeyeye, O.A., Butler, J.F., 1989. Population structure and seasonal intra-burrow movement of *Ornithodoros turicata* (Acari: Argasidae) in gopher tortoise burrows. *J. Med. Entomol.* 26, 279–283.
- Adéyeyè, O., Phillips, J.S., 1996. Photoperiodic response in the soft tick, *Ornithodoros turicata*. *Int. J. Parasitol.* 26, 629–635. doi:10.1016/0020-7519(96)87214-6
- Arthur, D.R., 1962. *Ticks and Disease*.
- Bagneres, A.-G., Killian, A., Clement, J.-L., Lange, C., 1991. Interspecific recognition among termites of the genus *Reticulitermes*: Evidence for a role for the cuticular hydrocarbons. *J. Chem. Ecol.* 17, 2397–2420. doi:10.1007/BF00994590
- Barluenga, M., Stölting, K.N., Salzburger, W., Muschick, M., Meyer, A., 2006. Sympatric speciation in Nicaraguan crater lake cichlid fish. *Nature* 439, 719–723. doi:10.1038/nature04325
- Barrett, R., Erikstad, K.E., 2011. Key-site monitoring on Hornøya in 2010, SEAPOP Short report, 3.
- Barton, T.R., Harris, M.P., Wanless, S., Elston, D.A., 1995. The activity periods and life-cycle of the tick *Ixodes uriae* (Acari: Ixodidae) in relation to host breeding strategies. *Parasitology* 112, 571–580. doi:10.1017/S0031182000066154
- Basu, A.K., Shinggu, P.A., Ahmed, M.I., 2015. Studies on diurnal abundance of the soft tick *Ornithodoros savignyi* in semi-arid Nigeria. *West Indian Vet. J.* 11.
- Benoit, J.B., Yoder, J.A., Lopez-Martinez, G., Elnitsky, M.A., Lee, R.E., Denlinger, D.L., 2007. Habitat requirements of the seabird tick, *Ixodes uriae* (Acari: Ixodidae), from the Antarctic Peninsula in relation to water balance characteristics of eggs, nonfed and engorged stages. *J. Comp. Physiol. B* 177, 205–215. doi:10.1007/s00360-006-0122-7
- Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K., Das, I., 2007. Cryptic species as a window on diversity and conservation. *Trends Ecol. Evol.* 22, 148–155. doi:10.1016/j.tree.2006.11.004
- Bird, C.E., Fernandez-Silva, I., Skillings, D.J., Toonen, R.J., 2012. Sympatric Speciation in the Post “Modern Synthesis” Era of Evolutionary Biology. *Evol. Biol.* 39, 158–180. doi:10.1007/s11692-012-9183-6
- Blackledge, T.A., Gillespie, R.G., 2004. Convergent evolution of behavior in an adaptive radiation of Hawaiian web-building spiders. *Proc. Natl. Acad. Sci. U. S. A.* 101, 16228–16233. doi:10.1073/pnas.0407395101
- Blomquist, G.J., Bagnères, A.-G., 2010. *Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology*. Cambridge University Press.
- Bookstein, F.L., 1997. *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge University Press.



- Boulanger, N., Rais, O., Vial, L., 2015. Annexe 3 Elevage de tiques dures et de tiques molles, in: Tiques et Maladies À Tiques. McCoy K. D., Boulanger N., pp. 295–300.
- Boulinier, T., Danchin, E., 1996. Population trends in Kittiwake *Rissa tridactyla* colonies in relation to tick infestation. *Ibis* 138, 326–334. doi:10.1111/j.1474-919X.1996.tb04345.x
- Boulinier, T., Kada, S., Ponchon, A., Dupraz, M., Dietrich, M., Gamble, A., Bourret, V., Duriez, O., Bazire, R., Tornos, J., Tveraa, T., Chambert, T., Garnier, R., McCoy, K.D., 2016. Migration, Prospecting, Dispersal? What Host Movement Matters for Infectious Agent Circulation? *Integr. Comp. Biol.* icw015. doi:10.1093/icb/icw015
- Boulinier, T., McCoy, K.D., Yoccoz, N.G., Gasparini, J., Tveraa, T., 2008. Public information affects breeding dispersal in a colonial bird: kittiwakes cue on neighbours. *Biol. Lett.* 4, 538–540. doi:10.1098/rsbl.2008.0291
- Brossard, M., Wikel, S.K., 2004. Tick immunobiology. *Parasitology* 129, S161–S176. doi:10.1017/S0031182004004834
- Bush, G.L., Butlin, R.K., 2004. Sympatric Speciation in Insects, in: Dieckmann, U., Doebeli, M., Metz, J.A.J., Tautz, D. (Eds.), *Adaptive Speciation*. Cambridge University Press, Cambridge, pp. 229–248.
- Camicas, J.-L., 1980. Les arbovirus à tiques en zone tropicale. *Médecine Trop.* 40, 499–508.
- Camicas, J.-L., Morel, P.C., 1977. Position systématique et classification des tiques (Acarida : Ixodida). *Acarologia* 18, 410–420.
- Chamberlin, R.V., 1920. *South American Arachnida, chiefly from the guano islands of Peru.*, Brooklyn, N.Y.,.
- Chambert, T., Staszewski, V., Lobato, E., Choquet, R., Carrie, C., McCoy, K.D., Tveraa, T., Boulinier, T., 2012. Exposure of black-legged kittiwakes to Lyme disease spirochetes: dynamics of the immune status of adult hosts and effects on their survival. *J. Anim. Ecol.* 81, 986–995. doi:10.1111/j.1365-2656.2012.01979.x
- Chastel, C., Main, A.J., Guiguen, C., Lay, G. le, Quillien, M.C., Monnat, J.Y., Beaucournu, J.C., 1985. The isolation of Meaban virus, a new Flavivirus from the seabird tick *Ornithodoros (Alectorobius) maritimus* in France. *Arch. Virol.* 83, 129–140. doi:10.1007/BF01309911
- Clayton, D.H., Bush, S.E., Johnson, K.P., 2015. *Coevolution of Life on Hosts: Integrating Ecology and History*. University of Chicago Press.
- Clayton, D.H., Cotgreave, P., 1994. Relationship of bill morphology to grooming behaviour in birds. *Anim. Behav.* 47, 195–201. doi:10.1006/anbe.1994.1022
- Clayton, D.H., Koop, J.A., Harbison, C.W., Moyer, B.R., Bush, S.E., 2010. How birds combat ectoparasites. *Open Ornithol J* 3, 41–71.
- Clayton, D.H., Lee, P.L.M., Tompkins, III, E.D.B., 1999. Reciprocal Natural Selection on Host-Parasite Phenotypes. *Am. Nat.* 154, 261–270. doi:10.1086/303237

- Clayton, D.H., Moyer, B.R., Bush, S.E., Jones, T.G., Gardiner, D.W., Rhodes, B.B., Goller, F., 2005. Adaptive significance of avian beak morphology for ectoparasite control. *Proc. R. Soc. Lond. B Biol. Sci.* 272, 811–817. doi:10.1098/rspb.2004.3036
- Cleaveland, S., Laurenson, M.K., Taylor, L.H., 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. *Philos. Trans. R. Soc. B Biol. Sci.* 356, 991–999. doi:10.1098/rstb.2001.0889
- Colas-Belcour, J., Rageau, J., 1960. Biologie et élevage d'*Ornithodoros capensis* Neumann (acarien Argasidae). *Bull. Société Pathol. Exot.* 53, 723–727.
- Collins, F.H., Paskewitz, S.M., 1996. A review of the use of ribosomal DNA (rDNA) to differentiate among cryptic *Anopheles* species. *Insect Mol. Biol.* 5, 1–9. doi:10.1111/j.1365-2583.1996.tb00034.x
- Colosimo, P.F., Hosemann, K.E., Balabhadra, S., Villarreal, G., Dickson, M., Grimwood, J., Schmutz, J., Myers, R.M., Schluter, D., Kingsley, D.M., 2005. Widespread Parallel Evolution in Sticklebacks by Repeated Fixation of Ectodysplasin Alleles. *Science* 307, 1928–1933. doi:10.1126/science.1107239
- Converse, J.D., Hoogstraal, H., Moussa, M.I., Feare, C.J., Kaiser, M.N., 1975. Soldado virus (Hughes group) from *Ornithodoros (Alectorobius) capensis* (Ixodoidea: Argasidae) infesting Sooty Tern colonies in the Seychelles, Indian Ocean. *Am. J. Trop. Med. Hyg.* 24, 1010–1018.
- Coulson, JohnC, 2001. Colonial Breeding in Seabirds, in: *Biology of Marine Birds, Marine Biology*. CRC Press, pp. 87–113.
- Cumming, G.S., 1999. Host distributions do not limit the species ranges of most African ticks (Acari: Ixodida). *Bull. Entomol. Res.* 89, 303–327. doi:10.1017/S0007485399000450
- Dambroski, H.R., Linn, C., Berlocher, S.H., Forbes, A.A., Roelofs, W., Feder, J.L., 2005. The Genetic Basis for Fruit Odor Discrimination in *Rhagoletis* Flies and Its Significance for Sympatric Host Shifts. *Evolution* 59, 1953–1964. doi:10.1111/j.0014-3820.2005.tb01065.x
- Danchin, E., 1992. The incidence of the tick parasite *Ixodes uriae* in Kittiwake *Rissa tridactyla* colonies in relation to the age of the colony, and a mechanism of infecting new colonies. *Ibis* 134, 134–141. doi:10.1111/j.1474-919X.1992.tb08390.x
- De Meeûs, T., Michalakis, Y., Renaud, F., 1998. Santa Rosalia Revisited: or Why Are There So Many Kinds of Parasites in 'The Garden of Earthly Delights'? *Parasitol. Today* 14, 10–13. doi:10.1016/S0169-4758(97)01163-0
- de Meeûs, T., Renaud, F., 2002. Parasites within the new phylogeny of eukaryotes. *Trends Parasitol.* 18, 247–251. doi:10.1016/S1471-4922(02)02269-9
- Debat, V., Peronnet, F., 2013. Asymmetric flies: The control of developmental noise in *Drosophila*. *Fly (Austin)* 7, 70–77. doi:10.4161/fly.23558
- Dietrich, M., Beati, L., Elguero, E., Boulinier, T., McCoy, K.D., 2013. Body size and shape evolution in host races of the tick *Ixodes uriae*. *Biol. J. Linn. Soc.* 108, 323–334. doi:10.1111/j.1095-8312.2012.02021.x

- Dietrich, M., Gómez-Díaz, E., McCoy, K.D., 2011. Worldwide Distribution and Diversity of Seabird Ticks: Implications for the Ecology and Epidemiology of Tick-Borne Pathogens. *Vector-Borne Zoonotic Dis.* 11, 453–470. doi:10.1089/vbz.2010.0009
- Dietrich, M., Kempf, F., Boulinier, T., McCoy, K.D., 2014a. Tracing the colonization and diversification of the worldwide seabird ectoparasite *Ixodes uriae*. *Mol. Ecol.* 23, 3292–3305. doi:10.1111/mec.12815
- Dietrich, M., Kempf, F., Gómez-Díaz, E., Kitaysky, A.S., Hipfner, J.M., Boulinier, T., McCoy, K.D., 2012. Inter-oceanic variation in patterns of host-associated divergence in a seabird ectoparasite. *J. Biogeogr.* 39, 545–555. doi:10.1111/j.1365-2699.2011.02620.x
- Dietrich, M., Lobato, E., Boulinier, T., McCoy, K.D., 2014b. An experimental test of host specialization in a ubiquitous polar ectoparasite: a role for adaptation? *J. Anim. Ecol.* 83, 576–587. doi:10.1111/1365-2656.12170
- Dif, G., Hainard, R., Lamouche, T., Dorst, J., 1982. *Les oiseaux de mer d'Europe*. Arthaud, Paris.
- Drès, M., Mallet, J., 2002. Host races in plant-feeding insects and their importance in sympatric speciation. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 357, 471–492. doi:10.1098/rstb.2002.1059
- Duffy, D.C., 1983. The Ecology of Tick Parasitism on Densely Nesting Peruvian Seabirds. *Ecology* 64, 110. doi:10.2307/1937334
- Dujardin, J.-P., 2011. Modern Morphometrics of Medically Important Insects, in: *Genetics and Evolution of Infectious Disease*. Elsevier, pp. 473–501.
- Dujardin, J.-P., 2008. Morphometrics applied to medical entomology. *Infect. Genet. Evol.* 8, 875–890. doi:10.1016/j.meegid.2008.07.011
- Dujardin, J.P., Bermudez, H., Casini, C., Schofield, C.J., Tibayrenc, M., 1997. Metric Differences Between Silvatic and Domestic *Triatoma infestans* (Heteroptera: Reduviidae) in Bolivia. *J. Med. Entomol.* 34, 544–551. doi:10.1093/jmedent/34.5.544
- Dunbar, H.E., Wilson, A.C.C., Ferguson, N.R., Moran, N.A., 2007. Aphid Thermal Tolerance Is Governed by a Point Mutation in Bacterial Symbionts. *PLOS Biol* 5, e96. doi:10.1371/journal.pbio.0050096
- Duron, O., Noël, V., McCoy, K.D., Bonazzi, M., Sidi-Boumedine, K., Morel, O., Vavre, F., Zenner, L., Jourdain, E., Durand, P., Arnathau, C., Renaud, F., Trape, J.-F., Biguezoton, A.S., Cremaschi, J., Dietrich, M., Léger, E., Appelgren, A., Dupraz, M., Gómez-Díaz, E., Diatta, G., Dayo, G.-K., Adakal, H., Zoungrana, S., Vial, L., Chevillon, C., 2015. The Recent Evolution of a Maternally-Inherited Endosymbiont of Ticks Led to the Emergence of the Q Fever Pathogen, *Coxiella burnetii*. *PLoS Pathog* 11, e1004892. doi:10.1371/journal.ppat.1004892
- Dusbábek, F., Šimek, P., Jegorov, A., Tříska, J., 1991. Identification of xanthine and hypoxanthine as components of assembly pheromone in excreta of argasid ticks. *Exp. Appl. Acarol.* 11, 307–316. doi:10.1007/BF01202877
- Estrada-Peña, A., 2001. Forecasting habitat suitability for ticks and prevention of tick-borne diseases. *Vet. Parasitol., Promotion Advancement, Preserving Tradition* 98, 111–132. doi:10.1016/S0304-4017(01)00426-5

- Estrada-Peña, A., 1993. Climate and cuticular hydrocarbon variation in *Rhipicephalus sanguineus* ticks (Acari: Ixodidae). *Parasitol. Res.* 79, 512–516. doi:10.1007/BF00931594
- Estrada-Peña, A., Dusbabek, F., 1993. Cuticular hydrocarbon gas chromatography analysis of *Argas vulgaris*, *A. polonicus*, and their hybrids. *Exp. Appl. Acarol.* 17, 365–376. doi:10.1007/BF00058599
- Estrada-Peña, A., Gray, J.S., Kahl, O., 1996. Variability in cuticular hydrocarbons and phenotypic discrimination of *Ixodes ricinus* populations (Acarina: Ixodidae) from Europe. *Exp. Appl. Acarol.* 20, 457–466. doi:10.1007/BF00053309
- Estrada-Pena, A., Mangold, A.J., Nava, S., Venzal, J.M., Labruna, M., Guglielmone, A.A., 2010. A review of the systematics of the tick family Argasidae (Ixodida). *Acarologia* 50, 317–333. doi:10.1051/acarologia/20101975
- Eveleigh, E.S., Threlfall, W., 1974. The biology of *Ixodes (Ceraticodes) uriae* White, 1852 in Newfoundland. *Acarologia* 16, 621–635.
- Falconer, D.S., 1960. Introduction to quantitative genetics. New York,: Ronald Press Co.
- Feare, C.J., 1976. Desertion and Abnormal Development in a Colony of Sooty Terns *Sterna Fuscata* Infested by Virus-Infected Ticks. *Ibis* 118, 112–115. doi:10.1111/j.1474-919X.1976.tb02015.x
- Foster, D.J., Podos, J., Hendry, A.P., 2008. A geometric morphometric appraisal of beak shape in Darwin's finches. *J. Evol. Biol.* 21, 263–275. doi:10.1111/j.1420-9101.2007.01449.x
- Futuyma, D.J., Moreno, G., 1988. The Evolution of Ecological Specialization. *Annu. Rev. Ecol. Syst.* 19, 207–233.
- Gandon, S., 2002. Local adaptation and the geometry of host–parasite coevolution. *Ecol. Lett.* 5, 246–256. doi:10.1046/j.1461-0248.2002.00305.x
- Garros, C., Dujardin, J.-P., 2013. Genetic and Phenetic Approaches to Anopheles Systematics, in: Manguin, S. (Ed.), *Anopheles Mosquitoes - New Insights into Malaria Vectors*. InTech.
- Gasparini, J., McCoy, K.D., Haussy, C., Tveraa, T., Boulinier, T., 2001. Induced maternal response to the Lyme disease spirochaete *Borrelia burgdorferi* sensu lato in a colonial seabird, the kittiwake *Rissa tridactyla*. *Proc. R. Soc. Lond. B Biol. Sci.* 268, 647–650. doi:10.1098/rspb.2000.1411
- Gibbs, A., Mousseau, T.A., Crowe, J.H., 1991. Genetic and acclimatory variation in biophysical properties of insect cuticle lipids. *Proc. Natl. Acad. Sci.* 88, 7257–7260. doi:10.1073/pnas.88.16.7257
- Gołębiowski, M., Boguś, M.I., Paszkiewicz, M., Stepnowski, P., 2010. Cuticular lipids of insects as potential biofungicides: methods of lipid composition analysis. *Anal. Bioanal. Chem.* 399, 3177–3191. doi:10.1007/s00216-010-4439-4
- Gómez-Díaz, E., Doherty Jr, P.F., Duneau, D., McCoy, K.D., 2010. Cryptic vector divergence masks vector-specific patterns of infection: an example from the marine cycle of Lyme borreliosis: Divergence and vector-specific infection. *Evol. Appl.* 3, 391–401. doi:10.1111/j.1752-4571.2010.00127.x

- Gómez-Díaz, E., Morris-Pocock, J.A., González-Solís, J., McCoy, K.D., 2012. Trans-oceanic host dispersal explains high seabird tick diversity on Cape Verde islands. *Biol. Lett.* 8, 616–619. doi:10.1098/rsbl.2012.0179
- Grant, P.R., Grant, B.R., 2008. Pedigrees, assortative mating and speciation in Darwin's finches. *Proc. R. Soc. Lond. B Biol. Sci.* 275, 661–668. doi:10.1098/rspb.2007.0898
- Grémillet, D., Chauvin, C., Wilson, R.P., Le Maho, Y., Wanless, S., 2005. Unusual feather structure allows partial plumage wettability in diving great cormorants *Phalacrocorax carbo*. *J. Avian Biol.* 36, 57–63. doi:10.1111/j.0908-8857.2005.03331.x
- Guglielmone, A.A., Robbins, R.G., Apanaskevich, D.A., Petney, T.N., Estrada-Pena, A., Horak, I.G., Shao, R., Barker, S.C., 2010. The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida) of the world : a list of valid species names.
- Hoberg, E.P., Brooks, D.R., 2015. Evolution in action: climate change, biodiversity dynamics and emerging infectious disease. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 370, 20130553. doi:10.1098/rstb.2013.0553
- Hochberg, M.E., Baalen, M. van, 1998. Antagonistic Coevolution over Productivity Gradients. *Am. Nat.* 152, 620–634. doi:10.1086/286194
- Hoogstraal, H., 1985. Argasid and Nuttalliellid Ticks as Parasites and Vectors, in: J.R. Baker and R. Muller (Ed.), *Advances in Parasitology*. Academic Press, pp. 135–238.
- Hoogstraal, H., 1969. *Ornithodoros (Alectorobius) muesebecki* n. sp., a parasite of the blue-faced booby (*Sula dactylatra melanops*) on Hasikiya Island, Arabian Sea. *Proc. Entomol. Soc. Wash.* 71, 368–374.
- Hoogstraal, H., Wassef, H.Y., Hays, C., Keirans, J.E., 1985. *Ornithodoros (Alectorobius) spheniscus* n. sp. [Acarina: Ixodoidea: Argasidae: *Ornithodoros (Alectorobius) capensis* group], a tick parasite of the Humboldt penguin in Peru. *J. Parasitol.* 71, 635–644.
- Horak, I.G., Camicas, J.-L., Keirans, J.E., 2002. The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida): A World List of Valid Tick Names. *Exp. Appl. Acarol.* 28, 27–54. doi:10.1023/A:1025381712339
- Hosokawa, T., Kikuchi, Y., Shimada, M., Fukatsu, T., 2007. Obligate symbiont involved in pest status of host insect. *Proc. R. Soc. Lond. B Biol. Sci.* 274, 1979–1984. doi:10.1098/rspb.2007.0620
- Jaenike, J., 1981. Criteria for Ascertaining the Existence of Host Races. *Am. Nat.* 117, 830–834.
- Jallon, J.-M., David, J.R., 1987. Variation in Cuticular Hydrocarbons Among the Eight Species of the *Drosophila melanogaster* Subgroup. *Evolution* 41, 294–302. doi:10.2307/2409139
- Johnson, K.P., Shreve, S.M., Smith, V.S., 2012. Repeated adaptive divergence of microhabitat specialization in avian feather lice. *BMC Biol.* 10, 52. doi:10.1186/1741-7007-10-52
- Jongejan, F., Uilenberg, G., 2004. The global importance of ticks. *Parasitology* 129, S3–S14. doi:10.1017/S0031182004005967
- Kaba, D., Ravel, S., Acapovi-Yao, G., Solano, P., Allou, K., Bosson-Vanga, H., Gardes, L., N'Goran, E.K., Schofield, C.J., Kone, M., Dujardin, J.-P., 2012. Phenetic and genetic structure of tsetse fly



- populations (*Glossina palpalis palpalis*) in southern Ivory Coast. *Parasit. Vectors* 5, 153. doi:10.1186/1756-3305-5-153
- Keirans, J.E., Clifford, C.M., Hoogstraal, H., 1984. *ORNITHODOROS (ALECTOROBIUS) YUNKERI*, NEW SPECIES (ACARI: IXODOIDEA: ARGASIDAE), FROM SEABIRDS AND NESTING SITES IN THE GALAPAGOS ISLANDS. *J. Med. Entomol.* 21, 344–350.
- Kempf, F., Boulinier, T., De Meeûs, T., Arnathau, C., McCOY, K.D., 2009. Recent evolution of host-associated divergence in the seabird tick *Ixodes uriae*. *Mol. Ecol.* 18, 4450–4462. doi:10.1111/j.1365-294X.2009.04356.x
- Kitaoka, S., Suzuki, H., 1973. *Ornithodoros (Alectorobius) sawaii* sp. n. (Ixodoidea, Argasidae) associated with the streaked shear-water, *Colonectris leucomelas*, from the Amami-oshima Islands, Japan. *Natl. Inst. Anim. Health Q. (Tokyo)* 13, 142–148.
- Klompen, J.S.H., Black, W.C., Keirans, J.E., J. H. Oliver, J., 1996. Evolution of Ticks. *Annu. Rev. Entomol.* 41, 141–161. doi:10.1146/annurev.en.41.010196.001041
- Klompen, J.S.H., Oliver, J.H., 1993. Systematic relationships in the soft ticks (Acari: Ixodida: Argasidae). *Syst. Entomol.* 18, 313–331. doi:10.1111/j.1365-3113.1993.tb00669.x
- Kocher, T.D., Conroy, J.A., McKaye, K.R., Stauffer, J.R., 1993. Similar Morphologies of Cichlid Fish in Lakes Tanganyika and Malawi Are Due to Convergence. *Mol. Phylogenet. Evol.* 2, 158–165. doi:10.1006/mpev.1993.1016
- Kohls, G.M., Sonenshine, D.E., Clifford, C.M., 1965. The systematics of the subfamily Ornithodorinae (Acarina: Argasidae). II. Identification of the larvae of the Western Hemisphere and descriptions of three new species. *Ann. Entomol. Soc. Am.* 58, 331–364.
- Lawing, A.M., Polly, P.D., 2010. Geometric morphometrics: recent applications to the study of evolution and development. *J. Zool.* 280, 1–7. doi:10.1111/j.1469-7998.2009.00620.x
- Lecointre, G., Guyader, H.L., 2001. *Classification phylogénétique du vivant*. Belin.
- Ledevin, R., Michaux, J.R., Deffontaine, V., Henttonen, H., Renaud, S., 2010. Evolutionary history of the bank vole *Myodes glareolus*: a morphometric perspective. *Biol. J. Linn. Soc.* 100, 681–694.
- Lehmann, T., 1993. Ectoparasites: Direct impact on host fitness. *Parasitol. Today* 9, 8–13. doi:10.1016/0169-4758(93)90153-7
- Levene, H., 1953. Genetic Equilibrium When More Than One Ecological Niche is Available. *Am. Nat.* 87, 331–333.
- Levins, R., MacArthur, R., 1966. The Maintenance of Genetic Polymorphism in a Spatially Heterogeneous Environment: Variations on a Theme by Howard Levene. *Am. Nat.* 100, 585–589.
- Lockey, K.H., 1988. Lipids of the insect cuticle: origin, composition and function. *Comp. Biochem. Physiol. Part B Comp. Biochem.* 89, 595–645.
- Losos, J.B., 1990. Concordant evolution of locomotor behaviour, display rate and morphology in *Anolis* lizards. *Anim. Behav.* 39, 879–890. doi:10.1016/S0003-3472(05)80952-2

- Magalhães, S., Forbes, M.R., Skoracka, A., Osakabe, M., Chevillon, C., McCoy, K.D., 2007. Host race formation in the Acari. *Exp. Appl. Acarol.* 42, 225–238. doi:10.1007/s10493-007-9091-0
- Mangin, S., Gauthier-Clerc, M., Frenot, Y., Gendner, J.-P., Le Maho, Y., 2003. Ticks *Ixodes uriae* and the breeding performance of a colonial seabird, king penguin *Aptenodytes patagonicus*. *J. Avian Biol.* 34, 30–34. doi:10.1034/j.1600-048X.2003.02916.x
- Mans, B.J., Neitz, A.W.H., 2004. The sand tampan, *Ornithodoros savignyi*, as a model for tick-host interactions. *South Afr. J. Sci.* 100, 283–288.
- Mans, B.J., Steinmann, C.M.L., Venter, J.D., Louw, A.I., Neitz, A.W.H., 2002. Pathogenic mechanisms of sand tampan toxicoses induced by the tick, *Ornithodoros savignyi*. *Toxicon* 40, 1007–1016. doi:10.1016/S0041-0101(02)00098-3
- McCoy, K., Beis, P., Barbosa, A., Cuervo, J., Fraser, W., González-Solís, J., Jourdain, E., Poisbleau, M., Quillfeldt, P., Léger, E., Dietrich, M., 2012. Population genetic structure and colonisation of the western Antarctic Peninsula by the seabird tick *Ixodes uriae*. *Mar. Ecol. Prog. Ser.* 459, 109–120. doi:10.3354/meps09749
- McCoy, K.D., Boulinier, T., Chardine, J.W., Danchin, E., Michalakis, Y., 1999. Dispersal and Distribution of the Tick *Ixodes uriae* within and among Seabird Host Populations: The Need for a Population Genetic Approach. *J. Parasitol.* 85, 196. doi:10.2307/3285619
- McCoy, K.D., Boulinier, T., Schjørring, S., Michalakis, Y., 2002. Local adaptation of the ectoparasite *Ixodes uriae* to its seabird host. *Evol. Ecol. Res.* 4, 441–456.
- McCoy, K.D., Boulinier, T., Tirard, C., Michalakis, Y., 2003. Host-Dependent Genetic Structure of Parasite Populations: Differential Dispersal of Seabird Tick Host Races. *Evolution* 57, 288–296.
- McCoy, K.D., Boulinier, T., Tirard, C., Michalakis, Y., 2001. Host specificity of a generalist parasite: genetic evidence of sympatric host races in the seabird tick *Ixodes uriae*. *J. Evol. Biol.* 14, 395–405. doi:10.1046/j.1420-9101.2001.00290.x
- McCoy, K.D., Chapuis, E., Tirard, C., Boulinier, T., Michalakis, Y., Bohec, C.L., Maho, Y.L., Gauthier-Clerc, M., 2005. Recurrent evolution of host-specialized races in a globally distributed parasite. *Proc. R. Soc. B Biol. Sci.* 272, 2389–2395. doi:10.1098/rspb.2005.3230
- McCoy, K.D., Dietrich, M., Jaeger, A., Wilkinson, D.A., Bastien, M., Lagadec, E., Boulinier, T., Pascalis, H., Tortosa, P., Le Corre, M., Dellagi, K., Lebarbenchon, C., 2016. The role of seabirds of the Iles Éparses as reservoirs and disseminators of parasites and pathogens. *Acta Oecologica, Îles Éparses (French Scattered Islands, SW Indian Ocean) as reference ecosystems for environmental research.* 72, 98–109. doi:10.1016/j.actao.2015.12.013
- McCoy, K.D., Leger, E., Dietrich, M., 2013. Host specialization in ticks and transmission of tick-borne diseases: a review. *Front. Cell. Infect. Microbiol.* 3. doi:10.3389/fcimb.2013.00057
- Meyers, L.A., Bull, J.J., 2002. Fighting change with change: adaptive variation in an uncertain world. *Trends Ecol. Evol.* 17, 551–557. doi:10.1016/S0169-5347(02)02633-2
- Michaux, J., Chevret, P., Renaud, S., 2007. Morphological diversity of Old World rats and mice (Rodentia, Muridae) mandible in relation with phylogeny and adaptation. *J. Zool. Syst. Evol. Res.* 45, 263–279. doi:10.1111/j.1439-0469.2006.00390.x



- Mopper, S., Strauss, S.Y., 2013. Genetic Structure and Local Adaptation in Natural Insect Populations: Effects of Ecology, Life History, and Behavior. Springer Science & Business Media.
- Muschick, M., Indermaur, A., Salzburger, W., 2012. Convergent Evolution within an Adaptive Radiation of Cichlid Fishes. *Curr. Biol.* 22, 2362–2368. doi:10.1016/j.cub.2012.10.048
- Nava, S., Guglielmono, A.A., 2013. A meta-analysis of host specificity in Neotropical hard ticks (Acari: Ixodidae). *Bull. Entomol. Res.* 103, 216–224. doi:10.1017/S0007485312000557
- Navia, D., Mendonça, R.S., Ferragut, F., Miranda, L.C., Trincado, R.C., Michaux, J., Navajas, M., 2013. Cryptic diversity in *Brevipalpus* mites (Tenuipalpidae). *Zool. Scr.* 42, 406–426. doi:10.1111/zsc.12013
- Nene, V., 2009. Tick Genomics - Coming of age. *Front. Biosci.* 14, 2666–2673.
- Neumann, G., 1901. Révision de la Famille des Ixodidés (4e Mémoire), in: *Mémoires de La Société Zoologique de France*. pp. 249–372.
- Ostfeld, R.S., Glass, G.E., Keesing, F., 2005. Spatial epidemiology: an emerging (or re-emerging) discipline. *Trends Ecol. Evol.*, SPECIAL ISSUE: BUMPER BOOK REVIEW 20, 328–336. doi:10.1016/j.tree.2005.03.009
- Pappers, S.M., Velde, G. van der, Ouborg, N.J., Groenendael, J.M. van, 2002. Genetically Based Polymorphisms in Morphology and Life History Associated with Putative Host Races of the Water Lily Leaf Beetle, *Galerucella Nymphaeae*. *Evolution* 56, 1610–1621. doi:10.1111/j.0014-3820.2002.tb01473.x
- Parola, P., Raoult, D., 2001. Tick-borne bacterial diseases emerging in Europe. *Clin. Microbiol. Infect.* 7, 80–83. doi:10.1046/j.1469-0691.2001.00200.x
- Patterson, J.S., Schofield, C.J., Dujardin, J.P., Miles, M.A., 2001. Population morphometric analysis of the tropicopolitan bug *Triatoma rubrofasciata* and relationships with Old World species of *Triatoma*: evidence of New World ancestry. *Med. Vet. Entomol.* 15, 443–451. doi:10.1046/j.0269-283x.2001.00333.x
- PÉREZ-EID, C., 2007. Les tiques : identification, biologie, importance médicale et vétérinaire. Lavoisier.
- Ponchon, A., Grémillet, D., Christensen-Dalsgaard, S., Erikstad, K.E., Barrett, R.T., Reiertsen, T.K., McCoy, K.D., Tveraa, T., Boulinier, T., 2014. When things go wrong: intra-season dynamics of breeding failure in a seabird. *Ecosphere* 5, 1–19. doi:10.1890/ES13-00233.1
- Pospelova-Shtrom, M.V., 1969. On the system of classification of ticks of the family Argasidae Can., 1890. *Acarologia* 11, 1–22.
- Poulin, R., Morand, S., 2000. The Diversity of Parasites. *Q. Rev. Biol.* 75, 277–293.
- Raup, D.M., 1966. Geometric analysis of shell coiling: general problems. *J. Paleontol.* 1178–1190.
- Ravigné, V., Barberousse, A., Bierne, N., Britton-Davidian, J., Capy, P., Desdevises, Y., Giraud, T., Jousselin, E., Mouliat, C., Smadja, C., Samadi, S., 2010. La spéciation, in: Thomas, F., Lefèvre, T., Raymond, M. (Eds.), *Biologie évolutive*. De Boeck, Bruxelles, pp. 79–122.

- Ribeiro, J., 1995. Blood-feeding arthropods: live syringes or invertebrate pharmacologists? *Infect. Agents Dis.* 4, 143–152.
- Rice, W.R., 1987. Speciation via habitat specialization: the evolution of reproductive isolation as a correlated character. *Evol. Ecol.* 1, 301–314. doi:10.1007/BF02071555
- Rundell, R.J., Price, T.D., 2009. Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends Ecol. Evol.* 24, 394–399. doi:10.1016/j.tree.2009.02.007
- Russell, J.A., Moran, N.A., 2006. Costs and benefits of symbiont infection in aphids: variation among symbionts and across temperatures. *Proc. R. Soc. Lond. B Biol. Sci.* 273, 603–610. doi:10.1098/rspb.2005.3348
- Schluter, D., 2009. Evidence for ecological speciation and its alternative. *Science* 323, 737–741. doi:10.1126/science.1160006
- Shimshoni, J.A., Erster, O., Rot, A., Cuneah, O., Soback, S., Shkap, V., 2013. Cuticular fatty acid profile analysis of three *Rhipicephalus* tick species (Acari: Ixodidae). *Exp. Appl. Acarol.* 61, 481–489. doi:10.1007/s10493-013-9713-7
- Simmons, L.W., Thomas, M.L., Gray, B., Zuk, M., 2014. Replicated evolutionary divergence in the cuticular hydrocarbon profile of male crickets associated with the loss of song in the Hawaiian archipelago. *J. Evol. Biol.* n/a-n/a. doi:10.1111/jeb.12478
- Sonenshine, D.E., 1993. *Biology of ticks*. Volume 2. xvii + 465 pp.
- Sonenshine, D.E., 1985. Pheromones and Other Semiochemicals of the Acari. *Annu. Rev. Entomol.* 30, 1–28. doi:10.1146/annurev.en.30.010185.000245
- Sonenshine, D.E., Roe, R.M. (Eds.), 2014a. *Biology of ticks*, 2nd ed. ed. Oxford University Press, New York.
- Sonenshine, D.E., Roe, R.M., 2014b. *Biology of Ticks Vol.2*, Second edition. ed. Oxford University Press.
- Stern, D.L., 2013. The genetic causes of convergent evolution. *Nat. Rev. Genet.* 14, 751–764. doi:10.1038/nrg3483
- Takano, A., Muto, M., Sakata, A., Ogasawara, Y., Ando, S., Hanaoka, N., Tsurumi, M., Sato, F., Nakamura, N., Fujita, H., Watanabe, H., Kawabata, H., 2009. Relapsing Fever Spirochete in Seabird Tick, Japan. *Emerg. Infect. Dis.* 15, 1528–1530. doi:10.3201/eid1509.090459
- Thomas, F., Lefevre, T., Raymond, M., 2010. *Biologie évolutive*, Première édition. ed. DE BOECK UNIVERSITE, Bruxelles.
- Thompson, D.W., 1945. *On growth and form*. Cambridge : University Press ; New York : Macmillan.
- Thompson, J.N., 1997. Evaluating the Dynamics of Coevolution Among Geographically Structured Populations. *Ecology* 78, 1619–1623. doi:10.1890/0012-9658(1997)078[1619:ETDOCA]2.0.CO;2

- Tylianakis, J.M., Didham, R.K., Bascompte, J., Wardle, D.A., 2008. Global change and species interactions in terrestrial ecosystems. *Ecol. Lett.* 11, 1351–1363. doi:10.1111/j.1461-0248.2008.01250.x
- Van Valen, L., 1977. The Red Queen. *Am. Nat.* 111, 809–810. doi:10.1086/283213
- van Zweden, J.S., d’Ettorre, P., 2010. Nestmate recognition in social insects and the role of hydrocarbons. *Insect Hydrocarb. Biol. Biochem. Chem. Ecol.* 11, 222–243.
- van Zweden, J.S., Dreier, S., d’Ettorre, P., 2009. Disentangling environmental and heritable nestmate recognition cues in a carpenter ant. *J. Insect Physiol.* 55, 159–164. doi:10.1016/j.jinsphys.2008.11.001
- Vermeil, C., Marjolet, M., Vermeil, F., 1997. *Ornithodoros (Alectorobius) cheikhi* n.sp. (Acarina, Ixodoidea, Argasidae, groupe *Ornithodoros (Alectorobius) capensis*) ; Tique parasite de sternes au banc d’Arguin - Mauritanie. *Bull. Société Sci. Nat. Ouest Fr.* 19, 66–76.
- Vermeil, Merguet, 1967. Sur le diagnostic des larves d’*Ornithodoros* du complexe *coniceps-capensis* (Acarina : Argasidae). *Ornithodoros coniceps* (Canestrini, 1890) *maritimus* n. ssp. prévaut dans les îles de Basse-Bretagne. *Acarologia* 9, p.557-565.
- Wang, S., Meyer, E., McKay, J.K., Matz, M.V., 2012. 2b-RAD: a simple and flexible method for genome-wide genotyping. *Nat. Methods* 9, 808–810. doi:10.1038/nmeth.2023
- Wang, Z., Gerstein, M., Snyder, M., 2009. RNA-Seq: a revolutionary tool for transcriptomics. *Nat. Rev. Genet.* 10, 57–63. doi:10.1038/nrg2484
- Wiens, J.J., 2004. What Is Speciation and How Should We Study It? *Am. Nat.* 163, 914–923. doi:10.1086/an.2004.163.issue-6
- Wikel, S.K., 1984. Immunomodulation of host responses to ectoparasite infestation – an overview. *Vet. Parasitol., Special Issue Veterinary Immunoparasitology* 14, 321–339. doi:10.1016/0304-4017(84)90099-2
- Yang, S., Xu, Y., Zhang, D., 2006. Morphological basis for the waterproof characteristic of bird plumage. *J. For. Res.* 17, 163–166. doi:10.1007/s11676-006-0039-8
- Young, F.N., Goff, C.C., 1939. An Annotated List of the Arthropods Found in the Burrows of the Florida Gopher Tortoise, *Gopherus polyphemus* (Daudin). *Fla. Entomol.* 22, 53–62. doi:10.2307/3492191
- ZUMPT, F., 1952. The ticks of seabirds.



# Annexes

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## Annexe 1

Duron, O., Noël, V., McCoy, K.D., Bonazzi, M., Sidi-Boumedine, K., Morel, O., Vavre, F., Zenner, L., Jourdain, E., Durand, P., Arnathau, C., Renaud, F., Trape, J.-F., Biguezoton, A.S., Cremaschi, J., Dietrich, M., Léger, E., Appelgren, A., Dupraz, M., Gómez-Díaz, E., Diatta, G., Dayo, G.-K., Adakal, H., Zoungrana, S., Vial, L., Chevillon, C., 2015. The Recent Evolution of a Maternally-Inherited Endosymbiont of Ticks Led to the Emergence of the Q Fever Pathogen, *Coxiella burnetii*. PLoS Pathog 11, e1004892. doi:10.1371/journal.ppat.1004892





## RESEARCH ARTICLE

# The Recent Evolution of a Maternally-Inherited Endosymbiont of Ticks Led to the Emergence of the Q Fever Pathogen, *Coxiella burnetii*

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**Data Availability Statement:** Nucleotide sequences of PCR-amplified fragments of tick-borne *Coxiella* and *Rickettsiella* genes have been deposited in the GenBank nucleotide database under accession codes KP994768-KP994862 (16S rRNA), KP994678-KP994767 (23S rRNA), KP985445-KP985537 (GroEL), KP985265-KP985357 (rpoB) and KP985358-KP985444 (dnaK).

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## Abstract

Q fever is a highly infectious disease with a worldwide distribution. Its causative agent, the intracellular bacterium *Coxiella burnetii*, infects a variety of vertebrate species, including humans. Its evolutionary origin remains almost entirely unknown and uncertainty persists regarding the identity and lifestyle of its ancestors. A few tick species were recently found to harbor maternally-inherited *Coxiella*-like organisms engaged in symbiotic interactions, but their relationships to the Q fever pathogen remain unclear. Here, we extensively sampled ticks, identifying new and atypical *Coxiella* strains from 40 of 58 examined species, and used this data to infer the evolutionary processes leading to the emergence of *C. burnetii*. Phylogenetic analyses of multi-locus typing and whole-genome sequencing data revealed that *Coxiella*-like organisms represent an ancient and monophyletic group allied to ticks. Remarkably, all known *C. burnetii* strains originate within this group and are the descendants of a *Coxiella*-like progenitor hosted by ticks. Using both colony-reared and field-collected

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gravid females, we further establish the presence of highly efficient maternal transmission of these *Coxiella*-like organisms in four examined tick species, a pattern coherent with an endosymbiotic lifestyle. Our laboratory culture assays also showed that these *Coxiella*-like organisms were not amenable to culture in the vertebrate cell environment, suggesting different metabolic requirements compared to *C. burnetii*. Altogether, this corpus of data demonstrates that *C. burnetii* recently evolved from an inherited symbiont of ticks which succeeded in infecting vertebrate cells, likely by the acquisition of novel virulence factors.

### Author Summary

How virulent infectious diseases emerge from non-pathogenic organisms is a challenging question. Here, we address this evolutionary issue in the case of Q fever. Its causative agent, the intracellular bacterium *Coxiella burnetii*, is extremely infectious to humans and a variety of animals. However, uncertainty persists regarding its evolutionary origin, including the identity and lifestyle of its ancestors. In this article, we show that *C. burnetii* arose from a rare evolutionary transformation of a maternally-inherited endosymbiont of ticks into a specialized and virulent pathogen of vertebrates. While arthropod symbionts are typically transmitted maternally and thought not to be infectious to vertebrates, we establish here that one *Coxiella* symbiont has evolved the necessary adaptations to exploit the vertebrate cell, leading to the emergence of Q fever.

### Introduction

‘Query fever’ (Q fever) is a highly infectious zoonotic disease first identified in 1937 [1,2,3,4,5]. The causative agent, the obligate intracellular bacterium *Coxiella burnetii*, infects a variety of vertebrate species, including humans. Sporadic cases in humans occur annually worldwide, but occasional outbreaks are also common [1,2,3,4]. For example, in the Netherlands more than 4,000 human cases were reported between 2007 and 2010 [6]. While most human cases are self-limiting with fever and fatigue, acute forms range from mild flu-like symptoms to pneumonia or hepatitis. The disease can also become chronic (mainly endocarditis), and, though rarely fatal, remains highly debilitating even when treated with antibiotics [1,2]. Most human cases are linked to contact with infected livestock, especially goats and sheep, which suffer abortion and reproductive disorders. Infection usually occurs by the inhalation of aerosolized resistant small cell variants that are present in the excretions of infected animals. Other modes of transmission including ingestion of unpasteurized milk or dairy products; human-to-human contact is also possible but considered rare [1,2,4,5]. One of the most virulent reference strains of *C. burnetii* (strain RSA 493 / Nine Mile I [7]) was isolated from a guinea pig on which field-collected Rocky Mountain wood ticks *Dermacentor andersoni* had fed, suggesting that transmission through tick bites may also occur [8]. The small cell variants of the bacterium can survive and remain highly infectious for long periods in the environment, leading to the classification of *C. burnetii* as potential bioterrorism agent [9].

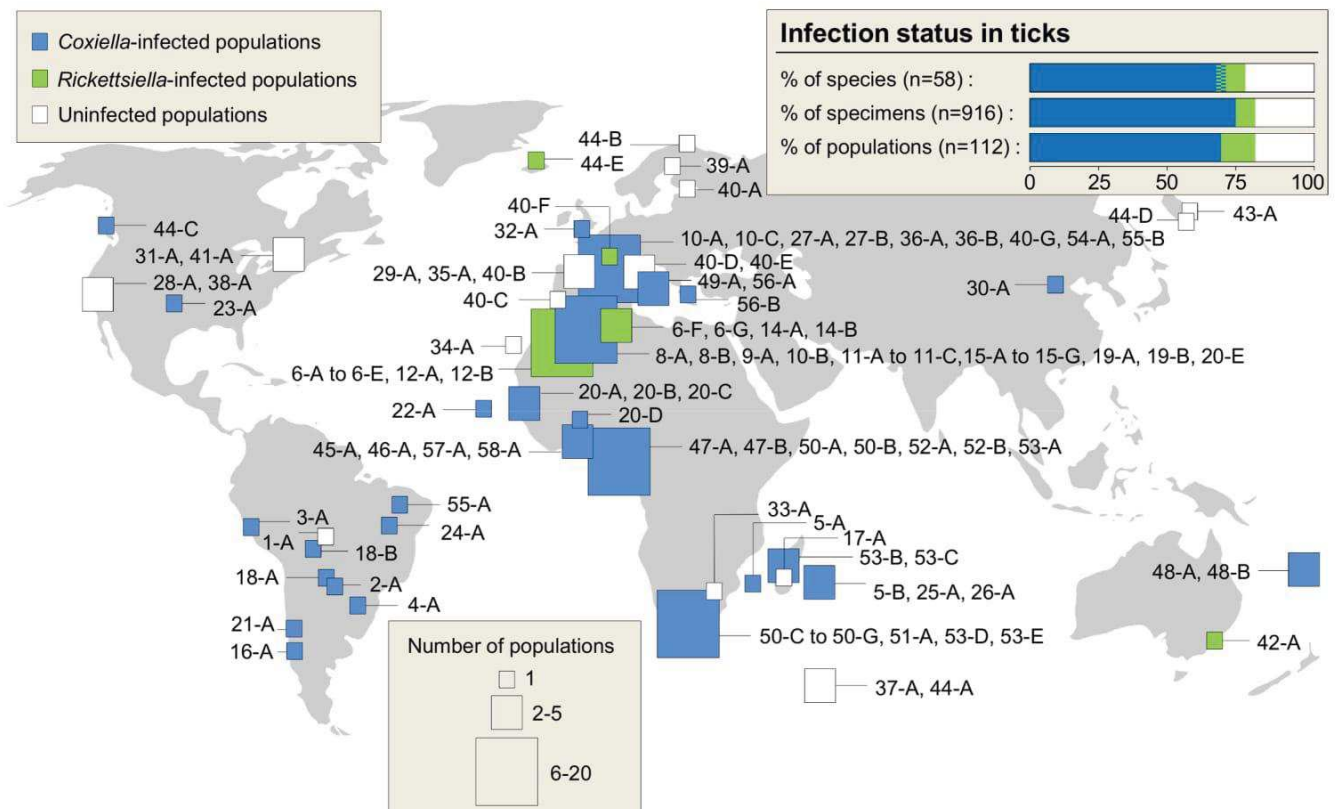
The evolutionary origin of Q fever is unclear since the *C. burnetii* ancestor and its primary lifestyle remain entirely unknown. Historically, *C. burnetii* was assigned to the taxonomic order Rickettsiales (Alphaproteobacteria), but it has been recently considered more closely related to the Legionellales order (Gammaproteobacteria) because of its genetic proximity to the Legionnaires’ disease agent, *Legionella pneumophila* [10]. The Legionellales order includes



many other intracellular bacteria infecting non-vertebrate species, such as, for instance, *Rickettsiella* species that are both widespread and biologically diverse in arthropods [11,12,13]. Within the *Coxiella* genus, the only known relative of *C. burnetii* which has been formally identified is *C. cheraxi*, a pathogen of crayfishes [14]. Many past descriptions of *Coxiella* were likely biased toward the detection of pathogenic strains since most *C. burnetii* isolates were collected from humans or domestic ruminants during Q fever outbreaks [1,5,15]. However, the advent of 16S rRNA gene sequencing as a universal DNA barcoding marker in bacteria has led to the description of a few novel *Coxiella*-like organisms in non-vertebrate species (listed in [16]), and particularly in ticks [17,18,19,20,21,22,23,24,25]. All these *Coxiella*-like organisms are closely related, but genetically distinct to *C. burnetii*, suggesting that some diversity exists within the *Coxiella* genus. The highly conserved nature of the 16S rRNA gene sequences has prevented researchers from establishing the exact relationship between *C. burnetii* and *Coxiella*-like organisms, and a sister clade relationship is commonly assumed [16,18,21,22,23].

The *Coxiella*-like organisms differ from *C. burnetii* in their biological traits and some may behave as subtle symbionts engaged in intricate interactions with ticks. In ticks belonging to *Ornithodoros*, *Amblyomma* and *Rhipicephalus* genera, *Coxiella*-like organisms were found to massively infect ovaries and to be maternally inherited through the egg cytoplasm [18,20,21,26]. In these tick species, the presence of the bacteria in the Malpighian tubules further suggests a possible role in nutrition by potentially provisioning their hosts with essential nutrients [20,21,26]. Indeed, the elimination of these bacteria with an antibiotic treatment was shown to negatively impact the fitness of the lone star tick *A. americanum* [27]. Accordingly, when the *Coxiella*-like bacterium found in *A. americanum* was recently sequenced [16], no recognizable virulence genes were found, indicating that this bacterium is likely non-pathogenic. In contrast, its genome encodes major vitamin and cofactor biosynthesis pathways, suggesting that it may be a vitamin-provisioning endosymbiont. This interaction exhibits the typical hallmarks of maternally-inherited symbionts with essential roles in arthropod biology [28,29]. Such patterns have been found in other exclusive blood-feeding species like bedbugs [30] and tsetse flies [31], two insect groups which rely on a single food source throughout their developmental cycle and harbor beneficial microbes that provide nutrients absent from their restricted diets. The *Coxiella*-like organisms of ticks share obvious similarities with these beneficial endosymbionts.

Here, we examine the origin of the Q fever pathogen, *C. burnetii*, by inferring the evolutionary processes that have shaped diversity within the entire *Coxiella* genus. To this aim, we first sampled an extensive range of ticks, with 58 tick species examined, and developed a sensitive detection method that reveals a wider *Coxiella* diversity than recognized in past studies. Second, instead of relying solely on the 16S rRNA gene, a molecular marker that is notoriously inadequate for inferring reliable fine-scale phylogenies [32], we used a novel multilocus typing method, allied to Whole Genome Sequencing (WGS) data, and conducted phylogenetic analyses on a large amount of DNA sequence data. Third, we examined two major ecological features of *Coxiella*-like organisms, i.e. their ability to be maternally-inherited through the tick egg cytoplasm and to grow in a vertebrate cell environment suitable to *C. burnetii*. Altogether, this corpus of data has led to the characterization of a large genetic and ecological diversity within the *Coxiella* genus, far beyond the *C. burnetii* type species. The *Coxiella*-like organisms of ticks form an ancient lineage of maternally-inherited tick endosymbionts that do not lie as a sister-clade to *C. burnetii* but rather form a basal lineage illustrative of the ancestral *Coxiella* life style.



**Fig 1. Geographic origin of the sampled ticks and distribution of *Coxiella* and *Rickettsiella* infections.** Square size indicates the number of populations sampled per geographic area. Numbers refer to the tick species whereas letters discriminate the different populations screened within a species; this nomenclature is detailed in Table 1. The colors within squares indicate the infection status of populations. Two tick species were infected by both *Coxiella* and *Rickettsiella* at the species level, but not at individual and population levels.

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## Results

### Ticks commonly harbor *Coxiella*-like organisms

We performed an extensive screening for the presence of *Coxiella* in 916 tick specimens from 58 species belonging to the two main tick families, Ixodidae (hard ticks, 36 species) and Argasidae (soft ticks, 22 species) (Fig 1 and Table 1). Except for 37 specimens (6 species) derived from laboratory colonies, all other tick specimens were sampled from natural populations in Europe, Americas, Africa, Oceania and Asia ( $n = 112$  localities). In these populations, ticks were collected either in the host habitat or directly on hosts (Table 1). To detect *C. burnetii* and its relatives, we developed a detection method based on a nested polymerase chain reaction (PCR) using total tick DNA extracts to amplify a 539–542 base-pair (bp) fragment of the *Coxiella rpoB* gene (Table A in S1 Text).

Using this procedure, all the tick-borne bacteria we detected belong to the Legionellales order and can be unambiguously assigned either to *Coxiella* or to its sister genus, *Rickettsiella*. Whole tick DNA extracts from more than two thirds of the specimens (637 out of 916, 69.6%) and the species (40 out of 58, 70.0%) were found to be positive for *Coxiella* (Fig 1 and Table 1). *Coxiella* was found in most tested genera of hard ticks (*Rhipicephalus*, *Ixodes*, *Amblyomma*, *Dermacentor*, *Haemaphysalis*) and soft ticks (*Ornithodoros*, *Argas*). In almost all infected



**Table 1. List of tick species and populations included in the analysis, with details on their origin, the population sample size, and the prevalence of *Coxiella* spp. and *Rickettsiella* spp.**

Tick species	Sample sites	Tick host species or habitat	n	<i>Coxiella</i> prevalence (number of infected tick specimens)	<i>Rickettsiella</i> prevalence (number of infected tick specimens)
Argasidae (soft ticks)					
1 - <i>Antricola guglielmonei</i> Estrada-Peña, Barros-Battesti and Venzal, 2004	1-A, Porto Velho, Rondonia, Brazil	Bat guano in cave	4	0.00 (0)	0.00 (0)
2 - <i>Argas monachus</i> Keirans, Radovsky and Clifford, 1973	2-A, Chaco, Argentina	Unknown	3	1.00 (3)	0.00 (0)
3 - <i>Ornithodoros amblus</i> Chamberlin, 1920	3-A, Lobos de Tierra Island, Peru, 2009	Peruvian Pelican ( <i>Pelecanus thagus</i> ), Peruvian Booby ( <i>Sula variegata</i> )	5	1.00 (5)	0.00 (0)
4 - <i>Ornithodoros brasiliensis</i> Aragão, 1923	4-A, Sao Francisco de Paula, Brazil	Unknown	3	1.00 (3)	0.00 (0)
5 - <i>Ornithodoros capensis</i> Neumann, 1901	5-A, Juan de Nova Island, Mozambic Channel, 2011	Sooty Tern ( <i>Onychoprion fuscatus</i> )	28	1.00 (28)	0.00 (0)
	5-B, Réunion Island	Sea bird nests	3	1.00 (3)	0.00 (0)
6 - <i>Ornithodoros costalis</i> Diatta, Bouattour, Durand, Renaud and Trape, 2013	6-A, Kenitra, Morocco, 2006	Rodent burrows	7	0.00 (0)	1.00 (7)
	6-B, Sidi Akhfennir, Morocco, 2006	Rodent burrows	18	0.00 (0)	1.00 (18)
	6-C, Boujdour, Morocco, 2006	Rodent burrows	2	0.00 (0)	1.00 (2)
	6-D, El Argoub, Morocco, 2006	Rodent burrows	2	0.00 (0)	1.00 (2)
	6-E, Lahmiris, Morocco, 2010	Rodent burrows	1	0.00 (0)	1.00 (1)
	6-F, Mostaganem, Algeria, 2012	Rodent burrows	4	0.00 (0)	1.00 (4)
	6-G, Oudhna, Tunisia, 2010	Rodent burrows	4	0.00 (0)	1.00 (4)
7 - <i>Ornithodoros denmarki</i> Kohls, Sonenshine and Clifford, 1965	7-H, unknown	Sea bird nests	1	1.00 (1)	0.00 (0)
8 - <i>Ornithodoros erraticus</i> Lucas, 1849	8-A, La Calle, Tunisia, 2009	Rodent burrows	1	1.00 (1)	0.00 (0)
	8-B, Taher, Algeria, 2010	Rodent burrows	2	1.00 (2)	0.00 (0)
9 - <i>Ornithodoros kairouanensis</i> Trape, Diatta, Bouattour, Durand and Renaud, 2013	9-A, Kairouan, Tunisia, 2010	Rodent burrows	3	1.00 (3)	0.00 (0)
10 - <i>Ornithodoros maritimus</i> Vermeil and Marguet, 1967	10-A, Medes Island, Spain, 2009	Yellow-legged Gull ( <i>Larus michahellis</i> )	20	1.00 (20)	0.00 (0)
	10-B, Zembra Island, Tunisia, 2009	Yellow-legged Gull ( <i>Larus michahellis</i> )	20	1.00 (20)	0.00 (0)
	10-C, Carteau, France, 2014	Yellow-legged Gull ( <i>Larus michahellis</i> )	8	1.00 (8)	0.00 (0)
11 - <i>Ornithodoros maroccanus</i> Velu, 1919	11-A, Izemmourèn, Morocco, 2009	Rodent burrows	1	1.00 (1)	0.00 (0)
	11-B, Berkane Oued Kiss, Morocco, 2006	Rodent burrows	1	1.00 (1)	0.00 (0)

(Continued)

Table 1. (Continued)

Tick species	Sample sites	Tick host species or habitat	n	<i>Coxiella</i> prevalence (number of infected tick specimens)	<i>Rickettsiella</i> prevalence (number of infected tick specimens)
	11-C, Bir-Jdid, Morocco, 2009	Rodent burrows	1	1.00 (1)	0.00 (0)
12 <i>Ornithodoros merionesi</i> - Trape, Diatta, Belghyti, Sarih, Durand and Renaud, 2013	12-A, Guelmin, Morocco, 2006	Rodent burrows	1	0.00 (0)	1.00 (1)
	12-B, Sidi Akhfennir, Morocco, 2006	Rodent burrows	2	0.00 (0)	1.00 (2)
13 <i>Ornithodoros moubata</i> - Murray, 1877	13-A, Laboratory strain derived from field specimens of unknown origin	Unknown	3	0.00 (0)	0.00 (0)
14 <i>Ornithodoros normandi</i> - Larrousse, 1923	14-A, Bizerte, Tunisia, 2010	Rodent burrows	1	0.00 (0)	1.00 (1)
	14-B, Oudhna, Tunisia, 2010	Rodent burrows	2	0.00 (0)	1.00 (2)
15 <i>Ornithodoros occidentalis</i> - Trape, Diatta, Durand and Renaud, 2013	15-A, Fes, Morocco, 2010	Rodent burrows	4	1.00 (4)	0.00 (0)
	15-B, Kenitra, Morocco, 2006	Rodent burrows	4	1.00 (4)	0.00 (0)
	15-C, Beb-Lerba, Morocco, 2010	Rodent burrows	4	1.00 (4)	0.00 (0)
	15-D, Oued Choufcherk, Morocco, 2010	Rodent burrows	4	1.00 (4)	0.00 (0)
	15-E, Bouira, Algeria, 2010	Rodent burrows	4	1.00 (4)	0.00 (0)
	15-F, Berrouaghia, Algeria, 2010	Rodent burrows	4	1.00 (4)	0.00 (0)
	15-G, Chlef, Algeria, 2010	Rodent burrows	4	1.00 (4)	0.00 (0)
16 <i>Ornithodoros peruvianus</i> - Kohls, Clifford and Jones, 1969	16-A, Chile	Common vampire Bat ( <i>Desmodus rotundus</i> )	3	1.00 (3)	0.00 (0)
17 <i>Ornithodoros porcinus</i> - Walton, 1962	17-A, Laboratory strain derived from field specimens collected in Mahitsy, Madagascar, 2008–2010	Unknown	3	0.00 (0)	0.00 (0)
18 <i>Ornithodoros rostratus</i> - Aragão, 1911	18-A, Salta, Argentina	Environement	18	1.00 (18)	0.00 (0)
	18-B, Laboratory strain derived from field specimens collected in Nhecolandi, Pantanal, Brazil	Environement	4	1.00 (4)	0.00 (0)
19 <i>Ornithodoros rupestris</i> - Trape, Bitam, Renaud and Durand, 2013	19-A, Saïda Mt Daïa, Algeria, 2012	Rodent burrows	1	1.00 (1)	0.00 (0)
	19-B, Mostaganem, Algeria, 2012	Rodent burrows	2	1.00 (2)	0.00 (0)
20 <i>Ornithodoros sonrai</i> - Sautet and Witkowski, 1943	20-A, Dielmo, Senegal, 2002	Rodent burrows	20	1.00 (20)	0.00 (0)
	20-B, Kanène Khar, Senegal, 2003	Rodent burrows	17	1.00 (17)	0.00 (0)
	20-C, Richard-Toll, Senegal, 2003	Rodent burrows	19	1.00 (19)	0.00 (0)

(Continued)



Table 1. (Continued)

Tick species	Sample sites	Tick host species or habitat	n	<i>Coxiella</i> prevalence (number of infected tick specimens)	<i>Rickettsiella</i> prevalence (number of infected tick specimens)
	20-D, Sogoli, Mali, 2007	Rodent burrows	10	1.00 (10)	0.00 (0)
	20-E, M'Chounèche, Algeria, 2009	Rodent burrows	37	1.00 (37)	0.00 (0)
21 <i>Ornithodoros spheniscus</i> - Hoogstraal, Wassef, Hays and Keirans, 1985	21-A, Pan de Azucar, Chile, 2010–2013	Humboldt penguin ( <i>Spheniscus humboldti</i> )	3	1.00 (3)	0.00 (0)
22- <i>Ornithodoros</i> sp. ( <i>capensis</i> species complex)	22-A, Boa Vista Island, Cape Verde, 2008	Cape Verde Shearwater ( <i>Calonectris edwardsii</i> ) and Brown Booby ( <i>Sula leucogaster</i> )	16	1.00 (16)	0.00 (0)
		Ixodidae (hard ticks)			
23 <i>Amblyomma americanum</i> - (Linnaeus, 1758)	23-A, Laboratory strain derived from field engorged females collected in Oklahoma, USA, 1976–2004	Unknown	20	1.00 (20)	0.00 (0)
24 <i>Amblyomma cajennense</i> - (Fabricius, 1787)	24-A, Chapada Gaucha, Brazil, 2013	Unknown	3	1.00 (3)	0.00 (0)
25 <i>Amblyomma loculosum</i> - Neumann, 1907	25-A, Petite Ile, La Réunion, 2012	Wedge-Tailed Shearwater ( <i>Puffinus pacificus</i> ) nests	3	1.00 (3)	0.00 (0)
26 <i>Amblyomma variegatum</i> - (Fabricius, 1794)	26-A, La Réunion	Unknown	2	1.00 (2)	0.00 (0)
27 <i>Dermacentor marginatus</i> - (Sulzer, 1776)	27-A, Cavaillon, France, 2011	Vegetation	1	1.00 (1)	0.00 (0)
	27-B, Les Plantiers, France, 2013	Vegetation	1	1.00 (1)	0.00 (0)
28 <i>Dermacentor occidentalis</i> - Marx, 1892	28-A, Hopland, Mendocino Co, California, 1985	Environement	6	0.00 (0)	0.00 (0)
29 <i>Dermacentor reticulatus</i> - (Fabricius, 1794)	29-A, Proveysieux, France, 2011	Vegetation	1	0.00 (0)	0.00 (0)
30 <i>Dermacentor silvarum</i> - Olenev, 1931	30-A, Laboratory strain derived from field specimens collected in Xiaowutai National Natural Reserve Area, China		2	1.00 (2)	0.00 (0)
31 <i>Dermacentor variabilis</i> - (Say, 1821)	31-A, Queen's biology station, Ontario, Canada, 2013	Vegetation	2	0.00 (0)	0.00 (0)
32 <i>Haemaphysalis punctata</i> - Canestrini and Fanzago, 1878	32-A, East Sussex, England, 2011	Vegetation	5	1.00 (5)	0.00 (0)
33 <i>Hyalomma impeltatum</i> - Schulze and Schlottko, 1930	33-A, Zimbabwe, 1998	Zebu ( <i>Bos indicus</i> )	3	0.00 (0)	0.00 (0)
34 <i>Hyalomma lusitanicum</i> - Koch, 1844	34-A, Veneguera, Canaries, 2010	Vegetation	2	0.00 (0)	0.00 (0)
35 <i>Ixodes frontalis</i> (Panzer, 1798)	35-A, Bretagne, France, 2008	Eurasian Collared Dove ( <i>Streptopelia decaocto</i> )	1	0.00 (0)	0.00 (0)
36- <i>Ixodes hexagonus</i> Leach, 1815	36-A, Bretagne, France, 2008	European Red Fox ( <i>Vulpes vulpes</i> )	1	1.00 (1)	0.00 (0)
	36-B, Beaumont-Montoux, France, 2008	European Hedgehog ( <i>Erinaceus europaeus</i> )	2	1.00 (1)	0.00 (0)
37 <i>Ixodes kerguelenensis</i> - André and Colas-Belcour, 1942	37-A, Crozet Archipelago, 2003	Sea bird nests	2	0.00 (0)	0.00 (0)

(Continued)

Table 1. (Continued)

	Tick species	Sample sites	Tick host species or habitat	n	<i>Coxiella</i> prevalence (number of infected tick specimens)	<i>Rickettsiella</i> prevalence (number of infected tick specimens)
38	<i>Ixodes pacificus</i> Cooley and Kohls, 1943	38-A, Hopland, Mendocino Co, California, 1985	Environement	6	0.00 (0)	0.00 (0)
39	<i>Ixodes persulcatus</i> Schulze, 1930	39-A, Oulu, Finland, 2011	Vegetation	3	0.00 (0)	0.00 (0)
40	<i>Ixodes ricinus</i> (Linnaeus, 1758)	40-A, Lehmäsaari, Finland, 2011	Vegetation	20	0.00 (0)	0.00 (0)
		40-B, Rioja, Spain, 2011	Vegetation	20	0.00 (0)	0.00 (0)
		40-C, Mafra, Portugal, 2013	Vegetation	16	0.00 (0)	0.00 (0)
		40-D, Neuchâtel, Switzerland, 2012	Vegetation	16	0.00 (0)	0.00 (0)
		40-E, Chur, Switzerland, 2012	Hazel grouse ( <i>Tetrastes bonasia</i> )	4	0.00 (0)	0.00 (0)
		40-F, Sénart, France, 2010	European Roe Deer ( <i>Capreolus capreolus</i> ), rodents and passerines	22	0.00 (0)	0.05 (1)
		40-G, Ain, France, 2004	Vegetation	2	1.00 (1)	0.00 (0)
41	<i>Ixodes scapularis</i> Say, 1821	41-A, Queen's biology station, Ontario, Canada, 2013	Vegetation	3	0.00 (0)	0.00 (0)
42	<i>Ixodes tasmani</i> Neumann, 1899	42-A, Kilarney Circuit, Coolangubra, Australia, 1982	Common Wombat ( <i>Vombatus ursinus</i> )	2	0.00 (0)	1.00 (2)
43	<i>Ixodes unicavatus</i> Neumann, 1908	43-A, Ariy Kamen Islet, Kamchatka, Russia, 2008	Black-legged Kittiwake ( <i>Rissa tridactyla</i> ) and Red-legged Kittiwake ( <i>Rissa brevirostris</i> )	4	0.00 (0)	0.00 (0)
44	<i>Ixodes uriae</i> White, 1852	44-A, Possession Island, Crozet Archipelago, 2003	King Penguin ( <i>Aptenodytes patagonicus</i> )	20	0.00 (0)	0.00 (0)
		44-B, Hornøya Island, Norway, 2010	Brünnich's Guillemot ( <i>Uria lomvia</i> )	20	0.00 (0)	0.00 (0)
		44-C, Triangle Island, Canada, 2010	Rhinoceros Auklet ( <i>Cerorhinca monocerata</i> )	14	0.50 (7)	0.00 (0)
		44-D, Pitchie2, Kamchatka, Russia, 2008	Red-faced Cormorant ( <i>Phalacrocorax urile</i> )	20	0.00 (0)	0.00 (0)
		44-E, Grimsey, Iceland, 2003	Atlantic Puffin ( <i>Fratercula arctica</i> ), Common Guillemot ( <i>Uria aalge</i> ) and Black-legged Kittiwake ( <i>Rissa tridactyla</i> )	25	0.00 (0)	0.20 (5)
45	<i>Ixodes</i> sp.1	45-A, Guiglo, Ivory Coast, 1994	Leopard ( <i>Panthera pardus</i> )	12	1.00 (12)	0.00 (0)
46	<i>Ixodes</i> sp.2	46-A, Guiglo, Ivory Coast, 1994	Dog ( <i>Canis familiaris</i> )	12	1.00 (12)	0.00 (0)
47	<i>Rhipicephalus annulatus</i> (Say, 1821)	47-A, Burkina-Faso, 2013	Zebu ( <i>Bos indicus</i> )	17	1.00 (17)	0.00 (0)
		47-B, Gogonou, Benin, 2012	Zebu ( <i>Bos indicus</i> )	5	1.00 (5)	0.00 (0)
48	<i>Rhipicephalus australis</i> Fuller, 1899	48-A, CCA Farm, New Caledonia, 2003	Cattle ( <i>Bos taurus</i> )	12	1.00 (12)	0.00 (0)
		48-B, BMMMM Farm, New Caledonia, 2003	Cattle ( <i>Bos taurus</i> )	12	1.00 (12)	0.00 (0)
49	<i>Rhipicephalus bursa</i> Canestrini and Fanzago, 1878	49-A, Italia	Cattle ( <i>Bos taurus</i> ) and Sheep ( <i>Ovis aries</i> )	2	1.00 (2)	0.00 (0)

(Continued)

Table 1. (Continued)

Tick species	Sample sites	Tick host species or habitat	n	<i>Coxiella</i> prevalence (number of infected tick specimens)	<i>Rickettsiella</i> prevalence (number of infected tick specimens)
50- <i>Rhipicephalus decoloratus</i> Koch, 1844	50-A, Burkina-Faso, 2013	Zebu ( <i>Bos indicus</i> )	20	0.95 (19)	0.00 (0)
	50-B, Gogonou, Benin, 2012	Zebu ( <i>Bos indicus</i> )	9	1.00 (9)	0.00 (0)
	50-C, Sandvelt, South Africa, 2011	Blue Wildebeest ( <i>Connochaetes taurinus</i> ), Greater Kudu ( <i>Tragelaphus strepsiceros</i> ) and Southern Eland ( <i>Taurotragus oryx</i> )	20	1.00 (20)	0.00 (0)
	50-D, Queenstown, South Africa, 2011	Zebu ( <i>Bos indicus</i> )	17	1.00 (17)	0.00 (0)
	50-E, Vaalwater, South Africa, 2010	Zebu ( <i>Bos indicus</i> )	19	1.00 (19)	0.00 (0)
	50-F, Lephalale, South Africa, 2010	South African Giraffe ( <i>Giraffa camelopardalis giraffa</i> )	9	1.00 (9)	0.00 (0)
	50-G, Zimbabwe, 1998	Impala ( <i>Aepyceros melampus</i> )	11	1.00 (11)	0.00 (0)
51- <i>Rhipicephalus evertsi</i> Neumann, 1897	51-A, Zimbabwe, 1998	Zebu ( <i>Bos indicus</i> )	8	1.00 (8)	0.00 (0)
52- <i>Rhipicephalus geigy</i> Aeschlimann and Morel, 1965	52-A, Burkina-Faso, 2013	Zebu ( <i>Bos indicus</i> )	18	0.94 (17)	0.00 (0)
	52-B, Gogonou, Benin, 2012	Zebu ( <i>Bos indicus</i> )	3	1.00 (3)	0.00 (0)
53- <i>Rhipicephalus microplus</i> (Canestrini, 1888)	53-A, Kpinnou, Benin, 2012	Zebu ( <i>Bos indicus</i> )	20	1.00 (20)	0.00 (0)
	53-B, Ambalanirana, Madagascar, 2013	Zebu ( <i>Bos indicus</i> )	18	1.00 (18)	0.00 (0)
	53-C, Imeritsiatosika, Madagascar, 2013	Zebu ( <i>Bos indicus</i> )	14	1.00 (14)	0.00 (0)
	53-D, Eglinton, South Africa, 2011	Zebu ( <i>Bos indicus</i> )	20	1.00 (20)	0.00 (0)
	53-E, Welverdiemda, South Africa, 2011	Zebu ( <i>Bos indicus</i> )	11	1.00 (11)	0.00 (0)
	53-F, Laboratory strain derived from field engorged females collected in Kpinnou, Benin, 2012	Girolando (Cattle x Zebu hybrid)	7	1.00 (7)	0.00 (0)
54- <i>Rhipicephalus pusillus</i> Gil Collado, 1936	54-A, Gard, France, 2006	Vegetation	2	1.00 (2)	0.00 (0)
55- <i>Rhipicephalus sanguineus</i> (Latreille, 1806)	55-A, Brazil	Dog ( <i>Canis familiaris</i> )	1	1.00 (1)	0.00 (0)
	55-B, Montferrier-sur-Lez, France, 2013	Dog ( <i>Canis familiaris</i> )	1	1.00 (1)	0.00 (0)
56- <i>Rhipicephalus turanicus</i> Pomerantzev, 1940	56-A, Italia	Vegetation	2	1.00 (2)	0.00 (0)
	56-B, Kerkyra, Greece, 2012	Human ( <i>Homo sapiens</i> )	3	1.00 (3)	0.00 (0)
57- <i>Rhipicephalus</i> sp.1	57-A, Guiglo area, Ivory Coast, 1994	Leopard ( <i>Panthera pardus</i> )	3	1.00 (3)	0.00 (0)
58- <i>Rhipicephalus</i> sp.2	58-A, Guiglo, Ivory Coast, 1994	Dog ( <i>Canis familiaris</i> )	2	1.00 (2)	0.00 (0)

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species, *Coxiella* was detected in >90% of the examined specimens, indicating high *Coxiella* prevalence in diverse tick species. For example, infection was apparently fixed in populations of most *Rhipicephalus* and *Ornithodoros* species (Table 1). In contrast, *Coxiella* was frequently absent in *Ixodes* species and displayed highly variable prevalence in the five infected species (out of 12 screened).

Other Legionellales bacteria of the genus *Rickettsiella* were found in 52 specimens (5.7%) from six *Ornithodoros* species and three *Ixodes* species (Fig 1 and Table 1). In two of the three *Rickettsiella*-infected *Ixodes* species, i.e. *I. ricinus* and *I. uriae*, *Coxiella* was also found, but in different individuals and in distinct populations (i.e., no co-infection by *Coxiella* and *Rickettsiella* occurred at individual and population levels; Table 1). Adding the *Rickettsiella*-positive samples, we found that 689 of the 916 examined tick specimens (75.2%) and 44 of 58 screened species (76%) harbored either *Coxiella* or one of its relatives.

### High genetic diversity among tick-borne *Coxiella*

To characterize *Coxiella* genetic diversity, we developed a multi-locus typing method based on five conserved bacterial genes including *rpoB* and four other housekeeping genes: 16S rRNA, 23S rRNA, *GroEL* and *dnaK* (Table A and Fig A in S1 Text). Multi-locus sequences were obtained from a subsample of 85 *Coxiella*- and 12 *Rickettsiella*-positive tick specimens (one to four specimens per infected species were examined). All five bacterial genes were successfully amplified from 71 *Coxiella*- and 12- *Rickettsiella* positive specimens representing 35 *Coxiella*- and six *Rickettsiella*-infected tick species. For five other *Coxiella*-infected species (i.e., 14 individual ticks), only three to four bacterial genes were successfully amplified. The sequences were easily readable without double peaks, indicating that there was no coinfection of *Coxiella*/*Rickettsiella* strains in any specimen.

The overall dataset included 33 to 40 alleles per bacterial gene (Table 2) and 51 new multi-locus genotypes (43 in *Coxiella* and eight in *Rickettsiella*). Within the *Coxiella* genus, all pairs of 16S rRNA gene sequences are at least 93% identical (Table 2) and range in threshold values typically used to delineate other Legionellales genera such as *Legionella* [33] and *Rickettsiella* [34]. Each of the infected tick species harbored a specific bacterial genotype or a set of closely related genotypes. None of the *Coxiella* multi-locus genotypes identified in ticks was identical to those of the 15 *C. burnetii* reference strains (Table B in S1 Text), although some showed moderate levels of nucleotide identity: pairwise identity between the two groups ranged from 77.8% to 97.7%. For each bacterial gene, the genetic diversity was significantly higher in the *Coxiella* strains of ticks than in *C. burnetii* as illustrated by the metrics on their respective genetic diversity (Table 2, paired t test, all  $P < 0.02$ ).

### *Coxiella burnetii* originated from a tick-borne *Coxiella* ancestor

We constructed a multi-gene phylogeny of the entire *Coxiella* genus using a dataset that included the *Coxiella* and *Rickettsiella* sequences from ticks, the 15 *C. burnetii* reference genomes, as well as sequences from *Legionella* spp. and more distant outgroups that were available in GenBank (Table B in S1 Text). The concatenated sequences included 3009 unambiguously aligned base pairs (bp). Prior recombination tests showed that *Coxiella* and *Rickettsiella* strains did not exhibit a strictly clonal structure, but rather experienced significant genetic exchanges. We thus applied a sequence-based network approach that does not force relationships to be tree-like but rather incorporates recombination into the phylogenetic reconstruction. The network results (Fig 2), as well as the results from the Maximum Likelihood (ML) tree-based analysis (Fig B in S1 Text), consistently showed that the *Coxiella* genus can be split into four main clades (labeled A-to-D hereafter) with each clade clustering the *Coxiella* genotypes found in five to 15

tick species. The phylogenetic analyses also highlight that all *C. burnetii* isolates cluster into a unique subclade embedded within the A clade (Fig B and C in S1 Text and Fig 2). Notably, the closest relatives of *C. burnetii* are the *Coxiella* strains from soft ticks of the *Ornithodoros* and *Argas* genera, suggesting that the common ancestor of *C. burnetii* originated from a *Coxiella* hosted by soft ticks.

The partitioning of *Coxiella* diversity among tick species revealed a complex structure, indicating a role for both co-divergence and horizontal transfer events in the evolution of this bacterial group. Closely related *Coxiella*-like organisms were frequently found in closely related tick species, a pattern suggestive of co-divergence between *Coxiella* and ticks (Fig B in S1 Text and Fig 2). For instance, all the *Coxiella*-like organisms found in the 12 examined *Rhipicephalus* tick species cluster together within the C clade, whereas all the *Coxiella*-like organisms in the *Ixodes* species cluster within the B clade (Fig B in S1 Text and Fig 2). Conversely, some *Coxiella*-like organisms found in related tick species are only distantly related and do not cluster together (e.g., the *Coxiella*-like organisms of *Ornithodoros* soft ticks are scattered among the A, B and C clades), a pattern suggestive of horizontal transfers among tick species.

Further analyses were conducted by examining public repositories of DNA sequencing data generated by the whole genome sequencing (WGS) projects of the cattle tick *R. microplus* and the deer tick *I. scapularis*. Using the 1,995,281 bp *C. burnetii* (str. Nine Mile I RSA 493) genome as a probe, we found clear evidence of *Coxiella* infections in *R. microplus* but not in *I. scapularis*. A total of 31 contigs (514–2,349 bp, totaling 34,990 bp) from *R. microplus* sequencing were uniquely attributable to *Coxiella*. They matched 50 genes of *C. burnetii* with 68-to-100% nucleotide identity (Fig A and Table C in S1 Text and Fig 3A). Alignment of the 31 *Coxiella* contigs to other bacterial genomes, including the 15 *C. burnetii* reference genomes (19,304 unambiguously aligned bp), corroborates the finding of our prior five loci-based analyses: the *Coxiella* strain identified in *R. microplus* is evolutionarily related, but distinct, to *C. burnetii* (Fig 3B).

### *Coxiella*-like organisms are maternally inherited in ticks

It should be noted that the *R. microplus* WGS DNA examined above was extracted from eggs of an inbred strain of ticks (Deutsch strain), first derived from a few field specimens sampled in Texas in 2001, and reared for at least seven generations in the laboratory. The presence of *Coxiella* DNA in the WGS of *R. microplus* eggs thus raised the issue of their maternal inheritance in ticks. To address this question, 24 gravid females of four *Coxiella*-positive tick species were collected either from seabird nests (*O. maritimus*,  $n = 8$  females), from a dog (*R. sanguineus*,  $n = 1$ ) or from laboratory colonies (*R. microplus*,  $n = 7$ ; *A. americanum*,  $n = 8$ ) in order to test for the presence of *Coxiella*-infection in the cytoplasm of their progeny (8 to 14 surface-sterilized eggs per female were individually examined; i.e., 244 eggs in total). The occurrence of maternal transmission was detected in all four tick species and in almost all eggs: *O. maritimus*—79 *Coxiella*-positive eggs out of 80, *R. sanguineus*—14 out of 14, *R. microplus*—68 out of 70, and *A. americanum*—80 of 80. The mean transmission rate can thus be estimated at 0.988 (95% confidence interval, 0.965–0.994), demonstrating highly efficient maternal transmission of *Coxiella* in ticks. Maternal inheritance is thus widespread in the *Coxiella* genera, being found in three different clades (A: *Coxiella*-like organism of *O. maritimus*; C: *R. sanguineus* and *R. microplus*; D: *A. americanum*).



**Table 2. Genetic estimates for 85 *Coxiella*-like strains and for 15 *Coxiella burnetii* reference strains.**

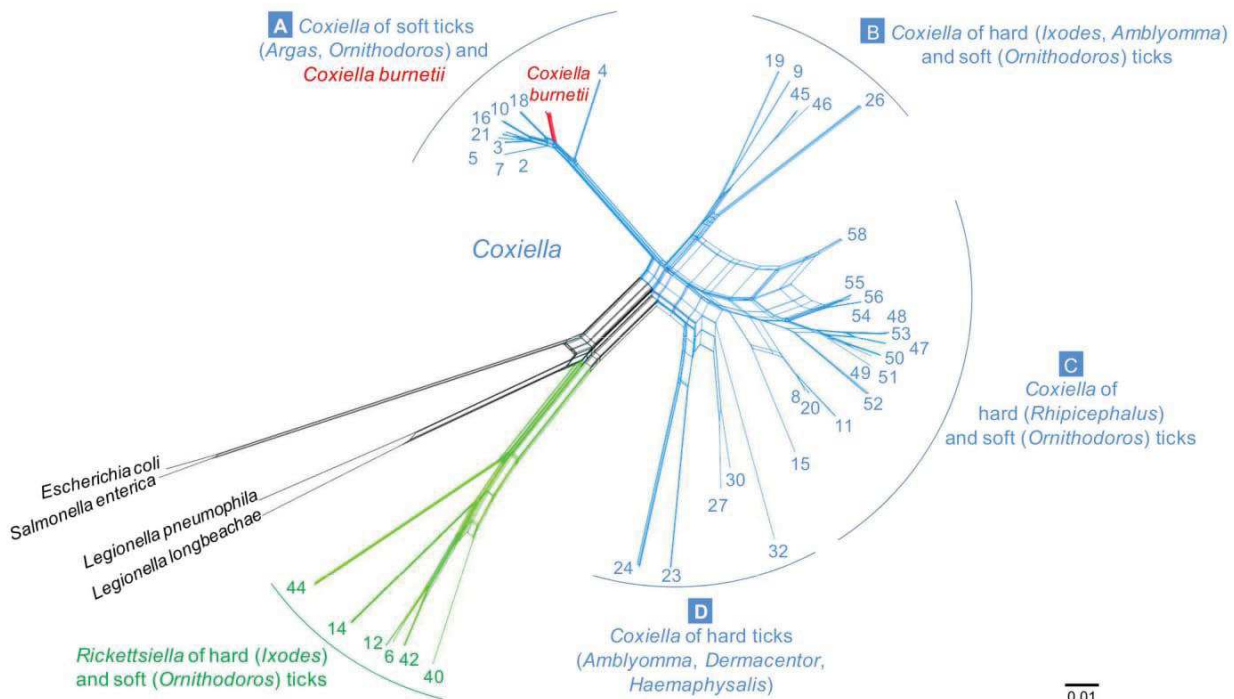
Locus	Function	L	Strains	N <sub>i</sub>	P <sub>nsi</sub>	N <sub>a</sub>	P <sub>s</sub>	A <sub>d</sub>	π	D
16S rRNA	Small ribosomal subunit	1066	<i>Coxiella</i> -like organisms	85	93.0–100	40	262	0.985	0.038	39.960
			<i>Coxiella burnetii</i>	15	99.7–100	4	4	0.552	0.001	1.224
23S rRNA	Large ribosomal subunit	496	<i>Coxiella</i> -like organisms	82	84.2–100	34	177	0.978	0.079	39.393
			<i>Coxiella burnetii</i>	15	99.4–100	2	2	0.133	0.001	0.267
<i>GroEL</i>	Chaperone protein GROEL	550	<i>Coxiella</i> -like organisms	82	68.7–100	37	292	0.982	0.179	98.527
			<i>Coxiella burnetii</i>	15	99.4–100	5	5	0.562	0.002	0.895
<i>rpoB</i>	DNA-directed RNA polymerase beta chain	474	<i>Coxiella</i> -like organisms	85	68.7–100	38	250	0.981	0.205	97.136
			<i>Coxiella burnetii</i>	15	99.3–100	4	4	0.467	0.001	0.648
<i>dnaK</i>	Chaperone protein DNAK	423	<i>Coxiella</i> -like organisms	74	69.6–100	33	227	0.979	0.177	75.789
			<i>Coxiella burnetii</i>	15	99.8–100	3	1	0.362	0.001	0.381
Full concatenated data set		3009	<i>Coxiella</i> -like organisms	71	81.8–100	36	1139	0.984	0.115	346.990
			<i>Coxiella burnetii</i>	15	99.6–100	7	16	0.781	0.001	2.933

Analyses are based on nucleotide sequences of five housekeeping genes, excluding sites with alignment gaps and/or missing data. L, sequence fragment length in base pairs; N<sub>i</sub>, Number of examined strains; P<sub>nsi</sub>, Pairwise nucleotide sequence identity (%); N<sub>a</sub>, number of alleles; P<sub>s</sub>, number of polymorphic sites; A<sub>d</sub>, allelic diversity; π, nucleotide diversity; D, average number of nucleotide differences between sequences.

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### Differences in metabolic requirements of *Coxiella*-like organisms and *C. burnetii*

We next compared the metabolic requirements of *Coxiella*-like organisms with those of *C. burnetii* by assessing their ability to replicate in both an axenic medium ACCM2 (mimicking the environment of the acidified lysosome-like vacuoles of phagocytes typically colonized by *C. burnetii*; [35]) and directly inside vertebrate host cells. First, ACCM2 was inoculated with *Coxiella*-like organisms extracted from eggs of either *O. maritimus*, *R. microplus* or *A. americanum*. Inoculated media were incubated for 10 days under standard conditions used to amplify *C. burnetii*. Although our *C. burnetii* positive controls readily replicated in the media, the *Coxiella* obtained from eggs of the three tick species did not grow. We then incubated egg homogenates from ticks of *O. maritimus* and *R. microplus* with mammalian cell cultures for seven days. Similar to results under axenic conditions, the incubation of vertebrate cell lines with *Coxiella*-like organisms failed to produce *Coxiella*-containing vacuoles, whereas the same cell lines incubated with *C. burnetii* under the same conditions were readily infected. The apparent inability to amplify tick-borne *Coxiella* through standardized protocols, well-characterized for *C. burnetii*, suggests that, despite their phylogenetic proximity, the *Coxiella*-like bacteria are adapted to radically different environments.



**Fig 2. Phylogenetic network with concatenated 16S rRNA, 23S rRNA, *GroEL*, *rpoB* and *dnaK* sequences (3009 unambiguously aligned bp), including 71 *Coxiella*-like strains of ticks, 15 *C. burnetii* reference strains, and bacterial outgroups.** The four *Coxiella* clades are labeled A to D. A zoom on the A clade which contains *C. burnetii* isolates is shown in Supplementary Fig C in S1 Text. Each number corresponds to one tick species as detailed in Table 1. Blue—*Coxiella*-like organisms; red—*C. burnetii*; green—*Rickettsiella*; black—other bacteria. All multi-locus typing of *Coxiella* and *Rickettsiella* of ticks are new sequences from this study. The scale bar is in units of substitution/site.

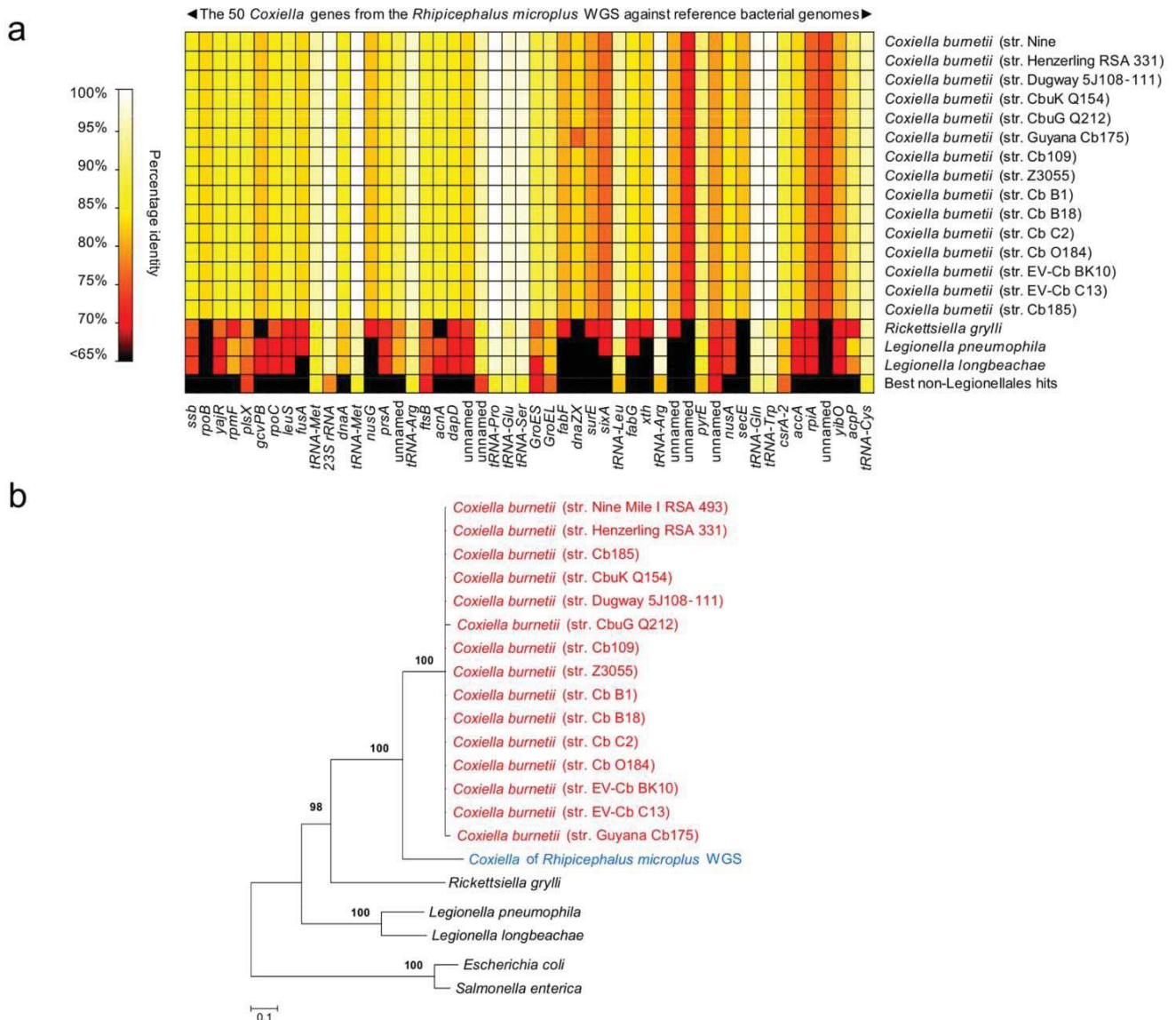
doi:10.1371/journal.ppat.1004892.g002

## Discussion

Since its original description, *C. burnetii* infections have been characterized in a wide variety of hosts. While only two species have been formally identified within the *Coxiella* genus, we show here that a far greater diversity of *Coxiella* exists in ticks. We detect the presence of *Coxiella*-like organisms in many more tick species than previously known [17,18,19,20,21,22,23,24,25] and describe a far wider genetic diversity among these bacteria than previously suspected. The incidence of *Coxiella*, as well as of its sister genus *Rickettsiella*, in ticks is exceptionally high, with approximately three quarters of tick species infected. Although possible tick-borne transmission of *C. burnetii* has been reported [1,2,8], none of the 43 new *Coxiella* genotypes identified here are identical to *C. burnetii*. We also demonstrate for genetically divergent *Coxiella* strains (i.e., members of the A, C and D clades) found in four tick species that infection is primarily transmitted maternally via the egg cytoplasm. These results converge to support the hypothesis that these *Coxiella*-like organisms are specific endosymbionts of ticks. Phylogenetic evidence further shows that one of the *Coxiella*-like organisms belonging to the A clade and primarily hosted by soft ticks has served as the progenitor of *C. burnetii*.

Three complementary lines of argument indicate a much longer evolutionary history for *Coxiella*-tick associations than for vertebrate-*Coxiella* associations. The first lies in the broad distribution of *Coxiella* and *Rickettsiella* bacteria across tick species, genera and families. The second concerns the extensive genetic diversity found in tick-borne *Coxiella* strains compared to *C. burnetii* strains, as illustrated by the clear subdivision of this genus into four highly divergent clades





**Fig 3. Characterization of new *Coxiella* strains derived from whole-genome sequencing (WGS) of the cattle tick *Rhipicephalus microplus*.** (a) Percent identity of 50 genes uniquely attributable to *Coxiella* from *R. microplus* WGS versus 15 sequenced *C. burnetii* genomes and other reference genomes. (b) Bacterial phylogeny, comprising the *Coxiella* found in the *R. microplus* WGS data, reconstructed from the concatenated sequences of Fig 3A (19,304 unambiguously aligned bp) using maximum-likelihood (ML). Branch numbers indicate percent bootstrap support for major branches (1,000 replicates; only bootstrap values >90% are shown). The scale bar is in units of substitution/site.

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(A-D). Finally, the clustering of all *C. burnetii* strains within one of the clades of tick-borne *Coxiella* shows that the ancestor of *C. burnetii* was a tick-associated bacterium which succeeded in infecting vertebrate cells. The remarkably low genetic diversity of *C. burnetii*, previously noted in other studies [36,37], indicates a unique and recent emergence of this highly infectious vertebrate pathogen. Interestingly, this hypothesis was initially raised a decade ago from observations of the profound differences in genome architecture of *C. burnetii* relative to other pathogenic intracellular bacteria [38]. It was again emphasized from the genome sequencing of new *C. burnetii*

strains [39]. Our data brings further support to this hypothesis by demonstrating that *C. burnetii* roots within the *Coxiella* phylogeny. Comparative genome sequences of *C. burnetii* [38, 39] and of the *Coxiella*-like organism from *A. americanum* [16] also suggest that *Coxiella* bacteria differ substantially in terms of genome size and gene content. The *C. burnetii* genome (A clade) has a size of ca 2Mb [38, 39], whereas the genome of the *Coxiella*-like organism isolated in *A. americanum* (D clade) is only about a 1/3 of this size (ie. 0.66 Mb) with a large percentage of missing genes [16]. This reduction in genome size may limit the transition to pathogenicity, and suggests that some *Coxiella*-like organisms may have evolved towards exclusive and irreversibly specialized interactions with their tick hosts. Overall, the diversity of genome sizes emphasizes that members of the different *Coxiella* clades may have retained a variety of evolutionary strategies to favour their spread and persistence in their hosts.

We identified *Coxiella* as a major emerging clade of bacterial endosymbionts allied to ticks. *Coxiella*-like organisms are maternally-transmitted through the egg cytoplasm at high frequency with 98–100% mother-to-offspring transmission, a pattern also reported in previous studies [20,21,26]. This transmission pattern is the rule for a variety of bacterial endosymbionts that live exclusively within arthropod cells [28,29,40]. While some, like *Wolbachia*, are globally common symbionts estimated to infect ca. 40% of insect species [41,42], others are globally rare, but common and important in particular arthropod groups [28]. This is precisely the case for *Coxiella*-like organisms; although they have not been found in other arthropod species, they are commonly associated with ticks. This leads to the obvious question of the phenotypic consequences of *Coxiella*-tick interactions. In some cases, *Coxiella*-like organisms of ticks likely act as obligate mutualistic symbionts required to support normal tick development, potentially provisioning their hosts with essential nutrients absent in vertebrate blood [16,20,21,26,27]. The ubiquity of *Coxiella* in some tick groups—such as in the *Rhipicephalus* genus in which infection is at fixation—corroborates the hypothesis of an obligate endosymbiont. This is not, however, the case for all tick species since some, such as *I. ricinus* and *I. uriae*, harbour *Coxiella*-like organisms at much lower frequencies. In these tick species, *Coxiella* is more likely to behave as a conditional mutualist—i.e., that confers advantages under certain environmental conditions—or as a reproductive parasite—i.e., that manipulates host reproduction toward the production of daughters (the transmitting sex), as commonly observed in arthropods with a variety of facultative symbionts [28,40]. It should also be noted that other endosymbionts also occur in ticks and may have evolved under complex multispecific interactions [17,24]. For instance, whereas the soft tick *O. moubata* was not found to be infected by a *Coxiella*-like organism in the present study, this tick species has been found to be infected by an endosymbiont belonging to the *Francisella* genus [17]. Endosymbionts other than *Coxiella* may thus interact with ticks, a pattern suggesting that endosymbiotic systems can be dynamic across tick lineages. These different hypotheses will now require specific testing.

Another question remains concerning the degree of vertebrate infection risk by the *Coxiella*-like organisms of ticks. Ticks are found worldwide and blood-feed on many different hosts; a combination of traits that may facilitate tick-to-vertebrate transfers of *Coxiella*. However, the bacteria observed in this study seem confined to ticks and, to our knowledge, none have ever been isolated from a vertebrate or associated with clinical symptoms. This suggests that these tick-associated bacteria currently pose a much lower infection risk to vertebrates than *C. burnetii*. As discussed above, the genome reduction of the *Coxiella*-like organism isolated in *A. americanum*, with the lack of nearly all the genes associated with pathogenicity [16], corroborates this view. Moreover, the inability to grow tick-borne bacteria in vertebrate cells highlights the significant barrier that must be overcome by the bacteria to successively achieve tick-to-vertebrate transmission. This type of transmission may, nonetheless, occasionally occur; an avian *Coxiella*-like organism was recently reported to induce fatal systematic



infections in domestic birds [43,44,45]. A very similar infection pattern was found for another maternally inherited endosymbiont, *Arsenophonus*, a widespread bacterium in different insect groups [41,46,47,48,49]. In particular, some *Arsenophonus* strains were detected in the phloem of plants fed on by infected phytophagous insects and were assumed to be opportunistic plant pathogens [50]. In such cases, the plant host may act as an ecological arena for the global exchange of endosymbionts like *Arsenophonus*, serving as a possible intermediate host for the horizontal transfer of bacteria among insect species [48]. In the case of *Coxiella*-like organisms, the extent of exchange between different tick species via the vertebrate host is yet to be established, but could be favoured by tick co-feeding (ticks feeding in close proximity on the host). The genetic similarity between *Coxiella*-like organisms found in unrelated tick species highlights the capacity to shift tick host species. Future research is now needed to assess the potential of different *Coxiella*-like organisms to infect vertebrates.

The reasons why *C. burnetii* is a highly virulent pathogen of vertebrates, but not *Coxiella*-like organisms (especially those from the A clade) remain unknown. As an intracellular pathogen with airborne transmission, *C. burnetii* has evolved specific mechanisms to survive in the abiotic environment, as well as to infect and exploit vertebrate cells [15]. Several evolutionary pathways may explain the acquisition of the genetic material necessary for this major lifestyle transition; this includes spontaneous genetic mutations in the genome of a tick-*Coxiella* ancestor, or the more likely transfer and integration of virulence genes from a co-infecting pathogen. The opportunity of gene transfer among bacteria, irrespective of their pathogenic or symbiotic properties, relies on their frequent co-occurrence within the same tick host [25,51,52]. The *Coxiella*-like organisms of the A clade may have dynamic genomes as observed in many arthropod symbionts: although they reside in confined intracellular environments, arthropod symbionts commonly experience variable degrees of recombination and gene transfer [53,54,55,56,57]. These gene transfers have served as immediate and powerful mechanisms of rapid adaptation in many endosymbionts, such as *Wolbachia* [56] and *Hamiltonella* [55,57]. This mechanism may explain the evolutionary transition from a *Coxiella* tick-symbiont of the A clade to the vertebrate pathogen *C. burnetii*. Other genetic connections are also possible; several *C. burnetii* genes that may contribute to major virulence traits, such as tissue tropism, are similar to eukaryotic genes and may have been acquired through lateral gene transfers from eukaryotes [38,39]. Detailed studies of virulence genes in *C. burnetii* and their homology with *Coxiella*-like organisms of the A clade will now be necessary to understand the remarkable emergence of the Q fever agent.

The evolutionary transition observed within the *Coxiella* genus is one of the rare cases reported to date of an arthropod-inherited symbiont evolving metabolic adaptations leading to the emergence of a vertebrate infectious disease. Another such transition occurred in the *Rickettsia* genus. The best-known members of this genus are transmitted by blood-feeding arthropods and are pathogenic in the vertebrate host. However, in recent years, many maternally-inherited *Rickettsia* endosymbionts found exclusively in arthropods have been discovered [58,59]. The examination of the evolutionary history of the *Rickettsia* genus revealed that this bacterium originated from endosymbionts of invertebrates and only secondarily became vertebrate pathogens [58,59]. Like *Coxiella*, some *Rickettsia* species of blood-feeding hosts have undergone a horizontal transmission through a vertebrate host, leading to pathogen emergence. Other bacteria, such as *Arsenophonus* [47,48] and *Sodalis* [60] may have had similar life cycle transitions, but the case of *Coxiella* is unique in that the arthropod host is no longer required to complete its life cycle.

In conclusion, we show that *C. burnetii* arose from a rare and recent event: the evolutionary transformation of a maternally inherited endosymbiont of ticks into a specialized and virulent pathogen of vertebrates. This raises a series of exciting questions related to both how *Coxiella*

endosymbionts made the major evolutionary transition leading to the emergence of Q fever and their role in the population dynamics of ticks. Identifying the evolutionary processes that transform symbiotic bacteria into emerging pathogens will require further exploration into the biology of the entire *Coxiella* genus.

## Methods

### Tick collection

The examined specimens represent the two main tick families, nine genera, 58 species and 112 populations from around the world (Table 1). Field specimens were sampled on various host species belonging to major mammal and bird families or from their habitats. We also used specimens from laboratory colonies reared in captivity for at least three generations for six tick species (derived from field specimens collected in North America, South America, Africa and China). All samples were preserved in 70–90% ethanol at room temperature until use. Before storage, tick eggs collected under laboratory conditions were surface-sterilized with 2.6% sodium hypochlorite and 0.5% SDS for 1 min and washed with sterile water to avoid external bacterial contamination.

### *Coxiella* screening and typing

Tick DNA was individually extracted using the DNeasy Blood & Tissue Kit (QIAGEN) following manufacturer instructions. DNA template quality was systematically verified by PCR amplification of the 18S ribosomal RNA (18S rRNA) or the cytochrome oxidase 1 (*C01*) arthropod primers (Table A in S1 Text). Tick DNA samples were then tested for *Coxiella* presence using a nested PCR assay and sequencing of the *rpoB* gene using *Coxiella*-specific primers. The use of nested PCR was efficient at decreasing the probability of contamination from unwanted amplification products. Additional PCR assays on the 16S rRNA, 23S rRNA, *GroEL* and *dnaK* genes were conducted on a subsample of *Coxiella*-positive tick DNA to obtain additional DNA sequences for phylogenetic analyses. We used 15 recently published genomes of *C. burnetii* (mainly isolated from humans and ruminants) and the genome of *Rickettsiella grylli* from woodlice (listed in Table B in S1 Text) as references to design PCR primers. The efficiency of our typing method was ascertained through positive PCR amplification and clear sequences for the five loci in four cultured reference strains of *C. burnetii* (Table B in S1 Text). Gene features, primers and PCR conditions are detailed in Table A in S1 Text. All PCR products were visualized through electrophoresis in a 1.5% agarose gel. Positive PCR products were purified and sequenced in both directions (EUROFINS). The chromatograms were manually inspected and cleaned with CHROMAS LITE ([http://www.technelysium.com.au/chromas\\_lite.html](http://www.technelysium.com.au/chromas_lite.html)) and sequence alignments were done using CLUSTALW [61], both implemented in MEGA [62].

*Coxiella* sequences were also searched for in the whole genome sequence (WGS) data of *R. microplus* and *I. scapularis* (GenBank accession numbers ADMZ02000000 and ABJB000000000, respectively) using the 1,995,281 bp *C. burnetii* genome (str. Nine Mile I RSA 493, GenBank accession number NC002971) as a probe and the Basic Local Alignment Search Tool (BLAST) with default parameters. Table C in S1 Text reports the number and content of *Coxiella* contigs that were detected in the *R. microplus* WGS data.

### Molecular and phylogenetic analyses

The GBLOCKS program [63] with default parameters was used to remove poorly aligned positions and to obtain non-ambiguous sequence alignments. Sequences of individual genes that differed by one or more nucleotides were assigned distinct allele numbers using DNASP [64],



with the option of excluding sites with alignment gaps and/or missing data. Tick-borne *Coxiella* strains are defined as each unique combination of alleles. The genetic diversity estimates ( $P_s$ , number of polymorphic sites;  $A_d$ , allelic diversity;  $\pi$ , nucleotide diversity;  $D$ , average number of nucleotide differences between sequences) were computed using DNASP. Other statistical analyses were carried out using the R statistical package. All sequence alignments were checked for putative recombinant regions using the GENECONV [65] and RDP [66] methods available in the RDP3 computer analysis package [67].

Phylogenetic analyses were based on single and concatenated sequences of the five bacterial genes used in the multi-locus typing scheme and on the 50 *Coxiella* genes found in the *R. microplus* WGS data. Sequence alignments included *Coxiella* and *Rickettsiella* sequences obtained in this study from tick DNA, as well as sequences available in GenBank from reference strains of *C. burnetii*, *Rickettsiella grylli*, *Legionella pneumophila*, *L. longbeachae*, and two more distantly related bacteria, *Escherichia coli* and *Salmonella enterica* (Table B in S1 Text). The evolutionary models fitting the sequence data most closely were determined using the Akaike information criterion with the program MEGA. For each data set examined, the best-fit approximation was the general time reversible model with gamma distribution and invariant sites (GTR+G+I). Network-based phylogenetic analyses were done using SplitsTree, implementing the evolutionary model under the agglomerating NeighborNet algorithm [68]. Tree-based phylogenetic analyses were done using maximum-likelihood (ML) analyses. A ML heuristic search using a starting tree obtained by neighbor-joining was conducted in MEGA. Clade robustness was assessed by bootstrap analysis using 1,000 replicates.

## Culture assays

We first assessed the ability of tick-borne *Coxiella* to replicate in an axenic medium as follows. Tick eggs were surface-sterilized as described above and homogenized by hand in sterile water. Eggs homogenates were used to inoculate 2ml of the axenic medium ACCM2 [35] and incubated three days in a humidified atmosphere of 5% CO<sub>2</sub> and 2.5% O<sub>2</sub> at 37°C. 50µl of each culture were then diluted in 2ml of fresh ACCM2 and further incubated under the same conditions for 10 days to assess bacterial growth. We then assessed the ability of tick-borne *Coxiella* to replicate inside vertebrate host cells as follows. Surface-sterilized tick eggs were homogenized by hand in 1 ml of 10% Foetal Bovine Serum (FBS) supplemented MEM medium (GIBCO). The homogenate (0.5 ml) was diluted in 25 ml of 10% SVF-MEM and centrifuged at 4000 rpm (2000g) at 4°C for 30 min. Ten ml of the supernatant was mixed with 10 ml of 10% FSB-MEM and again centrifuged at 2000g at 4°C for 30 min. Ten ml of the supernatant was harvested and filtered through a sterile 0.45 µm pore size filter (MILLIPORE). Two flasks containing confluent Sheep Foetal Thymus cells (SFT) cells were inoculated with 5 ml of the obtained filtrate and incubated at 35°C and allowed to grow for 12 weeks. Cell culture flasks were observed daily for the presence of contamination or growth signs such as vacuoles containing *Coxiella*, during the first week then once a week. As a positive control, a homogenate of *C. burnetii* was used following the same protocol.

## Accession codes

Nucleotide sequences of PCR-amplified fragments of tick-borne *Coxiella* and *Rickettsiella* genes have been deposited in the GenBank nucleotide database under accession codes KP994768-KP994862 (16S rRNA), KP994678-KP994767 (23S rRNA), KP985445-KP985537 (*GroEL*), KP985265-KP985357 (*rpoB*) and KP985358-KP985444 (*dnaK*).

## Supporting Information

**S1 Text. Table A.** Genes and primers used in polymerase chain reaction (PCR) assays to detect *Coxiella* and relatives and to control tick DNA quality. The same primers were used for the *Coxiella* and *Rickettsiella* typing, with the exception of the 16S rRNA gene for which different primers were designed for the two bacteria. Nested PCR amplifications (16S rRNA, 23S rRNA, *GroEL*, *rpoB* and *dnaK*) were performed as follows: the first PCR run with the external primers was performed in a 10  $\mu$ L volume containing 20–50 ng of genomic DNA, 3 mM of each dNTP (Thermo Scientific), 8 mM of MgCl<sub>2</sub> (Roche Diagnostics), 3  $\mu$ M of each primer, 1  $\mu$ L of 10 $\times$  PCR buffer (Roche Diagnostics), and 0.5 U of Taq DNA polymerase (Roche Diagnostics). A 1- $\mu$ L aliquot of the PCR product from the first reaction was then used as a template for the second round of amplification. The second PCR was performed in a total volume of 25  $\mu$ L and contained 8 mM of each dNTP (Thermo Scientific), 10 mM of MgCl<sub>2</sub> (ThermoScientific), 7.5  $\mu$ M of each of the internal primers, 2.5  $\mu$ L of 10 $\times$ PCR buffer (Thermo Scientific), and 1.25 U of Taq DNA polymerase (Thermo Scientific). Non-nested PCR amplifications (*CO1* and *18S rRNA*) were performed following conditions similar to the first PCR run used in the nested PCR assays. All PCR amplifications were performed under the following conditions: initial denaturation at 93°C for 3 min, 35 cycles of denaturation (93°C, 30 s), annealing ( $T_m = 50$ –56°C, depending on primers, 30 s), extension (72°C, 1–2 min), and a final extension at 72°C for 5 min. **Table B.** List, biological features and GenBank accession numbers of the bacterial strains used as references in molecular and phylogenetic analyses.\* reference strains of *C. burnetii* used for primer testing. **Table C.** List, sequence accession numbers and features of the 31 *Coxiella* contigs from the whole-genome shotgun sequencing (WGS) of the cattle tick *Rhipicephalus microplus*. **Fig A.** Map of the *Coxiella burnetii* genome (strain Nine Mile I RSA 493) showing the position of the genetic markers (in blue) used in this study. The arrows indicate the position along the chromosome of the five housekeeping genes (16S rRNA, 23S rRNA, *GroEL*, *rpoB* and *dnaK*) used in the multi-locus typing of tick-borne *Coxiella* infections. The numbered boxes (1–31) indicate the position of the 31 *Coxiella* contigs (listed in Table C in S1 Text) detected from the whole genome sequencing of the hard tick *Rhipicephalus microplus*. **Fig B.** *Coxiella* and *Rickettsiella* phylogeny constructed using maximum-likelihood (ML) estimations based on 16S rRNA, 23S rRNA, *GroEL*, *rpoB* and *dnaK* concatenated sequences (3009bp), including 71 *Coxiella*-like strains of ticks, 15 *C. burnetii* reference strains and outgroups. The four *Coxiella* clades are labeled A to D. Each number corresponds to one tick species as detailed in Table 1. Blue, *Coxiella*-line organisms; red, *C. burnetii*; green, *Rickettsiella*; black, other bacteria. All multi-locus typing of tick-borne *Coxiella* and *Rickettsiella* of ticks are new sequences from this study. Branch numbers indicate percentage bootstrap support for major branches (1000 replicates; only bootstrap values >90% are shown). **Fig C.** Inset of *Coxiella* network from Fig 2 with focus on the A clade (*Coxiella* of soft ticks and *C. burnetii*). Each number corresponds to one tick species as detailed in Table 1. Blue, *Coxiella*-line organisms; red, *C. burnetii*. The scale bar is in units of substitution/site. (DOCX)

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## Author Contributions

Conceived and designed the experiments: OD CC. Performed the experiments: OD VN JC OM. Analyzed the data: OD CC. Contributed reagents/materials/analysis tools: OD VN KDM MB KSB OM FV LZ EJ PD CA FR JFT ASB JC MDi EL AA MDu EGD GD GKD HA SZ LV CC. Wrote the paper: OD KDM CC.

## References

1. Madariaga MG, Rezaei K, Trenholme GM, Weinstein RA. Q fever: a biological weapon in your backyard. *Lancet Infect Dis*. 2003; 3: 709–721. PMID: 14592601
2. Raoult D, Marrie T, Mege J. Natural history and pathophysiology of Q fever. *Lancet Infect Dis*. 2005; 5: 219–226. PMID: 15792739
3. Vanderburg S, Rubach MP, Halliday JE, Cleaveland S, Reddy EA, Crump JA. Epidemiology of *Coxiella burnetii* infection in Africa: a OneHealth systematic review. *PLoS Negl Trop Dis*. 2014; 8: e2787. doi: 10.1371/journal.pntd.0002787 PMID: 24722554
4. Angelakis E, Raoult D. Q Fever. *Vet Microbiol*. 2010; 140: 297–309. doi: 10.1016/j.vetmic.2009.07.016 PMID: 19875249
5. Bewley KR. Animal models of Q fever (*Coxiella burnetii*). *Comp Med*. 2013; 63: 469–476. PMID: 24326221
6. van der Hoek W, Dijkstra F, Schimmer B, Schneeberger PM, Vellema P, Wijkmans C, et al. Q fever in the Netherlands: an update on the epidemiology and control measures. *Euro Surveill* 2010; 15.
7. Russell-Lodrigue KE, Andoh M, Poels MWJ, Shive HR, Weeks BR, Zhang GQ, et al. *Coxiella burnetii* isolates cause genogroup-specific virulence in mouse and guinea pig models of acute Q fever. *Infect Immun*. 2009; 77: 5640–5650. doi: 10.1128/IAI.00851-09 PMID: 19786560
8. McDade JE. Historical aspects of Q fever. In: Marie TJ, editor. *Q Fever Volume 1: The Disease*. Boca Raton: CRC Press; 1990. pp. 5–21.
9. Oyston PC, Davies C. Q fever: the neglected biothreat agent. *J Med Microbiol*. 2011; 60: 9–21. doi: 10.1099/jmm.0.024778-0 PMID: 21030501
10. Weisburg WG, Dobson ME, Samuel JE, Dasch GA, Mallavia LP, Baca O, et al. Phylogenetic diversity of the Rickettsiae. *J Bacteriol*. 1989; 171: 4202–4206. PMID: 2753854
11. Leclercq A, Kleespies RG. Type IV secretion system components as phylogenetic markers of entomopathogenic bacteria of the genus *Rickettsiella*. *FEMS Microbiol Ecol*. 2008; 279: 167–173. doi: 10.1111/j.1574-6968.2007.01025.x PMID: 18179586
12. Bouchon D, Cordaux R, Grève P. *Rickettsiella*, intracellular pathogens of arthropods. In: Zchori-Fein E, Bourtzis K, editors. *Manipulative Tenants*. Boca Raton: CRC Press; 2012. pp. 127–148.
13. Tsuchida T, Koga R, Horikawa M, Tsunoda T, Maoka T, Matsumoto S, et al. Symbiotic bacterium modifies aphid body color. *Science* 2010; 330: 1102–1104. doi: 10.1126/science.1195463 PMID: 21097935
14. Tan CK, Owens L. Infectivity, transmission and 16S rRNA sequencing of a rickettsia, *Coxiella cheraxi* sp. nov., from the freshwater crayfish *Cherax quadricarinatus*. *Dis Aquat Organ*. 2000; 41: 115–122. PMID: 10918979
15. van Schaik EJ, Chen C, Mertens K, Weber MM, Samuel JE. Molecular pathogenesis of the obligate intracellular bacterium *Coxiella burnetii*. *Nat Rev Microbiol*. 2013; 11: 561–573. doi: 10.1038/nrmicro3049 PMID: 23797173
16. Smith TA, Driscoll T, Gillespie JJ, Raghavan R. A *Coxiella*-like Endosymbiont is a potential vitamin source for the Lone Star Tick. *Genome Biol Evol*. 2015; 7: 831–838. doi: 10.1093/gbe/evv016 PMID: 25618142
17. Noda H, Munderloh UG, Kurtti TJ. Endosymbionts of ticks and their relationship to *Wolbachia* spp. and tick-borne pathogens of humans and animals. *Appl Environ Microbiol*. 1997; 63: 3926–3932. PMID: 9327557
18. Almeida AP, Marcili A, Leite RC, Nieri-Bastos FA, Domingues LN, Ricardo Martins J, et al. *Coxiella* symbiont in the tick *Ornithodoros rostratus* (Acari: Argasidae). *Ticks Tick Borne Dis*. 2012; 3: 203–206. doi: 10.1016/j.ttbdis.2012.02.003 PMID: 22480930



19. Duron O, Jourdain E, McCoy KD. Diversity and global distribution of the *Coxiella* intracellular bacterium in seabird ticks. *Ticks Tick Borne Dis.* 2014; 5: 557–563. doi: 10.1016/j.ttbdis.2014.04.003 PMID: 24915875
20. Klyachko O, Stein BD, Grindle N, Clay K, Fuqua C. Localization and visualization of a *Coxiella*-type symbiont within the lone star tick, *Amblyomma americanum*. *Appl Environ Microbiol.* 2007; 73: 6584–6594. PMID: 17720830
21. Machado-Ferreira E, Dietrich G, Hojgaard A, Levin M, Piesman J, Zeidner NS, et al. *Coxiella* symbionts in the Cayenne tick *Amblyomma cajennense*. *Microb Ecol.* 2011; 62: 134–142. doi: 10.1007/s00248-011-9868-x PMID: 21611689
22. Lalzar I, Harrus S, Mumcuoglu KY, Gottlieb Y. Composition and seasonal variation of *Rhipicephalus turanicus* and *Rhipicephalus sanguineus* bacterial Communities. *Appl Environ Microbiol.* 2012; 78: 4110–4116. doi: 10.1128/AEM.00323-12 PMID: 22467507
23. Jasinskas A, Zhong J, Barbour AG. Highly prevalent *Coxiella* sp. bacterium in the tick vector *Amblyomma americanum*. *Appl Environ Microbiol.* 2007; 73: 334–336. PMID: 17085709
24. Clay K, Klyachko O, Grindle N, Civitello D, Oleske D, Fuqua C. Microbial communities and interactions in the lone star tick, *Amblyomma americanum*. *Mol Ecol.* 2008; 17: 4371–4381. PMID: 19378409
25. Wilkinson DA, Dietrich M, Lebarbenchon C, Jaeger A, Le Rouzic C, Lagadec E, et al. Massive infection of seabird ticks with bacterial species related to *Coxiella burnetii*. *Appl Environ Microbiol.* 2014; 80: 3327–3333. doi: 10.1128/AEM.00477-14 PMID: 24657860
26. Lalzar I, Friedmann Y, Gottlieb Y. Tissue tropism and vertical transmission of *Coxiella* in *Rhipicephalus sanguineus* and *Rhipicephalus turanicus* ticks. *Environ Microbiol.* 2014; 16: 3657–3668. doi: 10.1111/1462-2920.12455 PMID: 24650112
27. Zhong J, Jasinskas A, Barbour AG. Antibiotic treatment of the tick vector *Amblyomma americanum* reduced reproductive fitness. *PLoS One.* 2007; 2: e405. PMID: 17476327
28. Moran NA, McCutcheon JP, Nakabachi A. Genomics and evolution of heritable bacterial symbionts. *Annu Rev Genet.* 2008; 42: 165–190. doi: 10.1146/annurev.genet.41.110306.130119 PMID: 18983256
29. Wernegreen JJ. Endosymbiosis. *Curr Biol.* 2012; 22: R555–561. doi: 10.1016/j.cub.2012.06.010 PMID: 22835786
30. Hosokawa T, Koga R, Kikuchi Y, Meng XY, Fukatsu T. *Wolbachia* as a bacteriocyte-associated nutritional mutualist. *Proc Natl Acad Sci USA.* 2010; 107: 769–774. doi: 10.1073/pnas.0911476107 PMID: 20080750
31. Akman L, Yamashita A, Watanabe H, Oshima K, Shiba T, Hattori M, et al. Genome sequence of the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia*. *Nat Genet.* 2002; 32: 402–407. PMID: 12219091
32. Husnik F, Chrudimsky T, Hypsa V. Multiple origins of endosymbiosis within the Enterobacteriaceae (gamma-Proteobacteria): convergence of complex phylogenetic approaches. *BMC Biol.* 2011; 9: 87. doi: 10.1186/1741-7007-9-87 PMID: 22201529
33. Gomez-Valero L, Rusniok C, Buchrieser C. *Legionella pneumophila*: population genetics, phylogeny and genomics. *Infect Genet Evol.* 2009; 9: 727–739. doi: 10.1016/j.meegid.2009.05.004 PMID: 19450709
34. Leclercq A, Kleespies RG. A *Rickettsiella* bacterium from the hard tick, *Ixodes woodi*: molecular taxonomy combining multilocus sequence typing (MLST) with significance testing. *PLoS One.* 2012; 7: e38062. doi: 10.1371/journal.pone.0038062 PMID: 22675436
35. Omsland A, Beare PA, Hill J, Cockrell DC, Howe D, Virtaneva K, et al. Isolation from animal tissue and genetic transformation of *Coxiella burnetii* are facilitated by an improved axenic growth medium. *Appl Environ Microbiol.* 2011; 77: 3720–3725. doi: 10.1128/AEM.02826-10 PMID: 21478315
36. Sekeyová Z, Roux V, Raoult D. Intraspecies diversity of *Coxiella burnetii* as revealed by *com1* and *mucZ* sequence comparison. *FEMS Microbiol Lett.* 1999; 180: 61–67. PMID: 10547445
37. Pearson T, Hornstra HM, Sahl JW, Schaack S, Schupp JM, Beckstrom-Sternberg SM, et al. When outgroups fail; phylogenomics of rooting the emerging pathogen, *Coxiella burnetii*. *Syst Biol.* 2013; 62: 752–762. doi: 10.1093/sysbio/syt038 PMID: 23736103
38. Seshadri R, Paulsen IT, Eisen JA, Read TD, Nelson KE, Nelson WC, et al. Complete genome sequence of the Q-fever pathogen *Coxiella burnetii*. *Proc Natl Acad Sci USA.* 2003; 100: 5455–5460. PMID: 12704232
39. Beare PA, Unsworth N, Andoh M, Voth DE, Omsland A, Gilk SD, et al. Comparative genomics reveal extensive transposon-mediated genomic plasticity and diversity among potential effector proteins within the genus *Coxiella*. *Infect Immun.* 2009; 77: 642–656. doi: 10.1128/IAI.01141-08 PMID: 19047403

40. Engelstadter J, Hurst GDD. The ecology and evolution of microbes that manipulate host reproduction. *Annu Rev Ecol Evol Syst.* 2009; 40: 127–149.
41. Duron O, Bouchon D, Boutin S, Bellamy L, Zhou L, Engelstadter J, et al. The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone. *BMC Biol.* 2008; 6: 27. doi: 10.1186/1741-7007-6-27 PMID: 18577218
42. Zug R, Hammerstein P. Still a host of hosts for *Wolbachia*: analysis of recent data suggests that 40% of terrestrial arthropod species are infected. *PLoS One.* 2012; 7: e38544. doi: 10.1371/journal.pone.0038544 PMID: 22685581
43. Shivaprasad HL, Cadenas MB, Diab SS, Nordhausen R, Bradway D, Crespo R, et al. *Coxiella*-like infection in psittacines and a toucan. *Avian Dis.* 2008; 52: 426–432. PMID: 18939630
44. Woc-Colburn AM, Garner MM, Bradway D, West G, D'Agostino J, Trupkiewicz J, et al. Fatal coxiellosis in Swainson's Blue Mountain Rainbow Lorikeets (*Trichoglossus haematodus moluccanus*). *Vet Pathol.* 2008; 45: 247–254. doi: 10.1354/vp.45-2-247 PMID: 18424842
45. Vapniarsky N, Barr BC, Murphy B. Systemic *Coxiella*-like infection with myocarditis and hepatitis in an eclectus parrot (*Eclectus roratus*). *Vet Pathol.* 2012; 49: 717–722. doi: 10.1177/0300985811409251 PMID: 21712515
46. Duron O, Wilkes TE, Hurst GDD. Interspecific transmission of a male-killing bacterium on an ecological timescale. *Ecol Lett.* 2010; 13: 1139–1148. doi: 10.1111/j.1461-0248.2010.01502.x PMID: 20545734
47. Duron O, Schnepat UE, Berthomieu A, Goodman SE, Droz B, Paupy C, et al. Origin, acquisition and diversification of heritable bacterial endosymbionts in louse flies and bat flies. *Mol Ecol.* 2014; 23: 2105–2017. doi: 10.1111/mec.12704 PMID: 24612422
48. Jouselin E, Coeur d'Acier A, Vanlerberghe-Masutti F, Duron O. Evolution and diversity of *Arsenophonus* endosymbionts in aphids. *Mol Ecol.* 2013; 22: 260–270. doi: 10.1111/mec.12092 PMID: 23106652
49. Novakova E, Hypsa V, Moran NA. *Arsenophonus*, an emerging clade of intracellular symbionts with a broad host distribution. *BMC Microbiol.* 2009; 9: 143. doi: 10.1186/1471-2180-9-143 PMID: 19619300
50. Bressan A. Emergence and evolution of *Arsenophonus* bacteria as insect-vectorized plant pathogens. *Infect Genet Evol.* 2014; 22: 81–90. doi: 10.1016/j.meegid.2014.01.004 PMID: 24444593
51. Benson MJ, Gawronski JD, Eveleigh DE, Benson DR. Intracellular symbionts and other bacteria associated with deer ticks (*Ixodes scapularis*) from Nantucket and Wellfleet, Cape Cod, Massachusetts. *Appl Environ Microbiol.* 2004; 70: 616–620. PMID: 14711698
52. van Overbeek L, Gassner F, van der Plas CL, Kastelein P, Nunes-da Rocha U, Takken W. Diversity of *Ixodes ricinus* tick-associated bacterial communities from different forests. *FEMS Microbiol Ecol.* 2008; 66: 72–84. doi: 10.1111/j.1574-6941.2008.00468.x PMID: 18355299
53. Duron O. Lateral transfers of insertion sequences between *Wolbachia*, *Cardinium* and *Rickettsia* bacterial endosymbionts. *Heredity* 2013; 111: 330–337. doi: 10.1038/hdy.2013.56 PMID: 23759724
54. Baldo L, Werren JH. Revisiting *Wolbachia* supergroup typing based on WSP: spurious lineages and discordance with MLST. *Curr Microbiol.* 2007; 55: 81–87. PMID: 17551786
55. Degnan PH, Moran NA. Evolutionary genetics of a defensive facultative symbiont of insects: exchange of toxin-encoding bacteriophage. *Mol Ecol.* 2008; 17: 916–929. doi: 10.1111/j.1365-294X.2007.03616.x PMID: 18179430
56. Nikoh N, Hosokawa T, Moriyama M, Oshima K, Hattori M, Fukatsu T. Evolutionary origin of insect-*Wolbachia* nutritional mutualism. *Proc Natl Acad Sci USA.* 2014; 111: 10257–10262. doi: 10.1073/pnas.1409284111 PMID: 24982177
57. Duron O. *Arsenophonus* insect symbionts are commonly infected with APSE, a bacteriophage involved in protective symbiosis. *FEMS Microbiol Ecol.* 2014; 90: 184–194. doi: 10.1111/1574-6941.12381 PMID: 25041857
58. Perlman SJ, Hunter MS, Zchori-Fein E. The emerging diversity of *Rickettsia*. *Proc R Soc Lond B Biol Sci.* 2006; 273: 2097–106.
59. Weinert LA, Werren JH, Aebi A, Stone GN, Jiggins FM. Evolution and diversity of *Rickettsia* bacteria. *BMC Biol.* 2009; 7: 6. doi: 10.1186/1741-7007-7-6 PMID: 19187530
60. Clayton AL, Oakeson KF, Gutin M, Pontes A, Dunn DM, von Niederhausern AC, et al. A novel human-infection-derived bacterium provides insights into the evolutionary origins of mutualistic insect–bacterial symbioses. *PLoS Genet.* 2012; 8: e1002990. doi: 10.1371/journal.pgen.1002990 PMID: 23166503
61. Thompson JD, Gibson TJ, Higgins DG. Multiple sequence alignment using ClustalW and ClustalX. *Curr Protoc Bioinformatics* 2002; 2: 3. doi: 10.1002/0471250953.bi0203s00 PMID: 18792934
62. Kumar S, Tamura K, Nei M. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform.* 2004; 5: 150–163. PMID: 15260895

63. Castresana J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol.* 2000; 17: 540–552. PMID: [10742046](#)
64. Librado P, Rozas J. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 2009; 25: 1451–1452. doi: [10.1093/bioinformatics/btp187](#) PMID: [19346325](#)
65. Sawyer SA. GENECONV: A computer package for the statistical detection of gene conversion. <http://www.math.wustl.edu/~sawyer>.
66. Martin D, Rybicki E. RDP: detection of recombination amongst aligned sequences. *Bioinformatics.* 2000; 16: 562–563. PMID: [10980155](#)
67. Martin D, Lemey P, Lott M, Moulton V, Posada D, Lefevre P. RDP3: a flexible and fast computer program for analyzing recombination. *Bioinformatics.* 2010; 26: 2462–2463. doi: [10.1093/bioinformatics/btq467](#) PMID: [20798170](#)
68. Huson DH, Bryant D. Application of phylogenetic networks in evolutionary studies. *Mol Biol Evol.* 2006; 23: 254–267. PMID: [16221896](#)





## Annexe 2

Boulinier, T., Kada, S., Ponchon, A., Dupraz, M., Dietrich, M., Gamble, A., Bourret, V., Duriez, O., Bazire, R., Tornos, J., Tveraa, T., Chambert, T., Garnier, R., McCoy, K.D., 2016. Migration, Prospecting, Dispersal? What Host Movement Matters for Infectious Agent Circulation? *Integr. Comp. Biol.* icw015. doi:10.1093/icb/icw015







## SYMPOSIUM

### Migration, Prospecting, Dispersal? What Host Movement Matters for Infectious Agent Circulation?

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**Synopsis** Spatial disease ecology is emerging as a new field that requires the integration of complementary approaches to address how the distribution and movements of hosts and parasites may condition the dynamics of their interactions. In this context, migration, the seasonal movement of animals to different zones of their distribution, is assumed to play a key role in the broad scale circulation of parasites and pathogens. Nevertheless, migration is not the only type of host movement that can influence the spatial ecology, evolution, and epidemiology of infectious diseases. Dispersal, the movement of individuals between the location where they were born or bred to a location where they breed, has attracted attention as another important type of movement for the spatial dynamics of infectious diseases. Host dispersal has notably been identified as a key factor for the evolution of host–parasite interactions as it implies gene flow among local host populations and thus can alter patterns of coevolution with infectious agents across spatial scales. However, not all movements between host populations lead to dispersal per se. One type of host movement that has been neglected, but that may also play a role in parasite spread is prospecting, i.e., movements targeted at selecting and securing new habitat for future breeding. Prospecting movements, which have been studied in detail in certain social species, could result in the dispersal of infectious agents among different host populations without necessarily involving host dispersal. In this article, we outline how these various types of host movements might influence the circulation of infectious disease agents and discuss methodological approaches that could be used to assess their importance. We specifically focus on examples from work on colonial seabirds, ticks, and tick-borne infectious agents. These are convenient biological models because they are strongly spatially structured and involve relatively simple communities of interacting species. Overall, this review emphasizes that explicit consideration of the behavioral and population ecology of hosts and parasites is required to disentangle the relative roles of different types of movement for the spread of infectious diseases.

#### Introduction

Although migration, the seasonal movement of animals to different areas of their distribution, is assumed to play a key role in the broad scale circulation of parasites and pathogens (Altizer et al. 2011; Bauer and Hoyer 2014, Fritzsche McKay and Hoyer 2016), other types of host movements may

also come into play in determining eco-epidemiological patterns across spatial scales. In the context of increasing interest for integrative approaches to infectious disease biology (Daszak et al. 2000; Cleaveland et al. 2014), spatial disease ecology builds on a vast array of approaches to address how the distribution and movements of hosts and

parasites may condition the dynamics of their interactions within ecosystems (Hess et al. 2002; Holt and Boulinier 2005). The long-standing interest in the roles of host dispersal and spatially structured contact networks in the dynamics of epidemics and the persistence of infectious agents within host communities has led to the development of diverse statistical approaches (Diggle 2000; Keeling et al. 2004; Craft et al. 2009; Fenner et al. 2011), including the analysis of dispersal gradients and the spatial spread of parasites via travelling waves (Bjørnstad et al. 2002; Mundt et al. 2009). However, the role of different types of host movement in the spreading of diseases remains understudied (Boulinier et al. 2001; Perkins et al. 2009; Plowright et al. 2011, Blackwood et al. 2013). Strong heterogeneity in individual movement patterns can contribute considerably to heterogeneity in the transmission of infectious agents at different scales (Lloyd-Smith et al. 2005; McCallum et al. 2007). With the recent development of the field of movement ecology (Nathan et al. 2008; Morales et al. 2010; Fieberg et al. 2010; Jeltsch et al. 2013), the question of the potential role of non-migratory host movements for disease spread can increasingly include ecological and evolutionary dimensions. Movement ecology has notably stressed that key features in the movements of individuals can be linked to the life histories of the species considered and the landscapes in which they have evolved (Johnson et al. 1992; Boulinier et al. 2008; Morales et al. 2010), and that these features may have critical implications for the dynamics and evolution of subdivided populations (Pulliam 1988; Clobert et al. 2001; Hanski and Gaggiotti 2004; Bowler and Benton 2005).

In this article, after reviewing the importance of the spatial dimension of parasite circulation, we outline which type of host movements may especially matter for the dispersal and spread of infectious agents. We then focus on the relatively neglected case of prospecting movements, i.e., visits to breeding patches where an individual is not currently breeding, illustrating insights that have been gained on this topic through the study of seabird-parasites systems. After briefly reviewing other systems in which non-migratory movements may require particular attention, we outline timely research perspectives, potential study designs and available tools to examine questions related to the impact of these alternative types of movement. Here, we exclude human-mediated movements, which are also well known to lead to the transmission of infectious agents or their vectors over long distances (Cunningham 1996; Adams and Kapan 2009; Stoddard et al. 2009).

## Why does the spatial dimension of host-parasite interactions matter?

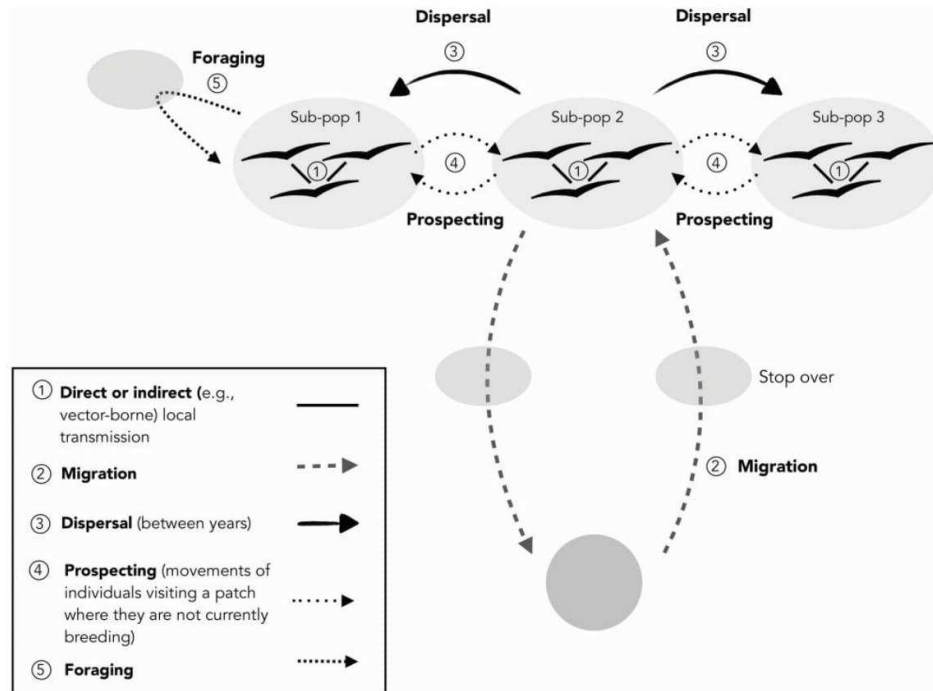
The spatial dimension is important for the epidemiology, ecology, and evolution of the interactions between infectious agents and their hosts. From an eco-epidemiological standpoint, the continental spread of West Nile Virus (Rappole et al. 2000) and *Mycoplasma gallisepticum* (Hochachka and Dhondt 2000) in susceptible bird populations, the northward expansion of Lyme disease in eastern North America (Leighton et al. 2012) or the rapid spread of Phocine Distemper Virus along European coastal areas (Harding et al. 2002) represent examples stressing the need to determine the factors contributing to the spatial dynamics of infectious agents. In the context of climate change, habitat quality can also change and new environments may become available for reservoir hosts, vectors and their parasites. Because of individual differences in exposure and susceptibility, the circulation and local persistence of infectious agents within and among host populations will depend on variation in individual movements and behaviors, as outlined by modeling approaches (Keeling and Rohani 2007; Plowright et al. 2011). Finally, because of effects they will have on host-parasite gene flow, the relative dispersal rates of hosts and parasites in spatially structured populations will also affect the coevolution of populations (Thompson 2005; Louhi et al. 2010; Mazé-Guilmo et al. 2016). These epidemiological, ecological, and evolutionary processes may all be at play when infectious agents can infect several host species; in that case, the spatial context of the interactions is also very likely to be important to consider, notably when reservoir species are to be identified and potential management decisions to be taken (Viana et al. 2014).

## What types of host movement are important for the spread of infectious disease agents?

### Migration

Migratory movements have been clearly identified as being important for the spread of infectious agents over broad spatial scales (Altizer et al. 2011; Bauer and Hoyer 2014). This is notable because of the large geographic scale they often encompass and the fact that they can contribute to the contact among individuals from various populations at remotely located stop-over and/or wintering sites (Fig. 1). Migration often occurs between high latitude areas, where reproduction takes place in the summer, and wintering





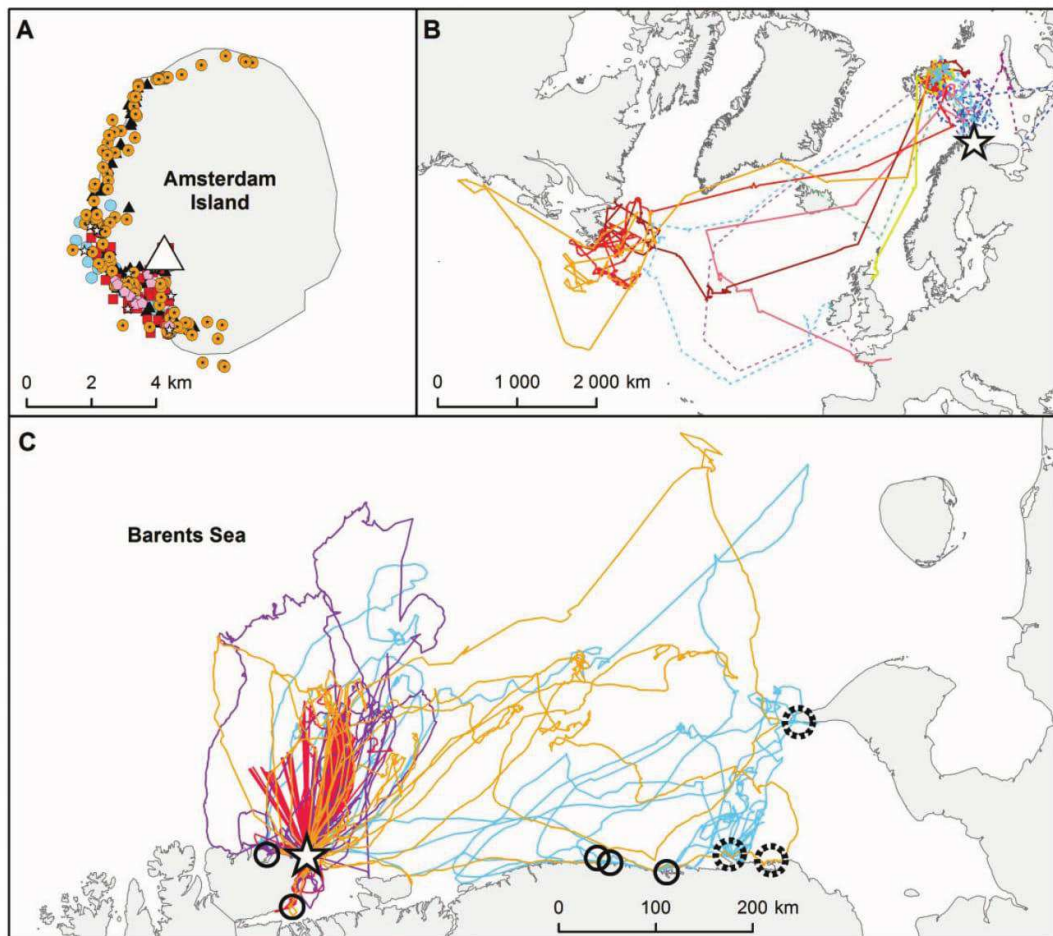
**Fig. 1** Different types of movements may lead to superspreading events. Such movements need to be made between locations where transmission among hosts can occur, thus they often involve the movements of infected hosts towards patches containing susceptible hosts (or the reverse). The timing of movements is also critical to consider in relation to opportunities for superspreading events. Migration as well as dispersal and foraging may be responsible for key spreading events in some cases, but a largely neglected type of movement, prospecting (i.e., visits among breeding groups within a season), may be a critical type of movement for the spatial epidemiology, ecology and evolution of host–parasite interactions, notably in social species.

areas at latitudes closer to the equator, although seasonal migratory movements may take various other forms (Dingle 2014). Migratory movements often involve large numbers of individuals that gather in high densities at sites where the transmission of infectious agents can occur between infected and susceptible individuals. Migration has thus been identified as a key process in the spread of both directly and environmentally transmitted agents (e.g., avian influenza viruses: Olsen et al. 2006; Gilbert et al. 2006, Hill et al. 2012), and vector-borne agents (e.g., tick-transmitted Lyme disease bacteria, Leighton et al. 2012; or mosquito-transmitted blood parasites, Fuller et al. 2012). Geographic and temporal characteristics of movements are important to consider in this context because the course of infection may simultaneously affect the likelihood of transmission and the ability of sick individuals to move long distances (Gaidet et al. 2010). The physiological stress associated with migration may also affect host susceptibility, and thus, potentially, disease transmission (Dingle 2014). The fact that a

disease involves migratory birds is nevertheless not sufficient in itself to assume that migratory behavior *per se* is the factor responsible for the spread of the infectious agent. Considering where and when transmission is likely to occur in relation to other types of movements and events may also be important (Fig. 1).

**Foraging**

Foraging movements can affect the spatial spread of many infectious agents if they are food-borne agents, trophically transmitted parasites, or directly transmissible when individuals compete for a focal food resource (Fig. 1). Although foraging movements usually occur at relatively much smaller scales than migratory movements, this is not always the case. Like many other seabirds, Scopoli’s shearwaters (*Calonectris borealis*) can forage several hundred kilometers from their colony; this is only a slightly smaller spatial scale than that of their winter migration (Péron and Grémillet 2013). Although such distances are impressive, it is unlikely that this mode of



**Fig. 2** Different types of movements potentially responsible for superspreading events: **(A)** Foraging locations of 6 Subantarctic skuas (*Catharacta antarctica*) recorded with GPS-UHF during the breeding season 2015 on Amsterdam Island, southern Indian Ocean. Over 3 days, the tracked individuals foraged close to their breeding colony (white triangle) among several marine vertebrate colonies they are preying upon and where they may disperse infectious agents; **(B)** Migratory movements of Black-legged kittiwakes (*Rissa tridactyla*) breeding on Hornøya, Norway (white star) recorded with satellite transmitters for the winter 2010–2011 (solid lines,  $n=5$ ) and 2011–2012 (dashed lines,  $n=3$ ). Despite such large scale movements, these pelagic seabirds are unlikely to contribute to the spread of infectious agents because most of them remain at sea during migration and wintering; **(C)** Movements of 4 failed breeding Black-legged kittiwakes recorded with GPS-UHF during the breeding season 2015. They show prospecting visits to known (solid circles) or suspected (dashed circles) kittiwake colonies situated  $> 40$  km away from their nesting colony, in Hornøya, Norway (white star). These often overlooked movements may significantly contribute to the spread of parasite vectors and infectious agents.

foraging will lead to the broad circulation of parasites and infectious agents because the birds do not necessarily interact closely with conspecifics when they forage. Conversely, in some cases, small scale foraging movements could indirectly result in the spread of some agents to another species that, in turn, spreads them on a broader scale. For example, scavenging or predatory birds, such as skuas, may

connect host prey populations (Fig. 2A) and transfer an infectious agent that may then have a dramatic effect on the dynamics of the prey species and/or spread over long distances via migration.

#### Dispersal

Dispersal is defined as the movement of individuals between a location where they were born or bred to



a new location where they breed (Clobert et al. 2001; Ronce, 2007; Fig. 1). Dispersal has attracted attention as another important type of movement for the spatial dynamics of infectious diseases, although empirical work in wild populations has lagged behind theory, possibly because many factors may affect dispersal (Boulinier et al. 2001; Blackwood et al. 2013). In addition to its contribution to understanding the dynamics of host–parasite interactions within meta-populations, where the dispersal of an infected host can lead to inter-patch colonization (Hess et al. 2002; Bjørnstad et al. 2004), host dispersal has been identified as a key factor in the evolution of host–parasite interactions as it implies gene flow among local host populations, which alters local standing genetic diversity and, thus, coevolution with infectious agents (Gandon et al. 1996; Gandon and Michalakis 2002; Thompson 2005). It has been suggested that the relative dispersal rates of hosts and parasites among local populations directly affect local adaptation of the host to the parasites and *vice versa* (Gandon et al. 1996, Lion and Gandon, 2015). But not all movements between host populations lead to dispersal *per se*; an overlooked detail when making generalizations about the dynamics of parasite circulation in subdivided populations (McCoy et al. 2005a; Mazé-Guilmo et al. 2016). For example, some exploratory movements, like prospecting, may not lead to the dispersal of a host individual, but may contribute to the large scale dispersal of a parasite among host populations.

### Prospecting

Prospecting movements are defined as visits made by individuals to locations where they are not currently breeding, but where they may settle in the future to reproduce (Reed et al. 1999; Fig. 1). Described for many bird species (Reed et al. 1999), these types of forays outside the birth location or main current breeding site of an individual are usually reported in a context of breeding habitat selection, in which such visits may allow individuals to gather information on the suitability of potential future breeding sites and to initiate access to these sites. Prospecting movements made by individuals before they first breed are reported to occur at much larger scales than prospecting forays made by adult birds that failed their breeding attempt (Reed et al. 1999), although the sometime much more nomadic movements of pre-breeders, especially in very young age classes of long-lived species, are especially difficult to track. Attendance at breeding areas by prospecting individuals usually occurs late in the breeding season in

colonially breeding bird species; at that time, individuals are possibly preparing their local recruitment in the next season (Boulinier et al. 1996; Reed et al. 1999). Prospectors often land, walk, enter nesting burrows, or land on nests or chicks (in birds) or directly interact with conspecifics (in birds and mammals), although it is often difficult to infer where the prospecting individuals came from. Prospecting forays have also been reported in other taxa, such as in social mammals, in which they are associated with attempts by individuals to seek mating opportunities outside their social group (Young et al. 2005, 2007). In an epidemiological context, these movements have been neglected because they are often secretive and difficult to track, also because they can be brief and involve young and/or unmarked individuals coming from outside a study area. They may however be especially important for the circulation of infectious agents because, as opposed to most dispersal events, they occur within a single breeding season and can involve intimate contact between individuals with very different exposure histories, favoring pathogen exchange.

### Transmission mode of infectious agents and the role of host movement in their dispersal

The transmission mode of infectious agents will determine the potential implications of host movement for their dispersal and transmission. Directly transmitted agents, and in particular sexually transmitted diseases, will require direct contact between susceptible and infectious hosts which can occur at any time, during any type of movement. When environmental transmission is required (for instance, for food-borne infectious agents), transmission chains require individuals to visit specific sites (Roche and Rohani 2010). While these agents may be less likely to be transferred by certain large-scale movements, their shedding along a migratory pathway or during visits with another social group could lead to a successful transmission event. The spread of environmentally transmitted infections may also be favored by scavengers or predators whose foraging grounds can be very large and encompass different sub-populations of infected and susceptible hosts (Fig. 2A). Their role may be reinforced by the year-round nature of their interactions with their prey, as opposed to the more seasonally variable contact rates that some (especially migratory) species display. Finally, vector-borne agents require the local presence of the vector for transmission to occur, but this condition is not in itself sufficient to ensure transmission, as hosts may not

transmit the infectious agent to the vector during close contact, and even a very close contact, such as a bite, may not result in successful infection of a new host. In some cases, when the vector is also a parasite (e.g., ticks, fleas, lice), it can be dispersed by a vertebrate along with the infectious agent (e.g., Comstedt et al. 2006). However, the successful colonization of a new habitat by a vector requires a specific set of conditions, such as suitable environmental conditions and the long-term presence of suitable hosts. Depending on the dispersed life stage of the vector, one may also expect very different rates of successful colonization.

### The case of prospecting: insight using a highly spatially structured seabird system

#### Why seabirds?

The role of birds as reservoirs and disseminators of parasites and pathogens has received much attention over the past several years due to their high mobility. However, the large spatial scales involved and the numerous species that share migratory flyways and breeding or wintering grounds make it difficult to disentangle the potential role of different types of movements for pathogen dissemination. Seabirds are particularly interesting hosts in this respect. In addition to incredible long-distance movements during migration, foraging and prospecting, these birds are long-lived, site faithful and breed in dense aggregations in specific colony locations with a limited diversity of host species (Furness and Monaghan 1987; McCoy et al. 2016). These characteristics can not only favor both the local maintenance and large-scale dissemination of parasites and pathogens, but also makes them ideal model systems to assess the role of different types of movements in spreading disease agents.

Even when breeding in polar areas, seabird populations are exposed to a high diversity of parasites and infections. Here, we focus on a particular system involving *Ixodes uriae*, a tick that commonly exploits colonial seabirds in temperate and polar regions, and the different infectious agents it transmits (McCoy et al. 2005b; Dietrich et al. 2011; Dietrich et al. 2014). Indeed, this tick hosts a suite of viruses (Chastel 1988), among which are several flaviviruses, that are extensively distributed among seabird species over a wide geographic area (Heinze et al. 2012). The tick *I. uriae* also transmits Lyme disease bacteria to seabirds in both hemispheres (Olsen et al. 1995; Gasparini et al. 2001; Gomez-Diaz et al. 2011). Local infestation levels by *I. uriae* can vary strongly among breeding cliffs (Gasparini et al. 2001) and colonies (McCoy et al.

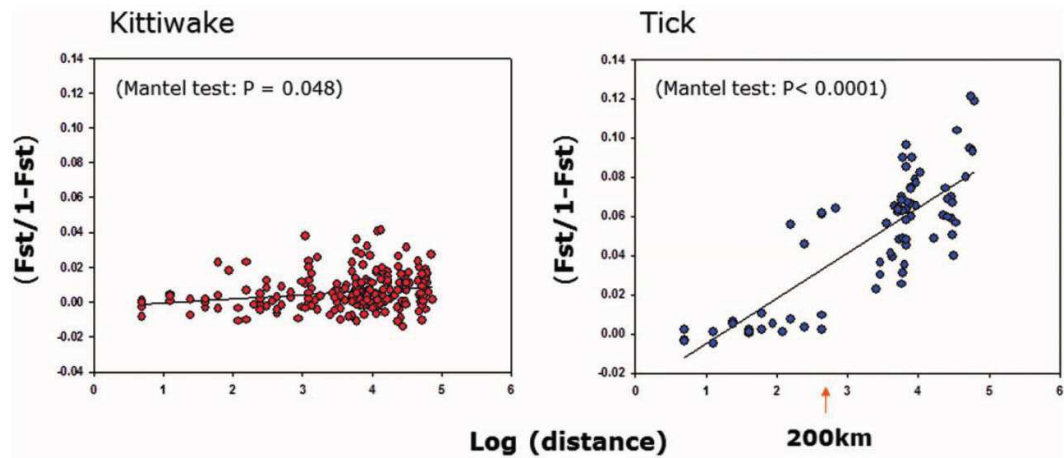
1999), and the tick relies entirely on its seabird host for inter-colony dispersal; given that seabird breeding phenology is highly seasonal at high latitudes, and that these ticks require about a week to complete a blood meal, inter-colony dispersal of ticks can only occur via the movements made by birds within the breeding season. Indeed, most seabirds, such as the black-legged kittiwake *Rissa tridactyla*, spend the winter at sea, far from land (Fig. 2B and Frederiksen et al. 2012).

Which individuals move among colonies during a breeding season should thus be paramount to the transmission of ticks and infectious agents. Young birds at fledging may frequently wander among colonies, and are often heavily infested by ticks. This was noted by Danchin (1992), who reported the occurrence of non-local fledglings parasitized by ticks in nests that were otherwise uninfested. These observations were possible due to detailed survey work of all the nests within a study colony where all the nestlings had been ringed (Danchin 1992). Another category of individuals that may visit different colonies within a breeding season are prospecting individuals that have not bred or that have failed their breeding attempt (Cadiou et al. 1994). Prospecting has been extensively studied in the kittiwake because it is relatively easy to track local movements of marked individuals that breed on vertical cliff faces and because these movements may be associated with the gathering of public information used by individuals to choose a future breeding site (Boulinier and Danchin 1997; Danchin et al. 1998; Boulinier et al. 2008). Prospecting visits occur late in the breeding season (Boulinier et al. 1996), when successful areas typically attract more individuals, potentially favoring the dispersal of parasites among breeding groups. This type of movement could be especially important in a metapopulation context, among colonies at regional scales. However, tracking marked individuals over large areas and within colonies where thousands of birds breed cannot be easily achieved by traditional capture-mark-resighting methods.

#### An indirect approach to the role of prospecting

As an indirect way to explore the potential role of prospecting in the dispersal of parasites, the population genetic structure of the kittiwake and its tick *I. uriae* was investigated using a comparable set of genetic markers for each species (microsatellites; McCoy et al. 2005a). For the tick, population genetic differentiation varied over the range of scales considered; little structure among tick populations exploiting kittiwakes was found among colonies separated by up to 200 km, whereas significant structure was





**Fig. 3** Exploration of the population genetic structure of a host species (A), the black-legged kittiwake (*Rissa tridactyla*), and of its tick (*Ixodes uriae*) (B) as an indirect way of inferring the spatial scale of host movements and their potential implication for the circulation of tick-borne agents. The figure shows pairwise genetic distance ( $F_{ST}/1-F_{ST}$ ) as a function of the geographical distance between populations sampled at the scale of the North Atlantic. Both host and parasite showed significant patterns of isolation by distance, but tick populations were not structured when colonies were separated by less than  $\sim 200$  km ( $\sim \log 2.3$ ), suggesting tick dispersal occurs via the prospecting of individual hosts among colonies situated from ten to a few hundred kilometers apart (redrawn from McCoy et al. 2005a).

observed beyond this limit and increased with the geographic distance between colonies (Fig. 3B). Host populations, in contrast, showed very little evidence of differentiation at a comparable scale (Fig. 3A). This suggests prospecting birds move frequently enough between colonies up to 200 km apart that tick populations are able to remain genetically mixed at this spatial scale. When exploring the population genetic structure of ticks sampled from other seabird species across the same scales, for example, the Atlantic puffin (*Fratercula arctica*), no structure was found among tick populations isolated by more than 700 km. This might be due to differences in behavior and breeding biology among seabird species. Puffins breed in burrows on the slopes above cliffs (rather on the vertical faces of cliffs) and might be more likely to drop-off or pick-up ticks while prospecting within colonies than kittiwakes (McCoy et al. 2003). The impact of frequent, large scale movements in seabirds and their ticks can be seen in patterns of geographic structure in the pathogens they transmit. For example, a phylogeographic study of the most frequently occurring Lyme disease bacterium in seabirds, *Borrelia garinii*, demonstrated large-scale exchange of strains both within and across ocean basins (Gomez-Diaz et al. 2011).

In contrast to hard ticks such as *I. uriae*, soft ticks infesting seabirds, such as *Ornithodoros maritimus* infesting yellow-legged gulls (*Larus michahellis*), may move less among colonies. Indeed, stable

differences in the seroprevalence of a tick-borne flavivirus have been recorded between colonies less than 100 km apart (Arnal et al. 2014). In soft ticks, nymphal and adult ticks take very brief blood meals ( $\sim 10$  min), typically at night when birds do not move. Because these ticks may thus rarely be dispersed, even by short movements of prospecting individuals, the study of their population genetic structure is likely to provide less information on the distance and frequency of host prospecting movements than that of hard ticks. Overall, the study of the genetic structure of vector populations and that of infectious agents is important as it allows one not only to infer dispersal rates at different scales, but also to explore whether host specialization plays a role in the circulation of infectious agents. In communities of host species with various levels of mobility, the degree of specialization of vectors could indeed potentially (and dramatically) affect the spread of infectious agents (e.g., see McCoy et al. 2003 and 2005b for the seabird-tick system).

#### A more direct approach to the role of prospecting

Although prospecting movements were suspected to occur over large spatial scales, the use of GPS tracking tools to record these movements was only recently proposed (Ponchon et al. 2013). GPS loggers are commonly used to track the foraging movements of breeding birds during incubation or chick rearing (e.g., Ponchon et al. 2014). However, their

deployment for studying prospecting movements has lagged, mainly because failed breeders, who are more likely to prospect, are also more difficult to recapture, especially if they bred in an area where local breeding success was low (Chambert et al. 2012; Ponchon et al. 2013). The tracking of individuals that failed their breeding attempt (e.g., that lost their eggs) has nevertheless been successful (Ponchon et al. 2015 and Fig. 2C), notably using UHF-GPS loggers that do not require the individuals to be recaptured in order to gather location data. Evidence of repeated prospecting visits by failed breeders to colonies situated tens of kilometers away is now accumulating, and suggests that those movements may connect colonies in a broad social network within which parasites/pathogens may be dispersed.

### The case of other (social) species

We have shown that prospecting movements may be responsible for disease spread in seabirds, much more likely than migratory movements, which, even if they occur at much broader scales might not necessarily lead to successful parasite dispersal given that most seabird species overwinter at sea. But could such prospecting movements be involved in superspreading events in other systems? Relatively, little is known about prospecting in non-social bird species (Pärt and Doligez 2003), but it is likely that prospecting movements by birds may contribute to the spread of parasites between local host populations, independently of migratory movements.

In social mammals, forays of individual meerkats (*Suricata suricatta*) among groups other than their own have been identified as prospecting events (Young et al. 2005, 2008; Drewe et al. 2009), and are suspected of leading to the dispersal of infectious agents such as tuberculosis bacteria (Drewe et al. 2010). In such cases, movements appear to be related to opportunities to mate outside the group, which if successful lead to direct contact among individuals (Doolan and Macdonald, 1996; Drewe et al. 2009). More generally, the movements of individual mammals among social groups have been related to an increased risk of spreading diseases (Altizer et al. 2003; Craft et al. 2011). Although the spatial scale involved is much smaller than that of highly mobile and colonially breeding mammals, such as some species of bats and seals, this issue has also specific implications in relation to disease management. When the structure of social groups is disrupted, some individuals may venture further from the group, leading to increased transmission of infectious agents

among groups and species. This is thought to be the case for tuberculosis bacteria among badgers (*Meles meles*). In fact, badger control efforts in the UK have been suspected to increase dispersal rates of individuals and lead to tuberculosis dissemination (Rogers et al. 1998 ; Vicente et al. 2007; McDonald et al. 2008). In the case of disease transmission between wild and domestic animals, the behavioral processes affecting the spatial movements of individuals may thus also be critical to consider (Miguel et al. 2013).

## Research Perspectives

### Pending questions

Many challenging questions remain to be addressed in the field of disease ecology (Tompkins et al. 2011) and we hope that our review emphasizes that integrating movement ecology and parasite transmission will open up new avenues of research. We have suggested that a relatively neglected type of movement, i.e., prospecting, may play a significant role in some instances, but its importance for the spread of disease agents in relation to other movements, notably in complex communities of hosts and parasites, remains to be determined. Even in the relatively simple cases we considered, much work is still needed to explore whether we can relate the movements of individuals to transmission events and eco-epidemiological dynamics. We have shown that some movements, conditioned by the experience of individuals, have the potential to spread infectious agents, but we still do not fully understand how much this depends on other plastic responses, such as the development of acquired immunity following exposure to infectious agents (Cross et al. 2005). In addition to their current involvement in reproduction, individuals may vary in their propensity to prospect, which could have direct implications for the spread of infectious agents. In particular, their individual exposure history and the timing of events affecting the quality of the environment may be critical. In this context, approaches considering the dynamic dimension of social network could be especially useful (Sih et al. 2009; Craft et al. 2011) and may prove powerful to address related community dynamic questions in heterogeneous landscapes. In addition, one may wonder how the effects of natural versus human-induced movements can be related to habitat selection and disease agent transmission.

### Available tools

The availability of tools to explore the roles of various types of movements on the dispersal of



infectious agents has increased dramatically over the last two decades. In addition to classical capture–mark–recapture/resighting approaches, miniaturized GPS loggers are widely available for use in tracking the movements of vertebrates; these enable research teams to track the movements of individuals with high spatial and temporal precision without requiring recapture (Nathan et al. 2008). This is especially useful as we have seen that roaming movements by non-breeding individuals or individuals that failed their current breeding attempt may be critical when studying transmission of some infectious agents, even if these are not the easiest to track (Ponchon et al. 2013). The development of radio-frequency identification technology, which allows the efficient tracking of large numbers of individuals simultaneously, will likely continue to generate very useful data to infer social contact networks and enable us to better infer the contribution of different types of movements to disease dissemination (Perkins et al. 2009). Networks of antennas, set-up within breeding groups, may also inform us about the occurrence of key movements, particularly for burrow nesting species which are difficult to directly survey. In some situations, loggers recording the proximity among individuals may be especially useful. More indirect tools are also becoming increasingly available. In addition to classical population genetic approaches for host and parasite populations, the use of microbial genetics may provide useful complementary data about transmission networks (e.g., VanderWaal et al. 2014). More classical methods, such as serological studies or the use of isotope analyses, can also be powerful to address questions related to the histories of individuals in terms of exposure to a community of infectious agents and particular habitats.

#### Including disease ecology in movement ecology studies

To obtain the most from data gathered to answer questions about movement and disease agent circulation, carefully designed studies should be set-up and implemented in the field. Such designs should allow researchers to account for potential biases that may limit the strength of the inferences to be made. A key issue in disease ecology is, for instance, accounting for the fact that it is difficult to be certain whether an individual has been exposed or not to specific infectious agents; this can now be dealt with using relevant statistical tools in addition to powerful biomedical tools (McClintock et al. 2010). Indeed, it is not because a field sample does not lead to the positive detection of an infectious agent or

specific antibodies that the sampled individuals are not infected or have not been exposed to the agent. Hierarchical sampling designs at appropriate temporal and spatial scales are especially important in this context as they can be used to account for detectability issues across scales. Statistical approaches originally developed in the context of capture–mark–recapture analyses are now increasingly applied to eco-epidemiological situations (Cooch et al. 2012; Choquet et al. 2013), and these should prove useful in such contexts.

Another important issue is the need for replication at relevant spatial scales; this may be especially critical when we consider movements among locations at broad scales (the focal study units become the patches, not necessarily the individuals within patches). As mentioned above, the design of field studies would benefit from a priori conceptual work involving participants with complementary skills, notably integrating modeling, laboratory and field-based approaches (Restif et al. 2012). Experimental approaches solidly grounded on detailed observational work with the study system may be especially powerful to address some specific questions. In the case of the potential importance of prospecting movements, we have seen that particular environmental and/or social conditions may favor prospecting; the manipulation of these conditions may thus provide a way to address the potential causal link between degradation of local conditions and the occurrence of prospecting movements. In cases where massive breeding failures may be due to local disease outbreaks, vaccination on some host patches could for instance enable one to address local epidemiological issues, along with issues related to the dispersal of the infectious agent among patches.

#### Conclusion

We believe that our review of the various types of movements that may be important for the super-spreading of infectious agents highlights that, in addition to gathering data on migratory species as part of extensive ringing programs, the specific design and implementation of field studies addressing original questions about the complex processes that underlie the dynamics of infectious agents in wild populations is required. Such studies must rely on integrative population approaches, and will likely be especially valuable if they are conducted in a spatially explicit context and involve, in addition to classical disease ecology methodologies, the tracking of the

individual host histories across environmental conditions.

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### References

- Adams B, Kapan, DD. 2009. Man bites mosquito: understanding the contribution of human movement to vector-borne disease dynamics. *PLoS ONE* 4:e6763.
- Altizer S, Nunn CL, Thrall PH, Gittleman JL, Antonovics J, Cunningham AA, Dobson AP, Ezenwa VO, Jones KE, Pedersen AB et al. 2003. Social organization and parasite risk in mammals: integrating theory and empirical studies. *Annu Rev Ecol Evol Syst* 34:517–47.
- Altizer S, Bartel R, Han BA. 2011. Animal migration and infectious disease risk. *Science* 331:296–302.
- Arnal A, Gómez-Díaz E, Cerdà-Cuëllar M, Lecollinet S, Pearce-Duvel J, Busquets N, García-Bocanegra I, Pagès N, Vittecoq M, Hammouda A et al. 2014. Circulation of a Meaban-like virus in yellow-legged gulls and seabird ticks in the western Mediterranean Basin. *PLOS One* 9:e89601.
- Bauer S, Hoye BJ. 2014. Migratory animals couple biodiversity and ecosystem functioning worldwide. *Science* 344:1242552.
- Bjørnstad ON, Peltonen M, Liebhold AM, Baltensweiler W. 2002. Waves of larch budmoth outbreaks in the European alps. *Science* 298:1020–23.
- Blackwood JC, Streicker DG, Altizer S, Rohani P. 2013. Resolving the roles of immunity, pathogenesis, and immigration for rabies persistence in vampire bats. *Proc Natl Acad Sci USA* 110:20837–42.
- Boulinier T, Danchin E, Monnat J-Y, Doutrelant C, Cadiou B. 1996. Timing of prospecting and the value of information in a colonial breeding bird. *J Avian Biol* 27:252–56.
- Boulinier T, Danchin E. 1997. The use of conspecific reproductive success for breeding patch selection in territorial migratory species. *Evol Ecol* 11:505–17.
- Boulinier T, McCoy KD, Sorci G. 2001. Dispersal and parasitism. In Clobert J, Danchin E, Dhondt A, Nichols JD, editors. *Dispersal*. Oxford: Oxford University Press Inc. p. 169–79.
- Boulinier T, Mariette M, Danchin E, Doligez B. 2008. Choosing where to breed: breeding habitat choice. In Danchin E, Giraldeau L-A, Cézilly F, editors. *Behavioral ecology*. London: Oxford University Press Inc. p. 285–321.
- Boulinier T, McCoy KD, Yoccoz NG, Gasparini J, Tveraa T. 2008. Public information affects breeding dispersal in a colonial bird: kittiwakes cue on neighbours. *Biol Lett* 4:538–40.
- Bowler DE, Benton TG. 2005. Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. *Biol Rev* 80:205–25.
- Chambert T, Pardo D, Staszewski V, McCoy KD, Tveraa T, Boulinier T. 2012. Heterogeneity in detection probability along the breeding season in Black-legged kittiwakes: implications for sampling design. *J Ornithol* 152:371–80.
- Chastel CE. 1988. Tick-borne virus infection of marine birds. In Harris K, editor. *Advances in disease vector Research* 5. New York: Springer Inc. p. 25–60.
- Choquet R, Carrie C, Chambert T, Boulinier T. 2013. Estimating transitions between states using measurements with imperfect detection: application to serological data. *Ecology* 94:2160–65.
- Cleaveland S, Borner M, Gislason M. 2014. Ecology and conservation: contributions to One Health. *Rev Sci Tech Office Intl Epizoot* 33:615–27.
- Clobert J., Danchin E., Dhondt A, Nichols JD. 2001. eds. *Dispersal*. Oxford: Oxford University Press Inc.
- Comstedt P, Bergström S, Olsen B, Garpmo U, Marjavaara L, Mejlon H, Barbour AG, Bunikis J. 2006. Migratory passerine birds as reservoirs of Lyme borreliosis in Europe. *Emerg Infect Disease* 12:1087–95.
- Cooch E, Conn PB, Ellner SP, Dobson AP, Pollock KP. 2012. Disease dynamics in wild populations: modeling and estimation: a review. *J Ornithol* 152:S485–509.
- Craft ME, Volz E, Packer C, Meyers LA. 2009. Distinguishing epidemic waves from disease spillover in a wildlife population. *Proc Roy Soc Lond B Biol Sci* 276:1777–85.
- Craft ME., Volz E, Packer C, Meyers LA. 2011. Disease transmission in territorial populations: the small-world network of Serengeti lions. *J Roy Soc Interface* 8:776–86.
- Cross PC, Lloyd-Smith JO, Johnson PLF, Getz WM. 2005. Duelling timescales of host movement and disease recovery determine invasion of disease in structured populations. *Ecol Lett* 8:587–59.
- Cunningham AA. 1996. Disease risks of wildlife translocations. *Conserv Biol* 10:349–53.
- Danchin E. 1992. The incidence of the tick parasite *Ixodes uriae* in Kittiwake *Rissa tridactyla* colonies in relation to the age of the colony, and a mechanism of infecting new colonies. *Ibis* 134:134–41.



- Danchin E, Boulinier T, Massot M. 1998. Conspecific reproductive success and breeding habitat selection: implications for the study of coloniality. *Ecology* 79:2415–28.
- Daszak P, Cunningham AA, Hyatt AD. 2000. Emerging infectious diseases of wildlife - threats to biodiversity and human health. *Science* 287:443–49.
- Dietrich M, Gómez-Díaz E, McCoy KD. 2011. Worldwide distribution and diversity of seabird ticks: implications for the ecology and epidemiology of tick-borne pathogens. *Vector-Borne Zoon Disease* 11:453–70.
- Dietrich M, Kempf F, Boulinier T, McCoy KD. 2014. Tracing the colonisation and diversification of the worldwide seabird ectoparasite *Ixodes uriae*. *Mol Ecol* 23:3292–305.
- Diggle PJ. 2000. Overview of statistical methods for disease mapping and its relationship to cluster detection. In Elliott P, Wakefield, JC, Best NG, Briggs DJ, editors. *Spatial epidemiology: methods and applications*. London: Oxford University Press Inc. p. 87–103.
- Dingle H. 2014. *Migration. The biology of life on the move*, 2nd edition. London: Oxford University Press Inc.
- Doolan SP, Macdonald DW. 1996. Dispersal and extra-territorial prospecting by slender-tailed meerkats (*Suricata suricatta*) in the south-western Kalahari. *Journal of Zoology* 240: 59–73.
- Drewe JA, Madden JR, Pearce GP. 2009. The social network structure of a wild meerkat population: 1. Inter-group interactions. *Behav Ecol Sociobiol* 63:1295–306.
- Drewe JA. 2010. Who infects whom? Social networks and tuberculosis transmission in wild meerkats. *Proc Roy Soc Lond B Biol Sci* 277:633–42.
- Fenner AL, Godfrey SS, Bull CM. 2011. Using social networks to deduce whether residents or dispersers spread parasites in a lizard population. *J Animal Ecol* 80:835–38.
- Fieberg J, Matthiopoulos J, Hebblewhite M, Boyce MS, Frair JL. 2010. Correlation and studies of habitat selection: problem, red herring or opportunity? *Phil Trans Roy Soc Lond B* 365:2233–44.
- Frederiksen M, Moe B, Daunt F, Phillips RA, Barrett RT, Bogdanova MI, Boulinier T, Chardine JW, Chastel O, Chivers LS et al. 2012. Multi-colony tracking reveals the non-breeding distribution of a pelagic seabird on an ocean basin scale. *Diversity Distrib* 18:530–42.
- Fritzsche McKay A, Hoye, 2016. Are migratory animals super-spreaders of infection?. *Integrative Comparative Biology*.
- Fuller T, Bensch S, Muller I, Novembre J, Perez-Tris J, Ricklefs RE, Smith TB, Waldenstrom J. 2012. The ecology of emerging infectious diseases in migratory birds: an assessment of the role of climate change and priorities for future research. *EcoHealth* 9:80–88.
- Furness RW, Monaghan P. 1987. *Seabird ecology*. Glasgow: Blackie.
- Gaidet N, Cappelle J, Takekawa JY, Prosser DJ, Iverson SA, Douglas DC, Perry WM, Mundkur T, Newman SH. 2010. Potential spread of highly pathogenic avian influenza H5N1 by wildfowl: dispersal ranges and rates determined from large-scale satellite telemetry. *J Appl Ecol* 47:1147–57.
- Gandon S, Capowiez Y, Dubois Y, Michalakis Y, Olivieri I. 1996. Local adaptation and gene-for-gene coevolution in a metapopulation model. *Proc Roy Soc Lond B* 263:1003–9.
- Gandon S, Michalakis Y. 2002. Local adaptation, evolutionary potential and host-parasite coevolution: interactions between migration, mutation, population size and generation time. *J Evol Biol* 15:451–62.
- Gasparini J, McCoy KD, Haussy C, Tveraa T, Boulinier T. 2001. Induced maternal response to the Lyme disease spirochaete *Borrelia burgdorferi sensu lato* in a colonial seabird, the Kittiwake *Rissa tridactyla*. *Proc Roy Soc Lond B* 1467:647–50.
- Gilbert M, Xiao X, Domenech J, Lubroth J, Martin V, Slingenbergh J. 2006. Anatidae migration in the Western Palearctic and spread of highly pathogenic Avian Influenza H5N1 virus. *Emerg Infect Disease* 12:1650–56.
- Gomez-Diaz E, Boulinier T, Cornet M, Ferquel E, Sertour N, McCoy KD. 2011. Genetic structure of marine *Borrelia garinii* and population admixture with the terrestrial cycle of *Lyme borreliosis*. *Environ Microbiol* 13:2453–67.
- Hanski I, Gaggiotti OE. 2004. eds. *Ecology, genetics and evolution of metapopulations*. London: Elsevier Academic Press Inc.
- Harding KC, Härkönen T, Caswell H. 2002. The 2002 European seal plague: epidemiology and population consequences. *Ecol Lett* 5:727–32.
- Heinze DM, Gould EA, Forrester NL. 2012. Revisiting the clinal concept of evolution and dispersal for the tick-borne flaviviruses by using phylogenetic and biogeographic analyses. *J Virol* 86:8663–71.
- Hess GR, Randolph SE, Arneberg P, Chemini C, Furlanello C, Harwood J, Roberts MG, Swinton J. 2002. Spatial aspects of disease dynamics. In Hudson PJ, Rizzoli A, Grenfell BT, Heesterbeek H, Dobson AP, editors. *The ecology of wildlife disease*. London: Oxford University Press Inc.
- Hill NJ, Takekawa JY, Ackerman JT, Hobson KA, Herring G, Cardona CJ, Runstadler JA, Boyce, WM. 2012. Migration strategy affects avian influenza dynamics in mallards (*Anas platyrhynchos*). *Mol Ecol* 21:5986–99.
- Hochachka WM, Dhondt AA. 2000. Density-dependent decline of host abundance resulting from a new infectious disease. *Proc Natl Acad Sci USA* 97:5303–06.
- Holt R, Boulinier T. 2005. Ecosystem and parasitism: the spatial dimension. In Thomas F, Renaud F, Guegan, J-F, editors. *Ecosystem and parasitism*. Oxford: Oxford University Press, Inc. p. 68–84.
- Jeltsch F, Bonte D, Pe'er G, Reineking B, Leimgruber P, Balkenhol N, Schröder B, Buchmann CM, Mueller T, Blaum N et al. 2013. Integrating movement ecology with biodiversity research - exploring new avenues to address spatiotemporal biodiversity dynamics. *Mov Ecol* 1:6.
- Johnson AR, Wiens JA, Milne BT, Crist TO. 1992. Animal movements and population dynamics in heterogeneous landscapes. *Landscape Ecol* 7:63–75.
- Keeling MJ, Bjornstad, ON, Grenfell BT. 2004. Metapopulation dynamics of infectious diseases. In Hanski I, Gaggiotti O, editors. *Ecology, genetics and evolution of metapopulations*. San Diego: Elsevier Academic Press Inc. p. pages 415–45.
- Keeling MJ, Rohani P. 2007. *Modeling infectious diseases in humans and animals*. Princeton: Princeton University Press Inc.
- Leighton PA, Koffi JK, Pelcat Y, Lindsay LR, Ogden NH. 2012. Predicting the speed of tick invasion: an empirical model of range expansion for the Lyme disease vector *Ixodes scapularis* in Canada. *J Appl Ecol* 49:457–64.

- Lion S, Gandon S. 2015. Evolution of spatially structured host-parasite interactions. *J Evol Biol* 28:10–28.
- Lloyd-Smith JO, Schreiber SJ, Kopp PE, Getz WM. 2005. Superspreading and the effect of individual variation on disease emergence. *Nature* 438:355–59.
- Lobato E, Pearce J, Staszewski V, Gómez-Díaz E, González-Solís J, Kitaysky AS, McCoy KD, Boulinier T. 2011. Species and location effects on the prevalence of antibodies anti Lyme disease *Borrelia spirochaetes* in seabirds breeding in North Pacific islands. *Vector-Borne Zoon Disease* 11:1521–27.
- Louhi K-R, Karvonen A, Rellstab C, Jokela J. 2010. Is the population genetic structure of complex life cycle parasites determined by the geographic range of the most motile host? *Infect Genet Evol* 10:1271–77.
- Mazé-Guilmo E, Blanchet S, McCoy KD, Loot G. 2016. Host dispersal as the driver of parasite genetic structure: a paradigm lost? *Ecol Lett* 19:336–47.
- McCallum H, Barlow N, Hone J. 2001. How should pathogen transmission be modelled? *Trends Ecol Evol* 16:295–300.
- McClintock BT, Nichols JD, Bailey LL, MacKenzie DI, Kendall WL. 2010. Seeking a second opinion: uncertainty in disease ecology. *Ecol Lett* 13:659–74.
- McCoy KD, Boulinier T, Chardine J, Michalakis Y, Danchin E. 1999. Dispersal of the tick *Ixodes uriae* within and among seabird host populations: the need for a population genetic approach. *J Parasitol* 85:196–202.
- McCoy KD, Boulinier T, Tirard C, Michalakis Y. 2003. Differential host-associated dispersal of the seabird ectoparasite *Ixodes uriae*. *Evolution* 57:288–96.
- McCoy KD, Boulinier T, Tirard C. 2005a. Comparative host-parasite population structures: disentangling prospecting and dispersal in the Black-legged kittiwake *Rissa tridactyla*. *Mol Ecol* 14:2825–38.
- McCoy KD, Chapuis E, Tirard C, Boulinier T, Michalakis Y, Le Bohec C, Le Maho Y, Gauthier-Clerc M. 2005b. Recurrent evolution of host-specialized races in a globally-distributed parasite. *Proc Roy Soc Lond B* 272:2389–95.
- McCoy KD, Dietrich M, Jaeger A, Wilkinson DA, Bastien M, Lagadee E, Boulinier T, Pascalis H, Tortosa P, Le Corre M et al. 2016. The role of seabirds of the Iles Eparses as reservoirs and disseminators of parasites and pathogens. *Acta Oecologica* 72: 98–109.
- McDonald RA, Delahay RJ, Carter SP, Smith GC, Cheeseman CL. 2008. Perturbing implications of wildlife ecology for disease control. *Trends Ecol Evol* 23:53–56.
- Miguel E, Grosbois V, Caron A, Boulinier T, Fritz H, Cornelis D, Foggin C, Makaya PV, Tshabalala PT, De Garine-Wichatitsky M. 2013. Contacts and foot and mouth disease transmission from wild to domestic bovines in Africa. *Ecosphere* 4:art51.
- Morales JM, Moorcroft PR, Matthiopoulos J, Frair JL, Kie JG, Powell RA, Merrill EH, Haydon DT. 2010. Building the bridge between animal movement and population dynamics. *Phil Trans Roy Soc Lond B Biol Sci* 365:2289–301.
- Mundt CC, Sackett KE, Wallace LD, Cowger C, Dudley JP. 2009. Long-distance dispersal and accelerating waves of disease: empirical relationships. *Am Nat* 173:456–66.
- Nathan R, Getz WM, Revilla E, Holyoak M, Kadmon R, Saltz D, Smouse PE. 2008. A movement ecology paradigm for unifying organismal movement research. *Proc Natl Acad Sci USA* 105:19052–59.
- Olsen B, Duffy DC, Jaenson TG, Gylfe A, Bonnedahl J, Bergström S. 1995. Transhemispheric exchange of Lyme disease spirochetes by seabirds. *J Clin Microbiol* 33:270–274.
- Olsen B, Munster VJ, Wallensten A, Waldenström J, Osterhaus AD, Fouchier RA. 2006. Global patterns of influenza A virus in wild birds. *Science* 312:384–88.
- Pärt T, Doligez B. 2003. Gathering public information for habitat selection: prospecting birds cue on parental activity. *Proc Roy Soc Lond B Biol Sci* 270:1809–13.
- Perkins SE, Cagnacci F, Stradiotto A, Arnoldi D, Hudson PJ. 2009. Comparison of social networks derived from ecological data: implications for inferring infectious disease dynamics. *J Animal Ecol* 78:1015–22.
- Péron C, Grémillet D. 2013. Tracking through life stages: adult, immature and juvenile autumn migration in a long-lived seabird. *PLoS ONE* 8:e72713.
- Plowright RK, Foley P, Field HE, Dobson AP, Foley JE, Eby P, Daszak P. 2011. Urban habituation, ecological connectivity and epidemic dampening: the emergence of Hendra virus from flying foxes (*Pteropus* spp.). *Proc Roy Soc Lond B* 278:3703–12.
- Ponchon A, Grémillet D, Doligez B, Chambert T, Tveraa T, Gonzales-Solis J, Boulinier T. 2013. Tracking prospecting movements involved in breeding habitat selection: insights, pitfalls and perspectives. *Method Ecol Evol* 4:143–50.
- Ponchon A, Grémillet D, Christensen-Dalsgaard S, Erikstad KE, Barrett RT, Reiertsein TK, McCoy KD, Tveraa T, Boulinier, T. 2014. When things go wrong: intra-season dynamics of breeding failure in a seabird. *Ecosphere* 5:art4.
- Ponchon A, Chambert T, Lobato E, Tveraa T, Grémillet D, Boulinier T. 2015. Breeding failure induces large scale prospecting movements in the black-legged kittiwake. *J Exp Marine Biol Ecol* 473:138–45.
- Pulliam HR. 1988. Sources, sinks, and population regulation. *Am Nat* 132:652–61.
- Rappole JH, Derrickson SR, Hubálek Z. 2000. Migratory birds and spread of West Nile virus in the Western Hemisphere. *Emerg Infect Disease* 6:319–28.
- Reed JM, Boulinier T, Danchin E, Oring L. 1999. Informed dispersal: prospecting by birds for breeding sites. *Curr Ornithol* 15:189–259.
- Restif O, Hayman DT, Pulliam JR, Plowright RK, George DB, Luis AD, Cunningham AA, Bowen RA, Fooks AR, O'Shea TJ, Wood JL, Webb CT. 2012. Model-guided fieldwork: practical guidelines for multidisciplinary research on wildlife ecological and epidemiological dynamics. *Ecol Lett* 15:1083–94.
- Roche B, Rohani P. 2010. Environmental transmission scrambles coexistence patterns of avian influenza viruses. *Epidemics* 2:92–8.
- Rogers LM, Delahay R, Cheeseman CL, Langton S, Smith GC, Clifton-Hadley RS. 1998. Movement of badgers (*Meles meles*) in a high-density population: individual, population and disease effects. *Proc Roy Soc Lond B* 265:1269–76.
- Ronce O. 2007. How does it feel to be like a rolling stone? Ten questions about dispersal evolution. *Annu Rev Ecol Syst* 38:231–253.



- Sih A, Hanser SF, McHugh KA. 2009. Social network theory: new insights and issues for behavioral ecologists. *Behav Ecol Sociobiol* 63:975–88.
- Stoddard ST, Morrison AC, Vazquez-Prokopec GM, Soldan VP, Kochel TJ, Kitron U, Elder JP, Scott, TW. 2009. The role of human movement in the transmission of vector-borne pathogens. *PLoS Negl Trop Disease* 3:e481.
- Thompson JN. 2005. *The geographic mosaic of coevolution*. Chicago: University of Chicago Press Inc.
- Tompkins DM, Dunn AM, Smith MJ, Telfer S. 2011. Wildlife diseases: from individuals to ecosystems. *J Animal Ecol* 80:19–38.
- VanderWaal KL, Atwill ER, Isbell LA, McCowan B. 2014. Linking social and pathogen transmission networks using microbial genetics in giraffe (*Giraffa camelopardalis*). *J Animal Ecol* 83:406–14.
- Viana M, Mancy R, Biek R, Cleaveland S, Cross PC, Lloyd-Smith JO, Haydon DT. 2014. Assembling evidence for identifying reservoirs of infection. *Trends Ecology Evol* 29:270–79.
- Vicente J, Delahay RJ, Walker NJ, Cheeseman CL. 2007. Social organization and movement influence the incidence of bovine tuberculosis in an undisturbed high-density badger *Meles meles* population. *J Animal Ecol* 76:348–60.
- Young AJ, Carlson AA, Clutton-Brock T. 2005 Trade-offs between extraterritorial prospecting and helping in a cooperative mammal. *Animal Behav* 70:829–37.
- Young AJ, Spong, G, Clutton-Brock T. 2007 Subordinate male meerkats prospect for extra-group paternity: alternative reproductive tactics in a cooperative mammal. *Proc Roy Soc Lond B* 274:1603–09.









## **Convergence dans l'évolution de la spécialisation d'hôte chez des tiques : modèle tiques-oiseaux de mers à distribution mondiale.**

Les interactions intimes et répétées entre hôtes et parasites peuvent engendrer la spécialisation d'un parasite à son hôte, grâce à des adaptations comportementales, morphologiques et/ou génétiques, combinées avec un flux de gènes limité. C'est un processus clef car il participe à l'évolution de la biodiversité parasitaire et peut ainsi permettre de mieux comprendre l'émergence d'organismes pathogènes. Encore peu étudié, une spécialisation d'hôte a néanmoins été démontrée lors de précédentes études chez deux espèces de tiques nidicoles : chez *Ixodes uriae* une tique dure, parasite des oiseaux marins coloniaux en zone arctique, et dans un complexe de tiques molles *Ornithodoros capensis* sensu lato, parasitant aussi de nombreuses espèces d'oiseaux marins, mais cette fois-ci en zones tempérées et tropicales. Ces espèces sont vectrices d'une grande diversité d'agents pathogènes incluant des virus, des bactéries et des protozoaires. Cependant, les facteurs impliqués dans le phénomène de spécialisation d'hôte restent inconnus. Dans ce cadre, le but de ma thèse était donc de déterminer 1) si l'évolution des divergences en fonction des hôtes est toujours accompagnée par les mêmes changements phénotypiques et 2) si ces changements pourraient permettre d'identifier les facteurs de sélection sous-jacents. Dans ce contexte, des campagnes d'échantillonnage de tiques ont été menées durant la période de reproduction des hôtes oiseaux dans les différentes zones de leur répartition et nous avons réalisé des analyses morphométriques, basées sur l'utilisation de landmarks et de contours sur chaque individu tique et des analyses phylogénétiques et génétiques des populations sur les mêmes individus. L'ensemble de ces résultats suggère la présence de convergences morphologiques au sein de ces systèmes et souligne un rôle de la sélection dans ce processus de divergence. En effet, les caractéristiques écologiques des hôtes mais aussi le micro-habitat exercent des pressions sélectives importantes dans ces deux systèmes pouvant être à l'origine de la divergence observée entre les populations. De plus, les caractéristiques biologiques de chaque espèce de tiques, telle que la capacité de dispersion, entrent également en jeu et peuvent fortement modifier l'épidémiologie des agents infectieux dont elles sont vectrices.

Mots clés : Argasidae, écologie de la transmission, évolution convergente, interactions hôte-parasite, Ixodidae, oiseaux marins.

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### **Convergence in the evolution of host specialization of ticks: insights from two worldwide tick-seabird model systems**

Intimate and repeated interactions between hosts and parasites can lead to parasite specialization to a given host via behavioral, morphological and/or genetic adaptations that act in combination with restricted gene flow. Specialization is a key process leading to the generation of parasite biodiversity and can help us understand the emergence of pathogenic organisms. Although little studied, host specialization has already been demonstrated to occur in previous studies of two nidicolous tick species: *Ixodes uriae* a hard tick parasitizing colonial seabirds in polar regions, and soft ticks of the complex *Ornithodoros capensis* sensu lato, that also exploit colonial seabirds, but this time in temperate and tropical zones. These species act as vector to a wide variety of pathogenic organisms, including viruses, bacteria and protozoa. However, the factors involved in host specialization remain unknown. In this context, the aim of my thesis was to determine 1) whether the evolution of host specialization is always accompanied by the same phenotypic changes and 2) whether these changes could help to identify the selective factors that influence this phenomenon. In this context, tick collections were conducted during the breeding period of the host birds in different areas of their distribution and morphometric analyses, based on landmark and contour methods, were performed on each individual tick. Phylogenetic and population genetic analyses were also carried out using the same individuals. Overall, the results demonstrate that morphological convergence occurs within these systems, highlighting the role of selection in the divergence process. Indeed, the ecological characteristics of the hosts, but also their micro-habitat, may exert significant selective pressures on ticks and may cause the observed divergence among populations. Likewise, the biological characteristics of each tick species, particularly in relation to dispersal capacity, may also come into play and will greatly modify the epidemiology of associated infectious agents.

Keywords: Argasidae, convergent evolution, host-parasite interactions, Ixodidae, transmission ecology, seabirds

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**Spécialité:** Biologie des Interactions ; **Discipline:** Evolution des systèmes infectieux

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# THÈSE

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Presented by  
**Marlène Dupraz**

**Convergence in the evolution of host  
specialization of ticks: insights from two  
worldwide tick-seabird model systems**

Thursday, December 15, 2016

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## Abstract

### Convergence in the evolution of host specialization of ticks: insights from two worldwide tick-seabird model systems

Intimate and repeated interactions between hosts and parasites can lead to parasite specialization to a given host via behavioral, morphological and/or genetic adaptations that act in combination with restricted gene flow. Specialization is a key process leading to the generation of parasite biodiversity and can help us understand the emergence of pathogenic organisms. Although little studied, host specialization has already been demonstrated to occur in previous studies of two nidicolous tick species: *Ixodes uriae* a hard tick parasitizing colonial seabirds in polar regions, and soft ticks of the complex *Ornithodoros capensis* sensu lato, that also exploit colonial seabirds, but this time in temperate and tropical zones. These species act as vector to a wide variety of pathogenic organisms, including viruses, bacteria and protozoa. However, the factors involved in host specialization remain unknown. In this context, the aim of my thesis was to determine 1) whether the evolution of host specialization is always accompanied by the same phenotypic changes and 2) whether these changes could help to identify the selective factors that influence this phenomenon. In this context, tick collections were conducted during the breeding period of the host birds in different areas of their distribution and morphometric analyses, based on landmark and contour methods, were performed on each individual tick. Phylogenetic and population genetic analyses were also carried out using the same individuals. Overall, the results demonstrate that morphological convergence occurs within these systems, highlighting the role of selection in the divergence process. Indeed, the ecological characteristics of the hosts, but also their micro-habitat, may exert significant selective pressures on ticks and may cause the observed divergence among populations. Likewise, the biological characteristics of each tick species, particularly in relation to dispersal capacity, may also come into play and will greatly modify the epidemiology of associated infectious agents.

Keywords: Argasidae, convergent evolution, host-parasite interactions, Ixodidae, transmission ecology, seabirds.

# Summary

▪ <b>Chapter 1 – Introduction</b>	<b>1</b>
♦ The evolution of parasite diversity	3
♦ Convergence in host race formation	4
♦ Study systems	8
× <i>Ixodes uriae</i>	
× <i>Ornithodoros capensis</i> sensu lato	
♦ Objectives	13
▪ <b>Chapter 2 – Species identification and discrimination</b>	<b>16</b>
♦ 2.1 Morphological description of ticks	18
♦ 2.2 Geometric morphometrics	20
<i>Article 1</i>	22
<b>Estrada-Peña et al.</b> The results of a blind test of ixodid tick identification by European experts. <i>Submitted</i> .	
<i>Article 2</i>	46
<b>Dujardin et al, 2014.</b> Outline based morphometrics, an overlooked method in arthropod studies? <i>Infection, Genetics and Evolution</i> . 28: 704-714.	
▪ <b>Chapter 3 – Convergence in diversification patterns</b>	<b>60</b>
<i>Article 3</i>	64
<b>Dupraz et al, 2016.</b> Linking morphometric and genetic divergence with host use in the tick complex <i>Ornithodoros capensis</i> s.l. <i>Infection, Genetics and Evolution</i> . <i>In press</i> .	
<i>Article 4</i>	120
<b>Dupraz et al.</b> Morphological convergence in <i>Ixodes uriae</i> host races. <i>In prep</i> .	
▪ <b>Chapter 4 – Host-associated divergence factors and the role of local isolation</b>	<b>140</b>
<i>Article 5</i>	143
<b>Dupraz et al.</b> Circulation of vector-borne infectious agents at local scales: structure of the soft tick <i>Ornithodoros maritimus</i> within a colony of its seabird host <i>Larus michahellis</i> . <i>In prep</i> .	
▪ <b>Chapter 5 – Discussion générale et perspectives</b>	<b>171</b>
▪ <b>Bibliography</b>	<b>184</b>
▪ <b>Annexes</b>	<b>198</b>
<b>Duron et al, 2015.</b> The Recent Evolution of a Maternally-Inherited Endosymbiont of Ticks Led to the Emergence of the Q Fever Pathogen, <i>Coxiella burnetii</i> .	
<b>Boulinier et al, 2016.</b> Migration, Prospecting, Dispersal? What Host Movement Matters for Infectious Agent Circulation?	

# Chapter 1 - Introduction

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*The Evolution of parasite diversity*

Speciation is a key process in the diversification of living organisms, leading to the formation of two distinct species from a single species. Speciation studies focus on examining the mechanism of population divergence. Population divergence can arise by two main processes, the details of which are still debated today (Bird et al, 2012). Allopatric speciation occurs when populations are spatially or temporally separated from each other by a barrier (geographic or temporal). Due to this barrier, populations are not able to reproduce together leading to a reduction or disappearance of gene flow (Wiens, 2004). Genetic drift and differential selection pressures may then occur leading to the genetic divergence and the potential fixation of different gene variants. The second speciation process, sympatric speciation, does not rely on isolation by a physical barrier, but rather arises following a disruption in gene flow due to some other type of phenomenon (Rice, 1987). For example, two sub-populations differentiate in sympatry if disruptive selection leads to distinct optimums for exploiting different habitats or niches in a spatially heterogeneous environment (Levene, 1953). In theory, adaptation to one type of habitat may result in a reduction in fitness in other habitats. Mate choice occurring in relation to habitat use will reinforce sub-population divergence (Ravigné et al, 2010). Environmental variation, population history and evolutionary forces such as gene flow, selection and drift can result in a continuum of adaptation, ranging from phenotypic polymorphism to complete speciation, and includes intermediate stages such as the formation of host races (Magalhães et al, 2007).

Parasites constitute an ideal biological model to study the evolutionary processes of speciation due to their high diversity and many transitions from a free-living lifestyle to parasitism (de Meeûs and Renaud, 2002; Poulin and Moran, 2000). Selection pressures exerted on parasites come from two sources: the biotic environment, e.g. host, and the abiotic environment in which the host lives. . Abiotic factors include temperature, humidity, circadian cycle and the chemical and physical properties of the environment. Biotic factors are determined by the presence and availability of competent hosts, competitors or predators, which themselves are conditioned by abiotic factors (Meyers and Bull, 2002). The nature of these factors can be cyclic or periodic and can operate at different spatial scales. Environment and climate variation, for example, tend to act at large spatial scales modifying the distribution and evolution of parasites because of their impacts on host and parasite population abundance, community composition and organismal physiology (Hochberg and Brooks, 2015; Tylianakis et al, 2008). At a smaller spatial scale, antagonistic interactions between host and parasite can also be more or less intense inducing differential selection pressures on mutual defensive mechanisms depending on local conditions (Hochberg and Baalen, 1998). Consequently, the combination of these

different factors can create a geographic mosaic in the nature of interspecific interactions at local scales which will modify overall co-evolutionary trajectories of the species involved (Thompson, 1997).

Among the factors acting on parasite evolution, the strongest selection pressures should come from the host because it represents the principal ecological needs of the parasite (habitat, food, reproduction). A host is a spatially (individual, population) and temporally (phenology) heterogeneous environment. Parasite success is therefore dependant on the degree of adaptation to these conditions (De Meeûs et al, 1998). By definition, a host wants to avoid or eliminate its parasites. Defense mechanisms (immunology, physiology, behavior) are thus developed by the host to fight against parasite exploitation (Van Valen, 1977). A generalist parasite, i.e. using different types of hosts, needs to invest in broad spectrum adaptations to exploit different habitats and resources and to counter different host defense responses. However, the quality of resources is often unequal among hosts and host constraints can be strong. Parasites may therefore specialize on a given host type to optimize fitness (Wikel, 1984; Combes, 2001). However, this adaptation may have a cost on the capacity of the parasite to efficiently exploit other host types; this is generally considered to result from a trade-off between performance and the spectrum of exploitable resources (Futuyma and Moreno, 1988).

Discerning the link between diversity, preference and performance of parasites with respect to their hosts and determining the factors affecting or maintaining these interactions are necessary to predict parasite population dynamics and evolutionary trajectories. In the case of parasites that also vector micropathogens, the capacity to use different hosts will largely influence the epidemiology of the associated diseases (Ostfeld et al, 2005) and is thus essential for understanding disease dynamics (Cleaveland et al, 2001).

### *Convergence in host-race formation*

Intimate and repeated interactions between hosts and parasites can lead to parasite specialization and, when combined with reduced gene flow, host race formation may be expected to occur (box 1). Host races are reproductively isolated populations which differ in their biological characteristics and may show subtle morphological variation, but are not considered as distinct species (Jaenike, 1981). The steps in host race formation are described in box 1 (Bush and Butlin, 2004). This phenomenon can be considered as a stage along the divergence continuum leading to speciation and is a key mechanism for sympatric speciation (Mopper and Strauss, 2013). Nevertheless, establishing the line between a host

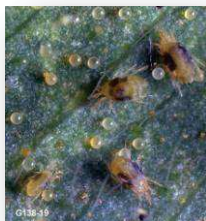
race and a full species is difficult because gene flow may become more limited with time and the definition of a species varies among researchers.

**Box 1 Stages of host race formation**

1. Mutation and/or recombination in a parasite population generate individuals able to use a novel host
2. Assortative mating occurs among parasites on the novel host reducing gene flow with the original population and allows adaptive genes to be maintained in the new host-associated population
3. During adaptation to a new host, a genetically distinct host-race evolves; fitness differences and host fidelity increase over time
4. For loci not involved in host-associated adaptations, genetic similarity is maintained with original population by low gene flow, particularly during the early stages of host-race formation
5. Speciation is completed by reinforcement or because continued divergence reduces gene flow to zero

Phytophagous insects have been the main focal model systems for studying sympatric speciation and host race formation studies for several reasons: they are a highly diverse group of organisms, there is a close association between feeding preference and reproduction in this group and they are easily usable in experimental settings (Drès and Mallet, 2002). The water lily leaf beetle, *Galerucella nymphaeae*, a herbivore of the family Chrysomelidae, was, for example, shown to form host races in association with two host families (Nymphaeaceae and Polygonaceae). In this species, performance differences result from distinct morphological traits and feeding preferences (Pappers et al, 2002). In the case of the apple maggot fly, *Rhagoletis pomonella*, which shows host races specialized on hawthorn and apple trees, it was demonstrated that differences in host choice are based on fruit odor discrimination and host phenology that lead to assortative mating. This reproductive isolation should facilitate complete speciation (Feder et al, 1994; Dambroski et al, 2005).

The Acari (ticks and mites) also present characteristics that may favour host race formation: they have close interactions with their hosts during exploitation, they show relatively strong philopatry and they have weak independent dispersion capacities. However, few studies have focused on this group to date and the majority that exist have used *Tetranychus urticae*, a plant generalist parasite (Magalhães et al, 2007) (see box 2).

**Box 2 - Host races example: the case of *Tetranychus urticae***

*T. urticae* of the family Tetranychidae is a generalist parasite of plants, ravaging cultured and wild plants. Web formation on the plant host allows the mite to maintain humidity insures protection and can favour local dispersal, but affects host plant viability. The biological cycle includes a series of life history stages: egg, larvae, two nymphal stages, one adult stage. The duration of each life stage is temperature-dependant. Females can live from 2 to 4 weeks and lay several hundred eggs (Van de Vrie et al, 1972). This mite presents ideal characteristics for studying host specialization: a wide host range, and the ability to be reared and manipulated in the field and the laboratory. Host races have been identified in association with tomato, cotton and cucumber hosts and tend to be morphologically similar. In this system, experimental work has showed that host race formation is based on differences in performance and preference (oviposition, survival, development) according to host use. These differences principally come from differences in host plant phenology (Agrawal, 2000; Egas and Sabelis, 2001) but also in relation to the production of volatile compounds that allow the plant to fight against parasites (Gotoh et al, 1993; Takabayashi et al, 2000).

Despite these classic examples, the demonstration of host specialization and its origin may often be hindered by phenotypic similarity among populations. Through the development of genetic and genomic tools, we now have access to better information on host specificity and its evolution. Combined with phenotypic analyses, genetic information can enable us to identify convergences in species that are constrained by the same ecological pressures. Phenotypic convergence, or homoplasy, corresponds to the presence of analogous characteristics within two distinct species or populations that result from the independent evolution of the same adaptation in response to natural or sexual selection pressure. These convergences can be behavioral (Blackledge and Gillespie, 2004), morphological (Kocher et al, 1993) or functional (Ribeiro, 1995) and can be based on genetic or molecular mechanisms (Stern, 2013). The relationship of phenotypic convergence to host specialization can thus be evaluated by direct measures of phenotypic variation within genetically differentiated sub-populations or host races. I used this approach to study the evolution of host specialization in ticks (order Ixodida), a widespread arthropod group of major veterinary and medical importance.

Ticks are hematophagous arthropods found in every region of the world and include about 900 described species (~700 species of hard ticks and ~200 species of soft ticks). A large number of factors (host availability and predictability) and selection pressures exerted by the host (behavior, immunity)

may favor tick specialization. Close physiological and immunological interactions are maintained with a broad spectrum of hosts including birds, reptiles, small and large mammals, including humans (Jongejan and Uilenberg, 2004). Ticks can have a direct impact on both host reproductive success and population dynamics, particularly when infestation levels are high (Boulinier and Danchin, 1996; Duffy, 1983; Feare, 1976; Jongejan and Uilenberg, 2004; Lehmann, 1993). A strong selective pressure is thus exerted on the hosts to resist exploitation. Moreover, ticks are important vectors of infectious agents (Parola and Raoult, 2001), transmitting bacteria, viruses and protozoan parasites. The socio-economic concerns related to animal and human health are thus considerable (Sonenshine and Roe, 2014). Given this, understanding the evolutionary mechanisms associated with host use and its consequences for disease epidemiology are of both fundamental and applied interest.

Tick-host interactions are diverse: some species are specialized on one host type while others are considered as host generalists. However, the degree of specificity can be difficult to establish because observations of host use can be opportunistic on the part of the tick and may not represent the true host range, sampling is often insufficient and identifications can be erroneous (McCoy et al, 2013). In most ticks, feeding times on the host tends to be relatively short compared to the time spent off-host, so it was suggested that tick distributions are not limited by the distributions of their hosts, but rather by environmental conditions (climate, vegetation) (Estrada-Pena, 2001; Klompen et al, 1996). Indeed, using distributional data from 229 African tick species, Cumming (1999) observed that half of the species showed more limited distributions than their associated hosts (the other half were not well-enough studied to provide strong conclusions). However, this study did not take into account relative tick abundance on each host type nor host abundance, factors known to modify tick distributions (McCoy et al, 2013). More recently, Nava and Guglielmone (2013) found similar results for tick-host interactions in the Neotropical zone. They concluded that tick distributions were principally due to ecological similarities in host habitat rather than to the presence of the host itself. In contrast to these results, population genetic analyses have revealed several cases of divergence in relation to host use (McCoy et al, 2013) and suggest that this type of divergence could be frequent in ticks. To evaluate this hypothesis and better understand the underlying factors associated with host use, I have focused on two complementary tick systems in this dissertation. The first one involves the hard tick *Ixodes uriae*, which exploits a wide range of colonial seabird species in polar regions of the world. In this system, genetically-distinct groups have been detected in relation to host use using population genetic tools. These host races have arisen repeatedly and independently in different areas of tick's distribution (McCoy et al, 2005). However, the factors at the origin of this divergence have not yet been identified. The second system involves soft ticks of the *Ornithodoros capensis* sensu lato complex. These ticks also exploit

colonial seabirds but in temperate and tropical zones. An indication of potential host-associated diversification in this system was revealed by an initial study in the Cape Verde and Canary Islands (Gomez-Diaz et al, 2012), but large scale patterns and the factors at their origin are again unknown. The biology of these two systems is detailed in the following sections.

## Study systems

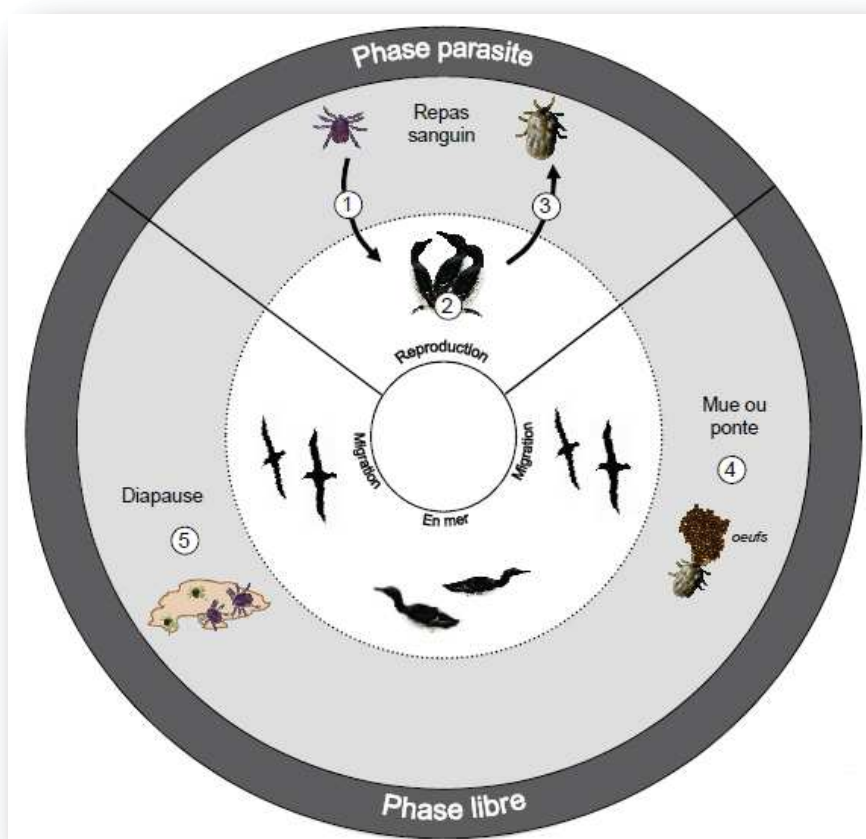
### *Ixodes uriae*



**Figure 1:** Pictures representing the tick *Ixodes uriae*. From left to right: an engorged female, feeding individuals on an Albatross, fed individuals assembled under a rock. Photos Credits: M. Dupraz, C. McKenzie and P. Bize.

*Ixodes uriae* (White, 1852) is an ectoparasite of the Ixodidae family. Ticks of this family are referred to as hard ticks because of an anterior chitinised plate on the dorsal surface. This species is widely distributed in cold and polar areas: North Atlantic, North Pacific, Arctic Ocean, Sub-Antarctic Islands and Antarctic (Dietrich et al, 2011). At least sixty colonial seabird species have been observed to carry this tick including species of the families Laridae, Alcidae, Procellariidae and Sulidae (Arthur, 1962; Zumpt, 1952). These birds all breed on land in colonies for a few months and then return to sea to overwinter (Coulson, 2001). The tick life cycle includes three active stages (Larvae-Nymph-Adult), with only a single nymphal instar. The time to complete a bloodmeal on the host is relatively long (3 to 12 days) compared to other hematophagous arthropods, but the duration varies with the life stage. Males don't feed in the adult stage (Eveleigh and Threlfall, 1974). As for all hard ticks, the bloodmeal involves an intense molecular dialogue between the hosts's hemostatic and immune systems and the tick's arsenal of anticoagulant and immuno-suppressive molecules used to avoid host responses (Brossard and Wikel, 2004). After completing the bloodmeal, these ticks return to protected shelters to moult and overwinter, or for females, to lay around a hundred eggs before dying (Eveleigh and Threlfall, 1974)(Figure 2).





**Figure 2:** Life cycle of *Ixodes uriae* (grey circle) dependant on host availability (white circle)(from PhD report of M. Dietrich, 2011). 1: non engorged ticks (larvae, nymph and female) find a host during the breeding season; 2 : Bloodmeal duration is dependant on the tick stages; 3 : After bloodmeal, ticks drop off in the environment; 4: ticks find shelters under rocks to lay eggs or moult; 5 : ticks overwinter in diapause waiting for the host to return.

As a nidicolous species, i.e. habitat restricted to the host nest or surrounding area, *I. uriae* spends the major part of its life cycle in the environment of host colony. While off-host, this tick forms aggregates in rock crevices or in the bottom of the host nest (Barton et al, 1995) to ensure more stable environmental conditions. The overall duration of the life cycle is strongly dependent on host presence and external temperatures and is generally achieved in 4 to 5 years (Eveleigh and Threlfall, 1974). Host use is facilitated by the life history of the seabirds themselves: colonial seabirds reproduce in high densities at the same time and same area from one year to another in mono- or heterospecific colonies. They are therefore both spatially and temporally predictable for their parasites. Moreover, they can easily live 15-20 years (Coulson, 2001), returning to the same nest site to breed. In the case of heavy infestations, *I. uriae* can directly impact host populations by reducing the body condition of both adult and juvenile birds via blood loss and therefore altering reproductive success (Boulinier and Danchin, 1996; Mangin et al, 2003). This tick also transmits a range of microparasites such as bacteria of the complex *Borrelia burgdorferi* responsible for Lyme disease in humans. However, the impact of different infectious agents on birds themselves remains largely unknown (but see Chambert et al, 2012). Because of the long off-

host phase in its life cycle, the survival of *I. uriae* also depends strongly on the temperature and humidity conditions of available microhabitats within the seabird colony. Dispersion is only possible via the host birds, particularly at the inter-colony scale. During the breeding season, bird movements are restricted to feeding areas at sea and the nest site. It is therefore unlikely that ticks disperse among colonies with breeding adult birds. However, failed individuals and young birds searching for a new breeding site have been proposed to be involved in tick dispersion (Boulinier et al, 2008, 2016; Danchin, 1992). The broad geographic distribution of this tick species and its capacity to exploit diverse seabird species led to its classification as a host generalist. Nevertheless, detailed sampling in mono and heterospecific colonies, combined with the use of population genetic tools, has resulted in the demonstration of host races in this species, races that have independently evolved in different areas of its worldwide distribution (McCoy et al, 2001, 2005, 2012). In the North Atlantic, this evolution should be relatively recent because of the time since colonization (Kempf et al, 2009). However, even in the ancestral areas of the distribution, levels of divergence are present at the host race level (Dietrich et al, 2014a). A transplantation experiment showed that performance differs significantly on alternative hosts (Dietrich et al, 2014b) and detailed morphometrical analyses have revealed that genetic divergence is supported by subtle morphological differences among host races (Dietrich et al, 2013). Nevertheless, these two studies were conducted at a single colony. During my PhD, I therefore tested if morphological convergence occurs among independently evolved host races exploiting the same host type, focusing specifically on different populations from the North Atlantic. From these patterns, we made inferences on the traits potentially implicated in the divergence process.

### ***Ornithodoros capensis* sensu lato complex**



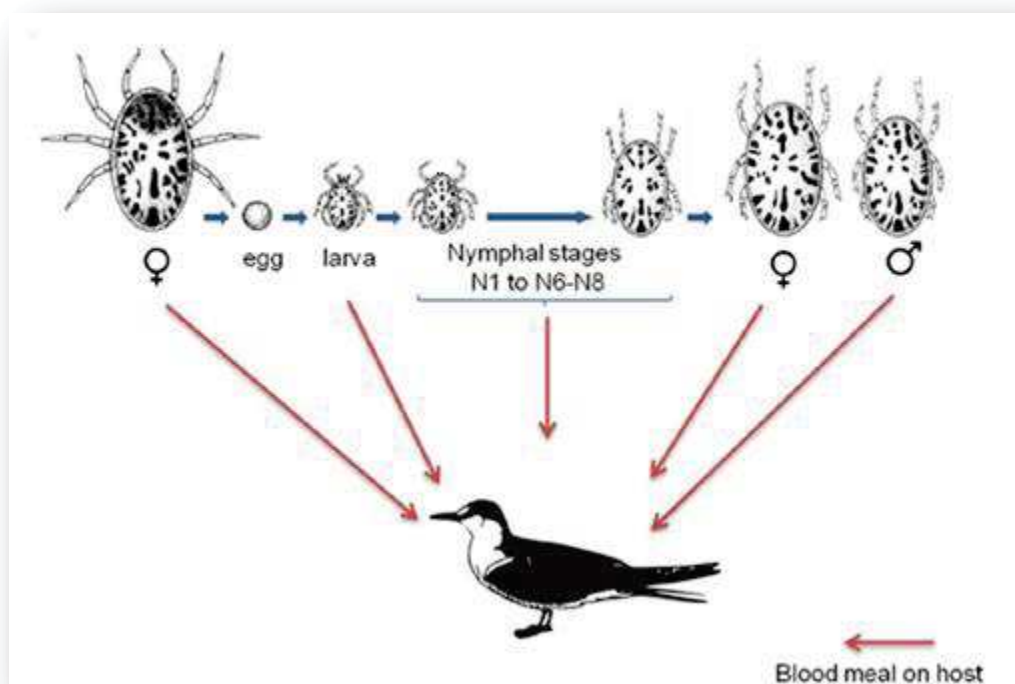
**Figure 3:** Pictures representing different species of the *O. capensis* s.l. complex.

*Ornithodoros capensis* sensu lato is a soft tick complex containing eight species of the family Argasidae. The ticks of this family are referred to as soft ticks because of their wrinkled body and springy, granulated cuticle. Contrary to hard ticks, the capitulum (structure containing the mouthparts) is situated on the ventral surface in the nymphal and adult stages of these ticks (Sonenshine, 1993). The *O. capensis* group is largely distributed across tropical and sub-tropical areas of the world. The different species were initially described from specimens opportunistically sampled and were almost exclusively based on larval morphological characteristics:

- *O. capensis* sensu stricto (Neumann, 1901)
- *O. maritimus* (Vermeil and Marguet, 1967)
- *O. amblus* (Chamberlin, 1920)
- *O. denmarki* (Kohls, Sonenshine et Clifford 1965)
- *O. muesebecki* (Hoogstraal, 1969)
- *O. sawaii* (Kitaoka and Suzuki, 1973)
- *O. spheniscus* (Hoogstraal, Wassef, Hays et Keirans, 1985)
- *O. yunkerii* (Keirans, Clifford et Hoogstraal, 1984)

More recently, Vermeil et al (1997) described an additional species in the complex collected on terns in Mauritania. The authors considered this species to be similar to *O. capensis* sensu stricto in larval stage, but with adult females resembling *O. maritimus*. However, after this initial description, no further information has been published concerning the taxonomic status and host affinities of this tick. Its inclusion in the *O. capensis* sensu lato complex therefore requires confirmation.

Like *I. uriae*, the ticks of the *O. capensis* complex are nidicolous and exploit a broad range of colonial seabirds including Alcidae, Laridae, Procellariidae, Sulidae and Spheniscidae. These seabirds breed on or close to the ground, generally in dry coastal areas and remote islands (Hoogstraal, 1985). The tick life cycle is multiphasic including one bloodmeal for larvae and one to several bloodmeals for each of the four to eight nymphal stages and adults. Compared to hard ticks, bloodmeal duration is very short, from few hours for larvae to few minutes for nymphs and adults. Males generally feed little or not at all. Consequently, we often collect nymphs and adults in the habitat and larvae on hosts. Females are able to repeatedly feed and lay a few hundred eggs each time (Pérez-Eid, 2007). The life cycle of *O. capensis* sensu stricto is described in figure 4.



**Figure 4:** Life cycle of *O. capensis sensu stricto*. Bloodmeal duration is dependant on the tick stages. Several short bloodmeals are taken on hosts by nymphs and adults.

Although different schools have tried to clarify the systematics of this group in the classification of soft ticks (Camicas and Morel, 1977; Hoogstraal, 1985; Klompen and Olivier, 1993; Pospelova-Shtrom, 1969) using morphological data, its position remains unclear (Guglielmone et al, 2010) and some authors refer to the ticks of this complex under the genera *Carios* (Horak et al, 2002). However, if we adopt the genus *Carios*, then *Ornithodoros* becomes a paraphyletic genus. We thus decided to be conservative concerning generic status of this group and refer to it as *Ornithodoros*. However, it is clear that effort is still needed to clarify the systematics of the Argasidae family (Estrada-Pena et al, 2010). In the same way, the geographic distribution and relatedness among species of the complex require clarification. Indeed, a preliminary genetic study based on ticks sampled in the Cape Verde archipelago has called into question the overall description of the complex (Gomez-Diaz et al, 2012). The problems associated with the description of this complex likely come from the fact that adult ticks are frequently collected in the field, but this life stage is difficult to identify morphologically to the species level and no genetic information of type specimens is currently available. We also know little to nothing about the ecology and evolution of this tick complex. We do not know whether these ticks may transmit pathogenic agents including, for example, relapsing fever bacteria of the genus *Borrelia* (Takano et al, 2009), or viruses such as Soldado virus which may induce high mortality in host colonies, breeding area desertion (Converse et al, 1975), or human puriginous infections (Camicas, 1980, Feare, 1976). Flaviviruses are

also frequently found such as Meaban or Kemerovo viruses (Chastel et al, 1985). Because of the wide range of exploited seabird species, the ticks of this complex are also considered to be host generalists. Nevertheless, the results of Gomez-Diaz et al (2012) also raised questions about this classification. Based on mitochondrial markers (RNAr 16S and 18S), the authors showed the coexistence of different tick lineages associated with specific host species within islands. As little information is available on the general biology and host use of the *O. capensis* sensu lato complex, more work is now required to understand how selection may act in generating these patterns. My work thus consisted in the identification and clarification of the distribution and interactions of the different tick species of the complex with different host species at a global scale.

## Objectives

My PhD project has focused on describing patterns of host specialization and identifying the factors that promote it in two tick-seabird systems. The specific objectives were to determine if the evolution of host specialization is always accompanied by the same phenotypic changes and to identify the underlying selective forces. I thus used morphometric methods to quantify subtle morphometrical variation among tick populations in relation to host use, and combined this data with phylogenetic and population genetic data. I used a local scale approach, based on *in situ* study, to try to understand the role of local isolation in this divergence process.

In chapter 2, I address technical questions associated with using morphological and morphometrical tools to characterize tick species and populations. The first article evaluates the reliability of classical descriptions to identify ticks. The second article focuses on testing for population-level differences using geometric morphometrics. I used this method to compare different tick populations in chapter 3.

In chapter 3, I examine the relationships between morphometrics and host use in the two tick-seabird systems to identify potential convergence in relation to host specialization in ticks. Thus, the third article of the thesis presents genetic and morphological divergence patterns in the *O. capensis* sensu lato complex at a global scale. The fourth manuscript explicitly tests for morphological convergence among *I. uriae* host races to identify the underlying factors promoting the repeated divergence observed in this system.

Chapter 4 is dedicated to the role of local isolation, and particularly local dispersal, of ticks within colonies in promoting local divergence patterns. I treat this question by a field-based study to determine

among nest differences in tick dynamics, tick movements within the colony (manuscript 5) and how this movement may impact pathogen circulation.

Finally, I conclude this dissertation with a general discussion (chapter 5) about the different factors that may generate tick specialization in the two systems, including perspectives for better understanding the evolutionary history of this process. The results from the tick-seabird systems are notably discussed in a more general context to evaluate if they could be informative for other tick systems and more generally for other vector systems.



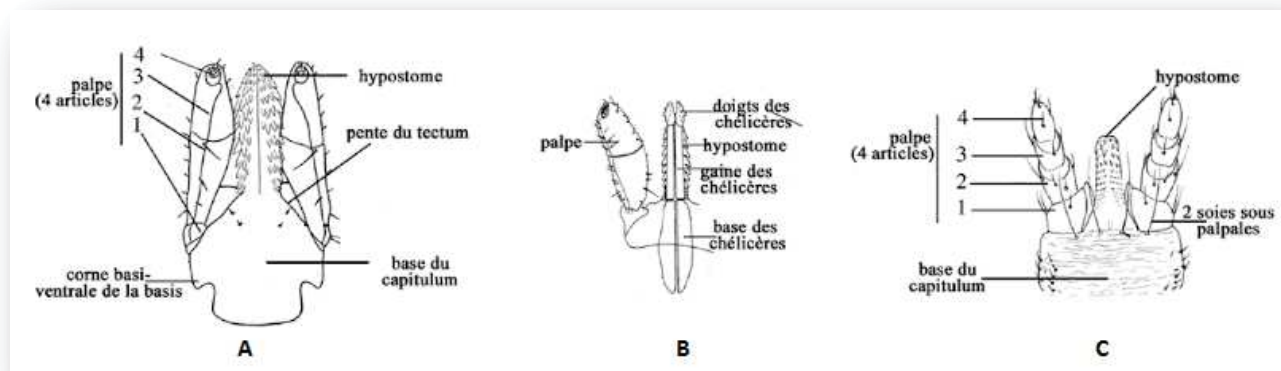


## **Chapter 2 – Species identification and discrimination**

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## 2.1 Morphological description of ticks

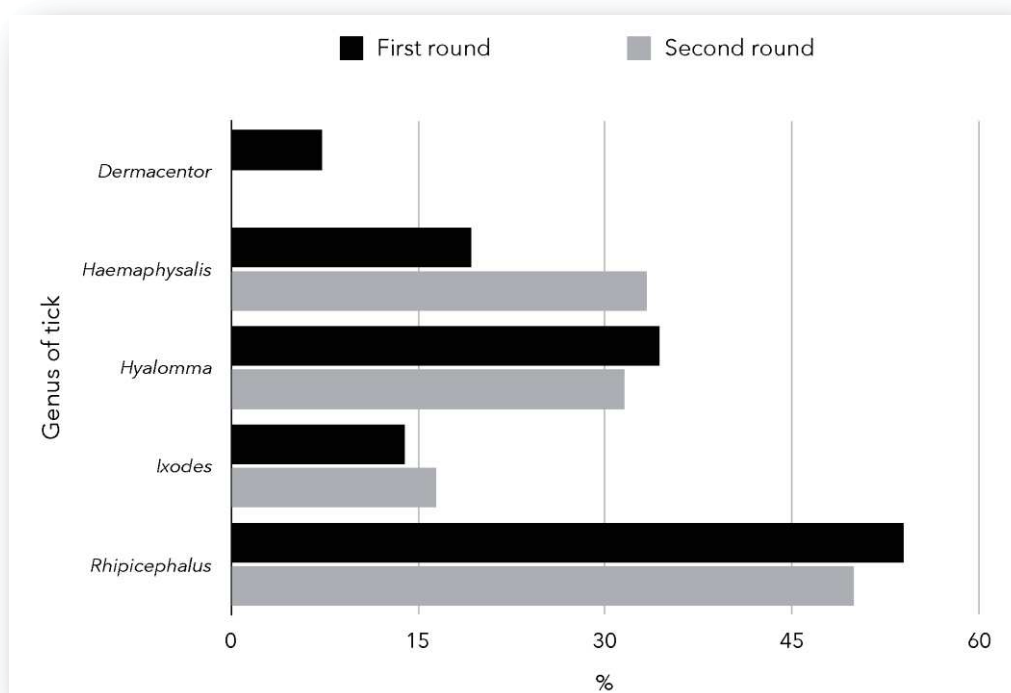


**Figure 5:** Tick capitulum morphology used as identification criteria. A and B: Ixodina, C: Argasina (Pérez-Eid, 2007).

Morphological descriptions, based on structural diversity, constitute the first step in species identification and classification (Adams et al, 2004). A primarily fixist view defining a species as a “set of resembling individuals”, has given way to an evolutionary vision instilled by Charles Darwin in the 19<sup>th</sup> century. This view considers a species as “a group of interbreeding individuals sharing common characters” (Thomas et al, 2010) and raises the notion of the intra-species variability. With this in mind, taxonomists have thus taken on the difficult task of defining or redefining species based on shared characters. This work is extensive and particularly so for arthropods which represent a significant proportion of the world’s biodiversity, with 1.5 million described species at present (Lecointre and Guyader, 2001). Cryptic species, which are not morphologically distinguishable from each other, are challenging and contribute today to the growing list of newly described species due to the increasing use of molecular tools (e.g., *Anopheles* mosquitos (Collins and Paskewitz, 1996) or plant mites (Navia et al, 2013)).

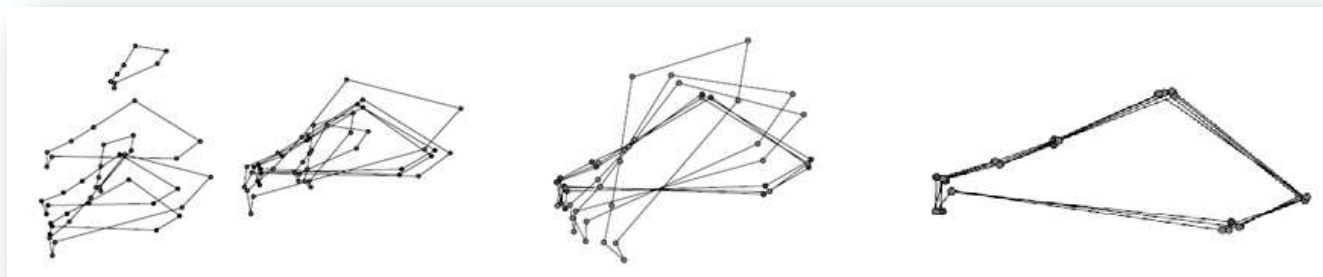
In ticks, species identification is traditionally based on morphological criteria such as the position of the capitulum, the relative size of different mouthparts, and the position of the anal groove (Bonnet et al, 2015) (Figure 5). Today, several classifications are available based on morphological keys or genetic markers. The repeated publication of different keys highlights the difficulty of researchers in correctly classifying the species of this arthropod group, especially for soft ticks (Estrada-Pena et al, 2010). Three tick families are currently recognized: *Nuttalliellidae*, represented by only one species, *Nuttammiella namaqua*, *Argasidae* or soft ticks containing 193 potential species including the genera *Antricola*, *Argas*, *Nothoapsis*, *Ornithodoros* and *Otobius* and, *Ixodidae* or hard ticks containing 702 species of 14 different genera such as *Ixodes*, *Amblyomma*, *Hyalomma*, *Rhipicephalus* and *Dermacentor* (Guglielmone et al,

2010). Within families, subtle morphological differences can lead to species misidentification and thus incorrect classifications. The development of molecular identification tools has helped clarify tick systematics and regularly leads to nomenclature changes (Plantard et al, 2015). The capacity of a specialist community to identify different tick species within the genera *Dermacentor*, *Ixodes*, *Haemaphysalis*, *Hyalomma* and *Rhipicephalus* was tested via a blind test (Article 1). Two rounds were done: the first one focused on identifying ticks with morphological keys or genetic tools and the second with a specifically-designed morphological key without illustrations. Results showed variable identification rates, from 0 to 54% according to the genus (Figure 6) and revealed an overall weakness in our ability to identify ticks using classical morphology. Thus, it appears important to rely on both correctly defined morphological keys and genetic tools to reliably differentiate tick species.



**Figure 6:** Misidentification rates of the tick genera during the two rounds.

## 2.2 Geometric morphometrics



**Figure 7:** Different steps of the Generalized Procrustes analysis based on landmarks (scaling, translation, rotation).

The evolutionary concept of a species allows for the existence of high intra-specific morphological variability, for example among juveniles and adults, males and females and also within an age class. In the last case, subtle morphological variability can be quantified using morphometric methods which involve studying the form (size + shape) of an object and its variations. Contrary to morphology used to describe and identify a unique entity (typically a species), morphometry compares entities (Thompson, 1945). Classical morphometry is based on a set of linear measures of surface or volume, as illustrated by the Raup model used to describe gastropod shells (Raup, 1966). However, there are some inconveniences concerning object configuration (shape): linear distance measures are strongly correlated with individual size and therefore need to be corrected, and two objects with different shapes can present identical linear measures. In the 80s, Bookstein's work revolutionized the discipline of morphometrics. He established the geometric morphometric concept based on the superposition of landmarks to compare the variability of forms using the raw coordinates of each landmark (Bookstein, 1997). The first step of this method relies on positioning landmarks on the anatomical structures of each studied individual. Landmarks have to be in same position and in sufficient number to be able to correctly describe the form. Landmark measures can be collected from images, radiography or in 3D with lasers or touch arms. Once the landmarks are positioned, the second step consists in comparing the geometric configuration generated by landmarks by superposing configurations from different individuals. This can be realized by different approaches, but the best suited for the comparison of several individuals is using a Generalized Procrustes Analysis (GPA) based on centroid size, i.e, the mean coordinates of the landmark configuration. This step includes different stages of scaling, translation and rotation (Dujardin, 2011) such that new coordinates can be defined to describe landmark configurations and compare shape without the influence of size (Figure 7). The degree of allometry, the relationship between size and shape, remaining after correction can be calculated using linear regression. The final



step consists in the statistical analyses of the landmark configurations to generate a graphic representation of the variability among individuals. Different tools can be used, for example a principal component analysis (PCA), a thin-plate spline method or a discriminant analysis (DA). As geometric morphometrics allows one to measure continuous characters that can be impacted by micro-evolutionary forces, such as traits associated with biological activities (feeding, locomotion, reproduction), this method is a particularly good tool to use when studying phenotypic changes. Indeed, it has now been frequently used to distinguish closely-related species (Barluenga et al, 2006; Michaux et al, 2007; Patterson et al, 2001), sub-populations (Dietrich et al, 2013; Kaba et al, 2012; Ledevin et al, 2010) or re-infestation sources after insecticide treatments (Dujardin, 2011; Dujardin et al, 1997). This method can also permit one to relate size and shape differences to environmental variables and therefore identify the mechanisms promoting divergence (Dujardin, 2008). Fast, cheap and non-destructive, geometric morphometrics can readily complement genetic analyses and can be used on precious collection specimens (Garros and Dujardin, 2013). Moreover, geometric morphometrics can even be used on non-articulated body parts by employing outline methods in the place of more classical landmarks. For example, the Fourier analysis uses the coordinates of a set of landmark, positioned on the contour of a body form of each individual to create an outline, in order to compare the relative shapes (Lawing and Polly, 2010). This outline method was compared to the landmark method using closely-related or cryptic species including bugs, anopheles, flies and soft ticks of the *O. capensis* complex. The two types of morphometrical analyses revealed similar population discrimination scores. Outline analysis is thus particularly adapted to use on soft ticks with their flexible cuticle (Article 2).

## Article 1

A. Estrada-Peña, G. D'Amico, A. M. Palomar, M. Dupraz, M. Fonville, D. Heylen, M.A. Habela, S. Hornok, L. Lempereur, M. Madder, M. S. Nuncio, D. Otranto, M. Pfaffle, O. Plantard, M. M. Santos-Silva, H. Sprong, Z. Vatansever, L. Vial, A.D. Mihalca. *Submitted to Ticks and Tick-borne Diseases*. The results of a blind test of ixodid tick identification 1 by European experts.



The results of a blind test of ixodid tick identification by European experts.

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## Abstract.

This study reports the results of a blind test of identification of ticks occurring in Western Europe and Northern Africa. A total of 14 laboratories were voluntarily enrolled in the test. Each participant received between 22 and 25 specimens of adult and nymphal ticks of the species *Dermacentor marginatus*, *D. reticulatus*, *Haemaphysalis punctata*, *Hyalomma lusitanicum*, *Hy. marginatum*, *Ixodes ricinus*, *I. hexagonus*, *Rhipicephalus annulatus*, *R. bursa*, *R. rossicus*, and/or *R. sanguineus* s.l. Ticks were morphologically identified by three of the co-authors and the identification confirmed by a fourth co-author who used molecular methods based on several genes. Then ticks were randomly selected and blindly distributed among participants, together with a questionnaire. Only specimens collected while questing and, if possible, in the same survey, were circulated. Because of the random nature of the test, a participant could receive several specimens of the same species. Species in the different genera had variable misidentification rates (MR) of 7% (*Dermacentor*), 14% (*Ixodes*), 19% (*Haemaphysalis*), 36% (*Hyalomma*), and 54% (*Rhipicephalus*). Within genera, the MR was also variable ranging from 5.4% for *I. ricinus* or 7.4% for *D. marginatus* or *D. reticulatus* to 100% for *R. rossicus*. The test provided a total misidentification rate of 29.6% of the species of ticks. There are no significant differences in MR according to the sex of the tick. Participants were requested to perform a second round of identifications on the same set of ticks, using only purposely prepared keys (without illustrations), circulated to the enrolled participants, including 2 species of the genus *Dermacentor*, 8 of *Haemaphysalis*, 10 of *Hyalomma*, 23 of *Ixodes*, and 6 of *Rhipicephalus*. The average MR in the second round was 28%: 0% (*Dermacentor*), 33% (*Haemaphysalis*), 30% (*Hyalomma*) 18% (*Ixodes*), and 50% (*Rhipicephalus*). Species which are not reported in the countries of a participating laboratory had always highest MR, i.e. purely Mediterranean species had highest MR by laboratories in Central and Northern Europe. Participants expressed their concerns about a correct identification for almost 50% of the ticks of the genera *Hyalomma* and *Rhipicephalus*. The results revealed the lack of total confidence identifying the most prominent species of ticks in the Western Palearctic, and underpin the need of adequate reference libraries for specialists involved in this task. Results also showed that an adequate combination of several genes may adequately identify the target species of ticks.

Keywords: blind test; identification; morphology; molecular; Ixodid ticks; Western Palearctic



## Background

Ticks are known to transmit a large variety of pathogens of medical and veterinary concern and are among the most important disease-transmitting arthropods (Estrada-Peña and de la Fuente, 2014). Most of the field studies on ticks are based on a correct identification of the specimens collected, as a prerequisite for further research. For example, in a recent literature review on the occurrence of ticks and tick-borne pathogens in Europe and the Mediterranean Basin (Maioli et al., 2012), only studies that used morphological keys for tick identification were considered. More than 60 species of the family Ixodidae can be found in Europe, and its morphological identification is not trivial, as some species form complexes with cryptic or sibling species, i.e. *Rhipicephalus sanguineus* group (Dantas-Torres et al., 2013) or have relatively complex morphological features for an accurate determination, which may require a deep training, like the genus *Hyalomma*. Even in a thoroughly surveyed area like Europe, new species have been recently described (i.e. *Ixodes inopinatus* Estrada-Peña, Nava & Petney, or *I. ariadnae* Hornok). Interspecific hybridization between two species of *Ixodes* has also been reported, a biological event that may pose additional concerns for specific identification, even using molecular markers (Kovalev et al. 2015). Moreover, morphological keys for ticks commonly cover only the species with medical interest, not every stages are included (Arthur, 1963; Nosek and Sixl, 1972; Cordas et al., 1993; Hillyard, 1996; Filippova, 1997; Manilla, 1998; Estrada-Peña et al., 2004; Cringoli et al., 2005; Pérez-Eid, 2007) and some of them may be unreliable because they do not include the most recent concepts about the species identity. Therefore, recent studies on comparative morphology of ticks in Europe are rare (Heylen et al., 2014). The availability of different methods has provided insights in the use of cuticular hydrocarbons composition (Estrada-Peña et al., 1996) or MALDI-TOF (Yssouf et al., 2013) for the identification of ticks, while most reports focused on the use of adequate genetic markers, like 16S rRNA, 12S rRNA, or *cytochrome c oxidase I (coxI)*. Although these technologies are useful, they will always be based on the use of reference specimens for which morphological species-specific identification need to be correctly conducted (Nava et al., 2009; Araya-Ancheta et al., 2015). “Garbage sequences” obtained from unreliably identified specimens that accumulate in databanks like GenBank are a source of misidentification. They may introduce a background noise when included in the context of a phylogenetic reconstruction of species of ticks (Zhang and Zhang, 2014) or produce an incorrect identification of separate specimens.

Further concerns on the topic include the possible spread of some tick species beyond their historical ranges because natural factors such as weather (Jaenson et al., 2012; Medlock et al., 2013; Jore et al., 2014), the peculiarities of the landscape (Li et al., 2012; Vanwambeke et al., 2016), or an increase of the density of adequate hosts (Li et al., 2014). If some species are prone to spread and colonize areas where they were previously unreported, there is a pressure for a correct identification to promote the adequate measures of control and evaluate the potential impact on either human or domestic animals.

Blind tests are often applied for the unbiased determination of events in different facets of the science, including very different events like cut marks versus carnivore tooth marks on bone surfaces (Blumenschine et al., 1996), plant or animal residues in archeological research (Leach, 1998; Lombard and Wadley, 2007) or the degree of anonymity of the blind or double-blind review process for scholarly research articles (Hill and Provost, 2003). Biological sciences have benefited of blind tests for the identification of organisms, the procedure being generally applied to compare the degree of similarity between the opinion of several specialists about the classification of organisms. In other cases, the classification obtained by both morphological and molecular methods has been compared (i.e. Kim et al., 1999; Coupe et al., 2005; Druzhinina et al., 2005). Most of the non-morphological methods used for the identification of some parasitic arthropods always used the blind approach, comparing the results of a candidate method against the background of morphological identification by experts (i.e. Dieme et al., 2014; Yssouf et al., 2013). Examples of a blind test approach to ticks so far cover the use of DNA-barcoding for the detection of blood remnants on specimens collected while feeding (Garipey et al., 2012). Nevertheless, the morphological identification of ticks is more routinely used in laboratories around the world than molecular methods, mainly in large sample sets. However, a comparative test on the opinion of experts about the specific identity on batches of ticks in a large region has never been done. In summary, there is no objective measure about the comparative reliability of experts in the field to recognize widely distributed and common species of ticks in a large territory.

We aimed to candidly and openly test the reliability of a blind test of morphological identification of ticks reported as established in Europe and Northern Africa, by several teams of experts, based on specimens which were previously selected to fit some requirements, carefully identified by their morphology, confirmed by molecular tools,

and circulated to the participants. We derived statistics of the rates of correct determinations compared with the genus and the sex/stage of each tick. Special attention was paid to the reliability of the identification according to the distribution of the ticks (e.g. species existing in the country of residence of the participant, and therefore familiar to him/her) and the approach used by participants in the identification of the specimens (i.e. using dedicated books, reprints, voucher specimens, etc.). A secondary aim was to estimate the rate of confidence of the participants with their identifications as compared with the rate of mismatches, a ratio that express how efficiently the experts can manage the threat derived by ticks. Finally, our last aim was to evaluate the reliability and usability of a comprehensive key for all the species of ticks reported in the Western Palearctic as a synthetic tool specifically aimed to improve the task.

#### Material and Methods

For an adequate representation of the most common species reported in the Western Palearctic (including Northern Africa), the following species have been included in the test: *Dermacentor marginatus* (Panzer) (14♀, 13♂), *Dermacentor reticulatus* (Fabricius) (14♀, 14♂), *Haemaphysalis punctata* Canestrini & Fanzago (14♀, 12♂), *Hyalomma lusitanicum* Koch (7♀, 11♂), *Hyalomma marginatum* Koch (14♀, 12♂), *Ixodes hexagonus* Leach (14♀, 13♂, and 15 nymphs), *Ixodes ricinus* (Linnaeus) (14♀, 11♂, and 12 nymphs), *Rhipicephalus annulatus* (Say) (14♀, 11♂), *Rhipicephalus bursa* Canestrini & Fanzago (12♀, 12♂), *Rhipicephalus rossicus* Yakimov and Kohl-Yakimova (12♀, 15♂), and *Rhipicephalus sanguineus* s.l. (9♀, 17♂). The test was explicitly focused on the tick fauna from the two large biogeographical regions of the target territory: the countries bordering the Mediterranean basin, which also includes species from Northern Africa, and countries in Central and Northern Europe. We did not include species of ticks which are monoxenous (like *Ha. hispanica* Gil-Collado *R. pusillus* Gil-Collado or *I. ventalloi* Gil-Collado, restricted to the Mediterranean wild rabbit, or *I. lividus* Koch, restricted to the European sand martin) or species inadequately described or scarcely reported (i.e. *I. eldaricus* Dzhaparidze, *I. festai* Tonelli-Rondelli, *I. kaiseri* Arthur). The species chosen for the study have special significance in both human and animal health, and they are well known vectors of pathogens to humans or livestock (Jongejan and Uilenberg, 2004).

The standardization of the batches of ticks circulated to the participants was a point of special concern. This interest prevented the inclusion of some species of potential importance in the test, like *I. frontalis* Panzer, *I. persulcatus* Schulze, or *I. acuminatus* Neumann, because the available specimens were in a variable degree of repletion, or obtained from a wide variety of sources, far from the standards adhered in the protocol. Only unengorged ticks were used. Specimens used for the test were always collected while questing, never feeding, to avoid the distortion of the morphological proportions. In the case of *R. annulatus*, which is a one host species, specimens were collected as engorged nymphs on cattle, and allowed to molt in the laboratory to have flat adults. All the specimens of the same species were collected in the same place and, when possible, they were collected in the same survey, site and date, to have the most homogeneous sample set. Every specimen which did not conform to the description of the species, or with morphological abnormalities was removed from the test. Specimens of *R. sanguineus* s.l. were collected on the walls of a kennel in Mediterranean Spain. In this case, we included in the test only specimens conforming to the classic description by Filippova (1997). This description overlaps with the morphology of the “type II” specimens reported by Dantas-Torres et al. (2013) and it intends to represent the Mediterranean populations of this complex of species. All specimens were determined by one of three co-authors (none of these participated in the blind test) and confirmed by the two others. A fourth author enrolled in the blind test, performed a molecular determination of every species and results were 100% in agreement with the morphological determination of the three authors mentioned above. Tick species were confirmed by PCR targeting the tick mitochondrial 16S ribosomal RNA (16S rRNA) (Black and Piesman, 1994). For some specimens, e.g. tick species in which this fragment gene had not been characterized, or was not specific enough, PCR assays for the mitochondrial 12S rRNA gene and the nuclear 5.8S-28S rRNA intergenic transcribed spacer 2 (ITS2) were also performed (Beati and Keirans, 2001; Labruna et al., 2002). Again, this is of special interest in the case of *R. sanguineus* s.l. for which molecular identity of the specimens included in the test was confirmed to belong to the so-called temperate clade. The genes targeted and the primers used for molecular identification are included in Table 1. The Table 2 includes the maximum identities of sequences obtained in this study for the molecular identification of the ticks.

The assignation of samples to each participant was blind and random. Once each individual tick was placed separately in a small vial containing 70% ethanol, a unique

Table 1. PCR primer pairs and conditions used for the genetic identification of tick, together with the original references.

Target gene	Primer sequence (5'→3')	Melting temperature (°C)	Fragment size (base pairs)	Reference
16S rRNA	16S+1: CTGCTCAATGATTTTTTAAATTGCTGT GG	48 54	456	Black and Piesman, 1994
	16S-1: CCGGTCTGAACTCAGATCAAGT			
12S rRNA	T1B: AACTAGGATTAGATACCCT	51	Approx.. 360	Beati and Keirans, 2001
	T2A: AATGAGAGCGACGGGCGATGT	53		
ITS2	RIB-4F: CCATCGATGTGAAYTGCAGGACA	55	variable, 800-1,100	Zahler et al. 1995; McLain et al. 1995 (from Labruna et al., 2002)
	RIB-R: GTGAATTCTATGCTTAAATTCAGGGG GT			

Table 2. Maximum identities of sequences obtained in this study for the genetic characterization of tick species.

Tick species	Gene sequences; % identity & GenBank no (no of sequences)		
	16S rRNA	12S rRNA	ITS2
<i>Dermacentor marginatus</i>	99.5 JX051098 (1)	-	-
	99.5 JX051097 (1)	100 AF031848 (1)	99.6 FN296278 (1)
<i>Dermacentor reticulatus</i>	99.8 JF928493 (1)	-	-
<i>Haemaphysalis punctata</i>	99.5 KR870978 (3)	100 AF483218 <sup>1</sup> (3)	-
<i>Hyalomma lusitanicum</i>	99.7-100 Z97881 (3)	-	-
<i>Hyalomma marginatum</i>	99.5 L34307 (1)	-	-
<i>Ixodes hexagonus</i>	100 JF928502 (1)	99.6 AF081828 (1)	100 GQ924083 (1)
<i>Ixodes ricinus</i>	100 GU074616 (1)	100 AF150029 (1)	-
	100 GU074606 (1)	100 KF197118 (1)	-
	99.8 GU074590 (1)	100 JN248424 (1)	-
	100 GU074589 (1)	-	-
<i>Rhipicephalus annulatus</i>	99.8 L34311 (2)	-	-
<i>Rhipicephalus bursa</i>	100 KU664351 (4)	100 KC243833 (2)	-
<i>Rhipicephalus rossicus</i>	100 KP866202 <sup>2</sup> ; 99.6 KU848178 (1)	100 AF150021 (1)	99.3 AF271282 <sup>3</sup> (1)
<i>Rhipicephalus sanguineus</i> s.l.	100 KT382469 (2)	-	-

<sup>1</sup> Identified as *Haemaphysalis* sp.; <sup>2</sup> *R. sanguineus* sequence, only a short sequence from *R. rossicus* available in GenBank (KU848178); <sup>3</sup> Sequence identified as *Rhipicephalus pumilio*. No ITS2 sequence from *R. rossicus* available in GenBank.

number was randomly allocated to each specimen, which was sent to one of the participants. Participating laboratories also were identified by a random number. Each participant received 22 to 25 specimens. Because of the random nature of the test, a participant could receive several specimens of the same species, without a balance of gender or stage of the ticks in the samples. Participants were informed of this characteristic of the test, and instructed that even if a species was already identified in the set of material, the same species could appear again, or not, in the received material.

Participants enrolled voluntarily in the study. All of them have a longstanding experience in the identification of ticks, and have a background of peer-reviewed publications on the topic. Only three of the authors (the ones which did the primary identification of ticks) knew the names of the other participants, to avoid the exchange of information among enrolled experts. In any case, the random identification numbers for the ticks were unique to avoid any attempt of information exchange among participants. Only one of the authors knew the complete correspondence between species of ticks, identification numbers for ticks, and details of the enrolled laboratories. Sixteen experts, from 14 laboratories (one of them involved in the molecular information of the specific identity of the specimens), were finally enrolled in the test.

The samples were circulated together with a questionnaire to be filled individually for each identified specimen. These forms were labelled with the number of the tick, and included questions about the identity of the specimen, its gender or stage. We specifically aimed to collect details about the process of identification, involving the procedures adhered by the participant regarding the use of keys/reprints/monographs and how confident he/she felt about his/her identification. The complete questionnaire is included in the supplementary material. After the first round of identification, participants received a key tailored for every stage and species of Ixodidae found in the Palearctic region, without illustrations. The keys included the adults of 2 species of the genus *Dermacentor*, 8 species of *Haemaphysalis*, 10 of *Hyalomma*, 23 of *Ixodes*, and 6 of *Rhipicephalus*, as well as keys for nymphs of 23 species of *Ixodes*. Participants were requested to perform a second round of identifications on the same set of ticks, using only the purposely prepared keys, and submit again the results. We received the response from the 14 laboratories in the first round but only 11 sets of responses in the



second round. This resulted in 306 ticks identified by morphological methods in the first round and 259 in the second.

Every participant was confidentially informed of his/her rates of success in the determination of the batches of ticks in both rounds. We did not consider that some species could be more difficult to identify than others, and therefore the rate of success is a crude, unweighted percentage. The compared performance of each laboratory is not included in this study because obvious rules of ethics and confidentiality. We calculated total rates of correct identifications, in both the first and the second round, as well as the same figures split by genera, to evaluate if some genera had poorer identification rates than others, and by sex, to check if males or females had different identification rates. We further compared if the rates of wrong identifications are higher for species that do not exist in the geographical zone of each participating laboratory. In other words, we tested if participating laboratories are able to identify potentially invasive ticks. We also compiled the list of references used to identify each species.

## Results

All the species of ticks classified on morphological grounds and circulated to the participants hit a 100% of correct identification rates for every species when determined by molecular methods (see Table 2). Regarding the identifications made on morphological grounds, every genera of ticks were adequately identified but for one specimen, and misclassification rates (MR) were at the level of species or stage. The total MR in the first round was of 29.6%, which decreased to 28.5% in the second round. The MR of stages was 1.6% (5 out of 306) and 0.8% (2 out of 259) in the first and second rounds, respectively. Two males of *D. marginatus* were initially considered as females during the first round of identification while there were in fact females; because numerous morphological characters allow to distinguish males and females in ixodid ticks, this mistake illustrates the existence of errors of inattention (possibly during the filling of the individual form for each tick specimen) among the misidentifications, which are not possible to address in the statistical assessment. Figure 1 shows the MR split by genera, in both the first and second rounds. It must be noted that the number of responses by participants was lower in the second round than in the first: 306 vs. 259 ticks were identified in each round. The species in the genera *Dermacentor* and *Ixodes* obtained the lowest misidentification rates, 7.27% and 13.92% in the first round, respectively. However, every specimen of *Dermacentor* was correctly identified in the second round but the misidentification rate of *Ixodes* increased to 18.03%. Within

genera, the misidentification rate varied from 5.4% for *I. ricinus* or 7.4% for *D. marginatus* or *D. reticulatus* (the three most studied tick species in Europe). Species belonging to the genus *Haemaphysalis* had misclassification rates of 19.23% and 33.33% in the first and second rounds, respectively. The species in *Hyalomma* and *Rhipicephalus* were those that had highest rates of misidentifications in both rounds, with similar figures, around 36% in *Hyalomma* and 54% in *Rhipicephalus*. The MR by species are included in Figure 2. The species was adequately identified if the specimen was a male in 71% and 72% of cases (first and second round), or in 68% in the case of females (both rounds). The misidentification rate was 100% for *R. rossicus* in the first round, a species rarely included in most morphological keys used by european tick taxonomy experts.

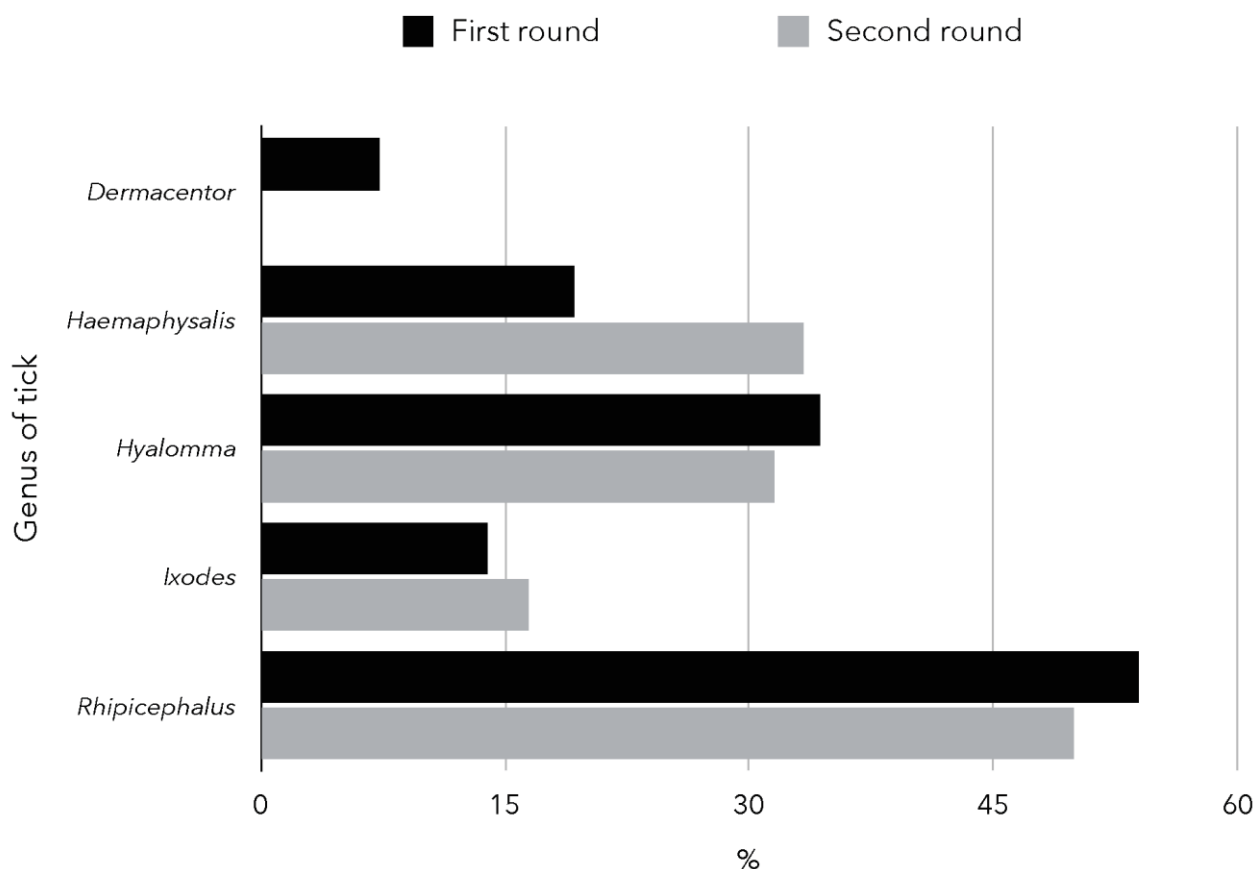


Figure 1: The misclassifications rates of ticks, split by genera (in %) in both the first and second round of morphological identification.

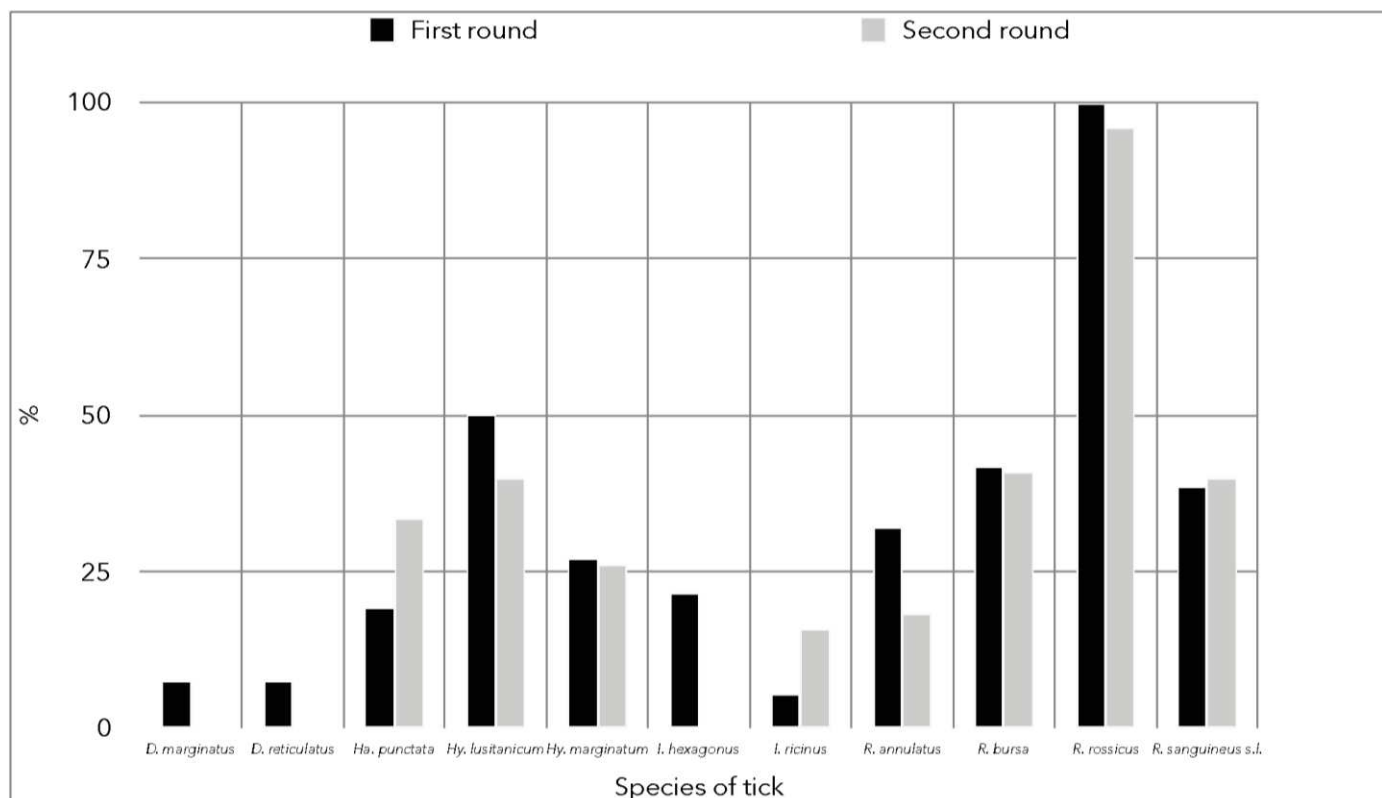


Figure 2: The misclassification rates of species of ticks (in %) in both the first and second round.

We compared the performance rate of identifications with the presence/absence of the tick in the national territory of each participating laboratory. We refer to these specimens as “endemic” or “non-endemic” if they have been reported or not in the national territory of the participating researcher. Only 8.49% of “endemic” ticks were misidentified in the first round, a value that increased to 10.45% in the second round of identifications. However, the misidentification rates of “non-endemic” ticks were 21.1% and 13.6%, in the first and second rounds, respectively. Regarding the use of bibliographical resources, 21% of specimens were identified without the help of further references, because participants were familiar with the tick, 49% used reprints for the identification (listed separately in the supplementary material), 5% used generalist book(s) that compile(s) data on species from a large territory, and 24% used both reprints and books. The self-perception of the participants about the reliability of identifications was very variable. The participants had the idea that 27% of specimens were reliably identified after a first look because they were familiar with the tick, and that 49% of specimens were identified after checking the bibliographical references. The participants had “serious concerns about the reliability of the morphological identification” of 23% of the specimens (see Figure 2). Comparing these figures about self-perception with the MR, 2% of specimens were erroneously identified in the first category, 7% in the second, and 15% in the third. However, these rough figures were

category, 7% in the second, and 15% in the third. However, these rough figures were highly variable when split by tick genera (see Figures 3 and 4). Most participants felt that identifications of *Dermacentor* and *Ixodes* were reliable. The degree of self-perception of identification reliability decreased in the species of *Haemaphysalis*. Most participants expressed “serious concerns” about the reliability of the identification of species in both *Hyalomma* and *Rhipicephalus*. The comparison of these crude figures

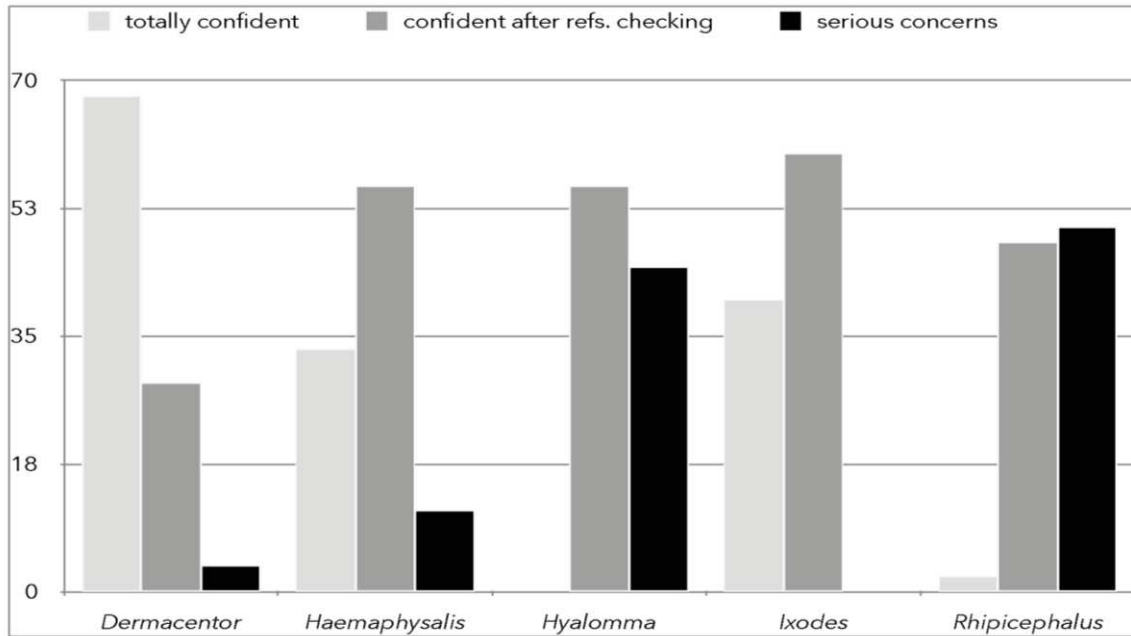


Figure 3: The degree of self-perception by participants about the reliability of the identification of ticks, split by the genus of the tick (in %).

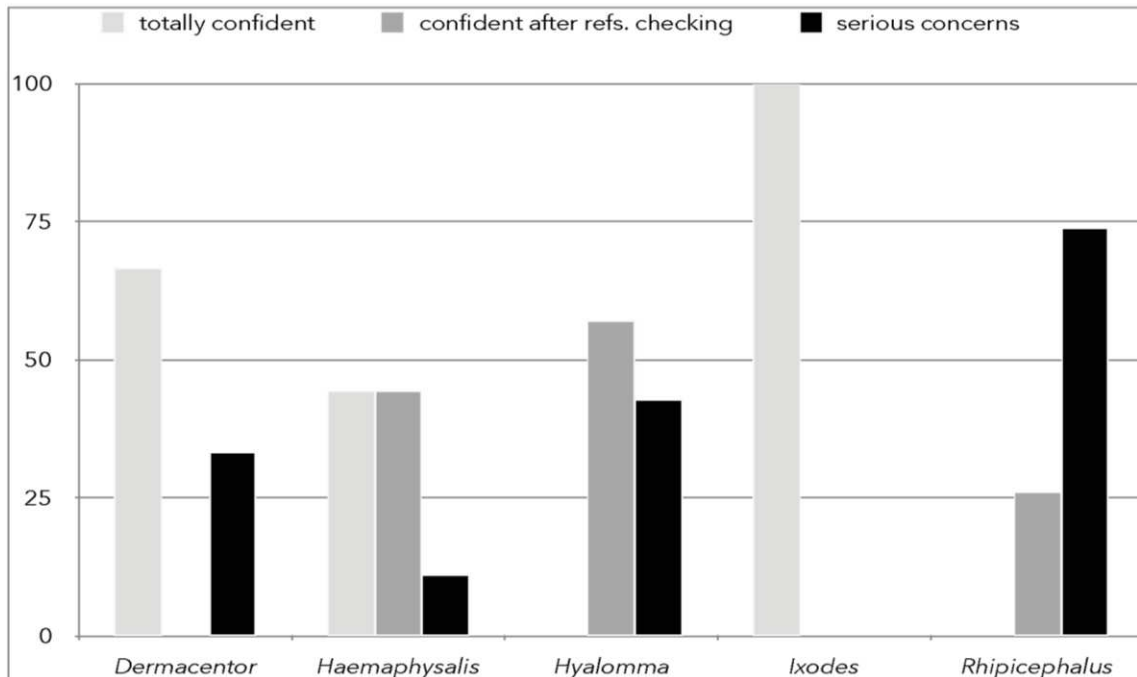


Figure 4: The percent of erroneously identified ticks (grouped by genera) according to the self-perception by participants about the reliability of the identification of ticks.

with the accuracy of the identifications provide some significant findings: participants were aware that a high percentage of the ticks in the genera *Rhipicephalus* and *Hyalomma* were probably wrongly determined.

#### Discussion

This paper reports the results of a blind test of identification of ticks carried out by 14 voluntarily enrolled laboratories in Europe. The study was intended to evaluate our capacity to identify both the well-established species of ticks which are common in the country of the participants, or to quantify the competences of the European groups to cope with potentially invasive species. The test included species of the genera *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* and *Rhipicephalus*, distributed in Europe and Northern Africa. The study was intended to focus on the most prominent species of ixodid ticks of the target region to clearly delineate the potential of the experts in the management of tick species with the highest impact on human or animal health. Results showed that species in the genera *Ixodes* and *Dermacentor* had the lowest misidentification rates, while species of *Hyalomma* and *Rhipicephalus* had the highest rates of unreliable identifications. Crude results showed about 30% of misidentifications, which decreased merely to 28% when a purposely prepared key without illustrations was circulated among participants in a second round. Claims about the possibility of spread of ticks currently reported in the Mediterranean to northern latitudes (Jaenson et al., 2012) are thus a concern after these results, since 21% of these “non-endemic” species were unreliably identified.

There are several factors that could bias the results reported here. The most obvious is the “gold standard” established for the identity of each specimen. This was done by three participants in the study, something which could arguable lead to different diagnostic criteria when the specimens were examined by other participants. The three co-authors initiating the study, identifying, and circulating the ticks were not involved in any other step of the study. Additionally, these three authors were aware also about the exact geographical origin of the ticks. However, the morphological identification of the batches of ticks matched at 100% when identified by molecular methods. For every other step, we adhered to the randomness of both batches of ticks and participants, therefore eliminating potential biases. Even at the stage of manuscript editing and final agreement of the submitted paper, the co-authors were unaware of the rates obtained by the other participants.

It is interesting to notice that the genera *Dermacentor*, *Ixodes* and *Haemaphysalis* were well known by almost every participant, with low rates of misidentifications. However, the genera *Rhipicephalus* and *Hyalomma* accumulated the highest rates of mistakes. In other words, the purely Mediterranean species were inadequately identified by most of the participating laboratories. It is however important to mention that only 6 laboratories in Mediterranean countries were involved in the test, of a total of 14. In the case of *Rhipicephalus*, the errors in classification were mainly due to the lack of a harmonized definition of the *R. sanguineus* s.l. group, which needs a re-description and the designation of a neotype of *R. sanguineus* s.s. (Nava et al., 2015). We circulated adults of both *R. rossicus*, from large batches obtained in Romania (Sándor et al., 2014) and *R. sanguineus* s.l., collected in a heavily infested kennel in Spain, which closely overlapped with the classic description of the “species” (Filippova, 1997), had highest similarity with the molecular signature reported for the Mediterranean populations (Nava et al., 2015) and closely adhered to the specimens mentioned as clade II by Dantas-Torres et al. (2013). As a further proof of the inherent difficulties of the genus *Rhipicephalus*, nobody was able to identify correctly the adults of the neglected *R. rossicus* (rarely included in most of the books used by European experts) in the first round, and only 1 laboratory managed a correct identification after a key containing the species was circulated among the participants.

Of particular interest is the fact that the circulation of the key without illustrations did not improve significantly the rate of reliable identifications, and, in some cases, introduced even higher rates of misidentifications. The second round produced poorer rates by some participants who already identified reliably a specimen in the first round. The obvious translation is that: (i) the inclusion of more species of ticks in the key produced a background noise that confused the participants, (ii) the lack of illustrations is a serious issue when only the crude text is used for the identification. The key included 2 species of the genus *Dermacentor*, 8 species of *Haemaphysalis*, 10 of *Hyalomma*, 23 of *Ixodes*, and 6 of *Rhipicephalus*. Whereas most mistakes using the keys were observed for the genera *Ixodes*, *Hyalomma*, and *Rhipicephalus*, we conclude that the “noise” generated by the new information regarding species of ticks which are strange to the participants, produced additional confusion. Being unfamiliar with these “new” species, researchers tended to identify ticks by close similarity rather than by complete identity. This explanation is further confirmed by the misclassification rates of “endemic” versus “non-endemic” species. The keys could probably help the participants



to identify the ticks with which they were not familiar, but introduced a further complication when dealing with species which they already knew, because several species (reported in the target territory) were included in the keys adding a “background noise” to the efforts of researcher.

It is necessary to stress that participants were aware in most cases of their unreliable identifications. Highest rates of confidence were obtained for the genera *Dermacentor* and *Ixodes*, meaning that participants were confident about the reliability of their identifications. Highest rates of concern about the validity of identification were obtained for species of *Hyalomma* and *Rhipicephalus*. Again, there is a high agreement between the rates of concern about the validity of classifications and the actual unreliable identifications for species of these genera.

Results from this blind test show the importance of an adequate source of information for the experts involved in the identification of ticks, both to cope with a reliable determination of the risk by a pathogen, and to adequately address further studies devoted to the epidemiology of tick-borne pathogens. Studies in multiple fields would benefit from adequate identifications of ticks. We believe that the best approach would be a curated library of specimens for reference, including every species and stages in the target territory. Such a reference collection does not exist and the forecasted difficulties in assembling such large number of species and stages render the procedure unpractical. Molecular approaches for the identification of ticks were reliable in this study, if performed with adequate primers and a careful selection of reference sequences (either produced in house or obtained from GenBank). The 5' region of the mitochondrial DNA gene *cytochrome c oxidase subunit I (coxI)* is the standard marker for DNA barcoding (Hebert, 2004). Nevertheless, 16S rRNA is a reliable marker for the tick identification at the species level (Lv et al., 2014a,b), and sequences of this fragment gene are the most available on GenBank. Moreover, other markers such as 12S rRNA or internal transcribed spacers (ITS) can be complementary for the tick classification (Lv et al., 2014a,b).

Undoubtedly, the success of DNA barcoding for any parasite species identification highly relies on the accurate morphological identification of reference specimens. Indeed, barcoding of ticks using alone the molecular approach could lead to inconsistent results (Lv et al., 2014a) and a combination of three DNA markers (*coxI*, 16S rDNA, and 18S rDNA) could efficiently separate several species of ticks (Lv et al., 2014a, b). The specific identification of ticks by molecular methods should not be considered as

definitive since it requires personal experience and adequate libraries, leading to the need of deposition of voucher specimens (i.e. Beati et al., 2013; Nava et al., 2014). The procedures used in this study show that an adequate combination of several genes and of the portions that produce the highest phylogenetic information is adequate for identification of ticks.

While the awareness about ticks and tick-borne pathogens increases worldwide, there is a lack of adequate knowledge about the identity of the most prominent species colonizing a large territory in the Western Palearctic. As far as we know, a similar blind test has never been performed in other parts of the world. It thus remains of interest to check how specialists of other regions can address the issue, or how other less known species are identified by the experts. Although other species of ticks may lack interest in circulating pathogens, they must be considered potentially confusing entities when compared with the focal species, introducing noise in reporting. We wanted to candidly show these results while claiming about the need of adequate training of experts involved in the identification of ticks, a step necessary either to address epidemiological studies or to cope with the risk by invasive species.

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## References

- Araya-Anchetta, A., Busch, J.D., Scoles, G.A., Wagner, D.M., 2015. Thirty years of tick population genetics: A comprehensive review. *Inf. Gen. Evol.* 29, 164-179.
- Arthur, D.R., 1963. *British Ticks*, London, Butterworth & Co. (Publishers) Ltd. 218 pp.
- Beati, L., Keirans, J.E., 2001. Analysis of the systematic relationships among ticks of the genera *Rhipicephalus* and *Boophilus* (Acari: Ixodidae) based on mitochondrial 12S ribosomal DNA gene sequences and morphological characters. *J. Parasitol.* 87, 32-48.
- Beati, L., Nava, S., Burkman, E. J., Barros-Battesti, D.M., Labruna, M.B., Guglielmone, A. A., Faccini, J. L., 2013. *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae), the Cayenne tick: phylogeography and evidence for allopatric speciation. *BMC Evol. Biol.* 13, 1.
- Black, W.C., Piesman, J., 1994. Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. *Proc. Nat. Acad. Sci. USA* 91, 10034-10038.
- Blumenschine, R.J., Marean, C.W., Capaldo, S.D., 1996. Blind tests of inter-analyst correspondence and accuracy in the identification of cut marks, percussion marks, and carnivore tooth marks on bone surfaces. *J. Archaeol. Sci.* 23, 493-507.
- Cordas, T., Aeschlimann, A., Morel, P.C., 1993. Étude morphologique des ixodidae s. str. (Schulze, 1937) de Suisse au microscope électronique à balayage. *Acarologia.* 34, 21-46.
- Coupe, S., Sarfati, C., Hamane, S., Derouin, F., 2005. Detection of *Cryptosporidium* and identification to the species level by nested PCR and restriction fragment length polymorphism. *J. Clin. Microbiol.* 43, 1017-1023.
- Cringoli, G., Lori, A., Rinaldi, L., Veneziano, V., Genchi, C., 2005. *Mappe parassitologiche - Zecche*. Series Edit. Giuseppe Cringoli, Napoli.
- Dantas-Torres, F., Latrofa, M. S., Annoscia, G., Giannelli, A., Parisi, A., Otranto, D., 2013. Morphological and genetic diversity of *Rhipicephalus sanguineus* sensu lato from the New and Old Worlds. *Parasit Vectors*, 6, 213.
- Dieme, C., Yssouf, A., Vega-Rúa, A., Berenger, J. M., Failloux, A. B., Raoult, D., Almeras, L. ,2014. Accurate identification of Culicidae at aquatic developmental stages by MALDI-TOF MS profiling. *Parasit Vectors.* 7, 544.
- Druzhinina, I.S., Kopchinskiy, A.G., Komoń, M., Bissett, J., Szakacs, G., Kubicek, C.P., 2005. An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea*. *Fungal Gen. Biol.* 42, 813-828.

- Estrada-Peña, A., de la Fuente, J., 2014. The ecology of ticks and epidemiology of tick-borne viral diseases. *Antiviral Res.* 108, 104-128.
- Estrada-Peña, A., Gray, J.S., Kahl, O., 1996. Variability in cuticular hydrocarbons and phenotypic discrimination of *Ixodes ricinus* populations (Acarina: Ixodidae) from Europe. *Exp. Appl. Acarol.* 20, 457-466.
- Estrada-Peña, A., Bouattour, A., Camicas, J.-L., Walker, A.R., 2004. Ticks of domestic animals in the Mediterranean region. University of Zaragoza.
- Filippova, N.A., 1997. Fauna of Russia and neighbouring countries. Arachnoidea, Ixodid ticks of subfamily Amblyomminae, Vol IV (5), Russian Academy of Sciences, Zoological Institute, New Series 145, Nauka Publishing House, St Petersburg.
- Gariepy, T.D., Lindsay, R., Ogden, N., Gregory, T.R., 2012. Identifying the last supper: utility of the DNA barcode library for bloodmeal identification in ticks. *Mol. Ecol. Res.* 12, 646-652.
- Hebert, P.D., Stoeckle, M.Y., Zemlak, T.S., Francis, C.M., 2004. Identification of Birds through DNA Barcodes. *PLoS Biol.* 2:e312.
- Heylen, D., De Coninck, E., Jansen, F., Madder, M., 2014. Differential diagnosis of three common *Ixodes* spp. ticks infesting songbirds of Western Europe: *Ixodes arboricola*, *I. frontalis* and *I. ricinus*. *Ticks Tick-borne Dis.* 5, 693-700.
- Hill, S., Provost, F., 2003. The myth of the double-blind review?: author identification using only citations. *ACM SIGKDD Explorations Newsl.* 5, 179-184.
- Hillyard, P.D., 1996. Ticks of North-West Europe. Field Studies Council, UK.
- Jaenson, T.G., Jaenson, D.G., Eisen, L., Petersson, E., Lindgren, E., 2012. Changes in the geographical distribution and abundance of the tick *Ixodes ricinus* during the past 30 years in Sweden. *Parasit. Vectors.* 5, 1
- Jongejan, F., Uilenberg, G., 2004. The global importance of ticks. *Parasitol.* 129(S1), S3-S14.
- Jore, S., Vanwambeke, S.O., Viljugrein, H., Isaksen, K., Kristoffersen, A.B., Woldehiwet, Z., Larsen, I. L., 2014. Climate and environmental change drives *Ixodes ricinus* geographical expansion at the northern range margin. *Parasit Vectors.* 7(1), 1-14.
- Kim, B.J., Lee, S.H., Lyu, M.A., Kim, S.J., Bai, G.H., Kim, S.J., Kook, Y.H., 1999. Identification of mycobacterial species by comparative sequence analysis of the RNA polymerase gene (*rpoB*). *J. Clin. Microbiol.* 37, 1714-1720.

- Kovalev, S.Y., Mikhaylishcheva, M.S., Mukhacheva, T.A., 2015. Natural hybridization of the ticks *Ixodes persulcatus* and *Ixodes pavlovskyi* in their sympatric populations in Western Siberia. *Infection. Gen. Evol.* 32, 388-395.
- Labruna, M.B., Marrelli, M.T., Heinemann, J.M., Fava, A.B., Cortez, A., Soares, R.M., Sakamoto, S.M., Richtzenhain, L.J., Marinotti, O., Schumaker, T.T., 2002. Taxonomic status of *Ixodes didelphidis* (Acari:Ixodidae). *J. Med. Entomol.* 39, 135-142.
- Leach, J.D., 1998. A brief comment on the immunological identification of plant residues on prehistoric stone tools and ceramics: results of a blind test. *J. Archaeol. Sci.* 25, 171-175.
- Li, S., Heyman, P., Cochez, C., Simons, L., Vanwambeke, S.O., 2012. A multi-level analysis of the relationship between environmental factors and questing *Ixodes ricinus* dynamics in Belgium. *Parasit Vectors* 5, 149.
- Li, S., Vanwambeke, S.O., Licoppe, A.M., Speybroeck, N., 2014. Impacts of deer management practices on the spatial dynamics of the tick *Ixodes ricinus*: A scenario analysis. *Ecol. Modell.* 276, 1-13.
- Lombard, M., Wadley, L., 2007. The morphological identification of micro-residues on stone tools using light microscopy: progress and difficulties based on blind tests *J. Archaeol. Sci.* 34, 155-165.
- Lv, J., Wu, S., Zhang, Y., Chen, Y., Feng, C., Yuan, X., Mei, L., 2014a. Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari: Ixodida). *Parasit Vectors.* 7, 93.
- Lv, J., Wu, S., Zhang, Y., Zhang, T., Feng, C., Jia, G., Lin, X., 2014b. Development of a DNA barcoding system for the Ixodida (Acari: Ixodida). *Mitochondrial DNA.* 25, 142-149.
- Maioli, M., Galletti, G., Calzolari, M., Biasi, G., Galletti, E., Dottori, M., 2012. Systematic literature review on the occurrence of ticks and tick-borne pathogens in the EU and Mediterranean Basin, EFSA Supporting Publications 9.
- Manilla, G., 1998. Fauna d'Italia (Vol. 36). Edizioni Calderini.
- Mclain, D.K., Wesson, D.M., Oliver, J.H., Collins, F.H., 1995. Variation in ribosomal DNA internal transcribed spacers 1 among eastern populations of *Ixodes scapularis* (Acari: Ixodidae). *J. Med. Entomol.* 32, 353-360.

- Medlock, J. M., Hansford, K. M., Bormane, A., Derdakova, M., Estrada-Peña, A., George, J. C., Kazimirova, M., 2013. Driving forces for changes in geographical distribution of *Ixodes ricinus* ticks in Europe. *Parasit Vectors*. 6, 1-11.
- Nava, S., Beati, L., Labruna, M.B., Cáceres, A.G., Mangold, A.J., Guglielmone, A.A., 2014. Reassessment of the taxonomic status of *Amblyomma cajennense* (L) with the description of three new species, *Amblyomma tonelliae* n. sp., *Amblyomma interandinum* n. sp. and *Amblyomma patinoi* n. sp., and reinstatement of *Amblyomma mixtum*, and *Amblyomma sculptum* (Ixodida: Ixodidae). *Ticks Tick-borne Dis*. 5, 252-276.
- Nava, S., Estrada-Peña, A., Petney, T., Beati, L., Labruna, M. B., Szabó, M. P., Guglielmone, A. A., 2015. The taxonomic status of *Rhipicephalus sanguineus* (Latreille, 1806). *Vet. Parasitol*. 208, 2-8.
- Nava, S., Guglielmone, A.A., Mangold, A.J., 2009. An overview of systematics and evolution of ticks. *Frontiers Biosci*. 14, 2857-2877.
- Nosek, J., Sixl, W., 1972. Central European ticks (Ixodoidea). Key for determination. *Jahrbuch der naturwissenschaftlichen Abteilung Joanneum* 1, 61-92.
- Pérez-Eid, C., 2007. Les tiques: identification, biologie, importance médicale et vétérinaire. Lavoisier Eds. 314pp.
- Sándor, A.D., Dumitrache, M.O., D'amico, G., Kiss, B.J., Mihalca, A.D., 2014. *Rhipicephalus rossicus* and not *R. sanguineus* is the dominant tick species of dogs in the wetlands of the Danube Delta, Romania. *Vet. Parasitol*. 204, 430-432.
- Vanwambeke, S.O., Artois, J., Davidson, R.K., Meyfroidt, P., Jore, S., 2016. Forest classes and tree cover gradient: tick habitat in encroached areas of southern Norway. *Exp. Appl. Acarol*. 68, 375-385.
- Yssouf, A., Flaudrops, C., Drali, R., Kernif, T., Socolovschi, C., Berenger, J. M., Parola, P., 2013. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for rapid identification of tick vectors. *J. Clin. Microbiol*. 51, 522-528.
- Zahler. M., Gothe, R., Rinder, H., 1995. Genetic evidence against a morphologically suggestive conspecificity of *Dermacentor reticulatus* and *D. marginatus* (Acari: Ixodidae). *Int. J. Parasitol*. 25, 1413-1419.
- Zhang, R.L., Zhang, B., 2014. Prospects of using DNA barcoding for species identification and evaluation of the accuracy of sequence databases for ticks (Acari: Ixodida). *Ticks Tick-borne Dis*. 5, 352-358.





Article 2

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## Outline-based morphometrics, an overlooked method in arthropod studies?

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### ABSTRACT

Modern methods allow a geometric representation of forms, separating size and shape. In entomology, as well as in many other fields involving arthropod studies, shape variation has proved useful for species identification and population characterization. In medical entomology, it has been applied to very specific questions such as population structure, reinfestation of insecticide-treated areas and cryptic species recognition. For shape comparisons, great importance is given to the quality of landmarks in terms of comparability. Two conceptually and statistically separate approaches are: (i) landmark-based morphometrics, based on the relative position of a few anatomical “true” or “traditional” landmarks, and (ii) outline-based morphometrics, which captures the contour of forms through a sequence of close “pseudo-landmarks”.

Most of the studies on insects of medical, veterinary or economic importance make use of the landmark approach. The present survey makes a case for the outline method, here based on elliptic Fourier analysis. The collection of pseudo-landmarks may require the manual digitization of many points and, for this reason, might appear less attractive. It, however, has the ability to compare homologous organs or structures having no landmarks at all. This strength offers the possibility to study a wider range of anatomical structures and thus, a larger range of arthropods.

We present a few examples highlighting its interest for separating close or cryptic species, or characterizing conspecific geographic populations, in a series of different vector organisms. In this simple application, i.e. the recognition of close or cryptic forms, the outline approach provided similar scores as those obtained by the landmark-based approach.

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### 1. Introduction

Contrary to classical morphology, the main objective of morphometrics is not to describe organisms, but to compare them. Because of the geometric constraints of modern morphometrics, data are generally collected on a non-articulated part, often a single organ (but see David et al. (1996) and Adams (1999)). A few characters are enough, provided that they are homologous.

Insect species studies generally made use of the wings because these structures are almost bidimensional and relatively rigid, reducing digitizing error; also, importantly, because the wings of many groups of insects provide a large number of landmarks. Not only are they many, they also are of good quality, called “true”

(or “anatomical”, or “traditional”) landmarks. True landmarks are considered homologous. Homology here refers to the positional equivalence of a small biological structure, as small as a point at the requisite scale. The level of homology of a landmark is governed by the precision with which it can be localized from one organism to another. Thus, true, anatomical landmarks are homologous in the sense that they are relocatable points, and according to this criterion various levels of homology have been recognized (see type I, II and III landmarks, Bookstein (1991)). A special development of type III landmarks, called “semi-landmarks” (also “sliding semi-landmarks”) allows the description of curved lines between two classical landmarks (Bookstein, 1997).

Anatomical, true landmarks are opposed to “pseudo-landmarks” used in the outline-based approach. Pseudo-landmarks describing contours or boundary outlines do not depend on the presence of true anatomical landmarks, they can exist with no

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anatomical landmark at all, or can include one or more of them. Obviously, pseudo-landmarks are of another nature than true landmarks: comparability is not expected from them separately, but from the structure they describe. Thus, if carefully chosen, like the mandible of a beetle, the genital paramere of a sandfly or an internal cell of the wing, a contour represents an homologous structure allowing interindividual and/or intergroup comparisons.

It is quite possible to develop both approaches on the same organ (Baylac and Frieß, 2005). One can see the wings of an insect as a configuration of anatomical landmarks, but the wing can be seen also as an assemblage of closed contours, i.e. the internal cells designated by the intersecting veins (Francoy et al., 2012). Moreover, other anatomical parts, because they are generally deficient in true landmarks, are only amenable to an outline analysis. For instance, many insect species, including very close species, are separated by the shape of some genital pieces. Such parts often lack anatomical landmarks, like the paramere of sandflies, the pronotum of Coleoptera (Faille et al., 2007), the genital leaflet of *Culex neavei* (Boussès et al., 2013; Garros and Dujardin, 2013) or the mandible parts of beetles (Tatsuta et al., 2009). Eggs and juvenile instars which also provide important taxonomic characters generally lack acceptable configurations of true landmarks: they can be considered through outline analyzes. Also good or natural candidates for the outline approach are the many wingless forms of insects, as for instance fleas, lice, ants, many of them of great veterinary, medical or agronomic importance. Finally, arthropods other than insects may present poorly defined landmarks but a particular anatomy which can be submitted to outline analyzes, as for instance the rounded body of ticks. In sum, the outline approach offers the possibility to study a wider range of organs, along with a wider range of arthropods.

The question is not limited however to technical considerations about presence or absence of landmarks (Rohlf, 1986), it also concerns the biological relevance of outlines. For entomologists, the primary needs are to distinguish species, especially cryptic species, and to detect them where they are not expected. Conspecific, but geographically or ecologically separated populations, are of interest too, in order to design relevant control strategies (Kaba et al., 2012) or to detect the origin of reinfestation after treatment (Felicangeli et al., 2007; Hernández et al., 2013; Gaspé et al., 2013). Does the outline approach distinguish species and populations as well as the landmark approach? We present here a few examples showing no significant difference between the two methods when it is about discriminating between close species or between conspecific populations.

## 2. Materials and methods

### 2.1. Material

#### 2.1.1. *Rhodnius prolixus* and *Rhodnius robustus*

*R. prolixus* Stal, 1859 and *R. robustus* Larrouse, 1927 are morphologically very close species with a long history of controversy among taxonomists (Bargues et al., 2010). *R. prolixus* is a major vector of Chagas disease in the northern part of Latin America, recently eradicated from Central America (Hashimoto and Schofield, 2012) but still active in Venezuela and Colombia (Gorla et al., 2010). The two species generally are found in different ecological conditions: in domestic and peridomestic structures for *R. prolixus*, in the crown of palm trees for *R. robustus*. Because of its silvatic habit preferences, *R. robustus* is not considered as an important vector of Chagas disease, but it is involved in some local transmission (Felicangeli et al., 2002). The overall morphology of the two species is very similar, although striking size differences are often observed, with *R. robustus* being generally the larger species. In

the field, there is frequently some size overlap, so specimens collected from trees are assumed to be *R. robustus*, and those collected from houses are assumed to be *R. prolixus*. Our material contains three groups previously identified by molecular tools (Monteiro et al., 2003): one is *R. prolixus* from houses in the Pampanito village (State of Trujillo, Venezuela), the second one is *R. prolixus* from palm trees collected in another locality, San José Tiznados (State of Guárico, Venezuela), and the last one is *R. robustus* from two localities of Venezuela: Pampanito and Candelaria (State of Trujillo).

We performed analyzes both combining the two *R. prolixus* habitats, as well as the two *R. robustus* origins, into single samples and considering them as separated groups. A total of 7 landmarks could be used, thus excluding the eighth used in Felicangeli et al. (2007) because of the inconsistency of the clavum position on the pictures. The outline submitted to the elliptical Fourier analysis (EFA, see Section 2.2.2) contained 5 of them, as well as the external boundary of the membranous part of the wing (Fig. 1).

#### 2.1.2. *Glossina palpalis palpalis* and *Glossina palpalis gambiensis*

*G. p. palpalis* (Robineau-Desvoidy), 1830 and *G. p. gambiensis* Vanderplank 1949 (Diptera: Glossinidae) are important vectors of sleeping sickness in West Africa. Both species hybridize readily in the laboratory but hybridized females produce fewer offspring and hybrid males are sterile (Gooding, 1988). These two subspecies are difficult to separate on morphological ground. Although males show consistent differences in the terminal dilatation of inferior claspers of their genitalia (Machado, 1954), morphological differentiation of female flies is not conclusive. The material we used here is from Ivory Coast and was collected in 2007. Male and female specimens of *G. p. palpalis* were collected in Aniassee, South of Ivory Coast. The *G. p. gambiensis* specimens came from Ganse, North of Ivory Coast. The subspecies identity of all the female and male specimens of the present study had no morphological nor molecular confirmation.

Ten landmarks were used as in Kaba et al. (2012). The EFA method was applied to the comparison of the central cell of the wing which includes five landmarks (Fig. 2). This cell is considered as having an important taxonomic significance for tsetse flies (De la Rocque et al., 2002).

#### 2.1.3. *Anopheles strodei* and *Anopheles oswaldoi*

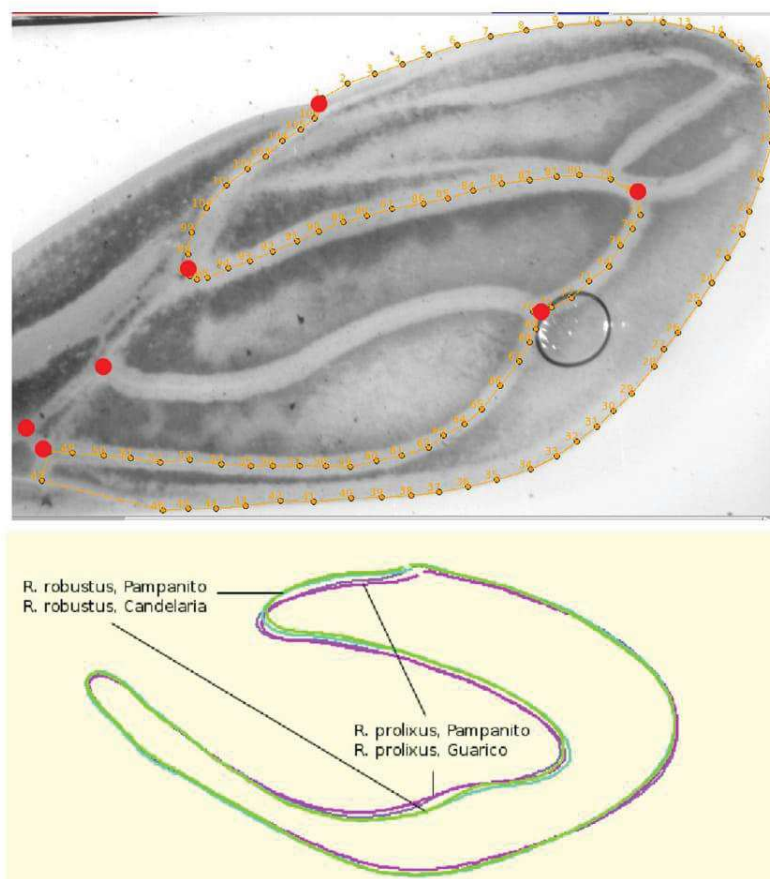
*A. oswaldoi* (Peryassú) has an unclear taxonomic and vectorial status in South America (Ruiz-Lopez et al., 2013), and *A. strodei* Root shows a high levels of morphological polymorphism (Bourke et al., 2013). Both species are difficult to distinguish based on existing dichotomous keys (Faran, 1980; Faran and Linthicum, 1981; Gonzales and Carrejo, 2007). Females were collected from the municipality of Puerto Asis, department of Putumayo, Colombia. They were induced to oviposit in the laboratory, where the male genitalia and associated immature stages of offspring were used for taxonomic identification. *A. oswaldoi* specimens were confirmed by PCR-RFLP of the rDNA marker ITS-2 (Ruiz et al., 2005). From *A. oswaldoi*, 30 adult F1 females and from *A. strodei*, 32 adult F1 females were randomly chosen for the morphometric studies.

Eleven anatomical landmarks were selected on the crossing of wing veins, while the EFA method used the contour of a small cell at the internal base of the wing, a contour which did not include any of the eleven landmarks (Fig. 3).

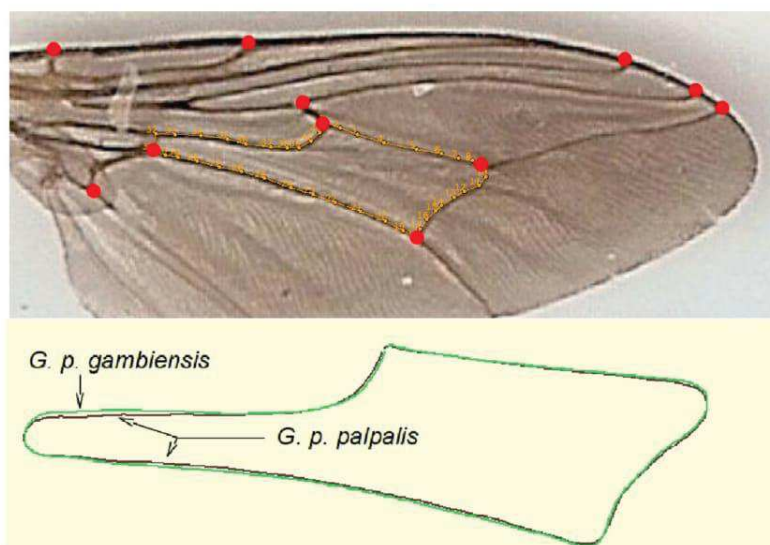
#### 2.1.4. *Ornithodoros maritimus*

*O. maritimus* is a soft tick (Family Argasidae) and part of the widespread species complex, *Ornithodoros capensis* sensu lato, exploiting colonial seabirds in tropical and sub-tropical areas of the world (Dietrich et al., 2011). The different members of this complex are known vectors of several viruses and bacteria,





**Fig. 1.** Top: Outline as digitized for a *Rhodnius* sp. wing, and seven landmarks (red dots). Bottom: Shape differences between outlines of *R. prolixus* and *R. robustus*. It can be seen that the selected outline remains stable within each species. The two samples of *R. prolixus* are almost identical in spite of having different sizes (see Fig. 5), different geographic origins (Pampanito, Guarico) and habitats (domestic, silvatic). A similar observation can be made for the two samples of *R. robustus*.



**Fig. 2.** Top: Outline as digitized for a *Glossina* sp. wing, and 10 landmarks (red dots). Bottom: Shape differences between outlines of female *Glossina palpalis palpalis* and *G. p. gambiensis*. The contours are very similar, with the elongated part of the cell thinner for *G. p. palpalis*.



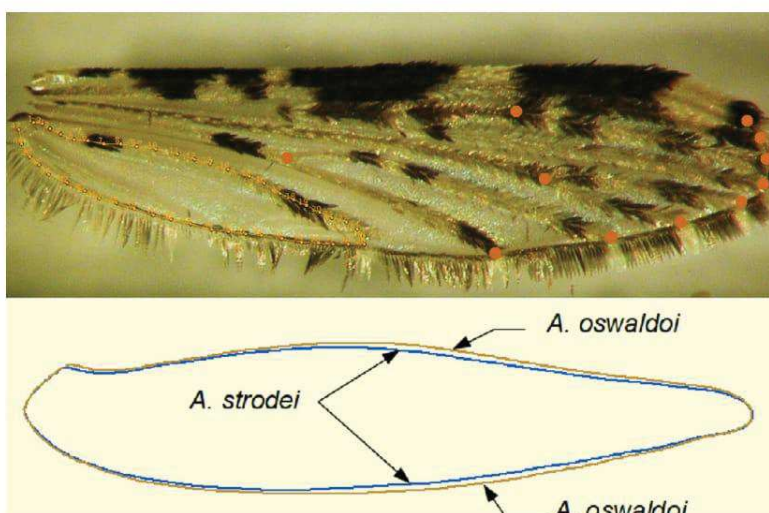


Fig. 3. Top: Outline as digitized for an *Anopheles* sp. wing, and 11 landmarks (red dots). Bottom: Shape differences between outlines of *A. strodei* and *A. oswaldoi*.

including the agents responsible for tick-borne relapsing fever and African tick bite fever (Takano et al., 2009; Dietrich et al., 2011; Dietrich et al., 2014). Although the *O. capensis* complex currently includes eight species, the actual number of species and their geographical distribution have been recently called into question (Gómez-Díaz et al., 2012). Given the lack of reliable morphological characters for identifying soft tick species in the adult life stage (Estrada-Pena et al., 2010), alternative methods are greatly needed. In the present study, two distant French populations were analyzed, both within the described distribution of *O. maritimus*: one from Rouzic, one of the islands of the Sept-Iles archipelago off the northern coast of Brittany and one from Riou, a Mediterranean island lying near the city of Marseilles. In Rouzic, 18 adult ticks were collected from around the nests of the Northern gannet (*Morus bassanus*), whereas in Riou, the 20 adult ticks analyzed came from nests of the Yellow-legged gull (*Larus michahellis*).

Fifteen landmarks were used on the ventral face of the ticks. The EFA method used the contour of the external boundary of the dorsal face (Fig. 4).

## 2.2. Statistical approaches

### 2.2.1. Landmark-based analyzes

Shape variables were obtained through the generalized Procrustes analysis (GPA) superimposition algorithm and subsequent projection of the Procrustes residuals into an euclidean space (Rohlf, 1990b). Both non-uniform and uniform components were used as shape variables. The shape variables were produced separately for males and females. These variables – also called “partial warps” (Slice et al., 1996) – actually describe the differences in shape as deviations from an average configuration of landmarks, and their principal components or a subset of them (see Section 2.2.3) were used as input for the discriminant analysis.

### 2.2.2. Outline-based analyzes

For each organism, except for the ticks, a previous study was necessary to select the outline to be used in the comparisons. Various outlines are possible on the same wing, and their discriminating power can be different (Francoy et al., 2012). We tried to select outlines containing the fewest possible landmarks, since the justification for using the outline-based approach is just that: no landmarks, or not enough of them. In each comparison, we used the elliptic Fourier analysis (EFA) (Kuhl and Giardina,

1982). Briefly, the observed contour is decomposed in terms of sine and cosine curves of successive frequencies called harmonics, and each harmonic is described by four coefficients. With this method (Kuhl and Giardina, 1982), the first harmonic ellipse parameters are used to standardize the Fourier coefficients so that they are invariant to size, rotation and the starting position of the outline trace. By doing this, the three first coefficients become constant (1, 0 and 0) and are not used in the remaining analyses. The fourth coefficient, the one related to the width-on-length ratio of the outline (Lestrel, 1989), has been used in our study.

As for the conventional Fourier analysis, the maximum number of harmonics is half the number of sample points (pseudo-landmarks). The optimal number of harmonics can be selected in various ways: by appraising the goodness-of fit using the sum of squared distances between the original data and reconstructed outline, by examining the spectrum of harmonic Fourier power, or simply by visualizing the reconstructed outlines (Lestrel, 1989, 1997; Claude, 2008). Ideally, more harmonics should be able to capture more shape parameters and produce a higher level of discrimination. However, more harmonics also inflate the digitization error and an optimal number should be selected (Crampton, 1995; Renaud et al., 1999; Firmat et al., 2010). In our study, the threshold regarding the number of harmonics was governed by the problem of high-dimensionality in the resulting data set (see next section).

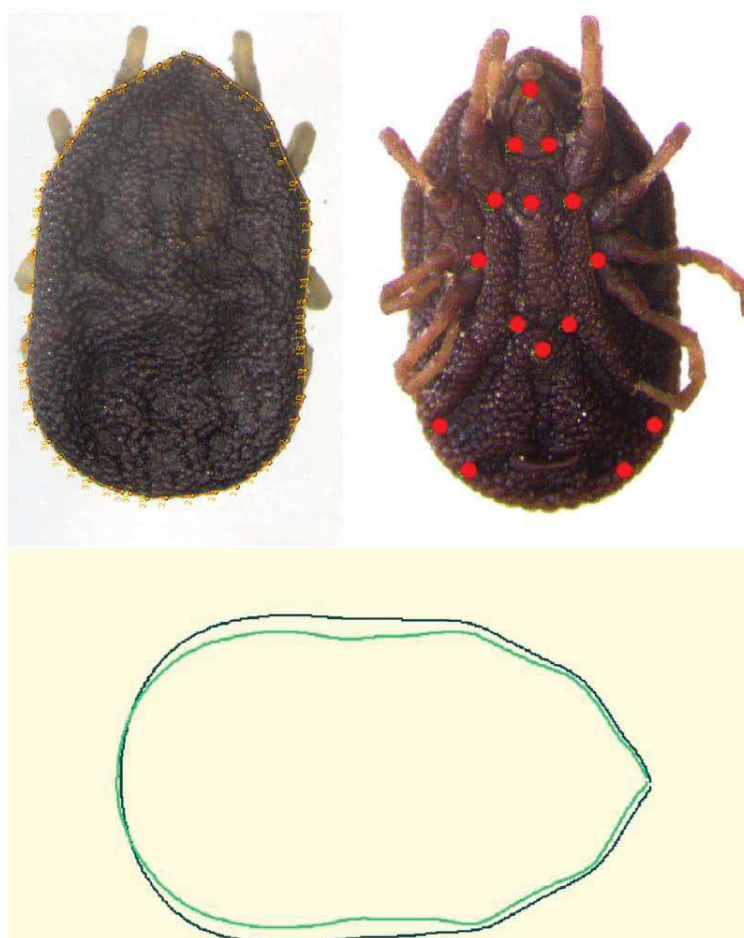
The superiority of the elliptic Fourier algorithm over other methods (Rohlf, 1990a) is now commonly accepted: the algorithm does not require the points to be equidistant (as in eigenshape analysis (Lohmann, 1983)), and the use of the normalized coefficients “saves the investigator from having to worry about aligning the images in a standard fashion” (Rohlf and Archie, 1984).

### 2.2.3. Assigning individuals to their corresponding groups

Each individual was classified after a discriminant analysis of shape variables according to a validated classification procedure, also called the jackknife classification (Manly, 2004), where each individual is allocated to its closest group without being used to help determine a group center.

Because of statistical constraints (Sheets et al., 2006), the discriminant analysis did not use the original shape variables as input. The number of shape variables produced by a two-dimensional landmark approach is two times the number of landmarks less four (Rohlf, 1996), and can be larger than the number of individuals. To circumvent the problem of high-dimensionality in the data set, a





**Fig. 4.** Top, left: Outline as digitized for *Ornithodoros maritimus*. Top, right: 15 landmarks on the ventral face (red dots). Bottom: Riou and Rouzic localities have different outlines for *Ornithodoros maritimus*.

few first principal components (PC) of shape variables were used as input for the discriminant analysis and subsequent validated classification. The size of this subset of PC was simply the number of specimens of the smallest group less one. There are other, more sophisticated ways to select for an adequate morphological subspace, but most rules of selection are heuristic or ad hoc methods (Jolliffe, 1986; Krzanowski, 1987). One of the least subjective is to select the number of retained components in each analysis that minimize the total cross-validated misclassification percentages (Baylac et al., 2003; Baylac and Frieß, 2005; Sheets et al., 2006). We used here a much simpler approach by placing the two morphometric techniques in similar statistical conditions, even if these conditions were not optimal for obtaining the best scores.

The use of the outline method also poses difficulties for the discriminant analysis. To accurately represent a curve many harmonics may be needed, each with four coefficients, so that the analyzes would require the measurement of numerous samples (Iwata, 2011). In each of our examples, the PC of the (normalized) Fourier coefficients were not derived from the total number of coefficients because it was frequently larger than the total sample. Thus, only a part of the Fourier coefficients was submitted to the PCA, and only a part of the first PC was used as input for the DA, where the same rule as above (“the smallest group less one”) was adopted.

#### 2.2.4. Size estimation and its contribution to shape discrimination

Whichever way shape is computed, the estimate of size in modern morphometrics is restricted to the geometric area

examined and is contained in a single variable: the root-squared area of a contour (instead of its perimeter (Lestrel, 1997)), or the “centroid size” for a configuration of landmarks (Bookstein, 1991). The centroid size is defined as the square root of the sum of the squared distances between the center of the configuration of landmarks and each separate landmark (Bookstein, 1991). The root-squared area of a contour was actually the one of the starting ellipse in the Fourier decomposition (Rohlf, 1990a).

The estimate of size was then used to evaluate its contribution to species discrimination based on shape: in each specific study, the coefficient of determination was computed after regressing the first (and generally unique) discriminant function on size.

#### 2.2.5. Measurement error

The precision of both techniques was estimated using the repeatability index. For each sample, we used an ANOVA design on repeated measurement performed on the totality the sample. The repeatability was measured using different variance components: the among-measurement ( $V_a$ ) and the within-measurement ( $V_w$ ) components. It was expressed in percentage, and computed as the ratio of  $V_a/(V_a + V_w)$  (Arnqvist and Mårtensson, 1998), which is the reverse of the measurement error as suggested by Yezerinac et al. (1992). The input variables for the ANOVA were the four first principal components of shape. We only report the average and standard deviation of the repeatability across species and comparisons for each morphometric approach.

### 2.3. Software

All analyzes and graphical output were obtained through the various modules of the CLIC package (Dujardin and Slice, 2006; Dujardin et al., 2010): the COO module to digitize either landmarks or curves, the MOG and PAD modules for the landmark-based approach, the FOG module for EFA analyzes and subsequent validated classifications, and the VAR module for estimating the repeatability of size and shape.

## 3. Results

The quality of group discrimination was generally satisfactory in both methods (Table 1, see the “%” columns) (86% of correct assignment on average versus 78% for landmarks). On average, the repeatability was slightly higher for outlines ( $85 \pm 11\%$ ) than for landmarks ( $79 \pm 17\%$ ). The contribution of size to the discrimination appears in Table 1 as a percentage in the “r2%” column. On average, it was lower in the landmark-based approach ( $23 \pm 18\%$  versus  $33 \pm 23\%$ ).

### 3.1. Group distinction

#### 3.1.1. *R. prolixus* and *R. robustus*

The landmark approach produced relatively satisfactory results: 15 (out of 17) *R. robustus* were correctly assigned, while 2 (out of 24) *R. prolixus* were wrongly attributed to *R. robustus*. More impressive results were obtained from outlines, where no *R. prolixus* was assigned to the *R. robustus* species, and only one *R. robustus* (out of 18) was confounded with *R. prolixus*. The *R. robustus* outline was not affected by the different geographic areas (Fig. 1) and the different sizes (Fig. 5). Similarly, the two samples of *R. prolixus*

showed a very close outline in spite of different habitats (Fig. 1) and very different sizes (Fig. 5).

#### 3.1.2. *G. p. palpalis* and *G. p. gambiensis*

There was a notable difference in the morphometric discrimination of subspecies according to the sex. Both morphometric techniques produced comparable and unsatisfactory results for male specimens (67–70% versus 66–77%, for landmarks and outlines, respectively). For females, only the outline comparisons produced satisfactory results (80–85%, versus 70–73% for landmark comparisons). Thus, outlines could only discriminate the two forms for females.

#### 3.1.3. *A. strodei* and *A. oswaldoi*

In this simple pairwise comparison of female mosquitoes, both landmarks and outlines performed satisfactorily (more than 83% were correctly classified). The scores obtained using landmarks (84%–86%) were slightly inferior to the ones obtained using the contour of a single internal cell of the wing (87%–94%).

#### 3.1.4. *O. maritimus*

The geographic origin of the 39 specimens of *O. maritimus* was almost perfectly recognized by the body contour (94%–100%), while imperfectly – but still consistently – by the landmark approach (80%–83%).

## 4. Discussion

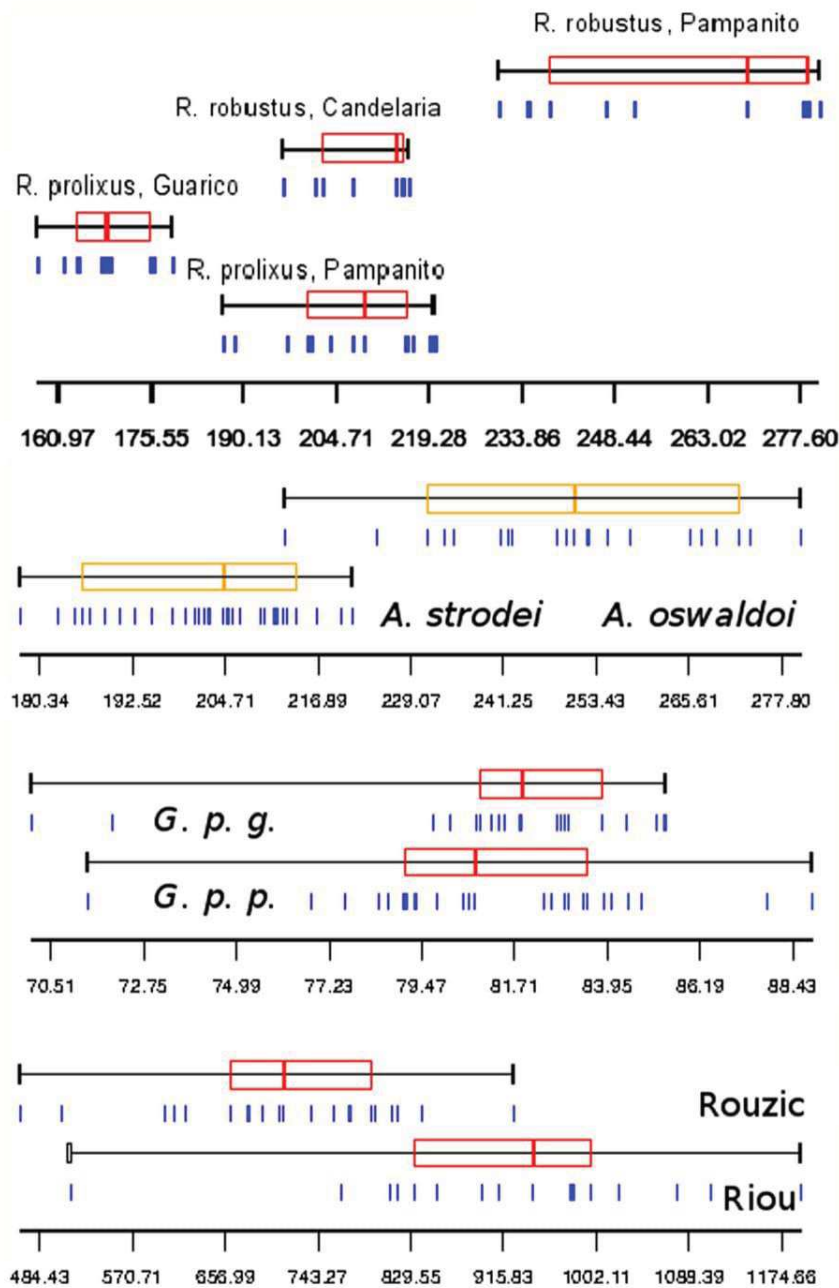
For analyzing curves containing anatomical landmarks, as is the case for most of the material used in this study, and in spite of some unresolved drawbacks (Sheets et al., 2004), the sliding semi-landmark approach becomes more frequently adopted in

**Table 1**

Validated classification based on shape. “n”, sample sizes; “%”, percentages of correct assignment of individuals to relevant species or group. “LM”, the number of true landmarks used; “H”, the number of harmonics corresponding to the number of normalized Fourier coefficients used as input for the principal component analysis; “r2%”, percent contribution of size to the first discriminant factor based on shape. The analyzes comparing *R. robustus* and *R. prolixus* were repeated with two different sampling distribution: either 14, 10 (both *R. prolixus*) and 18 *R. robustus* (mixing their geographic origins, i.e. Candelaria and Pampanito), or 14, 10, 8 *R. robustus* from Candelaria and 10 from Pampanito. 23/22, 30/22, 17/18 or 7/8 mean a different sample according to the approach: the first number refers to the sample size analyzed with landmarks, the second number to the one analyzed with outlines; F, females; M, males.

Species and groups	n	Validated classifications					
		Landmarks			Outlines		
		LM	%	r2%	H	%	r2%
Tsetse flies, females		10		1	11		0
<i>G. p. palpalis</i>	26		73			80	
<i>G. p. gambiensis</i>	20		70			85	
Tsetse flies, males		10		2	9		2
<i>G. p. palpalis</i>	15		67			66	
<i>G. p. gambiensis</i>	23/22		70			77	
Mosquitoes		11		27	13		59
<i>A. strodei</i>	32		87			87	
<i>A. oswaldoi</i>	30/22		93			94	
Kissing bugs, 2 groups		7		45	10		41
<i>R. prolixus</i> (2 habitats)	24		83			100	
<i>R. robustus</i>	17/18		94			94	
Kissing bugs, 3 groups		7		39	10		40
<i>R. prolixus</i> (domestic)	14		86			92	
<i>R. prolixus</i> (silvatic)	10		70			80	
<i>R. robustus</i>	17/18		88			94	
Kissing bugs, 4 groups		7		11	10		52
<i>R. prolixus</i> (domestic)	14		86			92	
<i>R. prolixus</i> (silvatic)	10		70			80	
<i>R. robustus</i> (Candelaria)	7/8		100			87	
<i>R. robustus</i> (Pampanito)	10		77			100	
Soft ticks		15		34	9		40
<i>O. maritimus</i> Rouzic	18		80			94	
<i>O. maritimus</i> Riou	20		83			100	





**Fig. 5.** Size variation as estimated by the square root of outline areas. From top to bottom: kissing bugs (*Rhodnius robustus* and *R. prolixus*), mosquitoes (*Anopheles strodei* and *A. oswaldoi*), tsetse flies (*G. p. p.* = *Glossina palpalis palpalis*, and *G. p. g.* = *Glossina p. gambiensis*) and ticks (*Ornithodoros maritimus* from two localities, Riou and Rouzic).

arthropods (Yee et al., 2011; Palestrini et al., 2012; Stephens and Juliano, 2012). The main benefit is that both landmarks and semi-landmarks can be arranged in one data set submitted to a single analysis. The discriminating power has been shown to be similar to the elliptic Fourier analysis (EFA) and some other outline methods (Sheets et al., 2006). The semi-landmark approach considers both curves and landmarks. As long as it requires true landmarks in order to describe the curves, it is a landmark-dependent method. The outline-based approach is completely different, as outlines may or may not include landmarks; it is a landmark-free method. Thus, for corresponding structures having very few landmarks or simply lacking landmarks, it is advisable to turn to the classical outline analysis based on EFA. We showed here that this option does not mean lower discriminating power. The EFA applies to closed contours only. Other statistical techniques can be applied

to curves, closed or not (Lohmann, 1983), as reviewed by Rohlf (1990a) and, more recently, by Sheets et al. (2006). The attractive feature of the EFA algorithm, which is used here, is that points need not to be equidistant nor equal in number.

#### 4.1. Data collection

In both approaches, we used manual data collection: a manual collection of landmarks, and a manual digitization of contours. For the landmark approach, it is not advisable to mix data from different users because of the “user effect”: small but persistent differences may arise between different users in pointing to the exact location of some landmarks, with effects on the final output (Dujardin et al., 2010). Because of the high comparability of each separate landmark, it is mandatory to collect each one very



carefully. Since the comparability of points (pseudolandmarks) is not a problem in outline digitization, it might be expected that the user effect is lower. However, it has been shown that different users, hence different ways to capture the contour, may have a more (Firmat et al., 2010) or less (Qing-Bin and Xin-Li, 2012) significant effect on the final output. We did not test for the compared magnitude of the user effect between landmark and outline techniques, but we show that, for the same images, both techniques suffered from a more or less similar amount of measurement error.

For both landmark and outline approaches, the automated capture of data could appear as the solution, but no such solution has been widely adopted so far. Automated identification of landmarks was recently proposed for Dipteran wings (Houle et al., 2003; Palaniswamy et al., 2010), but it may be a very difficult task when applied to wings covered with scales (the *Anopheles* in this study) or when crosses between veins are wide zones instead of points (kissing bugs). Automated curve tracing is already part of many imagery software and might look easier to generalize. It is a possible task when the contour is the external boundary of the body, as can be the case for rounded bodies as for instance in mussels (Ferson et al., 1985; Claude, 2008). The problem becomes more difficult when the perimeter to be digitized is a substructure of the main image, an internal part of the body. These and other obstacles in performing automatic captures of outlines have led some authors to rely on the more flexible manual collection (Sheets et al., 2006; Firmat et al., 2010).

Even if adopting the same starting point is not mandatory for the EFA method we used, we digitized the contour trying to start from the same point. This strategy, when possible, allows other methods to be applied to the same set of pseudolandmarks, as for instance the Procrustes superposition (after equalizing the number of points) used for our graphical output (Figs. 1–4).

#### 4.2. Statistical analyzes

The reclassification procedure used in the present study was based on the discriminant analysis. This analysis requires that there be more specimens than variables in the smallest group. As we were unable to increase the sample sizes, we used a principal component analysis (PCA) to reduce the dimensionality of the data: the scores of a limited number of PC was used instead of the original data. The criterion to determine the number of PC selected was simply the number of specimens in the smallest group less one. Thus, in our study, the selected number of PC can be deduced from Table 1 by looking at the sample sizes in each comparison (see the “n” column). More sophisticated procedures exist (Baylac and Frieß, 2005), some of them not based on the PCA method (Sheets et al., 2006).

Because of the PCA-based dimension reduction method, only a part of shape variation, although generally a consistent part, was used to classify individuals, which probably lowered the power of the analyzes. There are two more reasons to think that low sample sizes probably represented a heavier handicap for the outline approach than for the landmark one. First, according to Baylac and Frieß (2005), the first PCA axes of Fourier coefficients rarely account for a significant proportion of the between-group differences. Second, we were not even able to use the maximum possible number of harmonics because the total sample was too low for the PCA to include them all. Table 1 indicates the number of harmonics used for the PCA (see the column “H”; which can also be deduced by computing the total sample of each group less 1, and divided by 4). Between close or cryptic species, and *a fortiori* between conspecific populations, we expect to have discrete, localized differences, if any. Such differences are captured by the higher level harmonics, while the first few ones generally describe more global differences in shape (Lestrel, 1989).

Thus, poor sample sizes could have reduced the discriminating power of outlines relative to landmarks. In spite of that, the outline classification showed satisfactory results.

#### 4.3. Compared scores

The morphometric methods used here to build shape representation remove the isometric change of size, but were not designed to remove the allometric effect of size on shape variation. Thus, shape variables, be they partial warps or normalized Fourier coefficients, contain an allometric residue. Here, the effect of this residue on shape discrimination was estimated by regressing the shape-derived discriminant factor on size variation; which is reported in the column “r2%” of Table 1. On average, it was slightly less present in the landmark approach, which suggests the landmark-based approach might provide a slightly greater independence between size and shape.

##### 4.3.1. *R. prolixus* and *R. robustus*

Previous morphometric studies based on landmarks could distinguish the two species to some extent, but in the favorable situation of allopatry (samples coming from distinct geographic areas) (Matias et al., 2001; Villegas et al., 2002; Márquez et al., 2011). In our material, we compared the two species from samples collected in the same village (Pampanito, Venezuela).

Morphological differences between the two taxa were frequently thought to be related to their separate environment, domestic for *prolixus* and silvatic for *robustus*. In our material, we could compare a sample of domestic *R. prolixus* (Pampanito) with another one collected in silvatic conditions in another locality (Guárico). In spite of coming from different habitats and localities, the two *R. prolixus* samples were similar, and remained distinct from *R. robustus*.

Both landmarks and outlines did a relatively good job, with again better results obtained from outlines. Actually, the outline-based distinction was almost perfect, since only one *R. robustus* out of 18 was confounded with *R. prolixus*, and no *R. prolixus* were confounded with *R. robustus*. Moreover, these outline-scores were not affected by subdividing the *R. robustus* sample according to its geographic origin. Within both species, the stability of the outline was remarkable: it was constant whatever the size (Fig. 5), the habitat and the geographic origin (Table 1).

As for previous studies on these two close species, the shape differences could not be clearly separated from the size variation (see the column “r2%” of Table 1). Feliciangeli et al. (2007) showed that in silvatic conditions size variance of *R. prolixus* may be significantly smaller. Size is generally considered more evolutionarily labile than shape, but it seems to have played a major role in the evolutionary divergence between *R. prolixus* and *R. robustus* (Fig. 5).

##### 4.3.2. *Tsetse flies*

Males of *G. p. palpalis* and *G. p. gambiensis* were unsatisfactorily discriminated by both landmarks and outlines. Since each individual of this study was not identified by a more accurate criterion than its geographic origin, we cannot certify that the two groups were completely homogeneous. However, in the case of these two subspecies, for which the taxonomic status is still discussed, the problem concerns particularly the females. Males indeed can be recognized by observable variations of the genitalia (terminal dilatations of inferior clusters). Fortunately, it appears that, for females, modern morphometrics can contribute to their identification, especially by using the boundary of the central cell (80%–85%).



#### 4.3.3. Mosquitoes

Mosquitoes represent a group of insects where very few outline-based morphometric analyses have been published (Garros and Dujardin, 2013). Yet the first application of EFA to the shape of living organisms was the quantitative analysis of the boundary of mosquito wings (Rohlf and Archie, 1984). The material of this seminal study was a set of drawings coming from an entomological book (plates 1 to 127 in Carpenter and LaCasse (1955)). As far as we know, no other outline studies have been performed on mosquitoes, except a study of the genital leaflet of *C. neavei* (Boussès et al., 2013; Garros and Dujardin, 2013), and the use of sliding-landmarks combining the posterior edge of the wing with a set of true landmarks in *Aedes aegypti* and *Aedes albopictus* (Stephens and Juliano, 2012). The outline used in our mosquito sample was this posterior edge united to the anal vein of the wing (Fig. 3). None of the landmarks used for the landmark-based approach was included in this contour, but it could still discriminate the two close species.

#### 4.3.4. Soft ticks

Fifteen landmarks of the ventral face were used, all of them however of type II, i.e. landmarks providing less homology (Bookstein, 1991). In spite using low quality landmarks, good scores were obtained, but the contour of the dorsal face of the tick provided even better scores. Whatever the approach, significant shape divergence was disclosed between geographic populations of the same species. These differences could reflect adaptation to different hosts and/or geographic isolation (two remote islands). From these initial results, we expect this approach may perform well in species discrimination within the *O. capensis* group.

### 5. Conclusion

Our four case-studies do not (and cannot) claim superiority of one technique over another, but do suggest that one should not overlook the outline approach for discriminating close forms. It produced high scores of correct group allocation, it suffered from a relatively low measurement error and could be applied to various organs of the body, including or not relocatable landmarks. Our study suggests that if the main objective is to distinguish morphologically close entities, and even if a landmark-based approach is possible, one should consider also to test the outline technique.

In some organisms other than arthropods where such comparisons have been performed, either the two methods were considered as equivalent (Jensen et al., 2002) or the outlines were said to perform better (Loy et al., 2000; Pavlinov, 2000; Baylac and Frieß, 2005; Albutra et al., 2012). A recent study on Hymenoptera wings showed that landmarks performed better than (internal wing cells) outlines, and that their combination provided the best results (Francoy et al., 2012). In our various examples involving arthropods, similar or even better discrimination scores were attained using the outline-based approach. Although our sample sizes were generally low, similar performances of outlines in four comparisons involving quite different organisms (ticks, tsetse flies, mosquitoes and kissing bugs) is a significant signal. It suggests that for discriminating close forms, the choice of an outline-based analysis can be as relevant as the choice of a landmark-based one. Ideally, the outline should be selected in areas of the body where landmarks are lacking. This is not to say that, in other applications than simple discrimination, the outline approach would be less informative. In general, there was a powerful biological signal in the variation of outlines for arthropods (Foster and Kaesler, 1988; Monti et al., 2001; Baltanas et al., 2002; Faille et al., 2007; Tatsuta et al., 2009), as well as for other organisms like fishes (Loy et al., 2000), birds (Sheets et al., 2006), mammals (Renaud

et al., 1996, 1999; Pavlinov, 2000; Renaud and Michaux, 2003; Friess and Baylac, 2003) and plants (Yamanaka et al., 2001; Jensen et al., 2002; Iwata et al., 2010; Iwata, 2011).

The objective of this study was to illustrate the interest of a relatively neglected method in modern morphometrics as applied to arthropods. We show that outline analyses can be applied in complement or as an alternative to the landmark-based approach.

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### References

- Adams, D.C., 1999. Methods for shape analysis of landmark data from articulated structures. *Evol. Ecol. Res.* 1 (8), 959–970.
- Albutra, Q.B., Torres, M.A.J., Demayo, C.G., 2012. Outline and landmark based geometric morphometric analysis in describing sexual dimorphism in wings of the white stem borer (*Schirpophaga innotata* walker). *ABAH* 4 (1), 5–14.
- Arngqvist, G., Mårtensson, T., 1998. Measurement error in geometric morphometrics: empirical strategies to assess and reduce its impact on measure of shape. *Acta Zool. Acad. Sci. Hung.* 44 (1–2), 73–96.
- Baltanas, A., Alcorlo, P., Danielopol, D.L., 2002. Morphological disparity in populations with and without sexual reproduction: a case study in *Eucypris virens* (Crustacea: Ostracoda). *Biol. J. Linn. Soc.* 75 (1), 9–19.
- Bargues, M.D., Schofield, C., Dujardin, J.P., 2010. The phylogeny and classification of the triatominae. In: Telleria, J., Tibayrenc, M. (Eds.), *American Trypanosomiasis: Chagas disease, One hundred years of research*.
- Baylac, M., Frieß, M., 2005. Fourier descriptors, Procrustes superimposition, and data dimensionality: an example of cranial shape analysis in modern human populations. In: *Modern Morphometrics in Physical Anthropology*, Slice ed. p. 145–165 (Chapter 6).
- Baylac, M., Villemant, C., Simbolotti, G., 2003. Combining geometric morphometrics with pattern recognition for the investigation of species complexes. *Biol. J. Linn. Soc.* 80 (1), 89–98.
- Bookstein, F.L., 1991. *Morphometric Tools for Landmark Data. Geometry and Biology*. Cambridge University Press, NY.
- Bookstein, F.L., 1997. Landmark methods for forms without landmarks: localizing group differences in outline shape. *Med. Image Anal.* 1, 225–243.
- Bourke, B.P., Oliveira, T.P., Suesdek, L., Bergamo, E.S., Sallum, M.A., 2013. A multi-locus approach to barcoding in the *Anopheles strodei* subgroup (Diptera: Culicidae). *Parasites Vectors* 6. <http://dx.doi.org/10.1186/1756-3305-6-111>.
- Boussès, P., Dehecq, J.S., Brengues, C., Fontenille, D., 2013. Updated inventory of mosquitoes (Diptera: Culicidae) of the island of La Réunion, Indian ocean. *Bull. Soc. Pathol. Exot.* 106, 113–125.
- Carpenter, S.J., LaCasse, W.J., 1955. *Mosquitoes of North America (north of Mexico)*. Univ. California Press, Los Angeles.
- Claude, J., 2008. *Morphometrics with R*. Springer Science+Business Media, LLC, ISBN 978-0-387-77789-4, p. 330.
- Crampton, J.S., 1995. Elliptic Fourier shape analysis of fossil bivalves: some practical considerations. *Lethaia* 28, 179–186.
- David, B., Dommergues, J.L., Magniez-Jannin, F., 1996. Exploration d'un espace morphologique les carabes (Coleoptera) du nord-est de la France. *C. R. Acad. Sci., Life Sci.* 319, 835–842.
- De la Rocque, S., Geoffroy, B., Michel, J.F., Borne, F., Solano, P., Meunier, J.Y., Cuisance, D., 2002. Tsetse flies wings, an identity card of the insect? *Parasite* 9 (3), 275–281.
- Dietrich, M., Gómez-Daz, E., McCoy, K.D., 2011. Worldwide distribution and diversity of seabird ticks: implications for the ecology and epidemiology of tick-borne pathogens. *Vector-Borne Zoonotic Dis.* 11 (5), 447–453.
- Dietrich, M., Lebarbenchon, C., Jaeger, A., Le Rouzic, C., Bastien, M., Lagadec, E., McCoy, K.D., Pascalis, H., Le Corre, M., Dellagi, K., Tortosa, P., 2014. *Rickettsia* spp. in Seabird Ticks from Western Indian Ocean Islands, 2011–2012. *Emerg. Infect. Dis.* 20 (5), 838–842.
- Dujardin, J.P., Kaba, D., Henry, A.B., 2010. The exchangeability of shape. *BMC Res. Notes* 3, 266. <http://dx.doi.org/10.1186/1756-0500-3-266>.
- Dujardin, J.-P., Slice, D.E., 2006. *Contributions of Morphometrics to Medical Entomology*. John Wiley & Sons, Inc. <<http://dx.doi.org/10.1002/9780470114209.ch25>>, p. 435–447.
- Estrada-Pena, A., Mangold, A.J., Nava, S., Venzal, J.M., Labruna, M., Guglielmo, A.A., 2010. A review of the systematics of the tick family Argasidae (Ixodida). *Acarologia* 50, 317–334.
- Faille, A., Déliot, P., Quéinnec, E., 2007. A new cryptic species of Aphaenops (Coleoptera: Carabidae: Trechinae) from a french Pyrenean cave: Congruence between morphometrical and geographical data confirm species isolation. *Ann. soc. entomol. Fr.* 43 (3), 363–370.



- Faran, M.E., 1980. Mosquito studies (Diptera: Culicidae) XXXIV a revision of the *Albimanus* section of the subgenus *Nyssorhynchus* of *Anopheles*. *Contrib. Am. Entomol. Inst.* 15, 1–214.
- Faran, M.E., Linthicum, K.J., 1981. A handbook of the species of *Anopheles* (*Nyssorhynchus*) (Diptera: Culicidae). *Mosq. Syst.* 13 (1), 1–81.
- Feliciangeli, M.D., Dujardin, J.P., Bastrenta, B., Mazzarri, M., Villegas, J., Flores, M., Muñoz, M., 2002. Is *Rhodnius robustus* (Hemiptera: Reduviidae) responsible for Chagas disease transmission in Western Venezuela? *Trop. Med. Int. Health* 7 (3), 280–287.
- Feliciangeli, M.D., Sanchez-Martin, M., Marrero, R., Davies, C., Dujardin, J.P., 2007. Morphometric evidence for a possible role of *Rhodnius prolixus* from palm trees in house re-infestation in the State of Barinas (Venezuela). *Acta Trop.* 101, 169–177.
- Ferson, S., Rohlf, F.J., Koehn, R.K., 1985. Measuring shape variation of two-dimensional outlines. *Syst. Zool.* 34 (1), 59–68.
- Firmat, C., Gomes-Rodrigues, H., Renaud, S., Claude, J., Hutterer, R., Garcia-Talavera, F., Michaux, J., 2010. Mandible morphology, dental microwear, and diet of the extinct giant rats *Canariomys* (Rodentia: Murinae) of the Canary Islands (Spain). *Biol. J. Linn. Soc.* 101, 28–40.
- Foster, D.W., Kaesler, R.L., 1988. Shape analysis, ideas from the Ostracoda. In: McKinney (Ed.), *Heterochrony in Evolution: A Multidisciplinary Approach*, N.Y. Topics in Geobiology.
- Francoy, T.M., Faria Franco, F., Roubik, D.W., 2012. Integrated landmark and outline-based morphometric methods efficiently distinguish species of *Euglossa* (Hymenoptera, Apidae, Euglossini). *Apidologie* 43, 609–617.
- Friess, M., Baylac, M., 2003. Exploring artificial cranial deformation using elliptic fourier analysis of procrustes aligned outlines. *Am. J. Phys. Anthropol.* 122 (1), 11–22.
- Garros, C., Dujardin, J.P., 2013. Genetic and phenetic approaches to *Anopheles* systematics. In: *Anopheles mosquitoes – New insights into malaria vectors*. <<http://www.intechopen.com/books/export/citation/BibTex/anopheles-mosquitoes-new-insights-into-malaria-vectors/genetic-and-phenetic-approaches-to-anopheles-systematics>>.
- Gaspe, M.S., Gurevitz, J.M., Gürtler, R.E., Dujardin, J.P., 2013. Origins of house reinfestation with *Triatoma infestans* after insecticide spraying in the Argentine Chaco using wing geometric morphometry. *Infect Genet Evol.*, p. 93–100.
- Gómez-Díaz, E., Morris-Pocock, J.A., Gonzalez-Solis, J., McCoy, K.D., 2012. Trans-oceanic host dispersal explains high seabird tick diversity on cape verde islands. *Biol. Lett.* 8, 616–619.
- Gonzales, R., Carrejo, N., 2007. Introducción al estudio taxonómico de *Anopheles* de Colombia. Claves y notas de distribución. Cali: Editorial Litocencia, p. 237.
- Gooding, R.H., 1988. Preliminary analysis of genetics of hybrid sterility in crosses of *Glossina palpalis palpalis* (robineau-desvoidy) and *Glossina palpalis gambienseis vanderplanck*. *The Can. Entomol.* 120 (11), 997–1001.
- Gorla, D., Ponce, C., Dujardin, J.P., Schofield, C.J., 2010. Control strategies against triatominae. In: Telleria, J., Tibayrenc, M. (Eds.), *American Trypanosomiasis Chagas Disease. One Hundred Years of Research*. Elsevier, pp. 233–245 [Chapter 10].
- Hashimoto, K., Schofield, C., 2012. Elimination of *Rhodnius prolixus* in Central America. *Parasites Vectors* 5 (1), 45 <<http://www.parasitesandvectors.com/content/5/1/45>>.
- Hernández, M.L., Dujardin, J.P., Gorla, D.E., Catalá, S.S., 2013. Potential sources of *Triatoma infestans* reinfesting peridomestic identified by morphological characterization in los Llanos, La Rioja, Argentina. *Mem. Inst. Oswaldo Cruz*, 91–97.
- Houle, D., Mezey, J., Galpern, P., Carter, A., 2003. Automated measurement of *Drosophila* wings. *BMC Evol. Biol.* 3, 25.
- Iwata, H., 2011. Quantitative genetic analyses of crop organ shape based on principal components of elliptic fourier descriptors (p. 50–66). In: Lestrel, P. (Ed.), *Biological Shape Analysis, Proceedings of the 1st International Symposium, Tsukuba Japan 3–6 June 2009*, p. 305.
- Iwata, H., Ebana, K., Uga, Y., Hayashi, T., Jannink, J.L., 2010. Genome-wide association study of grain shape variation among *Oryza sativa* 1 germplasms based on elliptic fourier analysis. *Mol. Breed.* 25, 203–215.
- Jensen, R.J., Ciofani, K.M., Miramontes, L.C., 2002. Lines, outlines, and landmarks: morphometric analyses of leaves of *Acer rubrum*, *Acer saccharinum* (aceraceae) and their hybrid. *Taxon* 51 (3), 475–492.
- Jolliffe, I.T., 1986. *Principal Component Analysis. Series in Statistics*. Springer, New York.
- Kaba, D., Ravel, S., Acapovi-Yao, G., Solano, P., Allou, K., Bosson-Vanga, H., Gardes, L., N'Goran, E.K., Schofield, C.J., Koné, M., Dujardin, J., 2012. Phenetic and genetic structure of tsetse fly populations (*Glossina palpalis palpalis*) in southern Ivory Coast. *Parasites Vectors* 5, 153.
- Krzyszowski, W.J., 1987. Cross-validation in principal component analysis. *Biometrics* 43, 575–584.
- Kuhl, F.P., Giardina, C.R., 1982. Elliptic fourier features of a closed contour. *Comput. Graphics Image Process.* 18, 236–258.
- Lestrel, P.E., 1989. Methods for analyzing complex two-dimensional forms: elliptic fourier functions. *Am. J. Hum. Biol.* 1, 149–164.
- Lestrel, P.E., 1997. Introduction and overview of Fourier descriptors. In: *Fourier Descriptors and their Applications in Biology*. Cambridge University Press, Cambridge, pp. 22–44 [Chapter 2].
- Lohmann, G.P., 1983. Eigenshape analysis of microfossils: a general morphometric procedure for describing changes in shape. *Math. Geol.* 15, 659–672.
- Loy, A., Busilacchi, S., Costa, C., Ferlin, L., Cataudella, S., 2000. Comparing geometric morphometrics and outline fitting methods to monitor fish shape variability of *Diplodus puntazzo* (Teleostea: Sparidae). *Aquacult. Eng.* 21 (4), 271–283.
- Machado, A., 1954. Révision systématique des glossines du groupe *palpalis* (Diptera). *Publ. cult. Co. Diam. Angola* 22, 189.
- Manly, B.F.J., 2004. *Multivariate Statistical Methods: A primer*, third ed. Chapman & Hall Statistics Texts.
- Márquez, E.J., Jaramillo-O, N., Gómez-Palacio, A., Dujardin, J.P., 2011. Morphometric and molecular differentiation of a *Rhodnius robustus*-like form from *R. robustus* Lrousse, 1927 and *R. prolixus* Stal, 1859 (Hemiptera, Reduviidae). *Acta Trop.* 120: 103–120: 109.
- Matias, A., De la Riva, J.X., Torrez, M., Dujardin, J.P., 2001. *Rhodnius robustus* in Bolivia identified by its wings. *Mem. Inst. Oswaldo Cruz* 96 (7), 947–950.
- Monteiro, F.A., Barrett, T.V., Fitzpatrick, S., Cordon-Rosales, C., Feliciangeli, D., Beard, C.B., 2003. Molecular phylogeography of the Amazonian Chagas disease vectors *Rhodnius prolixus* and *R. robustus*. *Mol. Ecol.* 12 (4), 997–1006.
- Monti, L., Baylac, M., Lalanne Cassou, B., 2001. Elliptic Fourier analysis of the form of genitalia in two *Spodoptera* species and their hybrids (Lepidoptera: Noctuidae). *Biol. J. Linn. Soc.* 72 (3), 391–400.
- Palaniswamy, S., Thacker, N.A., Klingenberg, C.P., 2010. Automatic identification of morphometric landmarks in digital images. *IET Comput. Vis.* 4 (4), 247–260 <<http://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.105.7188>>.
- Palestrini, C., Roggero, A., Lindsay Karen Hernández, N., Giachino, M.P., Rolando, A., 2012. On the evolution of shape and size divergence in *Nebria* (Nebriola) ground beetles (Coleoptera, Carabidae). *Systematics and Biodiversity*, 147–157 <<http://dx.doi.org/10.1080/14772000.2012.685775>>.
- Pavlinov, I.Y., 2000. Supplementary data on application of geometric morphometrics in analyzing variation of molar shape in voles (Rodentia, Alticola). *Zoologicheskoy Zhurnal* 79 (1), 104–107.
- Qing-Bin, Z., Xin-Li, W., 2012. Elliptic fourier analysis of the wing outline shape of five species of antlion (Neuroptera: Myrmeleontidae: Myrmeleontini). *Zool. Stud.* 51 (3), 399–405.
- Renaud, S., Michaux, J., Jaeger, J.-J., Aufray, C., 1996. Fourier analysis applied to *Stephanomys* (rodentia: Muridae) molars: non-progressive evolutionary patterns in a gradual lineage. *Paleobiology* 22, 251–261.
- Renaud, S., Michaux, J., Mein, P., Aguilar, J.-P., Aufray, C., 1999. Patterns of size and shape differentiation during the evolutionary radiation of the european miocene murine rodents. *Lethaia* 32, 61–71.
- Renaud, S., Michaux, J.R., 2003. Adaptive latitudinal trends in the mandible shape of *Apodemus* wood mice. *J. Biogeog.* 30 (10), 1617–1628.
- Rohlf, F.J., 1986. Relationships among eigenshape analysis, Fourier analysis and analysis of coordinates. *Math. Geol.* 18 (8), 845–854.
- Rohlf, F.J., 1990a. Fitting curves to outlines. In: Rohlf, F.J., Bookstein, F.L. (Eds.), *Proceedings of the Michigan Morphometrics Workshop*. University of Michigan Museums, Ann Arbor, pp. 168–177, ISBN 0-9628499-01.
- Rohlf, F.J., 1990b. Rotational fit (Procrustes) methods. In: Rohlf, F.J., Bookstein, F.L. (Eds.), *Proceedings of the Michigan morphometrics workshop*, Special Publication No. 2. University of Michigan Museums, Ann Arbor, pp. 227–236, The University of Michigan Museum of Zoology, Ann Arbor, MI, p. 380.
- Rohlf, F.J., 1996. Morphometric spaces, shape components and the effects of linear transformations. In: Marcus, L.F., Corti, M., Loy, A., Naylor, G., Slice, D. (Eds.), *Advances in Morphometrics. Proceedings of the 1993 NATO-ASI on Morphometrics*, NATO ASI, Ser. A, Life Sciences. Plenum Publ., New York, pp. 117–129.
- Rohlf, F.J., Archie, J.W., 1984. A comparison of Fourier methods for the description of wing shape in mosquitoes (Diptera: Culicidae). *Syst. Zool.* 33, 302–317.
- Ruiz, F., Quiñones, M.L., Erazo, H.F., Calle, D.A., Alzate, J.F., Linton, Y.M., 2005. Molecular differentiation of *Anopheles* (*Nyssorhynchus*) *benarrochi* and *A* (N.) *oswaldoi* from Southern Colombia. *Mem. Inst. Oswaldo Cruz* 100, 155–160.
- Ruiz-Lopez, F., Wilkerson, R.C., Ponsonby, D.J., Herrera, M., Sallum, M.A.M., Dario Velez, I., Quiñones, M.L., Flores-Mendoza, C., Chadee, D.D., Alarcon-Ormasa, J., Linton, Y.-M., 2013. Systematics of the Oswaldo complex (*Anopheles*, *Nyssorhynchus*) in South America. *Parasites Vectors* 6, 324.
- Sheets, D.H., Kim, K., Mitchell, C.E., 2004. A combined landmark and outline-based approach to ontogenetic shape change in the Ordovician trilobite *Triarthrus becki*. In: *Morphometrics. Applications in Biology and Paleontology*. Springer-Verlag, Berlin, Heidelberg, pp. 67–82 [Chapter 6].
- Sheets, H.D., Covino, K.M., Panasiewicz, J.M., Morris, S.M., 2006. Comparison of geometric morphometric outline methods in the discrimination of age-related differences in feather shape. *Front. Zool.* 3, 15.
- Slice, D.E., Bookstein, F.L., Marcus, L.F., Rohlf, F.J., 1996. A glossary for morphometrics. pp 531–551. In: Naylor, G.J.P., Slice, D. (Eds.), *Advances in Morphometrics. Proceedings of the 1993 NATO-ASI on Morphometrics*, NATO ASI, ser. A, Life Sciences. Plenum Publ., New York, pp. 387–398.
- Stephens, C.R., Juliano, S.A., 2012. Wing shape as an indicator of larval rearing conditions for *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). *J. Med. Entomol.* 49 (4), 927–938.
- Takano, A., Muto, M., Sakata, A., Ogasawara, Y., Ando, S., Hanaoka, N., Tsurumi, M., Sato, F., Nakamura, N., Fujita, H., Watanabe, H., Kawabata, H., 2009. Relapsing fever spirochete in seabird tick, Japan. *Emerg. Infect. Dis.* 15, 1528–1530.
- Tatsuta, H., Iwata, H., Goka, K., 2009. Morphometric studies of male Lucanid beetle mandibles: shape variation between hybrid subspecies. In: Lestrel, P.E. (Ed.), *Biological Shape Analysis*. World Scientific Publishing Co. Pte. Ltd., London, ISBN 10-981-4355-23-2.



- Villegas, J., Feliciangeli, M.D., Dujardin, J.P., 2002. Wing shape divergence between *Rhodnius prolixus* from Cojedes (Venezuela) and *R. robustus* from Mérida (Venezuela). *Infect. Genet. Evol.* 2, 121–128.
- Yamanaka, N., Ninomiya, S., Hoshi, M., Tsukura, Y., Yano, M., Nagamura, Y., Sasaki, T., Harada, K., 2001. An informative linkage map of soybean reveals qtls for flowering time, leaflet morphology and regions of segregating distortion. *DNA Res.* 8, 61–72.
- Yee, W.L., Sheets, H.D., Chapman, P.S., 2011. Analysis of surstylus and aculeus shape and size using geometric morphometrics to discriminate *Rhagoletis pomonella* and *Rhagoletis zephyria* (Diptera: Tephritidae). *Ann. Entomol. Soc. Am.* 104 (2), 105–114. <http://dx.doi.org/10.1603/AN10029>.
- Yezerinac, S.M., Loogheed, S.C., Handford, P., 1992. Measurement error and morphometric studies: statistical power and observer experience. *Syst. Biol.* 41, 471–482.



## Chapter 3 - **Convergence in diversification patterns**

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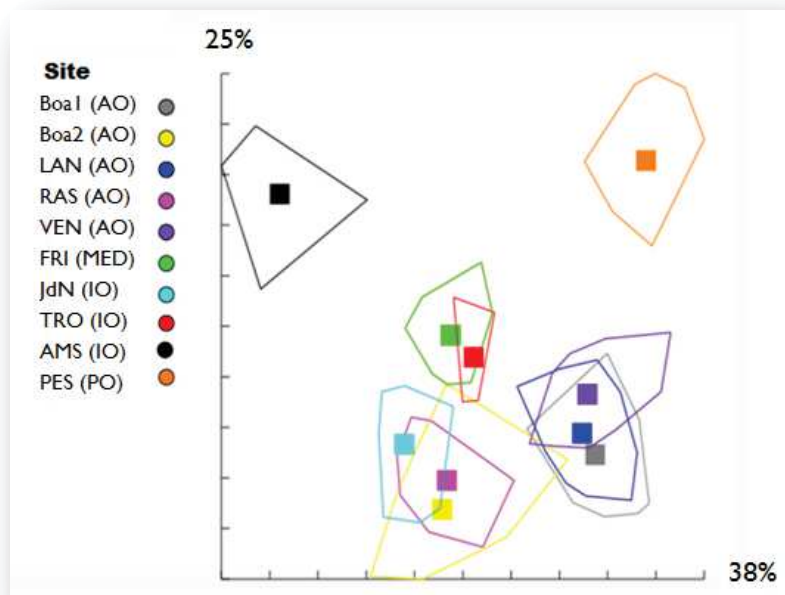


Strong sexual or divergent natural selective pressures in different environments can rapidly generate the ecological and morphological divergence of sympatric populations and lead to speciation (Rundell and Price, 2009). Divergent selection can come from biotic or abiotic factors such as food resources, environmental conditions or inter-specific interactions such as competition or predation (Schluter, 2009). Biological models of adaptive divergence in the wild, such as Darwin's finches (*Geospiza fortis*) in the Galapagos Islands or *Anolis* lizards of the Caribbean Islands, have demonstrated the coexistence of different species harboring size and microhabitat differences in several localities (Grant and Grant, 2008; Losos 1990). Likewise, the wide diversity of Cichlid fishes in East African lakes has resulted from adaptation to specific habitats and resources, leading to morphological convergence (Muschick et al, 2012). Some of these examples of morphological convergence have been directly linked to genetic mechanisms, as shown for the development of protective plates in stickleback fish species (Colosimo et al, 2005).

Host-parasite systems are similar to island systems in which host species form isolated islands which may result in the adaptive divergence of their parasites. For example, Johnson et al (2012) studied the morphology of different louse species with respect to their escape behavior on different bird-hosts. They showed that lice were specialized to different host body regions, presenting morphological differences specific to each body part and this due to a process of repeated adaptive divergence within a host group. Moreover, these authors highlight morphological convergence where similar selective pressures on the same host body part lead to the independent evolution of the same morphological trait in the lice.

Within tick-seabird systems, I used two morphometric approaches, along with genetic analyses, to study phenotypic variability of different tick species and host races to document patterns of host specialization and identify underlying selective factors. Using population genetic and morphometric analyses, we show in Article 3 that size and shape variation are closely related to genetic structure in the ticks of the *O. capensis* s.l. complex and that these patterns are closely linked to host use, even when they are breeding in sympatry. Distant tick populations, collected in Sulidae host populations, presented similar phenotypes (Figure 8). We discuss the impact of selective pressures associated with host use.





**Figure 8:** Results of a discriminant analysis based on outlines and showing populations grouped in relation to host use in the *O. capensis* s. l. complex. See article 3 for population abbreviations.

Article 4 deals with the morphometrical discrimination (outlines and landmarks) of *I. uriae* host races collected on different hosts in Canada, Iceland and Norway. A signal of morphological convergence was found, but was relatively weak. We discuss observed patterns of phenotypic variation in relation to selective pressures coming from hosts or microhabitats.

Article 3

Dupraz M, Toty C, Noël V, Estrada-Peña A, González-Solís J, Boulinier T, Dujardin J-P, McCoy K D.2016. Linking morphometric and genetic divergence with host use in the tick complex *Ornithodoros capensis* s.l. *Infection, genetics and evolution*. doi: 10.1016/j.meegid.2016.10.005



Linking morphometric and genetic divergence with host use in the tick complex, *Ornithodoros capensis* sensu lato.

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**Abstract**

Host specific adaptations in parasites can lead to isolation and divergence of conspecific populations. However, divergence can be difficult to measure because morphological changes may not be expressed or because obvious changes may simply reflect phenotypic plasticity. Combining both genetic and phenotypic information can enable a better understanding of the divergence process and help identify the underlying selective forces, particularly in closely related species groups. Here, we link genetic and phenotypic data to understand divergence patterns within the *Ornithodoros (Carios) capensis* complex, a group of soft ticks (Argasidae) exploiting colonial seabirds across the globe. Species designations in this complex were historically based on larval morphology and geographic location. However, recent work has suggested that divergence within the group may be at least partially linked to host specificity. Using genetic and geometric morphometrics (landmark and outline analyses), we test whether host use in the complex is always accompanied by the same phenotypic changes. Genetic and morphometric analyses revealed strong structure in relation to host use, both when populations were sympatric and widely allopatric. Tick size varied among populations, along with relative body width after correcting for size. Shape and size variation was closely linked to genetic structure, but only weakly with geography. These results support the hypothesis that speciation in this tick group has been more strongly shaped by host use than by geographic barriers per se. The revealed phenetic patterns now require detailed investigation to link them with host-specific selective forces.

Keywords: adaptation, host specificity, population genetic structure, morphometry, ectoparasite, tropical seabirds

## 1 Introduction

Being a food resource and principal habitat, the host often represents the majority of a parasite's ecological needs. The optimal exploitation of one host type may require specific adaptations that can lead to the divergence of conspecific populations (Ravigné et al. 2009). However, host-associated divergence is sometimes difficult to measure in natural populations, as adaptation may not require morphological changes (Bickford et al, 2007) or because these changes may simply represent phenotypic plasticity (Agrawal, 2001). Molecular techniques, such as DNA barcoding (Hajibabaei et al, 2007) and genome-wide typing (Manske et al, 2012), have become increasingly popular and accessible allowing us to measure levels of genetic isolation, but identifying the drivers of this divergence remains difficult, particularly in non-model organisms. One tool that can be used to help understand the link between genetic divergence and host specific adaptations is morphometry, the quantitative analysis of form. Morphometric methods, although developed in the 1980's, have gained in popularity over the last few years (Adams et al, 2004) because they represent a cheap, rapid and biologically significant way of measuring phenotypic changes (Lawing and Polly, 2010). As these methods are also non-destructive, they can be used in cases where genetic analyses are impossible, for example with certain collection specimens. Morphometric tools not only complement more traditional morphological species descriptions, but are also able to quantify subtle population-level variation (eg. Barluenga et al, 2006) and can help identify the factors underlying divergence. Although rarely employed for parasitic organisms, coherent results have been obtained for several taxa, including blood-feeding arthropods (Dujardin, 2008, Dietrich et al. 2013) and ectoparasitic trematodes (Huyse and Volckaert, 2002). For example, morphometric analyses of different sympatric host-associated populations of the hard tick *Ixodes uriae* showed that the overall body shape of ticks differed with host use (Dietrich et al. 2013) and correlated with the genetic divergence among tick subpopulations and the relative parasite fitness on alternative host types (McCoy et al. 2005, Dietrich et al, 2012, Dietrich et al, 2014). However, the morphometrical analysis in this case was limited to patterns within a single location and it remains unknown whether the



same morphological changes occur on the same host types in other areas of the distribution. The ability to make inferences on host traits associated with these changes is therefore limited.

The *Ornithodoros (Carios) capensis* complex is a soft tick (Argasidae) taxon that includes eight described species (Hoogstraal et al, 1976). These species are considered to be host generalists parasitizing a range of tropical and temperate colonial seabird species, including at least 35 species of 12 avian families from across the Pacific, Atlantic and Indian Oceans. Although poorly studied to date, these ectoparasites are considered to have direct effects on host reproductive success when occurring in high density (e.g., Duffy, 1983) and are known to harbour numerous pathogenic agents for humans including Soldado and West Nile viruses and relapsing fever bacteria (Dietrich et al, 2011). Like other soft ticks, their endophilic life style should restrict encounters with alternative host species (Vial, 2009), which may favor the evolution of local adaptation and host specialization (Whitlock 1996), processes already reported for other tick species (McCoy et al, 2013).

The members of the *O. capensis* complex share a high degree of morphological similarity. Species descriptions in this taxon have been based on a combination of larval characteristics and geography (Colas-Belcour and Rageau, 1960, Hoogstraal et al, 1976, Clifford et al, 1980). However, recent work on the Cape Verde Islands suggested that both these descriptions and the generalist nature of these species may be erroneous; surprisingly high genetic diversity was found within islands, with several putative species occurring on different sympatric seabird species (Gómez-Díaz et al, 2012). The authors suggest that this diversity is due to a combination of long-distance colonization events via seabird movement and adaptations to specific host genera. Here, we examine this hypothesis at a global scale, by combining genetic and morphometric data to compare the diversity and structure of *O. capensis* ticks associated with different hosts in the Cape-Verde and Canary Islands with ticks collected from across the distribution of the complex. We expected that genetic results would reveal isolation and divergence among tick populations in relation to the host genus and that this factor would better explain genetic distance than geography. We also expected that patterns of host-associated divergence would match with differences in tick size and shape, potentially revealing adaptive variation. We discuss our results in light of the current

systematics of this species group and the relative importance of different evolutionary forces in driving parasite divergence.

## 2 Material and methods

### 2.1 Biological system

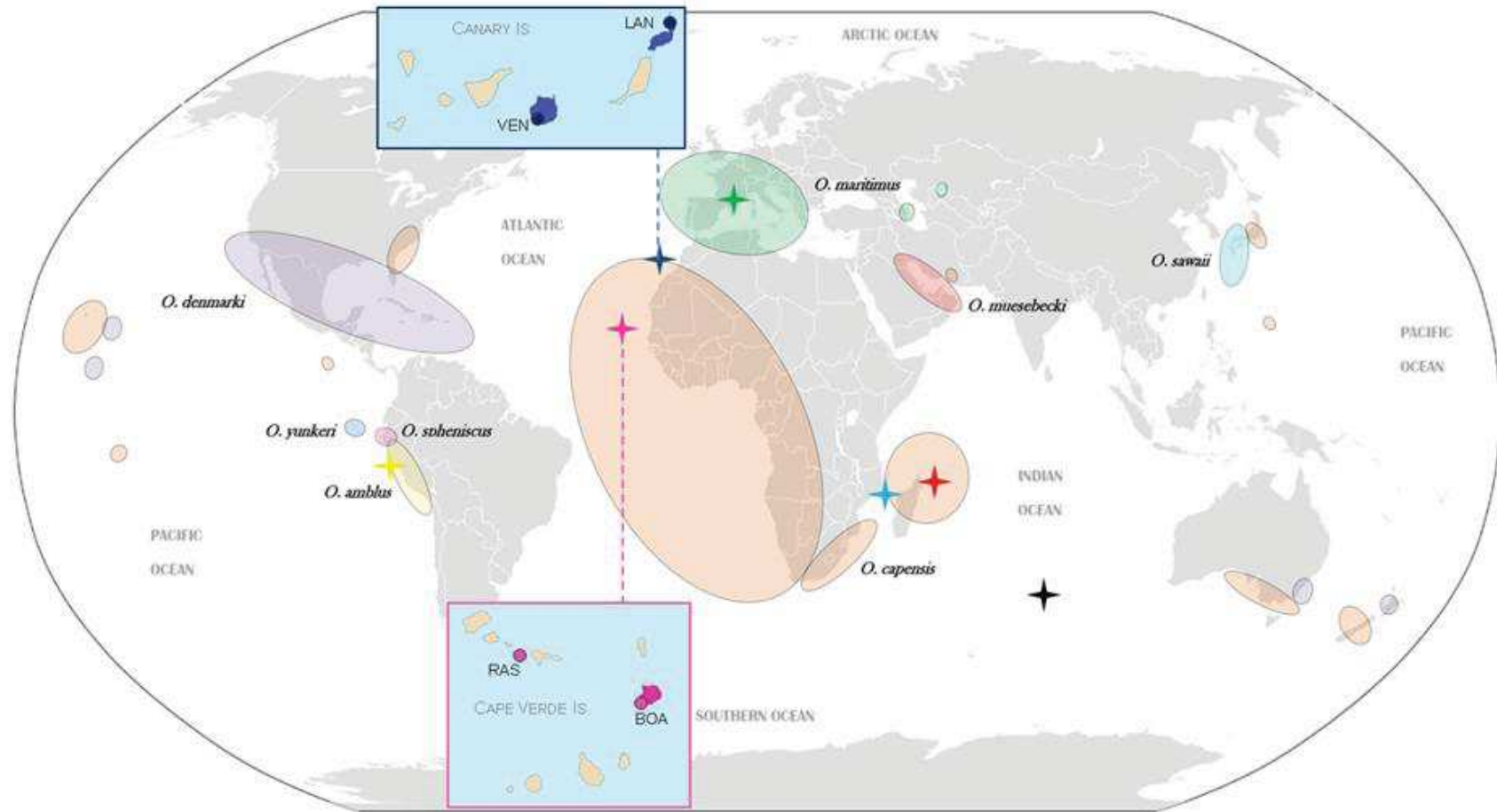
The species of the *O. capensis* complex, their geographic ranges, and host associations have been described somewhat opportunistically by different authors over the past 100 years. Eight species are currently recognized within the complex (Hoogstraal et al. 1985), with a possible ninth species that remains poorly studied as yet (Vermeil et al, 1997): *O. capensis sensu stricto* Neumann 1901 has been recorded on albatrosses, boobies, cormorants, gulls, penguins and terns, and is widely distributed within tropical, subtropical and southern temperate islands and along the coasts of the Atlantic, Pacific and Indian Oceans, the Caribbean and Corral seas and lakes of the East African Rift Valley (Hoogstraal et al, 1985); *O. amblus* Chamberlin 1920 is associated with boobies, cormorants and pelicans and found on islands off the coasts of Peru (Clifford et al, 1980); *O. denmarki* Kohls, Sonenshine & Clifford 1965 was described from boobies and terns in Florida, California, Hawaii and Mexico (Kohls et al, 1965, Amerson, 1968); *O. muesebecki* Hoogstraal 1969 is associated with boobies, cormorants and terns in the Arabian gulf; *O. maritimus* Vermeil and Marguet 1966 is found on numerous species of cormorants, gulls, alcids and terns in Great Britain, Ireland, France, Italy, Tunisia (Hoogstraal et al, 1976); *O. sawaii* Kitaoka and Suzuki 1973 was initially collected from Streaked Shearwaters (*Calonectris leucomelas*) on the Amani-Oshima Islands in Japan, but has since also been found on Swinhoe's Storm Petrels (*Oceanodroma monorhis*) (Kawabata et al, 2006; Takano et al, 2009); *O. yunkerii* Keirans, Clifford & Hoogstraal 1984 is associated with albatrosses, boobies, cormorants and penguins of the Galapagos Islands and *O. spheniscus* Hoogstraal, Wassef, Hays & Keirans 1985 was found to be associated with the Humboldt Penguin in different localities in Peru and has been more recently described on these hosts in Chile (González-Acuña et al, 2008). *O. cheikhi*, a species morphologically close to *O. capensis s.s* and *O.*

*maritimus* was described from tern nests in Mauritania (Vermeil et al, 1997), but no additional data concerning its taxonomic status or host affinities have since been reported.

Like other argasid ticks, species of the *O. capensis* complex have three developmental stages: larva, nymph and adult, with a variable number of nymphal instars (Pérez-Eid, 2007). Although obligate parasites, these species only associate with the host for the time of their relatively short blood meal, taking from a few hours to engorge for larval ticks to several minutes for nymphal and adult stages. The greatest part of the life cycle is therefore confined to the microhabitat of the host nesting area (within nests, burrows or under nearby rocks and vegetation) (Hoogstraal, 1985) and we generally refer to these species as being endophilic. Feeding occurs multiple times for nymphal and adult stages, typically at night, and depends on the presence of the host at the nest site (Duffy & Daturi, 1987). Colonial seabirds are particular as hosts since many species are only on land for a few months each year to breed. However, as they tend to show high natal and breeding fidelity to their colony and nest site, their presence is highly predictable for their nest parasites (McCoy et al, 2016).

## 2.2 Tick sampling

Ticks were collected on different seabird species or from host nests from a series of islands (see Fig.1 and Table.1 for details) and were preserved in 70% ethanol. They were initially assigned to the species level based on historical records of geographic distribution (Fig.1). For genetic analyses, 206 collected ticks were sequenced at three genes (16S, CO1, 18S) and 12 additional 16S rDNA sequences were added from GenBank (Table 1). The same 206 ticks (94 females and 112 males) used for sequencing were also analyzed for morphometry, with a mean of 20 ticks per population (Table 1). All analyzed ticks were in the adult stage and non-engorged to avoid body distortion from the blood meal in the morphometrical analyses. For tick populations in which larvae were collected, species identifications were verified based on classical morphological traits using available keys: Hoogstraal et al, 1976; Hoogstraal, 1969; Hoogstraal et al, 1985; Keirans et al, 1984; Kitaoka and Suzuki, 1973; Kohls et al, 1965; and Clifford et al, 1980.



**Fig.1:** Sampled sites in seabird colonies across the range of the *Ornithodoros capensis sensu lato* complex. The distribution of the eight currently described species is indicated by colored ellipses. Purple: *O. denmarki*; Blue: *O. yunkeri*; Pink: *O. spheniscus*; Yellow: *O. amblus*; Orange: *O. capensis*; Green: *O. maritimus*; Red: *O. muesebecki*; Light blue: *O. sawaii*. The sampled sites are indicated by stars. Yellow: Pescadore Island (PES) on *S. variegata*; Dark blue (inset map): Canary Islands on *C. borealis* (LAN and VEN); Pink (inset map): Cape-Verde Islands on *C. edwardsii* (Boa1) and *S. leucogaster* (Boa2 and RAS); Green: Frioul Island (FRI) on *L. michahellis*; Light blue: Juan de Nova Island (JdN) on *O. fuscatus*; Red: Tromelin Island (TRO) on *S. sula*; Black: Amsterdam Island (AMS) on *E. chrysocome*. Distributional data from Dietrich et al, 2011. See Table 1 for more detail on sampling locations.

**Table.1:** Tick populations used in this study, with collection localities, geographic coordinates, population abbreviations, presumed tick species based on geographic distributions, host species and the number of ticks used in morphometric and genetic (16S, COI and 18S genes) analyses. F: female; M: male. Accession numbers for 16S gene sequences are also provided (See SM for accession numbers for 18S and COI gene sequences).

Origin	Sample location (Lat. Lon.)	Pop	Presumed tick species	Host	N° ticks morphometry	N° ticks 16S	16S Accession number	Reference study	N° ticks COI	N° ticks 18S
Atlantic Ocean - Cape-Verde - Boavista1	15.993700, -22.790108	Boa1	<i>O. capensis</i>	Cape Verde shearwater <i>Calonectris edwardsii</i>	12F - 12M	22	KX825947-50	present study	9	16
Atlantic Ocean - Cape-Verde - Boavista1	15.993700, -22.790108	Boa1	<i>O. capensis</i>	Cape Verde shearwater <i>Calonectris edwardsii</i>	-	1	JQ824298.1	Gómez-Díaz et al, 2012	-	-
Atlantic Ocean - Cape-Verde - Boavista2	15.993700, -22.790108	Boa2	<i>O. capensis</i>	Brown booby <i>Sula leucogaster</i>	13F - 12M	9	KX825953	present study	8	14
Atlantic Ocean - Cape-Verde - Boavista2	15.993700, -22.790108	Boa2	<i>O. capensis</i>	Brown booby <i>Sula leucogaster</i>	-	2	JQ824315.1	Gómez-Díaz et al, 2012	-	-
Atlantic Ocean - Cape-Verde - Raso	16.616844, -24.586501	RAS	<i>O. capensis</i>	Brown booby <i>Sula leucogaster</i>	8F - 16M	21	KX825954-56	present study	21	11
Atlantic Ocean - Cape-Verde - Raso	16.616844, -24.586501	RAS	<i>O. capensis</i>	Brown booby <i>Sula leucogaster</i>	-	2	JQ824308.1	Gómez-Díaz et al, 2012	-	-
Atlantic Ocean - Canary - Lanzarote	29.297243, -13.534957	LAN	<i>O. capensis</i>	Cory's shearwater <i>Calonectris borealis</i>	7F - 20M	24	KX825951,52,58	present study	10	10
Atlantic Ocean - Canary - Lanzarote	29.297243, -13.534957	LAN	<i>O. capensis</i>	gull <i>Larus michahellis</i>	-	3	KX825970	present study	-	-

Atlantic Ocean - Canary - Veneguera	27.905011, -15.730907	VEN	<i>O. capensis</i>	Cory's shearwater <i>Calonectris borealis</i>	14F - 10M	13	KX825957	present study	17	22
Atlantic Ocean – Canary - Tenerife	28.372326, -16.76808	TEN	<i>O. capensis</i>	Cory's shearwater <i>Calonectris borealis</i>	-	3	JQ824316.1	Gómez-Díaz et al, 2012	-	-
Mediterranean Sea - France - Frioul	43.274548, 5.302565	FRI	<i>O. maritimus</i>	Yellow-legged gull <i>Larus michahellis</i>	6F - 16M	20	KX825959	present study	6	20
Indian Ocean - TAAF - Juan de Nova	-17.055907, 42.724928	JdN	<i>O. capensis</i>	Sooty tern <i>Onychoprion fuscatus</i>	16F - 5M	18	KX825960,61	present study	10	9
Indian Ocean - TAAF - Amsterdam	-37.834645, 77.557291	AMS	?	Rockhopper penguin <i>Eudyptes chrysocome</i>	9F - 5M	15	KX825965-69	present study	5	12
Indian Ocean - TAAF - Tromelin	-15.891818, 54.523823	TRO	<i>O. capensis</i>	Red-footed booby <i>Sula sula</i>	8M	8	KX825962,63	present study -	6	2
Pacific Ocean - Peru - Pescadores	-11.756390, -77.284593	PES	<i>O. amblus</i>	Peruvian booby <i>Sula variegata</i>	9F - 8M	20	KX825964	present study	14	9
Pacific Ocean - Peru - Islas Chincha	-13.642826, -76.399884	Peru	<i>O. amblus</i>	Peruvian booby <i>Sula variegata</i>	-	2	KX825971	present study	-	-
Pacific Ocean - Mexico - Isabel Island	21.848529, -105.884897	Mex	<i>O. denmarki</i>	Blue-footed booby <i>Sula nebouxii</i>	-	2	KX825972	present study	-	-
Pacific Ocean - Japan - Hanmya Island	28.046214, 129.187829	HanmyaA	<i>O. capensis</i>	Streaked shearwater <i>Calonectris leucomelas</i>	-	1	AB057538	Kawabata et al, 2006	-	-



Pacific Ocean - Japan - Ishikawa	?	Ishikawa1	<i>O. sawaii</i>	Streaked shearwater <i>Calonectris leucomelas</i>	-	1	AB242427	Kawabata et al, 2006	-	-
Pacific Ocean – Hawaii – Laysan Island	25.768278, -171.732304	Hawaii1	<i>O. capensis</i>	Laysan albatross <i>Diomedea immutabilis</i>	-	1	AB057538	Ushijima et al, 2003	-	-
Atlantic Ocean – Texas – Longman reef Island	?	TexasA	<i>O. capensis</i>	Reddish egret <i>Egretta rufescens</i>	-	1	AB076081	Ushijima et al, 2003	-	-

### 2.3 Genetic analyses

*DNA isolation and PCR amplification:* After taking standardized images, the 206 newly collected ticks were washed in two water baths, placed individually in a sterile tube and crushed with a pestle. DNA extractions were performed with the DNeasy Tissue kit (Qiagen, Valencia, CA, USA) following manufacturer's instructions. Mitochondrial 16S and COI, and nuclear rDNA 18S gene fragments were PCR-amplified (Black and Piesman, 1994; Simon et al, 1994; Black et al, 1997) for each individual tick and sequenced using standard methods (see A1 in supplementary materials for detailed amplification protocols and sequencing methods).

*Genetic and statistical analyses:* DNA sequences were aligned with the MEGA software (version 6.0.5; Tamura et al, 2013) and used in DNAsp (version 5.10.01) (Librado and Rozas, 2009) to calculate haplotype ( $H_d$ ) and nucleotide ( $\pi$ ) diversities (Nei 1973) (see A2 in supplementary material for accession numbers of used DNA sequences).

In order to test for genetic differentiation among populations, we calculated KST\*, a distance index based on the number of nucleotide differences between sequences (Hudson et al, 1992). Both an overall estimate of KST\* (KST\*<sub>Tot</sub>) including all populations and pairwise population values were calculated for each gene fragment. Permutation tests, with 1000 repetitions, were used to assess the significance of KST\* estimates.

To visualize genetic differences among populations, cluster analyses were performed for each gene fragment and for concatenated sequences of the 16S and COI genes (800bp) using the UPGMA method with Splitstree (version 4.13.1; Huson and Bryant, 2006). Five 16S gene sequences from the study of Gómez-Díaz et al. (2012) and 4 additional sequences available from Genbank (See Table 1) were used to construct phylogenetic trees with MEGA (version

6.0.5) using maximum likelihood with sequences from the hard tick *Ixodes uriae* (Ixodidae) as an outgroup.

In order to attribute genetic structure to different sources (host versus geography), we carried out an analysis of molecular variance (AMOVA) on haplotypic data using ARLEQUIN (version 3.5.1.2; Excoffier and Lischer, 2010). For this analysis, we considered only those host groups containing at least two tick populations (i.e. shearwater/booby/gull). Consequently, we excluded the AMS, JdN, Hawaii1 and TexasA populations.

## 2.4 Morphometric analyses

We used both landmark and outline-based analyses to describe tick morphology. The first type enables us to obtain precise measures of morphological traits associated with different biological activities (i.e., feeding, reproduction, locomotion) as landmarks are positioned on genetically determined anatomical structures. The outline analysis, in contrast, provides an overall measure of differences in body shape and size because it can be used on body parts lacking clear anatomical structure, and is thus especially relevant for the soft tick body form (Dujardin et al, 2014). For both analyses, we used a standardized image of each tick taken using a binocular microscope (Leica M80). All morphometrical analyses were performed using the different modules of the CLIC package (Dujardin & Slice, 2006, Dujardin et al., 2010), freely available at <http://mome-clic.com/>.

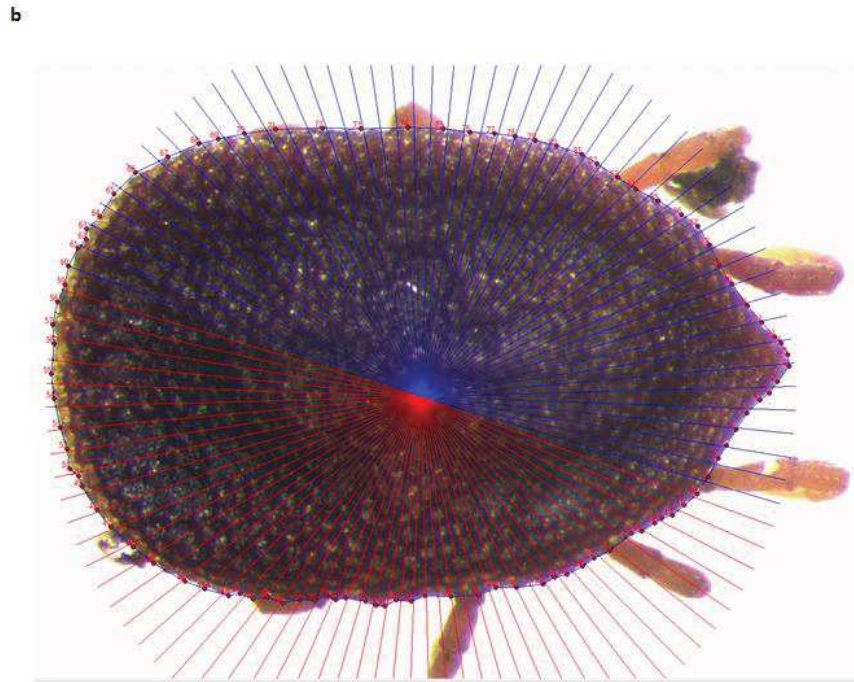
For the landmark analysis, a set of 15 type II (Bookstein, 1997) landmarks was established on the ventral face of the tick (Fig.2a). Shape variables (uniform and non-uniform components) were obtained by computing a generalized Procrustes analysis (GPA). Briefly, after removing the size factor through the Procrustes superimposition on the consensus shape, the residual coordinates were converted into “partial warps” (PW) to be used by standard multivariate

analyses (principal component and discriminant analyses). The connected residual coordinates of the 15 landmarks provided polygons allowing visual comparisons of mean shape among tick populations. A discriminant analysis was then performed using the principal components of the PW (i.e., the relative warps, or RW) as input.

For the outline-based analysis, we used the elliptic Fourier analysis (EFA) method (Kuhl & Giardina, 1982) to compute the contour of the dorsal face of each tick (Fig.2b) with the aim of comparing overall tick shape. The outline was decomposed into elliptic Fourier coefficients which were normalized (NEF) in order to be compared without the influence of size, orientation or starting point. A variance-covariance matrix was then constructed with these coefficients to summarize variation and was used as input for multivariate analyses.

a





**Fig.2 (a)** Image representing the 15 true landmarks used on the ventral face of each tick (here shown on a female tick from Juan de Nova). Each point represents one landmark. Landmarks N°1-2: width between landmarks 1 and 2 on extremities of the transverse post-anal groove; N°3-4: width between landmarks 3 and 4 on extremities of the preanal groove; N°5: anus; N°8-9: width between landmarks 8 and 9 at coxae III/IV; N°10-12: width between landmarks 10 and 12 at coxae I/II; N°11: genital pore; N°13-14: width between landmarks 13 and 14 on the lower extremity of the basis capitulum; N°15: capitulum extremity. **(b):** Image representing the outline method used on the dorsal face of ticks (an image of a female from Juan de Nova). The central point represents the centroid. The presence of radii is an illustrative feature of the software; landmarks were collected manually.

For size analyses, we computed the centroid size (CS) for the landmark analysis, defined as the square root of the sum of the squared distances between the center of the landmark configuration and each individual landmark (Bookstein, 1997). For outline analyses, we used the perimeter (P) as an estimate of body size. Size means and variances were analyzed using permutation tests due to low sample sizes in some populations. At each permutation cycle, individuals were randomly exchanged among populations, and the mean and variance was recalculated (1000 permutations in total). Then, we used a Bonferroni correction to determine the significance of size differences between populations. The permutation tests were also used to test size and shape differences between females and males for each tick population.

Shape differences among populations were quantified based on Mahalanobis distances (Dujardin et al, 2010) that were used to construct dendrograms in the R software (version 3.0.2) using the UPGMA method. Size variation among populations for landmarks and outlines was visualized using boxplots, showing values for each individual tick, the median value per population, and the 25<sup>th</sup> and 75<sup>th</sup> percentiles. If boxes overlap, landmark arrangements or outlines were considered similar among populations. A random validated classification method (CCC, for “cross checked classification”), based on the jackknife procedure, was used to test the affiliation of each individual tick to each population.

The effect of size (either CS or P) on the discrimination of shape (either RW or principal components of NEF) was estimated by linear regression, using the correlation ( $r$ ) and determination ( $r^2$ ) coefficients.

The morphometrical Mahalanobis distance matrix was compared statistically against the genetic distance matrix (KST\*) using Mantel tests to evaluate their relationship. All Mantel tests were performed using XLSTAT software (version 2015), with significance assigned based on 1000 matrix permutations and a critical level of  $\alpha = 0.05$ .

## **3 Results**

### **3.1 Population genetic analyses**

Gene fragments were successfully sequenced for the three genes (16S 351bp, COI 400bp and 18S 312bp), although some amplification problems occurred for the 18S gene. As generally expected, the COI gene fragment showed the highest polymorphism with 146 variable sites, followed by the mitochondrial 16S gene with 90 polymorphic sites and the 18S nuclear gene fragment, with 33 polymorphic sites (Table 2 and Table A1).



Haplotypic and nucleotidic diversities were relatively low within populations but high among tick populations (Table 2 and SM Table A1). This was supported by measures of population differentiation;

**Table.2:** Genetic indexes of population diversity for the 16S gene fragment. Seq: number of sequences; NS: number of variable sites; K: mean number of nucleotide differences; H: number of haplotypes; Hd  $\pm$  SD: Haplotype diversity and standard deviation;  $\pi$   $\pm$  SD: Nucleotide diversity and standard deviation.

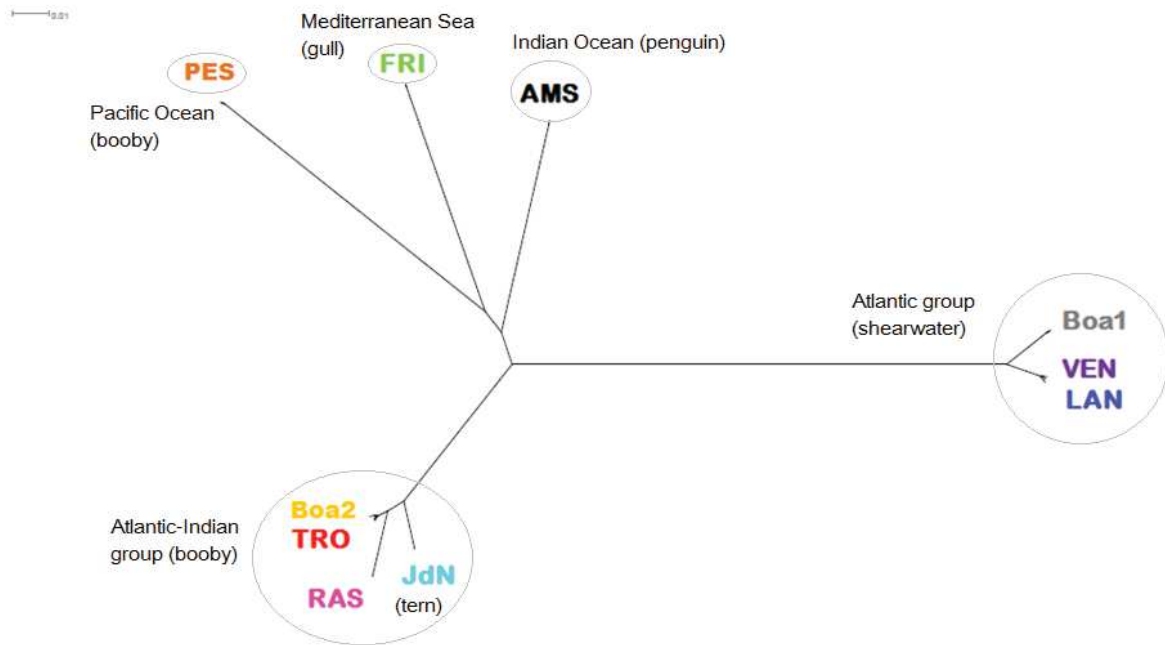
rDNA 16S – 351 bp						
Population	Seq	NS	K	H	Hd $\pm$ SD	$\pi$ $\pm$ SD
<b>Boavista 1</b>	22	67	6.255	4	0.333 $\pm$ 0.124	0.0183 $\pm$ 0.01557
<b>Boavista 2</b>	9	0	0.000	1	0 $\pm$ 0	0 $\pm$ 0
<b>Lanzarote</b>	24	2	0.373	3	0.359 $\pm$ 0.110	0.00109 $\pm$ 0.00036
<b>Raso</b>	21	1	0.267	3	0.267 $\pm$ 0.120	0.00077 $\pm$ 0.00035
<b>Veneguera</b>	13	0	0.000	1	0 $\pm$ 0	0 $\pm$ 0
<b>Frioul</b>	20	0	0.000	1	0 $\pm$ 0	0 $\pm$ 0
<b>Juan de Nova</b>	18	6	0.667	2	0.111 $\pm$ 0.096	0.00193 $\pm$ 0.00167
<b>Amsterdam</b>	15	4	1.829	7	0.857 $\pm$ 0.065	0.00530 $\pm$ 0.00082
<b>Pescadores</b>	20	1	0.100	2	0.100 $\pm$ 0.088	0.00029 $\pm$ 0.00026
<b>Tromelin</b>	8	1	0.429	2	0.429 $\pm$ 0.169	0.00124 $\pm$ 0.00049
<b>Total</b>	<b>170</b>	<b>90</b>	<b>35.392</b>	<b>20</b>	<b>0.888 <math>\pm</math> 0.009</b>	<b>0.1278 <math>\pm</math> 0.00304</b>

KST\* coefficients indicated strong genetic differentiation among all populations (Table 3). The high number of variable sites (67) observed for 16S mitochondrial gene fragments in the Boal tick population originated from two sequences that more closely matched sequences from the FRI tick population; interestingly, this was not the case for the other gene fragments, nor for morphometrical analyses suggesting a historical introgression event.

**Table.3:** Pairwise KST\* indexes between populations for the 16S gene fragment. Significance is indicated as follow: NS = not significant; \*0.01<P<0.05; \*\*0.001<P<0.01; \*\*\*P<0.001. Green and purple boxes represent respectively results within and between genetic identified groups.

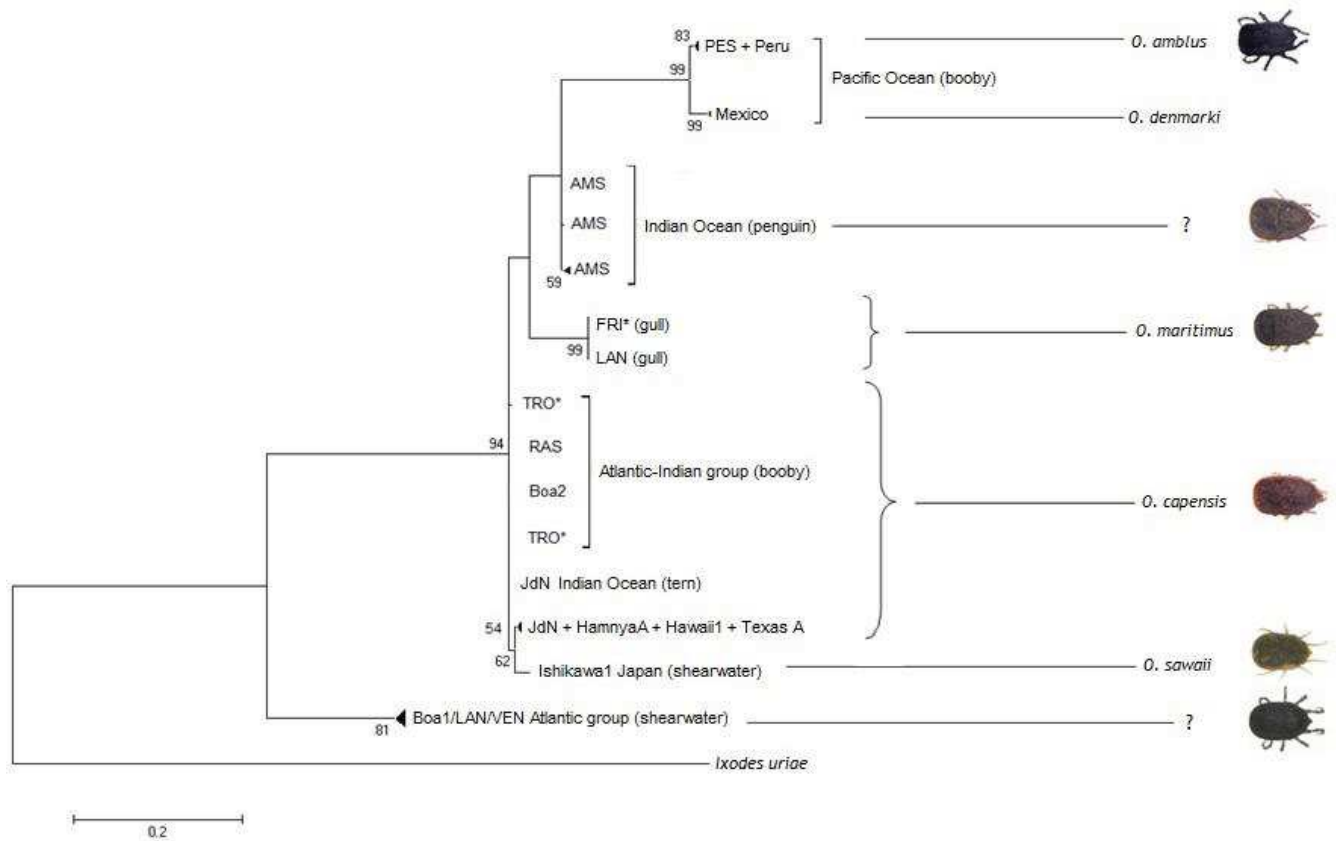
16S KST*		Boa 1	Boa 2	Lan	Ras	Ven	Fri	JdN	Ams	Pes
Cape Verde	Boavista 2	<b>0.79655</b> ***								
Canarias	Lanzarote	<b>0.69279</b> ***	<b>0.8932</b> 9 ***							
Cape Verde	Raso	<b>0.83639</b> ***	- 0.0213 2 NS	<b>0.9003</b> 6 ***						
Canary	Veneguera	<b>0.71604</b> ***	<b>1</b> ***	3 NS	<b>0.9438</b> 9 ***					
Mediterranean Sea	Frioul	<b>0.86840</b> ***	<b>1</b> ***	<b>7</b> ***	<b>8</b> ***	<b>1</b> ***				
Indian Ocean	Juan de Nova	<b>0.82440</b> ***	<b>0.8404</b> 1 ***	<b>0.8924</b> 1 ***	<b>0.8096</b> 7 ***	<b>0.9407</b> 8 ***	<b>0.9413</b> 1 ***			
Indian Ocean	Amsterda m	<b>0.70254</b> ***	<b>0.7338</b> 9 ***	<b>0.7735</b> 8 ***	<b>0.7058</b> 6 ***	<b>0.7884</b> 9 ***	<b>0.7887</b> 6 ***	<b>0.7005</b> 8 ***		
Peru	Pescadores	<b>0.86147</b> ***	<b>0.9694</b> 6 ***	<b>0.9244</b> 2 ***	<b>0.9328</b> 4 ***	<b>0.9798</b> 8 ***	<b>0.9817</b> 2 ***	<b>0.9282</b> 1 ***	<b>0.7785</b> 7 ***	
Indian Ocean	Tromelin	<b>0.75013</b> ***	<b>0.5924</b> 1 **	<b>0.8514</b> 0 ***	<b>0.4108</b> 1 ***	<b>0.9500</b> 1 ***	<b>0.9474</b> 4 ***	<b>0.7608</b> 7 ***	<b>0.5943</b> 7 ***	<b>0.9208</b> 1 ***
KST* Tot 16S			<b>0.9226</b> 9	***						

Cluster analyses showed the presence of two main population groups, one group of tick populations associated with shearwater hosts [Boa1, VEN, LAN] and a second with mainly booby hosts [Boa2, RAS, TRO, JdN]. The tick populations of AMS, FRI and PES (collected on Peruvian Boobies) fell outside of these groups (Fig.3). The shearwater group was the most genetically differentiated at all genes (See SM Fig A1.1, A1.2, and A1.3). It should be noted that the 18S gene fragments were not included in the concatenated tree due to missing sequences in some populations.



**Fig.3:** Cluster analysis of the concatenated sequences of the 16S and COI mitochondrial gene fragments. Populations are identified by abbreviations as outlined in Table 1.

Phylogenetic analyses of the three genes (including additional sequences for 16S gene) were well-resolved and concordant with the cluster analyses (Fig.4 and See SM Fig.A2.1 and A2.2). The shearwater group was the most isolated, with the Atlantic/Indian ocean booby group and the other populations showing more recent divergence. The PES tick population branched with *O. amblus* samples collected from Peru and were closely related to *O. denmarki* samples collected from Mexico, constituting a Pacific booby group. The FRI tick population branched with ticks collected on Lanzarote Island from gull nests identified as *O. capensis*. The AMS tick population was isolated from all other typed populations and no known species could be attributed to these ticks. The JdN tick population branched with *O. capensis* samples from Texas, Hawaii and Japan collected on different seabird species (Reddish Egret, Laysan Albatross and Streaked Shearwater) and grouped with *O. capensis s.s.* This large group tended to be related to *O. sawaii* collected from Streaked Shearwaters in Japan.



**Fig.4:** Phylogenetic tree based on sequences of the 16S mitochondrial marker. *Ixodes uriae* represents the outgroup. To the right, we have provided the expected species names based on historical descriptions and an associated image, when available. The population names with a star indicate that species identifications have been verified using classical morphological traits of larval ticks.

The AMOVA results showed that genetic differentiation was highly dependent on host (54.07% of the variation due to among host-group differences), but that geography also played a substantial role with 43% of the variation attributed to among population differences within groups (Table 4). Much of the variation within host groups can be attributed to differences among booby tick populations in the Atlantic and Pacific Oceans (see above).

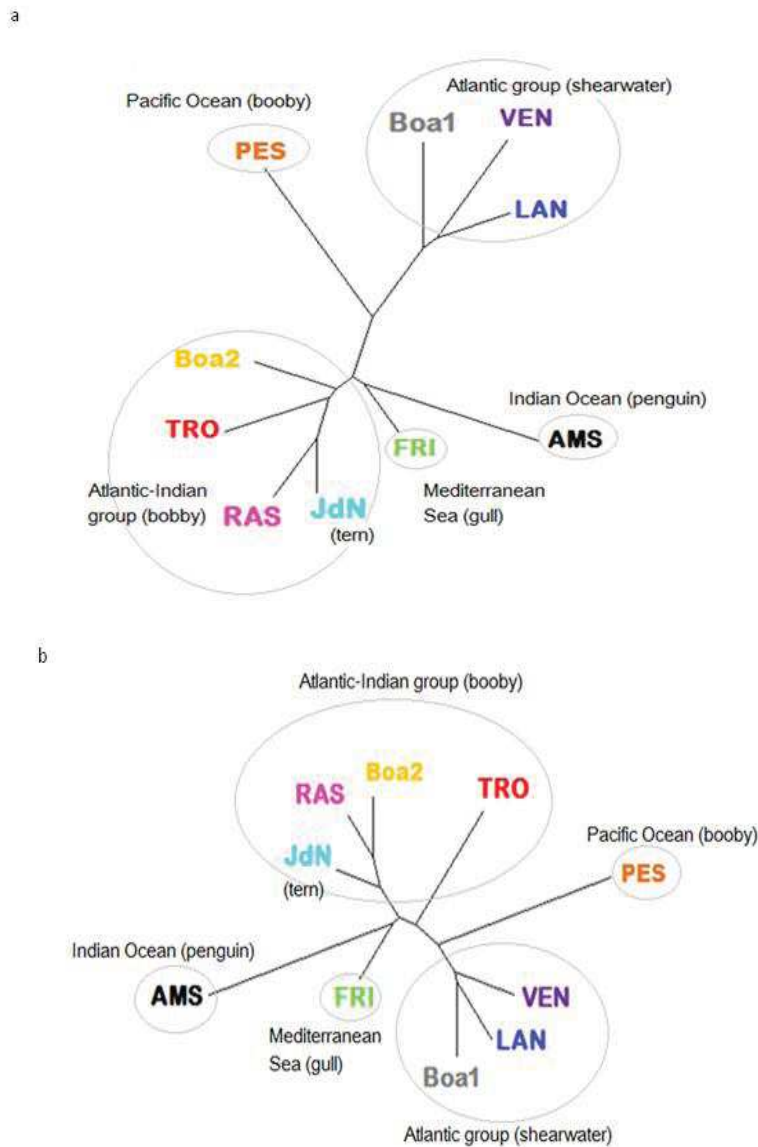
**Table.4:** Analysis of molecular variance (AMOVA) using haplotypic data from populations of three host groups (Shearwater, Booby, Gull) (see text for details).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P-value
Among host groups	2	1507.631	12.20141	54.07	0.00782
Among populations within groups	12	1013.299	9.64217	42.73	0.00000
Within populations	140	101.348	0.72391	3.21	0.00000
Total	154	2622.277	22.56750		

### 3.2 Morphometrical analyses

Similar results were obtained for males and females for both types of morphometrical analyses; we therefore combined the sexes for population level comparisons (pairwise differences among males and females of each population  $p > 0.0120$ : starting value for the Bonferroni test: 0.000292).

The UPGMA trees, based on Mahalanobis distances indicated the presence of two main population groups (Fig.5): one composed of shearwater tick populations [Boa1, LAN and VEN], and another formed by four populations [Boa2, RAS, TRO, JdN] with slightly more diverse host relationships (i.e. boobies and terns). The other three populations – AMS, FRI and PES (collected respectively on penguins, gulls and Peruvian boobies) - were largely isolated from these main groups and from each other. The same results were obtained for the discriminant analyses (See SM Fig.A3).



**Fig.5:** UPGMA tree based on Mahalanobis distances from the landmark **(a)** and outline **(b)** analyses of tick populations. See Table 1 for population abbreviations.

Boa1 and LAN showed the largest size variation and overall centroid size, whereas Boa2 and RAS were significantly smaller than the other populations (See SM Fig.A4 and A5 and Table.A4). The cross-check classification provided strong support for the distinctness of populations with a minimum of 50% for TRO based on the landmark analysis (similar to JdN and Boa2) and 48% for Boa2 with the outline analysis (similar to RAS). Maximal scores were obtained for PES at 88% (strongly conserved landmark configuration) and 95% for FRI



(strongly conserved outline configuration) (See SM Fig.A4 ad A5). AMS and PES showed the most distant inter-population morphologies based on both analyses (See Fig.5 and SM Fig.A6 and A7).

For the landmark analysis, the size effect on shape represented 18.3% of the overall variation in tick morphology (DF1 regression:  $r=-0.260$ ;  $r^2=6.8\%$ ;  $df=204$ ;  $t=3.85$ ,  $p=8.2 \times 10^{-5}$ ; DF2 regression:  $r=-0.339$ ;  $r^2=11.5\%$ ;  $df=204$ ;  $t=5.15$ ,  $p=3.1 \times 10^{-7}$ ), whereas for outline analyses, the size effect represented 22.5% of the overall shape variation (DF1 regression:  $r=0.444$ ;  $r^2=19.7\%$ ;  $df=204$ ;  $t=7.08$   $p=1.17 \times 10^{-11}$ ; DF2 regression  $r=0.167$ ;  $r^2=2.8\%$ ;  $df=204$ ;  $t=2.42$   $p=0.008$ ).

Landmark arrangement variation among populations seemed to be due to changes in the relative position of the genital aperture and the anus (See SM Fig.A6). The position of the anus, and thus the distance between the anus and the genital aperture, of shearwater tick populations [Boa1, LAN and VEN] and the PES population seemed to differ from the other populations. The distance between the posterior ends of the preanal groove also varied among tick populations, principally due to changes in tick body width (See SM Fig.A6). The majority of shape variation in the outline analyses originated from the form of anterior margin of the body and the relative width of the body contour (See SM Fig A7).

### **3.3 Correlation between genetic and morphological analyses**

Significant correlations were found between morphological and genetic distances among populations (See Table 5). However, the results from the Mantel tests of the 16S and CO1 suggest that genetic distance only explains a small part of the morphometric variation among tick populations; this relationship was stronger for the 18S gene fragment ( $r^2=65.6\%$

$p \leq 0.0001$  for the landmarks; See Table 5) and may be linked to ventral traits on the tick body.

**Table.5:** Mantel test results between morphometric (Mahalanobis distances) and genetic (KST\* distances) matrices. The corresponding graphs for each analysis for the landmark (LM) distances are shown in SM Table.A.3.

	16S			COI			18S		
	r-value	r <sup>2</sup> (%)	p-value	r-value	r <sup>2</sup> (%)	p-value	r-value	r <sup>2</sup> (%)	p-value
<b>Landmarks</b>	0.378	14.3	0.025	0.532	28.3	0.001	0.810	65.6	<0.0001
<b>Outline</b>	0.316	10.0	0.059	0.449	20.2	0.005	0.603	36.4	0.0003

## 4 Discussion

The *O. capensis sensu lato* complex is a diverse group of ticks found in seabird colonies across the globe. Previous descriptions of this complex were based on both classical morphology and geographic location, but included largely fragmented and opportunistic samples. As a consequence, host relationships are vague and most species have been considered to be seabird generalists, exploiting any locally available seabird (Dietrich et al, 2011). Here, we examined genetic structure among populations of this complex from across the globe to determine the relative role of host versus geography in driving divergence. We compared these patterns with detailed analyses of tick morphometry to test congruence and infer the potential phenotypic basis of divergence.

### 4.1 Genetic and associated morphometric structure

Our genetic analyses, based on three genes, were able to discriminate tick populations in relation to host use and/or locality and were in strong agreement with morphometric analyses, confirming the presence of at least five different species among the sampled ticks (Fig.4 and 5). Distant populations using the same or closely-related host species shared haplotypes across

large spatial scales and were morphologically similar. Moreover, differences among host-associated populations remained even when ticks were sampled from different hosts in complete sympatry (ex., Boa1 and Boa2). However, despite these global patterns, significant differences in haplotype frequencies were evident among populations within geographic regions. Indeed, pairwise population genetic analyses showed that almost all populations are genetically distinct. Therefore, although host-associated groups are evident at large spatial scales, gene flow is not frequent among populations within groups (Table 2 and 3). This is not surprising given the life history traits of soft ticks. Indeed, although birds can migrate over large distances, soft ticks only exploit their hosts for a short period of time (several minutes) and typically at night when most birds do not travel. Therefore, dispersal events should be rare. These events may occur during bird immigration from distant colonies (Thibault & Bretagnolle, 1998) or with the movements of young birds that prospect different colonies prior to breeding, a period that can last 2 to 6 years in these seabird species (Votier et al. 2011; Peron & Gremillet 2013, Boulinier et al, 2016).

Despite the presence of clear host-associated groups, some evidence of mixing among host groups was still apparent in the data. In the Boa1 population (shearwater hosts), haplotypes typically associated with the FRI Mediterranean population (gull hosts) were found at the 16S gene in two sampled ticks, but were not present at the other two genes. These two individuals were not morphological outliers, suggesting a signal of a past introgression events among ticks of these two species. Some degree of hybridization among distinct tick lineages may not be surprising given that different tick lineages are known to co-occur sympatrically on some islands (Gómez-Díaz et al, 2012). Indeed, gull ticks collected on Lanzarote Island matched the FRI tick population from the same host and co-occurred with the shearwater group on this island (LAN). However, given the relative distinctness of the different host-associated populations we found, hybridization events are presumably very rare and may depend on the

occurrence of specific ecological conditions (e.g., low tick population size, unavailability of typical host, etc). *O. spheniscus*, for example, is specialized on the Humboldt penguin, *Spheniscus humboldti*, which breeds in dense colonies, but has never been found to occur on other seabird species that breed sympatrically with these penguin hosts (Hoogstraal et al, 1985).

#### **4.2 Host-association and the geographic distribution of species within the *O. capensis* complex**

Based on genetic and morphometrical evidence, we suggest that at least five different species of the *O. capensis* complex have been sampled in our study. At least two tick species were sympatric on the Cape Verde Islands. One of these species, associated with booby hosts, is also found within the Indian Ocean. Given this large distribution and historical descriptions, these populations should be classified as *O. capensis* s.s. Indeed, larval ticks from the TRO tick population were identified as *O. capensis* s.s. using traditional morphological criteria (data not shown). The JdN tick population, collected on terns also grouped with *O. capensis* s.s., although some genetic divergence was evident between these ticks and those of booby hosts. This may suggest a recent shift of ticks between booby and tern hosts within this region of the world. The ticks from terns may also represent a distinct species: *O. cheikhi*; a species described on terns in Mauritania was suggested to be morphologically related to *O. capensis* (Vermeil et al, 1997), but no further work has been done on this species. The JdN tick population was genetically similar to the Ishikawa sample representing *O. sawaii*, a tick species found in Japanese seabird colonies and again reported to be closely related to *O. capensis* (Kitaoka and Suzuki, 1973). A more complete sampling of tern colonies within the

Indian Ocean and Japan, with representative samples of larval ticks, is now called for to clarify the specific status of these populations.

The second tick population present on the Cape-Verde Islands was strictly associated with shearwater hosts and was also found in the Canary Islands. This tick could not be identified to any described species using traditional morphology on larval ticks (data not shown), and likely represents a new species. The strong morphological and genetic distances between this group and the other ticks sampled suggest that this species is not part of the *O. capensis* complex at all, and may represent an independent shift to seabird hosts.

Like the shearwater tick group, ticks from AMS did not match any previously described species of the *O. capensis* complex, nor did sequence alignments match with available *O. sawaii* sequences (Kawabata et al, 2006), or *O. denmarki*, described from coastal regions of Northern and Central America (Fig.1). However, unlike the ticks associated with shearwaters, ticks from penguins on AMS seem to belong to the *O. capensis* complex, their clade being well-nested within the complex. Ticks from PES could be attributed to *O. amblus*, a species distributed along the west coast of Peru and known to exploit different host species (Clifford et al, 1980). The morphology and genetic differences of this species with respect to the Atlantic-Indian booby group is likely related to geographic isolation of the hosts. Ticks from FRI correspond to *O. maritimus* which occurs throughout gull colonies of the Mediterranean Sea and along the Atlantic coast of Europe and Africa (Hoogstraal et al, 1976; Guiguen et al, 1987; Vermeil et al, 1997). Larval ticks from this population were identified as *O. maritimus* using traditional morphological criteria, confirming this identification (data not shown). These ticks grouped with those collected on gulls on Lanzarote Island, indicating the presence of *O. maritimus* on the Canary Islands. As host affinities are subtle in the *O. capensis* complex and our samples reflect only part of its diversity, additional sampling and official descriptions,

notably of larval stages, will now be necessary to match existing descriptions with these new observations.

#### **4.3 Drivers of diversification: host versus geography**

Both landmark and outline analyses showed that ticks from boobies are typically smaller than the other tick populations. Following Harrison's Rule (Harrison, 1915), parasite size is thought to be directly correlated with host size, i.e. parasite size increases with increasing host size. Malenke et al. (2009), for example, showed a proportional relationship between parasite size and host size in *Columbicola* feather lice. However, Johnson et al (2005) found no such relationship in body lice. This rule does not seem to apply to the present tick populations, as boobies are larger overall (>70cm total length) than terns, gulls, penguins and shearwaters (<50cm total length). Reduced tick size could be due to several alternative factors. For example, Amin and Sonenshine (1969) demonstrated that size variation is directly linked to the differential engorgement success of immature stages in the American dog tick, *Dermacentor variabilis*. Engorgement success can depend on host traits associated with physiology and immunity that alter the quantity of blood imbibed. Tick size reduction could also come from differences in tick digestive capacities, reflecting intrinsic differences in the ability of ticks to exploit different host resources (Willadsen et al, 1984; Dietrich et al, 2013). However, in both of these cases, we would expect that adaptation to the host should enable a release from such constraints allowing for increased tick size. The smaller size of booby ticks could also be linked to a physical constraint imposed by the host, and more specifically the ability to remove ticks during preening (Clayton et al, 2010). Indeed, beak shape and size variation have been shown to play a major role in determining the size and load of ectoparasites (Clayton et al, 1999, Clayton et al, 2005). In our study, beak shape strongly



differs between shearwaters and boobies, with shearwaters having hooked beaks and boobies pointed; these differences may alter the ability of hosts to preen off ticks. High CCC scores based on Mahalanobis distances indicated that size is relatively constant within booby tick populations (Fig.A4 and A5), suggesting strong host-imposed size selection on this trait. Indeed, ticks exploiting boobies in the Pacific (PES population) were also among the smallest-sized populations, despite being only distantly related to the other booby tick lineage. These elements suggest preening rather than variation due to the quantity or quality of the bloodmeal may explain at least some of the size patterns in our data.

Tick shape was largely independent of body size variation (only 18-22.5% of shape variation was due to size effects). The dorsal boundary outline of the tick body showed strong population structure in relation to host use. Contrary to size, which can be influenced by environmental conditions, shape is thought to be more stable (Dujardin et al, 2003; Perrard et al, 2012), and can modify the ability of ticks to move within the feather environment and escape preening (Bush et al, 2011). As for size, variance in shape was lower for ticks on booby hosts compared to the other populations suggesting strong selective pressures on tick body shape. Comparative studies of bill morphology will now be required to test the role of preening in determining tick size and shape.

Aside from selective pressures directly related to feeding and survival on the host, geographic differences in climate, landscape or ecological perturbations may alter tick shape and size and play a role in their diversification. Indeed, ticks spend the major part of their lifecycle in the off-host environment and require specific conditions of temperature and humidity to survive (Gray et al, 2014). Such factors could present an alternative explanation for morphological differences observed among AMS, FRI and PES tick populations, but are unlikely to explain divergences observed within islands among hosts or populations located in similar climatic zones (ex. Cape-Verde and Canary archipelagos). It is possible that the microhabitats

associated with the use of different sympatric hosts (e.g., burrows versus rocky nests) exert off-host selection pressures on tick populations and can explain some of this variation. However, soft ticks seem to be able to readily move at the within colony scale, assuming environmental conditions are suitable. *O. savignyi*, for example, is able to cross more than twenty meters to find a host (Mans and Neitz, 2004).

Although genetic and morphometric analyses revealed tick population structure linked to host use and geographic location, correlations between morphometric and genetic distances were variable in strength. The relationship between the two distance measures was stronger for the 18S nuclear gene than for the mitochondrial genes. This stronger relationship may be logical if morphological structures have a genetic basis and represent gene flow of both tick sexes, rather than only female gene flow as is the case for the two mitochondrial genes. Likewise, Mahalanobis distances based on landmark measures of morphology were more strongly correlated with genetic distances than outline measures. These results suggest that the ventral traits measured by the landmark analysis are more genetically conserved than overall body shape.

## 5 Conclusions

Combining genetic and morphometric analyses enabled us to test specific predictions on the role of host specificity in the diversification of the *O. capensis* complex. Genetic-based analyses were able to discriminate the presence of different species and were supported by morphometric results, illustrating the need for more complete studies of the *O. capensis* complex in relation to host use if we are to fully understand their systematic and global diversity. Although not mutually exclusive from climate or distance-based isolation, divergence associated with host specific adaptations remains the most parsimonious

explanation for the morphometric and genetic structures that we observed among tick populations. Therefore, our results also highlight the utility of combining genetics and morphometrics for studying cryptic parasite biodiversity and the potential selective forces acting on this diversity. Finally, our results support previous hypotheses suggesting that ticks are rarely true host generalists and that host-associated adaptations likely play a major role in the diversification of these parasite vectors and in the circulation of tick-borne pathogens (McCoy et al, 2013).

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## References

- Adams, D.C., Rohlf, F.J., Slice, D.E., 2004. Geometric morphometrics: Ten years of progress following the “revolution.” *Italian Journal of Zoology* 71, 5–16. doi:10.1080/11250000409356545
- Agrawal, A.A., 2001. Phenotypic Plasticity in the Interactions and Evolution of Species. *Science* 294, 321–326. doi:10.1126/science.1060701
- Amerson, A.B., Jr, 1968. Tick distribution in the central Pacific as influenced by sea bird movement. *J. Med. Entomol.* 5, 332–339.
- Amin, O.M., Sonenshine, D.E., 1969. Development of the American Dog Tick, *Dermacentor variabilis*, Following Partial Feeding by Immatures. *Annals of the Entomological Society of America* 63, 128–133.
- Barluenga, M., Stölting, K.N., Salzburger, W., Muschick, M., Meyer, A., 2006. Sympatric speciation in Nicaraguan crater lake cichlid fish. *Nature* 439, 719–723. doi:10.1038/nature04325
- Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K., Das, I., 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution* 22, 148–155. doi:10.1016/j.tree.2006.11.004
- Black IV, W.C., Klompen, J.S.H., Keirans, J.E., 1997. Phylogenetic Relationships among Tick Subfamilies (Ixodida: Ixodidae: Argasidae) Based on the 18S Nuclear rDNA Gene. *Molecular Phylogenetics and Evolution* 7, 129–144. doi:10.1006/mpev.1996.0382
- Black, W.C., Piesman, J., 1994. Phylogeny of hard-and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. *Proceedings of the National Academy of Sciences* 91, 10034–10038.
- Bookstein, F.L., 1997. *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge University Press.
- Boulinier, T., Kada, S., Ponchon, A., Dupraz, M., Dietrich, M., Gamble, A., Bourret, V., Duriez, O., Bazire, R., Tornos, J., Tveraa, T., Chambert, T., Garnier, R., McCoy, K.D., 2016. Migration, Prospecting, Dispersal? What Host Movement Matters for Infectious Agent Circulation? *Integr. Comp. Biol.* icw015. doi:10.1093/icb/icw015
- Bush, S.E., Villa, S.M., Boves, T.J., Brewer, D., Belthoff, J.R., 2011. Influence of Bill and Foot Morphology on the Ectoparasites of Barn Owls. *Journal of Parasitology* 98, 256–261. doi:10.1645/GE-2888.1
- Clayton, D.H., Koop, J.A., Harbison, C.W., Moyer, B.R., Bush, S.E., 2010. How birds combat ectoparasites. *Open Ornithol J* 3, 41–71.
- Clayton, D.H., Lee, P.L.M., Tompkins, III, E.D.B., 1999. Reciprocal Natural Selection on Host-Parasite Phenotypes. *The American Naturalist* 154, 261–270. doi:10.1086/303237
- Clayton, D.H., Moyer, B.R., Bush, S.E., Jones, T.G., Gardiner, D.W., Rhodes, B.B., Goller, F., 2005. Adaptive significance of avian beak morphology for ectoparasite control.

- Proceedings of the Royal Society of London B: Biological Sciences 272, 811–817. doi:10.1098/rspb.2004.3036
- Clifford, C.M., Hoogstraal, H., Radovsky, F.J., Stiller, D., Keirans, J.E., 1980. *Ornithodoros (Alectorobius) amblus* (Acarina: Ixodoidea: Argasidae): Identity, Marine Bird and Human Hosts, Virus Infections, and Distribution in Peru. *The Journal of Parasitology* 66, 312–323. doi:10.2307/3280825
- Colas-Belcour, J., Rageau, J., 1960. Biologie et élevage d'*Ornithodoros capensis* Neumann (acarien Argasidae). *Bulletin de la Société de Pathologie Exotique* 53, 723–727.
- Dietrich, M., Beati, L., Elguero, E., Boulinier, T., McCoy, K.D., 2013. Body size and shape evolution in host races of the tick *Ixodes uriae*. *Biological Journal of the Linnean Society* 108, 323–334. doi:10.1111/j.1095-8312.2012.02021.x
- Dietrich, M., Gómez-Díaz, E., McCoy, K.D., 2011. Worldwide Distribution and Diversity of Seabird Ticks: Implications for the Ecology and Epidemiology of Tick-Borne Pathogens. *Vector-Borne and Zoonotic Diseases* 11, 453–470. doi:10.1089/vbz.2010.0009
- Dietrich, M., Kempf, F., Gómez-Díaz, E., Kitaysky, A.S., Hipfner, J.M., Boulinier, T., McCoy, K.D., 2012. Inter-oceanic variation in patterns of host-associated divergence in a seabird ectoparasite. *Journal of Biogeography* 39, 545–555. doi:10.1111/j.1365-2699.2011.02620.x
- Dietrich, M., Lobato, E., Boulinier, T., McCoy, K.D., 2014. An experimental test of host specialization in a ubiquitous polar ectoparasite: a role for adaptation? *J Anim Ecol* 83, 576–587. doi:10.1111/1365-2656.12170
- Duffy, D.C., 1983. The Ecology of Tick Parasitism on Densely Nesting Peruvian Seabirds. *Ecology* 64, 110. doi:10.2307/1937334
- Duffy, D.C., Daturi, A., 1987. Diel rhythms of tick parasitism on incubating African penguins. *Medical and Veterinary Entomology* 1, 103–106. doi:10.1111/j.1365-2915.1987.tb00329.x
- Dujardin, J.-P., 2008. Morphometrics applied to medical entomology. *Infection, Genetics and Evolution* 8, 875–890. doi:10.1016/j.meegid.2008.07.011
- Dujardin, J.-P.A., Kaba, D., Henry, A.B., 2010. The exchangeability of shape. *BMC Research Notes* 3, 266. doi:10.1186/1756-0500-3-266
- Dujardin, J.-P., Kaba, D., Solano, P., Dupraz, M., McCoy, K.D., Jaramillo-O, N., 2014. Outline-based morphometrics, an overlooked method in arthropod studies? *Infection, Genetics and Evolution* 28, 704–714. doi:10.1016/j.meegid.2014.07.035
- Dujardin, J.-P., Pont, F.L., Baylac, M., 2003. Geographical versus interspecific differentiation of sand flies (Diptera: Psychodidae): a landmark data analysis. *Bulletin of Entomological Research* 93, 87–90. doi:10.1079/BER2002206
- Dujardin, J.-P., Slice, D.E., 2006. Contributions of morphometrics to medical entomology. *Encyclopedia of infectious diseases: modern methodologies* 435–447.

- Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10, 564–567. doi:10.1111/j.1755-0998.2010.02847.x
- Gómez-Díaz, E., Morris-Pocock, J.A., González-Solís, J., McCoy, K.D., 2012. Trans-oceanic host dispersal explains high seabird tick diversity on Cape Verde islands. *Biol. Lett.* 8, 616–619. doi:10.1098/rsbl.2012.0179
- González-Acuña, D., Moreno, L., Guglielmone, A.A., 2008. First report of *Ornithodoros spheniscus* (Acari: Ixodoidea: Argasidae) from the Humboldt Penguin in Chile. *Systematic and Applied Acarology* 13, 120–122. doi:10.11158/saa.13.2.4
- Gray, J.S., Estrada-Pena, A., Vial, L., 2014. Ecology of nidicolous ticks, in: *Biology of Ticks*.
- Guiguen, C., Monnat, J., Launay, H., Beaucournu, J., 1987. Ectoparasites des oiseaux de Bretagne, III. Ixodoidea. *Cahiers Orstom, Ser. Ent. Méd. Parasitol.* 73–81.
- Hajibabaei, M., Singer, G.A.C., Hebert, P.D.N., Hickey, D.A., 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics* 23, 167–172. doi:10.1016/j.tig.2007.02.001
- Harrison, L., 1915. Mallophaga from Apteryx, and their Significance; with a Note on the Genus *Rallicola*. *Parasitology* 8, 88–100. doi:10.1017/S0031182000010428
- Hoogstraal, H., 1985. Argasid and Nuttalliellid Ticks as Parasites and Vectors, in: J.R. Baker and R. Muller (Ed.), *Advances in Parasitology*. Academic Press, pp. 135–238.
- Hoogstraal, H., 1969. *Ornithodoros (Alectorobius) muesebecki* n. sp., a parasite of the blue-faced booby (*Sula dactylatra melanops*) on Hasikiya Island, Arabian Sea. *Proceedings of the Entomological Society of Washington* 71, 368–374.
- Hoogstraal, H., Clifford, C.M., Keirans, J.E., Kaiser, M.N., Evans, D.E., 1976. The *Ornithodoros (Alectorobius) Capensis* Group (Acarina: Ixodoidea: Argasidae) of the Palearctic and Oriental Regions. O. (A.) *Maritimus*: Identity, Marine Bird Hosts, Virus Infections, and Distribution in Western Europe and Northwestern Africa. *The Journal of Parasitology* 62, 799. doi:10.2307/3278964
- Hoogstraal, H., Wassef, H.Y., Hays, C., Keirans, J.E., 1985. *Ornithodoros (Alectorobius) spheniscus* n. sp. [Acarina: Ixodoidea: Argasidae: *Ornithodoros (Alectorobius) capensis* group], a tick parasite of the Humboldt penguin in Peru. *J. Parasitol.* 71, 635–644.
- Hudson, R.R., Boos, D.D., Kaplan, N.L., 1992. A statistical test for detecting geographic subdivision. *Mol Biol Evol* 9, 138–151.
- Huson, D.H., Bryant, D., 2006. Application of Phylogenetic Networks in Evolutionary Studies. *Mol Biol Evol* 23, 254–267. doi:10.1093/molbev/msj030
- Huyse, T., Volckaert, F.A., 2002. Identification of a host-associated species complex using molecular and morphometric analyses, with the description of *Gyrodactylus rugiensoides* n. sp. (Gyrodactylidae, Monogenea). *International Journal for Parasitology* 32, 907–919. doi:10.1016/S0020-7519(02)00026-7



- Johnson, K.P., Bush, S.E., Clayton, D.H., 2005. Correlated Evolution of Host and Parasite Body Size: Tests of Harrioso's Rule Using Birds and Lice. *Evolution* 59, 1744–1753. doi:10.1111/j.0014-3820.2005.tb01823.x
- Kawabata, H., Ando, S., Kishimoto, T., Kurane, I., Takano, A., Nogami, S., Fujita, H., Tsurumi, M., Nakamura, N., Sato, F., Takahashi, M., Ushijima, Y., Fukunaga, M., Watanabe, H., 2006. First Detection of Rickettsia in Soft-Bodied Ticks Associated with Seabirds, Japan. *Microbiology and Immunology* 50, 403–406. doi:10.1111/j.1348-0421.2006.tb03807.x
- Keirans, J.E., Clifford, C.M., Hoogstraal, H., 1984. *Ornithodoros* (*Alectorobius*) *yunkeri*, new species (Acari: Ixodoidea: Argasidae), from seabirds and nesting sites in the Galapagos Islands. *Journal of Medical Entomology* 21, 344–350.
- Kitaoka, S., Suzuki, H., 1973. *Ornithodoros* (*Alectorobius*) *sawaii* sp. n. (Ixodoidea, Argasidae) associated with the streaked shear-water, *Colonectris leucomelas*, from the Amami-oshima Islands, Japan. *Natl Inst Anim Health Q* (Tokyo) 13, 142–148.
- Kohls, G.M., Sonenshine, D.E., Clifford, C.M., 1965. The systematics of the subfamily Ornithodorinae (Acarina: Argasidae). II. Identification of the larvae of the Western Hemisphere and descriptions of three new species. *Ann. Entomol. Soc. Am.* 58, 331–364.
- Kuhl, F.P., Giardina, C.R., 1982. Elliptic Fourier features of a closed contour. *Computer Graphics and Image Processing* 18, 236–258. doi:10.1016/0146-664X(82)90034-X
- Lawing, A.M., Polly, P.D., 2010. Geometric morphometrics: recent applications to the study of evolution and development. *Journal of Zoology* 280, 1–7. doi:10.1111/j.1469-7998.2009.00620.x
- Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25, 1451–1452. doi:10.1093/bioinformatics/btp187
- Malenke, J.R., Johnson, K.P., Clayton, D.H., 2009. Host Specialization Differentiates Cryptic Species of Feather-Feeding Lice. *Evolution* 63, 1427–1438. doi:10.1111/j.1558-5646.2009.00642.x
- Mans, B.J., Neitz, A.W.H., 2004. The sand tampan, *Ornithodoros savignyi*, as a model for tick-host interactions. *South African journal of science* 100, 283–288.
- Manske, M., Miotto, O., Campino, S., Auburn, S., Almagro-Garcia, J., Maslen, G., O'Brien, J., Djimde, A., Doumbo, O., Zongo, I., Ouedraogo, J.-B., Michon, P., Mueller, I., Siba, P., Nzila, A., Borrmann, S., Kiara, S.M., Marsh, K., Jiang, H., Su, X.-Z., Amaratunga, C., Fairhurst, R., Socheat, D., Nosten, F., Imwong, M., White, N.J., Sanders, M., Anastasi, E., Alcock, D., Drury, E., Oyola, S., Quail, M.A., Turner, D.J., Ruano-Rubio, V., Jyothi, D., Amenga-Etego, L., Hubbard, C., Jeffreys, A., Rowlands, K., Sutherland, C., Roper, C., Mangano, V., Modiano, D., Tan, J.C., Ferdig, M.T., Amambua-Ngwa, A., Conway, D.J., Takala-Harrison, S., Plowe, C.V., Rayner, J.C., Rockett, K.A., Clark, T.G., Newbold, C.I., Berriman, M., MacInnis, B., Kwiatkowski, D.P., 2012. Analysis of *Plasmodium falciparum* diversity in natural infections by deep sequencing. *Nature* 487, 375–379. doi:10.1038/nature11174

- McCoy, K.D., Chapuis, E., Tirard, C., Boulinier, T., Michalakis, Y., Bohec, C.L., Maho, Y.L., Gauthier-Clerc, M., 2005. Recurrent evolution of host-specialized races in a globally distributed parasite. *Proc. R. Soc. B* 272, 2389–2395. doi:10.1098/rspb.2005.3230
- McCoy, K.D., Dietrich, M., Jaeger, A., Wilkinson, D.A., Bastien, M., Lagadec, E., Boulinier, T., Pascalis, H., Tortosa, P., Le Corre, M., Dellagi, K., Lebarbenchon, C., 2016. The role of seabirds of the Iles Eparses as reservoirs and disseminators of parasites and pathogens. *Acta Oecologica* 72, 98–109. doi:10.1016/j.actao.2015.12.013
- McCoy, K.D., Leger, E., Dietrich, M., 2013. Host specialization in ticks and transmission of tick-borne diseases: a review. *Front Cell Infect Microbiol* 3. doi:10.3389/fcimb.2013.00057
- Nei, M., 1973. Analysis of Gene Diversity in Subdivided Populations. *PNAS* 70, 3321–3323.
- Pérez-Eid, C., 2007. Les tiques : identification, biologie, importance médicale et vétérinaire. Lavoisier.
- Perrard, A., Villemant, C., Carpenter, J.M., Baylac, M., 2012. Differences in caste dimorphism among three hornet species (Hymenoptera: Vespidae): forewing size, shape and allometry. *Journal of Evolutionary Biology* 25, 1389–1398. doi:10.1111/j.1420-9101.2012.02527.x
- Ravigné, V., Dieckmann, U., Olivieri, I., 2009. Live where you thrive: joint evolution of habitat choice and local adaptation facilitates specialization and promotes diversity. *Am. Nat.* 174, E141–169. doi:10.1086/605369
- Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, Weighting, and Phylogenetic Utility of Mitochondrial Gene Sequences and a Compilation of Conserved Polymerase Chain Reaction Primers. *Annals of the Entomological Society of America* 87, 651–701. doi:10.1093/aesa/87.6.651
- Takano, A., Muto, M., Sakata, A., Ogasawara, Y., Ando, S., Hanaoka, N., Tsurumi, M., Sato, F., Nakamura, N., Fujita, H., Watanabe, H., Kawabata, H., 2009. Relapsing Fever Spirochete in Seabird Tick, Japan. *Emerg Infect Dis* 15, 1528–1530. doi:10.3201/eid1509.090459
- Tamura, K., Stecher, G., Peterson, D., Filipowski, A., Kumar, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30, 2725–2729. doi:10.1093/molbev/mst197
- Thibault, J.-C., Bretagnolle, V., 1998. A Mediterranean breeding colony of Cory's Shearwater *Calonectris diomedea* in which individuals show behavioural and biometric characters of the Atlantic subspecies. *Ibis* 140, 523–528. doi:10.1111/j.1474-919X.1998.tb04615.x
- Ushijima, Y., Oliver, J.H., Keirans, J.E., Tsurumi, M., Kawabata, H., Watanabe, H., Fukunaga, M., 2003. Mitochondrial Sequence Variation in *Carios capensis* (Neumann), a Parasite of Seabirds, Collected on Torishima Island in Japan. *Journal of Parasitology* 89, 196–198. doi:10.1645/0022-3395(2003)089[0196:MSVICC]2.0.CO;2
- Vermeil, C., Marjolet, M., Vermeil, F., 1997. *Ornithodoros* (*Alectorobius*) *cheikhi* n.sp. (Acarina, Ixodoidea, Argasidae, groupe *Ornithodoros* (*Alectorobius*) *capensis*); Tique

parasite de sternes au banc d'Arguin - Mauritanie. Bulletin de la Société des sciences naturelles de l'Ouest de la France 19, 66–76.

Vial, L., 2009. Biological and ecological characteristics of soft ticks (Ixodida: Argasidae) and their impact for predicting tick and associated disease distribution. Parasite 16, 191–202.

Votier, S.C., Grecian, W.J., Patrick, S., Newton, J., 2010. Inter-colony movements, at-sea behaviour and foraging in an immature seabird: results from GPS-PPT tracking, radio-tracking and stable isotope analysis. Mar Biol 158, 355–362. doi:10.1007/s00227-010-1563-9

Whitlock, M.C., 1996. The Red Queen Beats the Jack-Of-All-Trades: The Limitations on the Evolution of Phenotypic Plasticity and Niche Breadth. The American Naturalist 148, S65–S77.

Willadsen, P., Kemp, D.H., McKenna, R.V., 1984. Bloodmeal ingestion and utilization as a component of host specificity in the tick, *Boophilus microplus*. Z. Parasitenkd. 70, 415–420. doi:10.1007/BF00927829

## Supplementary material

**A1:** PCR amplification conditions and information for mitochondrial 16S and COI and nuclear 18S gene fragments:

DNA extractions were performed with the DNeasy Tissue kit (Qiagen, Valencia, CA, USA) following manufacturer's instructions. Each fragment was amplified in a 25µl reaction mixture containing 2.5 µl of 10X reaction buffer (Qiagen, Courtaboeuf, France), 2.5mM of MgCl<sub>2</sub>, 200µM of each deoxynucleotide triphosphate (Eurogentec, France), 1 unit of Taq DNA polymerase (Cat. No 201205 Qiagen, Courtaboeuf, France), and 10 pmol of each forward and reverse primers.

For the amplification of the 16S gene fragment, we used the primers developed by Black and Piesman (1994): forward 5'-CTGCTCAATGATTTTTTAAATTGC-3', reverse 5'-CCGGTCTGAACTCAGATCATGTA-3'. For the COI gene fragment, we used the primers from Simon et al. (1994): forward 5'-GGAGGATTTGGAAATTGATTAGTTCC-3' and reverse 5'-ACTGTAAATATATGATGAGCTCA-3'. For the 18S gene fragment, we used the primers from Black et al. (1997): forward 5'-GCAAGTCTGGTGCCAGCAGCC-3', reverse 5'-CTCCGTCAATTCCTTTAAG-3'.

The PCR conditions included an initial denaturation step at 94°C for 2min, followed by 35 cycles at 94°C for 45s, 50°C for 45s and 72°C for 45s, with a final extension step at 72°C for 10min. Amplification products were sequenced in both directions using Sanger method by Eurofins Genomics (Courtaboeuf, France).

## References:

Black IV, W.C., Klompen, J.S.H., and Keirans, J.E. (1997). Phylogenetic Relationships among Tick Subfamilies (Ixodida: Ixodidae: Argasidae) Based on the 18S Nuclear rDNA Gene. *Molecular Phylogenetics and Evolution* 7, 129–144.

Black IV, W.C., and Piesman, J. (1994). Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. *Proceedings of the National Academy of Sciences, USA* 91, 10034–10038.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., and Flook, P. (1994). Evolution, Weighting, and Phylogenetic Utility of Mitochondrial Gene Sequences and a Compilation of Conserved Polymerase Chain Reaction Primers. *Annals of the Entomological Society of America* 87, 651–701.

**A2:** Accession numbers of the DNA sequences used in this study. Each number corresponds to distinct haplotypes seen in each population.

	<i>COI</i>	<i>16S</i>	<i>18S</i>
<b>Boa1</b>	KX826007	KX825947	KX825974
	KX826008	KX825948	
		KX825949	
		KX825950	
<b>Boa2</b>	KX826003	KX825953	KX825977
	KX826004		
	KX826005		
<b>LAN</b>	KX825994	KX825951	KX825975
	KX825995	KX825952	KX825986
	KX825996	KX825958	KX825987
		KX825970	
<b>VEN</b>	KX825991	KX825957	KX825973
	KX825992		
	KX825993		
<b>RAS</b>	KX825997	KX825954	KX825976
	KX825998	KX825955	
	KX825999	KX825956	
	KX826000		
	KX826001		
	KX826002		

	<i>COI</i>	<i>16S</i>	<i>18S</i>
<b>TRO</b>	KX826006	KX825962	KX825985
		KX825963	
<b>JdN</b>	KX826013	KX825960	KX825979
	KX826014	KX825961	KX825984
<b>AMS</b>	KX826015	KX825965	KX825988
		KX825966	KX825989
		KX825967	KX825990
		KX825968	
		KX825969	
<b>PES</b>	KX826009	KX825964	KX825983
	KX826010		
	KX826011		
	KX826012		
<b>FRI</b>	KX826016	KX825959	KX825978
	KX826017		KX825980
			KX825981
			KX825982
<b>Per</b>		KX825971	
<b>Mex</b>		KX825972	

**Table A1:** Genetic indexes of population diversity for COI and 18S gene fragments. Seq: number of sequences; NS: number of variable sites; K: mean number of nucleotide differences; H: number of haplotypes; Hd  $\pm$  SD: Haplotype diversity and standard deviation;  $\pi$   $\pm$  SD: Nucleotide diversity and standard deviation.

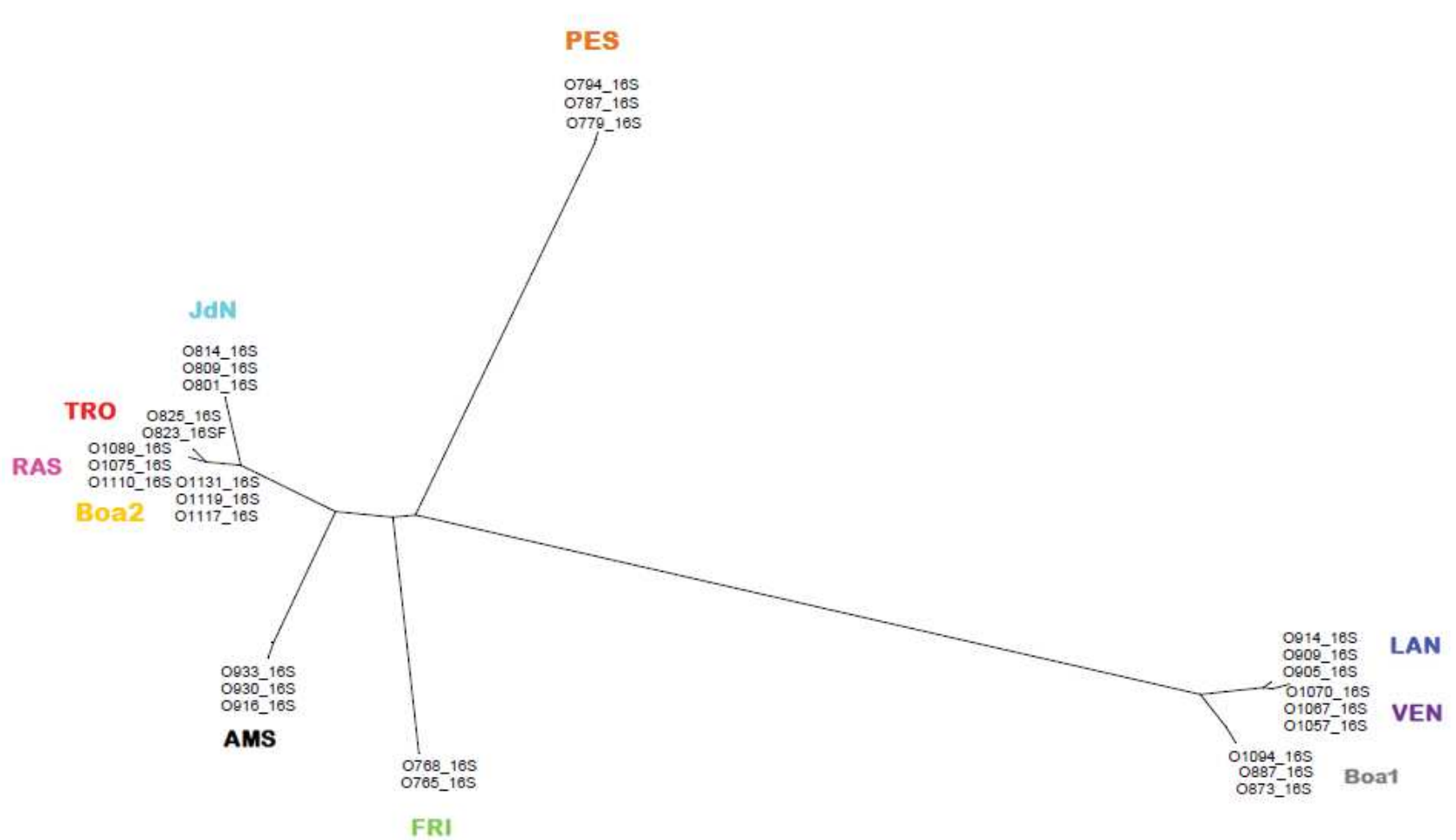
COI – 400 bp						
Population	Seq	NS	K	H	Hd $\pm$ SD	$\pi$ $\pm$ SD
Boavista 1	9	1	0.222	2	0.222 $\pm$ 0.166	0.00056 $\pm$ 0.00042
Boavista 2	8	2	1.071	3	0.750 $\pm$ 0.096	0.00311 $\pm$ 0.00048
Lanzarote	10	2	0.400	3	0.378 $\pm$ 0.181	0.00101 $\pm$ 0.00052
Raso	21	21	2.248	6	0.552 $\pm$ 0.122	0.00565 $\pm$ 0.00316
Veneguera	17	5	0.588	3	0.228 $\pm$ 0.129	0.00148 $\pm$ 0.00103
Frioul	6	2	0.867	3	0.733 $\pm$ 0.155	0.00218 $\pm$ 0.00062
Juan de Nova	10	1	0.200	2	0.200 $\pm$ 0.154	0.00050 $\pm$ 0.00039
Amsterdam	5	0	0.000	1	0 $\pm$ 0	0 $\pm$ 0
Pescadores	14	4	1.033	4	0.648 $\pm$ 0.116	0.00299 $\pm$ 0.00082
Tromelin	6	0	0.000	1	0 $\pm$ 0	0 $\pm$ 0
<b>Total</b>	<b>106</b>	<b>146</b>	<b>51.704</b>	<b>24</b>	<b>0.888 <math>\pm</math> 0.017</b>	<b>0.15030 <math>\pm</math> 0.00532</b>
rDNA 18S – 312 bp						
Population	Seq	NS	K	H	Hd $\pm$ SD	$\pi$ $\pm$ SD
Boavista 1	16	0	0.000	1	0 $\pm$ 0	0 $\pm$ 0
Boavista 2	14	10	1.429	2	0.143 $\pm$ 0.119	0.00468 $\pm$ 0.00390
Lanzarote	10	2	0.822	3	0.511 $\pm$ 0.164	0.00263 $\pm$ 0.00086
Raso	11	0	0.000	1	0 $\pm$ 0	0 $\pm$ 0
Veneguera	22	0	0.000	1	0 $\pm$ 0	0 $\pm$ 0
Frioul	20	11	1.384	4	0.284 $\pm$ 0.128	0.00452 $\pm$ 0.00290
Juan de Nova	9	2	0.444	2	0.222 $\pm$ 0.166	0.00144 $\pm$ 0.00108
Amsterdam	12	2	0.697	3	0.591 $\pm$ 0.108	0.00228 $\pm$ 0.00055
Pescadores	9	0	0.000	1	0 $\pm$ 0	0 $\pm$ 0
Tromelin	2	2	2.000	2	1 $\pm$ 0.5	0.00662 $\pm$ 0.00331
<b>Total</b>	<b>125</b>	<b>33</b>	<b>6.804</b>	<b>13</b>	<b>0.7001 <math>\pm</math> 0.026</b>	<b>0.02246 <math>\pm</math> 0.00111</b>



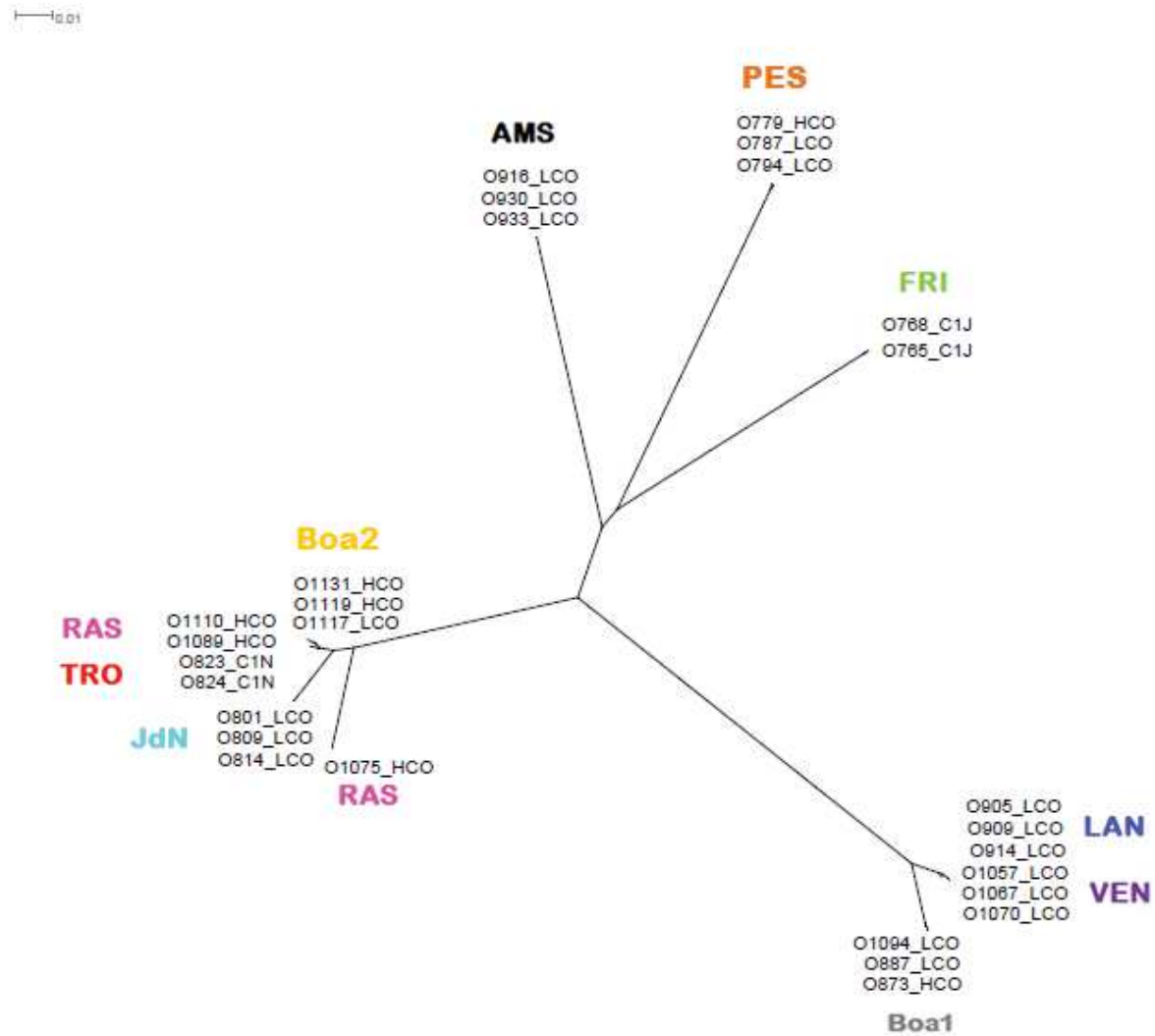
**Table A2:** KST\* indexes for the COI and 18S gene fragments. Significance is indicated as follow: NS = not significant; \*0.01<P<0.05; \*\*0.001<P<0.01; \*\*\*P<0.001

	COI KST*	Boavista 1	Boavista 2	Lanzarote	Raso	Veneguera	Frioul	Juan de Nova	Amsterdam	Pescadores
Cape Verde	Boavista 2	<b>0.84520-</b> ***								
Canary	Lanzarote	<b>0.84710 -</b> ***	<b>0.82358 - ***</b>							
Cape Verde	Raso	<b>0.76706 -</b> ***	<b>0.09014 - **</b>	<b>0.76297 - ***</b>						
Canary	Veneguera	<b>0.36976 -</b> ***	<b>0.83180 - ***</b>	(-0.00754 - NS)	<b>0.80310 - ***</b>					
Mediterranean Sea	Frioul	<b>0.87886 -</b> ***	<b>0.78129 - ***</b>	<b>0.85076 - ***</b>	<b>0.66309- ***</b>	<b>0.83751 -</b> ***				
Indian Ocean	Juan de Nova	<b>0.94017 -</b> ***	<b>0.70214 - ***</b>	<b>0.91596 - ***</b>	<b>0.62142 - ***</b>	<b>0.90223 -</b> ***	<b>0.87763 - ***</b>			
Indian Ocean	Amsterdam	<b>0.95378 -</b> ***	<b>0.81681 - **</b>	<b>0.91416 - ***</b>	<b>0.67154 - ***</b>	<b>0.87913 -</b> ***	<b>0.86800 - **</b>	<b>0.95249 - **</b>		
Peru	Pescadores	<b>0.82330 -</b> ***	<b>0.74313 - ***</b>	<b>0.81254 - ***</b>	<b>0.73578- ***</b>	<b>0.83738-</b> ***	<b>0.74885- ***</b>	<b>0.82518 -</b> ***	<b>0.77029 -</b> ***	
Indian Ocean	Tromelin	<b>0.95818 -</b> ***	<b>0.23322 - ***</b>	<b>0.92168 - ***</b>	(-0.03912 - NS)	<b>0.89025 -</b> ***	<b>0.88734 - ***</b>	<b>0.92350 -</b> ***	<b>1 - **</b>	<b>0.79485 -</b> ***
	KST* Tot COI		<b>0.88398</b>	***						

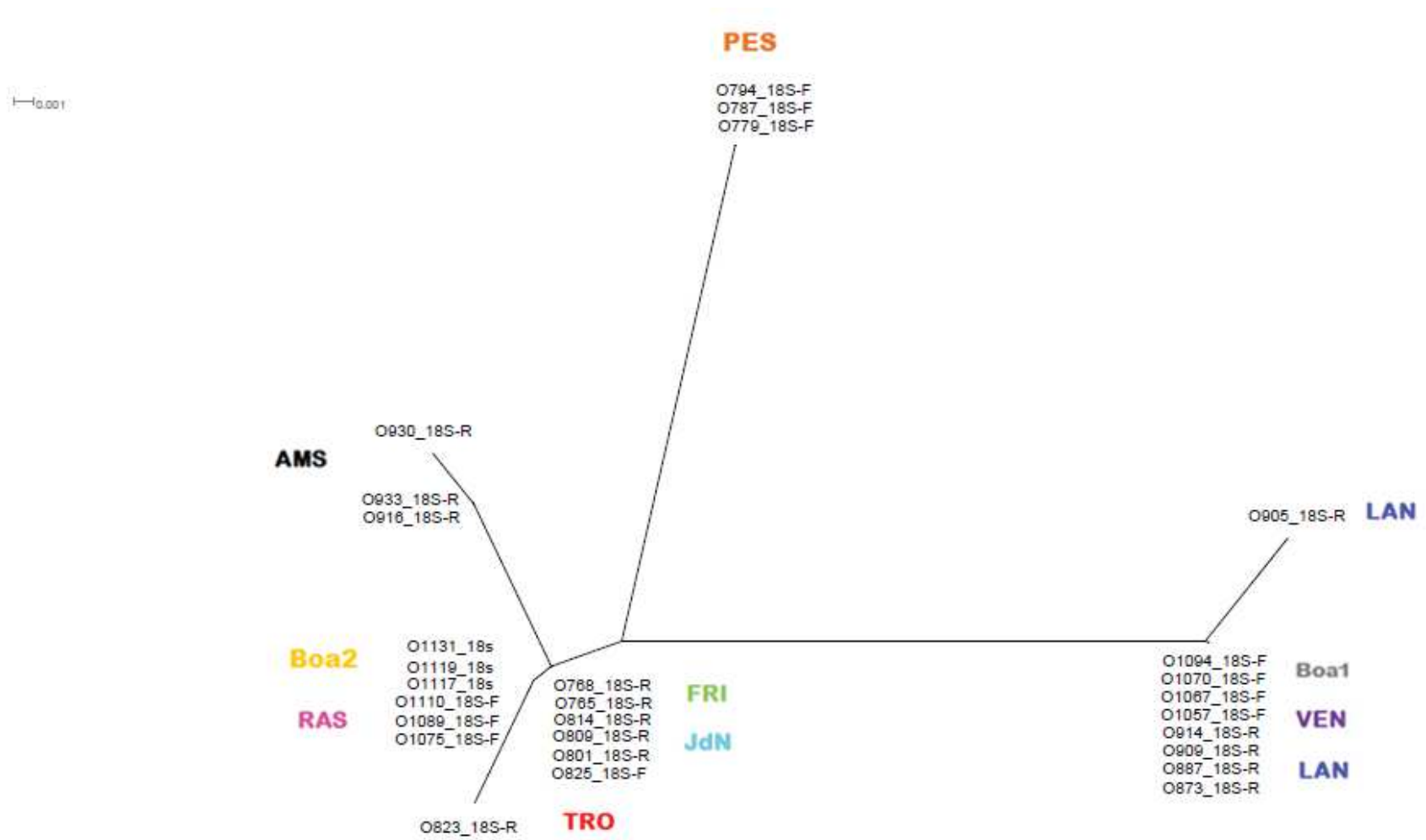
	<b>18S KST*</b>	Boavista 1	Boavista 2	Lanzarote	Raso	Veneguera	Frioul	Juan de Nova	Amsterdam	Pescadores
		<b>0.87022 -</b> ***								
Cape Verde	Boavista 2									
Canary	Lanzarote	<b>0.16338 - *</b>	<b>0.70563 - ***</b> (- 0.02041 - NS)	<b>0.83630 - ***</b>						
Cape Verde	Raso	<b>1 - ***</b>								
Canary	Veneguera	-	<b>0.88712 - ***</b>	<b>0.20098 - **</b>	<b>1 - ***</b>					
Mediterranean Sea	Frioul	<b>0.82183 -</b> ***	(-0.01062 - NS)	<b>0.67492 - ***</b>	(-0.02216 - NS)	<b>0.84688 -</b> ***				
Indian Ocean	Juan de Nova	<b>0.93157 -</b> ***	(-0.01976 - NS)	<b>0.74844 - ***</b>	(0.02778 - NS)	<b>0.93960 -</b> ***	(-0.02119 - NS)			
Indian Ocean	Amsterdam	<b>0.86875 -</b> ***	<b>0.60894 - ***</b>	<b>0.71304 - ***</b>	<b>0.73467 - ***</b>	<b>0.88494 -</b> ***	<b>0.55466 - ***</b>	<b>0.62344 -</b> ***		
Peru	Pescadores	<b>1 - ***</b>	<b>0.83213 - ***</b>	<b>0.84303 - ***</b>	<b>1 - **</b>	<b>1 - ***</b>	<b>0.74909 - ***</b>	<b>0.90517 -</b> ***	<b>0.81519 -</b> ***	
Indian Ocean	Tromelin	<b>1 - *</b>	(0.20206 - NS)	<b>0.56640 - **</b>	(1 - NS)	<b>1 - **</b>	(0.10675 - NS)	(0.37209 - NS)	<b>0.42243 - **</b>	<b>1 - *</b>
	<b>KST* Tot</b>									
	<b>18S</b>		<b>0.86822</b>							



**Fig.A1.1:** Cluster analysis based on sequences of 16S mitochondrial marker. Labels under population abbreviations represent sequence numbers.



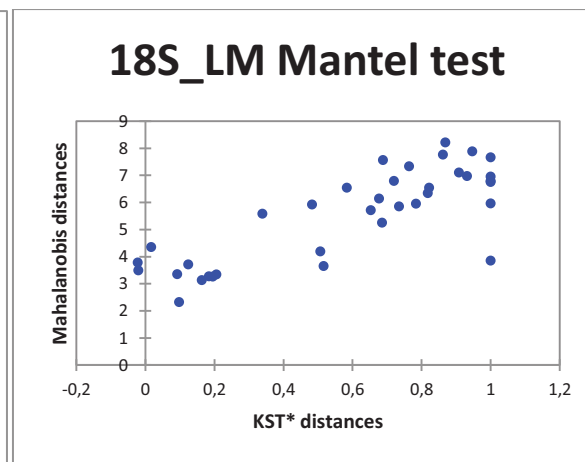
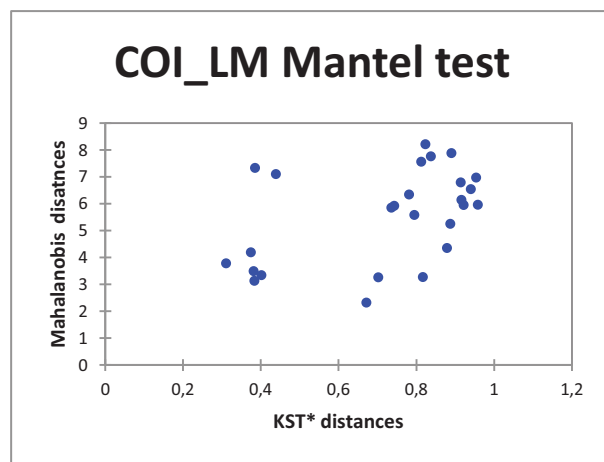
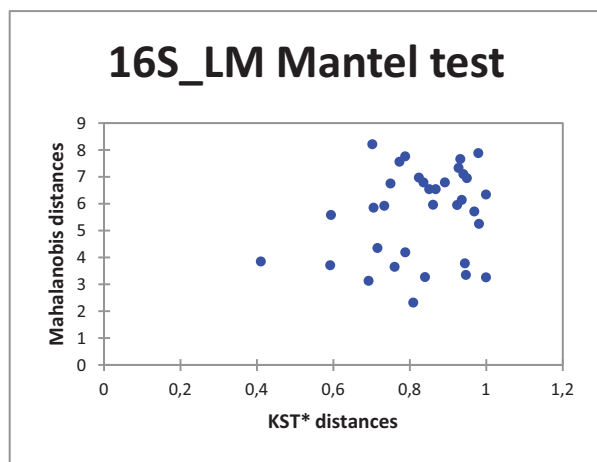
**Fig.A1.2:** Cluster analysis based on sequences of COI mitochondrial marker. Labels under population abbreviations represent sequence numbers.



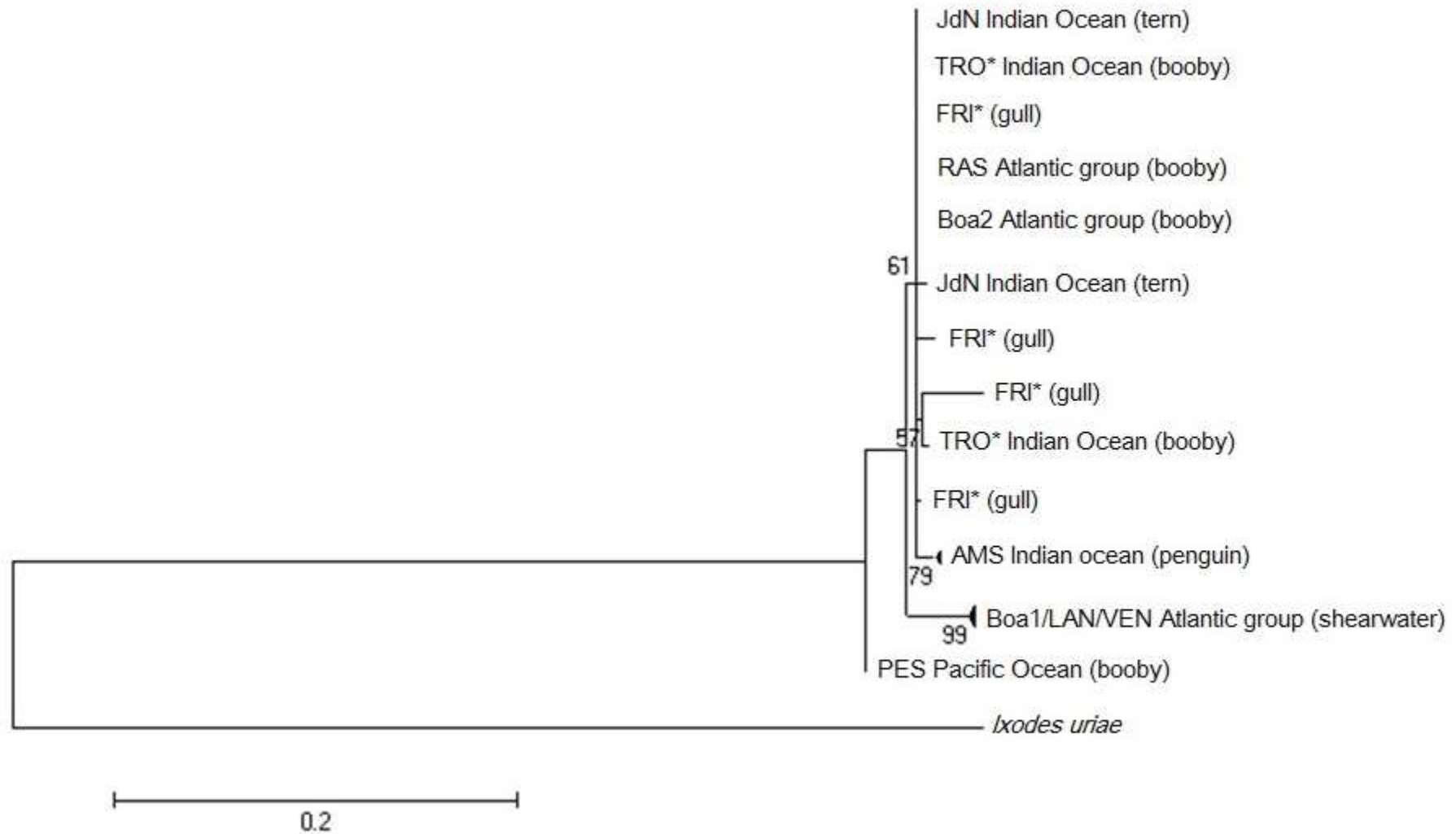
**Fig.A1.3:** Cluster analysis based on sequences of 18S nuclear marker.

**Table A3:** Mantel test results between morphometric (Mahalanobis distances) and genetic (KST\* distances) matrices. The corresponding graphs for each analysis for the landmark (LM) distances are shown below.

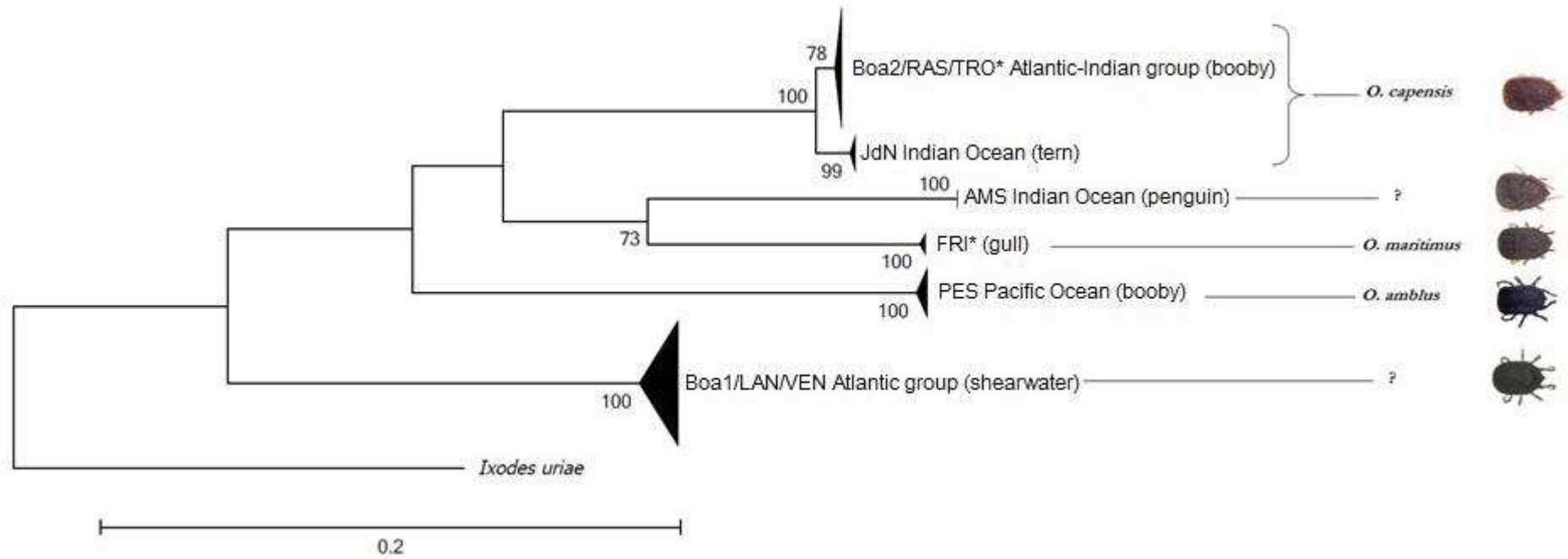
	<b>16S</b>			<b>COI</b>			<b>18S</b>		
	r-value	r <sup>2</sup> (%)	p-value	r-value	r <sup>2</sup> (%)	p-value	r-value	r <sup>2</sup> (%)	p-value
<b>Landmarks</b>	0.378	14.3	0.025	0.532	28.3	0.001	0.810	65.6	<0.0001
<b>Outline</b>	0.316	10.0	0.059	0.449	20.2	0.005	0.603	36.4	0.0003





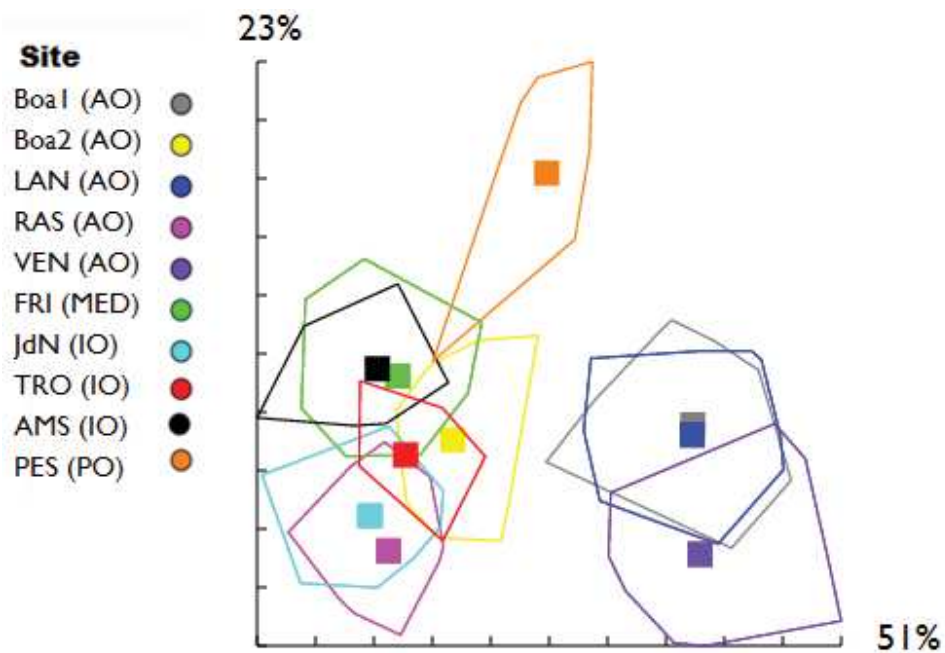


**Fig.A2.1:** Phylogenetic tree based on sequences of 18S nuclear marker. *Ixodes uriae* represents the outgroup. Population names with star have been verified based on classical morphological traits of larval ticks.

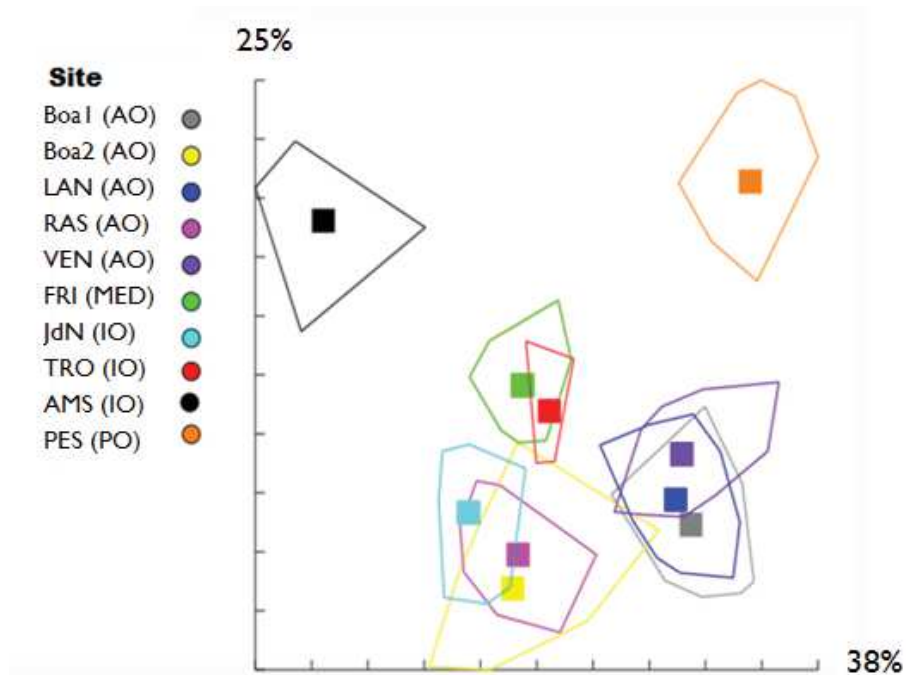


**Fig.A2.2:** Phylogenetic tree based on sequences of COI mitochondrial marker. *Ixodes uriae* represents the outgroup. Population names with star have been verified based on classical morphological traits of larval ticks.

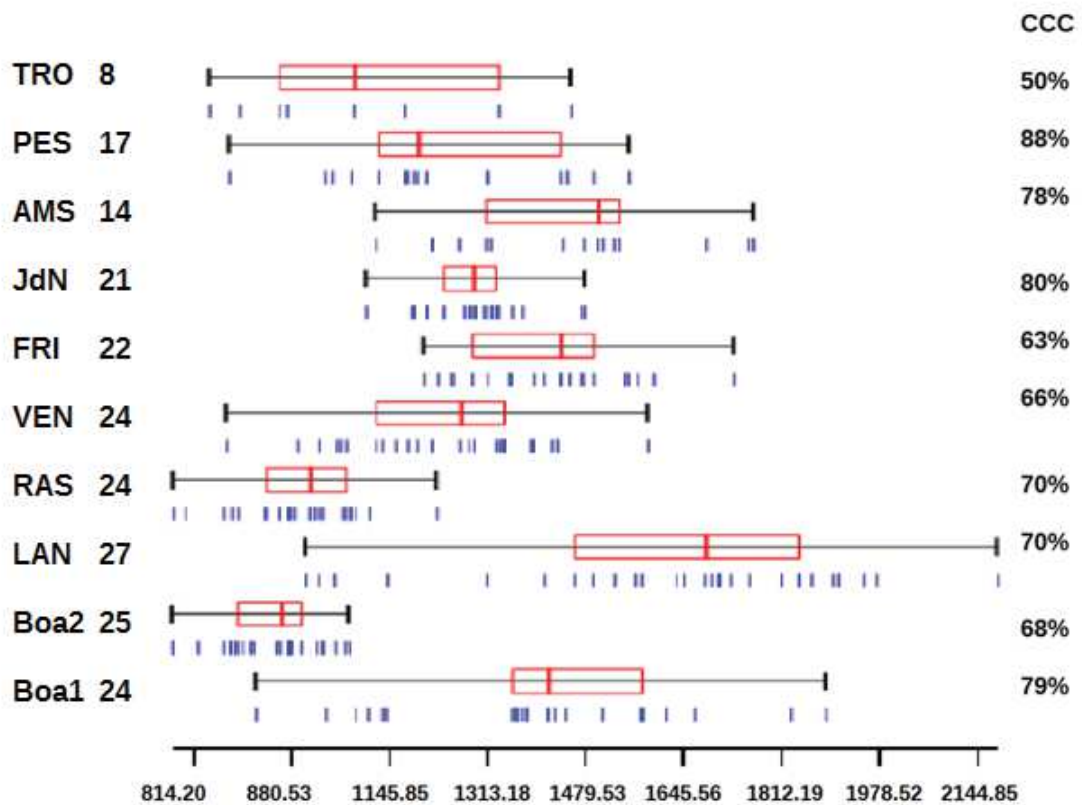
a



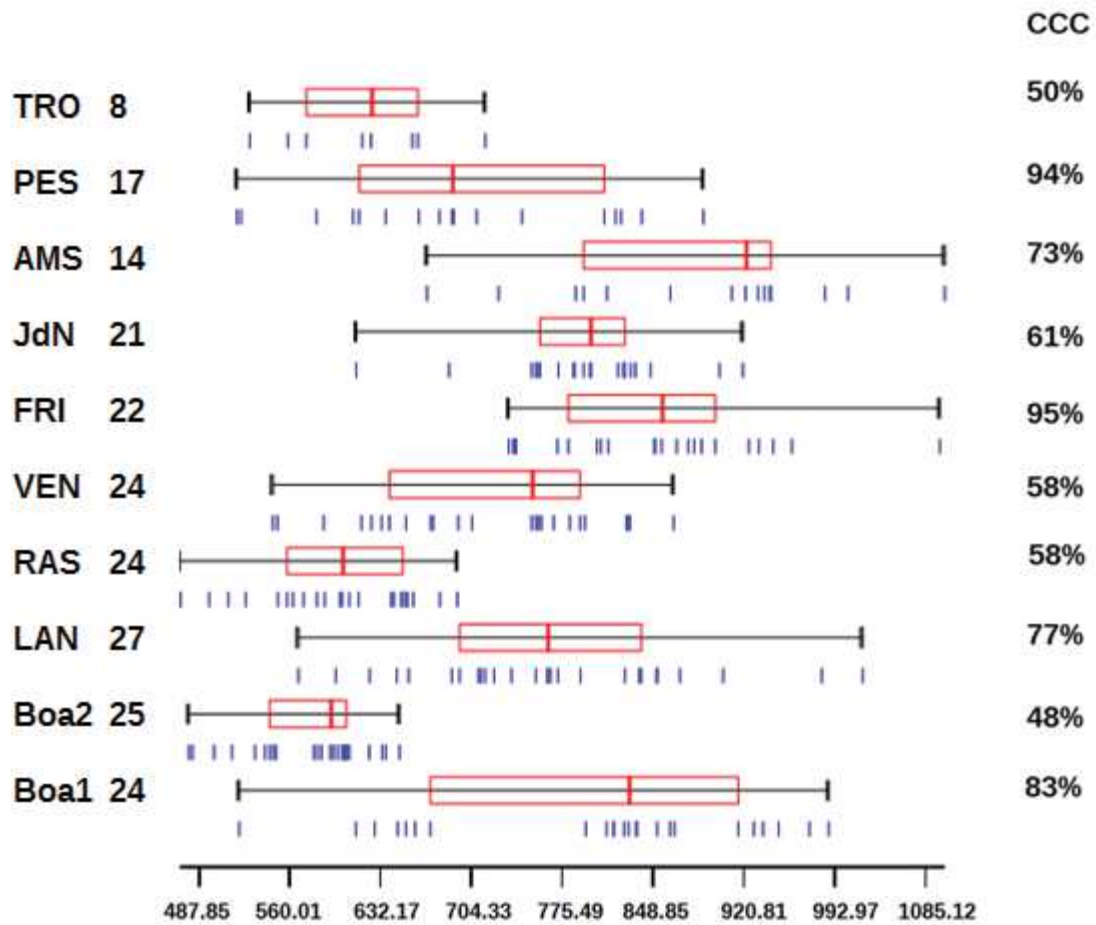
b



**Fig.A3:** First two functions of a discriminant analysis performed on **(a)**: a set of first RW based on the landmark arrangement and **(b)**: on normalized Fourier coefficients based on the outline analysis and showing the proportion of total variance explained. The polygons represent the maximal parameter space of each population and the squares the population mean. The site locations are: AO: Atlantic Ocean. MED: Mediterranean Sea. IO: Indian Ocean. PO: Pacific Ocean.



**Fig.A4:** Size variation (in pixels) presented as quantile plots for tick populations based on landmark arrangements. The box shows the median as a line across the middle and the quartiles (25<sup>th</sup> and 75<sup>th</sup> percentiles) as its ends. Extremities represent the minimal and maximal values. The right column represents the validated percent classification (CCC) result based on shape, used to test the affiliation of each individual tick to each population. The population abbreviations (Table 1) and the sample size for each population are indicated on the left.

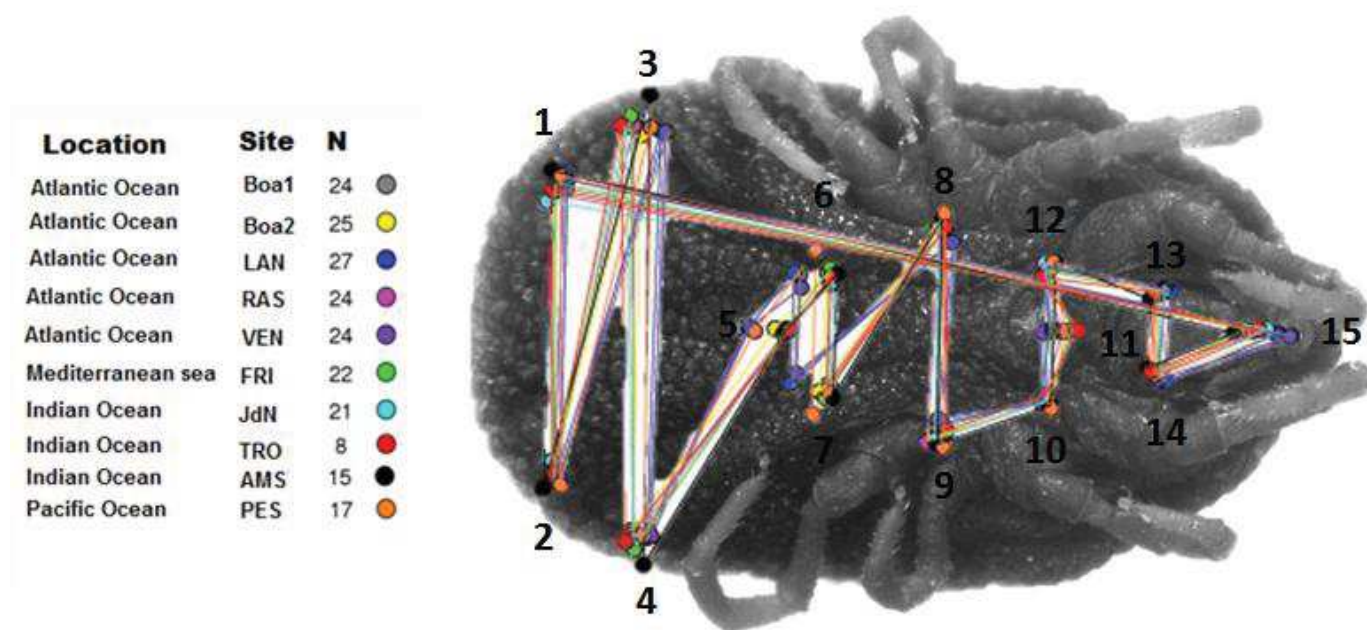


**Fig.A5:** Size variation (in pixels) presented as quantile plots for tick populations based on the outlines analysis. The box shows the median as a line across the middle and the quartiles (25<sup>th</sup> and 75<sup>th</sup> percentiles) as its ends. Extremities represent the minimal and maximal values. The right column represents the validated percent classification (CCC) result based on shape, used to test the affiliation of each individual tick to each population. The population abbreviations (Table 1) and the sample size for each population are indicated on the left.

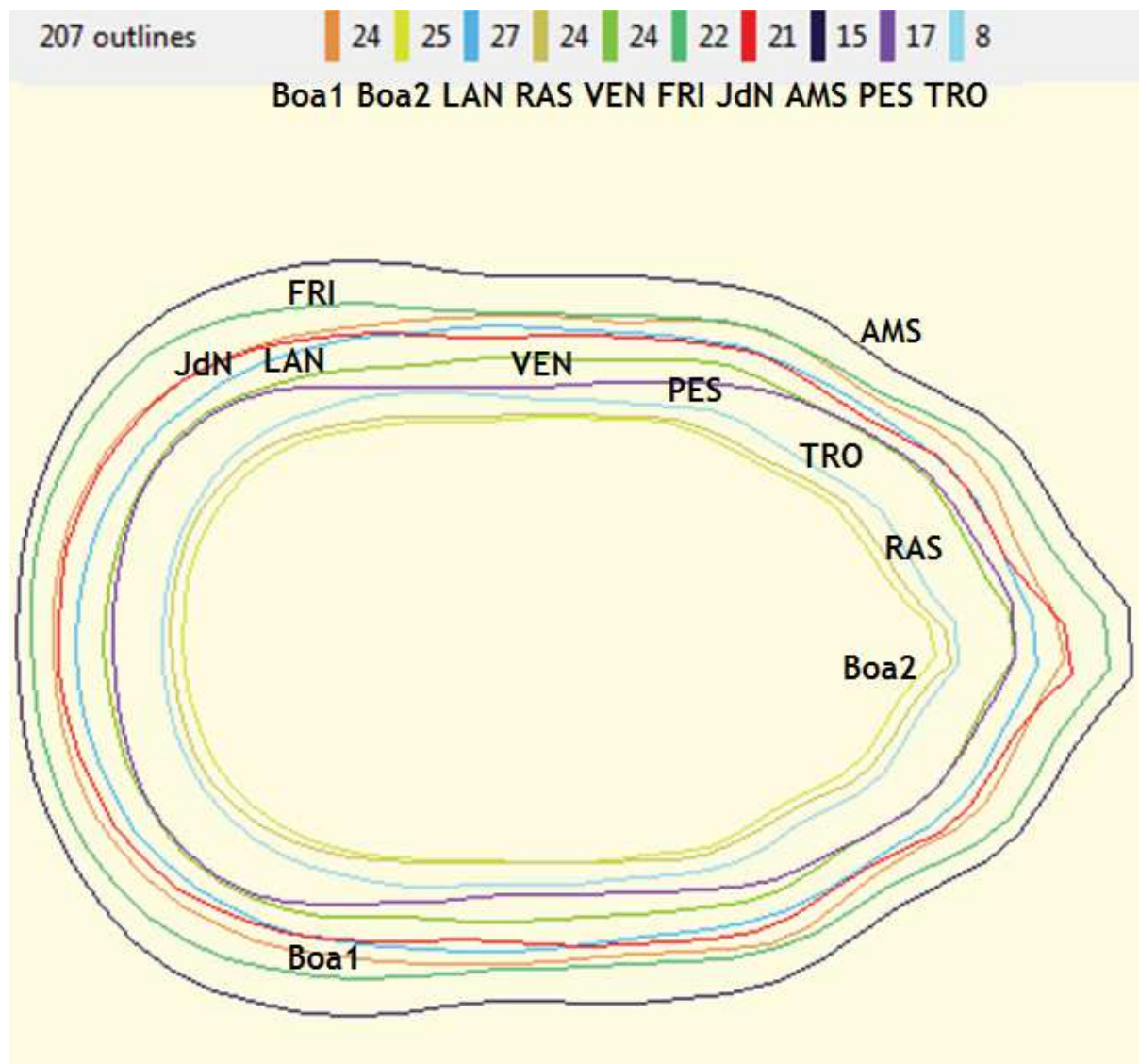
**Table A4:** P-value tables of the pairwise mean comparisons among populations after 1000 permutations, for the landmarks and outline analyses (starting value for the Bonferroni test: 0.00111111111111111111). Significant values are in bold.

Landmarks	Boa1	Boa2	LAN	RAS	VEN	FRI	JdN	AMS	PES
Boa2	<b>0.0000</b>								
LAN	0.0070	<b>0.0000</b>							
RAS	<b>0.0000</b>	0.1220	<b>0.0000</b>						
VEN	0.0040	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>					
FRI	0.8650	<b>0.0000</b>	0.0070	<b>0.0000</b>	<b>0.0000</b>				
JdN	0.0550	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	0.1490	<b>0.0000</b>			
AMS	0.4040	<b>0.0000</b>	0.0930	<b>0.0000</b>	<b>0.0000</b>	0.3820	0.0020		
PES	0.0290	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	0.8100	<b>0.0010</b>	0.3250	0.0050	
TRO	0.0040	0.0080	<b>0.0000</b>	0.0840	0.0690	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	0.0700
Outlines	Boa1	Boa2	LAN	RAS	VEN	FRI	JdN	AMS	PES
Boa2	<b>0.0000</b>								
LAN	0.1620	<b>0.0000</b>							
RAS	<b>0.0000</b>	0.3170	<b>0.0000</b>						
VEN	0.0090	<b>0.0000</b>	0.0590	<b>0.0000</b>					
FRI	0.3480	<b>0.0000</b>	0.0150	<b>0.0000</b>	<b>0.0000</b>				
JdN	0.2450	<b>0.0000</b>	0.7100	<b>0.0000</b>	0.0140	0.0030			
AMS	0.0400	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	0.0870	<b>0.0000</b>		
PES	0.0090	<b>0.0000</b>	0.0320	<b>0.0000</b>	0.5020	<b>0.0000</b>	0.0050	<b>0.0000</b>	
TRO	<b>0.0000</b>	0.0240	<b>0.0010</b>	0.2100	0.0110	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>	0.0780





**Fig.A6:** Mean landmark arrangements for each population as shown on a female tick from the JdN population. Each point represents one landmark. Landmark N°1-2: width between landmarks 1 and 2 on extremities of the transverse post-anal groove; N°3-4: width between landmarks 3 and 4 each located on extremities of the pre-anal groove; N°5: anus; N°8-9: width between landmarks 8 and 9 at coxae III/IV; N°10-12: width between landmarks 10 and 12 at coxae I/II; N°11: genital pore; N°13-14: width between landmarks 13 and 14 on the lower extremity of the basis capitulum; N°15: capitulum extremity.



**Fig.A7:** Mean contours for each population. Each colored bar represents one population: Orange: Boavista 1; Yellow: Boavista 2; Blue: Lanzarote; Light brown: Raso; Light green: Veneguera; Green: Frioul; Red: Juan de Nova; Black: Amsterdam; Purple: Pescadores; Light blue: Tromelin. Numbers beside colored bars indicate the number of ticks used for each population.



Article 4

Dupraz M, Toty C, Boulinier T, Dujardin J-P, McCoy K-D.  
Morphological convergence in *I. uriae* host races. En préparation.



Morphological convergence in *Ixodes uriae* host races.

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## Abstract

Parasite diversification occurs within a particular ecological context resulting from the dynamic equilibrium between extinction, colonization and divergence in different microhabitats. For ectoparasites, the host often represents the greatest part of the parasite's needs, i.e. alimentation, habitat and reproduction, and host-associated traits may exert strong selective pressures on the parasite to specialize. Among the parasite traits under pressure, morphology can be of primary importance because physical defenses used by the host can be very efficient at eliminating parasites, particularly ectoparasites. Previous studies in the widely distributed seabird tick, *Ixodes uriae*, demonstrated the occurrence of genetically distinct host races within multi-specific colonies and showed that a given host race could evolve independently in different isolated areas of the tick distribution. Here, we use geometric morphometrics to test whether host-associated selection pressure has also resulted in phenotypic congruence in two different tick host races associated with Atlantic puffins (*Fratercula arctica*) and common guillemots (*Uria aalge*). Using both landmark and outline analyses, we assessed the degree of morphological congruence among ticks using the same host species but in three distinct colony locations. We found that adult females showed some degree of congruence when exploiting puffins, but that female ticks on guillemots tended to be morphologically distinct in each colony location. These patterns were much less apparent in male ticks, which may not be surprising as this life stage does not feed on the host. These results suggest that some phenotypic divergence in relation to host use does occur, but that either selection is not strong enough to produce the same distinct traits associated with each host type and/or the populations that we have analyzed are not yet at equilibrium. We discuss the potential host factors that may drive the observed patterns and the future directions required to evaluate our alternative hypotheses.

## 1 Introduction

Parasite diversification occurs within a particular ecological context resulting from a dynamic equilibrium between extinction, colonization and divergence in the different microhabitats occupied by the parasite (Poulin, 2006). For parasites, the host represents the greatest part of its biological needs, i.e. food, habitat and site of reproduction. Because of this, we generally consider that high predictability in parasite-host encounters should favor the evolution of specialist parasites, parasites that avoid the cost of investing or maintaining multiple host-specific adaptations, for example to different immunological systems (Combes, 1995). For ectoparasites, like ticks, contact with the host can be more temporary, largely restricted to feeding (De Meeûs et al., 1998). However, although these contacts are short, the molecular dialogue can be intense at the host interface and may lead to the emergence of host specialization (Magalhães et al., 2007; Mans and Neitz, 2004; McCoy et al., 2013). For those parasites that spend a good part of their life cycle off-host, environmental constraints such as temperature and humidity can also greatly impact their fitness and may select for specific parasite traits related to parasite physiology, morphology or behavior (Clayton et al., 2010; Kuclu et al., 2011; Prudhomme et al., 2012). Depending on the interplay between selection in the on-host and off-host environments and its effects on assortative mating, the probability of parasite divergence among host types will either increase or decrease.

The involvement of the different host and environmental factors in population divergence are often complicated to distinguish, particularly when trait changes are not highly visible. Morphometrical analyses is one method that can be used to differentiate cryptic population divergence as it can detect and quantify subtle population-level variation and help identify factors implicated in the divergence process (Barluenga et al., 2006; Dujardin et al., 2014; Klimov et al., 2006).

*Ixodes uriae* is a hard tick (family Ixodidae) widely distributed across the circumpolar regions of both hemispheres and parasitizing a great diversity of seabird species breeding in dense and predictable colonies (Dietrich et al., 2011). This species is nidicolous, spending the major part of its life cycle off-host, in the nest or under rocks or crevices and interacting with the seabird host for a brief period of 5-10 days each year during the seabird breeding period (Sonenshine and Roe, 2014). Based on their large host spectra and wide distribution, this tick interacts within a diverse array of ecological conditions. Indeed, parasitized hosts have different life history traits and behaviors that can impose different selection pressures on infesting ticks. These hosts may also use different microhabitats within colonies, again imposing different abiotic conditions for tick survival and persistence. Previous studies in this model system demonstrated the existence of genetically distinct host races occurring within multi-host

colonies (Dietrich et al., 2012; Kempf et al., 2009; McCoy et al., 2005; McCoy et al., 2001) and that these races evolve recurrently, even for the same host species (Dietrich et al., 2014a; McCoy et al., 2013). Adaptive specialization of these races for different host species was tested by a field-cross-infestation experiment and revealed performance differences on alternative hosts, indicating that the genetic divergence observed was at least partially linked to host specialization (Dietrich et al., 2014b). An initial morphometric study of these tick races further revealed variation in tick size and shape in relation to host use (Dietrich et al., 2013). However, this study was limited to different tick life stages in a single multi-host colony. It is unknown as yet whether the specific morphological differences observed among the sympatric races reflect direct selection pressure from the host. If this is the case, we would expect the same morphological traits to occur for each race, even when host race formation has occurred independently.

Here, we test this hypothesis using two tick races of *Ixodes uriae* sampled from three multi-host colonies where sympatric host races were previously demonstrated to occur. We use two geometric morphometric techniques, landmarks and outlines, to determine whether morphometrical convergence has occurred in different locations in relation to host use. As understanding the pattern of morphological changes in specialized parasites can help us to identify the factors underlying the diversification process, we try to relate observed differences with selection factors coming from the host and the abiotic environment.

## 2 Material and methods

### 2.1 Tick sampling and predictions

During the 2016 breeding season, non engorged adult *Ixodes uriae* ticks were collected on seabirds or from the host nesting area in three multi-host seabird colonies on island in the North Atlantic and preserved in 70% ethanol: Hornoya in northern Norway (HOR), Grimsey (GRI) in northern Iceland and Gull Island (GUL) in Newfoundland, Canada. On each island, ticks were sampled from three sympatric seabird species: the black-legged kittiwake (KT) *Rissa tridactyla*, the common guillemot (CG) *Uria aalge* and the Atlantic puffin (PF) *Fratercula arctica* (Table 1).

**Table 1:** Sampled sites of *Ixodes uriae* in North Atlantic seabird colonies.

Origin	Lat. Long.	Pop	Host species	N° ticks
Iceland - Grimsey	66.549744, -17.992711	GRI CG	<i>Uria aalge</i>	10F – 10M
Iceland - Grimsey	66.549744, -17.992711	GRI PF	<i>Fratercula arctica</i>	10F – 10M
Canada - Gull	47.952609, -53.040384	GUL CG	<i>Uria aalge</i>	10F – 10M
Canada - Gull	47.952609, -53.040384	GUL PF	<i>Fratercula arctica</i>	4F – 10M
Norway - Hornøya	70.389348, 31.154289	HOR CG	<i>Uria aalge</i>	10F - 10M
Norway – Hornøya	70.389348, 31.154289	HOR PF	<i>Fratercula arctica</i>	10F - 10M

Previous studies suggested that the evolution of host race formation in the western North Atlantic was largely independent of this process in the eastern North Atlantic, with some exchange occurring between certain colonies in Iceland and Norway (McCoy et al., 2013). This is particularly the case for the PF tick race which tends to show more spatially extensive gene flow than the other tick races (McCoy et al., 2003). In 2016, KT populations were in failure or not synchronous with our passage in the colonies, so we were not able to collect enough ticks to include in our analyses. Consequently, we used only CG and PF populations to test for large-scale convergence in morphological traits in relation to host use. Based on previous genetic analyses and the initial morphometric study carried out on HOR (Dietrich et al., 2013), we expected that PF ticks should be larger overall and relatively wider than CG ticks in all three colony locations. Some direct gene flow may occur between the colonies of HOR and GRI which could account for similar phenotypic patterns (McCoy et al., 2013). However, this should not be the case for GUL where host race evolution has been independent. The occurrence of similar between-race patterns in this colony would provide evidence for morphological convergence related to host selection pressures. As male adult ticks do not feed on the host, we also expected that patterns of host-associated divergence would be stronger in female ticks than in male ticks.

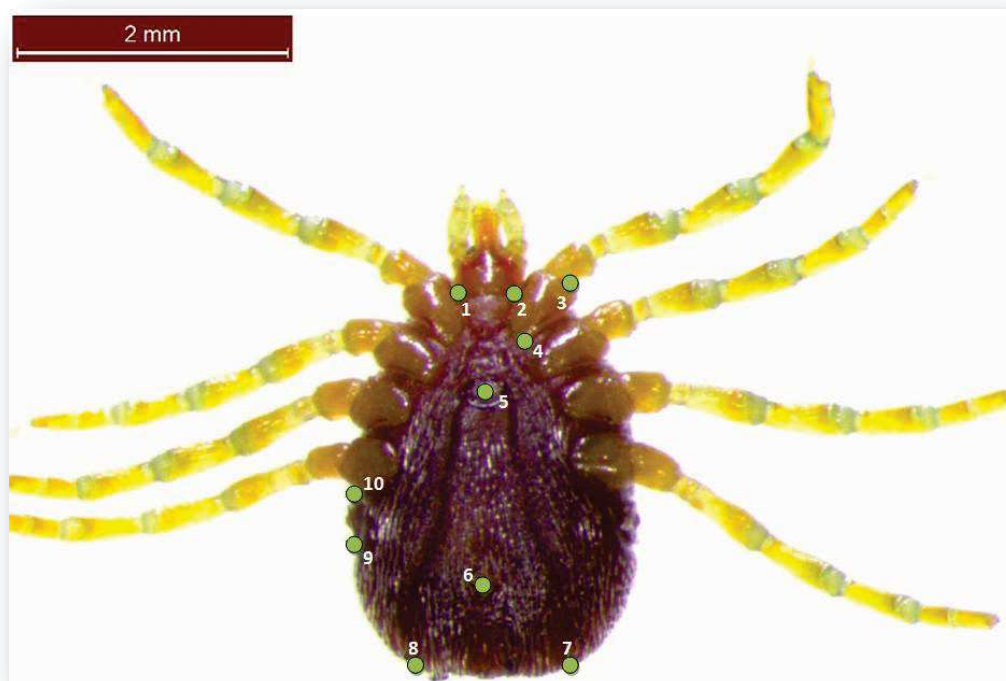
## 2.2 Morphometric analyses

Standardized images of 10 females and 10 males were taken using a binocular microscope (Leica M80) except for GUL PF female population containing only 4 ticks. Morphometrical analyses were performed using the CLIC package (Dujardin et al., 2010; Dujardin and Slice, 2006), freely available at <http://mome-clic.com/>. Landmark analyses were used to acquire measures of morphological features involved in biological activities (feeding, reproduction, locomotion) and outline analyses to obtain an overall measure of shape and size of each tick population. Females and males were treated separately.

*Landmark-based analyses:* We used a set of 10 landmarks for females and 12 for males on the ventral face (Fig. 1). Shape variables (uniform and non-uniform components) were obtained by computing a

generalized Procrustes analysis (GPA) with a superimposition algorithm using the landmark arrangement and removing the size factor. The connected residual coordinates of the landmarks were converted into “partial warps” (PW) to be used by standard multivariate analyses (principal component and discriminant analyses) allowing visual comparisons of mean shape between tick populations. The principal components of the PW (i.e., the relative warps, or RW) were computed to show the morphospace, represented by polygons, for each population separately. In each morphospace, the horizontal axis is the first relative warp (RW1), the vertical axis is the second one (RW2). Overlapping polygons share individuals for which outlines or landmark configurations are similar, while non-overlapping polygons are constituted by individuals sharing a particular conformation, typical of the population.

*Outline-based analysis:* The elliptic Fourier analysis (EFA) method (Kuhl and Giardina, 1982) was used to compute the contour of the dorsal face of each tick. The outline was decomposed into Fourier coefficients which were normalized (NEF) in order to be compared without the influence of size, orientation or starting point. A variance-covariance matrix was then constructed with these coefficients to summarize variation and principal components of NEF were used as input for multivariate analyses.





**Fig.1** : Images representing the 10 and 12 landmarks used on the ventral face of female and male ticks. Each point represents one landmark. Landmarks N°1-2: width between landmarks 1 and 2 on the lower extremity of the basis capitulum; N°3-4: width between landmarks 10 and 12 at coxae I; N°5: genital pore; N°6: anus; For female: N°7-8: width between landmarks 7 and 8 extremities of the genual groove; N°9-10: width between landmarks 9 and 10 on the spiracular plate; For male: N°7-8, 8-9, 9-10: width between landmarks of the festoons; N°11-12: width between landmarks 11 and 12 on the spiracular plate.

We computed the centroid size (CS) for each landmark configuration in size analyses, defined as the square root of the sum of the squared distances between the center of the landmark configuration and each individual landmark (Bookstein, 1997). We used the perimeter (P) as an estimate of body size in outlines analyses. Using the VAR module in CLIC software, we analyzed size means and variances among populations using permutation tests. At each permutation cycle, individuals were randomly exchanged among populations, and the mean and variance was recalculated (1000 permutations in total). Then, we used a Bonferroni correction to determine the significance of size differences between populations.

A random validated cross-check classification method (CCC), based on the jackknife method, was used to test the affiliations of each individual to each population.

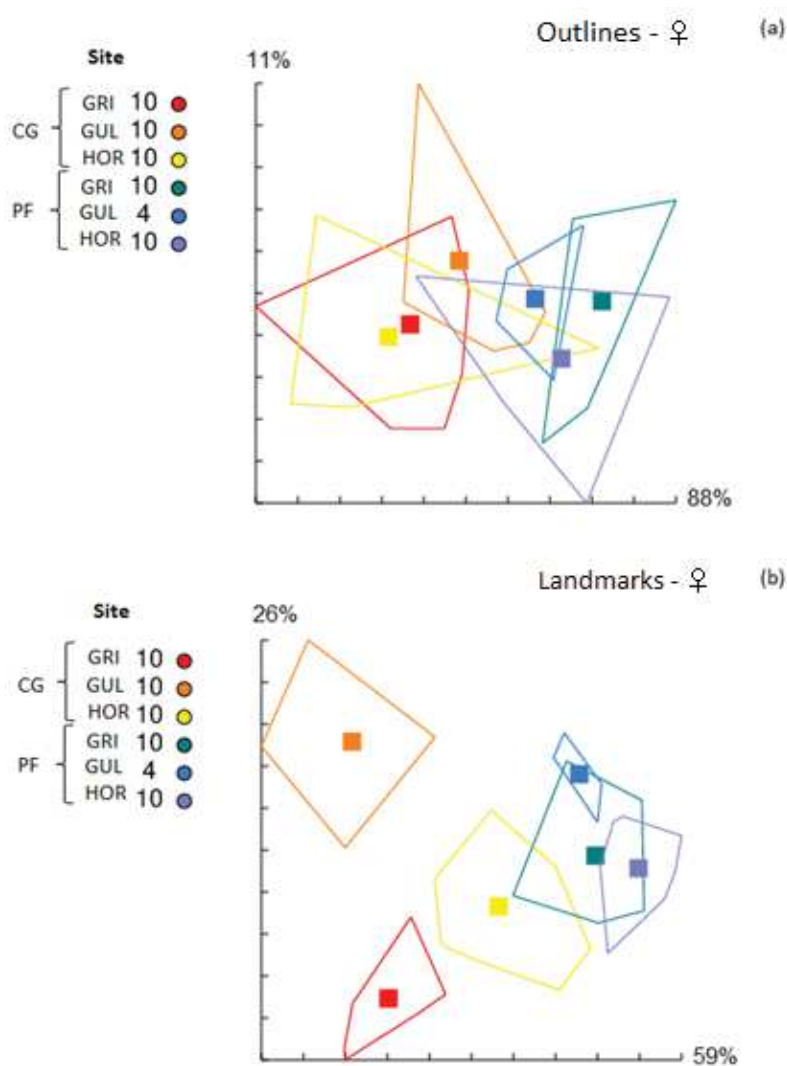
The size effect (CS or P) on body shape (RW or principal component of NEF), was estimated by a linear regression using the PAD module in CLIC software.



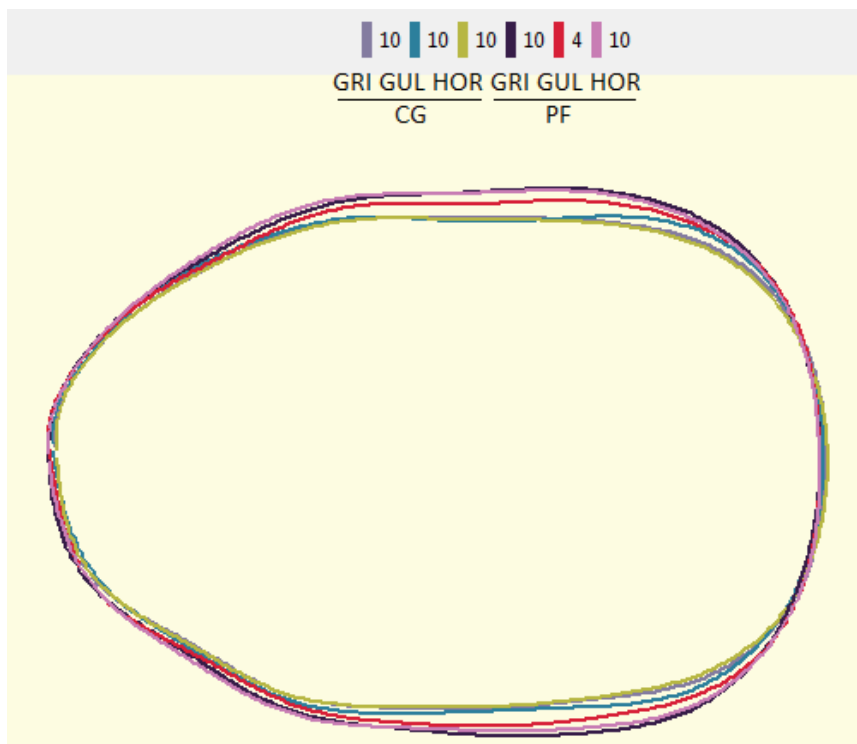
### 3 Results

#### Females

Outline analyses revealed that all tick populations were overlapping, sharing an overall body shape, with a slight tendency for CG and PF populations to be grouped together (Fig.2a). PF ticks showed significant size differences (Table 2) and tended to be larger than CG ticks (Fig.3). The size effect on shape represented 16.9% of the overall shape variation (DF1 regression:  $r=0.288$ ;  $r^2=8.3\%$ ;  $df=52$ ;  $t=2.17$ ,  $p=0.01737$ ; DF2 regression:  $r=-0.293$ ;  $r^2=8.6\%$ ;  $df=52$ ;  $t=2.21$ ,  $p=0.0007$ ). The cross-check classification showed that populations were not well-defined based on size alone, ranging from 10% correctly-assigned individuals for GRI CG to 60% for GRI PF (Fig.4a).



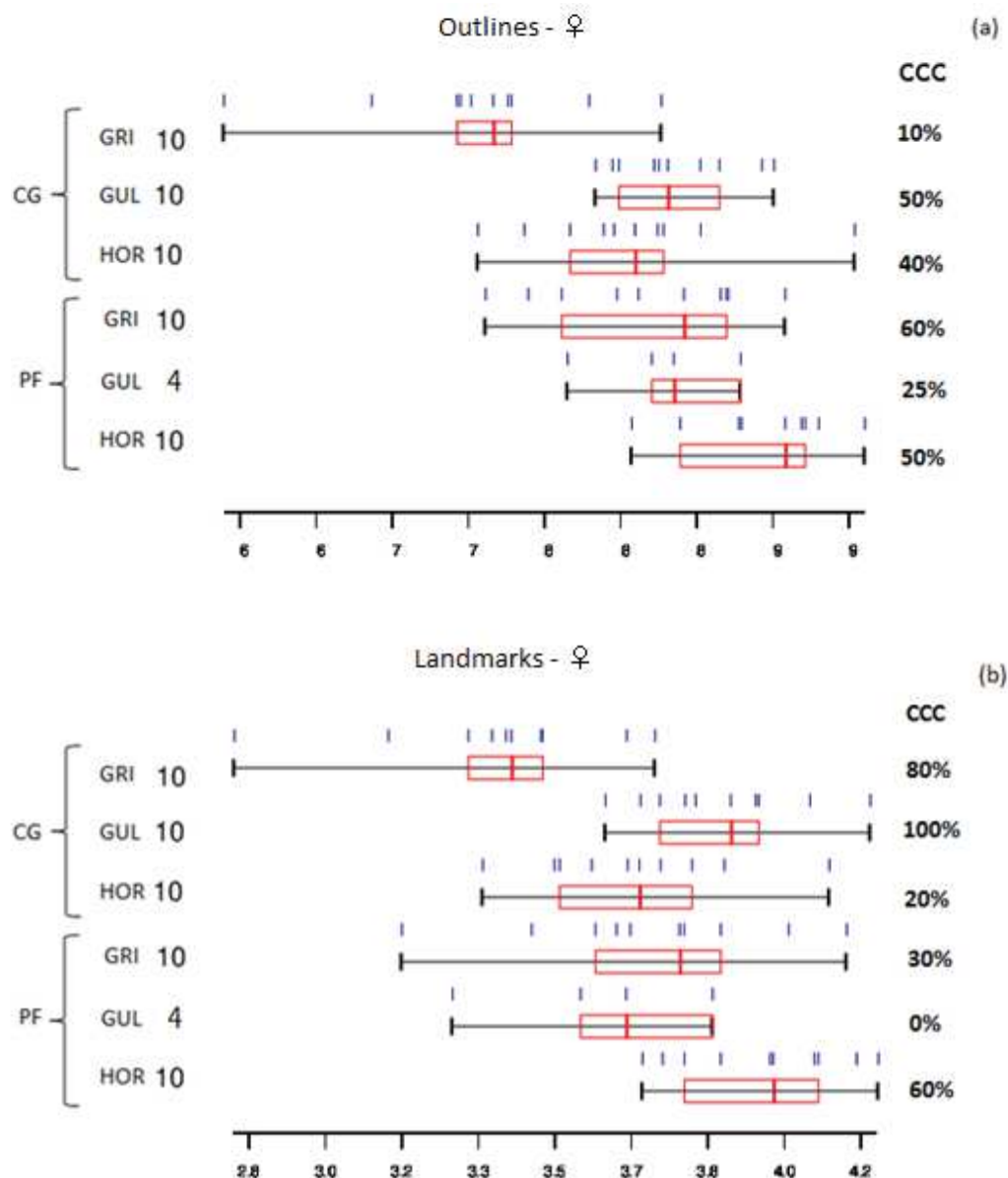
**Fig.2:** First two functions of a discriminant analysis performed on **(a)**: normalized Fourier coefficients based on the outline analysis and **(b)**: a set of first RW based on the landmark arrangement of females and showing the proportion of total variance explained. The polygons represent the maximal parameter space of each population and the squares the population mean. The site locations are: GRI: Grimsey; GUL: Gull; HOR: Hornoeya.



**Fig.3:** Mean contours for each female tick population. Each colored bar represents one population: grey: GRI CG; cyan: GUL CG; olive green: HOR CG; black: GRI PF; red: GUL PF; pink: HOR PF. Numbers beside colored bars indicate the number of ticks used for each population.

**Table 2:** Results of permutation tests of size differences in female tick outline contours (upper matrix) and landmark (lower matrix) configurations. The threshold value for significance using a Bonferroni correction is 0.0033. Significant values are in red. Blue cells indicate comparisons between sympatrically occurring host races.

	GRI CG	GUL CG	HOR CG	GRI PF	GUL PF
GUL CG	0.0000				
HOR CG	0.0040	0.1850			
GRI PF	0.0010	0.3360	0.7180		
GUL PF	0.0060	0.6180	0.5880	0.7850	
HOR PF	0.0000	0.0430	0.0060	0.0190	0.0760
	GRI CG	GUL CG	HOR CG	GRI PF	GUL PF
GUL CG	0.0010				
HOR CG	0.0090	0.0440			
GRI PF	0.0100	0.1360	0.8110		
GUL PF	0.1750	0.0370	0.4880	0.4470	
HOR PF	0.0000	0.2460	0.0030	0.0250	0.0120



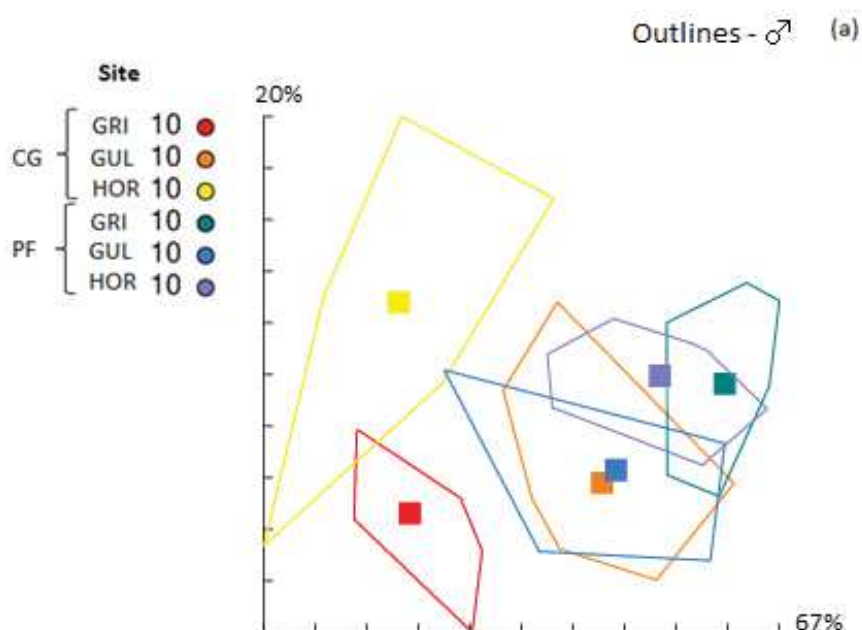
**Fig.4:** Size variation (in pixels) of females presented as quantile plots for tick populations based on (a) outlines and (b) landmark arrangements. The box shows the median as a line across the middle and the quartiles (25<sup>th</sup> and 75<sup>th</sup> percentiles) as its ends. Extremities represent the minimal and maximal values. The right column represents the validated classification (CCC) result based on shape in percentage, used to test the affiliation of each individual tick to each population. The site locations are: GRI: Grimsey; GUL: Gull; HOR: Hornoeya.

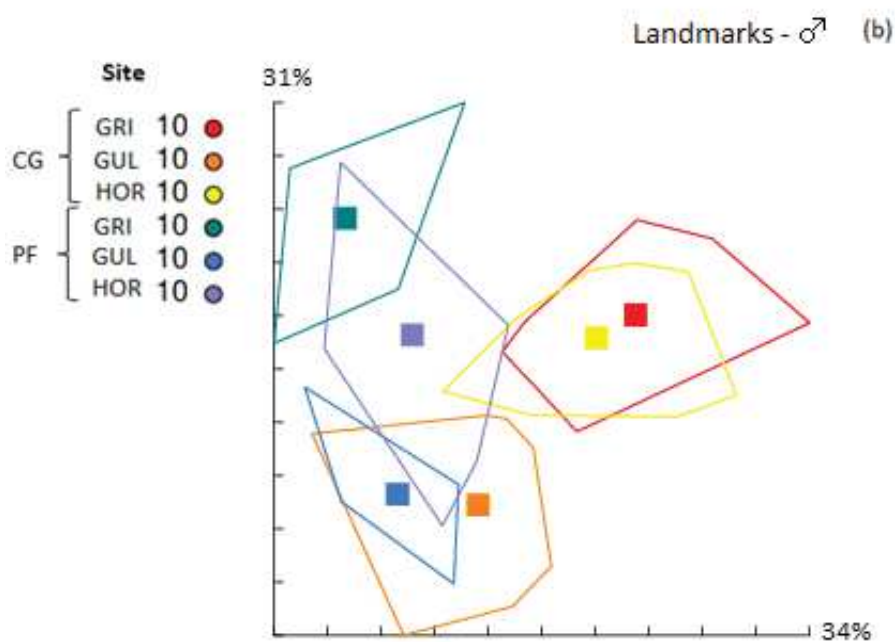
Landmark analyses showed greater discrimination among tick populations and tended to group PF ticks more closely than CG ticks, which were each highly distinct (Fig.2b). As for the outline analysis, size was significantly different between CG and PF ticks, except on GUL where this pattern seemed to be reversed (Fig.4a). The size effect on shape represented 10.7% of the overall variation in tick morphology (DF1 regression:  $r=0.101$ ;  $r^2=10\%$ ;  $df=52$ ;  $t=0.73$ ,  $p=0.23308$ ; DF2 regression:  $r=-0.083$ ;  $r^2=0.7\%$ ;  $df=52$ ;  $t=0.59$ ,  $p=0.0001$ ). The cross check classification showed that populations were differently supported,

ranging from 0% well-assigned individuals for GUL PF (but only 4 female ticks constituted this population) to 100% for GUL CG (Fig.4b).

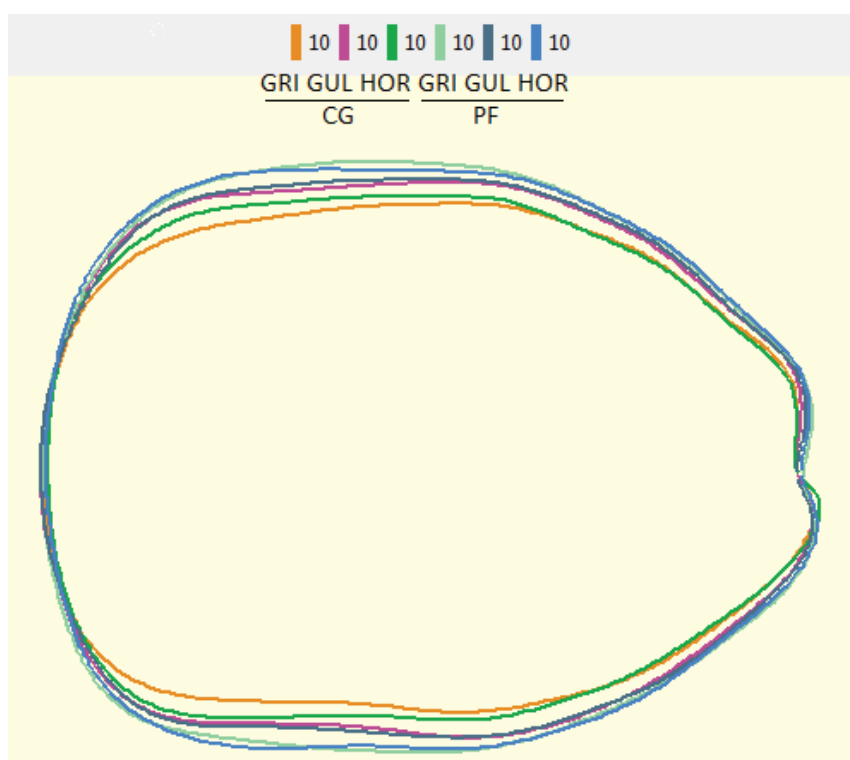
### Males

Male morphogroups were not as well discriminated as female morphogroups. The overall body shape of CG males differed between tick races in the HOR and GRI colonies with the populations of each race grouping together for the two PF populations, but this was not the case for the GUL population where ticks from CG and PF largely overlapped for both outline (Fig.5a) and landmark (Fig.5b) analyses. As for females, outlines showed that PF ticks tended to be wider than CG ticks (Fig.6). The GRI CG tick size was the smallest and was significantly different from all other populations (Fig.6, 7 and Table 3). The size effect on shape represented 36% of the overall shape variation for the outline analysis (DF1 regression:  $r=0.470$ ;  $r^2=22.1\%$ ;  $df=58$ ;  $t=4.06$ ,  $p=7.65 \times 10^{-5}$ ; DF2 regression:  $r=-0.373$ ;  $r^2=13.9\%$ ;  $df=58$ ;  $t=3.06$ ,  $p=0.0127$ ) and 12.3% of the overall variation in tick morphology for landmark analysis (DF1 regression:  $r=0.320$ ;  $r^2=10.2\%$ ;  $df=58$ ;  $t=2.57$ ,  $p=0.0064$ ; DF2 regression:  $r=-0.144$ ;  $r^2=2.1\%$ ;  $df=58$ ;  $t=1.11$ ,  $p=0.142$ ). The cross check classification showed that populations are differently supported with the GUL PF population showing the least support in both analyses and GRI PF population showing the most (Fig.7).

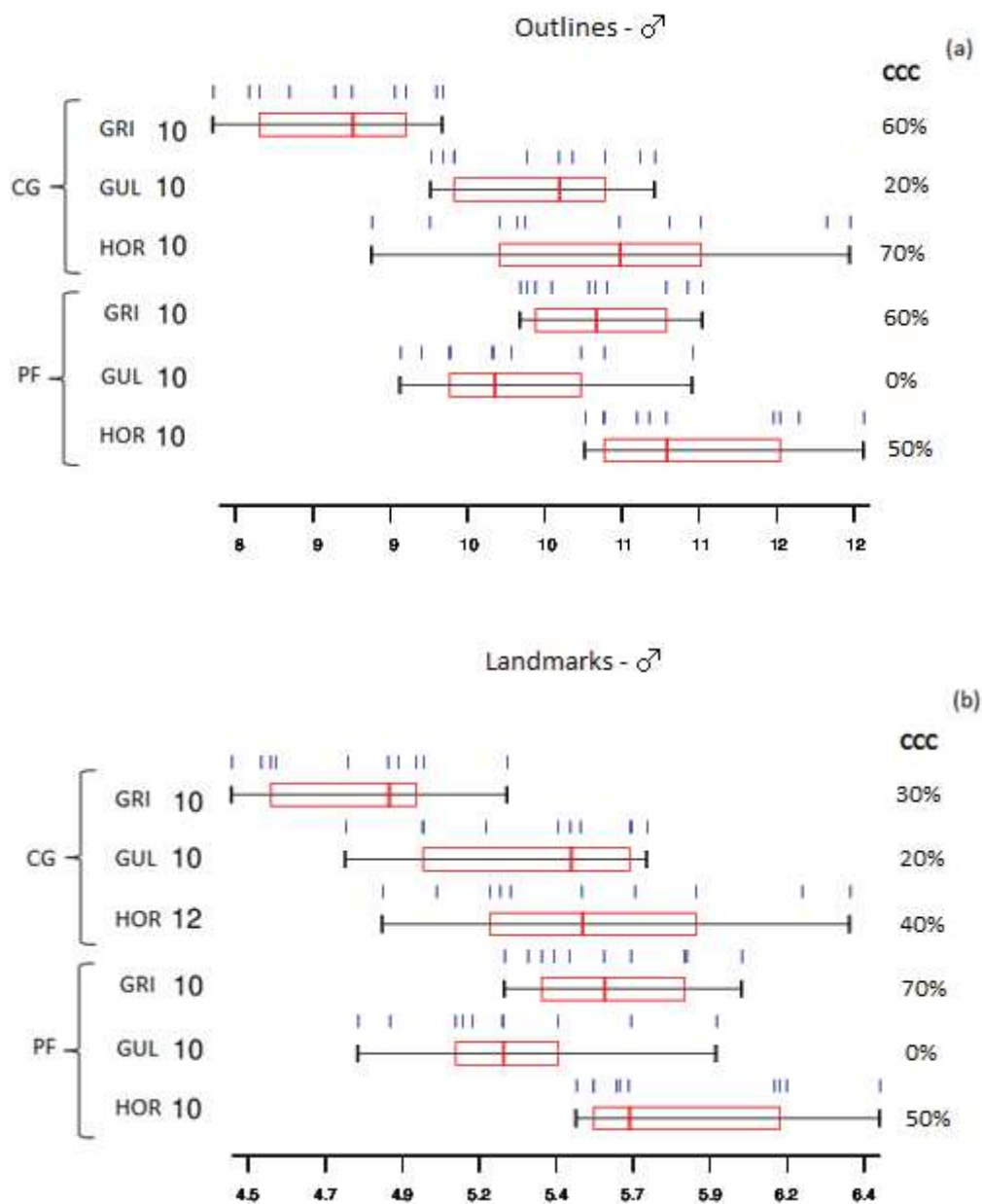




**Fig.5:** First two functions of a discriminant analysis performed on (a): normalized Fourier coefficients based on the outline analysis and (b): a set of first RW based on the landmark arrangement of males and showing the proportion of total variance explained. The polygons represent the maximal parameter space of each population and the squares the population mean. The site locations are: GRI: Grimsey; GUL: Gull; HOR: Hornoeya.



**Fig.6:** Mean contours for each male tick population. Each colored bar represents one population: orange: GRI CG; pink: GUL CG; dark green: HOR CG; light green: GRI PF; grey: GUL PF; blue: HOR PF. Numbers beside colored bars indicate the number of used ticks for each population.



**Fig.7:** Size variation (in pixels) of males presented as quantile plots for tick populations based on (a) outlines and (b) landmark arrangements. The box shows the median as a line across the middle and the quartiles (25<sup>th</sup> and 75<sup>th</sup> percentiles) as its ends. Extremities represent the minimal and maximal values. The right column represents the validated classification (CCC) result based on shape in percentage, used to test the affiliation of each individual tick to each population. The site locations are: GRI: Grimsey; GUL: Gull; HOR: Hornoeya.



**Table 3** Results of permutation tests of size differences in male tick outline contours (upper matrix) and landmark (lower matrix) configurations. The threshold value for significance using a Bonferroni correction is 0.0033. Significant values are in red. Blue cells indicate comparisons between sympatrically occurring host races.

	GRI CG	GUL CG	HOR CG	GRI PF	GUL PF
GUL CG	0.0000				
HOR CG	0.0000	0.2280			
GRI PF	0.0000	0.1120	0.7550		
GUL PF	0.0010	0.4740	0.0970	0.0200	
HOR PF	0.0000	0.0010	0.2030	0.0280	0.0010
	GRI CG	GUL CG	HOR CG	GRI PF	GUL PF
GUL CG	0.0000				
HOR CG	0.0000	0.3250			
GRI PF	0.0000	0.0980	0.7850		
GUL PF	0.0020	0.6130	0.1800	0.0310	
HOR PF	0.0000	0.0080	0.1320	0.0670	0.0000

## 4 Discussion

Host races were phenotypically distinct in each colony location in relation to both size and shape in two of the three colonies examined here. Congruent patterns in size and shape for each tick race were found for the HOR and GRI populations, but the morphological divergence of ticks from the GUL population was not clear. PF ticks tended to be wider than CG ticks, as observed in a previous study (Dietrich et al., 2013) but this pattern was less clear for male ticks of the GUL population. These results suggest that no one simple host-associated factor is responsible for the phenotypic divergence observed among co-occurring tick races, but rather that processes other than host-associated selection need to be considered.

Previous genetic analyses have suggested that CG and PF tick races on HOR and GRI population share a common origin (McCoy et al., 2013). We cannot therefore rule out that the observed patterns of phenotypic congruence in these colonies are due to selection *per se*. The similarity that we find may be largely associated with historical constraints and/or contemporary patterns of gene flow. However,

although the morphology of CG ticks was largely variable among colonies (with the same tendencies in HOR and GRI), this was not the case for PF ticks where morphology was relatively constant across colonies. This may suggest that within-colony divergence comes from asymmetric selection on ticks exploiting puffins to evolve a specific body form in order to escape host preening. Indeed, Clayton et al (1999) showed that efficient preening (grooming and scratching) selects for small body size in lice so they can better hide between feather barbs. In the *I. uriae*-seabird system, we observed that ticks on puffins tended to be wider than ticks on common guillemots. This does not correspond to host body size (28 to 35 cm in length for puffins and 40 to 46 cm in length for guillemots (Dif et al., 1982); suggesting that Harrison's rule (Harrison, 1915) does not apply to this system. Tick size differences could thus come from other sources, such as beak size and shape. Indeed, puffin beaks are large and slightly hooked measuring 23 to 32mm in length while common guillemot beaks are thin and pointed measuring 37 to 49mm in length. These differences could favor wider size in PF ticks and thinner size in CG ticks. Observations of seabird behavior while grooming and scratching and morphometrical analyses of the different seabird beaks could provide more support to this hypothesis, in particular by measuring gap widths between the mandibles.

Given the nidicolous lifestyle of *I. uriae*, size and shape differences among host races and colonies could also be the result of different selection pressures mediated by the abiotic environment. Indeed, as organisms physically interact with their environment, microhabitat conditions might induce morphological changes. The seabird species involved in this study use very different nesting substrates with potential differences in temperature and humidity conditions, known to affect tick distributions (Estrada-Peña, 2001). Common guillemots breed close to each other directly on rocks, whereas puffins breed in individual burrows which can be up to 5 meters deep. These different conditions may select for differences in tick size and shape. For example, thinner guillemot ticks may better fit into the rocky crevices of guillemot breeding ledges than the wider puffin ticks. More information concerning the abiotic conditions of each seabird nesting area and the use of temperature and humidity data loggers placed in the tick sheltered areas, are now needed to investigate this hypothesis.

Other factors may also explain the weak divergence patterns we observed in this study. For example, the pattern we observed for the GUL population could come from weak selection, the fitness consequences of tick size and shape are not strong enough to lead to population divergence. Alternatively, the patterns found in the GUL population may come from extinction-recolonization dynamics. Indeed, twenty years ago, infestation levels on kittiwake hosts were extremely high on GUL and the number of nesting guillemots was very low (McCoy et al., 1999). In 2016 almost no nestling or adult kittiwakes carried ticks in this colony and guillemots were found breeding throughout the island.

This suggests a potential shift in the local levels of infestation and host use. Such dynamics may mean that the ticks currently infesting the birds of this colony have shifted over time and may not yet have reached equilibrium. This can be tested by analyzing the sampled ticks in each colony at neutral genetic markers and determining whether the host-associated populations are still genetically distinct and whether there has been a recent host shift. These population dynamic events could also lead to host “mistakes” that add error to our measures of morphological divergence; genetic data can also allow us to estimate this error rate. If genetically-distinct host races are still present in each colony, we can use assignment tests to evaluate the degree to which host switching has blurred patterns of phenotypic divergence (see Dietrich et al., 2013). Finally, our results are based on relatively low sample sizes. Resampling efforts should therefore be made to improve the power of our analyses and make strong inferences concerning the divergence process of these *I. uriae* populations.

In conclusion, although our morphometrical analyses partially support congruent phenotypic divergence in the *I. uriae*-seabird system across the North Atlantic in relation to host use, this divergence is not homogeneous in all examined populations. Additional samples and supplementary analyses are now required to confirm this pattern and to distinguish among the different possible factors shaping divergence process.

## 5 Acknowledgements

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## References

- Barluenga, M., Stölting, K.N., Salzburger, W., Muschick, M., Meyer, A., 2006. Sympatric speciation in Nicaraguan crater lake cichlid fish. *Nature* 439, 719–723. doi:10.1038/nature04325
- Bookstein, F.L., 1997. *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge University Press.
- Clayton, D.H., Koop, J.A., Harbison, C.W., Moyer, B.R., Bush, S.E., 2010. How birds combat ectoparasites. *Open Ornithol J* 3, 41–71.
- Clayton, D.H., Lee, P.L.M., Tompkins, III, E.D.B., 1999. Reciprocal Natural Selection on Host-Parasite Phenotypes. *Am. Nat.* 154, 261–270. doi:10.1086/an.1999.154.issue-3
- Combes, C., 1995. *Interactions durables: écologie et évolution du parasitisme*. Masson.
- De Meeûs, T., Michalakis, Y., Renaud, F., 1998. Santa Rosalia Revisited: or Why Are There So Many Kinds of Parasites in 'The Garden of Earthly Delights'? *Parasitol. Today* 14, 10–13. doi:10.1016/S0169-4758(97)01163-0
- Dietrich, M., Beati, L., Elguero, E., Boulinier, T., McCoy, K.D., 2013. Body size and shape evolution in host races of the tick *Ixodes uriae*. *Biol. J. Linn. Soc.* 108, 323–334. doi:10.1111/j.1095-8312.2012.02021.x
- Dietrich, M., Gómez-Díaz, E., McCoy, K.D., 2011. Worldwide Distribution and Diversity of Seabird Ticks: Implications for the Ecology and Epidemiology of Tick-Borne Pathogens. *Vector-Borne Zoonotic Dis.* 11, 453–470. doi:10.1089/vbz.2010.0009
- Dietrich, M., Kempf, F., Boulinier, T., McCoy, K.D., 2014a. Tracing the colonization and diversification of the worldwide seabird ectoparasite *Ixodes uriae*. *Mol. Ecol.* 23, 3292–3305. doi:10.1111/mec.12815
- Dietrich, M., Kempf, F., Gómez-Díaz, E., Kitaysky, A.S., Hipfner, J.M., Boulinier, T., McCoy, K.D., 2012. Inter-oceanic variation in patterns of host-associated divergence in a seabird ectoparasite. *J. Biogeogr.* 39, 545–555. doi:10.1111/j.1365-2699.2011.02620.x
- Dietrich, M., Lobato, E., Boulinier, T., McCoy, K.D., 2014b. An experimental test of host specialization in a ubiquitous polar ectoparasite: a role for adaptation? *J. Anim. Ecol.* 83, 576–587. doi:10.1111/1365-2656.12170
- Dif, G., Hainard, R., Lamouche, T., Dorst, J., 1982. *Les oiseaux de mer d'Europe*. Arthaud, Paris.
- Dujardin, J.-P.A., Kaba, D., Henry, A.B., 2010. The exchangeability of shape. *BMC Res. Notes* 3, 266. doi:10.1186/1756-0500-3-266
- Dujardin, J.-P., Kaba, D., Solano, P., Dupraz, M., McCoy, K.D., Jaramillo-O, N., 2014. Outline-based morphometrics, an overlooked method in arthropod studies? *Infect. Genet. Evol.* 28, 704–714. doi:10.1016/j.meegid.2014.07.035
- Dujardin, J.-P., Slice, D.E., 2006. Contributions of morphometrics to medical entomology. *Encycl. Infect. Dis. Mod. Methodol.* 435–447.
- Estrada-Peña, A., 2001. Forecasting habitat suitability for ticks and prevention of tick-borne diseases. *Vet. Parasitol., Promotion Advancement, Preserving Tradition* 98, 111–132. doi:10.1016/S0304-4017(01)00426-5

- Harrison, L., 1915. Mallophaga from Apteryx, and their Significance; with a Note on the Genus Rallicola. *Parasitology* 8, 88–100. doi:10.1017/S0031182000010428
- Kempf, F., Boulinier, T., De Meeûs, T., Arnathau, C., McCOY, K.D., 2009. Recent evolution of host-associated divergence in the seabird tick *Ixodes uriae*. *Mol. Ecol.* 18, 4450–4462. doi:10.1111/j.1365-294X.2009.04356.x
- Klimov, P.B., Bochkov, A.V., Oconnor, B.M., 2006. Host specificity and multivariate diagnostics of cryptic species in predacious cheyletid mites of the genus *Cheletophyes* (Acari: Cheyletidae) associated with large carpenter bees. *Biol. J. Linn. Soc.* 87, 45–58. doi:10.1111/j.1095-8312.2006.00554.x
- Kuclu, O., Aldemir, A., Demirci, B., 2011. Altitudinal variation in the morphometric characteristics of *Aedes vexans* Meigen from northeastern Turkey. *J. Vector Ecol. J. Soc. Vector Ecol.* 36, 30–41. doi:10.1111/j.1948-7134.2011.00138.x
- Kuhl, F.P., Giardina, C.R., 1982. Elliptic Fourier features of a closed contour. *Comput. Graph. Image Process.* 18, 236–258. doi:10.1016/0146-664X(82)90034-X
- Magalhães, S., Forbes, M.R., Skoracka, A., Osakabe, M., Chevillon, C., McCoy, K.D., 2007. Host race formation in the Acari. *Exp. Appl. Acarol.* 42, 225–238. doi:10.1007/s10493-007-9091-0
- Mans, B.J., Neitz, A.W.H., 2004. Adaptation of ticks to a blood-feeding environment: evolution from a functional perspective. *Insect Biochem. Mol. Biol.* 34, 1–17. doi:10.1016/j.ibmb.2003.09.002
- McCoy, K.D., Boulinier, T., Chardine, J.W., Danchin, E., Michalakis, Y., 1999. Dispersal and Distribution of the Tick *Ixodes uriae* within and among Seabird Host Populations: The Need for a Population Genetic Approach. *J. Parasitol.* 85, 196. doi:10.2307/3285619
- McCoy, K.D., Boulinier, T., Tirard, C., Michalakis, Y., 2003. Host-Dependent Genetic Structure of Parasite Populations: Differential Dispersal of Seabird Tick Host Races. *Evolution* 57, 288–296.
- Mccoy, K.D., Boulinier, T., Tirard, C., Michalakis, Y., 2001. Host specificity of a generalist parasite: genetic evidence of sympatric host races in the seabird tick *Ixodes uriae*. *J. Evol. Biol.* 14, 395–405. doi:10.1046/j.1420-9101.2001.00290.x
- McCoy, K.D., Chapuis, E., Tirard, C., Boulinier, T., Michalakis, Y., Bohec, C.L., Maho, Y.L., Gauthier-Clerc, M., 2005. Recurrent evolution of host-specialized races in a globally distributed parasite. *Proc. R. Soc. B Biol. Sci.* 272, 2389–2395. doi:10.1098/rspb.2005.3230
- McCoy, K.D., Léger, E., Dietrich, M., 2013. Host specialization in ticks and transmission of tick-borne diseases: a review. *Front. Cell. Infect. Microbiol.* 3. doi:10.3389/fcimb.2013.00057
- Poulin, R., 2006. *Evolutionary Ecology of Parasites*., 2nd edition, New Second edition. ed. Princeton University Press, Princeton, N.J.
- Prudhomme, J., Gunay, F., Rahola, N., Ouanaimi, F., Guernaoui, S., Boumezzough, A., Bañuls, A.-L., Sereno, D., Alten, B., 2012. Wing size and shape variation of *Phlebotomus papatasi* (Diptera: Psychodidae) populations from the south and north slopes of the Atlas Mountains in Morocco. *J. Vector Ecol.* 37, 137–147. doi:10.1111/j.1948-7134.2012.00210.x
- Sonenshine, D.E., Roe, R.M., 2014. *Biology of Ticks Vol.2*, Second edition. ed. Oxford University Press.

## **Chapter 4 – Host-associated divergence factors and the role of local isolation**

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Dispersal is a central topic in ecology and evolution because it mediates gene flow and thus the distribution of genetic variation. Parasites are not distributed in a uniform manner across spatial scales and local adaptation strongly depends on dispersal capacity (Gandon, 2002). In the case of *I. uriae*, for example, four genetically differentiated large groups were shown within its global distribution, and are considered to be associated with constraints on host movements (Dietrich et al, 2014a). Boulinier et al. (2016, see annexes) highlight the importance of different seabird movements (migration, dispersal, prospecting) for the dispersal of *I. uriae* at different spatial scales (Gasparini et al, 2001; McCoy et al, 1999). Using population genetic tools, it was shown that tick population structure starts to be seen at a distance of 200 km around a focal colony for some tick host races (McCoy et al, 2003). Indeed, the use of GPS loggers on ringed birds demonstrates that prospecting behavior can occur hundreds of kilometers from the original colony, allowing the dispersal of ticks and other potentially infectious agents (Ponchon et al, 2014). These prospecting movements can play a major role in hard tick dispersal because each developmental stage requires several days to feed to repletion. Moreover, in this system, it is important to evaluate tick population structure in order to understand involvement in epidemiology. In fact, it was demonstrated that *I. uriae* host races harbored variable prevalence and infection intensities of *Borrelia burgdorferi* s. l. bacteria and that these differences can impact local infection dynamics. Furthermore, tick host races carried different bacterial strains within colonies, highlighting a cascading effect of vector divergence on pathogen epidemiology (Gomez-Diaz et al, 2010). In the case of soft ticks, we can hypothesize that host movements will impact tick dispersal, depending on the life stage, as adults and nymphs feed very rapidly, generally at night when the birds are not moving, while larval ticks can stay attached to the host for many hours, increasing dispersal probability (Kada et al, in prep). We therefore expect that the population genetic structure of soft ticks should be higher than that of hard ticks, reducing in turn the degree of pathogen circulation.

To test this hypothesis, I performed a field experiment to test for the local isolation of soft ticks at the scale of a single seabird colony and combined information from both the ticks themselves and their pathogenic agents. Infectious agents were detected with a previously-developed Fluidigm dynamic array. Results revealed weak spatial structure of ticks and pathogens, suggesting relatively frequent tick circulation within the colony. This indicates dispersal capacity may be more difficult to predict than expected and that species-specific characteristics should be taken into account if we are to better understand tick population dynamics and pathogen circulation.

## Article 5

Dupraz M, Toty C, Devillers E, Blanchon T, Elguero E, Vittecoq M, Moutailler S, McCoy K-D. Circulation of vector-borne infectious agents at local scales: structure of the soft tick *Ornithodoros maritimus* within a colony of its seabird host *Larus michahellis*. En preparation.



Circulation of vector-borne infectious agents at local scales: structure of the soft tick *Ornithodoros maritimus* within a colony of its seabird host *Larus michahellis*.

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## Abstract

The epidemiology of vector-borne zoonoses depends on the movements of both hosts and vectors at different spatial scales, with the role of vectors being potentially constrained at local spatial scales. However, very little information is currently available on local scale movements of vector organisms, and particularly so for ticks, the vector group transmitting the greatest diversity of infectious agents of medical and veterinary importance. Here, we investigated the dynamics and structure of the soft tick *Ornithodoros maritimus* over the course of a breeding season at the scale of a single seabird host colony to understand the degree to which ticks disperse among nests and are responsible for local scale dissemination of tick-borne infectious agents. As the host species, the Yellow-legged gull *Larus michahellis*, is highly territorial, we expected ticks to be the main agent of local pathogen dissemination. Ticks were sampled within a series of nests across the colony at regular intervals over the course of the season; in half of the nests, all ticks were removed (destructive sampling) and in the other half they were counted and returned to the nest. At the last sampling event, all ticks were removed and a sample of all collected ticks was screened for a wide array of known infectious agents (bacteria, viruses, parasites) using a high throughput real-time PCR system. We then quantified tick dynamics over the course of the season in relation to the sampling treatment and tested for spatial auto-correlation in the presence of ticks and tick-borne agents among nests. The results show a temporal dynamic in the presence of the different tick stages over the season. Adult females and males were present throughout the reproductive season, increasing linearly until the second last visit. The appearance of nymphs and larvae coincided with chick hatching suggesting a potential adaptation to host phenology. No tick spatial structure was detected and the effect of destructive sampling reduced tick abundance in the nest from the fourth visit onward, suggesting that ticks move frequently to find shelter and/or an available host within the colony. The spatial distribution of bacteria carried by the ticks supported this view. Three symbiotic bacteria, four potentially pathogenic bacteria, one parasite and one virus were found in the sampled ticks, but none showed evidence of spatial structure within the colony. These results highlight the importance of considering vector movement patterns to predict and control pathogen circulation.

Keywords: Argasidae, within-nest dynamics, dispersal, epidemiology



## 1 Introduction

Vector-borne zoonoses are often maintained in complex transmission cycles including different vector and reservoir host species (Sonenshine and Mather, 1994; Brisson et al, 2008; Keesing et al, 2006). Locally, vector spatial dynamics mediate pathogen population structure among hosts, the outcome of host-parasite co-evolutionary interactions (Gandon and Michalakis, 2002) and ultimately the dynamics of disease emergence (Ostfeld et al, 2005). Understanding the spatial and temporal dynamics of vectors is thus of major importance to prevent or control potential outbreaks, especially as global change favors the redistribution of biodiversity (Tylianakis et al, 2008; Ogden et al, 2013). Ticks are among the most important vectors of diseases worldwide and are able to transmit bacteria, viruses and eukaryotic parasites (Jongejan and Uilenberg, 2004) to a wide variety of hosts including birds, reptiles and mammals (Sonenshine and Roe, 2014). However, very little information is currently available on local scale movements of this vector organism. Indeed, few studies have focused on vector dispersal. Abraham et al. (2011) showed that different Triatominae species use active movement to disperse locally, rather than passive transportation with more wide ranging vertebrate hosts. McCoy et al. 2003b found that cliff topography and nest site organization are of key importance for predicting the local population structure of the seabird tick *Ixodes uriae*. Likewise, soft ticks of the *Ornithodoros turicata* species showed low interburrow movement and specific depth preferences for tortoise burrows (Adeyeye and Butler, 1989). These few studies highlight the variety of different biotic and abiotic factors that may come into play when trying to understand local scale vector structure.

*Ornithodoros maritimus* Vermeil and Marguet 1966 is a member of the soft tick (Argasidae) complex *Ornithodoros capensis sensu lato* containing 8 described species exploiting colonial seabirds in tropical and sub-tropical areas of the globe (Perez-Eid, 2007). *O. maritimus* has been recorded in association with a wide range of seabird host species including cormorants, gulls, alcids and terns in Great Britain, Ireland, France, Italy, Spain and Tunisia (Hoogstraal et al, 1976). It is also known to vector numerous viruses such as Meaban and West Nile viruses (Arnal et al, 2014; Dietrich et al, 2011), and Soldado virus which can induce high mortality rates in bird populations and pruritus in humans (Converse et al, 1975; Feare et al, 1976). More recently, these ticks have also been found to harbor endosymbiotic bacteria that, in addition to playing a potentially important role in tick biology, may favor the emergence of medically important pathogens, such as the Q fever agent *Coxiella burnetii* (Duron et al, 2015). *O. maritimus* is largely nidicolous, feeding on the host rapidly at night in the nymphal and adult life stages. This limited contact with the host should result in low tick dispersal among colonies, and should have a cascading effect on pathogen spread (Kada et al, submitted).

Here, we investigate the dynamics of the different tick stages in bird-host nests over the course of a seabird breeding season and test for spatial structuring among nests at the scale of an island. To examine the degree to which ticks move among nests, we destructively sampled ticks in half of our study nests to determine whether we could alter within-nest population dynamics by removing ticks. As the study location is situated in a biodiversity hotspot, we used a high throughput real-time PCR system to test for the presence of the most common tick-borne infectious agents including bacteria, parasites and viruses potentially harbored by the ticks and examined their spatial structure within the colony.

## 2 Material and methods

### 2.1 Biological system

Like other soft ticks, the life cycle of *O. maritimus* is polyphasic, composed of three active stages: a single larval stage, several nymphal instars and a sexual adult stage. As larvae, the ticks attach and feed for several hours. In nymphal and adult stages, the ticks feed to repletion in a few minutes during periods when the host is resting – typically at night. Females oviposit after each blood meal, depositing a few hundred eggs each time (Vial, 2009). While not feeding, the ticks of all life stages are found in the nest or surrounding area (nidicolous), where they benefit from stable temperature and humidity conditions (Vial, 2009). Ticks can be found in nests during the host breeding season, but spend the rest of the year in diapause in shelters or deeper in the ground waiting for the return of their host the following season.

The breeding range of the yellow-legged gull *Larus michahellis* is centered on the Mediterranean Sea. These gulls breed in dense colonies (pairs usually nest several meters apart) laying eggs in nests built with a sparse mound of vegetation on the ground or on cliff ledges (Olsen, 2004). These birds are very territorial, with relatively limited movements during the breeding season, mainly from feeding areas to the nest area. Outside the breeding season, the species remains gregarious, congregating around ports, harbours and dumps (Olsen, 2004).

### 2.2 Tick sampling and study location

Ticks were collected in the Camargue area of southern France from March to May 2015 on Carteau, a flat island in the Gulf of Fos of 210 meters long and 65 meters wide, entirely occupied by a breeding colony of yellow-legged gulls *L. michahellis*. In 2015, 385 breeding pairs occupied the island over the reproduction season (February to June). We selected, marked and took the gps coordinates of 30 nests across the island (Fig.1). At each visit, a nest was searched for 3 minutes by two people; one person

examined the upper nest materials in a white tray and, the other searched directly inside each nest and all ticks found were removed. For 15 of the 30 focal nests, ticks were returned to the nest once all life stages were identified and counted (non-engorged females, engorged females, males, non-engorged nymphs, engorged nymphs and larvae); we refer to these nests as “count nests”. In the other 15 nests, all ticks were collected and kept alive in the laboratory; we refer to these as “collected nests” (Fig.1). The sampling protocol was repeated once per week over six weeks on the same nests. Only during the last visit were all ticks were collected from all nests. After collection, ticks were identified and used for DNA/RNA extractions. At each visit, the number of gull eggs, hatching eggs or chicks in each nest was noted. In 2015, eggs started hatching during the fourth visit (16.04.15).

### **2.3 RNA and genomic DNA extraction**

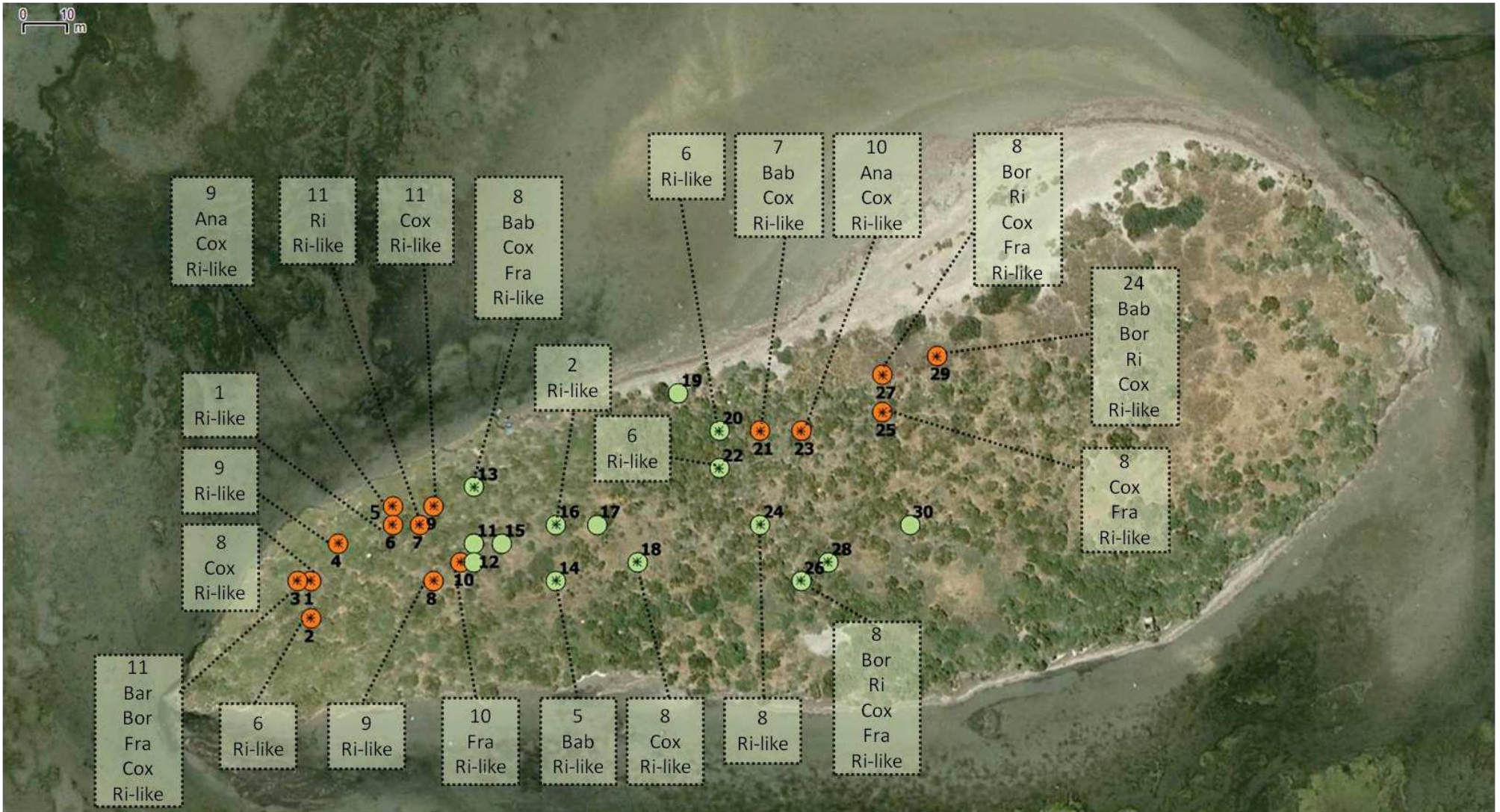
Extraction protocols followed those outlined in Moutailler et al. (2016) and Michelet et al. (2014). 201 adult ticks (162 females and 39 males) collected at the second, fourth, fifth and sixth visits were selected from 24 study nests to test for the presence of infectious agents (~7ticks/nest). All ticks were washed during 5 min in an ethanol bath, 10 min in two successive water baths and placed individually in sterile tubes and crushed in 300  $\mu$ L of DMEM with 10% fetal calf serum using the Precellys®24 Dual homogenizer (Bertin, France). The supernatant was divided into 3 fractions: 100  $\mu$ L for the DNA extraction, 100  $\mu$ L for the RNA extraction and the rest was used as back-up and conserved at -80°C. Genomic tick DNA was then extracted using the Wizard genomic DNA purification kit (Promega, France) according to the manufacturer’s instructions. RNA was extracted using the Nucleospin RNA II kit (Macherey Nagel, Germany) according to the manufacturer’s instructions. Purified DNA was eluted into 50  $\mu$ L elution buffer and stored at -20°C, whereas RNA was eluted into 50  $\mu$ L of RNase-free water and conserved at -80°C. Tick DNA and RNA quality was assessed, via the amplification of the ITS2 and COI respectively (Michelet et al, 2014; Moutailler et al, 2016).

### **2.4 High throughput real-time PCR system**

Ticks were analyzed for the most common tick-borne infectious agents using the BioMark real-time PCR system (Fluidigm, USA) for high-throughput microfluidic real-time PCR amplification. The DNA primer chip developed by Michelet et al. (2014) includes primers for detecting 28 bacterial species, 12 parasite species and 25 viruses (SM Table A1).

All RNAs were reverse transcribed into cDNAs using random primers and oligos (dT). The remaining methods followed those of Michelet et al, 2014 and Moutailler et al, 2016.





**Fig.1:** Map showing the position of the 30 tracked nests on Carteau Island, Camargue, France. Orange points represent the 15 nests in which ticks were counted and released. The green points are those nests where all ticks were counted and collected. Stars within the points represent the nests in which ticks were used for pathogen screening. Boxes indicate the number of ticks screened and the detected infectious agents: Ana: *Anaplasma* spp; Bab: *Babesia* spp; Bar: *Bartonella* spp; Bor: *Borrelia* spp; Cox: *Coxiella*-like symbiont; Fra: *Francisella*-like symbiont; Ri: *Rickettsia helvetica*; Ri-like: *Rickettsia*-like symbiont.

**Table 1:** List of pathogens detected using the high-throughput microfluidic real-time PCR amplification. The target gene refers to the gene used for the initial detection of the infectious agent and the confirmation method, the gene and procedure used for verifying the correction identification of the organism. NP: Not performed.

Pathogens	Species	Target Gene (HT)	Positive ticks - Prevalence % (n=201)	Confirmation method (gene targeted)	Reference confirmation technique	Confirmation	Sequence reference
Bacterium	<i>Anaplasma</i> spp.	msp2	3 – 1.5	PCR (16S)	Hornok et al, 2008	<i>Anaplasma</i> spp.	Sequence obtained without known species match
Bacterium	<i>Bartonella henselae</i>	pap31	1 – 0.5	PCR (gltA)	Norman et al, 1995	none	No sequence obtained
Bacteria	<i>Borrelia</i> spp.	23s	3 – 1.5	Nested PCR (IGS and p66)	Bunikis et al, 2004	none	No sequence obtained
Bacterium	<i>Coxiella</i> spp.	icd	25 – 12.4	NP	-	<i>Coxiella</i> -like symbiont	-
Bacterium	<i>Francisella</i> spp	tul4	3 – 1.5	NP	-	<i>Francisella</i> like symbiont	-
Bacterium	<i>Rickettsia helvetica</i>	ITS	6 - 3	NP	-	<i>R. helvetica</i>	-
Bacteria	<i>Rickettsia endosymbiont</i>	gltA	164 – 81.6	PCR (gltA)	Regnery et al, 1991	<i>R. lusitaniae</i>	gb JQ771933.1 (Milhano et al, 2014)
Parasite	<i>Babesia</i> spp.	hsp70	6 - 3	PCR (18S)	Bonnet et al, 2007	<i>Babesia</i> spp.	Sequence obtained without known species match
Virus	West Nile (WNV)	polyprotein	6 - 3	RT-PCR (NS5)	Scaramozzino et al, 2001	none	No sequence obtained

DNA and cDNA pre-amplifications were performed using the TaqMan PreAmp Master Mix (Applied Biosystems, France) according to the manufacturer's instructions. Primers for bacteria or viruses were pooled by combining equal volume of primers (200nM final each). This step was performed in a final volume of 5  $\mu$ L containing 2.5  $\mu$ L TaqMan PreAmp Master Mix (2X), 1.2  $\mu$ L pooled primer mix (0.2X) and 1.3  $\mu$ L DNA. Thermal cycling conditions were as follows: one cycle at 95°C for 10min, 14 cycles at 95°C for 15s and 4min at 60°C (Michelet et al., 2014).

The qPCR reactions were then performed using 6-carboxyfluorescein (FAM) and black hole quencher (BHQ1)-labeled TaqMan probes (Michelet et al, 2014) with TaqMan Gene expression Master Mix, in accordance to the manufacturer's instructions (Applied Biosystem, France). PCR cycling comprised 5min at 95°C, 45 cycles at 95°C for 10s, 15s at 60°C and 10s at 40°C.

Data were acquired on the BioMark Real-Time PCR system and analyzed using the Fluidigm Real-Time PCR Analysis software to obtain crossing point (CP) values. The assays were performed in duplicate using two negative water controls per chip and *Escherichia coli* strain EDL933 was added in each run to control for internal inhibition (Michelet et al, 2014).

## 2.5 Validation of detected infectious agents

Conventional and nested PCRs using different primers than those of the BioMark® system were used to confirm the presence of the detected infectious agents in the samples (Table 1). Amplicons were sequenced by Eurofins MWG Operon (Germany) and assembled using the BioEdit software (Ibis Biosciences, Carlsbad). An online BLAST (National Center for Biotechnology Information) was used to identify the sequenced organisms.

## 2.6 Statistical analyses

To evaluate the impact of destructive sampling on tick dynamics, we fit Poisson generalized linear mixed models to the tick numbers over time. The explanatory variables were i) nest treatment, ie whether ticks were collected or counted and ii) an exponential time trend. The effect of collecting on tick dynamics was assessed via the interaction between these two variables. Note that exponential trends are standard in Poisson models, as what is modeled is actually the logarithm of the response. To complete the models, a random effect nest was introduced to take into account unobserved differences between nests. Females, males and nymphs were studied separately. Calculations were performed with the R software (R-core team, 2015).

To test for spatial structure in tick numbers among nests, we calculated Moran's I index using the *correlogram* function in pgirmess package for R software using total tick numbers and female tick

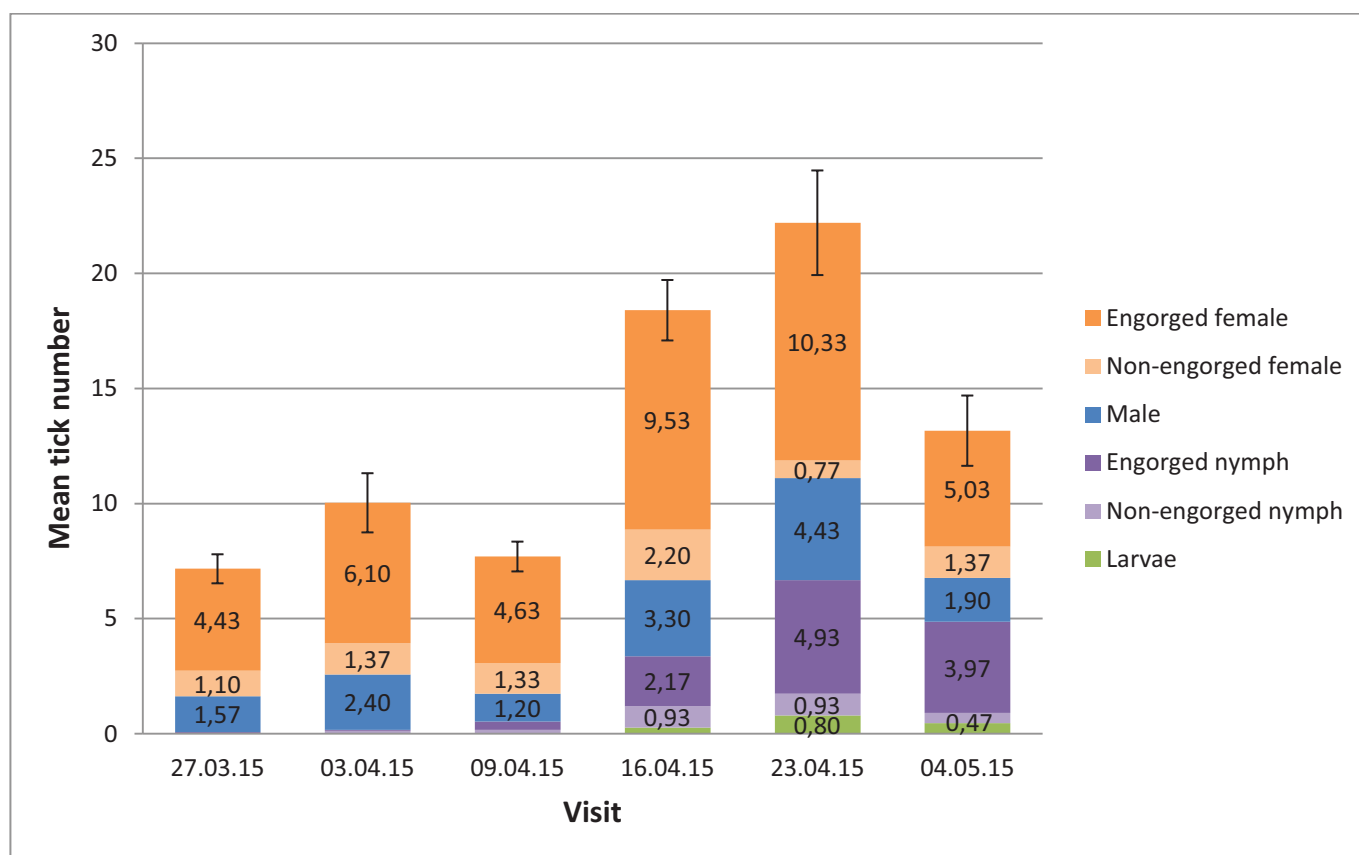


numbers only; this was not done for the other life stages due to low numbers. Ten distance classes among nests were defined at the first visit to test for step-by-step tick dispersal. Three distance classes were then defined for counted nests at each subsequent visit to test for short, medium and large-scale patterns of tick movement. The presence of structure in the distribution of the detected microorganisms was also tested using infected nests and ten distance classes.

### 3 Results

#### 3.1 Within season tick dynamics

Ticks were found in all nests, but not at each visit; the prevalence of infested nests per visit varied from 86 to 100%, with all nests infested during visits 4 and 5. The total number of ticks per nest increased over time up to visit 5 and then started to decline (Fig. 2). During the first three visits, we observed predominantly adult ticks (Fig. 3a and 3b). From the third visit onward, we started to observe engorged and non-engorged nymphs (Fig. 3c) and larvae. The most stable tick life stage was engorged females (mean abundance over time:  $6.68 \pm 8.89$  or see Fig. 2). At the fifth visit, one week after egg hatching, tick abundance was maximal and all life stages were found.

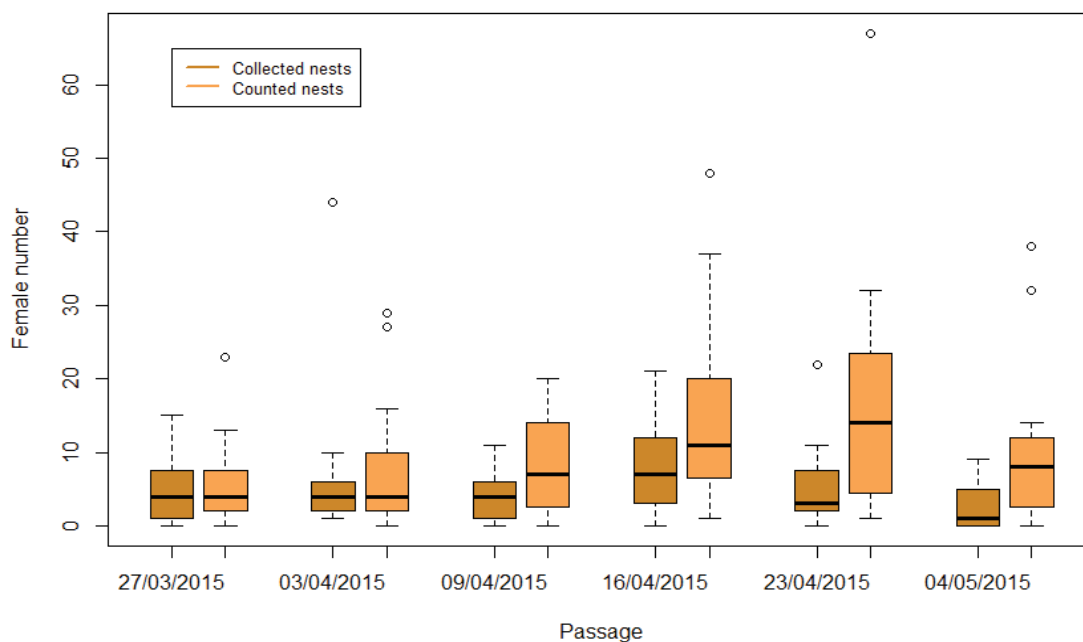


**Fig.2:** Histogram presenting the mean number of ticks observed in all nests over time. Bars represent mean standard errors of the total number of ticks.

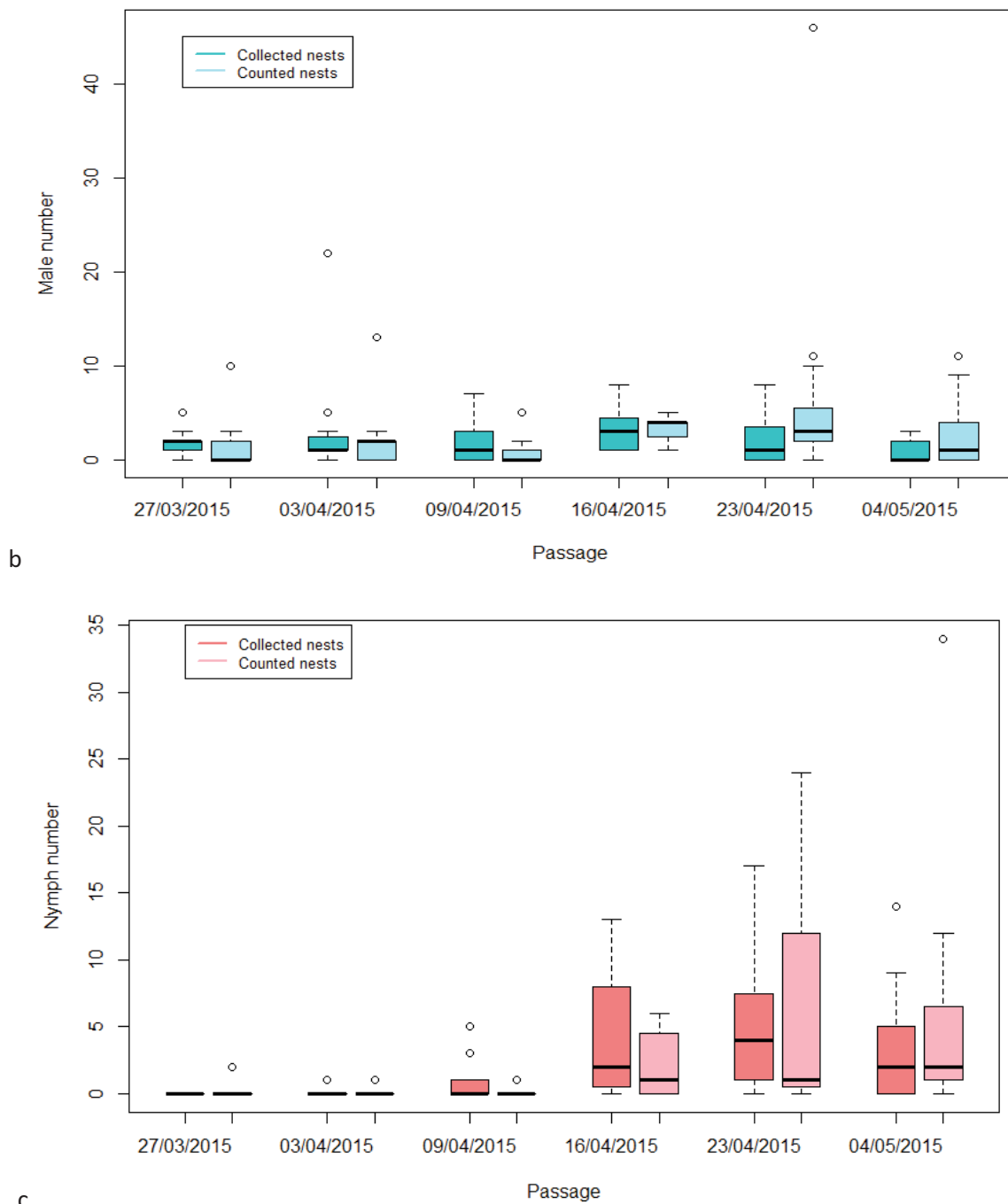
Table 2 shows the results of the generalized linear models. All slopes were significant, except for the male population of the collected nests. For females, males and nymphs, collecting had a significant effect on the population size (ie, significant interaction). There was also an interesting trend in nymphal ticks, where abundance seemed to be higher in the collected nests prior to visit 4 and, after this time, this trend reversed itself, suggesting a marked reduction in the presence of this life stage with destructive sampling (Fig. 3).

**Table 2:** Generalized linear model results. As tick number was modeled by an exponential trend, a time slope above 1 indicates an increase in the population size, whereas a time slope less than 1 indicates a decrease compared to expectations. A significant interaction effect denotes a difference in the population size between the two sampling treatments.

Nest type	Time slope	95% confidence interval	P-value	Interaction p-value
<b>Females</b>				
Counted	1.15	1.11 – 1.20	<1.0E-7	
Collected	0.94	0.89 – 0.99	0.023	<1.0E-7
<b>Males</b>				
Counted	1.30	1.20 – 1.40	<1.0E-7	
Collected	0.94	0.86 – 1.02	0.11	<1.0E-7
<b>Nymphs</b>				
Counted	2.04	1.82 – 2.28	<1.0E-7	
Collected	1.67	1.51 – 1.84	<1.0E-7	0.0089



a.



**Fig.3:** Boxplot representations of tick numbers in counted and collected nests over time: a, females only; b, males only; c, nymphs. The box shows the median as a line across the middle and the quartiles (25<sup>th</sup> and 75<sup>th</sup> percentiles) at either end. Extremities represent the minimal and maximal values and circles represent outliers.

### 3.2 Infectious agents in ticks

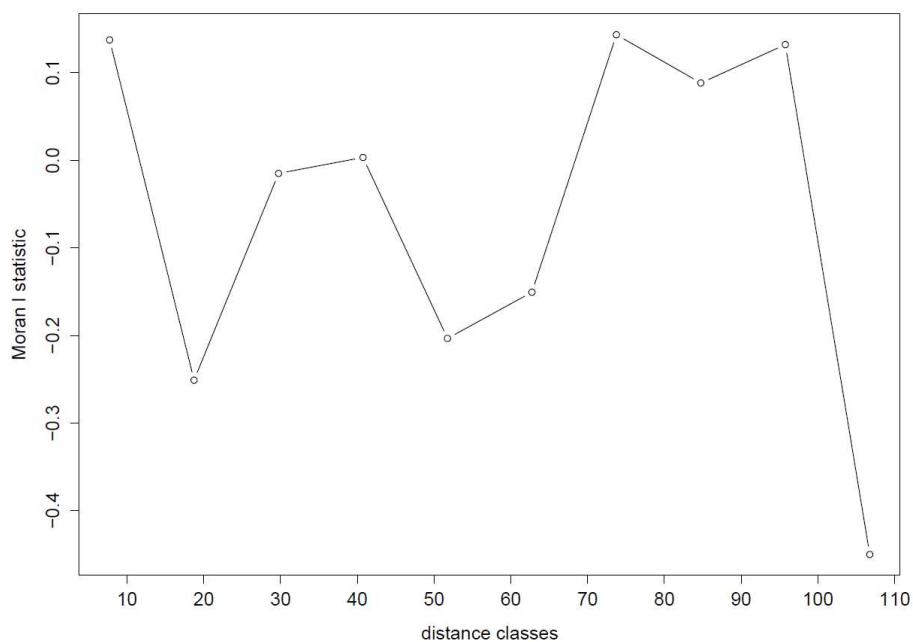
Among the 24 nests and 201 tick individuals analyzed by real-time PCR, 177 (88%) harbored at least one micro-organism including 7 bacteria, 1 parasite and 1 virus (Table 1). After subsequent amplification and sequencing, 159 ticks (79%) were found to harbor only symbiotic bacteria, 4 (2%) were infected by known pathogenic agents, 14 (7%) ticks were co-infected by both symbiotic bacteria and pathogens and 24 (12%) were found uninfected. Three presumably symbiotic bacteria were detected: the *Rickettsia*-like

bacterium was detected in all nests in high prevalence (50-100% of sampled ticks) and matched sequences of *Rickettsia lusitaniae* (Milhano et al, 2014) (99% Blast identity), one *Coxiella*-like bacterium was found in 10 nests with variable prevalence among ticks (9-50%), and one *Francisella*-like bacterium was detected in 3 nests in low prevalence (10-12.5%). Four potentially pathogenic bacteria were detected in low prevalence. *Anaplasma* spp was found in three nests (2-11%), but the reamplified sequence did not match on GenBank. *Bartonella henselae* was detected in a single nest with species-specific primers (9%), but could not be reamplified for confirmation. *Borrelia* spp. was also detected in three nests at low prevalence (4-12.5%), but could not be reamplified for confirmation; we do not yet know what bacterium species was initially amplified. Finally, *Rickettsia helvetica* was detected and confirmed to occur in five nests (4-25%). Only one parasite was detected, *Babesia* spp. and occurred in five nests (8-20%). However, the reamplified sequences of this *Babesia* spp. did not match any known sequences on GenBank and may represent a new species. Two tick co-infections were observed for potential pathogens: *B. henselae* and *R. helvetica* in one female tick (nest 3) and *Borrelia* spp and *Rickettsia helvetica* in a different female tick (nest 26). In addition, co-infections of presumed pathogens also occurred at the nest level: *Anaplasma* spp., *B. henselae* and *R. helvetica* (nest 3); *R. helvetica* and *Borrelia* spp. in nests 26 and 27; *Babesia* spp., *R. helvetica* and *Borrelia* spp. (nest 29).

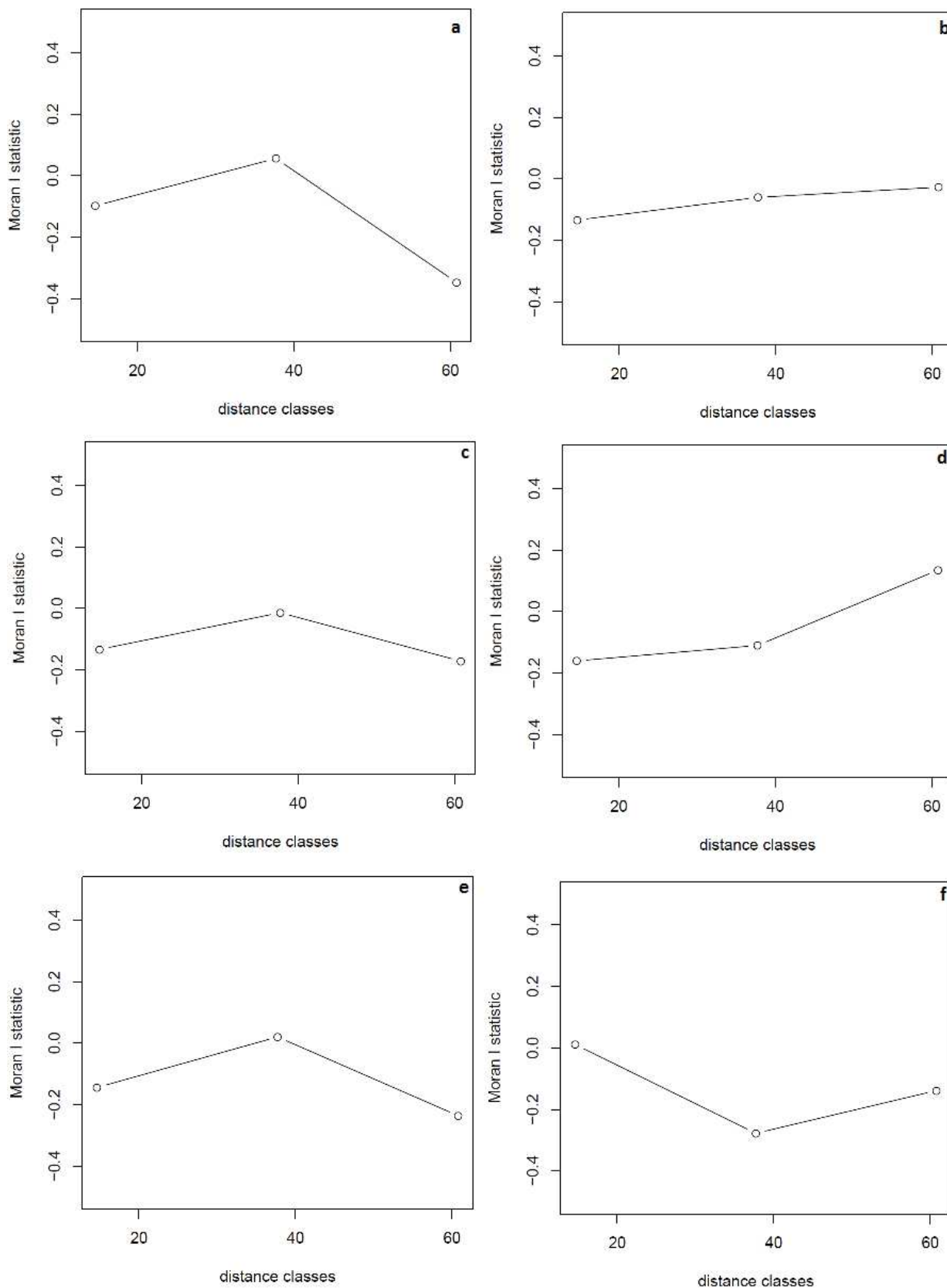
Among the 25 tested viruses, only one, the West Nile virus (WNV), occurred on Carteau in 6 individual ticks from 5 different nests. Two individual ticks infected by WNV were also infected by *Babesia* spp. (nest 13) and by *R. helvetica* (nest 29). Nevertheless, we were not able to obtain a sequence of this virus with RT-PCR or nested PCRs and the presence of this particular virus remains to be confirmed.

### 3.3 Spatial and temporal structuring of ticks and their infectious agents

The tests for spatial auto-correlation in tick abundance at the initial visit were non-significant (Fig. 4) and no clear spatial patterns in tick presence could be found for counted nests at each visit (Fig. 5). These patterns were similar when we tested for within-colony structure in the occurrence of infectious agents in the ticks (data not shown).



**Fig.4:** Spatial autocorrelation in total tick number estimated by Moran's I (Sokal and Oden, 1978). Data are from the first visit in the colony and include nests of both treatments. Ten distance classes have been defined in metres. No index value was significantly different from zero. The same results were obtained using female count data only (results not shown).



**Fig.5:** Spatial autocorrelation in total tick number and distance between counted nests at each visit with three distance classes based on Moran's I: a, 1<sup>st</sup> visit; b, 2<sup>nd</sup> visit; c, 3<sup>rd</sup> visit; d, 4<sup>th</sup> visit; e, 5<sup>th</sup> visit; f, 6<sup>th</sup> visit. Circles indicate the autocorrelation coefficients. The same results were obtained with female count numbers.



## 4 Discussion

To understand the degree to which ticks disperse among nests and are responsible for pathogen dissemination at local scales, we investigated the dynamics and structure of a soft tick *Ornithodoros maritimus* over the course of a host breeding season at the scale of a single island colony. We found a temporal dynamic in the presence of the different tick stages over the season, with the simultaneous appearance of juvenile stages and hatched chicks. Destructive sampling reduced tick abundance in the nest from the fourth visit onward, suggesting the presence of some localized populations, but no significant spatial structure within the colony was found for either tick abundance or pathogen presence.

### 4.1 Within-nest tick dynamics and impact of destructive sampling

In this study, one of the goals was to understand and describe the dynamics of *O. maritimus* ticks within host nests during the breeding season. What we observed is that adult ticks were present from the start of the season, with females feeding on brooding adult birds. As engorged females continued to occur and increased in number over time after chick hatching, it is likely that this life stage also feeds on chicks (Fig. 2 and Fig.3a). The proportion of males increased in line with females, potentially to maximize copulation opportunities (Fig. 3b). Nymphs and larvae only started to appear at the third and fourth visits respectively. However, observations of nymphs and larvae in the nest are extremely difficult due to their small size, particularly when unengorged: 1 to 5mm for nymphs and 0.5 to 1 mm for larvae (Hoogstraal et al, 1976). The abundance data associated with these life stages should therefore be considered with caution as the relative quantities of these stages are likely greatly underestimated compared to the larger and more conspicuous adult ticks. The number of juvenile ticks was maximal at the fifth visit (Fig. 3c) which may represent an adaptation to host phenology due to their intimate and repeated interactions (Magalhães et al, 2007). In effect, larvae were found feeding on chicks during the fourth visit. These larvae may have been produced from eggs laid a few weeks prior by females that starting feeding at the start of incubation. Due to their longer blood meal (several hours), larvae that feed on stationary chicks may have a higher probability of finding suitable off-host habitat post-bloodmeal than larvae that feed on the mobile adult birds. This kind of adaptation to host phenology is already known in other parasite-host systems, and notably in phytophagous insects (Filchack et al, 2000; Rouault et al, 2004). To further test the hypothesis that life stage appearance in ticks has evolved to

match breeding phenology, detailed sampling in other colonies that show different timing in breeding will be necessary.

The decrease in all tick stages at the sixth visit was surprising, but may be explained by the fact that chicks are no longer restricted to the nest site. Indeed, at this point, the young birds start to use the entire territory and notably sheltered areas in and around the vegetation. This behavior may favor the return of ticks to overwintering microhabitats where temperature and humidity conditions may be more stable (Hoogstraal, 1985).

At the scale of the seabird colony, we tested whether the repeated collection of ticks from nests over six weeks would affect within-nest dynamics of the tick population. On the whole, tick dynamics were relatively homogenous between collected and counted nests despite destructive sampling (Fig.3). Nevertheless, tick number (female, male and nymph) was significantly reduced in collected nests (Table 2). Although the number of ticks observed in collected nests was lower than that of counted nests, collected nests were still recolonized week after week by all tick stages suggesting that tick dispersal at the within colony level is frequent. Even though ticks were surely missed during sampling, it is difficult to imagine that the number of uncollected ticks in a nest was the sole contributor to ticks retrieved at the next visit (ie, some movement into the nest must be occurring).

#### 4.2 Diversity and structure of symbionts and infectious agents

Micro-organisms present in ticks of Carteau Island included both presumed endosymbionts and pathogens. The presence of *Coxiella* symbionts in *O. maritimus* was expected based on previous results obtained from this island colony (Duron et al, 2015) and has been recently reported in members of the *O. capensis* complex from across the globe (Al Deeb et al, 2015; Duron et al, 2014; Reeves et al, 2006). The *Rickettsia*-like symbiont was initially isolated from a soft tick parasitizing birds in Portugal, *O. erraticus* (Milhano et al, 2014) and from cell lines of *O. capensis* collected from coastal Georgia, USA (Mattila et al, 2007). Likewise, the *Francisella*-like symbiont has been previously described in both soft ticks, *O. moubata* (Noda et al, 1997) and *Argas persicus* (Suitor et Weiss, 1961), and hard ticks (Niebylski et al, 1997; Sun et al, 2000, Michelet et al, 2013). These different endosymbiotic bacteria are suggested to perform essential functions required for the completion of the tick life cycle, but details on their physiological impacts are scarce for now and we cannot yet explain the high diversity of these organisms within our population (Duron et al, 2015). Although they likely have no impact on the bird host, recent work has suggested that mutations in endosymbiotic bacteria can directly lead to the emergence of novel pathogenic organisms (Duron et al, 2015).

The prevalence of suspected pathogenic agents was much lower within the tick population, as would be expected. Different pathogenic species of *Anaplasma* are transmitted by hard ticks to mammals (including humans) (Dantas-Torres et al, 2012) and by a soft tick *Argas reflexus* to pigeons (Jongejan and Uilenberg, 2004). The *Anaplasma* species detected in our study did not match any known species available on public databases and may represent a new species. Although the msp2 target gene shows high intra and interspecies polymorphism, it is typically used to identify the species of this genus (Rymaszewska, 2010). We also detected a bacterium that matches with *Bartonella henselae*, the known agent of cat-scratch fever. The first identification of a *Bartonella* species, related to *B. henselae*, in a tick was in the soft tick *O. sonrai* in Senegal but the principal vectors of *B. henselae* are typically lice of wild felids (Mediannikov et al, 2013). The primers used in our real-time PCR assay were specific to this species, but we were not able to reamplify this bacterium using an alternative gene. This may have been due to low DNA quantity or to the fact that this bacterium may represent a genetic variant of the species. These issues require further investigation. *Rickettsia helvetica*, an infectious agent causing meningitis in humans, is well-known to be transmitted by hard ticks (Parola et al, 2005) and notably to wild birds by *I. ventalloi* in Portugal (Santos-Silva et al, 2006). Our study represents the first confirmed report of this bacterium in association with soft ticks to our knowledge. We also found an unknown species of *Babesia* in our *O. maritimus* colony. These apicomplexan parasites cause different forms of hemolytic disease, or babesiosis, in mammals. Most species of *Babesia* are vectored by hard ticks. Only one soft tick species has so far been recorded to vector these parasites; *O. erraticus* transmitting *Babesia meri* to sand rats in Africa (Yabsley and Shock, 2013). The *Babesia* spp. we found in *O. maritimus* could not be identified using data available on Genbank. Further work will therefore be required to determine the relationship of this species to other described species of *Babesia*. The presence of *Borrelia* spp. and a type of West Nile Virus (a Flavivirus) were also detected in our ticks, but could not be confirmed. Both agents are known to be transmitted by different species of soft ticks (Cutler, 2010; Lawrie et al, 2004) and WNV has been previously reported in the Camargue region (Balança et al, 2009; Pradier et al, 2014; Vittecoq et al 2013). However, a previous study of Yellow-legged gulls on Carteau did not find evidence for the presence of Flavivirus antibodies in eggs, suggesting that WNV and other related viruses did not circulate in gulls (Arnal et al, 2014). These results therefore also require further confirmation.

Compared to results obtained using the same molecular detection assay and 267 females of the hard tick *Ixodes ricinus* from nine sites in France (Moutailler et al, 2016), we found many fewer pathogens and in lower prevalence (Table 1) in our soft ticks. This may be a direct consequence of the soft tick lifestyle; soft ticks feed on the host for only a short time compared to hard ticks and therefore may be more isolated because they have fewer opportunities for host-associated dispersal (Kada et al.

submitted). Alternatively, soft ticks or host-birds may be more efficient at clearing infections compared to hard ticks and their hosts (Brown and O'Brien, 2011). It should also be noted that the detection of pathogen DNA in the ticks does not mean that these pathogens were still alive and transmissible to a new host. Laboratory tests are therefore required now to determine true vector competence. Likewise, data from different colonies across the Mediterranean are needed to evaluate the role of *O. maritimus* in the circulation of these different infectious agents at larger spatial scales.

### 4.3 Vector movement at the within-colony scale

Removal sampling and the analysis of spatial structure reveal little to no structuring for ticks or their infectious agents in the gull colony of Carteau Island, even among nests separated by several meters. Topography is known to be an important factor impacting the isolation of hard tick populations and may modify pathogen circulation at small spatial scales (McCoy et al, 2003b). However, no apparent spatio-temporal structuring was observed for the Meaban virus transmitted by *O. maritimus* in Spain (Arnal et al, 2014) suggesting that it could be a less important factor in soft ticks especially on a flat and densely occupied island as Carteau. Hosts are considered to be the major driver of tick dispersal and may transport them over long distances (e.g., Smith et al, 1996; McCoy et al, 2003a). In the case of soft ticks however, feeding times are so short that the role of host movements in structuring tick populations and their infectious agents may be minimal. Indeed, Arnal et al. (2014) found that the seroprevalence of the Meaban virus was highly stable at the colony level, but variable at the nest level. This suggests little intercolony dispersal of either birds or ticks, but rather shifts in either nest use by birds and/or movement of infected ticks within the colony. In relation to our results, we suggest that it is the latter mechanism at play and that ticks are disseminating from one nest to another relatively easily. Moreover, on Carteau Island, we observed that exact nest sites are not necessarily reused by hosts from one year to the next. Ticks must therefore move at least over the area of the territory each season in order to find a suitable host. Indeed, the within-nest patterns of infection that we found suggest frequent within-colony movements; surprisingly few ticks from a same nest were infected with the same infectious agents, which would have been expected if all ticks in a nest repeatedly fed on the same individual bird. Analyses of tick genetic structure are now required to determine the degree to which ticks from different nests represent single, panmictic populations. Further analyses on the presence of infectious agents or agent-specific antibodies in birds (Arnal et al, 2014) would also provide additional information on exposure rates to ticks at the within-colony level. As soft ticks differ substantially in life history traits from hard ticks, these results highlight the need to understand the spatial and temporal dynamics of this

vector at local scales in order to better to predict and control pathogen circulation at larger spatial scales.

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## References

- Abrahan, L.B., Gorla, D.E., Catalá, S.S., 2011. Dispersal of *Triatoma infestans* and other Triatominae species in the arid Chaco of Argentina: Flying, walking or passive carriage? The importance of walking females. *Memórias do Instituto Oswaldo Cruz* 106, 232–239. doi:10.1590/S0074-02762011000200019
- Adeyeye, O.A., Butler, J.F., 1989. Population structure and seasonal intra-burrow movement of *Ornithodoros turicata* (Acari: Argasidae) in gopher tortoise burrows. *Journal of medical entomology* 26, 279–283.
- Al-Deeb, M.A., Frangoulidis, D., Walter, M.C., Kömpf, D., Fischer, S.F., Petney, T., Muzaffar, S.B., n.d. Coxiella-like endosymbiont in argasid ticks (*Ornithodoros muesebecki*) from a Socotra Cormorant colony in Umm Al Quwain, United Arab Emirates. *Ticks and Tick-borne Diseases*. doi:10.1016/j.ttbdis.2015.10.012
- Arnal, A., Gómez-Díaz, E., Cerdà-Cuellar, M., Lecollinet, S., Pearce-Duvet, J., Busquets, N., García-Bocanegra, I., Pagès, N., Vittecoq, M., Hammouda, A., Samraoui, B., Garnier, R., Ramos, R., Selmi, S., González-Solís, J., Jourdain, E., Boulinier, T., 2014. Circulation of a Meaban-Like Virus in Yellow-Legged Gulls and Seabird Ticks in the Western Mediterranean Basin. *PLoS ONE* 9, e89601. doi:10.1371/journal.pone.0089601
- Balança, G., Gaidet, N., Savini, G., Vollot, B., Foucart, A., Reiter, P., Boutonnier, A., Lelli, R., Monicat, F., 2009. Low West Nile Virus Circulation in Wild Birds in an Area of Recurring Outbreaks in Southern France. *Vector-Borne and Zoonotic Diseases* 9, 737–741. doi:10.1089/vbz.2008.0147
- Bonnet, S., Jouglin, M., Malandrin, L., Becker, C., Agoulon, A., Hostis, M., Chauvin, A., 2007. Transstadial and transovarial persistence of *Babesia divergens* DNA in *Ixodes ricinus* ticks fed on infected blood in a new skin-feeding technique. *Parasitology* 134, 197–207. doi:10.1017/S0031182006001545
- Brisson, D., Dykhuizen, D.E., Ostfeld, R.S., 2008. Conspicuous impacts of inconspicuous hosts on the Lyme disease epidemic. *Proceedings of the Royal Society of London B: Biological Sciences* 275, 227–235. doi:10.1098/rspb.2007.1208
- Brown, C., O'Brien, V., 2011. Are Wild Birds Important in the Transport of Arthropod-Borne Viruses?, in: *Ornithological Monographs No. 71, Ornithological Monographs*. American Ornithologists' Union, pp. 1–64.
- Bunikis, J., Garpmo, U., Tsao, J., Berglund, J., Fish, D., Barbour, A.G., 2004. Sequence typing reveals extensive strain diversity of the Lyme borreliosis agents *Borrelia burgdorferi* in North America and *Borrelia afzelii* in Europe. *Microbiology* 150, 1741–1755. doi:10.1099/mic.0.26944-0
- Converse, J.D., Hoogstraal, H., Moussa, M.I., Feare, C.J., Kaiser, M.N., 1975. Soldado virus (Hughes group) from *Ornithodoros (Alectorobius) capensis* (Ixodoidea: Argasidae) infesting Sooty Tern colonies in the Seychelles, Indian Ocean. *Am J Trop Med Hyg* 24, 1010–1018.
- Cutler, S. j., 2010. Relapsing fever – a forgotten disease revealed. *Journal of Applied Microbiology* 108, 1115–1122. doi:10.1111/j.1365-2672.2009.04598.x
- Dantas-Torres, F., Chomel, B.B., Otranto, D., 2012. Ticks and tick-borne diseases: a One Health perspective. *Trends in Parasitology* 28, 437–446. doi:10.1016/j.pt.2012.07.003



- Dietrich, M., Gómez-Díaz, E., McCoy, K.D., 2011. Worldwide Distribution and Diversity of Seabird Ticks: Implications for the Ecology and Epidemiology of Tick-Borne Pathogens. *Vector-Borne and Zoonotic Diseases* 11, 453–470. doi:10.1089/vbz.2010.0009
- Duron, O., Jourdain, E., McCoy, K.D., 2014. Diversity and global distribution of the *Coxiella* intracellular bacterium in seabird ticks. *Ticks and Tick-borne Diseases* 5, 557–563. doi:10.1016/j.ttbdis.2014.04.003
- Duron, O., Noël, V., McCoy, K.D., Bonazzi, M., Sidi-Boumedine, K., Morel, O., Vavre, F., Zenner, L., Jourdain, E., Durand, P., Arnathau, C., Renaud, F., Trape, J.-F., Biguezoton, A.S., Cremaschi, J., Dietrich, M., Léger, E., Appelgren, A., Dupraz, M., Gómez-Díaz, E., Diatta, G., Dayo, G.-K., Adakal, H., Zoungrana, S., Vial, L., Chevillon, C., 2015. The Recent Evolution of a Maternally-Inherited Endosymbiont of Ticks Led to the Emergence of the Q Fever Pathogen, *Coxiella burnetii*. *PLoS Pathog* 11, e1004892. doi:10.1371/journal.ppat.1004892
- Feare, C.J., 1976. Desertion and Abnormal Development in a Colony of Sooty Terns *Sterna Fuscata* Infested by Virus-Infected Ticks. *Ibis* 118, 112–115. doi:10.1111/j.1474-919X.1976.tb02015.x
- Filchak, K.E., Roethele, J.B., Feder, J.L., 2000. Natural selection and sympatric divergence in the apple maggot *Rhagoletis pomonella*. *Nature* 407, 739–742. doi:10.1038/35037578.
- Gandon, S., Michalakis, Y., 2002. Local adaptation, evolutionary potential and host–parasite coevolution: interactions between migration, mutation, population size and generation time. *Journal of Evolutionary Biology* 15, 451–462. doi:10.1046/j.1420-9101.2002.00402.x
- Hoogstraal, H., 1985. Argasid and Nuttalliellid Ticks as Parasites and Vectors, in: J.R. Baker and R. Muller (Ed.), *Advances in Parasitology*. Academic Press, pp. 135–238.
- Hoogstraal, H., Clifford, C.M., Keirans, J.E., Kaiser, M.N., Evans, D.E., 1976. The *Ornithodoros* (*Alectorobius*) *Capensis* Group (Acarina: Ixodoidea: Argasidae) of the Palearctic and Oriental Regions. O. (A.) *Maritimus*: Identity, Marine Bird Hosts, Virus Infections, and Distribution in Western Europe and Northwestern Africa. *The Journal of Parasitology* 62, 799. doi:10.2307/3278964
- Hornok, S., Földvári, G., Elek, V., Naranjo, V., Farkas, R., de la Fuente, J., 2008. Molecular identification of *Anaplasma marginale* and rickettsial endosymbionts in blood-sucking flies (Diptera: Tabanidae, Muscidae) and hard ticks (Acari: Ixodidae). *Veterinary Parasitology* 154, 354–359. doi:10.1016/j.vetpar.2008.03.019
- Johnson, K.P., Shreve, S.M., Smith, V.S., 2012. Repeated adaptive divergence of microhabitat specialization in avian feather lice. *BMC Biology* 10, 52. doi:10.1186/1741-7007-10-52
- Jongejan, F., Uilenberg, G., 2004. The global importance of ticks. *Parasitology* 129, S3–S14. doi:10.1017/S0031182004005967
- Lawrie, C.H., Uzcátegui, N.Y., Gould, E.A., Nuttall, P.A., 2004. Ixodid and argasid tick species and West Nile virus. *Emerg Infect Dis* 10, 653–657.
- Keesing, F., Holt, R.D., Ostfeld, R.S., 2006. Effects of species diversity on disease risk. *Ecology Letters* 9, 485–498. doi:10.1111/j.1461-0248.2006.00885.x
- Magalhães, S., Forbes, M.R., Skoracka, A., Osakabe, M., Chevillon, C., McCoy, K.D., 2007. Host race formation in the Acari. *Exp Appl Acarol* 42, 225–238. doi:10.1007/s10493-007-9091-0

- Mattila, J.T., Burkhardt, N.Y., Hutcheson, H.J., Munderloh, U.G., Kurtti, T.J., 2007. Isolation of Cell Lines and a Rickettsial Endosymbiont from the Soft Tick *Carios capensis* (Acari: Argasidae: Ornithodorinae). *Journal of Medical Entomology* 44, 1091–1101. doi:10.1093/jmedent/44.6.1091
- McCoy, K.D., Boulinier, T., Tirard, C., Michalakis, Y., 2003a. Host-Dependent Genetic Structure of Parasite Populations: Differential Dispersal of Seabird Tick Host Races. *Evolution* 57, 288–296.
- McCoy, K.D., Tirard, C., Michalakis, Y., 2003b. Spatial genetic structure of the ectoparasite *Ixodes uriae* within breeding cliffs of its colonial seabird host. *Heredity* 91, 422–429. doi:10.1038/sj.hdy.6800339
- Mediannikov, O., Diatta, G., Kasongo, K., Raoult, D., 2013. Identification of Bartonellae in the Soft Tick Species *Ornithodoros sonrai* in Senegal. *Vector-Borne and Zoonotic Diseases* 14, 26–32. doi:10.1089/vbz.2013.1326
- Michelet, L., Bonnet, S., Madani, N., Moutailler, S., 2013. Discriminating *Francisella tularensis* and *Francisella*-like endosymbionts in *Dermacentor reticulatus* ticks: Evaluation of current molecular techniques. *Veterinary Microbiology* 163, 399–403. doi:10.1016/j.vetmic.2013.01.014
- Michelet, L., Delannoy, S., Devillers, E., Umhang, G., Aspan, A., Juremalm, M., Chirico, J., van der Wal, F.J., Sprong, H., Boye Pihl, T.P., Klitgaard, K., BÃ,cker, R., Fach, P., Moutailler, S., 2014. High-throughput screening of tick-borne pathogens in Europe. *Frontiers in Cellular and Infection Microbiology* 4. doi:10.3389/fcimb.2014.00103
- Milhano, N., Palma, M., Marcili, A., Nuncio, M.S., de Carvalho, I.L., de Sousa, R., 2014. *Rickettsia lusitaniae* sp. nov. isolated from the soft tick *Ornithodoros erraticus* (Acarina: Argasidae). *Comparative Immunology, Microbiology and Infectious Diseases* 37, 189–193. doi:10.1016/j.cimid.2014.01.006
- Moutailler, S., Moro, C.V., Vaumourin, E., Michelet, L., Tran, F.H., Devillers, E., Cosson, J.-F., Gasqui, P., Van, V.T., Mavingui, P., Vourc'h, G., Vayssier-Taussat, M., 2016. Co-infection of Ticks: The Rule Rather Than the Exception. *PLOS Negl Trop Dis* 10, e0004539. doi:10.1371/journal.pntd.0004539
- Niebylski, M.L., Peacock, M.G., Fischer, E.R., Porcella, S.F., Schwan, T.G., 1997. Characterization of an endosymbiont infecting wood ticks, *Dermacentor andersoni*, as a member of the genus *Francisella*. *Appl. Environ. Microbiol.* 63, 3933–3940.
- Noda, H., Munderloh, U.G., Kurtti, T.J., 1997. Endosymbionts of ticks and their relationship to *Wolbachia* spp. and tick-borne pathogens of humans and animals. *Appl. Environ. Microbiol.* 63, 3926–3932.
- Norman, A.F., Regnery, R., Jameson, P., Greene, C., Krause, D.C., 1995. Differentiation of *Bartonella*-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. *J. Clin. Microbiol.* 33, 1797–1803.
- Ogden, N., 2013. Changing geographic ranges of ticks and tick-borne pathogens: drivers, mechanisms and consequences for pathogen diversity. *Front. Cell. Infect. Microbiol* 3, 46. doi:10.3389/fcimb.2013.00046
- Olsen, K.M., 2004. *Gulls of Europe, Asia and North America*. Christopher Helm Publishers Ltd, London.

- Ostfeld, R.S., Glass, G.E., Keesing, F., 2005. Spatial epidemiology: an emerging (or re-emerging) discipline. *Trends in Ecology & Evolution*, Special Issue: Bumper Book Review 20, 328–336. doi:10.1016/j.tree.2005.03.009
- Parola, P., Paddock, C.D., Raoult, D., 2005. Tick-Borne Rickettsioses around the World: Emerging Diseases Challenging Old Concepts. *Clin. Microbiol. Rev.* 18, 719–756. doi:10.1128/CMR.18.4.719-756.2005
- Perez-Eid, C., 2007. Les tiques : identification, biologie, importance médicale et vétérinaire. Lavoisier.
- Pradier, S., Sandoz, A., Paul, M.C., Lefebvre, G., Tran, A., Maingault, J., Lecollinet, S., Leblond, A., 2014. Importance of Wetlands Management for West Nile Virus Circulation Risk, Camargue, Southern France. *International Journal of Environmental Research and Public Health* 11, 7740–7754. doi:10.3390/ijerph110807740
- R Core Team, 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Reeves, W.K., Loftis, A.D., Sanders, F., Spinks, M.D., Wills, W., Denison, A.M., Dasch, G.A., 2006. *Borrelia*, *Coxiella*, and *Rickettsia* in *Carios capensis* (Acari: Argasidae) from a brown pelican (*Pelecanus occidentalis*) rookery in South Carolina, USA. *Exp Appl Acarol* 39, 321–329. doi:10.1007/s10493-006-9012-7
- Regnery, R.L., Spruill, C.L., Plikaytis, B.D., 1991. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. *J. Bacteriol.* 173, 1576–1589.
- Rouault, G., Turgeon, J., Candau, J.-N., Roques, A., Aderkas, P. von, 2004. Oviposition strategies of conifer seed chalcids in relation to host phenology. *Naturwissenschaften* 91, 472–480. doi:10.1007/s00114-004-0554-4
- Rymaszewska, A., 2010. Variability within the *msp2* gene in populations of *Anaplasma phagocytophilum*. *Folia Biol. (Praha)* 56, 269–275.
- Santos-Silva, M.M., Sousa, R., Santos, A.S., Melo, P., Encarnação, V., Bacellar, F., 2006. Ticks Parasitizing Wild Birds in Portugal: Detection of *Rickettsia aeschlimannii*, *R. helvetica* and *R. massiliae*. *Exp Appl Acarol* 39, 331. doi:10.1007/s10493-006-9008-3
- Scaramozzino, N., Crance, J.-M., Jouan, A., DeBriel, D.A., Stoll, F., Garin, D., 2001. Comparison of Flavivirus Universal Primer Pairs and Development of a Rapid, Highly Sensitive Heminested Reverse Transcription-PCR Assay for Detection of Flaviviruses Targeted to a Conserved Region of the NS5 Gene Sequences. *J. Clin. Microbiol.* 39, 1922–1927. doi:10.1128/JCM.39.5.1922-1927.2001
- Smith, R.P., Rand, P.W., Lacombe, E.H., Morris, S.R., Holmes, D.W., Caporale, D.A., 1996. Role of Bird Migration in the Long-Distance Dispersal of *Ixodes dammini*, the Vector of Lyme Disease. *J Infect Dis.* 174, 221–224. doi:10.1093/infdis/174.1.221
- Sokal, R.R., Oden, N.L., 1978. Spatial autocorrelation in biology: 1. Methodology. *Biological Journal of the Linnean Society* 10, 199–228. doi:10.1111/j.1095-8312.1978.tb00013.x

- Sonenshine, D.E., Mather, T.N., 1994. *Ecological Dynamics of Tick-Borne Zoonoses*. Oxford University Press.
- Sonenshine, D.E., Roe, R.M., 2014. *Biology of Ticks Vol.2, Second edition*. ed. Oxford University Press.
- Suitor, E.C., Weiss, E., 1961. Isolation of a Rickettsialike Microorganism (*Wolbachia Persica*, N. SP.) From *Argas Persicus* (Oken). *J Infect Dis.* 108, 95–106. doi:10.1093/infdis/108.1.95
- Sun, L.V., Scoles, G.A., Fish, D., O'Neill, S.L., 2000. Francisella-like Endosymbionts of Ticks. *Journal of Invertebrate Pathology* 76, 301–303. doi:10.1006/jipa.2000.4983
- Tylianakis, J.M., Didham, R.K., Bascompte, J., Wardle, D.A., 2008. Global change and species interactions in terrestrial ecosystems. *Ecology Letters* 11, 1351–1363. doi:10.1111/j.1461-0248.2008.01250.x
- Vial, L., 2009. Biological and ecological characteristics of soft ticks (Ixodida: Argasidae) and their impact for predicting tick and associated disease distribution. *Parasite* 16, 191–202.
- Vittecoq, M., Lecollinet, S., Jourdain, E., Thomas, F., Blanchon, T., Arnal, A., Lowenski, S., Gauthier-Clerc, M., 2013. Recent Circulation of West Nile Virus and Potentially Other Closely Related Flaviviruses in Southern France. *Vector-Borne and Zoonotic Diseases* 13, 610–613. doi:10.1089/vbz.2012.1166
- Yabsley, M.J., Shock, B.C., 2013. Natural history of Zoonotic Babesia: Role of wildlife reservoirs. *International Journal for Parasitology: Parasites and Wildlife* 2, 18–31. doi:10.1016/j.ijppaw.2012.11.003

## Supplementary material

Table A1: List of the pathogens screened for, but non-detected.

Pathogen	Species	Target gene	Pathogen	Species	Target gene	Pathogen	Species
Bacterium	<i>A. marginale</i>	msp1	Parasite	<i>Babesia divergens</i>	hsp70	Virus	Tick Borne Encephalitis (TBEV)
Bacterium	<i>A. platys</i>	groEL	Parasite	<i>B. microti</i>	CCTeta	Virus	Langat (LGTV)
Bacterium	<i>A. ovis</i>	msp4	Parasite	<i>B. venatorum EU1</i>	18s	Virus	Louping ill (LIV)
Bacterium	<i>A. centrale</i>	groEL	Parasite	<i>B. canis</i>	18s	Virus	Deer tick virus
Bacterium	<i>B. quintana</i>	btq	Parasite	<i>B. bovis</i>	CCTeta	Virus	Powassan virus (POW)
Bacterium	<i>Borrelia burgdorferi ss</i>	rpoB	Parasite	<i>B. caballi</i>	rap1	Virus	Meaban (MEAV)
Bacterium	<i>B. garinii</i>	rpoB	Parasite	<i>B. bigemina</i>	18s	Virus	Kyasanur Forest disease (KFDV)
Bacterium	<i>B. afzelii</i>	fla	Parasite	<i>B. major</i>	CCTeta	Virus	Omsk hemorrhagic fever (OHFV)
Bacterium	<i>B. valaisiana</i>	ospA	Parasite	<i>B. ovis</i>	18s	Virus	African swine fever (AFSV)
Bacterium	<i>B. lusitaniae</i>	rpoB	Parasite	<i>Theileria equi</i>	ema1 R2	Virus	Thogoto virus
Bacterium	<i>B. spielmanii</i>	fla	Parasite	<i>T. annulata</i>	18s	Virus	Bourbon (BOUV)
Bacterium	<i>B. bissetii</i>	rpoB				Virus	Dhori (DHOV)
Bacterium	<i>B. miyamotoi</i>	glpQ				Virus	Heartland (HRTV)
Bacterium	<i>Coxiella burnetii</i>	IS1111				Virus	Kemerovo (KEMV)
Bacterium	<i>Francisella tularensis</i>	fopA				Virus	Colorado (CTF)
Bacterium	<i>Ehrlichia chaffeensis</i>	dsb				Virus	Eyach virus
Bacterium	<i>E. ruminantium</i>	dsb				Virus	Crimean Congo haemorrhagic fever (CCHF)
Bacterium	<i>E. canis</i>	dsb				Virus	Dugbe virus
Bacterium	<i>Neoehrlichia mikurensis</i>	groEL				Virus	Nairobi (NSD)
Bacterium	<i>Rickettsia conorii</i>	ITS				Virus	Uukuniemi (UUKV)
Bacterium	<i>R. slovaca</i>	ITS				Virus	Schmallenberg (SBV)
Bacterium	<i>R. massiliae</i>	ITS				Virus	Severe fever thrombocytopenia syndrome (SFTSV)





## **Chapter 5- General discussion and perspectives**

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Studying host specialization is of particular interest because of its role in generating biodiversity. Nevertheless, this process is sometimes difficult to study, especially in natural systems in which experiments are often complicated or impossible. Detailed analyses are required to reveal subtle phenotypic variation in relation to population differentiation, variation that may be associated with varying selective regimes across populations. Studying convergent evolution can provide evidence to support population divergence linked with specific selection factors. Indeed, observing the same divergence patterns between genetically distant populations can be a signal of similar selective pressures in action. The ultimate aim of this dissertation was to determine if host specialization in tick-seabird systems is always accompanied by the same phenotypic changes and to identify the underlying factors affecting phenotypic convergence. The main results of this work show that signs of phenotypic convergence are present in the studied systems, but are weaker at large scales than initially expected based on local patterns. Several sources of selection from hosts and/or the colony environment in relation to the biological characteristics and history of each system participate to population structure were considered.

### **Selective pressures within each studied systems**

Host race divergence in *I. uriae* results from different factors specific to each population (host-parasite interaction dynamics, life-history traits) combined with selective pressures exerted by host and/or environments and/or evolution mechanisms such as drift modifying adaptive responses of parasites. For example, recognition of host chemical signals (kairomones)(Benoit et al, 2007), host differences in phenology (Barret and Erikstad, 2011) and host resistance mechanisms against parasites (behavior, immunity) or micro-habitat likely play a role in host race divergence. In this system, patterns of morphological variation with respect to size and shape correlate with host use across different zones of its distribution (Article 4) and are in general agreement with previous results (Dietrich et al, 2013). Differential performance of host races on alternative hosts was shown experimentally and suggested to be due to different capacities to resist diverse host immune systems and/or to digest host blood (Dietrich et al, 2014b). These factors may impact tick size but should not modify shape per se, suggesting that other factors are implicated in the patterns of morphological variation we observed. Host physical defenses may be the most parsimonious explanation to explain shape differences. Indeed, beak morphology, closely associated with the use of specific food resources, is also an important tool for preening and removing unwanted ectoparasites (Clayton et al, 2010; Clayton and Cotgreave, 1994). Size and shape differences in the beak (particularly the meeting zone of the upper and lower mandibles and

the nature of the beak extremity) can influence parasite size and load (Clayton et al, 1999, 2005). The beak shapes of hosts examined in *I. uriae* study is variable. Indeed, puffins have a short, wide and slightly hooked beak, kittiwakes have a medium, thin and slightly hooked beak, and guillemots have a long, thin and pointed beak (Dif et al, 1982) (Figure 9).



**Figure 9:** Pictures illustrating beak morphology of the hosts of *Ixodes uriae*. From left to right: Atlantic puffin (*Fratercula arctica*), Black-legged kittiwake (*Rissa tridactyla*) and common guillemot (*Uria aalge*). Photo credits: M. Dupraz.

Size and shape differences beak morphology could thus be implicated in the phenotypic differences observed among different *I. uriae* host races. Likewise, host plumage can act as a barrier to parasites (Clayton et al, 2010) but also permits temperature regulation and protection to external conditions. For example, in seabirds, it provides a waterproof layer during foraging at sea (Grémillet et al, 2005). Yang et al (2006) showed plumage density differences between terrestrial, swimming and diving birds. The plumage type and density differs strongly among the seabird host species we studied and could thus have an impact on tick morphology selecting for ideal tick size and shape to favour movement among feathers to reach optimal feeding zones and to maintain plumage integrity during foraging. Detailed studied of feather structure are now required to further examine these hypotheses and to evaluate its potential involvement in host race divergence.

As mentioned above, in this system the micro-habitats used by the different seabird hosts during the breeding period differ and may play a role in divergence. In ticks, the ability to successfully exploit a host depends on its ability to survive local environmental conditions (Estrada-Pena, 2001). Cuticle permeability, based on cuticular hydrocarbon production, constitutes a major limiting factor in this respect. In the *I. uriae* system, bird-hosts use different nesting substrates within the colony: puffins use deep individual burrows, kittiwakes breed in individual nests on steep cliff faces and guillemots breed side by side directly on rocks. Variation in temperature and humidity and the presence of available off-host shelters therefore vary with host use. Moreover, mating in *I. uriae* occurs off-host (Eveleigh and

Threlfall, 1974), which may lead to non-random mating and reduced gene flow between host races. To understand the differential impact of micro-habitats, temperature and humidity data loggers could be placed inside host breeding areas to monitor daily and seasonal variations. Using these data, resistance tests in controlled conditions could then be performed on the different host races, imitating micro-habitat conditions and evaluating the role of host micro-habitat on host-associated divergence.

In the second model system that I examined during my PhD work, the *O. capensis* s. l. complex that exploits a wide range of seabird species in tropical and sub-tropical regions, we found evidence of genetically and morphologically different groups in relation to host use. Although our samples of the different host and tick species were incomplete and the genetic markers we employed were conservative in terms of their ability to detect recent divergence events, host preferences in the complex are apparent. Two tick species, clearly distinguished using the two types of analyses, are present sympatrically in the Cape-Verde Islands. The first one is associated with booby host populations and has been identified as *O. capensis* sensu stricto, a species known to parasitize colonial seabirds in the Atlantic and Indian Oceans (Dietrich et al, 2011). However, the genetic distance observed among ticks of this species exploiting boobies and terns in the Indian Ocean (Article 3) and the description of a potential closely-related species, *O. cheikhi* parasitizing terns in Mauritania (Vermeil et al, 1997), suggests that further host-associated divergence may occur in this wide-spread species. Initial attempts to develop microsatellite markers for *O. capensis* s.l. were unsuccessful. Thus, to better identify and discriminate species, we are currently developing SNP markers in expressed zones of the genome. The second species detected on the Cape-Verde islands exploits shearwaters and seems to lie outside of the *O. capensis* s. l. complex. We do not yet know the evolutionary origin of this species but based on its discovery, it is clear that particular care is needed when identifying ticks in this system. Indeed, blind test results (Article 1) for commonly encountered ticks highlighted the difficulty in correctly identifying tick species based on classical morphological characteristics. In this study, combining genetic tools and morphological descriptions led to correct identification of species and should thus become the method of choice for discriminating morphologically similar species such as soft ticks of seabirds.

As in the system involving *I. uriae*, different parameters may exert selection pressures in this system. For example, the shearwaters and boobies breeding in hetero-specific colonies in the Cape-Verde Islands on which the two soft tick species were collected, show very different life history traits. Beak shape, respectively hooked and pointed, and habitat, respectively constituted by sandy burrows and rocky nests, could lead to the isolation in relation to host use. Nevertheless, the host preferences highlighted by our analyses represent only part of tick biology and supplementary sampling in other colonies, in

combination with morphological and genetic analyses, are now needed to describe the species distribution and host use of these two tick species.

However, the comparison of the two tick seabird systems studied in this dissertation work already provides an indication on the potential impact of selection coming from host beak morphology. Indeed, in the *I. uriae* system, the long and pointed beaks of guillemots seem to select for thinner tick body forms (Article 4). This result is repeated in *O. capensis* s. l. complex system, where the long and pointed beaks of boobies were associated with thinner and smaller ticks (Article 3). This hypothesis could now be tested using bird specimens captured in nature or available in museums, on which detailed morphometric measures could be taken. These measures could be treated in parallel with the morphometrical analyses realized on the ticks to determine their association (see Foster et al, 2008 for morphometrical measures of beaks).

Observed patterns of tick population divergence with respect to host use will be largely conditioned by gene flow at different spatial scales. Even if we admit that tick dispersal is realized by the host at large spatial scales, this is not necessarily so at smaller scales. Local tick dispersal should be based on species' characteristics and their ecological niche. For example, Adeyeye and Butler (1989) studied the movements of *Ornithodoros turicata* inside gopher tortoise (*Gopherus polyphemus*) burrows during 14 months using different color fluorescent powders. This tick is an ectoparasite of amphibians, reptiles and mammals (Adeyeye and Phillips, 1996) but is not known to feed on tortoises. It only enjoys suitable temperature and humidity conditions in the burrows of these animals whose depth can reach 5 meters (Young and Goff, 1939). The authors observed that tick depth preferences fluctuated strongly with season but they did not observe any inter-burrow movements. Other soft ticks such as *O. savignyi* (Andouin, 1826) inducing sand tampan toxicosis (Mans et al, 2002), live in semi-arid areas and take refuge in sand under trees or next to livestock waiting for a novel host (Basu et al, 2015). Because of its short bloodmeal in nymphal and adult stages, tick dispersal is considered as strongly limited. Nevertheless, Mans and Neitz (2004b) observed that this species is able to move over a distance of more than twenty meters to find a host. Our field observations showed weak local structure among nests within a colony for the soft tick *O. maritimus* (Article 5). Indeed, the gull hosts do not necessarily reuse the same nests from a year to another, obligating tick movement at least within the breeding territory. Moreover, the studied colony occurs on a low-lying island that is subject to strong storms in the winter that may destroy remnant nests. Ticks therefore need to find a suitable shelter to survive overwinter. These observations suggest that tick dispersal is conditioned by its own biology and local external factors. The weak pattern of structure in the ticks was also mirrored in the spatial structure of tick-borne infectious agents. Three symbiotic bacteria, four potentially pathogenic bacteria, one parasite and one



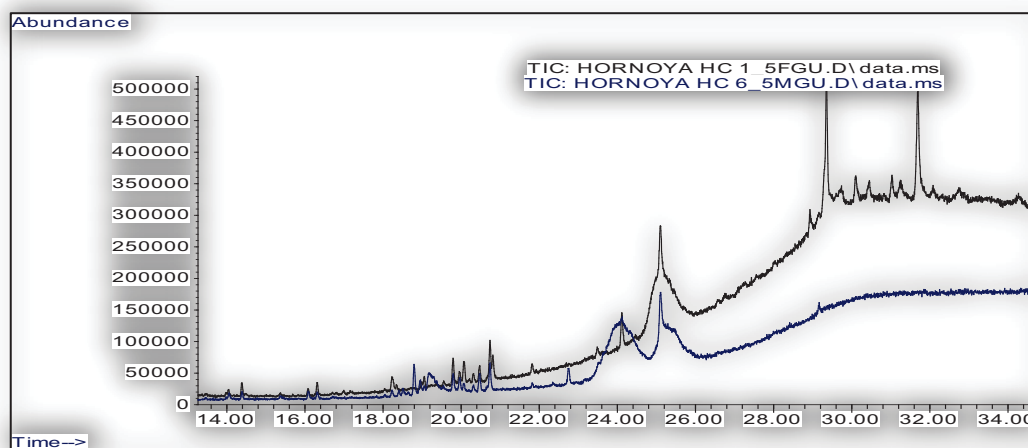
virus were detected in the collected ticks but no spatial structure was found in the colony. This suggests that tick dispersal may go beyond just neighboring nests within the colony. This hypothesis can now be tested using population genetic analyses to determine the degree of tick relatedness within and among nests. A mark-recapture experiment such as the one used by Adeyeye and Butler (1989) could also be employed to directly quantify tick movements at different life stages. This type of information will be crucial if we are to understand host-associated adaptation and its role in pathogen circulation. Indeed, the degree of tick movements within colonies will alter patterns of adaptation to specific host species and will modify the probability of transmitting infectious agents among colonies.

The presence of endosymbionts constitutes another factor which could potentially modify arthropod population dynamics and impact host-associated selection and gene flow. We know that these endosymbionts are able to complement their host in vitamins and can modify host reproductive success, but they have also been demonstrated to participate in the rapid adaptation of an arthropod host to a novel resource (Hosokawa et al, 2007). Infected insects can also show optimal performance under different temperature conditions (Dunbar et al, 2007; Russell and Moran, 2006). In ticks, the role of endosymbionts in tick biology is not well known. However, some endosymbionts are almost always present and highly diverse. For example, a broad diversity of *Coxiella* bacteria have been detected in different tick species collected in the field, including *O. maritimus*. This group of bacteria could be the source of new pathogenic bacteria, such as the causative agent of Q fever, *C. burnetti* (Duron et al, 2015; see annexes), but may also play a role in determining tick distributions and host use.

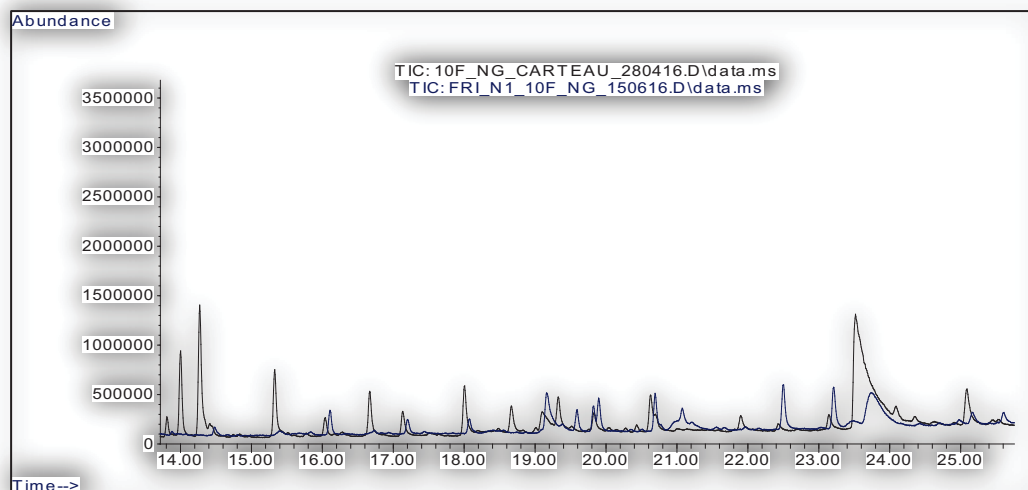
### **Divergence and phenotypic analyses**

To be relevant for studies in ecology and evolution, phenotypic analyses have to be able to quantify intra- and inter-species variability. Geometric morphometrics constitute a good tool to discriminate species of the *O. capensis* s. l. complex and *I. uriae* host races. Outline analyses were shown to be particularly appropriate when landmark use was complicated or impossible as for the dorsal surface of soft ticks (Article 2 and 3) and were found to be as discriminating as landmarks in the case of *I. uriae* host races (Article 4). Cheap and rapid, geometric morphometrics represent an alternative method for the rapid detection of distinct species and for testing for population structure. Moreover, this method can be used to measure fluctuating asymmetry (FA) in individuals coming from different populations. FA is generally measured as the deviation of a trait compared to perfect symmetry, i.e. variability of a trait between right and left sides (Debat and Peronnet, 2013). It was proposed that FA could increase when organisms are stressed by environment. This stress could lead to shape differences among individuals that are detectable by landmark analyses.

Other phenotypic analyses can be used to investigate selection pressures that result in the differentiation of arthropod populations. For example, cuticular hydrocarbons (HC) are carbon and hydrogen volatile chains constituting the lipid layer of the arthropod cuticle (Lockey, 1988). HC analyses are realized by HC extraction in a solvent. They are then separated by high performance liquid chromatography (HPLC) and analyzed by gas chromatography-mass spectrometry (GC-MS)(Golebiowski et al, 2010). HC represent a chemical signature that varies qualitatively between species and quantitatively within species. These molecules participate in water retention, aggregation, communication and mate choice (Blomquist and Bagnères, 2010; van Zweden and d’Ettorre, 2010). This chemical signature is under genetic control, but production can be modified by environmental variation (Estrada-Pena, 1993; Gibbs et al, 1991). In tick-seabird systems, HC production could thus be potentially linked to host use and survival under the different temperature and humidity conditions of the host micro-habitats. To test this hypothesis, we carried out HC extractions from hard and soft ticks, individually from living larvae, nymphs and adults. The HC concentrations from these initial extractions were too weak with high background noise to enable component identification. We therefore performed extractions on pools containing 5 and 10 ticks. However, again and surprisingly, HC concentrations were still too weak for analysis (Figures 10a, b).



a.



b.

**Figure 10:** Profiles of the extracted hydrocarbons: **(a)** from pools of 5 female ticks (black curve) and 5 male ticks (blue curve) of *I. uriae* collected in a common guillemot colony on Hornøya Island in Norway; **(b)** from pools of 10 female ticks of *O. maritimus* collected in the Carteau colony in Camargue (black curve) and on Frioul Island near to Marseille (blue curve).

The extraction method we used has already been used on organisms such as ants and spiders (Lenoir et al, 2001; van Zweden et al, 2009) and HC profiles have enabled the discrimination of arthropod sub-populations (Bagnères et al, 1991; Jallon and David, 1987; Simmons et al, 2014) including for soft and hard ticks (Estrada-Pena et al, 1996; Estrada-Pena and Dusbabek, 1993). HC composition according to host use was also demonstrated in tick species like *Rhipicephalus bursa* and *R. sanguineus* (Shimshoni et al, 2013). It was therefore difficult to understand the lack of HC in our study. *I. uriae* and *O. capensis* are both nidicolous species and, as such, are restricted to compartmentalized environments in which conditions are relatively stable. As producing HC may be metabolically costly (Sonenshine and Roe, 2014b) and the usefulness of HC in nidicolous species may be low, it may be that these ticks do not produce these molecules to the same degree as non-nidicolous tick species. This hypothesis is supported by particularly low concentrations of HC for male ticks of *I. uriae*, who do not take bloodmeals and therefore do not expose themselves to external conditions (Figure 10a). The endophilic lifestyle could therefore initiate alternative communication pathways among individuals. For example, it was shown that *I. uriae* can use a mixture of guanine and other components present in tick feces as an assembly pheromone (Benoit et al, 2008). Assembly pheromones are typically weakly volatile, require contact to be perceived and remain durably in environment (Sonenshine, 1985). Moreover, Eveleigh and Threlfall (1974) observed that mating is initiated in this species after a direct physical contact which permits the female to be attracted to the male. These types of pheromones (guanine, xanthine and hypoxanthine) are also present in soft ticks (Dusbabek et al, 1991). To find a suitable host, *I. uriae* (notably unfed stage) uses bird guano (uric acid and other components) as kairomone (Benoit et al, 2008). How fine-tuned this detection is remains unknown. Based on these initial publications, attraction tests could be performed

to test tick response while facing different tick and host feces. These data could provide information about the potential role of these products in host specialization. These tests could also be realized on soft ticks collected on different hosts. Such tests would be complicated to implement in nature, but could be easily done in laboratory using laboratory reared ticks. However, only one study to date has tried to rear *I. uriae* in laboratory, using chickens as surrogate hosts (Eveleigh and Threlfall, 1974). Unfortunately, this endeavor was a failure, suggesting a strong specificity for seabirds. *O. capensis* species, on the other hand, are easier to maintain in the laboratory and readily feed on chicken and pigeons (Colas-Belcour and Rageau, 1960). Indeed, over the course of my thesis, we started to rear different soft tick populations of the *O. capensis* s. l. complex in the laboratory. These ticks are maintained at stable temperature (24°C) and humidity (90%) conditions. Natural conditions are mimicked with a cycle of 12h of day and 12h of night. Tick feeding is realized on artificial membranes as described in Boulanger et al (2015). Ticks are put on the membrane to feed on cow blood heated to bird blood temperature, approximately 41°C. The bloodmeal takes place in dark during the night. Engorged females are later isolated and lay eggs in about 10 days. This type of rearing system will allow us to test the degree of host specificity and its potential underlying factors.

### **Genetic basis of phenotypic variation**

Showing phenotypic variation is not enough to understand divergent evolution. An important part in determining the implication of different factors in tick diversification is also understanding the degree to which observed phenotypic differences have a genetic basis. Indeed, trait evolution is the result of both the intensity of selection on this trait and its heritability (Falconer, 1960). A given trait has to be selected and transmitted to descendants to be maintained in a population. The impact of selection will depend on other genetic factors such as number of implicated genes affecting the trait and its interaction with other traits (pleiotropy, epistasis), mating type (assortative, random), dispersal between populations and the generation time of the species (Clayton et al, 2015). Thus, to confirm observed patterns of morphological variation in North Atlantic populations of *I. uriae* (Article 4), morphometrical analyses have to be combined with population genetic analyses to determine if the observed variations have a genetic basis. Genomic scans such as RADseq (Wang et al, 2009) could help to identify zones under selection and remain an important perspective of the work that I present in this dissertation.

Concerning the *O. capensis* s. l. complex, genetic studies employed to date involve only very conserved genetic markers (Article 3). Genome studies in this species and other ticks are difficult to envision because of the complexity of tick genomes (large genome with a high percent of repeated sequences)(Nene, 2009). To go further in our understanding of genetic divergence in this species

complex, other methods such as RNAseq based on transcriptome sequencing (Wang et al, 2009) are required. Indeed, the development of SNP markers based on the transcriptome of two species within the *O. capensis* complex is currently underway. Via these markers, we may identify markers under selection that enable us to discriminate tick species in this complex and identify the traits linked to speciation process.

### **The endpoint of host-associated divergence**

During this and previous work, we have shown that host-associated divergence has evolved recurrently and particularly so in the *I. uriae* system. In this system, we are currently evaluating the stability of the host race state by carrying out a temporal study that investigates divergence of sympatric populations in different independent locations collected at different time intervals on the same host types. These analyses will test the current idea that host races are highly stable over time and do not readily move to complete species in this system (Dietrich et al 2014a). However, in the *O. capensis* s. l. complex divergence may have occurred more rapidly and lead to the species diversity we currently observe. To test this idea, we require more detailed information on current micro-evolutionary patterns of divergence in this system and to take into account dispersal factors that may impact extinction and colonization dynamics. Depending on these factors, the formation of new species or host races may be highly localized and rapid or may spread among colonies and be relatively slow.

### **General conclusions**

Many factors may be implicated in host specialization in ticks and can impact the divergence process at different spatial scales. Host-associated selection pressures (immunity, behavior, micro-habitat), the biological characteristics of the ticks themselves (lifestyle, bloodmeal duration, dispersal) but also macro-environmental conditions all constitute important parameters that determine adaptive potential and spatial distribution. An understanding of these factors combined with the use of phenotypic and genetic analyses can thus help us to understand the host specialization process in these systems and to define associated local epidemiological risks. Our results suggest that morphological convergence among tick populations can result from selection pressures associated with host behavior, but that other factors also play a major role in determining these patterns. These factors include the characteristics of each tick species, such as lifestyle or dispersal capacities, and probably the use of different micro-habitats. As outlined above, the work presented in this dissertation has opened the way for many future perspectives. In particular, it appears particularly pressing to investigate the nature of host-based selection pressures exerted on tick populations and to define the genetic basis of observed phenotypic

variation if we are to better understand the process of host specialization in these two tick-seabird systems and apply this understanding more widely to other arthropod and vector systems.





# Bibliography

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- A. Lenoir, P. D'Etterre, Errard, and C., Hefetz, A., 2001. Chemical Ecology and Social Parasitism in Ants. *Annu. Rev. Entomol.* 46, 573–599. doi:10.1146/annurev.ento.46.1.573
- Adams, D.C., Rohlf, F.J., Slice, D.E., 2004. Geometric morphometrics: Ten years of progress following the “revolution.” *Ital. J. Zool.* 71, 5–16. doi:10.1080/11250000409356545
- Adeyeye, O.A., Butler, J.F., 1989. Population structure and seasonal intra-burrow movement of *Ornithodoros turicata* (Acari: Argasidae) in gopher tortoise burrows. *J. Med. Entomol.* 26, 279–283.
- Adéyèyè, O., Phillips, J.S., 1996. Photoperiodic response in the soft tick, *Ornithodoros turicata*. *Int. J. Parasitol.* 26, 629–635. doi:10.1016/0020-7519(96)87214-6
- Arthur, D.R., 1962. Ticks and Disease.
- Bagneres, A.-G., Killian, A., Clement, J.-L., Lange, C., 1991. Interspecific recognition among termites of the genus *Reticulitermes*: Evidence for a role for the cuticular hydrocarbons. *J. Chem. Ecol.* 17, 2397–2420. doi:10.1007/BF00994590
- Barluenga, M., Stölting, K.N., Salzburger, W., Muschick, M., Meyer, A., 2006. Sympatric speciation in Nicaraguan crater lake cichlid fish. *Nature* 439, 719–723. doi:10.1038/nature04325
- Barrett, R., Erikstad, K.E., 2011. Key-site monitoring on Hornøya in 2010, SEAPOP Short report, 3.
- Barton, T.R., Harris, M.P., Wanless, S., Elston, D.A., 1995. The activity periods and life-cycle of the tick *Ixodes uriae* (Acari: Ixodidae) in relation to host breeding strategies. *Parasitology* 112, 571–580. doi:10.1017/S0031182000066154
- Basu, A.K., Shinggu, P.A., Ahmed, M.I., 2015. Studies on diurnal abundance of the soft tick *Ornithodoros savignyi* in semi-arid Nigeria. *West Indian Vet. J.* 11.
- Benoit, J.B., Lopez-Martinez, G., Philips, S.A., Elnitsky, M.A., Yoder, J.A., Jr, R.E.L., Denlinger, D.L., 2008. The seabird tick, *Ixodes uriae*, uses uric acid in penguin guano as a kairomone and guanine in tick feces as an assembly pheromone on the Antarctic Peninsula. *Polar Biol* 31, 1445–1451. doi:10.1007/s00300-008-0485-1
- Benoit, J.B., Yoder, J.A., Lopez-Martinez, G., Elnitsky, M.A., Lee, R.E., Denlinger, D.L., 2007. Habitat requirements of the seabird tick, *Ixodes uriae* (Acari: Ixodidae), from the Antarctic Peninsula in relation to water balance characteristics of eggs, nonfed and engorged stages. *J. Comp. Physiol. B* 177, 205–215. doi:10.1007/s00360-006-0122-7
- Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K., Das, I., 2007. Cryptic species as a window on diversity and conservation. *Trends Ecol. Evol.* 22, 148–155. doi:10.1016/j.tree.2006.11.004
- Bird, C.E., Fernandez-Silva, I., Skillings, D.J., Toonen, R.J., 2012. Sympatric Speciation in the Post “Modern Synthesis” Era of Evolutionary Biology. *Evol. Biol.* 39, 158–180. doi:10.1007/s11692-012-9183-6

- Blackledge, T.A., Gillespie, R.G., 2004. Convergent evolution of behavior in an adaptive radiation of Hawaiian web-building spiders. *Proc. Natl. Acad. Sci. U. S. A.* 101, 16228–16233. doi:10.1073/pnas.0407395101
- Blomquist, G.J., Bagnères, A.-G., 2010. *Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology*. Cambridge University Press.
- Bookstein, F.L., 1997. *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge University Press.
- Boulanger, N., Rais, O., Vial, L., 2015. Annexe 3 Elevage de tiques dures et de tiques molles, in: *Tiques et Maladies À Tiques*. McCoy K. D., Boulanger N., pp. 295–300.
- Boulinier, T., Danchin, E., 1996. Population trends in Kittiwake *Rissa tridactyla* colonies in relation to tick infestation. *Ibis* 138, 326–334. doi:10.1111/j.1474-919X.1996.tb04345.x
- Boulinier, T., Kada, S., Ponchon, A., Dupraz, M., Dietrich, M., Gamble, A., Bourret, V., Duriez, O., Bazire, R., Tornos, J., Tveraa, T., Chambert, T., Garnier, R., McCoy, K.D., 2016. Migration, Prospecting, Dispersal? What Host Movement Matters for Infectious Agent Circulation? *Integr. Comp. Biol.* icw015. doi:10.1093/icb/icw015
- Boulinier, T., McCoy, K.D., Yoccoz, N.G., Gasparini, J., Tveraa, T., 2008. Public information affects breeding dispersal in a colonial bird: kittiwakes cue on neighbours. *Biol. Lett.* 4, 538–540. doi:10.1098/rsbl.2008.0291
- Brossard, M., Wikel, S.K., 2004. Tick immunobiology. *Parasitology* 129, S161–S176. doi:10.1017/S0031182004004834
- Bush, G.L., Butlin, R.K., 2004. Sympatric Speciation in Insects, in: Dieckmann, U., Doebeli, M., Metz, J.A.J., Tautz, D. (Eds.), *Adaptive Speciation*. Cambridge University Press, Cambridge, pp. 229–248.
- Camicas, J.-L., 1980. Les arbovirus à tiques en zone tropicale. *Médecine Trop.* 40, 499–508.
- Camicas, J.-L., Morel, P.C., 1977. Position systématique et classification des tiques (Acarida : Ixodida). *Acarologia* 18, 410–420.
- Chamberlin, R.V., 1920. *South American Arachnida, chiefly from the guano islands of Peru.*, Brooklyn, N.Y.,
- Chambert, T., Staszewski, V., Lobato, E., Choquet, R., Carrie, C., McCoy, K.D., Tveraa, T., Boulinier, T., 2012. Exposure of black-legged kittiwakes to Lyme disease spirochetes: dynamics of the immune status of adult hosts and effects on their survival. *J. Anim. Ecol.* 81, 986–995. doi:10.1111/j.1365-2656.2012.01979.x
- Chastel, C., Main, A.J., Guiguen, C., Lay, G. le, Quillien, M.C., Monnat, J.Y., Beaucournu, J.C., 1985. The isolation of Meaban virus, a new Flavivirus from the seabird tick *Ornithodoros (Alectorobius) maritimus* in France. *Arch. Virol.* 83, 129–140. doi:10.1007/BF01309911
- Clayton, D.H., Bush, S.E., Johnson, K.P., 2015. *Coevolution of Life on Hosts: Integrating Ecology and History*. University of Chicago Press.

- Clayton, D.H., Cotgreave, P., 1994. Relationship of bill morphology to grooming behaviour in birds. *Anim. Behav.* 47, 195–201. doi:10.1006/anbe.1994.1022
- Clayton, D.H., Koop, J.A., Harbison, C.W., Moyer, B.R., Bush, S.E., 2010. How birds combat ectoparasites. *Open Ornithol J* 3, 41–71.
- Clayton, D.H., Lee, P.L.M., Tompkins, III, E.D.B., 1999. Reciprocal Natural Selection on Host-Parasite Phenotypes. *Am. Nat.* 154, 261–270. doi:10.1086/303237
- Clayton, D.H., Moyer, B.R., Bush, S.E., Jones, T.G., Gardiner, D.W., Rhodes, B.B., Goller, F., 2005. Adaptive significance of avian beak morphology for ectoparasite control. *Proc. R. Soc. Lond. B Biol. Sci.* 272, 811–817. doi:10.1098/rspb.2004.3036
- Cleaveland, S., Laurenson, M.K., Taylor, L.H., 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. *Philos. Trans. R. Soc. B Biol. Sci.* 356, 991–999. doi:10.1098/rstb.2001.0889
- Colas-Belcour, J., Rageau, J., 1960. Biologie et élevage d'*Ornithodoros capensis* Neumann (acarien Argasidae). *Bull. Société Pathol. Exot.* 53, 723–727.
- Collins, F.H., Paskewitz, S.M., 1996. A review of the use of ribosomal DNA (rDNA) to differentiate among cryptic *Anopheles* species. *Insect Mol. Biol.* 5, 1–9. doi:10.1111/j.1365-2583.1996.tb00034.x
- Colosimo, P.F., Hosemann, K.E., Balabhadra, S., Villarreal, G., Dickson, M., Grimwood, J., Schmutz, J., Myers, R.M., Schluter, D., Kingsley, D.M., 2005. Widespread Parallel Evolution in Sticklebacks by Repeated Fixation of Ectodysplasin Alleles. *Science* 307, 1928–1933. doi:10.1126/science.1107239
- Converse, J.D., Hoogstraal, H., Moussa, M.I., Feare, C.J., Kaiser, M.N., 1975. Soldado virus (Hughes group) from *Ornithodoros (Alectorobius) capensis* (Ixodoidea: Argasidae) infesting Sooty Tern colonies in the Seychelles, Indian Ocean. *Am. J. Trop. Med. Hyg.* 24, 1010–1018.
- Coulson, JohnC, 2001. Colonial Breeding in Seabirds, in: *Biology of Marine Birds, Marine Biology*. CRC Press, pp. 87–113.
- Cumming, G.S., 1999. Host distributions do not limit the species ranges of most African ticks (Acari: Ixodida). *Bull. Entomol. Res.* 89, 303–327. doi:10.1017/S0007485399000450
- Dambroski, H.R., Linn, C., Berlocher, S.H., Forbes, A.A., Roelofs, W., Feder, J.L., 2005. The Genetic Basis for Fruit Odor Discrimination in *Rhagoletis* Flies and Its Significance for Sympatric Host Shifts. *Evolution* 59, 1953–1964. doi:10.1111/j.0014-3820.2005.tb01065.x
- Danchin, E., 1992. The incidence of the tick parasite *Ixodes uriae* in Kittiwake *Rissa tridactyla* colonies in relation to the age of the colony, and a mechanism of infecting new colonies. *Ibis* 134, 134–141. doi:10.1111/j.1474-919X.1992.tb08390.x
- De Meeûs, T., Michalakis, Y., Renaud, F., 1998. Santa Rosalia Revisited: or Why Are There So Many Kinds of Parasites in 'The Garden of Earthly Delights'? *Parasitol. Today* 14, 10–13. doi:10.1016/S0169-4758(97)01163-0



- de Meeûs, T., Renaud, F., 2002. Parasites within the new phylogeny of eukaryotes. *Trends Parasitol.* 18, 247–251. doi:10.1016/S1471-4922(02)02269-9
- Debat, V., Peronnet, F., 2013. Asymmetric flies: The control of developmental noise in *Drosophila*. *Fly* (Austin) 7, 70–77. doi:10.4161/fly.23558
- Dietrich, M., Beati, L., Elguero, E., Boulinier, T., McCoy, K.D., 2013. Body size and shape evolution in host races of the tick *Ixodes uriae*. *Biol. J. Linn. Soc.* 108, 323–334. doi:10.1111/j.1095-8312.2012.02021.x
- Dietrich, M., Gómez-Díaz, E., McCoy, K.D., 2011. Worldwide Distribution and Diversity of Seabird Ticks: Implications for the Ecology and Epidemiology of Tick-Borne Pathogens. *Vector-Borne Zoonotic Dis.* 11, 453–470. doi:10.1089/vbz.2010.0009
- Dietrich, M., Kempf, F., Boulinier, T., McCoy, K.D., 2014a. Tracing the colonization and diversification of the worldwide seabird ectoparasite *Ixodes uriae*. *Mol. Ecol.* 23, 3292–3305. doi:10.1111/mec.12815
- Dietrich, M., Kempf, F., Gómez-Díaz, E., Kitaysky, A.S., Hipfner, J.M., Boulinier, T., McCoy, K.D., 2012. Inter-oceanic variation in patterns of host-associated divergence in a seabird ectoparasite. *J. Biogeogr.* 39, 545–555. doi:10.1111/j.1365-2699.2011.02620.x
- Dietrich, M., Lobato, E., Boulinier, T., McCoy, K.D., 2014b. An experimental test of host specialization in a ubiquitous polar ectoparasite: a role for adaptation? *J. Anim. Ecol.* 83, 576–587. doi:10.1111/1365-2656.12170
- Dif, G., Hainard, R., Lamouche, T., Dorst, J., 1982. *Les oiseaux de mer d'Europe*. Arthaud, Paris.
- Drès, M., Mallet, J., 2002. Host races in plant-feeding insects and their importance in sympatric speciation. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 357, 471–492. doi:10.1098/rstb.2002.1059
- Duffy, D.C., 1983. The Ecology of Tick Parasitism on Densely Nesting Peruvian Seabirds. *Ecology* 64, 110. doi:10.2307/1937334
- Dujardin, J.-P., 2011. Modern Morphometrics of Medically Important Insects, in: *Genetics and Evolution of Infectious Disease*. Elsevier, pp. 473–501.
- Dujardin, J.-P., 2008. Morphometrics applied to medical entomology. *Infect. Genet. Evol.* 8, 875–890. doi:10.1016/j.meegid.2008.07.011
- Dujardin, J.P., Bermudez, H., Casini, C., Schofield, C.J., Tibayrenc, M., 1997. Metric Differences Between Silvatic and Domestic *Triatoma infestans* (Heteroptera: Reduviidae) in Bolivia. *J. Med. Entomol.* 34, 544–551. doi:10.1093/jmedent/34.5.544
- Dunbar, H.E., Wilson, A.C.C., Ferguson, N.R., Moran, N.A., 2007. Aphid Thermal Tolerance Is Governed by a Point Mutation in Bacterial Symbionts. *PLOS Biol* 5, e96. doi:10.1371/journal.pbio.0050096
- Duron, O., Noël, V., McCoy, K.D., Bonazzi, M., Sidi-Boumedine, K., Morel, O., Vavre, F., Zenner, L., Jourdain, E., Durand, P., Arnathau, C., Renaud, F., Trape, J.-F., Biguezoton, A.S., Cremaschi, J., Dietrich, M., Léger, E., Appelgren, A., Dupraz, M., Gómez-Díaz, E., Diatta, G., Dayo, G.-K.,

- Adakal, H., Zoungrana, S., Vial, L., Chevillon, C., 2015. The Recent Evolution of a Maternally-Inherited Endosymbiont of Ticks Led to the Emergence of the Q Fever Pathogen, *Coxiella burnetii*. *PLoS Pathog* 11, e1004892. doi:10.1371/journal.ppat.1004892
- Dusbábek, F., Šimek, P., Jegorov, A., Tříska, J., 1991. Identification of xanthine and hypoxanthine as components of assembly pheromone in excreta of argasid ticks. *Exp. Appl. Acarol.* 11, 307–316. doi:10.1007/BF01202877
- Estrada-Peña, A., 2001. Forecasting habitat suitability for ticks and prevention of tick-borne diseases. *Vet. Parasitol., Promotion Advancement, Preserving Tradition* 98, 111–132. doi:10.1016/S0304-4017(01)00426-5
- Estrada-Peña, A., 1993. Climate and cuticular hydrocarbon variation in *Rhipicephalus sanguineus* ticks (Acar: Ixodidae). *Parasitol. Res.* 79, 512–516. doi:10.1007/BF00931594
- Estrada-Peña, A., Dusbábek, F., 1993. Cuticular hydrocarbon gas chromatography analysis of *Argas vulgaris*, *A. polonicus*, and their hybrids. *Exp. Appl. Acarol.* 17, 365–376. doi:10.1007/BF00058599
- Estrada-Peña, A., Gray, J.S., Kahl, O., 1996. Variability in cuticular hydrocarbons and phenotypic discrimination of *Ixodes ricinus* populations (Acarina: Ixodidae) from Europe. *Exp. Appl. Acarol.* 20, 457–466. doi:10.1007/BF00053309
- Estrada-Peña, A., Mangold, A.J., Nava, S., Venzal, J.M., Labruna, M., Guglielmone, A.A., 2010. A review of the systematics of the tick family Argasidae (Ixodida). *Acarologia* 50, 317–333. doi:10.1051/acarologia/20101975
- Eveleigh, E.S., Threlfall, W., 1974. The biology of *Ixodes (Ceraticodes) uriae* White, 1852 in Newfoundland. *Acarologia* 16, 621–635.
- Falconer, D.S., 1960. Introduction to quantitative genetics. New York,: Ronald Press Co.
- Feare, C.J., 1976. Desertion and Abnormal Development in a Colony of Sooty Terns *Sterna Fuscata* Infested by Virus-Infected Ticks. *Ibis* 118, 112–115. doi:10.1111/j.1474-919X.1976.tb02015.x
- Feder, J.L., Opp, S.B., Wlazlo, B., Reynolds, K., Go, W., Spisak, S., 1994. Host fidelity is an effective premating barrier between sympatric races of the apple maggot fly. *PNAS* 91, 7990–7994.
- Foster, D.J., Podos, J., Hendry, A.P., 2008. A geometric morphometric appraisal of beak shape in Darwin's finches. *J. Evol. Biol.* 21, 263–275. doi:10.1111/j.1420-9101.2007.01449.x
- Futuyma, D.J., Moreno, G., 1988. The Evolution of Ecological Specialization. *Annu. Rev. Ecol. Syst.* 19, 207–233.
- Gandon, S., 2002. Local adaptation and the geometry of host–parasite coevolution. *Ecol. Lett.* 5, 246–256. doi:10.1046/j.1461-0248.2002.00305.x
- Garros, C., Dujardin, J.-P., 2013. Genetic and Phenetic Approaches to Anopheles Systematics, in: Manguin, S. (Ed.), *Anopheles Mosquitoes - New Insights into Malaria Vectors*. InTech.
- Gasparini, J., McCoy, K.D., Haussy, C., Tveraa, T., Boulinier, T., 2001. Induced maternal response to the Lyme disease spirochaete *Borrelia burgdorferi sensu lato* in a colonial seabird, the kittiwake *Rissa tridactyla*. *Proc. R. Soc. Lond. B Biol. Sci.* 268, 647–650. doi:10.1098/rspb.2000.1411

- Gibbs, A., Mousseau, T.A., Crowe, J.H., 1991. Genetic and acclimatory variation in biophysical properties of insect cuticle lipids. *Proc. Natl. Acad. Sci.* 88, 7257–7260. doi:10.1073/pnas.88.16.7257
- Gołębiowski, M., Boguś, M.I., Paszkiewicz, M., Stepnowski, P., 2010. Cuticular lipids of insects as potential biofungicides: methods of lipid composition analysis. *Anal. Bioanal. Chem.* 399, 3177–3191. doi:10.1007/s00216-010-4439-4
- Gómez-Díaz, E., Doherty Jr, P.F., Duneau, D., McCoy, K.D., 2010. Cryptic vector divergence masks vector-specific patterns of infection: an example from the marine cycle of Lyme borreliosis: Divergence and vector-specific infection. *Evol. Appl.* 3, 391–401. doi:10.1111/j.1752-4571.2010.00127.x
- Gómez-Díaz, E., Morris-Pocock, J.A., González-Solís, J., McCoy, K.D., 2012. Trans-oceanic host dispersal explains high seabird tick diversity on Cape Verde islands. *Biol. Lett.* 8, 616–619. doi:10.1098/rsbl.2012.0179
- Grant, P.R., Grant, B.R., 2008. Pedigrees, assortative mating and speciation in Darwin's finches. *Proc. R. Soc. Lond. B Biol. Sci.* 275, 661–668. doi:10.1098/rspb.2007.0898
- Grémillet, D., Chauvin, C., Wilson, R.P., Le Maho, Y., Wanless, S., 2005. Unusual feather structure allows partial plumage wettability in diving great cormorants *Phalacrocorax carbo*. *J. Avian Biol.* 36, 57–63. doi:10.1111/j.0908-8857.2005.03331.x
- Guglielmone, A.A., Robbins, R.G., Apanaskevich, D.A., Petney, T.N., Estrada-Pena, A., Horak, I.G., Shao, R., Barker, S.C., 2010. The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida) of the world : a list of valid species names.
- Hoberg, E.P., Brooks, D.R., 2015. Evolution in action: climate change, biodiversity dynamics and emerging infectious disease. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 370, 20130553. doi:10.1098/rstb.2013.0553
- Hochberg, M.E., Baalen, M. van, 1998. Antagonistic Coevolution over Productivity Gradients. *Am. Nat.* 152, 620–634. doi:10.1086/286194
- Hoogstraal, H., 1985. Argasid and Nuttalliellid Ticks as Parasites and Vectors, in: J.R. Baker and R. Muller (Ed.), *Advances in Parasitology*. Academic Press, pp. 135–238.
- Hoogstraal, H., 1969. *Ornithodoros (Alectorobius) muesebecki* n. sp., a parasite of the blue-faced booby (*Sula dactylatra melanops*) on Hasikiya Island, Arabian Sea. *Proc. Entomol. Soc. Wash.* 71, 368–374.
- Hoogstraal, H., Wassef, H.Y., Hays, C., Keirans, J.E., 1985. *Ornithodoros (Alectorobius) spheniscus* n. sp. [Acarina: Ixodoidea: Argasidae: *Ornithodoros (Alectorobius) capensis* group], a tick parasite of the Humboldt penguin in Peru. *J. Parasitol.* 71, 635–644.
- Horak, I.G., Camicas, J.-L., Keirans, J.E., 2002. The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida): A World List of Valid Tick Names. *Exp. Appl. Acarol.* 28, 27–54. doi:10.1023/A:1025381712339

- Hosokawa, T., Kikuchi, Y., Shimada, M., Fukatsu, T., 2007. Obligate symbiont involved in pest status of host insect. *Proc. R. Soc. Lond. B Biol. Sci.* 274, 1979–1984. doi:10.1098/rspb.2007.0620
- Jaenike, J., 1981. Criteria for Ascertaining the Existence of Host Races. *Am. Nat.* 117, 830–834.
- Jallon, J.-M., David, J.R., 1987. Variation in Cuticular Hydrocarbons Among the Eight Species of the *Drosophila melanogaster* Subgroup. *Evolution* 41, 294–302. doi:10.2307/2409139
- Johnson, K.P., Shreve, S.M., Smith, V.S., 2012. Repeated adaptive divergence of microhabitat specialization in avian feather lice. *BMC Biol.* 10, 52. doi:10.1186/1741-7007-10-52
- Jongejan, F., Uilenberg, G., 2004. The global importance of ticks. *Parasitology* 129, S3–S14. doi:10.1017/S0031182004005967
- Kaba, D., Ravel, S., Acapovi-Yao, G., Solano, P., Allou, K., Bosson-Vanga, H., Gardes, L., N’Goran, E.K., Schofield, C.J., Kone, M., Dujardin, J.-P., 2012. Phenetic and genetic structure of tsetse fly populations (*Glossina palpalis palpalis*) in southern Ivory Coast. *Parasit. Vectors* 5, 153. doi:10.1186/1756-3305-5-153
- Keirans, J.E., Clifford, C.M., Hoogstraal, H., 1984. ORNITHODOROS (ALECTOROBIUS) YUNKERI, NEW SPECIES (ACARI: IXODOIDEA: ARGASIDAE), FROM SEABIRDS AND NESTING SITES IN THE GALAPAGOS ISLANDS. *J. Med. Entomol.* 21, 344–350.
- Kempf, F., Boulinier, T., De Meeûs, T., Arnathau, C., McCOY, K.D., 2009. Recent evolution of host-associated divergence in the seabird tick *Ixodes uriae*. *Mol. Ecol.* 18, 4450–4462. doi:10.1111/j.1365-294X.2009.04356.x
- Kitaoka, S., Suzuki, H., 1973. *Ornithodoros (Alectorobius) sawaii* sp. n. (Ixodoidea, Argasidae) associated with the streaked shear-water, *Colonectris leucomelas*, from the Amami-oshima Islands, Japan. *Natl. Inst. Anim. Health Q. (Tokyo)* 13, 142–148.
- Klompen, J.S.H., Black, W.C., Keirans, J.E., J. H. Oliver, J., 1996. Evolution of Ticks. *Annu. Rev. Entomol.* 41, 141–161. doi:10.1146/annurev.en.41.010196.001041
- Klompen, J.S.H., Oliver, J.H., 1993. Systematic relationships in the soft ticks (Acari: Ixodida: Argasidae). *Syst. Entomol.* 18, 313–331. doi:10.1111/j.1365-3113.1993.tb00669.x
- Kocher, T.D., Conroy, J.A., McKaye, K.R., Stauffer, J.R., 1993. Similar Morphologies of Cichlid Fish in Lakes Tanganyika and Malawi Are Due to Convergence. *Mol. Phylogenet. Evol.* 2, 158–165. doi:10.1006/mpev.1993.1016
- Kohls, G.M., Sonenshine, D.E., Clifford, C.M., 1965. The systematics of the subfamily Ornithodorinae (Acarina: Argasidae). II. Identification of the larvae of the Western Hemisphere and descriptions of three new species. *Ann. Entomol. Soc. Am.* 58, 331–364.
- Lawing, A.M., Polly, P.D., 2010. Geometric morphometrics: recent applications to the study of evolution and development. *J. Zool.* 280, 1–7. doi:10.1111/j.1469-7998.2009.00620.x
- Lecointre, G., Guyader, H.L., 2001. *Classification phylogénétique du vivant*. Belin.
- Ledevin, R., Michaux, J.R., Deffontaine, V., Henttonen, H., Renaud, S., 2010. Evolutionary history of the bank vole *Myodes glareolus*: a morphometric perspective. *Biol. J. Linn. Soc.* 100, 681–694.

- Lehmann, T., 1993. Ectoparasites: Direct impact on host fitness. *Parasitol. Today* 9, 8–13. doi:10.1016/0169-4758(93)90153-7
- Levene, H., 1953. Genetic Equilibrium When More Than One Ecological Niche is Available. *Am. Nat.* 87, 331–333.
- Levins, R., MacArthur, R., 1966. The Maintenance of Genetic Polymorphism in a Spatially Heterogeneous Environment: Variations on a Theme by Howard Levene. *Am. Nat.* 100, 585–589.
- Lockey, K.H., 1988. Lipids of the insect cuticle: origin, composition and function. *Comp. Biochem. Physiol. Part B Comp. Biochem.* 89, 595–645.
- Losos, J.B., 1990. Concordant evolution of locomotor behaviour, display rate and morphology in Anolis lizards. *Anim. Behav.* 39, 879–890. doi:10.1016/S0003-3472(05)80952-2
- Magalhães, S., Forbes, M.R., Skoracka, A., Osakabe, M., Chevillon, C., McCoy, K.D., 2007. Host race formation in the Acari. *Exp. Appl. Acarol.* 42, 225–238. doi:10.1007/s10493-007-9091-0
- Mangin, S., Gauthier-Clerc, M., Frenot, Y., Gendner, J.-P., Le Maho, Y., 2003. Ticks *Ixodes uriae* and the breeding performance of a colonial seabird, king penguin *Aptenodytes patagonicus*. *J. Avian Biol.* 34, 30–34. doi:10.1034/j.1600-048X.2003.02916.x
- Mans, B.J., Neitz, A.W.H., 2004. The sand tampan, *Ornithodoros savignyi*, as a model for tick-host interactions. *South Afr. J. Sci.* 100, 283–288.
- Mans, B.J., Steinmann, C.M.L., Venter, J.D., Louw, A.I., Neitz, A.W.H., 2002. Pathogenic mechanisms of sand tampan toxicoses induced by the tick, *Ornithodoros savignyi*. *Toxicon* 40, 1007–1016. doi:10.1016/S0041-0101(02)00098-3
- McCoy, K., Beis, P., Barbosa, A., Cuervo, J., Fraser, W., González-Solís, J., Jourdain, E., Poisbleau, M., Quillfeldt, P., Léger, E., Dietrich, M., 2012. Population genetic structure and colonisation of the western Antarctic Peninsula by the seabird tick *Ixodes uriae*. *Mar. Ecol. Prog. Ser.* 459, 109–120. doi:10.3354/meps09749
- McCoy, K.D., Boulinier, T., Chardine, J.W., Danchin, E., Michalakis, Y., 1999. Dispersal and Distribution of the Tick *Ixodes uriae* within and among Seabird Host Populations: The Need for a Population Genetic Approach. *J. Parasitol.* 85, 196. doi:10.2307/3285619
- McCoy, K.D., Boulinier, T., Schjørring, S., Michalakis, Y., 2002. Local adaptation of the ectoparasite *Ixodes uriae* to its seabird host. *Evol. Ecol. Res.* 4, 441–456.
- McCoy, K.D., Boulinier, T., Tirard, C., Michalakis, Y., 2003. Host-Dependent Genetic Structure of Parasite Populations: Differential Dispersal of Seabird Tick Host Races. *Evolution* 57, 288–296.
- McCoy, K.D., Boulinier, T., Tirard, C., Michalakis, Y., 2001. Host specificity of a generalist parasite: genetic evidence of sympatric host races in the seabird tick *Ixodes uriae*. *J. Evol. Biol.* 14, 395–405. doi:10.1046/j.1420-9101.2001.00290.x
- McCoy, K.D., Chapuis, E., Tirard, C., Boulinier, T., Michalakis, Y., Bohec, C.L., Maho, Y.L., Gauthier-Clerc, M., 2005. Recurrent evolution of host-specialized races in a globally distributed parasite. *Proc. R. Soc. B Biol. Sci.* 272, 2389–2395. doi:10.1098/rspb.2005.3230



- McCoy, K.D., Dietrich, M., Jaeger, A., Wilkinson, D.A., Bastien, M., Lagadec, E., Boulinier, T., Pascalis, H., Tortosa, P., Le Corre, M., Dellagi, K., Lebarbenchon, C., 2016. The role of seabirds of the Îles Éparses as reservoirs and disseminators of parasites and pathogens. *Acta Oecologica, Îles Éparses (French Scattered Islands, SW Indian Ocean) as reference ecosystems for environmental research*. 72, 98–109. doi:10.1016/j.actao.2015.12.013
- McCoy, K.D., Leger, E., Dietrich, M., 2013. Host specialization in ticks and transmission of tick-borne diseases: a review. *Front. Cell. Infect. Microbiol.* 3. doi:10.3389/fcimb.2013.00057
- Meyers, L.A., Bull, J.J., 2002. Fighting change with change: adaptive variation in an uncertain world. *Trends Ecol. Evol.* 17, 551–557. doi:10.1016/S0169-5347(02)02633-2
- Michaux, J., Chevret, P., Renaud, S., 2007. Morphological diversity of Old World rats and mice (Rodentia, Muridae) mandible in relation with phylogeny and adaptation. *J. Zool. Syst. Evol. Res.* 45, 263–279. doi:10.1111/j.1439-0469.2006.00390.x
- Mopper, S., Strauss, S.Y., 2013. *Genetic Structure and Local Adaptation in Natural Insect Populations: Effects of Ecology, Life History, and Behavior*. Springer Science & Business Media.
- Muschick, M., Indermaur, A., Salzburger, W., 2012. Convergent Evolution within an Adaptive Radiation of Cichlid Fishes. *Curr. Biol.* 22, 2362–2368. doi:10.1016/j.cub.2012.10.048
- Nava, S., Guglielmo, A.A., 2013. A meta-analysis of host specificity in Neotropical hard ticks (Acari: Ixodidae). *Bull. Entomol. Res.* 103, 216–224. doi:10.1017/S0007485312000557
- Navia, D., Mendonça, R.S., Ferragut, F., Miranda, L.C., Trincado, R.C., Michaux, J., Navajas, M., 2013. Cryptic diversity in *Brevipalpus* mites (Tenuipalpidae). *Zool. Scr.* 42, 406–426. doi:10.1111/zsc.12013
- Nene, V., 2009. Tick Genomics - Coming of age. *Front. Biosci.* 14, 2666–2673.
- Neumann, G., 1901. Révision de la Famille des Ixodidés (4e Mémoire), in: *Mémoires de La Société Zoologique de France*. pp. 249–372.
- Ostfeld, R.S., Glass, G.E., Keesing, F., 2005. Spatial epidemiology: an emerging (or re-emerging) discipline. *Trends Ecol. Evol.*, SPECIAL ISSUE: BUMPER BOOK REVIEW 20, 328–336. doi:10.1016/j.tree.2005.03.009
- Pappers, S.M., Velde, G. van der, Ouborg, N.J., Groenendaal, J.M. van, 2002. Genetically Based Polymorphisms in Morphology and Life History Associated with Putative Host Races of the Water Lily Leaf Beetle, *Galerucella Nymphaeae*. *Evolution* 56, 1610–1621. doi:10.1111/j.0014-3820.2002.tb01473.x
- Parola, P., Raoult, D., 2001. Tick-borne bacterial diseases emerging in Europe. *Clin. Microbiol. Infect.* 7, 80–83. doi:10.1046/j.1469-0691.2001.00200.x
- Patterson, J.S., Schofield, C.J., Dujardin, J.P., Miles, M.A., 2001. Population morphometric analysis of the tropicopolitan bug *Triatoma rubrofasciata* and relationships with Old World species of *Triatoma*: evidence of New World ancestry. *Med. Vet. Entomol.* 15, 443–451. doi:10.1046/j.0269-283x.2001.00333.x
- PÉREZ-EID, C., 2007. *Les tiques : identification, biologie, importance médicale et vétérinaire*. Lavoisier.



- Ponchon, A., Grémillet, D., Christensen-Dalsgaard, S., Erikstad, K.E., Barrett, R.T., Reiertsen, T.K., McCoy, K.D., Tveraa, T., Boulinier, T., 2014. When things go wrong: intra-season dynamics of breeding failure in a seabird. *Ecosphere* 5, 1–19. doi:10.1890/ES13-00233.1
- Pospelova-Shtrom, M.V., 1969. On the system of classification of ticks of the family Argasidae Can., 1890. *Acarologia* 11, 1–22.
- Poulin, R., Morand, S., 2000. The Diversity of Parasites. *Q. Rev. Biol.* 75, 277–293.
- Raup, D.M., 1966. Geometric analysis of shell coiling: general problems. *J. Paleontol.* 1178–1190.
- Ravigné, V., Barberousse, A., Bierne, N., Britton-Davidian, J., Capy, P., Desdevises, Y., Giraud, T., Jousset, E., Mouliat, C., Smadja, C., Samadi, S., 2010. La spéciation, in: Thomas, F., Lefèvre, T., Raymond, M. (Eds.), *Biologie évolutive*. De Boeck, Bruxelles, pp. 79–122.
- Ribeiro, J., 1995. Blood-feeding arthropods: live syringes or invertebrate pharmacologists? *Infect. Agents Dis.* 4, 143–152.
- Rice, W.R., 1987. Speciation via habitat specialization: the evolution of reproductive isolation as a correlated character. *Evol. Ecol.* 1, 301–314. doi:10.1007/BF02071555
- Rundell, R.J., Price, T.D., 2009. Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends Ecol. Evol.* 24, 394–399. doi:10.1016/j.tree.2009.02.007
- Russell, J.A., Moran, N.A., 2006. Costs and benefits of symbiont infection in aphids: variation among symbionts and across temperatures. *Proc. R. Soc. Lond. B Biol. Sci.* 273, 603–610. doi:10.1098/rspb.2005.3348
- Schluter, D., 2009. Evidence for ecological speciation and its alternative. *Science* 323, 737–741. doi:10.1126/science.1160006
- Shimshoni, J.A., Erster, O., Rot, A., Cuneah, O., Soback, S., Shkap, V., 2013. Cuticular fatty acid profile analysis of three *Rhipicephalus* tick species (Acari: Ixodidae). *Exp. Appl. Acarol.* 61, 481–489. doi:10.1007/s10493-013-9713-7
- Simmons, L.W., Thomas, M.L., Gray, B., Zuk, M., 2014. Replicated evolutionary divergence in the cuticular hydrocarbon profile of male crickets associated with the loss of song in the Hawaiian archipelago. *J. Evol. Biol.* n/a-n/a. doi:10.1111/jeb.12478
- Sonenshine, D.E., 1993. *Biology of ticks*. Volume 2. xvii + 465 pp.
- Sonenshine, D.E., 1985. Pheromones and Other Semiochemicals of the Acari. *Annu. Rev. Entomol.* 30, 1–28. doi:10.1146/annurev.en.30.010185.000245
- Sonenshine, D.E., Roe, R.M. (Eds.), 2014a. *Biology of ticks*, 2nd ed. ed. Oxford University Press, New York.
- Sonenshine, D.E., Roe, R.M., 2014b. *Biology of Ticks Vol.2*, Second edition. ed. Oxford University Press.

- Stern, D.L., 2013. The genetic causes of convergent evolution. *Nat. Rev. Genet.* 14, 751–764. doi:10.1038/nrg3483
- Takano, A., Muto, M., Sakata, A., Ogasawara, Y., Ando, S., Hanaoka, N., Tsurumi, M., Sato, F., Nakamura, N., Fujita, H., Watanabe, H., Kawabata, H., 2009. Relapsing Fever Spirochete in Seabird Tick, Japan. *Emerg. Infect. Dis.* 15, 1528–1530. doi:10.3201/eid1509.090459
- Thomas, F., Lefevre, T., Raymond, M., 2010. *Biologie évolutive*, Première édition. ed. DE BOECK UNIVERSITE, Bruxelles.
- Thompson, D.W., 1945. *On growth and form*. Cambridge : University Press ; New York : Macmillan.
- Thompson, J.N., 1997. Evaluating the Dynamics of Coevolution Among Geographically Structured Populations. *Ecology* 78, 1619–1623. doi:10.1890/0012-9658(1997)078[1619:ETDOCA]2.0.CO;2
- Tylianakis, J.M., Didham, R.K., Bascompte, J., Wardle, D.A., 2008. Global change and species interactions in terrestrial ecosystems. *Ecol. Lett.* 11, 1351–1363. doi:10.1111/j.1461-0248.2008.01250.x
- Van Valen, L., 1977. The Red Queen. *Am. Nat.* 111, 809–810. doi:10.1086/283213
- van Zweden, J.S., d’Ettorre, P., 2010. Nestmate recognition in social insects and the role of hydrocarbons. *Insect Hydrocarb. Biol. Biochem. Chem. Ecol.* 11, 222–243.
- van Zweden, J.S., Dreier, S., d’Ettorre, P., 2009. Disentangling environmental and heritable nestmate recognition cues in a carpenter ant. *J. Insect Physiol.* 55, 159–164. doi:10.1016/j.jinsphys.2008.11.001
- Vermeil, C., Marjolet, M., Vermeil, F., 1997. *Ornithodoros (Alectorobius) cheikhi* n.sp. (Acarina, Ixodoidea, Argasidae, groupe *Ornithodoros (Alectorobius) capensis*) ; Tique parasite de sternes au banc d’Arguin - Mauritanie. *Bull. Société Sci. Nat. Ouest Fr.* 19, 66–76.
- Vermeil, Merguet, 1967. Sur le diagnostic des larves d’*Ornithodoros* du complexe *coniceps-capensis* (Acarina : Argasidae). *Ornithodoros coniceps* (Canestrini, 1890) *maritimus* n. ssp. prévaut dans les îles de Basse-Bretagne. *Acarologia* 9, p.557-565.
- Wang, S., Meyer, E., McKay, J.K., Matz, M.V., 2012. 2b-RAD: a simple and flexible method for genome-wide genotyping. *Nat. Methods* 9, 808–810. doi:10.1038/nmeth.2023
- Wang, Z., Gerstein, M., Snyder, M., 2009. RNA-Seq: a revolutionary tool for transcriptomics. *Nat. Rev. Genet.* 10, 57–63. doi:10.1038/nrg2484
- Wiens, J.J., 2004. What Is Speciation and How Should We Study It? *Am. Nat.* 163, 914–923. doi:10.1086/an.2004.163.issue-6
- Wikel, S.K., 1984. Immunomodulation of host responses to ectoparasite infestation – an overview. *Vet. Parasitol., Special Issue Veterinary Immunoparasitology* 14, 321–339. doi:10.1016/0304-4017(84)90099-2

- Yang, S., Xu, Y., Zhang, D., 2006. Morphological basis for the waterproof characteristic of bird plumage. *J. For. Res.* 17, 163–166. doi:10.1007/s11676-006-0039-8
- Young, F.N., Goff, C.C., 1939. An Annotated List of the Arthropods Found in the Burrows of the Florida Gopher Tortoise, *Gopherus polyphemus* (Daudin). *Fla. Entomol.* 22, 53–62. doi:10.2307/3492191
- ZUMPT, F., 1952. The ticks of seabirds.



# Annexes

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## Annexe 1

Duron, O., Noël, V., McCoy, K.D., Bonazzi, M., Sidi-Boumedine, K., Morel, O., Vavre, F., Zenner, L., Jourdain, E., Durand, P., Arnathau, C., Renaud, F., Trape, J.-F., Biguezoton, A.S., Cremaschi, J., Dietrich, M., Léger, E., Appelgren, A., Dupraz, M., Gómez-Díaz, E., Diatta, G., Dayo, G.-K., Adakal, H., Zoungrana, S., Vial, L., Chevillon, C., 2015. The Recent Evolution of a Maternally-Inherited Endosymbiont of Ticks Led to the Emergence of the Q Fever Pathogen, *Coxiella burnetii*. PLoS Pathog 11, e1004892. doi:10.1371/journal.ppat.1004892



## RESEARCH ARTICLE

# The Recent Evolution of a Maternally-Inherited Endosymbiont of Ticks Led to the Emergence of the Q Fever Pathogen, *Coxiella burnetii*

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**Data Availability Statement:** Nucleotide sequences of PCR-amplified fragments of tick-borne *Coxiella* and *Rickettsiella* genes have been deposited in the GenBank nucleotide database under accession codes KP994768-KP994862 (16S rRNA), KP994678-KP994767 (23S rRNA), KP985445-KP985537 (GroEL), KP985265-KP985357 (rpoB) and KP985358-KP985444 (dnaK).

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## Abstract

Q fever is a highly infectious disease with a worldwide distribution. Its causative agent, the intracellular bacterium *Coxiella burnetii*, infects a variety of vertebrate species, including humans. Its evolutionary origin remains almost entirely unknown and uncertainty persists regarding the identity and lifestyle of its ancestors. A few tick species were recently found to harbor maternally-inherited *Coxiella*-like organisms engaged in symbiotic interactions, but their relationships to the Q fever pathogen remain unclear. Here, we extensively sampled ticks, identifying new and atypical *Coxiella* strains from 40 of 58 examined species, and used this data to infer the evolutionary processes leading to the emergence of *C. burnetii*. Phylogenetic analyses of multi-locus typing and whole-genome sequencing data revealed that *Coxiella*-like organisms represent an ancient and monophyletic group allied to ticks. Remarkably, all known *C. burnetii* strains originate within this group and are the descendants of a *Coxiella*-like progenitor hosted by ticks. Using both colony-reared and field-collected

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gravid females, we further establish the presence of highly efficient maternal transmission of these *Coxiella*-like organisms in four examined tick species, a pattern coherent with an endosymbiotic lifestyle. Our laboratory culture assays also showed that these *Coxiella*-like organisms were not amenable to culture in the vertebrate cell environment, suggesting different metabolic requirements compared to *C. burnetii*. Altogether, this corpus of data demonstrates that *C. burnetii* recently evolved from an inherited symbiont of ticks which succeeded in infecting vertebrate cells, likely by the acquisition of novel virulence factors.

### Author Summary

How virulent infectious diseases emerge from non-pathogenic organisms is a challenging question. Here, we address this evolutionary issue in the case of Q fever. Its causative agent, the intracellular bacterium *Coxiella burnetii*, is extremely infectious to humans and a variety of animals. However, uncertainty persists regarding its evolutionary origin, including the identity and lifestyle of its ancestors. In this article, we show that *C. burnetii* arose from a rare evolutionary transformation of a maternally-inherited endosymbiont of ticks into a specialized and virulent pathogen of vertebrates. While arthropod symbionts are typically transmitted maternally and thought not to be infectious to vertebrates, we establish here that one *Coxiella* symbiont has evolved the necessary adaptations to exploit the vertebrate cell, leading to the emergence of Q fever.

### Introduction

‘Query fever’ (Q fever) is a highly infectious zoonotic disease first identified in 1937 [1,2,3,4,5]. The causative agent, the obligate intracellular bacterium *Coxiella burnetii*, infects a variety of vertebrate species, including humans. Sporadic cases in humans occur annually worldwide, but occasional outbreaks are also common [1,2,3,4]. For example, in the Netherlands more than 4,000 human cases were reported between 2007 and 2010 [6]. While most human cases are self-limiting with fever and fatigue, acute forms range from mild flu-like symptoms to pneumonia or hepatitis. The disease can also become chronic (mainly endocarditis), and, though rarely fatal, remains highly debilitating even when treated with antibiotics [1,2]. Most human cases are linked to contact with infected livestock, especially goats and sheep, which suffer abortion and reproductive disorders. Infection usually occurs by the inhalation of aerosolized resistant small cell variants that are present in the excretions of infected animals. Other modes of transmission including ingestion of unpasteurized milk or dairy products; human-to-human contact is also possible but considered rare [1,2,4,5]. One of the most virulent reference strains of *C. burnetii* (strain RSA 493 / Nine Mile I [7]) was isolated from a guinea pig on which field-collected Rocky Mountain wood ticks *Dermacentor andersoni* had fed, suggesting that transmission through tick bites may also occur [8]. The small cell variants of the bacterium can survive and remain highly infectious for long periods in the environment, leading to the classification of *C. burnetii* as potential bioterrorism agent [9].

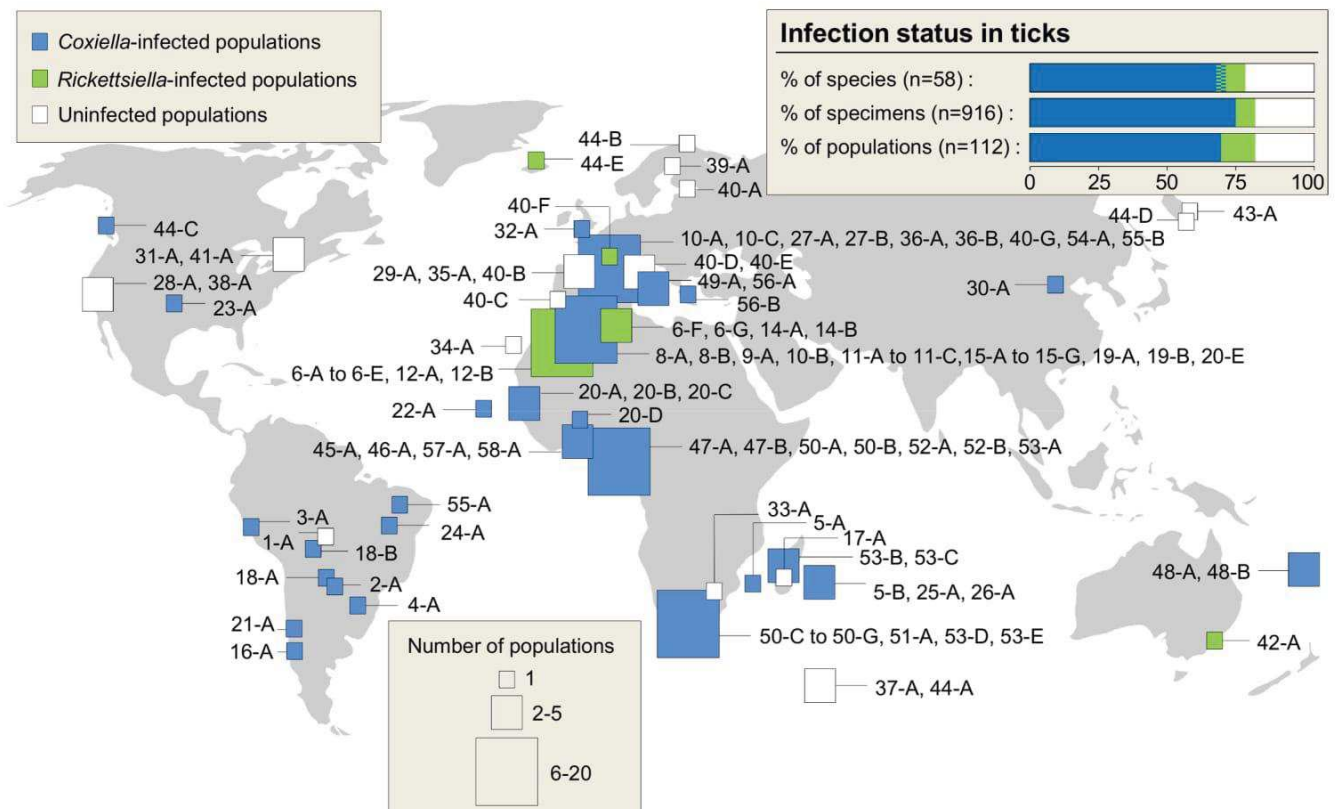
The evolutionary origin of Q fever is unclear since the *C. burnetii* ancestor and its primary lifestyle remain entirely unknown. Historically, *C. burnetii* was assigned to the taxonomic order Rickettsiales (Alphaproteobacteria), but it has been recently considered more closely related to the Legionellales order (Gammaproteobacteria) because of its genetic proximity to the Legionnaires’ disease agent, *Legionella pneumophila* [10]. The Legionellales order includes



many other intracellular bacteria infecting non-vertebrate species, such as, for instance, *Rickettsiella* species that are both widespread and biologically diverse in arthropods [11,12,13]. Within the *Coxiella* genus, the only known relative of *C. burnetii* which has been formally identified is *C. cheraxi*, a pathogen of crayfishes [14]. Many past descriptions of *Coxiella* were likely biased toward the detection of pathogenic strains since most *C. burnetii* isolates were collected from humans or domestic ruminants during Q fever outbreaks [1,5,15]. However, the advent of 16S rRNA gene sequencing as a universal DNA barcoding marker in bacteria has led to the description of a few novel *Coxiella*-like organisms in non-vertebrate species (listed in [16]), and particularly in ticks [17,18,19,20,21,22,23,24,25]. All these *Coxiella*-like organisms are closely related, but genetically distinct to *C. burnetii*, suggesting that some diversity exists within the *Coxiella* genus. The highly conserved nature of the 16S rRNA gene sequences has prevented researchers from establishing the exact relationship between *C. burnetii* and *Coxiella*-like organisms, and a sister clade relationship is commonly assumed [16,18,21,22,23].

The *Coxiella*-like organisms differ from *C. burnetii* in their biological traits and some may behave as subtle symbionts engaged in intricate interactions with ticks. In ticks belonging to *Ornithodoros*, *Amblyomma* and *Rhipicephalus* genera, *Coxiella*-like organisms were found to massively infect ovaries and to be maternally inherited through the egg cytoplasm [18,20,21,26]. In these tick species, the presence of the bacteria in the Malpighian tubules further suggests a possible role in nutrition by potentially provisioning their hosts with essential nutrients [20,21,26]. Indeed, the elimination of these bacteria with an antibiotic treatment was shown to negatively impact the fitness of the lone star tick *A. americanum* [27]. Accordingly, when the *Coxiella*-like bacterium found in *A. americanum* was recently sequenced [16], no recognizable virulence genes were found, indicating that this bacterium is likely non-pathogenic. In contrast, its genome encodes major vitamin and cofactor biosynthesis pathways, suggesting that it may be a vitamin-provisioning endosymbiont. This interaction exhibits the typical hallmarks of maternally-inherited symbionts with essential roles in arthropod biology [28,29]. Such patterns have been found in other exclusive blood-feeding species like bedbugs [30] and tsetse flies [31], two insect groups which rely on a single food source throughout their developmental cycle and harbor beneficial microbes that provide nutrients absent from their restricted diets. The *Coxiella*-like organisms of ticks share obvious similarities with these beneficial endosymbionts.

Here, we examine the origin of the Q fever pathogen, *C. burnetii*, by inferring the evolutionary processes that have shaped diversity within the entire *Coxiella* genus. To this aim, we first sampled an extensive range of ticks, with 58 tick species examined, and developed a sensitive detection method that reveals a wider *Coxiella* diversity than recognized in past studies. Second, instead of relying solely on the 16S rRNA gene, a molecular marker that is notoriously inadequate for inferring reliable fine-scale phylogenies [32], we used a novel multilocus typing method, allied to Whole Genome Sequencing (WGS) data, and conducted phylogenetic analyses on a large amount of DNA sequence data. Third, we examined two major ecological features of *Coxiella*-like organisms, i.e. their ability to be maternally-inherited through the tick egg cytoplasm and to grow in a vertebrate cell environment suitable to *C. burnetii*. Altogether, this corpus of data has led to the characterization of a large genetic and ecological diversity within the *Coxiella* genus, far beyond the *C. burnetii* type species. The *Coxiella*-like organisms of ticks form an ancient lineage of maternally-inherited tick endosymbionts that do not lie as a sister-clade to *C. burnetii* but rather form a basal lineage illustrative of the ancestral *Coxiella* life style.



**Fig 1. Geographic origin of the sampled ticks and distribution of *Coxiella* and *Rickettsiella* infections.** Square size indicates the number of populations sampled per geographic area. Numbers refer to the tick species whereas letters discriminate the different populations screened within a species; this nomenclature is detailed in Table 1. The colors within squares indicate the infection status of populations. Two tick species were infected by both *Coxiella* and *Rickettsiella* at the species level, but not at individual and population levels.

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## Results

### Ticks commonly harbor *Coxiella*-like organisms

We performed an extensive screening for the presence of *Coxiella* in 916 tick specimens from 58 species belonging to the two main tick families, Ixodidae (hard ticks, 36 species) and Argasidae (soft ticks, 22 species) (Fig 1 and Table 1). Except for 37 specimens (6 species) derived from laboratory colonies, all other tick specimens were sampled from natural populations in Europe, Americas, Africa, Oceania and Asia ( $n = 112$  localities). In these populations, ticks were collected either in the host habitat or directly on hosts (Table 1). To detect *C. burnetii* and its relatives, we developed a detection method based on a nested polymerase chain reaction (PCR) using total tick DNA extracts to amplify a 539–542 base-pair (bp) fragment of the *Coxiella rpoB* gene (Table A in S1 Text).

Using this procedure, all the tick-borne bacteria we detected belong to the Legionellales order and can be unambiguously assigned either to *Coxiella* or to its sister genus, *Rickettsiella*. Whole tick DNA extracts from more than two thirds of the specimens (637 out of 916, 69.6%) and the species (40 out of 58, 70.0%) were found to be positive for *Coxiella* (Fig 1 and Table 1). *Coxiella* was found in most tested genera of hard ticks (*Rhipicephalus*, *Ixodes*, *Amblyomma*, *Dermacentor*, *Haemaphysalis*) and soft ticks (*Ornithodoros*, *Argas*). In almost all infected



**Table 1. List of tick species and populations included in the analysis, with details on their origin, the population sample size, and the prevalence of *Coxiella* spp. and *Rickettsiella* spp.**

Tick species	Sample sites	Tick host species or habitat	n	<i>Coxiella</i> prevalence (number of infected tick specimens)	<i>Rickettsiella</i> prevalence (number of infected tick specimens)
Argasidae (soft ticks)					
1 - <i>Antricola guglielmonei</i> Estrada-Peña, Barros-Battesti and Venzal, 2004	1-A, Porto Velho, Rondonia, Brazil	Bat guano in cave	4	0.00 (0)	0.00 (0)
2 - <i>Argas monachus</i> Keirans, Radovsky and Clifford, 1973	2-A, Chaco, Argentina	Unknown	3	1.00 (3)	0.00 (0)
3 - <i>Ornithodoros amblus</i> Chamberlin, 1920	3-A, Lobos de Tierra Island, Peru, 2009	Peruvian Pelican ( <i>Pelecanus thagus</i> ), Peruvian Booby ( <i>Sula variegata</i> )	5	1.00 (5)	0.00 (0)
4 - <i>Ornithodoros brasiliensis</i> Aragão, 1923	4-A, Sao Francisco de Paula, Brazil	Unknown	3	1.00 (3)	0.00 (0)
5 - <i>Ornithodoros capensis</i> Neumann, 1901	5-A, Juan de Nova Island, Mozambic Channel, 2011	Sooty Tern ( <i>Onychoprion fuscatus</i> )	28	1.00 (28)	0.00 (0)
	5-B, Réunion Island	Sea bird nests	3	1.00 (3)	0.00 (0)
6 - <i>Ornithodoros costalis</i> Diatta, Bouattour, Durand, Renaud and Trape, 2013	6-A, Kenitra, Morocco, 2006	Rodent burrows	7	0.00 (0)	1.00 (7)
	6-B, Sidi Akhfennir, Morocco, 2006	Rodent burrows	18	0.00 (0)	1.00 (18)
	6-C, Boujdour, Morocco, 2006	Rodent burrows	2	0.00 (0)	1.00 (2)
	6-D, El Argoub, Morocco, 2006	Rodent burrows	2	0.00 (0)	1.00 (2)
	6-E, Lahmiris, Morocco, 2010	Rodent burrows	1	0.00 (0)	1.00 (1)
	6-F, Mostaganem, Algeria, 2012	Rodent burrows	4	0.00 (0)	1.00 (4)
	6-G, Oudhna, Tunisia, 2010	Rodent burrows	4	0.00 (0)	1.00 (4)
7 - <i>Ornithodoros denmarki</i> Kohls, Sonenshine and Clifford, 1965	7-H, unknown	Sea bird nests	1	1.00 (1)	0.00 (0)
8 - <i>Ornithodoros erraticus</i> Lucas, 1849	8-A, La Calle, Tunisia, 2009	Rodent burrows	1	1.00 (1)	0.00 (0)
	8-B, Taher, Algeria, 2010	Rodent burrows	2	1.00 (2)	0.00 (0)
9 - <i>Ornithodoros kairouanensis</i> Trape, Diatta, Bouattour, Durand and Renaud, 2013	9-A, Kairouan, Tunisia, 2010	Rodent burrows	3	1.00 (3)	0.00 (0)
10 - <i>Ornithodoros maritimus</i> Vermeil and Marguet, 1967	10-A, Medes Island, Spain, 2009	Yellow-legged Gull ( <i>Larus michahellis</i> )	20	1.00 (20)	0.00 (0)
	10-B, Zembra Island, Tunisia, 2009	Yellow-legged Gull ( <i>Larus michahellis</i> )	20	1.00 (20)	0.00 (0)
	10-C, Carteau, France, 2014	Yellow-legged Gull ( <i>Larus michahellis</i> )	8	1.00 (8)	0.00 (0)
11 - <i>Ornithodoros maroccanus</i> Velu, 1919	11-A, Izemmourèn, Morocco, 2009	Rodent burrows	1	1.00 (1)	0.00 (0)
	11-B, Berkane Oued Kiss, Morocco, 2006	Rodent burrows	1	1.00 (1)	0.00 (0)

(Continued)

Table 1. (Continued)

Tick species	Sample sites	Tick host species or habitat	n	<i>Coxiella</i> prevalence (number of infected tick specimens)	<i>Rickettsiella</i> prevalence (number of infected tick specimens)
	11-C, Bir-Jdid, Morocco, 2009	Rodent burrows	1	1.00 (1)	0.00 (0)
12 <i>Ornithodoros merionesi</i> - Trape, Diatta, Belghyti, Sarih, Durand and Renaud, 2013	12-A, Guelmin, Morocco, 2006	Rodent burrows	1	0.00 (0)	1.00 (1)
	12-B, Sidi Akhfennir, Morocco, 2006	Rodent burrows	2	0.00 (0)	1.00 (2)
13 <i>Ornithodoros moubata</i> - Murray, 1877	13-A, Laboratory strain derived from field specimens of unknown origin	Unknown	3	0.00 (0)	0.00 (0)
14 <i>Ornithodoros normandi</i> - Larrousse, 1923	14-A, Bizerte, Tunisia, 2010	Rodent burrows	1	0.00 (0)	1.00 (1)
	14-B, Oudhna, Tunisia, 2010	Rodent burrows	2	0.00 (0)	1.00 (2)
15 <i>Ornithodoros occidentalis</i> - Trape, Diatta, Durand and Renaud, 2013	15-A, Fes, Morocco, 2010	Rodent burrows	4	1.00 (4)	0.00 (0)
	15-B, Kenitra, Morocco, 2006	Rodent burrows	4	1.00 (4)	0.00 (0)
	15-C, Beb-Lerba, Morocco, 2010	Rodent burrows	4	1.00 (4)	0.00 (0)
	15-D, Oued Choufcherk, Morocco, 2010	Rodent burrows	4	1.00 (4)	0.00 (0)
	15-E, Bouira, Algeria, 2010	Rodent burrows	4	1.00 (4)	0.00 (0)
	15-F, Berrouaghia, Algeria, 2010	Rodent burrows	4	1.00 (4)	0.00 (0)
	15-G, Chlef, Algeria, 2010	Rodent burrows	4	1.00 (4)	0.00 (0)
16 <i>Ornithodoros peruvianus</i> - Kohls, Clifford and Jones, 1969	16-A, Chile	Common vampire Bat ( <i>Desmodus rotundus</i> )	3	1.00 (3)	0.00 (0)
17 <i>Ornithodoros porcinus</i> - Walton, 1962	17-A, Laboratory strain derived from field specimens collected in Mahitsy, Madagascar, 2008–2010	Unknown	3	0.00 (0)	0.00 (0)
18 <i>Ornithodoros rostratus</i> - Aragão, 1911	18-A, Salta, Argentina	Environement	18	1.00 (18)	0.00 (0)
	18-B, Laboratory strain derived from field specimens collected in Nhecolandi, Pantanal, Brazil	Environement	4	1.00 (4)	0.00 (0)
19 <i>Ornithodoros rupestris</i> - Trape, Bitam, Renaud and Durand, 2013	19-A, Saïda Mt Daïa, Algeria, 2012	Rodent burrows	1	1.00 (1)	0.00 (0)
	19-B, Mostaganem, Algeria, 2012	Rodent burrows	2	1.00 (2)	0.00 (0)
20 <i>Ornithodoros sonrai</i> - Sautet and Witkowski, 1943	20-A, Dielmo, Senegal, 2002	Rodent burrows	20	1.00 (20)	0.00 (0)
	20-B, Kanène Khar, Senegal, 2003	Rodent burrows	17	1.00 (17)	0.00 (0)
	20-C, Richard-Toll, Senegal, 2003	Rodent burrows	19	1.00 (19)	0.00 (0)

(Continued)

Table 1. (Continued)

Tick species	Sample sites	Tick host species or habitat	n	<i>Coxiella</i> prevalence (number of infected tick specimens)	<i>Rickettsiella</i> prevalence (number of infected tick specimens)
	20-D, Sogoli, Mali, 2007	Rodent burrows	10	1.00 (10)	0.00 (0)
	20-E, M'Chounèche, Algeria, 2009	Rodent burrows	37	1.00 (37)	0.00 (0)
21 <i>Ornithodoros spheniscus</i> - Hoogstraal, Wassef, Hays and Keirans, 1985	21-A, Pan de Azucar, Chile, 2010–2013	Humboldt penguin ( <i>Spheniscus humboldti</i> )	3	1.00 (3)	0.00 (0)
22- <i>Ornithodoros</i> sp. ( <i>capensis</i> species complex)	22-A, Boa Vista Island, Cape Verde, 2008	Cape Verde Shearwater ( <i>Calonectris edwardsii</i> ) and Brown Booby ( <i>Sula leucogaster</i> )	16	1.00 (16)	0.00 (0)
		Ixodidae (hard ticks)			
23 <i>Amblyomma americanum</i> - (Linnaeus, 1758)	23-A, Laboratory strain derived from field engorged females collected in Oklahoma, USA, 1976–2004	Unknown	20	1.00 (20)	0.00 (0)
24 <i>Amblyomma cajennense</i> - (Fabricius, 1787)	24-A, Chapada Gaucha, Brazil, 2013	Unknown	3	1.00 (3)	0.00 (0)
25 <i>Amblyomma loculosum</i> - Neumann, 1907	25-A, Petite Ile, La Réunion, 2012	Wedge-Tailed Shearwater ( <i>Puffinus pacificus</i> ) nests	3	1.00 (3)	0.00 (0)
26 <i>Amblyomma variegatum</i> - (Fabricius, 1794)	26-A, La Réunion	Unknown	2	1.00 (2)	0.00 (0)
27 <i>Dermacentor marginatus</i> - (Sulzer, 1776)	27-A, Cavaillon, France, 2011	Vegetation	1	1.00 (1)	0.00 (0)
	27-B, Les Plantiers, France, 2013	Vegetation	1	1.00 (1)	0.00 (0)
28 <i>Dermacentor occidentalis</i> - Marx, 1892	28-A, Hopland, Mendocino Co, California, 1985	Environement	6	0.00 (0)	0.00 (0)
29 <i>Dermacentor reticulatus</i> - (Fabricius, 1794)	29-A, Proveysieux, France, 2011	Vegetation	1	0.00 (0)	0.00 (0)
30 <i>Dermacentor silvarum</i> - Olenev, 1931	30-A, Laboratory strain derived from field specimens collected in Xiaowutai National Natural Reserve Area, China		2	1.00 (2)	0.00 (0)
31 <i>Dermacentor variabilis</i> - (Say, 1821)	31-A, Queen's biology station, Ontario, Canada, 2013	Vegetation	2	0.00 (0)	0.00 (0)
32 <i>Haemaphysalis punctata</i> - Canestrini and Fanzago, 1878	32-A, East Sussex, England, 2011	Vegetation	5	1.00 (5)	0.00 (0)
33 <i>Hyalomma impeltatum</i> - Schulze and Schlottko, 1930	33-A, Zimbabwe, 1998	Zebu ( <i>Bos indicus</i> )	3	0.00 (0)	0.00 (0)
34 <i>Hyalomma lusitanicum</i> - Koch, 1844	34-A, Veneguera, Canaries, 2010	Vegetation	2	0.00 (0)	0.00 (0)
35 <i>Ixodes frontalis</i> (Panzer, 1798)	35-A, Bretagne, France, 2008	Eurasian Collared Dove ( <i>Streptopelia decaocto</i> )	1	0.00 (0)	0.00 (0)
36- <i>Ixodes hexagonus</i> Leach, 1815	36-A, Bretagne, France, 2008	European Red Fox ( <i>Vulpes vulpes</i> )	1	1.00 (1)	0.00 (0)
	36-B, Beaumont-Monteux, France, 2008	European Hedgehog ( <i>Erinaceus europaeus</i> )	2	1.00 (1)	0.00 (0)
37 <i>Ixodes kerguelenensis</i> - André and Colas-Belcour, 1942	37-A, Crozet Archipelago, 2003	Sea bird nests	2	0.00 (0)	0.00 (0)

(Continued)



Table 1. (Continued)

	Tick species	Sample sites	Tick host species or habitat	n	<i>Coxiella</i> prevalence (number of infected tick specimens)	<i>Rickettsiella</i> prevalence (number of infected tick specimens)
38	<i>Ixodes pacificus</i> Cooley and Kohls, 1943	38-A, Hopland, Mendocino Co, California, 1985	Environement	6	0.00 (0)	0.00 (0)
39	<i>Ixodes persulcatus</i> Schulze, 1930	39-A, Oulu, Finland, 2011	Vegetation	3	0.00 (0)	0.00 (0)
40	<i>Ixodes ricinus</i> (Linnaeus, 1758)	40-A, Lehmäsaari, Finland, 2011	Vegetation	20	0.00 (0)	0.00 (0)
		40-B, Rioja, Spain, 2011	Vegetation	20	0.00 (0)	0.00 (0)
		40-C, Mafra, Portugal, 2013	Vegetation	16	0.00 (0)	0.00 (0)
		40-D, Neuchâtel, Switzerland, 2012	Vegetation	16	0.00 (0)	0.00 (0)
		40-E, Chur, Switzerland, 2012	Hazel grouse ( <i>Tetrastes bonasia</i> )	4	0.00 (0)	0.00 (0)
		40-F, S�nart, France, 2010	European Roe Deer ( <i>Capreolus capreolus</i> ), rodents and passerines	22	0.00 (0)	0.05 (1)
		40-G, Ain, France, 2004	Vegetation	2	1.00 (1)	0.00 (0)
41	<i>Ixodes scapularis</i> Say, 1821	41-A, Queen's biology station, Ontario, Canada, 2013	Vegetation	3	0.00 (0)	0.00 (0)
42	<i>Ixodes tasmani</i> Neumann, 1899	42-A, Kilarney Circuit, Coolangubra, Australia, 1982	Common Wombat ( <i>Vombatus ursinus</i> )	2	0.00 (0)	1.00 (2)
43	<i>Ixodes unicavatus</i> Neumann, 1908	43-A, Ariy Kamen Islet, Kamchatka, Russia, 2008	Black-legged Kittiwake ( <i>Rissa tridactyla</i> ) and Red-legged Kittiwake ( <i>Rissa brevirostris</i> )	4	0.00 (0)	0.00 (0)
44	<i>Ixodes uriae</i> White, 1852	44-A, Possession Island, Crozet Archipelago, 2003	King Penguin ( <i>Aptenodytes patagonicus</i> )	20	0.00 (0)	0.00 (0)
		44-B, Hornoeya Island, Norway, 2010	Br�nnich's Guillemot ( <i>Uria lomvia</i> )	20	0.00 (0)	0.00 (0)
		44-C, Triangle Island, Canada, 2010	Rhinoceros Auklet ( <i>Cerorhinca monocerata</i> )	14	0.50 (7)	0.00 (0)
		44-D, Pitchie2, Kamchatka, Russia, 2008	Red-faced Cormorant ( <i>Phalacrocorax urile</i> )	20	0.00 (0)	0.00 (0)
		44-E, Grimsey, Iceland, 2003	Atlantic Puffin ( <i>Fratercula arctica</i> ), Common Guillemot ( <i>Uria aalge</i> ) and Black-legged Kittiwake ( <i>Rissa tridactyla</i> )	25	0.00 (0)	0.20 (5)
45	<i>Ixodes</i> sp.1	45-A, Guiglo, Ivory Coast, 1994	Leopard ( <i>Panthera pardus</i> )	12	1.00 (12)	0.00 (0)
46	<i>Ixodes</i> sp.2	46-A, Guiglo, Ivory Coast, 1994	Dog ( <i>Canis familiaris</i> )	12	1.00 (12)	0.00 (0)
47	<i>Rhipicephalus annulatus</i> (Say, 1821)	47-A, Burkina-Faso, 2013	Zebu ( <i>Bos indicus</i> )	17	1.00 (17)	0.00 (0)
		47-B, Gogonou, Benin, 2012	Zebu ( <i>Bos indicus</i> )	5	1.00 (5)	0.00 (0)
48	<i>Rhipicephalus australis</i> Fuller, 1899	48-A, CCA Farm, New Caledonia, 2003	Cattle ( <i>Bos taurus</i> )	12	1.00 (12)	0.00 (0)
		48-B, BMMMM Farm, New Caledonia, 2003	Cattle ( <i>Bos taurus</i> )	12	1.00 (12)	0.00 (0)
49	<i>Rhipicephalus bursa</i> Canestrini and Fanzago, 1878	49-A, Italia	Cattle ( <i>Bos taurus</i> ) and Sheep ( <i>Ovis aries</i> )	2	1.00 (2)	0.00 (0)

(Continued)

Table 1. (Continued)

Tick species	Sample sites	Tick host species or habitat	n	<i>Coxiella</i> prevalence (number of infected tick specimens)	<i>Rickettsiella</i> prevalence (number of infected tick specimens)
50- <i>Rhipicephalus decoloratus</i> Koch, 1844	50-A, Burkina-Faso, 2013	Zebu ( <i>Bos indicus</i> )	20	0.95 (19)	0.00 (0)
	50-B, Gogonou, Benin, 2012	Zebu ( <i>Bos indicus</i> )	9	1.00 (9)	0.00 (0)
	50-C, Sandvelt, South Africa, 2011	Blue Wildebeest ( <i>Connochaetes taurinus</i> ), Greater Kudu ( <i>Tragelaphus strepsiceros</i> ) and Southern Eland ( <i>Taurotragus oryx</i> )	20	1.00 (20)	0.00 (0)
	50-D, Queenstown, South Africa, 2011	Zebu ( <i>Bos indicus</i> )	17	1.00 (17)	0.00 (0)
	50-E, Vaalwater, South Africa, 2010	Zebu ( <i>Bos indicus</i> )	19	1.00 (19)	0.00 (0)
	50-F, Lephalale, South Africa, 2010	South African Giraffe ( <i>Giraffa camelopardalis giraffa</i> )	9	1.00 (9)	0.00 (0)
	50-G, Zimbabwe, 1998	Impala ( <i>Aepyceros melampus</i> )	11	1.00 (11)	0.00 (0)
51- <i>Rhipicephalus evertsi</i> Neumann, 1897	51-A, Zimbabwe, 1998	Zebu ( <i>Bos indicus</i> )	8	1.00 (8)	0.00 (0)
52- <i>Rhipicephalus geigy</i> Aeschlimann and Morel, 1965	52-A, Burkina-Faso, 2013	Zebu ( <i>Bos indicus</i> )	18	0.94 (17)	0.00 (0)
	52-B, Gogonou, Benin, 2012	Zebu ( <i>Bos indicus</i> )	3	1.00 (3)	0.00 (0)
53- <i>Rhipicephalus microplus</i> (Canestrini, 1888)	53-A, Kpinnou, Benin, 2012	Zebu ( <i>Bos indicus</i> )	20	1.00 (20)	0.00 (0)
	53-B, Ambalanirana, Madagascar, 2013	Zebu ( <i>Bos indicus</i> )	18	1.00 (18)	0.00 (0)
	53-C, Imeritsiatosika, Madagascar, 2013	Zebu ( <i>Bos indicus</i> )	14	1.00 (14)	0.00 (0)
	53-D, Eglinton, South Africa, 2011	Zebu ( <i>Bos indicus</i> )	20	1.00 (20)	0.00 (0)
	53-E, Welverdiemda, South Africa, 2011	Zebu ( <i>Bos indicus</i> )	11	1.00 (11)	0.00 (0)
	53-F, Laboratory strain derived from field engorged females collected in Kpinnou, Benin, 2012	Girolando (Cattle x Zebu hybrid)	7	1.00 (7)	0.00 (0)
54- <i>Rhipicephalus pusillus</i> Gil Collado, 1936	54-A, Gard, France, 2006	Vegetation	2	1.00 (2)	0.00 (0)
55- <i>Rhipicephalus sanguineus</i> (Latreille, 1806)	55-A, Brazil	Dog ( <i>Canis familiaris</i> )	1	1.00 (1)	0.00 (0)
	55-B, Montferrier-sur-Lez, France, 2013	Dog ( <i>Canis familiaris</i> )	1	1.00 (1)	0.00 (0)
56- <i>Rhipicephalus turanicus</i> Pomerantzev, 1940	56-A, Italia	Vegetation	2	1.00 (2)	0.00 (0)
	56-B, Kerkyra, Greece, 2012	Human ( <i>Homo sapiens</i> )	3	1.00 (3)	0.00 (0)
57- <i>Rhipicephalus</i> sp.1	57-A, Guiglo area, Ivory Coast, 1994	Leopard ( <i>Panthera pardus</i> )	3	1.00 (3)	0.00 (0)
58- <i>Rhipicephalus</i> sp.2	58-A, Guiglo, Ivory Coast, 1994	Dog ( <i>Canis familiaris</i> )	2	1.00 (2)	0.00 (0)

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species, *Coxiella* was detected in >90% of the examined specimens, indicating high *Coxiella* prevalence in diverse tick species. For example, infection was apparently fixed in populations of most *Rhipicephalus* and *Ornithodoros* species (Table 1). In contrast, *Coxiella* was frequently absent in *Ixodes* species and displayed highly variable prevalence in the five infected species (out of 12 screened).

Other Legionellales bacteria of the genus *Rickettsiella* were found in 52 specimens (5.7%) from six *Ornithodoros* species and three *Ixodes* species (Fig 1 and Table 1). In two of the three *Rickettsiella*-infected *Ixodes* species, i.e. *I. ricinus* and *I. uriae*, *Coxiella* was also found, but in different individuals and in distinct populations (i.e., no co-infection by *Coxiella* and *Rickettsiella* occurred at individual and population levels; Table 1). Adding the *Rickettsiella*-positive samples, we found that 689 of the 916 examined tick specimens (75.2%) and 44 of 58 screened species (76%) harbored either *Coxiella* or one of its relatives.

### High genetic diversity among tick-borne *Coxiella*

To characterize *Coxiella* genetic diversity, we developed a multi-locus typing method based on five conserved bacterial genes including *rpoB* and four other housekeeping genes: 16S rRNA, 23S rRNA, *GroEL* and *dnaK* (Table A and Fig A in S1 Text). Multi-locus sequences were obtained from a subsample of 85 *Coxiella*- and 12 *Rickettsiella*-positive tick specimens (one to four specimens per infected species were examined). All five bacterial genes were successfully amplified from 71 *Coxiella*- and 12- *Rickettsiella* positive specimens representing 35 *Coxiella*- and six *Rickettsiella*-infected tick species. For five other *Coxiella*-infected species (i.e., 14 individual ticks), only three to four bacterial genes were successfully amplified. The sequences were easily readable without double peaks, indicating that there was no coinfection of *Coxiella*/*Rickettsiella* strains in any specimen.

The overall dataset included 33 to 40 alleles per bacterial gene (Table 2) and 51 new multi-locus genotypes (43 in *Coxiella* and eight in *Rickettsiella*). Within the *Coxiella* genus, all pairs of 16S rRNA gene sequences are at least 93% identical (Table 2) and range in threshold values typically used to delineate other Legionellales genera such as *Legionella* [33] and *Rickettsiella* [34]. Each of the infected tick species harbored a specific bacterial genotype or a set of closely related genotypes. None of the *Coxiella* multi-locus genotypes identified in ticks was identical to those of the 15 *C. burnetii* reference strains (Table B in S1 Text), although some showed moderate levels of nucleotide identity: pairwise identity between the two groups ranged from 77.8% to 97.7%. For each bacterial gene, the genetic diversity was significantly higher in the *Coxiella* strains of ticks than in *C. burnetii* as illustrated by the metrics on their respective genetic diversity (Table 2, paired t test, all  $P < 0.02$ ).

### *Coxiella burnetii* originated from a tick-borne *Coxiella* ancestor

We constructed a multi-gene phylogeny of the entire *Coxiella* genus using a dataset that included the *Coxiella* and *Rickettsiella* sequences from ticks, the 15 *C. burnetii* reference genomes, as well as sequences from *Legionella* spp. and more distant outgroups that were available in GenBank (Table B in S1 Text). The concatenated sequences included 3009 unambiguously aligned base pairs (bp). Prior recombination tests showed that *Coxiella* and *Rickettsiella* strains did not exhibit a strictly clonal structure, but rather experienced significant genetic exchanges. We thus applied a sequence-based network approach that does not force relationships to be tree-like but rather incorporates recombination into the phylogenetic reconstruction. The network results (Fig 2), as well as the results from the Maximum Likelihood (ML) tree-based analysis (Fig B in S1 Text), consistently showed that the *Coxiella* genus can be split into four main clades (labeled A-to-D hereafter) with each clade clustering the *Coxiella* genotypes found in five to 15



tick species. The phylogenetic analyses also highlight that all *C. burnetii* isolates cluster into a unique subclade embedded within the A clade (Fig B and C in S1 Text and Fig 2). Notably, the closest relatives of *C. burnetii* are the *Coxiella* strains from soft ticks of the *Ornithodoros* and *Argas* genera, suggesting that the common ancestor of *C. burnetii* originated from a *Coxiella* hosted by soft ticks.

The partitioning of *Coxiella* diversity among tick species revealed a complex structure, indicating a role for both co-divergence and horizontal transfer events in the evolution of this bacterial group. Closely related *Coxiella*-like organisms were frequently found in closely related tick species, a pattern suggestive of co-divergence between *Coxiella* and ticks (Fig B in S1 Text and Fig 2). For instance, all the *Coxiella*-like organisms found in the 12 examined *Rhipicephalus* tick species cluster together within the C clade, whereas all the *Coxiella*-like organisms in the *Ixodes* species cluster within the B clade (Fig B in S1 Text and Fig 2). Conversely, some *Coxiella*-like organisms found in related tick species are only distantly related and do not cluster together (e.g., the *Coxiella*-like organisms of *Ornithodoros* soft ticks are scattered among the A, B and C clades), a pattern suggestive of horizontal transfers among tick species.

Further analyses were conducted by examining public repositories of DNA sequencing data generated by the whole genome sequencing (WGS) projects of the cattle tick *R. microplus* and the deer tick *I. scapularis*. Using the 1,995,281 bp *C. burnetii* (str. Nine Mile I RSA 493) genome as a probe, we found clear evidence of *Coxiella* infections in *R. microplus* but not in *I. scapularis*. A total of 31 contigs (514–2,349 bp, totaling 34,990 bp) from *R. microplus* sequencing were uniquely attributable to *Coxiella*. They matched 50 genes of *C. burnetii* with 68-to-100% nucleotide identity (Fig A and Table C in S1 Text and Fig 3A). Alignment of the 31 *Coxiella* contigs to other bacterial genomes, including the 15 *C. burnetii* reference genomes (19,304 unambiguously aligned bp), corroborates the finding of our prior five loci-based analyses: the *Coxiella* strain identified in *R. microplus* is evolutionarily related, but distinct, to *C. burnetii* (Fig 3B).

### *Coxiella*-like organisms are maternally inherited in ticks

It should be noted that the *R. microplus* WGS DNA examined above was extracted from eggs of an inbred strain of ticks (Deutsch strain), first derived from a few field specimens sampled in Texas in 2001, and reared for at least seven generations in the laboratory. The presence of *Coxiella* DNA in the WGS of *R. microplus* eggs thus raised the issue of their maternal inheritance in ticks. To address this question, 24 gravid females of four *Coxiella*-positive tick species were collected either from seabird nests (*O. maritimus*, n = 8 females), from a dog (*R. sanguineus*, n = 1) or from laboratory colonies (*R. microplus*, n = 7; *A. americanum*, n = 8) in order to test for the presence of *Coxiella*-infection in the cytoplasm of their progeny (8 to 14 surface-sterilized eggs per female were individually examined; i.e., 244 eggs in total). The occurrence of maternal transmission was detected in all four tick species and in almost all eggs: *O. maritimus*—79 *Coxiella*-positive eggs out of 80, *R. sanguineus*—14 out of 14, *R. microplus*—68 out of 70, and *A. americanum*—80 of 80. The mean transmission rate can thus be estimated at 0.988 (95% confidence interval, 0.965–0.994), demonstrating highly efficient maternal transmission of *Coxiella* in ticks. Maternal inheritance is thus widespread in the *Coxiella* genera, being found in three different clades (A: *Coxiella*-like organism of *O. maritimus*; C: *R. sanguineus* and *R. microplus*; D: *A. americanum*).

**Table 2. Genetic estimates for 85 *Coxiella*-like strains and for 15 *Coxiella burnetii* reference strains.**

Locus	Function	L	Strains	N <sub>i</sub>	P <sub>nsi</sub>	N <sub>a</sub>	P <sub>s</sub>	A <sub>d</sub>	π	D
16S rRNA	Small ribosomal subunit	1066	<i>Coxiella</i> -like organisms	85	93.0–100	40	262	0.985	0.038	39.960
			<i>Coxiella burnetii</i>	15	99.7–100	4	4	0.552	0.001	1.224
23S rRNA	Large ribosomal subunit	496	<i>Coxiella</i> -like organisms	82	84.2–100	34	177	0.978	0.079	39.393
			<i>Coxiella burnetii</i>	15	99.4–100	2	2	0.133	0.001	0.267
<i>GroEL</i>	Chaperone protein GROEL	550	<i>Coxiella</i> -like organisms	82	68.7–100	37	292	0.982	0.179	98.527
			<i>Coxiella burnetii</i>	15	99.4–100	5	5	0.562	0.002	0.895
<i>rpoB</i>	DNA-directed RNA polymerase beta chain	474	<i>Coxiella</i> -like organisms	85	68.7–100	38	250	0.981	0.205	97.136
			<i>Coxiella burnetii</i>	15	99.3–100	4	4	0.467	0.001	0.648
<i>dnaK</i>	Chaperone protein DnaK	423	<i>Coxiella</i> -like organisms	74	69.6–100	33	227	0.979	0.177	75.789
			<i>Coxiella burnetii</i>	15	99.8–100	3	1	0.362	0.001	0.381
Full concatenated data set		3009	<i>Coxiella</i> -like organisms	71	81.8–100	36	1139	0.984	0.115	346.990
			<i>Coxiella burnetii</i>	15	99.6–100	7	16	0.781	0.001	2.933

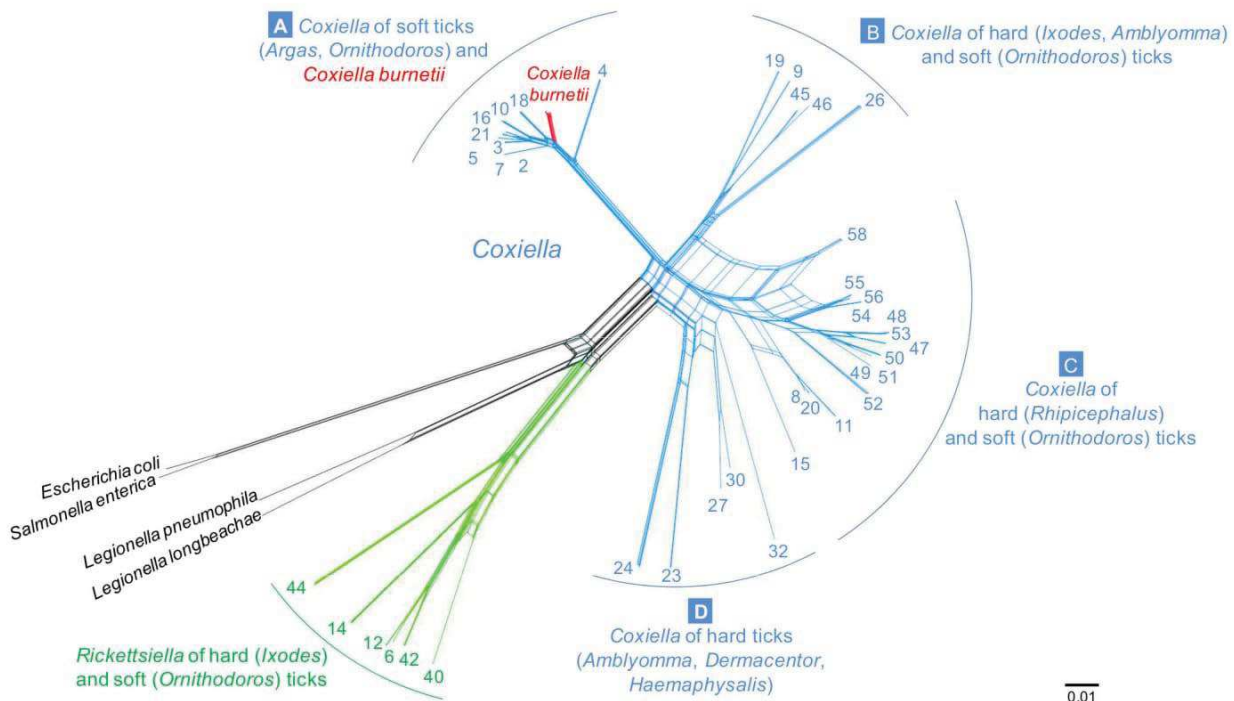
Analyses are based on nucleotide sequences of five housekeeping genes, excluding sites with alignment gaps and/or missing data. L, sequence fragment length in base pairs; N<sub>i</sub>, Number of examined strains; P<sub>nsi</sub>, Pairwise nucleotide sequence identity (%); N<sub>a</sub>, number of alleles; P<sub>s</sub>, number of polymorphic sites; A<sub>d</sub>, allelic diversity; π, nucleotide diversity; D, average number of nucleotide differences between sequences.

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### Differences in metabolic requirements of *Coxiella*-like organisms and *C. burnetii*

We next compared the metabolic requirements of *Coxiella*-like organisms with those of *C. burnetii* by assessing their ability to replicate in both an axenic medium ACCM2 (mimicking the environment of the acidified lysosome-like vacuoles of phagocytes typically colonized by *C. burnetii*; [35]) and directly inside vertebrate host cells. First, ACCM2 was inoculated with *Coxiella*-like organisms extracted from eggs of either *O. maritimus*, *R. microplus* or *A. americanum*. Inoculated media were incubated for 10 days under standard conditions used to amplify *C. burnetii*. Although our *C. burnetii* positive controls readily replicated in the media, the *Coxiella* obtained from eggs of the three tick species did not grow. We then incubated egg homogenates from ticks of *O. maritimus* and *R. microplus* with mammalian cell cultures for seven days. Similar to results under axenic conditions, the incubation of vertebrate cell lines with *Coxiella*-like organisms failed to produce *Coxiella*-containing vacuoles, whereas the same cell lines incubated with *C. burnetii* under the same conditions were readily infected. The apparent inability to amplify tick-borne *Coxiella* through standardized protocols, well-characterized for *C. burnetii*, suggests that, despite their phylogenetic proximity, the *Coxiella*-like bacteria are adapted to radically different environments.





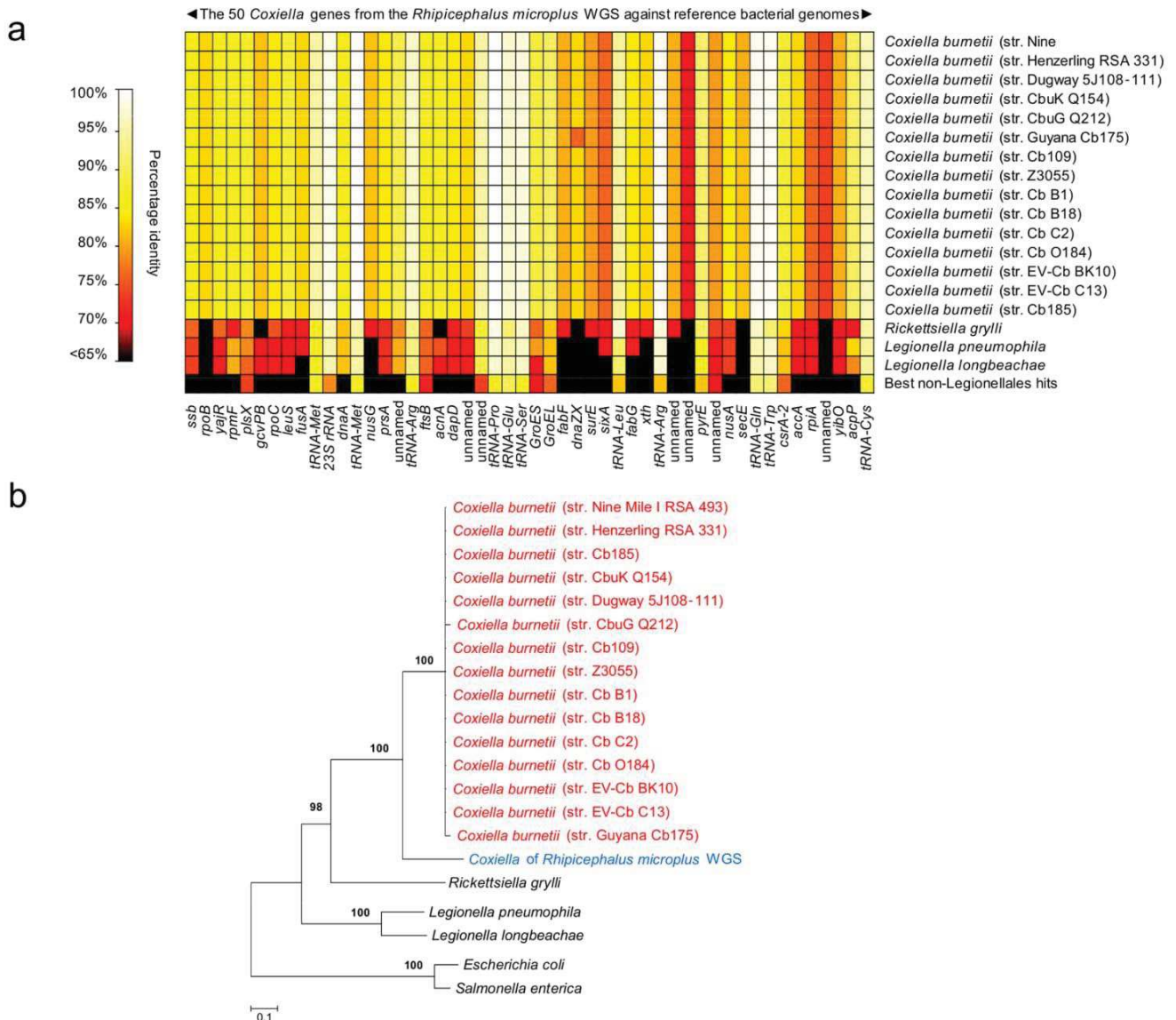
**Fig 2. Phylogenetic network with concatenated 16S rRNA, 23S rRNA, *GroEL*, *rpoB* and *dnaK* sequences (3009 unambiguously aligned bp), including 71 *Coxiella*-like strains of ticks, 15 *C. burnetii* reference strains, and bacterial outgroups.** The four *Coxiella* clades are labeled A to D. A zoom on the A clade which contains *C. burnetii* isolates is shown in Supplementary Fig C in S1 Text. Each number corresponds to one tick species as detailed in Table 1. Blue—*Coxiella*-like organisms; red—*C. burnetii*; green—*Rickettsiella*; black—other bacteria. All multi-locus typing of *Coxiella* and *Rickettsiella* of ticks are new sequences from this study. The scale bar is in units of substitution/site.

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## Discussion

Since its original description, *C. burnetii* infections have been characterized in a wide variety of hosts. While only two species have been formally identified within the *Coxiella* genus, we show here that a far greater diversity of *Coxiella* exists in ticks. We detect the presence of *Coxiella*-like organisms in many more tick species than previously known [17,18,19,20,21,22,23,24,25] and describe a far wider genetic diversity among these bacteria than previously suspected. The incidence of *Coxiella*, as well as of its sister genus *Rickettsiella*, in ticks is exceptionally high, with approximately three quarters of tick species infected. Although possible tick-borne transmission of *C. burnetii* has been reported [1,2,8], none of the 43 new *Coxiella* genotypes identified here are identical to *C. burnetii*. We also demonstrate for genetically divergent *Coxiella* strains (i.e., members of the A, C and D clades) found in four tick species that infection is primarily transmitted maternally via the egg cytoplasm. These results converge to support the hypothesis that these *Coxiella*-like organisms are specific endosymbionts of ticks. Phylogenetic evidence further shows that one of the *Coxiella*-like organisms belonging to the A clade and primarily hosted by soft ticks has served as the progenitor of *C. burnetii*.

Three complementary lines of argument indicate a much longer evolutionary history for *Coxiella*-tick associations than for vertebrate-*Coxiella* associations. The first lies in the broad distribution of *Coxiella* and *Rickettsiella* bacteria across tick species, genera and families. The second concerns the extensive genetic diversity found in tick-borne *Coxiella* strains compared to *C. burnetii* strains, as illustrated by the clear subdivision of this genus into four highly divergent clades



**Fig 3. Characterization of new *Coxiella* strains derived from whole-genome sequencing (WGS) of the cattle tick *Rhipicephalus microplus*.** (a) Percent identity of 50 genes uniquely attributable to *Coxiella* from *R. microplus* WGS versus 15 sequenced *C. burnetii* genomes and other reference genomes. (b) Bacterial phylogeny, comprising the *Coxiella* found in the *R. microplus* WGS data, reconstructed from the concatenated sequences of Fig 3A (19,304 unambiguously aligned bp) using maximum-likelihood (ML). Branch numbers indicate percent bootstrap support for major branches (1,000 replicates; only bootstrap values >90% are shown). The scale bar is in units of substitution/site.

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(A-D). Finally, the clustering of all *C. burnetii* strains within one of the clades of tick-borne *Coxiella* shows that the ancestor of *C. burnetii* was a tick-associated bacterium which succeeded in infecting vertebrate cells. The remarkably low genetic diversity of *C. burnetii*, previously noted in other studies [36,37], indicates a unique and recent emergence of this highly infectious vertebrate pathogen. Interestingly, this hypothesis was initially raised a decade ago from observations of the profound differences in genome architecture of *C. burnetii* relative to other pathogenic intracellular bacteria [38]. It was again emphasized from the genome sequencing of new *C. burnetii*



strains [39]. Our data brings further support to this hypothesis by demonstrating that *C. burnetii* roots within the *Coxiella* phylogeny. Comparative genome sequences of *C. burnetii* [38, 39] and of the *Coxiella*-like organism from *A. americanum* [16] also suggest that *Coxiella* bacteria differ substantially in terms of genome size and gene content. The *C. burnetii* genome (A clade) has a size of ca 2Mb [38, 39], whereas the genome of the *Coxiella*-like organism isolated in *A. americanum* (D clade) is only about a 1/3 of this size (ie. 0.66 Mb) with a large percentage of missing genes [16]. This reduction in genome size may limit the transition to pathogenicity, and suggests that some *Coxiella*-like organisms may have evolved towards exclusive and irreversibly specialized interactions with their tick hosts. Overall, the diversity of genome sizes emphasizes that members of the different *Coxiella* clades may have retained a variety of evolutionary strategies to favour their spread and persistence in their hosts.

We identified *Coxiella* as a major emerging clade of bacterial endosymbionts allied to ticks. *Coxiella*-like organisms are maternally-transmitted through the egg cytoplasm at high frequency with 98–100% mother-to-offspring transmission, a pattern also reported in previous studies [20,21,26]. This transmission pattern is the rule for a variety of bacterial endosymbionts that live exclusively within arthropod cells [28,29,40]. While some, like *Wolbachia*, are globally common symbionts estimated to infect ca. 40% of insect species [41,42], others are globally rare, but common and important in particular arthropod groups [28]. This is precisely the case for *Coxiella*-like organisms; although they have not been found in other arthropod species, they are commonly associated with ticks. This leads to the obvious question of the phenotypic consequences of *Coxiella*-tick interactions. In some cases, *Coxiella*-like organisms of ticks likely act as obligate mutualistic symbionts required to support normal tick development, potentially provisioning their hosts with essential nutrients absent in vertebrate blood [16,20,21,26,27]. The ubiquity of *Coxiella* in some tick groups—such as in the *Rhipicephalus* genus in which infection is at fixation—corroborates the hypothesis of an obligate endosymbiont. This is not, however, the case for all tick species since some, such as *I. ricinus* and *I. uriae*, harbour *Coxiella*-like organisms at much lower frequencies. In these tick species, *Coxiella* is more likely to behave as a conditional mutualist—i.e., that confers advantages under certain environmental conditions—or as a reproductive parasite—i.e., that manipulates host reproduction toward the production of daughters (the transmitting sex), as commonly observed in arthropods with a variety of facultative symbionts [28,40]. It should also be noted that other endosymbionts also occur in ticks and may have evolved under complex multispecific interactions [17,24]. For instance, whereas the soft tick *O. moubata* was not found to be infected by a *Coxiella*-like organism in the present study, this tick species has been found to be infected by an endosymbiont belonging to the *Francisella* genus [17]. Endosymbionts other than *Coxiella* may thus interact with ticks, a pattern suggesting that endosymbiotic systems can be dynamic across tick lineages. These different hypotheses will now require specific testing.

Another question remains concerning the degree of vertebrate infection risk by the *Coxiella*-like organisms of ticks. Ticks are found worldwide and blood-feed on many different hosts; a combination of traits that may facilitate tick-to-vertebrate transfers of *Coxiella*. However, the bacteria observed in this study seem confined to ticks and, to our knowledge, none have ever been isolated from a vertebrate or associated with clinical symptoms. This suggests that these tick-associated bacteria currently pose a much lower infection risk to vertebrates than *C. burnetii*. As discussed above, the genome reduction of the *Coxiella*-like organism isolated in *A. americanum*, with the lack of nearly all the genes associated with pathogenicity [16], corroborates this view. Moreover, the inability to grow tick-borne bacteria in vertebrate cells highlights the significant barrier that must be overcome by the bacteria to successively achieve tick-to-vertebrate transmission. This type of transmission may, nonetheless, occasionally occur; an avian *Coxiella*-like organism was recently reported to induce fatal systematic

infections in domestic birds [43,44,45]. A very similar infection pattern was found for another maternally inherited endosymbiont, *Arsenophonus*, a widespread bacterium in different insect groups [41,46,47,48,49]. In particular, some *Arsenophonus* strains were detected in the phloem of plants fed on by infected phytophagous insects and were assumed to be opportunistic plant pathogens [50]. In such cases, the plant host may act as an ecological arena for the global exchange of endosymbionts like *Arsenophonus*, serving as a possible intermediate host for the horizontal transfer of bacteria among insect species [48]. In the case of *Coxiella*-like organisms, the extent of exchange between different tick species via the vertebrate host is yet to be established, but could be favoured by tick co-feeding (ticks feeding in close proximity on the host). The genetic similarity between *Coxiella*-like organisms found in unrelated tick species highlights the capacity to shift tick host species. Future research is now needed to assess the potential of different *Coxiella*-like organisms to infect vertebrates.

The reasons why *C. burnetii* is a highly virulent pathogen of vertebrates, but not *Coxiella*-like organisms (especially those from the A clade) remain unknown. As an intracellular pathogen with airborne transmission, *C. burnetii* has evolved specific mechanisms to survive in the abiotic environment, as well as to infect and exploit vertebrate cells [15]. Several evolutionary pathways may explain the acquisition of the genetic material necessary for this major lifestyle transition; this includes spontaneous genetic mutations in the genome of a tick-*Coxiella* ancestor, or the more likely transfer and integration of virulence genes from a co-infecting pathogen. The opportunity of gene transfer among bacteria, irrespective of their pathogenic or symbiotic properties, relies on their frequent co-occurrence within the same tick host [25,51,52]. The *Coxiella*-like organisms of the A clade may have dynamic genomes as observed in many arthropod symbionts: although they reside in confined intracellular environments, arthropod symbionts commonly experience variable degrees of recombination and gene transfer [53,54,55,56,57]. These gene transfers have served as immediate and powerful mechanisms of rapid adaptation in many endosymbionts, such as *Wolbachia* [56] and *Hamiltonella* [55,57]. This mechanism may explain the evolutionary transition from a *Coxiella* tick-symbiont of the A clade to the vertebrate pathogen *C. burnetii*. Other genetic connections are also possible; several *C. burnetii* genes that may contribute to major virulence traits, such as tissue tropism, are similar to eukaryotic genes and may have been acquired through lateral gene transfers from eukaryotes [38,39]. Detailed studies of virulence genes in *C. burnetii* and their homology with *Coxiella*-like organisms of the A clade will now be necessary to understand the remarkable emergence of the Q fever agent.

The evolutionary transition observed within the *Coxiella* genus is one of the rare cases reported to date of an arthropod-inherited symbiont evolving metabolic adaptations leading to the emergence of a vertebrate infectious disease. Another such transition occurred in the *Rickettsia* genus. The best-known members of this genus are transmitted by blood-feeding arthropods and are pathogenic in the vertebrate host. However, in recent years, many maternally-inherited *Rickettsia* endosymbionts found exclusively in arthropods have been discovered [58,59]. The examination of the evolutionary history of the *Rickettsia* genus revealed that this bacterium originated from endosymbionts of invertebrates and only secondarily became vertebrate pathogens [58,59]. Like *Coxiella*, some *Rickettsia* species of blood-feeding hosts have undergone a horizontal transmission through a vertebrate host, leading to pathogen emergence. Other bacteria, such as *Arsenophonus* [47,48] and *Sodalis* [60] may have had similar life cycle transitions, but the case of *Coxiella* is unique in that the arthropod host is no longer required to complete its life cycle.

In conclusion, we show that *C. burnetii* arose from a rare and recent event: the evolutionary transformation of a maternally inherited endosymbiont of ticks into a specialized and virulent pathogen of vertebrates. This raises a series of exciting questions related to both how *Coxiella*



endosymbionts made the major evolutionary transition leading to the emergence of Q fever and their role in the population dynamics of ticks. Identifying the evolutionary processes that transform symbiotic bacteria into emerging pathogens will require further exploration into the biology of the entire *Coxiella* genus.

## Methods

### Tick collection

The examined specimens represent the two main tick families, nine genera, 58 species and 112 populations from around the world (Table 1). Field specimens were sampled on various host species belonging to major mammal and bird families or from their habitats. We also used specimens from laboratory colonies reared in captivity for at least three generations for six tick species (derived from field specimens collected in North America, South America, Africa and China). All samples were preserved in 70–90% ethanol at room temperature until use. Before storage, tick eggs collected under laboratory conditions were surface-sterilized with 2.6% sodium hypochlorite and 0.5% SDS for 1 min and washed with sterile water to avoid external bacterial contamination.

### *Coxiella* screening and typing

Tick DNA was individually extracted using the DNeasy Blood & Tissue Kit (QIAGEN) following manufacturer instructions. DNA template quality was systematically verified by PCR amplification of the 18S ribosomal RNA (18S rRNA) or the cytochrome oxidase 1 (*C01*) arthropod primers (Table A in S1 Text). Tick DNA samples were then tested for *Coxiella* presence using a nested PCR assay and sequencing of the *rpoB* gene using *Coxiella*-specific primers. The use of nested PCR was efficient at decreasing the probability of contamination from unwanted amplification products. Additional PCR assays on the 16S rRNA, 23S rRNA, *GroEL* and *dnaK* genes were conducted on a subsample of *Coxiella*-positive tick DNA to obtain additional DNA sequences for phylogenetic analyses. We used 15 recently published genomes of *C. burnetii* (mainly isolated from humans and ruminants) and the genome of *Rickettsiella grylli* from woodlice (listed in Table B in S1 Text) as references to design PCR primers. The efficiency of our typing method was ascertained through positive PCR amplification and clear sequences for the five loci in four cultured reference strains of *C. burnetii* (Table B in S1 Text). Gene features, primers and PCR conditions are detailed in Table A in S1 Text. All PCR products were visualized through electrophoresis in a 1.5% agarose gel. Positive PCR products were purified and sequenced in both directions (EUROFINS). The chromatograms were manually inspected and cleaned with CHROMAS LITE ([http://www.technelysium.com.au/chromas\\_lite.html](http://www.technelysium.com.au/chromas_lite.html)) and sequence alignments were done using CLUSTALW [61], both implemented in MEGA [62].

*Coxiella* sequences were also searched for in the whole genome sequence (WGS) data of *R. microplus* and *I. scapularis* (GenBank accession numbers ADMZ02000000 and ABJB000000000, respectively) using the 1,995,281 bp *C. burnetii* genome (str. Nine Mile I RSA 493, GenBank accession number NC002971) as a probe and the Basic Local Alignment Search Tool (BLAST) with default parameters. Table C in S1 Text reports the number and content of *Coxiella* contigs that were detected in the *R. microplus* WGS data.

### Molecular and phylogenetic analyses

The GBLOCKS program [63] with default parameters was used to remove poorly aligned positions and to obtain non-ambiguous sequence alignments. Sequences of individual genes that differed by one or more nucleotides were assigned distinct allele numbers using DNASP [64],

with the option of excluding sites with alignment gaps and/or missing data. Tick-borne *Coxiella* strains are defined as each unique combination of alleles. The genetic diversity estimates ( $P_s$ , number of polymorphic sites;  $A_d$ , allelic diversity;  $\pi$ , nucleotide diversity;  $D$ , average number of nucleotide differences between sequences) were computed using DNASP. Other statistical analyses were carried out using the R statistical package. All sequence alignments were checked for putative recombinant regions using the GENECONV [65] and RDP [66] methods available in the RDP3 computer analysis package [67].

Phylogenetic analyses were based on single and concatenated sequences of the five bacterial genes used in the multi-locus typing scheme and on the 50 *Coxiella* genes found in the *R. microplus* WGS data. Sequence alignments included *Coxiella* and *Rickettsiella* sequences obtained in this study from tick DNA, as well as sequences available in GenBank from reference strains of *C. burnetii*, *Rickettsiella grylli*, *Legionella pneumophila*, *L. longbeachae*, and two more distantly related bacteria, *Escherichia coli* and *Salmonella enterica* (Table B in S1 Text). The evolutionary models fitting the sequence data most closely were determined using the Akaike information criterion with the program MEGA. For each data set examined, the best-fit approximation was the general time reversible model with gamma distribution and invariant sites (GTR+G+I). Network-based phylogenetic analyses were done using SplitsTree, implementing the evolutionary model under the agglomerating NeighborNet algorithm [68]. Tree-based phylogenetic analyses were done using maximum-likelihood (ML) analyses. A ML heuristic search using a starting tree obtained by neighbor-joining was conducted in MEGA. Clade robustness was assessed by bootstrap analysis using 1,000 replicates.

## Culture assays

We first assessed the ability of tick-borne *Coxiella* to replicate in an axenic medium as follows. Tick eggs were surface-sterilized as described above and homogenized by hand in sterile water. Eggs homogenates were used to inoculate 2ml of the axenic medium ACCM2 [35] and incubated three days in a humidified atmosphere of 5% CO<sub>2</sub> and 2.5% O<sub>2</sub> at 37°C. 50µl of each culture were then diluted in 2ml of fresh ACCM2 and further incubated under the same conditions for 10 days to assess bacterial growth. We then assessed the ability of tick-borne *Coxiella* to replicate inside vertebrate host cells as follows. Surface-sterilized tick eggs were homogenized by hand in 1 ml of 10% Foetal Bovine Serum (FBS) supplemented MEM medium (GIBCO). The homogenate (0.5 ml) was diluted in 25 ml of 10% SVF-MEM and centrifuged at 4000 rpm (2000g) at 4°C for 30 min. Ten ml of the supernatant was mixed with 10 ml of 10% FSB-MEM and again centrifuged at 2000g at 4°C for 30 min. Ten ml of the supernatant was harvested and filtered through a sterile 0.45 µm pore size filter (MILLIPORE). Two flasks containing confluent Sheep Foetal Thymus cells (SFT) cells were inoculated with 5 ml of the obtained filtrate and incubated at 35°C and allowed to grow for 12 weeks. Cell culture flasks were observed daily for the presence of contamination or growth signs such as vacuoles containing *Coxiella*, during the first week then once a week. As a positive control, a homogenate of *C. burnetii* was used following the same protocol.

## Accession codes

Nucleotide sequences of PCR-amplified fragments of tick-borne *Coxiella* and *Rickettsiella* genes have been deposited in the GenBank nucleotide database under accession codes KP994768-KP994862 (16S rRNA), KP994678-KP994767 (23S rRNA), KP985445-KP985537 (*GroEL*), KP985265-KP985357 (*rpoB*) and KP985358-KP985444 (*dnaK*).



## Supporting Information

**S1 Text. Table A.** Genes and primers used in polymerase chain reaction (PCR) assays to detect *Coxiella* and relatives and to control tick DNA quality. The same primers were used for the *Coxiella* and *Rickettsiella* typing, with the exception of the 16S rRNA gene for which different primers were designed for the two bacteria. Nested PCR amplifications (16S rRNA, 23S rRNA, *GroEL*, *rpoB* and *dnaK*) were performed as follows: the first PCR run with the external primers was performed in a 10  $\mu$ L volume containing 20–50 ng of genomic DNA, 3 mM of each dNTP (Thermo Scientific), 8 mM of MgCl<sub>2</sub> (Roche Diagnostics), 3  $\mu$ M of each primer, 1  $\mu$ L of 10 $\times$  PCR buffer (Roche Diagnostics), and 0.5 U of Taq DNA polymerase (Roche Diagnostics). A 1- $\mu$ L aliquot of the PCR product from the first reaction was then used as a template for the second round of amplification. The second PCR was performed in a total volume of 25  $\mu$ L and contained 8 mM of each dNTP (Thermo Scientific), 10 mM of MgCl<sub>2</sub> (ThermoScientific), 7.5  $\mu$ M of each of the internal primers, 2.5  $\mu$ L of 10 $\times$ PCR buffer (Thermo Scientific), and 1.25 U of Taq DNA polymerase (Thermo Scientific). Non-nested PCR amplifications (*CO1* and *18S rRNA*) were performed following conditions similar to the first PCR run used in the nested PCR assays. All PCR amplifications were performed under the following conditions: initial denaturation at 93°C for 3 min, 35 cycles of denaturation (93°C, 30 s), annealing ( $T_m$  = 50–56°C, depending on primers, 30 s), extension (72°C, 1–2 min), and a final extension at 72°C for 5 min. **Table B.** List, biological features and GenBank accession numbers of the bacterial strains used as references in molecular and phylogenetic analyses.\* reference strains of *C. burnetii* used for primer testing. **Table C.** List, sequence accession numbers and features of the 31 *Coxiella* contigs from the whole-genome shotgun sequencing (WGS) of the cattle tick *Rhipicephalus microplus*. **Fig A.** Map of the *Coxiella burnetii* genome (strain Nine Mile I RSA 493) showing the position of the genetic markers (in blue) used in this study. The arrows indicate the position along the chromosome of the five housekeeping genes (16S rRNA, 23S rRNA, *GroEL*, *rpoB* and *dnaK*) used in the multi-locus typing of tick-borne *Coxiella* infections. The numbered boxes (1–31) indicate the position of the 31 *Coxiella* contigs (listed in Table C in S1 Text) detected from the whole genome sequencing of the hard tick *Rhipicephalus microplus*. **Fig B.** *Coxiella* and *Rickettsiella* phylogeny constructed using maximum-likelihood (ML) estimations based on 16S rRNA, 23S rRNA, *GroEL*, *rpoB* and *dnaK* concatenated sequences (3009bp), including 71 *Coxiella*-like strains of ticks, 15 *C. burnetii* reference strains and outgroups. The four *Coxiella* clades are labeled A to D. Each number corresponds to one tick species as detailed in Table 1. Blue, *Coxiella*-line organisms; red, *C. burnetii*; green, *Rickettsiella*; black, other bacteria. All multi-locus typing of tick-borne *Coxiella* and *Rickettsiella* of ticks are new sequences from this study. Branch numbers indicate percentage bootstrap support for major branches (1000 replicates; only bootstrap values >90% are shown). **Fig C.** Inset of *Coxiella* network from Fig 2 with focus on the A clade (*Coxiella* of soft ticks and *C. burnetii*). Each number corresponds to one tick species as detailed in Table 1. Blue, *Coxiella*-line organisms; red, *C. burnetii*. The scale bar is in units of substitution/site. (DOCX)

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## Author Contributions

Conceived and designed the experiments: OD CC. Performed the experiments: OD VN JC OM. Analyzed the data: OD CC. Contributed reagents/materials/analysis tools: OD VN KDM MB KSB OM FV LZ EJ PD CA FR JFT ASB JC MDi EL AA MDu EGD GD GKD HA SZ LV CC. Wrote the paper: OD KDM CC.

## References

1. Madariaga MG, Rezai K, Trenholme GM, Weinstein RA. Q fever: a biological weapon in your backyard. *Lancet Infect Dis*. 2003; 3: 709–721. PMID: [14592601](#)
2. Raoult D, Marrie T, Mege J. Natural history and pathophysiology of Q fever. *Lancet Infect Dis*. 2005; 5: 219–226. PMID: [15792739](#)
3. Vanderburg S, Rubach MP, Halliday JE, Cleaveland S, Reddy EA, Crump JA. Epidemiology of *Coxiella burnetii* infection in Africa: a OneHealth systematic review. *PLoS Negl Trop Dis*. 2014; 8: e2787. doi: [10.1371/journal.pntd.0002787](#) PMID: [24722554](#)
4. Angelakis E, Raoult D. Q Fever. *Vet Microbiol*. 2010; 140: 297–309. doi: [10.1016/j.vetmic.2009.07.016](#) PMID: [19875249](#)
5. Bewley KR. Animal models of Q fever (*Coxiella burnetii*). *Comp Med*. 2013; 63: 469–476. PMID: [24326221](#)
6. van der Hoek W, Dijkstra F, Schimmer B, Schneeberger PM, Vellema P, Wijkmans C, et al. Q fever in the Netherlands: an update on the epidemiology and control measures. *Euro Surveill* 2010; 15.
7. Russell-Lodrigue KE, Andoh M, Poels MWJ, Shive HR, Weeks BR, Zhang GQ, et al. *Coxiella burnetii* isolates cause genogroup-specific virulence in mouse and guinea pig models of acute Q fever. *Infect Immun*. 2009; 77: 5640–5650. doi: [10.1128/IAI.00851-09](#) PMID: [19786560](#)
8. McDade JE. Historical aspects of Q fever. In: Marie TJ, editor. *Q Fever Volume 1: The Disease*. Boca Raton: CRC Press; 1990. pp. 5–21.
9. Oyston PC, Davies C. Q fever: the neglected biothreat agent. *J Med Microbiol*. 2011; 60: 9–21. doi: [10.1099/jmm.0.024778-0](#) PMID: [21030501](#)
10. Weisburg WG, Dobson ME, Samuel JE, Dasch GA, Mallavia LP, Baca O, et al. Phylogenetic diversity of the Rickettsiae. *J Bacteriol*. 1989; 171: 4202–4206. PMID: [2753854](#)
11. Leclercq A, Kleespies RG. Type IV secretion system components as phylogenetic markers of entomopathogenic bacteria of the genus *Rickettsiella*. *FEMS Microbiol Ecol*. 2008; 279: 167–173. doi: [10.1111/j.1574-6968.2007.01025.x](#) PMID: [18179586](#)
12. Bouchon D, Cordaux R, Grève P. *Rickettsiella*, intracellular pathogens of arthropods. In: Zchori-Fein E, Bourtzis K, editors. *Manipulative Tenants*. Boca Raton: CRC Press; 2012. pp. 127–148.
13. Tsuchida T, Koga R, Horikawa M, Tsunoda T, Maoka T, Matsumoto S, et al. Symbiotic bacterium modifies aphid body color. *Science* 2010; 330: 1102–1104. doi: [10.1126/science.1195463](#) PMID: [21097935](#)
14. Tan CK, Owens L. Infectivity, transmission and 16S rRNA sequencing of a rickettsia, *Coxiella cheraxi* sp. nov., from the freshwater crayfish *Cherax quadricarinatus*. *Dis Aquat Organ*. 2000; 41: 115–122. PMID: [10918979](#)
15. van Schaik EJ, Chen C, Mertens K, Weber MM, Samuel JE. Molecular pathogenesis of the obligate intracellular bacterium *Coxiella burnetii*. *Nat Rev Microbiol*. 2013; 11: 561–573. doi: [10.1038/nrmicro3049](#) PMID: [23797173](#)
16. Smith TA, Driscoll T, Gillespie JJ, Raghavan R. A *Coxiella*-like Endosymbiont is a potential vitamin source for the Lone Star Tick. *Genome Biol Evol*. 2015; 7: 831–838. doi: [10.1093/gbe/evv016](#) PMID: [25618142](#)
17. Noda H, Munderloh UG, Kurtti TJ. Endosymbionts of ticks and their relationship to *Wolbachia* spp. and tick-borne pathogens of humans and animals. *Appl Environ Microbiol*. 1997; 63: 3926–3932. PMID: [9327557](#)
18. Almeida AP, Marcili A, Leite RC, Nieri-Bastos FA, Domingues LN, Ricardo Martins J, et al. *Coxiella* symbiont in the tick *Ornithodoros rostratus* (Acari: Argasidae). *Ticks Tick Borne Dis*. 2012; 3: 203–206. doi: [10.1016/j.ttbdis.2012.02.003](#) PMID: [22480930](#)



19. Duron O, Jourdain E, McCoy KD. Diversity and global distribution of the *Coxiella* intracellular bacterium in seabird ticks. *Ticks Tick Borne Dis.* 2014; 5: 557–563. doi: 10.1016/j.ttbdis.2014.04.003 PMID: 24915875
20. Klyachko O, Stein BD, Grindle N, Clay K, Fuqua C. Localization and visualization of a *Coxiella*-type symbiont within the lone star tick, *Amblyomma americanum*. *Appl Environ Microbiol.* 2007; 73: 6584–6594. PMID: 17720830
21. Machado-Ferreira E, Dietrich G, Hojgaard A, Levin M, Piesman J, Zeidner NS, et al. *Coxiella* symbionts in the Cayenne tick *Amblyomma cajennense*. *Microb Ecol.* 2011; 62: 134–142. doi: 10.1007/s00248-011-9868-x PMID: 21611689
22. Lalzar I, Harrus S, Mumcuoglu KY, Gottlieb Y. Composition and seasonal variation of *Rhipicephalus turanicus* and *Rhipicephalus sanguineus* bacterial Communities. *Appl Environ Microbiol.* 2012; 78: 4110–4116. doi: 10.1128/AEM.00323-12 PMID: 22467507
23. Jasinskas A, Zhong J, Barbour AG. Highly prevalent *Coxiella* sp. bacterium in the tick vector *Amblyomma americanum*. *Appl Environ Microbiol.* 2007; 73: 334–336. PMID: 17085709
24. Clay K, Klyachko O, Grindle N, Civitello D, Oleske D, Fuqua C. Microbial communities and interactions in the lone star tick, *Amblyomma americanum*. *Mol Ecol.* 2008; 17: 4371–4381. PMID: 19378409
25. Wilkinson DA, Dietrich M, Lebarbenchon C, Jaeger A, Le Rouzic C, Lagadec E, et al. Massive infection of seabird ticks with bacterial species related to *Coxiella burnetii*. *Appl Environ Microbiol.* 2014; 80: 3327–3333. doi: 10.1128/AEM.00477-14 PMID: 24657860
26. Lalzar I, Friedmann Y, Gottlieb Y. Tissue tropism and vertical transmission of *Coxiella* in *Rhipicephalus sanguineus* and *Rhipicephalus turanicus* ticks. *Environ Microbiol.* 2014; 16: 3657–3668. doi: 10.1111/1462-2920.12455 PMID: 24650112
27. Zhong J, Jasinskas A, Barbour AG. Antibiotic treatment of the tick vector *Amblyomma americanum* reduced reproductive fitness. *PLoS One.* 2007; 2: e405. PMID: 17476327
28. Moran NA, McCutcheon JP, Nakabachi A. Genomics and evolution of heritable bacterial symbionts. *Annu Rev Genet.* 2008; 42: 165–190. doi: 10.1146/annurev.genet.41.110306.130119 PMID: 18983256
29. Wernegreen JJ. Endosymbiosis. *Curr Biol.* 2012; 22: R555–561. doi: 10.1016/j.cub.2012.06.010 PMID: 22835786
30. Hosokawa T, Koga R, Kikuchi Y, Meng XY, Fukatsu T. *Wolbachia* as a bacteriocyte-associated nutritional mutualist. *Proc Natl Acad Sci USA.* 2010; 107: 769–774. doi: 10.1073/pnas.0911476107 PMID: 20080750
31. Akman L, Yamashita A, Watanabe H, Oshima K, Shiba T, Hattori M, et al. Genome sequence of the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia*. *Nat Genet.* 2002; 32: 402–407. PMID: 12219091
32. Husnik F, Chrudimsky T, Hypsa V. Multiple origins of endosymbiosis within the Enterobacteriaceae (gamma-Proteobacteria): convergence of complex phylogenetic approaches. *BMC Biol.* 2011; 9: 87. doi: 10.1186/1741-7007-9-87 PMID: 22201529
33. Gomez-Valero L, Rusniok C, Buchrieser C. *Legionella pneumophila*: population genetics, phylogeny and genomics. *Infect Genet Evol.* 2009; 9: 727–739. doi: 10.1016/j.meegid.2009.05.004 PMID: 19450709
34. Leclercq A, Kleespies RG. A *Rickettsiella* bacterium from the hard tick, *Ixodes woodi*: molecular taxonomy combining multilocus sequence typing (MLST) with significance testing. *PLoS One.* 2012; 7: e38062. doi: 10.1371/journal.pone.0038062 PMID: 22675436
35. Omsland A, Beare PA, Hill J, Cockrell DC, Howe D, Virtaneva K, et al. Isolation from animal tissue and genetic transformation of *Coxiella burnetii* are facilitated by an improved axenic growth medium. *Appl Environ Microbiol.* 2011; 77: 3720–3725. doi: 10.1128/AEM.02826-10 PMID: 21478315
36. Sekeyová Z, Roux V, Raoult D. Intraspecific diversity of *Coxiella burnetii* as revealed by *com1* and *mucZ* sequence comparison. *FEMS Microbiol Lett.* 1999; 180: 61–67. PMID: 10547445
37. Pearson T, Hornstra HM, Sahl JW, Schaack S, Schupp JM, Beckstrom-Sternberg SM, et al. When outgroups fail; phylogenomics of rooting the emerging pathogen, *Coxiella burnetii*. *Syst Biol.* 2013; 62: 752–762. doi: 10.1093/sysbio/syt038 PMID: 23736103
38. Seshadri R, Paulsen IT, Eisen JA, Read TD, Nelson KE, Nelson WC, et al. Complete genome sequence of the Q-fever pathogen *Coxiella burnetii*. *Proc Natl Acad Sci USA.* 2003; 100: 5455–5460. PMID: 12704232
39. Beare PA, Unsworth N, Andoh M, Voth DE, Omsland A, Gilk SD, et al. Comparative genomics reveal extensive transposon-mediated genomic plasticity and diversity among potential effector proteins within the genus *Coxiella*. *Infect Immun.* 2009; 77: 642–656. doi: 10.1128/IAI.01141-08 PMID: 19047403

40. Engelstadter J, Hurst GDD. The ecology and evolution of microbes that manipulate host reproduction. *Annu Rev Ecol Evol Syst.* 2009; 40: 127–149.
41. Duron O, Bouchon D, Boutin S, Bellamy L, Zhou L, Engelstadter J, et al. The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone. *BMC Biol.* 2008; 6: 27. doi: 10.1186/1741-7007-6-27 PMID: 18577218
42. Zug R, Hammerstein P. Still a host of hosts for *Wolbachia*: analysis of recent data suggests that 40% of terrestrial arthropod species are infected. *PLoS One.* 2012; 7: e38544. doi: 10.1371/journal.pone.0038544 PMID: 22685581
43. Shivaprasad HL, Cadenas MB, Diab SS, Nordhausen R, Bradway D, Crespo R, et al. *Coxiella*-like infection in psittacines and a toucan. *Avian Dis.* 2008; 52: 426–432. PMID: 18939630
44. Woc-Colburn AM, Garner MM, Bradway D, West G, D'Agostino J, Trupkiewicz J, et al. Fatal coxiellosis in Swainson's Blue Mountain Rainbow Lorikeets (*Trichoglossus haematodus moluccanus*). *Vet Pathol.* 2008; 45: 247–254. doi: 10.1354/vp.45-2-247 PMID: 18424842
45. Vapniarsky N, Barr BC, Murphy B. Systemic *Coxiella*-like infection with myocarditis and hepatitis in an eclectus parrot (*Eclectus roratus*). *Vet Pathol.* 2012; 49: 717–722. doi: 10.1177/0300985811409251 PMID: 21712515
46. Duron O, Wilkes TE, Hurst GDD. Interspecific transmission of a male-killing bacterium on an ecological timescale. *Ecol Lett.* 2010; 13: 1139–1148. doi: 10.1111/j.1461-0248.2010.01502.x PMID: 20545734
47. Duron O, Schnepat UE, Berthomieu A, Goodman SE, Droz B, Paupy C, et al. Origin, acquisition and diversification of heritable bacterial endosymbionts in louse flies and bat flies. *Mol Ecol.* 2014; 23: 2105–2017. doi: 10.1111/mec.12704 PMID: 24612422
48. Jouselin E, Coeur d'Acier A, Vanlerberghe-Masutti F, Duron O. Evolution and diversity of *Arsenophonus* endosymbionts in aphids. *Mol Ecol.* 2013; 22: 260–270. doi: 10.1111/mec.12092 PMID: 23106652
49. Novakova E, Hyspa V, Moran NA. *Arsenophonus*, an emerging clade of intracellular symbionts with a broad host distribution. *BMC Microbiol.* 2009; 9: 143. doi: 10.1186/1471-2180-9-143 PMID: 19619300
50. Bressan A. Emergence and evolution of *Arsenophonus* bacteria as insect-vectoring plant pathogens. *Infect Genet Evol.* 2014; 22: 81–90. doi: 10.1016/j.meegid.2014.01.004 PMID: 24444593
51. Benson MJ, Gawronski JD, Eveleigh DE, Benson DR. Intracellular symbionts and other bacteria associated with deer ticks (*Ixodes scapularis*) from Nantucket and Wellfleet, Cape Cod, Massachusetts. *Appl Environ Microbiol.* 2004; 70: 616–620. PMID: 14711698
52. van Overbeek L, Gassner F, van der Plas CL, Kastelein P, Nunes-da Rocha U, Takken W. Diversity of *Ixodes ricinus* tick-associated bacterial communities from different forests. *FEMS Microbiol Ecol.* 2008; 66: 72–84. doi: 10.1111/j.1574-6941.2008.00468.x PMID: 18355299
53. Duron O. Lateral transfers of insertion sequences between *Wolbachia*, *Cardinium* and *Rickettsia* bacterial endosymbionts. *Heredity* 2013; 111: 330–337. doi: 10.1038/hdy.2013.56 PMID: 23759724
54. Baldo L, Werren JH. Revisiting *Wolbachia* supergroup typing based on WSP: spurious lineages and discordance with MLST. *Curr Microbiol.* 2007; 55: 81–87. PMID: 17551786
55. Degnan PH, Moran NA. Evolutionary genetics of a defensive facultative symbiont of insects: exchange of toxin-encoding bacteriophage. *Mol Ecol.* 2008; 17: 916–929. doi: 10.1111/j.1365-294X.2007.03616.x PMID: 18179430
56. Nikoh N, Hosokawa T, Moriyama M, Oshima K, Hattori M, Fukatsu T. Evolutionary origin of insect-*Wolbachia* nutritional mutualism. *Proc Natl Acad Sci USA.* 2014; 111: 10257–10262. doi: 10.1073/pnas.1409284111 PMID: 24982177
57. Duron O. *Arsenophonus* insect symbionts are commonly infected with APSE, a bacteriophage involved in protective symbiosis. *FEMS Microbiol Ecol.* 2014; 90: 184–194. doi: 10.1111/1574-6941.12381 PMID: 25041857
58. Perlman SJ, Hunter MS, Zchori-Fein E. The emerging diversity of *Rickettsia*. *Proc R Soc Lond B Biol Sci.* 2006; 273: 2097–106.
59. Weinert LA, Werren JH, Aebi A, Stone GN, Jiggins FM. Evolution and diversity of *Rickettsia* bacteria. *BMC Biol.* 2009; 7: 6. doi: 10.1186/1741-7007-7-6 PMID: 19187530
60. Clayton AL, Oakeson KF, Gutin M, Pontes A, Dunn DM, von Niederhausern AC, et al. A novel human-infection-derived bacterium provides insights into the evolutionary origins of mutualistic insect–bacterial symbioses. *PLoS Genet.* 2012; 8: e1002990. doi: 10.1371/journal.pgen.1002990 PMID: 23166503
61. Thompson JD, Gibson TJ, Higgins DG. Multiple sequence alignment using ClustalW and ClustalX. *Curr Protoc Bioinformatics* 2002; 2: 3. doi: 10.1002/0471250953.bi0203s00 PMID: 18792934
62. Kumar S, Tamura K, Nei M. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform.* 2004; 5: 150–163. PMID: 15260895



63. Castresana J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol.* 2000; 17: 540–552. PMID: [10742046](#)
64. Librado P, Rozas J. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 2009; 25: 1451–1452. doi: [10.1093/bioinformatics/btp187](#) PMID: [19346325](#)
65. Sawyer SA. GENECONV: A computer package for the statistical detection of gene conversion. <http://www.math.wustl.edu/~sawyer>.
66. Martin D, Rybicki E. RDP: detection of recombination amongst aligned sequences. *Bioinformatics.* 2000; 16: 562–563. PMID: [10980155](#)
67. Martin D, Lemey P, Lott M, Moulton V, Posada D, Lefevre P. RDP3: a flexible and fast computer program for analyzing recombination. *Bioinformatics.* 2010; 26: 2462–2463. doi: [10.1093/bioinformatics/btq467](#) PMID: [20798170](#)
68. Huson DH, Bryant D. Application of phylogenetic networks in evolutionary studies. *Mol Biol Evol.* 2006; 23: 254–267. PMID: [16221896](#)



## Annexe 2

Boulinier, T., Kada, S., Ponchon, A., Dupraz, M., Dietrich, M., Gamble, A., Bourret, V., Duriez, O., Bazire, R., Tornos, J., Tveraa, T., Chambert, T., Garnier, R., McCoy, K.D., 2016. Migration, Prospecting, Dispersal? What Host Movement Matters for Infectious Agent Circulation? *Integr. Comp. Biol.* icw015. doi:10.1093/icb/icw015





## SYMPOSIUM

### Migration, Prospecting, Dispersal? What Host Movement Matters for Infectious Agent Circulation?

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**Synopsis** Spatial disease ecology is emerging as a new field that requires the integration of complementary approaches to address how the distribution and movements of hosts and parasites may condition the dynamics of their interactions. In this context, migration, the seasonal movement of animals to different zones of their distribution, is assumed to play a key role in the broad scale circulation of parasites and pathogens. Nevertheless, migration is not the only type of host movement that can influence the spatial ecology, evolution, and epidemiology of infectious diseases. Dispersal, the movement of individuals between the location where they were born or bred to a location where they breed, has attracted attention as another important type of movement for the spatial dynamics of infectious diseases. Host dispersal has notably been identified as a key factor for the evolution of host–parasite interactions as it implies gene flow among local host populations and thus can alter patterns of coevolution with infectious agents across spatial scales. However, not all movements between host populations lead to dispersal per se. One type of host movement that has been neglected, but that may also play a role in parasite spread is prospecting, i.e., movements targeted at selecting and securing new habitat for future breeding. Prospecting movements, which have been studied in detail in certain social species, could result in the dispersal of infectious agents among different host populations without necessarily involving host dispersal. In this article, we outline how these various types of host movements might influence the circulation of infectious disease agents and discuss methodological approaches that could be used to assess their importance. We specifically focus on examples from work on colonial seabirds, ticks, and tick-borne infectious agents. These are convenient biological models because they are strongly spatially structured and involve relatively simple communities of interacting species. Overall, this review emphasizes that explicit consideration of the behavioral and population ecology of hosts and parasites is required to disentangle the relative roles of different types of movement for the spread of infectious diseases.

#### Introduction

Although migration, the seasonal movement of animals to different areas of their distribution, is assumed to play a key role in the broad scale circulation of parasites and pathogens (Altizer et al. 2011; Bauer and Hoyer 2014, Fritzsche McKay and Hoyer 2016), other types of host movements may

also come into play in determining eco-epidemiological patterns across spatial scales. In the context of increasing interest for integrative approaches to infectious disease biology (Daszak et al. 2000; Cleaveland et al. 2014), spatial disease ecology builds on a vast array of approaches to address how the distribution and movements of hosts and



parasites may condition the dynamics of their interactions within ecosystems (Hess et al. 2002; Holt and Boulinier 2005). The long-standing interest in the roles of host dispersal and spatially structured contact networks in the dynamics of epidemics and the persistence of infectious agents within host communities has led to the development of diverse statistical approaches (Diggle 2000; Keeling et al. 2004; Craft et al. 2009; Fenner et al. 2011), including the analysis of dispersal gradients and the spatial spread of parasites via travelling waves (Bjørnstad et al. 2002; Mundt et al. 2009). However, the role of different types of host movement in the spreading of diseases remains understudied (Boulinier et al. 2001; Perkins et al. 2009; Plowright et al. 2011, Blackwood et al. 2013). Strong heterogeneity in individual movement patterns can contribute considerably to heterogeneity in the transmission of infectious agents at different scales (Lloyd-Smith et al. 2005; McCallum et al. 2007). With the recent development of the field of movement ecology (Nathan et al. 2008; Morales et al. 2010; Fieberg et al. 2010; Jeltsch et al. 2013), the question of the potential role of non-migratory host movements for disease spread can increasingly include ecological and evolutionary dimensions. Movement ecology has notably stressed that key features in the movements of individuals can be linked to the life histories of the species considered and the landscapes in which they have evolved (Johnson et al. 1992; Boulinier et al. 2008; Morales et al. 2010), and that these features may have critical implications for the dynamics and evolution of subdivided populations (Pulliam 1988; Clobert et al. 2001; Hanski and Gaggiotti 2004; Bowler and Benton 2005).

In this article, after reviewing the importance of the spatial dimension of parasite circulation, we outline which type of host movements may especially matter for the dispersal and spread of infectious agents. We then focus on the relatively neglected case of prospecting movements, i.e., visits to breeding patches where an individual is not currently breeding, illustrating insights that have been gained on this topic through the study of seabird-parasites systems. After briefly reviewing other systems in which non-migratory movements may require particular attention, we outline timely research perspectives, potential study designs and available tools to examine questions related to the impact of these alternative types of movement. Here, we exclude human-mediated movements, which are also well known to lead to the transmission of infectious agents or their vectors over long distances (Cunningham 1996; Adams and Kapan 2009; Stoddard et al. 2009).

## Why does the spatial dimension of host-parasite interactions matter?

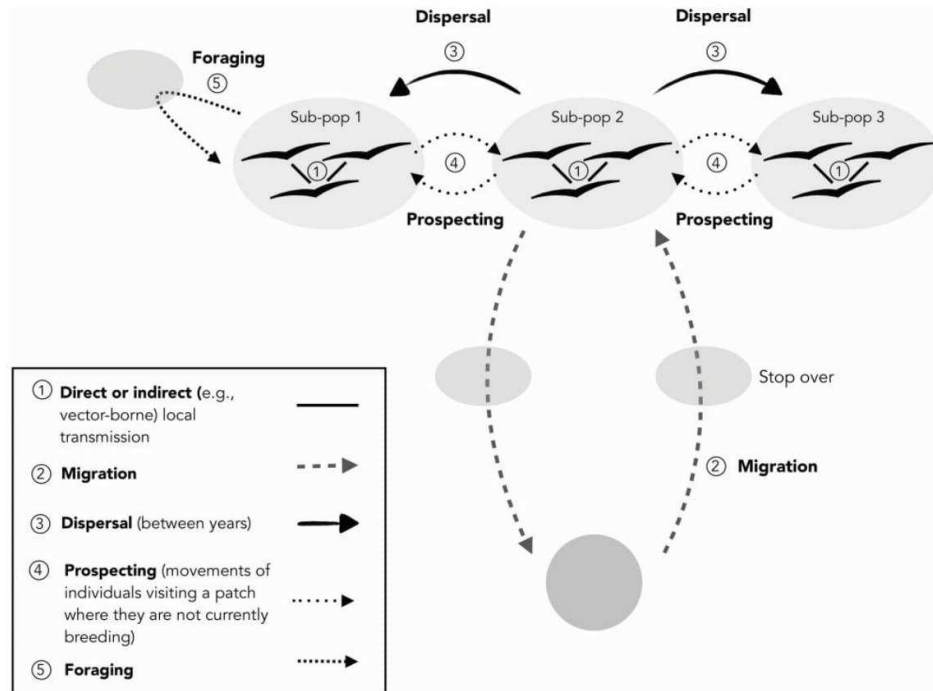
The spatial dimension is important for the epidemiology, ecology, and evolution of the interactions between infectious agents and their hosts. From an eco-epidemiological standpoint, the continental spread of West Nile Virus (Rappole et al. 2000) and *Mycoplasma gallisepticum* (Hochachka and Dhondt 2000) in susceptible bird populations, the northward expansion of Lyme disease in eastern North America (Leighton et al. 2012) or the rapid spread of Phocine Distemper Virus along European coastal areas (Harding et al. 2002) represent examples stressing the need to determine the factors contributing to the spatial dynamics of infectious agents. In the context of climate change, habitat quality can also change and new environments may become available for reservoir hosts, vectors and their parasites. Because of individual differences in exposure and susceptibility, the circulation and local persistence of infectious agents within and among host populations will depend on variation in individual movements and behaviors, as outlined by modeling approaches (Keeling and Rohani 2007; Plowright et al. 2011). Finally, because of effects they will have on host-parasite gene flow, the relative dispersal rates of hosts and parasites in spatially structured populations will also affect the coevolution of populations (Thompson 2005; Louhi et al. 2010; Mazé-Guilmo et al. 2016). These epidemiological, ecological, and evolutionary processes may all be at play when infectious agents can infect several host species; in that case, the spatial context of the interactions is also very likely to be important to consider, notably when reservoir species are to be identified and potential management decisions to be taken (Viana et al. 2014).

## What types of host movement are important for the spread of infectious disease agents?

### Migration

Migratory movements have been clearly identified as being important for the spread of infectious agents over broad spatial scales (Altizer et al. 2011; Bauer and Hoyer 2014). This is notable because of the large geographic scale they often encompass and the fact that they can contribute to the contact among individuals from various populations at remotely located stop-over and/or wintering sites (Fig. 1). Migration often occurs between high latitude areas, where reproduction takes place in the summer, and wintering





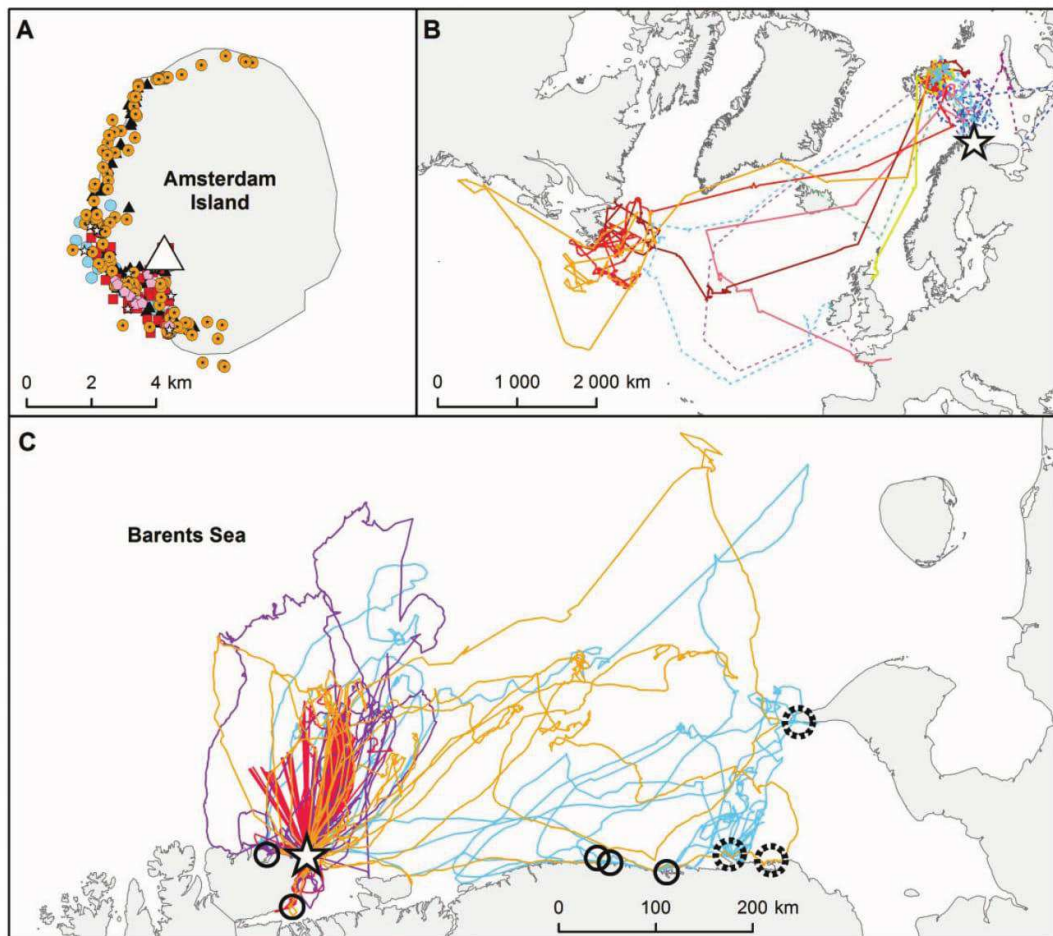
**Fig. 1** Different types of movements may lead to superspreading events. Such movements need to be made between locations where transmission among hosts can occur, thus they often involve the movements of infected hosts towards patches containing susceptible hosts (or the reverse). The timing of movements is also critical to consider in relation to opportunities for superspreading events. Migration as well as dispersal and foraging may be responsible for key spreading events in some cases, but a largely neglected type of movement, prospecting (i.e., visits among breeding groups within a season), may be a critical type of movement for the spatial epidemiology, ecology and evolution of host–parasite interactions, notably in social species.

areas at latitudes closer to the equator, although seasonal migratory movements may take various other forms (Dingle 2014). Migratory movements often involve large numbers of individuals that gather in high densities at sites where the transmission of infectious agents can occur between infected and susceptible individuals. Migration has thus been identified as a key process in the spread of both directly and environmentally transmitted agents (e.g., avian influenza viruses: Olsen et al. 2006; Gilbert et al. 2006, Hill et al. 2012), and vector-borne agents (e.g., tick-transmitted Lyme disease bacteria, Leighton et al. 2012; or mosquito-transmitted blood parasites, Fuller et al. 2012). Geographic and temporal characteristics of movements are important to consider in this context because the course of infection may simultaneously affect the likelihood of transmission and the ability of sick individuals to move long distances (Gaidet et al. 2010). The physiological stress associated with migration may also affect host susceptibility, and thus, potentially, disease transmission (Dingle 2014). The fact that a

disease involves migratory birds is nevertheless not sufficient in itself to assume that migratory behavior *per se* is the factor responsible for the spread of the infectious agent. Considering where and when transmission is likely to occur in relation to other types of movements and events may also be important (Fig. 1).

**Foraging**

Foraging movements can affect the spatial spread of many infectious agents if they are food-borne agents, trophically transmitted parasites, or directly transmissible when individuals compete for a focal food resource (Fig. 1). Although foraging movements usually occur at relatively much smaller scales than migratory movements, this is not always the case. Like many other seabirds, Scopoli’s shearwaters (*Calonectris borealis*) can forage several hundred kilometers from their colony; this is only a slightly smaller spatial scale than that of their winter migration (Péron and Grémillet 2013). Although such distances are impressive, it is unlikely that this mode of



**Fig. 2** Different types of movements potentially responsible for superspreading events: **(A)** Foraging locations of 6 Subantarctic skuas (*Catharacta antarctica*) recorded with GPS-UHF during the breeding season 2015 on Amsterdam Island, southern Indian Ocean. Over 3 days, the tracked individuals foraged close to their breeding colony (white triangle) among several marine vertebrate colonies they are preying upon and where they may disperse infectious agents; **(B)** Migratory movements of Black-legged kittiwakes (*Rissa tridactyla*) breeding on Hornøya, Norway (white star) recorded with satellite transmitters for the winter 2010–2011 (solid lines,  $n=5$ ) and 2011–2012 (dashed lines,  $n=3$ ). Despite such large scale movements, these pelagic seabirds are unlikely to contribute to the spread of infectious agents because most of them remain at sea during migration and wintering; **(C)** Movements of 4 failed breeding Black-legged kittiwakes recorded with GPS-UHF during the breeding season 2015. They show prospecting visits to known (solid circles) or suspected (dashed circles) kittiwake colonies situated  $> 40$  km away from their nesting colony, in Hornøya, Norway (white star). These often overlooked movements may significantly contribute to the spread of parasite vectors and infectious agents.

foraging will lead to the broad circulation of parasites and infectious agents because the birds do not necessarily interact closely with conspecifics when they forage. Conversely, in some cases, small scale foraging movements could indirectly result in the spread of some agents to another species that, in turn, spreads them on a broader scale. For example, scavenging or predatory birds, such as skuas, may

connect host prey populations (Fig. 2A) and transfer an infectious agent that may then have a dramatic effect on the dynamics of the prey species and/or spread over long distances via migration.

#### Dispersal

Dispersal is defined as the movement of individuals between a location where they were born or bred to



a new location where they breed (Clobert et al. 2001; Ronce, 2007; Fig. 1). Dispersal has attracted attention as another important type of movement for the spatial dynamics of infectious diseases, although empirical work in wild populations has lagged behind theory, possibly because many factors may affect dispersal (Boulinier et al. 2001; Blackwood et al. 2013). In addition to its contribution to understanding the dynamics of host–parasite interactions within meta-populations, where the dispersal of an infected host can lead to inter-patch colonization (Hess et al. 2002; Bjørnstad et al. 2004), host dispersal has been identified as a key factor in the evolution of host–parasite interactions as it implies gene flow among local host populations, which alters local standing genetic diversity and, thus, coevolution with infectious agents (Gandon et al. 1996; Gandon and Michalakis 2002; Thompson 2005). It has been suggested that the relative dispersal rates of hosts and parasites among local populations directly affect local adaptation of the host to the parasites and *vice versa* (Gandon et al. 1996, Lion and Gandon, 2015). But not all movements between host populations lead to dispersal *per se*; an overlooked detail when making generalizations about the dynamics of parasite circulation in subdivided populations (McCoy et al. 2005a; Mazé-Guilmo et al. 2016). For example, some exploratory movements, like prospecting, may not lead to the dispersal of a host individual, but may contribute to the large scale dispersal of a parasite among host populations.

### Prospecting

Prospecting movements are defined as visits made by individuals to locations where they are not currently breeding, but where they may settle in the future to reproduce (Reed et al. 1999; Fig. 1). Described for many bird species (Reed et al. 1999), these types of forays outside the birth location or main current breeding site of an individual are usually reported in a context of breeding habitat selection, in which such visits may allow individuals to gather information on the suitability of potential future breeding sites and to initiate access to these sites. Prospecting movements made by individuals before they first breed are reported to occur at much larger scales than prospecting forays made by adult birds that failed their breeding attempt (Reed et al. 1999), although the sometime much more nomadic movements of pre-breeders, especially in very young age classes of long-lived species, are especially difficult to track. Attendance at breeding areas by prospecting individuals usually occurs late in the breeding season in

colonially breeding bird species; at that time, individuals are possibly preparing their local recruitment in the next season (Boulinier et al. 1996; Reed et al. 1999). Prospectors often land, walk, enter nesting burrows, or land on nests or chicks (in birds) or directly interact with conspecifics (in birds and mammals), although it is often difficult to infer where the prospecting individuals came from. Prospecting forays have also been reported in other taxa, such as in social mammals, in which they are associated with attempts by individuals to seek mating opportunities outside their social group (Young et al. 2005, 2007). In an epidemiological context, these movements have been neglected because they are often secretive and difficult to track, also because they can be brief and involve young and/or unmarked individuals coming from outside a study area. They may however be especially important for the circulation of infectious agents because, as opposed to most dispersal events, they occur within a single breeding season and can involve intimate contact between individuals with very different exposure histories, favoring pathogen exchange.

### Transmission mode of infectious agents and the role of host movement in their dispersal

The transmission mode of infectious agents will determine the potential implications of host movement for their dispersal and transmission. Directly transmitted agents, and in particular sexually transmitted diseases, will require direct contact between susceptible and infectious hosts which can occur at any time, during any type of movement. When environmental transmission is required (for instance, for food-borne infectious agents), transmission chains require individuals to visit specific sites (Roche and Rohani 2010). While these agents may be less likely to be transferred by certain large-scale movements, their shedding along a migratory pathway or during visits with another social group could lead to a successful transmission event. The spread of environmentally transmitted infections may also be favored by scavengers or predators whose foraging grounds can be very large and encompass different sub-populations of infected and susceptible hosts (Fig. 2A). Their role may be reinforced by the year-round nature of their interactions with their prey, as opposed to the more seasonally variable contact rates that some (especially migratory) species display. Finally, vector-borne agents require the local presence of the vector for transmission to occur, but this condition is not in itself sufficient to ensure transmission, as hosts may not

transmit the infectious agent to the vector during close contact, and even a very close contact, such as a bite, may not result in successful infection of a new host. In some cases, when the vector is also a parasite (e.g., ticks, fleas, lice), it can be dispersed by a vertebrate along with the infectious agent (e.g., Comstedt et al. 2006). However, the successful colonization of a new habitat by a vector requires a specific set of conditions, such as suitable environmental conditions and the long-term presence of suitable hosts. Depending on the dispersed life stage of the vector, one may also expect very different rates of successful colonization.

### The case of prospecting: insight using a highly spatially structured seabird system

#### Why seabirds?

The role of birds as reservoirs and disseminators of parasites and pathogens has received much attention over the past several years due their high mobility. However, the large spatial scales involved and the numerous species that share migratory flyways and breeding or wintering grounds make it difficult to disentangle the potential role of different types of movements for pathogen dissemination. Seabirds are particularly interesting hosts in this respect. In addition to incredible long-distance movements during migration, foraging and prospecting, these birds are long-lived, site faithful and breed in dense aggregations in specific colony locations with a limited diversity of host species (Furness and Monaghan 1987; McCoy et al. 2016). These characteristics can not only favor both the local maintenance and large-scale dissemination of parasites and pathogens, but also makes them ideal model systems to assess the role of different types of movements in spreading disease agents.

Even when breeding in polar areas, seabird populations are exposed to a high diversity of parasites and infections. Here, we focus on a particular system involving *Ixodes uriae*, a tick that commonly exploits colonial seabirds in temperate and polar regions, and the different infectious agents it transmits (McCoy et al. 2005b; Dietrich et al. 2011; Dietrich et al. 2014). Indeed, this tick hosts a suite of viruses (Chastel 1988), among which are several flaviviruses, that are extensively distributed among seabird species over a wide geographic area (Heinze et al. 2012). The tick *I. uriae* also transmits Lyme disease bacteria to seabirds in both hemispheres (Olsen et al. 1995; Gasparini et al. 2001; Gomez-Diaz et al. 2011). Local infestation levels by *I. uriae* can vary strongly among breeding cliffs (Gasparini et al. 2001) and colonies (McCoy et al.

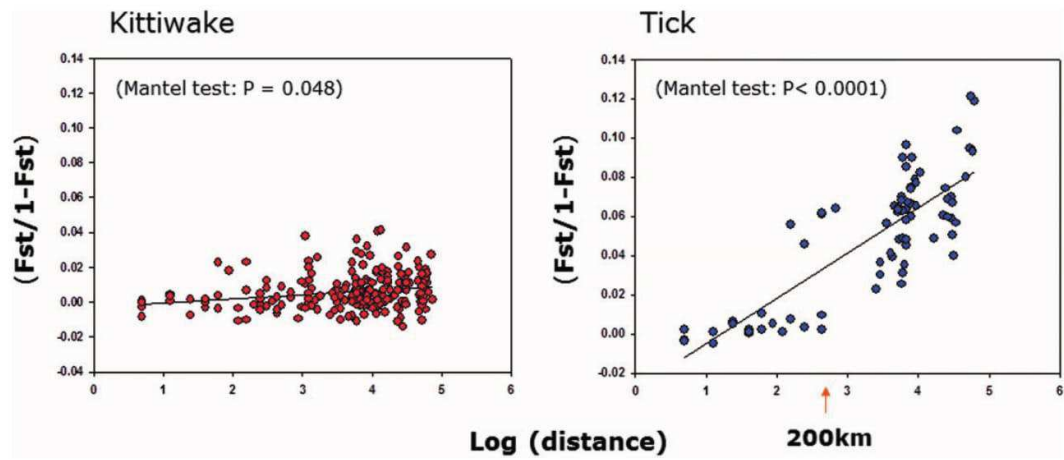
1999), and the tick relies entirely on its seabird host for inter-colony dispersal; given that seabird breeding phenology is highly seasonal at high latitudes, and that these ticks require about a week to complete a blood meal, inter-colony dispersal of ticks can only occur via the movements made by birds within the breeding season. Indeed, most seabirds, such as the black-legged kittiwake *Rissa tridactyla*, spend the winter at sea, far from land (Fig. 2B and Frederiksen et al. 2012).

Which individuals move among colonies during a breeding season should thus be paramount to the transmission of ticks and infectious agents. Young birds at fledging may frequently wander among colonies, and are often heavily infested by ticks. This was noted by Danchin (1992), who reported the occurrence of non-local fledglings parasitized by ticks in nests that were otherwise uninfested. These observations were possible due to detailed survey work of all the nests within a study colony where all the nestlings had been ringed (Danchin 1992). Another category of individuals that may visit different colonies within a breeding season are prospecting individuals that have not bred or that have failed their breeding attempt (Cadiou et al. 1994). Prospecting has been extensively studied in the kittiwake because it is relatively easy to track local movements of marked individuals that breed on vertical cliff faces and because these movements may be associated with the gathering of public information used by individuals to choose a future breeding site (Boulinier and Danchin 1997; Danchin et al. 1998; Boulinier et al. 2008). Prospecting visits occur late in the breeding season (Boulinier et al. 1996), when successful areas typically attract more individuals, potentially favoring the dispersal of parasites among breeding groups. This type of movement could be especially important in a metapopulation context, among colonies at regional scales. However, tracking marked individuals over large areas and within colonies where thousands of birds breed cannot be easily achieved by traditional capture-mark-resighting methods.

#### An indirect approach to the role of prospecting

As an indirect way to explore the potential role of prospecting in the dispersal of parasites, the population genetic structure of the kittiwake and its tick *I. uriae* was investigated using a comparable set of genetic markers for each species (microsatellites; McCoy et al. 2005a). For the tick, population genetic differentiation varied over the range of scales considered; little structure among tick populations exploiting kittiwakes was found among colonies separated by up to 200 km, whereas significant structure was





**Fig. 3** Exploration of the population genetic structure of a host species (A), the black-legged kittiwake (*Rissa tridactyla*), and of its tick (*Ixodes uriae*) (B) as an indirect way of inferring the spatial scale of host movements and their potential implication for the circulation of tick-borne agents. The figure shows pairwise genetic distance ( $F_{ST}/1-F_{ST}$ ) as a function of the geographical distance between populations sampled at the scale of the North Atlantic. Both host and parasite showed significant patterns of isolation by distance, but tick populations were not structured when colonies were separated by less than  $\sim 200$  km ( $\sim \log 2.3$ ), suggesting tick dispersal occurs via the prospecting of individual hosts among colonies situated from ten to a few hundred kilometers apart (redrawn from McCoy et al. 2005a).

observed beyond this limit and increased with the geographic distance between colonies (Fig. 3B). Host populations, in contrast, showed very little evidence of differentiation at a comparable scale (Fig. 3A). This suggests prospecting birds move frequently enough between colonies up to 200 km apart that tick populations are able to remain genetically mixed at this spatial scale. When exploring the population genetic structure of ticks sampled from other seabird species across the same scales, for example, the Atlantic puffin (*Fratercula arctica*), no structure was found among tick populations isolated by more than 700 km. This might be due to differences in behavior and breeding biology among seabird species. Puffins breed in burrows on the slopes above cliffs (rather on the vertical faces of cliffs) and might be more likely to drop-off or pick-up ticks while prospecting within colonies than kittiwakes (McCoy et al. 2003). The impact of frequent, large scale movements in seabirds and their ticks can be seen in patterns of geographic structure in the pathogens they transmit. For example, a phylogeographic study of the most frequently occurring Lyme disease bacterium in seabirds, *Borrelia garinii*, demonstrated large-scale exchange of strains both within and across ocean basins (Gomez-Diaz et al. 2011).

In contrast to hard ticks such as *I. uriae*, soft ticks infesting seabirds, such as *Ornithodoros maritimus* infesting yellow-legged gulls (*Larus michahellis*), may move less among colonies. Indeed, stable

differences in the seroprevalence of a tick-borne flavivirus have been recorded between colonies less than 100 km apart (Arnal et al. 2014). In soft ticks, nymphal and adult ticks take very brief blood meals ( $\sim 10$  min), typically at night when birds do not move. Because these ticks may thus rarely be dispersed, even by short movements of prospecting individuals, the study of their population genetic structure is likely to provide less information on the distance and frequency of host prospecting movements than that of hard ticks. Overall, the study of the genetic structure of vector populations and that of infectious agents is important as it allows one not only to infer dispersal rates at different scales, but also to explore whether host specialization plays a role in the circulation of infectious agents. In communities of host species with various levels of mobility, the degree of specialization of vectors could indeed potentially (and dramatically) affect the spread of infectious agents (e.g., see McCoy et al. 2003 and 2005b for the seabird-tick system).

#### A more direct approach to the role of prospecting

Although prospecting movements were suspected to occur over large spatial scales, the use of GPS tracking tools to record these movements was only recently proposed (Ponchon et al. 2013). GPS loggers are commonly used to track the foraging movements of breeding birds during incubation or chick rearing (e.g., Ponchon et al. 2014). However, their

deployment for studying prospecting movements has lagged, mainly because failed breeders, who are more likely to prospect, are also more difficult to recapture, especially if they bred in an area where local breeding success was low (Chambert et al. 2012; Ponchon et al. 2013). The tracking of individuals that failed their breeding attempt (e.g., that lost their eggs) has nevertheless been successful (Ponchon et al. 2015 and Fig. 2C), notably using UHF-GPS loggers that do not require the individuals to be recaptured in order to gather location data. Evidence of repeated prospecting visits by failed breeders to colonies situated tens of kilometers away is now accumulating, and suggests that those movements may connect colonies in a broad social network within which parasites/pathogens may be dispersed.

### The case of other (social) species

We have shown that prospecting movements may be responsible for disease spread in seabirds, much more likely than migratory movements, which, even if they occur at much broader scales might not necessarily lead to successful parasite dispersal given that most seabird species overwinter at sea. But could such prospecting movements be involved in superspreading events in other systems? Relatively, little is known about prospecting in non-social bird species (Pärt and Doligez 2003), but it is likely that prospecting movements by birds may contribute to the spread of parasites between local host populations, independently of migratory movements.

In social mammals, forays of individual meerkats (*Suricata suricatta*) among groups other than their own have been identified as prospecting events (Young et al. 2005, 2008; Drewe et al. 2009), and are suspected of leading to the dispersal of infectious agents such as tuberculosis bacteria (Drewe et al. 2010). In such cases, movements appear to be related to opportunities to mate outside the group, which if successful lead to direct contact among individuals (Doolan and Macdonald, 1996; Drewe et al. 2009). More generally, the movements of individual mammals among social groups have been related to an increased risk of spreading diseases (Altizer et al. 2003; Craft et al. 2011). Although the spatial scale involved is much smaller than that of highly mobile and colonially breeding mammals, such as some species of bats and seals, this issue has also specific implications in relation to disease management. When the structure of social groups is disrupted, some individuals may venture further from the group, leading to increased transmission of infectious agents

among groups and species. This is thought to be the case for tuberculosis bacteria among badgers (*Meles meles*). In fact, badger control efforts in the UK have been suspected to increase dispersal rates of individuals and lead to tuberculosis dissemination (Rogers et al. 1998 ; Vicente et al. 2007; McDonald et al. 2008). In the case of disease transmission between wild and domestic animals, the behavioral processes affecting the spatial movements of individuals may thus also be critical to consider (Miguel et al. 2013).

## Research Perspectives

### Pending questions

Many challenging questions remain to be addressed in the field of disease ecology (Tompkins et al. 2011) and we hope that our review emphasizes that integrating movement ecology and parasite transmission will open up new avenues of research. We have suggested that a relatively neglected type of movement, i.e., prospecting, may play a significant role in some instances, but its importance for the spread of disease agents in relation to other movements, notably in complex communities of hosts and parasites, remains to be determined. Even in the relatively simple cases we considered, much work is still needed to explore whether we can relate the movements of individuals to transmission events and eco-epidemiological dynamics. We have shown that some movements, conditioned by the experience of individuals, have the potential to spread infectious agents, but we still do not fully understand how much this depends on other plastic responses, such as the development of acquired immunity following exposure to infectious agents (Cross et al. 2005). In addition to their current involvement in reproduction, individuals may vary in their propensity to prospect, which could have direct implications for the spread of infectious agents. In particular, their individual exposure history and the timing of events affecting the quality of the environment may be critical. In this context, approaches considering the dynamic dimension of social network could be especially useful (Sih et al. 2009; Craft et al. 2011) and may prove powerful to address related community dynamic questions in heterogeneous landscapes. In addition, one may wonder how the effects of natural versus human-induced movements can be related to habitat selection and disease agent transmission.

### Available tools

The availability of tools to explore the roles of various types of movements on the dispersal of



infectious agents has increased dramatically over the last two decades. In addition to classical capture–mark–recapture/resighting approaches, miniaturized GPS loggers are widely available for use in tracking the movements of vertebrates; these enable research teams to track the movements of individuals with high spatial and temporal precision without requiring recapture (Nathan et al. 2008). This is especially useful as we have seen that roaming movements by non-breeding individuals or individuals that failed their current breeding attempt may be critical when studying transmission of some infectious agents, even if these are not the easiest to track (Ponchon et al. 2013). The development of radio-frequency identification technology, which allows the efficient tracking of large numbers of individuals simultaneously, will likely continue to generate very useful data to infer social contact networks and enable us to better infer the contribution of different types of movements to disease dissemination (Perkins et al. 2009). Networks of antennas, set-up within breeding groups, may also inform us about the occurrence of key movements, particularly for burrow nesting species which are difficult to directly survey. In some situations, loggers recording the proximity among individuals may be especially useful. More indirect tools are also becoming increasingly available. In addition to classical population genetic approaches for host and parasite populations, the use of microbial genetics may provide useful complementary data about transmission networks (e.g., VanderWaal et al. 2014). More classical methods, such as serological studies or the use of isotope analyses, can also be powerful to address questions related to the histories of individuals in terms of exposure to a community of infectious agents and particular habitats.

#### **Including disease ecology in movement ecology studies**

To obtain the most from data gathered to answer questions about movement and disease agent circulation, carefully designed studies should be set-up and implemented in the field. Such designs should allow researchers to account for potential biases that may limit the strength of the inferences to be made. A key issue in disease ecology is, for instance, accounting for the fact that it is difficult to be certain whether an individual has been exposed or not to specific infectious agents; this can now be dealt with using relevant statistical tools in addition to powerful biomedical tools (McClintock et al. 2010). Indeed, it is not because a field sample does not lead to the positive detection of an infectious agent or

specific antibodies that the sampled individuals are not infected or have not been exposed to the agent. Hierarchical sampling designs at appropriate temporal and spatial scales are especially important in this context as they can be used to account for detectability issues across scales. Statistical approaches originally developed in the context of capture–mark–recapture analyses are now increasingly applied to eco-epidemiological situations (Cooch et al. 2012; Choquet et al. 2013), and these should prove useful in such contexts.

Another important issue is the need for replication at relevant spatial scales; this may be especially critical when we consider movements among locations at broad scales (the focal study units become the patches, not necessarily the individuals within patches). As mentioned above, the design of field studies would benefit from a priori conceptual work involving participants with complementary skills, notably integrating modeling, laboratory and field-based approaches (Restif et al. 2012). Experimental approaches solidly grounded on detailed observational work with the study system may be especially powerful to address some specific questions. In the case of the potential importance of prospecting movements, we have seen that particular environmental and/or social conditions may favor prospecting; the manipulation of these conditions may thus provide a way to address the potential causal link between degradation of local conditions and the occurrence of prospecting movements. In cases where massive breeding failures may be due to local disease outbreaks, vaccination on some host patches could for instance enable one to address local epidemiological issues, along with issues related to the dispersal of the infectious agent among patches.

#### **Conclusion**

We believe that our review of the various types of movements that may be important for the super-spreading of infectious agents highlights that, in addition to gathering data on migratory species as part of extensive ringing programs, the specific design and implementation of field studies addressing original questions about the complex processes that underlie the dynamics of infectious agents in wild populations is required. Such studies must rely on integrative population approaches, and will likely be especially valuable if they are conducted in a spatially explicit context and involve, in addition to classical disease ecology methodologies, the tracking of the

individual host histories across environmental conditions.

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### References

- Adams B, Kapan, DD. 2009. Man bites mosquito: understanding the contribution of human movement to vector-borne disease dynamics. *PLoS ONE* 4:e6763.
- Altizer S, Nunn CL, Thrall PH, Gittleman JL, Antonovics J, Cunningham AA, Dobson AP, Ezenwa VO, Jones KE, Pedersen AB et al. 2003. Social organization and parasite risk in mammals: integrating theory and empirical studies. *Annu Rev Ecol Evol Syst* 34:517–47.
- Altizer S, Bartel R, Han BA. 2011. Animal migration and infectious disease risk. *Science* 331:296–302.
- Arnal A, Gómez-Díaz E, Cerdà-Cuëllar M, Lecollinet S, Pearce-Duvel J, Busquets N, García-Bocanegra I, Pagès N, Vittecoq M, Hammouda A et al. 2014. Circulation of a Meaban-like virus in yellow-legged gulls and seabird ticks in the western Mediterranean Basin. *PLOS One* 9:e89601.
- Bauer S, Hoye BJ. 2014. Migratory animals couple biodiversity and ecosystem functioning worldwide. *Science* 344:1242552.
- Bjørnstad ON, Peltonen M, Liebhold AM, Baltensweiler W. 2002. Waves of larch budmoth outbreaks in the European alps. *Science* 298:1020–23.
- Blackwood JC, Streicker DG, Altizer S, Rohani P. 2013. Resolving the roles of immunity, pathogenesis, and immigration for rabies persistence in vampire bats. *Proc Natl Acad Sci USA* 110:20837–42.
- Boulinier T, Danchin E, Monnat J-Y, Doutrelant C, Cadiou B. 1996. Timing of prospecting and the value of information in a colonial breeding bird. *J Avian Biol* 27:252–56.
- Boulinier T, Danchin E. 1997. The use of conspecific reproductive success for breeding patch selection in territorial migratory species. *Evol Ecol* 11:505–17.
- Boulinier T, McCoy KD, Sorci G. 2001. Dispersal and parasitism. In Clobert J, Danchin E, Dhondt A, Nichols JD, editors. *Dispersal*. Oxford: Oxford University Press Inc. p. 169–79.
- Boulinier T, Mariette M, Danchin E, Doligez B. 2008. Choosing where to breed: breeding habitat choice. In Danchin E, Giraldeau L-A, Cezilly F, editors. *Behavioral ecology*. London: Oxford University Press Inc. p. 285–321.
- Boulinier T, McCoy KD, Yoccoz NG, Gasparini J, Tveraa T. 2008. Public information affects breeding dispersal in a colonial bird: kittiwakes cue on neighbours. *Biol Lett* 4:538–40.
- Bowler DE, Benton TG. 2005. Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. *Biol Rev* 80:205–25.
- Chambert T, Pardo D, Staszewski V, McCoy KD, Tveraa T, Boulinier T. 2012. Heterogeneity in detection probability along the breeding season in Black-legged kittiwakes: implications for sampling design. *J Ornithol* 152:371–80.
- Chastel CE. 1988. Tick-borne virus infection of marine birds. In Harris K, editor. *Advances in disease vector Research* 5. New York: Springer Inc. p. 25–60.
- Choquet R, Carrie C, Chambert T, Boulinier T. 2013. Estimating transitions between states using measurements with imperfect detection: application to serological data. *Ecology* 94:2160–65.
- Cleaveland S, Borner M, Gislason M. 2014. Ecology and conservation: contributions to One Health. *Rev Sci Tech Office Intl Epizoot* 33:615–27.
- Clobert J., Danchin E., Dhondt A, Nichols JD. 2001. eds. *Dispersal*. Oxford: Oxford University Press Inc.
- Comstedt P, Bergström S, Olsen B, Garpmo U, Marjavaara L, Mejlon H, Barbour AG, Bunikis J. 2006. Migratory passerine birds as reservoirs of Lyme borreliosis in Europe. *Emerg Infect Disease* 12:1087–95.
- Cooch E, Conn PB, Ellner SP, Dobson AP, Pollock KP. 2012. Disease dynamics in wild populations: modeling and estimation: a review. *J Ornithol* 152:S485–509.
- Craft ME, Volz E, Packer C, Meyers LA. 2009. Distinguishing epidemic waves from disease spillover in a wildlife population. *Proc Roy Soc Lond B Biol Sci* 276:1777–85.
- Craft ME., Volz E, Packer C, Meyers LA. 2011. Disease transmission in territorial populations: the small-world network of Serengeti lions. *J Roy Soc Interface* 8:776–86.
- Cross PC, Lloyd-Smith JO, Johnson PLF, Getz WM. 2005. Duelling timescales of host movement and disease recovery determine invasion of disease in structured populations. *Ecol Lett* 8:587–59.
- Cunningham AA. 1996. Disease risks of wildlife translocations. *Conserv Biol* 10:349–53.
- Danchin E. 1992. The incidence of the tick parasite *Ixodes uriae* in Kittiwake *Rissa tridactyla* colonies in relation to the age of the colony, and a mechanism of infecting new colonies. *Ibis* 134:134–41.



- Danchin E, Boulinier T, Massot M. 1998. Conspecific reproductive success and breeding habitat selection: implications for the study of coloniality. *Ecology* 79:2415–28.
- Daszak P, Cunningham AA, Hyatt AD. 2000. Emerging infectious diseases of wildlife - threats to biodiversity and human health. *Science* 287:443–49.
- Dietrich M, Gómez-Díaz E, McCoy KD. 2011. Worldwide distribution and diversity of seabird ticks: implications for the ecology and epidemiology of tick-borne pathogens. *Vector-Borne Zoon Disease* 11:453–70.
- Dietrich M, Kempf F, Boulinier T, McCoy KD. 2014. Tracing the colonisation and diversification of the worldwide seabird ectoparasite *Ixodes uriae*. *Mol Ecol* 23:3292–305.
- Diggle PJ. 2000. Overview of statistical methods for disease mapping and its relationship to cluster detection. In Elliott P, Wakefield, JC, Best NG, Briggs DJ, editors. *Spatial epidemiology: methods and applications*. London: Oxford University Press Inc. p. 87–103.
- Dingle H. 2014. *Migration. The biology of life on the move*, 2nd edition. London: Oxford University Press Inc.
- Doolan SP, Macdonald DW. 1996. Dispersal and extra-territorial prospecting by slender-tailed meerkats (*Suricata suricatta*) in the south-western Kalahari. *Journal of Zoology* 240: 59–73.
- Drewe JA, Madden JR, Pearce GP. 2009. The social network structure of a wild meerkat population: 1. Inter-group interactions. *Behav Ecol Sociobiol* 63:1295–306.
- Drewe JA. 2010. Who infects whom? Social networks and tuberculosis transmission in wild meerkats. *Proc Roy Soc Lond B Biol Sci* 277:633–42.
- Fenner AL, Godfrey SS, Bull CM. 2011. Using social networks to deduce whether residents or dispersers spread parasites in a lizard population. *J Animal Ecol* 80:835–38.
- Fieberg J, Matthiopoulos J, Hebblewhite M, Boyce MS, Frair JL. 2010. Correlation and studies of habitat selection: problem, red herring or opportunity? *Phil Trans Roy Soc Lond B* 365:2233–44.
- Frederiksen M, Moe B, Daunt F, Phillips RA, Barrett RT, Bogdanova MI, Boulinier T, Chardine JW, Chastel O, Chivers LS et al. 2012. Multi-colony tracking reveals the non-breeding distribution of a pelagic seabird on an ocean basin scale. *Diversity Distrib* 18:530–42.
- Fritzsche McKay A, Hoye, 2016. Are migratory animals super-spreaders of infection?. *Integrative Comparative Biology*.
- Fuller T, Bensch S, Muller I, Novembre J, Perez-Tris J, Ricklefs RE, Smith TB, Waldenstrom J. 2012. The ecology of emerging infectious diseases in migratory birds: an assessment of the role of climate change and priorities for future research. *EcoHealth* 9:80–88.
- Furness RW, Monaghan P. 1987. *Seabird ecology*. Glasgow: Blackie.
- Gaidet N, Cappelle J, Takekawa JY, Prosser DJ, Iverson SA, Douglas DC, Perry WM, Mundkur T, Newman SH. 2010. Potential spread of highly pathogenic avian influenza H5N1 by wildfowl: dispersal ranges and rates determined from large-scale satellite telemetry. *J Appl Ecol* 47:1147–57.
- Gandon S, Capowiez Y, Dubois Y, Michalakis Y, Olivieri I. 1996. Local adaptation and gene-for-gene coevolution in a metapopulation model. *Proc Roy Soc Lond B* 263:1003–9.
- Gandon S, Michalakis Y. 2002. Local adaptation, evolutionary potential and host-parasite coevolution: interactions between migration, mutation, population size and generation time. *J Evol Biol* 15:451–62.
- Gasparini J, McCoy KD, Haussy C, Tveraa T, Boulinier T. 2001. Induced maternal response to the Lyme disease spirochaete *Borrelia burgdorferi sensu lato* in a colonial seabird, the Kittiwake *Rissa tridactyla*. *Proc Roy Soc Lond B* 1467:647–50.
- Gilbert M, Xiao X, Domenech J, Lubroth J, Martin V, Slingenbergh J. 2006. Anatidae migration in the Western Palearctic and spread of highly pathogenic Avian Influenza H5N1 virus. *Emerg Infect Disease* 12:1650–56.
- Gomez-Diaz E, Boulinier T, Cornet M, Ferquel E, Sertour N, McCoy KD. 2011. Genetic structure of marine *Borrelia garinii* and population admixture with the terrestrial cycle of *Lyme borreliosis*. *Environ Microbiol* 13:2453–67.
- Hanski I, Gaggiotti OE. 2004. eds. *Ecology, genetics and evolution of metapopulations*. London: Elsevier Academic Press Inc.
- Harding KC, Härkönen T, Caswell H. 2002. The 2002 European seal plague: epidemiology and population consequences. *Ecol Lett* 5:727–32.
- Heinze DM, Gould EA, Forrester NL. 2012. Revisiting the clinal concept of evolution and dispersal for the tick-borne flaviviruses by using phylogenetic and biogeographic analyses. *J Virol* 86:8663–71.
- Hess GR, Randolph SE, Arneberg P, Chemini C, Furlanello C, Harwood J, Roberts MG, Swinton J. 2002. Spatial aspects of disease dynamics. In Hudson PJ, Rizzoli A, Grenfell BT, Heesterbeek H, Dobson AP, editors. *The ecology of wildlife disease*. London: Oxford University Press Inc.
- Hill NJ, Takekawa JY, Ackerman JT, Hobson KA, Herring G, Cardona CJ, Runstadler JA, Boyce, WM. 2012. Migration strategy affects avian influenza dynamics in mallards (*Anas platyrhynchos*). *Mol Ecol* 21:5986–99.
- Hochachka WM, Dhondt AA. 2000. Density-dependent decline of host abundance resulting from a new infectious disease. *Proc Natl Acad Sci USA* 97:5303–06.
- Holt R, Boulinier T. 2005. Ecosystem and parasitism: the spatial dimension. In Thomas F, Renaud F, Guegan, J-F, editors. *Ecosystem and parasitism*. Oxford: Oxford University Press, Inc. p. 68–84.
- Jeltsch F, Bonte D, Pe'er G, Reineking B, Leimgruber P, Balkenhol N, Schröder B, Buchmann CM, Mueller T, Blaum N et al. 2013. Integrating movement ecology with biodiversity research - exploring new avenues to address spatiotemporal biodiversity dynamics. *Mov Ecol* 1:6.
- Johnson AR, Wiens JA, Milne BT, Crist TO. 1992. Animal movements and population dynamics in heterogeneous landscapes. *Landscape Ecol* 7:63–75.
- Keeling MJ, Bjornstad, ON, Grenfell BT. 2004. Metapopulation dynamics of infectious diseases. In Hanski I, Gaggiotti O, editors. *Ecology, genetics and evolution of metapopulations*. San Diego: Elsevier Academic Press Inc. p. pages 415–45.
- Keeling MJ, Rohani P. 2007. *Modeling infectious diseases in humans and animals*. Princeton: Princeton University Press Inc.
- Leighton PA, Koffi JK, Pelcat Y, Lindsay LR, Ogden NH. 2012. Predicting the speed of tick invasion: an empirical model of range expansion for the Lyme disease vector *Ixodes scapularis* in Canada. *J Appl Ecol* 49:457–64.

- Lion S, Gandon S. 2015. Evolution of spatially structured host-parasite interactions. *J Evol Biol* 28:10–28.
- Lloyd-Smith JO, Schreiber SJ, Kopp PE, Getz WM. 2005. Superspreading and the effect of individual variation on disease emergence. *Nature* 438:355–59.
- Lobato E, Pearce J, Staszewski V, Gómez-Díaz E, González-Solís J, Kitaysky AS, McCoy KD, Boulinier T. 2011. Species and location effects on the prevalence of antibodies anti Lyme disease *Borrelia spirochaetes* in seabirds breeding in North Pacific islands. *Vector-Borne Zoon Disease* 11:1521–27.
- Louhi K-R, Karvonen A, Rellstab C, Jokela J. 2010. Is the population genetic structure of complex life cycle parasites determined by the geographic range of the most motile host? *Infect Genet Evol* 10:1271–77.
- Mazé-Guilmo E, Blanchet S, McCoy KD, Loot G. 2016. Host dispersal as the driver of parasite genetic structure: a paradigm lost? *Ecol Lett* 19:336–47.
- McCallum H, Barlow N, Hone J. 2001. How should pathogen transmission be modelled? *Trends Ecol Evol* 16:295–300.
- McClintock BT, Nichols JD, Bailey LL, MacKenzie DI, Kendall WL. 2010. Seeking a second opinion: uncertainty in disease ecology. *Ecol Lett* 13:659–74.
- McCoy KD, Boulinier T, Chardine J, Michalakis Y, Danchin E. 1999. Dispersal of the tick *Ixodes uriae* within and among seabird host populations: the need for a population genetic approach. *J Parasitol* 85:196–202.
- McCoy KD, Boulinier T, Tirard C, Michalakis Y. 2003. Differential host-associated dispersal of the seabird ectoparasite *Ixodes uriae*. *Evolution* 57:288–96.
- McCoy KD, Boulinier T, Tirard C. 2005a. Comparative host-parasite population structures: disentangling prospecting and dispersal in the Black-legged kittiwake *Rissa tridactyla*. *Mol Ecol* 14:2825–38.
- McCoy KD, Chapuis E, Tirard C, Boulinier T, Michalakis Y, Le Bohec C, Le Maho Y, Gauthier-Clerc M. 2005b. Recurrent evolution of host-specialized races in a globally-distributed parasite. *Proc Roy Soc Lond B* 272:2389–95.
- McCoy KD, Dietrich M, Jaeger A, Wilkinson DA, Bastien M, Lagadee E, Boulinier T, Pascalis H, Tortosa P, Le Corre M et al. 2016. The role of seabirds of the Iles Eparses as reservoirs and disseminators of parasites and pathogens. *Acta Oecologica* 72: 98–109.
- McDonald RA, Delahay RJ, Carter SP, Smith GC, Cheeseman CL. 2008. Perturbing implications of wildlife ecology for disease control. *Trends Ecol Evol* 23:53–56.
- Miguel E, Grosbois V, Caron A, Boulinier T, Fritz H, Cornelis D, Foggini C, Makaya PV, Tshabalala PT, De Garine-Wichatitsky M. 2013. Contacts and foot and mouth disease transmission from wild to domestic bovines in Africa. *Ecosphere* 4:art51.
- Morales JM, Moorcroft PR, Matthiopoulos J, Frair JL, Kie JG, Powell RA, Merrill EH, Haydon DT. 2010. Building the bridge between animal movement and population dynamics. *Phil Trans Roy Soc Lond B Biol Sci* 365:2289–301.
- Mundt CC, Sackett KE, Wallace LD, Cowger C, Dudley JP. 2009. Long-distance dispersal and accelerating waves of disease: empirical relationships. *Am Nat* 173:456–66.
- Nathan R, Getz WM, Revilla E, Holyoak M, Kadmon R, Saltz D, Smouse PE. 2008. A movement ecology paradigm for unifying organismal movement research. *Proc Natl Acad Sci USA* 105:19052–59.
- Olsen B, Duffy DC, Jaenson TG, Gylfe A, Bonnedahl J, Bergström S. 1995. Transhemispheric exchange of Lyme disease spirochetes by seabirds. *J Clin Microbiol* 33:270–274.
- Olsen B, Munster VJ, Wallensten A, Waldenström J, Osterhaus AD, Fouchier RA. 2006. Global patterns of influenza A virus in wild birds. *Science* 312:384–88.
- Pärt T, Doligez B. 2003. Gathering public information for habitat selection: prospecting birds cue on parental activity. *Proc Roy Soc Lond B Biol Sci* 270:1809–13.
- Perkins SE, Cagnacci F, Stradiotto A, Arnoldi D, Hudson PJ. 2009. Comparison of social networks derived from ecological data: implications for inferring infectious disease dynamics. *J Animal Ecol* 78:1015–22.
- Péron C, Grémillet D. 2013. Tracking through life stages: adult, immature and juvenile autumn migration in a long-lived seabird. *PLoS ONE* 8:e72713.
- Plowright RK, Foley P, Field HE, Dobson AP, Foley JE, Eby P, Daszak P. 2011. Urban habituation, ecological connectivity and epidemic dampening: the emergence of Hendra virus from flying foxes (*Pteropus* spp.). *Proc Roy Soc Lond B* 278:3703–12.
- Ponchon A, Grémillet D, Doligez B, Chambert T, Tveraa T, Gonzales-Solis J, Boulinier T. 2013. Tracking prospecting movements involved in breeding habitat selection: insights, pitfalls and perspectives. *Method Ecol Evol* 4:143–50.
- Ponchon A, Grémillet D, Christensen-Dalsgaard S, Erikstad KE, Barrett RT, Reiertsein TK, McCoy KD, Tveraa T, Boulinier T. 2014. When things go wrong: intra-season dynamics of breeding failure in a seabird. *Ecosphere* 5:art4.
- Ponchon A, Chambert T, Lobato E, Tveraa T, Grémillet D, Boulinier T. 2015. Breeding failure induces large scale prospecting movements in the black-legged kittiwake. *J Exp Marine Biol Ecol* 473:138–45.
- Pulliam HR. 1988. Sources, sinks, and population regulation. *Am Nat* 132:652–61.
- Rappole JH, Derrickson SR, Hubálek Z. 2000. Migratory birds and spread of West Nile virus in the Western Hemisphere. *Emerg Infect Disease* 6:319–28.
- Reed JM, Boulinier T, Danchin E, Oring L. 1999. Informed dispersal: prospecting by birds for breeding sites. *Curr Ornithol* 15:189–259.
- Restif O, Hayman DT, Pulliam JR, Plowright RK, George DB, Luis AD, Cunningham AA, Bowen RA, Fooks AR, O'Shea TJ, Wood JL, Webb CT. 2012. Model-guided fieldwork: practical guidelines for multidisciplinary research on wildlife ecological and epidemiological dynamics. *Ecol Lett* 15:1083–94.
- Roche B, Rohani P. 2010. Environmental transmission scrambles coexistence patterns of avian influenza viruses. *Epidemics* 2:92–8.
- Rogers LM, Delahay R, Cheeseman CL, Langton S, Smith GC, Clifton-Hadley RS. 1998. Movement of badgers (*Meles meles*) in a high-density population: individual, population and disease effects. *Proc Roy Soc Lond B* 265:1269–76.
- Ronce O. 2007. How does it feel to be like a rolling stone? Ten questions about dispersal evolution. *Annu Rev Ecol Syst* 38:231–253.



- Sih A, Hanser SF, McHugh KA. 2009. Social network theory: new insights and issues for behavioral ecologists. *Behav Ecol Sociobiol* 63:975–88.
- Stoddard ST, Morrison AC, Vazquez-Prokopec GM, Soldan VP, Kochel TJ, Kitron U, Elder JP, Scott, TW. 2009. The role of human movement in the transmission of vector-borne pathogens. *PLoS Negl Trop Disease* 3:e481.
- Thompson JN. 2005. *The geographic mosaic of coevolution*. Chicago: University of Chicago Press Inc.
- Tompkins DM, Dunn AM, Smith MJ, Telfer S. 2011. Wildlife diseases: from individuals to ecosystems. *J Animal Ecol* 80:19–38.
- VanderWaal KL, Atwill ER, Isbell LA, McCowan B. 2014. Linking social and pathogen transmission networks using microbial genetics in giraffe (*Giraffa camelopardalis*). *J Animal Ecol* 83:406–14.
- Viana M, Mancy R, Biek R, Cleaveland S, Cross PC, Lloyd-Smith JO, Haydon DT. 2014. Assembling evidence for identifying reservoirs of infection. *Trends Ecology Evol* 29:270–79.
- Vicente J, Delahay RJ, Walker NJ, Cheeseman CL. 2007. Social organization and movement influence the incidence of bovine tuberculosis in an undisturbed high-density badger *Meles meles* population. *J Animal Ecol* 76:348–60.
- Young AJ, Carlson AA, Clutton-Brock T. 2005 Trade-offs between extraterritorial prospecting and helping in a cooperative mammal. *Animal Behav* 70:829–37.
- Young AJ, Spong, G, Clutton-Brock T. 2007 Subordinate male meerkats prospect for extra-group paternity: alternative reproductive tactics in a cooperative mammal. *Proc Roy Soc Lond B* 274:1603–09.











## **Convergence dans l'évolution de la spécialisation d'hôte chez des tiques : modèle tiques-oiseaux de mers à distribution mondiale.**

Les interactions intimes et répétées entre hôtes et parasites peuvent engendrer la spécialisation d'un parasite à son hôte, grâce à des adaptations comportementales, morphologiques et/ou génétiques, combinées avec un flux de gènes limité. C'est un processus clef car il participe à l'évolution de la biodiversité parasitaire et peut ainsi permettre de mieux comprendre l'émergence d'organismes pathogènes. Encore peu étudié, une spécialisation d'hôte a néanmoins été démontrée lors de précédentes études chez deux espèces de tiques nidicoles : chez *Ixodes uriae* une tique dure, parasite des oiseaux marins coloniaux en zone arctique, et dans un complexe de tiques molles *Ornithodoros capensis* sensu lato, parasitant aussi de nombreuses espèces d'oiseaux marins, mais cette fois-ci en zones tempérées et tropicales. Ces espèces sont vectrices d'une grande diversité d'agents pathogènes incluant des virus, des bactéries et des protozoaires. Cependant, les facteurs impliqués dans le phénomène de spécialisation d'hôte restent inconnus. Dans ce cadre, le but de ma thèse était donc de déterminer 1) si l'évolution des divergences en fonction des hôtes est toujours accompagnée par les mêmes changements phénotypiques et 2) si ces changements pourraient permettre d'identifier les facteurs de sélection sous-jacents. Dans ce contexte, des campagnes d'échantillonnage de tiques ont été menées durant la période de reproduction des hôtes oiseaux dans les différentes zones de leur répartition et nous avons réalisé des analyses morphométriques, basées sur l'utilisation de landmarks et de contours sur chaque individu tique et des analyses phylogénétiques et génétiques des populations sur les mêmes individus. L'ensemble de ces résultats suggère la présence de convergences morphologiques au sein de ces systèmes et souligne un rôle de la sélection dans ce processus de divergence. En effet, les caractéristiques écologiques des hôtes mais aussi le micro-habitat exercent des pressions sélectives importantes dans ces deux systèmes pouvant être à l'origine de la divergence observée entre les populations. De plus, les caractéristiques biologiques de chaque espèce de tiques, telle que la capacité de dispersion, entrent également en jeu et peuvent fortement modifier l'épidémiologie des agents infectieux dont elles sont vectrices.

Mots clés : Argasidae, écologie de la transmission, évolution convergente, interactions hôte-parasite, Ixodidae, oiseaux marins.

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### **Convergence in the evolution of host specialization of ticks: insights from two worldwide tick-seabird model systems**

Intimate and repeated interactions between hosts and parasites can lead to parasite specialization to a given host via behavioral, morphological and/or genetic adaptations that act in combination with restricted gene flow. Specialization is a key process leading to the generation of parasite biodiversity and can help us understand the emergence of pathogenic organisms. Although little studied, host specialization has already been demonstrated to occur in previous studies of two nidicolous tick species: *Ixodes uriae* a hard tick parasitizing colonial seabirds in polar regions, and soft ticks of the complex *Ornithodoros capensis* sensu lato, that also exploit colonial seabirds, but this time in temperate and tropical zones. These species act as vector to a wide variety of pathogenic organisms, including viruses, bacteria and protozoa. However, the factors involved in host specialization remain unknown. In this context, the aim of my thesis was to determine 1) whether the evolution of host specialization is always accompanied by the same phenotypic changes and 2) whether these changes could help to identify the selective factors that influence this phenomenon. In this context, tick collections were conducted during the breeding period of the host birds in different areas of their distribution and morphometric analyses, based on landmark and contour methods, were performed on each individual tick. Phylogenetic and population genetic analyses were also carried out using the same individuals. Overall, the results demonstrate that morphological convergence occurs within these systems, highlighting the role of selection in the divergence process. Indeed, the ecological characteristics of the hosts, but also their micro-habitat, may exert significant selective pressures on ticks and may cause the observed divergence among populations. Likewise, the biological characteristics of each tick species, particularly in relation to dispersal capacity, may also come into play and will greatly modify the epidemiology of associated infectious agents.

Keywords: Argasidae, convergent evolution, host-parasite interactions, Ixodidae, transmission ecology, seabirds

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**Spécialité:** Biologie des Interactions ; **Discipline:** Evolution des systèmes infectieux

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