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Assessing the conservation status of pangolins in the dahomey gap using spatial and molecular ecology, ethnozoology and conservation genetics

Stanislas Zanvo

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THÈSE

En vue de l'obtention du **DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE**

Délivré par l'Université Toulouse 3 - Paul Sabatier

Cotutelle internationale: Université d'Abomey-Calavi(UAC)

Présentée et soutenue par
Stanislas ZANVO

Le 10 mars 2022

**Evaluation du statut de conservation des pangolins dans le
Dahomey Gap à travers l'écologie spatiale et moléculaire,
l'ethnozologie et la génétique de conservation**

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M. Clément AGBANGLA, Président

Dedication

I dedicate this to my folks, Désiré Zanvo and Tohossi Ganhounouto, with love and admiration for their lives' accomplishments.

To my wife Nadia Houéfa Hounkponou and my children Sèdjro, Donan and Yélognissè for their patience and sacrifice during these four years of almost permanent absence.

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Abbreviations

%	percent
°C	Celcius degree
ACPBA	Association des Chasseurs Professionnels du Borgou Alibori
AIC	Akaike Information Criterion
ANOSIM	Analysis of Similarity
ANOVA	Analysis of Variance
AVIGREF	Associations Villageoises de Gestion des Réserves de Faune
CENAGREF	Centre National de Gestion des Réserves de Faune
CENATEL	Centre National de Télédétection et de Suivi Ecologique
CITES	Convention on Internrtional Trade in Endangered Species
cm	centimetre
CR1	Control Region 1
DG	Dahomey Gap
DGEFC	Direction Générale des Eaux-Forêts et Chasse
DGL	Dahomey Gap Lineage
DTT	Dithiothreitol
eDNA	environmental DNA
e.g.	Exempli Gratia (for exemple)
i.e.	id est (that is)
FAO	Food and Agriculture Organization of the United Nations
GLM	General Linear Model
IBD	Isolation by Distance

ICD	International Classification of Diseases
INRAE	Institut National de la Recherche pour l'Alimentation et l'Environnement
IUCN	International Union for Conservation of Nature
km	kilometer
km²	square kilometer
LEK	Local Ecological Knowledge
MCMC	Markov Chain Monte Carlo
mtDNA	Mitochondrial DNA
NCBI	National Center of Biotechnology Information
NMDS	Non-metric Multidimensional Scaling
PAPE	Programme d'Appui aux Parcs de l'Entente
PCoA	Principal Coordinate Analysis
SMM	Single Mutation Model
SNP	Single Nucleotide Polymorphism
sPCA	Spatial Principal Component Analysis
TCM	Traditional Chinese Medicine
TMM	Traditional Medicine Market
TPM	Two Phase Model
UV	Use Value
USD	United States Dollar
WBP	White-bellied pangolin

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Summary

Pangolins are on the top of conservation priority due mainly to their unprecedented level of harvesting. The four African species are under great gap of baseline data that hampers the set up of a well-designed evidence-based strategy for tackling their alarming conservation status. My PhD dissertation focused on the biogeographically delineated Dahomey Gap region (West Africa) using the white-bellied pangolin as a case study to: (i) assess the geographic distribution and population trends of pangolin species across Benin, (ii) Model pangolin population extirpation rates using validated local ecological knowledge, (iii) assess the ethnozoological and commercial values of pangolins in Benin, (iv) investigate the population genetics of the white-bellied pangolin and set up the molecular tracing of their fine scale trade across the Dahomey Gap, and (v) assess the precise dietary composition of the white-bellied pangolin in Benin through eDNA metabarcoding.

The introduction presents an overview on pangolin species in general and the conservation context of the white-bellied and giant pangolins within the Dahomey Gap in particular. It describes the main objectives and the structure of the dissertation.

Chapter 1, titled *Study area*, clearly defined the traditional Dahomey Gap and the alternative delineation to which this thesis refers, especially in chapter 5. It describes the sampling sites comprising the forest habitats, bushmeat and traditional medicine markets.

Chapter 2, titled *Assessing the spatiotemporal dynamics of endangered mammals through local ecological knowledge combined with direct evidence: The case of pangolins in Benin (West Africa)* shed the light on the geographic distribution and population trends of the white-bellied and giant pangolins over the last two decades across Benin using LEK and direct evidence. LEK

revealed a 31 and 93 % contraction of the occurrence areas of the white-bellied and giant pangolins respectively, and highlighted habitat degradation and overexploitation as the main drivers of population decline for both species.

Chapter 3, titled *Modelling pangolin population extirpation rates using validated local ecological knowledge*, modelled the probability of persistence for each pangolin species in Benin over the last two decades and predicted future occurrence patterns using LEK, direct evidence and landscape metadata. Generalized linear model revealed that species identity, abundance in 1998, distances from main roads and protected areas to villages, land use changes and deforestation over time were factors that significantly explained the current persistence of pangolins in Benin. Prediction models suggested decreasing trends for the white-bellied pangolin over the next two decades and a total extirpation of the giant pangolin whatever the scenario considered (deforestation versus no deforestation).

Chapter 4, titled *Ethnozoological and commercial values of pangolins in Benin*, assessed the ethnozoology of pangolins across their occurrence ranges, estimated the pricing of pangolins and deciphered the trade network in Benin through a crossed analysis of LEK collected in the traditional medicine markets and rural areas. Pangolins are valuable animals for local people and ethnozoological knowledge varied significantly from southern to northern Benin. The trade network included traditional healers, restorers, vendors in the traditional medicine markets, citizens from neighbor countries and Chinese diaspora. Pangolins in rural areas and traditional medicine markets are relatively cheaper in Benin compared to other countries in West and central Africa, but their price is 3-8 times higher when clients are from the Chinese diaspora.

Chapter 5, titled *Can DNA help trace the local trade of pangolins? A genetic assessment of white-bellied pangolins from the Dahomey Gap (West Africa)*, used nuclear and mitochondrial markers to understand the conservation genetics of the white-bellied pangolin within the Dahomey Gap. Microsatellites and mitochondrial DNA-based analyses suggested low genetic diversity, inbred populations, absence of clear geographic subdivision and isolation by distance between populations. Demographic history assessment suggested a drastic decrease in effective population size 100-250 generations ago. Overall, mitochondrial DNA-based phylogenetic analysis suggested an endemic trade within the Dahomey Gap affecting the Dahomey Gap lineage of white-bellied pangolins, although microsatellite markers pinpointed a long-distance trade within and between countries in the Dahomey Gap.

Chapter 6, titled *Assessing the precise dietary spectrum of the white-bellied pangolin in Benin using DNA metabarcoding*, experienced for the first time the eDNA metabarcoding method to investigate the feeding ecology of a pangolin species. This study revealed that the eDNA metabarcoding approach is appropriate for determining the diet composition of pangolins. The white-bellied pangolin preys mainly on ants and termites but also other insect taxa belonging to Anisembiidae, Bethylidae, Endomychidae, Liposcelididae, Cicadellidae, Tephritidae, Acrididae, Thripidae, Cecidomyiidae, Chrysididae, Curculionidae and Pyrrhocoridae. The Shannon diversity of detected prey items was significantly higher ($p < 0.001$) for the gut content (*versus* scat) samples. Bray-Curtis dissimilarity index showed highly significant dietary profiles dissimilarities ($p < 0.001$) between southern and central regions. Spatial visualization showed high heterogeneity of distances between samples from central region, especially in the Mont Kouffé forest inversely to samples from southern region where distances tend to be almost homogeneous or null

between samples, suggest intra (central region) and inter variation of prey spectrum between region.

The last section of this dissertation discusses the major and relevant findings, their implications for the conservation of pangolins in Benin and perspectives for future investigations. Data on distribution should be considered to update the IUCN global map and the red list for Benin. The delineated spatial context could be crossed with population inbreeding and genetic diversity erosion to establish conservation priority and management strategies in the Dahomey Gap. The trafficking network and pangolin pricing represent a cornerstone tool to tackle the illegal trade. Diet investigations inform more on the ecological role of pangolins in natural ecosystems and could improve the decision making in managed feeding programs for the white-bellied pangolin. However, the giant pangolin presence/absence within the Alibori forest needs to be confirmed via direct evidence, and population size census estimates of both species is lacking. Our reference database needs to be improved for a better molecular identification of the termite preys detected in pangolin scats. Single nucleotide polymorphism markers should be used to investigate the genetic structure and illegal trade in the Dahomey Gap.

General introduction

General introduction

1. Background

Pangolins are placental mammals belonging to the order Pholidota and comprise eight species geographically restricted to African and Asian continents (Gaubert et al. 2020). Four species occur in Asia (Chinese pangolin, Indian pangolin, Philippines pangolin and Sunda pangolin) and other four species range in Africa (giant pangolin, Temminck's ground pangolin, black-bellied pangolin and white-bellied pangolin). They are edentates with an elongate tongue and represent the only mammalian group with a body covered by scales. All pangolin species excavate burrow, to varying extents and for shelter, except the black-bellied pangolin. All the species prey mainly on ants and termites (Pages 1968; Pietersen et al. 2016; Cabana et al. 2017) and occur within a diversity of habitats, ranged from primary to semi-degraded forests (Hoffmann et al. 2020; Jansen et al. 2020).

The wildlife trade is worth billions of dollars annually and affects mostly mammals (Morton et al. 2021). Pangolins are the most traded mammals in the world even during the first peak of the Covid-19 pandemic (March to August 2020, Aditya et al. 2021), and this raised up a worldwide concern over the taxonomic group. All eight species are listed on the Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2017) and their conservation status on the IUCN Red List of Threatened Species is either Vulnerable, Endangered or Critically Endangered (IUCN, 2019). Several tons of pangolins have been harvested from their natural habitats to feed legally and illegally an international trade since the turn of the 20th century, with unequal impacts following species and continents (Challender et al. 2020). Because the Asian pangolins have been poached to near extinction during the 20th century, the route of trafficking changed since the turn of the 21st century and an escalating growth in the illegal international

trade of African pangolins took place, now targeting mainly white-bellied and giant pangolins from Africa, for their scales in particular (Challender et al. 2015; Mwale et al. 2017; Ingram et al. 2019; Zhang et al. 2020). Recent estimates revealed that around 900 000 African pangolins were illegally traded over the last two decades (Challender et al. 2020). An assessment of the illegal trade chain estimated between 0.2 and 0.4 million, the number of pangolins annually harvested in Central Africa with increasing trends over time (Ingram et al. 2018). This intercontinental trade has been confirmed using the CITES database (Challender et al. 2015; Heinrich et al. 2016; Ingram et al. 2018), surveys in the bushmeat and traditional medicine markets (Boakye et al. 2016; Mambeya et al. 2018; Ingram et al. 2019) but also through DNA-profiling of specimens seized in Asia in particular (Mwale et al. 2017; Zhang et al. 2020). Some studies clearly established a connection between the Gulf of Guinea and Asia (Boakye et al. 2016; Mambeya et al. 2018; Ingram et al. 2019). In response to the high demands of African pangolins and their derivatives, an inflation of their purchase prices was recorded in Central Africa (Mwale et al. 2017; Ingram et al. 2018). The selling prices of pangolins increases about 73 and 211 % for the white-bellied and giant pangolins, respectively, in Gabon (Mambeya et al. 2018), and 2 - 6 times respectively on the urban wildlife markets in the central Africa (Ingram et al. 2018). Such a context highlights the major role that international trade plays on the depletion of African pangolins, the white-bellied and giant pangolins in particular from their natural habitats.

In Africa, wild meat (non-domesticated animals) is an important source of animal protein and income for local people (Fa et al. 2002; Codjia & Assogbadjo 2004; McNamara et al. 2015; Ingram et al. 2021). Wild animals are openly sold in the bushmeat and traditional medicine markets (Djagoun et al. 2013; D’Cruze et al. 2020) that represent a great threat to African’s wildlife

(D’Cruze et al. 2020; Ingram et al. 2021) but also to human health (Zanvo et al. 2021a). Among specimens sold in these markets occur pangolin species and many other threatened species present on the IUCN Red List (Djagoun et al. 2013; D’Cruze et al. 2020; Ingram et al. 2021). National and international trade can drive a species to extinction (Morton et al. 2021). Local use in pangolins for consumption and traditional medicine combined with inter-regional and international trade in an unregulated context certainly emphasize the depletion of African pangolins. The latter may also suffer from fragmented and isolated populations by infrastructure development and agriculture that could rapidly lead to irreversible local extinctions (Reed et al. 2003; Clabby 2010).

In the Dahomey Gap, the white-bellied pangolin (*Phataginus tricuspis*) and giant pangolin (*Smutsia gigantea*) have been previously reported (Sayer & Green 1984; Akpona et al. 2008; Akpona & Daouda 2011; Nixon et al. 2019; Pietersen et al. 2019). Benin remains the only country in the Dahomey Gap where the giant pangolin has been reported but without any direct or recent evidence (Sayer & Green 1984; Akpona & Daouda 2011; Amori et al. 2016; Nixon et al. 2019). The Dahomey Gap encompasses one of six phylogenetically delineated lineage of the white-bellied pangolin (Gaubert et al. 2016). This lineage has been named the “Dahomey Gap lineage” even if the phylogenetic analysis clustered individuals from Benin and Togo (Dahomey Gap countries) with individuals originated from Nigeria belonging to the lower Guinean rainforest block (White 1983). The white-bellied and giant pangolins are Endangered and Critically Endangered respectively on the Red List for Benin. The both two species are Endangered on the IUCN Red List of threatened species. As most of threatened species, natives and non-natives, pangolins are openly traded in the wildlife markets despite their national status of protected species (see (Djagoun et al. 2013; D’Cruze et al. 2020). Pangolins in the Dahomey Gap are equally targeted by the international trade and has

been facing unprecedented poaching pressures to feed the growing local needs fueled by the high international demands (Ingram et al. 2019). If Togo was the most targeted country by the international trade in pangolins within the traditional Dahomey Gap (Heinrich et al. 2016; Wasser et al. 2018), 513 kg of pangolin's scales on destination to Vietnam were recently seized by the forest officers at the international airport of Cotonou in Benin (Ingram et al. 2019). This represents an unprecedented seizure in Benin and an evidence that Beninese pangolins are also involved in the international trafficking. All the above information shows clearly that pangolins within the Dahomey Gap have been undergoing unprecedented harvesting levels, harmful to long term conservation of the endemic Dahomey Gap lineage.

Within the Dahomey Gap, pangolins occur in a human-dominated landscape (Akpona & Daouda 2011; Segniagbeto et al. 2021). The natural vegetation covers 26 % of the land area with 23.3 and 14 % of terrestrial Protected Areas patchily distributed in a farmland-dominated landscape matrix in Benin and Togo (Bogaert et al. 2011; Mama et al. 2013; Alohou et al. 2017; Padonou et al. 2017; Atsri et al. 2018; Tente et al. 2019; Akodéwou et al. 2020) respectively . Some of the natural strongholds of pangolins are invaded by human settlements and therefore under high anthropogenic pressures (Amegadjè 2007; Akpona & Daouda 2011; Atsri et al. 2018; Koubodana et al. 2019). The human-dominated landscape has been fragmented by the development of agriculture, road infrastructures and urbanization (Ahlonko et al. 2015; Atsri et al. 2018; Koubodana et al. 2019). Several forest patches have been severely degraded by logging and geographically isolated by large agricultural surfaces, road network and growing cities that could impede the movements of pangolins like all other wild animals across the landscape. In this human-mediated landscape, the use of transportation corridors by pangolins may cause direct mortality through collision with vehicules (Moreli

2017). Such a spatial configuration of physically disconnected habitat patches, may induce an absence of functional connectivity between populations due probably to the limited dispersal ability of pangolins (Pietersen et al. 2014). The absence of a functional connectivity between forest patches can impact the genetic diversity, the dynamics of pangolin populations and their survival (Wilson et al. 2015; Roffler et al. 2016; Creech et al. 2017). From northern to southern Benin and Togo occur three climatic zones (White 1983) and this could accelerate the genetic divergence process between populations confined to the different climatic zones.

Although the illegal pangolin trade is widely acknowledged to be the major threat to the conservation of pangolins globally and African pangolins in particular (Challender et al. 2015, 2020), these data are not sufficient to mitigate the ongoing decline of all eight species. They are still ecologically and genetically data poor species with a great gap of knowledge (Ingram et al. 2019; Heighton & Gaubert 2021) that hampers effective conservation actions. The lack of ecological knowledge is certainly related to the cryptic and elusive behaviour of pangolins (Heath & Coulson 1997; Willcox et al. 2019) making challenging the biomonitoring of the species (Cunningham & Lindenmayer 2005; Lomba et al. 2010; Singhal et al. 2018). The phylogeny and population genetics of African pangolins have been previously addressed (Gaubert et al. 2018; Du Toit et al. 2020), but these studies were based on limited data globally without precisions of sampled forest habitats. The phylogeographic investigations and population genetic using Single Nucleotide Polymorphism markers have been performed on Asian pangolins (*Manis javanica* and *Manis pentadactyla*). These studies were conducted at global scale (Nash et al. 2018; Hu et al. 2020). The illegal international trade of pangolins has been traced from seized specimens using DNA-barcoding (Mwale et al. 2017; Zhang et al. 2020) and to our knowledge, never the illegal trade of African pangolins has

been investigated using population genetic markers and at fine scale, identifying the source forests targeted by local, regional and international trade. Few microsatellite markers-based studies focused on Asian pangolins (Ching-Min Sun et al. 2020) and African pangolins (Du Toit et al. 2020; Aguillon et al. 2020) and the main objective of these studies was the development of markers for the tracing of the illegal trade and the population genetics.

Effective and modern wildlife conservation implementation requires informed decision-making (Rose et al. 2018; Ausden & Walsh 2020). In the Dahomey Gap, knowledge on pangolins was extremely poor with only one study on ecology and ethnozoology of the white-bellied pangolin in southern Benin (Akpona et al. 2008). The paucity of scientific knowledge makes challenging the conservation decision-making in the Dahomey Gap. Apart from the pioneer study in Lama forest reserve (Akpona et al. 2008), no participatory management plans, dissertation thesis and scientific papers did document the presence of pangolins in Benin and a similar gap of knowledge was observed in Togo before a very recent investigation on the distribution of the white-bellied pangolin (Segniagbeto et al. 2021). The white-bellied and giant pangolins in both two countries were orphan species. The available data on the geographic distribution of both species were not reliable. A comparison of the global maps of IUCN Red List (Pietersen et al. 2019) for the white-bellied and giant pangolins with the local maps of the Red List for Benin (Akpona & Daouda 2011) reflected some incongruences about the geographic distribution of both species and call for scientific surveys to fill the gap on spatial ecology and population abundance over time, but also to identify the spatial and intrinsic drivers of the current geographic distribution for each species. Despite the local demand in pangolins openly sold as wild meat and their derivatives highly used in the traditional medicine, the ethnozoology of pangolins has little been

investigated (Akpona et al. 2008) and the commercial value of the species was unknown in Benin. Although an accurate knowledge on the source habitats of specimens openly sold in the local wildlife markets can help design a sound strategy to combat the illegal trade at a fine scale, the DNA profiling-based tracing of specimens traded on local wildlife markets and an identification of the trade network of pangolins have never been investigated in parallel in any country around the world. In the modern context of wildlife conservation, genetic knowledge is also applied to reduce the risk of population and species extinction (Frankham 2019; Teixeira & Huber 2021). Within such a heterogeneous and severely fragmented landscape as in the Dahomey Gap where populations of pangolins are under overhunting and experienced a particular climatic history (Salzmann & Hoelzmann 2005), population genetics and demographic history of each pangolin species can help anticipate the extinction of the Dahomey Gap lineage of the white-bellied pangolin. Even if the feeding ecology of Asian pangolins (Wu et al. 2005; Lim 2007; Irshad et al. 2015; Lee et al. 2017; Ashokkumar et al. 2017; Willcox et al. 2019; Sun et al. 2020; Karawita et al. 2020) and the Temminck's ground pangolin (Pietersen et al. 2016b) has been investigated using direct and indirect observations, none of these studies has reached a high taxonomic resolution identification of consumed preys. This data is essential to understand the role of pangolins in their wild habitats and for managed breeding programs. The high taxonomic resolution of the dietary preference of pangolins could be reached using molecular approach, the metabarcoding analysis of fecals, which allows detecting the elusive and cryptic species (Thomsen & Willerslev 2015).

This dissertation aims at filling a part of the knowledge gap highlighted above for pangolins using a multidisciplinary approach that combines macro-ecology, Local Ecological Knowledge (LEK), ethnozoology, feeding ecology through environmental DNA metabarcoding, population genetics and DNA

profiling-based trade tracing. The dissertation provides a package of scientific knowledge that we hope will be essential for long-term conservation of African pangolins globally and the white-bellied pangolin Dahomey Gap lineage in particular.

2. Research objectives

The present dissertation aims at promoting the conservation of pangolins in the Dahomey Gap via scientific knowledge. More specifically, this research project aims at:

- (i) assessing the geographic distribution and population trends of pangolin species across Benin;
- (ii) modelling pangolin population extirpation rates using validated LEK;
- (iii) assessing the ethnozoological and commercial values of pangolins in Benin;
- (iv) investigating the population genetics of white-bellied pangolins and setting up the molecular tracing of their fine scale trade across the Dahomey Gap;
- (v) assessing the precise dietary spectrum of the white-bellied pangolin in Benin using DNA metabarcoding.

3. Dissertation structure

The dissertation comprises six chapters (Fig. 1). It starts by a general introduction and ends with a general discussion. The general introduction gives an overview on the conservation status and threats to the conservation of all eight species of pangolins with a focus on the white-bellied and giant pangolins in the Dahomey Gap, outlines the objectives of the dissertation and the organisation of this document. The general discussion highlights the main

findings of the dissertation, compares them with previous studies on the focal taxonomic group and other studies focused on the same perspectives related to long-term conservation of pangolins. **Chapter 1** describes the phylogenetically delineated Dahomey Gap considered in **chapter 5**, and the sampling sites including the forest habitats, bushmeat and traditional medicine markets sampled for the five chapters. In **Chapter 2**, LEK combined with direct evidence (scales of pangolins) is used to shed the light on the geographic distribution and population trends over the last two decades of the white-bellied and giant pangolins across Benin. **Chapter 3** uses metadata, LEK and direct evidence to determine the drivers of the current spatial distribution of the white-bellied and giant pangolin delineated in **Chapter 2**, and predicts the spatial persistence of both species over the next two decades using two different spatial scenarios. Like **Chapter 3**, **Chapter 4** allows understanding the ethnozoological drivers of the decreasing trends observed of the white-bellied pangolin and the almost extirpation of the giant pangolin highlighted in **Chapter 1** using the LEK from stakeholders in the traditional medicine markets and local people across the occurrence habitats of both two species in Benin. **Chapter 5** uses a molecular approach to assess the consequences of the spatial context delineated in **Chapters 2 and 3** on the evolutionary process within the white-bellied pangolins, and attempts at tracing the trade revealed in **Chapter 4** by identifying the source forests of traded specimens in the wildlife markets at the Dahomey Gap scale. **Chapter 6** fills an important data gap on pangolins in general and the white-bellied pangolin in particular. It provides detailed knowledge on the feeding ecology of the species across its occurrence areas in Benin using eDNA metabarcoding. This chapter provides details on the prey spectrum of the focal species and further knowledge on the role of pangolins in their natural habitats that can help guide the decision-making and design managed breeding programs.

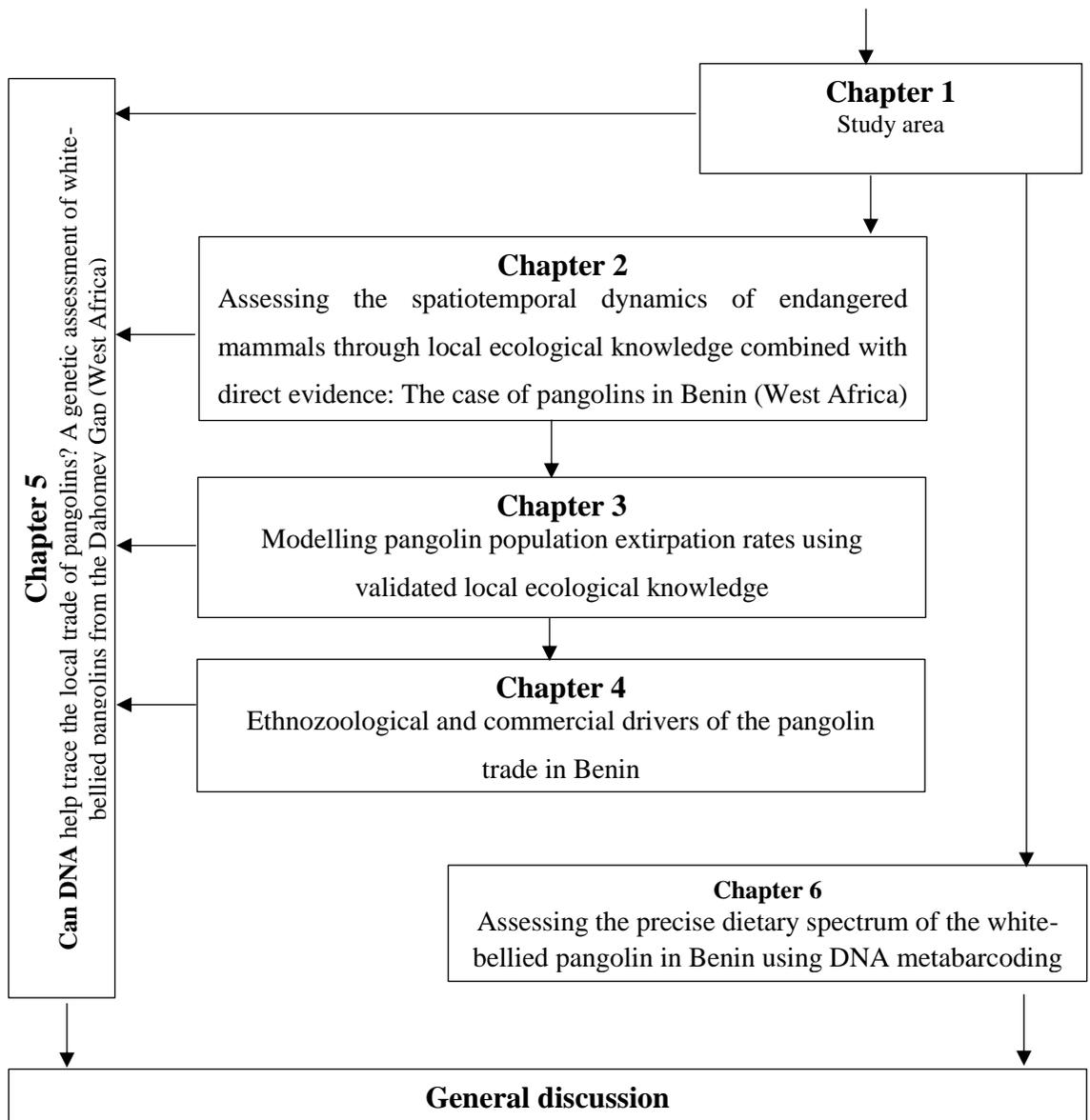


Figure 1. Diagram outlining the dissertation structure. Links between chapters are indicated by arrows

Chapter 1

Study area

CHAPTER 1

Study area

1.1. Geographic location and data: the Dahomey Gap as considered in this dissertation and samples used for each chapter

The Dahomey Gap

The Dahomey Gap, a savannah corridor that reaches the coast of southern Benin, Togo and eastern Ghana, separates currently the Upper Guinean (West Africa) and Lower Guinean (Central Africa) rain forest blocks between 0°-3° East of longitude. The Dahomey Gap in its current form appeared probably at the onset of the late Holocene period between 4500-3400 years ago (Salzmann & Hoelzmann 2005) and is considered to be an ecogeographical barrier to plants and animals (van Bruggen 1989). According to White (1983), the vegetation within the Dahomey Gap belongs to the Guinean transition zone with a mosaic of forests and savannas. In this dissertation, we will use the term “Dahomey Gap lineage” for white-bellied pangolins as referring to the occurrence areas of a phylogenetically delineated lineage endemic to the sub-region and including Benin, Togo and south-eastern Nigeria (Gaubert et al. 2016).

Sample type and size

Chapters 2 and 3 include the LEK and scale samples termed “direct evidence” in this dissertation. LEK was collected with local hunters in 312 villages, among which 156 were considered in Chapter 3 for a total of 52 villages where LEK was supported by direct evidence. Chapter 4 includes LEK from local people in 54 villages and vendors in five wildlife markets. In Chapters 5 and 6, genetic samples were used to achieve our objectives. A total of 189 pieces of tissue and 52 fecal/gut contents were used for Chapters 5 and 6 respectively.

1.2. Sampling sites

1.2.1. Forest habitats

Benin and Togo are the two countries in which forest habitats have been sampled for this dissertation. In Benin and Togo occur three climatic zones: Guineo-Congolian, Sudano-Guinean and Sudanian (White 1983). In the Guineo-Congolian zone, in the South, natural vegetation is represented by the patches of semi-deciduous, lowland and swamp forests. The native plant species include *Triplochiton scleroxylon*, *Celtis zenkeri*, *Cola gigantea*, *Milicia excelsa*, *Antiaris toxicaria*, *Ceiba pentandra*, *Albizia spp.*, *Alstonia congensis*, *Xylopia rubescens* and *Syzygium owariense*. In the Sudano-Guinean zone, in the Centre, occur the patches of woodland, dry and riparian forest whereas only woodland occurs in the Sudanian zone, in the North. The common plant species in the Sudano-Guinean zone are *Vitellaria paradoxa*, *Parkia biglobosa*, *Khaya senegalensis* and *Azelia africana* and *Combretum spp.*, *Acacia spp.*, *Hyparrhenia spp.*, *Loudetia spp.* and *Andropogon spp.*, the commonest plant species in the Sudanian zone. In these three climatic zones, the natural vegetation has been transformed by human activities such as shifting agriculture and logging (Amegadje 2007, Bogaert et al. 2011; Foussemi et al. 2012; Mama et al. 2013; Alohou et al. 2017; Padonou et al. 2017; Tente et al. 2019; Akodéwou et al. 2020). Most of the landscape in the Dahomey Gap is under cultivation with fields, fallows and plantations. The islands of natural vegetation are mostly protected either by local communities for religious reasons (hereafter called community forest) or by forest services (forest reserves, wildlife reserves and National Parks) for biodiversity conservation. According to Sinsin et al. (2010), such a mixture of community forests and forest reserves provide habitats for populations of many threatened and rare species in the southern and central regions including the red-bellied monkey

Cercopithecus erythrogaster erythrogaster endemic to the Dahomey Gap; *Colobus vellerosus*, *Procolobus verus*, *Hippopotamus amphibius*, *Funiscirus leucogenys*, *Funiscirus substriatus*, *Anomalurus derbianus*, *Phataginus tricuspis* and *Smutsia gigantea*, *Philantomba walteri*, *Ictonyx striatus*, *Mellivora capensis*, *Genetta genetta*, *Genetta tigrina*, *Ichneumia albicauda*, *Aonyx capensis*, *Lutra maculicollis*, *Viverra civetta*, *Atilax paludinoscus*, *Herpestes sanguinea*, *Dendrohyrax dorsalis*, *Tragelaphus spekii* and *Tragelaphus scriptus*. In the national Parks (North) occur the large mammals and carnivores: *Syncerus caffer*, *Hippotragus equinus*, *Aelaphus buselaphus major*, *Kobus kob*, *Kobus ellipsiprymnus defassa*, *Redunca redunca*, *Sylvicarpa grimmia*, *Cephalophus rufilatus*, *Ourebia ourebi*, *Tragelaphus scriptus*, *Damaliscus lunatus korrigum*, *Phachocoerus aethiopicus*, *Papio anubis*, *Cercopithecus aethiops*, *Erythrocebus patas*, *Panthera leo*, *Acynonix jubatus*, *Panthera pardus*, *Crocuta crocuta*, *Lycaon pictus* and *Loxodonta africana*. Small carnivores are also found in the Parks, including: *Canis aureus*, *Canis adustus*, *Ictonyx striatus*, *Mellivora capensis*, *Aonyx capensis*, *Lutra maculicollis*, *Viverra civetta*, *Atilax paludinoscus*, *Herpestes sanguinea*, *Felis lybica*, *Felis caracal*, *Felis serval*, *Genetta genetta*, “*Genetta tigrina*”, *Ichneumia albicauda*.

1.2.2. Bushmeat markets

Bushmeat refers to wild meat, the meat derived from non-domesticated animals hunted for consumption or trade in many regions of the world (Fa et al. 2002). The bushmeat markets are the public places where the wild meat is openly traded by local people. In Africa, the bushmeat markets are more developed in West and Central Africa where the phenomenon constitutes a major threat to biodiversity (Bennett et al. 2002; Ingram et al. 2021). These markets established in both rural and urban areas, generate substantial income

to local people whom depend on the trade for their survival (Fa et al. 2002, 2009). In West and Central Africa, most of the bushmeat markets are distributed along the road networks (Fa et al. 2014; Fargeot et al. 2017). In Benin where the bushmeat markets have been surveyed as part of this work, some of these markets operate only in the hunting season when the game is available. The largest and permanent bushmeat market in Benin is located near the Lama forest reserve at Tègon but other small markets are randomly distributed in the landscape from southern to northern areas.

1.2.3. Traditional medicine markets

Traditional medicine markets (TMMs) are the markets where plants and animal derivatives are openly sold for traditional medicine purposes. On these markets, native and non-native species, threatened species and even some species protected under national legislation are traded (Djagoun et al. 2013; D’Cruze et al. 2020). These markets are well known in West Africa, especially in Benin, Togo and Nigeria where they support cultural practices (medicinal and spiritual purposes). Nigeria, Togo and Benin in particular are widely known for their Vodoun culture, which is strongly linked to a diversity of traditional practices (medicinal and spiritual) using various animal derivatives (Djagoun et al. 2013; D’Cruze et al. 2020). Vodoun practices have driven the development of a dense TMM (also called “fetish markets”) network in Benin, with at least 42 markets identified in southern and central Benin (Zanvo et al. 2021a). In Togo occurs the large fetish market of Akodessèwa in Lomé. Most of these markets are permanent and operate every day. The traditional medicine markets have been sampled within the occurrence areas of the Dahomey Gap lineage of the white-bellied pangolin especially for the tracing of the trade in pangolins.

Chapter 2

Assessing the spatiotemporal dynamics of endangered mammals through local ecological knowledge combined with direct evidence: The case of pangolins in Benin (West Africa)

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CHAPTER 2

Assessing the spatiotemporal dynamics of endangered mammals through local ecological knowledge combined with direct evidence: The case of pangolins in Benin (West Africa)

Abstract

Although pangolins are considered to be one of the most trafficked wild mammals in the world, their conservation status remains uncertain through most of their ranges, and notably in western Africa. Using local ecological knowledge in combination with direct occurrence evidence, we assessed the distribution and abundance of the white-bellied pangolin (*Phataginus tricuspis*) and the giant pangolin (*Smutsia gigantea*) in Benin over the last two decades (1998-2018). We organised focus groups with local hunters in 312 villages within sampling units of 25 km × 25 km covering the whole country. Participatory maps of past and current geographic ranges of pangolins combined with direct evidence (96 collected scales) suggested that the white-bellied pangolin had a wide distribution and reached higher latitudes than previously known, whereas the giant pangolin could be restricted to a single forest and has been absent from the northern part of the country over at least the last two decades. Local hunters perceived a significant decline of the white-bellied pangolin (range contraction = 31% in 20 years) and almost unanimously an extirpation of the giant pangolin (93%), the latter being restricted to the Alibori forest reserve (northern Benin). Protected areas were identified as the main potential occurrence zones for pangolins: 77 and 100% of the potential geographic ranges of the white-bellied and the giant pangolins, respectively, overlapped partially or totally with the protected area network of Benin. Using a generalized linear model, we showed that the probability of reporting recent observations of the white-bellied pangolin was significantly higher for villages near protected areas and distant from main roads. The forests perceived as

having sustainable populations of white-bellied pangolins were the Lama Forest reserve (southern Benin) and the complex comprising Monts Kouffe - Wari Maro - Ouémé supérieur (central Benin), a view that was supported by the great number of direct occurrence evidence collected in those areas. Overall, our study highlights an important geographic range contraction of the white-bellied pangolin and a possible entire extirpation of the giant pangolin over the last two decades in Benin.

Key words: geographic range, local ecological knowledge, pangolins, population decline, local extirpation, Benin

2.1. Introduction

Pangolins (Pholidota, Mammalia), or scaly anteaters, are one of the most trafficked wild mammals in the world (Heinrich et al. 2017; Challender & Waterman 2017). They are facing unprecedented levels of harvesting to supply local bushmeat market networks (Mambeya et al. 2018) and, more importantly, to feed the ever-increasing demand from the Chinese Traditional Medicine market (Challender et al. 2015; Heinrich et al. 2016; Mwale et al. 2017; Ingram et al. 2019). Because of a recent decline of the Asian pangolin populations, an illegal, international trade sourcing from Africa into Asia has emerged (Baker 2014; Challender et al. 2020), likely facilitated by the increasing economic ties between East Asia (China) and many African countries (Ingram et al. 2019). These trafficking routes probably follow the same criminal networks as the ivory trade (TRAFFIC 2019).

Globally, around 895,000 pangolins were trafficked since the early 19th century (Challender et al. 2020) and the Gulf of Guinea is one of the main hub of the trade (Ingram et al. 2019). Although recent investigations using CITES trade data (Challender et al. 2015; Heinrich et al. 2016), bushmeat and

traditional medicine market surveys (Boakye et al. 2016; Ingram et al. 2018, 2019; Mambeya et al. 2018) and DNA profiling of seized scales (Mwale et al. 2017; Zhang et al. 2020) have shed light on the extent of the pangolin trade in Africa, the ecology, distribution and population trends of the four species occurring across the continent remain poorly studied.

Understanding spatiotemporal changes in distribution and abundance, and their drivers is a prerequisite for implementing successful wildlife conservation actions (Parry & Peres 2015). Such investigations have been carried out in part of the ranges for *Manis pentadactyla*, *M. crassicaudata*, and *M. javanica* in China and Bangladesh (Nash et al. 2016; Trageser et al. 2017) and *Smutsia temminckii* in southern African (Pietersen et al. 2016a). Ingram et al. (2019) highlighted the need of baseline ecological data for African pangolins in West Africa to inform policy makers and implement efficient conservation efforts. As such, investigating the spatiotemporal dynamics of African pangolins can help design effective conservation strategies at the country and continental scales.

Two species of pangolins are reported to occur in Benin: the white-bellied pangolin (*Phataginus tricuspis*) and the giant pangolin (*Smutsia gigantea*). The white-bellied pangolin is considered Endangered on both the IUCN Red List and the Red List for Benin, whereas the giant pangolin is considered Endangered on the IUCN Red List and Critically Endangered on the Red List for Benin. A certain number of discrepancies exist between the geographic range delineated for each species on the Red List for Benin (Akpona & Daouda 2011) and the global IUCN Red List assessments (Nixon et al. 2019; Pietersen et al. 2019c). Indeed, the IUCN Red List suggested the uncertain presence (i.e. possible extinction, no recent observations) of the giant pangolin in northern Benin (citing Akpona and Daouda 2011) and a latitudinally limited (6100 -

1000 N) distribution of the white-bellied pangolin, whereas the Red List for Benin showed a widespread distribution of both species in the national parks and forest reserves from southern to northern Benin.

In West Africa, pangolins are used for nutritional, medicinal and spiritual purposes, those having constituted drivers of population decline since the last three decades (Soewu et al. 2020). There is an urgent need to re-assess the distribution and population trends of the two species occurring in Benin, given the human-induced pressures that pangolins are facing, which combine agricultural activities and road infrastructure development (Mama et al. 2013; Alohou et al. 2017) and the local trade for bushmeat and traditional medicine (Codjia & Assogbadjo 2004, 2004; Akpona et al. 2008; Djagoun & Gaubert 2009; Djagoun et al. 2013). In addition, the international trafficking network has probably reached pangolin populations in Benin, with 513 kg of pangolin scales recently seized at the international airport of Cotonou (March 2018; Ingram et al. 2019). So far, only one local-scale study has investigated the ecological aspect of the white-bellied pangolin in Benin (in the Lama forest reserve; Akpona et al. 2008).

Large-scale (here, countrywide) assessments of species' distribution and population trends are especially challenging for nocturnal and elusive species such as pangolins (Thompson 2004; Pillay et al. 2011). The lack of country-scale and long-term data in Benin hampers a literature-based analysis, which can provide an alternative to surveys in countries where there is a paucity of research (Mota-Vargas & Rojas-Soto 2012). In this context, the use of LEK represents a cost-effective, reliable approach for monitoring past and current species distribution and population trends at large scales (Silvertown 2009; Turvey et al. 2015). LEK has received growing interest in ecology over the last two decades (Brook & McLachlan 2008; Bélisle et al. 2018). This approach is

especially appropriate in tropical regions where people depend mainly on natural resources (Pardo-de-Santayana & Macía 2015) and where collection of biological data is scarce (Lima et al. 2017; Martinez-Levasseur et al. 2017), such as in Benin (Gaillard 2010). LEK has been recommended for studying the distribution and population trends of cryptic and threatened species (Turvey et al. 2015), and has been used to study the distribution and conservation status of pangolins in Asia (Nash et al. 2016; Trageser et al. 2017) and southern Africa (Pietersen et al. 2016a).

Our study combines LEK with sampling of direct occurrence evidence to assess over the last two decades (1) the distribution of the white-bellied and giant pangolins in Benin, (2) the perceptions of local hunters on their population trends, and (3) the landscape drivers influencing the current geographic distribution of pangolins.

2.2. Material and methods

2.2.1. Sampling design and data collection

Benin was subdivided into 228 grids of 25 km x 25 km using ArcMap 10.1 (ESRI Inc.) (Fig. 2). At this grid size, 80% of the sampling units included a core of natural or semi-natural vegetation (potential hunting zones) with human presence, essential for collecting LEK. Forty-six grids were not considered due to the absence of villages within their boundaries. In southern, central and a part of northern regions, grid-based surveys were conducted within 122 grids. This sampling approach was not carried out for the remaining 48 grids in northern Benin and two incomplete grids (i.e. a small portion of grid cell without any potential habitat) in central Benin. We restricted our grid-based sampling efforts in northern Benin since we were able to gather grid level data for these areas from the Pendjari and W National Parks staff, in addition to professional hunters' associations (wildlife hunting management

organizations, AVIGREF and professional hunter organizations from the departments of Borgou and Alibori, ACPBA) and former poachers. These structures (AVIGREF and ACPBA) are affiliated to the two National Parks (Pendjari and W respectively). The two small portions of grid located in the central were covered by discussions in neighbouring grid cells.

We gathered information on the past and current occurrence of pangolins on the basis of key-respondent surveys in villages surrounded by habitats potentially favourable to pangolins (forest, tree and shrub savannah, gallery forest, wetland, swamped forest and old plantation). Target villages were selected using the source origins of the pangolins found in 49 bushmeat and traditional medicine markets (Zanvo et al. 2021a) and the potential habitats identified during meetings with stakeholder groups (and sometimes through informal interviews when we did not detain any prior information). At least one village per grid was sampled, except in the northern region where we were able to collect data for many grids from a reduced grid number. The sampling efforts varied from a grid to another according to the spatial extent of hunters' LEK, potential habitat diversity and size, and the presence of direct evidence. We used a focus group technique to collect data with different stakeholder groups (hunters, rangers and former poachers), except the wildlife-monitoring officers with whom we performed individual interviews before starting data collection in the Pendjari and W National Parks. Focus group technique is a cost-effective approach that has been widely used for addressing biodiversity conservation issues, notably in surveys of local communities with a similar hunting background to ours (Nyumba et al. 2018). Such an approach was relevant in the Beninese context where local hunters are likely to avoid giving their opinion individually, fearing negative repercussions from forest management authorities (S. Zanvo, pers. obs. March 2018). Moreover, hunting fraternities are known to exist across Benin, with memberships following well-

defined internal rules to keep secrets and knowledge (Kougniazondé et al. 2014).

Focus groups participants were identified through informal interviews with villagers and mediated by local community leaders and/or gatekeepers (the authorities of particular divinities 'Vodoun' or a secret organization in villages) whom assisted us during exchanges but not as respondents. Single focus groups (8 - 15 persons) were organized in 312 villages. Respondents selected to participate in the focus groups were local hunters, at least thirty years old generally, volunteers and having long-term (>10 years) residency in the area (Kotschwar Logan et al. 2015). However, in some villages, twenty years old hunters were also involved in the focus groups because they were strongly recommended as knowledgeable persons for recent observations of pangolins. They have just provided information on the frequency of observing/harvesting pangolins and potential habitats during the last five years. Snowball sampling approach (Berg 2001) was used to collect additional, potential occurrence sites of pangolins with non-participants (i.e. people not included in the focus groups) who provided us direct evidence at the end of focus groups. A local translator was used in some villages, generally a student able to speak French in order to facilitate discussion with participants. All participants were guaranteed confidentiality and anonymity before starting discussion. Groups of participants were all composed of adult males, with at least one experienced hunter (over 50 years old) able to give us accurate trends on species dynamics over the last two decades. We started surveys in southern Benin around two reference habitats close to our home institution: the forest reserves of Pahou (unknown absence/presence) and Lama (confirmed presence). These first two 'pilot' focus groups allowed us to validate our protocol before the countrywide survey.

A colour poster and an identification guide comprising the four African pangolin species were used to guide the debate during the focus groups (Beaudreau et al. 2011; Kotschwar Logan et al. 2015). The poster comprised nine photographs of native mammals, with two photographs of pangolins, and three photographs of non-native mammals. The combination of native and non-native mammals on the poster was necessary to be sure that local hunters were able to genuinely identify pangolins, in order to avoid any social desirability bias (Krumpal 2013). For each pangolin species, the identification guide showed the different parts (head, belly, tail and scales) of the animal in order to optimize identification by participants. Discussions were conducted following three steps: 1) identification of mammalian species by respondents on the poster; 2) identification of pangolin species occurring in the local environment (and based on the identification guide); 3) answer to a short questionnaire. During the focus groups, participants were asked to identify the mammals that occur in their local environment and give their local names. If pangolins were included in the list, participants were asked to spot the species they refer to among the four African species on the identification guide. When the species was accurately identified, participants were asked to give their perceptions on the abundance trends over the past 20 years (2018, 2013, 2008 and 1998), and to list the drivers of these trends and the potential occurrence sites within the grid extent. The proposed response options for characterizing the abundance trends were defined as follows: ‘missing’- probability of observing an individual at the target period is zero in the local environment; ‘scarce’- probability of observing an individual at the target period is near to zero in the local environment; ‘medium’ high probability of observing an individual every month in the hunting season for the target period; and ‘abundant’ high probability of observing many individuals every month in the hunting season at the target period in the local environment. Questions on drivers and potential habitats were open. We delimited a period of two decades in order to avoid the bias related to the probable loss of LEK with time

(Saynes-Vásquez et al. 2013; Aswani et al. 2018). The geographic coordinates of each village were recorded. For each step, the consensual decision of participants was recorded, except for questions about drivers of abundance trends and potential habitats where we recorded all responses.

The presence of pangolins determined during the focus groups was confirmed by the collection of direct evidence –whenever possible– when respondents evoked opportunistic encounters with a recently deceased animal or when scales collected some time ago were said to be kept in the village. The ‘snowball’ technique allowed us to collect the scale samples from both participants and non-participants of focus groups, when the latter were recognized by participants as possessing the scales. Three to five scales were collected in a plastic zip bag on which the provider’s code, harvesting year and habitat were recorded. The scale samples were deposited in the collections of the Laboratory of Applied Ecology, University of Abomey-Calavi, Benin. A monthly collection of direct evidence was conducted during the dry season (January, February and March) in the villages where the presence of pangolins was declared by local hunters during focus groups without providing direct evidence.

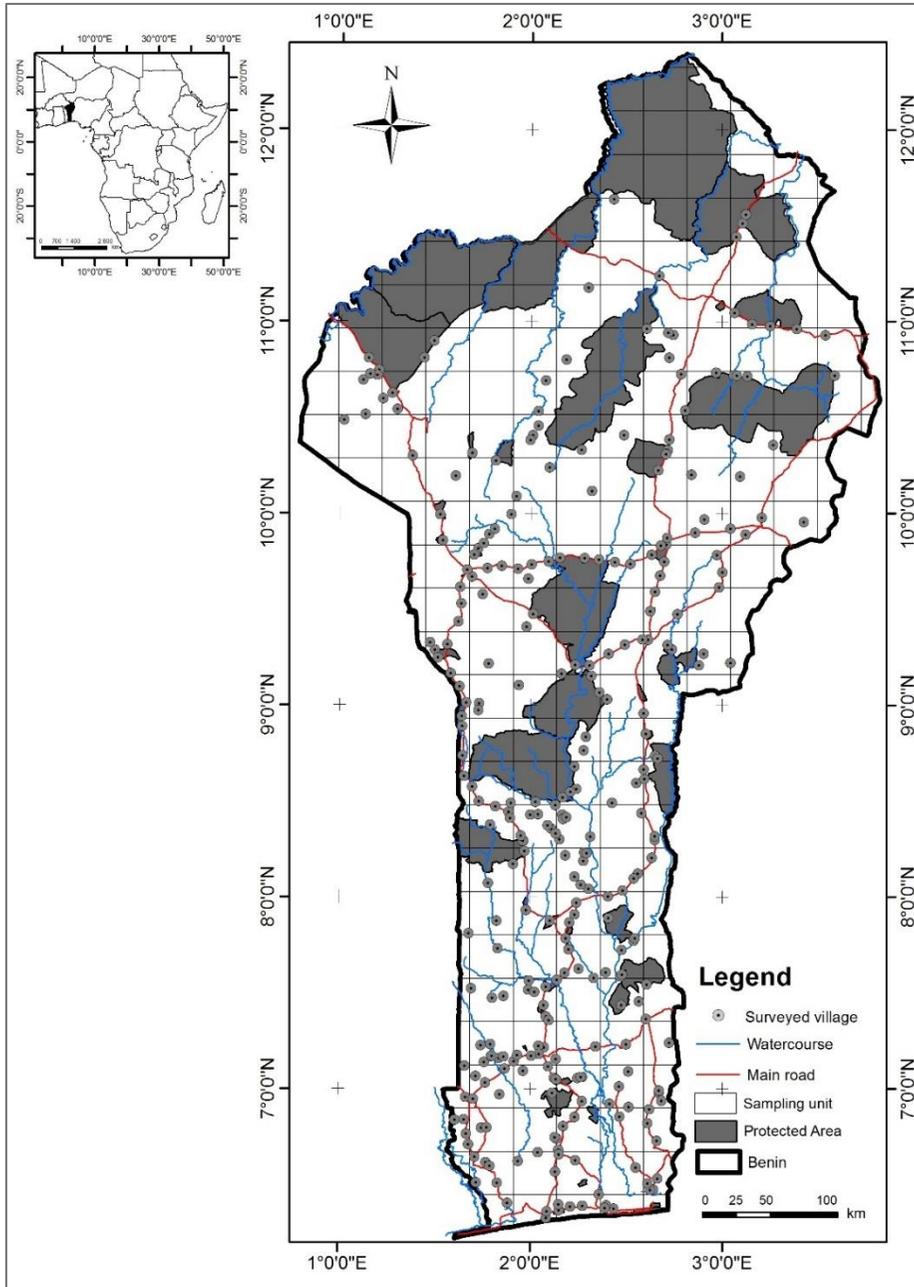


Figure 1. Location of the 312 villages in which the focus groups were held with local hunters from April 2018 to March 2019 in combination with direct evidence collection. Grids (sample units) are 25 x 25 km. ‘Absence’: villages in which focus group participants mentioned the absence of pangolins; ‘Presence’: villages in which focus group participants mentioned the presence of pangolins without providing direct evidence (scales); ‘Presence + direct evidence’: villages in which focus group participants supported their statement with direct evidence (scales)

2.2.2. Data analysis

In order to build the distribution maps of the white-bellied and giant pangolins, we considered the geographic coordinates of all the villages ($N = 235$) where focus group participants successfully and in a consensual manner (100% of participants when there is no direct evidence) agreed on a pangolin species presence, with or without collecting direct evidence (scales). Whenever the above conditions were not met, the species were considered absent from the village. As such, only the villages where respondents reported pangolins to be “scarce”, “medium” or “abundant” for a given time period were considered as valid occurrences. We used ArcMap 10.1 (ESRI Inc.) to generate superimposed layers (25 km x 25 km grids) representing each period/species, where the grids containing villages reporting the presence of pangolins were considered as occupancy areas of the targeted species. Occupancy area is here defined as the probability that a grid was occupied at a given time period (over the past 20 years) by a pangolin species (see Latif et al. 2016). For each reported potential habitat with or without direct evidence collection, the total area was calculated using the recent shapefile in ArcMap 10.1 (ESRI Inc.). The management modality for each forest and its national classification were extracted from official documents (FAO 2010, CENAGREF/PAPE 2013, DGEFC 2017).

In order to assess local people’s perceptions about trends in the abundance of the white-bellied and giant pangolins over the last two decades and identify factors causing these trends, each focus group was considered as an independent observation. R version 3.5.1 (*R Core development team*) was used for all the statistical analyses. Local hunters’ perceptions regarding pangolin abundance level (missing, scarce, medium and abundant) were plotted for each species using the cumulative barplot in the ggplot2 R package for four different periods (2018, 2013, 2008 and 1998). Differences among abundance levels across time periods were tested with the Pearson’s χ^2 test for the white-bellied

pangolin and the Fisher exact test for the giant pangolin due to low observation numbers. The drivers of pangolin decline as extracted from the respondents were classified into the six following categories, for which frequencies of citations were calculated: wood energy harvesting (deforestation), hunting, agriculture, transhumance (livestock), fire and urbanization.

In order to understand the landscape characteristics that may influence the current geographic distribution of the white-bellied pangolin, we computed in ArcMap the Euclidean distances from villages to the nearest (i) protected areas (*DistAP*), (ii) permanent watercourses (*DistWater*), and (iii) main roads (*DistRoad*). For all the villages considered ($N = 235$), focus group participants were hunters with similar hunting background. So, we assume an equal understanding of the white-bellied pangolin distribution in the local areas among villages. Only 2018 was considered and presence/absence data were defined as described above, and were modelled with these landscape variables (*DistAP*, *DistWater* and *DistRoad*) using a generalized linear model. We did not model the current distribution of the giant pangolin because only two villages reported its presence in 2018, without any direct evidence of occurrence.

2.3. Results

Focus groups gathered 3,282 respondents (hunters, rangers and former poachers), 291 local community leaders, 21 gatekeepers. The average age per focus group varied between 33 and 52 years old and all the participants were men. All the collected pangolin scales (96 samples) belonged to the white-bellied pangolin with 6, 38, 15 and 47 % harvested respectively in 2016, 2017, 2018 and 2019 by local people.

2.3.1. Geographic range over the last two decades

The geographic distribution of the two species of pangolins as extrapolated from the focus groups extended between 6°10' and 11°00' N, with the giant pangolin being restricted to 10° 20'-11°00' N (Fig. 3a and b). Our investigations suggested that the white-bellied pangolin over the last two decades (from 1998 to 2018) underwent a 31 % (14,169 km²) contraction of its occupancy area while that of the giant pangolin was reduced by 93 % (15,955 km²) (Table1).

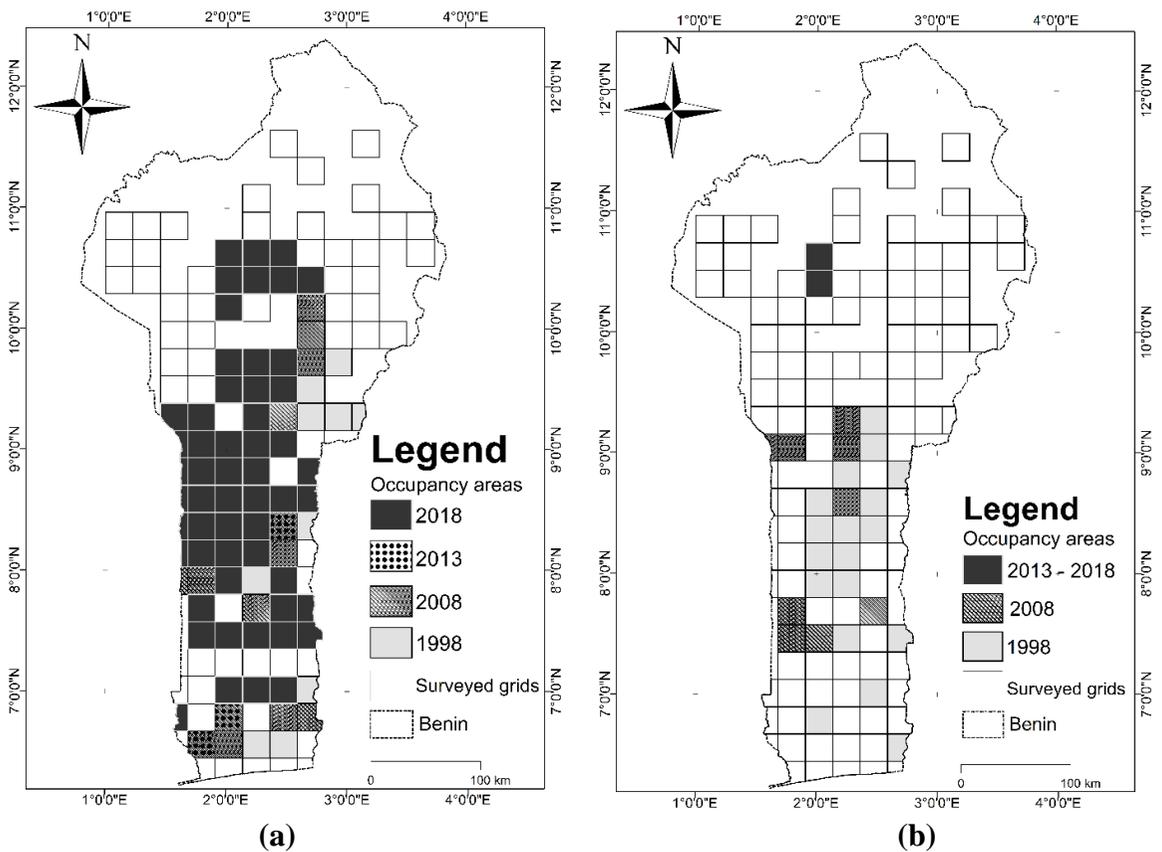


Figure 2. Geographical range dynamics of (a) the white-bellied pangolin and (b) the giant pangolin in Benin from 1998 to 2018. Each grid cell represents an occupancy area for one or several time period(s) by overlaying sampled units (grids) and responses of focus groups. During this period, no new colonization and recolonization events have been mentioned by participants. Thus, the time of occupancy of a grid cell implies that the species has been present since 1998

In total, c. 77 and 100% of the occupancy areas (Table 1) of the white-bellied pangolin and the giant pangolin, respectively, were partially or totally included in protected areas in 2018, against c. 60% two decades ago (1998). Twelve forest reserves and two game ranches out of the 23 forest islands cited by local hunters are the current potential habitats of the white-bellied pangolin with only one out of 23 forests cited by local hunters for the giant pangolin (Table S1, Appendix 1). These forest reserves and ranches are managed as wildlife reserves and for wood energy harvesting (Table S1, Appendix 1). Forest reserves and ranches (protected areas) supplied 76% of the pangolin scales collected as direct evidence (66% from wildlife reserves and 10% from wood energy forests). The wildlife reserve complex constituted by Monts Kouffé, Wari-Marou and Ouémé supérieur in the central part of Benin provided 46% of the scale samples and the Lama forest in southern Benin provided 13%.

Table 1. Twenty-year temporal trend in occupancy areas (global and protected) between the two species of pangolins in Benin, as expressed by the focus groups

Year	<i>Phataginus tricuspis</i>		<i>Smutsia gigantea</i>	
	Occupancy area (km ²)	Occupancy area including a protected area (km ²)	Occupancy area (km ²)	Occupancy area including a protected area (km ²)
2018	30960.797	23820.235 (77 %)	1250	1250 (100 %)
2013	32813.046	24445.235 (74 %)	-	-
2008	39693.748	26945.235 (68 %)	6443.909	3943.909 (61 %)
1998	45129.603	27934.588 (62 %)	17205.278	10540.684 (61 %)

2.3.2. Abundance trends according to respondents

Respondents perceived a decline in the abundance level of the white-bellied pangolin in Benin (Fig. 3a), and their perceptions depended significantly on time periods ($\chi^2=126.7, p < 0.001$) over the last two decades. The species was

seen as abundant for 37 % of the focus groups in 1998, in comparison with 4 % in 2018. Inversely, its extirpation (missing) was mentioned in 33 % of the focus groups in 1998 against 54 % in 2018. The extirpation of the giant pangolin was perceived almost unanimously over the last period of our study (2013-2018; Fig. 3b), and this perception was also related to time periods ($p < 0.001$).

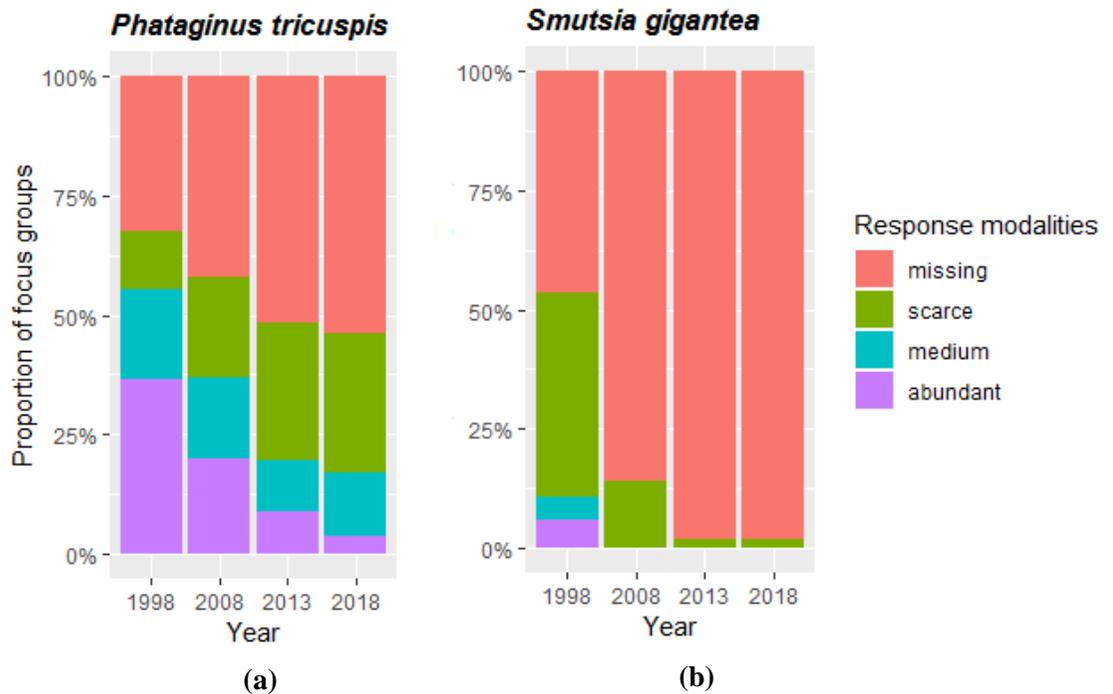


Figure 3. Focus group representations of abundance trends of pangolins in Benin over the last two decades (1998-2018)

Respondents perceived wood energy harvesting (81.5 %), hunting (77.08 %) and agriculture (70.84 %) (Table 3) as the major drivers of the white-bellied pangolin’s decline, and hunting (91.2 %) as mainly driving the extirpation of the giant pangolin.

Table 2. Local hunters’ perceptions about the drivers of pangolins’ decline ($N = 235$ focus groups)

Threats	Frequency of citations (%)	
	<i>P. tricuspis</i>	<i>S. gigantea</i>
Deforestation (wood energy)	81.25	59.88
Hunting	77.08	91.2
Agriculture	70.84	45.05
Transhumance	45.83	55.36
Fire	29.16	3.16
Urbanization	8.33	-

2.3.3. Drivers of geographic distribution of the white-bellied pangolin

GLM analysis revealed that distance from, villages to protected areas ($a = -0.0001$, $p < 0.0001$) and villages to main roads ($a = 0.0001$, $p < 0.0001$) were the main landscape variables explaining the current distribution of the white-bellied pangolin. The probability that hunters made recent observations of the white-bellied pangolin increased when villages were (i) closer to protected areas and (ii) further away from main roads.

2.4. Discussion

Our national-scale study offers an unprecedented reassessment of the distribution, population trends and landscape occupancy drivers of pangolins in Benin. Such a detailed level of investigation on pangolins at the regional or country-scale have so far only been done in Asia (Nash et al. 2016; Trageser et al. 2017) and southern Africa (Pietersen et al. 2016a) on other species.

Our approach combining LEK and direct evidence of occurrence (pangolin scales) yielded a reliable assessment of the current ranges of pangolins in

Benin, and likely improved the accuracy of the current ranges delineated in global or national red lists that serve as references for the community involved in wildlife conservation (Akpona & Daouda 2011; Nixon et al. 2019b; Pietersen et al. 2019c). From the respondents and direct occurrence evidence collected in this study, it appears that the ranges of both the white-bellied and giant pangolins probably never occurred beyond a latitude of 11°00'N, at least within the last 20 years. The national parks occurring beyond that latitude are the Biosphere Reserve of Pendjari and the Transboundary Park W, where wildlife biomonitoring done in recent years (camera traps and wildlife census, 2012-2015) by park managers has failed to report any sightings of pangolins (S. Zanvo, pers. obs. December 2018). Unanimously, the oldest hunters, together with rangers and former poachers stated that the two species of pangolins had never occurred in the National Parks of Pendjari and W, and their surrounding areas, contradicting what was suggested in the Red List for Benin on the basis of vague assumptions without photographic evidence or voucher specimen (Akpona & Daouda 2011). This contradiction is equally observed with the IUCN Red List (Nixon et al. 2019), which listed the giant pangolin as “probably extant” in those two national Parks (citing Akpona and Daouda 2011; but the supposed record from Pendjari comes from Sayer and Green (1984). Although LEK may be subject to erosion through time (Saynes-Vásquez et al. 2013; Aswani et al. 2018), we believe that the absence of the giant pangolins from those two national parks is plausible since such northern latitudes may represent unfavourable climatic conditions for the species. Our study suggests that the giant pangolin was subject to a rapid decline and total extirpation from southern and central Benin, representing 93 % of range contraction in the last 20 years. In the last five years, the giant pangolin was only mentioned by a focus group from the Alibori forest reserve, showing a very restricted potential range in Benin (10°20' - 11°00' N; c. 259,000 km²).

No direct occurrence evidence (scales) from giant pangolin was retained by villagers confirming the rarity of the species in the country as reported in other parts of its range (Bräutigam et al. 1994), but is at odds with the relatively widespread occurrence of the species in Benin suggested by Akpona and Douada (2011).

Our survey suggested a more stable distribution dynamics of the white-bellied pangolin over the past 20 years from southern to north-central Benin (6°10' N to 11°00' N), although the species was identified as undergoing an important contraction of its range (31 %) over the last two decades. Our survey indicates that the white-bellied pangolin is likely present in at least 23 Beninese forests, and this was confirmed by direct occurrence evidence in 17 (74 %) of the cases (Table S1, Appendix 1). The current distribution of the white-bellied pangolin as delineated from our interviews shows a more fragmented range in southern and central Benin, and demonstrates the occurrence of the species at higher latitudes, increasing the range exposed by the IUCN Red List (reaching approximately 10°20' N in Pietersen et al. (2019) *versus* 11°00' N in the present study).

Globally, the two species of pangolins in Benin were seen as undergoing a significant decrease of their distribution range over the last 20 years (see above), associated with an increase in the proportion of their existing range falling in protected areas, reaching 100 % (one reserve) in the case of the giant pangolin. Such a pattern of range loss and “refugial” occupancy into protected areas is typical of populations undergoing rapid extirpation due to habitat deterioration and targeted hunting activities (Bauer et al. 2015). Our survey also suggested an important decrease in the abundance of the two pangolin species over the last two decades in Benin, as perceived by local hunters. For the giant pangolin, 100 % of the focus groups declared the species either rare

(15 %) or missing (85 %) ten years ago, with the species being reported as missing in 2018 by 98 % of the groups. Such perceptions are consistent with general population trends for the species, again supporting the view that the giant pangolin is generally rare and declining throughout its range (Nixon et al. 2019). In Benin, traders from traditional medicine markets also confirmed this trend (S. Zanvo, unpublished data). A decrease in abundance was also perceived in the white-bellied pangolin over the last two decades, the species being declared abundant in 1998 by 37 % of the focal groups but only by 4 % in 2018. Although the species is considered the most common African pangolin (Pagès 1975; Angelici et al. 1999; Gaubert 2011), it is believed to be declining in Ghana and Guinea, and to near extinction in Rwanda (Pietersen et al. 2019c). Soewu and Ayodele (2009) also reported that 92 % of the traditional practitioners in the nearby Ogun State, Nigeria, stated that the abundance of white-bellied pangolin was continuously decreasing. Although a previous study from the Lama Forest Reserve in southern Benin reported a density of 0.84 individuals per km² (Akpona et al. 2008), our study suggests that such relatively high density estimates may not be common or reflective of the current situation in the country. Khwaja et al. (2019) suggested that degraded habitats could lead to a greater detection probability of the white-bellied pangolin, amplifying its time of activity on exposed parcels. So, in the current context of highly degraded forests in Benin, the general decline perceived by local communities over the last two decades could be considered as a genuine representation of the population trends in pangolins.

Nixon et al. (2019) associated the decline of the giant pangolin to habitat alteration and unsustainable exploitation for bushmeat and traditional medicine, feeding both local and international markets (Djagoun et al. 2013; Heinrich et al. 2016; Ingram et al. 2019). Its large size, low reproductive rate and terrestrial habits make it particularly vulnerable to overexploitation

(Kingdon et al. 2013). In Benin, the major cause for the species' depletion was identified as hunting by c. 91 % of the focus groups, followed to a lesser extent by habitat alteration (45 - 60 %), in agreement with Akpona and Douada (2011). Because the species is restricted to forested habitats (Gaubert 2011), deforestation and agricultural activities might have played a significant role in its local extinction and range fragmentation in Benin. Despite almost one year of survey at the country scale, no direct occurrence evidence (scales) of giant pangolin could be collected (whereas 96 items were collected for the white-bellied pangolin). We posit that the giant pangolin, which was already considered rare in Benin 20 years ago, is now at best restrained to the forest reserve of Alibori and is facing high extinction risk, especially since its former presence in southern and central forest reserves did not prevent from its rapid extirpation. Field investigations using camera trapping (Khwaja et al. 2019; Morin et al. 2020) are urgently needed to confirm the existence and status of the remaining populations and to investigate their habitat use in order to propose concrete solutions for the conservation of the giant pangolin in Benin.

Pietersen et al. (2019) linked the decline of the white-bellied pangolin across its range to habitat alteration, wood plantation and unsustainable hunting. In Benin, the major causes of decline were identified as deforestation (81 %), agriculture (70 %) and hunting (77 %). Although the species predominantly occurs in moist tropical lowland forests, it can also be found in dense woodlands and secondary growth forests such as abandoned oil palm or even teak plantations (Sodeinde & Adedipe 1994; Angelici et al. 1999; Akpona et al. 2008; Gaubert 2011), thus suggesting a certain level of adaptability to human-induced habitat transformation. However, severe fragmentation of forested habitats by intense agriculture activities, growing urbanization and forest exploitation for wood energy (FAO 2010, Alohou et al. 2017) are likely to significantly reduce the coverage of suitable habitats for the white-bellied

pangolin in Benin. The species is subject to intense exploitation across its range for bushmeat and traditional medicine, and is the most represented pangolin species in bushmeat markets (Djagoun & Gaubert 2009; Djagoun et al. 2013). The important quantity of direct occurrence evidence (96 samples of scales) collected during our survey showed that hunters stored pangolin scales in their houses either as trophies or for uses in traditional medicine (Akpona et al. 2008; Djagoun et al. 2013), and possibly for supplying traditional medicine markets.

The expanding scale of the illegal international trade towards Asia (Challender & Hywood 2012; Xu et al. 2016) is likely contributing to the depletion of white-bellied pangolin populations. In Benin, international trafficking has been confirmed by a recent seizure of 513 kg of pangolin scales at the airport of Cotonou (Ingram et al. 2019). This potentially highlights an extension of the western African pangolin trafficking network towards Asia, which was so far preeminent in Sierra Leone, Ivory Coast, Ghana, and Nigeria (Xu et al. 2016). Our study suggested that the white-bellied pangolin in Benin mostly occurred far from the road network and near or within protected areas, emphasizing avoidance of the most disturbed human-dominated landscapes by road networks (Shanley & Pyare 2011) and the crucial role played by protected areas for the survival of the species. The road networks would have facilitated defaunation from impacted areas due to the good access to pangolin's habitats for hunting activities. However, the high percentage (76 %) of scales collected from protected areas is a token of the ongoing poaching pressure within those reserves. Besides, the classification objective into wood energy production of almost half of the forest reserves where the species was reported to occur may prove rapidly unsuitable relative to the ecological requirements of the species, which involve large trees for sheltering.

In the current context, conservation actions should be urgently engaged to design a sound species-conservation and land-management plan in Benin. The classification of the main reserves potentially housing pangolins to limit anthropogenic disturbance –especially deforestation– should be encouraged. An upstream regulation of local bushmeat and traditional medicine markets should also be implemented, in order to limit local trafficking (Djagoun & Gaubert 2009; Djagoun et al. 2013). Because the Lama forest reserve in southern Benin and the complex Monts Kouffé - Wari Maro - Ouémé supérieur in central Benin are the two sole forest relics that have been identified as housing large populations of white-bellied pangolins, those two areas, together with the Alibori forest reserve as the last potential stronghold for the giant pangolin, should be urgently investigated.

2.5. Conclusion

Our study cross-validating LEK data with direct evidence constitutes a useful reference framework for future field-based investigations on pangolin populations in Benin and the establishment of a national strategy for pangolin conservation. Despite the reliability of our methodological approach, further field surveys based on newly validated methods (Khwaja et al. 2019) will be essential to confirm occurrences, population status and population trends of pangolins in Benin.

Chapter 3

Modelling pangolin population extirpation rates using validated local ecological knowledge

This chapter will be submitted as:

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CHAPTER 3

Modelling pangolin population extirpation rates using validated local ecological knowledge

Abstract

Informed decision-making is a prerequisite to the long-term conservation of endangered species. Ecological data are lacking decision tools to achieve effective conservation of pangolins, highly threatened by the illegal international trade habitat fragmentation and degradation. This study used local ecological knowledge combined with direct evidence in 156 villages and landscape metadata to model the probability of persistence and predict the persistence of the *Phataginus tricuspis* and *Smutsia gigantea* in Benin. The best-fitted binomial generalized linear model of persistence was obtained for a 15 km buffer size surrounding the sampled villages. Our model was highly efficient in predicting the presence of the white-bellied pangolin in villages where actual presence was confirmed from the observation of scales. The best fitted binomial generalized linear model revealed species identity and abundance levels two decades ago (1998) as drivers that explained with the highest significance levels ($p < 0.001$) the current persistence of white-bellied and giant pangolins in Benin. Temporal trends of deforestation area (2001-2019), land-use suitability changes (1998-2015), together with distance of villages from protected areas and asphalted roads in 2018 also significantly explained ($p < 0.01$) the current persistence of both species. The probability of current persistence increased with abundance levels estimated two decades ago, distance of villages from asphalted roads and land-use suitability. On the other hand, it decreased with deforestation trends over time and distance of villages from protected areas. The white-bellied pangolin had the higher probability of persistence compared to the giant pangolin. The occupancy area of both species was predicted to decrease in the next two decades whatever the

scenarios considered: landuse change and deforestation vs. no change. The projection is particularly alarming for the giant pangolin, which was predicted to be extinct in Benin by 2038. Our study suggested an unsustainable spatial context to the current and future persistence of pangolins in Benin, that highly dependent on their abundance levels. It calls for urgent conservation actions against overhunting and habitat degradation in order to maintain sustainable abundance levels of pangolins in their last forest refuges.

Keywords: local ecological knowledge, modelling, probability of persistence, landscape drivers, pangolins, predictions

3.1. Introduction

Pangolins are mammals that have been facing high extinction risk due to increasing levels of poaching of different species to feed local/regional wildlife markets, fueled by the exponential international demand of African species in particular (Heinrich et al. 2016; Challender et al. 2020). The trade, mainly focused on Asian species in late 20th century, has shifted to African pangolins in the 21th century since Asian pangolins severely declined during pre-2000 period (Challender et al. 2020). During the last decade (2010 – 2019), more than 400 000 individuals of African pangolins were involved in the illegal international trade (Challender et al. 2020). The white-bellied (*Phataginus tricuspis*) and giant (*Smutsia gigantea*) pangolins were the most targeted species (Mwale et al. 2017; Mambeya et al. 2018; Ingram et al. 2019; Challender et al. 2020; Zhang et al. 2020). Beside the illegal international trade (Mambeya et al. 2018; Ingram et al. 2019; Challender et al. 2020; Zavo et al. 2021), African pangolins underwent an unprecedented degradation of their habitats, especially in Western Africa where > 80 % of forest cover vanished over the last century (Aleman et al. 2018).

Endangered on the IUCN Red List of threatened species (Nixon et al. 2019; Pietersen et al. 2019), the white-bellied and giant pangolins are respectively classified as Endangered and Critically Endangered on the Red List for Benin (Akpona & Daouda 2011). They are threatened by local trade in bushmeat and traditional medicine markets (Zanvo et al. 2021), and more recently by international trafficking (Ingram et al. 2019; Zanvo et al. 2021). LEK-based survey suggested declining trends in occupancy area for both species. The white-bellied pangolin underwent a contraction of one third of its occurrence range and the giant pangolin experienced a quasi-total extirpation with 93 % of its occurrence range lost over the last two decades (Zanvo et al. 2020). Habitat degradation and overexploitation were the major drivers of population decline according to local hunters (Zanvo et al. 2020). The two species are currently distributed in a human-dominated landscape within patchily distributed habitats (Zanvo et al. 2020), and this spatial configuration certainly has an influence on the persistence of the populations of many mammals (see Schneider 2001; Fischer & Lindenmayer 2007; Collinge 2010; Horváth et al. 2019).

In a rapidly changing Benin's landscape (Bogaert et al. 2011; Mama et al. 2013; Alohou et al. 2017; Padonou et al. 2017; Tente et al. 2019), understanding abiotic drivers of species distribution across space and over time is an essential step for establishing spatial conservation priorities (Addison et al. 2013; Mouquet et al. 2015). In this context, the long-term persistence of pangolins may be negatively affected by infrastructure development (e. g. roads network), deforestation and land cover changes but also by the natural permeability of the landscape to dispersing individuals (e.g. watercourses). Given that overexploitation is a driver of pangolin population decline in Benin (Zanvo et al. 2020), the spatial distribution of wildlife markets where pangolins are sold in Benin (Zanvo et al. 2021) could be negatively correlated to their

geographic persistence. Within such a matrix of degraded habitats by human activities, protected areas should act as refuges for wildlife (Macedo et al. 2019; Pacifici et al. 2020). However, the efficiency of protected areas in Benin has received little attention with regard to pangolin conservation.

Despite the high concern status of pangolin species in Benin (Akpona & Daouda 2011), little is known about key features of their ecology (Akpona et al. 2008; Zanzo et al. 2020) and this hampers informed decision-making, essential to reverse the ongoing declining trends. Pangolins are cryptic and elusive species and using conventional monitoring methods to assess population status across space and over time is therefore challenging (Heath & Coulson 1997; Willcox et al. 2019). This is even more difficult in developing countries such as Benin where scientific research has been facing financial constraints (Field et al. 2005; Gaillard 2010) and long-term scientific data are scarce (Early-Capistrán et al. 2020). In such context, it becomes important to find innovative affordable solutions to survey high concern species such as pangolins. LEK is one of such alternative approaches recently used to study Asian and African pangolins (Nash et al. 2016; Zanzo et al. 2020; Archer et al. 2020; Segniagbeto et al. 2021; Cheng et al. 2021). Some authors have raised concerns about the uncritical use of LEK in environmental and conservation studies (McKelvey et al. 2008; Davis & Ruddle 2010). When population status is assessed via LEK it is therefore important to validate the approach by comparing its output with direct evidence of occurrence or population size, particularly when modelling is involved (Gilchrist et al. 2005; Lima et al. 2017; Bélisle et al. 2018;). It is the case of this study that combines LEK with pangolin's scales as direct evidence of species occurrence.

In Benin, the decline of the two pangolin species is quite well documented from a previous study (Zanzo et al. 2020) but this general pattern can mask

spatial heterogeneity in population trends and extirpation/colonization dynamics. The aim of the present study is to understand why some pangolin populations have been extirpated within a two decades period (1998-2018) while the others are still extant, a question of paramount importance to design effective local conservation actions. The extirpation status of the focal populations come from the LEK survey conducted by Zanvo et al. (2020) and so it is important to know whether reliable statistical models can be built from such data. Fortunately, an important result of our study is to show that modelling extirpation rate from LEK accurately predicted population persistence in an independent set of populations for which pangolin presence is known with certainty. Note that it would have been informative to study the colonisations, but none have been recorded in Zanvo et al. (2020). Armed with a validated model, we explored the likelihood of population extirpation in relation to landscape changes (e. g. deforestation) and indirect indices of anthropogenic pressure (e. g. distance to road) to account for the two main factors incriminated in pangolin population decline according to LEK (Zanvo et al. 2020). The effectiveness of protected areas in preventing or mitigating population decline has been tested too. Because it is well established that the persistence of a population increases with its size (Borregaard & Rahbek 2010), the contribution of initial population size (abundance index at 1998) has been accounted for in models. Given its larger body size and longer generation time, it is predicted that the Giant pangolin is more threatened with extinction than the white bellied pangolin and interspecific differences in extirpation rate has been considered.

3.2. Material and methods

3.2.1. Sampling design and data collection

LEK survey was conducted in 312 villages across Benin from April 2018 to March 2019 and direct evidence collection was extended to June 2020. The villages were selected within 25 km × 25 km grid cells across Benin (see details in Zanvo et al. 2020). The survey was conducted using: (i) a poster including nine native species with three photos of our focal species, white bellied and Giant pangolins and three photos of non-native species (absent in Benin) and (ii) an identification guide including the different body parts of four African pangolins. Data collection was carried out using focus groups (8-15 persons) with local hunters following three steps: (i) poster-based presence/absence of pangolins within local environment, (ii) identification of species using the identification guide of pangolin species and (iii) perception of local hunters on the abundance levels of each identified species over the last two decades (1998, 2008, 2013 and 2018) using a pre-established questionnaire (more details in Zanvo et al. 2020). The abundance levels over time were measured using the following response options: ‘missing’- probability of observing an individual at the target period is zero in the local environment; ‘scarce’- probability of observing an individual at the target period is near to zero in the local environment; ‘medium’- high probability of observing an individual every month in the hunting season for the target period; ‘abundant’ - high probability of observing many individuals every month in the hunting season at the target period in the local environment and ‘highly abundant’ - high probability of observing many individuals every month at target period in the local environment. To ensure the reliability of local hunters’ responses, direct evidence of recent pangolin presence (scales) were collected (if possible) with focus group participants but also with non-participants identified as holders of

direct evidence within each sampled village using the snowball technique. Focus group participants and direct evidence donors were men and adult hunters (> 18 years, with at least one experienced hunter > 50 years), volunteers, with at least 10 years residency in the village (Kotschwar Logan et al. 2015). They were selected through informal interviews with villagers. The wildlife markets were equally georeferenced through an exhaustive survey from the southern to central Benin and covering the distribution areas of both species.

3.2.2. Data analysis

3.2.2.1. Model, variables and tested hypotheses

From the original survey data, we discarded the villages where pangolins have never occurred and the one for which both species have already disappeared in 1998 according to local hunter. We focused analyses on the villages (N=208) having at least one species present in 1998 (Fig. 1). Two subsets of this dataset have been created: with and without direct evidence of pangolin presence (respectively N=52 and N=156; Fig. 1a). The latter (N=156) is used to calibrate the model whereas the former (N=52) acts as a validating set. The modelled variable is binary: 0 if a pangolin population has been extirpated (absence in 2018), 1 if it has persisted until 2018 (presence in 2018). Presence-absence status is based either on LEK (calibration) or on LEK+ direct evidence (validation). The information obtained from hunters for intermediate periods (2008 and 2013) is discarded because scales are evidence of recent presence (scales of individuals hunted between 2016–2020) and cannot be used to validate more ancient reported presence.

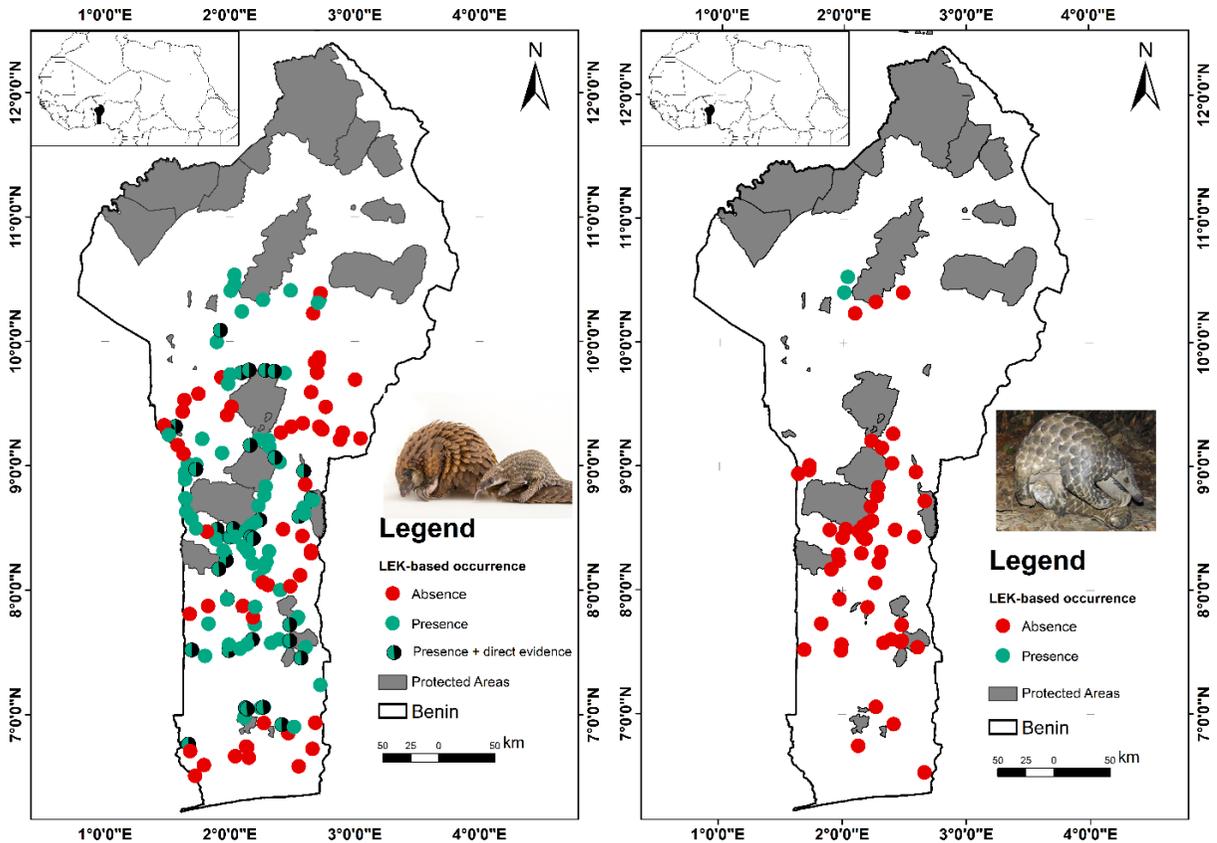


Figure 1. Local ecological knowledge and direct-evidence-based distribution trends of pangolins from 1998 to 2018 in Benin. (a): white-bellied pangolin and (b): giant pangolin. Green circles (presence) indicates the villages were the species still occur in 2018 according to local hunters; red circles (absence) represent the former (1998) occurrence villages where the species disappeared in 2018. Green and black circles (presence +direct evidence) represent the current occurrence villages in 2018 where the presence of white-bellied pangolins was confirmed with direct evidence (pangolins' scales)

To model the probability of persistence (Pr_{2018}), the following explanatory variables have been considered to test hypotheses related to human exploitation, landscape changes, initial population abundance and interspecific differences.

Species: this binary variable referred to our focal species: the white-bellied pangolin (coded 0) vs. giant pangolin (coded 1). Large bodied species are more vulnerable to increased mortality rates (as induced by human exploitation in

our case) than small bodied species and as result they are more likely to decline, and eventually to be extirpated. Indeed, large body size is positively correlated with the extinction risk in mammals (Johnson 2002; Lyons et al. 2004; Van Valkenburgh et al. 2004; Liow et al. 2008). The giant pangolin is by far the largest of our focal species: it is 12 to 14 times heavier than the white-bellied pangolin (see Kingdon and Hoffmann 2013; Mambeya et al. 2018). Therefore, all else being equal, we expect this species to be extirpated at a higher rate than the white-bellied pangolin. It would have been preferable to model each species independently, but there is not enough data on the giant pangolin to run a reliable model for this species (Fig 1b). The joint model proposed allows comparing the two species in their average extirpation probabilities but makes the debatable assumption that, otherwise, they have identical responses (regression coefficients) to the tested variables. However, the two species are sufficiently close in their ecological requirements so that it is very unlikely that they display an opposite response to an important variable (e. g. a positive correlation with deforestation for one species and a negative one for the second).

ab1998 represents the initial (circa 1998) population abundance index as given by LEK surveys. This is an ordinal variable scaled as follows: 1 (scarce), 2 (medium), 3 (abundant) and 4 (highly abundant). The data collection section describes the rules used to estimate abundance levels. All else being equal, the persistence of a population is positively related to its size, irrespective of the nature of extinction, whether it is deterministic (extinction resulting from a declining population size) or stochastic (extinction resulting from the temporal fluctuation in population size). Therefore, we hypothesized that the current persistence of a pangolin species in a local environment is strongly dependent on its abundance level two decades ago (1998).

In a changing landscape, spatial variability across space and over time may affect the spatial persistence of species. In a human-dominated landscape as Benin, pangolins may be sensitive to the disturbances (habitat fragmentation, creation of resistance surfaces to dispersal ability, natural components of landscape, and overexploitation) within their occurrence environment. We considered in our model the following spatial variables: *distPA*, *distRoad*, *distMarkets*, *distWater*, dft_i and LSI_i . These spatial variables were metadata generated from georeferenced villages (surveyed villages) with other spatial information following the considered variables.

distPA is the Euclidian distance in kilometre (km) from each village to the nearest protected area (CENATEL, 2012). If the protected areas are efficient in maintaining viable populations of pangolin, we hypothesized that the probabilities of persistence of the white-bellied and giant pangolins are higher near protected areas, acting as sources for immigration.

distRoad is the Euclidian distance in kilometre (km) from each village to the nearest asphalted road. The road development was identified among important drivers to the high extraction of wildlife for the trade in the local and urban tropical wildlife markets (Espinosa et al. 2014; Constantino 2016). We hypothesized that the easy access to local and urban wildlife markets in the villages close to asphalted roads had heightened the hunting pressures on pangolins in the local environments that decreased the probability of local persistence of white-bellied and giant pangolins conversely to the villages far away from asphalted roads. A positive estimate is expected and it means that the probability of persistence is highest in the local environment far away from asphalted roads.

distMarkets is the Euclidian distance in kilometre (km) from each village to the nearest wildlife market (bushmeat and traditional medicine markets). The

wildlife markets were those we sampled from southern to central Benin and the northern markets drawn from Djagoun et al. (2013). Wildlife markets are one of indirect factor of high extinction risk for wild animals in general (Ingram et al. 2021) and pangolins species in particular (Boakye et al. 2016; Mambeya et al. 2018b; Ingram et al. 2019; Zanzo et al. 2021b). We hypothesized that the probability of persistence highly decreases for both two pangolins species at proximity of the wildlife markets in Benin.

distWater is the Euclidian distance in kilometre (km) from each village to the nearest main watercourse. The white-bellied and giant pangolins may occur in dense woodlands with nearby water courses (Kingdon & Hoffmann 2013). We hypothesized that the probability of spatial persistence of both two species is higher near water courses. We expected a negative estimate which means that the probability of observing pangolins is higher near the main water courses in Benin.

dft_i is the total deforestation area in kilometre (km) around each sampled village within a buffer size of radius *i* (see below) between 2001 and 2019. The deforested areas were extracted from the Global Forest Change database (<https://google.earthengine.app/view/forest-change> ; Hansen et al. 2013). For each buffer size, we clipped for each village the extent of cumulated deforested areas (km²) from 2001 to 2019 using ArcGis 10.8.1 (Ensi France). 2001 is the oldest year available while being sufficiently close to 1998 to provide a meaningful trend over our period of interest. The ecological requirements of white-bellied and giant pangolins are strongly related to forest habitats, primary and secondary forests with large trees in particular. Accordingly, we hypothesized that the strength of population decline, and in turn the probability of extirpation, is positively related to the rate of deforestation.

LSI_i is the change in habitat suitability index between 1998 and 2015 within a buffer of radius i . The suitability index is based on the land-use categories included in the Global Land cover data base (<http://maps.elie.ucl.ac.be/CCI/viewer/download.php>). According to the literature (Pages 1968; Pagès 1975; Angelici et al. 1999; Fischer et al. 2002; Akpona et al. 2008; Kingdon & Hoffmann 2013; Zanvo et al. 2020; Segniagbeto et al. 2021) on pangolin habitat requirements, each land-use category received a suitability score (Table S1, Appendix 2). Not suitable habitat (score 1) includes landscapes where the species do not occur and all resistance surfaces able to impede the movement of pangolins. Suitable habitat (score 2) encompasses mosaic of natural vegetation with trees or shrubs and cropland within which pangolins may occur. Highly suitable habitat (score 3) includes evergreen or deciduous tree covers with open or closed canopy. We applied the same scoring to the two species given that the white-bellied and giant pangolins prefer almost the same ecosystems (Lowland forests, primary and secondary forests, forest-savanna and savanna mosaic, dense woodland, gallery forests, riparian forests, wooded savanna and wet grassland; Hoffmann et al. 2020; Jansen et al. 2020), but also because in our study area, LEK-based past and current distribution revealed an overlapping of occurrence habitats for both two species (Zanvo et al. 2020). For each village, an average of the score values ($SI_{i,t}$) is computed for year t , weighted by the area occupied within the buffer of radius i . Whether or not the landscape changed in a favourable way to pangolins during the period of interest, the land-use suitability change index over time (1998-2015) was calculated as follows: $LSI_i = SI_{i,1998} - SI_{i,2015}$. A positive value indicating that the suitability of the landscape increased over time and a negative one the opposite. Land-use data were not available after 2015, but the latter is sufficiently close to 2018 to provide meaningful trends over our period of interest. We hypothesized a positive correlation between

pangolin population trends and habitat suitability trends and therefore that population extirpation will be more likely if *LSI* is negative.

Two of the explanatory variables (dft_i and LSI_i) depend on a buffer size for their computation. The buffer is the area defined by a circle centred on the village of interest. Five buffers of radius i have been considered: 5, 10, 15, 20 and 25 km. The choice of a minimum 5 km buffer size referred to 7.7 km mean hunting effort previously determined in West Africa (Alexander et al. 2014) and the maximum 25 km buffer size was based on the extent of our sampling unit (25 km \times 25 km grid-cells, see above).

3.2.2.2. Statistical analysis

Correlation between variables (Table S2, Appendix 2) was tested using the package *correlation* (Makowski et al. 2020). The following generalized linear model with binomial error and logit link has been fitted to the calibration data set for each buffer size:

$$Pr1998 \sim species + ab1998 + distPA + distRoad + distMarkets + distWater + dft_i + LSI_i$$

Based on the Akaike's Information Criterion (AICs) of models, we selected the buffer size providing the best fit.

For 52 villages, direct evidences (scales) of the recent presence of white-bellied pangolin were available allowing us to test how accurate were prediction models based on LEK only. As no scales were collected for the giant pangolin, the model cannot be validated for this species.

From the model retained in above step, the probability of presence of white-bellied pangolin was computed for each village for which scales are available

and if this value is higher than 0.5, then the species is predicted present, absent otherwise.

We then fitted the model using all the sampled villages in 2018, LEK only and LEK + direct evidence villages ($N=208$; both two species) to assess whether the explanatory variables contributed significantly to the model and acted in the anticipated way. We also fitted a second regression model using only the observations of white-bellied pangolin.

Based on the modelled trends of species status over the last twenty years, projections were made for the next twenty years following two possible scenarios of landscape changes. The first scenario assumes that the landscape will not change in the future, meaning no more deforestation ($dft=0$) and no land-use changes ($LSI=0$). The second scenario assumes that the observed trends over the past twenty years will be maintained for the next twenty years. This implies that the observed values of dft and LSI for the last twenty years (1998-2018) were kept unchanged for the 2018-2038 period. In both cases, population abundance is updated by using the one in 2018 values instead of the abundance in 1998.

Statistical analyses and mapping of distributions were carried out in R version 4.0.5 (*R core development team2021*) and ArcGis 10.8.1 (Ensi France) respectively.

3.3. Result

3.3.1. Drivers of spatial persistence of pangolins in Benin

AIC-based analysis following buffer size revealed that the binomial GLM model developed using 15 km buffer had the lowest AIC value (Fig. 1).

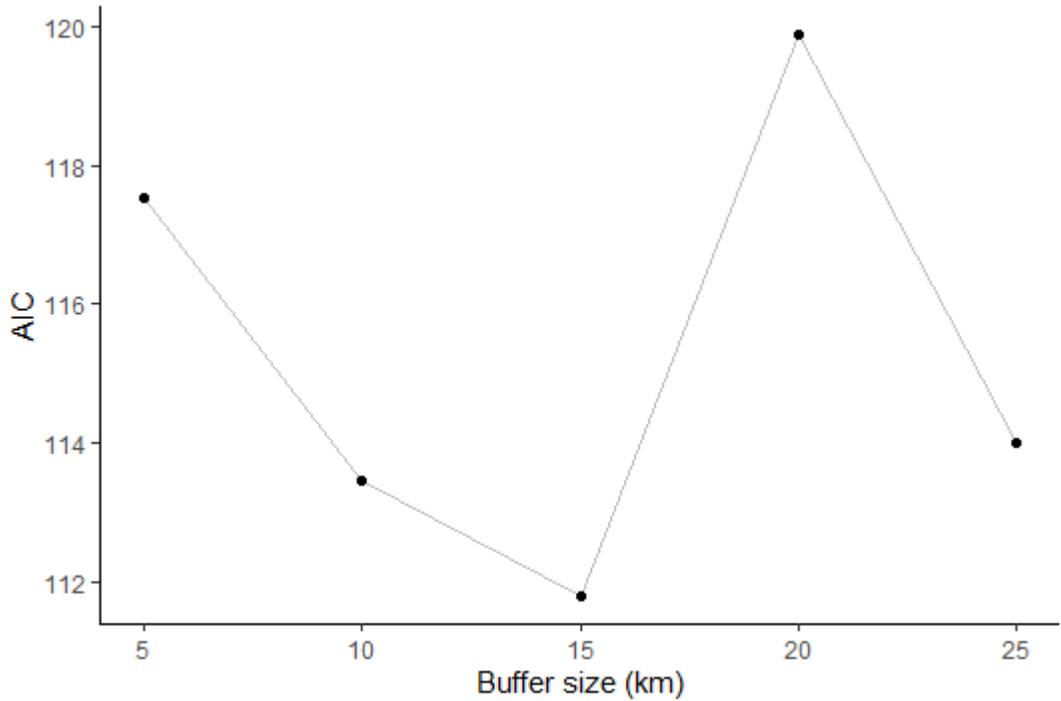


Figure 2. Variation of the Akaike's An Information Criterion following a gradient of buffer size

The LEK-based model is highly efficient in predicting the presence of white-bellied pangolin in the villages where actual presence has been confirmed with scales. Only one village has been mis-classified, resulting in 97% of correct classification (Fig. 3).

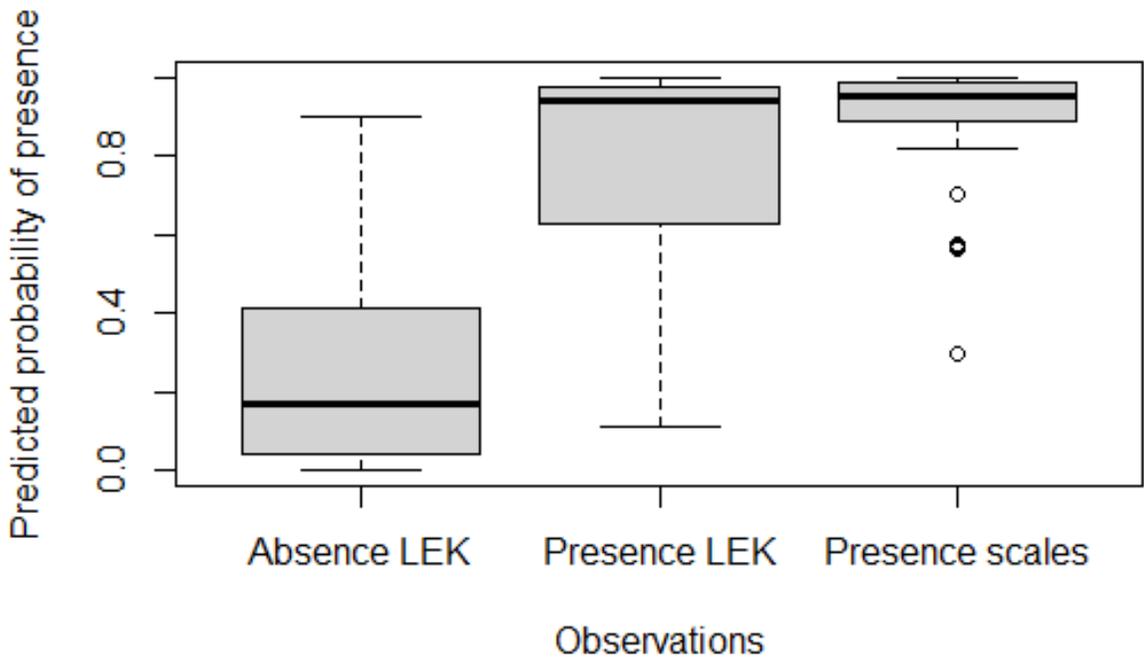


Figure 3. Direct evidence-base prediction model of the Local Ecological knowledge

The obtained results by fitting the binomial regression to all the data (N=208) and using a 15 km buffer size are shown in Table 1 and those by considering the white-bellied pangolin alone in the Table S2 (Appendix 2). All the variables contributing significantly to the fit were acting as anticipated (regression coefficients of the anticipated sign) and only two variables were not significant (*distMarkets* and *distWater*). The variables contributing the most to the model were species identity and initial abundance (both with $p < 0.001$). The persistence probability for the white bellied pangolin displayed strong spatial heterogeneity, from almost 1 in average for villages in the upper quartile of the predictions to about 0.1 in the lower quartile (Fig. 4a). In contrast, for the giant pangolin, the persistence probability was low all over the studied area (Fig. 4b).

Table 1. GLM model using 15 km buffer size ($N= 208$ villages)

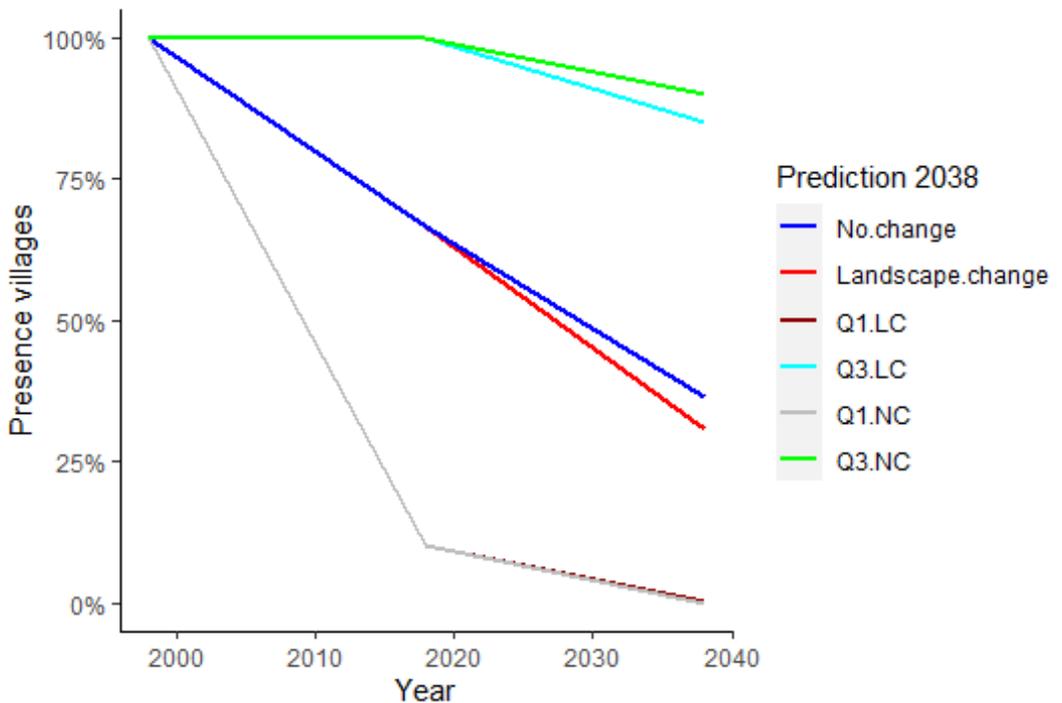
Variables	Estimate	p -values
<i>Intercept</i>	-2.976e+00	0.00326**
<i>ab1998</i>	2.333e+00	8.62e-08***
<i>species</i>	-5.851e+00	1.17e-06***
<i>dft₁₅</i>	-3.659e-02	0.00902**
<i>LSI₁₅</i>	1.000e+01	0.00160**
<i>distPA</i>	-7.858e-05	0.00706**
<i>distRoad</i>	6.951e-05	0.00547**
<i>distWater</i>	-2.176e-05	0.47819
<i>distMarkets</i>	-2.809e-06	0.55882

Significance : ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05

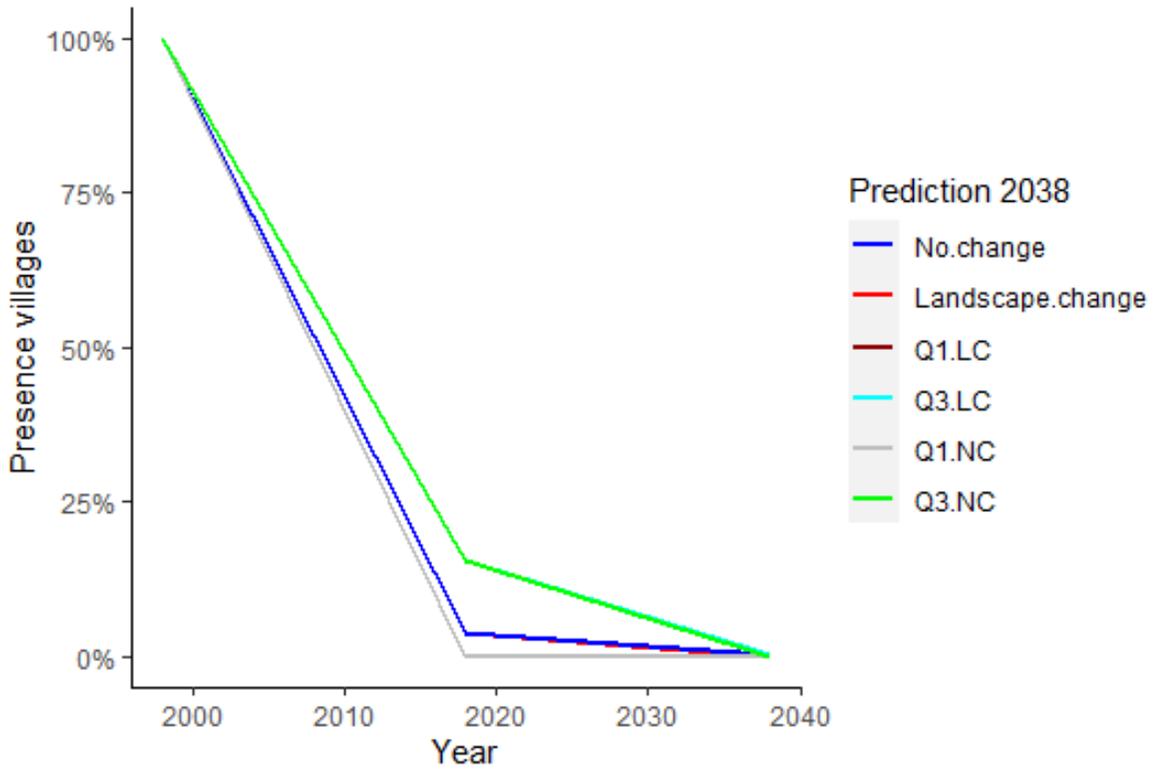
3.3.2. Prediction of spatial persistence of pangolins the next two decades (2038)

The same interspecific difference in spatial variability is observed when persistence was predicted for 2038. For both species, assuming either a status quo in habitat suitability or an habitat change continuing at the ongoing rate had a negligible effect on the predicted decline (Fig. 4). The area of occupancy of the two species was predicted to decrease in the coming years but the projection is particularly alarming for the giant pangolin which was predicted to be virtually extinct from Benin by 2038. The same variables of interest were observed when the binomial model was fitted to the white-bellied pangolin only, with a little difference of the significance levels for land-use suitability over time and the distance from villages to asphalted that increases and decreases respectively (Table S2, Appendix 2).

Spatial visualization of presence in 2018 and the predicted probability of persistence in 2038 showed that all the points of presence of the white-bellied pangolin were located on the limits of protected areas with the hotspots of high presence probability near the forest patches between 6°5'0'' and 7°5'0'' (*Lama and Gnanhouizoumè forests*) and a central block of protected areas located between 8°5'0'' and 10°0'0'' (*Complex Mont kouffé-Wari Maro-Ouémé supérieur*), whatever the model considered (Fig. 5). The same spatial configuration was observed with the white-bellied pangolin only (Fig S2, Appendix 2). Inversely, a total extirpation of the giant pangolin was observed in its last refuge (Fig. 6).



(a)



(b)

Figure 4. Trends over the next two decades of the probability of presence for the white-bellied pangolin in Benin. (a) the white-bellied pangolin and (b) the giant pangolin. The blue, grey and green lines correspond to the model assuming no deforestation and change of Land-use suitability over the next two decades based on the global, first quartile and third quartiles respectively. The red, darkred and cyan lines correspond to the model assuming similar changes as in the last two decades

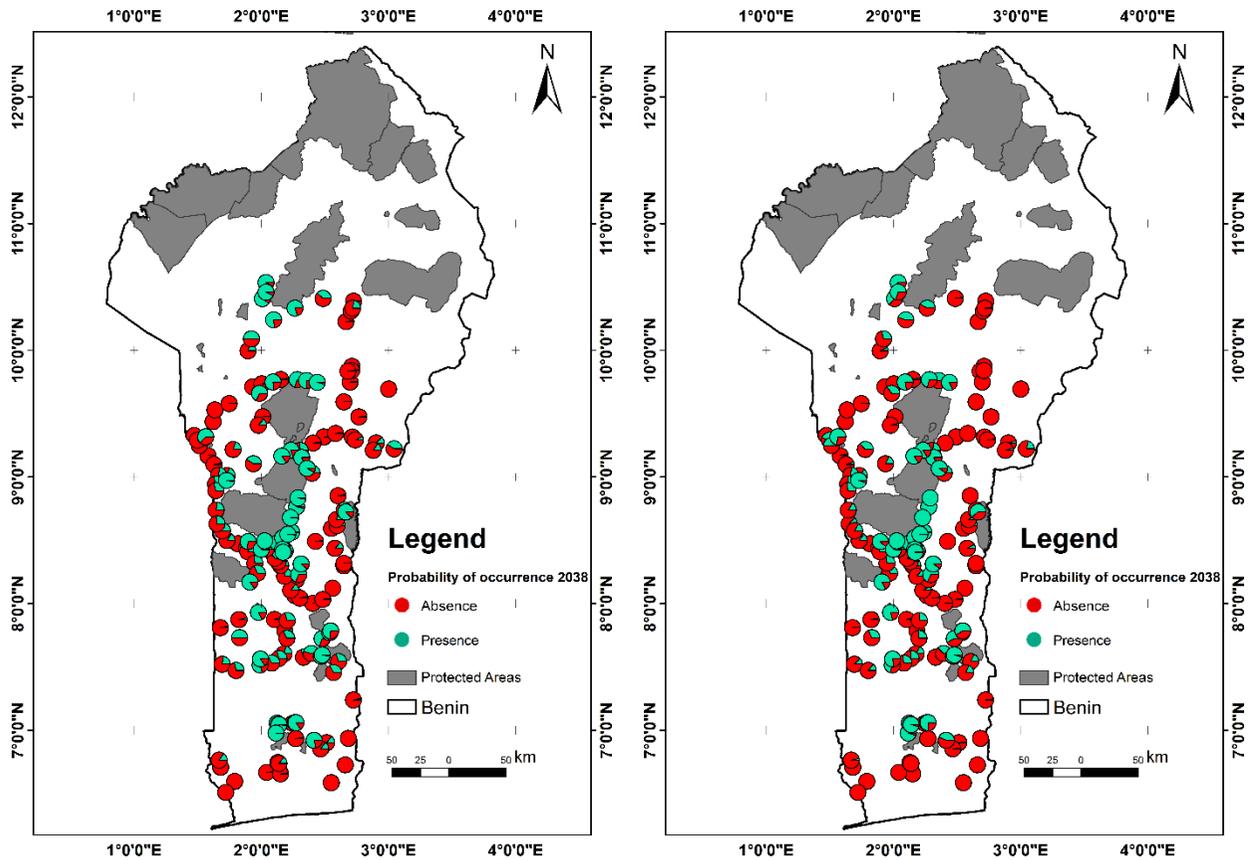


Figure 5. Prediction maps of the probability of occurrence for the white-bellied pangolin in 2038. (a) assuming no deforestation and change of the land-use suitability over the next two decades (2018-2038) and (b) assuming similar trends of deforestation and change of land-use suitability as in the last two decades (1998-2018)

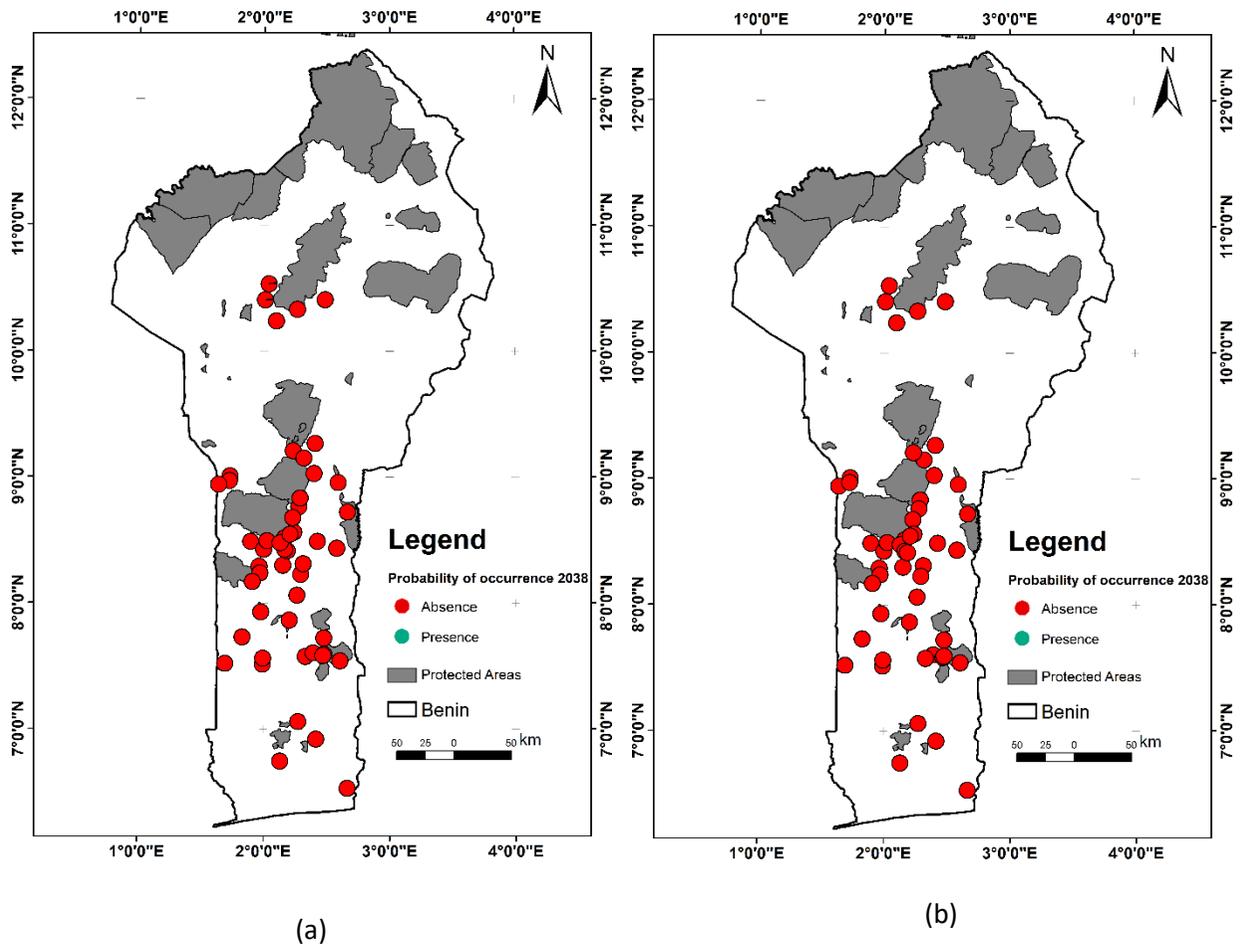


Figure 6. Prediction maps of the probability of occurrence for the giant pangolin in 2038. (a) assuming no deforestation and change of the land-use suitability over the next two decades (2018-2038) and (b) assuming similar trends of deforestation and change of land-use suitability as in the last two decades (1998-2018)

3.4. Discussion

Effective conservation implies informed decision-making which is not achievable in data lacking context. African pangolins (Pietersen & Challender 2020; Heighton & Gaubert 2021), and, in particular, the white-bellied and giant pangolins in Benin (Zanvo et al. 2020) are data poor species under extinction risk. Although LEK is subject to some controversies, since its international

recognition by the *Convention on Biological Diversity*, its combination with scientific ecological knowledge is increasingly encouraged in ecology for the reliability of studies. It has been increasingly integrating in all disciplines of ecology, especially in ecological modeling (Bélisle et al.2018) where it heightens the legitimacy and reliability of models that may be used for decision-making. Our study using LEK combined with direct evidence partly fills the ecological knowledge gap by pinpointing the spatial context, species identity and their abundance levels on the probability of persistence that may guide the conservation actions for long-term persistence of pangolins in Benin.

The GLM model revealed a high probability of pangolin population persistence in the villages where abundant populations were reported two decades ago. This result is coherent with the expectation that large populations are more likely to go extinct than small ones, whatever the extinction processes and drivers. However, the fact that, when initial population size is controlled for, additional variables contribute to extirpation rate strongly suggests that most of the populations are declining either because of negative population growth rate (e. g. overexploitation) or because of decreasing carrying capacity (e.g. habitat loss), as discussed below.

Considering the model structure (no interaction between species and the other variables), the low number of giant pangolin occurrences, and a validation test performed for the white-bellied pangolin only, detailed inter-specific comparison of model outputs should be done with caution. However, our study strongly suggests that giant pangolin populations have been extirpated at a higher rate than white-bellied pangolin populations. This is an expected pattern considering the positive correlation between the body size and the extinction risk in mammals (Johnson 2002; Lyons et al. 2004; Van Valkenburgh et al. 2004; Liow et al. 2008). Several factors explain the sensibility of large species

to human impacts: they are easy targets for hunters, they generally occur at low density and they can't afford high rates of mortality (Tomiya 2013; Ripple et al. 2017). In our data the population abundance has been accounted for and, therefore, cannot explain alone why the giant pangolin is so vulnerable. In addition, the giant pangolin is terrestrial making the species easily visible in the Benin's landscape dominated by open vegetation and more vulnerable to hunting in contrast with the arboreal and more elusive white-bellied pangolin that was commonly observed in modified, degraded and artificial ecosystems (Angelici et al. 1999; Akpona et al. 2008; Jansen et al. 2020). A similar explanation has been put forward to account for differences in hunting pressure between two pangolin species in Vietnam (Newton et al. 2008).

According to our model, the probability of persistence of a pangolin population increases with the distance from villages to roads. The negative effect of asphalted road network on the persistence of pangolins is in line with previous studies that demonstrated the road development as a driver of high extraction of wildlife in natural habitats (Espinosa et al. 2014; Constantino 2016), including natural reserves (Brashares et al. 2001). Pangolins are the most heavily trafficked mammals in the world for subsistence, medicinal and commercial purposes. In Benin they have been experiencing a heavy poaching pressure, in particular to supply local trade occurring in wildlife markets (Zanvo et al. 2021). The proximity of asphalted roads may facilitate the access of hunters to markets where they can sell pangolins. Under this hypothesis it is also expected that the proximity to a wildlife market increases hunting pressure and in turn leads to higher extirpation rate, a pattern that did not receive a statistical support in our model. An alternate explanation is that an asphalted road may facilitate the access to pangolin populations and, hence, increase the hunting pressure (Brashares et al. 2001). Another possible scenario is that roads density is an indirect measure of other human perturbations (e. g. habitat

fragmentation) that could negatively affect the persistence of pangolin populations. Although, there is no data on road kills of wildlife in Benin, the human transportation corridors may also be a key driver in direct mortality of pangolins by collision with the traffic (Moreli 2017). The combined effects of the above scenarios would certainly have contributed to the extirpation of pangolin populations in areas with heavy road density.

The GLM model pinpointed negative impacts of deforestation and reduction of the suitable habitat over the last two decades on pangolins, leading to the conclusion that habitat degradation has contributed to the decline of pangolin populations. Other studies pointed out that land-use changes lead to local extinction in mammals (Visconti et al. 2011; Newbold 2018; Magioli et al. 2021). Given the ecological requirements of the white-bellied and giant pangolins, strongly linked to the tree-dominated habitats (Pages 1968; Pagès 1975; Angelici et al. 1999; Akpona et al. 2008; Hoffmann et al. 2020; Jansen et al. 2020; Zanzo et al. 2020; Segniagbeto et al. 2021), the fast change of land-use in Benin converting 53% of forests, woodlands and tree savannas into farmlands and degraded savannas over time (1990-2010; Padonou et al. 2017) could explain pangolin population decline. Extirpation following habitat loss may result from the extinction proneness of small (meta)populations occupying a small amount of favourable habitat. According to theory, metapopulation extinction is certain when the amount of habitat loss has been so important that local extinctions are no more balanced by colonizations (Hanski 2011; Rueda et al. 2015; Horváth et al. 2019) and evidence-based ecological relationship between habitat and species loss has been demonstrated (Hoavah et al. 2019). With our model, we were not able to assess whether or not this threshold has already been reached in some areas and this point deserves further attention. Anyway, the fact that the prediction models for both species and the white-bellied pangolin only exhibited almost the same decreasing

trends with and without deforestation and land-use changes for the next two decades (Fig 3.) suggesting that habitat degradation is probably of secondary importance in explaining population extirpations. The possibility that the temporal changes in pangolin habitat suitability have been imperfectly captured in our approach cannot be entirely discarded. However, in light of the available information about pangolin habitat requirements, it seems unlikely that major landscape features have been overlooked in our habitat suitability index and future efforts should be devoted to a better modelling of micro-habitat requirements.

Among the variables included in our model, there is the distance from villages to watercourses. Although the expected negative estimate was observed, this variable did not explain significantly the probability of persistence of pangolins in Benin, rejecting a correlated persistence of pangolins to the watercourses. The persistence of pangolins is probably not related to the gallery forest in Benin even if the species may occur in this type of habitat (Fischer et al. 2002; Kingdon & Hoffmann 2013; Zavo et al. 2020).

Apart from the above-mentioned deleterious spatial factors for pangolins, our model showed significant probability of persistence near protected areas for the white-bellied and giant pangolins in Benin. This result supports previous studies that highlighted the cornerstone importance of protected areas in the conservation of imperilled mammals (Macedo et al. 2019; Pacifici et al. 2020). The forest reserve patches have been displaying the role of last strongholds in the conservation of pangolins in Benin mitigating the combined effects of environment changes (road development, deforestation, land-use changes) and the overexploitation. This role is more perceptible in the Lama forests (6°5'0'' and 7°5'0'') and in the central block of protected Areas, including the Complex Mont kouffé-Wari Maro-Ouémé supérieur (8°5'0'' and 10°0'0''). It is also

worth noting that, at the northern limit of the occurrence range of the white bellied pangolin in Benin (circa 11°N, Zanvo et al. 2020), the species persists mainly in or in the vicinity of protected areas emphasizing their efficiency at sustaining populations when environmental conditions are probably not optimal. In the same vein, extant populations of giant pangolin are reported by local hunters only in the vicinity of the Alibori reserve without providing direct evidence. An important question is to know whether the existing reserve network will be sufficient to preserve self-sustaining populations of pangolins in Benin. Protected areas are not panacea in preventing populations extirpations (Macedo et al. 2019; Pacifici et al. 2020), particularly if they are small or not well managed (Brashares et al. 2001, Craigie et al. 2010). If the financial and personnel resources are lacking, the resistance of protected areas to anthropogenic landscape changes (conversion of forests, woodlands and tree savannas into farmlands; Padonou et al. 2017) and overhunting (see Zanvo et al. 2021), could be compromised. In addition, it is likely that some reserves are too small to insure long-term viability of pangolin populations, even in the absence of any detrimental human impacts. Refer to the low effective population size (78-135 individuals, contemporaneous) suggested by Zanvo et al. (2021) in the Dahomey Gap lineage of white-bellied pangolin (Benin, Togo and southeastern of Nigeria) and the high number of occurrence forest reserves within this occupancy areas (Zanvo et al. 2020; Segniagbeto et al. 2021), it appears that even within protected areas occur probably populations under the threshold of a minimum viable population suggesting by Traill et al. (2007).

Direct evidence of presence (scales) highly validated LEK-based data we used to understand the drivers of spatial persistence of the white-bellied and giant pangolins in Benin. These results demonstrate that the LEK could be a precious decision tool in the management of wildlife (Gilchrist et al. 2005; Lima et al. 2017; Bélisle et al. 2018; Skroblin et al. 2019), in our case, by providing

reliable data about the spatio-temporal occurrence of the focal species. The LEK-based abundance index is among the most important variables explaining the persistence of pangolins in Benin, and therefore it would be important to assess its reliability too. However, in contrast with occurrence data, few information exists about pangolin abundance in Benin (Akpona et al. 2008) and, more generally, assessing population status of pangolin is challenging (Wilcox et 2019). Therefore, designing a protocol to validate LEK based abundances indices is expected to be faced with many constraints.

3.5. Conclusion

Our results strongly suggest that the combined effects of an unprecedented hunting pressure and human-driven landscape changes resulted in numerous pangolin population extirpations. It resonates an heterogenous distribution of pangolins across space with some forest reserve patches which seem to encompass sustainable populations of the white-bellied pangolin. As no colonization have been reported over the last two decades in the LEK survey, the spatial occupancy is decreasing for the two species and, without efficient conservation actions, this decline is expected to persist in the near future. The observed and predicted decline is particularly strong for the giant pangolin and in the absence of recent evidence of its presence its extinction or quasi-extinction from Benin cannot be discarded. It is thus urgent to confirm the presence of this species in the localities where, according to LEK, it is supposed to occur. The white-bellied pangolin is also declining but at a very spatially heterogeneous rate so that the great majority of the populations benefiting from the best conditions (the upper quartile, comprising the villages located on the limits of protected areas mainly) are expected to persist over the next decades. However even for those favoured populations the projected extirpation rate is still too high to qualify them as viable. Anyway,

the populations with the highest predicted persistence time should be considered for reliable spatial prioritization, an essential step for long term suitability conservation plans (Grantham et al. 2020). In particular, the protected areas enhance significantly the persistence of pangolins populations in Benin and this efficiency should be maintained (and preferably improved) in the future to balance an anticipated increasing international trade. According to our model, halting deforestation and habitat degradation will not be enough to prevent the decline of pangolin populations, even in the most favourable environments (e.g. in the vicinity of a protected area). This observation calls for more efficient measures and rigorous management (strict enforcement of anti-poaching laws) than the one already implemented to reduce the hunting pressure on pangolin (including within protected areas), otherwise populations are likely to keep declining at an alarming rate.

Chapter 4

Ethnozoological and commercial drivers of the pangolin trade in Benin

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CHAPTER 4

Ethnozoological and commercial drivers of the pangolin trade in Benin

Abstract

Pangolins are trafficked in unsustainable volumes to feed both local and global trade networks for their meat and the medicinal properties of their derivatives, including scales. We focus on a West African country (Benin) to assess the medicinal and spiritual values of pangolins among different ethnic groups and identify the cohort of buyers involved in the pangolin trade and related economic values along the chain, notably from local diasporas. We organised 54 focus groups in villages surrounding occurrence habitats of pangolins across Benin and conducted 35 individual interviews with vendors from five major traditional medicine markets (TMMs). Our questionnaire addressed the different uses of pangolins, the commercial value of pangolin items, the categories of clients and the related selling prices. Pangolin meat was strictly consumed as food. Scales, head, bones, tongue, blood, heart and xiphisternum were the items used by local communities as part of medicinal (65 % of the focus groups) and spiritual (37 %) practices. Scales were the most frequently used item (use value index = 1.56). A total of 42 medicinal and spiritual uses, covering 15 International Classification of Diseases (ICD) categories, were recorded among ethnic groups. The ICD and spiritual categories-based analyses of similarity showed a partial overlapping of ethnozoological knowledge across Benin, although knowledge was significantly influenced by ethnicity and geographic location. The pricing of pangolins both varied with the category of stakeholders (local communities *vs.* stakeholders of TMMs) and clients (local and West African clients *vs.* Chinese community), and the type of items sold. The Chinese community was reported to only buy pangolins alive and average selling prices were 3-8 times higher than those to West

African clients. Our results confirm that pangolins in Africa are valuable and versatile resources for consumption and medicinal / spiritual practices. The pangolin trade in Benin is based on an endogenous and complex network of actors that now appears influenced by the specific, high-valued demand from the Chinese diaspora. Further investigations are required to assess the growing impact of the Chinese demand on the African wildlife trade.

Keywords: Ethnozoological knowledge, Spiritual use, Traditional medicine market, Wildlife trade, Pangolins, Benin, West Africa

4.1. Introduction

Bushmeat –i.e., the wild game from the tropics– constitute the main animal protein and income sources for rural people in sub-Saharan Africa (Petrozzi et al. 2016). In the Congo Basin, individual consumption amounts several dozens of kg per year (e.g., Fargeot & Dieval 2000; Starkey 2004). Bushmeat often represents the cheapest animal protein alternative for poor rural households (Brashares et al. 2011). Bushmeat hunting also stands among the prime income-generating activities in rural areas of tropical Africa, where the bushmeat trade can generate more than 500 USD per year for a single household hunter (e.g., Kümpel et al. 2010).

Bushmeat species also play a vital role in traditional African medicine where animal-derived body parts (items) are used for the treatment of diseases, ailments and spiritual purposes (e.g., Djagoun et al. 2012, D’Cruze et al. 2020). The specific markets, mostly urban, where such items are sold add to the bushmeat selling network already connecting rural to main urban centres (Luiselli et al. 2018). As a consequence, bushmeat consumption and use, which occur at unsustainable rates in Africa (Bennett et al. 2002), have so far

remained an intractable issue, contributing at the same time to household wealthiness and biodiversity extinction (Wilkie et al. 2016; Ripple et al. 2017). Pangolins (Pholidota, Mammalia), or scaly anteaters, have recently emerged as the flagship taxon of the bushmeat crisis. They are trafficked in unsustainable volumes to feed the local and international –mostly driven by the Traditional Chinese Medicine (TCM)– demands for both their meat and the medicinal properties of their scales and other items (Challender et al. 2015; Heinrich et al. 2016; Ingram et al. 2019). Pangolins have also been suggested as the intermediate host responsible for the COVID-19 pandemic (Zhang et al. 2020), despite the lack of concrete evidence for this claim. In Africa, pangolins have recently seen their trafficking volumes and market prices increase, in line with the trends observed for the Asian species (Ingram et al. 2018). Effective trading networks are now connecting Africa and Asia to feed the TCM demand for pangolin scales (Ingram et al. 2019).

Among the four species of African pangolins, the white-bellied pangolin (*Phataginus tricuspis*) is the most frequently found on the bushmeat and traditional medicine markets (e.g., Boakye et al. 2016, Soewu et al. 2020), notably in West Africa (Djagoun et al. 2012, Mambeya et al. 2018, Ingram et al. 2018, D’Cruze et al. 2020). Ethnozoological knowledge on the species shows a diversity of uses by local communities involved in medicinal and spiritual practices, to treat convulsion, rheumatism, hiccups, healing wounds, woman unfaithfulness and impotence (Boakye 2018; Soewu et al. 2020). Scales are the most commonly used, although various items such as tongue, bones and head are also regularly found on the traditional medicine market (TMM; Djagoun et al. 2012, D’Cruze et al. 2020).

Benin, situated in the Dahomey Gap (West Africa) and often designated as the cradle of the “Vodoun culture”, harbours a vibrant market network for animal-based medicinal and spiritual practices, likely to have deleterious impacts on

biodiversity conservation in the whole sub-region (Djagoun et al. 2012, D’Cruze et al. 2020). Although the ethnozoology of pangolins has received much attention in neighbouring countries (Soewu and Ayodele 2009, Boakye et al. 2014, Boakye et al. 2016, Boakye, 2018, D’Cruze et al. 2020), the situation in Benin, where the white-bellied pangolin’s range has been contracted by 1/3 over the last two decades (Zanvo et al. 2020), remains understudied. Akpona et al. (2008) found seven different items of pangolins used by southern communities for 13 medicinal and spiritual purposes, with scales as the most frequently cited. However, this study was restricted to southern Benin and included only two ethnic groups (*Hôli* and *Fon*), despite the larger extent of pangolin’s distribution in Benin (Zanvo et al. 2020).

Investigating on the ethnozoology of pangolins should help understand the causes and extent of the species decline as related to the medicinal and spiritual practices that prevail in Benin. In this study, we propose a country-scale survey of the main ethnozoological drivers of the pangolin trade encompassing four major Beninese ethnic groups, and incorporating the recently raised issue of the international demand from local diasporas (Ingram et al. 2019). Our main objectives are to (i) assess the different uses of pangolin items among ethnic groups in Benin, and (ii) identify the cohort of buyers involved in the pangolin trade and related economic values along the chain, including the local demand from the Chinese community.

4.2. Material and methods

4.2.1. Data collection

We targeted (i) 54 villages neighbouring all the potential areas of occurrence of pangolins in Benin and (ii) the five major TMMs in southern and central Benin (*Avogbannan*, *Calavi*, *Dantokpa*, *Gbèdagba* and *Zobè*) (Fig. 1). The target local communities were identified after Zanvo et al. (2020) from

occurrence areas providing pangolin scales. Focus group participants and vendors were adults at least eighteen years old. Focus group participants were volunteers, identified through informal interviews with villagers whereas in the TMMs, availability and trust towards the interviewer were the criteria of participation. In each village, we organized a single focus group with 7-11 local people using a pre-established questionnaire and a poster figuring pangolins. The questionnaire was conducted as a semi-structured interview addressing the names used for pangolins in local languages, the different uses of pangolins and which items were involved, the selling prices of pangolins, the categories of clients and the time period at which the trade with each category of clients started. For each question, all the answers were recorded without discrimination among participants of the focus group. In the TMMs, we generally had to use a modified approach focusing on the questions related to the commercial value of pangolins. Such a strategy was necessary because we were not able to gather simultaneously several vendors and the latter were not available for long-time interviews, due to their activity. However, six interviewees out of 35 were able to fully answer the questionnaire. Their responses on the ethnozoology of pangolins were treated as a single focus group, as they all belonged to the same *Fon* ethnic group.

4.2.2. Data analysis

We used the International Classification of Diseases (ICD-11; version 09/2020) to group the recorded medicinal uses into ICD categories for each item of pangolins. Given the important number of spiritual uses recorded, we created an additional, dedicated category. Each focus group was considered as a single observation for the analysis. Descriptive statistics were used to assess the frequency of citation of the items used, ICD categories mentioned by focus group participants and type of clients buying pangolin items. We used the Use

Value index (UV; adapted from Rossato et al. (1999) to assess the spectrum of use of each pangolin item for medicinal and spiritual purposes, as follows:

$$UV = \frac{\sum U_p}{n}$$

where U_p represents the number of uses mentioned by focus groups for each pangolin part and n the total number of focus groups.

One-way analysis of similarities (ANOSIM test) based on 9,999 permutations was carried out using the *vegan* package in *R* version 4.0.0 (R Core Team 2017) on a matrix of ICD and spiritual categories including the ethnic groups partitioned into geographic regions (South, Centre and North Benin). The *R* statistics was interpreted according to Clarke (1993): (i) $R < 0.25$ means no separation between groups, (ii) $0.25 < R < 0.5$ some level of separation between groups despite a degree of overlap, $R > 0.75$ well separated groups, and $R = 1$ total separation between groups. The ANOSIM test was used to assess variation of knowledge among ethnic groups (recorded at least four times; *Bariba, Fon, Mahi and Nagot*) and geographic regions using medicinal and spiritual use patterns. We used Non-metric Multidimensional Scaling (NMDS) analysis to visualize in a Cartesian space the dissimilarity between ethnic groups and geographic regions. We removed meat from ANOSIM and NMDS analyses due to the absence of medicinal and spiritual uses for this pangolin item that was solely and unanimously cited for consumption by all the focus group participants. We calculated the average selling prices among local communities and in the TMMs for the different categories of clients. We used the *t* test to compare average selling prices according to the categories of clients in rural areas (local communities) and in TMMs, and between rural areas and TMMs.

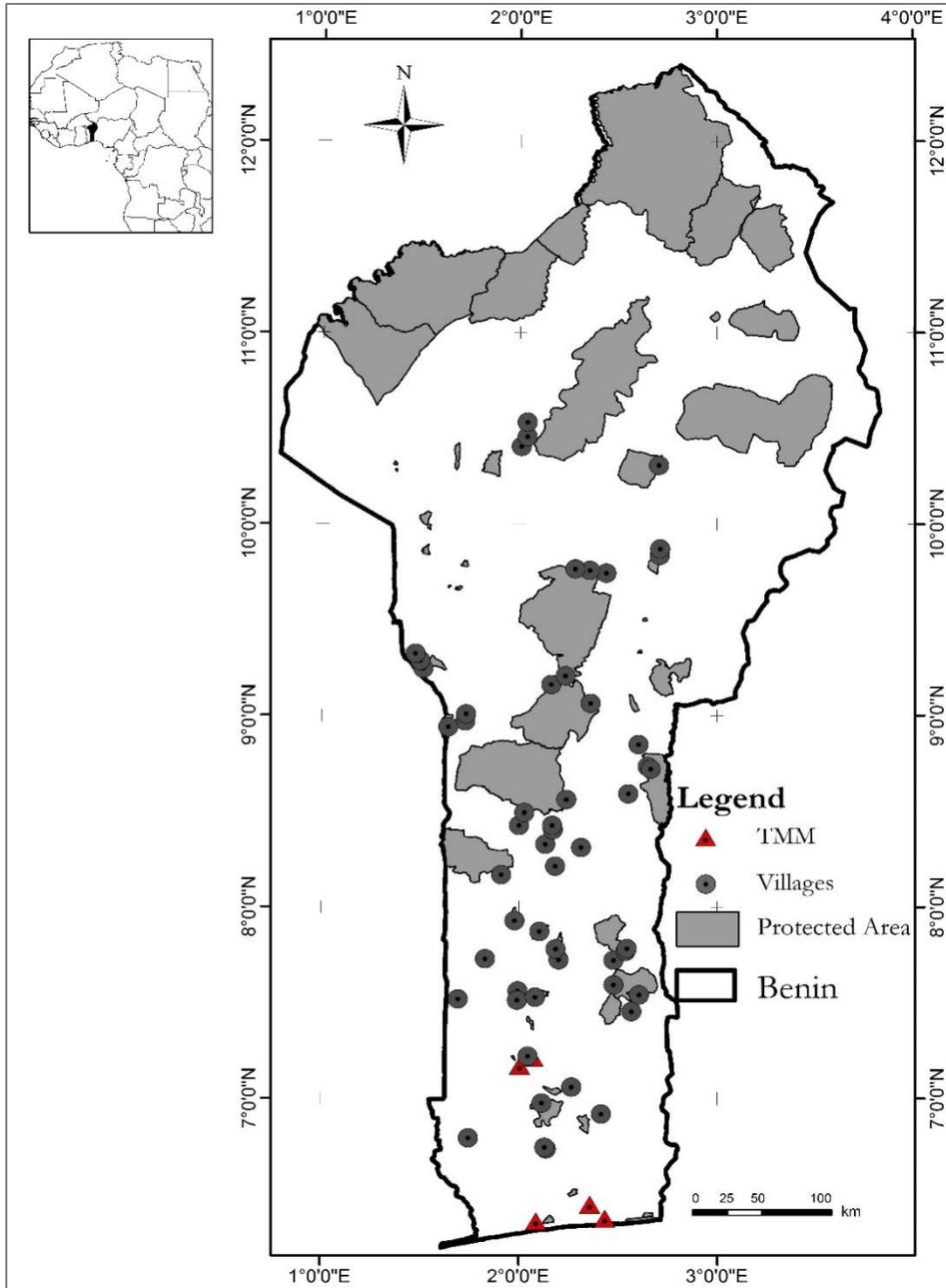


Figure 1. Villages and traditional medicine markets (TMM) in Benin surveyed as part of this study

4.3. Results

4.3.1. Frequency of citation of items, use value and ICD categories

Fifty-four focus groups were carried out across the occurrence zone of pangolins in Benin with 18, 22 and 16 focus groups in southern, central and northern regions respectively. Thirty-five individual interviews were performed in the TMMs. Focus group participants were mostly men (84 %), between 22 and 76 years (mean = 36 years), and included farmers (36 %), farmers-hunters (54%) and housewives (8%), whereas all the interviewees from the TMMs were men, aged 26 to 54 (mean = 37). *Lihoui* was the common name almost unanimously used for pangolins among ethnic groups in southern Benin whereas in central Benin, Nagot, Ifè, and Idatcha named pangolins either *Aïka* or *Akikan*. In northern Benin, the common name of pangolins changed from an ethnic group to another (Table 1).

Table 1. Common names used for the white-bellied pangolin among ethnic groups and geographic regions in Benin

Region	Ethnic Groups	Local names
	<i>Adja, Agouna, Aïzo, Gun</i>	<i>Lihoui</i>
South	<i>Fon</i>	<i>Lihoui, Houékin</i>
	<i>Yoruba, Holi</i>	<i>Iwô</i>
Centre	<i>Mahi</i>	<i>Kosso Lihoui</i>
	<i>Nagot, Ifè, Idatcha</i>	<i>Aïka, Akikan</i>
North	<i>Anii</i>	<i>Sassabokourou</i>
	<i>Bariba</i>	<i>Kokowaka</i>
	<i>Nagot</i>	<i>loufiloufi</i>
	<i>Lokpa</i>	<i>Libilibi</i>
	<i>Kotokoli</i>	<i>Kaminakpara</i>
	<i>Ditamaré</i>	<i>Tempétakpara</i>
	<i>N'tcha</i>	<i>Akonkon</i>
<i>Yom</i>	<i>Narm</i>	

Pangolins were unanimously cited by all the focus group participants for their use as an animal protein source. Medicinal and spiritual uses were cited by 65 % and 37 % of the focus groups, respectively. Scales, tongue, head, bones, xiphisternum (a specific prolongation of the sternum to attach the tongue muscles), blood and heart were the eight pangolin items cited by focus group participants (Fig. 2). Scales (64%), tongue (18%), bones (15%) and head (13%) were the items most cited by the focus groups for medicinal and spiritual uses.

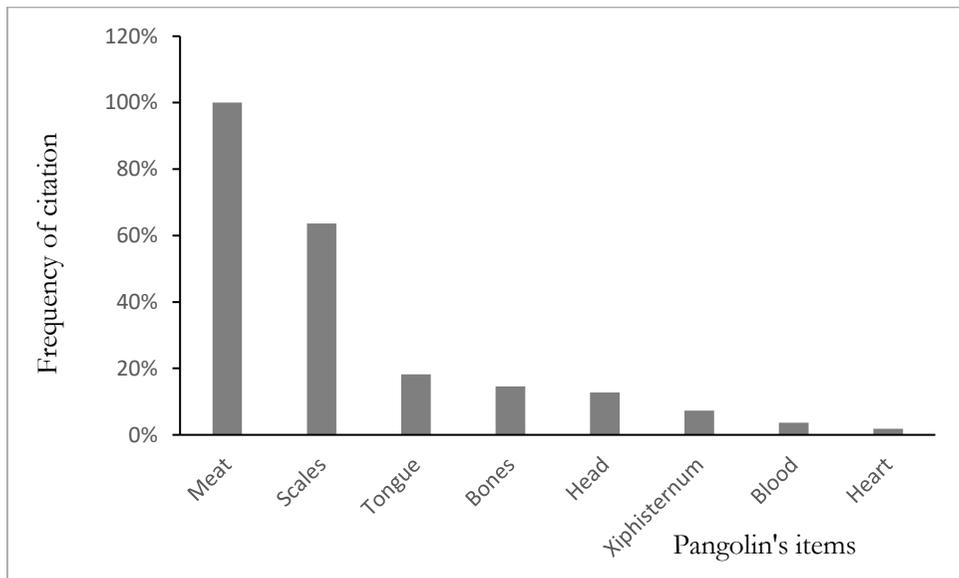


Figure 2. Frequency of citations of pangolin’s items used in local communities

A total of 42 medicinal (n = 31) and spiritual (n = 11) uses were recorded. The medicinal uses fell into 15 out of 26 ICD categories. The scales were the item of pangolins that had the highest use reported (56), number of ICD categories (14) and use value (UV = 1.56), followed by tongue, bones, head, xiphisternum, blood and heart (Table 2).

Table 2. Pangolin's items used in traditional medicine and spiritual practices in Benin

Spiritual purposes are in bold

Items	Conditions treated	UR	ICD	UV
Scales	Physical strength , oedema, cough, hiccups, rheumatism, convulsion of new born, pharyngitis, healing wound, snake bite, measles, man dominance in household , healing fire burns, defeating the opponent in case of litigation , facilitate delivery, infertility, scabies, sexual bewitchment , ringworm, joint pain, stomach aches, vigour of new born, breast cancer, dizziness, heartbeat, late infantile spinal muscular atrophy, osteoarthritis and asthma	56	14	1.56
Tongue	Stomach aches, pneumonia, hip pain, developmental language disorders, protection against sexual bewitchment , Epilepsy, incantation , defeating the opponent in case of litigation , sterility, thief sickness and stop women's infidelity	12	7	0.33
Bones	Hiccups, cough, osteoarthritis, healing wound, ataxia, asthma, coxarthrosis, developmental language disorders and late infantile spinal muscular atrophy.	11	6	0.31
Head	Oedema, pharyngitis, epilepsy, defeating the opponent in case of litigation , vigour of new born, unnatural power and luck	9	5	0.25
Xiphisternum	Impotence, sexual bewitchment	4	2	0.11
Blood	Asthma and convulsion of new born	3	2	0.08
Heart	Man dominance in household	1	1	0.03

The spiritual use category was the most mentioned by focus group participants (69%), superior to any ICD categories (Fig. 3). Sexual bewitchment was the spiritual use most frequently reported by focus groups, followed by the control of women’s infidelity. Among the 15 recorded ICD categories, Neoplasms (ICD-2; 31%), Traditional Medicine conditions (ICD-26; 25%), and certain infectious or parasitic diseases (ICD-1; 22%) were the most cited ICD categories by focus group participants (Fig. 3).

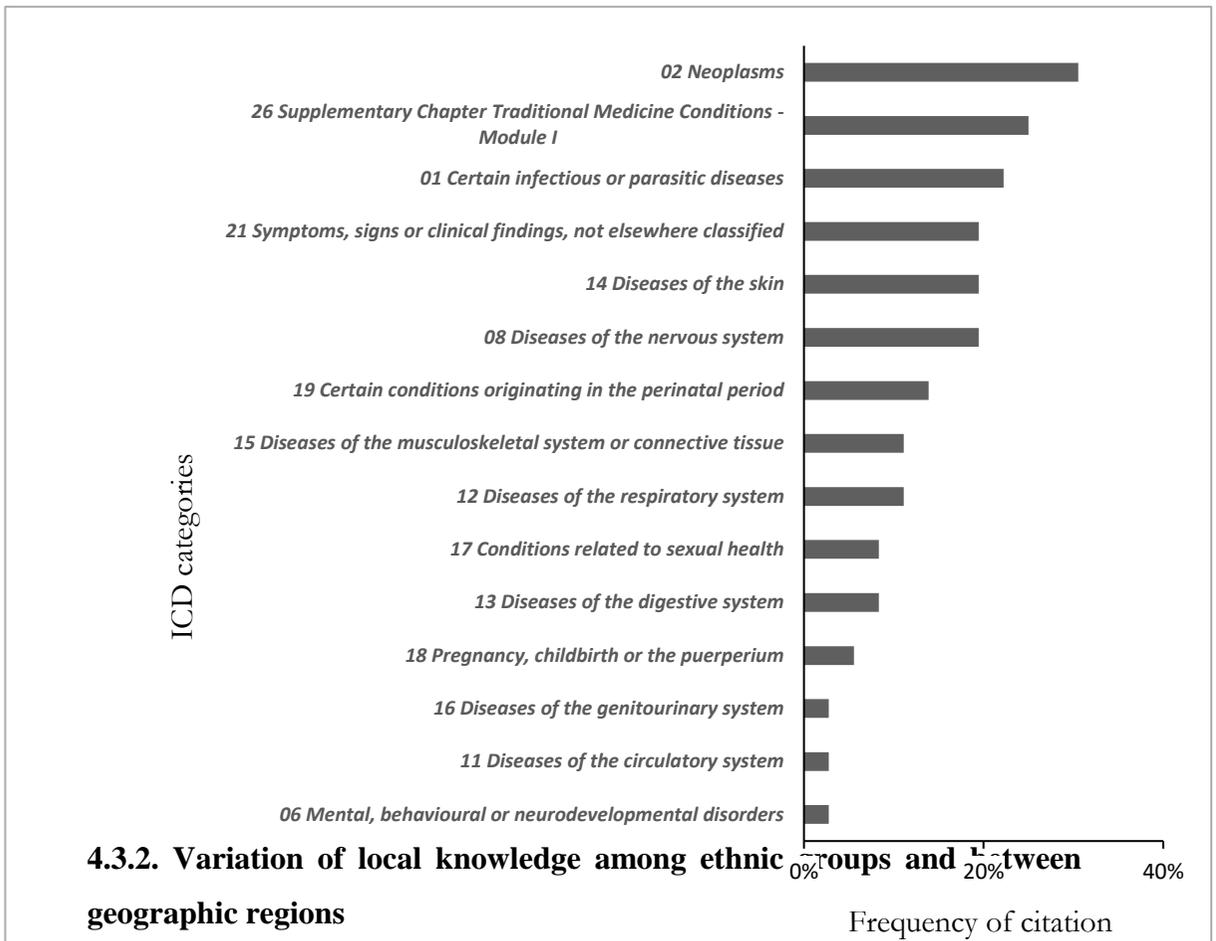


Figure 3. Frequency of citations of the conditions treated with pangolin items according to ICD categories

regions ($R= 0.48; p <0.001$). All the R statistic values ranged between 0.25 and 0.5,

indicating that the ethnic groups shared some level of ethnozoological knowledge. The NMDS plot showed some variation of the distance within and between *Fon*, *Nagot* and *Mahi* ethnic groups located in southern and central (Fig. 4). Conversely, the *Bariba* were close to each other, and generally remote from and opposite to all the *Fon*, *Mahi* and almost all the *Nagot*. We observed some overlapping between focus groups among *Bariba*, *Mahi* and *Nagot* ethnic groups. Moreover, some focus groups among *Mahi* and *Nagot* ethnic groups overlapped with *Fon* whereas others were remotely distributed in space.

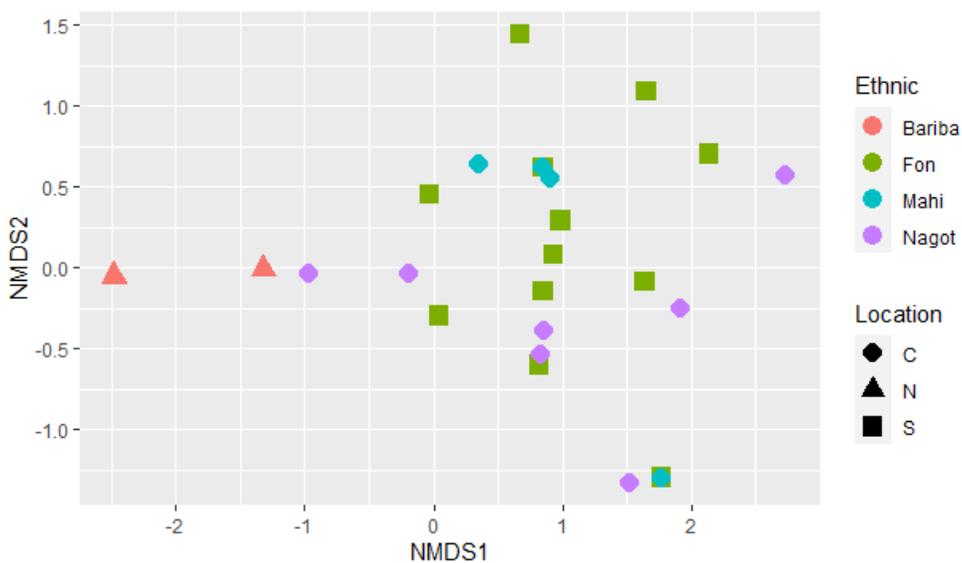


Figure 4. Spatial distribution of ethnic groups according to their ICD and spiritual categories-based knowledge. Euclidean distances between ethnic groups and geographic regions reflect the degree of similarity of knowledge. Overlapping implies the sharing of the same knowledge

to traditional medicine markets

Local communities cited five client types involved in the trade of pangolins in Benin (restorers [i.e. managers of restaurants], traditional healers, traders of traditional medicine markets, foreigners from West African countries and Chinese community), while only two client types (traditional healers and

Chinese community) were reported by TMM stakeholders (Fig. 5). Restorers, traders from TMMs and foreigners from West Africa (Togo and Ghana, according to focus group participants) were cited by 74, 48 and 30 % of the focus groups, respectively, as clients buying pangolins to local communities. Traditional healers (4 %) and Chinese community (9 %) were the client categories less cited by local communities. Within the TMM stakeholders, traditional healers (96 %) and Chinese community (88 %) were the only clients cited as buying pangolins. Local communities and the TMM stakeholders highlighted that the trade of pangolins with local people exists since the development of wildlife markets whereas the trade with West African and Chinese communities started approximately one decade ago.

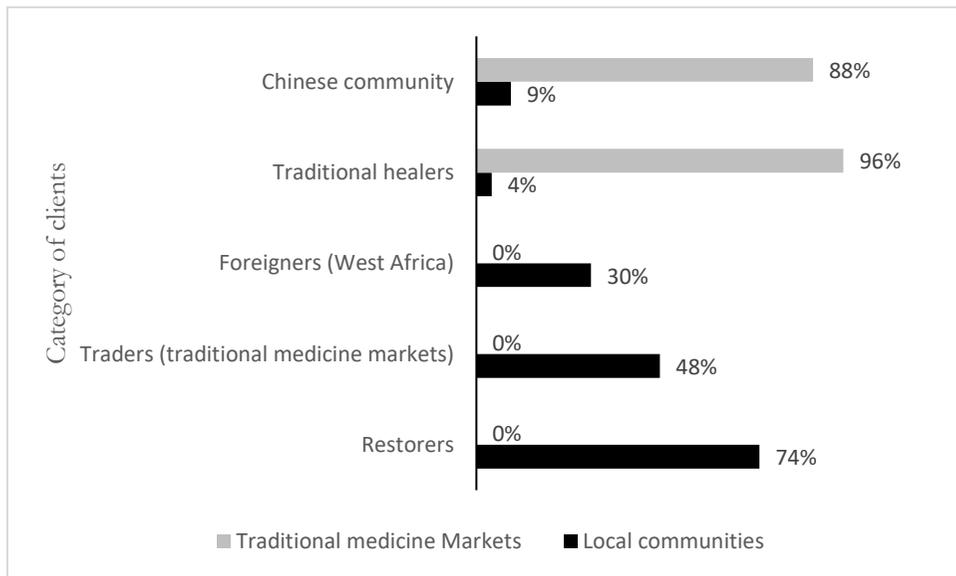


Figure 5. Frequency of citations of client categories within traditional medicine markets and local communities in Benin. West African foreigners originate from Ghana and Togo

Pangolin pricing changed according to the category of stakeholders (local community vs trader in TMMs) and clients (local and West African clients vs Chinese community) (Table 3). The average selling prices were higher in the TMMs

than in local communities. The Chinese community bought only pangolins alive, while local and West African customers both bought alive and dead pangolins or pangolin items. Head and scales were only sold in the TMMs to local and West African clients. The highest selling price (73.38 USD) for a pangolin (alive) was recorded in a TMM for a client from China. The average selling price of pangolins sold on the TMMs to the Chinese community was significantly higher than the selling prices to local and West African clients ($t = -34.089$; $p < 0.001$). The same trend ($t = -16.238$; $p < 0.001$) was recorded at the local community level with the Chinese community buying pangolins alive at higher prices than local and West African clients. There was no significant difference between the average selling prices to Chinese community in local communities and TMMs.

Table 3. Variation in pangolin pricing between local communities and traditional medicine markets in Benin, after client categories. African clients other than local originate from Togo and Ghana. Prices are given in USD (conversion: 30 July 2020). TMM refers to traditional medicine markets

		Pangolin pricing (USD)			
		Local communities		TMMs	
Type of item	Average	Local and West African clients	Chinese clients	Local and West African clients	Chinese clients
Alive	min	5.01	42.9	18.96	55.35
	max	7.84	60.05	24.83	73.38
Dead	min	4.52	-	18.96	
	max	6.68	-	24.83	
Head	min	-	-	3.88	
	max	-	-	4.73	
Scales (whole body)	min	-	-	8.62	
	max	-	-	12.06	

4.4. Discussion

Relative to the unique study that had been conducted on the ethnozoology of pangolins in southern Benin (Akpona et al. 2008), our investigations provide a deeper understanding of ethnozoological values across a diversity of ethnic groups in combination with the economic incentives possibly motivating the overexploitation of pangolins in Benin (Zanvo et al. 2020).

Lihoui was the common name for pangolins among most of the ethnic groups in southern Benin, whereas *Aïka* / *Akika* was used in central Benin. This uniformity of common names among ethnic groups located within the same geographic region (*Fon*, *Aizo*, *Adja* and *Gun* in South, and *Nago*, *Ifè* and *Idatcha* in Centre) could be due to the genealogical relationships between the sampled ethnic groups, which originate from the same ancestors and share similar languages (Sanni 2017). The *Fon* ethnic group is believed to be the initiator of the trade in animal derivatives since the Dahomey Kingdom (from c. 1600 to 1904 AD) and represents the vast majority of the actors currently operating in this sector in Benin (SZ, unpubl. data). The common name *Lihoui* could have originated from this dominant ethnic group, and through the colonization of other ethnic groups have diffused since centuries in all the TMMs of southern Benin (see Stevens et al. 2014). Conversely, northern Benin showed eight different names used for pangolins. This is likely because (i) this region has been colonized by ethnic groups from different origins with socio-linguistic divergences (Bio Bigou 1995; Debourou 2015) and (ii) the TMM network is almost inexistent, thus preventing from any diffusion of a dominant pangolin name.

Pangolins are used for food, medicinal and spiritual purposes in Benin, in line with the literature record for tropical Africa (Soewu & Ayodele 2009; Wright & Priston 2010; Boakye et al. 2014, 2015; Baiyewu et al. 2018; Malimbo et al.

2020). More specifically, pangolins in Benin constitute at the same time a bushmeat resource and an important input to traditional medicine and cultural practices (Akpona et al. 2008). Pangolin meat is unanimously consumed as food in Benin, whereas no medicinal and spiritual use is recorded, in line with Akpona et al. (2008) but contrary to Boakye et al. (2014 and 2015) and Baiyewu et al. (2018) who found that meat was used in traditional medicine by local people in several countries of western and southern Africa.

Among the eight recorded pangolin items, scales were by far the most commonly used by local communities for medicinal and spiritual purposes. Scales had the highest use value (UV = 1.56) and were mentioned in 14 out of the 15 ICD categories recorded for 56 different use reports. These results show that the scales possess a great traditional value for local people in Benin, probably justifying the storage of old scale samples in rural households (Zanvo et al. 2020) and the great number of scales present in the TMM stalls (SZ; unpubl. data). Our results corroborate those of previous studies in tropical Africa pointing out the high use value and versatility of use of pangolin scales in comparison with other items (Bräutigam et al. 1994, Boakye et al. 2014, 2015). Although the diversity of items for ethnozoological use was generally lower in Benin than in other African countries (Boakye et al. 2014, 2015; D’Cruze et al. 2020; but higher than in Akpona et al. 2008 and Setlalekgomo 2014), we observed the use of a so far unreported pangolin item, the xiphisternum. The latter is involved in impotence and sexual bewitching treatments, and could be a particular knowledge of *Fon* and *Mahi* ethnic groups.

A large proportion of the disease and ailment treatments where pangolin items are involved had never been described for Benin (Akpona et al. 2008), although almost all of them had already been recorded in West Africa (Boakye et al. 2014, 2015). The ratio between the diversity of medicinal / spiritual uses and

items was higher in Benin (42 medicinal and spiritual purposes for eight items) than what was recorded from traditional healers and fetish markets in other West African countries (Boakye et al. 2014, 2015, D’Cruze et al. 2020). In Benin, spiritual uses (69%) and the ICD categories Neoplasms (31%) and Traditional Medicine conditions (25%) were the most cited by local communities. Overall, this indicates that pangolin items are used against many diseases and ailments generally uncovered by conventional medicine, revealing the high endogenous value of pangolins in a country where Vodoun practices are thriving (Médiouhouan 1993).

Our results suggested slight differences of ethnozoological knowledge within and between ethnic groups and geographic regions, and at the same time indicated partial overlapping of ethnozoological knowledge among the major ethnic groups (*Fon*, *Nagots*, *Bariba* and *Mahi*) in Benin. Socio-cultural distances between ethnic groups in Benin (see Bio-Bigou 1995, Débourou 2015) could be a factor explaining the divergences of knowledge on medicinal and spiritual uses of pangolins. For instance, the use of scales to treat breast cancer was only cited by the *Bariba* ethnic group whereas pneumonia, epilepsy, rheumatism and measles were only recorded among *Fon*. The *Nagot* were the only ethnic group that mentioned the use of pangolin items for the treatment of ringworm, scabies and heart palpitations. Thus, our results show a partial influence of ethnicity on ethnozoological knowledge of pangolins, in line with what was observed in Ghana (Boakye et al. 2018). However, *Fon*, *Mahi* and *Nagot* ethnic groups also share ethnozoological knowledge on pangolins such as healing wound, treatment of sterility, hiccups, easy delivery, snakebite, pharyngitis, sexual bewitchment, defeating the opponent in case of litigation, and sterility. South and central Benin count 42 TMMs (SZ, unpubl. data) mainly dominated by the *Fon* ethnic group, whose beliefs are strongly linked to Vodoun practices. Through this market network, the *Fon* ethnic group

has probably diffused or gained medicinal and spiritual knowledge of pangolins in the central region of Benin.

As part of the bushmeat species spectrum sold in Central and West Africa, pangolins constitute a traditional source of income for rural communities, restorers and TMM vendors (Mambeya et al. 2018, Ingram et al. 2018; D’Cruze et al. 2020). In Benin, we showed that the pricing of pangolins both varied with the category of stakeholders (local communities vs. stakeholders of TMMs) and clients (local and West African clients vs. Chinese community), and the type of items sold (from alive to scales). Average selling prices for live and dead pangolins were higher in TMMs (18.96 - 73.38 USD), probably to write off the costs of being at the tip of the supply chain and to benefit from the higher wealthiness of urban households (Brashares et al. 2011). However, excluding buyers from the Chinese community, average selling prices of pangolins in rural areas (4.52 - 7.84 USD) and in TMMs (18.96 - 24.83 USD) were lower than those recorded in the bushmeat markets from central Africa one decade ago (mean = 34.27 – 35.66 USD; Ingram et al. 2019). The relatively cheap cost of pangolins on the Beninese markets means that the species is economically reachable by West African consumers, which may not facilitate the mitigation of the volumes of pangolins extracted each year in Benin as part of the wildlife trade.

Our study highlighted the international component of the pangolin trade in Benin through the diversity of foreign clients, involving West African and Chinese nationals since c. one decade (probably also involving Vietnamese nationals, after a case of recent seizure at Cotonou airport; Ingram et al. 2019). Whether pangolins are consumed locally or brought back to the clients’ home countries is unknown, and would deserve further investigations. We revealed different practices according to the type of clients, the Chinese community only buying pangolins alive whereas local and West African clients would also

buy dead pangolins and various items (head, scales). The average selling prices of pangolins to the Chinese community was 3-8 times higher than the selling prices to local and West African clients, confirming that Chinese diasporas in Africa has brought further economic incentives to the endemic pangolin trade (Mambeya et al. 2018, Ingram et al. 2018). Further investigations are required to assess whether the Chinese diaspora demand has modified the practices of wildlife traders and is constitutive of an “overexploitation vortex” of pangolins (see Aisher 2016) in Benin.

Assuming a mean body weight of 2.48 kg for the white-bellied pangolin (2.36 ♂- 2.6 ♀ kg; in Kingdon & Hoffmann 2013) and a 1/4-1/3 contribution of the scales to the total weight of the species (Gaubert 2011), scales bought by local and West African clients would cost 10.39 – 19.45 USD per kg. Because there is a possibility that the scales of pangolins bought by the Chinese community in Benin end up feeding the global pangolin trade (e.g., 513 kg of scales were seized in Cotonou in 2018, Ingram et al. 2019), we also estimated the average price of pangolin scales per kg if scales were extracted from live pangolins (17.30 – 29.59 USD per kg). Such prices confirm that pangolin scales constitute a valuable source of income on West African markets (Boakye et al. 2014). Compared to the prices of pangolin scales sold in China and Vietnam (485 - 759 USD per kg) (Challender et al. 2015), both paths of acquiring scales in Benin (either directly or from a live animal) would remain highly profitable to traffickers that feed the illegal pangolin trade to –mostly– China (Volpato et al. 2020).

Studying client practices allowed us to delineate a complex network for the pangolin trade in Benin, including (i) the TMM that mostly supplies traditional healers and the Chinese community and (ii) a less urbanized and more diffused market network where local and West African clients, together with restorers and TMM stakeholders, would be the main buyers. The low contribution of

the Chinese community as mentioned by local communities (9 %; only from the Lama forest reserve, the main forest island from southern Benin) may imply that intermediates collect pangolins from more proximal sources than the TMMs, but this will require further investigations.

4.5. Conclusion

Our study addressed the ethnozoology of pangolins across Benin and its major ethnic groups, and revealed the importance of the traditional (medicinal and spiritual) and economic values of pangolins for local communities and TMM vendors. Our results suggest that the pangolin trade in Benin is based on endogenous practices now influenced by economic drivers (higher prices and change of selling practices) from the local Chinese demand, and that a number of actors are involved in an intricate, multi-scale network. Conserving pangolins in Benin will require considering the multiple, cultural and economic drivers of the market and engaging synergic efforts against both endemic and international trafficking. Long term monitoring of offtake and trafficking network in Beninese markets and targeted habitats, together with higher law enforcement, will be essential to reverse the decline of the white-bellied pangolin as witnessed by Beninese rural communities (Zanvo et al. 2020).

Chapter 5

Can DNA help trace the local trade of pangolins? Conservation genetics of white-bellied pangolins from the Dahomey Gap (West Africa)

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CHAPTER 5

Can DNA help trace the local trade of pangolins? Conservation genetics of white-bellied pangolins from the Dahomey Gap (West Africa)

Abstract

African pangolins are currently experiencing unprecedented levels of harvesting, feeding both local demands and the illegal international trade. So far, the lack of knowledge on the population genetics of African pangolins has hampered any attempts at assessing their demographic status and tracing their trade at the local scale. We conducted a pioneer study on the genetic tracing of the African pangolin trade in the Dahomey Gap (DG). We sequenced and genotyped 189 white-bellied pangolins from 18 forests and 12 wildlife markets using one mitochondrial fragment and 20 microsatellite loci. Tree-based assignment procedure showed that the pangolin trade is endemic to the DG region, as it was strictly fed by the the Dahomey Gap lineage (DGL). DGL populations were characterized by low levels of genetic diversity, an overall absence of equilibrium, inbreeding depression and lack of geographic structure. We identified a 92-98% decline in DGL effective population size 200-500 ya –concomitant with major political transformations along the ‘Slave Coast’– leading to contemporaneous estimates being inferior to minimum

viable population size (< 500). Genetic tracing suggested that wildlife markets from the DG sourced pangolins through the entire DGL range. Our loci provided the necessary power to distinguish among all the genotyped pangolins, tracing the dispatch of same individuals on the markets and within local communities. We developed an approach combining rarefaction analysis of private allele frequencies with cross-validation of observed data that traced five traded pangolins to their forest origin, c. 200-300 km away from the markets. Although the genetic toolkit that we designed from traditional markers can prove helpful to trace the illegal trade in pangolins, our tracing ability was limited by the lack of population structure within the DGL. Given the deleterious combination of genetic, demographic and trade-related factors affecting DGL populations, the conservation status of white-bellied pangolins in the DG should be urgently re-evaluated.

Keywords: Microsatellites, conservation genetics, demographic decline, trade tracing, white-bellied pangolin, Dahomey Gap

5.1. Introduction

Pangolins (Order Pholidota) are considered the most trafficked wild mammals in the world, with c. 900,000 individuals seized over the last 20 years (Challender et al. 2020). Although pangolins have been –mistakenly– highlighted as potential intermediary hosts of the COVID-19 pandemic (Frutos et al. 2020), the volumes traded have remained unsustainably high (Aditya et al. 2021). As the exponential demand from the Traditional Chinese Medicine (TCM) market has reduced the populations of Asian pangolins, new trafficking routes have emerged from Africa (Ingram et al. 2019). Between 2015 and 2019, an estimated > 400,000 African pangolins were seized en route to Asian markets (Pietersen et al. 2019; Challender et al. 2020). Consequently, African

pangolins are currently experiencing unprecedented levels of harvesting, for both local demands and the illegal international trade, with a possible influence of the Chinese diaspora on the African trade networks and dynamics (Mambeya et al. 2018; Ingram et al. 2019; Zanzo et al. 2021b).

The white-bellied pangolin (WBP; *Phataginus tricuspis*) is the species with the largest range in tropical Africa and is the pangolin most frequently sold in the African bushmeat markets (Pietersen et al. 2019). It is also the most highly represented African species in international seizures of pangolin scales (Ingram et al. 2019; Zhang et al. 2020). Recent genetic investigations have shown that WBP consisted of six cryptic, geographically traceable lineages (Gaubert et al. 2016), one of which occurs ‘outside’ the rainforest blocks, in a West African savannah corridor interspersed with highly fragmented forest cover, the Dahomey Gap (Salzmann & Hoelzmann 2005). The Dahomey Gap lineage (DGL) is of particular importance as it is endemic to a unique biogeographical zone in western Africa (from Togo to Benin and southwestern Nigeria; (Gaubert et al. 2016)) and likely is the only pangolin species surviving in the Dahomey Gap (Zanzo et al. 2020; Segniabeto et al. 2021).

DGL populations currently suffer from intense levels of deforestation and hunting. Populations are fragmented into –generally small– patches of forest islands, and have drastically decreased in abundance through the last decades (Zanzo et al. 2020; Segniabeto et al. 2021). In Togo and Benin, WBP is hunted for its meat and use in traditional medicine, both contributing to its overexploitation (Zanzo et al. 2020; Segniabeto et al. 2021). With recent seizures of scales intended for the international trade (Cotonou airport; (Ingram et al. 2019)), a prominent proportion of DGL in Asian seizures (Zhang et al. 2020), and the established trafficking hub in neighbouring Nigeria (Omifolaji et al. 2020), there is a serious risk that DGL populations are exploited at unsustainable levels.

The genetic toolkit has been successfully applied to trace the illegal wildlife trade, by providing accurate information on the species traded and their geographic origins (Lorenzini et al. 2020; Lorenzini & Garofalo 2021). Recently, the genetic distinctiveness among the different WBP lineages has been used to assign the regional origins of international seizures of pangolin scales (Zhang et al. 2020). However, the lack of knowledge on the population genetics of WBP has hampered any attempts at assessing their demographic status and tracing their local scale –e.g., country-scale– trade (see Heighton & Gaubert 2021). The stakes behind tracing the local WBP trade are high, as they reside in identifying the source populations feeding the market (and thus the market network), but also estimating the number of individuals traded (e.g., from scales on stalls and in seizures), and, therefore, better informing conservation actions to mitigate this trade. We propose a pioneering investigation on the utility of the genetic toolkit applied to the conservation genetics of WBP, based on recently developed microsatellite markers (Aguillon et al. 2020) in combination with a mitochondrial marker used to delineate among WBP lineages (Gaubert et al. 2016). Our general objective is to provide a detailed overview of the genetic status of DGL populations and their traceability on the local pangolin trade. Our specific objectives encompass the assessment of (i) population structure and diversity within the DGL, (ii) the demographic history of this endemic lineage, and (iii) the resolutive power of our genetic markers for tracing the pangolin trade in the Dahomey Gap.

5.2. Material and methods

5.2.1. Genetic sampling and wet laboratory procedures

We collected a total of 189 WBP samples across Benin (6°10' - 11°00' N), Togo (8°10' - 9°0') and southwestern Nigeria (6°10' - 11°00' N). Our sampling

effort included all of the species occurrence zones in Benin (Zanvo et al. 2020), five geographically proximate forest in Togo (111 samples from 18 forests), nine major traditional medicine markets (TMMs; 71) from Benin and Togo, and three bushmeat markets in Benin and Nigeria (7). Samples from forests (reference samples) and wildlife markets (individuals to be traced) were collected simultaneously from April 2018 to February 2020. For the reference samples, collection was done with local hunters from the villages surrounding the occurrence habitats of pangolins using a snowball technique (Zanvo et al. 2020). Sample types varied from fresh tissue, skin and tongue (59), to dried skin and tissue (60), and scale connective tissue (70) (see Table S1, Appendix 3). Thirty-six samples were taken from carcasses having received preservative chemical treatments (Zanvo et al. 2021b). Free consent from local hunters and market sellers was obtained before collecting samples. We relied on an opportunistic sampling strategy (Olayemi et al. 2011), without financial incentives. The samples collected from the forest were traced to their original location after information was provided by hunters (Zanvo et al. 2020b).

DNA extraction from fresh and scale connective tissues was performed using the NucleoSpin® Tissue Kit (Macherey-Nagel, Hoerd, France), following manufacturer's recommendations. The final elution step was repeated twice in 50 µl Elution buffer to increase DNA yield. The samples treated with chemicals were extracted following a modified CTAB protocol including upstream TE washing baths and Dithiothreitol (DTT; (Gaubert & Zenatello 2009)). Elution volumes varied from 30 to 100 µl nuclease free water, depending on the size of the DNA pellet. DNA concentrations were estimated on the NanoDrop 1000 Spectrophotometer (ThermoFisher Scientific, Illkirch-Graffenstaden, France).

We amplified a mitochondrial fragment of 432 bp from Control Region 1 (CR1) following Gaubert et al. (Gaubert et al. 2016). PCR products were sequenced at Genoscreen (<https://www.genoscreen.fr/en>; Lille, France) and Macrogen Europe (<https://dna.macrogen-europe.com/en>; Amsterdam, the Netherlands). Sequences were aligned manually with BioEdit v7.0.5 (Hall 1999) and the unique haplotypes were submitted to Genbank under accession numbers OK275650- OK275662.

We amplified 20 microsatellite markers developed from the genome of WBP in four multiplexes following Aguilon et al. (2020). PCR triplicates were conducted for dried and chemically treated samples to mitigate the potential issue of allelic dropout and false alleles (Taberlet et al. 1996). In our case, a consensus was considered met when at least two out of the three replicates indicated the presence of an allele. PCR products were separated on an automated sequencer at Genoscreen and GeT-PlaGe (<https://get.genotoul.fr/>; INRAE, Toulouse, France).

5.2.2. Data analysis – Control region 1

Clustering

We assessed the ‘endemicity’ of the pangolins from Togo, Benin and Nigeria (N = 168) through a distance-tree analysis including all the CR1 sequences of WBP available in Genbank (N = 100). All the sequences were aligned manually with BioEdit v7.0.5 (Hall 1999). Phylogenetic tree reconstruction was performed in MEGA-X v10.2.2 (Kumar et al. 2018) using Neighbor Joining, 1,000 bootstrap replicates, Kimura 2-parameter model (Kimura 1980) and Gamma distribution (G). A pangolin was considered endemic to the DGL if its sequence clustered within the Dahomey Gap lineage as defined by (Gaubert et al. 2016).

Genetic diversity and structure

Genetic diversity and structure in DGL were estimated from sequences without missing data (N = 126). We used DnaSP v6.12 (Rozas et al. 2017) to compute haplotype number (h), haplotype diversity (Hd) and nucleotide diversity (π) (Table S2, Appendix 3) for the six WBP lineages. We mapped the distribution of haplotypes in DGL using ArcGIS 10.1 (Esri France). We used Network v10.2.0.0 to build a median-joining haplotype network with $\epsilon = 0$ to minimize alternative median networks.

Demographic history

Mismatch analysis was performed in Arlequin 3.5.2 (Excoffier & Lischer 2015) to test for signatures of demographic and spatial expansion in DGL, by calculating the sum of squared deviations (SSD) between observed and expected distributions using 1,000 bootstrap replicates (Schneider & Excoffier 1999).

We also tested for deviation from neutrality by computing a series of statistics in DnaSP, including Tajima's D (Tajima 1989), Fu's F_s (Fu 1997), Harpending raggedness index r (Harpending 1994) and Ramos-Onsins and Rozas' R_2 (Ramos-Onsins & Rozas 2002). We ran 1,000 replicates assuming a coalescent process with a neutral, infinite-sites model and large constant population sizes (Hudson 1990), to calculate the P-value of each observed statistics.

5.2.3. Data analysis – Microsatellites

Genetic diversity

Genious 9.0.5 (Kearse et al. 2012) was used for allele scoring and genotype extraction through the Microsatellites plugin (<https://www.geneious.com/features/microsatellite-genotyping/>). Only the DGL individuals with at least 75% of genotyping success were considered for the analyses (N = 169). This arbitrary threshold coincides with ≥ 15

microsatellite markers, tripling the minimum number of loci needed to discriminate among individuals in the white-bellied pangolin lineage from Western Central Africa (Aguillon et al. 2020). Genetic diversity at each locus was characterized through (i) number of alleles (N_a), observed (H_o) and expected (H_e) heterozygosity as computed from GenAlEx 6.5 (Peakall & Smouse 2012), (ii) allelic richness (A_R) and F_{IS} as estimated from FSTAT 2.9.4 (Goudet 2003). Deviation from Hardy-Weinberg Equilibrium (HWE) was calculated for each locus in GenAlEx. Linkage Disequilibrium (LD) for all the pairs of loci was tested in FSTAT using 1000 randomisations with a Bonferroni correction. Null allele detection, assuming population at equilibrium, was done with Microchecker 2.2.3 (Oosterhout et al. 2004) using a Bonferroni correction.

Genetic structure

Global genetic variance within the DGL was visualized through a Principal Coordinates Analysis (PCoA), using pairwise population matrix unbiased genetic distances in GenAlEx.

Pairwise differentiation (F_{ST}) among forest populations ($N = 104$) was computed in Arlequin 3.5 (Excoffier & Lischer 2010) using three partition schemes (Fig. S3, Appendix 3): (i) a 6-partition scheme considering populations (i.e., from the same habitat patch) with ≥ 7 sampled individuals; (ii) a 3-partition scheme including a southern forest block radiating from the Lama forest (c. 50 km maximal radius), a central forest block radiating from the Mont Kouffé forest (c. 45 km maximal radius), and a central forest block in Togo radiating from the Assoukoko forest (c. 25 km maximal radius); and (iii) a 3-partition scheme using a latitudinal-based grouping in Benin (South, lower Centre, and upper Centre). Because the first partition scheme yielded the highest levels of differentiation among populations (see Results), we calculated their inbreeding coefficients (F_{IS}) in FSTAT.

We used STRUCTURE 2.3.4 (Pritchard et al. 2010) to conduct a clustering analysis on all the DGL individuals. We performed 20 independent runs for $K = 1-10$ using 10^5 Markov chain Monte Carlo (MCMC) iterations and burnin = 10^4 , assuming admixture model and correlated allele frequencies. STRUCTURE HARVESTER 0.6.94 (Earl & vonHoldt 2012) was used to detect the most likely number of populations (K) using the ΔK method (Evanno et al. 2005). We also ran STRUCTURE using the LOCPRIOR model (and same other parameters) to assess the amount of information carried by the geographic distribution of populations (r). For r values > 1 , the geographic information is considered uninformative (Pritchard et al. 2010).

We also inferred the number of populations, spatial locations of genetic discontinuities and population membership among georeferenced individuals using the *Geneland* package (Guillot et al. 2005) in R 4.0.5 (*R Team Development Core 2021*). Following Coulon et al. (2006), we first allowed K to vary from 1 to 10 and launched five runs of $5 \cdot 10^5$ MCMC iterations (500 thinning and 500 burn-in) under a frequency-correlated model and 1 km of uncertainty for spatial coordinates. Second, we fixed the number of estimated populations on the basis of the first analysis ($K = 6-7$), to perform 20 independent runs using the same parameters. We also performed 20 independent runs fixing $K = 3$ as obtained with from STRUCTURE (see Results). For both analyses, we assessed how stable the population assignment was for individuals among the best (i.e. with highest posterior probabilities) five runs. We used 500×500 pixels to map the posterior probabilities of population assignment.

Given the absence of any clear genetic structuring from the above analyses (see Results), we performed spatial Principal Component Analysis (sPCA) using the Delaunay triangulation connection network, which defines neighbouring

entities based on pairwise geographic distances. The sPCA is a spatially explicit multivariate approach capable of investigating complex and cryptic spatial patterns of genetic variability from allelic frequencies (Jombart 2008). Such an approach does not require data to meet Hardy–Weinberg or linkage equilibrium among loci. The sPCA was run with the DGL georeferenced individuals using the *adegenet* package (Jombart 2008) in R, with 9,999 MCMC resampling to infer global *vs.* local structuration levels. Threshold distance between any two neighbors was set to zero.

We tested isolation-by-distance (IBD) among (i) forest individuals and (ii) populations (6-partition scheme; see above) by running a Mantel test in the R package *pegas* (Paradis 2010), where we quantified the correlation (r) between genetic (Edward's) and geographic (Euclidean) distances through 10,000 permutations. The geographic distances among populations were calculated from the center of each forest.

Ability of the microsatellite data to trace the pangolin trade

The discriminating power of our microsatellite markers among market and non-market individuals was evaluated by: (i) counting the number of identical genotypes among samples with the Multilocus tagging option in GenALEx (suboption Matches), (ii) computing the probability of encountering the same genotype more than once by chance using the R package *poppr* (method=single; (Kamvar et al. 2014)), and (iii) calculating values of unbiased probability of identity and probability of identity among siblings (uPI and PIsibs) in Gimlet 1.3.3 (Valière 2002).

We used the generalized rarefaction approach implemented in ADZE (Szpiech et al. 2008) to compute private allele frequencies among various combinations

of populations. We delineated six original forest populations after the partition scheme (i), and let population number vary from 2 to 6 (sample size rarefied from 2 to 7). Because the original scheme of six populations yielded the greatest frequencies of inferred private alleles (see Results), we graphed the rarefaction curves of each locus for the maximum sample size in these six populations to assess their respective contributions in the identification of private alleles per population. These six original forest populations represent the reference populations that we will use to assign the individuals sampled from wildlife markets. Loci that reached a plateau or showed an exponential trend in their estimated private allele frequencies (selection thresholds were fixed to ≥ 50 and $\geq 45\%$ of private allele frequency, respectively) were considered as potentially useful for tracing the origin of pangolins found in the markets, whereas loci showing a decreasing trend were discarded. We then crossed these results with the private alleles actually observed for the six populations (GenAIEx), and only considered the loci that showed both observed private alleles (GenAIEx output) and a high potential for tracing (ADZE output). Finally, we manually screened the genotypes of the market individuals to retrieve said private alleles and attribute them to source populations.

Demographic history

We tested for bottleneck events in the DGL using the Single Mutation Model (SMM) and the Two Phase Model (TPM) in BOTTLENECK 1.2.02 (Piry et al. 1999). We applied the Wilcoxon sign-rank test to analyze the presence of heterozygote excess/deficit using 10,000 replications.

Demographic history was also assessed through the R package *varEff* (Nikolic & Chevalet 2014), an approximate-likelihood method that infers temporal changes in effective population size. Given the lack of data on sexual maturity

in WBP, we fixed a conservative generation time of 2 yrs based on estimates from Asian species (Zhang et al. 2015a, 2016). Mutation rate was fixed to 5.10^{-4} based on published average mutation rate (Schlötterer 2000). The analysis was run with the single mutation, geometric mutation and two phases mutation models, using 10,000 MCMC batches with a length of one thinned every 100 batches and JMAX = 3. The first 10,000 batches were discarded as part of the burn-in period. Confidence intervals for ancestral and current effective population size estimates were calculated from the harmonic means for each mutation model.

5.3. Results

5.3.1. Genetic diversity and structure of the white-bellied pangolin in the Dahomey Gap

Mitochondrial DNA

Our ML tree based on 268 mitochondrial DNA (mtDNA) sequences recovered the six WBP geographic lineages with robust nodal support, including Western Africa, Ghana, Dahomey Gap, western Central Africa, Gabon and Central Africa (Fig. 1). All the sequences produced from Togo, Benin and southwestern Nigeria clustered into the Dahomey Gap lineage (bootstrap support = 75%).

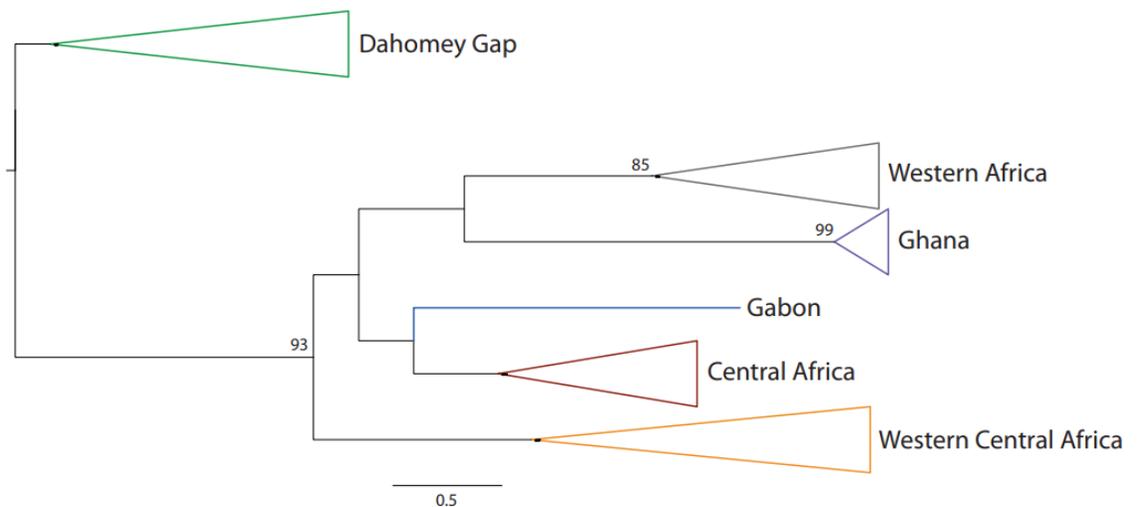


Figure 1. Neighbor-joining tree of white-bellied pangolins based on 268 control region sequences, showing the six main geographic lineages (following Gaubert et al. 2016) collapsed. Bootstrap supports are given at nodes. All the individuals from the Dahomey Gap belong to the Dahomey Gap lineage (see Fig S8, Appendix 3, for the expanded tree)

network did not show any specific geographic structure in haplotype distribution (Fig S1, Appendix 3). Two haplotypes were dominant in the DGL, with H5 (45%) being widely distributed and H1 (25%) located in the central and northern parts of the range (Fig. 2). Nine haplotypes were found in both forests and wildlife markets, while four were only found in wildlife markets. The proportion of H5 (40 %) and H1 (27%) in wildlife markets was reflective of their frequencies observed in forest populations.

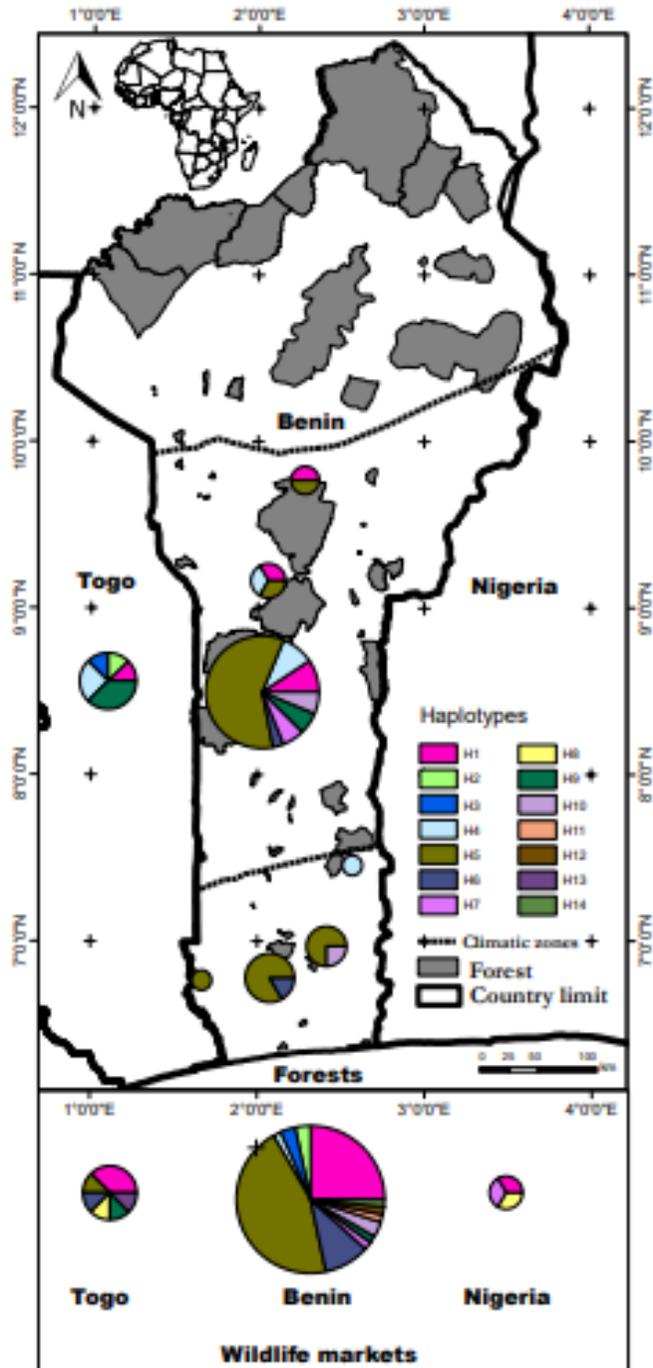


Figure 2. Distribution of control region haplotypes in white-bellied pangolins from the forests and wildlife markets of the Dahomey Gap. Haplotype numbers refer to Table S2 (Appendix 3). Top left shows the location of the study zone in Africa (in black)

Mismatch analysis of CR1 haplotypes showed a bimodal distribution significantly deviating from the sudden expansion demographic model ($P(\text{Sim. Rag.} \geq \text{Obs. Rag.}) = 0.005$), whereas the spatial expansion model could not be rejected ($P(\text{Sim. Rag.} \geq \text{Obs. Rag.}) = 0.15$). The statistics D (-0.56496), r (0.2057) and R^2 (0.0710) showed no significant deviation from a scenario of large and constant population size through time ($p > 0.10$), whereas F_s (-4.780, $p = 0.0431$) significantly rejected the model.

Microsatellites

Within the DGL, the number of alleles (N_a) varied from 2 to 11 (mean=5.3). Allelic richness (A_R) ranged from 1.86 to 8.04 (mean = 4.27; sample size = 169), and observed heterozygosity (H_o) and expected heterozygosity (H_e) from 0.072 to 0.775 (mean = 0.414) and 0.069 to 0.842 (mean = 0.498), respectively. Eleven loci deviated significantly from *HWE* ($P < 0.05$). Six of them showed significant levels of heterozygote deficiency ($P < 0.0025$) and three of them were involved in LD (PT_839522, PT_1453906 and PT_353755). Null alleles were identified in ten loci, including seven that deviated from *HWE* (Table 1).

Table 1. Genetic diversity estimates at the 20 microsatellite loci used in this study. N = number of genotyped individuals; Na = number of alleles; A_R = allelic richness; H_O = observed heterozygosity; H_E = expected heterozygosity; $P(HWE)$ = P -value for deviation from the null hypothesis of Hardy–Weinberg equilibrium; F_{IS} = inbreeding coefficient. P -values * <0.05 , *** <0.001

Locus	N	Na	A_R	H_O	H_E	$p(HWE)$	F_{IS}
PT_1162028	169	4	3.123	0.426	0.6	0.000***	0.292*
PT_1753627	162	11	7.993	0.642	0.689	0.019*	0.071
PT_796077	168	3	2.117	0.16	0.186	0.281	0.145
PT_1973508	169	5	4.339	0.562	0.48	0.028*	-0.167
PT_839522	168	7	6.168	0.524	0.788	0.000***	0.338*
PT_464918	168	8	7.025	0.56	0.766	0.000***	0.272*
PT_1453906	168	4	3.851	0.321	0.646	0.000***	0.505*
PT_34432	151	2	1.863	0.086	0.082	0.580	-0.042
PT_1594892	151	3	2.988	0.192	0.501	0.000***	0.619*
PT_308752	169	11	7.203	0.657	0.678	0.000***	0.034
PT_1669238	169	3	2.233	0.314	0.361	0.276	0.134
PT_1225378	167	3	1.903	0.072	0.069	0.972	-0.031
PT_739516	167	4	3.363	0.461	0.498	0.612	0.077
PT_619913	168	2	1.959	0.113	0.128	0.142	0.116
PT_338821	165	7	5.930	0.503	0.721	0.000***	0.305*
PT_18497228	168	4	3.848	0.595	0.6	0.611	0.01
PT_378852	168	2	1.969	0.113	0.138	0.020*	0.182
PT_353755	160	9	8.044	0.775	0.842	0.000*	0.083
PT_276641	166	5	4.168	0.699	0.696	0.535	-0.002
PT_2019332	169	8	5.319	0.503	0.498	0.309	-0.007

Genetic variance within the DGL individuals did not show any geographic structuring on the main PCoA axes (PC1 to PC3 = 12.36 % of total variance), whether individuals from wildlife markets were considered or not (Fig. 3).

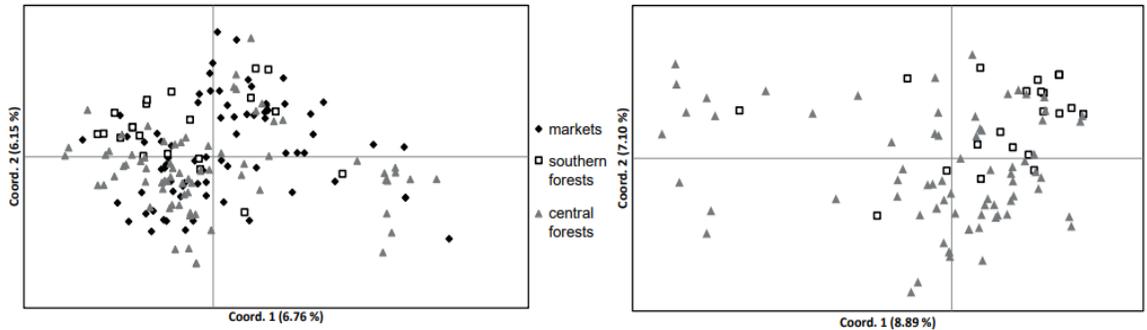


Figure 3. Distribution of genetic variance (PCoA) within white-bellied pangolins from the Dahomey Gap lineage. A- including forests and wildlife markets; B- excluding wildlife markets

Paiwise population differentiations using partition schemes (ii) and (iii) ranged from low ($F_{ST} = 0.0378$) to moderate ($F_{ST} = 0.116$; between central Togo and southern Benin). In partition scheme (i), differentiations among the six forest populations were the greatest, all significant ($p < 0.05$), and ranged from low/moderate ($F_{ST} = 0.0528$ - 0.1399 ; 67% of the F_{ST} values) to high ($0.166 < F_{ST} < 0.244$; 27% of the F_{ST} values) (Table S3, Appendix 3). The mean inbreeding coefficient (F_{IS}) in the six DGL populations was 0.172 and varied from 0.098 to 0.317 (Table S4, Appendix 3).

Bayesian clustering analysis with STRUCTURE –without prior information on locations– detected $K = 3$ most likely number of populations (Fig S2, Appendix 3). The three clusters did not correspond to exclusive geographic delineations, each being an admixture of individuals from southern and central forest regions together with wildlife markets. The assignment probabilities of

the individuals to their respective populations were generally low. Under the LOCPRIOR model, r was equal to 2.368.

Final inference of population number using *Geneland* was $K = 7$ (95% of the runs). The geographic delineation of the populations did not show any spatially exclusive distribution. Assignment probabilities to populations varied greatly among the best five runs (data not shown). Similar results were observed when fixing $K = 3$ (Fig S4, Appendix 3).

The eigenvalues observed from the spCA analysis (Fig. 4a) suggested a relatively strong signal of “local structure”, indicating negative autocorrelation between geographic and genetic distances in pangolins from the Dahomey Gap. However, we could not detect any significant global or local structure signal ($p = 0.602$ and 0.102 , respectively) across the study area.

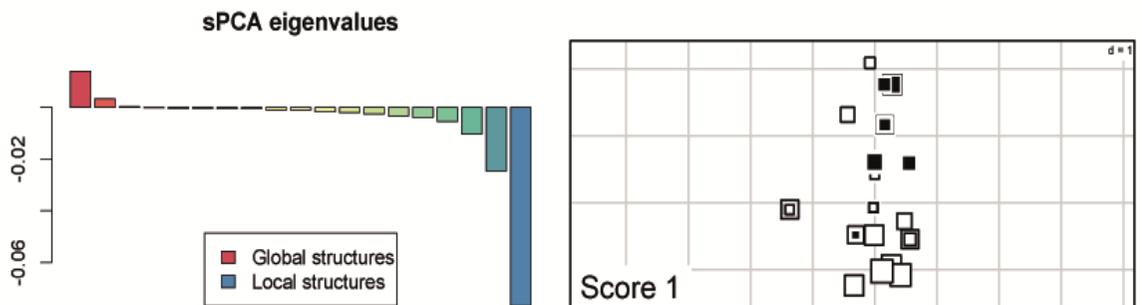


Figure 4. Spatial clustering in white-bellied pangolins from the Dahomey Gap obtained from spatial Principal Component Analysis (sPCA). Left: Positive and negative sPCA eigenvalues indicating global and local structures, respectively. Right: Map of the first global sPCA score among sampling localities. Large white and black squares stand for highly negative and positive scores respectively. Large white squares are genetically well differentiated from large black squares, while small squares are less differentiated

There was significant IBD effect among forest individuals ($r = 0.128$; $p = 0.001$) and between populations (forests with at least 7 individuals) ($r = 0.779$; $p = 0.009$) (Fig. 5).

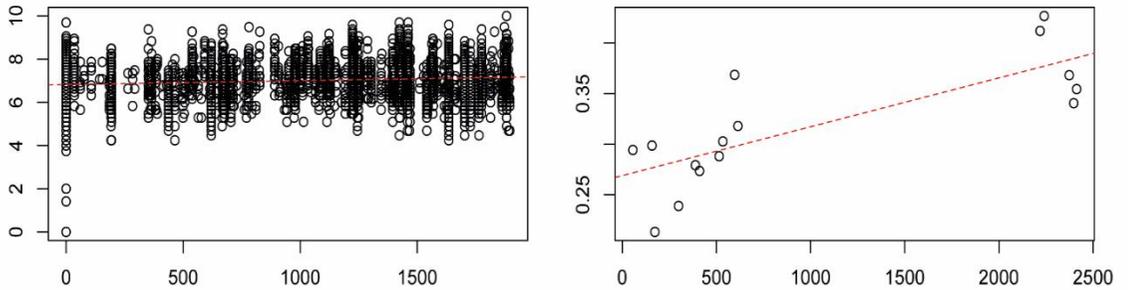


Figure 5. Isolation by distance among (left) individuals and (right) populations of white-bellied pangolins from the Dahomey Gap as inferred from 20 microsatellite loci. Dashed curve indicates linear regression

5.3.2. Demographic history of the white-bellied pangolin in the Dahomey Gap

The Bottleneck analysis on the DGL across 20 loci was not significant (Wilcoxon sign-rank test; $p > 0.05$) for both SMM and TPM models.

VarEff identified a pronounced and recent decline in the effective population size (N_e) of DGL regardless of the models (Fig. 6). Our results suggested a 92-98% reduction of N_e , from 1682-3440 (ancestral N_e) to 78-135 (contemporaneous N_e) individuals as harmonic means (95% CI: 263 to 487). The decrease in N_e was estimated to occur c. 200-500 years ago.

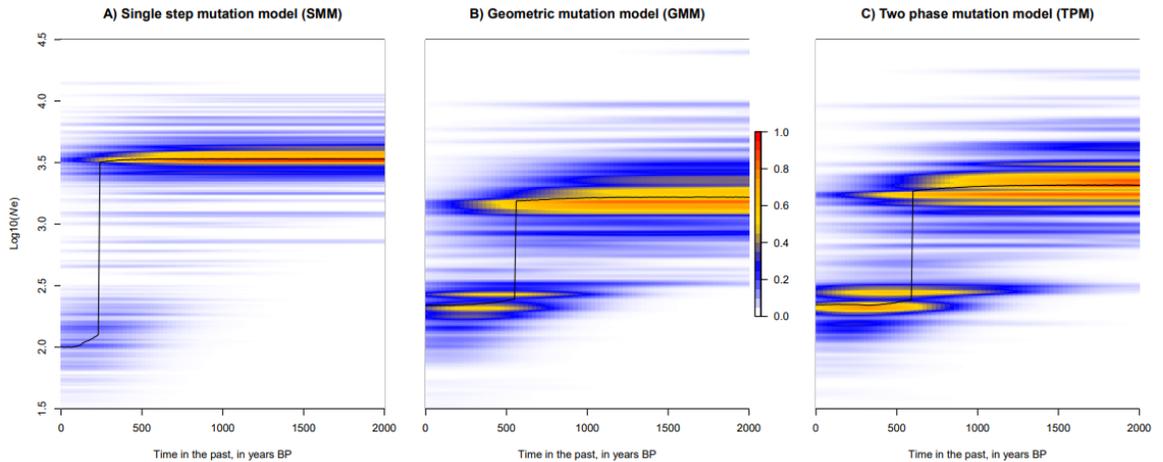


Figure 6. Temporal change in the effective population size of white-bellied pangolins in the Dahomey Gap, as estimated from *VarEff* under three different mutation models. Mode (black line) and kernel density (color scale) of effective population size (N_e) posterior distributions are given in years BP

5.3.3. Tracing of local trade: forest habitat-wildlife market

A total of 164 samples (97 %) had a unique genotype. Five samples shared a genotype with four other samples, including three pairs (A65-A66, B1-B12, B2-B15) and one triplet (D14-D15-D16; see Table S1, Appendix 3). The null hypothesis of encountering the different genotypes more than once by chance was rejected in all cases ($P < 0.0001$). The unbiased probability of identity (uPI) and the probability of identity among siblings (PIsibs) were both low (uPI = 8.12×10^{-13} ; PIsibs = 9.22×10^{-6}). At least seven microsatellite loci were needed to reach the conservative value of PIsibs < 0.01 (Fig S5, Appendix 3).

Estimated mean frequencies of private alleles across the 20 loci (sample size = 7) using ADZE ranged from 0.10 to 0.24. Within the 11 loci that presented appropriate private allele signatures for one or several populations (see Fig S6 and S7, Appendix 3), seven loci provided six observed private alleles in GenAlEx that could potentially differentiate among four populations. On this basis, five individuals found on wildlife markets could be traced back to their

forests of origin: A2 (Gbèdagba market, Central Benin) and A38 (Azovè market, southern Benin) to the Wari-Marò forest reserve, B50 (Azovè market, southern Benin) to the forests of central Togo, and D13 (Avogbannan market) and D26 (Dantokpa market) to the Ouémé supérieur forest reserve.

5.4. Discussion

The endemic lineage of white-bellied pangolins from the Dahomey Gap feeds an endemic trade

Our mtDNA tree-based assignment procedure indicated that all the white-bellied pangolins (WBP) collected and traded in the Dahomey Gap –from Togo, Benin and southwestern Nigeria– belong to the Dahomey Gap lineage (DGL; Gaubert et al. 2016). With 168 new samples sequenced from different forests and TMM, we have more accurately described the geographic delimitation of the DGL, from central Togo to northernmost and southernmost locations in Benin (Ouémé supérieur and Gnanhouizounmè, respectively), and Asejire in southwestern Nigeria. We have also confirmed the absence of range overlap with other WBP lineages, notably from neighboring Ghana. This last result is to be tempered by the fact that introgression between WBP lineages cannot be excluded –but has not been reported to date– and could have passed undetected for the few samples sequenced at a single locus (mtDNA) in our study. The endemic pattern of the DGL superimposes with DNA-based delineation recently found in mammals and plants from the Dahomey Gap (Colyn et al. 2010; Demenou et al. 2016), further emphasizing the patrimonial importance of the area for West African forest taxa.

Contrary to the bushmeat markets in Têgon (Benin), Hounkpogon (Benin) and Asejire (southwestern Nigeria) that are known to source the game from nearby forests (Olayemi et al. 2011), this study), the endemism of the pangolin trade was not expected for the traditional medicine markets (TMMs). This is because

the large geographic source from which the TMM network relies was shown in previous investigations from Benin and Nigeria, revealing the long-distance trade of non-native species (Saidu & Buij 2013; Djagoun et al. 2013). Moreover, there is a great demand for pangolins in the Dahomey Gap (D’Cruze et al. 2020), notably from the Chinese diaspora (Zanvo et al. 2021b), and the trade of pangolins across borders has been reported elsewhere in tropical Africa (Mambeya et al. 2018). The endemicity of the pangolin trade in the Dahomey Gap might translate into a huge hunting pressure on such a geographically restricted lineage. This is especially true as what we observed in the markets might not encompass the full scale of the pangolin trade, which may also feed the international market through alternative networks (Ingram et al. 2018; Mambeya et al. 2018; Zanvo et al. 2021b).

White-bellied pangolins in the Dahomey Gap show genetic diversity erosion and recent, sharp demographic decline

Overall, the DGL populations were characterized by low levels of genetic diversity. Mitochondrial (CR1) haplotype and nucleotide diversity was lower compared to all the other WBP lineages. Mean allelic richness based on microsatellites was also lower than what was found in WBP from Cameroon using the same markers ($A_R = 4.63$ vs. 6.74, respectively; minimum sample size = 37; see Aguillon et al. 2020). Compared to genetic diversity estimates based on ten WBP samples from Ghana, mean observed heterozygosity was again lower in the DGL ($H_o = 0.541$ vs. 0.414, respectively; see du Toit et al. 2020).

Low levels of genetic diversity are assumed to be negatively correlated with fitness and adaptability (Habel & Schmitt 2012). The overall absence of equilibrium detected from our microsatellite dataset suggests that inbreeding is one of the driving factors of low genetic diversity observed in the DGL (as

also observed from the mean inbreeding coefficient among the six populations). Although deviations from Hardy-Weinberg equilibrium (55% of the loci in this study) can be due to a number of factors including inbreeding, population structure and genotyping errors (Wigginton et al. 2005), we can reasonably discard the latter two given (i) the apparent lack of population structure in the DGL and (ii) the optimized loci and genotyping approach that we used. Besides, it is well known that populations going through inbreeding will produce upwardly biased estimates of null allele frequencies (Chybicki & Burczyk 2009), as observed in the DGL (50% of the loci). The deficit of heterozygotes observed in 75% of the loci, 30% of which have significant levels of deficiency, also supports the view that the DGL populations are subject to inbreeding depression, possibly indicative of non-random mating (Keller & Waller 2002).

Our demographic analyses based on microsatellites identified a sharp and recent decline in the effective population size (N_e) of the DGL c. 200-500 years ago (100-250 generations), leading to a 92-98 % reduction in the current N_e . This is below the conservative thresholds of minimum viable population size (500-5000; Reed et al. 2003; Clabby 2010). As variation in N_e is crucial to determining levels of genetic diversity (Charlesworth 2009), the state of genetic depauperation observed in the DGL may be directly linked to the recent demographic decline affecting the lineage. The time of decline corresponds to a period of major transformations along the 'Slave Coast', where from the 17th century the Dahomey kingdom expanded as a state bureaucracy benefiting from the growing trade of slaves and agricultural goods with Europeans (Law 1986; Monroe 2003). Whether such political growth was followed by agricultural expansion and deforestation causing the decline of pangolins in the region is uncertain, but similar declines have been observed in commercially exploited species of vertebrates through the last centuries (Monroe 2003;

Bishop et al. 2009; Stoffel et al. 2018). Because the area underwent drastic alternations of dry and humid periods since at least the last 150,000 yr (Dupont & Weinelt 1996), it is possible that the DGL populations were affected by early, successive founder effects and bottlenecks due to Late Pleistocene adverse climatic changes (Demenou et al. 2016). Such demographic events could also have shaped the genetic diversity and absence of population structure (see below) observed today in the DGL. Our results are important for the conservation of the DGL, because inbreeding depression together with high levels of genetic drift will potentially lead to the fixation of mildly deleterious alleles that could drive an extinction vortex in this lineage (Frankham 2005).

Earlier events such as the expansion of agriculture in West Africa c. 4,200 BP (Ozainne et al. 2014) and natural forest fragmentation caused by cyclical drier climatic conditions in the Dahomey Gap from 4,500 BP (Salzmann & Hoelzmann 2005) do not seem to have affected the demographic history of the DGL populations. This is due to mtDNA-based demographic analyses showing no deviation from a model of large, constant N_e through time, indicating long-term matrilineal stability. The only exception was the Fu's statistics, which has maximum power to detect sudden demographic decline events (Ramírez-Soriano et al. 2008) and thus can be related to the recent decline discussed above. However, further analyses based on nuclear genomic markers (SNPs) will have to be conducted to assess the ancient demographic history of the DGL, the origin of which dates back to 120-240 kya (Gaubert et al. 2016). The Dahomey Gap is a broad savannah corridor intermixed with forest patches that separates the two African rainforest blocks, and as such can be considered a sub-optimal habitat for WBP which heavily rely on rainforest cover (Gaubert et al. 2016).

The fragmented populations of white-bellied pangolins in the Dahomey Gap show no genetic structure

Our analyses generally suggested that there was no geographic structuring across the Dahomey Gap, against the expectation that habitat fragmentation leads to genetic isolation (Frankham et al. 2017). The same absence of structure was observed in the Chinese pangolin among four populations from mainland China (Ching-Min Sun et al. 2020). One potential explanation to the lack of population structure would involve long-range dispersals. Pangolins have been reported to disperse up to 300 km in four months, with a marked period of mobility for unestablished young individuals through –notably– anthropized areas (Sun et al. 2019; Ching-Min Sun et al. 2020) However, the dispersal ecology of pangolins remains poorly known, especially in WBP. The species seems to heavily rely on forest cover and old trees for its nocturnal activities (Pages 1968), exploring its home range up to 1.8 km per night (Pagès 1975). In the Dahomey Gap, WBP may occur in disturbed habitats including commercial plantations of teaks and palm trees, fallows and farmlands (Akpona et al. 2008). However, evidence of long-range dispersal is lacking and the general absence of structural connectivity among the remnant forest islands of the Dahomey Gap, especially in Benin see (see Mama et al. 2013; Alohou et al. 2017) does not support such a scenario.

Although we could not find any clear population structure in the DGL, we detected significant levels of differentiation and isolation-by-distance among both individuals and populations. We observed the strongest differentiations between the most distant Togolese and Beninese populations, and some cases of moderate differentiation between geographically close populations (e.g., the contiguous Ouémé supérieur and Wari-Marô protected areas). Genetic differentiation among populations is determined by the interplay between

homogenizing processes such as gene flow and differentiating processes including local adaptation, different adaptive responses to shared environmental conditions, and genetic drift (Whitlock 2011). If we posit that, in the case of the DGL, (i) gene flow between populations is not an option (see above), (ii) genetic drift in isolated, inbred populations should have resulted in detectable geographic structure (Lloyd et al. 2013), and (iii) environmental conditions are similar across the DGL range (Guineo-Congolian and Sudano-Guinean zones; White 1983), then different adaptive responses of populations to a similar environment could be a candidate scenario to explain the level of differentiation observed among populations from the Dahomey Gap. This could notably be expected in the case of populations trapped in sub-optimal environmental conditions and impacted by frequent climatic oscillations (see Gaubert et al. 2016). However, microsatellite markers generally reflect neutral genetic variation, and should not be affected by signatures of local adaptation (Holderegger et al. 2006).

A more plausible scenario would relate to a former, possibly recent, spatial expansion in subdivided populations across the DGL range, as mtDNA did not reject this model. It is possible that forest-restricted DGL populations underwent a spatial expansion following the last recent increase in rainforest cover during the last Interglacial or early Holocene periods (Salzmann & Hoelzmann 2005). Such event would explain the absence of population structure together with some level of population differentiation as observed in this study, provided that dispersal among populations would have been maintained long enough to counter-balance the effect of genetic drift in isolated populations later induced by drier climatic conditions, (Salzmann & Hoelzmann 2005) and anthropogenic pressure in the Dahomey Gap. Overall, our study has the merit to posit a number of hypotheses that could explain the puzzling pattern observed in the population structure of the DGL. Such

hypotheses will have to be tested through a demographic scenario-based strategy, preferentially using versatile and powerful genomic resources.

Tracing the pangolin trade in the Dahomey Gap: specimen dispatch and evidence for long-distance trade

The genetic diversity of WBP sold on the markets was reflective of the overall genetic diversity (haplotype and allelic frequencies) observed in DGL forest populations, suggesting a widespread sourcing of pangolins through the entire Dahomey Gap. Our 20 microsatellites loci provided the necessary power to confidently distinguish among all the DGL individuals, and only seven best microsatellite loci were needed to reach the conservative value of probability of identity < 0.01 (Waits et al. 2001). The probability that two individuals drawn at random from a population, including or not including siblings, will have the same genotype was low (but higher than in previous studies, Aguilon et al. 2020; Ching-Min Sun et al. 2020). This has important implications for the genetic tracing of the pangolin trade in the Dahomey Gap, as one of the main inputs of the genetic toolkit is its potential for tracing the trade at the individual level (K et al. 2017). For instance, our markers would be capable of estimating the exact number of individuals from scale seizures, a major challenge that conservationists are currently facing (Ullmann et al. 2019). We also demonstrated that our genotyping approach was useful in tracing the dispatch of a same pangolin sold on the market (one individual detected on two different stalls in Dantokpa) or kept by local communities (scales of two different individuals shared between villagers in Mont Kouffé and Lama forests). Such application is especially relevant for tracing the pangolin trade, which often forms into separate networks specialized in the selling of specific parts (scales, organs, meat), notably in Benin (Zanvo et al. 2021b).

Given the lack of population structure in the DGL and the negative autocorrelation between geographic and genetic distances (for individuals and populations), classical assignment procedures (Hansen et al. 2001; Ogden & Linacre 2015) are hardly usable to trace the pangolin trade in the Dahomey Gap. We have developed a conservative approach combining rarefaction analysis of private allele frequencies in each population with cross-validation from observed data that could partly circumvent that issue. Such method may be applicable to any taxon or lineage without observed genetic structure across its range, notably at the local scale. We identified seven private alleles (from seven loci) that could potentially differentiate among five DGL populations. Five pangolins were traced to their forest of origin using three private alleles, illustrating the long-distance trading routes that feed TMMs in the Dahomey Gap (see Zanvo et al. 2021b). For example, pangolins from the forests of central Togo, Wari-Marô and Ouémé Supérieur were found on the markets of Dantokpa, Gbèdagba, Azovè and Avogbannan, c. 200-300 km away from the source forests.

Despite a relatively fair number of loci (20) and an exhaustive sample set across Benin, we could only trace c. 8% of the WBP genotyped from TMMs. Such performance could be improved with additional geographic sampling, notably from Togo and southwestern Nigeria, and denser sampling of populations, although the ideal standards for reaching confident estimates of allele frequencies when applied to forensic use might be unreachable in our case (100-150 individuals per population; Chakraborty 1992). Future analyses based on bi-allelic markers such as SNPs will have to be considered as they significantly reduce the sample size required for reliable estimates of allele frequency distribution (B-Rao 2001).

5.5. Conclusion

Our study is the first to provide a comprehensive population genetic assessment of an African pangolin species/lineage, filling an important knowledge gap for the future conservation of pangolins in Africa (Heighton & Gaubert 2021). Overall, we showed that the DGL populations suffered from inbreeding, genetic diversity erosion and a drastic decline in effective population size. Given the multi-purpose trade that DGL populations are the target of (this study, Zanvo et al. 2021b), and the observed reduction of the DGL range during the last two decades (at least in Benin, Zanvo et al. 2020), the conservation status of WBP in the Dahomey Gap should be urgently re-assessed. Conservation measures are to be implemented before the species becomes locally extinct, as it may already be the case for the giant pangolin (Zanvo et al. 2020). Measures should include the reinforcement of national protection status, the creation of dedicated protected areas and forest corridors, campaigns of public awareness, and breeding programs.

Chapter 6

Assessing the precise dietary spectrum of the white-bellied pangolin in Benin through eDNA metabarcoding

This chapter will be submitted as:

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CHAPTER 6

Assessing the precise dietary spectrum of the white-bellied pangolin in Benin using DNA metabarcoding

Abstract

Accurate data on dietary profiles is a prerequisite to understanding how wild animals, especially cryptic and elusive species such as pangolins, make use of their habitats, in order to design effective conservation plans. Despite their conservation status, there exists limited data on the dietary intake of African pangolins, hampering effective conservation strategies and attempted managed feeding program. Using 23 rejected fecal samples and 29 gut content samples, this preliminary molecular analyses of the feeding ecology of white-bellied pangolin assesses the efficacy of a DNA metabarcoding approach to performing dietary analyses. This extends to the dietary profile and the variation in dietary preference of the focal species following a latitudinal gradient and across varied habitats. Our study demonstrated the potential of DNA metabarcoding in assessing the diet of the white-bellied pangolin when combined with the capture, amplification, sequencing and detection of prey species DNA barcodes within the fecal/stomach content samples. We observed a relatively wide prey spectrum for the white-bellied pangolin compared to that previously recorded through visual observation using radio-tracking. The detected prey species included *Camponotus acvapimensis*, *Camponotus conradti*, *Camponotus (Myrmotrema) carbo*, *Camponotus (Tanaemyrmex) maculatus*, *Cataulacus guineensis*, *Lepisiota capensis*, *Oecophylla longinoda*, *Pheidole megacephala*, *Platythyrea conradti*, *Polyrhachis militaris*, *Dorylus nigricans*, with eight other photo-based ant species (2 *Pheidole* sp. and 6 *Crematogaster* sp.) and eight termite species. *Pheidole* sp., *Camponotus* sp. and *Crematogaster* sp. were the genera with the most abundant reads. DNA metabarcoding analyses confirmed the prey

selective behaviour previously observed for pangolin species using traditional methods. The alpha diversity of detected prey items varied significantly ($p < 0.05$) following sample types, habitats and regions, with our limited data suggesting that individual sex, and a variation to sample pre-processing did not. Bray-Curtis dissimilarity index showed highly significant dietary profile dissimilarities ($p < 0.001$) between southern and central regions. Our study demonstrated that DNA metabarcoding is a promising tool for disentangling the diet intake of pangolins, providing substantial knowledge on their feeding ecology which can inform management decisions for the conservation of the species in its wild habitat. It supports the need for further investigations with a balanced number of sample types to clearly elucidate the dietary profile variation following the latitudinal gradient and ecosystems.

Keywords: DNA metabarcoding, fecal, gut content, white-bellied pangolin, dietary profile

6.1. Introduction

Feeding ecology provides fundamental information on the intricate interactions of species with their environment, and thus is an important aspect of wildlife ecology (Duffy et al. 2007). The feeding ecology of a species will affect its habitat use, functional role, population dynamics, mating system, and persistence in the environment (Corlett 2014). In the case of predatory species, accurate knowledge of prey spectrum is a prerequisite to understanding the ecological function of species relative to their prey dynamics and environmental/seasonal changes; such knowledge can in turn be applied to conservation planning (Corlett 2014).

The feeding ecology of elusive and rare species usually is poorly known, whereas acquiring such baseline biological data is important to support effective management and conservation strategies (Goldberg et al. 2020;

Kiszka et al. 2021). Pangolins are secretive, Afro-Asian scaled mammals with a specialized diet mostly restricted to ants and termites (myrmecophagy; Gaubert 2011). Because pangolins are solitary, nocturnal and mostly dependent on dense forest cover, precise data on their dietary spectrum have remained limited and consequently knowledge on their functional role in natural ecosystems (Willcox et al. 2019) (Chao et al. 2020). Conventional methods such as the direct observation of feeding activities and visual recognition of preys in gut or fecal contents had a limited reach when applied to pangolins, because ants and termites are hyper-diverse taxa (Chapman 2009) and digested insect remains are challenging to identify (Karawita et al. 2020).

Asian pangolins have so far received the greatest attention in terms of feeding ecology relative to the under-studied African species (Heighton & Gaubert 2021). Studies on the dietary composition in Chinese, Indian and Sunda pangolins (*Manis pentadactyla*, *M. crassicaudata* and *M. javanica*, respectively) suggested a myrmecophagous diet profile dominated by ant preys and including all life stages of ants and termites (eggs, larvae, imagoes and castes). All above species are prey selective and their diet composition varied by seasons (e.g. Irshad et al. 2015; Karawita et al. 2020 (e.g. Wu et al. 2005; Sun et al. 2017; Lee et al. 2017; Ashokkumar et al. 2017) Lim 2007). In Africa, only the dietary composition of the Temminck's pangolin (*Smutsia temminckii*) has properly been addressed, revealing high selectivity of preys within the myrmecophagous spectrum (Jacobsen et al. 1991; Swart et al. 1999; Pietersen et al. 2016). Despite pioneering investigations on the ecology of arboreal white-bellied pangolin and giant pangolin in Gabon (Pages 1968; Pagès 1975; Vincent 1964), no contemporary studies have yet explored the feeding ecology of these species.

Pangolins are the most heavily trafficked mammals in the world, with illegal trade networks now sourcing from Africa to feed the Asian demand for meat and pharmacopeia (Ingram et al. 2019). During the last decade (2010 – 2019), more than 400 000 African pangolins were involved in the illegal international trade (Challender et al. 2020), with the white-bellied pangolin (WBP) being the most targeted species (Mwale et al. 2017; Mambeya et al. 2018; Ingram et al. 2019; Challender et al. 2020; Zhang et al. 2020). In Benin (West Africa), populations of WBP are heavily impacted by local activities including poaching for meat and medicinal practices, deforestation and agriculture, resulting in c. 30% reduction of the species' occupancy area over the last 20 yrs, dramatic habitat fragmentation and high levels of inbreeding (Zanvo et al. 2020, 2021a, 2021b). As a consequence, WBP is in need of an urgent, scientifically-based conservation strategy.

Because we believe that accurate knowledge on the feeding ecology of WBP will contribute to best-informed conservation planning in Benin, we conducted an unprecedented study using environmental DNA (eDNA) metabarcoding from fecal and stomach content samples. Our general objective is to decipher the prey spectrum of the white-bellied pangolin using the resolution power of amplicon-based high-throughput sequencing. We determine the effectiveness of eDNA metabarcoding for the dietary investigation of white-bellied pangolin using a diversity of samples. Because we collected samples across a variety of habitats in southern and central Benin, we also assess the effect of habitats and sample types on the diversity of the prey spectrum characterizing WBP. Given the arborealism of WBP, we expect a high diversity and abundance of arboreal ants and termites in the species' dietary profile. We also posit that the alpha diversity of detected prey species will be lower in the fecal samples because of the digestion process through the gastrointestinal tract.

6.2. Material and methods

6.2.1. Fecal collection

Sample collection was done from December 2018 to May 2019 (28 samples) and November to October 2020 (24 samples) (Table 1). Samples consisted of either fecal collected directly from live animals or gut contents from freshly dead animals. Fecal samples collection entailed using a technique learnt from local people. “Live pangolins were raised up by their tail for two minutes until fecals were expelled as a result of stress”. We were able to collect 17, 5 and 1 fecals using this technique in LM, MK and Gna forests respectively. We equally collected through opportunistic encounters 24, 2 and 2 gut contents from individuals killed in the MK, Gna and OS respectively. Both sample types were collected with the free consent of local hunters. Further one gut content was collected from an individual killed by students at the university of Abomey-Calavi in Benin (April 2020) due to the statements that demonized pangolins in the COVID-19 pandemic. Both types of samples were fixed in 96% ethanol for 24 hours, and then dried and stored at room temperature.

Table 1. Characteristics of samples used for DNA metabarcoding

Forest habitats	Region	Gut content samples	Rejected fecal samples
Gnanhouizounmè	South	2	1
Lama	South	-	17
Mont Kouffé	Center	24	5
Ouémé supérieur	Center	2	-
UAC	South	1	-

6.2.2. Local reference database of potential prey

We decided to build a local reference database of potential preys for WBP because of the restricted availability of reference sequences for ants and termites in public databases (see (Meiklejohn et al. 2019)). We used the available literature to establish a tentative list of the species of ants and termites present in Benin. The most relevant literature was (i) Taylor et al. (2018), whom recorded a total of 133 species of Formicidae (ants) –representing 22 genera– from agricultural landscapes, and (ii) Attignon et al. (2004) and Lachat et al. (2006), whom recorded 19 termite species belonging to 13 genera and five families from LM. For these potential preys of WBP, only a few sequences (ants: 18 species in COX1 and two species in 16S; termites: 16 species in COX1 and nine species in 16S) were available in NCBI (<https://www.ncbi.nlm.nih.gov/>) and Bold (<http://www.boldsystems.org/>).

Sampling of potential preys was conducted between 28 March and 5 April 2020 in LM and MK, as guided by the preliminary results obtained from the shallow sequencing of the first batch of 28 fecal samples. A total of 23 and 16 quadrats of 5×2 m were installed in the MK and LM forests respectively following a modification of the original Jones and Eggleton (2000) protocol. The quadrats were distributed on both sides of three tracks (c. 69 km) crossing MK and one track (c. 39 km) surrounding the *Noyau central* of LM. Each quadrat was located at 50 meters from the nearest track. Within each quadrat, the searching of termites and ants from soil, ant and termite mounds, decayed wood, dead trees and dead branches of living trees were performed by two people for 30 min (Jones & Eggleton 2000). Three to seven individuals were sampled per morphospecies identified by the collector (SZ) and preserved in 96% ethanol. Given our preliminary results, we also extended the sampling of potential preys to other insects, including Gryllidae, Chrysomelidae, Apidae

and Fulgoridae. The morphological identification of preys was made at the International Institute of Tropical Agriculture in Benin (George Goergen; IITA-Bénin) and *Laboratoire écologie fonctionnelle et environnement* (Alain Dejean; EcoLab), Université Toulouse III- Paul Sabatier (France). Most ant species and bees, but not termites were (98 %, 87/89) was identified with consensus by both specialists.

6.2.3. Molecular and bioinformatic protocols

DNA extraction from fecal and gut content samples was performed in an eDNA-dedicated room isolated from the other lab rooms (ADN Trace-EDB). Two hundred grams per samples were processed with the QIAamp DNA Stool Mini Kit (Qiagen; Düren, Germany), generally following the manufacturer's recommendations. Given that we were not able to retrieve 600 µl of supernatant after adding 850 µl of buffer ST1 to some samples as indicated in the manufacturer's protocol, we hydrated the latter (eight samples) using 200 µl of nuclease free water before adding 850 µl of buffer ST1 and for other samples (24 samples) we added directly 1200 µl of buffer ST1. All above preprocessing steps were performed to optimized the extraction protocols in order to improve the DNA yields. A negative control was used for the DNA extraction. The DNA extracts were stored at -20°C before PCR-processing. The prey samples were processed in order to avoid co-extracting host and preys (notably in ants) by using appendices such as legs or antennas, and avoiding head, thorax or abdomen. DNA extraction was performed using a Chelex-based protocol (Casquet et al. (2012)). The DNA extracts were stored at -20 °C for five days before PCR-processing. We amplified a 150-bp 16S minibarcode fragment (5' TRRGACGAGAAGACCCTATA 3', 5' ACGCTGTTATCCCTAAGGTA 3') after Taberlet et al. (2018). These primers are specific to insect taxa and able to amplify degenerated DNA

fragments (Taberlet et al. 2018). Three PCR replicates were amplified per eDNA sample. Three insect mock community and three negative PCR controls were included in each of the two amplification plates (Table S1, Appendix 4). Prey samples were amplified separately, only once. DNA amplification for all types of samples was carried out in 20 µl volume reaction containing 16 µl of mix [5.84 µl UHQ water, 10 µl buffer (×2) and 0.16 µl BSA], 2 µl of tagged primers and 2 µl of DNA. The following thermal cycling conditions were used: initial denaturation at 95° C for 10 min, 40 cycles of denaturation at 95°C for 30 s, hybridization at 49° C for 30 s, and elongation at 72° C for 30 s, and final extension step at 72° C for 7 min. PCR products were visualized on 1.5 % agarose gel.

PCR products were pooled using 10 µl of each PCR and negative control, and 2 µl of positive control. A library of PCR pool was performed using 40 % of our PCR yields (39 % of fecal samples and 1 % of potential preys) together with other PCR yields belonging to other taxa on the same plates. These proportions were guided by our preliminary analysis on the 28 fecals crossed with the number of reads previously generated with Mi-Seq sequencing for each of target taxonomic group in the literature. With these proportions we were confident to catch up the maximum diversity within the different type of samples. This library allowed us to reach c. 5 752 261 16S eDNA sequences and 777 182 16S prey sequences for a Mi-Seq run.

The pre-processing of sequence reads obtained from the two libraries (16S eDNA and 16S prey) was carried out using OBITool v1.2.11 (Boyer et al. 2016) on the GenoToul bioinformatics platform (INRAE-Toulouse, France). Forward and reverse paired-end reads were assembled based on their overlapping 3'-end sequences using illumina paired-end utility, and then were de-multiplexed into their corresponding sample and de-replicated into unique sequences using the command obiuniq. Data curation was performed in order

to remove sequences with low paired-end alignment scores, unique sequences, short sequences (<140 bp), sequences containing ambiguous bases and PCR errors (alignment score below 50) using the *obiclean* command (Table S3). We built Operational Taxonomic Units (OTUs) using the *sumacrust* clustering algorithm (Mercier et al. 2013) with 97% similarity threshold. We removed cross-sample contaminations and reagent contaminants by referring to negative and empty controls as distributed on the plate plan, and dysfunctional PCRs (PCR corresponding to samples with > 10% of their reads corresponding to contaminants) following Zinger et al. (2019) using the *metabaR* package (Zinger et al. 2021) in R version 4.1.1 (R Development Core Team 2021). For 16S prey after demultiplexing and dereplication, the majority sequence for each sample was selected using *obiselect* function. We performed a blast in NCBI and phylogeny tree with MEGA-X using the selected majority sequences in order to cleaned the reference database (remove non-insect taxa and remote taxa clustered on the same branch of the tree).

Taxonomy was assigned to each OTU using a global database derived from EMBL7 196 (<https://www.ebi.ac.uk/ena/browser/home>; release 141) OBITOOL's *ecoTag* program (Boyer et al. 2016). After data curation, we discarded the non-targeted taxa (other than insects) and aggregated the PCR replicates for each sample. A complementary taxonomic assignment was achieved through the local blast of our cleaned reference database, using *makeblastdb* of module *bioinfo/ncbi_cxx--18_0_0*. The taxonomic assignments obtained from EMBL and local blast were combined: prey identification at species level were retained when their best hit identities were higher than 80 and 95 % for EMBL and local reference databases respectively, with a correspondence of order and family names for two databases.

6.2.4. Statistical analysis

The total number of reads (sequences) for each OTU within a given sample was normalized for the purpose of comparative analysis, by standardizing with the largest proportion of reads observed across all the samples for each OTU. We measured the diversity of OTUs within samples using the Shannon index, as the latter is considered the most appropriate index to assess alpha diversity in eDNA metabarcoding data (Calderón- Sanou et al. 2020). We compared diversity index values between pre-processed samples [hydrated ($y=22$), non-hydrated ($No=28$) and additional quantity of buffer ST1 ($No^+=24$)], genders, forests (Gna, LM, MK and OS), geographic region (South vs. Centre) and sample type (gut content vs. fecal samples). Analysis of variance (ANOVA) was used to assess the variables that explain significantly the measured diversity; results were plotted with *ggplot2* in R. We used the Bray-Curtis dissimilarity index to test differences among prey spectra in WBP. The dissimilarity between regions was computed from Hellinger-transformed community data to downweigh the effects of rarely occurring OTUs (Legendre & Legendre, 2012). Spatial visualization of variation in dietary preference were performed using Principal Coordinate Analysis with the *vegan* package in R.

6.3. Results

6.3.1. Diet composition

Assembling forward and reverse reads generated 5,752,261 sequences including 326,855 unique sequences. The clustering of the remaining 3,775,916 sequences after filtering revealed 1780 Operational Taxonomic Units (OTUs). Applying the 80% best hit identity threshold with the EMBL database, a final number of 1032 OTUs (58%) were retained. OTUs were assigned to 18 families comprising mainly Thripidae (49% of the filtered sequences) [Thysanoptera],

Formicidae (9%) [Hymenoptera] and Termitidae (<1%) [Blattodea], which represented <1%, 31% and 2% of the OTUs, respectively (Fig. 1a). *Crematogaster* (28%) was the most represented genus within ants, followed by *Pheidole* (20%) and *Camponotus* (7%). Sample-based OTUs accumulation curves reached asymptotes after different indices and estimates (Appendix 4, Fig S1), indicating sufficient sampling and sequencing efforts to achieve representative diversity of the prey spectrum of WBP.

Blast on our local reference database using the 95% best hit identity threshold allowed us to obtain more accurate taxonomic (species-level) identification for 583 OTUs. We detected four sub-families of Formicidae (Myrmicinae, Formicinae, Ponerinae and Dorylinae), Termitidae and Apidae [Hymenoptera] for 13 % of the total sequences (Fig. 1b). Myrmicinae, Termitidae and Formicinae represented 53, 14 and 11% of the total number of OTUs, respectively. Formicinae, Myrmicinae, Ponerinae and Termitidae were detected within all the samples, whereas Dorylinae were only detected in LM and MK and Apidae in MK. All the OTUs represented <1% of the total sequences, except in MK where Formicinae and Myrmicinae represented 1 and 11% of the total number of OTUs, respectively (Fig. 1b). Higher proportions of prey reads were observed in gut content samples (Fig. 1). *Camponotus acvapimensis*, *Camponotus conradti*, *Camponotus (Myrmotrema) carbo*, *Camponotus (Tanaemyrmex) maculatus*, *Cataulacus guineensis*, *Lepisiota capensis*, *Oecophylla longinoda*, *Pheidole megacephala*, *Platythyrea conradti*, *Polyrhachis militaris* and other eight morphospecies (*two Pheidole sp.* and six *Crematogaster sp.*) were detected at high taxonomic resolution ($\geq 97\%$ best hit identity) using EMBL and local reference databases respectively (Appendix 4; Table S2; Appendix 4). *Dorylus nigricans* was detected for 96 % of hit identities. Overall, eight morphospecies of termites species were equally detected for at least 97% hit identities (Appendix 4).

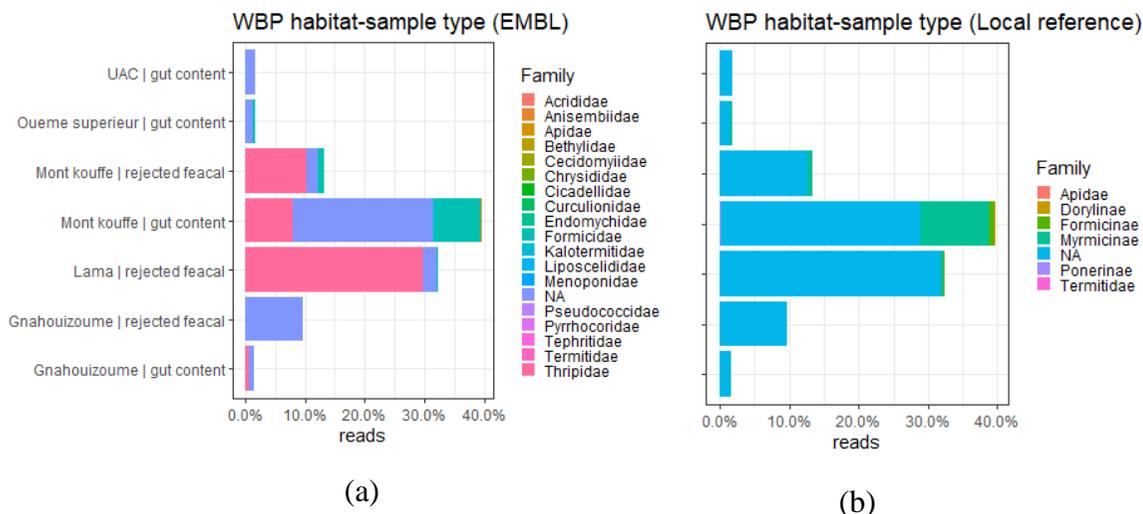


Figure 1. Diversity of preys detected using public (EMBL) and local reference databases among the different forests. (a) Proportions of reads assigned per family using EMBL; (b) proportions of reads assigned per family using the local reference database. NA=50%

6.3.2. Prey items detection following the pre-processing, sex, sample type, forest habitat and region

Sample-based species accumulation curves reached asymptotes following the richness, Shannon and Simpson diversity indices, and OTUS coverage (Fig S1, Appendix 4), indicating sufficient sampling efforts (eDNA collection and sequencing efforts).

The alpha diversity (Shannon diversity) of detected prey items within pre-processed samples by hydration (Fig. 2a) and samples from male individuals (Fig. 2b) was higher but not significant ($P > 0.05$) whereas this diversity was significantly higher ($p < 0.001$) within gut content samples (Fig. 2c, d). However sample type-based comparison of the Shannon diversity within Mont Kouffé and Gnanhouizoumè revealed no significant difference ($P > 0.05$) between gut content and reject fecal.

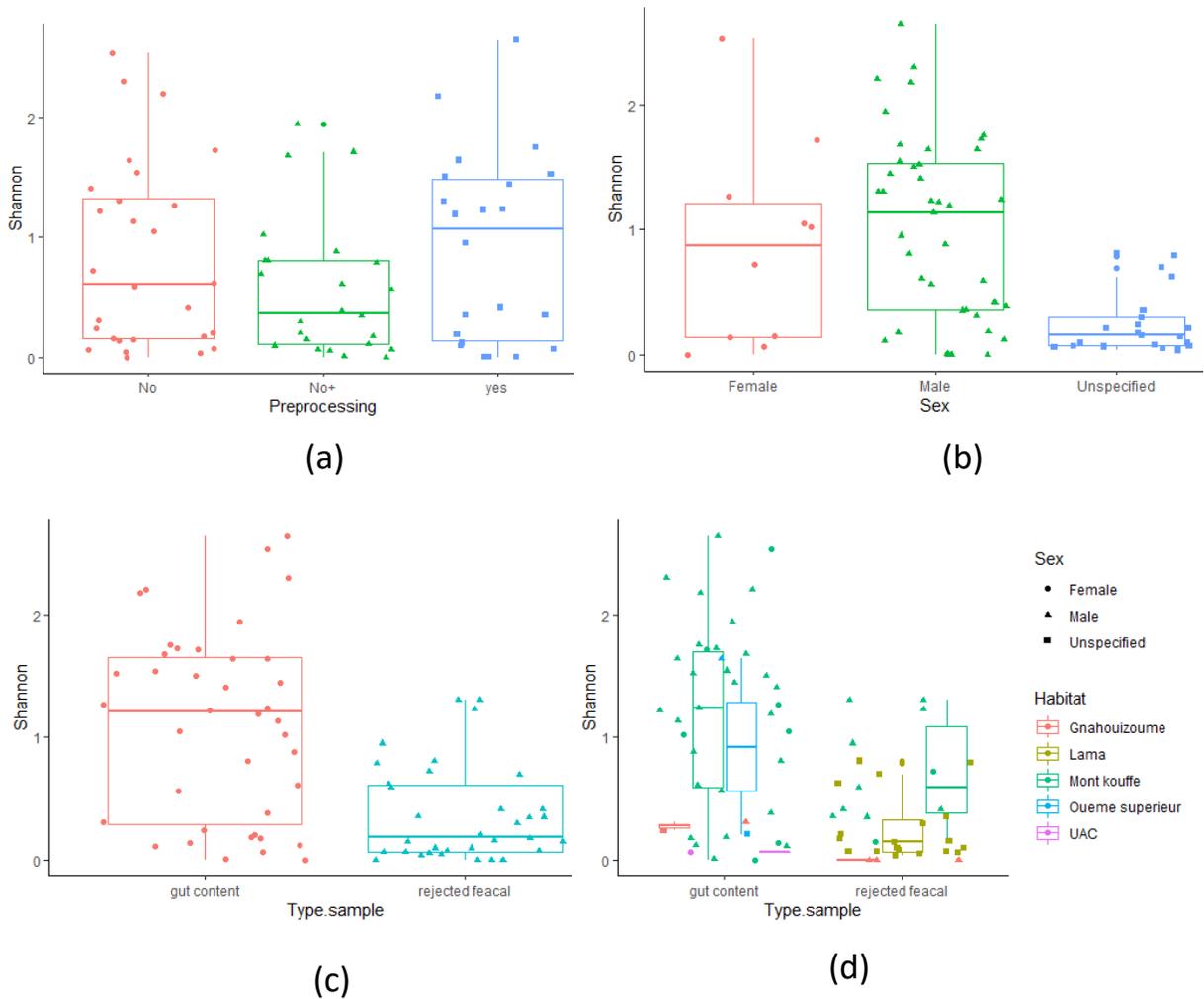


Figure 2. Prey spectrum variation in WBP according to different sample processing and type, gender and forests. (a) Pre-processing of the samples: yes=hydrated, No=non-hydrated, No⁺ =non-hydrated, with addition of 1200 μ l of buffer ST1; (b) Gender: unspecified = gender not identified; (c) Type of sample: gut content=samples collected in the gastrointestinal tract of dead individuals; fecal samples=obtained from alive individuals

Region and habitat-based analyses showed highly significant differences of the alpha diversity. The Shannon diversity in prey items was significantly higher in central region (Fig. 3b) due certainly to the significantly higher diversity in the Mont Kouffé forest compared to Lama and Gnahouizounmè forests (Fig. 3a).

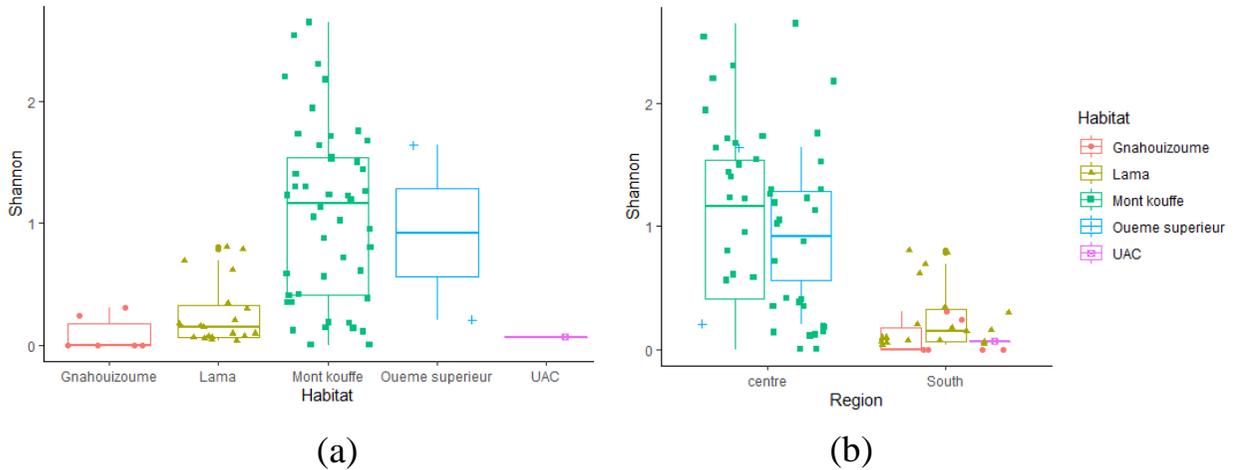


Figure 3. Shannon diversity following sample habitat and regions. UAC represent an individual sampled at the University of Abomey-Calavi, Benin. (a) shows the diversity of consumed preys by sampled habitat and (b) the shows the diversity of consumed preys following south-center gradient

Bray-Curtis dissimilarity index showed highly significant dissimilarities in dietary profiles ($p < 0.001$) between southern and central regions. Spatial visualization showed high heterogeneity of distances between samples from central region, especially in the Mont Kouffé inversely to samples from southern region where distances tend to be almost homogeneous and null between samples (Fig. 4).

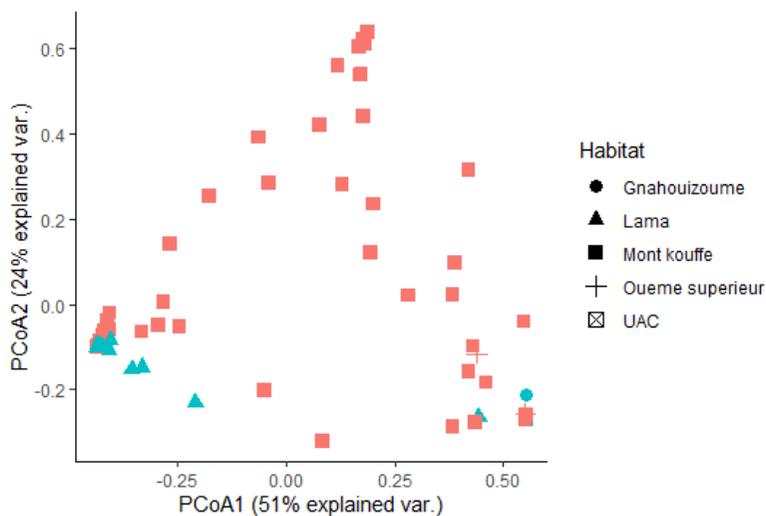


Figure 4. Principal Coordinate Analysis of consumed prey communities between region

6.4. Discussion

Environmental DNA metabarcoding has the potential to, overcome most of problems related to traditional methods of biodiversity monitoring and detect cryptic, elusive and rare animals in particular (Thomsen & Willerslev 2015). This technique is a noninvasive, time and cost-effective approach compared to traditional methods (Pompanon et al. 2012; Kress et al. 2015; Thomsen & Willerslev 2015) widely used in recent investigations for deciphering the dietary composition of prey items consumed by cryptic, elusive and endangered species (Soininen et al. 2009; Balestrieri et al. 2011; Berry et al. 2017). The study is a pioneering molecular scatology of pangolins that provides an insight into the efficiency of DNA metabarcoding method for the focal group, the prey spectrum, some potential factors that may influence the feeding behaviour of pangolins, especially the white-bellied pangolin and ecological role of pangolins. These data are essential for managed breeding of pangolins, especially the focal species and is a starting point of DNA metabarcoding-based investigations of the dietary composition of pangolins.

The 16S primers amplified successfully all the fecal samples; the rarefaction curves for the prey richness, Shannon diversity, Simpson diversity and the coverage reached the saturation and the detected prey species belong mostly to insect taxa. The DNA metabarcoding approach is a promising approach for deciphering the dietary composition of white-bellied pangolin, corroborating the importance of molecular scatology in animal ecology and its increasing application in mammals (Ando et al. 2020). Although our study suggested DNA metabarcoding method as a promising approach to investigate the diet of pangolins, a significant effect of sample type was observed in the prey detection success. The diversity of detected prey species was significantly higher in the gut content samples compared to rejected fecal samples. This result makes DNA metabarcoding method questionable for conserving

pangolins given it supports invasive sampling efficiency. Recent investigations highlighted the positive effect of sampling effort on the prey detection in DNA metabarcoding analyses (Grey et al. 2018; Cantera et al. 2019). The observed sample type-based differences of prey diversity may be due to the unbalanced sampling effort in this study including 29 gut contents vs. 23 rejected fecal samples. The influence of an unbalanced number of samples is probably emphasized by the highest quantity of biomass sampled per gut content sample inversely to the rejected fecal samples. According to Forin-Wiart et al. (2018), DNA integrity gradient is positively correlated to the prey detection using the DNA metabarcoding approach. The prey DNAs within the rejected fecal sample underwent profound transformation along the gastrointestinal tract (Karawita et al. 2020) that potentially degraded the DNA quality to a greater extent making prey detection less efficient inversely to gut content for which the digestion process is partial. The prey diversity does not vary significantly when samples have been pre-processed before extraction indicating that the pre-processing step does improve the prey detectability. The detection efficiency with rejected fecal samples could be improved by a sufficient sampling effort as previously recommended for traditional methods (Trites & Joy 2005)

Despite the limitations of our two reference databases (EMBL and local reference), we were able to detect OTUs belonging to 18 insect families, 10 ant species at high taxonomic resolution and identifications of morphospecies including further 8 ant and 8 termite species respectively (Appendix 4). All the ant species previously identified as prey items by the pioneer study using radio-tracking on the focal species (Pages 1968; Pagès 1975) are covered by the prey spectrum delineated in this study. Out of *Camponotus sp.*, *Cataulacus sp.*, *Oecophylla sp.*, *Crematogaster spp* and *Dorylus sp.* genera previously identified and for which at least one species is detected, DNA metabarcoding

approach revealed further ant taxa such as *Lepisiota capensis*, *Platythyrea conradti*, *Polyrhachis militaris* and *Pheidole megacephala* as prey species for the white-bellied pangolins. These results are in line with previous investigations that highlighted the performance of DNA metabarcoding approach compared to the traditional diet analysis through direct observations, macroscopy identifications of gastrointestinal tract contents and fecals (Thomsen & Willerslev 2015; Nichols et al. 2016; Horswill et al. 2018; Forin-Wiart et al. 2018). We detected abundant unassigned OTUs at the family level (64 % for EMBL vs 21% for local reference database). These results demonstrate the importance of the local reference database that improved substantially the assignment of OTUs (see Meredith et al. 2021; Polling et al. 2022) but also the gaps in the two reference for the DNA metabarcoding assessment in the pangolins's diet profile.

Within the relatively high diversity in insects especially ants and termites occurring in Benin (Eggleton et al. 2002; Attignon et al. 2004; Lachat et al. 2006; Taylor et al. 2018), the white-bellied pangolin preys on a relatively small number of species and mainly on the *Crematogaster*, genera that was revealed the most abundant prey followed by *Pheidole* and *Camponotus*. The relatively too limited prey spectrum including three majors genera is in line with previous investigations on other pangolin species (Wu et al. 2005; Lim 2007b; Irshad et al. 2015; Pietersen et al. 2016b). However, *Crematogaster*, *Pheidole* and *Camponotus* genera are the most diverse and common of all ant genera in West Africa (Yeo et al. 2017; Taylor et al. 2018). The white-bellied pangolin is not prey selective in its wild habitats in Benin, inversly to what was highlighted for other pangolin species (Wu et al. 2005; Lim 2007; Irshad et al. 2015; Pietersen et al. 2016b).

The detected prey species of white-bellied pangolins potentially includes the big-headed ant (*Pheidole megacephalla*) known as one of 100 worst invasive species (Lowe et al. 2019). This result is an evidence that the white-bellied pangolin plays an important regulation role of invasive insect species in the wild habitats and this represents the second case of invasive species predation among eight species after the Chinese pangolin that preys on *Anoplolepis gracilipes* (Lee et al. 2017).

Beyond ant and termite species, prey items belonging to Thripidae (abundant but represent less than 1% of OTUs), Menoponidae, Anisembiidae, Endomychidae, Bethylidae, Liposcelididae, Cicadellidae, Tephritidae, Acrididae, Cecidomyiidae, Chrysididae, Curculionidae, Pseudococcidae, Pyrrhocoridae and Apidae with (*Apis mellifera adansonii*; 90% hit identity) were detected in the diet intake. This diversity of detected taxa suggests that the prey spectrum of white-bellied pangolin is probably not restricted to ant and termites. It may prey on other species belonging to arthropods as previously highlighted for the Indian pangolin (Hutton 1949). However, the detected prey species (*Camponotus sp.*, *Crematogaster sp.*, *Oecophylla sp.*, *Lepisiota sp.*, *Polyrhachis sp.*, *Platythyrea sp.*, *Dorylus sp.* and *Pheidole sp.*) are arboreal predatory ants that prey on arthropods in the tropical forests (Floren et al. 2002; Cerdá & Dejean 2011). For example, formicidae was detected as one of predators of Thripidae (Rocha et al. 2015). Our primers 16S insect have certainly amplified both the prey species directly consumed by the white-bellied pangolin (ants and termites mainly) but also the prey species consumed by the ants. This make challenging a clear prey spectrum delimitation for the white-bellied pangolins using DNA metabarcoding approach.

In Benin, an anecdotal statement widely spreaded evokes that "pangolins within a hive in the hollows of trees erect their scales allowing to the bees to

enter beneath their scales, before quickly closing the scales, trapping the prey and then after climb down on the ground, re-erect the scales and release the killed bees that they consume. By referring to this popular anecdote and a similar amazing behaviour previously reported for the Chinese pangolin (Wang 1990, Wu et al. 2005), one is tempted to believe that the white-bellied pangolin preys on Apidae for which 80 % and 90% hit identities was observed for *Apis mellifera adansonii* using EMBL and local reference databases respectively.

There is no significant sex-related differences in diet of white-bellied pangolin. This result corroborates the absence of sex influence in the dietary composition of *Terminckii* (Pietersen et al. 2016b). In contrast, prey diversity varied significantly following latitudinal gradient (North-South) and following habitats. The prey diversity was higher in the central region and habitats sampled in this region compared to southern region. Recent investigations demonstrated a negatively correlated relationship between ant diversity and latitudinal gradient (Economato et al. 2018). It appears that our results do not support this finding validated for most of taxonomic groups and ant in particular (Economato et al. 2018). However, we are not able to draw active conclusions about diet variation following latitudinal gradient given the analyses were based on an unbalanced number of samples and different sample types between region and also habitats. Although higher diversity was observed in the Ouémé supérieur forest in central region (02 gut content samples) compared to the Lama forest in the southern (17 rejected fecal samples), this would be due to the samples types and call for further investigations.

6.5. Conclusion

To our knowledge this study is unique in assessing the dietary profile of pangolins using a molecular approach and represents an ideal framework that should guide future investigations on the diet of pangolins in general and the

white-bellied pangolin in particular. The study revealed the DNA metabarcoding as a promising tool to provide detailed data on the diet and ecological role of pangolins. It revealed a relatively wide prey spectrum than what was observed using traditional methods and gave some details about the ecological role of white-bellied pangolin that potentially prey on one of worst invasive ant species (*Pheidole megacephalla*). This approach showed significant differences in the prey diversity following sample type, region and habitat. Finally, the study raised some research questions that is important to focus on for further investigations in order to get in-depth accurate information on the dietary profile of white-bellied: (i) the necessary sampling effort of rejected fecal to cover all the prey diversity revealed by gut contents, (ii) latitudinal gradient and habitat-based variations of the dietary preference and (iii) the diversity and rate of indirectly consumed insect species (prey of ants).

General discussion

General discussion

1.1. Landscape context over the last two decades and past abundance levels affected negatively the spatio-temporal distribution and persistence of pangolins in Benin

Although mammals are the taxonomic group for which the most detailed distribution data are in general available (Ceballos & Ehrlich 2006), the distribution of flagship species such as pangolins in Benin was subject to controversies between the global maps of the IUCN Red List of threatened species (Nixon et al. 2019, Pietersen et al. 2019) and the distributions suggested by the Red List for Benin (Akpona and Dahouda 2011). LEK combined with direct evidence revealed different geographic distribution patterns to what was indicated previously in reference documents (Nixon et al. 2019, Pietersen et al. 2019, Akpona and Dahouda 2011). The current occurrence areas of pangolins include a diversity of natural, anthropogenic and degraded patches across Benin. The white-bellied pangolin is under declining trends within habitats patchily distributed across a human-dominated landscape from southern to central region (6°10' and 11°00' N) and the giant pangolin, almost extirpated from Benin, is probably restricted to the Alibori forest reserve between 10°20'-11°00' N. The current spatial configuration of white-bellied pangolins confirms adaptation ability to a diversity of natural and semi-degraded habitats (Pagès 1968, 1975; Angelici et al. 1999, Akpona et al. 2008, Seigniabeto et al. 2021). In addition to its large distribution, the white-bellied pangolin is the most abundant species in Benin compared to giant pangolin. These results corroborate the positive interspecific distribution-abundance relationships in mammals (Brown 1984; Collins & Glenn 1997; Blackburn et al. 1998; Johnson 1998). Despite our sampling efforts across Benin and in the wildlife markets, no direct evidence has been recorded for the

giant pangolin. The giant pangolin is very scarce or probably extirpated from Benin, contrary to the white-bellied pangolin. However, the persistence of both species (if the giant pangolin still exists) within the human-dominated Beninese's landscape is highly threatened by infrastructure development, deforestation and land use changes that severely contracted the home range of both species in Benin. This current spatial context, in addition to the decreasing trends of pangolin populations mainly induced by the overexploitation (hunting) due to their ethnozoological and commercial importance for local people, should drive the two species to a total extirpation from the country, in absence of substantial conservation efforts (See Visconti et al. 2011; Hanski 2011; Rueda et al. 2015; Newbold 2018; Horváth et al. 2019; Magioli et al. 2021).

Given that 77 and 100 % of the current ranges of the white-bellied and giant pangolins, respectively, overlap with formal protected areas that represent the only favourable driver to the persistence of both species, there is evidence that the conservation of pangolins strongly depends on protected areas in Benin (see, Macedo et al. 2019, Pacifici et al. 2020). However, the role of protected areas will certainly be mitigated if the populations of pangolins are not maintained at sustainable levels as revealed by the persistence model that we built (see Rija et al. 2020). The surveillance of protected areas should be improved and substantial conservation efforts should be undertaken to regulate pangolin extraction from their wild habitats. This is an unavoidable condition to achieve long-term persistence of pangolins, and more generally, of the wildlife fauna in Benin. Moreover, this role of protected areas toward pangolins and more generally wildlife fauna could be enhanced through the existence of forested corridors.

1.2. Pangolins are threatened by the versatile use and the cheap cost of their items sold in Benin

Ethnozoological assessment of white-bellied pangolins revealed that their meat is widely consumed among all the ethnic groups across Benin. Eight body parts of pangolins are used for 42 diseases belonging to 15 International Classification of Diseases categories and the scales were the body part with the highest use value. These results reflect the versatile use of pangolin items, especially the scales as previously highlighted for all eight pangolins species (Soewu et al. 2020; Xing et al. 2020). This is a further evidence that scales are the gold coin that makes pangolins unique but also vulnerable in all their occurrence areas (Challender et al. 2015, Boakye et al. 2016, Soewu et al. 2020, Xing et al. 2020). The high value of pangolins as unanimously recognized by all the ethnic groups across Benin represents one of the major drivers of the population trajectories in addition to the international trade (Heinrich et al. 2016, Ingram et al. 2019).

In Benin, pangolins are sold at lower prices than in the rest of West Africa (Mambeya et al. 2018, Ingram et al. 2018), and the selling price varied according to the type of client (the highest prices were for the Chinese diaspora). These results are to be linked with the molecular tracing of seized pangolins that highlighted the huge influence of China on the international trade of African pangolins (Zhang et al. 2015; Mwale et al. 2017). Low pricing of pangolins could make Benin the new destination of traffickers given that an inflation of prices has been observed in central Africa where highest volumes of pangolins are traded yearly (Ingram et al. 2018, Mambeya et al. 2018).

Prior to the recent seizure of pangolins destined to Asia (see Ingram et al. 2018), pangolins were already threatened by unregulated hunting for household consumption and local trade on bushmeat and traditional medicine markets (Akpona et al. 2008, Djagoun et al. 2013). This calls an informed

conservation strategy based on a rigorous law enforcement to combat the illegal trade at national scale in order to discourage all the actors involved in this trafficking.

1.3. The Dahomey Gap lineage of white-bellied pangolins experienced a drastic reduction of its effective population size and is affected by inbreeding depression and genetic erosion

Phylogenetic analyses confirmed the occurrence of an endemic lineage of white-bellied pangolins in the Dahomey Gap (Gaubert et al. 2016), including populations from Benin, Togo and southwestern Nigeria. This is further evidence of a unique evolutionary process in the Dahomey Gap (Colyn et al. 2010, Demenou et al. 2016), increasing endemism within this specific biogeographic region. However, a clear delimitation of this lineage remains to be elucidated, given that some individuals (only three individuals) from bushmeat markets in Nigeria were clustered together with populations from traditional Dahomey Gap.

Among the six phylogenetically delineated lineages (Gaubert et al. 2016), the Dahomey Gap lineage had the lowest levels of genetic diversity. The Dahomey Gap lineage had certainly lost a part of its genetic diversity as a result of abrupt climate change at the Holocene period (Salzmann and Hoelzmann 2005), contrary to the other lineages in the Lower and Upper Guinean forest blocks that were exempt from this climatic event (see Aguillon et al. 2020, du Toit et al. 2020). Such historic loss of genetic diversity has probably been aggravated by recent landscape modifications (agriculture, road development, human settlements, and cities) within the Dahomey Gap (See Bogaert et al. 2013, Padonou et al. 2017). Our results may suggest that the Dahomey Gap lineage shows less capacity to respond adaptatively to future climate change

(see Schmidt et al. 2020), supporting the high extinction risk projected by our persistence models.

Demographic inference over time revealed that the Dahomey Gap lineage experienced a drastic reduction of its effective population size c. 200-500 years ago (100-250 generations), leading to a 87-97% reduction to contemporaneous N_e below the conservative thresholds of minimum viable population size (500-5000; Reed et al. 2003; Clabby 2010). Considering that reduced effective population size might promote the loss of genetic diversity through genetic drift (Charlesworth 2009), it appears that the demographic history of the white-bellied pangolin is characteristic of a population at risk (Frankham 2019).

Given the human-dominated context in the Dahomey Gap with physically disconnected habitats across landscape, inbred populations, reduced effective population size, and limited dispersal ability of pangolins as suggested by previous studies (Pagès 1975, Pietersen et al. 2014), we expected highly structured populations in the Dahomey Gap. However, no clear geographic subdivision was observed using our twenty polymorphic markers, indicating an unsuspected dispersal ability for the white-bellied pangolin or a recent geographic expansion during the last interglacial period (Zanvo et al. *subm.*). Considering ecological conditions' similarity between southern and central region in the Dahomey Gap, a possibly similar evolutionary process may occur all over the Dahomey Gap, slowing down genetic differentiation between fragmented populations. Moreover, if we were not able to detect a structure in the Dahomey Gap lineage, this may be due to the resolution of our markers that is probably not sufficient enough to reveal a cryptic structure.

1.4. Long-distance and endemic trade in the Dahomey Gap?

Illegal wildlife trade is one of major drivers of the current biodiversity crisis (Maxwell et al. 2016). Among the most targeted taxa, pangolins have become

the most trafficked mammals in the world. Despite their status of nationally protected species within the in nearly all range states (Plowman 2020), pangolins –like many terrestrial mammals in tropical Africa– are highly threatened by hunting for local consumption and traditional medicine (Akpona et al. 2008, D’Cruze et al. 2020), but also for fueling the international wildlife trade (Challender et al. 2020). However, in the face of the decline of the different pangolin species, the lack of reliable data on source forests of the specimens sold on wildlife markets and those frequently seized in the international trafficking make conservation efforts ineffective.

Phylogenetic clustering revealed an endemic trade in the Dahomey Gap restricted to the Dahomey Gap lineage of white-bellied pangolins. The genetic assignment of specimens sampled in the wildlife markets using a newly designed tracing approach suggested intra- and inter-country (from Togo to Benin) trade as previously suggested for the wildlife trade in Benin (see Djagoun et al. 2013). All the specimens sampled on the wildlife markets in Benin, Togo and southwestern Nigeria were clustered in the Dahomey Gap lineage populations. This result indicates that local hunters still find enough individuals of white-bellied pangolins within the Dahomey Gap to supply local demands. The long-distance trade observed between Benin and Togo may be associated with the diversity of stakeholders in the traditional medicine in Lomé (*Akodéssawa* fetish market) including both Togolese and Beninese. This may be a source of exchanges between the two countries. The fact that the traced specimens from central Togo was sampled in the traditional medicine market of *Azove* in Benin (nearest market to the targeted forest; 200km) may be an evidence that the inter-country trade operates between geographically close forest habitats and wildlife markets. LEK-based investigations of the trade network broaden the spectrum of trafficking beyond the Dahomey Gap, including Ghana and the Chinese diaspora in Africa as intermediates of

intercontinental trafficking (Mambeya et al. 2018, Ingram et al. 2019, Challender et al. 2020). This is in line with previous investigations that highlighted the involvement of Benin, Togo and Nigeria in the international illegal trade, especially for the populations occurring in Nigeria and Togo (see Omifolaji et al. 2020, Heinrich et al. 2016, Ingram et al. 2019, Challender et al. 2020). A cross-analysis of results obtained from molecular and LEK-based tracing suggests an inter-country trade within the Dahomey Gap and a unidirectional international trade from Dahomey Gap to Asiain particular (see Heinrich et al. 2016, Ingram et al. 2019, Challender et al. 2020).

1.5. The feeding ecology of the with-bellied pangolin exhibits variation related to latitude, forests and sample type

The eDNA metabarcoding approach is a promising tool for deciphering the dietary composition of white-bellied pangolin, corroborating the importance of molecular scatology in animal ecology and its increasing application to mammals (Ando et al. 2020). Our study provides substantial data on the ant and termite species that the white-bellied pangolin preys on in its habitats. The eDNA metabarcoding method extends the previously recorded prey spectrum of white-bellied pangolins using direct observations (Pagès 1968, 1975) to *Lepisiota capensis*, *Platythyrea conradti*, *Polyrhachis militaris* and *Pheidole megacephala* with eight photo-based termite species. The high taxonomic resolution data provided on the nutrition components by this study can help improve the artificial diet of the white-bellied pangolin that was subject to high mortality rate in the early attempts of a captive breeding program in North America (Brookfield, Columbus Zoo, Pittsburgh Zoo, Memphis Zoo and Gladys Porter Zoo), due probably to the too limited knowledge of the species' dietary profile (Hua et al. 2015). It can also serve of framework for local breeding initiatives for *ex-situ* conservation.

The eDNA metabarcoding approach revealed a significantly high consumed prey diversity in the gut content compared with fecal samples. These results show that gut content is the more efficient sample type to cover the prey spectrum of the white-bellied pangolin. It appears that the eDNA metabarcoding method on the white-bellied pangolins is more efficacious for an invasive sampling scheme and this is in opposition to the non-invasive principle of the DNA metabarcoding method (Thomsen & Willerslev 2015; Nichols et al. 2016; Horswill et al. 2018; Forin-Wiart et al. 2018). Further research is needed to estimate the necessary sampling effort of rejected fecals to cover the prey spectrum of the white-bellied pangolin.

Although the dietary composition varied following sample type (gut content vs. rejected fecal), region (Center vs. South) and ecosystem (forest vs. savannah), which is in line with previous studies (Graham et al. 2019; Economo et al. 2018), we are not able to draw an active conclusion because of unbalanced sampling effort. This calls for more investigations to rebalance the data. Despite the performance of the eDNA metabarcoding in investigating the dietary composition of white-bellied pangolins, apart from ant and termite preys, it is difficult to separate direct preys from indirect (eaten by ants, for instance). This makes a clear delimitation of the preys directly consumed by the white-bellied pangolins challenging, and once again calls for further investigations by comparing the prey diversity in the gut content of detected ant and termite preys to the one recorded using gut content and fecal of pangolins in the sampled habitats.

Interestingly, the white-bellied pangolin preys on *Pheidole megacephala*, one of the 100 worst pest species in the world (Lowe et al. 2019). The white-bellied pangolin in its wild habitat acts as a regulator of populations of ants and termites, notably of invasive insect species (Chao et al 2020).

1.6. Implications for conservation

The present dissertation filled substantial knowledge gap on pangolins within the Dahomey Gap. The knowledge that we have produced could be used by national and international policymakers as decision tools for conserving the white-bellied and giant pangolins. This study provided evidence-based data on the geographic distribution, LEK-based population trajectories and the drivers of declining trends the Dahomey Gap lineage of white bellied pangolins. This information should be used to update the IUCN global map for the white-bellied and giant pangolins in Benin, the presence of which remains very uncertain in the country. At the national scale, conservation status and the distribution map of both species in the Red List for Benin have to be re-assessed using the findings of this dissertation. The geographic distribution, LEK-based population trends over time, drivers of persistence, predicted probability of persistence, ethnozoological value, pangolin pricing, trophic ecology, demographic history, population genetic and magnitude of the trade network represent a package of data that may guide the design of a national strategy for an effective conservation of pangolins in Benin and the suitable management of protected areas. The long-distance, endemic and inter-country trade in pangolins constitute an invaluable information that wildlife conservation actors may use via synergistic actions through transnational collaborations in the sub-region to tackle the pangolin trade. Our investigations revealed a significant role of protected areas in the persistence of pangolins in Benin. However, this role should be improved through the establishment of forested corridors and effective anti-poaching measures to expect long-term conservation of pangolins in Benin. Finally, more details on the diet composition have been achieved by our eDNA metabarcoding-based investigations on the feeding ecology of white-bellied pangolins. These investigations give more details on the regulation role that the white-bellied

pangolin plays in its habitat, notably against invasive insect species (e.g *Pheidole megacephala*, listed among the 100 worst invasive species in the world; Lowe et al. 2019). Data on prey spectrum is also a prerequisite for undertaking breeding programs on the focal species, which is under unprecedented anthropogenic pressures. Our dissertation experienced new methods for collecting LEK and molecular tracing of the illegal trade at local scale. These different methods represent invaluable tools that could be applied for other investigations in wildlife ecology and molecular tracing of the focal taxa but also other taxa than pangolins.

1.7. Future challenges

Although substantial baseline data have been generated through this dissertation, some key data remain lacking for pangolins in general, and the two species occurring within the Dahomey Gap in particular. Further research using camera traps is needed to confirm the presence/absence of giant pangolins within the Alibori forest reserve, its last forest habitat delineated by LEK. Estimating population size inform on an important indicator of current or/and future extinction risk level for a given species (Keith et al. 2015) and represents a key criterion in the IUCN assessment of the conservation status. Although LEK-based distribution and population trajectories are investigated for the white-bellied and giant pangolins in this work, evidence-based presence/absence on giant pangolins and population size of both species using validated conventional method (see Kharawi et al. 2010) is lacking. These data will allow improving the reliability of decision making towards pangolins in the Dahomey Gap.

The home range, habitat use and dispersal ability of pangolins previously investigated through a series of pioneer studies in Central Africa (Pagès 1968 and 1975) is particularly lacking in the Guinean Gulf (Ingram et al. 2019), but

essential to identify high priority areas in order to enhance conservation efforts. These data collected in a human-dominated landscape may inform us on the ecological requirements of pangolins in degraded habitats *versus* non-degraded habitats within the Dahomey Gap.

We were unable to detect genetic structure in the Dahomey Gap lineage and this may be due to the limitation of our markers, although a genuine lack of structure is also a legitimate option. Reliable knowledge on genetic structure is an important data for establishing conservation priorities over space and time. It will be important to develop other markers such as Single Nucleotide Polymorphisms (SNPs) that are expected to be more resolute to detect potential genetic structure (e.g., Nash et al. 2018) and decipher complex evolutionary patterns. Equally, such investigations will help improve the efficacy of the fine scale tracing of the pangolin trade within the Dahomey Gap.

High resolution data on the prey spectrum of pangolins are lacking and our reference database did not cover all the prey diversity sequenced in the white-bellied pangolins' faeces. These data are essential to know more about the function of pangolins in their ecosystem and is a cornerstone information for breeding programs. Some attempts of breeding programs using white-bellied pangolins from Togo in North American zoos faced a high mortality rate due mainly to inappropriate artificial diet, because accurate data on the dietary composition of pangolin is lacking (Hua et al. 2015). These programs could use our findings to refine the ongoing breeding program using the Dahomey Gap lineage in American Zoo.

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Appendix 1

Table 1. Characteristics of the current forests potentially housing the white-bellied and the giant pangolins, with frequency of collected scales (direct evidence)

Forest/Habitat	Management modality	Classification objective	Total area (km ²)	Number of collected scales
Agoua*	Forest reserve	Wood energy	67052.18	5
Agrimey*	Forest reserve	Wood energy	3056.877	0
Alibori supérieur**	Forest reserve	Wood energy	259135.158	0
Atchin*	Community forest	Social service	37.992	1
Bassila*	Forest reserve	Wood energy	3040.771	0
Djaloukou	Game ranch	Wildlife reserve	3880	0
Dévé*	Community forest	Social services	3.83	2
Dogo*	Forest reserve	Wood energy	31630.365	4
Felea*	Community forest	Social services	42.759	0
Gbadagba	Game ranch	Wildlife reserve	1415	2
Gnanhouizoun*	Community forest	Social services	6.261	8
Ikpokporo*	Community forest	Social services	32.368	0
Kouvizoun*	Community forest	Social services	529.588	2
Lama*	Forest reserve	Wildlife reserve	17604.197	13
Lokoli*	Community forest	Social services	2942.103	1
Monlouvé*	Community forest	Social services	18.382	2
Monts Kouffé*	Forest reserve	Wildlife reserve	191652.549	27
Ouéme Boukou*	Forest reserve	Wildlife reserve	22437.079	4
Ouéme supérieur*	Forest reserve	Wildlife reserve	184768.521	12
Penessoulou*	Forest reserve	Wood energy	5247.062	1
Toui-klibo*	Forest reserve	Wildlife reserve	44131.032	2
Wari-Marou*	Forest reserve	Wildlife reserve	117615.512	7
Gallery forests*	NA	Soil and water protection	NA	8

(*) white-bellied pangolin (**) white-bellied and giant pangolins

Appendix 2

Table S1. Land-use suitability partition based on ecological requirements of the white-bellied and giant pangolin in Benin

Land-use	Suitability level	Attributed Score
Bare areas		
Consolidated bare areas		
Cropland, irrigated or post-flooding		
Cropland, rainfed		
Grassland		
Herbaceous cover	Not suitable	1
Lichens and mosses		
Shrub or herbaceous cover, flooded, fresh/saline/brakish water		
Tree cover, flooded, saline water		
Unconsolidated bare areas		
Urban areas		
Sparse herbaceous cover (<15%)		
Shrub or herbaceous cover, flooded, fresh/saline/brakish water		
Mosaic cropland (>50%) / natural vegetation (tree, shrub, herbaceous cover) (<50%)		
Mosaic natural vegetation (tree, shrub, herbaceous cover) (>50%) / cropland (<50%)		
Mosaic tree and shrub (>50%) / herbaceous cover (<50%)	suitable	2
Mosaic herbaceous cover (>50%) / tree and shrub (<50%)		
Shrubland		
Shrubland deciduous		
Tree cover, broadleaved, evergreen, closed to open (>15%)		
Tree cover, broadleaved, deciduous, closed to open (>15%)		
Tree cover, broadleaved, deciduous, closed (>40%)		
Tree cover, broadleaved, deciduous, open (15-40%)		
Tree cover, needleleaved, evergreen, closed to open (>15%)		
Tree cover, needleleaved, evergreen, closed (>40%)	Highly suitable	3
Tree cover, needleleaved, evergreen, open (15-40%)		
Tree cover, needleleaved, deciduous, closed to open (>15%)		
Tree cover, needleleaved, deciduous, closed (>40%)		
Tree cover, needleleaved, deciduous, open (15-40%)		
Tree cover, mixed leaf type (broadleaved and needleleaved)		

Metadata computation

We computed the variables *distPA*, *distRoad*, *distWater* and *distMarkets* by joining the layer of georeferenced villages ($N=208$) to the layers of protected areas, asphalted roads, main watercourses and wildlife markets respectively using ArcGis 10.5 (Ensi France). The variables dft_i and LSI_i were estimated following a gradient of buffer size radius each village (5, 10, 15, 20 and 25 km of buffer size). The variable dft_i was computed using the geographic coordinates of sampled villages and Global Forest Change data from 2001 to 2019 (<https://google.earthengine.app/view/forest-change> ; Hansen et al. 2013). For each buffer size, we clipped for each villages the extent of deforested area (km) from 2001 to 2019 using ArcGis 10.5 (Ensi France).

To build the land-use suitability change index over time (LSI_i), we firstly used the Global Land cover 1998 and 2015 at 300 m resolution (<http://maps.elie.ucl.ac.be/CCI/viewer/download.php>) to calculate the land-use areas (km) for each village within each buffer size as above. The obtained land-use components were partitioned into three categories following the suitability level to pangolins (Table 1) referring to ecological requirements of the white-bellied and giant pangolins: (i) not suitable land-use (attributed score = 1) that included the non-occurrence landscape features and all resistance surfaces able to impede the movement of pangolins, (ii) suitable land-use (attributed score= 2), that encompassed mosaic of natural vegetation with trees or shrubs and cropland within which pangolins, especially the white-bellied adapt itself and may occurs and (iii) highly suitable land-use (attributed score = 3) included these occurrence landscape features of pangolins without or with negligible human fingerprints with tree covers. We defined these categories by referring to

previous studies on the distribution and activity pattern of the white-bellied pangolin, highly related to tree covers and its ability to occurs in semi-degraded habitats (Pages 1968a; Pagès 1975; Angelici et al. 1999; Akpona et al. 2008; Zanzo et al. 2020a; Segniagbeto et al. 2021a). We applied the same index to the two species given the white-bellied and giant pangolins prefer almost the same ecosystems (Lowland forests, primary and secondary forests, forest-savanna and savanna mosaic, dense woodland, gallery forests, riparian forests, wooded savanna and wet grassland; Hoffmann et al. 2020; Jansen et al. 2020), but also because in our study area, LEK-based past and current distribution revealed an overlapping of occurrence habitats for both two species (Zanzo et al. 2020a).

The Land-use suitability index (*SI*) was calculated for each buffer size, time periode and per village using the following formula:

$$SI = \frac{A_i \times S_i + A_{ii} \times S_{ii} + A_{iii} \times S_{iii}}{A_i + A_{ii} + A_{iii}}$$

S_i , S_{ii} , and S_{iii} were the attributed scores for the land-use types, unsuitable, suitable and highly suitable habitats respectively and A_i , A_{ii} and A_{iii} were the areas (km) of unsuitable, suitable and highly suitable habitats respectively.

The Land-use suitability change index over time (1998-2015) was calculated for each village and buffer size as followed:

$$LSI = SI_{1998} - SI_{2015}$$

Table S2. Correlation test between variables include in the GLM model

Parameter1	Parameter2	R	95% CI	p-value
	<i>dft₁₅</i>			
<i>ab1998</i>		-0.19	[-0.34, -0.03]	0.230
<i>ab1998</i>	<i>LSI₁₅</i>	0.08	[-0.08, 0.24]	> .999
<i>ab1998</i>	distAP	-0.14	[-0.29, 0.02]	0.525
<i>ab1998</i>	distRoad	0.24	[0.09, 0.38]	0.046*
<i>ab1998</i>	distWater	-0.24	[-0.39, -0.09]	0.039*
<i>ab1998</i>	distmarket	-0.17	[-0.31, -0.01]	0.369
<i>dft₁₅</i>				
	<i>LSI₁₅</i>	0.22	[0.07, 0.37]	0.077
<i>dft₁₅</i>				
	distAP	0.05	[-0.11, 0.20]	> .999
<i>dft₁₅</i>				
	distRoad	-0.12	[-0.27, 0.04]	0.683
<i>dft₁₅</i>				
	distWater	0.27	[0.12, 0.41]	0.014*
<i>dft₁₅</i>				
	distmarket	0.38	[0.24, 0.51]	< .001***
<i>LSI₁₅</i>				
<i>LSI₁₅</i>	distAP	-0.17	[-0.32, -0.01]	0.369
<i>LSI₁₅</i>	distRoad	2.62e-03	[-0.15, 0.16]	> .999
<i>LSI₁₅</i>	distWater	0.15	[-0.01, 0.30]	0.455
	distmarket	0.19	[0.03, 0.34]	0.230
distAP	distRoad	0.16	[0.01, 0.31]	0.369
distAP	distWater	0.06	[-0.10, 0.21]	> .999
distAP	distmarket	-0.23	[-0.37, -0.07]	0.069
distRoad	distWater	-0.19	[-0.34, -0.04]	0.224
distRoad	distmarket	0.18	[0.03, 0.33]	0.248
distWater	distmarket	0.20	[0.05, 0.35]	0.154

Table S3. GLM model using 15 km buffer size ($N= 156$ villages) for the White-bellied pangolin

Variables	Estimate	p -values
Intercept	-2.492e+00	0.013837*
<i>ab1998</i>	2.337e+00	3.81e-07***
distPA	-8.374e-05	0.005799**
distRoad	5.841e-05	0.014237*
distWater	-2.132e-05	0.489262
distMarkets	-5.665e-06	0.268218
dft_{15}	-4.085e-02	0.005199**
LSI_{15}	1.093e+01	0.000994***

Figure S1. Trends over the next two decades of the probability of presence for the white-bellied pangolin in Benin. The blue, grey and green lines correspond to the model assuming no deforestation and change of Land-use suitability over the next two decades based on the global, first quartile and third quartiles respectively. The red, darkred and cyan lines correspond to the model assuming similar changes as in the last two decades

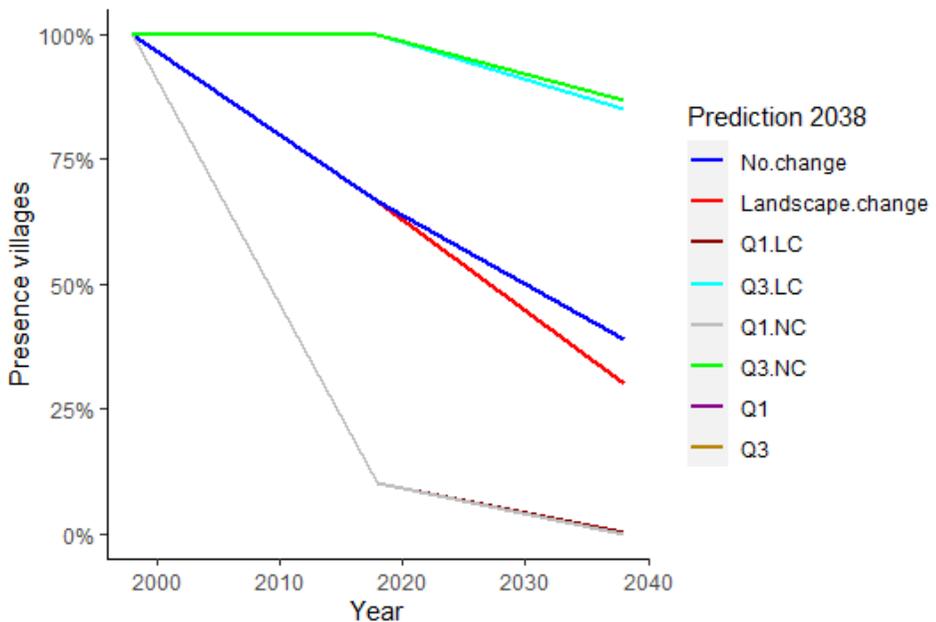
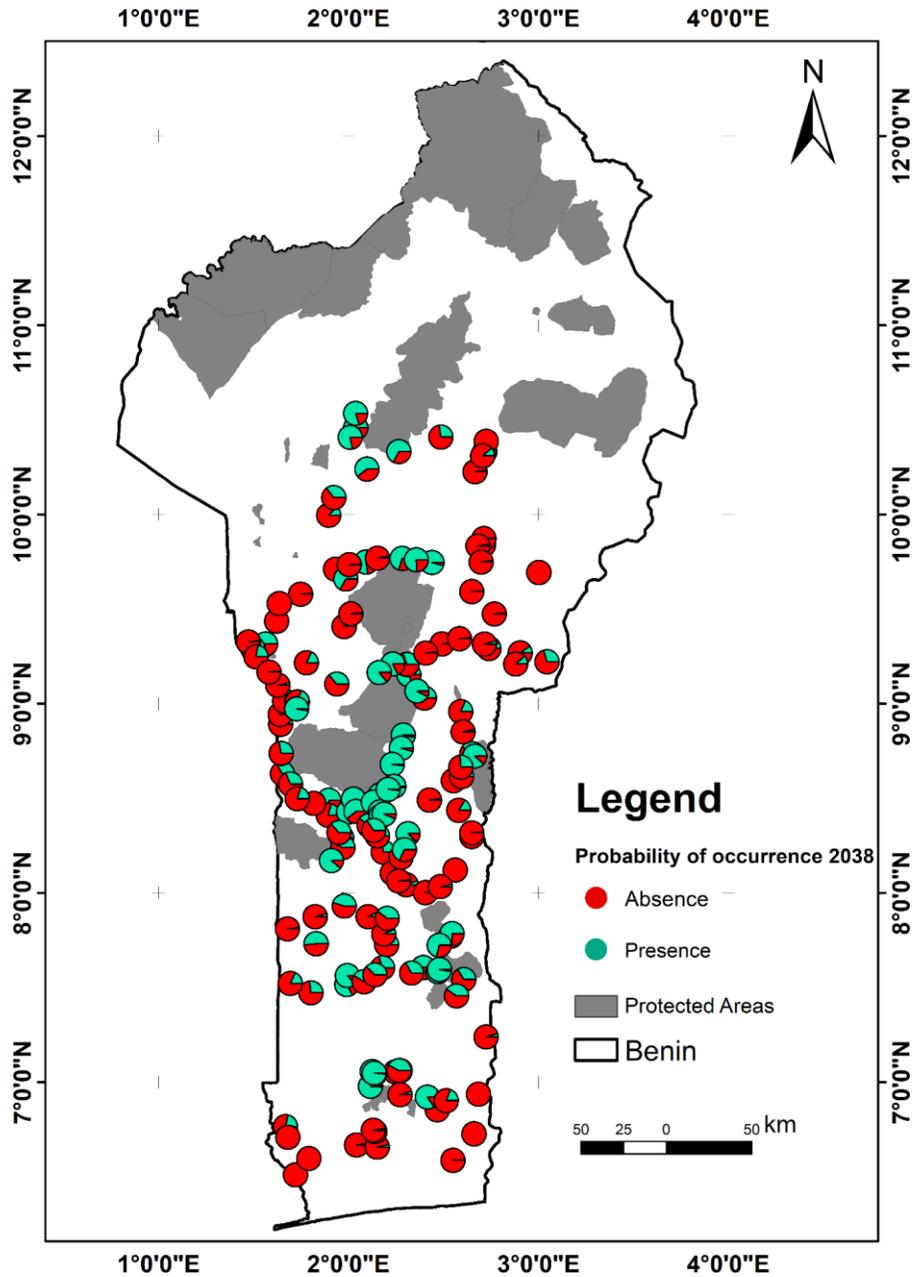
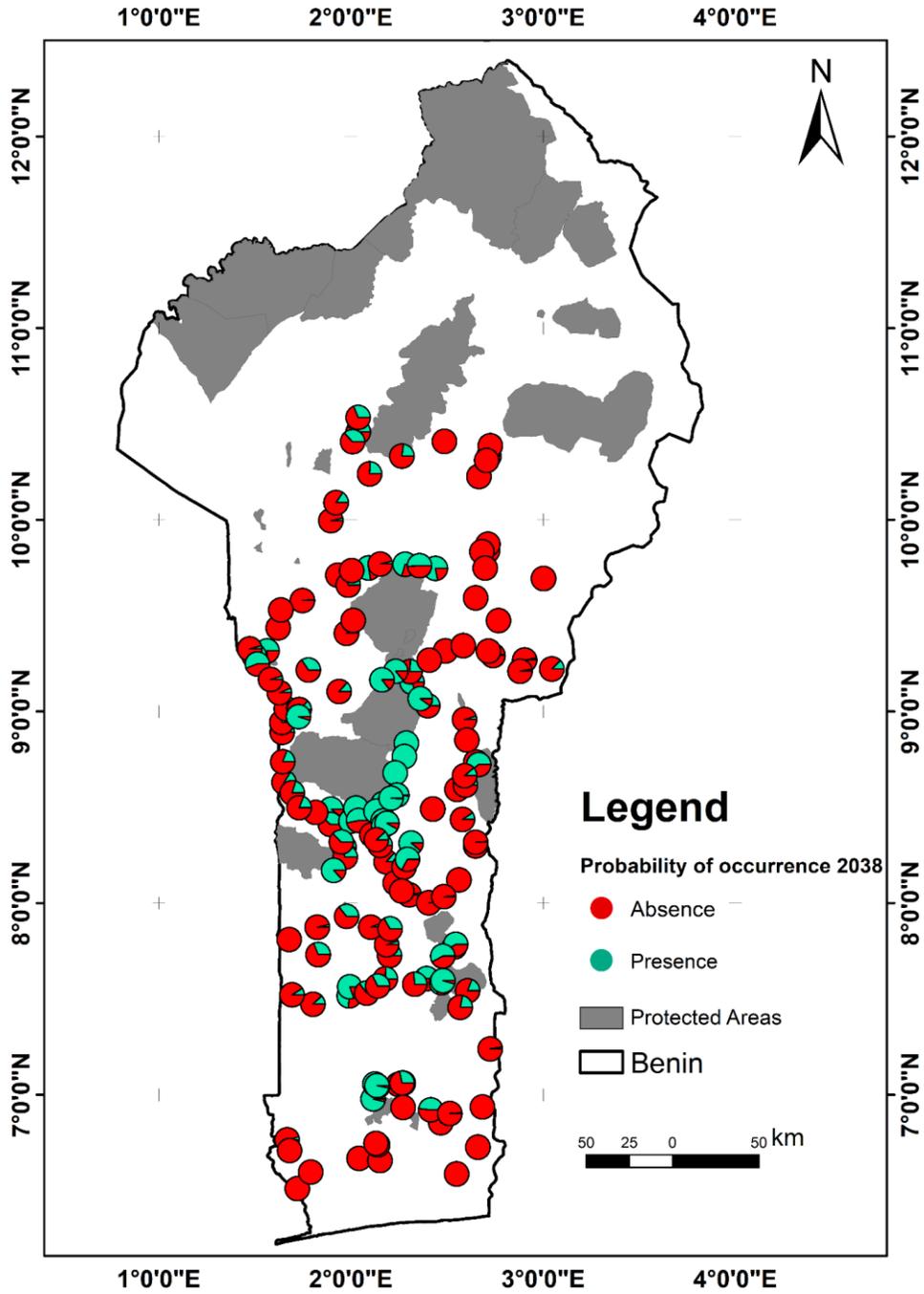


Figure S2. Prediction maps of the probability of occurrence for the white-bellied pangolin in 2038. (a) assuming no deforestation and change of the land-use suitability over the next two decades (2018-2038) and (b) assuming similar trends of deforestation and change of land-use suitability as in the last two decades (1998-2018)

(a)



(b)



Appendix 3

Table S1. General database including geographic references and detailed information on sequenced and genotyped individuals. Empty lines indicate unsuccessful sequencing / genotyping. Haplotypes of CR1 sequences used to compute genetic diversity are provided (H1 to H14). Individuals sequenced successfully but not used to compute genetic diversity –because of missing data– are indicated by “yes”. [separate Excel file]

Table S2: Diversity indices calculated from the control region (CR1) among the white-bellied pangolin lineages.

h: Number of haplotypes, *Hd*: Haplotype (gene) diversity, π : Nucleotide diversity, *SD*: Standard deviation of haplotype diversity.

Lineages	Number of individuals	<i>h</i>	<i>Hd</i>	<i>SD (Hd)</i>	π
Dahomey Gap	126	14	0.686	0.033	0.00297
Ghana	3	2	0.667	0.314	0.00154
Western Africa	12	6	0.818	0.096	0.00407
Western Central Africa	59	31	0.96	0.012	0.00847
Central Africa	9	6	0.833	0.127	0.00797

Table S3. F_{ST} values (below diagonal) and associated levels of significance (above diagonal) for the different partition schemes (i), (ii) and (iii). South and North in the partition schemes refer to Benin country

* $p < 0.05$

(i)

	1	2	3	4	5	6
1	0	*	*	*	*	*
2	0.08925	0	*	*	*	*
3	0.09822	0.0965	0	*	*	*
4	0.11262	0.16601	0.05282	0	*	*
5	0.10279	0.05366	0.02209	0.11546	0	*
6	0.24435	0.21227	0.13992	0.19121	0.13868	0

pop1 = Gnanhouizoun, pop2 = Lama, pop3 = Monts Kouffé, pop4 = Ouémé Supérieur, pop5 = Wari-Marou, pop6 = Togo.

(ii)

	1	2	3
1	0.00000	*	*
2	0.01137	0.00000	*
3	0.03921	0.01865	0.00000

pop1 = South, pop2 = North, pop3 = Togo.

(iii)

	1	2	3
1	0.00000	*	*
2	0.03783	0.00000	*
3	0.11619	0.06300	0.00000

pop1 = South, pop2 = North, pop3 = intermediate.

Table S4. Inbreeding coefficient in white-belled pangolin populations as delineated in the 6-partition scheme (i)

Forest	F_{is}
Gnanhouizounmè	0.098
Lama	0.098
Monts Kouffé	0.098
Ouémé Supérieur	0.131
Togo	0.317
Wari-Maró	0.101

Figure S1. Haplotype network (control region) within the Dahomey Gap lineage. Haplotype numbers refer to Table S1. Short bars correspond to mutation numbers

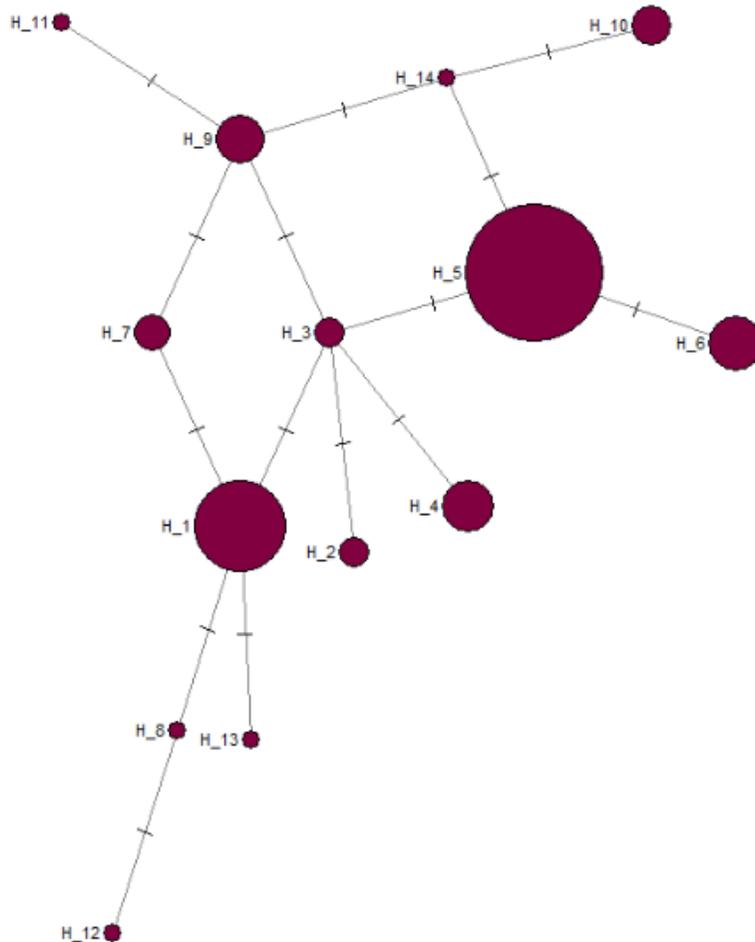


Figure S2. Assignment plots among the white-bellied pangolins from the Dahomey Gap as assessed with STRUCTURE for K=2 (top) and K=3 (bottom). Each individual is represented by a vertical bar

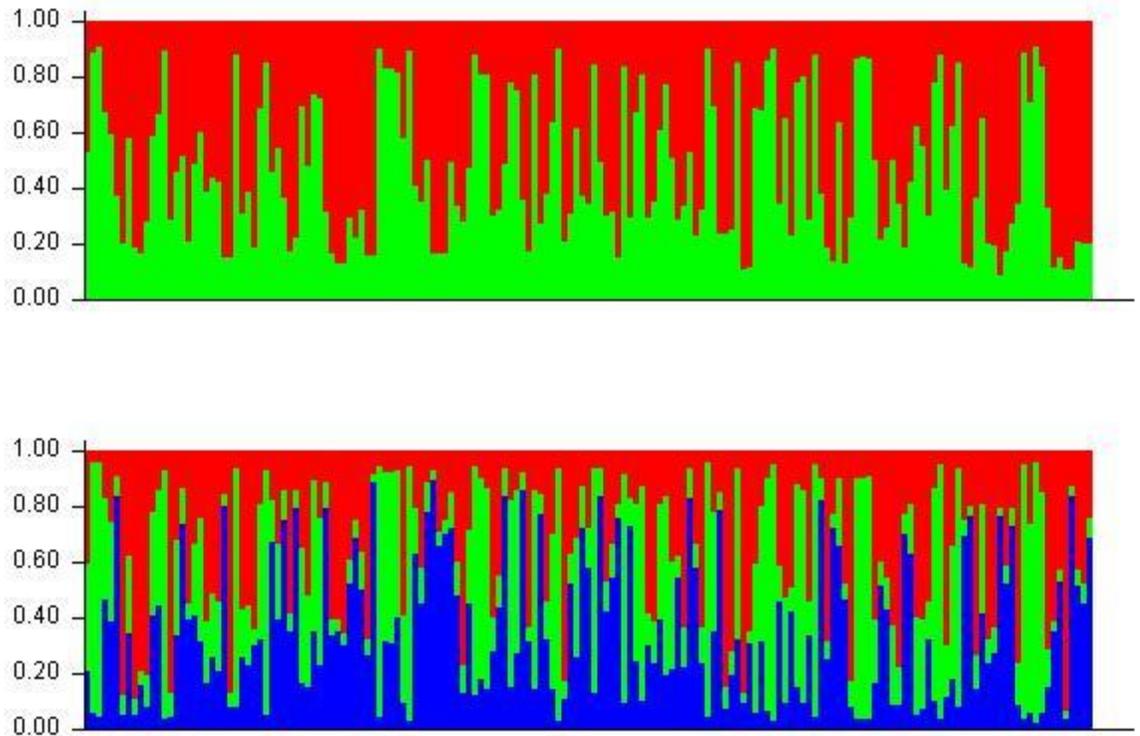
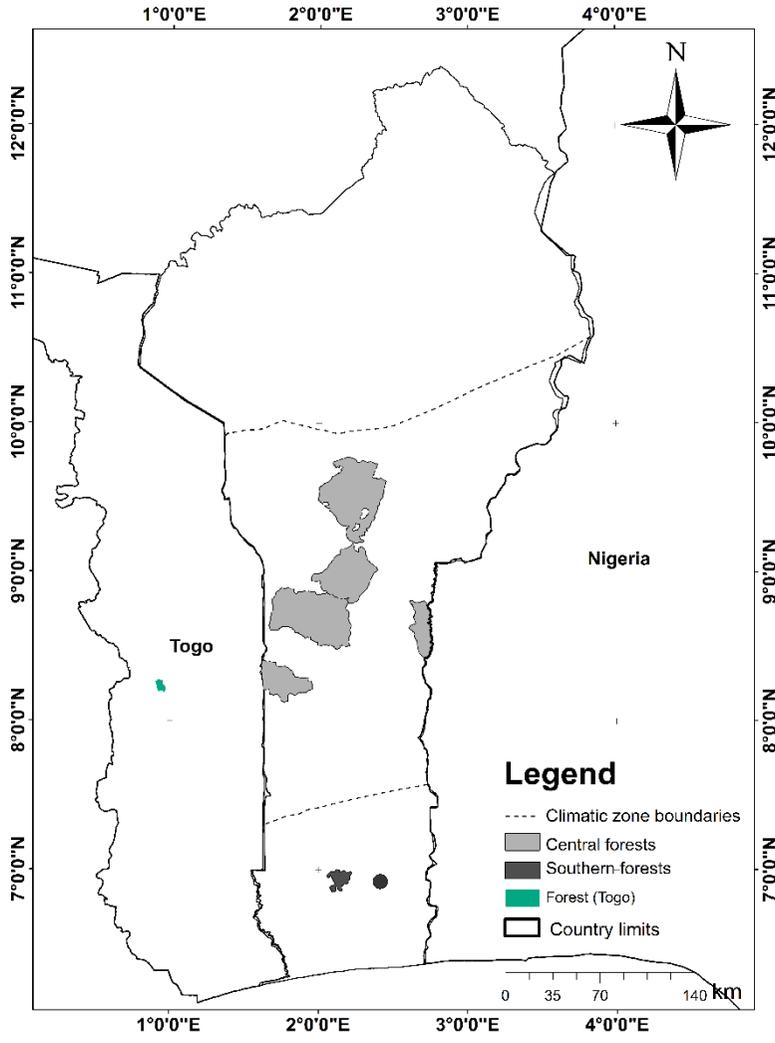
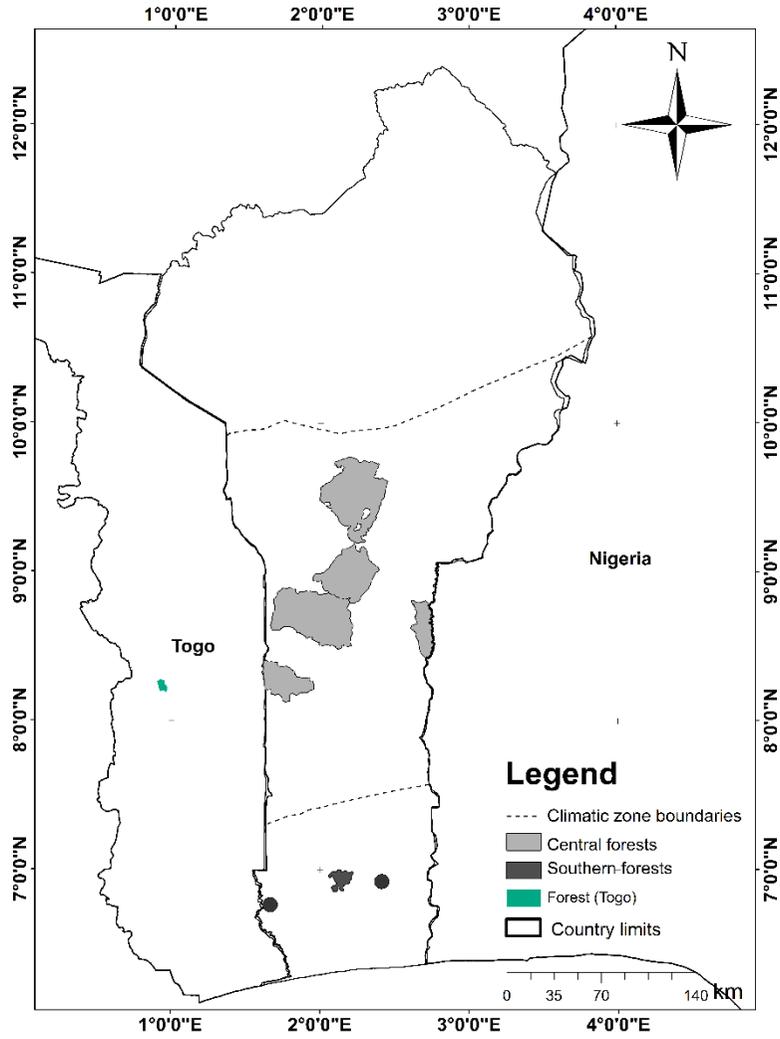


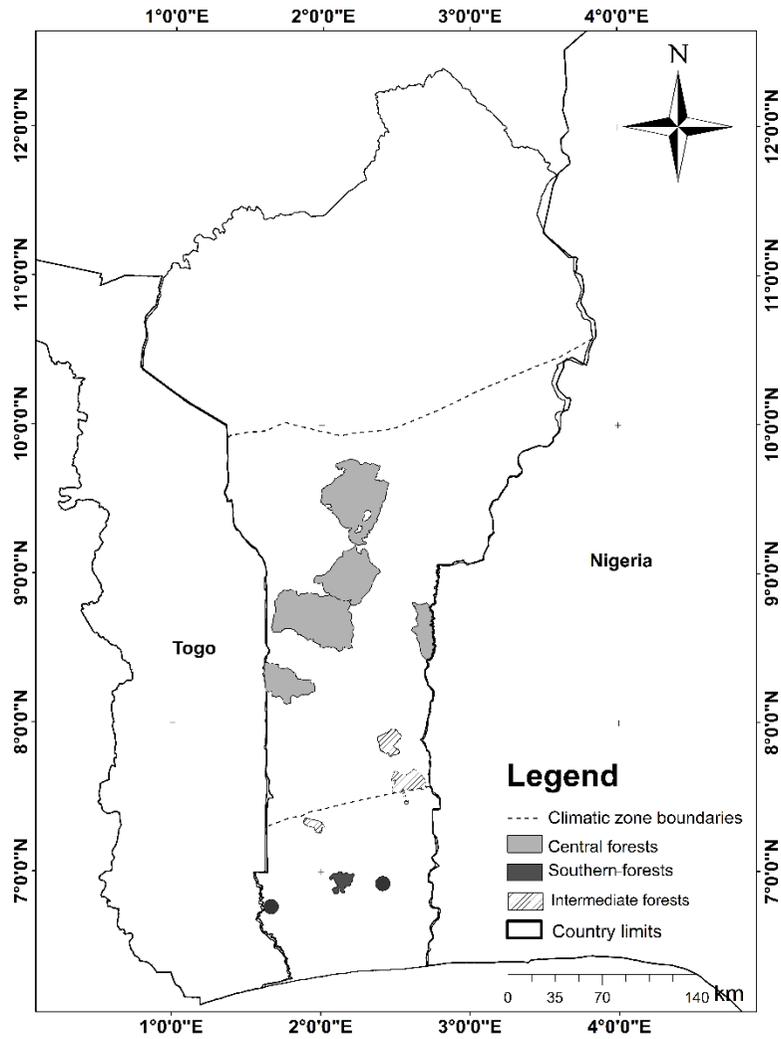
Figure S3. Geographic partition schemes used for computing pairwise differentiations (F_{ST}). (i) 6-partition scheme including the forest habitats with at least 7 individuals, (ii) 3-partition scheme among South Benin-Central Benin-Togo, (iii) gradient-based 3-partition scheme within Benin



(i)

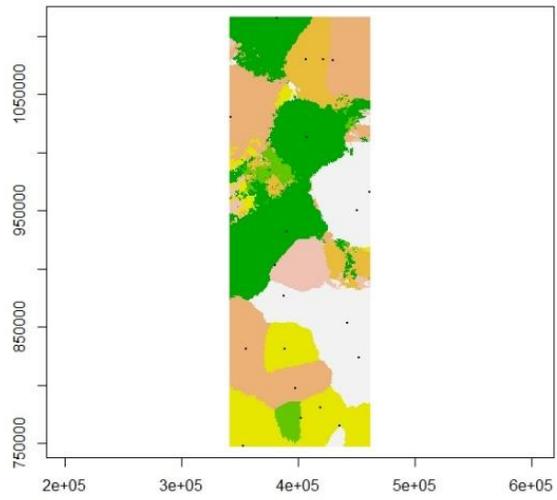


(ii)

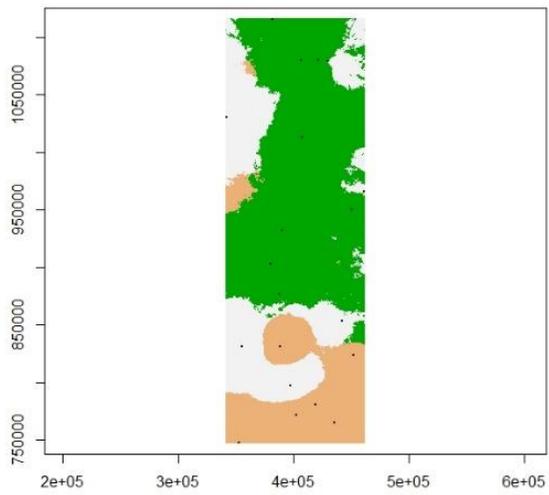


(iii)

Figure S4. Spatial clustering of white-bellied pangolins in the Dahomey Gap obtained using Geneland for $K=7$ (Geneland; above) and $K=3$ (Structure;



Estimated cluster membership



Estimated cluster membership

Figure S5. Unbiased probability of identity (uPI) and probability of identity among siblings (PIsibs) for increasing, optimized combinations among the 20 microsatellite markers

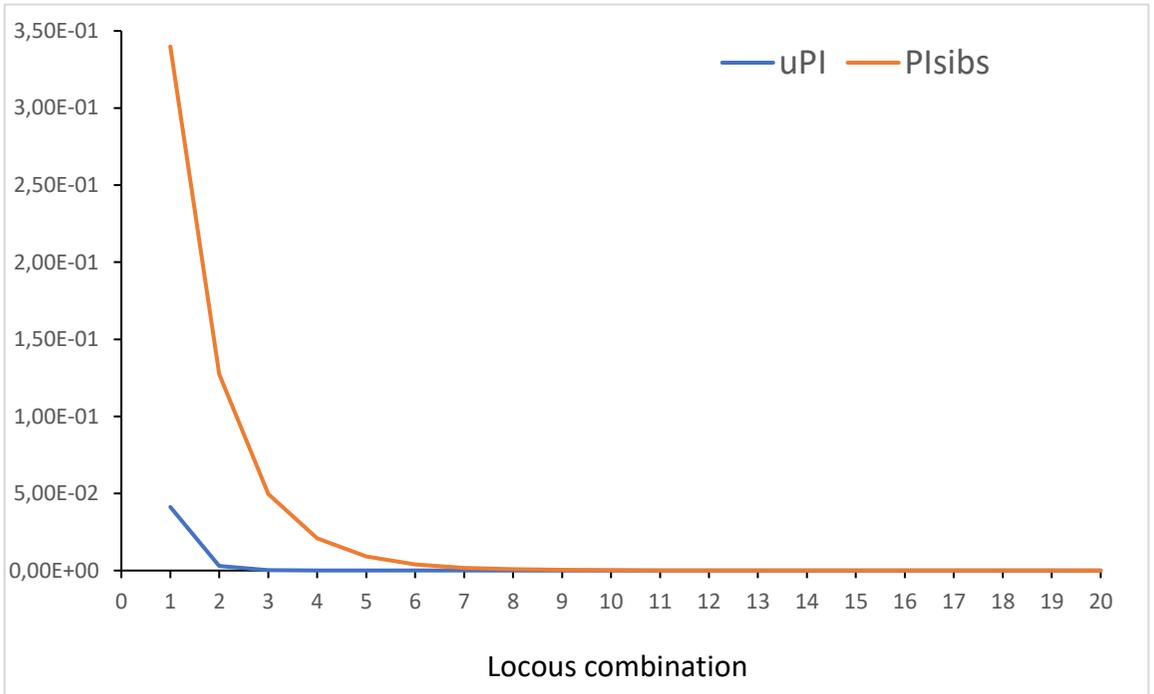


Figure S6. Mean private allelic richness per locus in white-bellied pangolin populations (6-partition scheme), as estimated from ADZE (N=7). FGN: Gnanhouzounmè, FL: Lama, FMK: Mont Koufé, FOS: Ouémé Supérieur, FWM: Wari Maro and TG: forests of central Togo

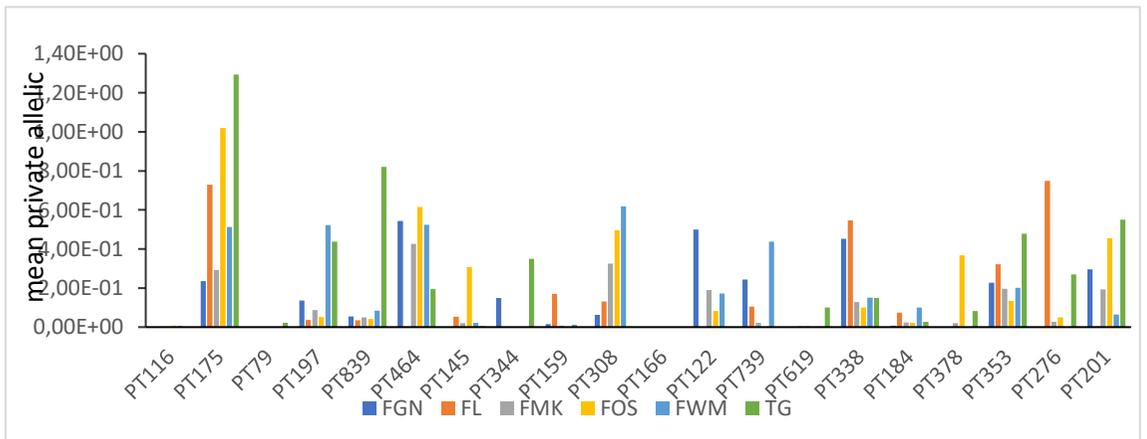
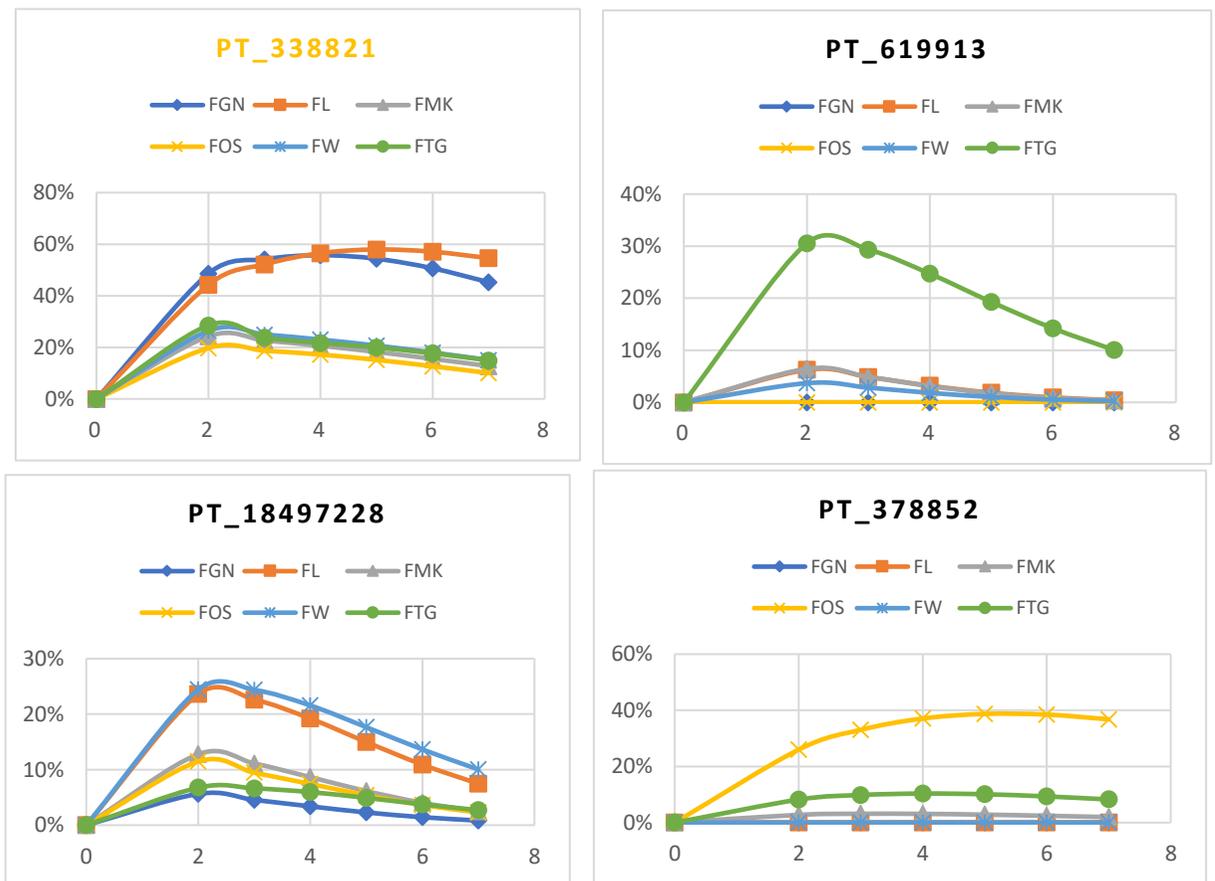
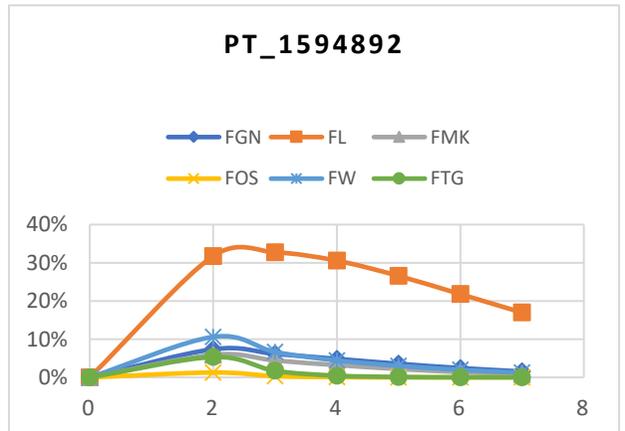
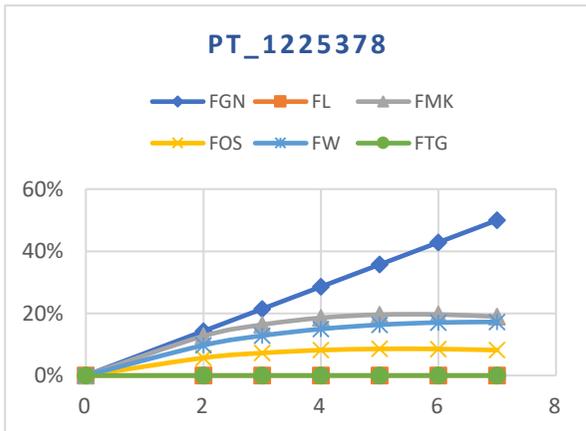
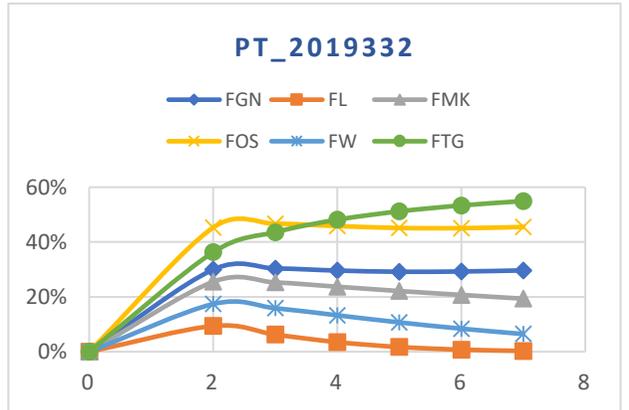
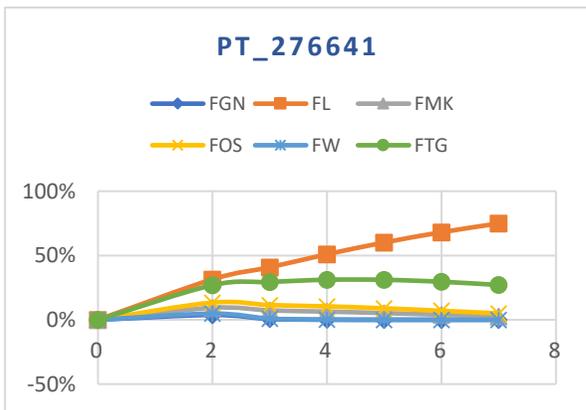
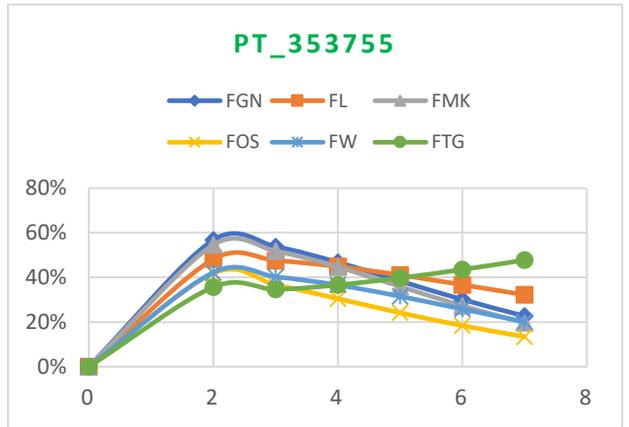
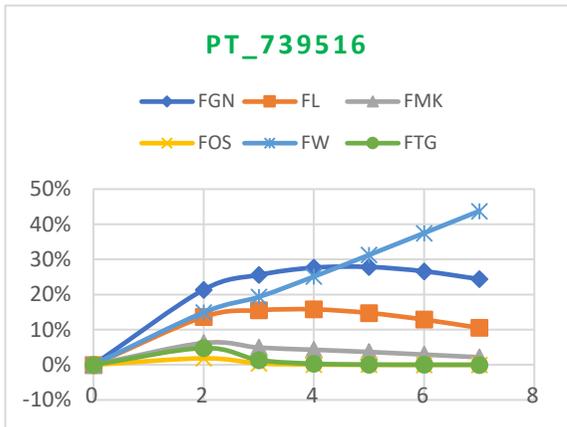
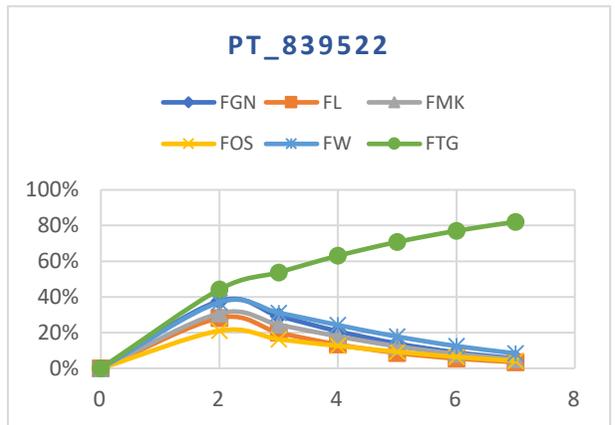
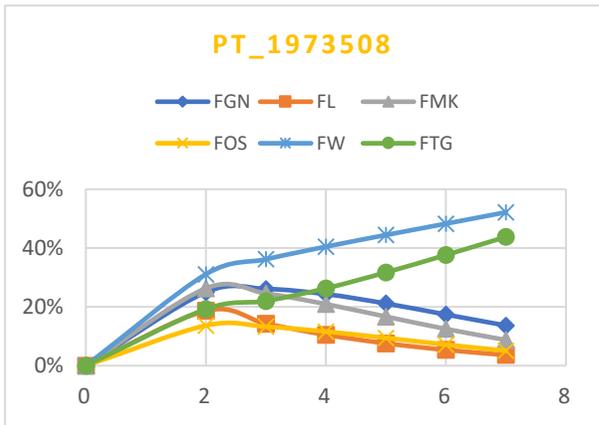
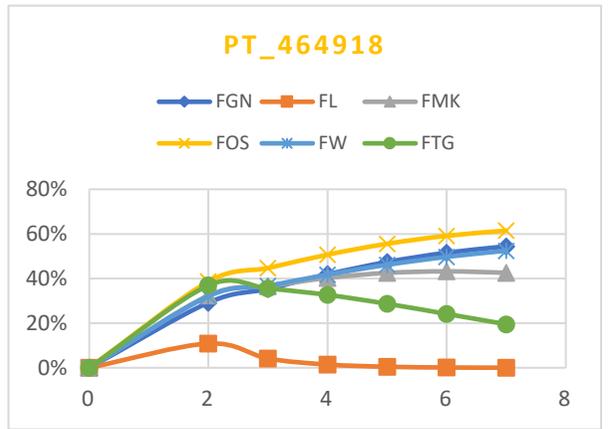
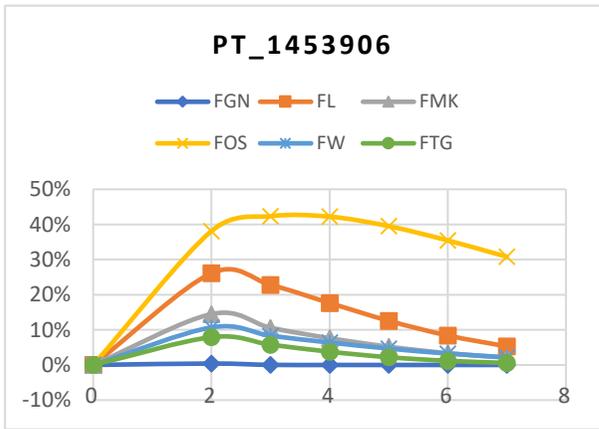
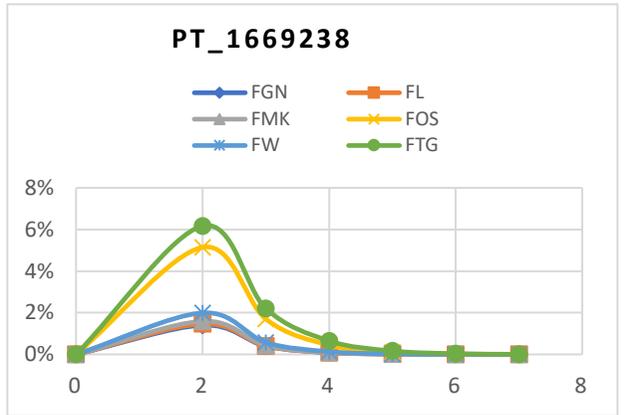
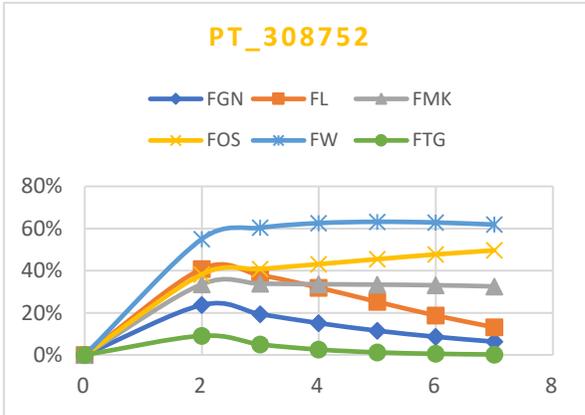


Figure S7. Mean private allelic frequencies per population (forest habitat), as estimated by rarefaction from ADZE. FGN: Gnanhouzounmè, FL: Lama, FMK: Mont Koufé, FOS: Ouémé Supérieur, FWM: Wari Maro and TG: forests of central Togo. The names of the three loci cross-validated (ADZE and GenAlEx) and used for the tracing (i.e. private alleles found in market individuals) appear in green. The four loci cross-validated (ADZE and GenAlEx) but not used for the tracing (i.e. private alleles not found in market individuals) appear in blue. The other four loci revealed using rarefaction but not observed in the actual dataset (GenAlEx) appear in orange







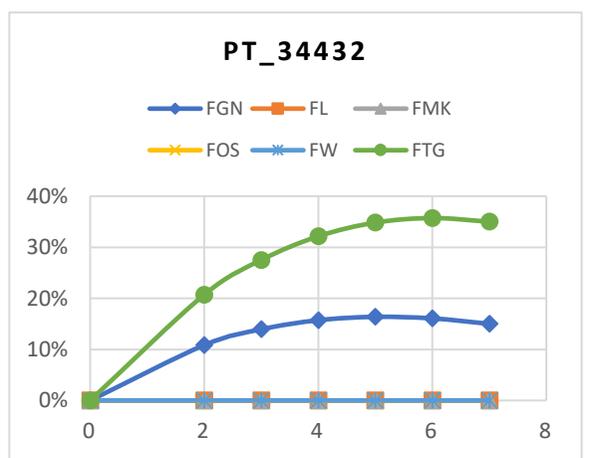
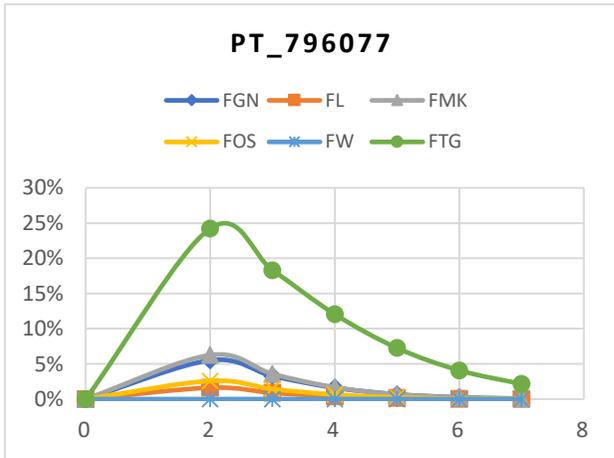
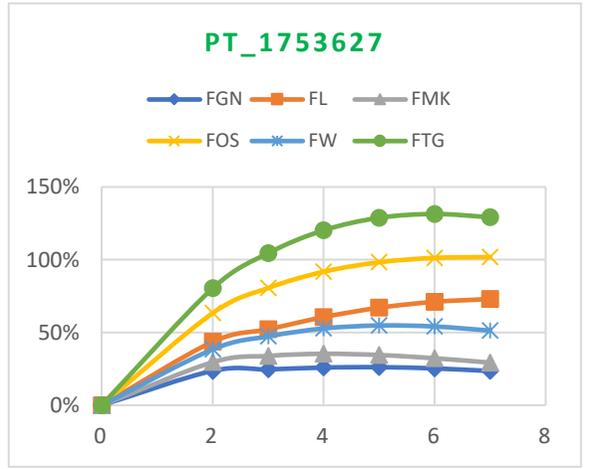
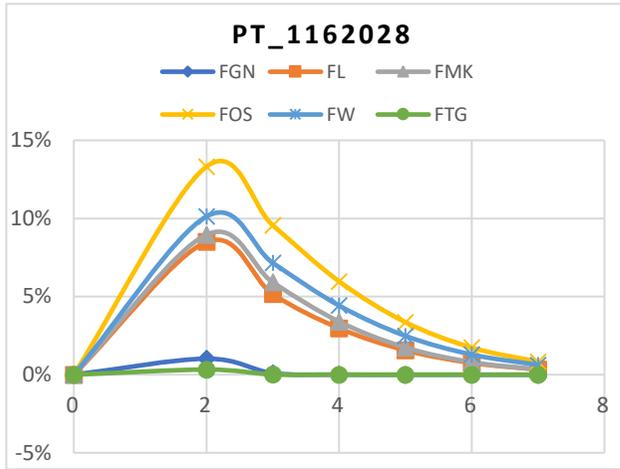


Figure S8. Neighbor joining tree inferred from the control region and including the six lineages of white-bellied pangolins. The tree includes 181 sequences from the Dahomey Gap, 59 sequences from Western Central Africa, 12 sequences from Western Africa, 9 sequences from Central Africa, 3 sequences from Ghana and 1 sequence from Gabon. [separate pdf]

Appendix 4

Figure S1. Rarefaction curves for samples of habitat types. Rarefaction curves drawn by randomly resampling a given number of reads 20 times, from which average diversity (DX) or sequencing coverage (line) and its SD value (shaded area) were computed. D0, D1, and D2 correspond to richness, exponential of the Shannon index, and inverse of the Simpson index respectively. Sequencing coverage corresponds to Good's coverage index

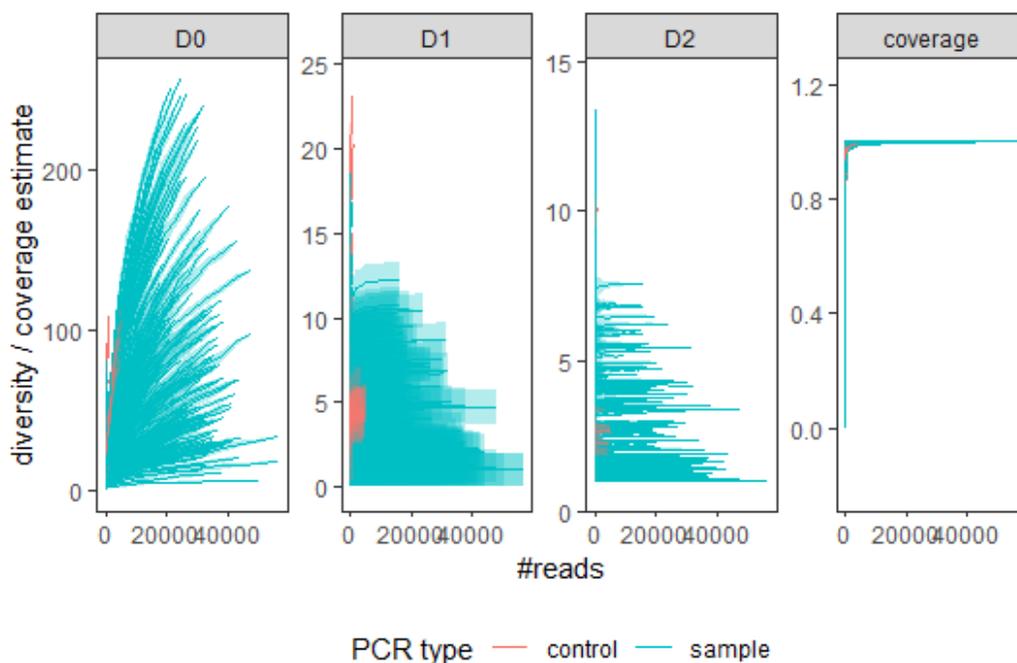


Table S1. The mock community used for positive PCR controls

Code	Ordre
313	Lepidoptera
PUC	Hemiptera (Homoptera)
330	Hemiptera (Homoptera)
450	Orthoptera
350	Thysanoptera
11_54	Hymenoptera
35	Coleoptera
DROSO	Diptera

Table S2. Crossed assignment of OTUs using public (EMBL) and Local reference databases

Family EMBL	Order EMBL	Family Local Ref	Genus EMBL	Species Local Ref	Hit Identity EMBL	Hit Identity Local Ref
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	80.00	100
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	80.28	100
NA	NA	Ponerinae	NA	<i>Platythyrea conradtiEM8</i>	80.36	100
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus Myrmotrema carboEM14</i>	80.43	100
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	80.47	100
NA	Hymenoptera	Hymenoptera	NA	<i>HymenopteraEM26</i>	80.53	100
NA	NA	Termitidae	NA	<i>TermiteEM19</i>	80.56	100
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole megacephalaEM25</i>	80.56	100
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL33</i>	80.75	100
Formicidae	Hymenoptera	Hymenoptera	NA	<i>HymenopteraEM26</i>	80.77	100
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	80.89	100
NA	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp7.EL1</i>	80.93	100
NA	NA	Termitidae	NA	<i>TermiteUAC16H10</i>	80.97	100
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus Myrmotrema carboEM14</i>	80.97	100
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp.1EM1</i>	81.00	100
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	81.02	100
Formicidae	Hymenoptera	Hymenoptera	NA	<i>HymenopteraEM26</i>	81.08	100
NA	Hymenoptera	Formicinae	NA	<i>Lepisiota capensisEM4</i>	81.19	100
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	81.19	100
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	81.25	100
NA	Blattodea	Termitidae	NA	<i>TermiteEM19</i>	81.25	100
NA	Blattodea	Termitidae	NA	<i>NasutitermitinaeEL6</i>	81.28	100
Termitidae	Blattodea	Termitidae	NA	<i>TermitesEL38</i>	81.31	100
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus Myrmopelta sp.EM51G12</i>	81.33	100
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus Myrmopelta sp.EM51G12</i>	81.42	100
Apidae	Hymenoptera	Formicinae	NA	<i>Lepisiota capensisEM4</i>	81.45	100
NA	Hymenoptera	Hymenoptera	NA	<i>HymenopteraEM26</i>	81.45	100
Formicidae	Hymenoptera	Hymenoptera	NA	<i>HymenopteraEM26</i>	81.46	100
NA	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp.1EM1</i>	81.82	100
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	82.19	100
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp7.EL1</i>	82.19	100
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole megacephalaEM25</i>	82.41	100
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus Myrmotrema carboEM14</i>	82.53	100
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus conradtiEM16</i>	82.67	100

Family EMBL	Order EMBL	Family Local Ref	Genus EMBL	Species Local Ref	Hit Identity EMBL	Hit Identity Local Ref
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus conradti</i> EM16	82.76	100
Formicidae	Hymenoptera	Myrmicinae	Cardiocondyla	<i>Pheidole sp</i> EL33	82.81	100
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp.</i> 1EM1	82.88	100
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus conradti</i> EM16	82.89	100
Formicidae	Hymenoptera	Formicinae	NA	<i>Lepisiota capensis</i> EM4	82.95	100
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp</i> 7.EL1	83.03	100
Formicidae	Hymenoptera	Myrmicinae	Cardiocondyla	<i>Crematogaster sp</i> 7.EL1	83.03	100
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus Myrmotrema carbo</i> EL20	83.18	100
NA	Blattodea	Termitidae	NA	<i>Termite</i> EL35	83.19	100
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole sp</i> EL10	83.33	100
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp.</i> EL37	83.56	100
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus conradti</i> EM16	83.56	100
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole sp</i> EL10	83.80	100
NA	Blattodea	Termitidae	NA	<i>Termites</i> UAC8F11	83.86	100
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole sp</i> EL10	83.94	100
Kalotermitidae	Blattodea	Termitidae	NA	<i>Termite</i> UAC16H10	84.00	100
Termitidae	Blattodea	Termitidae	NA	<i>Termite</i> EM19	84.02	100
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp.</i> 1EM1	84.47	100
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus Myrmotrema carbo</i> EL20	84.51	100
Formicidae	Hymenoptera	Myrmicinae	Cardiocondyla	<i>Crematogaster sp</i> EM30	84.58	100
Formicidae	Hymenoptera	Myrmicinae	Monomorium	<i>Crematogaster sp.</i> 6EL40	84.62	100
Formicidae	Hymenoptera	Myrmicinae	Pristomyrmex	<i>Crematogaster sp.</i> 1EM1	84.83	100
NA	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp.</i> 4EM28	82.33	99.524
NA	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp</i> 7.EL1	80.73	99.517
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole sp</i> EL10	80.00	99.495
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus Myrmotrema carbo</i> EM14	81.30	99.457
NA	Blattodea	Termitidae	NA	<i>Termite</i> EL35	82.59	99.451
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus conradti</i> EM16	83.56	99.438
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus acvapimensis</i> EM53E12	84.21	99.435
NA	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp.</i> 1EM1	80.00	99.425
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole sp</i> EL10	83.33	99.419
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole sp</i> EL10	81.86	99.415
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole sp</i> EL10	80.68	99.387
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole sp</i> EL10	82.19	99.387
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp</i> 7.EL1	82.65	99.375
NA	Blattodea	Termitidae	NA	<i>Termites</i> UAC8F11	84.07	99.286
NA	Blattodea	Termitidae	NA	<i>Termites</i> UAC8F11	81.22	99.225

Family EMBL	Order EMBL	Family Local Ref	Genus EMBL	Species Local Ref	Hit Identity EMBL	Hit Identity Local Ref
Termitidae	Blattodea	Termitidae	NA	<i>Termite</i> EM19	82.83	99.18
Termitidae	Blattodea	Termitidae	NA	<i>Termites</i> EL38	80.41	99.174
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus acvapimensis</i> EM53E12	83.84	99.099
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus conradti</i> EM16	83.70	99.095
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Cataulacus guineensis</i> EM11	81.00	99.052
Formicidae	Hymenoptera	Myrmicinae	Pheidole	<i>Pheidole</i> spEL10	83.19	99.052
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole</i> spEL10	80.18	99.015
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole</i> spEL10	80.09	99.005
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole</i> spEL10	81.39	98.985
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole</i> spEL10	80.09	98.98
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole</i> spEL10	81.40	98.925
Formicidae	Hymenoptera	Formicinae	NA	<i>Oecophylla longinoda</i> EL15	81.21	98.905
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus acvapimensis</i> EM53E12	81.86	98.901
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole</i> spEL10	81.86	98.857
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole</i> spEL10	81.40	98.837
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Crematogaster</i> sp.1EM1	83.78	98.824
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole</i> spEL10	82.87	98.817
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Crematogaster</i> sp7.EL1	83.03	98.817
Termitidae	Blattodea	Termitidae	NA	<i>Termite</i> EL35	82.99	98.75
NA	Hymenoptera	Hymenoptera	NA	<i>Hymenoptera</i> EM26	81.53	98.485
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus Myrmotrema carbo</i> EM14	81.22	98.37
NA	NA	Ponerinae	NA	<i>Platythyrea conradti</i> EM8	80.00	98.305
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus Myrmotrema carbo</i> EL20	82.74	98.276
Formicidae	Hymenoptera	Hymenoptera	NA	<i>Hymenoptera</i> EM26	81.66	98.256
Termitidae	Blattodea	Termitidae	NA	<i>Termites</i> EL38	84.58	98.171
NA	Hymenoptera	Myrmicinae	NA	<i>Crematogaster</i> sp7.EL1	81.74	98.137
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole</i> spEL10	80.45	98.113
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole</i> spEL10	82.03	98.101
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole</i> spEL10	80.95	97.714
Formicidae	Hymenoptera	Myrmicinae	Pheidole	<i>Pheidole megacephala</i> EM25	82.02	97.63
Formicidae	Hymenoptera	Myrmicinae	Pheidole	<i>Pheidole</i> spEL10	82.38	97.63
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole</i> spEL10	84.09	97.63
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole</i> spEL10	82.65	97.546
Kalotermitidae	Blattodea	Termitidae	NA	<i>Termite</i> UAC16H10	84.02	97.487
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus Myrmotrema carbo</i> EM14	80.69	97.297
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus conradti</i> EM16	81.66	97.207
Formicidae	Hymenoptera	Myrmicinae	Pheidole	<i>Pheidole</i> spEL10	81.66	97.17

Family EMBL	Order EMBL	Family Local Ref	Genus EMBL	Species Local Ref	Hit Identity EMBL	Hit Identity Local Ref
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	81.45	97.159
Formicidae	Hymenoptera	Formicinae	NA	<i>Polyrhachis militarisEL5</i>	80.00	97.143
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus Myrmotrema carboEM14</i>	81.47	97.143
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	83.03	97.093
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	82.95	97.059
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	82.41	97.041
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	82.95	97.041
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	80.91	97.03
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	81.36	97.03
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	82.51	96.939
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	80.45	96.933
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole megacephalaEM25</i>	83.87	96.933
Apidae	Hymenoptera	Formicinae	NA	<i>Lepisiota capensisEM4</i>	80.72	96.923
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	80.73	96.855
NA	Blattodea	Termitidae	NA	<i>TermitesEL38</i>	81.45	96.842
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus Myrmopelta sp.EM51G12</i>	81.42	96.774
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	83.11	96.698
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	83.18	96.698
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	83.26	96.698
NA	Hymenoptera	Hymenoptera	NA	<i>HymenopteraEM26</i>	81.28	96.682
NA	Hymenoptera	Dorylinae	NA	<i>Dorylus nigricansEM10</i>	81.70	96.682
NA	Hymenoptera	Hymenoptera	NA	<i>HymenopteraEM26</i>	81.82	96.682
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	82.11	96.682
NA	Blattodea	Termitidae	NA	<i>TermiteEL35</i>	80.09	96.667
Formicidae	Hymenoptera	Myrmicinae	Pristomyrmex	<i>Crematogaster sp.1EM1</i>	84.36	96.651
Termitidae	Blattodea	Termitidae	NA	<i>TermitesUAC8F11</i>	80.61	96.639
Termitidae	Blattodea	Termitidae	NA	<i>TermitesUAC8F11</i>	81.86	96.639
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus Myrmotrema carboEM14</i>	81.30	96.629
NA	Blattodea	Termitidae	NA	<i>TermiteUAC16H10</i>	82.81	96.585
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	81.82	96.552
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	81.53	96.535
NA	Blattodea	Termitidae	NA	<i>TermiteEL35</i>	83.11	96.535
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole megacephalaEM25</i>	82.11	96.491
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus Myrmotrema carboEM14</i>	80.26	96.429
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus Myrmotrema carboEM14</i>	81.97	96.396
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus conradtiEM16</i>	83.86	96.38
Termitidae	Blattodea	Termitidae	NA	<i>TermiteEM24</i>	82.11	96.33

Family EMBL	Order EMBL	Family Local Ref	Genus EMBL	Species Local Ref	Hit Identity EMBL	Hit Identity Local Ref
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp.1EM1</i>	83.87	96.296
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	82.81	96.262
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	83.41	96.244
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Cataulacus guineensisEM11</i>	80.18	96.226
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp7.EL1</i>	81.28	96.226
NA	NA	Termitidae	NA	<i>TermiteUAC16H10</i>	81.82	96.209
Formicidae	Hymenoptera	Myrmicinae	Pristomyrmex	<i>Crematogaster sp.1EM1</i>	83.89	96.172
NA	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp.1EM1</i>	82.71	96.154
NA	Blattodea	Termitidae	NA	<i>TermiteUAC16H10</i>	82.81	96.135
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus acvapimensisEM53E12</i>	84.89	96.133
Termitidae	Blattodea	Termitidae	NA	<i>NasutitermitinaeEL6</i>	81.00	96.078
NA	Blattodea	Termitidae	NA	<i>TermiteEL35</i>	83.48	96.078
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp.EL37</i>	83.41	96.067
NA	Blattodea	Termitidae	NA	<i>TermiteUAC16H10</i>	83.64	96.04
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole megacephalaEM25</i>	82.19	96.023
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus Myrmotrema carboEM14</i>	80.34	95.964
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus Myrmotrema carboEM14</i>	80.52	95.964
Termitidae	Blattodea	Termitidae	NA	<i>TermitesEL38</i>	83.86	95.96
Formicidae	Hymenoptera	Formicinae	Pheidole	<i>Camponotus Myrmotrema carboEM14</i>	80.08	95.946
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole megacephalaEM25</i>	83.03	95.93
NA	Hymenoptera	Hymenoptera	NA	<i>HymenopteraEM26</i>	81.65	95.897
Formicidae	Hymenoptera	Myrmicinae	Pheidole	<i>Pheidole spEL10</i>	82.46	95.794
Formicidae	Hymenoptera	Myrmicinae	Pheidole	<i>Pheidole spEL10</i>	83.77	95.775
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	82.35	95.755
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	82.81	95.755
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	82.27	95.735
NA	Hymenoptera	Hymenoptera	NA	<i>HymenopteraEM26</i>	82.65	95.735
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp.1EM1</i>	83.49	95.714
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	83.64	95.714
NA	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp7.EL1</i>	80.45	95.652
Termitidae	Blattodea	Termitidae	NA	<i>NasutitermitinaeEL6</i>	80.93	95.652
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus conradtiEM16</i>	83.62	95.516
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus conradtiEM16</i>	82.22	95.506
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole megacephalaEM25</i>	81.36	95.376
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole megacephalaEM25</i>	81.94	95.349
NA	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	80.79	95.305
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole spEL10</i>	82.03	95.294

Family EMBL	Order EMBL	Family Local Ref	Genus EMBL	Species Local Ref	Hit Identity EMBL	Hit Identity Local Ref
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp7.EL1</i>	82.81	95.294
Formicidae	Hymenoptera	Formicinae	Camponotus	<i>Camponotus conradiEM16</i>	81.74	95.288
Formicidae	Hymenoptera	Myrmicinae	Pheidole	<i>Pheidole megacephalaEM25</i>	82.53	95.283
Formicidae	Hymenoptera	Myrmicinae	Pheidole	<i>Pheidole spEL10</i>	82.67	95.283
Formicidae	Hymenoptera	Myrmicinae	NA	<i>Pheidole megacephalaEM25</i>	82.95	95.266
NA	Hymenoptera	Hymenoptera	NA	<i>HymenopteraEM26</i>	81.82	95.261
Formicidae	Hymenoptera	Myrmicinae	Pheidole	<i>Pheidole spEL10</i>	82.74	95.261
Formicidae	Hymenoptera	Myrmicinae	Pheidole	<i>Pheidole spEL10</i>	84.14	95.261
NA	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp7.EL1</i>	81.28	95.169
NA	Hymenoptera	Myrmicinae	NA	<i>Crematogaster sp.4EM28</i>	80.09	95.135
NA	Blattodea	Termitidae	NA	<i>NasutermitinaeEM33</i>	81.00	95.122
Termitidae	Blattodea	Termitidae	NA	<i>TermiteEM24</i>	82.59	95

Table S3. Number of reads, OTUs and PCRs included in the dataset following each stage of data filtering

Filtering Stage	Reads	OTUs	PCRs	Sequencing	Threshold
Step OBiTol					
Sequence assignment to samples	5752261	-	-	-	-
Low quality sequences and fragment size removal	5752261	-	-	-	-
Dereplication (US = Unique Sequence)	5752261	-	-	-	
Removal of singletons	5486436	-	-	-	
Clustering	5486436	-	-	-	97%
MetabaR					
Extraction control contaminants	0%	0	-		
PCR control contaminants	1%	2.462702e-04	2		> 10% read
Sequencing Contaminant removal	-	2.406193e-05	-	28	1%
Removal of OTUs below similarity threshold	28,49%	1123 (37,62%)	-	-	<85% with EMBL
Removal of non-target taxa		2.849805e-01			
Removal of PCRs below threshold for deficient	-	-	0.01315789		

Detected ants species using photography

Pheidole sp.EL10



Crematogaster sp.7.EL1



Crematogaster sp.1EM1



Crematogaster sp.EL37



Crematogaster sp.4EM28



Crematogaster spEM30



Crematogaster sp. 6EL40



Hymenoptera EM26



Detected termite species using photography

TermiteEM19



TermiteUAC16H10



TermitesEL38



TermiteEL35



TermiteEM24



NasutitermitinaeEL6



*Nasutitermitinae*EM33



*Termites*UAC8F11



Résumé

Les pangolins sont en tête des priorités en matière de conservation, principalement en raison du niveau de prélèvement sans précédent dont ils font l'objet. Les quatre espèces africaines manquent cruellement de données de référence, ce qui entrave la mise en place d'une stratégie de conservation bien conçue, basée sur des preuves, pour s'attaquer à leur état de conservation alarmant. Ma thèse de doctorat s'est concentrée sur la région biogéographiquement délimitée du Dahomey Gap (Afrique de l'Ouest) en utilisant le pangolin à ventre blanc comme étude de cas pour : (i) évaluer la distribution géographique et les tendances démographiques des espèces de pangolins au Bénin, (ii) modéliser la persistance actuelle et future des pangolins au Bénin, (iii) évaluer les valeurs ethnozoologiques et commerciales des pangolins au Bénin, (iv) étudier la génétique des populations de pangolins à ventre blanc et mettre en place le traçage moléculaire de leur commerce à petite échelle à travers le Dahomey Gap, et (v) évaluer avec précision la composition alimentaire du pangolin à ventre blanc au Bénin par métabarcodage de l'ADN électronique.

L'introduction présente une vue d'ensemble des espèces de pangolins en général et le contexte de conservation des pangolins à ventre blanc et des pangolins géants dans le Dahomey gap en particulier. Elle décrit les principaux objectifs et la structure de la thèse.

Le chapitre 1, intitulé *Zone d'étude*, définit clairement le Dahomey Gap traditionnel et celui biogéographiquement délimité à partir des pangolins à ventre blanc en utilisant les marqueurs mitochondriaux, et auquel cette thèse se réfère, notamment au chapitre 5. Il décrit les sites d'échantillonnage

comprenant les habitats forestiers, les marchés de viande de brousse et de médecine traditionnelle.

Le chapitre 2, intitulé *Évaluation de la dynamique spatio-temporelle des mammifères en danger grâce aux connaissances écologiques locales combinées à des évidences directes : cas des pangolins au Bénin (Afrique de l'Ouest)*, a mis en lumière la distribution géographique et les tendances démographiques des pangolins à ventre blanc et des pangolins géants au cours des deux dernières décennies au Bénin, en utilisant les connaissances écologiques locales et les preuves directes. Les connaissances locales ont révélé une contraction de 31 et 93 % des zones d'occurrence des pangolins à ventre blanc et des pangolins géants respectivement leux deux dernières decennies. Elles ont mis en évidence la dégradation de l'habitat et la surexploitation comme les principaux facteurs du déclin de la population des deux espèces.

Le chapitre 3, intitulé *Modélisation des taux de disparition des populations de pangolins à l'aide des connaissances écologiques locales validées*, a modélisé la probabilité de persistance de chaque espèce de pangolin au Bénin au cours des deux dernières décennies (1998-2018) et a prédit les modèles d'occurrence futurs à l'aide des connaissances écologiques locales, des évidences directes et des métadonnées du paysage. Le modèle linéaire généralisé a révélé que l'identité de l'espèce, l'abondance en 1998, les distances des villages aux routes principales et aux aires protégées, les changements d'utilisation des terres et la déforestation au fil du temps étaient les facteurs qui expliquaient de manière significative la persistance actuelle des pangolins au Bénin. Les modèles de prédiction suggéraient le déclin pour le pangolin à ventre blanc au cours des deux prochaines décennies et

une disparition totale du pangolin géant quel que soit le scénario envisagé (déforestation *versus* absence de déforestation).

Le chapitre 4, intitulé *Valeurs ethnozoologiques et commerciales des pangolins au Bénin*, a évalué l'ethnozoologie des pangolins, estimé le prix des pangolins et décrypté le réseau de commerce au Bénin par une analyse croisée des connaissances locales collectées sur les marchés de médecine traditionnelle et dans les zones rurales. Les pangolins sont des animaux de valeurs pour les populations locales et les connaissances ethnozoologiques varient considérablement du sud au nord du Bénin. Le réseau commercial comprenait des guérisseurs traditionnels, des restaurateurs, des vendeurs sur les marchés de médecine traditionnelle, des étrangers des pays voisins et la diaspora chinoise. Les pangolins dans les zones rurales et les marchés de médecine traditionnelle sont relativement moins chers au Bénin par rapport à d'autres pays d'Afrique centrale et de l'Ouest, mais leur prix est 3 à 8 fois plus élevé lorsque les clients sont issus de la diaspora chinoise.

Le chapitre 5, intitulé *L'ADN peut-elle aider au traçage du commerce des pangolins ? Génétique de conservation des pangolins à ventre blanc dans Dahomey Gap (Afrique de l'Ouest)*, a utilisé des marqueurs nucléaires et mitochondriaux pour comprendre la génétique de conservation du pangolin à ventre blanc dans le Dahomey Gap. Les analyses basées sur les microsatellites et l'ADN mitochondrial ont déterminé une faible diversité génétique, des populations consanguines, l'absence de subdivision géographique claire et un isolement par la distance entre les populations. L'inférence de l'histoire démographique a suggéré une diminution drastique de la taille effective de la population il y a 100-250 générations. Dans l'ensemble, l'analyse phylogénétique basée sur l'ADN mitochondrial a suggéré un commerce endémique au sein du Dahomey Gap affectant la

lignée des pangolins à ventre blanc du Dahomey Gap. Les marqueurs microsatellites ont mis en évidence un commerce de longue distance au sein et entre les pays du Dahomey Gap.

Le chapitre 6, intitulé *Évaluation précise du spectre alimentaire du pangolin à ventre blanc au Bénin en utilisant l'ADN environnementale*, a expérimenté pour la première fois la méthode de métabarcodage de l'ADN électronique pour étudier l'écologie alimentaire d'une espèce de pangolin. Cette étude a révélé que l'approche du code-barre ADN est appropriée pour déterminer la composition du régime alimentaire des pangolins. Le pangolin à ventre blanc se nourrit principalement de fourmis et de termites mais aussi probablement d'autres taxons d'insectes appartenant à la famille des Anisembiidae, Bethylidae, Endomychidae, Liposcelididae, Cicadellidae, Tephritidae, Acrididae, Thripidae, Cecidomyiidae, Chrysididae, Curculionidae et Pyrrhocoridae. La diversité de proies détectées était significativement plus élevée ($p < 0,001$) pour les échantillons de contenu intestinal (par rapport aux crottes). L'indice de dissimilarité de Bray-Curtis a montré des dissimilarités de profils alimentaires très significatives ($p < 0,001$) entre les régions du sud et du centre. La visualisation spatiale a montré une forte hétérogénéité des distances entre les échantillons de la région centrale, en particulier dans la forêt du Mont Kouffé, à l'inverse des échantillons de la région sud où les distances ont tendance à être presque homogènes ou nulles entre les échantillons, ce qui suggère une variation intra (région centrale) et inter du spectre des proies entre les régions.

La dernière section de cette thèse discute les résultats majeurs et pertinents, leurs implications pour la conservation des pangolins au Bénin et les perspectives pour les investigations futures. Les données sur la distribution devraient être prises en compte pour mettre à jour la carte de distribution

globale de l'UICN et la liste rouge pour le Bénin. Le contexte spatial mis en évidence pourrait être croisé avec les données de consanguinité des populations et l'érosion de la diversité génétique afin d'établir des priorités de conservation dans le Dahomey Gap. Le réseau de trafiquants et la tarification des pangolins représentent un outil essentiel pour lutter contre le commerce illégal. Les données sur le régime alimentaire renseignent davantage sur le rôle écologique des pangolins dans les écosystèmes naturels et pourraient améliorer la prise de décision dans les programmes d'alimentation artificielle pour le pangolin à ventre blanc. Cependant, la présence/absence du pangolin géant dans la forêt d'Alibori doit être confirmée par des évidences directes et les estimations de la taille des populations des deux espèces font défaut. Notre base de données de référence doit être améliorée pour une meilleure identification moléculaire des proies de termites détectées dans les crottes des pangolins. Des marqueurs de polymorphisme mononucléotidique devraient être utilisés pour étudier la structure génétique et le commerce illégal dans le Dahomey Gap.

Curriculum vitae

Stanislas was born on 28th May 1986 in Bohicon, Republic of Benin. He obtained his “Baccalaureat série D mention Assez-bien” at CEG 2 Abomey in 2006. He registered at Faculty of Technical Sciences, option Chemistry Biology and Geology. He graduated for a “Maitrise ès Science Natural” in 2011. He taught at secondary school from 2011 to 2014 before enrolled himself for a Master degree at Faculty of Agronomic Sciences. He graduated for a Master degree in Management of Natural Resources and Biodiversity “mention Très Bien”. He started his PhD research project on pangolins in April 2018 at Faculty of Agronomic Sciences and one year later (March 2019) at the University Toulouse 3-Paul Sabatier, “Laboratoire Evolution et Diversité Biologique”

Publications

In this dissertation

- **Zanvo S.**, Djagoun C.A.M.S., Azihou A.F., Djossa B., Afiademanyo K., Olayemi A., Agbangla C., Sinsin B., Gaubert P. 2022. Can DNA help trace the local trade of pangolins? Conservation genetics of white-bellied pangolins from the Dahomey Gap (West Africa). *BMC Ecology and Evolution* 22:16. <https://doi.org/10.1186/s12862-022-01971-5>
- **Zanvo S.**, Djagoun C.A.M.S., Azihou A.F., Djossa B., Sinsin B., Gaubert P. 2021. Ethnozoological and commercial drivers of the pangolin trade in Benin. *Journal of Ethnobiology and Ethnomedicine*, 17(1): 18. <https://doi.org/10.1186/s13002-021-0044446-z>.
- **Zanvo S.**, Gaubert P., Djagoun C.A.M.S., Azihou A.F., Djossa B., Sinsin B. 2020. Assessing the spatiotemporal dynamics of endangered mammals through local ecological knowledge combined with direct evidence: the case of pangolins in Benin (West Africa). *Global Ecology and Conservation*, 23: 1-12. <https://doi.org/10.1016/j.gecco.2020.e01085>.

- **Zanvo S.**, Djagoun C.A.M.S., Azihou A.F., Jeszequel C., Djossa B., Sinsin B., Gaubert P., Hugueny B. 2022. Modelling the current and future spatial persistence of pangolins in Benin (*Submitted to Conservation Biology*).
- **Zanvo S.**, Djagoun C.A.M.S., Pelozuelo A., Donald J., Azihou A.F., Djossa B., Sinsin B., Gaubert P. 2022. Assessing the precise dietary composition of the white-bellied pangolin in Benin through eDNA metabarcoding (*To be Submitted to Molecular Ecology and Resources*)

Satellite publications

- **Zanvo S.**, Djagoun C.A.M.S., Azihou A.F., Sinsin B., Gaubert P. 2021. Preservative chemicals as a new health risk related to traditional medicine markets in western Africa. *One Health*, 13:1-4. <https://doi.org/10.1016/j.onehlt.2021.100268>.
- Djagoun C.A.M.S., **Zanvo S.**, Padonou E.A., Sogbohossou E., Sinsin B. 2021. Perceptions of ecosystem services: A comparison between sacred and non-sacred forests in central Benin (West Africa). *Forest Ecology and Management*, 503: 119791.
- Djagoun C.A.M.S., **Zanvo S.**, Sogbohossou E., Sinsin B. 2020. Quantification and monitoring of terrestrial wildlife using line transect and camera trap methods. In: A. E. Assogbadjo, F. A. Y. Okou and B. Sinsin (Eds). Hand note for NTFP's Scientists. Abomey-Calavi, 2020. pp. 148-169
- **Zanvo S.** Appui à l'opérationnalisation des dispositifs de suivi écologique dans la Réserve de Biosphère Transfrontalière du Delta du Mono. Consultancy report of the project Réserve de Biosphère Transfrontalière du Delta du Mono. 65 pages.
- Houéhanou D.T., Assogbadjo A.E., Chadare F.J., **Zanvo S.**, Sinsin B. 2016. Approches méthodologiques synthétisées des études

d'ethnobotanique quantitative en milieu tropical. Annales des Sciences Agronomiques, 19: 187-206

Conferences and Workshops

- XV World Forestry Congress, South Korea, 2-6 May 2022 (Poster communication)
- International Conference on Pangolin, South Africa, 13-16 September 2021 (Virtual Oral Communication)
- Virtual meeting of the Association for Tropical Biology and Conservation, 21-23 July, 2021 (Oral communication)
- Ecolo'Tech Congress, Salon de l'Ecologie. Montpellier, 6 November 2020 (Poster communication)
- 1st workshops on forest and biodiversity conservation in Benin, Forest Services, Benin (June. 26-29, 2018). Oral communication
- Student Conference on Conservation Science (SCCS), 27-29 Mars 2018, University of Cambridge, U.K. Oral communication.
- XXII Congress of the Benin Association of Pastoralism (ABePa), November 20th, 2015.
- Mava Foundation grant for Participating in the training in Management of Protected Areas in West Africa IUCN-University of Alexandria, Ouagadougou, Burkina Faso, April 16-12th June 2016

Trainings

- **2021:** Assessing ecosystem services in agricultural and peri-urban spaces. Distance learning 2-4 March 2021.
- **2021:** FROGS: tools for bioinformatics and statistics analyses with amplicon metagenomics data. Toulouse, 15-18 march 2021
- **2021:** Theory and practice in metabarcoding for Biodiversity, iBioGen Consortium. Chypre, 7-13 January 2021 (online)
- **2020:** Bonnes pratiques pour une recherche reproductible en écologie numérique, Cesab-FRB et le GDR EcoStat. Montpellier, 2-6 Novembre 2020 (online).
- **2019 :** Ethique et recherche scientifique (Espace de Réflexion Ethique Occitanie). Toulouse, 28 Novembre 2019.

- **2018:** Life data statistics & spatial statistics: Applications to therapeutic trials, industrial reliability, epidemiology and climate change. Research School of International Centre of Pure and Applied Mathematics (CIMPA), Lomé 3rd-15th September 2018.
- **2018:** Short course in social survey, University of Cambridge, Department of Zoology, March 24-26, 2018. UK
- **2016:** Management of Protected Areas in West Africa, IUCN-University of Alexandria, Ouagadougou, Burkina Faso, April 16-12th June 2016

Research Grants

- **2019:** Pangolin Consortium Conservation Grant, Chicago Zoological Society.
<https://pangolinconsortium.org/grants/2018-pangolin-consortium-grantees/>
- **2016:** Rufford Small Grants for Nature Conservation Application for a research Project with
<https://www.rufford.org/projects/zanvo-stanislas/emerging-zoonotic-diseases-and-habitat-conservation-a-psychological-model-of-peoples-commitment-to-serve-forest-islands-in-the-dahomey-gap/>

Travel Grants

- **2018:** Research School of International Centre of Pure and Applied Mathematics (CIMPA), Lomé 3rd-15th September 2018.
- **2018:** Bursary from the Student Conference on Conservation Science, March 24-26, 2018. University of Cambridge, UK.
- **2016:** Management of Protected Areas in West Africa, IUCN-University of Alexandria, Ouagadougou, Burkina Faso, April 16-12th June 2016

Media events

- **2021 :** InFocus Podcast & Interviews : Interviews with Tropical Ecology Researchers.
<https://tropecol.wordpress.com/infocus/#stanislas-zanvo>

- **2021** : Emission radio le nid de pie (Toulouse FM) : 36_Fascinants termites & suivi du pangolin. <https://leniddepie.lepodcast.fr/36-fascinants-termites-et-suivi-du-pangolin-ft-din-dipita-et-stanislas-zanvo>