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**Systematics and biogeography of the genus
Cardiodactylus (Orthoptera: Eneopterinae: Lebinthini)
in the Southeast Asia**

Jiajia Dong

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Jiajia Dong

Le 24 Novembre 2017

**Systematics and biogeography of the genus *Cardiodactylus*
(Orthoptera: Eneopterinae: Lebinthini) in the Southeast Asia**

Sous la direction de : **Monsieur Robillard Tony, Maître de Conférences**
Monsieur Xu Shengquan, Professor

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Systematics and biogeography of the genus *Cardiodactylus* (Orthoptera: Eneopterinae: Lebinthini) in the Southeast Asia

Jiajia Dong

Abstract

Among the crickets of the subfamily Eneopterinae, *Cardiodactylus* Saussure, 1878 (Orthoptera: Eneopterinae: Lebinthini) is the most speciose and widely distributed genus. It consists of 82 species organized in two species groups (*Noveaguineae* and *Efordi* species groups). The species distributions encompass hundreds of islands and territories ranging from the coasts of Southeast Asia to the Samoan islands. *Cardiodactylus* species show contrastive distribution patterns, some being highly endemic while others are distributed in the whole Western Pacific region. This diversity coupled with the rich geological context of the Southeast Asian and Pacific regions, offer great opportunities to address biogeographical questions and investigate the dynamics of diversification in islands.

Despite recent efforts to improve the taxonomical knowledge of *Cardiodactylus*, their diversity is such high that many new species still continue to be discovered. In a first time, taxonomic studies based on morphological, molecular and acoustic data led to the description of five new species from Eastern New Guinea and to the redescription of two species.

In a second time, the molecular phylogeny of *Cardiodactylus* was reconstructed. The results support the monophyly of the genus, of the tested species, and of the *Novaeguineae* species group. The *Efordi* species group is clearly found paraphyletic, which tends to confirm the presence of a third species group, as suggested by the taxonomical study. According to the historical biogeographic analysis, the origin of *Cardiodactylus* was recovered in the Western Pacific during the Mid-Eocene (ca. 42 Ma). Through the transition zone of New Guinea, *Cardiodactylus* colonized Southeast Asia from East to West through three independent passageways: Java, Sulawesi and the Philippines. This work showed that the high diversity of Southeast Asian *Cardiodactylus* took place during the Miocene, as a result of accumulation of *in situ* diversification and immigration events.

In a third time, a protocol and sets of primers were designed to amplify mitogenomes in crickets. The method was exemplified by amplifying the mitogenome of the species *Cardiodactylus muiri* Otte, 2007 with a long-PCR plus NGS approach. The purpose of this work is to obtain more informative molecular data with relatively low-cost but high taxonomic coverage for future phylogenetic reconstructions.

Keywords: Biodiversity, Crickets, Geological history, Historical biogeography, Lebinthini, Mitogenome, Phylogeny, Southeast Asia, Systematics, Taxonomy.

Résumé

Parmi les grillons de la sous-famille Eneopterinae, *Cardiodactylus* Saussure, 1878 (Orthoptera : Eneopterinae : Lebinthini) est le genre avec le plus grand nombre d'espèces et le plus largement distribué de la sous-famille des Eneopterinae. Il comprend 82 espèces organisées en deux groupes d'espèces (groupes d'espèces *Novaeguineae* et *Efordi*). L'aire de distribution des espèces englobe des centaines d'îles et de territoires allant des côtes de l'Asie du Sud-Est aux îles Samoa. Les espèces de *Cardiodactylus* présentent des patrons de distribution contrastés, certaines étant fortement endémiques tandis que d'autres sont distribuées dans toute la région du Pacifique occidental. Cette diversité, conjuguée au riche contexte géologique des régions de l'Asie du Sud-Est et du Pacifique, offre de grandes opportunités pour aborder des questions biogéographiques et étudier la dynamique de la diversification insulaire.

Malgré les efforts récents pour améliorer les connaissances taxonomiques de *Cardiodactylus*, leur diversité est telle que de nombreuses nouvelles espèces restent encore à découvrir. Dans un premier temps, des études taxonomiques fondées sur des données morphologiques, moléculaires et acoustiques ont permis de décrire cinq nouvelles espèces de Nouvelle-Guinée orientale et de redécrire deux espèces.

Dans un second temps, la phylogénie moléculaire des *Cardiodactylus* a été reconstruite. Les résultats soutiennent la monophylie du genre, des espèces testées et du groupe d'espèces *Novaeguineae*. Le groupe d'espèces *Efordi* est clairement paraphylétique, ce qui tend à confirmer la présence d'un troisième groupe d'espèces, comme le suggère l'étude taxonomique. Selon l'analyse de biogéographie historique, l'origine de *Cardiodactylus* se situe dans le Pacifique Ouest au cours de l'Éocène moyen (environ 42 Ma). *Cardiodactylus* a ensuite colonisé l'Asie du Sud-Est depuis l'Est vers l'Ouest via une zone transition correspondant à la Nouvelle-Guinée, et ce en empruntant trois passages indépendants: Java, Sulawesi et les Philippines. Ce travail montre que la grande diversité de *Cardiodactylus* en Asie du Sud-Est s'est mise en place au Miocène à travers l'accumulation d'événements de diversification in situ et d'événements d'immigration.

Dans un troisième temps, un protocole et des jeux d'amorces ont été mis au point pour amplifier les mitogénomes de grillons. La méthode a été illustrée par l'amplification du mitogénome de l'espèce *Cardiodactylus muiri* Otte, 2007 par une approche PCR longue plus NGS. Le but de ce travail est d'obtenir des données moléculaires plus informatives à faible coût mais avec une couverture taxonomique élevée pour de futures reconstructions phylogénétiques.

Mots-clés : Biodiversité, Grillons, Histoire géologique, Biogéographie historique, Lebinthini, Mitogénome, Phylogénie, Asie du Sud-Est, Systématique, Taxonomie.

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General Introduction

1. Context and Aims of the Thesis

Biodiversity is the variety of life on earth and includes variation at all levels of biological organization from genes to species and ecosystems, involving terrestrial, marine, and other aquatic ecosystems and the ecological complexes of which they are part (Gaston & Spicer, 2004). Biodiversity boosts ecosystem productivity where each species, no matter how small, all have an important role to play. A healthy biodiversity offers many natural services, such as ecosystem services (protection of water resources, soils formation and protection, nutrient storage and recycling, pollution breakdown and absorption, contribution to climate stability, maintenance of ecosystems, recovery from unpredictable events), biological resources (food, medicinal resources and pharmaceutical drugs, wood products, ornamental plants, breeding stocks, population reservoirs, diversity in genes, species and ecosystems) and social benefits (research, education and monitoring, recreation and tourism and cultural values). Human cannot exist without biodiversity as we use it directly (e.g. biological resources) and indirectly (e.g. ecosystem services and social benefits) in various ways.

However, global biodiversity is changing at an unprecedented rate as a complex response to several human-induced changes in the global environment. For example, habitat change, climate change, invasive species, over-exploitation and pollution as the primary drivers lead to loss of biodiversity. Therefore, it is an emergency to study its components (from genes to species and ecosystems) and patterns (spatial and temporal) aiming at protecting biodiversity. Spatial patterns of biodiversity include hotspots, biomes, biogeographic realms, ecosystems, and ecoregions; and temporal patterns comprise background rates of extinction and biodiversity loss. These patterns involve not only the knowledge of taxonomic, functional, trophic, genetic, and other dimensions of biodiversity, but also the dynamics of changes in natural systems and the responses to those changes through time. In this context, biodiversity hotspots (Myers *et al.*, 2000) were defined as regions of the world where attention is needed to address biodiversity loss and to guide investments in terms of conservation. Understanding the historical biogeography of biodiversity hotspots is as important to long-term conservation

goals as ecology and evolution are to understanding current patterns and processes shaping biodiversity.

The purpose of this thesis is to review the cricket genus *Cardiodactylus* and describe new species based on information of morphology, distribution and calling songs (Chapter I); to detect the phylogenetic relationship within this genus using mitochondrial and nuclear markers, and explore the biogeographical patterns and dynamics of diversification of *Cardiodactylus* in Southeast Asia (Chapter II); and to develop using an easy and inexpensive access obtaining more informative molecular data and to reconstruct more robust phylogenies of *Cardiodactylus* and of crickets in general. .

2. Theoretical Context of Island Biogeography

2.1 Generalities about biogeography

Biogeography is the science studying the present and past patterns of distribution of the biological diversity and the processes resulting in these patterns (Crisci, 2001). It is a dynamic and burgeoning field that seeks to understand the role of historical factors in shaping biodiversity and, as importantly, to predict how biodiversity will respond to the rapidly changing world (Gelot, 2016). The definition and aims of biogeography are thus simple, but it is nonetheless very challenging to answer complex questions such as: Where do organisms come from? Why are organisms distributed as they do? How have faunas and floras assembled through time? (Crisci, 2001; Sanmartín, 2012).

In the process of studying biogeography, abiotic and biotic factor, and their interactions through time, have been attracting biogeographers' attention since the origin of the discipline (Figure 0.1). The abiotic factors include climate information, soil type, water chemistry, and a long list of other environmental conditions that vary in a highly nonrandom manner across geographic gradients of latitude, elevation, depth, and proximity to major landforms such as coastlines, islands and mountain ranges. Organisms can respond to this highly nonrandom

spatial variation through adaptations (either behavioral and/or physiological), dispersal, or speciation, or if unsuccessful at any of these responses, populations may suffer local extinction. All these responses, taken together, ultimately determine the geographic distributions and patterns of variation among populations, species, and communities across geographic gradients. On the other hand, not only are species distributions influenced by environmental conditions, but those distributions and related patterns are also influenced by interactions among the species themselves (e.g., mutualism, parasitism, predation and competitive exclusion). In addition, some species, called ecosystem engineers, can directly or indirectly modulate the availability of resources to other organisms by causing physical state changes in biotic or abiotic materials (Jones *et al.*, 1994); this is the case of large trees in the forest (Jones *et al.*, 1997) (Figure 0.2), human activities (Crisci *et al.*, 2006) (Figure 0.3), and micro-organisms in Earth's atmosphere (Womack *et al.*, 2010).

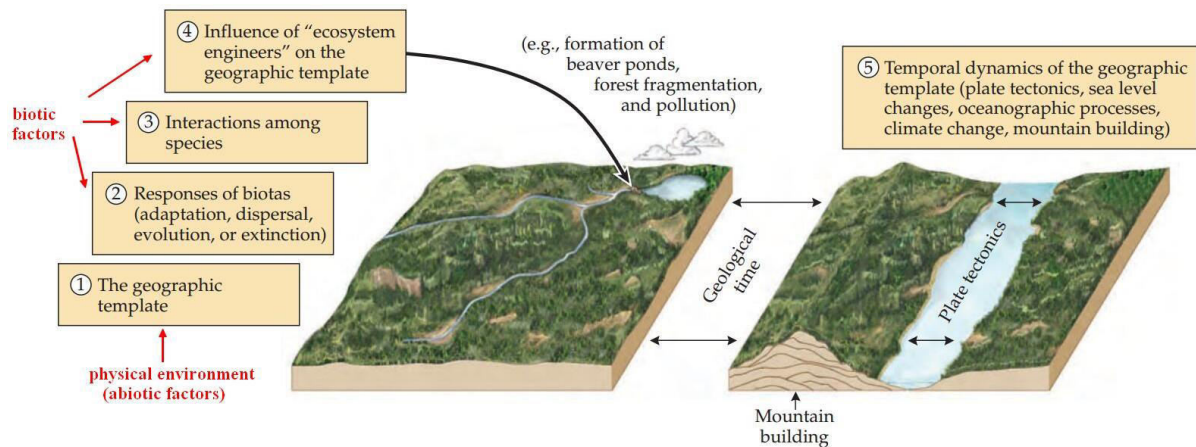


Figure 0.1. Interactions between abiotic and biotic factors in the process of biogeographical distribution diversity (modified from Lomolino *et al.*, 2010). Distributions and other patterns of geographic variation of life forms and communities result from (1) the highly non-random patterns of spatial variation in environmental characteristics across the Earth; (2) the responses of biotas (including adaptation, dispersal, evolution, or extinction) to this variation; (3) interactions among organisms (e.g., competitive exclusion and mutualistic interactions); (4) impacts of particular species- “ecosystem engineers” – such as humans on the geographic template; and (5) the temporal dynamics of the template (including the so-called TECO events – plate Tectonics, Eustatic changes in sea level, Climate change, and Orogeny [mountain building]).

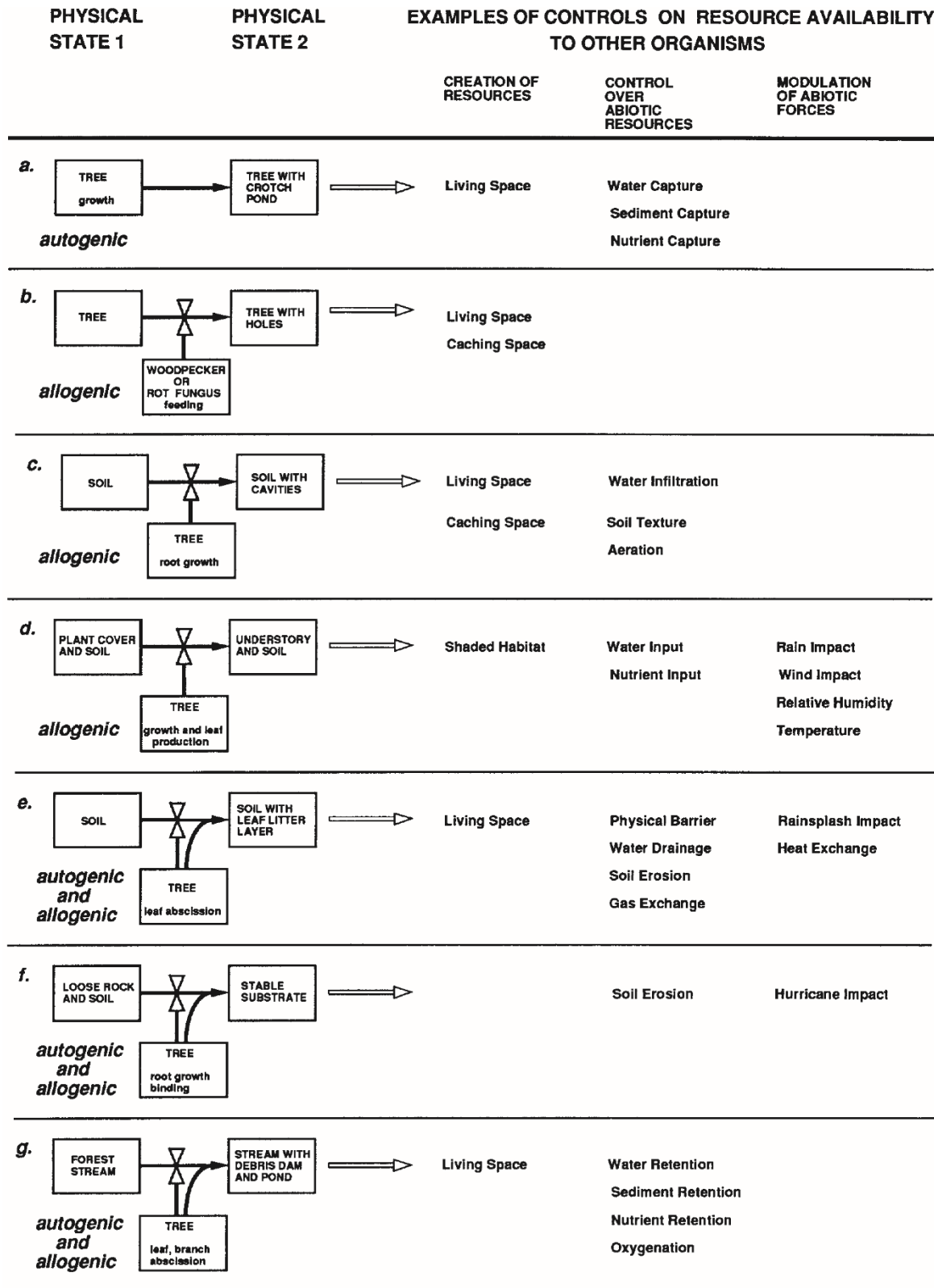


Figure 0.2. Trees as ecosystem engineers to the environment (from Jones *et al.*, 1997).

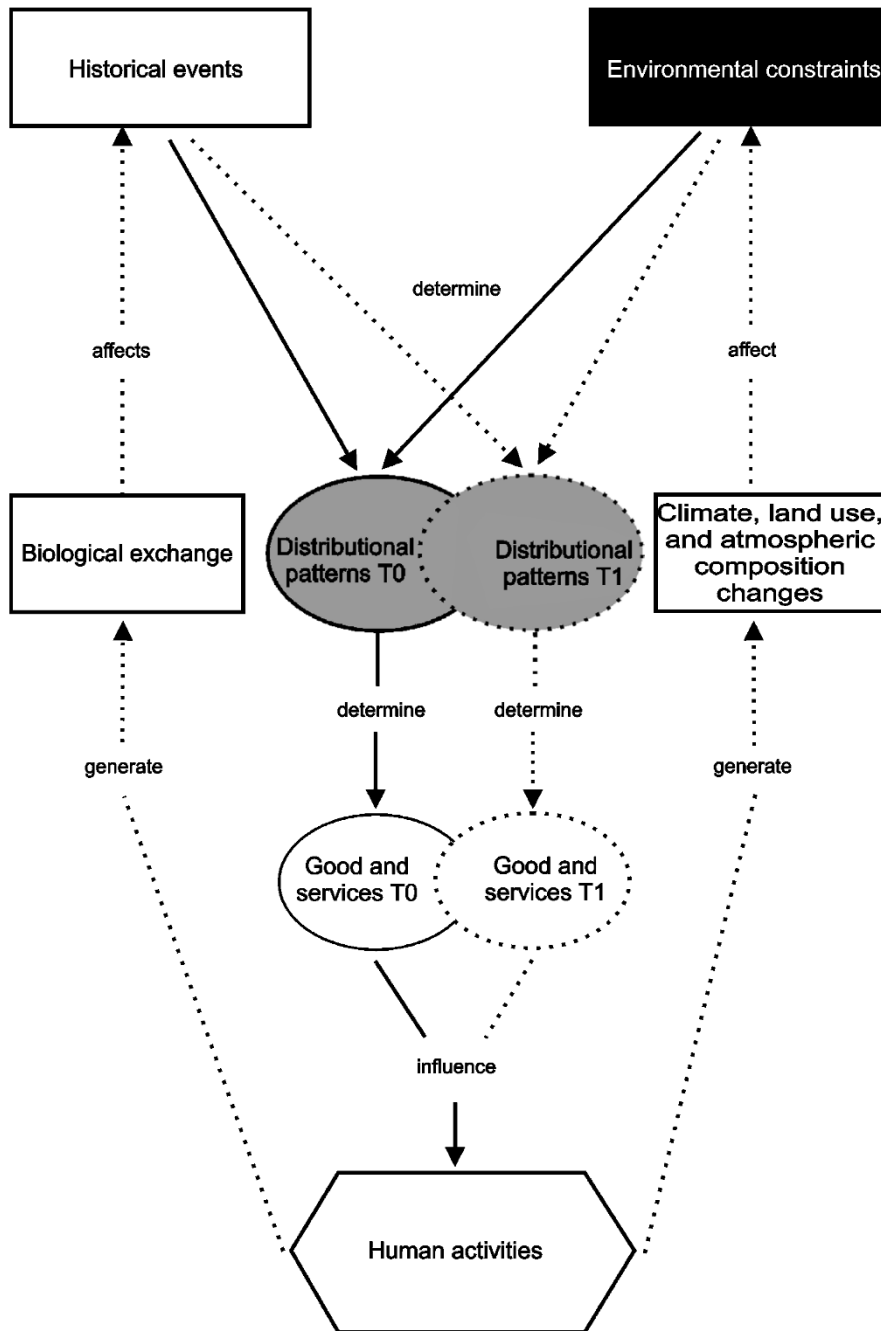


Figure 0.3. Concept map showing human activities influence and being influenced by distributional patterns of species through the changes in historical events and environmental constraints. Dotted lines indicate human-mediated changes on distributional patterns (from Crisci, 2006).

2.2. Two traditional approaches of biogeography

The first principle of biogeography was given by Buffon's law in 1761: "similar climatic and environmental conditions were often inhabited by distinct kinds of plants and animals" (Buffon and Comte de, 1761). Since the time of Buffon, biogeography has been divided into many branches and married within integrative concepts and information from ecology, evolutionary biology, geology and physical geography (Gehlot, 2016). However, regarding causal explanations, historical and ecological biogeography have been recognized as two traditional fields of the science of biogeography by the Swiss botanist Augustin Pyramus de Candolle in 1820 (Morrone & Crisci, 1995). Although both of two fields have the same objective of understanding the distribution of species in space and time, they use different epistemological approaches and focus on different scales (Table 0.1):

(1) spatiotemporal scales: ecological biogeography searches causal explanations of the distributional patterns in short temporal and small spatial scale, while historical biogeography concerns evolutionary processes occurring over millions of years, often on a large global scale;

(2) organization level: ecological biogeography is based on functional groups of species and environmental constraints, whereas historical biogeography focuses on taxonomic groups and historical biogeographical events (Crisci *et al.*, 2006).

During the last decades, the distinction between these two traditional approaches has become blurred, with the idea that distributional patterns cannot be fully understood without an integration of both subdisciplines, ecological and historical biogeography (Crisci *et al.*, 2006; Sanmartín, 2012) (Figure 0.4). In particular, the theory of both traditional biogeographical approaches has been directly or indirectly implemented on the study of island biotas (Santos *et al.*, 2016).

Table 0.1. Difference between ecological and historical biogeography.

	Spatial scale	Temporal scale	Functional group	Constraint factor
Ecological biogeography	small (e.g. limited area range)	short (e.g. years)	species	environmental constraints (e.g. niche)
Historical biogeography	large (e.g. global)	long (e.g. millions of years)	taxonomic groups	historical biogeographic events

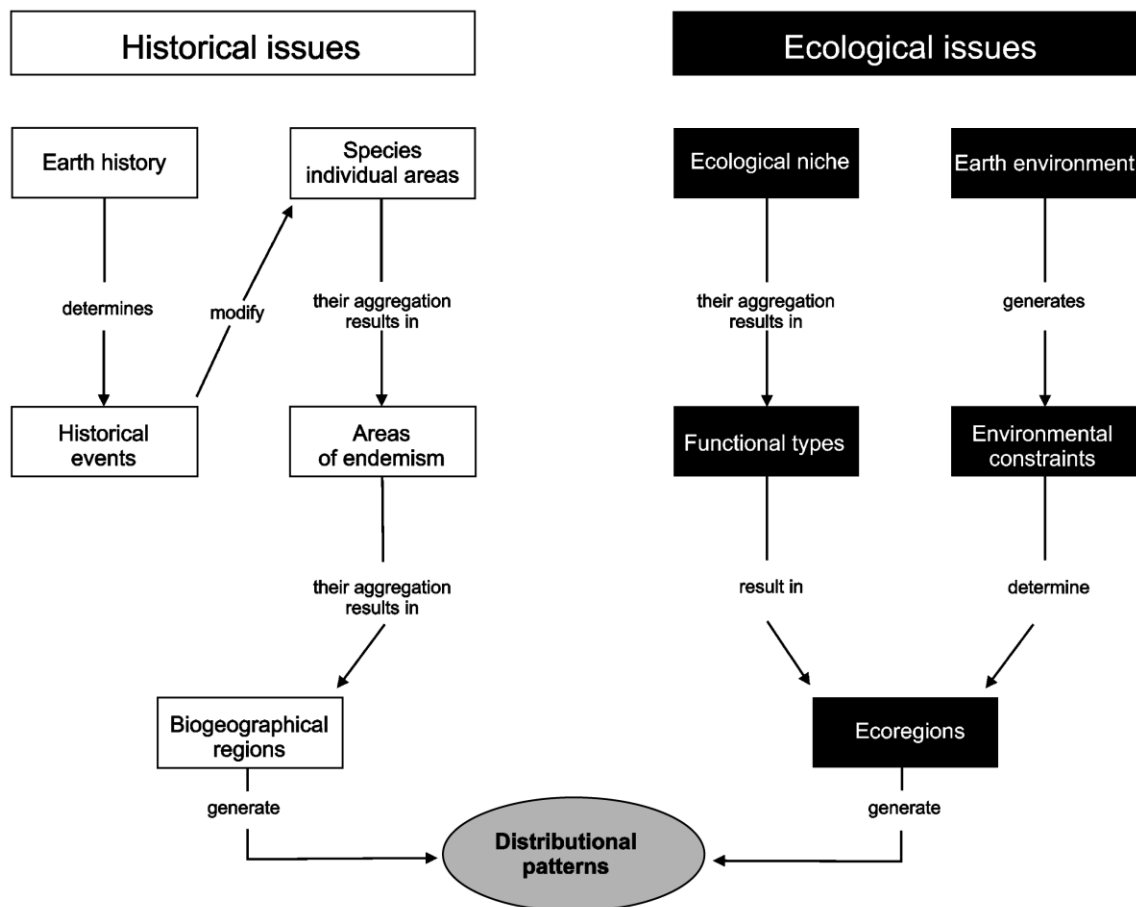


Figure 0.4. Concept map showing the integration between historical and ecological biogeography (from Crisci, 2006). Historical issues are indicated in white boxes and ecological issues in black boxes. Their interaction is indicated in gray.

2.3. Islands as models to study biogeography

Islands have been considered as natural laboratories to study evolution and the assets of islands as model systems for Darwin, Wallace, MacArthur and Wilson, are easily recognizable (e.g., Brown & Lomolino, 2000; Emerson, 2002; Losos & Ricklefs, 2009; Warren *et al.*, 2015). Island systems present ecological, evolutionary and geological processes on time-scales that are relatively similar, and often quite recent (Santos *et al.*, 2016). The habitats of islands often represent much more manageable study areas because they are more condensed than larger ecosystems on the mainland. Islands are also ideal locations because they allow scientists to look at habitats that invasive species have only recently colonized, in order to observe how they disperse throughout the island and impact it. They can then apply their understanding to similar, but more complex, mainland habitats. In addition, islands are very diverse in their biomes, ranging from tropical to arctic climates. This diversity of islands in terms of habitat allows for a wide range of studies in different parts of the world. The study of biogeography greatly benefited from the study of islands.

Based upon the ideas of Darwin and Wallace, three traditional categories of island systems were defined as follows: 1) recent continental island, 2) ancient continental island, and 3) oceanic island (Ali, 2017). The first two categories, recent and ancient continental islands, are classified by the time when they separated from the continent. The first category is found in shelf settings adjacent to a nearby continent, their current insularity resulting from the high global sea levels associated with Earth presently being in an interglacial state (e.g. Bintanja *et al.*, 2005). The biological suites on the islands and the adjacent hinterlands are largely identical having been separated only recently, e.g., Wallace listed the British Isles, Borneo and Java in Wallacean region, Taiwan Island from China. The second type of island bio-system, ancient continental, occurs on the continental blocks that are surrounded by deep waters, typically 2.5–5.5 km. The formation time of such landmasses could be dated back to several hundred million years ago, and such landmasses remain isolated even during the extreme lows in global sea level (90–125 m) below the present day (Bintanja *et al.*, 2005), e.g.

Madagascar, Seychelles and the Greater Antilles (Jamaica, Cuba, Hispaniola and Puerto Rico). One important characteristic is that the divergences between the assemblages of species on the islands and the nearest continents are wide and ancient, i.e. they often diverged at the family level (Goldberg *et al.*, 2008). The third category, oceanic island, is found on the volcanic constructs built atop deep sea floors. Having emerged from the waters as blank slates, the substrates are initially devoid of terrestrial life. In some instance, the active cone or extinct edifice is subaerial (e.g. Hawaii, Galapagos, Canaries, St Helena), in others the peak is inundated, but crowned by a coral atoll (e.g. Maldives, Carolines).

However, from a modern biological perspective, it is more useful to consider an island as any area in which a set of biota is isolated as a result of being surrounded by a dramatically different habitat matrix (Gillespie, 2017). Except for the oceanic island in traditional island bio-systems, three other island bio-systems were integrated and classified by the following types: (1) sky islands on mountaintops: The environments of mountaintops are often quite different from the surrounding slopes, and can include cloud forests, alpine grasslands, herb fields and paramo; these sites tend to be relictual fragments of previously more widespread habitats, isolated by the changing climate; (2) rock cavity islands: caves can be considered rock cavity island with the taxa adapted to these systems isolated both by the impenetrable rock surrounding them and by the surface environment; (3) fragmented habitat: the process of fragmentation has resulted in insularization at many different scales across space and time.

Thus, the main feature that make islands biologically interesting is their isolation, and the barriers that impose isolation, which varies according to the organisms in question.

2.4. Island Biogeography Theory

Since MacArthur and Wilson published their 'An equilibrium theory of insular zoogeography' (MacArthur & Wilson, 1963), island systems, being provided as model systems, have played an important role in the development of biogeographical, evolutionary and ecological theory (Warren *et al.*, 2015).

The basic model of island biogeography is one of the dynamic equilibrium between immigration of new species onto islands and the extinction of species previously established. There are two things to note immediately: 1) This is a dynamic equilibrium, not a static one. Species continue to immigrate over an indefinite period, not all are successful in becoming established on the island. Some that have been resident on the island go extinct. However, the model predicts only the equilibrium number of species, will remain 'fixed'. The species list for the island changes; those changes are called turnover. 2) The model only explicitly applies to the non-interactive phase of island history. Initially, at least, we will consider only events and dynamics over an ecological time scale, and one which assumes ecological interactions on the island occur as a result of random filling of niches, without adaptations to the presence of interacting species developing there.

The field of Island biogeography has provide some genuinely transformative insights for the biological sciences (Lomolino & Brown, 2009), it remains to contribute a number of biological questions that involve islands as key elements, which are related to: (1) priority effects for community assembly – changes in species abundance that might occur after colonization, the influence of existing species on the establishment of newly arrived species and the influence of island area and isolation on community assembly, equilibrium and evolution; (2) ecosystem functioning – particularly the contribution of *in situ* evolution to changes in rates of energy flux and nutrient cycling; and (3) speciation and diversification – the role of gene flow in speciation, and the more rapid diversification of some lineages in comparison with others (Santo *et al.*, 2016).

2.5. Studied region: Southeast Asia

This thesis focusses on the biogeography of a cricket genus diversified in the region of Southeast Asia (SEA). Southeast Asia covers only 4% of the earth's land area but is homes to 20-25% of the planet and animal species (Woodruff, 2010). It comprises four of 25 global biodiversity hotspots (Indo-Burma, Sundaland, Wallacea and the Philippines), three of the 17 global megadiverse countries (Indonesia, Malaysia, and the Philippines) and the most diverse coral reefs in the world, involving more than 20,000 islands straddling the equator (Myers *et al.*, 2000, Lohman *et al.*, 2011). Besides of that, SEA has been considered as one of the most geological dynamic regions in the world. Its geological history is a long-term complexity of continent-continent and continent-arc collisions and the geography of this region has changed considerably in the history (Metcalf, 2011a, b). The various continental pieces that now make up SEA were all derived from the southern hemisphere supercontinent Gondwana and travelled north to progressively collide and coalesce prior to the current ongoing collision with the northwards moving Australian continent (more details in Chapter II). The core of SEA was assembled from continental blocks that separated from Gondwana in the Paleozoic and amalgamated with Asian blocks in the Triassic. Fragments of these Gondwana/Cathaysia blocks rifted and separated from Asia and later reamalgamated with the Southeast Asian continental core. Mesozoic rifting of fragments from the Australian margins followed by Cretaceous collisions, and Cenozoic collision of Australia with the SE Asian margin added more continental crust (Hall, 2011).

The complex history of SEA has given rise to a diverse and highly endemic biota in this region. In turn, its complex geological fragmentation pattern and history, megadiverse and highly endemic biota attract more and more attentions, both on the study of geology and biogeography, especially in the field of evolutionary and island biogeography (Lohman *et al.*, 2011).

3. Study Group: the crickets *Cardiodactylus*

3.1. Crickets and island biogeography

The insects have had a long history since the divergence of the Hexapoda from the Crustacea hundreds of millions of years ago (Footitt & Adler, 2009; Misof *et al.*, 2014). During this time the Earth has undergone much evolution itself, from droughts to floods, from ice ages to arid heat. Extra-terrestrial objects have collided with the Earth, and major extinction events have occurred periodically. Through this long time, insects have changed their ranges, evolved to display the enormous modern diversity (including numbers of species and phylogenetic diversity), and adapted the environment successfully (Condamine *et al.*, 2016; Footitt & Adler, 2009; Gressitt, 1974; Grimaldi & Engel, 2005; Mayhew, 2007; Labandeira & Sepkoski, 1993).

Crickets (Orthoptera: Grylloidea) are an excellent group to study island biogeography (Otte & Cowper, 2009). On the one hand, they have high dispersal abilities and have reached some of the most remote oceanic archipelagos worldwide, by colonizing the islands on their own (i.e. randomly through wind or ocean currents) or carried by human (Otte, 1994; Otte, 2007a; Otte & Cowper, 2009). On the other hand, their omnivorous behavior, without specific food requirements in the new habitats, facilitates their ability to colonize distant habitats and adapt new environments (Otte & Cowper, 2009). In addition, although cricket species diversity is generally higher on older and larger islands, crickets are believed to be capable of speciating at small spatial scales and low levels of geographic isolation. For example, most of the diversity of flightless Caribbean cricket, *Amphiacusta*, was generated under a combination of intra-island diversification and island-island vicariance (Oneal *et al.*, 2010); divergence in courtship or sexual behavior driven rapid speciation in a group of forest-dwelling Hawaiian crickets, *Laupala* (Mendelson & Shaw, 2005); microendemism in New Caledonia explained the allopatric speciation in its endemic cricket genus, *Agnotecous* (Nattier *et al.*, 2012). And recent study on Indian ocean island crickets (Mogoplistinae: Ornebius) investigated an intra-island speciation scenario relying on the existence of genetic, song and ecological divergence

at intra-island scale, which in turns invoke the discussion about ecological differences between species and differences in environmental history between islands (Warren et al., 2016).

3.2. Presentation of the genus *Cardiodactylus*

This thesis focuses on one genus of crickets, *Cardiodactylus* Saussure, 1878, which belongs to the tribe Lebinthini in the subfamily Eneopterinae. This group has been the subject of many studies during the last 15 years about the evolution of their communication systems and calling songs (e.g. Desutter-Grandcolas, 2004; Robillard & Desutter-Grandcolas, 2004a, b, c). The Lebinthini crickets are a diversified tribe mostly distributed in Southeast Asia and in the Western Pacific archipelagos. It consists of a monophyletic group including two main groups of species: the long-winged species corresponding to the speciose genus *Cardiodactylus* Saussure, 1878 (e.g. Robillard *et al.*, 2014; Dong & Robillard, 2016), and the brachypterous species corresponding to several genera (Robillard *et al.*, 2016). An original system of communication was recently found in the Lebinthini, involving high-frequency calling songs (Robillard & Desutter-Grandcolas, 2004b), female vibrational responses to male call and loss of phonotaxis (ter Hofstede *et al.*, 2015). To investigate how these original traits evolved and diversified as a new system of communication, it is necessary to use a clear taxonomical, phylogenetic and biogeographical context. In turn, this necessitates taxonomic and biological studies for as many species as possible.

As currently the most speciose and diverse genus of the cricket subfamily Eneopterinae, *Cardiodactylus* is widely distributed, both in islands and continents, ranging from the northern Ryukyu Islands, to the coasts of Southeast Asia, Northern Australia and in many archipelagos in the Western Pacific encompassing Indonesia, Papua New-Guinea, the Philippines, the Solomon Islands, Vanuatu, New Caledonia, the Caroline Islands, Palau, Wallis and Futuna, Fidji and the Samoan Islands (Figure 0.5) (Otte, 2007a; Robillard & Ichikawa, 2009; Robillard 2009, 2011, 2014; Robillard *et al.*, 2014). Until now, a total 82 species are recorded in this genus, including 60 species in the species group *Novaeguineae*

and 22 in the species group *Efordi* (Cigliano *et al.*, 2017). Between the two species groups of *Cardiodactylus*, the distribution range of *Novaeguineae* is wider than the other, spreading from SEA to the Western Pacific. This is especially true in the case of the species *C. novaeguineae*, which sole distribution encompasses most of this area (Robillard & Ichikawa, 2009). In contrast, the species of the *Efordi* species group are only distributed from New Guinea to the Western Pacific, except for the species *C. pictus* which is found both in New Guinea and in Moluccas, i.e, east to the margin of the Lydekker Line. Therefore, Moluccas become the distribution boundary of the *Efordi* species group out of SEA. Despite the taxonomic revision of the genus has started several years ago, clarifications and further taxonomic descriptions are still necessary to improve our knowledge about the genus (see Chapter I).

Vicente *et al.* (2017) investigated the historical biogeography of the subfamily Eneopterinae and found that *Cardiodactylus* originated from SEA ca. 42 Ma. The wide scale of this study however did not allow to distinguish the detail within this complex area, which contains both continents and many archipelagoes and islands. While providing a spatio-temporal context to the study of *Cardiodactylus*, these results do not solve the question of the origin of *Cardiodactylus*. Did the genus originate from the continent and then colonize the island systems, or the reverse? Furthermore, how and when did *Cardiodactylus* colonize the Pacific from Southeast Asia? (see Chapter II).

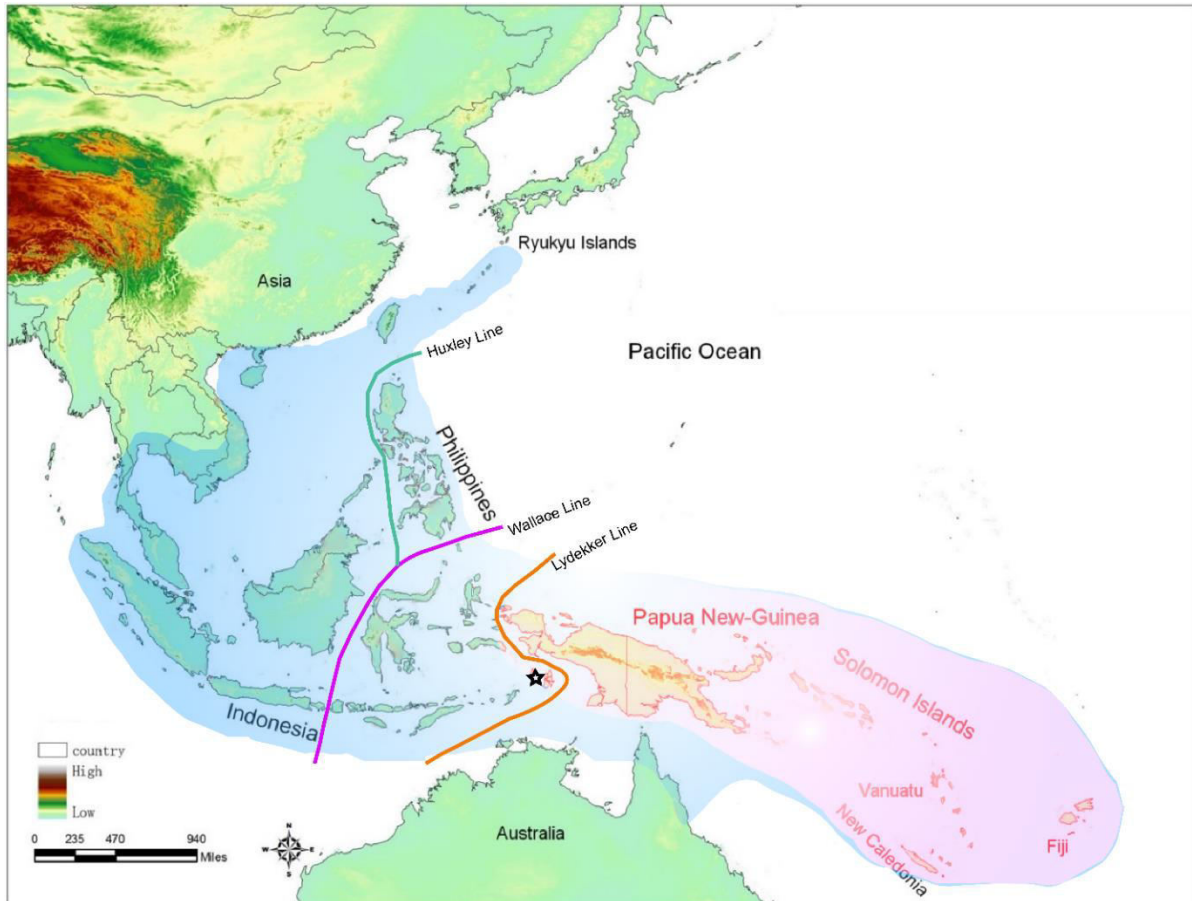


Figure 0.5. Distribution map of the genus *Cardiodactylus*. The purple line corresponds the Wallace line, and the orange line corresponds the Lydekker line. The shadow area indicates the distribution area of the genus *Cardiodactylus*, the red shadow area indicates the distribution region of *Efordi* species group. The star corresponds the locality of *Cardiodactylus pictus* in Moluccas. The Caroline Islands, the Samoan Islands and Wallis and Futuna are not shown on the map.

3.3 *Cardiodactylus* as a model to study the biogeography in Southeast Asia

In SEA 36 species of *Cardiodactylus* are present, among which 35 belong to the *Novaeguineae* species group and one to the *Efordi* species group (*C. pictus*) (Figure 0.5). It represents 58% of the diversity of the *Novaeguineae* species group (total number: 60) and 42% of the whole genus (total number: 82). Among them, the distribution area of *C. guttulus* covered from the northern Ryukyu Islands to the southeastern coast of China (Taiwan and Hainan), extending to the coast of Vietnam. But *C. guttulus* did not go further toward Chinese mainland. Another species, *C. thailandia* was recorded in the south of Thailand, not spreading to the north of Thailand based on the current data. This thesis aims at answering questions such as: Why did *Cardiodactylus* have ability to cross the sea spreading from the northern Ryukyu Islands to the coasts of Vietnam, but does not spread further in the continent? Moreover, *C. borneoe* was recovered both in Borneo and Malaysia (Tioman Island). Where did *Cardiodactylus* colonize first, Borneo or Malaysia? Or did the genus arrive in Borneo or Malaysia at the same time when these two areas were bound up together as a unique landmass (see Chapter II; Hall, 2009)? Similar case of interest concern *C. empagatao*, which is distributed both in the Philippines and in the north of Sulawesi, a distribution pattern crossing the Wallace line. Besides species such as mentioned above show a more or less widespread area of distribution, most *Cardiodactylus* species are more endemic, with a distribution restricted to one island (for example, *C. doloduo*, *C. contrarius*, *C. oeroe* and *C. fruhstorferi* in Sulawesi) or several adjacent small islands (i.e., *C. lombrinjani* in the Lesser Sunda islands, and *C. muiri* in Moluccas).

4. Main Results of the Thesis

In Chapter I, a taxonomic review of the genus *Cardiodactylus* is represented with description of five new species from Western New Guinea, *C. baitabensis* Dong & Robillard, 2016, *C. haddocki* Dong & Robillard, 2016, *C. lucus* Dong & Robillard, 2016, *C. niugini* Dong & Robillard, 2016 and *C. nobilis* Dong & Robillard, 2016, involving the morphological characters, distribution, habitat and calling songs. We also redescribed the species *C. javarere* Otte, 2007 and *C. busu* Otte, 2007 to complete the previous descriptions.

Interestingly, the comparison of the species *C. javarere* and *C. kolombangara* Otte, 2007 show intermediate character between the species groups *Novaeguineae* and *Efordi*, which suggests that these two species could be considered as a third species group, a hypothesis that will necessitate further tests with both on molecular and morphological analyses.

In Chapter II, I investigated the biogeographical patterns and dynamics of diversification of *Cardiodactylus* in SEA. The monophyly of the genus and of the species is recovered, as well as that of the *Novaeguineae* species group. However, the *Efordi* species group is clearly found paraphyletic in the molecular phylogenetic analysis, which tends to confirm the presence of a third species group, as suggested by the taxonomical study.

The origin of *Cardiodactylus* was recovered in Western Pacific during the Mid-Eocene (ca. 42 Ma). Through the transition zone of New Guinea, *Cardiodactylus* began to colonize SEA from east to west via three independent passageways: Java, Sulawesi and the Philippines. The colonization events happened more frequently during Miocene compared with the events that happened during the Eocene and the Plio-Pleistocene.

By summing the total numbers of emigration and immigration events through time in SEA, we find the following results: for three areas (Lesser Sunda Islands, the Moluccas and Palawan) only immigration events are inferred; the level of immigration and emigration are at equilibrium in Java, Sulawesi and New Guinea; emigration events are more frequent than immigrations for the Philippines and Western Pacific, while the opposite is recovered in all

remaining areas (Borneo, Indochina, Sumatra and the Thai-Malay Peninsula). By summing the total numbers of *in situ* diversification, the largest account is for Sulawesi, followed by the Lesser Sunda Islands.

According to the result of diversification analysis, there is no rate shift in diversification rates within the genus *Cardiodactylus*. Finally, the highest diversity of *Cardiodactylus* in SEA, found in Sulawesi, resulted by accumulation of both immigration and *in situ* diversification events.

In Chapter III, we defined a protocol and sets of primers to amplify mitogenomes in crickets, and more specifically in Eneopterinae and *Cardiodactylus*, with the purpose of increasing the size of molecular data sets at relatively low-cost and with high taxonomic coverage for future phylogenetic reconstructions. We exemplify this method by amplifying the mitogenome of the species *Cardiodactylus muiri* Otte, 2007 with a long-PCR approach, sequenced it on an Ion Torrent Personal Genome Machine (PGM) with the technology of next generation sequencing, assembly with high-coverage and reported its components (protein-coding genes, ribosomal RNA genes and noncoding region) and their orders.

We also compared *C. muiri* mitogenome with existing mitogenomes in the subfamily Gryllinae, closely related to the subfamily Eneopterinae. We compared the mitogenome lengths, nucleotide contents (A and T content, AT and GC-skew), gene order and orientation. Our results lead to the conclusion that the mitogenomes in crickets have a relatively stable structural and functional evolution, as suggested by previous studies made in Orthoptera and in insects in general.

CHAPTER I

A Taxonomic Review of the genus *Cardiodactylus*

Saussure, 1878 (Orthoptera: Gryllidae:

Eneopterinae), with description of five new species

I.1. Chapter Introduction

To investigate the origin and evolution of crickets on island, taxonomic and biological information is needed for as many species as possible. Under clear classification and definition, the precise descriptions and distribution pattern are necessary and helpful to estimate the patterns of diversification and evaluate the dynamics of radiation.

This chapter was carried out to review the taxonomic knowledge about the genus *Cardiodactylus* (section I.2) and described five new species from Eastern New Guinea (section I.3).

I.2. Taxonomic Background of *Cardiodactylus*

Successive exploration on Cardiodactylus taxonomic work

The genus *Cardiodactylus*, belonging to the tribe Lebinthini of the subfamily Eneopterinae (Robillard & Desutter-Grandcolas, 2008), was established by Saussure in 1878. Saussure included six species in the genus: he transferred two species from the genus *Platydactylus*, *C. novaeguineae* (Haan, 1842) (the type species), *C. gaimardi* (Serville, 1838), and described four new species (*C. pictus*, *C. canotus*, *C. haanii* and *C. rufidulus*) (Saussure 1878).

Until recently, the taxonomic work about this genus developed very slowly, since it included only nine valid species and two *nomina dubia* in 2000 (Table I and Figure I). Otte (2007a, b) contributed a lot in this genus by describing 39 new species from the western Pacific region. He also outlined the distribution map of the genus, and organized it in two species groups based on the structure of the male genitalia (*Novaeguineae* and *Efordi* species groups). Of these 39 species, 36 are still valid today: *C. malangona* was listed at the beginning of the paper but was missing from the main part of the study (nomen nudum); besides, *C. boharti* and *C. tathimani* were synonymized under *C. guttulus* and *C. rufidulus*, respectively (Robillard & Ichikawa, 2009; Robillard, 2014). Even though descriptions of species were rough and

missed a lot of detailed information, Otte's 2007 study marked a real progress in the knowledge of *Cardiodactylus* diversity, by increasing the number of valid species up to 45.

Robillard (2009) provided more information of *Cardiodactylus* in terms of morphology, especially focusing on genitalia and forewing venation of both male and female, and described four new species (*C. aobaensis*, *C. epiensis*, *C. pentecotensis* and *C. tankara*) from Vanuatu and redescribed two species, *C. cheesmani* Otte, 2007 and *C. enkraussi* Otte, 2007, which were previously simply described by Otte (2007a). Several expeditions in Southeast Asia and study of natural history museums led to the discovery of new *Cardiodactylus* species (or new records for existing species) (see Table I.1). Along with these detailed descriptions and taxonomic recent studies researches added as much more information, when available, about the natural history of the species, including information on habitat, mating behavior and acoustic communication.

On the other hand, the status of species described before 1915 were also reviewed critically and redescribed carefully, combining information from the old type specimens and newly collected material (Robillard & Ichikawa, 2009; Robillard, 2014): *C. novaeguineae* - redescribed and established a neotype; *C. guttulus* – validated and redescribed; *C. canotus* – validated and redescribed; *C. gaimardi* – validated and redescribed; *C. haani* – validated and redescribed; *C. pictus* – validated and redescribed; *C. rufidulus* – validated and redescribed; *C. philippinensis* and *C. praecipuus* – both confirmed as *nomen dubium* due to missing original type specimen (*C. philippinensis*) and potential label error for original single female type specimen (*C. praecipuus*). These works are very helpful for subsequent explorations of taxonomic study and further systematic research on this genus.

Until now, total 82 species are recorded in the genus, including 60 species which belong to the *Novaeguineae* species group and 22 in *Efordi* species group (Cigliano *et al.*, 2017). *Cardiodactylus* taxonomic achievements since it was established are presented in Table I.1 and the number of species increased through time is shown in Figure I.1.

TABLE I.1. The taxonomic achievements of the genus *Cardiodactylus* since it was established. The column named as number corresponds the total number of valid species during that year.

Year	Reference	Number	taxonomic achievements	Remark
1878	Saussure, 1878	6	Description of 4 new species, transfer of 2 species from <i>Platydictylus</i>	description of the genus
1906	Kirby, 1906	6		record of six valid species
1930	Shiraki, 1930	7	Shiraki moves <i>Gryllodes guttulus</i> to <i>C. guttulus</i>	
1936	Chopard, 1936	7	Chopard moves <i>Platydictylus praecipus</i> (Walker, 1969) to <i>C. praecipus</i>	currently nomen dubium (Robillard, 2014)
1951	Chopard, 1951	7	key to four <i>Cardiodactylus</i> (<i>C. rufidulus</i> , <i>C. gaimardi</i> , <i>C. novaeguineae</i> , <i>C. canous</i>)	
1967	Chopard, 1967	7	transfer <i>Gryllopsis guttula</i> (Chopard, 1967) to <i>C. guttulus</i>	but until 1995, <i>C. guttulus</i> was validated by Oshiro
1968	Chopard, 1968	7	records seven species: <i>C. novaeguineae</i> , <i>C. praecipuus</i> , <i>C. gaimardi</i> , <i>C. pictus</i> , <i>C. haanii</i> , <i>C. canotus</i> , <i>C. rufidulus</i>	<i>C. praecipuus</i> is nomen dubium
1969	Chopard, 1969	7	records of <i>C. novaeguineae</i> and <i>C. praecipuus</i>	<i>C. praecipuus</i> is nomen dubium
1981 (1979)	Bhowmik, 1981[1979]	9	records of <i>C. novaeguineae</i> and <i>C. praecipuus</i> , description of two new species: <i>C. minuta</i> and <i>C. nigris</i>	<i>C. praecipuus</i> is nomen dubium
1983	Otte & Alexander, 1983	9	Record of cognition of the genus; record of <i>C. novaeguineae</i> in Australia	<i>C. praecipuus</i> is nomen dubium
1994	Otte, 1994	9	10 species recorded	two nomina dubia: <i>C. philippinensis</i> and <i>C. praecipus</i>
1995	Oshiro, 1995	9	formally moved <i>Gryllodes guttulus</i> to <i>C. guttulus</i>	
	Yin & Liu, 1995	9	new record of <i>C. novaeguineae</i> in Taiwan, China	
1996	Rentz, 1996	9	record of <i>C. novaeguineae</i> in Australia	
2004	Robillard & Desutter-Grandcolas, 2004a	9	<i>Cardiodactylus</i> belongs to the tribe Lebinthini Robillard, 2004	first phylogeny of Eneopterinae
2007	Otte, 2007a, b	45	description of 39 new species; two species groups (<i>Novaeguineae</i> , <i>Efordi</i>)	<i>C. philippinensis</i> and <i>C. praecipus</i> are nomen dubium; <i>C. malangona</i> = nomen nudum; <i>C. manus</i> spelling mistake as <i>C. manusense</i> ,
2009	Robillard, 2009	49	description of four new species	First description of habitats and calling songs of <i>Cardiodactylus</i> species
	Robillard & Ichikawa, 2009	49	records of <i>C. novaeguineae</i> ; redescription of <i>C. guttulus</i>	<i>C. boharti</i> synonymized under <i>C. guttulus</i>
2010	Ma & Zhang, 2010	49	description of one new species (<i>C. hainanensis</i>)	synonymized under <i>C. guttulus</i> (Robillard et al. 2014)
2011	Robillard, 2011	51	description of two new species (<i>C. singapura</i> and <i>C. thailandia</i>)	
2014	Robillard, 2014	51	review and redescription of seven species	<i>C. tathimani</i> synonymized under <i>C. rufidulus</i>
	Robillard et al., 2014	75	description of 25 new species, redescription of three species, new record for five species	<i>C. hainanensis</i> synonymized under <i>C. guttulus</i> <i>C. buru</i> synonymized under <i>C. muiri</i> (Dong et al., 2017)
	Tan & Robillard, 2014	76	description of one new species (<i>C. admirabilis</i>)	

(Continued)

TABLE I.1. Continued

Year	Reference	Number	taxonomic achievements	Remark
2015	Robillard & Yap, 2015	76	redescription two species	
2016	Dong & Robillard, 2016 (section I.2 in this thesis)	81	description of five new species, redescription of <i>C. busu</i> and <i>C. javarene</i>	
2017	Dong & Robillard, 2017	81	complete mitochondrial genome of <i>C. muiri</i>	<i>C. buru</i> is synonymized under <i>C. muiri</i>
	Dong <i>et al.</i> , in preparation	82	one new species, <i>C. affinis lampongsi</i>	

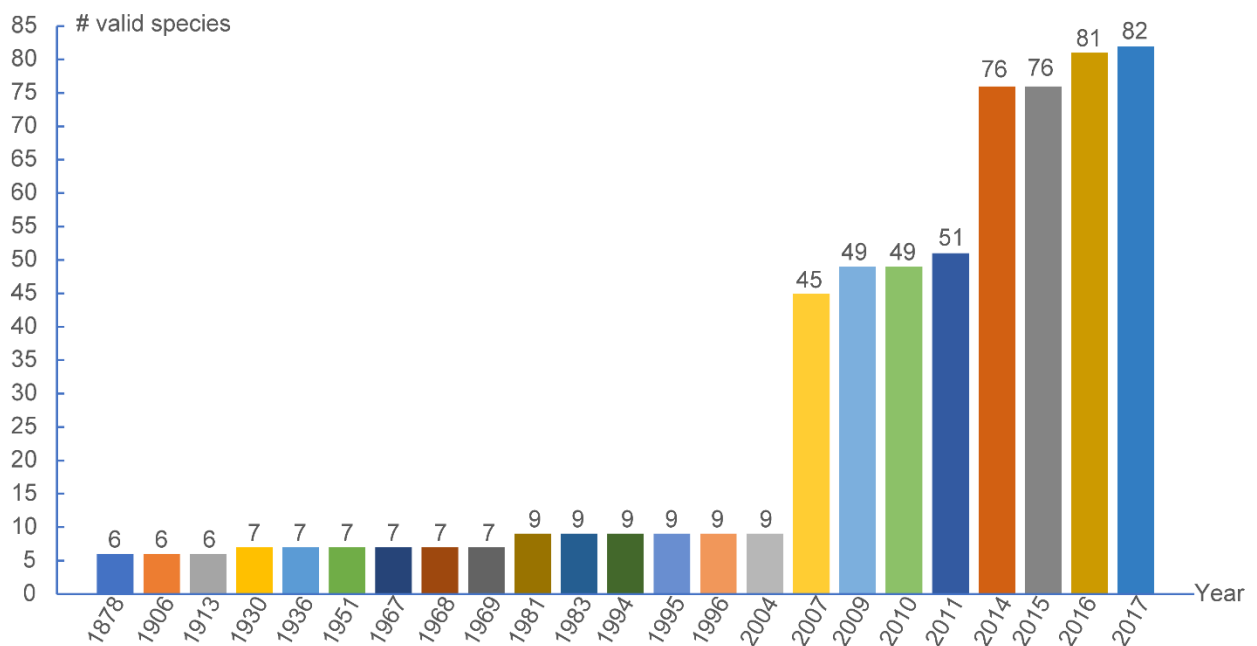


Figure I.1. The number of valid *Cardiodactylus* species increased since the genus was described in 1878. The different color columns correspond the years, and the number above the columns correspond the number of valid species in that year.

Taxonomic account

Subfamily Eneopterinae Saussure, 1874

Tribe Lebinthini Robillard, 2004

Genus *Cardiodactylus* Saussure, 1878

Type species. *Cardiodactylus novaeguineae* (Haan, 1842)

Diagnosis. – Emended from Robillard et al., 2014.

Among Eneopterinae and Lebinthini genera, *Cardiodactylus* is characterized by its average to large size, its long wings in both sexes, as in *Ponca*, and male forewing venation (Figure I.2 and I.3). It is larger in size than other genera, except *Centuriarus* Robillard, 2011. Furthermore, although wings of *Ponca* are developed, there is a big difference between *Cardiodactylus* and *Ponca* in male genitalia. For *Ponca*, pseudepiphallic sclerite is flat and its posterior apex is divided into two parallel lophi, while for *Cardiodactylus* the pseudepiphallus is narrowed at mid-length with two characteristic dorsal ridges in later view, and posterior apex of pseudepiphallus is not divided, forming a wide gutter shape with rounded apex (in *Novaeguineae* species group) or a flat gutter shape with a flat apex (in *Efordi* species group) (Figure I.4).

Classification. The species in *Cardiodactylus* are classified in two species groups, *Efordi* and *Novaeguineae*. The main differences between this two species groups are represented in Table I.2.

TABLE I.2. The difference between *Efordi* and *Novaeguineae* species groups.

Species group	Size	Hind wings	Forewings	Male genitalia	
				Pseudepiphallus	Apex of pseudepiphallus
<i>Efordi</i>	small to average	Long	brown coloration	dorsal ridges not fused	shallow gutter-like with flat apex
<i>Novaeguineae</i>	average to large	Shorter	with yellow or whitish areas around chords and posterior to mirror	dorsal ridges fused medially above a canal	spoon-like with rounded apex

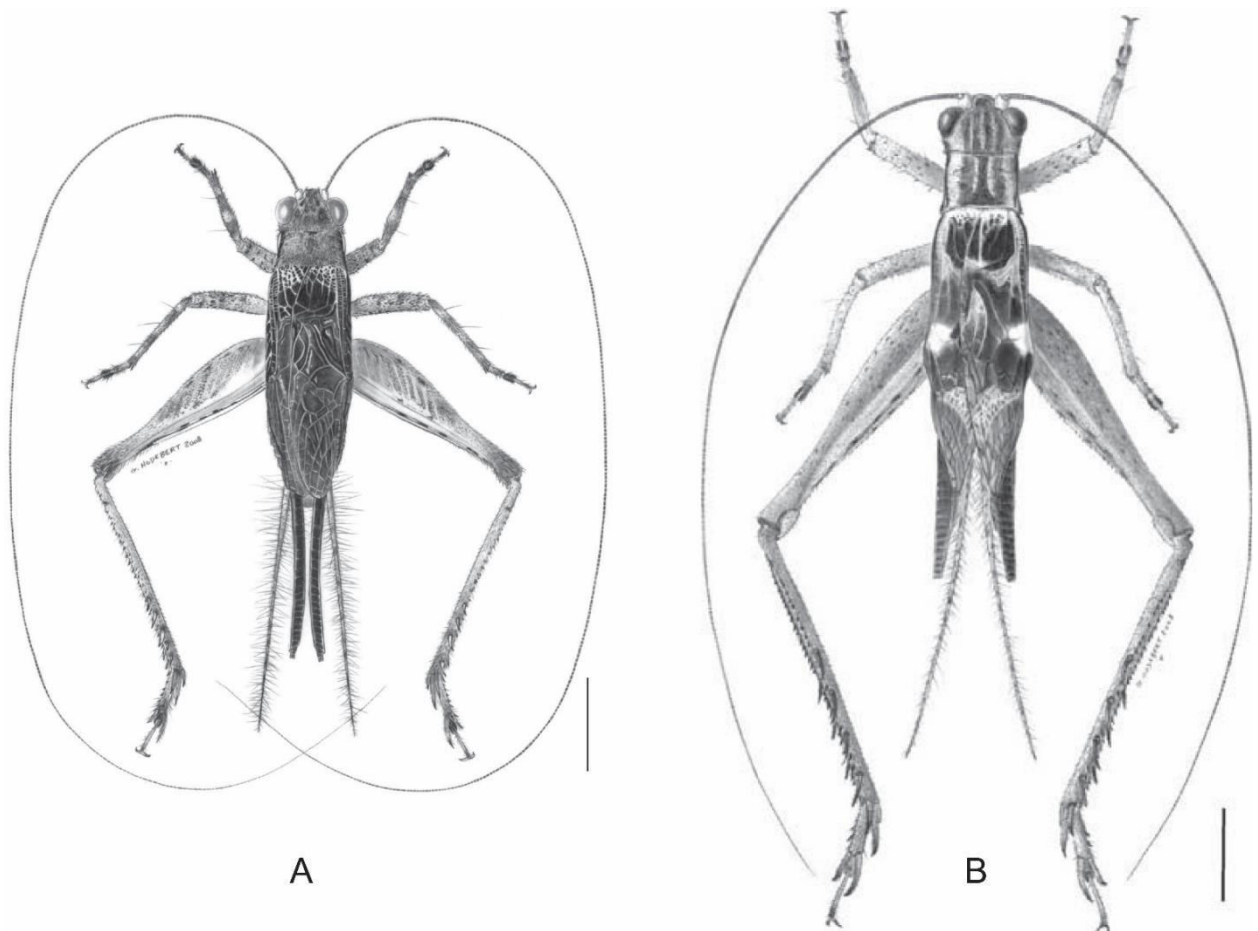


Figure 1.2. *Cardiodactylus*, male habitus. *Cardiodactylus enkraussi* Otte, 2007 exemplifies the *Efordi* species group (A) and paraneotype of *Cardiodactylus novaeguineae* (Haan, 1844) exemplifies the *Novaeguineae* species group (B). Scale bar: 5mm. Modified from Robillard, 2009 and Robillard & Ichikawa, 2009.

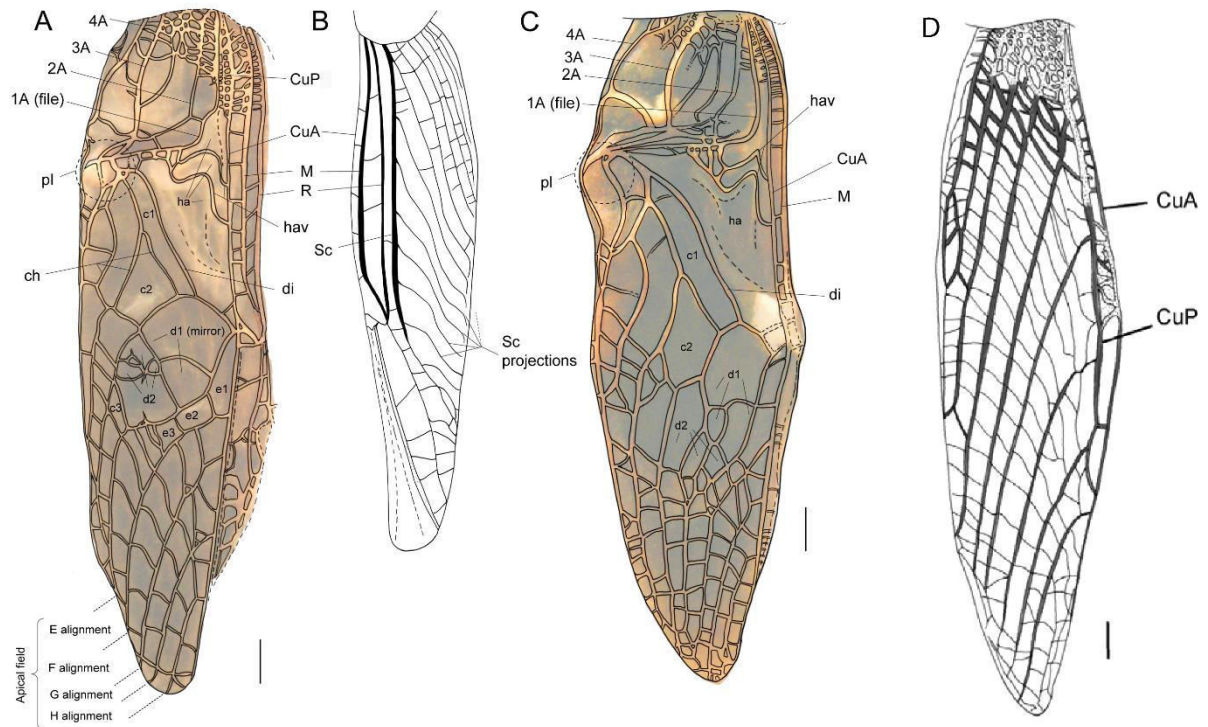


Figure I.3. Forewing venation of *Cardiodactylus*, male (A, B and C) and female (D). Dorsal field (A) with corresponding lateral field (B) of *C. enkraussi* Otte, 2007 exemplifies the *Efordi* species group; dorsal field (C) of *C. tankara* Robillard, 2009 exemplifies the *Novaeguineae* species group. Dorsal field (D) of *C. novaeguineae* exemplifies the female forewing venation. The list of abbreviations for veins and cells is given in materials and methods part of section I.2. Modified from Robillard & Ichikawa, 2009 and Robillard *et al.*, 2014. Scale bar: 1mm.

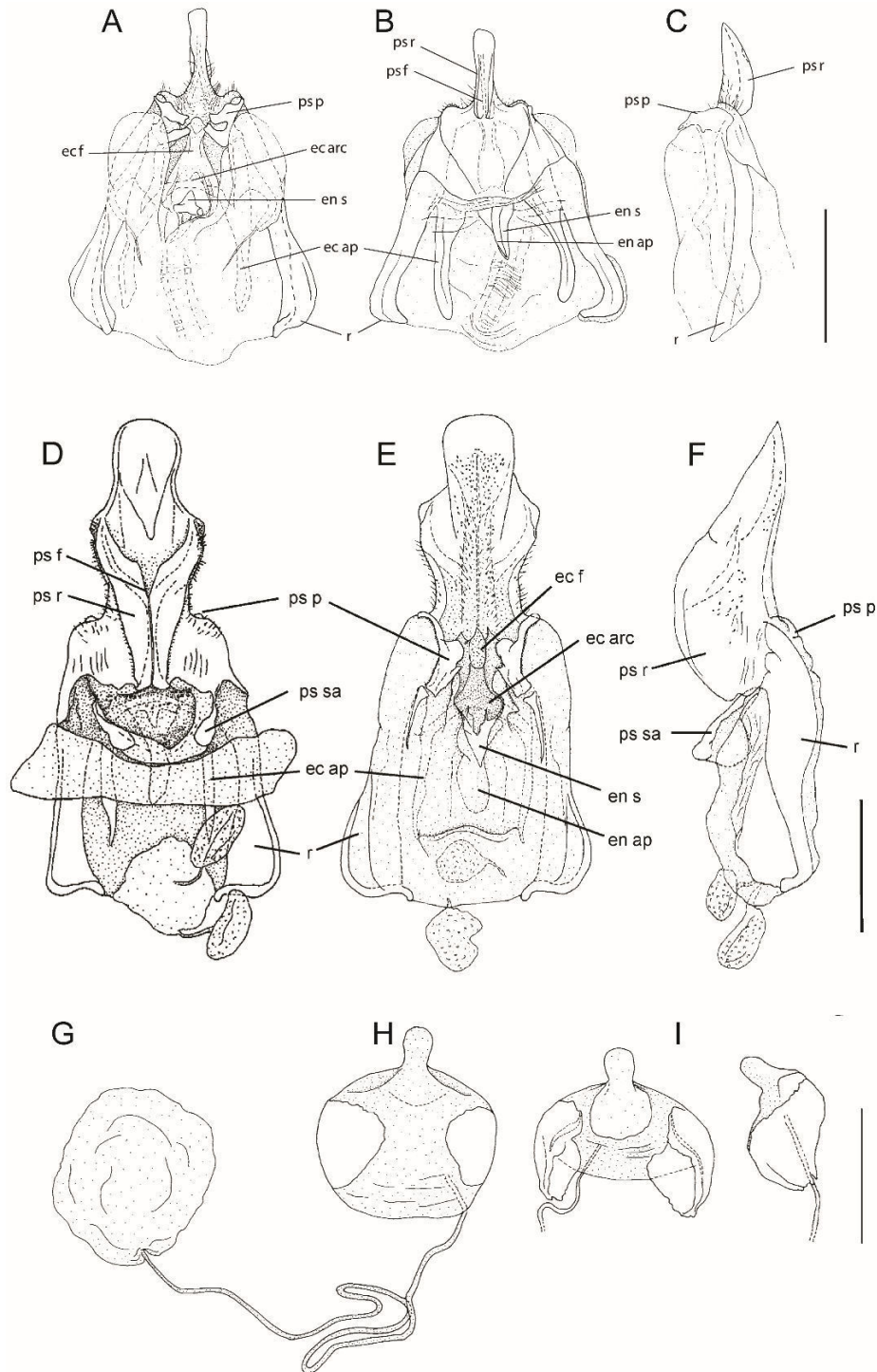


Figure 1.4. Male genitalia and female copulatory papilla of *Cardiodactylus*. Ventral (A), dorsal (B) and lateral (C) views of *C. enkraussi* Otte, 2007 exemplifies the *Efordi* species group; dorsal (D), ventral (E) and lateral (F) views of *C. novaeguineae* (Haan, 1844) exemplifies the *Novaeguineae* species group; dorsal (D), ventral (E) and lateral (F) views of *C. novaeguineae* (Haan, 1844) exemplifies the female copulatory papilla. Modified from Robillard & Ichikawa, 2009 and Robillard *et al.*, 2014. Scale bars: 1mm.

Habitat. Species lives in or near forested areas and are found on leaf litter, tree trunk and/or plant during day and night.

Remarks. The species *Cardiodactylus novaeguineae* was first described by De Haan in 1842 under name *Platydactylus novae-guineae*, later was moved to the genus *Cardiodactylus* and selected as the type species of this genus by Saussure (1878) under the named *C. novae-guineae* with vague morphological description. However, as the type species, it was cited and identified without type comparison with the spelling of *C. novae-guineae* or *C. novaeguineae* in the later documents (Kirby, 1906; Holdhaus, 1909; Chopard, 1951, 1968, 1969; Otte & Alexander, 1983; Otte, 1994, 2007; Oshiro, 1995; Yin & Liu, 1995; Rentz, 1996). Robillard and Ichikawa redescribed *C. novaeguineae* by referring to previous description and combining information from old type specimens and new materials.

The distribution area of *Cardiodactylus* is wide, ranging from the northern Ryukyu Island, to the coasts of Southeast Asia, Northern Australia and in many archipelagos in the Western Pacific encompassing Indonesia, Papua New-Guinea, the Philippines, the Solomon Islands, Vanuatu, New Caledonia, the Caroline Islands, Fidji and Samoan Islands (Robillard *et al.*, 2014). Despite the recent discoveries on this genus, many species remain to be described and discovered. In particular, the exploration of *Cardiodactylus* in New Guinea and in the Western Pacific did not progress since the work of Otte (2007) and Robillard (2009). In the next section, I will introduce the taxonomic work made in western New Guinea with new *Cardiodactylus* descriptions.

I.3. Five new *Cardiodactylus* species from Eastern New Guinea

Article 1: Dong, J. & Robillard, T. 2016. The *Cardiodactylus* crickets from Eastern New Guinea, with description of five new species (Orthoptera: Gryllidae: Eneopterinae). in Robillard, T., Legendre, F., Villemant, C. & Leponce, M. (eds), Insects of Mount Wilhelm, Papua New Guinea. Muséum national d'Histoire naturelle, Paris : 203-258 (*Mémoires du Muséum national d'Histoire naturelle* ; 209).

The *Cardiodactylus* crickets from Eastern New Guinea, with description of five new species (Orthoptera: Gryllidae: Eneopterinae)

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ABSTRACT

The genus *Cardiodactylus* is the most speciose genus of the cricket subfamily Eneopterinae and of the Lebinthini tribe. Along with diverse acoustic features, this genus is also characterized by a wide distribution area running from Japan to the archipelagos of the Western Pacific, with a high contrast in species distributions. In this paper we focus on the *Cardiodactylus* species from the eastern part of the island of New Guinea (Papua New Guinea). We list the existing species, describe five new species and redescribe or add new signalizations for three other species. Associations between male, female and juvenile forms are confirmed using a DNA barcoding approach. Descriptions are based on integrative information about general morphology, coloration, male and female genitalia and forewing venation. Whenever possible, information is provided about species distribution, male calling song, life history and habitats.

RÉSUMÉ

Les grillons *Cardiodactylus* de l'est de la Nouvelle-Guinée et description de cinq espèces nouvelles (Orthoptères : Gryllidae : Eneopterinae).

Le genre *Cardiodactylus* est le plus diversifié en nombre d'espèces parmi les genres de grillons de la sous-famille Eneopterinae et de la tribu Lebinthini. Outre les propriétés acoustiques très diverses de leurs chants, ces grillons sont également caractérisés par leur large aire de répartition, qui court du Japon jusqu'aux archipels du Pacifique Ouest, avec un fort contraste entre les aires de distribution des différentes espèces. Dans cette étude, nous nous intéressons aux espèces de *Cardiodactylus* de l'est de la Nouvelle-Guinée (Papouasie-Nouvelle-Guinée). Nous listons les espèces présentes, décrivons cinq nouvelles espèces et redécrivons ou présentons de nouvelles signalisations pour trois autres espèces précédemment décrites. Les associations entre les formes mâle, femelle et juvénile ont été confir-

mées par une approche de code barre moléculaire. Les descriptions s'appuient sur des informations intégrant la morphologie générale, la coloration, les genitalia et la nervation mâle et femelle. Lorsqu'elles sont disponibles, des informations sont fournies à propos de la distribution des espèces, du chant d'appel du mâle, de la biologie et des habitats.

INTRODUCTION

The Eneopterinae crickets are distributed in most tropical areas and are characterized by diverse shapes and original features related to acoustic communication (e.g., Robillard & Desutter-Grandcolas 2004a, 2011a; Robillard *et al.* 2007, 2013, 2015, ter Hofstede *et al.* 2015). The tribe Lebinthini Robillard, 2004 is the most diversified and speciose tribe of the subfamily, and it currently comprises a dozen of genera mostly distributed in Southeast Asia, in the north of South America, and in most archipelagos of the West Pacific (see Robillard *et al.*, this volume).

Cardiodactylus is currently the most speciose and widely distributed genus of the Eneopterinae and Lebinthini. The wide distribution area of the genus runs from Japan (northern Ryukyu Islands), to the coasts of Southeast Asia, Northern Australia and in many archipelagos in the Western Pacific across Indonesia, Papua New Guinea, the Philippines, the Solomon Islands, Vanuatu, New Caledonia, the Caroline Islands, Fiji, Tonga and the Samoan Islands (e.g., Otte 2007; Robillard & Ichikawa 2009; Robillard 2009, 2011; Robillard *et al.* 2014). To better understand the origin and evolution of their unique acoustic features and the processes explaining their rich distribution pattern, precise information are needed for as many species as possible. This necessitates taxonomic and biological studies. The revision of the genus has begun with a review of the old types of *Cardiodactylus* (Robillard 2014) and with a recent study focusing on the western part of the genus' distribution and the *Novaeguineae* species group (Robillard *et al.* 2014), where 25 new species were described, bringing the number of valid described species to 75.

In the present study, we focus on the species distributed in the western part of New Guinea Island (Papua New Guinea), and in particular on the Mount Wilhelm, which has been studied during a recent "Our Planet Reviewed - Papua New Guinea". We describe five new species, redescribe two existing species and bring new signalizations for *C. novaeguineae* (Haan, 1842). Whenever possible, information is provided about species distribution, male calling song and male and female genitalia, forewing venation and habitat.

MATERIALS AND METHODS

MATERIAL EXAMINED

Most crickets studied herein were collected during an expedition in Papua New Guinea in Oct–Dec 2012, organized by Our Planet Reviewed (Niugini 2012, MNHN-Pronatura International). New collected material was collected mostly by sight, by night and day, in order to observe species' habitat and type of activity. Specimens were deposited in the collections of the Muséum national d'Histoire naturelle, Paris (MNHN). Square brackets in lists of materials are used for additional information not mentioned on specimen labels or translated from French labels.

DNA SEQUENCING

Males, females and juveniles from a given morphotype were analyzed using a DNA barcoding approach to confirm that they belong to the same species, with a simple tree-based approach using a maximum likelihood tree reconstructed with the IQ-TREE web service (Nguyen *et al.* 2015). We used a fragment of the small mitochondrial ribosomal subunit (12S rRNA, ~ 400 bp), easy to amplify and which proved to be variable between closely related species in crickets (e.g., Robillard & Desutter-Grandcolas 2006; Nattier *et al.* 2012).

Molecular works were performed at the Service de Systématique Moléculaire of the MNHN. All sequences were generated by using the DNA extraction, amplification and sequencing protocols described in Nattier *et al.* (2012), with similar primers and annealing temperatures. DNA extractions were made from median legs of crickets using the Epmotion

5075 robot (Eppendorf). Newly generated sequences were edited in Sequencher v.4.9 (Gene Codes Co.) and BioEdit v.7.0.5.3 (Hall 1999), blasted with NCBI blast tools and compared; the data will be published as part of a forthcoming phylogenetic study of *Cardiodactylus* (Dong *et al.* in prep.).

OBSERVATIONS AND MORPHOLOGICAL ANALYSIS

Descriptions follow the standards of recent publications about new species of *Cardiodactylus* (e.g., Robillard *et al.* 2014; Tan & Robillard 2014). Direct observations and dissections have been made using a binocular microscope Leica MZ16 at magnifications up to 115. Male tegminal veins and cells follow terminology by Ragge (1955) and Robillard & Desutter-Grandcolas (2004a)

MALE TEGMINAL VENATION

Male tegminal veins and cells are named after Robillard *et al.* (2014) for the genus *Cardiodactylus*, Desutter-Grandcolas (2003) for Ensifera, and Robillard & Desutter-Grandcolas (2004a) for the subfamily Eneopterinae more specifically. For specimen studied with scanning electron microscopy, the right forewing was softened with cotton soaked in water before being cut. We then used environmental scanning electron microscope (ESEM) from “plateforme de microscopie électronique” of the MNHN to characterize the morphology of the stridulatory files.

MALE AND FEMALE GENITALIA

Whenever possible, male and female genitalia have been dissected in softened specimens by cutting the membranes between the paraprocts and the subgenital plate, or between the ovipositor and the subgenital plate, respectively; they have been cleaned with cold KOH and have been observed using a binocular microscope Leica MZ16 at magnifications up to 115, and then kept in glycerine in vials pinned under specimens. Male genitalia are named according to Desutter (1987), modified in Desutter-Grandcolas (2003) and Robillard & Desutter-Grandcolas (2004a). Photographs of male and female genitalia were obtained using an AmScope MU1000 digital camera (www.Amscope.com). Abbreviations: see below.

ACOUSTIC DATA

The basic cricket song terminology follows Ragge & Reynolds (1998). One song unit is called a syllable and corresponds to one opening-closure cycle of the male forewings. Four species described or redescribed here have been recorded in the field from specimens collected as adults or sub-adults. The recordings were made with a modified Condenser Microphone Capsule CM16 (Avisoft Bioacoustics, Berlin), with a flat frequency response from 3 to 150 kHz (R. Specht pers. comm.), connected to a Fostex FR-2LE digital field recorder (96 kHz sampling frequency, 16 bit). Song features were measured using the automatic commands under Avisoft-SASLab Pro version 4.40 (Specht, 2008). Voucher recordings are deposited in the Sound Library of the MNHN.

ABBREVIATIONS

DESCRIPTIONS

General morphology: FW, forewing; HW, hind wing; Tarsomere III-1, III-3: first and third segments of hind leg tarsomere.

Tegminal venation: 1A-4A, first to fourth anal veins; CuA, anterior cubitus; CuA1, CuA2, ... first, second, ... bifurcations of CuA; CuP, posterior cubitus; M, median vein; R, radial vein Sc, subcostal vein; c1-3, first to third cells of C alignment; d1 cell (mirror), first cell(s) of D alignment.

MEASUREMENTS

FIII L, length of hind femora; FIII W, width of hind femora; FW L, forewing length; FW W, forewing width (at the level of maximal width); Ias: inner spines on TIII dorsal side, above the spurs; Ibs: inner spines on TIII dorsal side, between

the spurs; Oas: outer spines on TIII dorsal side, above the spurs; Obs: outer spines on TIII dorsal side, between the spurs; OL, ovipositor length; PronL, pronotum length; PronW, pronotum width; TIIIL, length of hind tibiae.

INSTITUTIONS

BMNH, Natural History Museum, London, UK; BPBM: Bernice P. Bishop Museum, Department of Zoology, 1355 Kalihi Street, Honolulu, Hawaii 96818, USA; MHNG, Muséum d'Histoire Naturelle de Genève, Switzerland; MNHN, Muséum national d'Histoire naturelle, Paris; MZB, Museum Zoologicum Bogoriense, Bogor; NHMW, Naturhistorisches Museum, Vienna; NHRS: Swedish Museum of Natural History, Stockholm, Sweden; RMNH, Nationaal Natuurhistorisch Museum, Leiden; SAMA: South Australia Museum, Adelaide, Australia; ZIN, Zoological Institute, Russian Academy of Sciences, S. Petersburg.

SYSTEMATIC PART

Subfamily ENEOPTERINAE Saussure, 1874

Tribe LEBINTHINI Robillard, 2004a

Genus *CARDIODACTYLUS* Saussure, 1878

Type species. *Cardiodactylus novaeguineae* (Haan, 1842)

DIAGNOSIS AND CLASSIFICATION — See Robillard *et al.* (2014).

List of *Cardiodactylus* species in Eastern New Guinea (by alphabetic order; the species not addressed in this study are represented in brackets)

Species group *Novaeguineae* Otte, 2007a

Cardiodactylus baitabensis n. sp.

[*Cardiodactylus bulolo* Otte, 2007a]

Cardiodactylus haddocki n. sp.

Cardiodactylus lucus n. sp.

[*Cardiodactylus mamai* Otte, 2007a]

Cardiodactylus novaeguineae (Haan, 1842)

Species group *Efordi* Otte, 2007a

Cardiodactylus busu Otte, 2007a

Cardiodactylus javarere Otte, 2007a

[*Cardiodactylus minuta* Bhowmik, 1981]

[*Cardiodactylus nigris* Bhowmik, 1981]

Cardiodactylus niugini n. sp.

Cardiodactylus nobilis n. sp.

[*Cardiodactylus singuawa* Otte, 2007a]

Species group *Novaeguineae* Otte, 2007a***Cardiodactylus novaeguineae*** (Haan, 1842)

Figures 1-3

Gryllus (Platydictylus) novae-guineae Haan, 1842: 233.*Cardiodactylus novae-guineae* – Saussure 1878: 659 (type comparison); Kirby 1906: 88 (type species of *Cardiodactylus*); Chopard 1915: 149; 1929: 49; 1931b: 140; 1969: 312.*Cardiodactylus novaeguineae* – Otte 1994: 65; Robillard 2004: 29, 2006: 675 (morphological phylogeny); Robillard & Desutter-Grandcolas 2004a: 273, 2004b: 479 (calling song), 2006: 644 (molecular and morphological phylogeny); Eades *et al.* 2008 (Orthoptera Species File Online).*Cardiodactylus novaeguineae* – Chopard 1968: 351; Bhowmik 1979 (1981): 43; Otte & Alexander 1983: 310; Otte *et al.* 1987: 42; Oshiro 1995: 44; Yin & Liu 1995: 97; Rentz 1996: 140; Willemse 2001: 110; Otte 2007a: 345; Otte 2007b: 239; Robillard *et al.* 2007; Desutter-Grandcolas *et al.* 2010: 616; Robillard 2009: 587; Robillard & Ishikawa 2009: 879 (redescription and neotype designation); Robillard & Desutter-Grandcolas 2011: 637; Nattier *et al.* 2011: 2200 (molecular phylogeny); Robillard 2014: 104; Eades *et al.* 2015 (Orthoptera Species File Online).

TYPE MATERIAL — Neotype male: Indonesia. Papua Province, Biak Is., XI.1953, L.v.s. Hammen (RMNH). Paraneotypes (7♂, 14♀): Indonesia. Papua Province, Biak [Is.], 20.II.1952, 1♂, L.D. Brongersma (RMNH); [Biak Is.], Base Biak, 17/24. III.1952, 2♂, 1♀, L.D. Brongersma & W.J. Roosdorp (RMNH); [Biak Is.], Schouten Islands, Base Biak, W of Sorido, 24.I.1955, 1♀, L.D. Brongersma (RMNH); Biak [Is.], X-XI.1953, 1♀, Pers Kon Marine (RMNH). Papua New Guinea. [Manus Province] Manus Is., Lorengau, 6.VI.1967, 1♀, R.E. & R.M. Blackith (MNHN-EO-ENSIF2044). [Morobe Province, New Guinea Island] Lae, 29.V.1967, 2♂, R.E. & R.M. Blackith (MNHN-EO-ENSIF2045, 2046). Oro Province [New Guinea Island] Mt. Lamington, 1300-1500 ft., 2♂, 4♀, C.T. McNamara, identified *Cardiodactylus novae guineae* by L. Chopard (SAMA). [Milne Bay Province] Misima Is., Louisiade Arch., Narian, 0-500 m (No 8), 1956, 1♀, fifth Archbold Exped. to New Guinea, L.J. Brass (AMNH). Misima Is., Louisiade Arch., 1♀, Rev. R.J. Andrew, identified *Cardiodactylus novae guineae* by L. Chopard (SAMA). Rossel Is., Louisiade Arch., Abaleti, 0-500 m, 1♀, fifth Archbold Exped. to New Guinea, L.J. Brass (AMNH). [New Ireland Province] New Ireland [Island], 2♀, E.R. Waite, identified *Cardiodactylus novae guineae* by L. Chopard (SAMA).

TYPE LOCALITY — Indonesia, Biak Island.

NEW SIGNALISATION — Papua New Guinea. Loloata Island resort, SE Port Moresby, zone littorale, 9°32'04,8"S 147°17'25,5"E, 16 m, 11.X.2012: 1♂ (TR3), nuit, sur tronc (8 cm), F. Legendre & T. Robillard (MNHN); 1 juvenile (TR2), nuit, plante, échantillon moléculaire C60-CnoLo, F. Legendre & T. Robillard (MNHN-EO-ENSIF3550). Nouvelle Guinée, Kwalansai [Kwalansam], 14 km SW de Finschhafen, Huon Golf, Nahe Strand, 6.I.1973, 2♂, 1 juvenile, K.W. Ströder (MNHG). Nouvelle Guinée, Kolem, 15 km Nord de Finschhafen, faulichtem kokos und Baum, 23.I.1973, 1♀, K.W. Ströder (MNHG).

REDESCRIPTION AND DISTRIBUTION — See Robillard & Ichikawa (2009). The species presents a wide distribution area and varies slightly in size and colouration, but its characteristic structure of male genitalia, with fused dorsal ridges of pseudepiphallus, remain unchanged across its distribution (Figures 2-3).

Cardiodactylus haddocki n. sp.

Figures 4-16, 34-35, 47-58, 95

Cardiodactylus haani Saussure, 1878 – Otte 2007: 345 (misidentification, see Robillard 2014: 110).*Cardiodactylus* n. sp. – Robillard 2014: 110.



FIGURES 1-9

Cardiodactylus novaeguineae (Haan, 1842): **1**, male on dead leaf of banana tree, Oubatché, New Caledonia (photo by F. Muller); **2-3**, male genitalia in dorsal and lateral views. *Cardiodactylus haddocki* n. sp.: **4-6**, male genitalia, specimen from Mount Wilhelm in dorsal (**4**), ventral (**5**) and lateral (**6**) views; **7-9**, variation, specimen from Baitabag (MNHN-EO-ENSIF3548) in dorsal (**7**), ventral (**8**) and lateral (**9**) views. Scale bars = 1 mm



TYPE MATERIAL — Male holotype: Papua New Guinea. Nouvelle-Guinée, Mt. Wilhelm, 700 m, camp 5°43'40.4"S 145°15'28.7"E, 768 m, zone herbacée, 1-4.XI.2012, nuit (TR1081), F. Legendre & T. Robillard (MNHN-EO-ENSIF3540). Female allotype: Papua New Guinea. Nouvelle-Guinée, Madang, station Wanang3, rivière Wanang, zone herbacée, 5°13'31.8"S 145°04'42"E, 76 m, 11.XI-2.XII.2012, nuit (TR1689), T. Robillard (MNHN-EO-ENSIF3544). Paratypes (11♂): Papua New Guinea. Same locality, date and collector as holotype: Nouvelle-Guinée, 1♂ (TR921), nuit, zone herbacée, sur branche d'arbre mort, enregistrement appel (MNHN-EO-ENSIF3543); 1♂ (TR1210), mort en élevage (MZB); 1♂ (TR897) (ZIN); 1♂ (TR922), nuit, zone herbacée, sur arbuste (h = 1 m), enregistrement appel (MNHN-EO-ENSIF3541); 1♂ (TR1551), nuit, mort en élevage, enregistrement appel (MNHN-EO-ENSIF3545). Nouvelle-Guinée, Mt. Wilhelm, bord de route 1700-700 m, 29.X.2012, 1♂ (TR615), jour, lisière, sur plante (h = 1 m), F. Legendre & T. Robillard (MNHN-EO-ENSIF3542). Nouvelle-Guinée, Mt. Wilhelm, hors plot 200 m, 5°44'23.9"S 145°19'46.7"E, 5-7.XI.2012, 1♂ (TR1217), nuit, plante (h = 1.2 m), molecular sample C69-CnoMW200, F. Legendre & T. Robillard (MNHN-EO-ENSIF3549). Nouvelle-Guinée, Madang, Station Wanang3, rivière Wanang, forêt secondaire, 5°13'31.8"S 145°04'42"E, 76 m, 11.XI-2.XII.2012: 1♂ (TR1760), nuit, zone ouverte (h = 30 cm), molecular sample C89, T. Robillard (MNHN-EO-ENSIF3546). Nouvelle-Guinée, Madang, village Wanang1, forêt secondaire, 5°13'52.4"S 145°10'54.2"E, 142 m, 11.XI-2.XII.2012, 1♂ (TR1649), nuit, plante, molecular sample C90, T. Robillard (MNHN-EO-ENSIF3547). New Guinea, Madang Dist., Finis-terre Mts. Damanti 3,550 ft., 2-11.X.1964, 1♂, Stn. No.30, M.E. Bacchus, identified *Cardiodactylus* ? *gaimardi* (Serville) by B. C. Townsend, 1982 (BMNH -B.M.1965-120).

OTHER MATERIAL EXAMINED — Papua New Guinea. Nouvelle-Guinée, Madang, Station Wanang3, rivière Wanang, forêt secondaire, 5°13'31.8"S 145°04'42"E, 76 m, 11.XI-2.XII.2012, 1♂, nuit, mort en élevage, T. Robillard (MNHN). Nouvelle-Guinée, Madang, village Wanang1, forêt secondaire, 5°13'52.4"S 145°10'54.2"E 142 m, 11.XI-2.XII.2012, 2 juveniles (TR1650-1651), nuit, plante, T. Robillard (MNHN). Nouvelle-Guinée, Madang, forêt de Baitabag, 5°08'20.6"S 145°46'26.4"E, 86 m, 6.XII.2012, 1♂ (TR2472), nuit, plante (h = 1 m), molecular sample C66-CnoMa, T. Robillard (MNHN-EO-ENSIF3548). New Guinea, Madang: 1♂, identified *Cardiodactylus novaeguineae* by L. Chopard, W. Lohe (SAMA); 1♀, B.G. Chalis (SAMA). Indonesia. [Papua] Irian Jaya Hutan Nafri-Jayapura, 17-22.VII.1993, 1♀, Y. R. Suhardjono (MZB). [Papua] tuss. Waosi en Sowi, Z. van Manokwari, 25.IV.1952, 1♀, L. D. Brongersma & Z. J. Roosdorp (RMNH). Hollandia [Jayapura], 1911, 2♂, Ned. Nw. Guinea Exp, Dr. P. N. v. Kampen (RMNH). Nov. Guinea, Jaona [Yaona, Tanahmerah-Bai], 1910, 1♀, Ned. Nw. Guinea Exp, 1911, Dr. P. N. v. Kampen (RMNH). W. New Guinea, env. of Manokwari town, primary forest on hills near sea, 4-6. XI.2004, 2♂, molecular sample C134, A. Gorochov (ZIN). New Guinea I., Papua Prov., Bagusa vill[age], near mouth of Mamberamo River, lowlying forest, 22-24.I.2012, 1♂, molecular sample C125, A. Gorochov (ZIN). W. New Guinea [Papua], env. of Jayapura city, Cyclop Mts., ~500 m, primary forest, 17-19.XI.2004, 1♀, molecular sample C130, A. Gorochov (ZIN). New Guinea I., Papua Prov., Fawi (Faowi) vill. in upper part of Tariku River (tributary of Mamberamo River), partly lowlying forest and partly forest on hills, 29.I-17.II.2013, 1♂, molecular sample C139; 1♂, 2♀, A. Gorochov (ZIN).

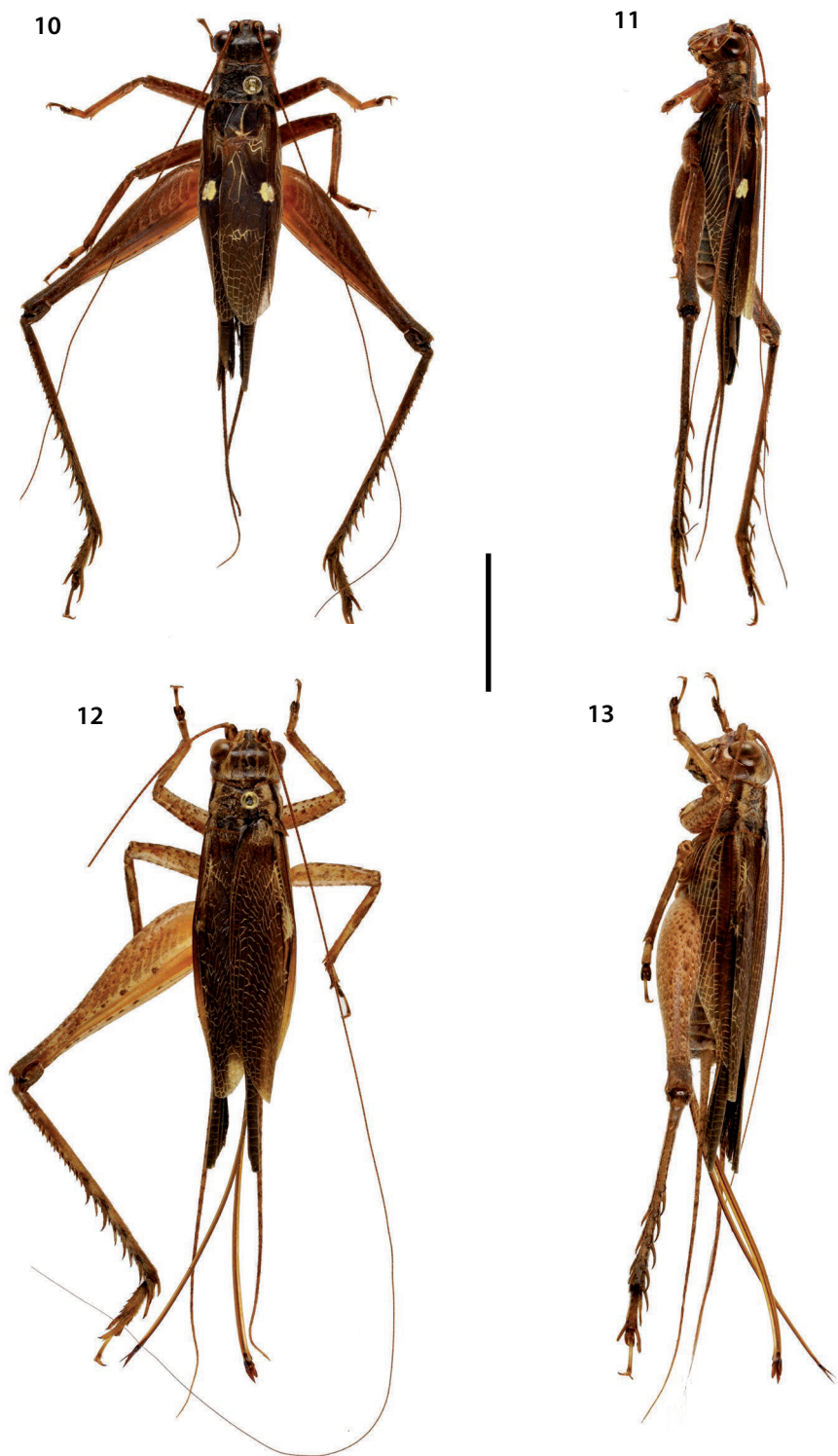
REMARK — Otte (2007) examined specimens of this species but erroneously identified them as *C. haani*, a large species from Gebe Island (Indonesia) located west from New Guinea and redescribed in Robillard (2014).

TYPE LOCALITY — Papua New Guinea, Mount Wilhelm, S5°43'40.4"S 145°15'28.7"E, 768 m.

DISTRIBUTION — Northern part of New Guinea Island (Indonesia and Papua New Guinea).

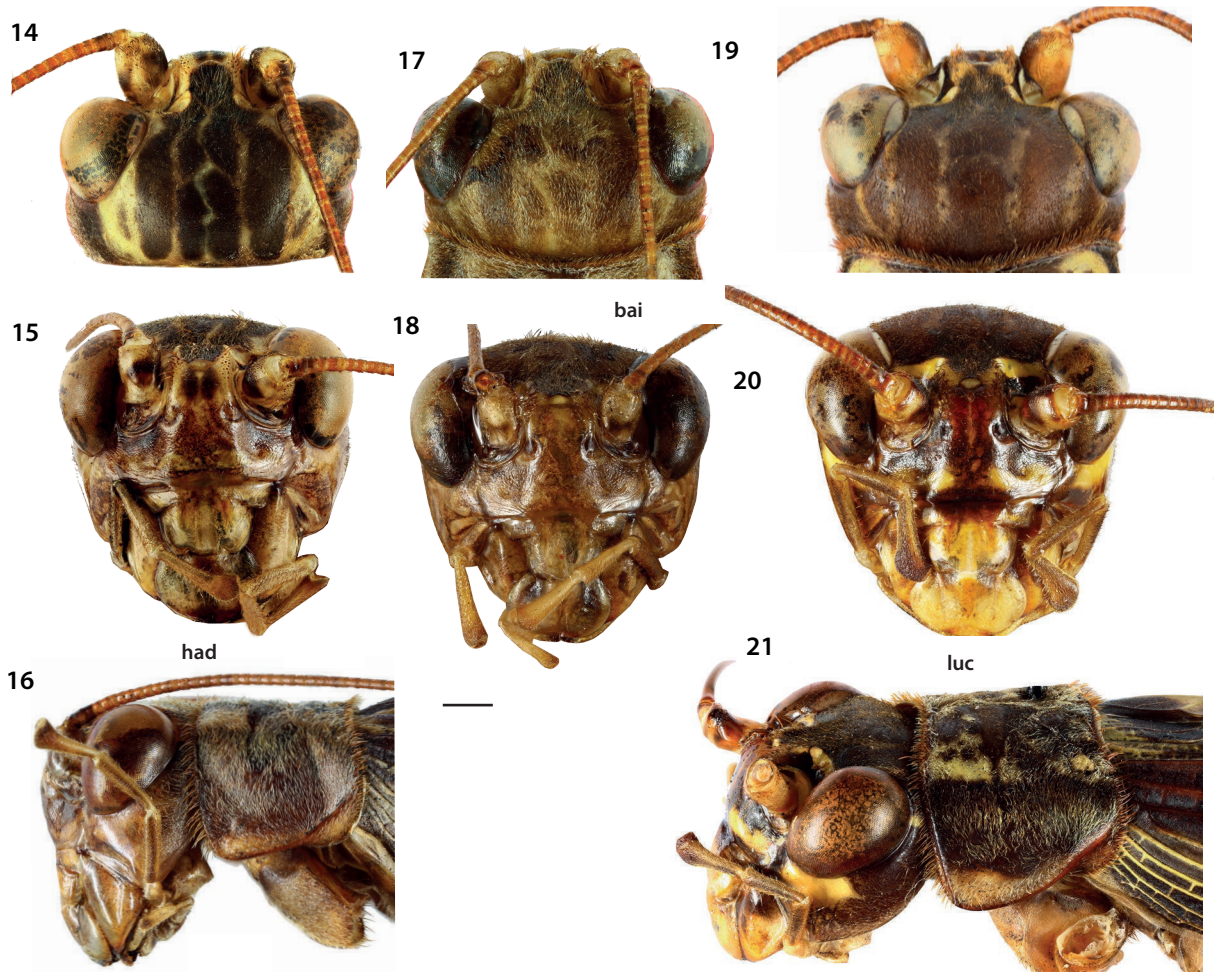
ETYMOLOGY — The species is dedicated to Olivier "captain Haddock" Pascal, co-organizer of the Our Planet Reviewed expeditions (MNHN/Pronatura International).

DIAGNOSIS — Species of average size for the species group, close to *C. novaeguineae* from which it differs by general coloration generally darker, male genitalia with partial V-shaped fusion of the pseudepiphallid ridges (complete fusion in *C. novaeguineae*) (compare Figures 2-3 and Figures 4-9).

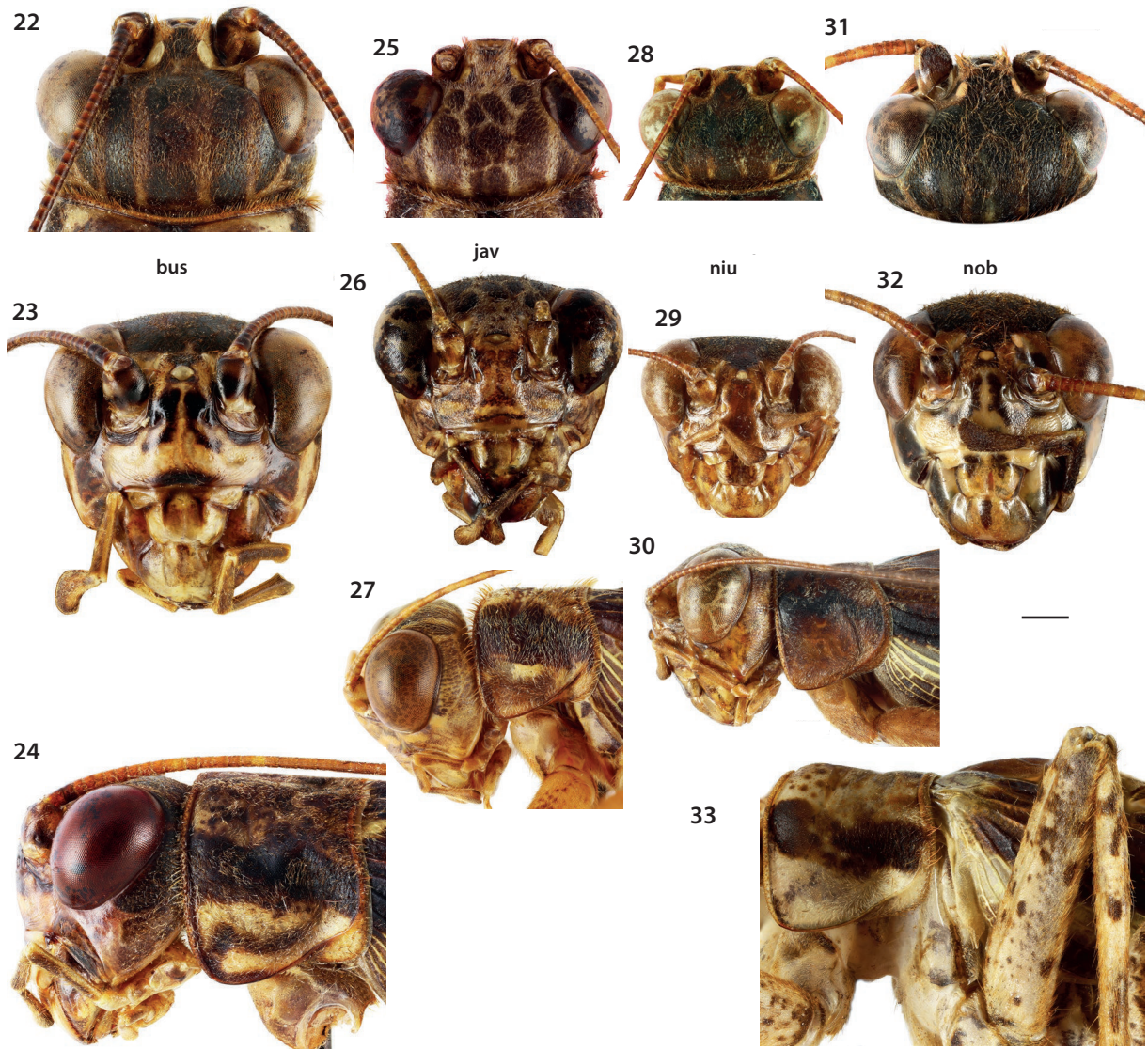


FIGURES 10-13

Cardiodactylus haddocki n. sp.;
10, male in dorsal view; **11**, in
 lateral view; **12**, female in dorsal
 view; **13**, in lateral view.
 Scale bar = 1 cm.



DESCRIPTION — Size variable, generally average for the species group. General coloration contrasted (Figures 10-13), mostly dark brown with very light areas ranging from yellow to whitish brown. Head (Figures 14-16): Dorsum brown with four longitudinal bands plus brown spots posterior to eyes, sometimes with a pale zigzag median suture. Fastigium brown, nearly as wide as scape, its margins yellow brown. Antennae homogeneously light brown, except their base dark brown. Cheeks brown dorsally and posterior to eyes, yellow brown ventrally. Scapes yellow brown. Face homogeneously yellow with two brown vertical stripes between the scapes. Maxillary palpi with a dark apical ring. Pronotum: Dorsal disk brown and trapezoidal; anterior margin more or less concave; posterior margin bisinuate; lateral margins yellow brown, median area brown, variably mottled with dark brown. Lateral lobes mostly dark brown, their ventral margin yellow. Legs almost homogeneously brown, from yellow brown to dark brown; fore and median legs faintly mottled with darker spots; TIII dark brown. FWs: Reaching tip of abdomen, shade variable from dark brown to light brown, but with a pattern of light areas almost constant (Figures 34-35). Lateral field: M/R area and veins brown; R/Sc area brown; Sc and its projections yellow, the cells between them translucent. Hind wings: Longer than FWs of a pronotum length; tail gray brown, sometimes with lighter transverse veins. Cerci: shorter than hind legs, gray brown.



FIGURES 14-33

14-16, *Cardiodactylus haddocki* n. sp., head in dorsal (14), facial (15) and lateral (16) views; 17-18, *Cardiodactylus baitabensis* n. sp., head in dorsal (17) and facial (18) views; 19-21, *Cardiodactylus lucus* n. sp., head in dorsal (19), facial (20) and lateral (21) views. 22-24, *Cardiodactylus busu* Otte, 2007a: head in dorsal (22), facial (23) and lateral (24) views; 25-27, *Cardiodactylus javarere* Otte, 2007a: head in dorsal (25), facial (26) and lateral (27) views; 28-30, *Cardiodactylus niugini* n. sp.: head in dorsal (28), facial (29) and lateral (30) views. 31-33, *Cardiodactylus nobilis* n. sp.: head in dorsal (31) and facial (32) views; lateral lobe of pronotum (33) Scale bar = 1 mm.



had



luc



bus



bai





FIGURES 34-46

Forewing venation of males (upper part) and females (lower part); **34-35**, *Cardiodactylus haddocki* n. sp.; **36-37**, *Cardiodactylus lucus* n. sp.; **38-40**, *Cardiodactylus busu* Otte, 2007 (**38, 39**, specimens from Mount Wilhelm; **40**, variation from near Jayapura); **41**, *Cardiodactylus baitabensis* n. sp.; **42-43**, *Cardiodactylus javarere* Otte, 2007; **44-45**, *Cardiodactylus niugini* n. sp.; **46**, *Cardiodactylus nobilis* n. sp. Scale bars = 5 mm.

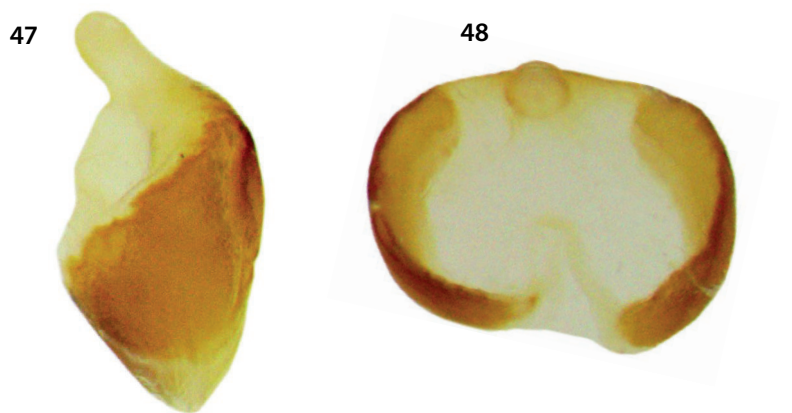
MALE: FW coloration (Figure 34) mostly dark brown to black with the following light pattern: strong yellow to white sclerotization on harp posterior angle, posterior M area, a transverse crescent of variable size including mirror posterior angle and basis of apical field, and a series of yellow to white sclerotized veins/areas including bases of anal veins up to file level, basal quarter of CuA and related M transverse veins, curved part of 1A, oblique veins and part of chordal veins. Diagonal bicolor, its anterior part orange, the rest dark brown. Area of plectrum with a strong orange sclerotization. FW venation: 1A bisinuate; stridulatory vein with 125 teeth (TR922), 88 being on the transverse part of the file, 23 on the angle area, and 14 near base of 1A (Figure 95). CuP absent. Harp narrow, 2 W-shaped oblique veins. Mirror not rounded, longer than wide, variably crossed by accessory veins. Apical field elongate; CuA bifurcations giving 3-4 cell alignments posterior to mirror. Lateral field: Latero-dorsal angle made by M. Sc with 6-9 projections.

Male genitalia: Pseudepiphallus narrowed at mid-length, setose laterally and ventrally, with two characteristic dorsal ridges fused anteriorly, the wide fusion forming a V shape. Anterior to dorsal fusion, two dorso-lateral sclerites surround a foldable membranous sack as in *C. novaeguineae*. Apex of pseudepiphallus spoon-like and rounded. Rami large, starting at level of pseudepiphallus narrowed part. Pseudepiphallic parameres trilobate, posterior lobe thin and divergent. Ectophallic arc complete, with a short posterior expansion. Ectophallic apodemes not reaching apex of rami. Apex of ectophallic fold trilobate, membranous. Endophallic sclerite small, V-shaped, sometimes with a median posterior expansion. Endophallic apodeme with both lateral lamellas and a large medio-dorsal crest extended anteriorly.

FEMALE: FW coloration (Figure 35) mostly brown, base of dorsal field whitish to yellow, including bases of anal and median veins and anterior half of CuA/M area. FW venation including nine strong longitudinal veins on dorsal field, mostly whitish to yellow. Ovipositor longer than hind femora; apex laterally flattened and lanceolate, dorsal edge of apex slightly denticulate, its ventral edge generally smooth.

Female genitalia (Figures 47-48): Copulatory papilla rounded, its apex narrowed and folded ventrally; two lateral sclerites and one near dorsal base of apex.

JUVENILE: Coloration lighter than adults, yellow molted with brown spots. Head and pronotum with similar banding patterns as in adults, ventral margin of pronotum lateral lobes yellow. Legs pale with grayish spots.



FIGURES 47-48

Cardiodactylus haddocki n. sp.;
47, female copulatory papilla in
 lateral view; **48**, in ventral view.

Scale bar = 0.5 mm.

VARIATION — Specimens examined in this study from northern New Guinea (Indonesia, material from SAMA) differ by their lighter coloration, possibly due to conservation method. On male FWs, transverse crescent area posterior to mirror appears variable: it is almost absent or very small in most specimens from Mount Wilhelm, but some specimens in the same populations present more developed yellow areas, as in the specimens from Indonesia. Accessory veins in mirror area also variable. We made similar observations on male genitalia: the V-shaped fusion of the dorsal ridges are variable in width between specimens from Mount Wilhelm, where the dorsal ridges are very flat (Figures 7-9), and some specimens from other localities where the dorsal ridges are very widened at the level of the fusion. This contrast is particularly visible when comparing with the male from the forest of Baitabag (MNHN-EO-ENSIF3548), near Madang, where the dorsal ridges are extremely wide. This male may in fact correspond to a different new species close to *C. novaeguineae* and *C. haddocki*, as it differs (Figures 7-9) by larger size and more contrasted coloration, in addition to this difference in male genitalia. Preliminary molecular results are not confirming this hypothesis, but more data will be needed, both morphologically and molecularly, to validate one hypothesis or the other.

MEASUREMENTS (in mm) — See Table 1.

TABLE 1

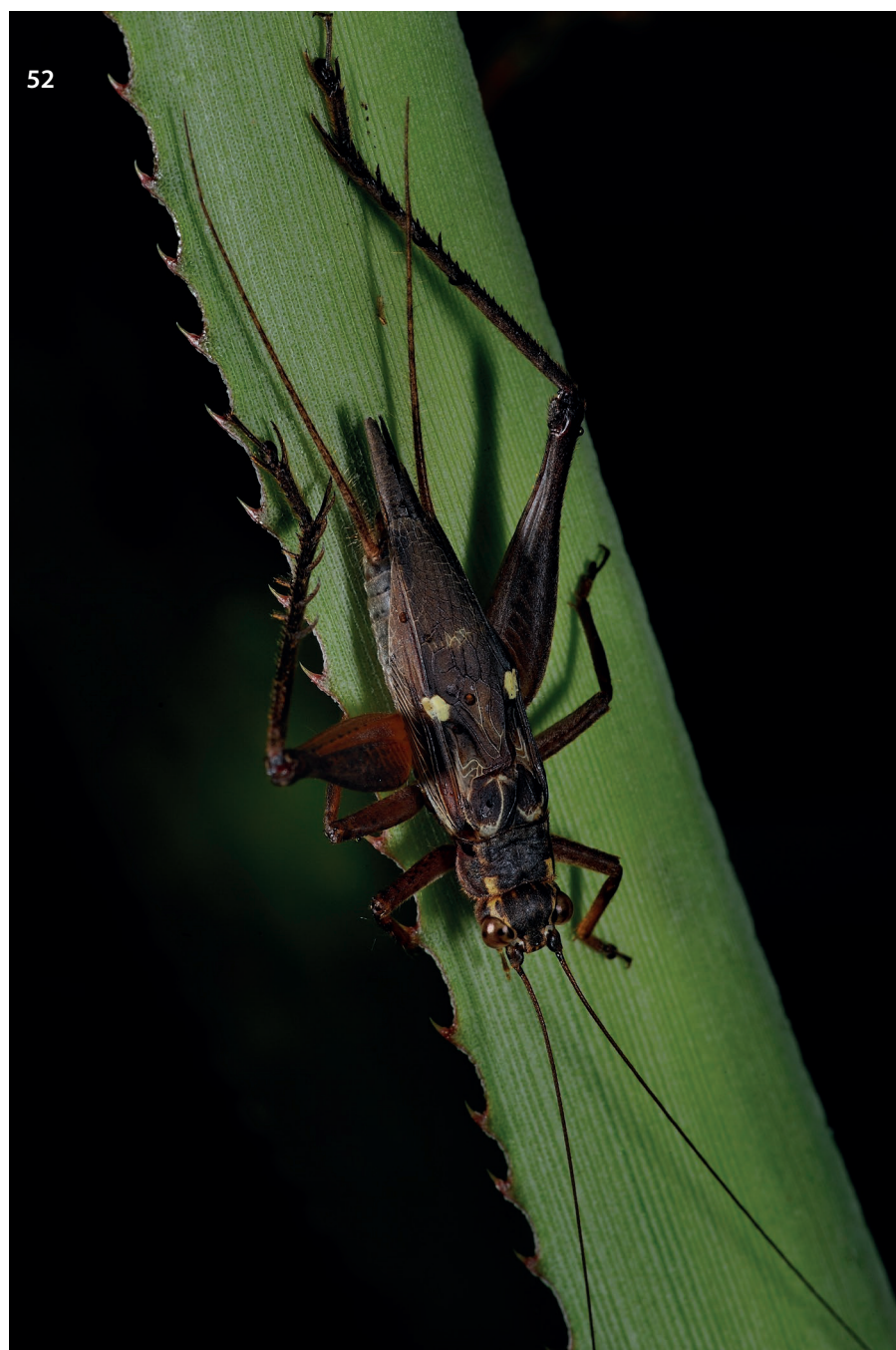
Measurements of *Cardiodactylus haddocki* n. sp.

Specimens from Eastern New Guinea (Papua New Guinea)														
	PronL	PronW	FWL	FWW	HWT	FIIL	FIIW	TIIL	TIII spines				Talls	OL
									las	lbs	Oas	Obs		
Holotype male	2.9	3.5	16.5	3.8	5.0	17.0	3.8	16.3	12	5	17	7	3	-
Males (n = 5)	2.9-3.1	3.5-4.0	15.9-17.8	3.8-4.0	5.1-6.0	17.0-18.5	3.8-4.1	16.0-16.9	10-13	4-6	15-19	7-9	2-3	-
(Male mean)	(3.0)	(3.7)	(16.8)	(3.9)	(5.4)	(17.6)	(4.0)	(16.3)	(12)	(5)	(17)	(7)	(3)	-
Allotype female	3.2	4.5	21.5	4.7	6.0	19.0	4.4	17.8	10	6	15	7	3	27.8
Specimens from Western New Guinea (Indonesia, Province Papua, localities Fawi, Manokwari, Bagusa)														
	PronL	PronW	FWL	FWW	HWT	FIIL	FIIW	TIIL	TIII spines				Talls	OL
									las	lbs	Oas	Obs		
Male (n = 5)	2.8-3.1	3.2-3.7	15.9-18.1	3.5-4.0	4.8-6.0	17.4-18.8	3.8-4.8	15.9-17.0	8-12	5-6	13-17	6-10	2-3	-
(Male mean)	(3.0)	(3.5)	(16.8)	(3.7)	(5.6)	(18.1)	(4.2)	(16.3)	(11)	(6)	(16)	(7)	(2)	-
Females (n=3)	3.4-3.7	4.0-4.4	19.2-20.1	4.2-4.3	6.4-7.6	21.7-23.0	4.6-5.3	18.4-20.5	10-12	6	15-17	6-8	2	26.9-29.8
(Female mean)	(3.6)	(4.2)	19.7	(4.2)	7.0	(23.4)	(4.9)	(19.5)	(11)	(6)	(16)	(7)	(3)	(28.4)

HABITAT AND LIFE HISTORY TRAITS — As *C. novaeguineae*, *C. haddocki* lives in open forested areas, near borders and hedges (Figures 49-52), where both adults and juveniles sit on leaves of bushes or trunks of low trees. Males call from dusk to late at night.

BEHAVIOR — The calling song of *C. haddocki* (Figures 53-58) is organized as in *C. novaeguineae*, each echeme being made of two parts separated by a short silence. At 23.3 °C (TR922, measurement of 10 echemes) the call duration is 2.1 ± 0.2 s (mean ± SD), with a period of 5.87 ± 1.32 s and a total of 63 ± 5.2 syllables organized as follows: The first sub-echeme (syllable rate = 26.5 syllables / s) lasts for 1.6 ± 0.2 s and consists of 44 ± 5.1 long syllables (syllable duration = 13.8 ± 2 ms; period = 37.7 ± 3.7 ms; duty cycle = 36.6 %). The second sub-echeme (syllable rate = 49.5 syllables / s) lasts for 386.8 ± 2.5 ms and consists of 19 ± 1 shorter syllables (syllable duration = 8.6 ± 1.8 ms; period = 20.2 ± 1.3 ms; duty cycle = 42.6 %). The difference between the two parts of the song is thus due to the longer syllable durations and



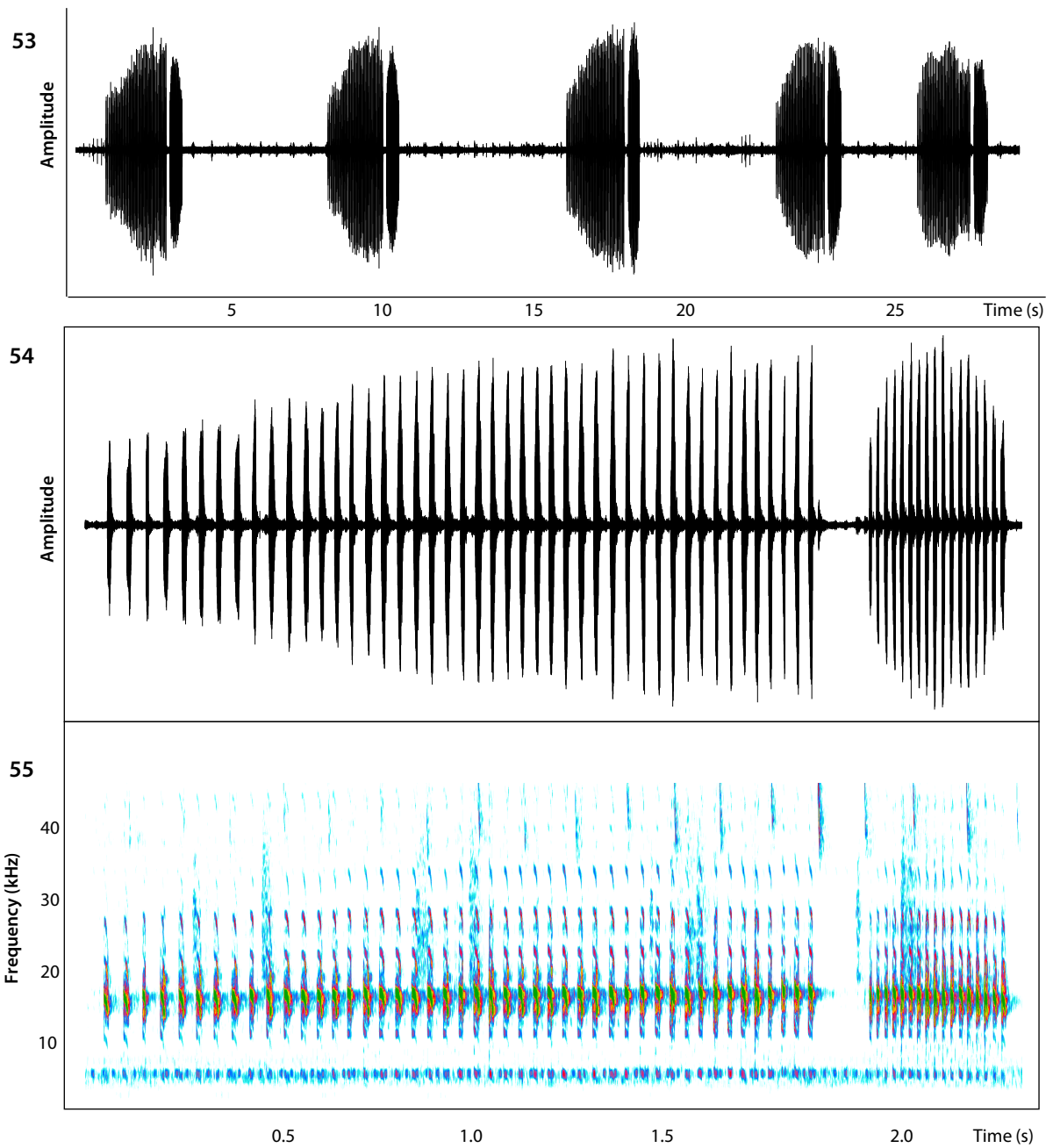


FIGURES 49-51

Cardiodactylus haddocki n. sp.;
49-50, male on banana tree in
 Mount Wilhelm during day;
51, male singing on low tree
 trunk in Mount Wilhelm at night.

FIGURE 52

52, *Cardiodactylus haddocki* n. sp.,
 male on *Pandanus* leaf in Mount Wilhelm at night.
 Photo by Xavier Desmier (MNHN-IRD).

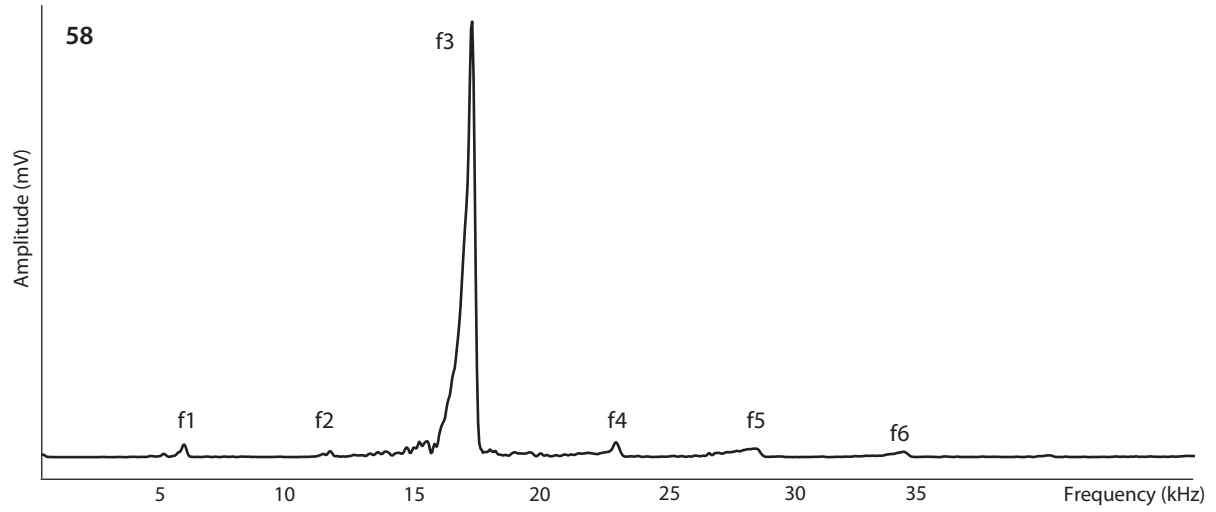
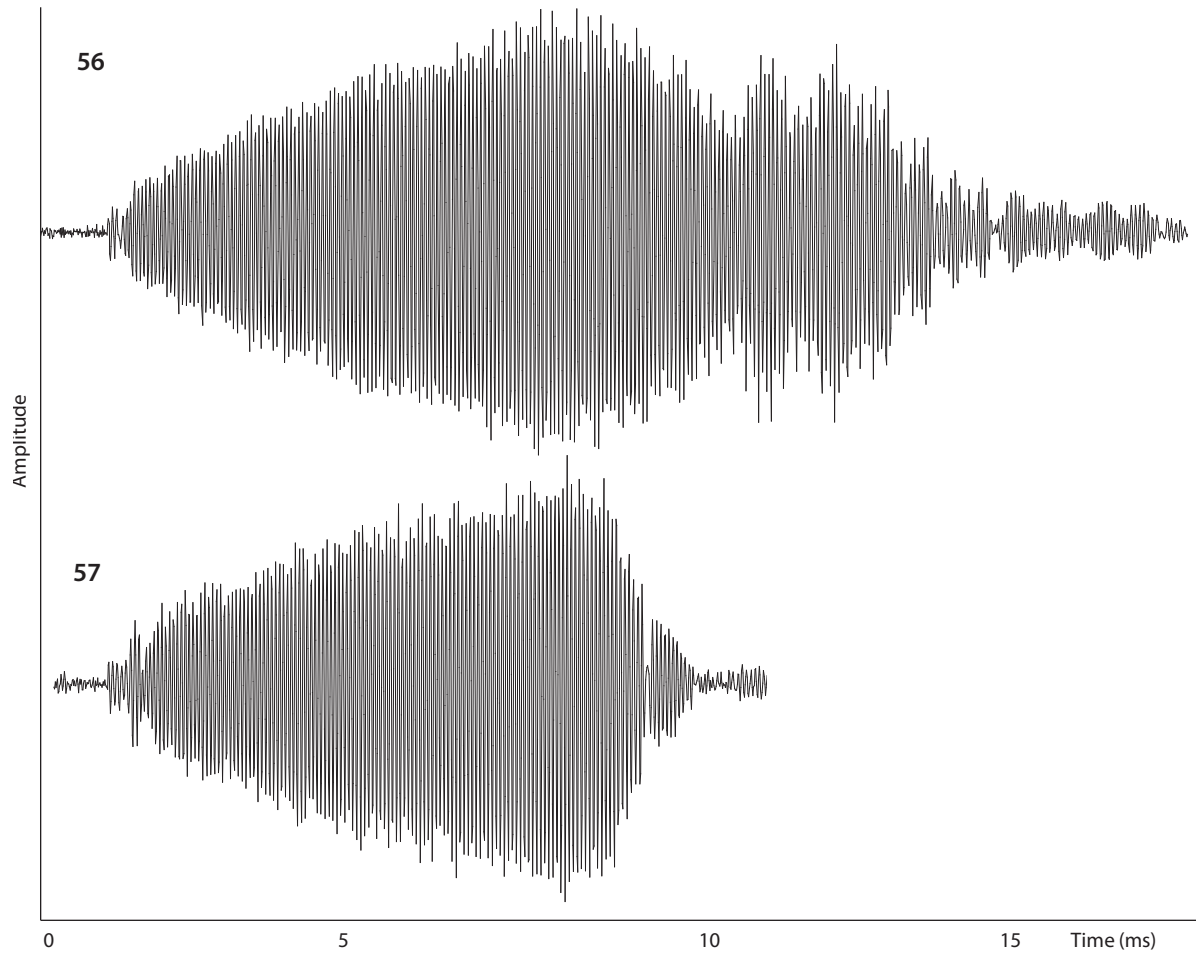


FIGURES 53-55

Calling song of *Cardiodactylus haddocki* n. sp.; **53**, oscillogram of 5 echemes; **54-55**, detailed oscillogram (**54**) and sonogram (**55**) of 1 echeme.

FIGURES 56-58

Calling song of *Cardiodactylus haddocki* n. sp.; **56-57**, detailed oscillogram of a syllable of first (**56**) and second sub-echeme (**57**); **58**, frequency spectrum of 1 syllable.



periods in the first sub-echeme, together with a slightly lower duty cycle (silence between syllables longer compared to syllable duration in first sub-echeme). The dominant frequency of the spectrum of *C. haddocki* is similar in both types of syllables (16.4 ± 0.4 kHz) and corresponds to the third harmonic of the song (Robillard *et al.* 2007); the mean fundamental frequency is about one-third as high (ca. 5.5 kHz).

***Cardiodactylus baitabensis* n. sp.**

Figures 17-18, 41, 59-62

TYPE MATERIAL — Female holotype: Papua New Guinea. Nouvelle-Guinée, Madang, forêt de Baitabag, 5°08'20,6"S 145°46'26,4"E, 86 m, 6.XII.2012, souche élevage F0, molecular sample C80-CnoBai, T. Robillard (MNHN-EO-ENSIF3539).

TYPE LOCALITY — Papua New Guinea, forest of Baitabag, 5°08'20,6"S 145°46'26,4"E, 86 m.

DISTRIBUTION — Eastern part of New Guinea Island.

ETYMOLOGY — Species named after type locality.

DIAGNOSIS — Species of average size for the species group, characterised by contrasted coloration on FWs (Figures 41, 59-60); close to *C. novaeguineae* and *C. haddocki*, from which it differs by the following characters: yellow bands on fastigium margins thinner; symmetrical dark brown patches on pronotum; whitish area on FWs small. Apex of ovipositor with both dorsal and ventral edges clearly denticulate, while it is smooth or slightly denticulate in *C. novaeguineae* and *C. haddocki*.

DESCRIPTION — Size large for the species group. General coloration brown with contrasting yellowish patterns on FWs (Figures 41, 59-60). Head (Figures 17-18): Dorsum yellow brown with four wide longitudinal bands, the median ones punctuated; area posterior to eyes with a brown patch and narrow yellow bands. Fastigium dark brown, slightly wider than scape, margins thin and yellow. Antennae homogeneously yellow brown. Scapes light brown with dark patterns. Face light brown. Mouthparts and maxillary palpi mostly yellow brown. Pronotum: Dorsal disk anterior margin concave; mostly yellow brown with contrasting symmetrical rounded patches; posterior margin bisinuate, mottled with dark brown. Lateral lobes light brown, ventral margin yellow. Legs brown, slightly mottled with dark brown. FWs exceeding tip of abdomen. Hind wings longer than FWs of a pronotum length; tail gray brown.

FEMALE: FWs with 10 strong longitudinal veins on dorsal field. FW coloration almost homogeneously brown, with a whitish band on external margin, including anterior half of veins CuA and M (Figure 41). Lateral field (Figure 60): R and Sc veins yellow; R/Sc area brown; Sc yellow, with 5 projections, the area between them brown. Ovipositor quite longer than hind femora (Figure 60), apex with both dorsal and ventral edges clearly denticulate.

Female genitalia (Figures 61-62): Copulatory papilla rounded, its apex narrowed and folded ventrally, slightly sclerotized; with two lateral triangular sclerites.

JUVENILE: Unknown.

MALE: Unknown.

MEASUREMENTS (in mm) — See Table 2.

HABITAT AND LIFE HISTORY TRAITS — Unknown.

BEHAVIOR — Unknown.

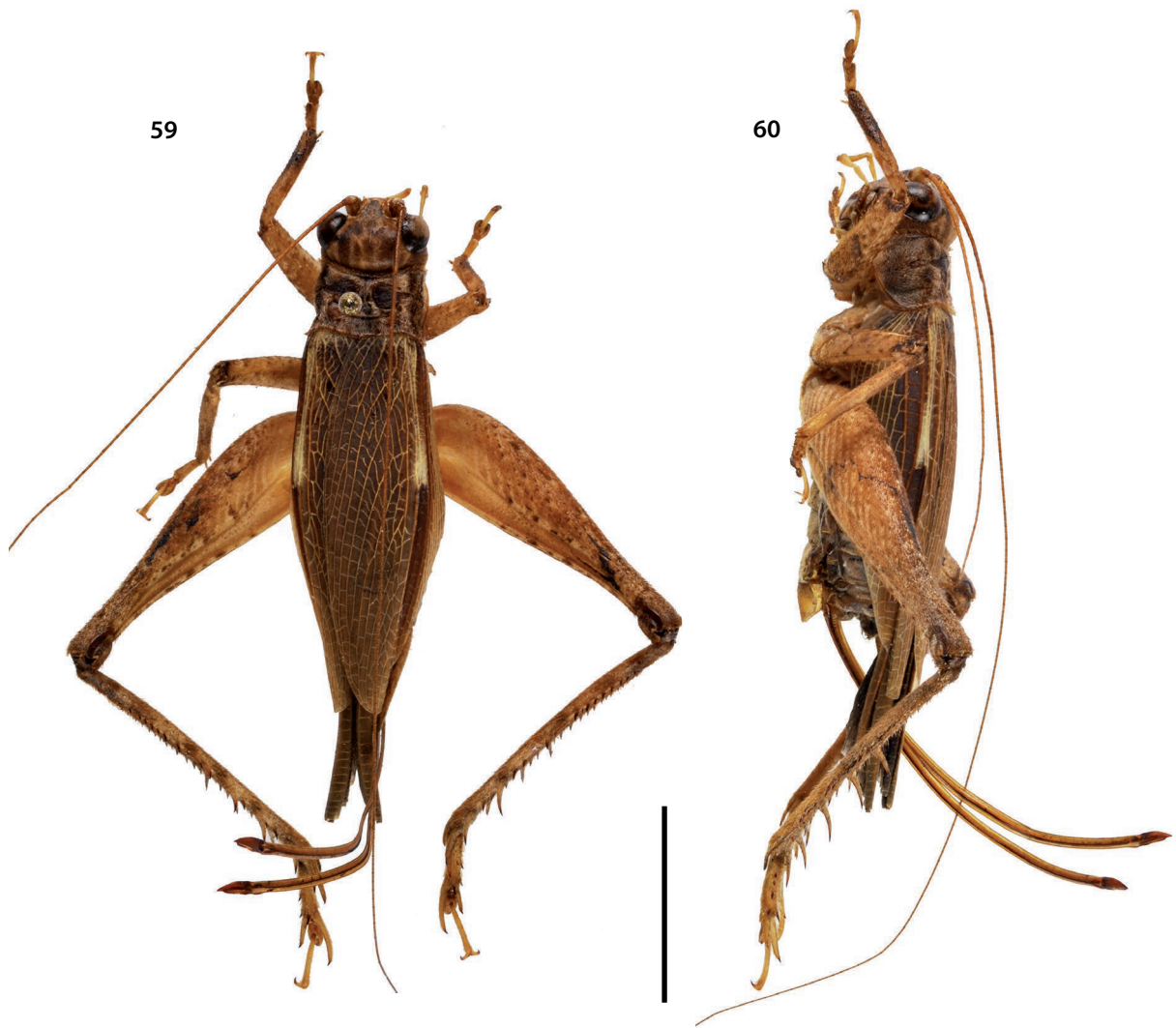
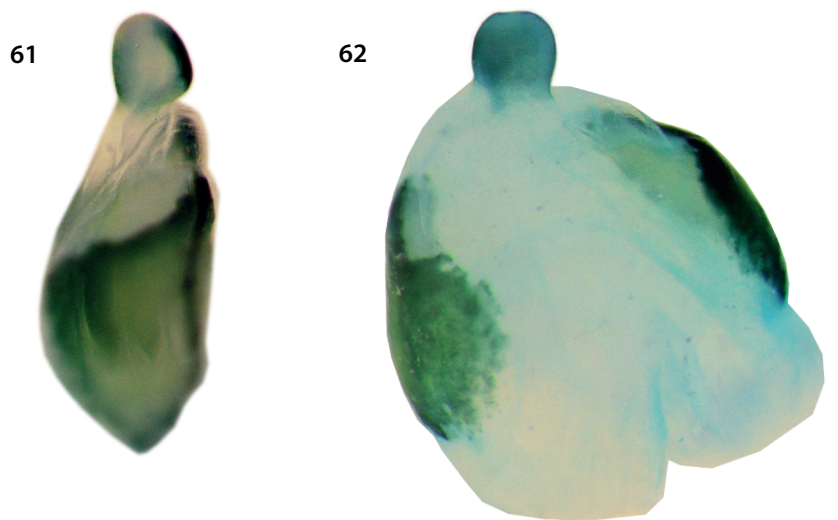


FIGURE 59-60
Cardiodactylus baitabensis n. sp.; **59**, female holotype in dorsal view;
60, in lateral view. Scale bar = 1 cm.

TABLE 2
 Measurements of *Cardiodactylus baitabensis* n. sp.

	PronL	PronW	FWL	FWW	HWT	FIILL	FIIIW	TIIIL	TIII spines				Tallls	OL
									las	lbs	Oas	Obs		
Holotype female	3.1	4.8	20.6	5.2	6.3	21.1	5.4	18.9	8	6	14	6	3	20.1



FIGURES 61-62

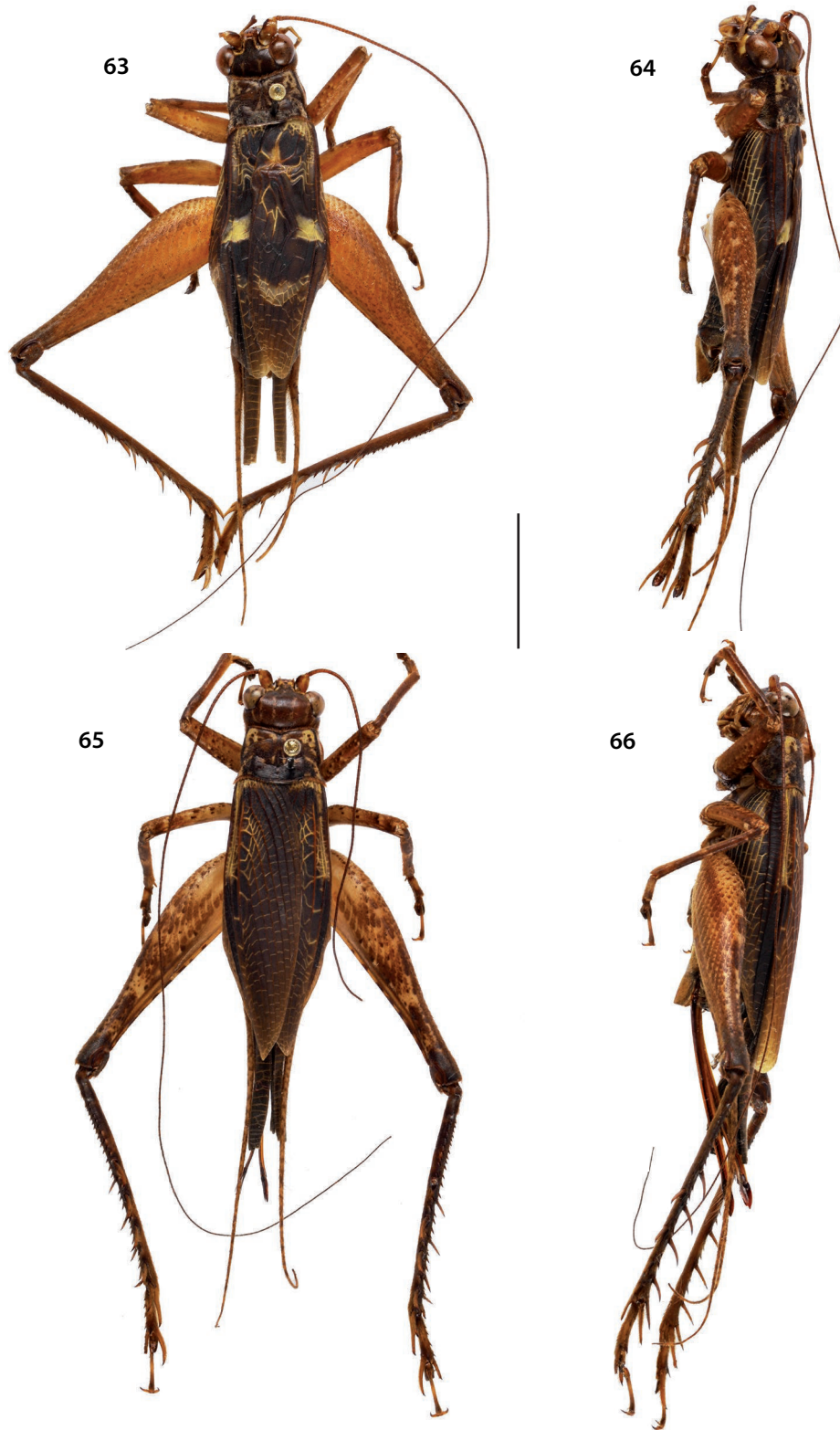
Cardiodyctylus baitabensis
n. sp.; **61**, female genitalia in
lateral view; **62**, in ventral view.
Scale bar = 0.5 mm.

***Cardiodyctylus lucus* n. sp.**

Figures 19-21, 36-37, 63-80, 96

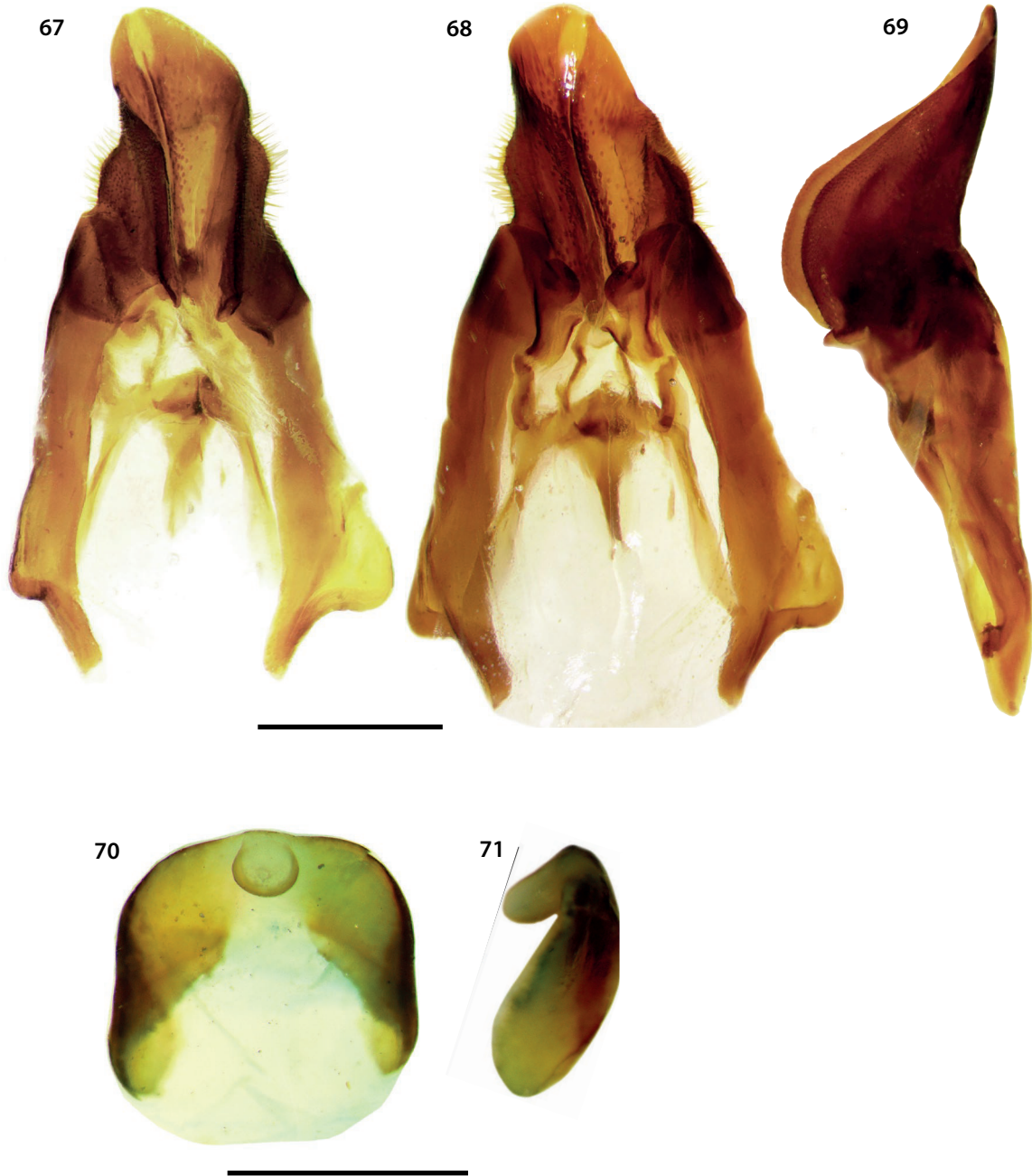
TYPE MATERIAL — Male holotype: Papua New Guinea. Nouvelle-Guinée, Madang, Station Wanang3, forêt autour du camp, 5°13'39,9"S 145°04'47,1"E, 199 m, 11.XI-2.XII.2012 (TR2420), jour, sur plante h = 1 m, T. Robillard (MNHN-EO-ENSIF126). Female allotype: Papua New Guinea. Same information as HT, morte en élevage (MNHN-EO-ENSIF107). Paratypes (1♂, 1♀): Papua New Guinea. Nouvelle-Guinée, Madang, Station Wanang3, PlotA, 5°13'34,6"S 145°04'51,6"E, 148 m, 19.XI.2012, T. Robillard: 1♂ (TR2162), jour, sur arbre, obs. copulation, mort en élevage, enregistrement appel Take Pap37-38, T. Robillard (MNHN-EO-ENSIF95); 1♀ (TR1843), jour, sur plante, arbuste h = 2,5 m, obs. copulation (PM), molecular sample CfoWa (MNHN-EO-ENSIF67).

OTHER MATERIAL EXAMINED. Papua New Guinea — Nouvelle-Guinée, Mt. Wilhelm, 200 m, PlotA 5°44'23,9"S 145°19'46,7"E, 246 m, 5-7.XI.2012, F. Legendre & T. Robillard: 1♂ (TR1648), jour, sur tronc h = 2,2 m, enregistrement appel Take Pap24, molecular sample CforMW, (MNHN-EO-ENSIF68); 1♀ (TR1741), jour, mort en élevage (MNHN). Nouvelle-Guinée, Mt. Wilhelm, 200 m, PlotC 5°44'41,5"S 145°19'38"E, 327 m, 5-7.XI.2012, 1♀ (TR1314), jour, sur plante, F. Legendre & T. Robillard (MNHN). Nouvelle-Guinée, Mt. Wilhelm, 200 m, hors plot, 5°44'23,9"S 145°19'46,7"E, 5-7.XI.2012, 1♀ (TR1959), mort en élevage, F. Legendre & T. Robillard (MNHN). Nouvelle-Guinée, Mt. Wilhelm, 700 m, camp 5°43'40,4"S 145°15'28,7"E, 768 m, 1-4.XI.2012, 1 juvénile (TR1550), nuit, F. Legendre & T. Robillard (MNHN). Nouvelle-Guinée, Mt. Wilhelm, 700 m, PlotC 5°43'56,6"S 145°15'24,8"E, 724 m, 1-4.XI.2012, 1 juvénile (TR1177), jour, plante, molecular sample C68-Csp700 (MNHN-ENSIF94). Nouvelle-Guinée, Mt. Wilhelm, 200 m, PlotA 5°44'23,9"S 145°19'46,7"E, 246 m, 5-7.XI.2012, 3 juvéniles (TR1660-1662), jour, F. Legendre & T. Robillard (MNHN). Nouvelle-Guinée, Mt. Wilhelm, 200 m, PlotB 5°44'27,3"S 145°19'46,1"E, 297 m, 5-7.XI.2012, 1 juvénile (TR1479), jour, sur plante, F. Legendre & T. Robillard (MNHN).



FIGURES 63-66

Cardiodactylus lucus n. sp.;
63, male in dorsal view; **64**, in
 lateral view; **65**, female in
 dorsal view; **66**, in lateral view.
 Scale bar = 1 cm.



FIGURES 67-71

Cardiodactylus lucus n. sp.;
67, male genitalia in dorsal view;
68, ventral view; **69**, lateral view;
70, female copulatory papilla in
ventral view; **71**, in lateral view.
Scale bars = 1 mm.

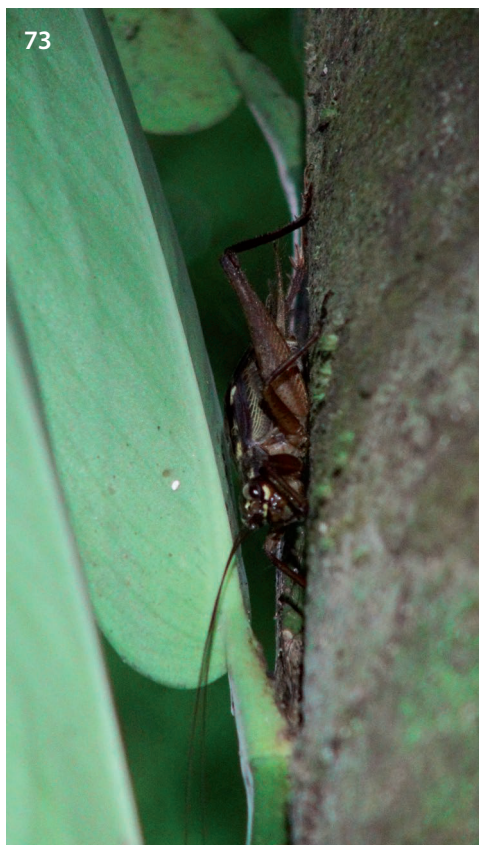
FIGURES 72-74

Cardiodactylus lucus n. sp.; **72**, female on large leaf in
Mount Wilhelm (200 m), at night; **73**, male on tree
trunk during day; **74**, juvenile on tree trunk at night.

72

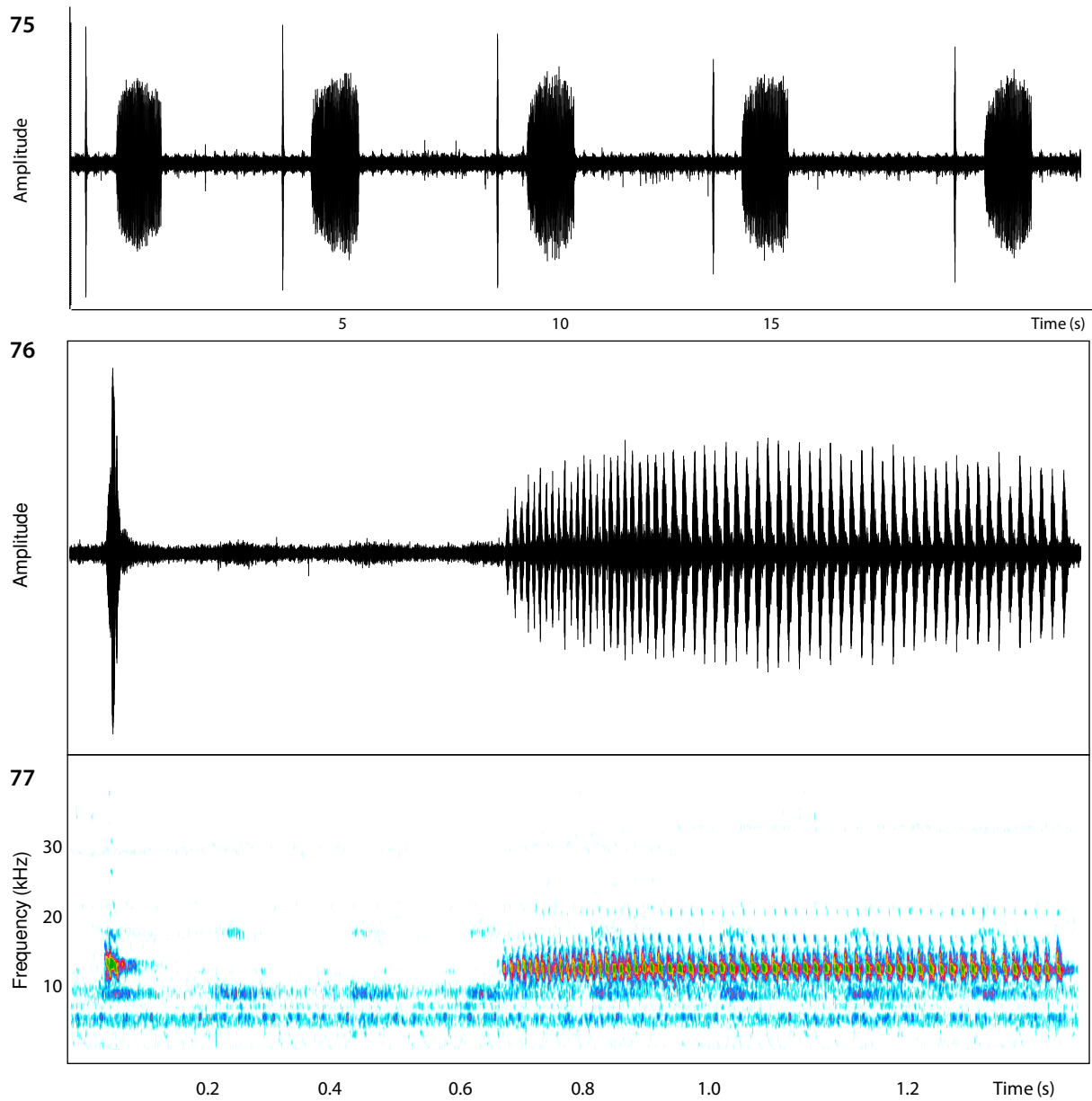


73



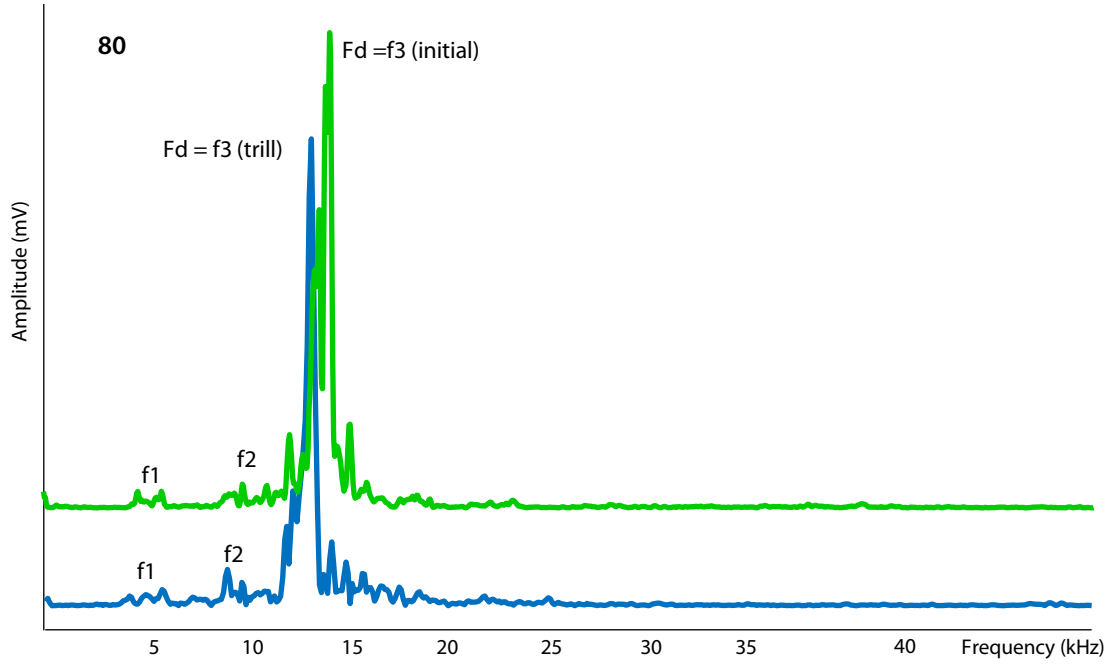
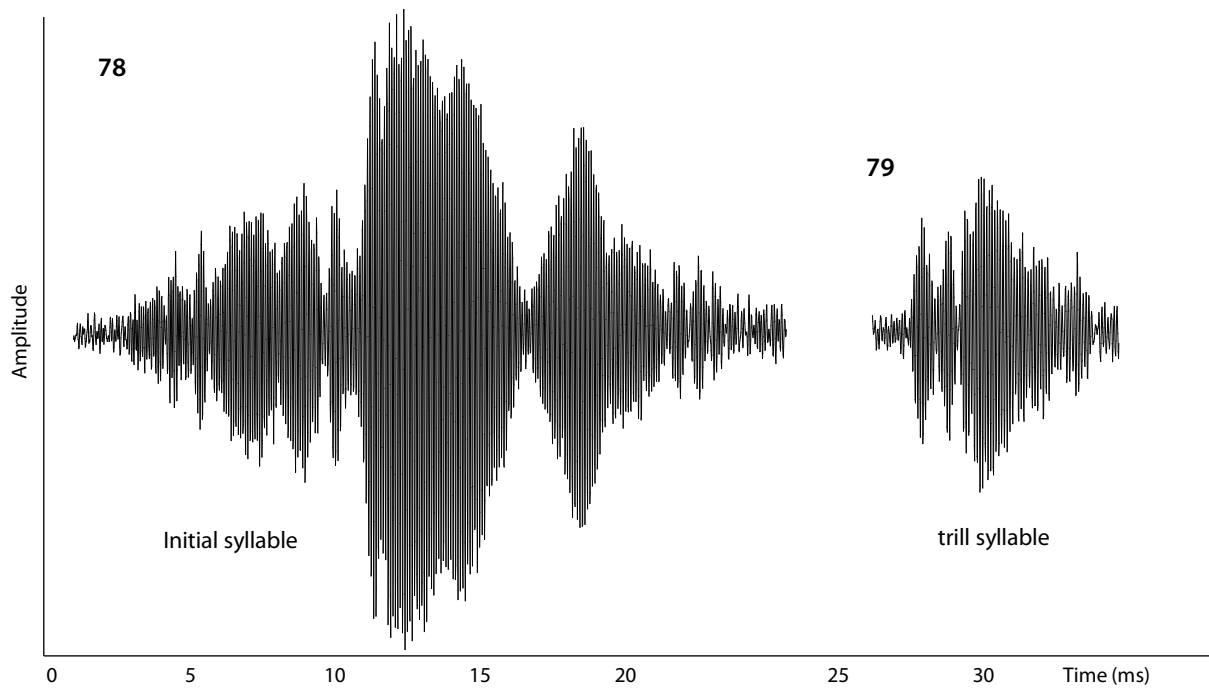
74





FIGURES 75-80

75-77. Calling song of *Cardiodactylus lucus* n. sp.; 75, oscillogram of 5 echemes; 76-77, detailed oscillogram (76) and sonogram (77) of 1 echeme. 78-80. Calling song of *Cardiodactylus lucus* n. sp.; 78, detailed oscillogram of an initial syllable; 79, trill syllable, showing relative amplitudes between both parts of the echeme; 80, frequency spectrum of initial syllable (green trace), and trill syllable (blue trace).



DIAGNOSIS — Species of large size even for the *Novaeguineae* group, characterized by contrasted coloration and asymmetrical male genitalia. Species very close to *Cardiodactylus bulolo* Otte, 2007, from which it differs by the following characters of male genitalia: dorsal ridges parallel (diverging posteriorly in *C. bulolo*), different in lateral profile; apical spoon asymmetrical, sclerotized and more extended posteriorly (very small and membranous in *C. bulolo*), apex of rami straight (hook-like in *C. bulolo*), posterior lobe of pseudepiphallallic parameres straight, pointing posteriorly (curved and pointing externally in *C. bulolo*).

DESCRIPTION — Size large for the species group. General coloration contrasted (Figures 63-66), dark brown with yellow patterns. Head (Figures 19-21): Dorsum almost entirely brown, with three wide bands almost fused together, separated by thin yellow bands; area posterior to eyes with brown triangles and a yellow line more externally. Fastigium as wide as scape, its anterior corners yellow, the rest dark brown. Scape yellowish brown with brown patterns. Antennae homogeneously orange brown. Face dark brown to brown, crossed by a transverse yellow band above epistomal suture, with a median interruption. Mouthparts yellow; bases of mandibles brown; maxillary palpi yellow brown, dark brown apically. Lateral part of head dark brown. Pronotum (Figures 21, 63-66): Dorsal disk trapezoidal, mottled with yellow and dark brown, its four corners yellow, with two symmetrical triangular yellow brown patches on median area; anterior margin slightly concave, posterior one bisinuate and dark brown. Lateral lobes almost homogeneously dark brown, ventral margin yellow. Hind and median legs brown, finely mottled with yellow and dark brown, femora with dark brown spots, tibiae with a faint yellow ring. FIII orange brown, variably mottled with dark brown, knees black (Figures 63-66). TIII dark brown, with faint yellowish rings at the level of spurs. TaIII-1 dark brown with a yellow ring. FWs reaching tip of abdomen, mostly dark brown with light patterns different in male and female (Figures 36-37). Lateral field: M, R and Sc veins dark orange; Sc projections and more ventral veins yellow, the cells between them dark brown. Hind wings: Longer than FWs of a pronotum length; tail dark brown, sometimes with lighter transverse veins. Cerci shorter than hind legs, gray brown mottled with dark spots. Tergites dark brown. Subgenital plate brown.

MALE: FW coloration (Figure 36): Dorsal field cells mostly dark brown, veins mostly yellow, with the following whitish areas: base of FWs, area anterior to file near plectrum, parts of chords, harp posterior angle, oblique veins and crescent region posterior to mirror. Base of chords with a dark brown sclerotization. FW venation (Figure 36): 1A bisinuate; stridulatory vein with 126 teeth (TR2126), 100 on the transverse part of the file, 15 on the angle area, and 11 near base of 1A (Figure 96). CuP absent. Harp narrow, longer than wide with 2 W-shaped oblique veins, with a straight one in anterior corner; posterior margin raised along diagonal vein. Mirror (d1) not rounded, longer than wide, variably crossed by one or several accessory veins. Apical field long and triangular, including 3-4 cell alignments posterior to mirror. Latero-dorsal angle made by M. Sc with 7-9 projections.

Male genitalia (Figures 67-69): Pseudepiphallus asymmetrical, narrowed at mid-length, setose laterally and ventrally. Dorsal ridges parallel, forming a wide gutter, external edges carinated, dorsal edges very narrow, not forming bean-like platforms; pseudepiphallus with only one level laterally. Pseudepiphallallic sclerite with latero-anterior triangular expansions, the left one longer anteriorly due to asymmetry; membrane between them with strong short setae. Apex of pseudepiphallus short, spoon-like and particularly asymmetrical, not rounded and slightly triangular, with a small median little-sclerotized area. Rami strong but narrow, with wide triangular preapical plates and long, straight apex. Pseudepiphallallic parameres trilobate, posterior lobe forming a wide triangle. Ectophallic arc complete, with a short posterior expansion. Ectophallic apodemes lamellate, not reaching rami apex. Apex of ectophallic fold trilobate, membranous. Endophallic sclerite small, with a medio-posterior expansion. Endophallic apodeme with both lateral lamellas and a large medio-dorsal crest extended anteriorly. Membrane of endophallic cavity smooth.

FEMALE: FW mostly dark brown with orange veins (Figure 37); base of FWs yellow; with a yellow area in antero-external part of FW including basal half of CuA and M (cells and veins) and extending dorsally around anterior part of CuP. Posterior half of CuA/M/R areas black. R and Sc orange brown, transverse veins and Sc projections yellow. FW venation with 10-12 (m = 11, n = 4) strong longitudinal veins on dorsal field. Ovipositor: shorter than hind femora; apex with both dorsal and ventral edges denticulate.

Female genitalia (Figures 70-71): Copulatory papilla almost square, wider than long, its apex narrowed and folded ventrally; two lateral sclerites.

JUVENILE: Coloration lighter than adults (Figures 72-74), yellow mottled with brown and black spots. Head and pronotum with similar banding patterns as in adults. Ventral edge of pronotum lateral lobes yellow.

MEASUREMENTS — See Table 3.

HABITAT AND LIFE HISTORY TRAITS — *C. lucus* lives in forested areas, where both adults and juveniles are generally found on tree trunks at variable heights (Figures 72-74). Juveniles are sometimes found on lower vegetation (Figure 74). Males start emitting their calling songs during late afternoon until late at night. Couples mate during day time, on high positions on tree trunks (Figure 73).

BEHAVIOR — The calling song of *C. lucus* (Figures 75-80) is made of short echemes composed of two parts (Figures 75-76). Echemes last for 1.41 ± 0.13 s with a period of 4.71 ± 1.33 s. The first part of the echeme consists of one powerful syllable (duration = 20.1 ± 6.4 ms), separated from the second part by a silence of 555 ± 123 ms; the second part is a short trill, made of 63.1 ± 5 syllables, with the following characteristics: syllable duration = 6.7 ± 1.5 ms; syllable period = 13.1 ± 2.9 ms; syllable duty cycle = 51.1 %; syllable rate = 76 /s. The dominant frequency of the spectrum has the same profile in both parts of the song and corresponds to the third harmonic peak (Robillard *et al.* 2007); however, the value of dominant frequency differs slightly between each part (Figure 80): In the initial syllable, the dominant frequency is 13.18 ± 0.25 kHz, while it is lower (12.27 ± 0.14 kHz) in the trill.

TABLE 3

Measurements of *Cardiodactylus lucus* n. sp.

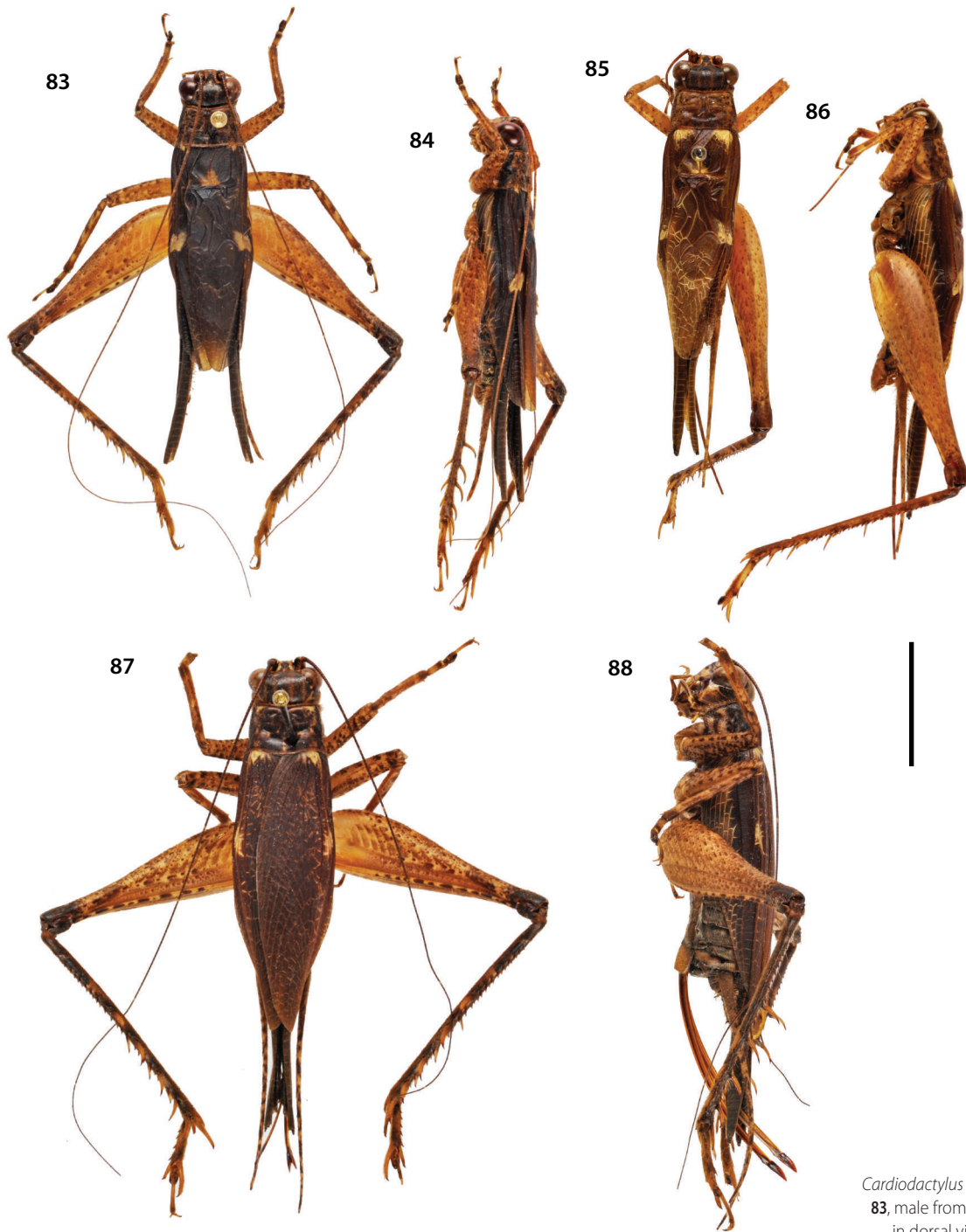
	PronL	PronW	FWL	FWW	HWT	FIIL	FIIW	TIIL	TIII spines				Tallls	OL
									las	lbs	Oas	Obs		
Holotype male	3.7	4.2	23.0	4.7	6.8	21.6	5.2	19.3	9	8	16	7	2	-
Males (n = 3)	3.4-3.7	4.2-5.4	18.8-23.0	4.7-5.1	6.4-7.3	20.1-21.6	4.9-5.4	18.0-19.3	8-10	7-8	14-16	7-8	2-3	-
(Male mean)	(3.6)	(4.9)	(20.6)	(4.9)	(6.8)	(21.0)	(5.2)	(18.5)	(9)	(7)	(15)	(7)	(3)	-
Allotype female	4.4	4.7	21.9	5.3	7.5	22.3	5.5	19.2	9	6	16	8	3	16.9
Females (n = 4)	4.1-4.4	4.7-5.5	20.5-22.2	4.7-5.5	6.7-7.5	20.8-22.3	5.1-5.7	19.0-19.8	8-11	5-6	14-16	6-8	3	16.6-16.9
(Female mean)	(4.3)	(5.1)	(21.4)	(5.1)	(7.2)	(21.8)	(5.4)	(19.4)	(9)	(6)	(15)	(7)	(3)	(16.8)

Species group *Efordi* Otte, 2007a***Cardiodactylus busu*** Otte, 2007a

Figures 22-24, 38-40, 81-94, 97, 98-105

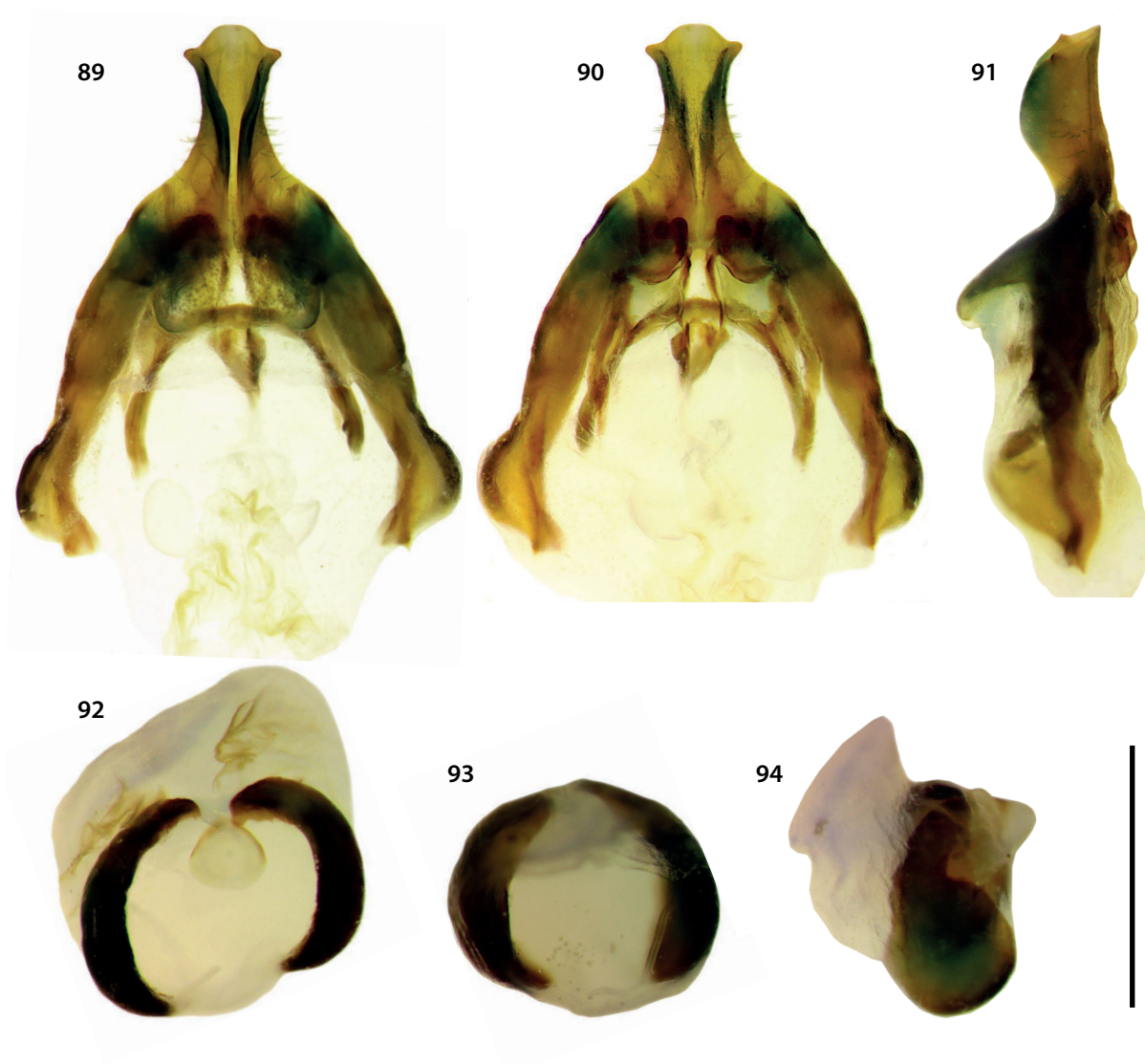
Cardiodactylus busu Otte 2007a: 351 – Otte 2007b: 31; Eades *et al.* 2015 (Orthoptera Species File Online).**TYPE MATERIAL** — Male holotype: Indonesia. New Guinea (NW), Japen Island, SSE of Sumberbaba, Dawai River, 10.X.1962, N. Wilson (BPBM) [not examined].**MATERIAL EXAMINED** — Papua New Guinea. Nouvelle-Guinée, Madang, forêt de Baitabag, 5°08'20,6"S 145°46'26,4"E, 86 m, 6.XII.2012, T. Robillard: 1 ♂ (TR2471), molecular sample C56CbuMa (MNHN-EO-ENSIF134). Mt. Wilhelm, 200 m, hors plot, 5°44'23,9"S 145°19'46,7"E, 5-7.XI.2012, morts en élevage, F. Legendre & T. Robillard: 2 ♀ (TR1938, 1959), 6 juveniles (TR1577, 1579, 1581, 1657, 1659, 1683). Nouvelle-Guinée, Mt. Wilhelm, 200 m, PlotB 5°44'27,3"S 145°19'46,1"E, 297 m, 5-7.XI.2012, nuit, 1 juvenile (TR1664), F. Legendre & T. Robillard (MNHN). Nouvelle-Guinée, Mt. Wilhelm, 200 m, PlotC 5°44'41,5"S 145°19'38"E, 327 m, 5-7.XI.2012, F. Legendre & T. Robillard: 1 ♂ (TR1740), mort en élevage, enregistrement appel, molecular sample C58 (MNHN-EO-ENSIF109). Nouvelle-Guinée, Madang, Station Wanang3, PlotA, 5°13'34,6"S 145°04'51,6"E, 148 m, T. Robillard: 19.XI.2012, 4 juveniles (TR1825-1828), jour, sur plante (MNHN). Nouvelle-Guinée, Madang, Station Wanang3, PlotB, 5°13'35"S 145°04'52"E, 154 m, T. Robillard: 24.XI.2012, 1 juvenile (TR1892), nuit, sur plante, molecular sample barcodePNG (MNHN-EO-ENSIF75); 20.XI.2012, 1 juveniles (TR1893), jour (MNHN). Nouvelle-Guinée, Madang, Station Wanang3, PlotC, 5°13'36,5"S 145°04'59,9"E, 126 m, T. Robillard: 30.XI.2012, 1 juvenile (TR2346), nuit (MNHN). Nouvelle-Guinée, Madang, Station Wanang3, forêt autour du camp, 5°13'39,9"S 145°04'47,1"E, 199 m, 11.XI-2.XII.2012, T. Robillard: 1 ♂, 7 ♀ (TR2432-2433, TR2442-2444), 4 juveniles (TR1770, 1791, 1933, 1934), mort en élevage (MNHN); 1 ♀ (TR2419), nuit, plante h = 20 cm (MNHN); 1 juvenile (TR1625), plante (MNHN); 1 ♂ (TR1777), nuit, arbuste h = 2.2 m, enregistrement appel Take Pap34 (MNHN-EO-ENSIF101); 1 ♂ (TR1863), nuit, sur plante h = 30 cm, enregistrement appel Take Pap30-32 (MNHN-EO-ENSIF116). Nouvelle-Guinée, Madang, station Wanang3, rivière Wanang, forêt secondaire, 5°13'31,8"S 145°04'42"E, 76 m, 11.XI-2.XII.2012, T. Robillard: 1 ♂ (TR2268), jour, sur tronc d = 70 cm (MNHN); 1 ♂ (TR2269), vidéo copulation sur tronc (MNHN-EO-ENSIF69); 2 ♀ (TR2205, 2479), mort en élevage (MNHN); 1 juvenile (TR2269), nuit, mort en élevage, échantillon moléculaire barcodePNG (MNHN-EO-ENSIF115); 3 juveniles (TR1932, 2182), morts en élevage (MNHN). Nouvelle-Guinée, Mount Wilhelm, 200 m, MW200FIT [flight interception trap], F. Legendre & T. Robillard, 1 juvenile (MNHN). Nouvelle-Guinée, Madang, Station Wanang3, WanangFIT [flight interception trap], F. Legendre & T. Robillard, 4 juveniles (MNHN). E. Dutch New Guinea, Jutefa Bay, Pim., sea level~1000ft, II.1936, 1 ♂, 1 ♀, L.E. Cheesman (BMNH-B.M.1936-271). New Guinea, 15 mls. S. of Paup, Torecella Mt., 1700 ft., 4-11.III.1939, 2 ♀, G.P. Moore, (BMNH-B.M.1939-479). N. Guinea, 1 ♀, identified *Cardiodactylus* sp. by R. L. Randell, Nyman (NHRS - NRM-ORTH-0011439). Indonesia. New Guinea, Stephansort [Astrolabe Bay], 1 ♂, 18-954 (NHMW). Ned. Nw Guinea exp., Hollandia, 1811, 1 ♀, Dr. P. N. v. Kampen (RMNH). W. New Guinea, Njau-limon, S. of Mt. Bougainville, 300 ft. II.1936, 1 ♀, E. Cheesman (BMNH-B.M.1936-271). [Papua], Dosay [village], 180-200 m, 21.V.1973, 1 ♂ (MZB-ORTH9556). New Guinea (NW), Hollandia [Jayapura], 100 m, 23.IX.1965, J. L. Gressitt (BPBM CAR60) [incorrectly labelled holotype].**TYPE LOCALITY** — Indonesia, Japen Island.**DISTRIBUTION** — Northern part of New Guinea Island and surrounding islands.**FIGURES 81-82**81-82, *Cardiodactylus busu* Otte, 2007, males on low vegetation in Mount Wilhelm.





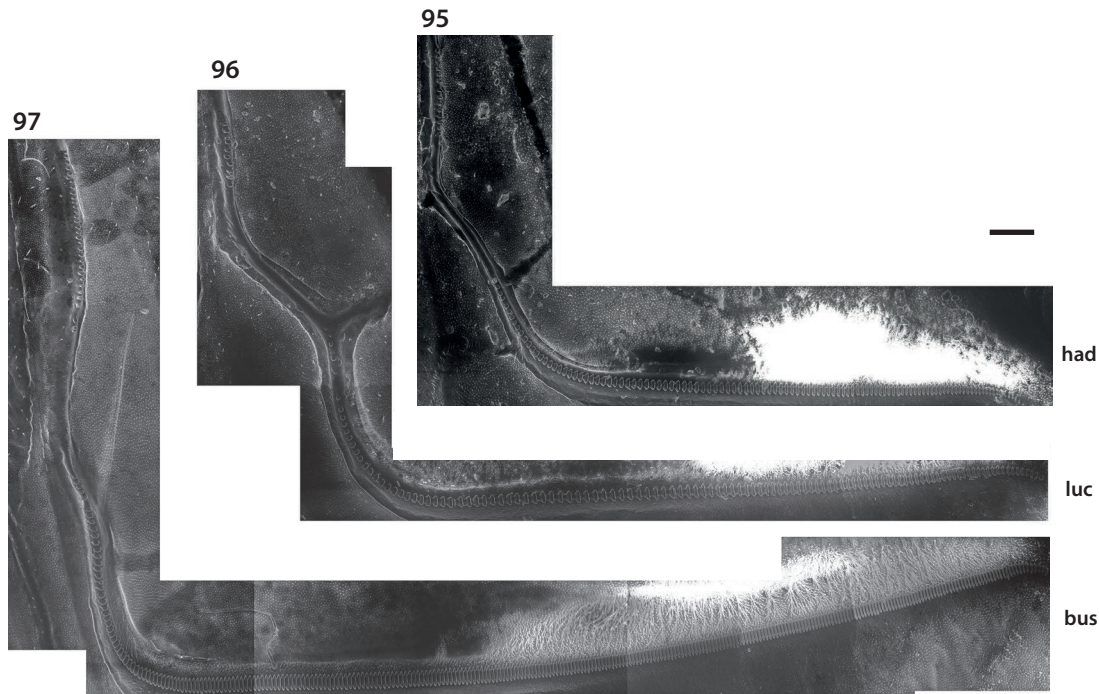
FIGURES 83-88

Cardiodyctylus busu Otte, 2007;
83, male from Mount Wilhelm
 in dorsal view; **84**, in lateral
 view; **85**, male from Jayapura in
 dorsal view; **86**, in lateral view;
87, female from Mount Wilhelm
 in dorsal view; **88**, in lateral
 view. Scale bar = 1 cm.



FIGURES 89-94

Cardiodactylus busu Otte, 2007; **89**, male genitalia in dorsal view; **90**, ventral view; **91**, lateral view; **92**, female copulatory papilla in ventral view; **93**, in dorsal view; **94**, in lateral view. Scale bars = 1 mm.

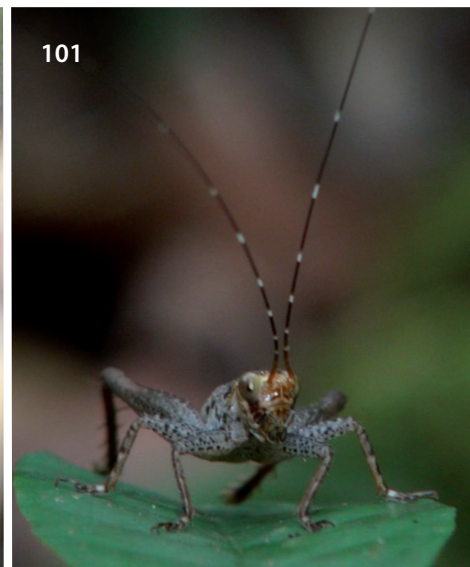


FIGURES 95-97

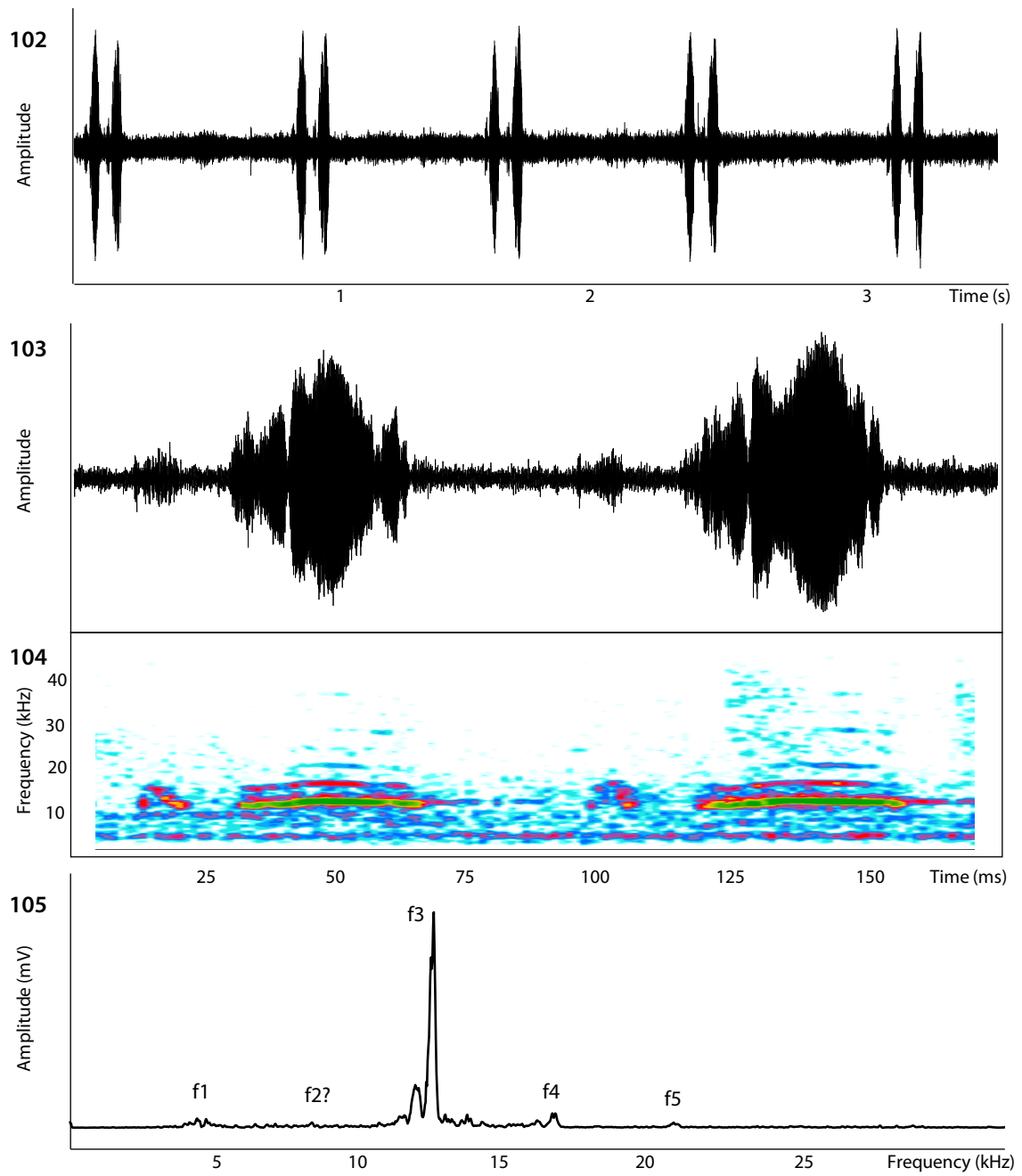
Stridulatory files photographed with SEM; **95**, *Cardiodactylus haddocki* n. sp.; **96**, *Cardiodactylus lucus* n. sp.; **97**, *Cardiodactylus busu* Otte, 2007. Scale bar = 200 μ m.

DIAGNOSIS — Size average. Species close to *Novaeguineae* group by its yellow-spotted coloration (Figures 81-88), but closer to *Efordi* group by male genitalia; close to *C. nobilis* n. sp. in terms of head and pronotum coloration, but differing by coloration of FWs and male genitalia. General coloration almost completely dark brown dorsally with contrasting yellow pattern on FW in both sexes (Figures 38-40), yellow and brown laterally; FW with long apical field. Face with vertical dark brown bands below eyes; lateral lobe of pronotum mostly yellow with a brown longitudinal line. Male genitalia with a triangular pseudepiphallus forming a gutter widely open posteriorly with characteristic lateral expansions.

REDESCRIPTION — Average to large size for the species group. General coloration dark brown with contrasting yellow area pattern (Figures 83-88). Head (Figures 22-24): Dorsum dark brown with four yellow brown longitudinal bands and two yellow bands suture posterior to eyes. Fastigium narrow, almost as wide as scape, dark brown with a triangular yellow spot between ocelli, margins yellow. Scapes brown, their base yellow. Antennae brown basally, then orange brown with yellow segments. Face yellow, with two lateral wide dark brown vertical stripes starting below eyes and continuing on mandibles; front part of fastigium with two symmetrical vertical dark brown stripes between the scapes. Lateral part of head dark brown dorsally, lighter ventrally. Mouthparts yellow with a brown ring on dorsal margin of labrum. Maxillary palpi brown. Pronotum: Dorsal disk mostly brown, with several symmetrical transverse sutures, its anterior corners yellow; anterior margin concave, posterior margin bisinuate; lateral margins dark brown. Lateral lobes dark brown to black near upper margin, rest yellow with thin longitudinal light brown stripes. Legs light brown, mottled with dark brown spots. Hind-wing tail brown, longer than pronotum. Cerci gray with brown rings.



FIGURES 98-101
Cardiodactylus busu Otte, 2007;
98, view of forest habitat in
 Wanang, near Wanang3 river;
99, couple mating on large tree
 trunk at day, male producing
 guarding song; **100-101**,
 juveniles on vegetation.



FIGURES 102-105

Calling song of *Cardiodactylus busu* Otte, 2007; **102**, oscillogram of 5 echemes; **103-104**, detailed oscillogram (**103**) and sonogram (**104**) of 1 echeme; **105**, frequency spectrum of 1 syllable.

MALE: FW coloration (Figures 38, 40, 81-82): Mostly dark brown with contrasting yellow pattern. Dorsal field: Yellow areas include base and most distal parts of anal veins and posterior part of harp. Lateral field: dark brown on M/R area and R/Sc area, yellow on Sc projection area with one posterior light brown stripe. FW venation: 1A bisinuate; stridulatory file with 320 teeth, located on both transverse part and basal longitudinal part of 1A. CuP absent. Harp wide with 2 W-shaped oblique veins. Mirror area variable, c1 narrow and long, c2 longer than wide. Mirror not rounded, longer than wide, variably crossed by accessory veins in posterior half. Apical field long and narrow, CuA bifurcations giving 4 or 5 longitudinal veins (CuA4-8) posterior to mirror. Sc with 10-12 projections.

Male genitalia (Figures 89-91): Pseudepiphallus triangular, narrowed twice, at mid-length and preapically. Apex of pseudepiphallus rounded, with two short preapical lateral extensions. Dorsal ridges divergent posteriorly, almost in contact anteriorly forming a gutter. Basal part of pseudepiphallus made of wide latero-anterior expansions raised basally, the membrane between them with thin setae. Rami with large preapical plates. Ectophallic arc complete, wide and curved, with a short but wide posterior expansion. Ectophallic apodemes extending to the middle of apex of rami preapical plates. Ectophallic fold membranous, with weak lateral sclerotizations; apex trilobate, membranous. Endophallic sclerite small, with a small posterior expansion; endophallic apodeme with wide lateral lamellas and a strong medio-dorsal crest.

FEMALE: FW coloration (Figure 39): Cells dark brown, veins orange brown, with whitish sclerotizations near bases of anal veins and laterally, near half of CuA. FW coloration with similar pattern as in male. FW venation with 10 strong longitudinal veins on dorsal field; lateral field with 8 longitudinal veins. Ovipositor: almost as long as hind femora; dorsal edge of apex slightly denticulate.

Female genitalia (Figures 92-94): Copulatory papilla rounded, its base with two strong lateral sclerotizations; apex rounded forming a transparent bubble folded ventrally.

JUVENILE: Coloration lighter than in adults, molted with dark spots, with similar color patterns on face and pronotum. Legs pale with dark gray spots (Figures 100-101).

MEASUREMENTS (in mm) — See Table 4.

HABITAT AND LIFE HISTORY TRAITS — *C. busu* lives in forested areas (Figure 98), on bushes and tree trunks. Males start calling at dusk and continues until late at night, sitting on vegetation. Couples are found during day time, mating on the trunks of large trees (Figure 99).

TABLE 4

Measurements of *Cardiodactylus busu* Otte, 2007a

	PronL	PronW	FWL	FWW	HWT	FIIL	FIIW	TIIL	TIII spines				TaIIIs	OL
									las	lbs	Oas	Obs		
Males (n = 5)	3.0-3.1	3.6-3.9	17.8-19.6	4.5-5.0	7.4-8.2	18.3-19.5	4.2-4.8	15.3-18.5	9-10	4-7	15-17	5-9	2-3	-
(Male mean)	(3.0)	(3.8)	(18.6)	(4.7)	(7.9)	(18.8)	(4.6)	(16.9)	(9)	(5)	(15)	(7)	(3)	-
Females (n = 4)	3.4-3.7	4.6-5.3	21.8-22.9	5.3-6.7	7.7-8.9	21.7-23.7	5.0-5.7	18.5-21.4	9-11	4-7	15	6-9	2-3	18.4-19.9
(Female mean)	(3.6)	(4.9)	(22.4)	(5.8)	(8.1)	(22.6)	(5.3)	(20.0)	(8)	(6)	(15)	(8)	(3)	(19.2)

BEHAVIOR — The calling song of *C. busu* (Figures 102-105) corresponds to doublets of syllables repeated almost continuously. At 24°C (TR1863, bout of 30 s measured), these doublets (= echemes) last for 120 ± 5 ms with a period of 760 ± 38 ms, and are thus emitted at a rate of 1.32 echemes / s (or 2.64 syllables / s). Syllables generally show several indentations in their amplitude profile, suggesting an irregular closing movement of the FWs. Syllables have the following characteristics: syllable duration = 35 ± 3.9 ms; syllable period = 83 ± 2 ms; syllable duty cycle = 42.2 %. The dominant frequency of the spectrum (Figure 105) is similar in both syllables within the doublets (12.34 ± 0.12 kHz) and corresponds to the third harmonic of the song (Robillard *et al.* 2007); the mean fundamental frequency is about one-third as high (ca. 4.1 kHz).

Cardiodactylus javarere Otte, 2007a

Figures 25-27, 42-43, 106-114

Cardiodactylus javarere Otte, 2007a: 354 – Otte 2007b: 32; Eades *et al.* 2015 (Orthoptera Species File Online).

TYPE MATERIAL — Male holotype: Papua New Guinea. Papua, Daradae, near Javarere, Musgrove River, 100 m?, 3.X.1958, Gressitt (BPBM 16,793) [examined].

OTHER MATERIAL EXAMINED — Papua New Guinea. Brit.[ish] New Guinea, Madew, St. Joseph R.[iver] 2000-3000 ft., 1909-22, W. Stalker, 1♂(BMNH). Nouvelle-Guinée, 1♀, Jolivet (MNHN). Nouvelle-Guinée, Madang, forêt de Baitabag, 5°08'20,6"S 145°46'26,4"E, 86 m, 6.XII.2012, 1♀, souche élevage F0, molecular sample C78-CspBai, T. Robillard (MNHN-EO-ENSIF3538). Nouvelle-Guinée, Madang, Station Wanang3, forêt autour du camp, 5°13'39,9"S 145°04'47,1"E, 199 m, 11.XI-2.XII.2012, nuit, T. Robillard: 1 juvenile (TR1931), mort en élevage (MNHN); 1 juvenile (TR1789), mort en élevage, molecular sample C63 (MNHN-EO-ENSIF103) ; 1 juvenile (TR1790), mort en élevage (MNHN); 1 juvenile (TR1930), molecular sample C164CnbWa, mort en élevage (MNHN-EO-ENSIF91). Nouvelle-Guinée, Madang, Station Wanang3, PlotB, 5°13'35"S 145°04'52"E, 154 m, 24.XI.2012, nuit, T. Robillard : 1 juvenile (TR2158). Papua New Guinea, Mount Wilhelm, PNG2012-MW200: 1 juvenile (FIT20, 11133j, P0772); 1 juvenile, (FIT19, 11165a, P0862), molecular sample C165-CnbMW (MNHN-EO-ENSIF60).

TYPE LOCALITY — Papua New Guinea, Daradae, S8°46'11.20"S, E147°10'52.00"

DISTRIBUTION — Eastern New Guinea Island.

EMENDED DIAGNOSIS — Species characterized by small size, stocky shape (Figures 106-109), wide head (Figure 25), male FWs with a thin and elongate mirror (Figure 42), harp with a whitish spot in posterior angle, male genitalia with a triangular apex and dorsal part of pseudepiphallus with strong setae (Figure 110). Species close to *C. kolombangara* Otte, 2007 in coloration and male genitalia, but differing by more slender body shape and smaller size, shape of pseudepiphallus in male genitalia and detail of coloration.

REMARK — Both *C. javarere* and *C. kolombangara* show intermediate characters between *Novaeguineae* and *Efordi* species group: their male FWs resemble of species of the *Novaeguineae* group, with a whitish spot in harp posterior angle, the male genitalia are clearly closer to the *Efordi* group, with a pseudepiphallus strongly narrowed at mid-length and with small or absent dorsal ridges.

REDESCRIPTION — In addition to the characters given by Otte (2007a): size average for the species group, stocky shape (Figures 106-109), general coloration brown with contrasting patterns on FWs. Head (Figures 25-27): Dorsum light brown with four wide longitudinal brown bands, the median ones almost fused, punctuated anteriorly; and with a brown band posterior to eyes. Eyes large and prominent. Fastigium light brown, wider than scape, its margins yellow. Antennae



FIGURES 106-109

Cardiodactylus javare Otte, 2007; **106**, male holotype in dorsal view; **107**, in lateral view; **108**, female from Baitabag in dorsal view; **109**, in lateral view. Scale bar = 1 cm.

110



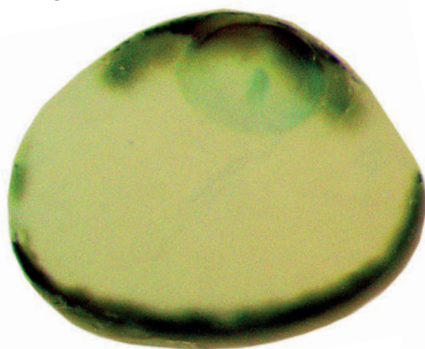
111



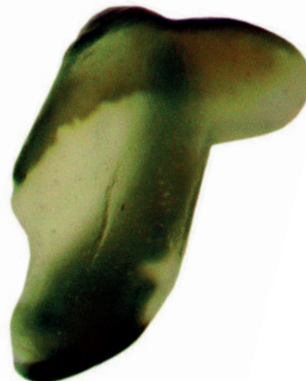
112



113



114



yellow brown. Scapes light brown. Face yellow brown variable mottled with dark brown, crossed by a yellow band starting below eyes. Mouthparts mostly yellow brown. Pronotum: Dorsal disk brown, almost rectangular, with several symmetrical sutures mottled with yellow and light brown coloration, anterior margin concave, posterior margin slightly bisinuate. Lateral lobes upper part brown, lower part yellow with a brown pattern. Legs yellow brown, faintly mottled with dark brown; FIII knee and TIII dark brown. FWs exceeding tip of abdomen, brown with light lateral patterns in both sexes (Figures 42-43). CuA/M/R areas with a whitish sclerotization anteriorly, veins brown; R/Sc area and veins brown. Hind wings longer than FWs of a pronotum length, tail gray brown. Cerci shorter than hind legs, gray brown.

MALE: FWs (Figure 42) mostly dark brown, with light patterns including a whitish sclerotized area near base of FWs, anterior parts of anal veins, middle part of CuA and posterior angle of harp; area posterior to plectrum with an orange sclerotization. CuP absent. 1A bisinuate, with a deep notch anterior to angle; file with ca. 400 stridulatory teeth ($n = 1$): nine on a small bump anterior to 1A notch, 29 on the angle part of 1A, and more than ca. 350 on the transverse part. Harp very narrow and elongate, about 3 times longer than wide, with one W-shaped oblique and 1-2 faint anterior ones. Cell c1 short and wide, c2 large; mirror (d1) very long and narrow, as long as harp, divided by a transverse accessory vein at 2/3 of its length; d2 small compared to d1, crossed by few accessory veins. Sc with 6 projections ($n = 2$).

Male genitalia (Figures 110-112): Pseudepiphallus narrowed at mid-length; posterior part forming almost a right angle with the rest of the sclerite laterally; setose laterally and dorsally; dorsal part of narrowed posterior part with two thin and narrow ridges forming a shallow gutter; posterior apex triangular, wider than preapical part, forming a fan. Anterior part of pseudepiphallus concave, with two lateral rounded triangular extensions; inner part of concavity covered with thick setae oriented anteriorly. Rami long and strong, with narrow preapical plates and long apical stems slightly convergent. Pseudepiphallic parameres trilobate: posterior and median lobes forming a straight angle, posterior lobe long and thin, median lobe forming a sclerotized spoon. Ectophallic arc complete, curved posteriorly. Ectophallic apodemes lamellate, reaching rami preapical plate. Endophallic fold with strong lateral sclerites, widened near membranous apex. Endophallic sclerite small, with a small posterior expansion and short latero-ventral arms; endophallic apodeme with both short lateral lamellas and a small dorsal crest.

FEMALE: FW coloration mostly brown (Figure 43), with similar pattern as in male including base of FW with a yellow sclerotization, whitish area along external margin at dorsal field, at FW mid-length, including part of CuP, CuA and M, this area followed posteriorly by a dark brown area. Anterior half of CuA and M whitish; M dark brown. Dorsal field with 8 strong longitudinal veins, lateral field with five longitudinal veins; Sc with five projections ($n = 2$). Ovipositor very short, shorter than FIII; apex dorsal and ventral edges denticulate.

Female genitalia (Figures 113-114): Copulatory papilla almost rounded, with a thin basal sclerotization; its apex rounded, folded ventrally, with a preapical dorsal sclerotization.

JUVENILE: Coloration mostly dark brown to black and whitish, variable during development, the earlier instars almost pitch black with whitish legs, later instars light brown dorsally, mottled with dark spots. Lateral lobes of pronotum black, with a yellow ventral margin. Legs pale with gray brown spots.

MEASUREMENTS (in mm) — See Table 5.

FIGURES 110-114

Cardiodactylus javarere Otte, 2007; **110**, male genitalia in dorsal view; **111**, in ventral view; **112**, in lateral view; **113**, female copulatory papilla in ventral view; **114**, in lateral view. Scale bars = 1 mm.

TABLE 5

Measurements of *Cardiodactylus javarere* Otte, 2007a

	PronL	PronW	FWL	FWW	HWT	FIIL	FIIW	TIIL	Till spines				Talls	OL
									las	lbs	Oas	Obs		
Holotype male	2.1	2.6	15.0	3.7	6.2	13.8	3.9	?	?	?	?	?	-	
Males (n = 2)	2.0-2.1	2.6-3.1	14.2-15.0	2.8-3.7	?	13.8-13.9	3.2-3.9	?	?	?	?	?	-	
(Male mean)	(2.1)	(2.9)	(14.6)	(3.3)	?	(13.9)	(3.6)	?	?	?	?	?	-	
Females (n = 2)	2.6-2.7	3.1-3.5	16.3-16.9	3.6-4.3	6.1-6.4	14.9	4.1-4.2	13.3-13.9	7-9	4-7	10-13	5-7	2-4	9.9-11.7
(Female mean)	(2.7)	(3.3)	(16.8)	(4.0)	(6.3)	(14.9)	(4.2)	(13.6)	(8)	(6)	(12)	(6)	(3)	(10.8)

HABITAT AND LIFE HISTORY TRAITS — The species has been collected in the field as juvenile only, at night, on low vegetation in forested area.

BEHAVIOR — Unknown.

***Cardiodactylus niugini* n. sp.**

Figures 28-30, 44-45, 119-132

TYPE MATERIAL — Male holotype: Papua New Guinea. Nouvelle-Guinée, Madang, Station Wanang3, forêt autour du camp, 5°13'39,9"S 145°04'47,1"E, 199 m, 11.XI-2.XII.2012 (TR1936), jour, T. Robillard (MNHN-EO-ENSIF148). Female allotype: Papua New Guinea. Nouvelle-Guinée, Madang, Station Wanang3, rivière Wanang, forêt secondaire, 5°13'31,8"S 145°04'42"E, 76 m, 11.XI-2.XII.2012, (TR2427), jour, T. Robillard (MNHN-EO-ENSIF135). Paratypes (4♂, 6♀): Papua New Guinea. Same information as AT: 1♂ (TR2264), jour, sur plante h = 50 cm, molecular sample C70-CbjWa (MNHN-EO-ENSIF73); 1♂ (TR2271), jour, sur arbuste h = 1,70 m, enregistrement appel Pap44 (MNHN-EO-ENSIF92); 1♂ (TR1960), mort en élevage, enregistrement appel Pap39 (MNHN-EO-ENSIF130); 1♂ (TR1786), mort en élevage (MNHN-EO-ENSIF1588); 4♀ (TR1921, 1925, no#), morte en élevage (MNHN-EO-ENSIF1569-1572). Nouvelle-Guinée, Madang, Station Wanang3, PlotA, 5°13'34,6"S 145°04'51,6"E, 148 m, T. Robillard: 23.XI.2012, 1♀ (TR1993), nuit, sur plante h = 1,2 m, (MNHN-EO-ENSIF1568); 19.XI.2012, 2♀ (TR1865-1866), jour (MNHN-EO-ENSIF1565-1566). Nouvelle-Guinée, Madang, Station Wanang3, WanangMal1 [malaise trap], [XI.2012], F. Legendre & T. Robillard: 2♂, 1♀ (P4909, P4912, P4908) (MNHN-EO-ENSIF1561, 1527, 1580). Nouvelle-Guinée, Madang, Station Wanang3, WanangMal4 [malaise trap], [XI.2012], F. Legendre & T. Robillard: 2♂ (P0539, P0545) (MNHN-EO-ENSIF1528-1529).

OTHER MATERIAL EXAMINED — Papua New Guinea. Nouvelle-Guinée, Madang, forêt de Baitabag, 5°08'20,6"S 145°46'26,4"E, 86 m, 6.XII.2012, T. Robillard: 1♂ (TR2455), molecular sample C54-CbjMa (MNHN-EO-ENSIF113); 1♂, mort en élevage; 2♀ (TR2456-2457) (MNHN). Nouvelle-Guinée, Mt. Wilhelm, 200 m, PlotA 5°44'23,9"S 145°19'46,7"E, 246 m, 5-7.XI.2012, jour, 1♀ (TR1492), sur plante, F. Legendre & T. Robillard (MNHN). Nouvelle-Guinée, Mt. Wilhelm, 200 m, PlotB 5°44'27,3"S 145°19'46,1"E, 297 m, 5-7.XI.2012, jour, 1♂, 1♀ (TR1491-1495), 6 juveniles (FL1190, 1219-1221, 1223, TR1478), sur plante, F. Legendre & T. Robillard (MNHN). Nouvelle-Guinée, Mt. Wilhelm, 200 m, PlotC 5°44'41,5"S 145°19'38"E, 327 m, 5-7.XI.2012, F. Legendre & T. Robillard: 1♂ (TR1316), nuit, arbuste h = 70 cm, enregistrement appel Pap20 (MNHN-EO-ENSIF1583); 1♂ (TR1388), nuit, sur plante h = 40 cm; 1♀ (TR1315); 1♀ (TR1389), nuit, sur plante h = 40 cm (MNHN); 3 juveniles, jour (FL1125, TR1582b, TR1549). Mt. Wilhelm, 200 m, hors plot, 5°44'23,9"S 145°19'46,7"E, 5-7.XI.2012, mort en élevage, F. Legendre & T. Robillard: 1♂ (TR1575), enregistrement appel Pap23

(MNHN-EO-ENSIF163); 1 ♂ (TR1744), enregistrement appel Pap26 (MNHN-EO-ENSIF1562); 2 ♀ (TR1940-1941) (MNHN). Nouvelle-Guinée, Madang, Station Wanang3, PlotA, 5°13'34,6"S 145°04'51,6"E, 148 m, 23.XI.2012, 1 juvenile (TR1990), nuit, plante, molecular sample barcode PNG, T. Robillard (MNHN-EO-ENSIF1590). Nouvelle-Guinée, Madang, Station Wanang3, rivière Wanang, forêt secondaire, 5°13'31,8"S 145°04'42"E, 76 m, 11.XI-2.XII.2012, 1 juvenile, jour (TR1735).

TYPE LOCALITY — Papua New Guinea, Madang, Station Wanang, S5°13'39.9 E145°4.47".

DISTRIBUTION — North East of New Guinea Island.

ETYMOLOGY — The species is named after the type locality.

DIAGNOSIS — Species of average size for the Efordi species group. Close to *Cardiodactylus pictus* Saussure, 1878 from which it differs by details of coloration and male genitalia. General coloration dark brown, with a characteristic lateral yellow band on FWs (Figures 115-118). Posterior apex of male genitalia rounded, with thin dorso-lateral ridges (Figure 122), while it is almost square with no ridges in *C. pictus* (see Robillard 2014).

DESCRIPTION — Size average to small for the species group. General coloration mostly dark brown with pale yellow or whitish longitudinal bands laterally (Figures 115-118). Head (Figures 28-30): Dorsum dark brown, with four wide dark brown bands almost fused together. Fastigium slightly wider than scapes, brown to yellow brown with yellow margins. Eyes large and prominent. Antennae homogeneously yellow brown, except base dark brown. Scapes yellowish brown, narrower than fastigium. Face mostly yellow brown, variably mottled with brown. Mouthparts yellow brown with a faint brown area on clypeus. Maxillary palpi yellow brown, apex darker. Lateral side of head yellow variably mottled with brown. Pronotum: Dorsal disk slightly trapezoidal, almost homogeneously brown, its posterior margin bisinuate; lateral lobes mostly yellow brown or light brown, dorsal third dark brown. Legs dark orange brown, more or less mottled with dark spots; hind knees black; TIII with dark brown rings. FWs reaching tip of abdomen, dark brown dorsally, some veins lighter (Figures 44-45); lateral field almost black, with a wide yellow or whitish longitudinal band crossing projections of Sc; CuA yellow brown, M orange brown, R and Sc dark brown; projections of Sc dark brown basally, then yellow; CuA/M/R areas brown, R/Sc area black. Hind wings: Longer than FWs of more than two pronotum length; tail dark brown with yellow brown transverse veins. Cerci: dark brown basally, apex with yellow rings.

MALE: FW venation (Figure 44): 1A slightly bisinuate, with 231 stridulatory teeth, 21 near base of 1A, 28 on angle part, and 186 on transverse part of 1A (n = 1). CuP absent. Harp narrow, longer than wide, with two W-shaped oblique veins. Mirror area variable, c2 longer than wide. Mirror not rounded, separated in two parts by an accessory vein, the anterior part larger than the posterior one, which is variably crossed by accessory veins. Cell d2 as wide as d1, filled with reticulated accessory veins. Apical field long and pointed, CuA bifurcations giving 4-5 longitudinal veins posterior to mirror. Sc with 6-8 projections.

Male genitalia (Figures 119-123): Pseudepiphallus narrowed at mid-length. Narrowed posterior part forming a right angle with the rest of the sclerite in lateral view; setose laterally and ventrally, with two thin dorsal ridges forming a wide gutter. Apex of pseudepiphallus rounded. Anterior part of pseudepiphallus concave dorsally, forming a wide sac with latero-anterior expansions. Rami long and strong, with narrow preapical plates, their apical stems long and slightly convergent. Pseudepiphallic parameres almost triangular. Ectophallic arc complete, curved posteriorly. Ectophallic fold with bisinuate lateral sclerites, its apex rounded. Ectophallic apodemes long, but not reaching apex of rami. Endophallic sclerite small, with short lateral arms and a small posterior expansion; endophallic apodeme with both lateral lamellas and a medio-dorsal crest.

FEMALE: FW coloration (Figure 45) with similar pattern as in male. Dorsal field with nine strong longitudinal veins. Ovipositor: almost as long as hind femora; dorsal edge of apex slightly denticulate.

115



116



117

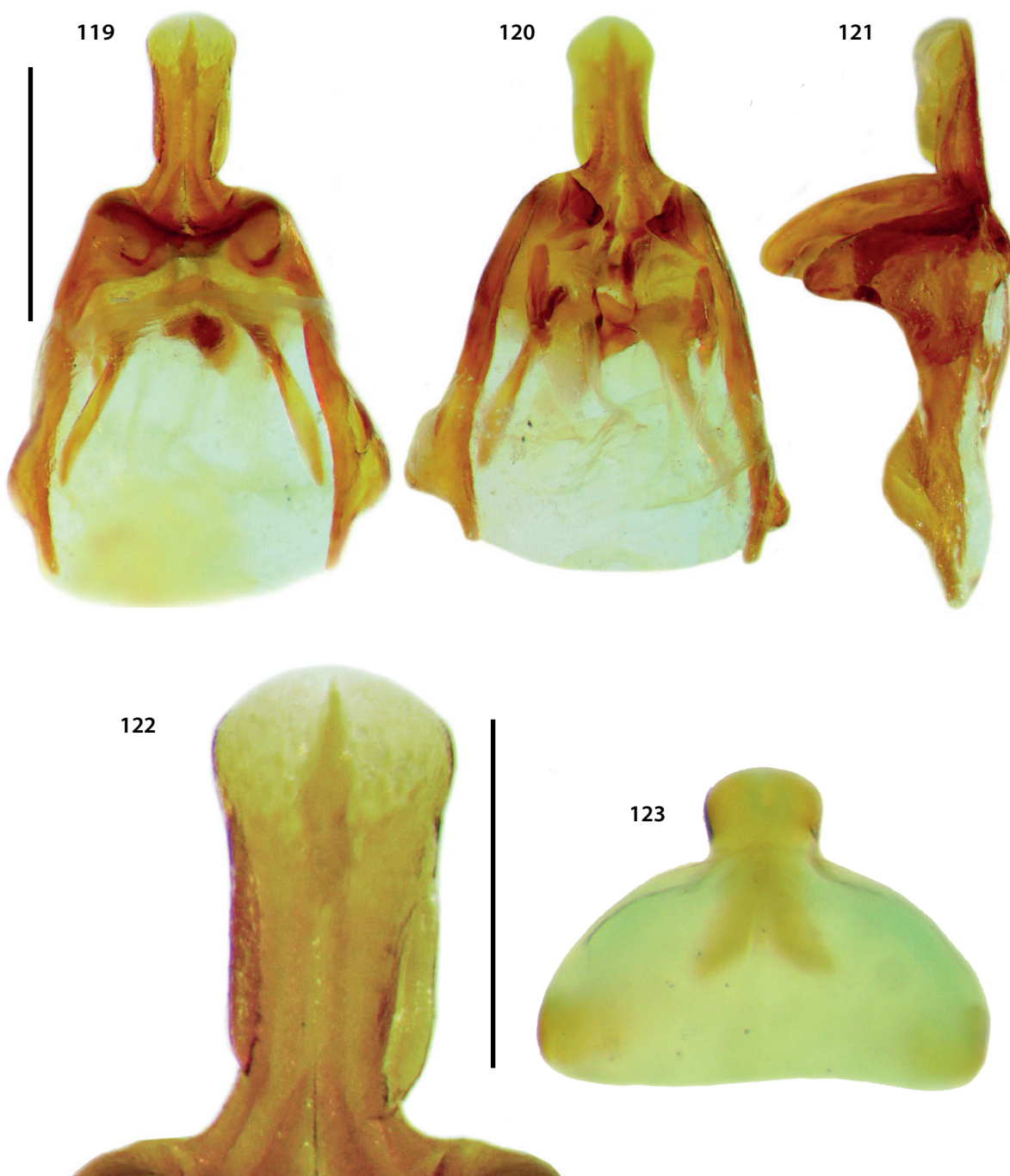


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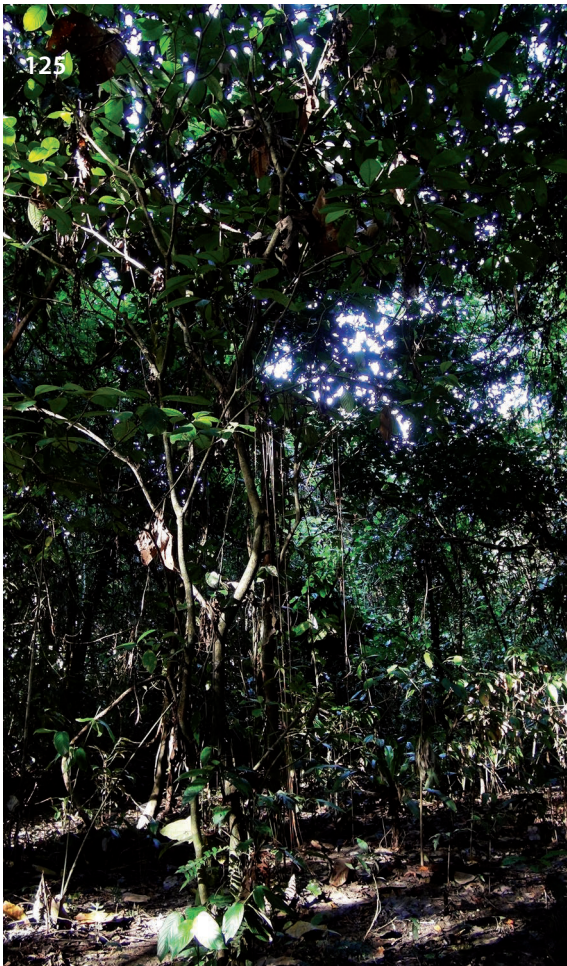
FIGURES 115-118

Cardiodactylus niugini n. sp.; **115**, male in dorsal view; **116**, in lateral view; **117**, female in dorsal view; **118**, in lateral view. Scale bar = 1 cm.

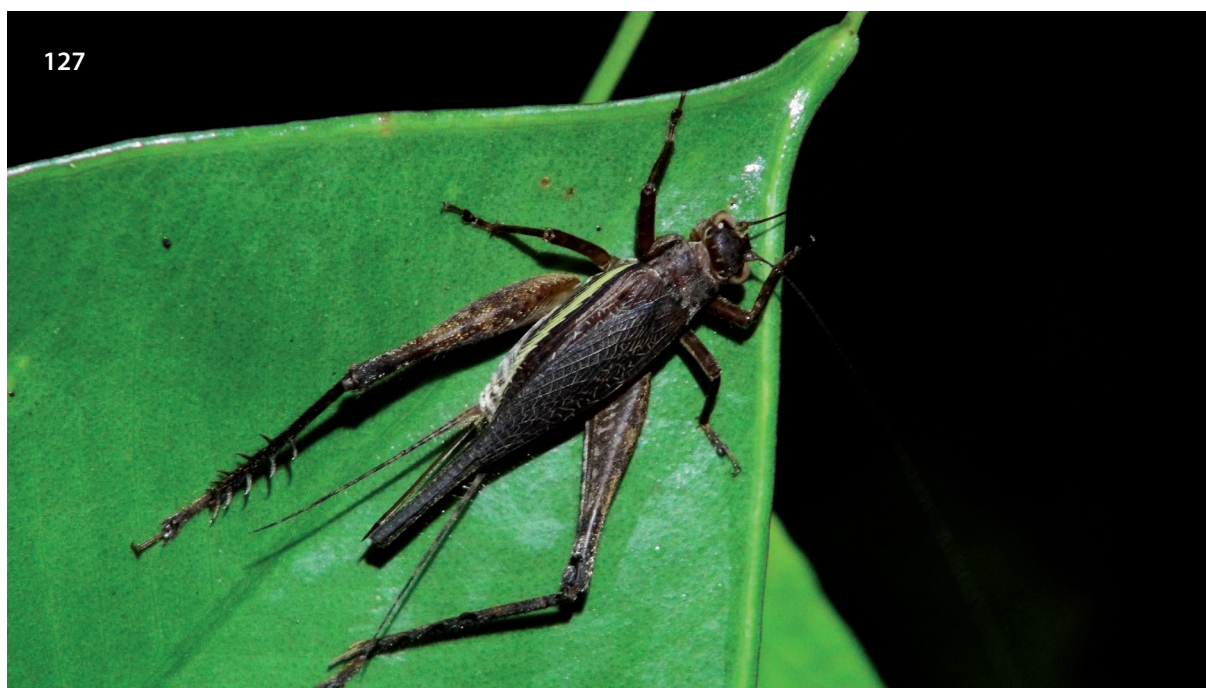


FIGURES 119-123

Cardiodactylus niugini n. sp.; **119**, male genitalia in dorsal view; **120**, in ventral view; **121**, in lateral view; **122**, zoom on posterior part of pseudepiphallus in ventral view; **123**, female copulatory papilla in dorsal view. Scale bars = 119-121, 1 mm; 122-123, 0.5 mm.

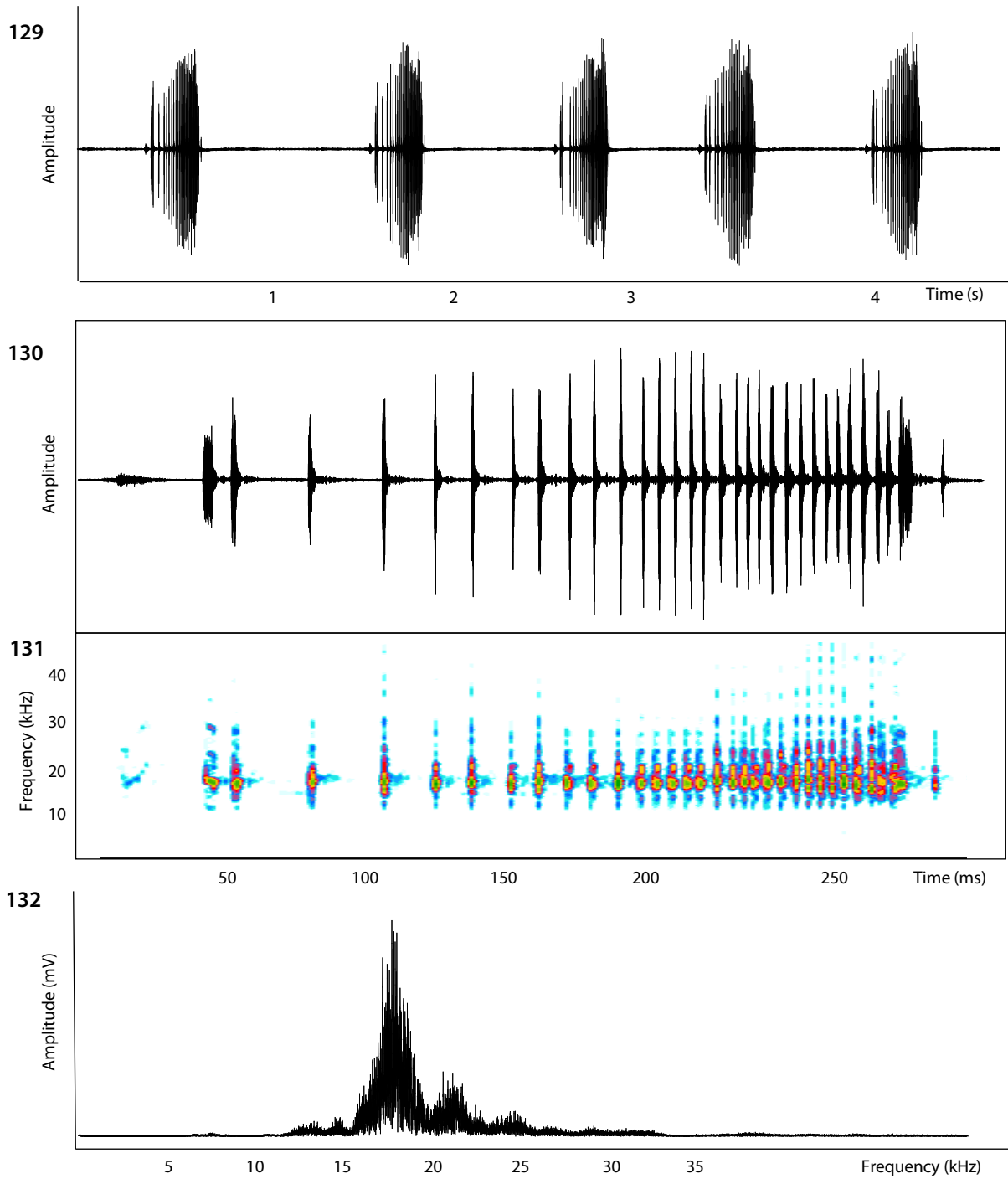


FIGURES 124-126
Cardiodactylus niugini n. sp.; **124-125**, view of forest habitat in Wanang, near Wanang3 river; **126**, male on top of vegetation at day.



FIGURES 127-128

Cardiodactylus niugini n. sp.; 127, female on vegetation at night; 128, male on vegetation at night.



FIGURES 129-132

Calling song of *Cardiodactylus niugini* n. sp.; **129**, oscillogram of 5 echemes (syllables); **130-132**, detailed oscillogram (**130**), sonogram (**131**), and frequency spectrum of 1 syllable (**132**).

Female genitalia: Copulatory papilla (Figure 123) wider than long, its apex narrowed and folded ventrally; with two baso-lateral sclerotizations and one at base of apex.

JUVENILE: Coloration typical, lighter than adults and molted with dark spots, the second tergite dark, and with two black stripes on the fifth segment of abdomen.

VARIATION — Specimens examined from Baitabag with yellow brown face and slightly wider pseudepiphallic gutter between thin dorsal ridges.

MEASUREMENTS (in mm) — See Table 6.

HABITAT AND LIFE HISTORY TRAITS — *C. niugini* adults and juveniles are found on low vegetation in forested areas (Figures 124-128), on bushes and small trees. Males start calling during the morning and formed couples sit on large leaves or on branches to mate during the day.

BEHAVIOR — The calling song of *C. niugini* (Figures 129-132) corresponds to only one FW closure (1 syllable), the gaps within the syllables being probably made by the jerking movement of the FW closure, as described before in other eneopterine species (*Cardiodactylus sumba* Robillard, 2014, in Robillard *et al.* 2014; *Lebinthus luae* Robillard & Tan, 2013, in Robillard *et al.* 2013). At 27°C (TR2271, bout of 80 s measured), these mono-syllabic calling songs are emitted continuously but not very homogeneously (variable period) at a rate of 1.04 syllables / second, and with the following characteristics: syllable duration = 316 ± 43 ms; syllable period = 908 ± 228 s; syllable duty cycle = 34.8 %. The power spectrum consists of a broad band dominating at 17.54 ± 1.4 kHz with no harmonic structure, suggesting that the system is not sustained as usually in crickets.

TABLE 6

Measurements of *Cardiodactylus niugini* n. sp.

	PronL	PronW	FWL	FWW	HWT	FIIL	FIIW	TIIIL	TIII spines				Tallls	OL
									las	lbs	Oas	Obs		
Holotype male	2.0	3.7	13.0	4.0	9.0	14.4	3.1	14.0	11	9	17	9	3	-
Males (n = 6)	2.0-2.2	3.4-3.8	12.0-13.2	3.7-4.0	8.5-9.2	13.5-14.8	3.1-3.7	12.5-14.4	8-15	6-9	13-18	5-9	3	-
(Male mean)	(2.1)	(3.6)	(12.5)	(3.9)	(9.0)	(14.3)	(3.3)	(13.4)	(10)	(7)	(15)	(8)	(3)	-
Allotype female	2.3	3.8	14.2	3.8	8.3	16.1	3.8	14.4	9	6	17	9	4	11.9
Female (n = 5)	2.3-2.7	3.8-4.4	13.9-14.8	3.5-4.0	8.2-11.6	14.5-16.7	3.3-4.1	14.0-14.4	8-16	6-9	14-17	7-9	3-4	10.2-11.9
(Female mean)	(2.5)	(4.0)	(14.3)	(3.8)	(9.3)	(16.0)	(3.7)	(14.2)	(11)	(8)	(16)	(8)	(4)	(10.8)

***Cardiodactylus nobilis* n. sp.**

Figures 31-33, 46, 133-140

TYPE MATERIAL — Male holotype: Papua New Guinea. Nouvelle-Guinée, Mt. Wilhelm, 1200 m, 5°43'17.1"S 145°16'13.1"E, 1211 m, P1584 Plot13, FIT-MW1200-M-1/8-d02, 26.X.2012-28.X.2012, molecular sample C59-Csp1MW1200, T. Robillard (MNHN-EO-ENSIF102).

OTHER MATERIAL EXAMINED — Papua New Guinea. Nouvelle-Guinée, Mt. Wilhelm, 1200 m, PlotA 5°43'14.6"S 145°16'09.5"E, 1193 m, 29-

31.X.2012, nuit, F. Legendre & T. Robillard: 1 juvenile (TR678) (MNHN). Nouvelle-Guinée, Mt. Wilhelm, 1200 m, PlotB 5°43'15.1"S 145°16'13.2"E, 1195 m, 29-31.X.2012, nuit, F. Legendre & T. Robillard: 1 juvenile (TR713), sur plante h = 20 cm, molecular sample C67-Csp2MW1200 (MNHN-EO-ENSIF131). Nouvelle-Guinée, Mt. Wilhelm, 1200 m, PlotC 5°43'15.8"S 145°16'17.4"E, 1193 m, 29-31.X.2012, nuit, F. Legendre & T. Robillard: 2 juveniles (TR830, 849), sur plante h = 20 cm; 1 juvenile (TR831), sur plante h = 30 cm (MNHN).

TYPE LOCALITY — Papua New Guinea, Mount Wilhelm, S5°43'17.1" E145°16'13.1", 1211 m.

DISTRIBUTION — Papua New Guinea, Mount Wilhelm, at 1200 m altitude.

ETYMOLOGY — Species named after the rarity of the specimens.

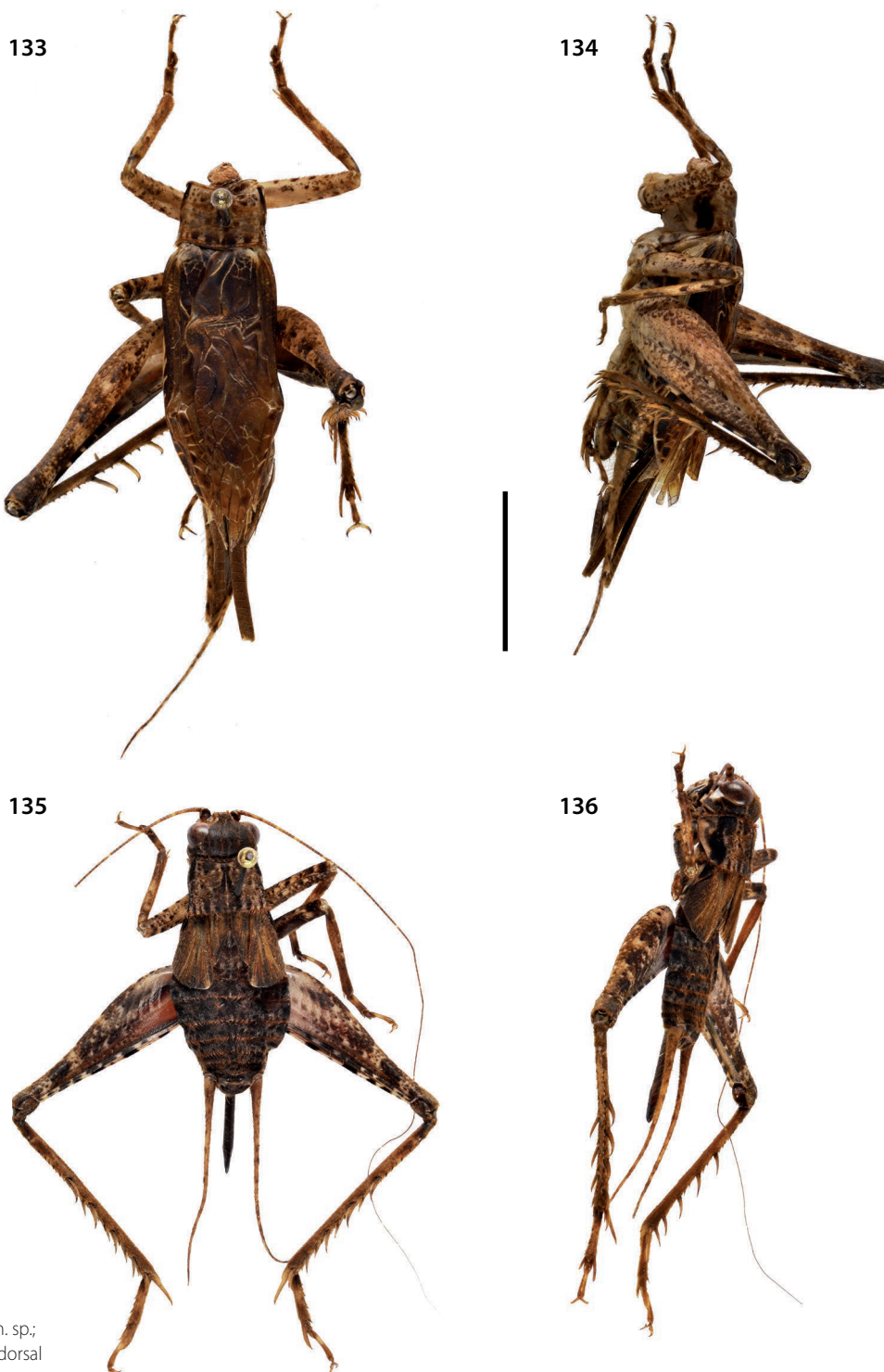
REMARK — The male HT was collected in flight-interception traps and was found headless during specimen sorting. DNA barcode analyses confirmed that the juveniles collected at this elevation on Mount Wilhelm belong to the same species as the adult form, and the oldest juvenile, a female sub-adult, was therefore used to describe the head color of the species, given that color patterns of head are usually consistent between late instars and adults in *Cardiodactylus* species (Robillard 2009; Robillard *et al.* 2014).

DIAGNOSIS — Species of average size, close to *C. busu* Otte, 2007 for head and pronotum coloration, but differing by coloration and venation of FWs, and by male genitalia. General coloration almost completely brown dorsally (Figure 133); FW yellow and brown laterally, characterised by absence of whitish spots on dorsal field. FWs with shorter apical field than in *C. busu*, lateral field with a characteristic longitudinal yellow band. Posterior part of pseudepiphallus narrow with a characteristic spatula shape, forming a right angle with rest of the sclerite.

DESCRIPTION — Size average. General coloration brown and yellow (Figures 133-136). Head (Figures 31-32) (examined in sub-adult specimens, HT being headless): Dorsum dark brown, with eight longitudinal bands almost fused together, with a yellow line posterior to eyes; fastigium trapezoidal, black, slightly wider than scape, with large whitish ocelli. Antennae light brown, some segments yellow. Cheeks brown, with longitudinal yellow stripes under eyes. Face homogeneously yellow brown, with two brown vertical stripes between scapes. Mouthparts yellow brown with black margin and two symmetrical vertical stripes on clypeus; maxillary palpi brown. Pronotum (Figure 33): Dorsal disk light brown mottled with brown and black, rectangular; anterior margin slightly concave, posterior margin slightly bisinuate; lateral margins yellow brown. Dorsal half of lateral lobes dark brown, ventral half yellow, with faint brown patterns. Legs yellow mottled with dark brown: FI-II with dark brown spots, TI-II with faint brown bands; FIII knees and TIII dark brown. FWs (Figure 46) reaching tip of abdomen, mostly brown with a few contrasting yellow veins. Hind wings: Longer than FWs of a pronotum length; tail gray brown. Cerci: almost as long as hind legs, gray brown mottled with dark spots.

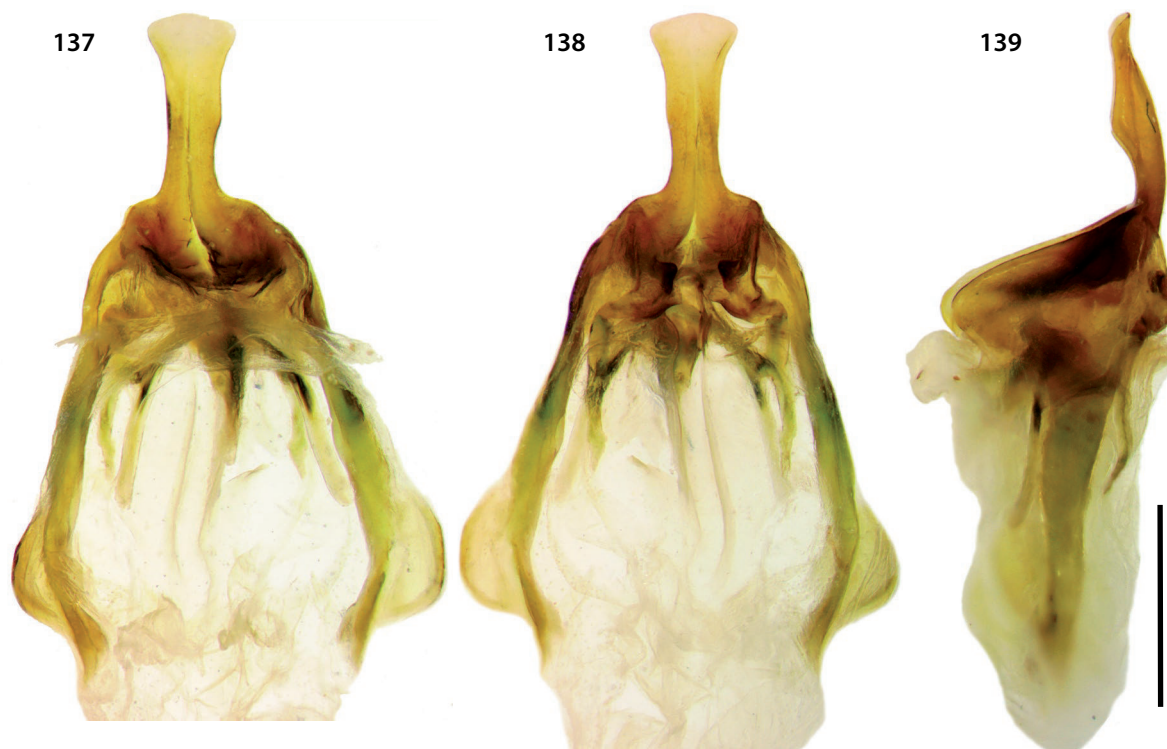
MALE: FWs coloration (Figure 46): almost completely brown, with few light patterns on dorsal field, including a whitish basal sclerotization; veins mostly brown, except yellow anterior parts of 3A and 4A, and several yellow intercalary veins in 2A/3A area and in apical field. FW venation: Dorsal field with 1A slightly bisinuate; harp narrow, two W-shaped oblique veins. Mirror large but not rounded, crossed by a yellow accessory vein. Apical field short, including four cell alignments posterior to mirror. Lateral field: Latero-dorsal angle made by M. Sc with eight bifurcating veins, black basally then yellow; CuA/M/R/Sc areas and veins orange brown, area ventral to Sc dark brown, crossed by a wide characteristic longitudinal yellow band.

Male genitalia (Figures 137-139): Pseudepiphallus narrowed at mid-length, the posterior part spatula shaped, with two short and thin dorsal ridges forming a narrow gutter; apex of spatula slightly widened and rounded. Anterior part of pseudepiphallus concave dorsally, forming a 90° angle with the spatula in lateral view. Rami long, with wide semicircular preapical plates, their base at level of pseudepiphallus narrowed part, near base of spatula. Pseudepiphallic parameres trilobate, the posterior lobe thin and pointing posteriorly. Ectophallic arc complete, with a short posterior expansion. Ectophallic apodemes rather short, their apex almost reaching rami preapical plate. Apex of ectophallic fold trilobate, membranous. Endophallic sclerite small, V shaped. Endophallic apodeme with narrow lateral lamellas and a medio-dorsal crest extended anteriorly.



FIGURES 133-136

Cardiodactylus nobilis n. sp.;
133, male holotype in dorsal
view; **134**, in lateral view;
135, female sub-adult in dorsal
view; **136**, in lateral view.
Scale bar = 1 cm.



FIGURES 137-139

Cardiodactylus nobilis n. sp.; **137**, male genitalia in dorsal view; **138**, in ventral view; **139**, in lateral view. Scale bar = 1 mm.

FEMALE: Unknown.

JUVENILE: Coloration darker than adults, mottled with brown spots (Figures 135-136, 140). Pronotum with similar banding patterns as in HT.

MEASUREMENTS (in mm) — See Table 7.

TABLE 7

Measurements of *Cardiodactylus nobilis* n. sp.

	PronL	PronW	FWL	FWW	HWT	FIIL	FIIW	TIIL	TIII spines				Talls	OL
									las	lbs	Oas	Obs		
Holotype male	3.3	4.0	17.1	5.1	5.5	18.1	3.1	15.9	6	7	9	5	4	-
Female (sub-adult)	3.3	4.0	-	-	-	16.1	4.1	14.0	5	6	9	7	3	-

HABITAT AND LIFE HISTORY TRAITS — The species was collected as juveniles only, which were found at night in forest on low vegetation (Figure 140).

BEHAVIOR — Unknown.

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FIGURES 140

Cardiodactylus nobilis n. sp.; 140, juvenile on leaf at night at 1200 m on Mount Wilhelm.

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I.4. Chapter Conclusion

In this chapter, I revise the taxonomic work in the genus of *Cardiodactylus*, describe with five new species from western New Guinea and redescribed two species. *Cardiodactylus* is distributed very widely and shows diverse morphologies which can vary among populations distributed in different places. For example, the species *C. muiri* distributed in Maluku Province (Indonesia) was first described as a new species under the name *C. buru* (Robillard et al., 2014), but after combining the information of specimens from the islands of Buru, Ambon, New Guinea and Kai Islands, the species was synonymised under *C. muiri* (see Dong *et al.*, 2017; Chapter III of this thesis). In turn, it encourages to explore as many species as possible across their distribution regions and it also stimulates to explore methods to help with species identification such as DNA barcoding and species delimitation methods.

CHAPTER II

Phylogenetics and historical biogeography of Southeast Asian *Cardiodactylus*

II.1. Chapter Introduction

With the clarification of the taxonomic knowledge about the genus *Cardiodactylus*, including the characterization of their distribution pattern (Chapter one), it becomes possible to address the evolutionary history of the genus. Considering the complex geological history of SEA and the diversity of *Cardiodactylus* in this region, understanding its biogeographical history in this region, a prerequisite to study questions about speciation and diversification of behavioral traits.

In this chapter, I present the geological background of the Southeast Asian region (section II.2), I reconstruct the phylogeny of *Cardiodactylus*, investigate its biogeographical history and infer its diversification pattern in order to understand how its diversity assembled through time (section II.3).

II.2. Geology and habitat reconstructions in Southeast Asia

In this thesis, I define Southeast Asia (SEA) as the region including the Ryukyu Islands (Japan), southeast China, the mainland states of Cambodia, Laos, Burma (Myanmar), Thailand, and Vietnam, and the territories and archipelagoes belonging to Malaysia, Indonesia, East Timor, the Philippines, and Singapore. Australia, Bangladesh, India, and their island territories are excluded. Papua New Guinea and the Solomon Islands are also excluded, except for the Indonesian part of New Guinea (Figure II.1).

SEA is rich in terms of wildlife. It includes four of the world's 34 biodiversity hotspots (Myers, 2000) – the areas of the world that contain a remarkable concentration of species while being exceptionally endangered (Hughes, 2017). The SEA region contains 20% of the planet's vertebrate and plant species and the world's third-largest tropical forest (Koh *et al.*, 2013). The region is uniquely and biologically complex, reflecting its complex biogeography and resulting in zonation of regional biotic patterns and complex biogeographic divisions (Lohman *et al.*, 2011).

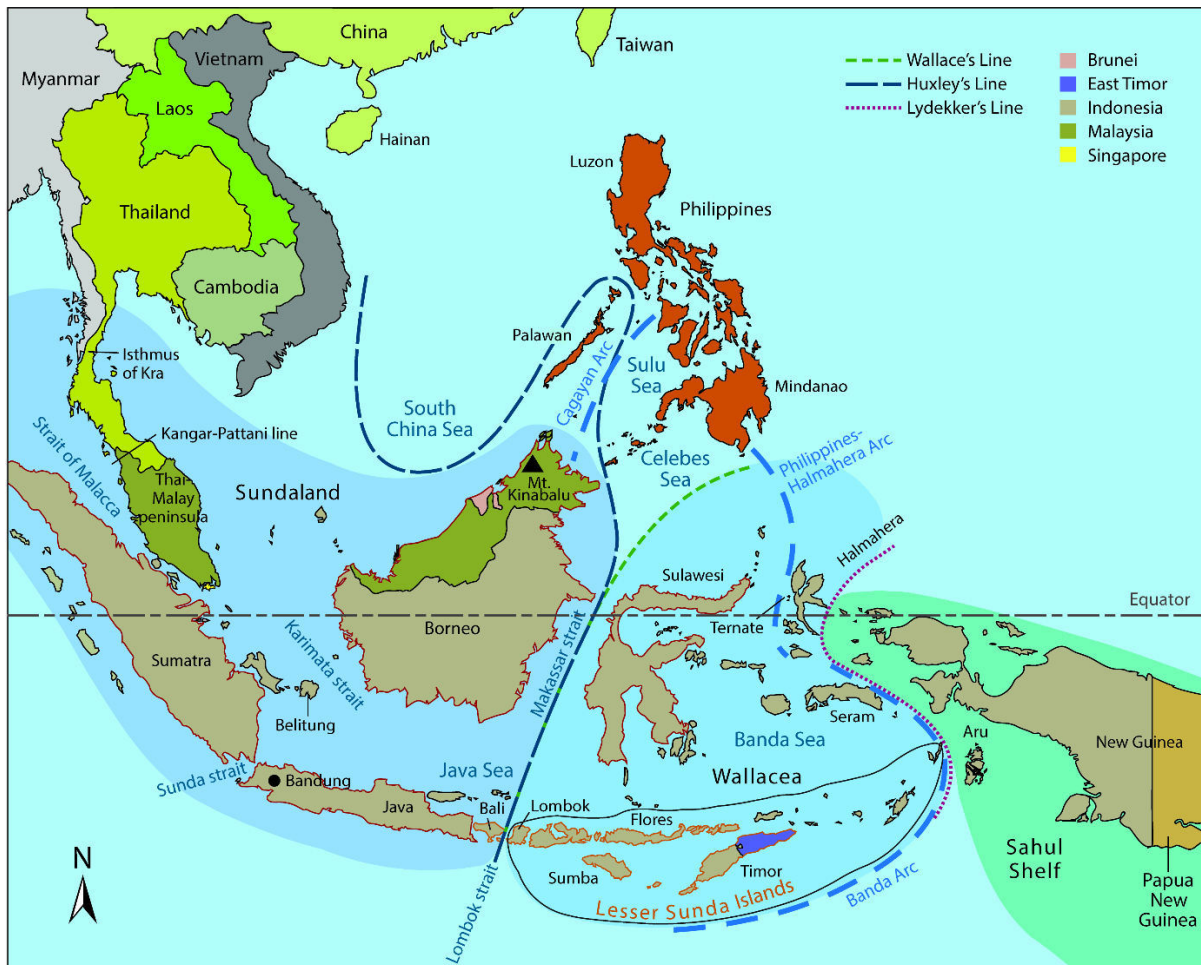


Figure II.1. Map of Southeast Asia as considered in this thesis. Contemporary landmasses, straits, seas, arcs and faunal lines are shown in map. Major islands are labeled; the countries are indicated by different colors. The Sundaland is surrounded by a dark blue area (comprising the Thai-Malay Peninsula, Sumatra, Java and Borneo) and the Wallacean region by a lighter blue area. In Wallacea, black borders around islands, e.g., Lombok, Flores, Sumba, Timor, indicate the group of islands corresponding to the Lesser Sunda Islands (modified from Lohman *et al.*, 2011).

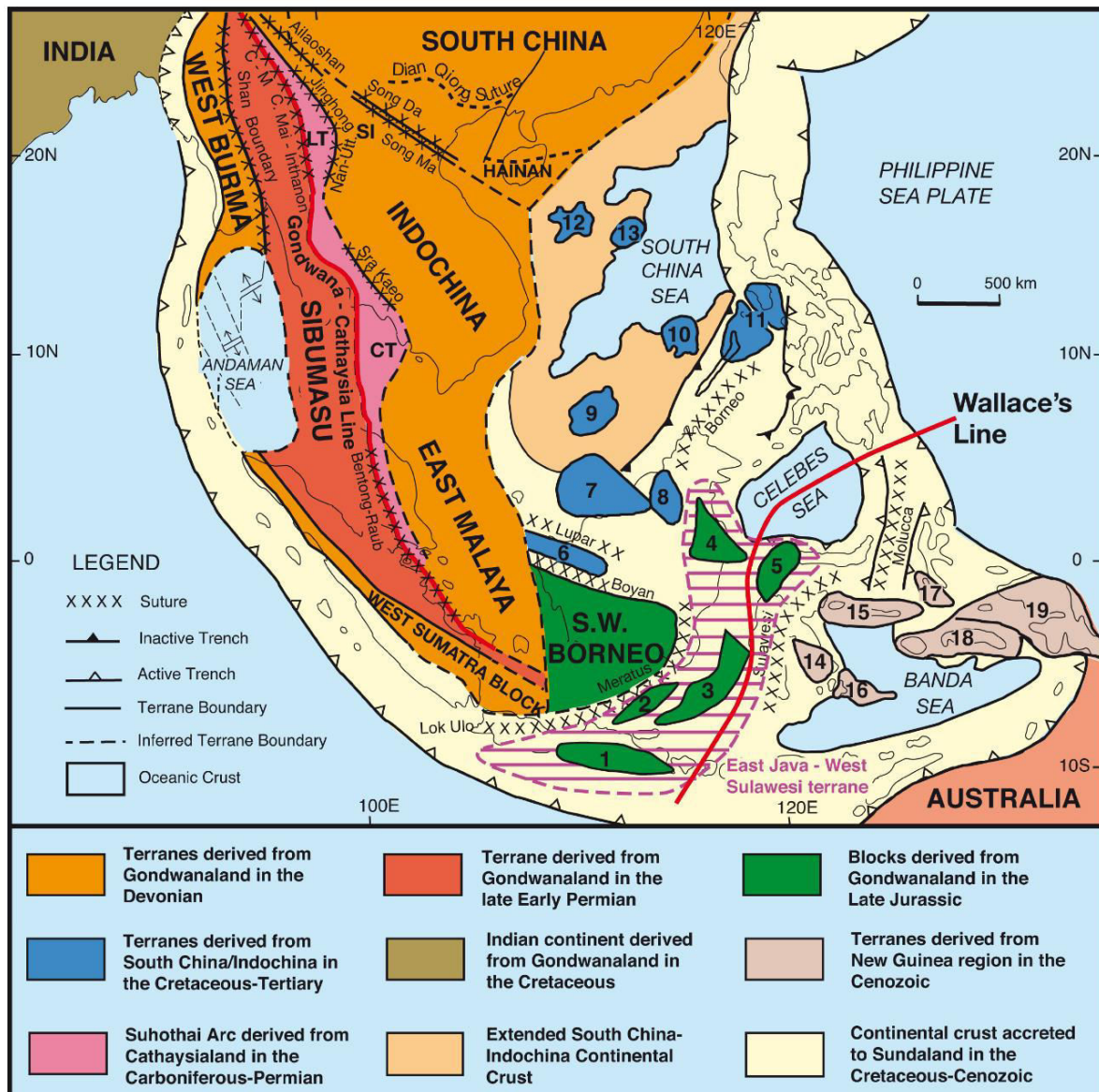


Figure II.2. Distribution of continental blocks, fragments and terranes, and principal sutures of SEA. Numbered microcontinental blocks: 1, East Java; 2, Bawean; 3, Paternoster; 4, Mangkalihat; 5, West Sulawesi; 6, Semitau; 7, Luconia; 8, KelabitLongbowan; 9, Spratly Islands-Dangerous Ground; 10, Reed Bank; 11, North Palawan; 12, Paracel Islands; 13, Macclesfield Bank; 14, East Sulawesi; 15, BangaiSula; 16, Buton; 17, Obi-Bacan; 18, Buru-Seram; 19, West Irian Jaya. LT, Lincang Terrane; CT, Chanthaburi Terrane; C-M, Changning-Menglian Suture; C-Mai –Inthanon, Chiang Mai – Inthanon Suture; Nan-Utt., Nan-Uttaradit Suture. Hatched area shows interpreted East Java – West Sulawesi terrane. The Paleozoic Gondwana - Cathaysia and extant Wallace biogeographic lines are shown in red (modified from Metcalfe, 2011b).

The various continental pieces that now make up SEA were all derived from the southern hemisphere supercontinent Gondwana and travelled north to progressively collide and coalesce prior to the current ongoing collision with the northwards moving Australian continent. The geological history of SEA has a long-term complexity of continent-continent and continent-arc collision (Hall, 2011).

The Paleozoic-Mesozoic tectonic evolution of SEA (400 Ma - 65 Ma)

The Gondwana-derived East Asia crustal fragments successively rifted and separated from the margin of eastern Gondwana. It occurred in three phases, linked with the successive opening and closure of three intervening Tethyan oceans before Cenozoic (Figure II.2) (Metcalf, 2006). The first group of continental blocks dispersed from Gondwana in the Devonian, opening the Palaeo-Tethys (Devonian-Triassic) behind them, and included the North China, Tarim, South China and Indochina blocks (including West Sumatra and West Burma). Remnants of the main Palaeo-Tethys ocean are now preserved within the Longmu Co-Shuanghu, Changning–Menglian, Chiang Mai/Inthanon and Bentong–Raub Suture Zones.

During the northwards subduction of the Palaeo-Tethys, the Sukhothai Arc, including the Lincang block in SW China, the Sukhothai block in central Thailand and the Chanthaburi block in southeast Thailand-Cambodia, was constructed on the margin of South China–Indochina and separated from those terranes by a short-lived back-arc basin now represented by the Jinghong, Nan–Uttaradit and Sra Kaeo Sutures. Concurrently, a second continental sliver or collage of blocks (Cimmerian continent) rifted and separated from northern Gondwana and the Meso-Tethys (Late Early Permian–Late Cretaceous) opened in the Late Early Permian between these separating blocks and Gondwana. The eastern Cimmerian continent, including the South Qiangtang block and Sibumasu Terrane (Baoshan and Tengchong blocks of Yunnan) collided with the Sukhothai Arc and South China/Indochina in the Triassic, closing the Palaeo-Tethys.

A third collage of continental blocks, including the Lhasa block, South West Borneo and East Java–West Sulawesi (now identified as the missing “Banda” and “Argoland” blocks)

separated from northwest Australia in the Late Triassic–Late Jurassic by opening of the Cenozoic Tethys (Late Triassic–Late Cretaceous) and accreted to southeast Sundaland by subduction of the Meso-Tethys in the Cretaceous. The addition of the continental fragments of Southwest Borneo and later East Java–West Sulawesi formed a larger emergent land area by the Late Cretaceous, that extended from Indochina to West Sulawesi. Subduction ceased around Sundaland during the Late Cretaceous; as a consequence, most of the Sundaland was emerged and physically connected to Asia ca. 80 Ma, but was separated from India and Australia by deep oceans (Hall, 2009, 2012b, 2013; Hall & Sevastjanova, 2012; Hutchison, 2014; Khin *et al.*, 2014; Metcalfe, 2011a, b, 2013). Plate boundaries shifted rapidly in the Cenozoic, and continent-continent and continent-arc collisions happened actively during this period (Figure II.2) (Hall, 2002).

The Cenozoic tectonic evolution of SE Asia (ca. 65 Ma to the present)

During the Cenozoic, Sundaland was largely surrounded by inactive margins with localized strike-slip deformation, extension and subduction (Figure II.3 a). Three major Cenozoic collision events had a great influence on the current archipelago setting and probably substantially affected both climate and the assembly of regional biota, possibly in tandem (Morley *et al.*, 2012).

India-Asia collision was related to the plate reorganization that occurred during the Eocene. About 45 Ma Australia began to move north, resulting in subduction and widespread rifting within Sundaland. The Makassar Strait became a wide marine barrier within Sundaland, and West Sulawesi separated from Sundaland, (Hall, 2013). East and north Borneo were largely submerged, and some major rivers flowed south and east, depositing clastic sediments across much of Java and Southeast Borneo (Figure II.3 b) (Hall, 2009).

The most important Cenozoic plate boundary reorganization occurred about 25 Ma, caused by Australia-Asia collision (Hall, 2002). The Australian margin drifted from the continent and formed the Bird's Head region of New Guinea, also called Sula Spur

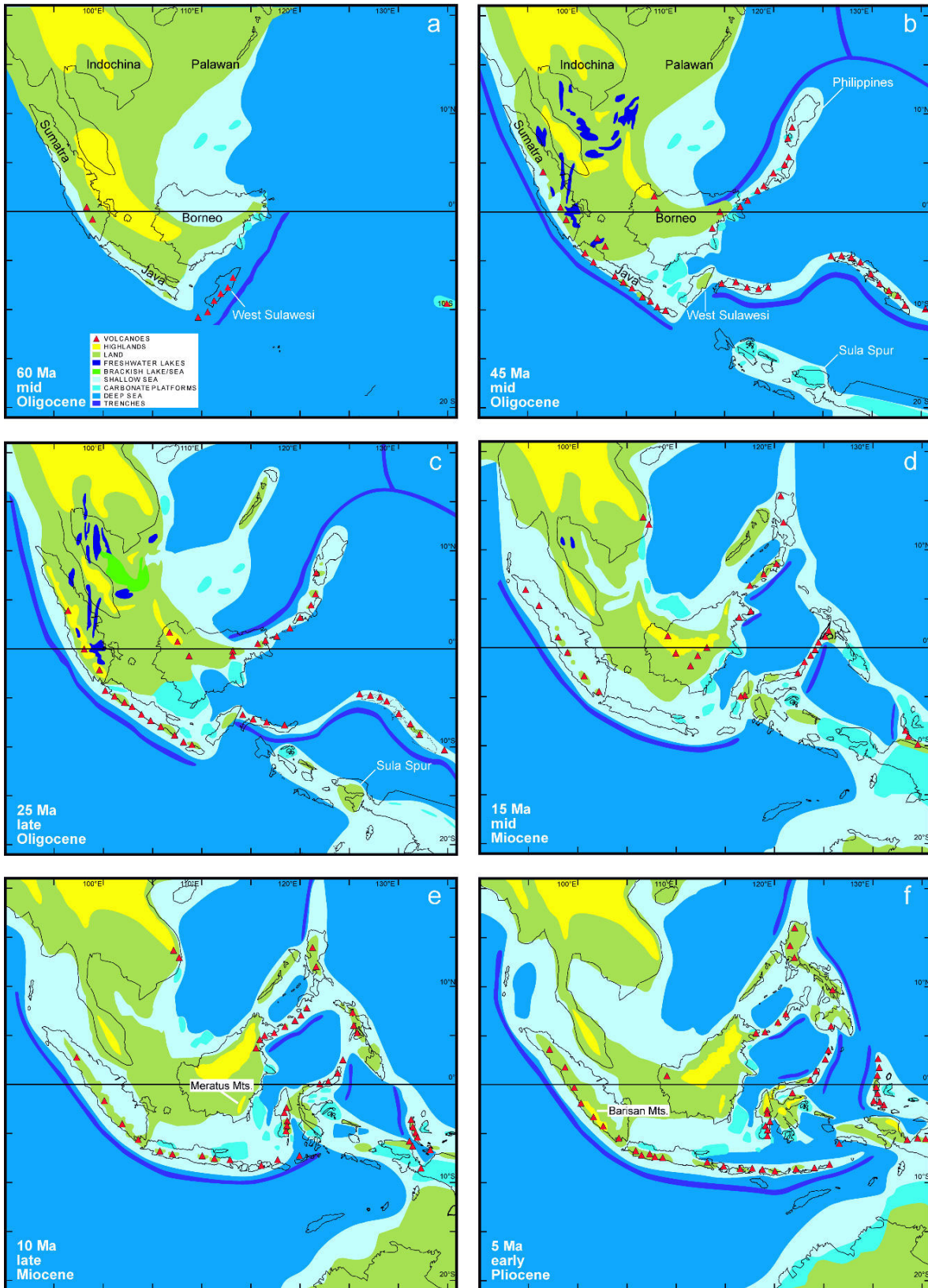


Figure II.3. Paleogene and Neogene maps of SEA paleogeography and key habitat availability. Modified after de Bruyn *et al.*, 2014 and Hall, 2012a.

promontory (Hall, 2011). The Palawan Continental Terrane (PCT) drifted from the margin of Southeast China mainland, caused by the opening of the South China Sea (Walia *et al.*, 2012). In addition, short-lived volcanic islands, which continued from North Borneo into the northern Philippines, arose by the subduction of the proto-South China Sea. Southeast Sulawesi began to uplift, other parts of Sulawesi started to emerge from the ocean and begin deformation. Widespread non-rigid counter-clockwise rotation of Sundaland (Borneo, West Sulawesi, Java) also began during this period (Figure II.3 c) (Hall, 2009).

The third collision involves the Sula Spur promontory and the Southeast Asian margin in Sulawesi during the Miocene, which formed the current Wallacean region (Figure II.3 d-f) (Hall, 2009). The Banda subduction began ca. 15 Ma when the active Java subduction tore eastwards into the embayment (Spakman & Hall, 2010). During the opening of the South Banda Sea, the arc and continental crust were further extended to form the Banda forearc and later by the accretion of several of the outer arc islands formed the Lesser Sunda islands (Figure II.3 e and f) (Hall, 2009). The present-day Molucca Sea double subduction system was initiated about 15 Ma (Figure II.3 d) (Baker & Malaihollo, 1996) and has been eliminated by subduction at both its eastern and western sides (Figure II.3 e). But the two arcs first came into contact ca. 3 Ma and began to build the central Molucca Sea accretionary complex (Figure II.3 f) (Hall, 2000).

Sea level change during Pleistocene in Southeast Asia (ca. 2.6 Ma)

Sea levels have fluctuated throughout the geological time, periodically encroaching or retreating across coastal plains, especially during the Later Pliocene and Pleistocene (Figure II.4 A) (Lambeck & Chappell, 2001). When sea levels were reduced by more than 120 m during glacial periods (Figure II.4 B), the present-day SEA mainland, Thai-Malaysia Peninsular, large islands of Sumatra, Java and Borneo were exposed and connected, forming the region known as Sudaland, with a region's land area much larger than that of the present day. When sea-levels were rising, but remained 60 m lower than that of present day, the land area of Sudaland was contracted, and the island within Wallacea were isolated by the sea

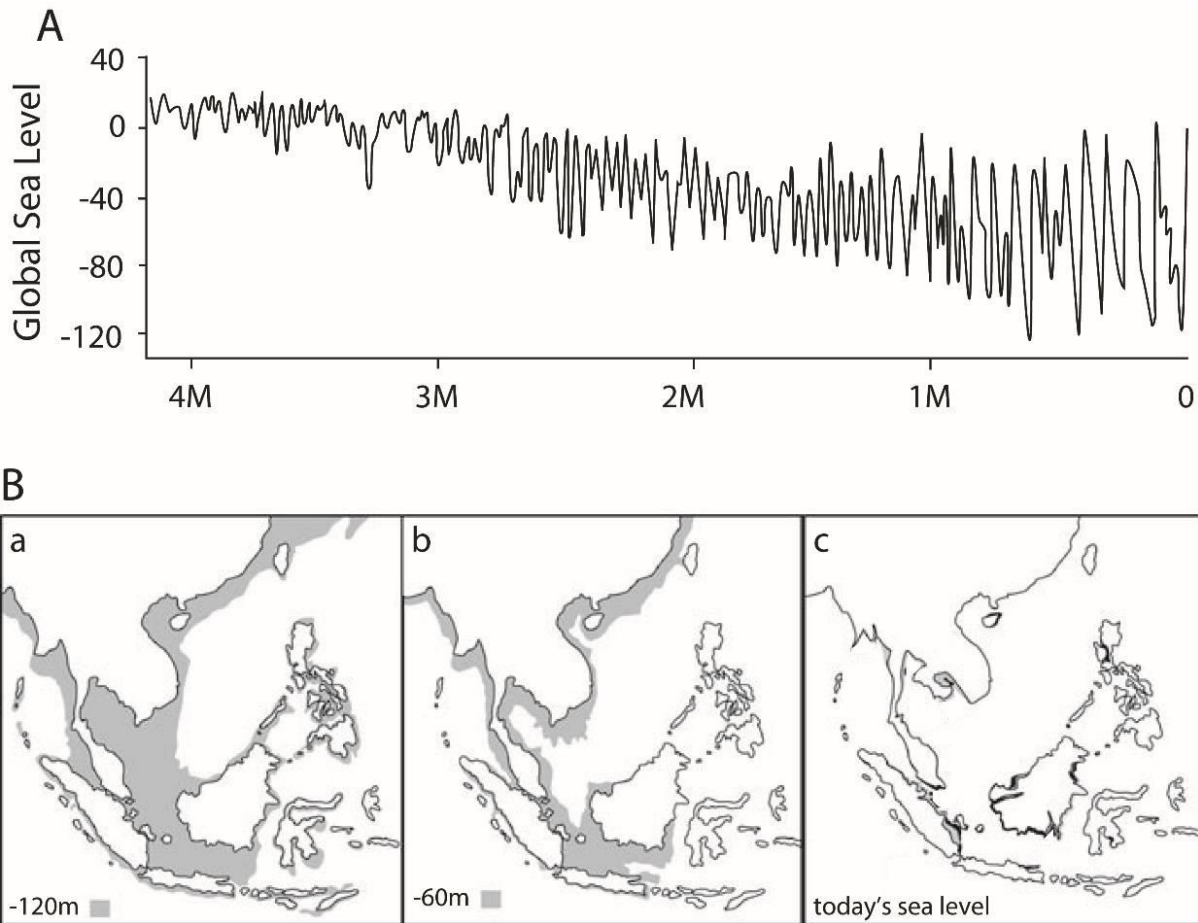


Figure II.4. Sea level change in SEA: A. Global sea level during Plio-Pleistocene; B. Outline maps of SEA when sea levels were at 120 m below (a), 60 m below (b) and 0 m from present-day sea level (c). (modified after Woodruff, 2010).

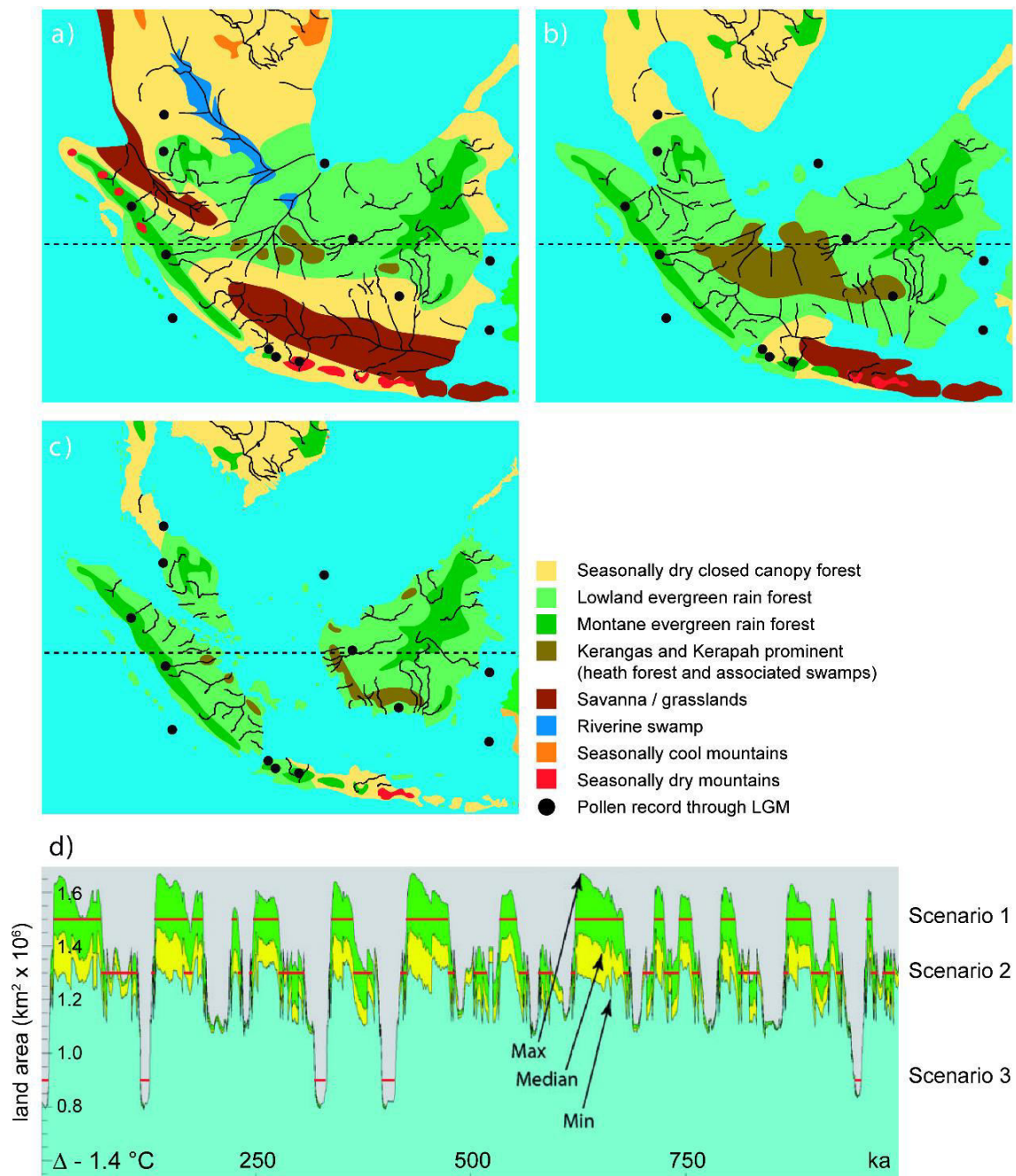


Figure II.5. The history of vegetation setting in Southeast Asia during three Quaternary climate sea-level scenarios: a) scenario 1: 25 ka at lowest sea levels, glacial maximum, Marine Isotope Stage (MIS) 2. b) Scenario 2: 12 ka as sea-flooded Sunda Shelf, intermediate sea level, MIS Stages 1, 3, 4, and 5a–d. c) Scenario 3: present-day, interglacial high sea level, MIS Stages 1, 5e. d) Land area of tropical evergreen broadleaf forest over last 1 Ma; horizontal red lines show times when scenarios 1–3 would likely have occurred (note that the blue line represents the most conservative model but not the most likely one here). Note that Borneo, in particular, has hosted extensive rain forests throughout all periods shown. Modified after de Bruyn *et al.*, 2014.

(Voris, 2000; Robles *et al.*, 2015). Glaciation and deglaciation and the accompanying lowering and rising of sea levels and climate change during the late Pleistocene are known to have greatly affected land mass configurations and biogeographical patterns in SEA (Cannon *et al.*, 2009; Harold, 2000; Verstappen, 1980; Voris, 2000).

History of the vegetation setting in SEA

The ancient vegetation in SEA was composed of gymnosperms during the early Cretaceous, then Angiosperms increased in number and diversity during the Late Cretaceous (Vakhrameev, 1991; Morley, 2000; de Bruyn *et al.*, 2014). Tropical rain forest in SEA has developed within an extensive archipelago during the Early Cenozoic, and extended much further north (to southern China and Japan) in the Mid-Miocene (ca. 15 Ma) due to the peak of warm, humid global climates (Heaney, 1991; Zachos *et al.*, 2001). During this period, both seasonal and ever wet rain forest emerged, and the open canopy woodland occupied at the high latitudes (Morley, 2012). However, the surface of tropical rain forest contracted during the Late Miocene and Early Pliocene as a result of global climate temperature decline (Hall, 2012a; Zachos *et al.*, 2001). Moreover, lower sea level, cooler temperatures, and modified rainfall patterns related with the development of continental glaciers during the Pleistocene (ca. 2.5 Ma) had a great impact on climate and vegetation setting in SEA (Figure II.5) (Heaney, 1991). Fossil pollen records demonstrate that Southeast Asian vegetation during the last glacial maximum (LGM, ca. 1.8 Ma) differed substantially from that of today, with an increase in the extent of montane vegetation and savannah (Figure II.5 a) (Bird *et al.*, 2005). At the LGM, with raising of the sea levels involving by the short warmer interglacials (Figure II.5 b), the Sundaland rainforests decreased and the upland forests reduced more rapidly than the lowland forests. The fragmentation of the land into islands and peninsulas formed step by step with higher sea levels through the LGM to the present (Figure II.5 c).

II.3. Biogeographical pattern and diversity dynamic of *Cardiodactylus* in Southeast Asia

Article 3: Biogeographical pattern and diversity dynamic of diversity dynamic of *Cardiodactylus* in Southeast Asia.

Jiajia Dong^{a,b,*}, Gael J. Kergoat^c, Natália Vicente^d, Cahyo Ramadi^e, Shengquan Xu^b, Tony Robillard^a. 2017. Biogeographical patterns and diversification dynamics of the genus *Cardiodactylus* Saussure (Orthoptera, Grylloidea, Eneopterinae) in Southeast Asia. (In preparation)

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Running title: Biogeography of *Cardiodactylus* crickets

Abstract

Southeast Asia harbors extraordinary species richness and endemism. It comprises four of the planet's 34 biodiversity hotspots, while only covering 4% of the earth's land area. Its complex geological history generated a megadiverse and highly endemic biota that has attracted a lot of attention, especially in the field of island biogeography. We used wide distributed *Cardiodactylus* species as a model, sampling taxon in SEA to (1) analyze phylogenetic relationship based on five mitochondrial and four nuclear markers, (2) estimate diverging time by BEAST analysis and examine biogeographical ancestral area in BioGeoBEARS, (3) depict colonization route, and summarize the events of emigration and immigration, and *in situ* diversification, and (4) determine where shifts in species diversification occurred using BAMM. The genus and the *Novaeguineae* species group proved monophyletic while the monophyly of the species group *Efordi* was not recovered. *Cardiodactylus* originated from the Western Pacific at the Mid-Eocene. It colonized SEA three times during the Early Miocene (ca. 19-16 Ma) by crossing New Guinea, which served as a transition zone, and passing through three different passageways from east to west: Java, Sulawesi and the Philippines. Sulawesi was the diversification hub in SEA combining high immigration and *in situ* diversification events, which is clearly linked to the geological history of in the Wallacean region.

Keywords: climate change, crickets, diversification analyses, geological change, molecular dating, Southeast Asia

Introduction

Southeast Asia (SEA) is considered as one of the most geologically dynamic regions of the planet (Lohman *et al.*, 2011). It aggregates four major biodiversity hotspots (de Bruyn *et al.*, 2014; Mittermeier *et al.*, 2004; Zachos and Habel, 2011) which are divided by sharp, yet porous biogeographic boundaries (Hearty *et al.*, 2007; Morley *et al.*, 2012; Wallace 1860). The complex geological history of SEA generated a megadiverse and highly endemic biota that has attracted a lot of attention, especially in the field of island biogeography (Condamine *et al.*, 2013, 2015; Hall, 2009; Lohman *et al.*, 2011; Metcalfe, 2006; Woodruff, 2010). In SEA, dynamic geological and climatic histories acted together to generate the world's largest island complex, in which the spatial distribution of terrestrial habitats has been altered extensively through time (Bird *et al.*, 2005; Hall 2009, 2011; Hall & Sevastjanova, 2012; Heaney 1991; Metcalfe, 2006; Voris 2000; Zahirovic *et al.*, 2014). Volcanic uplifts and repeated sea-level fluctuations promoted evolutionary diversification by providing countless opportunities for allopatric speciation (Heaney, 2000; Stepan *et al.*, 2003; Jansa *et al.*, 2006; Outlaw & Voelker, 2008).

The interplay between the movement of tectonic plates, oscillations in the Earth's orbit, and the variable configuration of land masses and ocean currents also affected the climate of SEA (Morley, 2012). As a result, the vegetation and the distribution of habitats of SEA were profoundly modified through time (Heaney, 1991). Tropical rainforest in SEA greatly expanded during the Paleogene Period (66.0-23.0 Million years ago (Ma)) and the beginning of the Neogene Period due to warmer temperatures and more humid climates. Tropical rainforests reached a peak during the mid-Miocene Climatic Optimum (MCO; 17-15 Ma), when their distribution extended further north to southern China and Japan (Heaney, 1991; Morley, 2012; Zachos *et al.*, 2001). It is not until the Late Miocene and Early Pliocene that tropical rainforests started to decrease in range as a result of global cooling (Hall, 2012; Zachos *et al.*, 2001). Lower sea levels, cooler temperatures, and modified rainfall patterns related with the development of continental glaciers during the Pleistocene (2.6-0.01 Ma) also had a great

impact on SEA vegetation setting (Heaney, 1991). Studies of fossil pollen records from the Last Glacial Maximum (20,000 years ago) indicate that SEA vegetation differed substantially from that of today, being characterized by an increase in the extent of montane vegetation and savannah, and by the presence of a continuous belt of evergreen rainforests across Sundaland (Bird *et al.*, 2005). During the Last Glacial Period (110,000-11,700 years ago), short warmer interglacial periods also resulted in raises of the global sea level, which led to the fragmentation of some parts of the Sundaland into islands and peninsulas; for instance, the Thai-Malay Peninsula, Borneo, Sumatra and Java were separated from each other every time the sea level rose.

The complex geological and climate history and the fragmented island habitats have raised many questions on the origins of the current biota of SEA. In a comprehensive study, de Bruyn *et al.* (2014) reviewed previous biogeographical works by conducting meta-analyses of geological, climatic and biological data. Based on inferences of colonization routes and ancestral areas derived from meta-analyses of phylogenetic data, they inferred that Borneo and Indochina acted as major evolutionary hotspots during the Cenozoic. One of the reasons invoked is the length of their emergent histories (more than 80 million years), which surpasses by far those of other regions in SEA. They also found out that within-area diversification (*in situ* diversification) and subsequent emigration played a major role in Borneo and Indochina, especially during the course of the Neogene and Quaternary sea-level fluctuations.

To investigate biogeographic and species diversification patterns in SEA, we use a species-rich clade of crickets from the subfamily Eneopterinae, the genus *Cardiodactylus* Saussure, 1878, as a model system. Eneopterinae crickets are mostly distributed in tropical areas (Vicente *et al.*, 2017) and are characterized by a diversity of traits in relation with acoustic communication (e.g. Robillard *et al.*, 2007, 2013; ter Hofstede *et al.*, 2015). With 82 known species, *Cardiodactylus* is currently the most speciose eneopterine genus. It is also the most widespread one, as its distribution runs from Japan (northern Ryukyu Islands), to the coasts of Southeast Asia, Northern Australia and the archipelagos of the Western Pacific across the Caroline Islands, Fiji, Indonesia, New Caledonia, Palau, Papua New Guinea, the

Philippines, the Samoan Islands, the Solomon Islands, the Mariana Islands, Tonga, Vanuatu and Wallis and Futuna (Otte, 2007; Robillard and Ichikawa, 2009; Robillard *et al.*, 2014). The genus is subdivided in two species groups, *Efordi* (22 known species) and *Novaeguineae* (60 known species), which show contrasted patterns of geographical distributions. Only one species of the *Efordi* group is distributed in SEA; the other species are distributed in New Guinea and in archipelagoes of the Western Pacific. In contrast, the *Novaeguineae* species group has a wider distribution, and is well diversified in SEA (35 species are found in SEA: Dong and Robillard, 2016; Robillard *et al.*, 2014). Interestingly, the distribution map of the *Novaeguineae* group indicates that species richness is inconsistent with the size of land masses (Appendix S1). For example, only one species occurs in the northern part of Indochina (*Cardiodactylus guttulus*, distributed from Japan to Northern Vietnam), while four species are endemic to Sulawesi. Some species are not endemic to a single island, for example *C. empagatao* is found in Philippines and Sulawesi, and *C. borneoe* is widely distributed in Borneo and in the Thai-Malay Peninsula. This diversity of patterns of distribution suggests the influence of intricate events of colonization that can be investigated with a biogeographical study.

According to Vicente *et al.* (2017) the genus *Cardiodactylus* did not originate in SEA but in the Southwest Pacific; however, this inference is potentially biased by the settings of the analysis in terms of time slices, and by the fact that it relies on a sparse sampling for the genus (only six *Cardiodactylus* species were sampled). Here, we used a more comprehensive sampling (41 species) for the genus and more appropriate time slices to infer the biogeographical patterns and diversification dynamics of the genus using a time-calibrated species tree. This allows us to assess with more precision the center of diversification of the genus, and to determine whether Borneo and Indochina played a major role in its diversification.

Materials and methods

Taxon sampling

The taxon sampling of *Cardiodactylus* is focused on species distributed in SEA, but also includes representatives of the whole distribution and taxonomic diversity of the genus. In this study, we sampled 113 individuals representing 41 species of *Cardiodactylus* crickets, including 28 species from SEA and 13 species from New Guinea and Western Pacific archipelagoes (New Caledonia, Solomon Islands and Vanuatu). For outgroup selection, we referred to a previous study of Eneopterinae crickets (Anso *et al.*, 2016), and selected 22 species representing the five tribes of the subfamily Eneopterinae, as well as two more distant species belonging to the subfamily Gryllinae. Detailed information on specimens is listed in Table 1.

Most specimens were collected during recent fieldworks and through cooperation works in several countries in SEA. In addition, some specimens used in the current study were obtained from the following institutions: the 'Muséum d'Histoire Naturelle de Genève' (MHNG), the 'Muséum National d'Histoire Naturelle, Paris' (MNHN), the 'Museum Zoologicum Bogoriense, Bogor' (MZB), the 'Nationaal Natuurhistorisch Museum, Leiden' (RMNH), the 'Natural History Museum, London' (BMNH), the 'Naturhistorisches Museum, Vienna' (NHMW) and the 'Zoological Institute, Russian Academy of Sciences, S. Petersburg' (ZIN).

DNA extraction, amplification and sequencing

The molecular work was performed at the 'Service de Systématique Moléculaire' of the MNHN. Whole genomic DNA was extracted from the median/hind femora of the dried, or alcohol-preserved specimens using the NucleoSpin® 96 Tissue Kit (Macherey-Nagel, Germany) and an automatic pipetting robot Eppendorf epMotion® 5075 TMX. Five mitochondrial markers and four nuclear gene markers used in previous phylogenetic studies on crickets (Chintauan-Marquier *et al.* 2016; Nattier *et al.*, 2011; Robillard & Desutter-Grandcolas, 2006) were amplified and sequenced. Mitochondrial markers were partial

fragments of 16S ribosomal RNA (16S), 12S ribosomal RNA (12S), cytochrome c oxidase subunit I (COI), cytochrome c oxidase subunit II (COII), cytochrome b (Cytb). Nuclear markers included partial fragments of two protein coding genes, elongation factor 1 alpha (EF1a) and histone H3 (H3), and two non-coding genes, 18S ribosomal subunit (18S) and 28S ribosomal subunit (28S). Whenever possible, we obtained molecular data from the same previous voucher specimens. We used the primers and settings detailed in Anso et al. (2016), Nattier et al. (2011) and Chintauan-Marquier et al. (2016). All sequences obtained in this study were cleaned, checked for sequencing errors and pseudogenes, and deposited in GenBank (see details in Table 1). The COI sequences for all sequenced specimens of *Cardiodactylus borneoe* were discarded because of suspicion of pseudogenes. The complete combined dataset consisted of 4,111 aligned base pairs (bp) for 145 terminals: 528 bp for partial 16S, 419 bp for partial 12S, 706 bp for partial COI, 340 bp for partial COII, 345 bp for partial Cytb, 653 bp for partial 18S, 375 bp for partial 28S, 327 bp for partial H3 and 405 bp for partial EF1a.

Phylogenetic analyses

To check for possible contaminations and artifacts, preliminary phylogenetic analyses were carried out for each marker using the IQ-TREE web server (<http://iqtree.cibiv.univie.ac.at/>; Trifinopoulos *et al.*, 2016) (see details in Appendix S5). Then the aligned sequences of all seven markers were concatenated in Geneious.

The resulting concatenated dataset was then analyzed using Bayesian inference (BI) with MrBayes 3.2.6 (Ronquist *et al.*, 2012) and maximum likelihood (ML) with RAxML 8.2.8 (Stamatakis, 2014). The best-fit partitioning scheme and substitution models were determined by PartitionFinder V1.1.1 (Lanfear *et al.*, 2012; Miller *et al.*, 2015), with the *greedy* algorithm, the *unlinked* option for branch lengths, and the corresponding set of models (*mrbayes* or *raxml*) (see details in Table 2). The Bayesian information criterion (BIC) was preferentially used to compare partitioning schemes and substitution models following the recommendation of Ripplinger and Sullivan (2008).

Table 1. Taxon sampling. List of all *Cardiodactylus* and outgroups used in this study with current geographical distribution and GenBank accession numbers of each marker. The abbreviations of taxonomic status indicate as following: subfamily Gryllinae (GRYL), subfamily Eneopterinae (ENEO), subfamily Gryllinae (Gry), tribe Lebinthini (Leb), tribe Eneopterini (Ene), tribe Eurepini (Eur), tribe Nisitrini (Nis), tribe Xenogryllini (Xen). NA means missing data. The voucher is missing or added later shown with mark (#####). The samples used in the dating analysis to represent each species are show with asterisk (*) in accession number.

Species	Taxonomic status	Voucher	Molecular code	Geographic area/code	16S	12S	COI	COII	Cytb	18S	28S	EF1a	H3
<i>Acheta domestica</i> (Linnaeus, 1758)	GRYL/Gr y	MNHN-EO-ENSIF3523	Adom	France (laboratory strain)	AF248 698	ADZ9 7611	JX897 403	JX897 439	AF248 682	AD18 SITS1	JX897 465	GO88 6692	KR90 3150
<i>Agnoteocus albifrons</i> Desutter-Grandcolas, 1997	ENEO/L eb	MNHN-EO-ENSIF-2767	AalCT	New Caledonia	JX897 353	JX897 394	JX897 418	JX897 446	JX897 314	JX897 583	JX897 490	JX897 527	JX897 572
<i>Agnoteocus albifrons</i> Desutter-Grandcolas, 1997	ENEO/L eb	MNHN-EO-ENSIF-1771	AalGe	New Caledonia	JX897 354	JX897 396	JX897 416	JX897 445	JX897 316	JX897 585	NA	JX897 529	JX897 574
<i>Agnoteocus azurensis</i> Desutter-Grandcolas, 2006	ENEO/L eb	MNHN-EO-ENSIF-2777	AazGK	New Caledonia	JX897 360	JX897 389	JX897 426	NA	JX897 331	JX897 593	JX897 472	JX897 505	JX897 565
<i>Agnoteocus azurensis</i> Desutter-Grandcolas, 2006	ENEO/L eb	MNHN-EO-ENSIF-2780	AazRB	New Caledonia	JX897 358	JX897 376	JX897 423	JX897 453	JX897 329	JX897 595	JX897 475	JX897 502	JX897 566
<i>Agnoteocus meridionalis</i> Desutter-Grandcolas, 2006	ENEO/L eb	MNHN-EO-ENSIF-2772	AmelDP	New Caledonia	JX897 349	JX897 401	JX897 420	NA	JX897 311	JX897 579	JX897 488	JX897 519	JX897 553
<i>Agnoteocus meridionalis</i> Desutter-Grandcolas, 2006	ENEO/L eb	MNHN-EO-ENSIF-2771	AmePB	New Caledonia	JX897 350	JX897 402	JX897 410	JX897 442	JX897 313	JX897 597	JX897 489	JX897 520	JX897 550
<i>Agnoteocus obscurus</i> (Chopard, 1970)	ENEO/L eb	MNHN-EO-ENSIF-2786	AobAo2	New Caledonia	JX897 356	JX897 398	JX897 415	JX897 449	JX897 319	JX897 591	NA	JX897 510	NA
<i>Agnoteocus obscurus</i> (Chopard, 1970)	ENEO/L eb	MNHN-EO-ENSIF-2785	AobMa	New Caledonia	JX897 357	JX897 393	JX897 412	JX897 450	JX897 320	JX897 587	JX897 487	JX897 525	JX897 576
<i>Agnoteocus robustus</i> (Chopard, 1915)	ENEO/L eb	MNHN-EO-ENSIF-2752	AroAo2	New Caledonia	JX897 359	JX897 375	JX897 406	JX897 443	JX897 333	JX897 588	NA	JX897 498	JX897 555
<i>Agnoteocus sarramea</i> Desutter-Grandcolas, 1997	ENEO/L eb	MNHN-EO-ENSIF-2764	AsaMA	New Caledonia	JX897 372	JX897 380	JX897 430	JX897 456	JX897 342	JX897 598	JX897 471	JX897 511	JX897 561
<i>Agnoteocus occidentalis</i> Desutter-Grandcolas, 2006	ENEO/L eb	MNHN-EO-ENSIF-2765	AspCR2	New Caledonia	JX897 362	JX897 386	JX897 434	JX897 461	JX897 322	JX897 589	NA	JX897 512	JX897 570
<i>Agnoteocus clarus</i> Desutter-Grandcolas, 2006	ENEO/L eb	MNHN-EO-ENSIF-2788	AspTRPP	New Caledonia	JX897 347	JX897 400	JX897 407	JX897 451	JX897 324	JX897 590	JX897 492	JX897 523	JX897 554
<i>Agnoteocus yahoue</i> Otte, 1987 Ko	ENEO/L eb	MNHN-EO-ENSIF-2766	AyaKo2	New Caledonia	JX897 366	JX897 387	JX897 438	JX897 463	JX897 334	JX897 602	JX897 478	JX897 500	JX897 549
<i>Agnoteocus yahoue</i> Otte, 1987 Mo	ENEO/L eb	MNHN-EO-ENSIF-2773	AyaMo5	New Caledonia	JX897 367	JX897 388	JX897 437	JX897 462	JX897 337	NA	JX897 479	JX897 501	NA
* <i>Cardiodactylus admirabilis</i> Tan & Robillard, 2014	ENEO/L eb	#####	Cadm_C84_Singapore	Singapore/B	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study
<i>Cardiodactylus borneo</i> Robillard & Gorochov, 2014	ENEO/L eb	RMNH	Cbor_C30_Borneo	Borneo/F	in this study	in this study	NA	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus borneo</i> Robillard & Gorochov, 2014	ENEO/L eb	#####	Cbor_C95_Sabah	Borneo/F	in this study	in this study	NA	NA	in this study	NA	NA	NA	in this study
<i>Cardiodactylus borneo</i> Robillard & Gorochov, 2014	ENEO/L eb	#####	Cbor_C96_Sabah	Borneo/F	in this study	in this study	NA	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus borneo</i> Robillard & Gorochov, 2014	ENEO/L eb	#####	Cbor_C98_Manukan	Borneo/F	in this study	in this study	NA	in this study	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus borneo</i> Robillard & Gorochov, 2014	ENEO/L eb	#####	Cbor_C99_Tioman	Malaysia/B	*in this study	*in this study	NA	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study
<i>Cardiodactylus busu</i> Otte, 2007	ENEO/L eb	#####	Cbus_C146_TritonBay	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	NA	in this study	in this study
<i>Cardiodactylus busu</i> Otte, 2007	ENEO/L eb	#####	Cbus_C162_Fawi	New Guinea/K	in this study	in this study	in this study	in this study	in this study	NA	NA	in this study	in this study
<i>Cardiodactylus busu</i> Otte, 2007	ENEO/L eb	MNHN-ENSIF184	Cbus_C56_Baitabag	New Guinea/K	NA	in this study	in this study	in this study	NA	in this study	in this study	NA	in this study
* <i>Cardiodactylus busu</i> Otte, 2007	ENEO/L eb	#####	Cbus_C57_Wanang	New Guinea/K	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study
<i>Cardiodactylus busu</i> Otte, 2007	ENEO/L eb	MNHN-ENSIF109	Cbus_C58_MW200	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus contrarius</i> Gorochov, 2014	ENEO/L eb	#####	Ccon_C100_Sulawesi	Sulawesi/H	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus contrarius</i> Gorochov, 2014	ENEO/L eb	#####	Ccon_C115_Sulawesi	Sulawesi/H	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study
* <i>Cardiodactylus doloduo</i> Gorochov, 2014	ENEO/L eb	#####	Cdol_C36_Sulawesi	Sulawesi/H	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study
<i>Cardiodactylus empagatao</i> Otte, 2007	ENEO/L eb	#####	Cemp_C35_Bunaken	Sulawesi/H	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus empagatao</i> Otte, 2007	ENEO/L eb	MNHN-ENSIF3353	Cemp_C79_Leyte	Philippines/D	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study
<i>Cardiodactylus empagatao</i> Otte, 2007	ENEO/L eb	#####	Cemp_C81_Barangay	Philippines/D	in this study	in this study	in this study	in this study	in this study	in this study	NA	in this study	in this study
<i>Cardiodactylus enkraussi</i> Otte, 2007	ENEO/L eb	#####	Cenk_C50_Lugarville	Western Pacific/L	in this study	in this study	in this study	in this study	NA	in this study	NA	NA	in this study

(Continued)

Table 1. Continued

Species	Taxonomic status	Voucher	Molecular code	Geographic area/code	16S	12S	COI	COII	Cytb	18S	28S	EF1a	H3
<i>Cardiodactylus enkraussi</i> Otte, 2007	ENEOL/eb	####	Cenk_C74_Peaoru	Western Pacific/L	NA	in this study	in this study	in this study	NA	in this study	NA	NA	in this study
<i>Cardiodactylus enkraussi</i> Otte, 2007	ENEOL/eb	####	Cenk_C75_Peavot	Western Pacific/L	NA	in this study	in this study	in this study	in this study	in this study	NA	NA	NA
* <i>Cardiodactylus enkraussi</i> Otte, 2007	ENEOL/eb	####	Cenk_C76_Butmas	Western Pacific/L	in this study	in this study	in this study	in this study	NA	in this study	in this study	in this study	in this study
<i>Cardiodactylus enkraussi</i> Otte, 2007	ENEOL/eb	MNHN-EO-ENSIF2366	Cenk_C9_Santo	Western Pacific/L	JF972518	JF972502	KU705561	KU705550	NA	JF972533	NA	NA	KU705595
<i>Cardiodactylus erniae</i> Robillard & Gorochov, 2014	ENEOL/eb	####	Cern_C110_Java	Java/G	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus erniae</i> Robillard & Gorochov, 2014	ENEOL/eb	MNHN-EO-ENSIF3489	Cern_C24_Java	Java/G	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus floresiensis</i> Robillard, 2014	ENEOL/eb	####	Cflo_C31_Flores	Flores island/I	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus floresiensis</i> Robillard, 2014	ENEOL/eb	####	Cflo_C48_Flores	Flores island/I	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus fruhstorferi</i> Gorochov & Robillard, 2014	ENEOL/eb	####	Cfru_C11_Sulawesi	Sulawesi/H	in this study	in this study	NA	in this study	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus fruhstorferi</i> Gorochov & Robillard, 2014	ENEOL/eb	MNHN-ENSIF1123	Cfru_C46_Sulawesi	Sulawesi/H	in this study	in this study	NA	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus guttulus</i> (Matsumura, 1913)	ENEOL/eb	####	Cgut_C101_Vietnam	Vietnam/A	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus guttulus</i> (Matsumura, 1913)	ENEOL/eb	####	Cgut_C103_Vietnam	Vietnam/A	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus guttulus</i> (Matsumura, 1913)	ENEOL/eb	MNHN-ENSIF1193	Cgut_C10_Japan	Japan/A	JF972519	JF972503	KU705562	NA	JF972487	JF972534	KU705580	KU705612	KU705596
<i>Cardiodactylus guttulus</i> (Matsumura, 1913)	ENEOL/eb	####	Cgut_C137_Vietnam	Vietnam/A	in this study	in this study	NA	in this study	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus guttulus</i> (Matsumura, 1913)	ENEOL/eb	####	Cgut_C45_Hainan	Hainan/A	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus guttulus</i> (Matsumura, 1913)	ENEOL/eb	####	Cgut_C86_Ryukyu	Ryukyu island/A	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus haddocki</i> Dong & Robillard, 2016	ENEOL/eb	(A.Gorochov) ZIN	Chad_C125_Bagusa	Bagusa/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus haddocki</i> Dong & Robillard, 2016	ENEOL/eb	(A.Gorochov) ZIN	Chad_C130_MCyclop	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus haddocki</i> Dong & Robillard, 2016	ENEOL/eb	####	Chad_C134_Manokwari	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus haddocki</i> Dong & Robillard, 2016	ENEOL/eb	(A.Gorochov) ZIN	Chad_C139_Fawi	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus haddocki</i> Dong & Robillard, 2016	ENEOL/eb	####	Chad_C64_MW700	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus haddocki</i> Dong & Robillard, 2016	ENEOL/eb	MNHN-EO-ENSIF3548	Chad_C66_Baitabag	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus haddocki</i> Dong & Robillard, 2016	ENEOL/eb	MNHN-EO-ENSIF3549	Chad_C69_MW200	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus haddocki</i> Dong & Robillard, 2016	ENEOL/eb	MNHN-EO-ENSIF3546	Chad_C89_Wanang	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	NA	in this study	in this study
<i>Cardiodactylus haddocki</i> Dong & Robillard, 2016	ENEOL/eb	MNHN-EO-ENSIF3547	Chad_C90_Wanang	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	NA	in this study	in this study
* <i>Cardiodactylus halmahera</i> Gorochov & Robillard, 2014	ENEOL/eb	####	Chalm_C38_Halmahera	Moluccas/J	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus javare</i> Otte, 2007	ENEOL/eb	MNHN-EO-ENSIF91	Cjav_C164_Wanang	New Guinea/K	NA	in this study	in this study	in this study	in this study	in this study	NA	in this study	NA
<i>Cardiodactylus javare</i> Otte, 2007	ENEOL/eb	MNHN-EO-ENSIF60	Cjav_C165_MW200	New Guinea/K	NA	in this study	in this study	NA	in this study	in this study	NA	in this study	in this study
<i>Cardiodactylus javare</i> Otte, 2007	ENEOL/eb	MNHN-EO-ENSIF103	Cjav_C63_Wanang	New Guinea/K	in this study	in this study	in this study	NA	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus javare</i> Otte, 2007	ENEOL/eb	MNHN-EO-ENSIF3538	Cjav_C78_Baitabag	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus kondoi</i> Otte, 2007	ENEOL/eb	####	Kkon_C41_Luzon	Philippines /D	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus kondoi</i> Otte, 2007	ENEOL/eb	####	Kkon_C42_Luzon	Philippines /D	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus kondoi</i> Otte, 2007	ENEOL/eb	####	Kkon_C51_Negros	Philippines /D	NA	in this study	in this study	in this study	in this study	in this study	in this study	NA	in this study
<i>Cardiodactylus kondoi</i> Otte, 2007	ENEOL/eb	MNHN-EO-ENSIF3160	Kkon_C53_Pollilo	Philippines /D	in this study	in this study	in this study	NA	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus kotandora</i> Robillard, 2014	ENEOL/eb	MNHN-EO-ENSIF3519	Ckot_C65_Flores	Flores island/I	in this study	in this study	in this study	in this study	in this study	NA	in this study	in this study	in this study
* <i>Cardiodactylus</i> affinis lampongsi Robillard & Gorochov, 2014	ENEOL/eb	####	Clam_C105_Sumatra	Sumatra/C	in this study	in this study	NA	in this study	in this study	NA	in this study	in this study	in this study
* <i>Cardiodactylus lampongsi</i> Robillard & Gorochov, 2014	ENEOL/eb	####	Clam_C117_Sumatra	Sumatra/C	in this study	in this study	in this study	NA	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus lombrinjani</i> Robillard, 2014	ENEOL/eb	MNHN-EO-ENSIF3675	Clom_C19_Sumbawa	Sumbawa/I	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus lombrinjani</i> Robillard, 2014	ENEOL/eb	MNHN-EO-ENSIF3673	Clom_C21_Lombok	Sumbawa/I	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study

(Continued)

Table 1. Continued

Species	Taxonomic status	Voucher	Molecular code	Geographic area/code	16S	12S	COI	COII	Cytb	18S	28S	EF1a	H3
<i>Cardiodactylus lucus</i> Dong & Robillard, 2016	ENEO/L eb	####	Cluc_C61_M W200	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	NA	in this study
<i>Cardiodactylus lucus</i> Dong & Robillard, 2016	ENEO/L eb	####	Cluc_C62_W anang	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	NA	in this study
* <i>Cardiodactylus lucus</i> Dong & Robillard, 2016	ENEO/L eb	MNHN-ENSIF94	Cluc_C68_M W700	New Guinea/K	in this study	in this study	in this study	NA	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus maai</i> Otte, 2007	ENEO/L eb	####	Cmaa_C119_Biak	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus maai</i> Otte, 2007	ENEO/L eb	####	Cmaa_C128_Supiori	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus manus</i> Otte, 2007	ENEO/L eb	####	Cman_C127_Rani	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus manus</i> Otte, 2007	ENEO/L eb	####	Cman_C37_Manokwari	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus muiri</i> Otte, 2007	ENEO/L eb	####	Cmui_C158_Kumawa	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	NA
* <i>Cardiodactylus muiri</i> Otte, 2007	ENEO/L eb	####	Cmui_C160_Kumawa	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus muria</i> Robillard, 2014	ENEO/L eb	MNHN-EO-ENSIF3481	Cmur_C18_Java	Java/G	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus niugini</i> Dong & Robillard, 2016	ENEO/L eb	MNHN-EO-ENSIF113	Cniu_C54_Baitabag	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus niugini</i> Dong & Robillard, 2016	ENEO/L eb	####	Cniu_C55_M W200	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus niugini</i> Dong & Robillard, 2016	ENEO/L eb	MNHN-EO-ENSIF73	Cniu_C70_W anang	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	NA	in this study	in this study
<i>Cardiodactylus nobilis</i> Dong & Robillard, 2016	ENEO/L eb	MNHN-EO-ENSIF102	Cnob_C59_MW1200	New Guinea/K	in this study	in this study	in this study	NA	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus nobilis</i> Dong & Robillard, 2016	ENEO/L eb	MNHN-EO-ENSIF131	Cnob_C67_MW1200	New Guinea/K	in this study	in this study	in this study	NA	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus novaeguineae</i> (Haan, 1842)	ENEO/L eb	####	Cnov_C141_Queensland	Australia/L	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus novaeguineae</i> (Haan, 1842)	ENEO/L eb	ZIN > MNHN-EO-ENSIF38	Cnov_C142_NewGeorgia	Western Pacific/L	in this study	in this study	in this study	in this study	in this study	NA	in this study	in this study	in this study
<i>Cardiodactylus novaeguineae</i> (Haan, 1842)	ENEO/L eb	####	Cnov_C143_Mhagen	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus novaeguineae</i> (Haan, 1842)	ENEO/L eb	####	Cnov_C151_TritonBay	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	NA	in this study	NA
<i>Cardiodactylus novaeguineae</i> (Haan, 1842)	ENEO/L eb	MNHN-EO-ENSIF1921	Cnov_C1_Lifou	New Caledonia/L	in this study	in this study	KU70 5564	KU70 5552	in this study	KU70 5588	KU70 5588	KU70 5614	KU70 5597
<i>Cardiodactylus novaeguineae</i> (Haan, 1842)	ENEO/L eb	####	Cnov_C27_Biak	New Guinea/K	in this study	in this study	in this study	in this study	in this study	NA	NA	NA	NA
<i>Cardiodactylus novaeguineae</i> (Haan, 1842)	ENEO/L eb	MNHN-EO-ENSIF2030	Cnov_C2_Peavot	Vanuatu/L	JF972 521	JF972 506	KU70 5563	KU70 5551	JF972 490	JF972 537	KR90 3500	KU70 5613	KR90 3151
<i>Cardiodactylus novaeguineae</i> (Haan, 1842)	ENEO/L eb	MNHN-ENSIF2038	Cnov_C3_Oubatche	New Caledonia/L	JF972 520	JF972 504	in this study	in this study	JF972 488	JF972 535	in this study	in this study	in this study
* <i>Cardiodactylus novaeguineae</i> (Haan, 1842)	ENEO/L eb	####	Cnov_C49_Wewec	New Caledonia/L	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus novaeguineae</i> (Haan, 1842)	ENEO/L eb	H.Song	Cnov_C5_NewIreland	New Guinea/K	in this study	NA	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus novaeguineae</i> (Haan, 1842)	ENEO/L eb	MNHN-EO-ENSIF3550	Cnov_C60_Loloata	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus novaeguineae</i> (Haan, 1842)	ENEO/L eb	Muséum Canberra	Cnov_C6_Queensland	Australia/L	in this study	in this study	in this study	NA	in this study	in this study	in this study	NA	in this study
<i>Cardiodactylus novaeguineae</i> (Haan, 1842)	ENEO/L eb	####	Cnov_C71_Penaoru	Vanuatu/L	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus novaeguineae</i> (Haan, 1842)	ENEO/L eb	####	Cnov_C72_Luganville	Vanuatu/L	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus novaeguineae</i> (Haan, 1842)	ENEO/L eb	####	Cnov_C73_BigBay	Vanuatu/L	in this study	in this study	in this study	NA	in this study	in this study	NA	NA	NA
<i>Cardiodactylus novaeguineae</i> (Haan, 1842)	ENEO/L eb	####	Cnov_C87_Tanna	Vanuatu/L	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus oeroe</i> Robillard, 2014	ENEO/L eb	RMNH	Coer_C43_Sulawesi	Sulawesi/H	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus palawan</i> Gorochov, 2014	ENEO/L eb	####	Cpal_C106_Palawan	Palawan/E	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus palawan</i> Gorochov, 2014	ENEO/L eb	####	Cpal_C107_Palawan	Palawan/E	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus palawan</i> Gorochov, 2014	ENEO/L eb	####	Cpal_C91_Turtle	Palawan/E	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus palawan</i> Gorochov, 2014	ENEO/L eb	####	Cpal_C92_Palawan	Palawan/E	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
<i>Cardiodactylus palawan</i> Gorochov, 2014	ENEO/L eb	####	Cpal_C93_Palawan	Palawan/E	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus pelagus</i> Otte, 2007	ENEO/L eb	####	Cpel_C109_Borneo	Borneo/F	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study

(Continued)

Table 1. Continued

Species	Taxonomic status	Voucher	Molecular code	Geographic area/code	16S	12S	COI	COII	Cytb	18S	28S	EF1a	H3
<i>Cardiodactylus quatei</i> Otte, 2007	ENEO/L eb	####	Cqua_C13_8_Fawi	New Guinea/K	in this study	in this study	in this study	in this study	in this study	NA	in this study	in this study	in this study
* <i>Cardiodactylus quatei</i> Otte, 2007	ENEO/L eb	####	Cqua_C15_6_Lobo	New Guinea/K	this study	this study	this study	this study	this study	this study	this study	this study	this study
<i>Cardiodactylus quatei</i> Otte, 2007	ENEO/L eb	####	Cqua_C15_7_Urisa	New Guinea/K	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus reticulatus</i> Gorochov, 2014	ENEO/L eb	####	Cret_C33_Tioman	Malaysia/B	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study
* <i>Cardiodactylus riga</i> Otte, 2007	ENEO/L eb	####	Crig_C77_Burauen	Philippines/D	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study
<i>Cardiodactylus riga</i> Otte, 2007	ENEO/L eb	MNHN-EO-ENSIF3352	Crig_C83_LeyteS	Philippines/D	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus rufidulus</i> Saussure, 1878	ENEO/L eb	####	Cruf_C44_Guadalcanal	Solomon/L	*in this study	*in this study	NA	this study	this study	NA	this study	NA	in this study
* <i>Cardiodactylus singapura</i> Robillard, 2011	ENEO/L eb	MNHN-EO-ENSIF2759	Csin_C12_Bukit	Singapour/B	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study
* <i>Cardiodactylus sumba</i> Robillard, 2014	ENEO/L eb	MNHN-EO-ENSIF3661	Csum_C20_Sumba	Sumba/I	this study	this study	this study	this study	this study	this study	this study	this study	this study
<i>Cardiodactylus sumba</i> Robillard, 2014	ENEO/L eb	####	Csum_C47_Sumba	Sumba/I	in this study	in this study	NA	in this study	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus tangkoko</i> Gorochov, 2014	ENEO/L eb	####	Ctang_C11_6_Sulawesi	Sulawesi/H	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study	in this study
* <i>Cardiodactylus tankara</i> Robillard, 2009	ENEO/L eb	MNHN-ENSIF2416	Ctank_C8_Espiritu	Vanuatu/L	*JF97_2522	*JF97_2508	*KU7_15286	*KU7_26590	*JF97_2491	*JF97_2538	*KU7_05581	this study	*KU7_05598
* <i>Cardiodactylus tello</i> Robillard, 2014	ENEO/L eb	MNHN-EO-ENSIF160	Ctel_C171_Siberut	Sumatra/C	*in this study	*in this study	NA	NA	NA	NA	NA	NA	NA
<i>Cardiodactylus thailandia</i> Robillard, 2011	ENEO/L eb	####	Ctha_C111_Thailand	Thailand/A	in this study	in this study	in this study	in this study	in this study	in this study	NA	in this study	in this study
* <i>Cardiodactylus thailandia</i> Robillard, 2011	ENEO/L eb	####	Ctha_C112_Thailand	Thailand/A	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study
<i>Cardiodactylus variegatus</i> Gorochov & Robillard, 2014	ENEO/L eb	####	Cvar_C113_Borneo	Borneo/F	in this study	in this study	in this study	NA	in this study	NA	in this study	in this study	in this study
<i>Cardiodactylus variegatus</i> Gorochov & Robillard, 2014	ENEO/L eb	MNHN-EO-ENSIF3510	Cvar_C28_Borneo	Borneo/F	in this study	in this study	NA	in this study	in this study	in this study	NA	in this study	in this study
* <i>Cardiodactylus variegatus</i> Gorochov & Robillard, 2014	ENEO/L eb	####	Cvar_C52_Borneo	Borneo/F	*in this study	*in this study	*in this study	*in this study	*in this study	*in this study	NA	*in this study	*in this study
<i>Eneoptera guyanensis</i> Chopard, 1931	ENEO/E ne	MNHN-EO-ENSIF2741	Egu	French Guiana	AY90_5301	AY90_5272	JX897_404	KU70_5553	AY90_5355	AY90_5331	KU70_5581	JX897_495	JX897_547
<i>Eurepini</i> sp.	ENEO/E ur	MNHN-EO-ENSIF3155	Eursp	Australia France (laboratory strain)	KR90_3674	KR90_3830	KU70_5565	KU70_5554	KR90_3331	KR90_4028	KR90_3503	NA	KR90_3153
<i>Gryllus bimaculatus</i> De Geer, 1773	GRYL/G ry	MNHN-EO-ENSIF3524/3404	Gbi		AF24_8685	AY90_5292	KY64_6234	KU70_5555	AF24_8659	AF51_4509	KR90_3002	NA	KR90_3154
<i>Lebinthus luae</i> Robillard & Tan, 2013	ENEO/L eb	MNHN-EO-ENSIF2740	LbiS1	Singapore	JF972_524	KR90_4017	KU70_5567	KU70_5557	JF972_493	KR90_4199	KR90_3665	KU70_5615	KR90_3321
<i>Lebinthus lifouensis</i> Desutter-Grandcolas, 1997	ENEO/L eb	MNHN-EO-ENSIF1346	Lli	New Caledonia	AY90_5309	AY90_5279	KU70_5566	KU70_5556	AY90_5364	AY90_5336	KU70_5583	NA	KU70_5599
<i>Lebinthus nattawa</i> Robillard, 2009	ENEO/L eb	MNHN-EO-ENSIF2564	LnaN	Vanuatu	JF972_525	JF972_510	KU70_5568	KU70_5558	JF972_494	JF972_541	KU70_5584	NA	KU70_5600
<i>Lebinthus</i> sp. PNG Stål, 1877	ENEO/L eb	MNHN-EO-ENSIF117	Lpng1	Papua New Guinea	JF972_528	JF972_513	KU71_5289	KU71_5288	JF972_5536	JF972_544	KU71_5290	KU71_5292	KU71_5291
<i>Lebinthus santoensis</i> Robillard, 2009	ENEO/L eb	MNHN-EO-ENSIF2484	LsaPe	Vanuatu	KU70_5528	KU70_8011	KU70_5569	NA	KU70_5535	KU70_5543	KU70_5585	NA	KU70_5601
<i>Lebinthus santoensis</i> Robillard, 2009	ENEO/L eb	MNHN-EO-ENSIF2437	LsaV	Vanuatu	JF972_527	JF972_511	JX897_405	JX897_441	JF972_495	JF972_542	JX897_467	NA	JX897_548
<i>Lebinthus villemantae</i> Robillard, 2010	ENEO/L eb	MNHN-EO-ENSIF2739	Lvil	Indonesia	JF972_526	JF972_512	KU70_5570	KU70_5559	JF972_496	JF972_543	KU70_5586	NA	KU70_5602
<i>Nisitrus vittatus</i> (Haan, 1842)	ENEO/N is	MNHN-EO-ENSIF2742	NviS	Singapore	SEA	SEA	KU70_5572	NA	SEA	AY90_5340	KR90_3667	JN88_7883	JX897_546
<i>Nisitrus vittatus</i> (Haan, 1842)	ENEO/N is	MNHN-EO-ENSIF3134	Nvith	Malaysia	AY90_5314	AY90_5284	KU70_5571	NA	AY90_5369	AY90_5340	KU70_5587	NA	KU70_5603
<i>Paranisitra longipes</i> Chopard, 1925	ENEO/N is	MNHN-EO-ENSIF3157	Plo2	Philippines	KR90_3827	KR90_4020	KU71_5287	NA	NA	KR90_4202	KR90_3668	NA	KR90_3325
<i>Pixibinthus sonicus</i> Anso & Robillard, 2016	ENEO/L eb	MNHN-EO-ENSIF99	L59LeNCRBa	New Caledonia	KU70_5530	KU70_8012	KU70_5577	NA	KU70_5538	KU70_5546	KU70_5592	NA	KU70_5608
<i>Pixibinthus sonicus</i> Anso & Robillard, 2016	ENEO/L eb	MNHN-EO-ENSIF83	L66LeNCFN2	New Caledonia	KU70_5532	KU70_8015	KU70_5574	NA	KU70_5540	KU70_5547	KU70_5590	NA	KU70_5605
<i>Swezwilderia</i> sp. Chopard, 1929	ENEO/L eb	MNHN-EO-ENSIF2737	Ssp	Fiji	JF972_529	JF972_514	KU70_5579	NA	JF972_498	JF972_545	NA	NA	KR90_3327
<i>Xenogryllus marmoratus</i> Bolivar, 1890	ENEO/X en	MNHN-EO-ENSIF3161	Xma2	Japan	KR90_3830	KR90_4024	NA	KU70_5560	KR90_3491	KR90_4206	NA	KU70_5610	KR90_3329

Both BI and ML analyses were performed on the CIPRES Science Gateway 3.3 (Miller *et al.*, 2015). For ML analyses the best-scoring tree was obtained using a heuristic search implementing 100 random-addition replicates. Clade support was then assessed using a non-parametric bootstrap procedure (500 replicates). Nodes supported by bootstrap support values (BS) $\geq 70\%$ were considered strongly supported following Hillis and Bull (1993). For the BI analyses we conducted two independent runs with eight Markov chain Monte Carlo (MCMC): one cold and seven incrementally heated that ran for 50 million generations with trees sampled every 1,000 generations. We used a conservative burn-in of 12.5 million generations per run after checking for stability on the log-likelihood curves and the split-frequencies of the runs in Tracer v.1.6 (Rambaut *et al.*, 2014). Support of nodes for MrBayes analyses was provided by clade posterior probabilities (PP) as directly estimated from the majority-rule consensus topology. A clade with a PP value higher than 0.95 was considered as well supported following Erixon *et al.* (2003). Whenever a *Cardiodactylus* species (for which multiple specimens were sequenced) or a specific taxonomic group of interest was recovered paraphyletic, we used Bayes factors (B_F) to assess whether there was statistical support for their non-monophyly. To do so, specific analyses (in which taxa of interest are constrained to be monophyletic) were carried out using MrBayes.

Divergence time estimation

We generated a species-level dataset (with one specimen per sampled species; see details in Table 1) to properly conduct dating and all subsequent analyses (i.e. historical biogeography and diversification analyses). Divergence times were estimated using Bayesian relaxed clocks as implemented in BEAST 1.8.4 (Drummond *et al.*, 2012). The partitions and substitution models were selected under PartitionFinder 1.1.1 following the settings above but with the *beast* set of models (see details in Table 2). BEAST analyses were performed on the CIPRES Science Gateway 3.3 (Miller *et al.*, 2015) using BEAGLE to improve and speed up the likelihood calculation (Ayres *et al.*, 2012). For each clock model/partitioning scheme an

uncorrelated lognormal relaxed clock was implemented. The *Tree Model* was set to a birth-death speciation process (Gernhard, 2008) to better account for extinct and missing lineages. The *ucl.d.mean* prior of each clock model was set to an uninformative interval (0.0001-1.0) with a uniform prior distribution.

Based on the worldwide biogeographical framework of Eneopterinae crickets inferred by Vicente et al. (2017) three secondary calibration points were enforced using normal distributions centered on the median ages from the study of Vicente et al. (2017): 1) the node for the most recent common ancestor (MRCA) of *Cardiodactylus* was assigned an interval of 33.60-55.19 Ma; 2) the node for the MRCA of the *Novaeguineae* species group was assigned an interval of 23.32-39.73 Ma, 3) and the node for the MRCA of *C. guttulus*, *C. oeroe*, *C. tankara* and *C. singapura* was assigned an interval of 17.83-31.17 Ma.

BEAST analyses consisted of 20 million generations of MCMC with the parameters and trees sampled every 2,000 generations. A burn-in of 25% was applied after checking the log-likelihood curves. Trees obtained from distinct analyses were combined using LogCombiner v1.8.3 (<http://beast.bio.ed.ac.uk/>). The maximum credibility tree, median ages and their 95% highest posterior density (HPD) were generated with TreeAnnotator v1.8.3 (<https://github.com/beast-dev/beast-mcmc/releases/tag/v1.8.4>).

Table 2. Best-fit models of sequence evolution and partitioning schemes selected in PartitionFinder for phylogenetic reconstructions using Bayesian Inference (MrBayes) and Maximum Likelihood (RAxML) and divergence dating using BEAST. Codon position is denoted by pos1, pos2 and pos3. Subsets are denoted by p1 and p2.

Analysis	Partitions	Models
Bayesian inference (BI) analysis	p1: 16S, COI_pos2, Cytb_pos2, 18S, 28S, H3_pos1, H3_pos2, H3_pos3, EF1a;	GTR+I+G
	p2: 12S, COI_pos1, COI_pos3, COII_pos1, COII_pos2, COII_pos3, Cytb_pos1, Cytb_pos3;	GTR+I+G
Maximum likelihood (ML) analysis	p1: 16S, COI_pos2, Cytb_pos2, 18S, 28S, H3_pos1, H3_pos2, H3_pos3, EF1a;	GTR+I+G
	p2: 12S, COI_pos1, COI_pos3, COII_pos1, COII_pos2, COII_pos3, Cytb_pos1, Cytb_pos3;	GTR+I+G
Bayesian evolutionary analysis by sampling trees (BEAST)	p1: 16S, COI_pos2, Cytb_pos2, 18S, 28S, H3_pos1, H3_pos2, H3_pos3, EF1a;	GTR+I+G
	p2: 12S, COI_pos1, COI_pos3, COII_pos1, COII_pos2, COII_pos3, Cytb_pos1, Cytb_pos3;	GTR+I+G

Ancestral area estimation

The package BioGeoBEARS (Matzke, 2014) implemented in R was used to infer the biogeographical history of *Cardiodactylus* crickets across SEA. The dispersal-extinction-cladogenesis (DEC; Ree and Smith, 2008) and additional founder-event speciation (DEC+j; Matzke, 2014) models were used to estimate ancestral areas. The analyses were conducted with the maximum clade credibility (MCC) tree inferred under BEAST. The geographical distribution of each species was estimated based on fieldwork data, extant literature, and information provided by museum specimens (see details in Table 1). Twelve operational areas were defined (Appendix S2) based on the distribution ranges of extant *Cardiodactylus* crickets (Robillard *et al.*, 2014): Indochina (A), Thai-Malay Peninsula (B), Sumatra (C), the Philippines (minus Palawan) (D), Palawan (E), Borneo (F), Java (G), Sulawesi (H), Lesser Sunda Islands (I), Moluccas (J), New Guinea (K) and the archipelagoes in the Western Pacific (L).

In previous studies (de Bruyn *et al.*, 2014; Hall, 2011; Morley, 2012), it has been shown that three major Cenozoic collision events had a great influence on the current archipelago setting: 1) the India-Asia collision at *ca.* 45 Ma facilitated the isolation of West Sulawesi from Sundaland; 2) the Australia-Asia collision at *ca.* 25 Ma generated the Bird's Head region of New Guinea; and 3) *ca.* 15 Ma the Sula Spur promontory collided with the Southeast Asian margin in Sulawesi, hereby forming the region now known as Wallacea (Hall, 2009). To account for these major geological events, three distinct time slices were enforced in our time-stratified biogeographical model: the first time slice runs from 45.0 to 25.0 Ma, the second one from 25.0 to 15.0 Ma and the last one from 15.0 Ma to the present (Hall, 2013; Hall & Sevastjanova, 2012) (see more details in Appendix S2). For each time slice, we constructed a matrix of scaling factors (0.01, 0.1, 0.25, 0.50, 0.75, 1.0) for the dispersal rates between areas according to their geographical position and changes in sea level. Alternative and more simplified sets of multipliers were also tested (0.01, 0.1, 0.25, 1.0; 0.01, 0.1, 0.50, 1.0; and 0.01, 0.1, 0.75, 1.0). Therefore, a total of eight processes, four distinct multipliers sets with

two speciation models (DEC and DEC+j), were implemented to estimate ancestral areas (the details of these processes are listed in Appendix S2).

Colonization events and in situ diversification

As mentioned above, only one member of the *Efordi* species group is distributed in SEA; in contrast, the *Novaeguineae* species group is more widely distributed, including in SEA, New Guinea and the Western Pacific. Therefore, we focus on colonization events and *in situ* diversification events of *Cardiodactylus* in SEA mainly based on the *Novaeguineae* species group. Within this clade, major colonization routes were inferred for each time slice. Emigration and immigration events for each area were analyzed by summarizing colonization events using SPSS Statistics 22 (SPSS Inc.). We also followed de Bruyn et al. (2014) to estimate *in situ* diversification events for the *Novaeguineae* species group using SPSS Statistics v.22 (SPSS Inc.).

Diversification analyses

Bayesian Analysis of Macroevolutionary Mixtures 2.5 (BAMM; Rabosky *et al.*, 2013) was used: 1) to estimate rates of speciation (λ), extinction (μ), and net diversification (γ) either for all *Cardiodactylus* species or only for the SEA species belonging to the *Novaeguineae* species group (which constitutes a monophyletic group), 2) conduct rate-through-time analysis of these rates, and 3) identify and visualize shifts in species rates across the *Cardiodactylus* phylogeny. BAMM accounts for non-random and incomplete taxon sampling in the phylogenetic trees by allowing all non-sampled species to be associated with a particular tip or more inclusive clade. Species numbers were obtained from the 'Orthoptera species file' website (Cigliano *et al.*, 2017) and published sources.

Priors for BAMM were generated using the R package BAMMtools v.2.5.0 (Mitchell *et al.*, 2017; Rabosky *et al.*, 2014) by providing the BEAST maximum clade credibility tree and the total species numbers across *Cardiodactylus* (Appendix S4). We used a gradient of prior

values ranging from 0.1 to 10 to test the sensitivity to the prior, to account for the recent debate on the reliability of BAMM estimates (Moore et al, 2016, but see Rabosky *et al.*, 2017). We ran BAMM by setting four independent MCMC running for 50 million generations and sampled every 50,000 generations; convergence was assessed by computing the ESS of log likelihoods. After removing 10% of trees as burn-in, the BAMM output were analyzed with employing BAMMtools package in R and the 95% credible rate shift configurations was estimated using Bayes factors. The best shift configuration with the highest maximum a posteriori probability was estimated in this analysis. Rates-through-time plots were generated for speciation (λ), extinction (μ) and net diversification (γ), either for all *Cardiodactylus* species or only for the SEA species. All visualization was performed using R and C code available through the R package BAMMtools.

Results

Data collection and phylogenetic analyses

The best-fit partition schemes and substitution models used in BI and ML analyses of the combined dataset are showed in Table 2. BI and ML phylogenetic analyses yielded robust and largely congruent topologies (for ML tree see Figure 1; for BI tree see Appendix S6;). In all analyses the subfamily Eneopterinae and the genus *Cardiodactylus* are recovered monophyletic with a high support (BS of 100%, PP of 1.0). All *Cardiodactylus* species are also recovered monophyletic, with the exception of *C. floresiensis*. However, additional analyses carried out under BI do not support the paraphyly of *C. floresiensis*: MCMC runs where *C. floresiensis* representatives are constrained to be monophyletic yield a harmonic mean estimate of -54171.49 versus -54167.57 for the unconstrained MCMC runs; hence the difference corresponds to a non-statistically significant B_F of 7.84 ($B_F < 10$; see Kass & Raftery, 1995).

Within the genus *Cardiodactylus*, the monophyly of the *Novaeguineae* species group is strongly supported in both analyses (BS of 100%, PP of 1.0). By contrast, the *Efordi* species

group is recovered paraphyletic in all analyses due to the placement of *C. javarere* and *C. enkraussi*. *Cardiodactylus javarere* is sister to the *Novaeguineae* species group, forming a well-supported clade (BS of 97%, PP of 1.0). *Cardiodactylus enkraussi* is sister to the (*C. javarere* + *Novaeguineae* species group) clade, but its position is less robust (BS of 83%, PP of 0.73). The remaining species of the *Efordi* species group constitute a well-supported clade (BS of 78% and PP of 0.96). Additional analyses carried out under BI support the paraphyly of the *Efordi* species group: MCMC runs where members of the *Efordi* species group are constrained to be monophyletic yield a harmonic mean estimate of -50162.07 versus -54167.57 for the unconstrained MCMC runs. Hence the difference corresponds to a statistically significant B_F of 8011.00 ($B_F > 10$; see Kass & Raftery, 1995).

Within the *Novaeguineae* species group, *C. haddocki* and *C. novaeguineae* form a robust clade (BS of 100%, PP of 1.0), sister to another well-supported clade (BS of 99%, PP of 1.0) comprising all the remaining species of the group. Within this clade, three species are the first lineages to branch off, sister to the remaining species of the group; these three species correspond to two species distributed in New Guinea (*C. lucus* and *C. maa'i*) and one in the Western Pacific (*C. tankara*). The remaining members of the species group form a well-supported clade (BS of 76%, PP of 0.97), which splits into two main clades (hereby referred as clade 1 and clade 2 on Figure 1). Clade 1 is well-supported (BS of 84%, PP of 1.0) and consists of lineages that are generally well-supported (BS comprised between 89% and 100%, PP comprised between 0.7 and 1.0). Clade 2 is only moderately supported, with a low BS of 40% and a moderate support under BI (PP of 0.91). Within clade 2, two main clades (clade 3 and clade 4) can be distinguished. All species belonging to the clade 4 are distributed in SEA, whereas only two species (*C. manus* and *C. quatei*), are distributed in SEA in the clade 3.

Divergence time estimation

BEAST analyses converged very rapidly and all parameters have ESS values > 200. The median ages derived from BEAST are presented in Figure 2 and Figure S15. Age estimates for the genus *Cardiodactylus* suggest an origin in the mid-Eocene 43.2 Ma (95% HPD: 33.47-52.93 Ma). Members from the *Efordi* species group started their diversification in the Late Eocene 40.9 Ma (95% HPD: 29.22-48.83 Ma), while members of the *Novaeguineae* species group started diversifying during the Late Oligocene 26.9 Ma (95% HPD: 20.39-33.67 Ma). The divergence time for the MRCA of *C. haddocki* and *C. novaeguineae*, which are sister to the remaining members of the *Novaeguineae* species group, is inferred at 8.4 Ma (95% HPD: 5.18-11.84 Ma), at the end of Miocene. Most speciation events within the *Novaeguineae* species group happened during the Miocene (*ca.* 23.0-5.3 Ma). Only a few lineages diverged recently (less than 4 Ma).

Ancestral area estimation

Model selections through LRT significantly support the DEC+j model over the DEC model for all tested dispersal multiplier matrices (see Appendix S2). Furthermore, the matrix of scaling factors involving five distinct rates implemented with a DEC+j model was supported as the best-fit model over more simplified sets of multipliers; the corresponding ancestral area evolution is presented in Figure 2. The corresponding inference of ancestral areas evolution suggests an origin of *Cardiodactylus* in Western Pacific during the Middle Eocene, followed by a complex and dynamic biogeographical history. Lineages belonging to the *Efordi* species group diverged earlier (*ca.* 40 Ma) than the *Novaeguineae* species group (*ca.* 27 Ma) and colonized New Guinea several times, once after 37.1 Ma (*C. niugini*), and once *ca.* 22.5 Ma (*C. busu* and *C. nobilis*). The members of the *Novaeguineae* species group colonized New Guinea three times from the Western Pacific, twice at the end of the Oligocene (*ca.* 24.0 Ma and 22.1 Ma) and once more recently with the divergence of *C. haddocki* and *C. novaeguineae* *ca.* 8.4 Ma. From New Guinea *Cardiodactylus* colonized SEA three times independently during

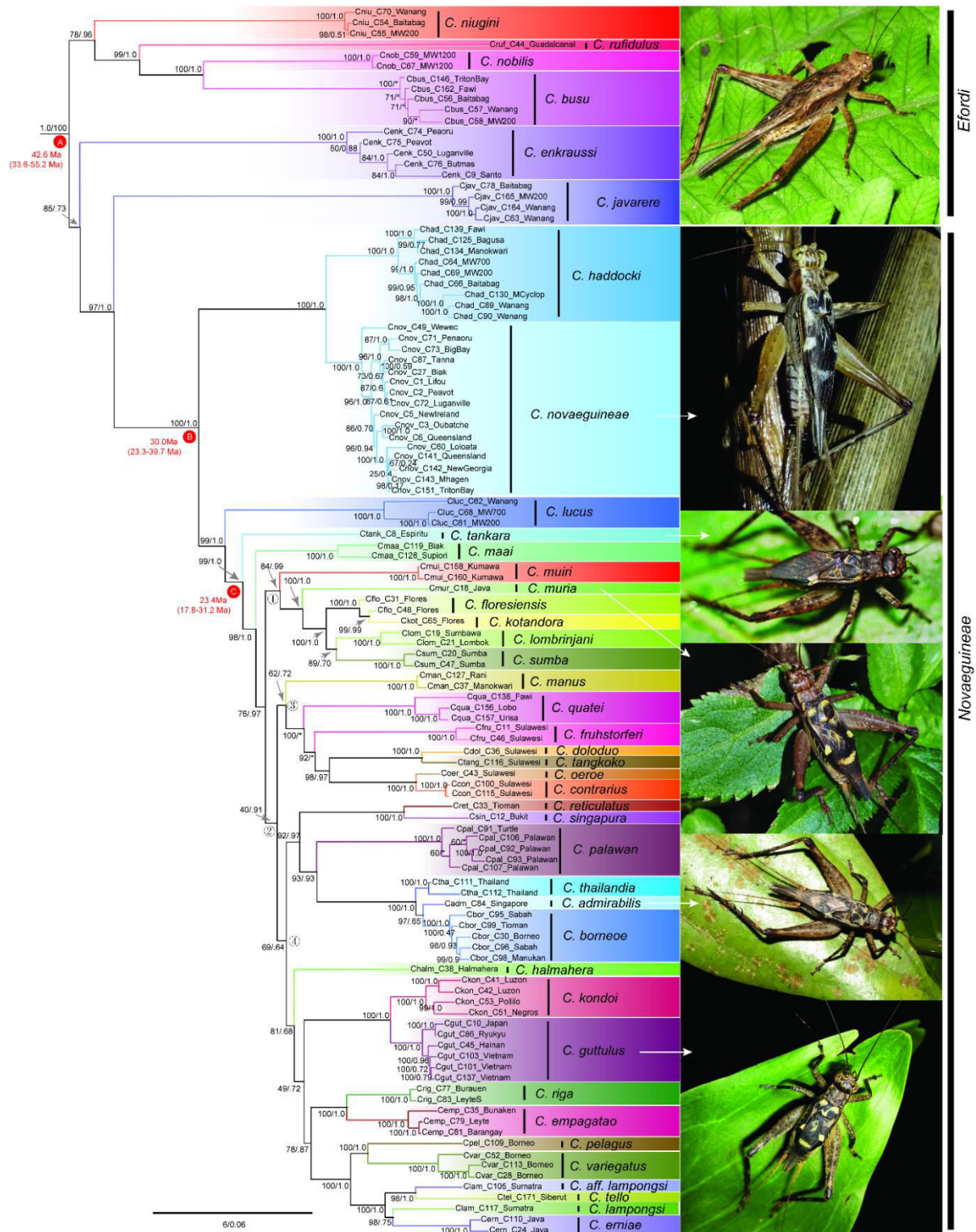


Figure 1. Phylogeny of the genus *Cardiodactylus* inferred from Maximum likelihood (ML) and Bayesian inference (BI). Numbers (** /**) next to nodes correspond to ML bootstrap scores (left of sign /, in percentage, values below 50% are discarded), and BI posterior probabilities (right of sign /, values below 0.5 are discarded). Signs (*) indicate topologies disparity between BI and ML, the topology in ML being represented. The bold red circles with letters indicate the secondary calibration points used in dating analysis; the circled numbers correspond to clades discussed in the text.

the Early Miocene (19.0-16.0 Ma), through three different passageways. The first one links New Guinea to SEA through the Philippines *ca.* 19 Ma; the second one through Sulawesi *ca.* 18 Ma, followed by colonization and recolonization events between *ca.* 18.0-16.0 Ma; the last one through Java *ca.* 16 Ma, which later allowed the colonization of the Lesser Sunda Islands. Through the first passageway, *Cardiodactylus* colonized Palawan through the Philippines, then the Thai-Malay Peninsula, Sulawesi and Borneo. From the Thai-Malay Peninsula, *Cardiodactylus* colonized Indochina twice, *ca.* 17 Ma and 4 Ma; meanwhile other lineages from the Thai-Malay Peninsula dispersed to Borneo *ca.* 3 Ma. From Sulawesi, *Cardiodactylus* dispersed to the Moluccas and the Philippines during the Late Miocene (11.6-5.3 Ma). From Borneo, the genus colonized Sumatra *ca.* 12 Ma and reached Java through Sumatra *ca.* 8 Ma.

Colonization routes, emigration / immigration events and in situ diversification

Colonization routes of the *Novaeguineae* species group are presented in Figure 3a. Before the Miocene only one route of colonization (linking Western Pacific and New Guinea) can be deduced from our biogeographical analyses. During the Miocene, 15 distinct colonization routes are inferred; interestingly the Philippines are recovered as a major biogeographical crossroad (five routes are inferred), followed by New Guinea (with three routes), Java (two routes), and Borneo, Sulawesi, Sumatra, the Thai-Malay Peninsula and Western Pacific (one route for each of them). Along these routes, two colonization events happened twice, i.e., from Western Pacific to New Guinea and from the Philippines to Sulawesi. During the Plio-Pleistocene eight colonization routes are identified; two depart from New Guinea and Sulawesi, and one from Borneo, Indochina, the Thai-Malay Peninsula and the Western Pacific.

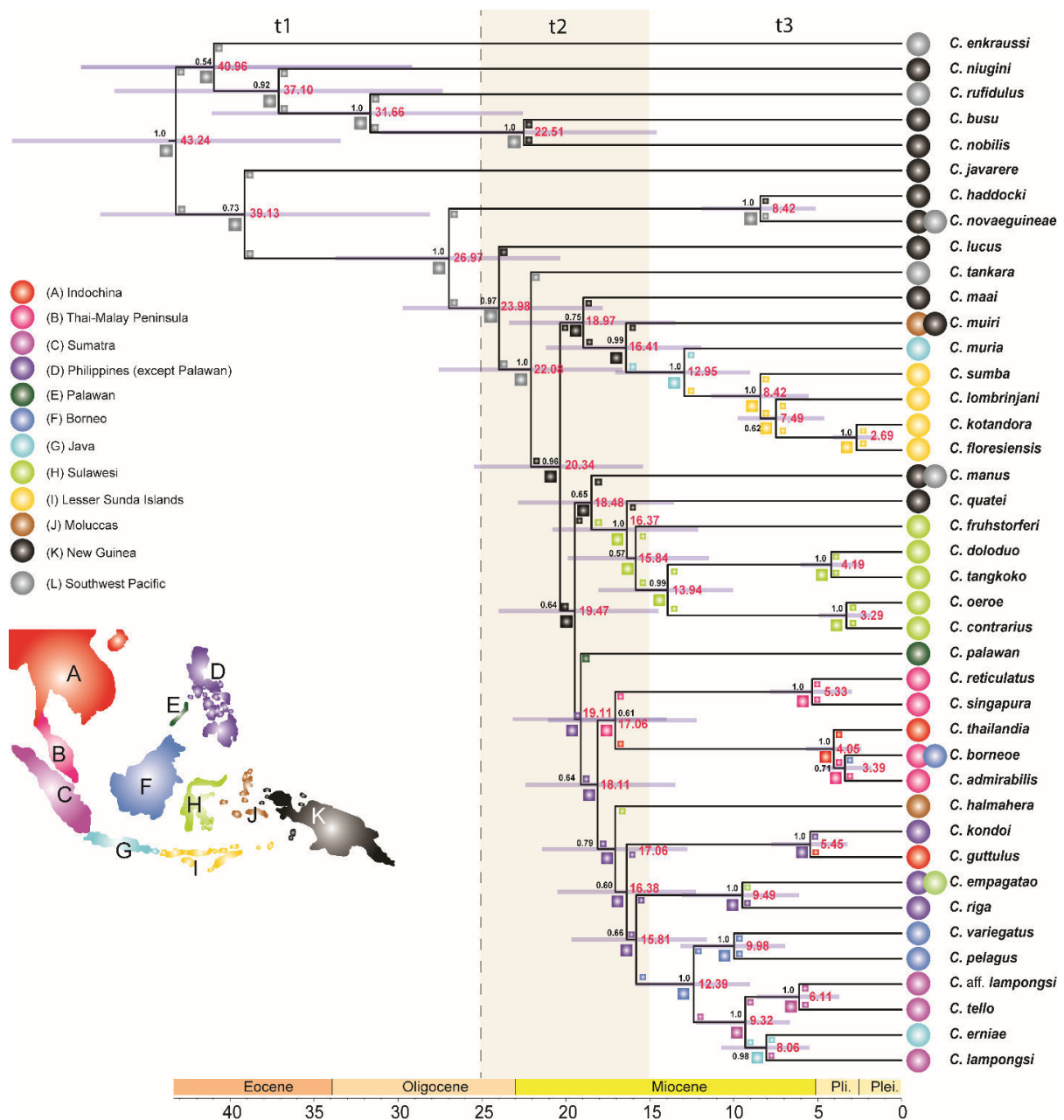


Figure 2. Reconstruction of historical biogeography for *Cardiodactylus* using a stratified dispersal-extinction-cladogenesis (DEC+j) model. The left panel and map represents the 12 areas implemented in the biogeographical model. Present-day distributions of each species are given at the tips by colored circles corresponding to colored areas on map; a colored square corresponding to the colored area on the map represents the inferred area(s) with the highest relative probability in the DEC+j analysis. The red numbers near each node correspond to the median age inferred in Beast analysis and the gray lines indicate its 95% height posterior distribution (HPD). The Bayesian posterior probabilities below 0.50 are not shown.

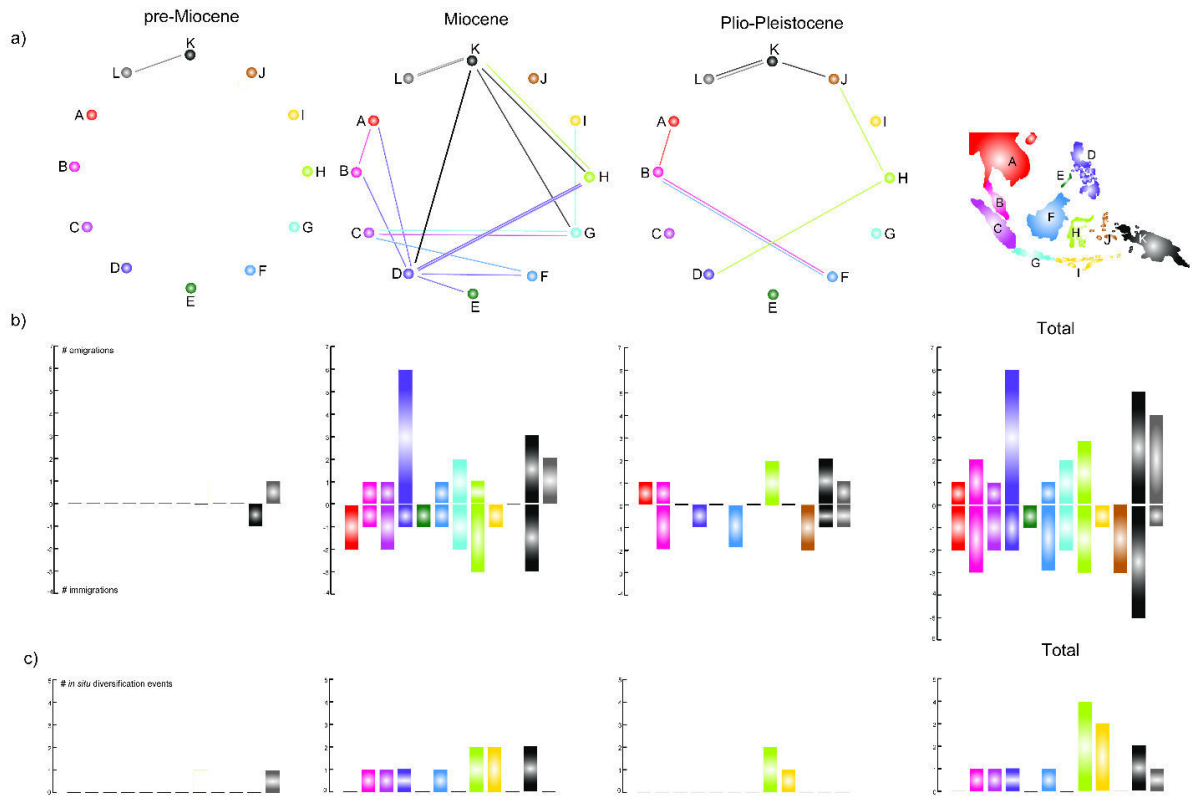


Figure 3. Colonization routes, numbers of emigration and immigration events and *in situ* diversification events inferred from ancestral area estimation (Fig. 2) for lineages of the *Novaeguineae* species group in pre-Miocene, Miocene and Plio-Pleistocene. a) Colonization routes; line colors correspond to inferred area of origin (same color code as in simplified map): multiple lines of the same color correspond to multiple colonization events. b) Number of emigration (positive bars) and immigration (negative bars) events inferred from colonization events (Fig. 3a) for each area in the pre-Miocene, Miocene and Plio-Pleistocene; column colors correspond to inferred areas as above. c) *In situ* diversification events inferred from ancestral area estimation for each area in the pre-Miocene, Miocene and Plio-Pleistocene.

Colonization routes, emigration / immigration events and in situ diversification

Colonization routes of the *Novaeguineae* species group are presented in Figure 3a. Before the Miocene only one route of colonization (linking Western Pacific and New Guinea) can be deduced from our biogeographical analyses. During the Miocene, 15 distinct colonization routes are inferred; interestingly the Philippines are recovered as a major biogeographical crossroad (five routes are inferred), followed by New Guinea (with three routes), Java (two routes), and Borneo, Sulawesi, Sumatra, the Thai-Malay Peninsula and Western Pacific (one route for each of them). Along these routes, two colonization events happened twice, i.e., from Western Pacific to New Guinea and from the Philippines to Sulawesi. During the Plio-Pleistocene eight colonization routes are identified; two depart from New Guinea and Sulawesi, and one from Borneo, Indochina, the Thai-Malay Peninsula and the Western Pacific.

Estimated emigration and immigration events through time are presented in Figure 3b. When summing the total numbers of events for each area through time, for three areas (Lesser Sunda Islands, the Moluccas and Palawan) only immigration events are inferred. In Java, Sulawesi and New Guinea the total number of emigration and immigration events is at equilibrium. For two areas (the Philippines and Western Pacific) the total number of reconstructed emigration events is higher than that of immigrations, while the opposite is recovered in all remaining areas (Borneo, Indochina, Sumatra and the Thai-Malay Peninsula).

In situ diversification events through time are presented in Figure 3c. When summing the total numbers of events for each area through time, Sulawesi is inferred to be the area with the highest number of *in situ* diversification events (four in total), followed by the Lesser Sunda Islands (three events), New Guinea (two events) and Borneo, the Philippines, Sumatra and the Thai-Malay Peninsula (one for each of them).

Diversification analyses

A *poissonRatePrior* of 0.5 was recovered as the best-fit prior by BAMMtools. Convergence of the MCMC chains in the BAMM analyses was observed after discarding the burn-in (ESS > 1000 for both the number of shifts and log likelihoods). The 95% credible set of rate shift configurations sampled with BAMM provides more support (probability of 0.69) for a scenario with no shift in diversification rates within the genus *Cardiodactylus* (Figure 4). BAMM analyses strongly support a diversity-dependent speciation process across *Cardiodactylus* genus with the following rates: 1) net diversification rate of 0.06 species/Myr (95% quantile=0.023-0.071), 2) speciation rate of 0.12 species/Myr (95% quantile=0.075-0.202) and 3) extinction rate of 0.06 species/Myr (95% quantile=0.003-0.170). Rate-through-time plots for the three corresponding rates are presented in Figure 5, for the whole genus and within the *Novaeguineae* species group. The resulting plots indicate that *Cardiodactylus* speciation and net diversification rates consistently decreased through time while extinction rates remained constant. A similar trend was recovered the *Novaeguineae* species group.

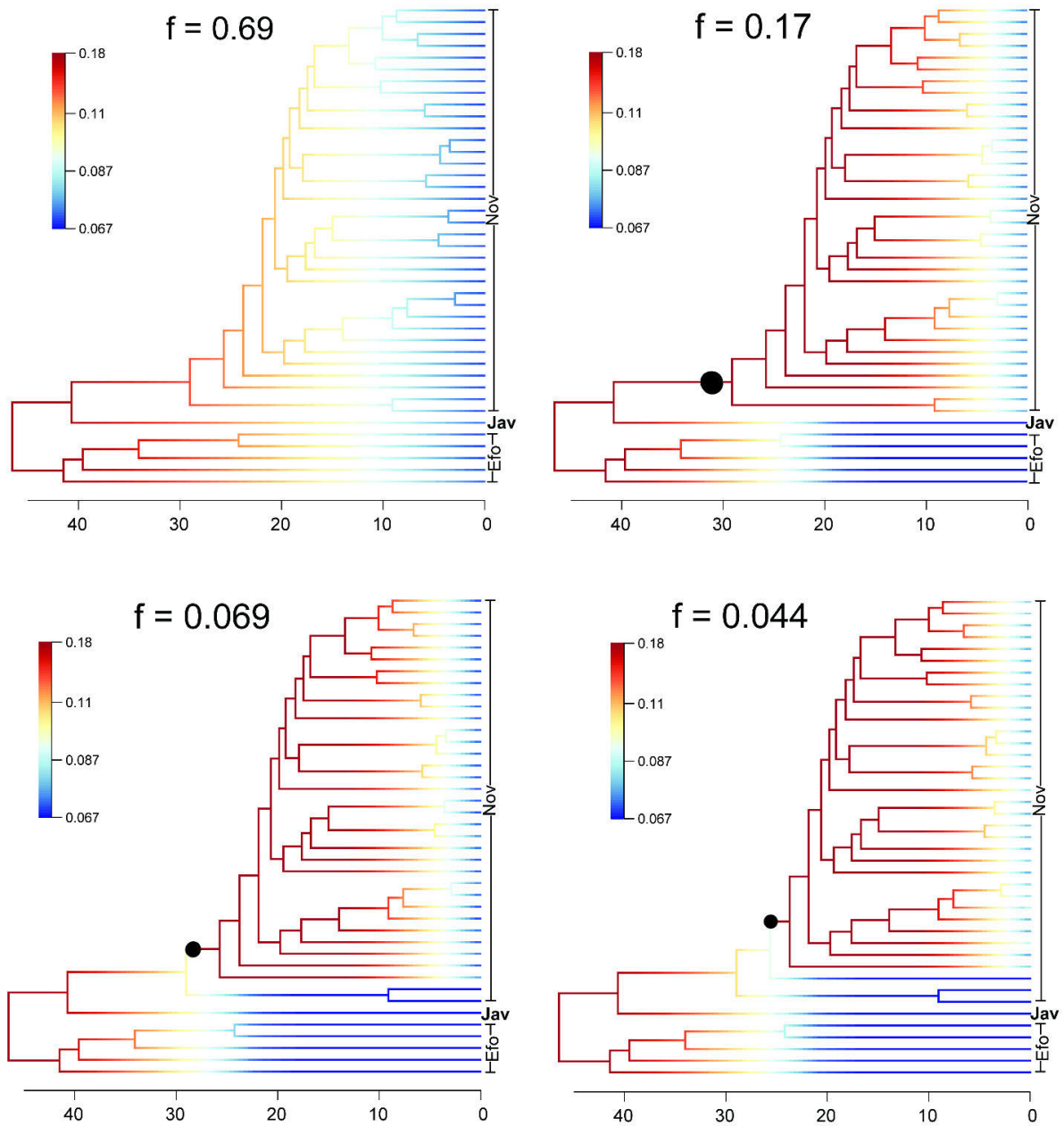
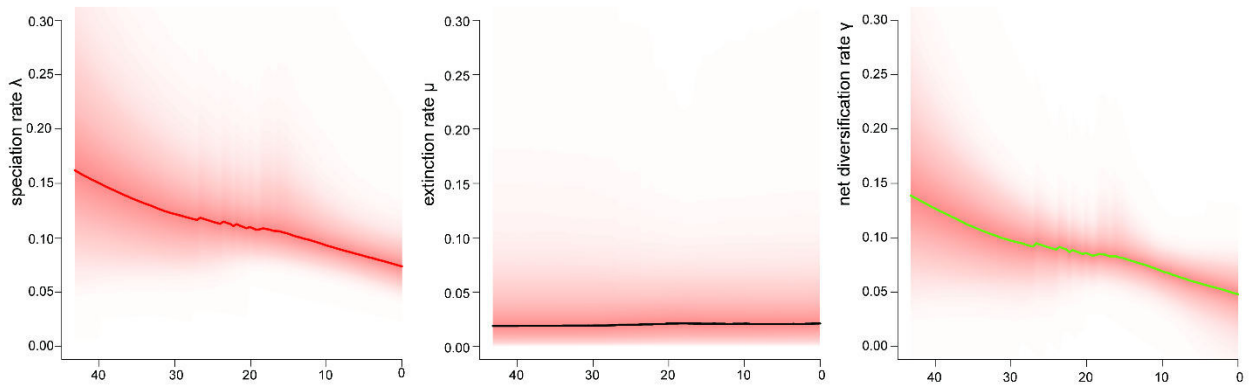


Figure 4. Rate shift configurations with the four highest posterior probabilities from the 95% credible set within *Cardiodactylus*. The tree topology referred to Figure 2. The colored histogram indicates the speciation (λ), the color gradient corresponds the speciation rate.

a) Rates-through-time analysis of speciation (λ), extinction (μ) and net diversification (γ) within genus *Cardiodactylus*



b) Rates-through-time analysis of speciation (λ), extinction (μ) and net diversification (γ) in *Novaeguineae* species group

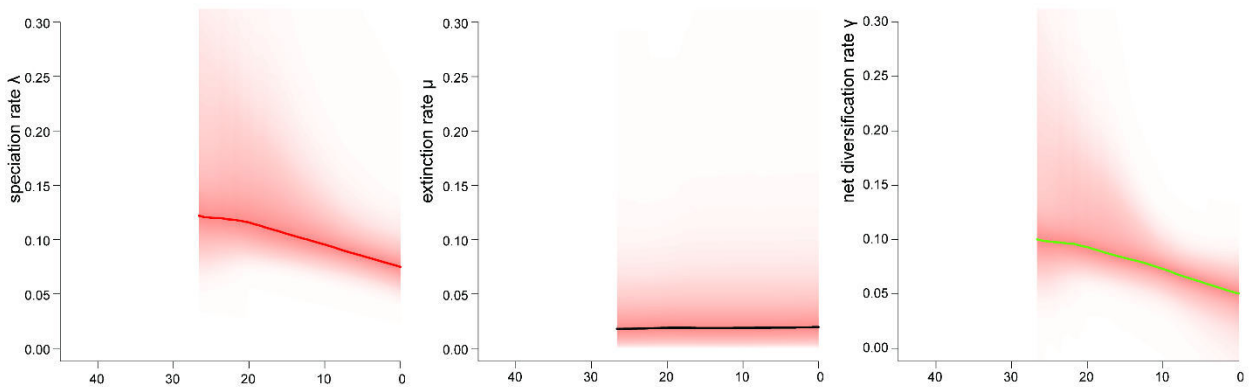


Figure 5. Rates-through-time analysis of speciation (λ), extinction (μ) and net diversification (γ) in *Cardiodactylus* (a) and within *Novaeguineae* species group (b).

Discussion

Phylogenetic relationships

Both BI and ML analyses recovered a similar placement of the genus *Cardiodactylus* within the subfamily Eneopterinae in the tribe Lebinthini (Figure 1), as in previous phylogenetic studies (Anso *et al.*, 2016; Nattier *et al.*, 2011; Robillard & Desutter-Grandcolas, 2005, 2006; Vicente *et al.*, 2017). Within *Cardiodactylus*, we were able to assess the status of the two species groups defined on morphological grounds by Otte (2007a).

Our analyses clearly support the monophyly of the species group *Novaeguineae* and the paraphyly of the *Efordi* species group. The paraphyly of the latter can be accounted for by the placement of two species: *C. javarere* and *C. enkraussi*. When using B_F we found out that the placement of *C. enkraussi* outside of the *Efordi* species group is not statistically supported whereas the opposite holds true for *C. javarere*. Interestingly *C. javarere*, along with another species, *C. kolombangara*, which is not included in our analyses, show combinations of characters that are intermediate between the *Novaeguineae* and *Efordi* species groups (Dong and Robillard, 2016). This provides some support to create a third species group which would include at least *C. javarere* and *C. kolombangara*. Further molecular analyses with a denser sampling will be likely necessary to reach a stable conclusion. Within the *Novaeguineae* species group, our results strongly support a clade grouping *C. novaeguineae* and *C. haddocki* as the sister group of all remaining species of the group. When comparing results of BI and ML analyses, similar relationships are inferred within the species group, except for the positions of *C. manus* and *C. quatei*, perhaps as a consequence of missing taxa from New Guinea and the Western Pacific. Nevertheless, the species distributed in SEA are clearly nested within a clade including species from New Guinea and the Western Pacific.

Origin of *Cardiodactylus* in the Western Pacific

Our study estimated the divergence time and ancestral areas of *Cardiodactylus* by implementing secondary calibrations based on the study of Vicente *et al.* (2017), which inferred that *Cardiodactylus* diverged from its sister clade within the tribe Lebinthini in SEA. As a result, similar age estimates for the MRCA of *Cardiodactylus* were recovered: 43.2 Ma in our study vs. 42.57 Ma in the study of Vicente *et al.* (2016).

Both DEC and DEC+j analyses infer an origin for *Cardiodactylus* in the Western Pacific. It seems to conflict with the widely accepted assertion that less diverse island communities are easier to invade. However, when we took overall comprehension of the biogeographical history for the tribe Lebinthini in the study of Vicente *et al.* (2017), *Cardiodactylus* diverged from its sister clade in SEA before colonizing the islands of the Western Pacific during the Mid-Eocene. During that time, species richness in SEA was much higher than that of the Western Pacific, since many islands in this region did not emerge yet (i.e. the Solomon and Fiji Islands) or were just formed with coral reef (i.e. New Caledonia) (Neall, 2008). In fact, the origin of *Cardiodactylus* in Western Pacific (island) and later recolonized SEA (continent) testified the “reverse colonization” (from island to continent) in Bellemain & Ricklefs (2008).

New Guinea as a transition zone between the Western Pacific and SEA

During the period ranging from its origination during the Mid-Eocene to the collision between Australia and Asia (*ca.* 25 Ma), there is no evidence that *Cardiodactylus* colonized any area other than New Guinea (Figure 2). After the emergence of the Bird’s Head region of New Guinea (*ca.* 25 Ma), our results inferred colonization events from the Western Pacific to New Guinea. New Guinea thus appeared to have been a significant passageway between the Western Pacific and SEA, even if this aspect of the biogeography of *Cardiodactylus* is likely not yet addressed with a sufficient taxonomic sampling.

Three independent colonizations of SEA from New Guinea were inferred before the emergence of the Wallacea (*ca.* 15 Ma). This result is consistent with the dispersal route

documented in flightless beetles which crossed Lydekker's line from New Guinea and reached the Moluccas ca. 5 Ma (Tänzler *et al.*, 2016). *Cardiodactylus* initially departed from New Guinea and colonized SEA by crossing the Wallace line, which is a good example of faunal boundaries permeation between Wallace's line and Lydekker's line (Lohman *et al.*, 2011).

Colonization routes from east to west in SEA and priority of adjacent area in colonization routes

Cardiodactylus colonized SEA three times independently from east to west across New Guinea, through three different passageways: the Philippines (ca. 19.1 Ma), Sulawesi (ca. 18.5 Ma), and Java (ca. 16.4 Ma), followed by dispersal to Moluccas.

During the early Miocene (ca. 19 Ma), the Philippines (minus Palawan), a true oceanic island archipelago, began to emerge, initially scattered along the margin of the Philippine Sea Plate next to the Asian Plate (Yumul *et al.*, 2004). *Cardiodactylus* colonized the Philippines early after they started emerging (ca. 19.1 Ma). From the Philippines, the genus it rapidly dispersed the rest of the region, first in Palawan, the Thai-Malay Peninsula and in Borneo, from where it dispersed toward Sumatra and Java. It also recolonized the continent multiple times, either through Indochina and the Thai-Malay Peninsula. It also colonized Sulawesi twice, first ca. 17 Ma, then during the Late Miocene ca. 9.5 Ma.

The colonization of Sulawesi ca. 18.5 Ma was followed by subsequent *in situ* diversification of this genus on this island, which is consistent with the "predominantly tectonic dispersal origin" of 20 taxa (27 individual datasets, including vertebrate and invertebrate) in Sulawesi as shown by the review of Stelbrink *et al.* (2012). In the study, Stelbrink *et al.* inferred that speciation on Sulawesi did not occur before the Miocene, which consistent with geological evidence for more extensive land on the island from that time.

During the Late Miocene, the colonization events by *Cardiodactylus* occurred less frequently and were only confined to adjacent areas: between the Thai-Malay Peninsula and Indochina, between Java and Sumatra, Philippines and Sulawesi, and Philippines and

Indochina (Figure 2 and Figure 3a). However, the frequent transgressing events crossing the Wallace's line (or modified Huxley's line) were not recovered in *Cardiodactylus*. These results are consistent with the conclusions of the study of flightless weevils's biogeographical history (Tänzler *et al.*, 2016), which proved that the Sunda Arc (region including Borneo, Java and Sumatra) could be a potential dispersal corridor between mainland Southeast Asia and Melanesia, in result of its continuous chain of islands.

De Bruyn *et al.* (2014), which inferred that colonization events happened more frequently between adjacent areas than between distant ones. This conclusion could however be tempered by the fact that the probabilities of transition amongst our areas were set proportionally with the distance between them.

Weak influence of recent sea level changes on *Cardiodactylus* diversification dynamics

The drastic changes of sea level during the Plio-Pleistocene have been considered a greater influence on species diversification in SEA, resulting in the fragmentation of areas when the sea level rose and the increasing landmass area when the sea level decreased (Guo *et al.*, 2015; Lohman *et al.*, 2011). Interestingly, colonization events of *Cardiodactylus* that happened during Plio-Pleistocene are far less common than expected. These recent events only happened from Indochina to the Thai-Malay Peninsula and from the Thai-Malay Peninsula to Borneo but did not generate high diversity (Figure 2 and 3a).

These results are consistent with the Thai-Malay Peninsular's biogeographic crossroad position between the Indochina and Sundaic faunal regions (region including Borneo, Java and Sumatra) (Lohman *et al.*, 2011). One interesting point in our results is the absence of immigrations of *Cardiodactylus* in Java and Sumatra (Figure 3b in Plio-Pleistocene), which differs from the conclusion of de Bruyn *et al.* (2014), where these two areas were characterized by the higher levels of immigration than emigration. This is related with the relative low diversity of the genus in these islands, which can in turn be linked with

missing knowledge of the species distributed in large areas of Sumatra and Java could not be sampled.

Dynamics of diversification through the history of *Cardiodactylus*

Our diversification analysis indicated that there was no distinct rate shift within *Cardiodactylus* and that the speciation rate of the genus changed slowly through time (Figure 4 and 5). However, when we focus on the diversity in each area of SEA, each lineage of *Cardiodactylus* shows a contrasted history.

According to the meta-analysis implemented by de Bruyn *et al.* (2014), the diversity of SEA biota may have arisen through the accumulation of immigrants, by *in situ* diversification, or some combination of the two. In our results (Figure 3b and 3c), the highest level of immigration occurred in Sulawesi, as well as Thai-Malay Peninsula, Borneo and Moluccas. They are followed by Indochina, Sumatra, the Philippines and Java. Palawan and Lesser Sunda Islands are characterized by similar low amounts of immigration.

The areas are however characterized by significantly different levels of *in situ* diversification. There was no event of *in situ* diversification in Indochina, Palawan, Java and Moluccas. One event of *in situ* diversification occurred in Thai-Malay Peninsula, Sumatra, the Philippines and Borneo. Sulawesi and the Lesser Sunda islands show the highest level of *in situ* diversification. The early colonization of Sulawesi from New Guinea and the fragmentation of the island from Miocene to the present may explain that level of *in situ* diversification in Sulawesi is larger compared with other areas. This result is consistent with the results of de Bruyn *et al.* (2014), who showed that the level of *in situ* diversification in ancient area (originating area) was higher than in others. However, the higher level of *in situ* diversification found in Lesser Sunda Islands contrasts with the conclusion of de Bruyn *et al.* (2014): The Lesser Sunda Islands are relatively small and recent islands that emerged during the Mid-Miocene. They partly come from microcontinental fragments sliced from Java or/and Australia, while some parts of these islands emerged truly from the ocean. During their history,

connection and disconnection between microcontinental fragments were very common (Hall, 2009). We sampled four different species from this small area: *C. lombrinjani* in Sumbawa and Lombok islands, *C. kotandora* and *C. floresiensis* in Flores island, and *C. sumba* in Sumba island. During the Late Miocene, *Cardiodactylus* colonized Sumba from Java, then dispersed to Sumbawa-Lombok, and at the Plio-Pleistocene in Flores. Linking the complex geological history in the Lesser Sunda Islands easily explains the species diversify found in this area despite its recent age and size. Our results confirm that higher levels of species diversification can be driven by frequent connection and disconnection between two areas at least in some parts of the genus distribution. However, we cannot exclude the hypothesis that this level of *in situ* diversification could possibly be linked to the fact that extensive field work has been done recently in this archipelago (Robillard *et al.*, 2014). Similarly, the low level of *in situ* diversification recovered in large islands colonized almost as early as Sulawesi, such as Java and Borneo, could partly be explained by biases in taxonomic sampling in these areas.

To summarize, the diversity of *Cardiodactylus* seems due to different factors in the different areas: it resulted through the accumulation of immigrants in Indochina, Palawan, Java and Moluccas; while in Thai-Malay Peninsula, Sumatra, Philippines, Borneo, Sulawesi and Lesser Sunda Islands, it resulted by the combination of both the accumulation of immigrants and *in situ* diversification. In the latter situation, the main factors for diversity in Sulawesi and Lesser Sunda Islands were *in situ* diversification events, while the main cause of diversify was the accumulation of immigrants in the remaining areas.

Conclusion

In summary, historical biogeography of *Cardiodactylus* is clearly linked to the early geological history of SEA when most of the current diversify of the genus originated. After originating from the Western Pacific (divergence from its sister group among the Lebinthini), *Cardiodactylus* crossed Lydekker's line multiple times, but more rarely transgressed the Wallace's line (or modified Huxley's line). New Guinea was the diversity hotspot of

Cardiodactylus in SEA, which was colonized three times independently by members of the *Novaeguineae* species group and once by one species of the *Efordi* species group. In addition, the Philippines were the major colonization sources of *Cardiodactylus* in SEA from east to west, and Thai-Malay Peninsula was an important transition zone between the Indochina and Borneo.

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II.4. Chapter Conclusion

In this chapter, I revised the geological history in the Southeast Asia, reconstructed the phylogeny of the genus *Cardiodactylus*, inferred its biogeographical history and discussed its dynamic diversification pattern in this region. The results of the phylogenetic analysis confirm the monophyly of the genus and of most of the species. The results also confirm the monophyly of the *Novaeguineae* species group, but not that of the Efordi species group; instead, they support the hypothesis of a potential third species group, at least including *Cardiodactylus javarere* and *C. kolombangara* as hypothesized based on morphology (see Chapter I). However, more information including morphological and molecular data for as many species as possible are needed to confirm this hypothesis. In turn, it is necessary to introduce more efficient method to obtain more phylogenetic information (see Chapter III). The results of biogeographical analysis of *Cardiodactylus* suggested the genus originated from the Western Pacific and colonized SEA via New Guinea several times through three different passageways, which is consistent with the geological history event in this region through time. However, the precise biogeographical history and diversity pattern of *Cardiodactylus* on New Guinea could not be detected because of missing data on the island. It would be the direction of New Guinea's transition role on the biogeographical study about the permeable faunal boundaries between Wallace's line and Lydekker's line.

CHAPTER III

Informative Mitogenomes for the Taxonomy and Systematics of Crickets

III.1. Chapter Introduction

Taxonomy and systematics provide the names and evolutionary framework for any biological study. Without these basic information, there is no access to the biological context where the evolutionary processes have given rise to a specific taxon: e.g. close relatives and sister species (hybridization), more distantly related taxa (ancestral states) (Koch & German, 2013). The methods used in taxonomy and systematics are numerous and varied, but they generally concern two main aspects, morphological and molecular technologies (Hillis *et al.*, 1996). Compare with the painstaking and time-consuming labor of morphological methods on observing similarities among organisms and reconstructing classifications according to those similarities, molecular methods (DNA sequencing) appear more efficient despite their inherent limitations. As such, they have been widely used since the beginning of the PCR revolution in the late 1980s (Crampton-Platt *et al.*, 2016). Except for fossils and when dealing with extinct species or rarely collected ones, for which the molecular data are unavailable, using molecular data can be applied to easily identify species, for example in polymorphic butterflies (Montagna *et al.*, 2016; White *et al.*, 2014) and stick insects (Forbes *et al.*, 2017). Molecular markers have revolutionized the field of phylogenetic reconstruction (Barton *et al.*, 2007; Singh, 2015). In this context, complete mitochondrial genomes (mitogenomes) is a convenient option for phylogenetic reconstruction for several reasons: 1) due to the high number of copies of this genome and the availability of conserved sets of primers, it is relatively easy to obtain mitochondrial molecular markers; 2) although variable enough to be used for barcoding initiatives, the mitochondrial genes are characterized by diverse rates of evolution, which make them informative markers for phylogenetic reconstruction at several taxonomic scales (Crampton-Platt *et al.*, 2015, 2016; Timmermans *et al.*, 2010).

Recently, studies using complete mitochondrial genomes have started being applied widely in the field of taxonomy, systematics, biodiversity and phylogeny (Dupuis *et al.*, 2012). On one hand, complete mitochondrial genomes provide more phylogenetic information than single markers (or small multi-locus markers). On the other hand, despite mitochondrial

genomes are becoming easy to obtain in virtue of technological advances, their use has remained limited in many research communities, for it may represent higher costs and time-consuming protocols of data acquisition. It is thus necessary to develop technical grounds to obtain information from mitogenomes in order to facilitate the use of this kind of data.

In this chapter, I first review the information available about ensiferan complete mitochondrial genomes and their use in phylogenetic reconstruction and dating analyses (section III.2). Next, I briefly review sequencing technics used to access complete mitochondrial genomes and explain the principles and processes for obtaining complete mitochondrial genomes developed in this thesis (section III.3). Finally, I reconstruct the first complete mitochondrial genome for the subfamily Eneopterinae in the species *Cardiodactylus muiri* Otte, 2007 and investigate its comparison within the cricket family Gryllidae (section III.4).

III.2. Introduction to ensiferan mitochondrial genome

The mitochondrial genome (mitogenome) of ensiferans, like typical insect mitogenomes, is a closed-circular, double-stranded molecule about 16 kb in size (15-18 kb in insects, Cameron, 2014a). It consists of 37 genes and one major noncoding region (Boore, 1999; Saito *et al.*, 2005; Yang *et al.*, 2016b; Zhang *et al.*, 2016). The 37 genes comprise 13 protein-coding genes (PCGs), which form part of the electron transport chain, and two ribosomal RNA (rRNA) genes and 22 transfer RNA (tRNA) genes, which are responsible for translating the mitochondrial PCGs (Osigus *et al.*, 2013). The major noncoding region, also known as the A+T-rich region because of its high content of Adenine and Thymine, has a role in the control of replication and transcription; as such it is also named control region (CR) (Simon *et al.*, 1994). Mitochondrial genes are transcribed on both strands; the strand including most genes is termed the “majority” strand and the other one the “minority” strand (Cameron, 2014b). In addition, insect mitogenomes are characterized by disproportions in base compositions: there is usually more Adenine than Thymine and more Cytosine than Guanine on the majority strand (Wei *et al.*, 2010). Besides strand asymmetry in nucleotide composition,

the arrangement of genes (e.g., deletions, genes order) within the mitogenome are highly variable in insects (Bernt *et al.*, 2013b; Borre & Brown, 1998; Borre *et al.*, 1998; Cameron, 2014a, b; Zhang & Hewitt, 1997). For example, the gene *trnP* is missing in Coleoptera (Timmermans & Vogler, 2012), and tRNAs and PCGs are rearranged in Hymenoptera (Song *et al.*, 2016; Wei *et al.*, 2014).

In Orthoptera, the mitogenome organization follows the putative ancestral insect mitogenome arrangement, which is presented in Figure III.1. Rare exceptions of rearrangement have been found: only a *D-K* (*D*: tRNA-Asp, *K*: tRNA-Lys) rearrangement in the lineage Acridomorpha (Flook & Rowell, 1995), inversion of the gene cluster *M-I-Q* (*M*: tRNA-Met, *I*: tRNA-Ile, *Q*: tRNA-Gln) in *Phyllomimus detersus* (Yang *et al.*, 2016a), and occasional inversion of *W* (tRNA-Trp) in the migratory locust (Ma *et al.*, 2012). Only few Orthopteran lineages have however been investigated in these respects (see below), which renders an attempt of generalization highly speculative.

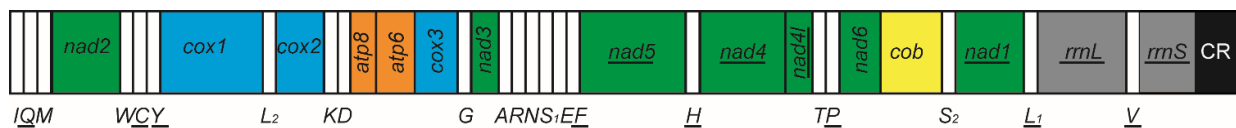


Figure III.1. Putative ancestral insect mitogenome arrangement, linearized between the control region (CR) and tRNA-Ile. The length of each gene is approximately proportional to its DNA length. Protein-coding genes are color-coded by OXPHOS complex (*cox*: blue; *nad*: green; *atp*: orange; *cob*: yellow); tRNAs: white; rRNAs: gray; and control region: black. Gene names correspond to the standard abbreviations used in this thesis; tRNA genes are indicated by the single letter IUPAC-IUB abbreviation for their corresponding amino acid; genes transcribed on the minority strand are underlined. Modified from Simon, 2014a, b.

Except for mitogenome arrangement order, single gene structure and function have been considered as useful sources of phylogenetic information and different types of mitochondrial DNA markers are available for phylogenetic analyses (Zhang & Hewitt, 1997). For example, cytochrome c oxidase subunit I (*cox1*) portions, and sometimes the two ribosomal RNA (*rrnS* and *rrnL*), have become powerful tools for inferring low-scale

phylogenetic relationships, investigating biodiversity and differentiating cryptic species (e.g. Crampton-Platt *et al.*, 2015; Muhammad Tahir & Akhtar, 2016). However, the inappropriate choice of single mitochondrial markers can bias the results of phylogenetic reconstruction, due to contrasting rates of substitution in different genes, e.g., *rnrS* is highly conserved in insects and used to study genetic diversity at higher level, but *rnrL* is often used for studies at low and intermediate levels such as in families or genera (Ferri *et al.*, 2009; Zhao *et al.*, 2010). Similarly, the barcoding marker, *cox1* is not appropriate when it comes to deep divergences in Orthoptera (Hawiltschek *et al.*, 2017, Taylor & Harris, 2012).

Considering the potential effect of nuclear copies of mitochondrial genes, or pseudogenes (numts), the complete mitogenomes in Orthoptera contain more accurate phylogenetic information than single or multi-locus data. They have been considered as a powerful source of data to reconstruct robust phylogenies with strong branch supports (Boore *et al.*, 2005; Fenn *et al.*, 2008; Zhou *et al.*, 2010).

Song *et al.* (2015) established a robust skeleton framework for the phylogeny of Orthoptera based on mitogenomes and four nuclear loci. He estimated the divergence time of the order around 250 Myr, based on fossil calibration of the molecular tree. The ensiferan phylogeny was similarly reconstructed based on complete mitogenome data in Song *et al.* (2015), but the deep-level relationships are not yet strongly supported, probably because of the insufficient number of mitogenomes available. More recently, Zhou *et al.* (2017) inferred a higher-level ensiferan phylogeny using only complete mitogenomes. However, compared with the large amount of extant species in Orthoptera (more than 20,000, Grimaldi & Engel, 2005; Tree of Life web project: <http://tolweb.org/Orthoptera>), the complete mitogenome datasets are still insufficient: only 140 complete orthopteran complete mitogenomes are represented in GenBank, among which only 61 belong to Ensifera. This limited amount of data demonstrates that efficient and cheap methods of sequencing mitogenomes are imminently demanded. Therefore, the sequencing methods available to obtain mitogenomes will be introduced in the next section, and the method used in this thesis is highlighted.

III.3. Methods to assemble complete mitogenomes with next-generation sequencing technologies

For the past three decades, mitogenome sequencing technologies have improved rapidly since the first insect mitogenome, *Drosophila yakuba*, obtained in 1985 (Boore *et al.*, 2005; Clary & Wolstenholme, 1985; Goodwin *et al.*, 2016). The initial methods of sequencing mitogenomes required purified mitochondrial DNA and consisted of sequencing cloned libraries, which was highly labor and time consuming (Clary *et al.*, 1982; Clary & Wolstenholme, 1985). With the advent of polymerase chain reaction and the access of primers for the amplification of mitogenomes, the process of purifying mt DNA from nuclear DNA and other plastid DNA was replaced by extraction of the genomic DNA (Kambhampati & Smith, 1995; Simon *et al.*, 1994).

To reduce risk of amplifying numts amplicon, long-PCR plus primer walking, whose main procedure is to amplify long amplicons spanning multiple genes of mitogenome and then design primer and sequencing long amplicons via Sanger sequencing, became prevalent before the appearance of next-generation sequencing (NGS) (Song *et al.*, 2008; Zhou *et al.*, 2008). With the development of NGS, the simplest approach which involves processing long-PCR amplicons removes the need for primer walking (Figure III.2). Therefore, the labor intensity of long-PCR plus NGS is lower but with far higher efficiency (Jia *et al.*, 2014). Beside of two above methods, RNA sequencing (RNAseq) plus gap filling and direct shotgun sequencing are also implemented in sequencing of insect mitogenomes (Cameron, 2014b). Overall consideration, the advantages and disadvantages of four viable insect mitogenomes sequencing approaches in terms of cost, speed, reliability and applicability to difficult templates are presented in Table III.1. The two “long-PCR” methods are widely used in Insecta and the long-PCR plus NGS has been considered as a promising approach to obtain complete mitogenomes efficiently (Song *et al.*, 2015; Yang *et al.*, 2016a, b; Zhou *et al.*, 2017).

TABLE III.1. Advantages and disadvantages of different mitogenome sequencing methods. The speed and cost of these different methods is relative among themselves. Modified from Cameron, 2014b.

	Long-PCR plus primer walking	Long-PCR plus NGS	RNAseq plus gap filling	Direct shotgun sequencing
Speed	Slow	Fast	Fast	Very fast
Cost	Moderate	Low	High	High
Acceptable template quality	Broad, ethanol or dried specimens successful	Broad, ethanol or dried specimens successful	Narrow, RNA extracts needed	Broad, ethanol or dried specimens successful
Ease of laboratory procedures	Very easy, standard PCR methods	Moderate, NGS template pre/library indexing	High, RNA extraction and sequencing	Moderate, NGS template pre/library indexing
Multiplexing	No	Yes	No	Yes
Specialized equipment	None	NGS platform	NGS platform and RNA extraction facilities	NGS platform
Assembly complexity	Low, any contig assembly software	Low, any contig assembly software	High, <i>de novo</i> transcriptome assembly required	High, <i>de novo</i> genome assembly required
Specialized requirement on computer	Normal speed	Normal speed	High speed	High speed

In this thesis, we employed the method of long-PCR plus NGS on an Ion Torrent Personal Genome Machine (PGM) to sequence mitogenomes (Dettai *et al.*, 2012; Hinsinger *et al.*, 2015) (see more details of the procedure in Figures III.3). With a few general recommendations in Gryllidae – only one complete mitogenome in Gryllidae, *Teleogryllus emma* (EU557269), recorded in GenBank before 2014, we designed three pairs of primers for long-PCR referring to other mitogenomes in Gryllidae (see details about the primers in section III.4, Table 1). Therefore, the complete mitogenomes was divided into three fragments with 500 to 1200 bp overlaps between each fragment. The long-PCR amplicons of each fragment were then respectively added into the bank of chip to sequence on PGM after checking length and concentration of amplification products. Each bank contained multiplex long-PCR fragments of mitogenomes involves in distantly-related species, e.g. mitogenome fragments from fish, different insect order and plants were mixed into one bank, in order to reduce the cost of individual sequencing. Unpaired reads obtained from NGS were checked in quality, and then trimmed and assembled to species-specific long contigs using reference gene in each fragment (Table III.2). Finally, the complete mitogenome was assembled from long contigs

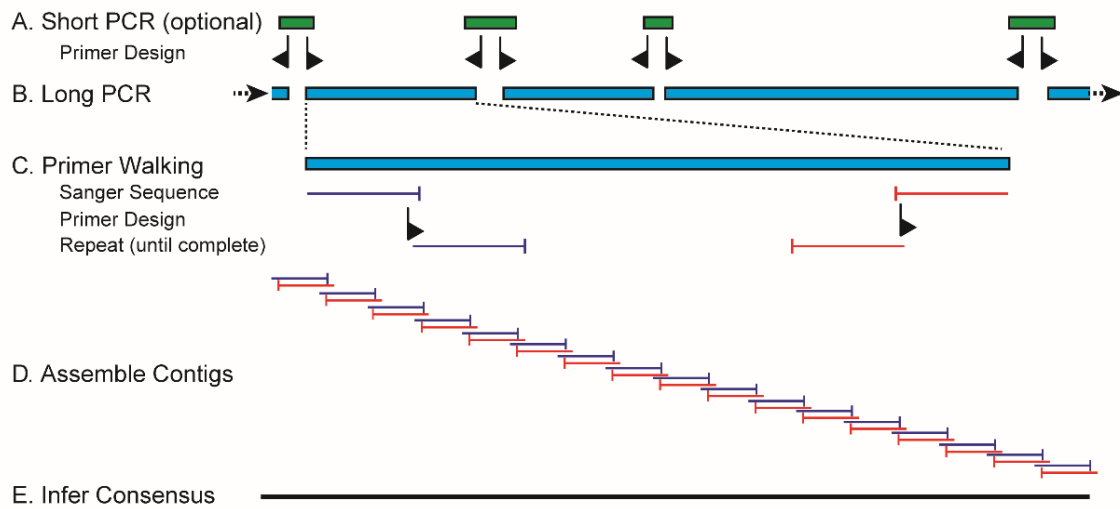
using *de novo* assembly in coverage and quality. All subsequent operations involving quality checking, trimming and assembly are applied in Geneious (Kearse *et al.*, 2012).

This method is more affordable and confident with high-coverage. It also reduces the risks of sequencing numts. In the next section, the first complete mitogenome of *Cardiodactylus* species was sequenced and described combining taxonomic revision and mitogenome comparison with other species in Gryllidae.

TABLE III.2. Genes distribution in the three long-PCR fragments of complete mitogenomes defined in this study.

Names of fragments	Genes
SET-1	<i>nd2, cox1, cox2, atp8, atp6, cox3, nd3,</i>
SET-2	<i>nd5, nd4, nd4l, nd6, cytb, nd1</i>
SET-3	<i>rrnL, rrnS,</i>

Method 1: Long PCR plus Primer Walking



Method 2: Long PCR plus Next-Gen Sequencing

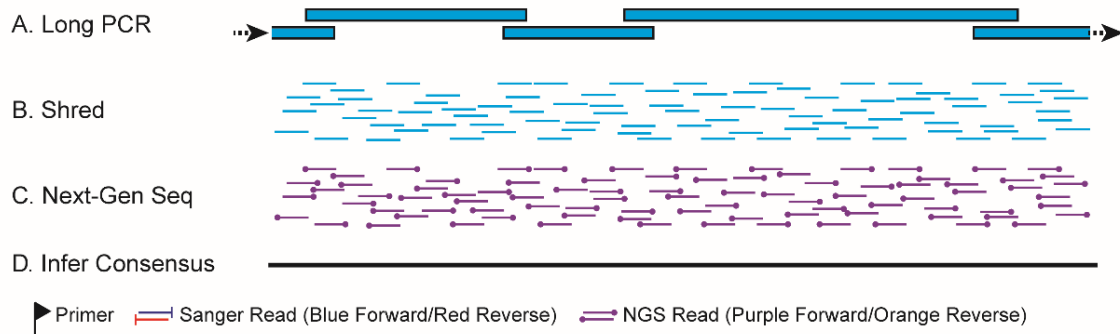


Figure III.2. Mitogenome sequencing procedures using long-PCR approach. Modified from Cameron, 2014b.

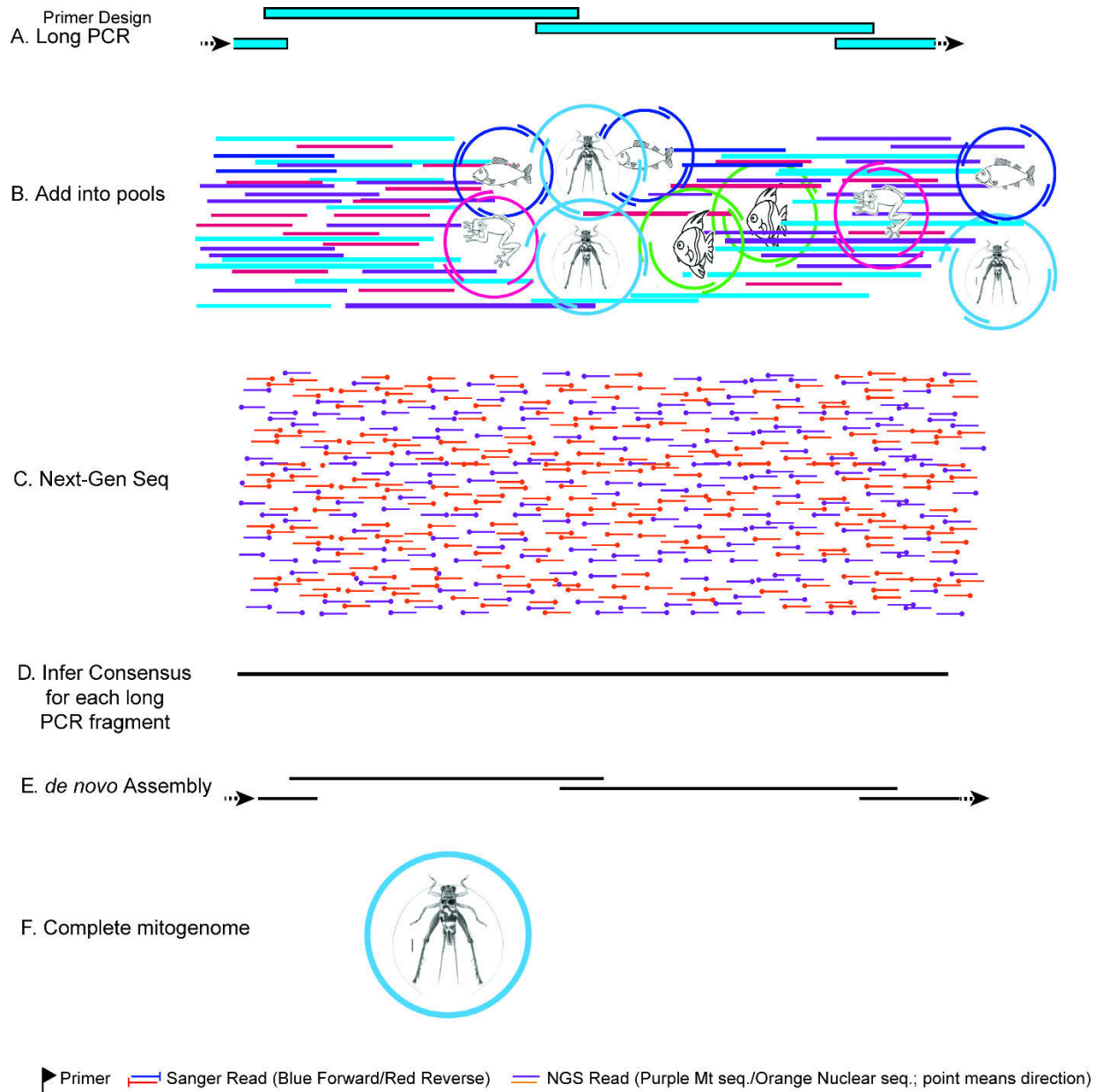


Figure III.3. Workflow of sequencing complete mitogenome in this thesis. In step B, incomplete circles and lines correspond mitogenomes and nuclear DNA fragments, respectively; different species are represented in different colors.

III.4. Complete mitochondrial genome and taxonomic revision of *Cardiodactylus mui* Otte, 2007 (Gryllidae: Eneopterinae: Lebinthini)

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Complete mitochondrial genome and taxonomic revision of *Cardiodactylus muiri* Otte, 2007 (Gryllidae: Eneopterinae: Lebinthini)

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Abstract

In the present study, we report the high-coverage complete mitochondrial genome (mitogenome) of the cricket *Cardiodactylus muiri* Otte, 2007. The mitogenome was sequenced using a long-PCR approach on an Ion Torrent Personal Genome Machine (PGM) for next generation sequencing technology. The total length of the amplified mitogenome is 16,328 bp, representing 13 protein-coding genes, 22 transfer RNA genes, two ribosomal RNA genes and one noncoding region (D-loop region). The new sets of long-PCR primers reported here are invaluable resources for future comparative evolutionary genomic studies in Orthopteran insects. The new mitogenome sequence is compared with published cricket mitogenomes. In the taxonomic part, we present new records for the species and describe life-history traits, habitat and male calling song of the species; based on observation of new material, the species *Cardiodactylus buru* Gorochoff & Robillard, 2014 is synonymized under *C. muiri*.

Key words: mitogenome, long-PCR approach, next generation sequencing technology, New Guinea, Indonesia

Introduction

In the era of next generation sequencing technologies, complete mitochondrial genomes (mitogenomes) are becoming a common and valuable resource for phylogenetic studies and molecular systematics (Crampton-Platt et al. 2015, 2016; Gomez-Rodriguez et al. 2015). In a recent phylogeny of the diverse and ancient insect order Orthoptera, Song et al. (2015) used both mitogenomes and traditional molecular markers as sources of phylogenetic information. They showed that despite the long evolutionary history of these insects, their mitogenome has remained relatively stable, making it a powerful tool to resolve phylogenetic relationships. However, mitogenome sequences have been little investigated in Orthopteran insects compared with other animal groups (Zhou et al. 2010), and large areas of their phylogenetic tree are barely or not documented when considering these data. This is particularly true in the superfamily Grylloidea. Despite the diversity of crickets and their allies, only a handful of mitogenomes are available today in databases (four in Song et al. 2015). The families Gryllotalpidae, Myrmecophilidae and Schizodactylidae are each documented by one species, while the "true crickets", comprising three large families, namely Gryllidae, Phalangopsidae and Trigonidiidae (ChintauanMarquier et al. 2016), are represented only by five species belonging to a single subfamily (Gryllinae): *Teleogryllus commodus* (Wolff et al. 2012), *Teleogryllus oceanicus* (Zhou et al. 2015), *Loxoblemmus equestris*, *Teleogryllus emma* and *Velarifictorus hemelytrus* (Yang et al. 2016a). The direct consequence of this is that the phylogenetic information of mitogenomes between and within cricket families has never been evaluated.

In this study, we present a new set of primers to obtain mitogenomes from long-range PCRs in next generation sequencing (NGS) (e.g., Jia *et al.* 2014, Hinsinger *et al.* 2015) and report the complete mitogenome of *Cardiodactylus muiri* Otte, 2007. This species belongs to the subfamily Eneopterinae, which is characterized by diverse shapes and original features related to acoustic communication (e.g., Robillard & Desutter-Grandcolas 2004a, b; Robillard *et al.* 2013; ter Hofstede *et al.* 2015). In the taxonomic part of the study, we revise *C. muiri* based on recent collections in New Guinea (Papua province, Indonesia) and *C. buru* Gorochoff & Robillard, 2014 is synonymized under *C. muiri*. We also present new geographic records for the species and describe life-history traits, habitats and male calling song.

Materials and methods

Materials. The new material was collected in the “Lengguru 2014” expedition in Papua, Indonesia and will be deposited in the collections of the Muséum national d’Histoire naturelle Paris, France (MNHN) and in the Museum Zoologicum Bogoriense, Bogor, Indonesia (MZB). The crickets were collected mostly by sight, night and day, in order to observe the habitat and activity of the species. Square brackets in the list of specimens are used for additional information not mentioned on the specimen labels or translated from French labels.

Molecular techniques. Total genomic DNA was extracted from the median femoral muscles using the NucleoSpin® 96 Tissue Kit (Macherey-Nagel, Germany) and the automatic pipetting robot Eppendorf epMotion 5075 TMX following the manufacturer’s manual. The complete mitochondrial genome sequence of *C. muiri* was obtained by overlapping three DNA fragments (> 5.5kb) amplified by long-range PCRs and the HotStart LongAmp® Taq DNA polymerase (New England BioLabs® Inc., Ipswich MA). The primers used for these PCRs are presented in Table 1. The amplicons were sequenced using the Ion Torrent Personal Genome Machine (PGM) sequencing platform with two level multiplexing (Hinsinger *et al.* 2015) at the "Service de Systématique Moléculaire" of the MNHN. The reads obtained from each long-range PCR were separately trimmed by error probability algorithm and assembled using the mapping to reference algorithm in GENEIOUS (8.1.7 version) (Table 1) (Kearse *et al.* 2012). The references used for mapping each long-range PCR were the partial mitochondrial gene markers of *C. muiri* obtained from a phylogenetic study of *Cardiodactylus* in preparation molecular data library (Dong *et al.* in prep., see details in Table 1). After this first assembly, the three contigs obtained were re-assembled into a circular mitochondrial genome with high coverage by the de novo assembly algorithm in GENEIOUS.

TABLE 1. List of primers used to amplify the mitogenome and mapping reference in assembly procedure of *Cardiodactylus muiri* Otte, 2007.

Set name	Primer name	Direction	Sequence (5'-3')	Mapping Reference	Length of the amplicon (bp)
SET-1	F146	Forward	GCTAATTAAAGCTAATGGGTTTCATACC	Partial <i>coxI</i> ,	~ 5.8 kb
	R5927	Reverse	CAGTTTCGACCTGAAAGAAAGATTAA	partial <i>cox2</i>	
SET-2	F5346	Forward	GAAGCAGCAGCATGATACTGACA	Partial <i>cytb</i>	~ 7.7 kb
	R13080	Reverse	CTAATGATTATGCTACCTTTGCACGGTCAATATA		
SET-3	F12775	Forward	AACATTACGCTGTTATCCCTAAGGTA	Partial <i>16S</i> ,	~ 5.8 kb
	R1281	Reverse	AGTAATGATNTAGACTGCAATTCTAAAG	partial <i>12S</i>	

Sequence analysis. Annotation of the mitogenome was performed using the MITO webserver with invertebrate genetic code (Bernt *et al.* 2013) and modified after comparisons with other mitogenomes from Grylloidea species. The validation of tRNA sequences was performed in tRNAscan-SE (<http://lowelab.ucsc.edu/tRNAscanSE/>) using the invertebrate mitogenome genetic codon (Schattner *et al.* 2005). The nucleotide base composition of the complete mitogenome was calculated in GENEIOUS.

Phylogenetic analysis. To validate the reliability of the mitogenome sequence and its identification, the *cytb* gene and *rrnS* sequences of this mitogenome were extracted and compared with other species of *Cardiodactylus*. In addition, a phylogenetic analysis of the family Gryllidae based on the sequences of *cytb* and *rrnS* was conducted using a Maximum Likelihood approach. The phylogenetic trees were reconstructed on the IQ-TREE web server (Nguyen *et al.* 2015).

The whole mitogenomes nucleotide base composition of *C. muiri* was calculated in GENEIOUS. The nucleotide compositional skew was calculated following the formula (Konstantionv *et al.*, 2016): AT-skew=(A-T)/(A+T) and GC-skew=(G-C)/(G+C), where A, T, C and G are the frequencies of the four bases. Six grylline mitogenomes are

currently present in GenBank: *Teleogryllus commodus* (JQ686193), *Teleogryllus emma* (EU557269), *Teleogryllus oceanicus* (KT824636), *Loxoblemmus equestris* (KU562919), *Teleogryllus emma* (KU562917) and *Velarifictorus hemelytrus* (KU562918)). Some of these sequences however include nuclear mitochondrial DNA sequences (numts), as reported from Wolff (2012) and Yang (2016) and were thus discarded here. We only considered the sequences of *L. equestris* (KU562919), *T. emma* (KU562917) *V. hemelytrus* (KU562918) and *C. muiri* which was obtained in this study. The nucleotide base composition comparison analysis for the whole mitogenomes, two ribosomal genes (the large and small ribosomal subunit, *rrnL* and *rrnS*) and the A+T-rich regions in grylline crickets were also calculated.

The percentage of pairwise identical bases (% identity) for the whole mitogenomes, each protein coding genes (PCGs) and two ribosomal genes (*rrnL* and *rrnS*) of grylline crickets were calculated in GENEIOUS. The average percentage of identical bases were then calculated between two subfamilies (Eneopterinae and Gryllinae) and within Gryllinae.

Besides, the comparison analysis of synonymous and non-synonymous substitutions (Ka/Ks) for all PCGs in grylline crickets were calculated using MEGA 7.0 (Kumar *et al.* 2016).

Acoustic analysis. The basic cricket song terminology follows Ragge & Reynolds (1998). One song unit is called a syllable and corresponds to one opening-closure cycle of the male forewings. Three males of *C. muiri* have been recorded in the field, in their natural habitat or in semi-captivity in a net cage hanged to a low tree. The recordings were made with a modified Condenser Microphone Capsule CM16 (Avisoft Bioacoustics, Berlin), with a flat frequency response from 3 to 150 kHz (R. Specht pers. comm.), connected to a Fostex FR-2LE digital field recorder (96 kilo-samples per second sampling frequency, 16 bit). Acoustic analyses were performed using the computer software Avisoft-SASLab Pro version 4.40 (Specht 2009). All recording files are deposited in the Sound Library of the MNHN.

Taxonomic part

Subfamily Eneopterinae Saussure, 1874

Tribe Lebinthini Robillard, 2004

Genus *Cardiodactylus* Saussure, 1878

Type species: *Cardiodactylus novaeguineae* (Haan, 1842)

Cardiodactylus muiri Otte, 2007a

(Figs 1–4)

Cardiodactylus muiri Otte, 2007a: 349–2007b: 30 (confirmation of depositary)—Robillard *et al.* 2014: 68 (redescription).

Synonym name. *Cardiodactylus buru* Gorochov & Robillard, 2014, in Robillard *et al.* 2014: 25. New synonymy. The differences between *C. buru* and *C. muiri* in male genitalia described in Robillard *et al.* (2014) are clearly present in specimens of the same populations in the newly examined material from New Guinea and Kai Islands. These differences must then be considered as intraspecific variation of *C. muiri*; *C. buru* is consequently a junior synonym of *C. muiri*.

Type material. Male holotype: Indonesia: Malaka Province, Ceram, Piroe [Seram I., Piru], II.1909, F. Muir (BPBM) [not examined].

Type locality. Indonesia, Seram Island, Piru.

Distribution. Indonesia, Maluku islands (Ambon, Seram, Buru), Kai Islands, South-west part of New Guinea (surroundings of Kumawa and Kaimana).

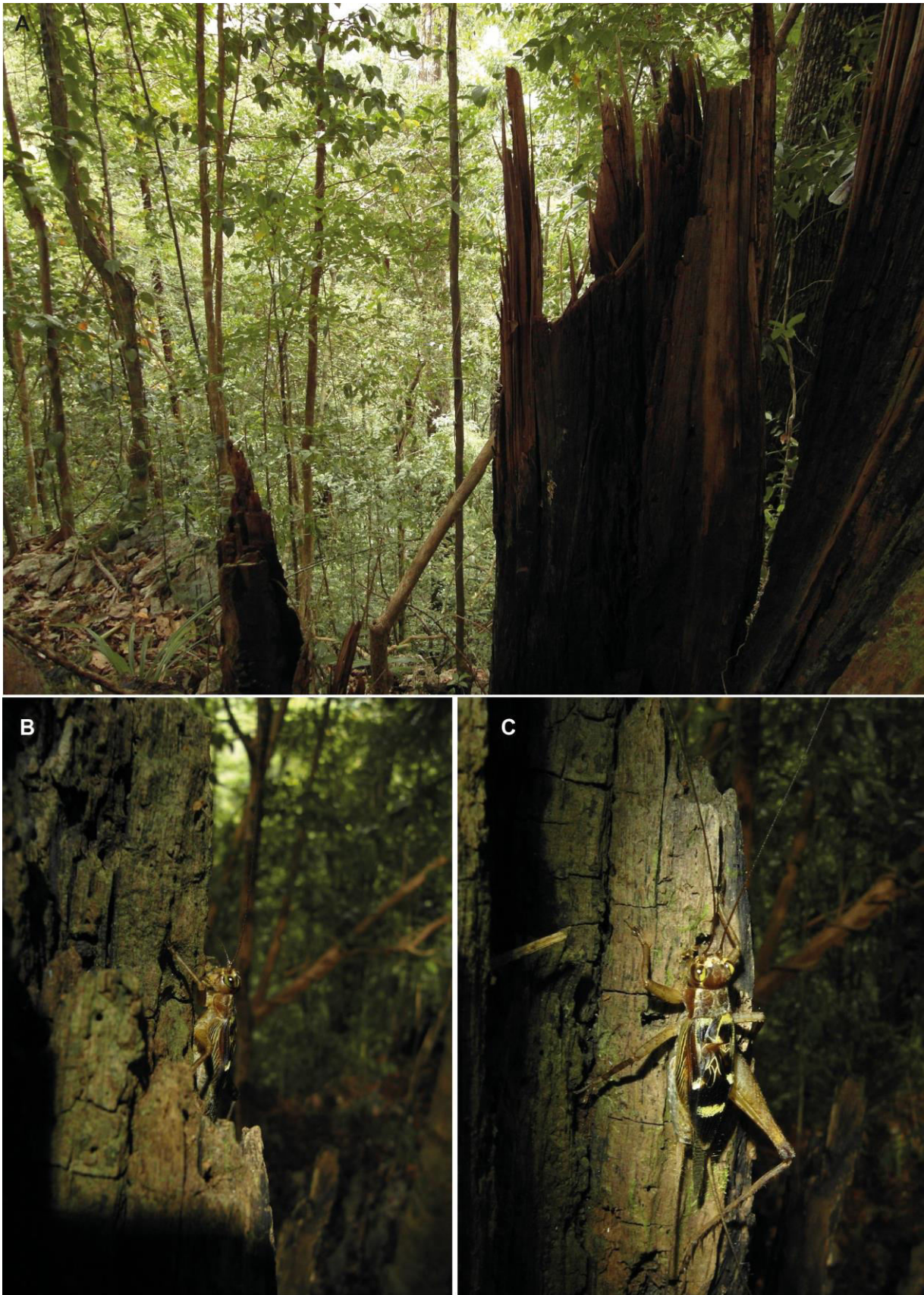


FIGURE 1. Habitat of *Cardiodactylus muiri* Otte, 2007. A, dead tree trunk in Kumawa (Papua, Indonesia); B, C, male sitting on dead tree (A) during the day.

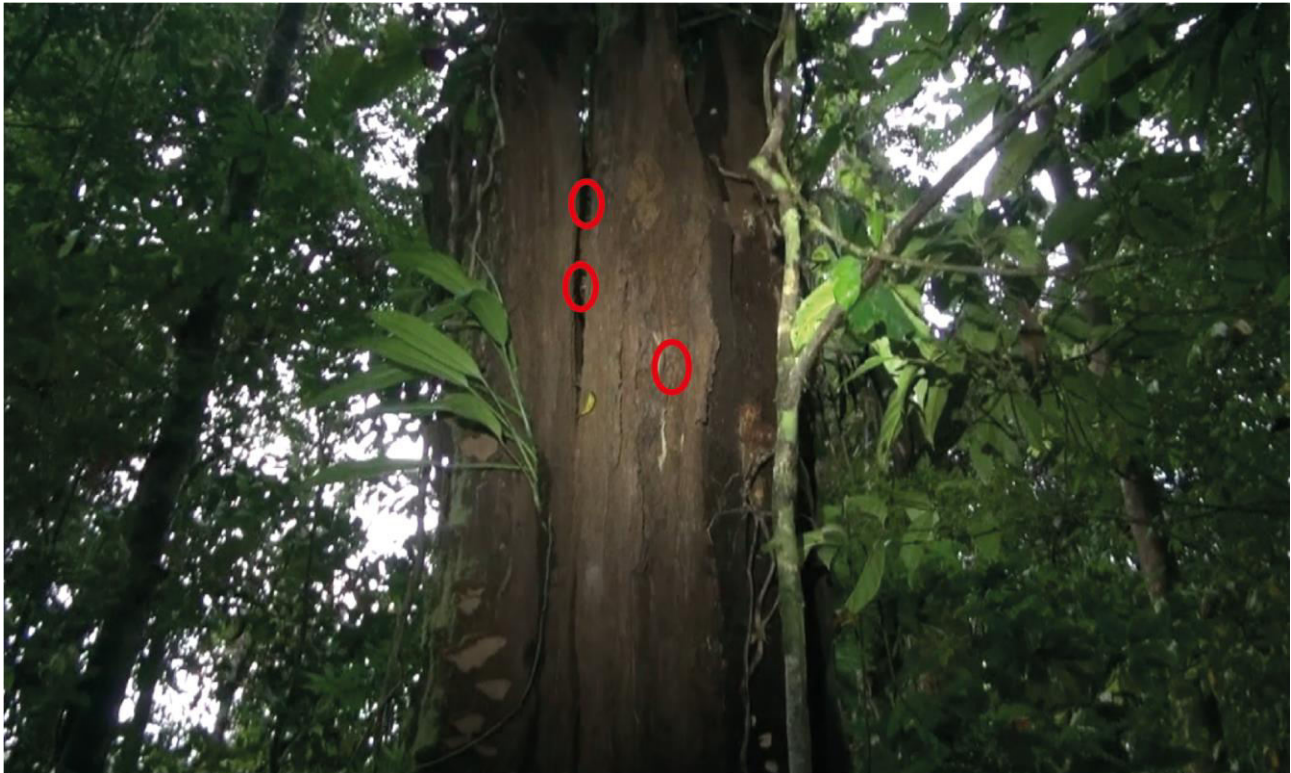


FIGURE 2. Tree trunk with males of *Cardiodactylus muiri* Otte, 2007 engaged in singing activity during late afternoon in Kumawa forest (Papua, Indonesia). Red circles mark males' positions; height ca. 4 m.

New records: Indonesia, West Papua: Nouvelle-Guinée [New Guinea], Kumawa, -4.0646111 133.037111 (KUM3), 82 m, forêt littorale sur karst [littoral forest on karst], ligne de crête, 13–15.XI.2014, T. Robillard: 1 male (LEN2014-TR606), jour [day], tronc chandelle [trunk of dead tree], molecular sample C158 (MNHN-EOENSIF4179); 1 female (LEN2014-TR582), nuit [night], litière [leaf litter], vidéo ponte dans racine 8 PM [video of oviposition in root] (MNHN); 1 female (LEN2014-TR583), nuit, litière, vidéo ponte dans racine 8 PM (MNHN); 1 juvenile (LEN2014-TR569), nuit (MNHN). Nouvelle-Guinée, Kumawa, -4.0688333 133.036444 (KUM2), 27 m, forêt littorale karstique sur pente, jour, tronc chandelle, 11–17.XI.2014, T. Robillard: 1 male (LEN2014-TR625) (MZB); 1 male (LEN2014-TR587), enregistrement appel en captivité [recording of calling song in captivity] Take Pap122 (MNHN-EO-ENSIF4403); 1 juvenile (LEN2014-TR595), jour, litière (MNHN); Nouvelle-Guinée, Kumawa, -4.0518611 133.066083 (KUM5), 87 m, forêt sur pente en amont de la rivière, 16–17.XI.2014, T. Robillard: 1 male (LEN2014-TR657), enregistrement appel en semi-captivité Takes Pap142-143 (MNHN-EOENSIF4404); 1 male (LEN2014-TR654), enregistrement appel en semi-captivité Takes Pap140-141 (MNHN-EOENSIF4405); Nouvelle-Guinée, Kumawa, -4.0555 133.066333 (KUM4), 47 m, forêt littorale karstique sur pente, 16.XI.2014, T. Robillard: 1 male (LEN2014-TR617), molecular sample C160 (MZB); 1 female (LEN2014TR655), mort élevage (MZB); 1 female (LEN2014-TR615), nuit, tronc de pandanus h=1.80 m (MZB); 1 female (LEN2014-TR616), nuit, tronc de pandanus h=1.80 m (MNHN); Nouvelle-Guinée, Kaimana, -3.643667 133.757028 (KAI3), 200 m, forêt littorale sur pente, 19.X.2014, 1 female (LEN2014-TR54), jour, plante, molecular sample C150 (MNHN-EO-ENSIF4164). Indonesia, Kei Eil. [Kai Islands], Gn. [Gunung] Daab [Pulau Kai-besar], 1922, H. C. Siebers: 4 males, #79, #131, #112, #121; 1 female #138 (MNHN); 1 male, #131, identified *Cardiodactylus haani* Ss [Saussure] by Lucien Chopard (MZB-ORTH10475).

Life history traits and habitats. *C. muiri* lives in dense forested habitats on tree trunks of various sizes or on dead trees still standing (Fig. 1). Groups of singing males (three or more) distant by ca. 50 cm to one meter, are commonly found during afternoon on large tree trunks above three meters high (Fig. 2). They sit on the bark surface and sometimes in crevices of the bark or of epiphytes. Songs were not heard during night, even when habitats with singing males heard during the day were visited after sunset. It could indicate that the species is diurnal or crepuscular and that the formed couples hide at night for mating. However, the species seems to have a low-light preference, given that light intensity under forest cover is limited during afternoon and in the confined habitats (Fig. 1). Females are found ovipositing during early night in the leaf litter or in roots near the ground (Fig. 3).

Calling song. The calling song of *C. muiri* (Fig. 4) consists of only one long syllable. The song bouts are rather irregular, which disqualifies them as echemes. At 26 °C (MNHN-EO-ENSIF4405, measurement of 28 syllables) the call duration is 38 ± 11.4 ms (mean \pm SD), with a period of $4.78 \text{ s} \pm 3.83 \text{ s}$, giving a syllable duty cycle of 8%. The dominant frequency is 11.98 ± 0.24 kHz, which is rather low for the genus and corresponds to the third peak of the frequency spectrum, the two first peaks being little marked.



FIGURE 3. Females of *Cardiodactylus muiri* Otte, 2007. A, walking on leaf litter during early night; B, ovipositing in a mossy tree root at night; in Kumawa (Papua, Indonesia).

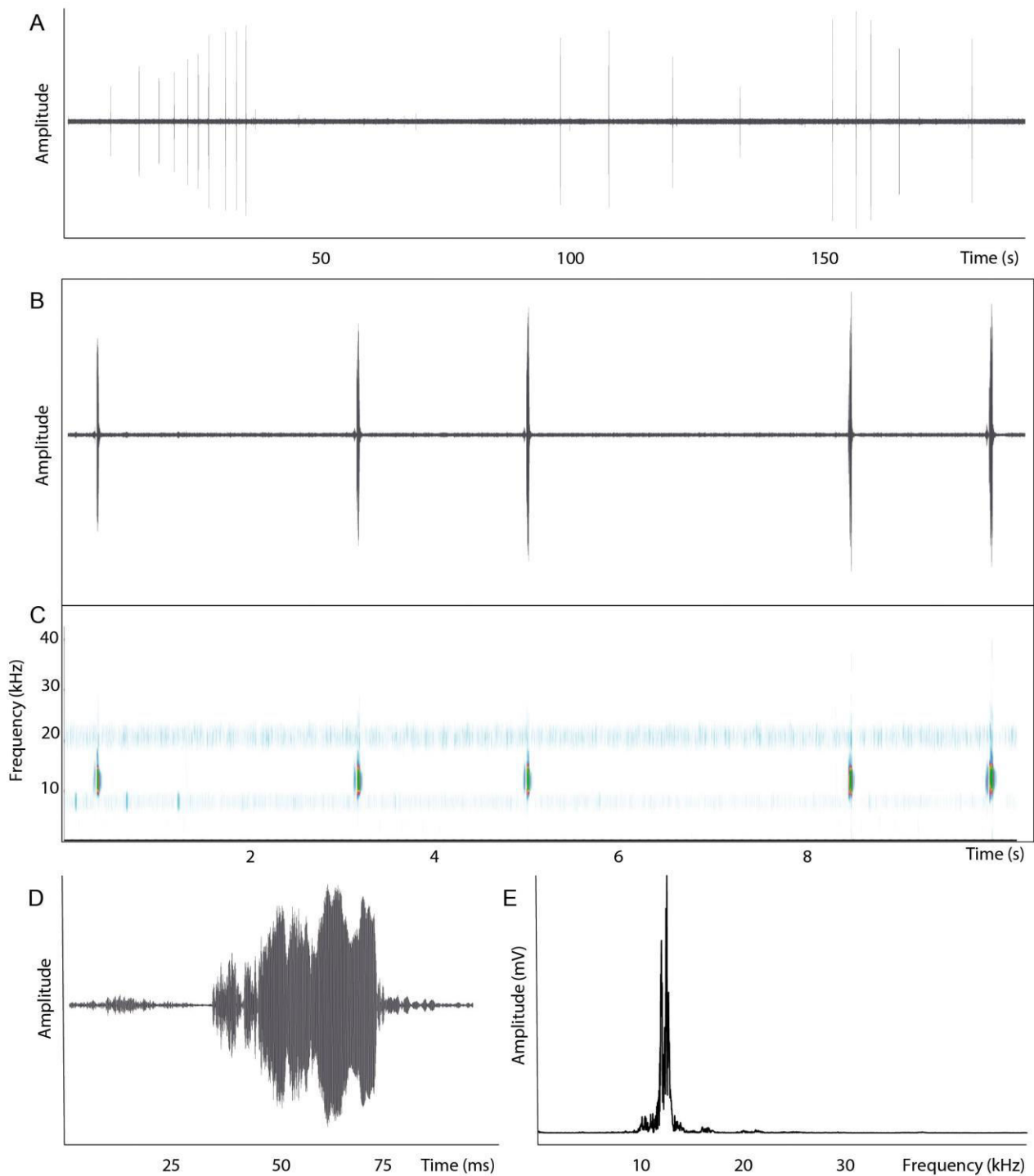


FIGURE 4. Calling song of *Cardiodactylus muiri* Otte, 2007. A, oscillogram of 19 syllables (= echemes); B–C, detailed oscillogram (B) and sonogram (C) of five syllables; D, detailed oscillogram of one syllable; E, frequency spectrum of one syllable.

Results of sequencing and phylogenetic analyses

The mitogenome is 16,328 bp long (Fig. 5), organized in the typical set of 37 genes (13 protein-coding genes, 22 transfer RNA genes and two ribosomal RNA genes) and one A+T-rich control region including two 279 bp repeated regions (Table 2).

TABLE 2. Annotation of the mitochondrial genome of *Cardiodactylus muiri* Otte, 2007.

Gene	Strand	Anticodon	Start codon	Stop codon	Position
tRNA-Ile	+	AUC			1–63 (0)
tRNA-Gln	-	UUG			61–129 (0)
tRNA-Met	+	AUG			143–211 (0)
nd2	+		ATT	T	212–1229 (0)
tRNA-Trp	+	UCA			1230–1294 (-8)
tRNA-Cys	-	GCA			1287–1348 (0)
tRNA-Tyr	-	GUA			1361–1425 (-8)
cox1	+		ATC	TAA	1418–2965 (0)
tRNA-Leu ²	+	UUA			2972–3036 (0)
cox2	+		ATG	T	3042–3720 (+3)
tRNA-Lys	+	AAG			3718–3788 (0)
tRNA-Asp	+	GAC			3789–3854 (0)
atp8	+		ATT	TAA	3855–4016 (+7)
atp6	+		ATG	TAA	4010–4693 (+1)
cox3	+		ATG	T	4693–5479 (0)
tRNA-Gly	+	GGA			5480–5542 (0)
nd3	+		ATA	TAA	5543–5896 (0)
tRNA-Ala	+	GCA			5904–5968 (0)
tRNA-Arg	+	CGA			5979–6041 (-1)
tRNA-Glu	-	UUC			6041–6105 (0)
tRNA-Ser ¹	-	GCU			6110–6176 (0)
tRNA-Asn	-	GUU			6177–6244 (0)
tRNA-Phe	-	GAA			6246–6307 (0)
nd5	-		ATT	T	6308–8021 (0)
tRNA-His	-	GUG			8040–8103 (-1)
nd4	-		ATG	T	8103–9445 (+7)
nd4l	-		ATG	TAA	9439–9735 (0)
tRNA-Thr	+	ACA			9747–9810 (0)
tRNA-Pro	-	UGG			9811–9877 (0)
nd6	+		ATC	TAA	9880–10404 (+1)
cytb	+		ATG	TAA	10404–11537 (0)
tRNA-Ser ²	+	UCA			11562–11624 (0)
nd1	-		ATA	T	11625–12594 (+6)
tRNA-Leu ¹	-	UAG			12589–12656 (+41)
rrnL	-				12616–13977 (+18)
tRNA-Val	-	UAC			13960–14027 (0)
rrnS	-				14028–14798 (0)
A+T-rich region	+	N/A			14857–16328 (0)

The ML gene trees, based on *cytb* and *rrnS* markers, are congruent and showed that the sequences issued from the present study clustered with published sequences of *Cardiodactylus* species and other representatives of the subfamily Eneopterinae (Fig. 6).

The nucleotide composition of *Cardiodactylus muiri* is typically insect A+T biased, with 76.5%, and is slightly A skewed (AT-skew=0.08) and strongly C skewed (GC-skew= -0.29). The nucleotide compositional skew of the whole mitogenomes, *rrnL* genes, *rrnS* genes and the A+T-rich regions of Gryllinae crickets are also represented (Table 3, Fig. 7). The conventional start/stop codons and incomplete stop codons are represented in the PCGs (see details in Table 2). All the protein-coding genes (PCGs) in the mitogenome start with a typical ATN codon but end with different stop codons. The complete stop codon TAA are determined in genes *cox2*, *atp8*, *atp6*, *nd3*, *nd4l*, *nd6* and *cytb*, while the incomplete stop codons (T) are found in genes *nd2*, *cox1*, *cox3*, *nd5*, *nd4*, *nd1*. The lengths of 22 tRNA genes range from 62 to 71 bp. Two rRNA genes (*rrnL* and *rrnS*), separated by tRNA-Val, are located between tRNA-Leu and A+T-rich region. The lengths of *rrnL* and *rrnS* are 1,362 and 771 bp, respectively. Their A+T contents are 78.5% and 74.9%, respectively. The A+T-rich region of 1,472 bp, with an A+T content of 80.1%, is located between *rrnS* and *trnI*.

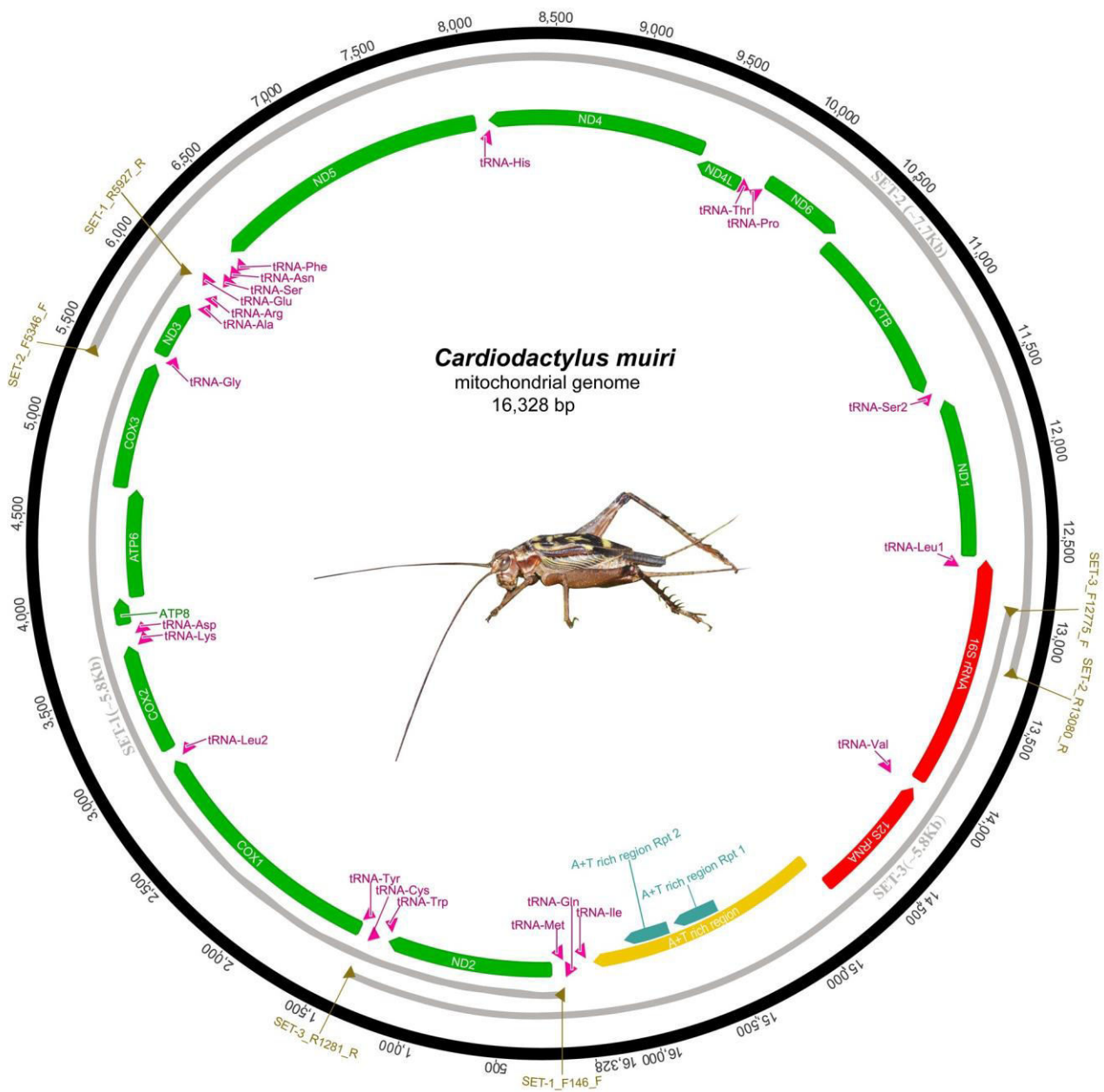


FIGURE 5. Map of the mitochondrial genome of *Cardiodactylus muiri* Otte, 2007.

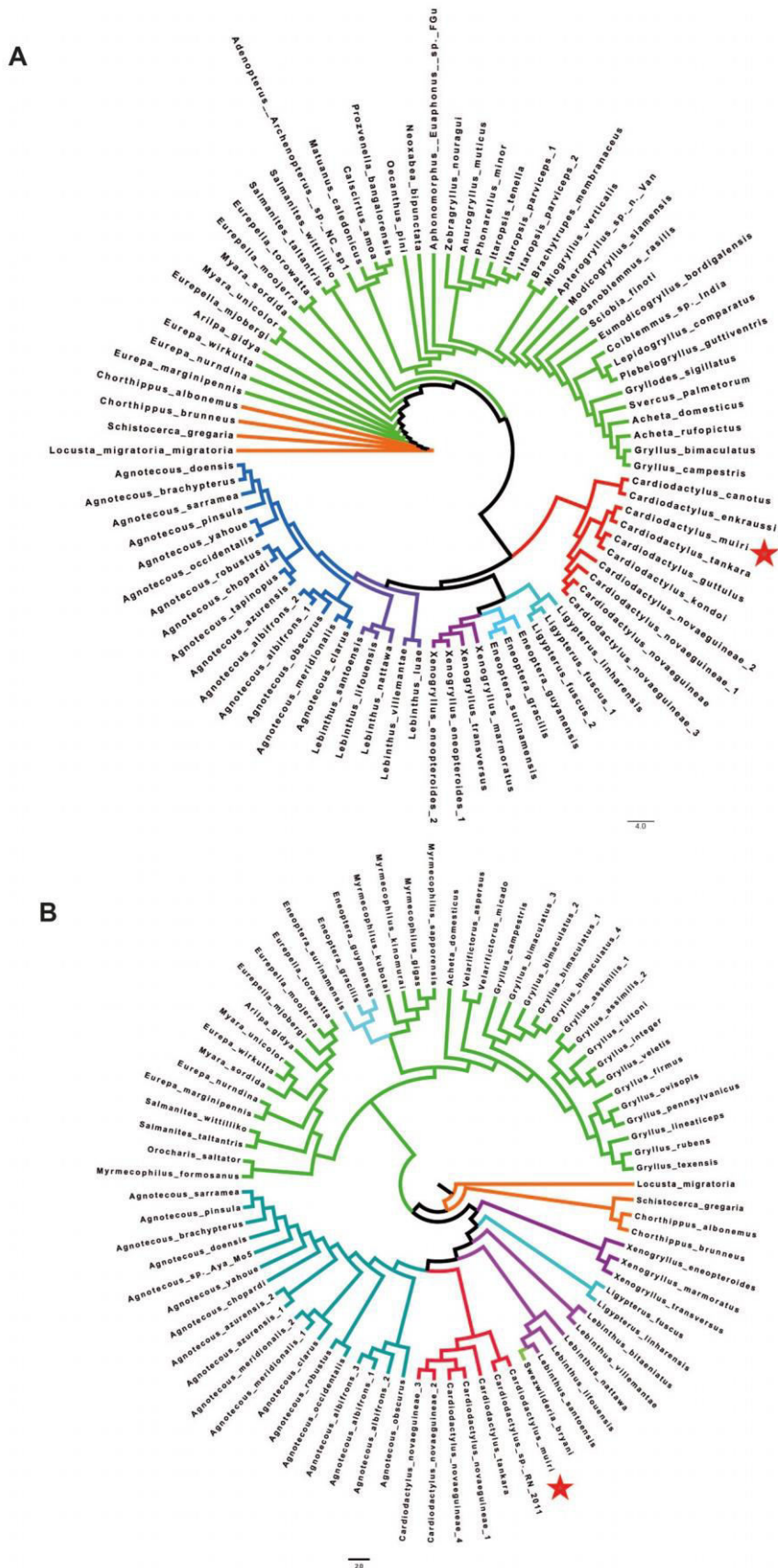


FIGURE 6. A, Maximum likelihood phylogeny of the family Gryllidae inferred from *rns* partial sequences available in GenBank. B, Maximum likelihood phylogeny of the family Gryllidae inferred from *cytb* partial sequences available in GenBank. The red star indicates the species under study.

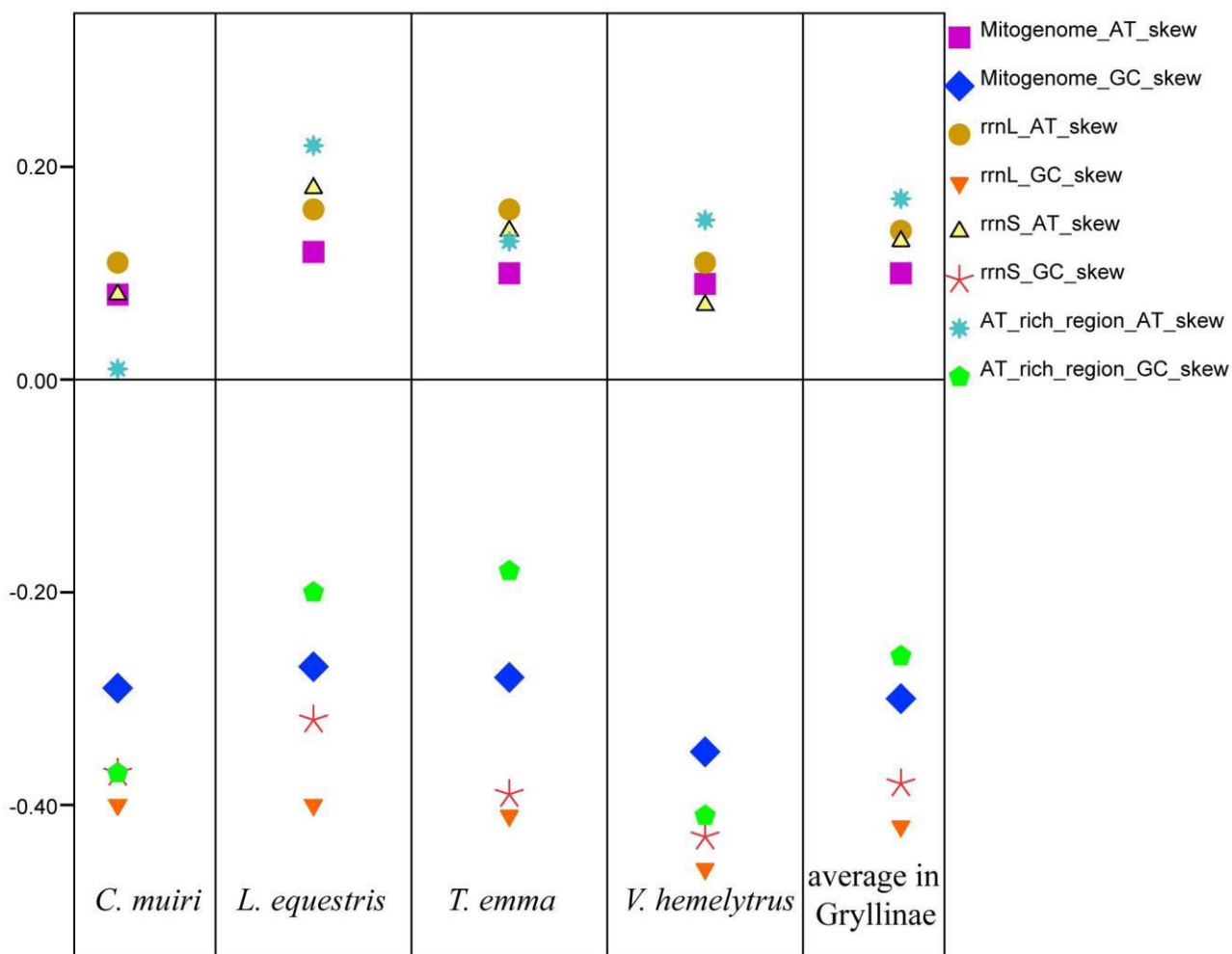


FIGURE 7. Comparison of AT-skews and GC-skews of Eneopterinae and Gryllinae.

TABLE 3. General nucleotide composition of complete mitogenome and nucleotide compositional skew of the Gryllidae mitogenomes, rrnL genes, rrnS genes and the A+T-rich regions in grylline crickets. A (%), T (%), G (%) and C (%) mean the percentage of adenine, thymine, guanine and cytosine. The number with asterisk (*) means the average nucleotide compositional skew in the subfamily Gryllinae.

Species	Length (bp)	Whole mitogenome				AT-skew	GC-skew	
		A (%)	T (%)	G (%)	C (%)			
<i>Cardiodactylus muiri</i>	16,328	41.4	35.1	8.4	15.2	0.08	-0.29	-
<i>Loxoblemmus equestris</i>	16,314	40.3	31.6	10.2	17.9	0.12	-0.27	-
<i>Teleogryllus emma</i>	15,697	40.2	33.0	9.6	17.2	0.10	-0.35	-
<i>Velarifictorus hemelytrus</i>	16,123	39.7	32.9	8.9	18.4	0.09	-0.35	-
						0.10*	0.30*	-

Continued

	<i>rrnL</i> genes		<i>rrnS</i> genes		A+T-rich region		
	AT-skew	GC-skew	AT-skew	GC-skew	AT-skew	GC-skew	
<i>Cardiodactylus muiri</i>	0.11	-0.40	0.08	-0.37	0.01	-0.37	-
<i>Loxoblemmus equestris</i>	0.16	-0.04	0.18	-0.32	0.22	-0.20	-
<i>Teleogryllus emma</i>	0.16	-0.41	0.14	-0.39	0.13	-0.18	-
<i>Velarifictorus hemelytrus</i>	0.11	-0.46	0.07	-0.43	0.15	-0.41	-
	0.14*	0.42*	0.13*	0.38*	0.17*	-0.26*	-

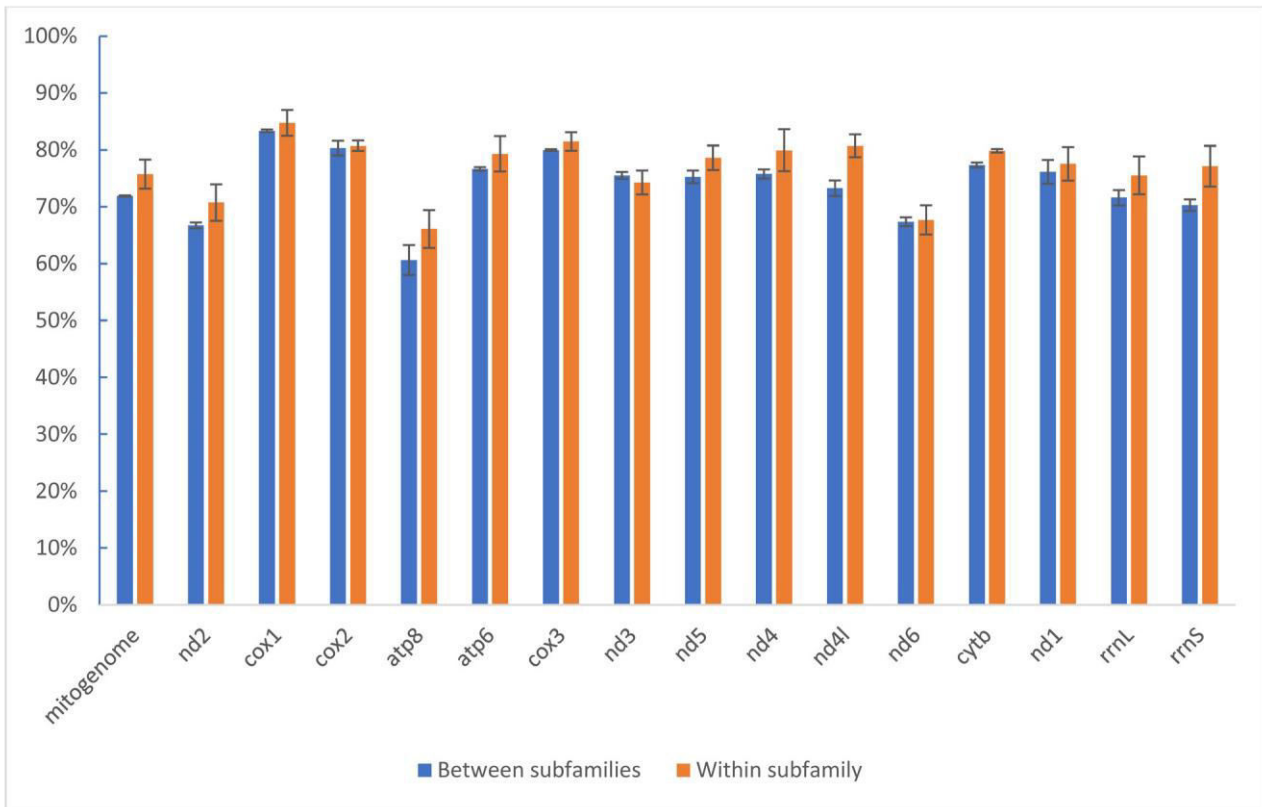


FIGURE 8. Percentage of pairwise identical bases (% identity) of mitogenome and each gene between the two subfamilies and within Gryllinae.

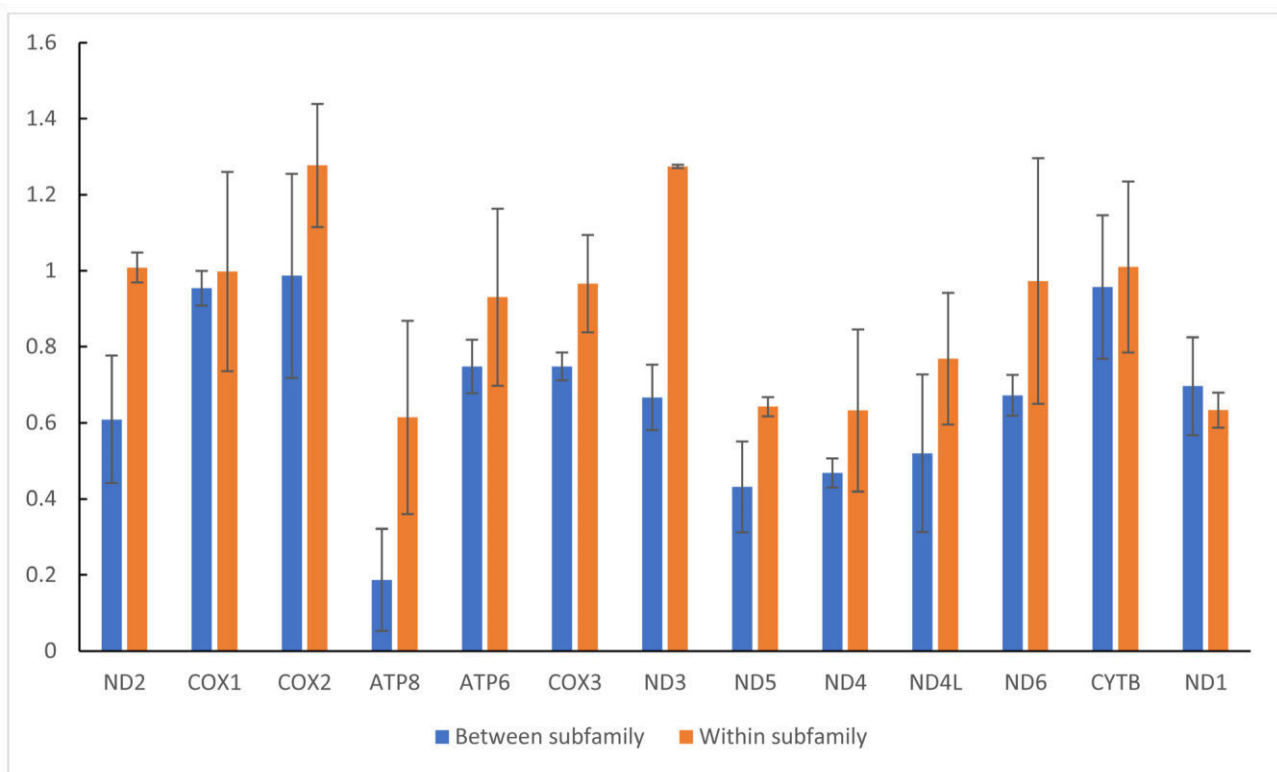


FIGURE 9. Evolutionary rate of each protein-coding (PCG) in the mitogenomes of Eneopterinae and Gryllinae.

The percentage of pairwise identical bases (% identity) for the whole mitogenomes, each PCG and two ribosomal genes, and the percentage of identity between the two subfamilies (Eneopterinae and Gryllinae) and within Gryllinae are presented in Table S1 and Fig. 8. Except for gene *nd3*, the percentage identities and the pairwise genetic distance within Gryllinae were higher than between the two subfamilies. Pairwise comparison of Ka/Ks for each PCG are also presented in Table S2 and Fig. 9. The overall Ka/Ks values are less than 1.0 between the two subfamilies, and higher than 1.0 in genes *nd1*, *cox1*, *cox2*, *nd3* and *cytb* within the subfamily Gryllinae. The values for Cytochrome Oxidase complex (*cox1*, *cox2* and *cox3*) and *cytb* are less than 1.0, but higher than for other genes between the two subfamilies. The smallest value between the two subfamilies is that for the *atp8*. The variability of Ka/Ks ratio within Gryllinae is similar with that between the two subfamilies.

Discussion

We generated the first mitogenome for the cricket subfamily Eneopterinae, which allows comparisons with existing mitogenomes from the subfamily Gryllinae. The gene order and orientation within the mitogenome of *C. muiri* are identical with those described for grylline crickets (*Loxoblemmus equestris*, *Teleogryllus emma* and *Velarifictorus hemelytrus*). The length of the whole mitogenome and the length range for tRNAs in *C. muiri* are also very close to Gryllinae (Yang *et al.* 2016b). The whole mitogenomes of these four crickets are weakly AT-skewed (0.08 to 0.10) and strongly GC-skewed (-0.35 to -0.27). The average AT- and GC-skew in the whole mitogenome of the Gryllinae are close to those of *C. muiri*. However, nucleotide compositional skew in *rrnL*, *rrnS* and A+T-rich region are stronger in Gryllinae than in *C. muiri*, with the exception of the GC-skew in A+T-rich region. It is logical that the nucleotide compositional skew in different genes of different subfamilies are slightly divergent, but more mitogenomes of other Eneopterinae crickets are necessary to test whether this tendency is consistent between the subfamilies.

There is no significant difference of the percentage identity, between the two subfamilies and within Gryllinae, for the mitogenome and the genes considered separately, except for the *atp8* and *nd6*. The percentage of pairwise identical bases for the mitogenome and for the individual genes are higher than 70%, both between the two subfamilies and within Gryllinae. The level of identity between the two subfamilies is higher or almost equal to that within Gryllinae in the genes *nd3* and *nd6*, respectively. According to the Ka/Ks ratio, the evolutionary rate of Cytochrome Oxidase complex (*cox1*, *cox2* and *cox3*) and *cytb* genes is high between the subfamilies and within Gryllinae. There is an obvious evolutionary rate discrepancy in NADH dehydrogenase complex genes and ATP synthase, since the genes *nd2* and *nd6* are less divergent than the other NADH dehydrogenase complex genes. In addition, the rate of evolution of *nd6* is almost the same, both between the two subfamilies and within Gryllinae. The gene *atp8* is found to have the slowest evolution rate between the two subfamilies, while the gene *cox2* has the highest rate of evolution within Gryllinae. Thus, the present study revealed these two genes as potential markers for future phylogenetic study at family and subfamily level in crickets. Our results lead to the conclusion that the mitogenomes in crickets have a relatively stable structural and functional evolution, as suggested by previous studies made in Orthoptera (Song *et al.* 2015) and in insects in general (e.g. Simon *et al.* (2006); Cameron (2014); Konstantinov *et al.* (2016)).

With the quick development of next generation sequencing (NGS) methods, the new sets of long-PCR primers defined here will allow to easily and cheaply acquire large amount of new data for large taxonomic samples. The new mitogenome described in the present study, combined with the existing datasets, arises as an invaluable resource for future comparative evolutionary genomic studies in crickets and insects, in general.

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III.5. Chapter Conclusion

In this chapter, I designed a set of primers to use for long-PCR amplification of complete mitogenomes in Gryllidae and applied this method to members of the subfamily Eneopterinae. I reconstructed the mitogenome of *Cardiodactylus muiri* (Section III.4) and obtained raw data for 44 other species (36 *Cardiodactylus* species and 8 other species in Eneopterinae) (Dong *et al.*, in preparation). This represents a promising and powerful tool to reconstruct phylogenetic relationship in Eneopterinae, and more generally in Gryllidae and Ensifera. This method highly relies on the successful amplification and high concentration of long-PCR amplicons. However, not all specimens in the collections are preserved well enough to allow long-PCR amplification. In this case, other methods such as shotgun sequencing will be needed to obtain mitogenomes from older specimens of collection, especially when a species is known from only one holotype preserved as a dry specimen.

Conclusion, Discussion and Perspective

1. Conclusions

The main goal of this thesis consisted of keeping exploring the taxonomic diversity of the genus *Cardiodactylus*, reconstructing its phylogeny, and estimating its divergence time and ancestral area, in order to infer its biogeographical history and answer questions about its origin, speciation rate and dispersal patterns. Here, I summarize the main results of this thesis.

1.1. Taxonomy of *Cardiodactylus*

It is impossible to understand the evolution and diversification patterns of species without considering the basic biological knowledge about them, such as morphological characters, behavior, habitat and distribution patterns. Thus, in the Chapter I of this thesis, I reviewed the taxonomic work about the genus *Cardiodactylus*, then described five new species from Eastern New Guinea, redescribed two existing species in order to complete the previous descriptions with as many information as possible about the species. Meanwhile, I also identified *C. buru* as a junior synonym of *C. mui* (Dong *et al.*, 2017; see Chapter III) after combining the information of specimens from different localities.

Most *Cardiodactylus* species are easy to categorize into the two species groups previously defined (*Novaeguineae* and *Efordi* species groups), based on the coloration of the forewings and male genitalia. However, some species such as *C. javarere* and *C. kolombangara* show intermediate characters between the two species groups. Male FWs of these species are similar to the *Novaeguineae* species group, with a whitish spot in harp posterior angle; while their male genitalia resemble that of the *Efordi* species group, with a pseudepiphallus strongly narrowed at mid-length and with small or absent dorsal ridges.

In addition, my taxonomic studies suggest that most *Cardiodactylus* species shows intraspecific variation on morphological characters among different populations across their geographic range, a variation that needs to be considered carefully, as it can lead to overestimating the species diversity. For example, the species *C. buru* described from Buru

Island in 2014, had to be synonymized under *C. muiri*, initially known from Seram Island in the Moluccas (Indonesia). The synonymy was revealed by considering additional material from Kai Islands and from Eastern New Guinea with both morphological and molecular evidence (Dong et al., 2017). This conclusion allowed me to consider with caution the morphological variation existing across the large island of New Guinea. For example, the material available for the new species *C. niugini* could have been divided into several species based on superficial examination of a subsample of the available samples; a close examination of the whole set of specimens however revealed that there were continuums of characters between distant localities (Dong & Robillard, 2016; Chapter I).

1.2. Phylogeny of *Cardiodactylus*

I reconstructed the phylogeny of the genus *Cardiodactylus* based on five mitochondrial and four nuclear markers (section II.3; in Chapter II). The monophyly of the genus is strongly supported, as well as that of most of the species and of the *Novaeguineae* species group. However, the result clearly indicate that the *Efordi* species group is paraphyletic.

Within the *Efordi* species group, the species *C. javarere* is sister to the *Novaeguineae* species group, forming a well-supported clade with high support. Combining this result with the morphological observations mentioned above, the position of *C. javarere* would support the hypothesis that there is a third monophyletic species group (Dong and Robillard, 2016; Chapter I). More material and molecular evidence are needed to test this hypothesis in further developments.

Interestingly, within the large *Novaeguineae* species group, a clade formed by *C. novaeguineae* and *C. haddocki* is strongly supported as the sister group of all remaining species of the group. Among all remaining the members of the *Novaeguineae* species group, the positions for the species distributed in New Guinea and the Western Pacific are less robust, i.e. *C. muiri*, *C. manus* and *C. quatei*, which probably results from the poor sampling in these regions. By contrast, all the species distributed in SEA are organized in well-supported clades.

1.3. Historical biogeography of *Cardiodactylus*

I inferred the biogeographical pattern of *Cardiodactylus* based on the divergence time and reconstruction of ancestral areas (Dong *et al.*, in preparation; in Chapter II).

The results suggest that *Cardiodactylus* originated from the Western Pacific in the mid-Eocene (ca. 43 Ma). The *Efordi* species group started its diversification first, during the late Eocene (ca. 40.9 Ma), while the *Novaeguineae* species group crown group started diversifying in the Late Oligocene (29 Ma). From Western Pacific, *Cardiodactylus* reached New Guinea while the island was not yet completely formed, which occurred ca. 23 Ma. New Guinea was first a transition zone, allowing *Cardiodactylus* to reach SEA from the Western Pacific. *Cardiodactylus* colonized SEA three times independently through three different passageways: the Philippines (19 Ma), Sulawesi (18 Ma) and Java (16 Ma). Through these passageways, *Cardiodactylus* colonized SEA from east to west, crossing the Wallace line to reach Sundaland, the southeast coast and north of Indochina, Palawan and the Philippines. However, most of the colonization events within SEA occurred before the Miocene, and few more recently, such as the colonization route from Indochina to the Thai-Malay Peninsula and from the Thai-Malay Peninsula to Borneo. My results also infer that the high diversity of *Cardiodactylus* took place during the period ranging from the Late Oligocene to the end of Miocene, and not during the Plio-Pleistocene, as expected by recent changes of sea level. It suggests that the diversification of *Cardiodactylus* was not affected by the recent changes of sea level. It is however strongly related with the geological events that occurred during the period from the Late Oligocene to the end of Miocene, which involved frequent connection and disconnection between continuous micro-fragments of islands.

1.4. Toward mitogenomic phylogenetic reconstruction of Ensifera

With rapid development of next generation sequencing technologies (NGS), mitogenomes are becoming a common and valuable resource for phylogenetic studies and molecular systematics. In Chapter III, I provided a set of primers for long-PCR amplification for reconstruction of complete mitogenomes in Gryllidae, and reported the complete mitogenome of *Cardiodactylus muiri* Otte, 2007 by using the method of long-PCR plus NGS. The mitogenome of *C. muiri* is 16,328 bp long, organized in the typical set of 37 genes (13 protein-coding genes, 22 transfer RNA genes and two ribosomal RNA genes) and one A+T-rich control region including two 279 bp repeated regions. The comparison of this mitogenome with the other published cricket mitogenomes show that, except for *nd1*, all genes forming part of the electron transport chain are very conserved between the subfamilies Eneopterinae and Gryllinae, probably because of the function of these genes. At the subfamily scale, the highest genetic variation was detected in the genes of *cox2* and *nd3*. However, it is too early to conclude that they are potential better candidates as barcoding gene in Ensifera than that *cox1*, more complete mitogenomes should be added to test in future work. On the other hand, complete mitogenomes could provide valuable phylogenetic information and represent a promising and powerful tool to reconstruct robust phylogenetic relationships in *Cardiodactylus* and Eneopterinae, and more generally in Gryllidae and Ensifera.

2. Further discussion on the evolution of *Cardiodactylus*

2.1. Origin and dispersal of *Cardiodactylus* in and from the Western Pacific

My results suggest that the origin of *Cardiodactylus* occurred in the Western Pacific. This conclusion contrasts with the study of Vicente *et al.* (2017), who investigated the historical biogeography of the subfamily Eneopterinae and found that *Cardiodactylus* diverged from its most recent common ancestor (MRCA) in SEA ca. 42 Ma, from a continental origin in the northern Boreotropical region. During this period, most islands in the Western Pacific did not emerge yet, which is for example the case of New Caledonia, that emerged 37 Ma (Grandcolas *et al.*, 2008) or the Fiji islands, which were formed by subduction during of late Eocene (34–37 Ma; Neall & Trewick, 2008).

It raises the question of how and when did the MRCA of *Cardiodactylus* disperse to the Pacific from the continent, through long distances. The following questions could be addressed: Did *Cardiodactylus* colonize the Western Pacific actively (i.e. through their flight ability) or passively (i.e. through occasional dispersal by wind or current)? If they colonized the Western Pacific actively, what factors contributed to this outstanding flight capability, long-wings and/or small size? If the MRCA of *Cardiodactylus* crossed the ocean passively, how could it happen, though which route of colonization, and how did the migrants adapt to the new environment? On the other hand, mega-landslides (fragments of island) has been considered as an important driver of island colonization of non-dispersive taxa (passive dispersal) (García-Olivares *et al.*, 2017). To test this hypothesis, a dense sampling in Western Pacific of the genus is required; but a comprehensive review of geological history of this region should be taken into account. To fully answer the question of the origin of *Cardiodactylus* in Western Pacific, it will have to be addressed at the wider scale of the whole tribe Lebinthini, considering a dense taxonomical sampling. It will in particular permit to take into account the various flight abilities existing within the tribe (from most genera being brachypterous to *Cardiodactylus*, which have variably long wings and are likely able to fly actively).

2.2. Ancestral states of the genus *Cardiodactylus*

According to the molecular phylogenetic analysis (details in Chapter II), the *Novaeguineae* species group is monophyletic, while *Efordi* is paraphyletic and *C. javarere* seems to be part of a third species group, sister to the *Novaeguineae* species group.

Interestingly, many features of *Cardiodactylus* species and species groups show contrasted states within the genus, which evolutionary history could be tracked back using the dated molecular phylogeny with ancestral state reconstructions.

Morphologically, the species are both characterized by color patterns on male forewings, and by highly modified male genitalia, the latter being supposed to play a major role in the speciation process in crickets as a possible pre-copulatory zygotic barrier. What is the ancestral state of male genitalia in *Cardiodactylus*, if the male genitalia shape of *Efordi* species group is an ancestral state and then underwent a transitional state (genitalia shape in *C. javarere*), finally evolved to the state of *Novaeguineae* species group?

The differences between *Cardiodactylus* species groups are also marked in terms of lengths of hind wings, which could potentially affect the flight abilities: the *Efordi* species group, for example, tends to have smaller sizes, narrow body shapes and longer wings, while the major part of the *Novaeguineae* species group are larger,

Finally, the taxonomic revision of the genus (Chapter I) allowed me to characterize their diversity in terms of calling songs, ecology and habitats, all of these aspects being good candidates for ancestral state reconstructions to address questions about the evolution of acoustic communication. For example, some species possess a very simple song made of one long syllable, while others have two distinct syllable types organized in complex calls.

3. Work in progress and perspectives

In this thesis, I presented the first comprehensive phylogeny of the genus *Cardiodactylus*, and its biogeography and dynamics diversification focusing on the region of Southeast Asia. However, additional work is needed to reconstruct a more complete phylogeny of the genus in order to address the biogeography of this clade accross its global distribution region to explain further its diversity. I will particularly focus my efforts on New Guinea in a first time. It is a large island with a complex geological history. Besides, my results showed that it was probably a bridge in the process of *Cardiodactylus* historical biogeography when it colonized SEA. Further biogeography study on New Guinea would be addressed to answer the question about adaptation and radiation.

First, and in parallel of the phylogenetic work, the taxonomic revision and exploration of *Cardiodactylus* will have to be pursued using all the tools available to the modern systematists. I am currently preparing the descriptions of several new species from the region of New Guinea using both my expertise as a taxonomist and morphological criteria, but also molecular data. In this study, I will use species delimitation methods based on molecular information to assess the putative new species. Such method will help dealing with the intraspecific variation existing in *Cardiodactylus* species. Moreover, complete mitogenomes, which have been considered as promising tool to study phylogeny and systematics, would be assembled to provide more useful information for species delimitation, but also would be added in the process of phylogenetic analyses. I have successfully sequenced 15 mitochondrial genomes following the protocol presented in Chapter 3 and am currently assembling the datasets in order to use in this context.

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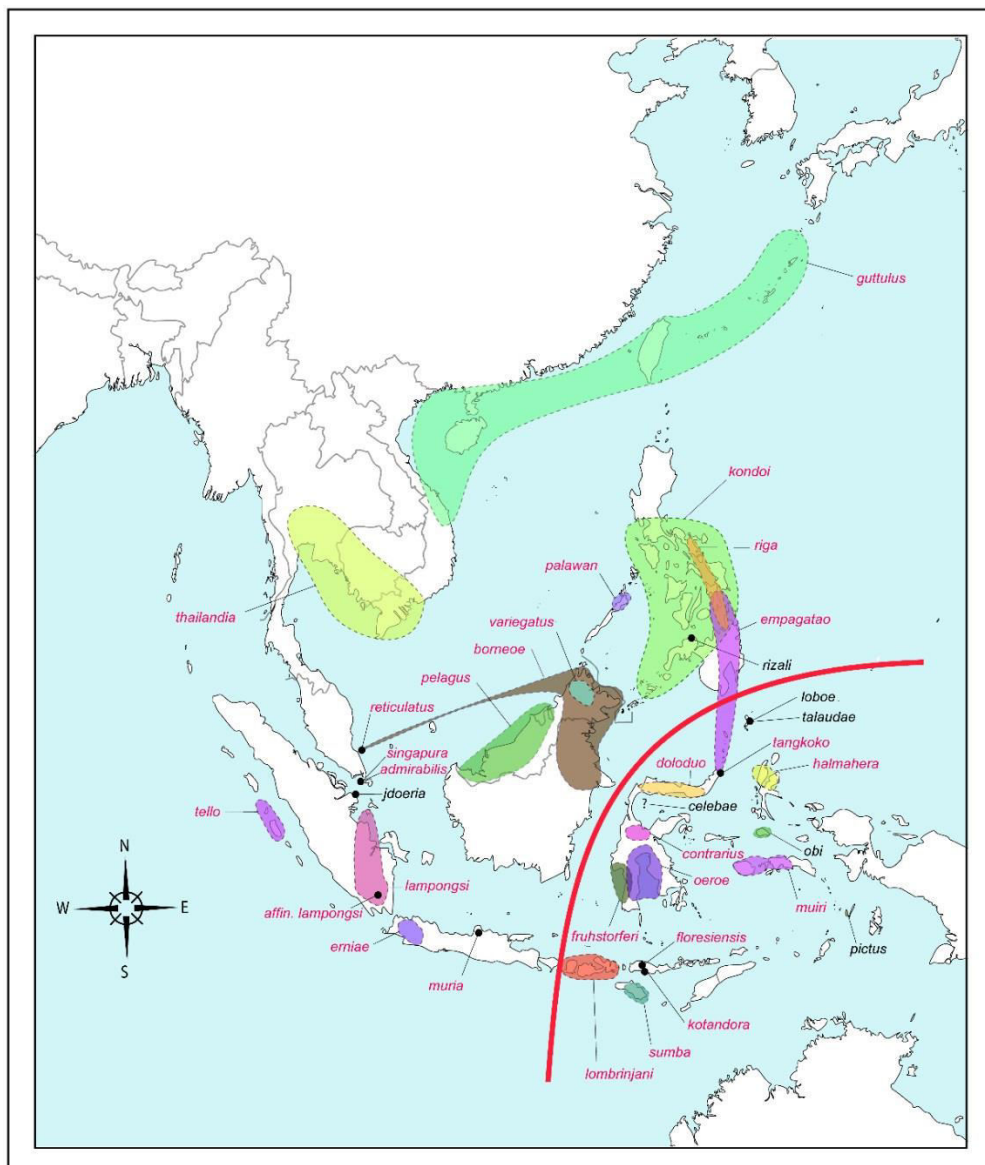
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Annexes

Annexes I - Supporting Information for Chapter II

Appendix S1. Distribution map of *Cardiodactylus* in Southeast Asia

The genus *Cardiodactylus* comprises two species groups, *Efordi* and *Novaeguineae* showing contrasted patterns of geographical distributions. Only one species of the *Efordi* species group is distributed in SEA, the other species being distributed in New Guinea and in archipelagoes of the Western Pacific. In contrast, the *Novaeguineae* species group has a wider distribution. In this study, we sampled 35 out of 41 species of the members of this species group distributed in SEA, which names are in pink on the map.

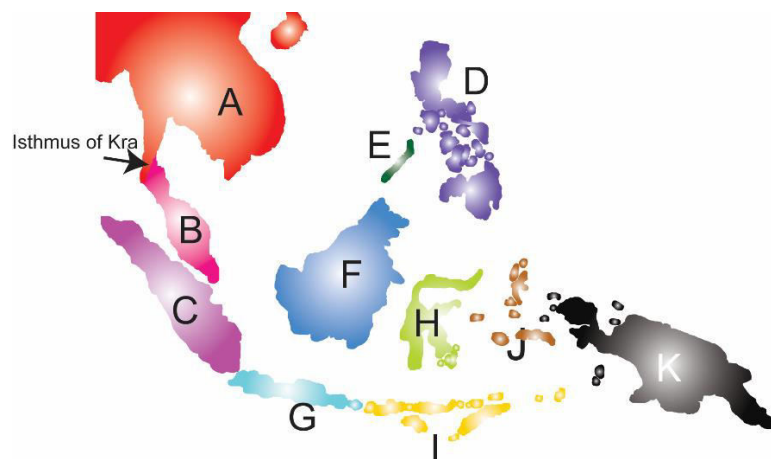


Appendix S2. Time-stratified biogeographical standard implemented in BioGeoBEARs analysis.

1. Definition of geographical areas

The map of SEA and surrounding regions was subdivided into 12 areas for the purpose of the biogeographic analysis: A: Indochina, B: Thai-Malay Peninsula, C: Sumatra, D: Philippines (except Palawan island), E: Palawan, F: Borneo, G: Java, H: Sulawesi, I: Lesser Sunda Islands, J: Moluccas, K: New Guinea, L: Western Pacific.

Here Indochina corresponds to the Southeast Asia mainland, including the northern Ryukyu Islands (but not showed on the simplified map), southeastern China, Taiwan, Vietnam and northern Thailand. The boundary between Indochina and Thai-Malay Peninsula correspond to the Isthmus of Kra which intersects the north and south of Thailand. Western Pacific is not shown on the map, but includes the archipelagoes located eastern from New Guinea.



2. Dispersal rates:

The matrix of scaling factors (between 0 and 1.0) for dispersal rates between areas were constructed according to the geographical position of the areas during a given time slice, interpreting greater distances and/or the extent of geographical barriers (inland river, shallow sea, deep sea) as being inversely proportional to the expected rate (Condamine et al., 2013; Toussaint & Balke, 2016). The factors were set as follows: no geological barrier between two connected areas: 1; minor geological barrier between two areas, e.g. inland river, shallow sea water: 0.75; large geological barrier between two areas, e.g. deep sea, strait, high mountain: 0.25; long-distance dispersal between two areas: 0.01.; absence of one area at a given time: 0. In addition, if two areas are separated by one area, the factor is given 0.5, 0.25 or 0.1: 0.5 when involving no minor barrier; 0.25 when the area in between involves a minor geological barriers; and 0.1 when the area in between involves a large geological barriers.

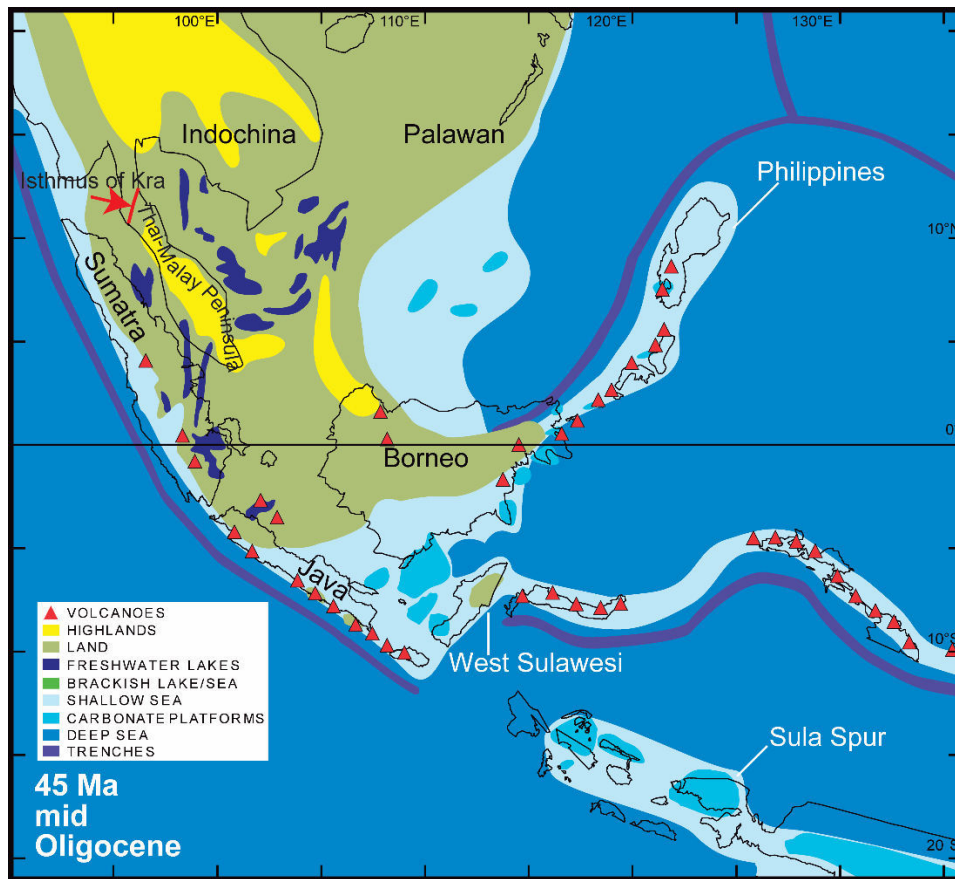
3. Time slices and dispersal multiplier matrix:

The core SEA continent comes from the Southern Hemisphere supercontinent Gondwana and initially assembled in the Late Paleozoic (300 Ma). The collision events and the resumption of subduction that happened during the Cenozoic had a great impact on the terrestrial biogeographic patterns and the current archipelago setting (Hall, 2013). Based on this, the time-stratified biogeographical models were defined according to three distinct time slices following three major paleogeological events in SEA):

3.1. Time slice 1: Eocene to Mid Oligocene (ca. 45-25 Ma)

The India-Asia collision was related to the 45 Ma plate reorganization during the Eocene, resulting in Australia's northward moving. The Makassar Straits became a wide marine barrier and separated west Sulawesi from core SEA. The Reed Bank-Palawan-Mindoro Block separated from the South China margin by the opening of the South China Sea during the Oligocene (Hall, 2002). However, at that period Palawan was still a part of Southeast China Mainland. In the Eocene, the southern part of the Philippines, with the Halmahera Arc, formed part of an arc system which extended into the western Pacific at an equatorial/southern hemisphere position. The northern Philippines, principally Luzon, was connected via the Sulu-Cagayan Arc to northern Borneo and Sabah before the Miocene (Hall, 2002). Although the collision and subduction event took actively, oceanic islands in the Wallacean region and New Guinea did not emerge from the sea (Hall, 2013). During this period, some islands or parts of current island in the Western Pacific began to emerge, which is for example the case of New Caledonia that emerged 37 Ma (Grandcolas *et al.*, 2008) or the Fiji islands, which were formed by subduction with the oldest rocks being from a volcanic island-arc of late Eocene (34–37 Ma; Neall & Trewick, 2008).

Therefore, the dispersal among Indochina, Palawan and Sundaland (comprising the Thai-Malay Peninsula [B], most of the present-day Sumatra [C], Java [G] and Borneo [F]) was facilitated, as well as within Sundaland. The factors of dispersal rate between above areas (except Borneo [F]) and the Philippines (except Palawan [E]) could be given 0.25 considering the ocean as a barrier. The factor of dispersal rate between Palawan [E] and Borneo (F) would be given 0.5 considering the presence of small continuous fragments of islands between these areas. In addition, the dispersal rate from Western Pacific to Southeast Asia could be taken as long-distance dispersal (probability of 0.01).



Reconstructed map of SEA ca. 45 Ma showing the areas corresponding to time slice 1 (modified from de Bruyn et al., 2014).

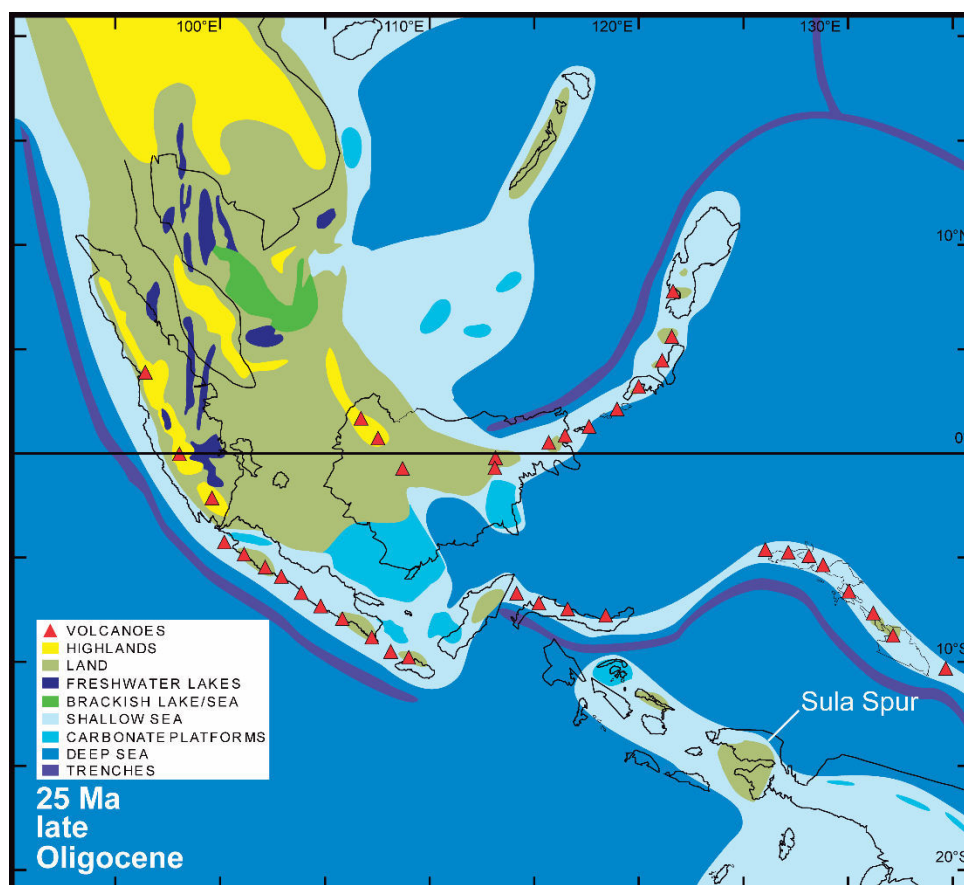
Matrix of scaling factors for dispersal rate in time slice 1 (45 to 25 Ma)

	A	B	C	D	E	F	G	H	I	J	K	L
A	1	1	0.5	0.25	1	0.5	0.25	0.1	0	0	0	0.01
B		1	1	0.25	0.75	0.75	0.75	0.1	0	0	0	0.01
C			1	0.25	0.75	1	1	0.1	0	0	0	0.01
D				1	0.1	0.5	0.25	0.1	0	0	0	0.01
E					1	0.75	0.5	0.1	0	0	0	0.01
F						1	1	0.25	0	0	0	0.01
G							1	0.25	0	0	0	0.01
H								1	0	0	0	0.01
I									0	0	0	0
J										0	0	0
K											0	0
L												1

3.2. Time slice 2: Mid Oligocene to Mid Miocene (ca. 25-15 Ma)

Between the Eocene and Early Miocene, the southern and southeast margin of Sundaland were volcanic arcs (Hall, 2002). The volcanic arcs located on the southern and southeast margin of Sundaland were active and these events made a minor inner barrier within Sundaland (e.g. inland river). The Palawan Continental Terrane isolated from the mainland because of the opening of Southeast China Sea and rifted towards Borneo (Walia et al., 2012). New Guinea began to emerge, probably as small islands along the northern edge, but most of the areas, which form mountains nowadays, were covered by shallow sea (Hall, 2009, 2013). Oceanic islands emerged from the sea and rift deformation formed some parts of the Philippines and Sulawesi. In addition, deformation and uplift began in Borneo, Java and Sulawesi.

Therefore, there are minor barriers within Sundaland (factor of dispersal of could 0.75). Long-distance dispersal was attributed to dispersal events between SEA and New Guinea, as well as between SEA and Western Pacific. However, the emergence of continuous small fragments / islands between Sulawesi and New Guinea probably increased the chances for dispersal events (dispersal factor 0.75).



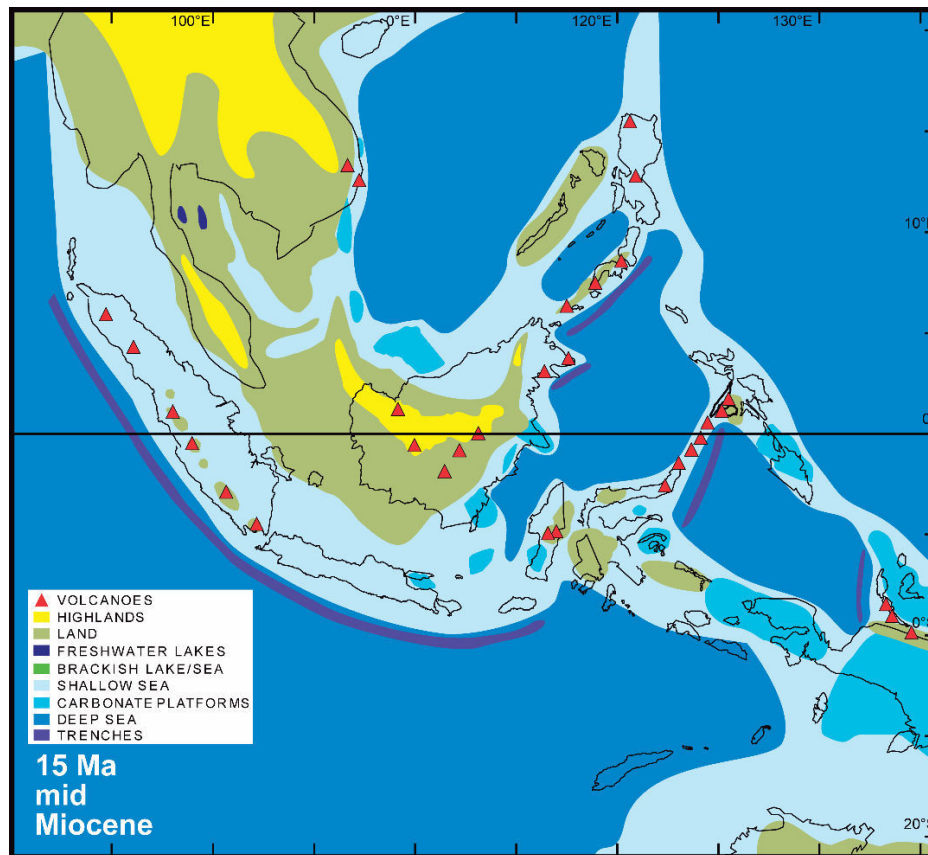
Reconstructed map of SEA ca. 25 Ma showing the areas corresponding to time slice 2 (modified from de Bruyn et al., 2014).

Matrix of scaling factors for dispersal rate in time slice 2 (25 to 15 Ma).

	A	B	C	D	E	F	G	H	I	J	K	L
A	1	1	0.5	0.1	0.25	0.75	0.25	0.1	0	0	0.01	0.01
B		1	0.75	0.25	0.5	0.75	0.25	0.1	0	0	0.01	0.01
C			1	0.25	0.5	0.75	0.75	0.1	0	0	0.01	0.01
D				1	0.25	0.5	0.25	0.5	0	0	0.1	0.01
E					1	0.75	0.25	0.1	0	0	0.01	0.01
F						1	0.75	0.25	0	0	0.01	0.01
G							1	0.1	0	0	0.01	0.01
H								1	0	0	0.75	0.01
I									0	0	0	0.01
J										0	0	0.01
K											1	0.1
L												1

3.3. Time slice 3: Mid Miocene to present-day (ca. 15-0 Ma)

The Australian margin drifted from the continent, forming the Bird's Head region of New Guinea, (Sula Spur promontory). By the Mid Miocene, about 15 Ma, the Sula Spur promontory collided with the margin of SEA in Sulawesi, forming the region now known as Wallacea, including Philippines, the Moluccas, completed Sulawesi and Lesser Sunda Islands (Hall, 2009, Spakman & Hall 2010). The geological pattern in SEA did not change so much since these events. Thus, the factor of dispersal rate within Sundaland were the same as that in the times slice of 25-15 Ma. Considering the continuous of the small islands involved in the origin of the Wallacean region, the factor of dispersal rate between the Philippines and Palawan, the Philippines and Borneo, the Philippines and Moluccas, and the Moluccas and New Guinea is set to 0.75.



Reconstructed map of SEA ca. 15 Ma showing the areas corresponding to time slice 3 (modified from de Bruyn et al., 2014).

Matrix of scaling factors for dispersal rate in time slice 3 (15 Ma to present day).

	A	B	C	D	E	F	G	H	I	J	K	L
A	1	1	0.5	0.1	0.25	0.75	0.1	0.1	0.01	0.01	0.01	0.01
B		1	0.75	0.1	0.25	0.75	0.25	0.1	0.1	0.01	0.01	0.01
C			1	0.25	0.25	0.75	0.75	0.1	0.25	0.01	0.01	0.01
D				1	0.75	0.75	0.25	0.5	0.25	0.75	0.25	0.01
E					1	0.75	0.25	0.1	0.25	0.1	0.01	0.01
F						1	0.75	0.25	0.5	0.1	0.01	0.01
G							1	0.1	0.75	0.1	0.01	0.01
H								1	0.5	0.75	0.25	0.01
I									1	0.5	0.25	0.01
J										1	0.75	0.25
K											1	0.5
L												1

4. Alternative dispersal multipliers

We implemented alternative sets of dispersal multiplier to test the robustness of the analysis and limit the impact of the arbitrariness of the probability attributed to each event. Three more simplified sets of multipliers were tested by following formulation: except the probabilities of 0, 0.01 and 1 that remained unchanged, the events with probabilities of 0.25, 0.5 and 0.75 were given homogenous values in the alternative sets of multipliers. The three simplified sets of multipliers are presented below.

Simplified set of dispersal multipliers 1 (0, 0.1, 0.25, 1.0) – probabilities of 0.25, 0.5 and 0.75 in the base matrix are given a probability of 0.25.

time slice 1: 45-25 Ma												
	A	B	C	D	E	F	G	H	I	J	K	L
A	1	1	0.25	0.25	1	0.25	0.25	0.1	0	0	0	0.01
B		1	1	0.25	0.25	0.25	0.25	0.1	0	0	0	0.01
C			1	0.25	0.25	1	1	0.1	0	0	0	0.01
D				1	0.1	0.25	0.25	0.1	0	0	0	0.01
E					1	0.25	0.25	0.1	0	0	0	0.01
F						1	1	0.25	0	0	0	0.01
G							1	0.25	0	0	0	0.01
H								1	0	0	0	0.01
I									0	0	0	0
J										0	0	0
K											0	0
L												1
time slice 2: 25-15 Ma												
	A	B	C	D	E	F	G	H	I	J	K	L
A	1	1	0.25	0.1	0.25	0.75	0.25	0.1	0	0	0.01	0.01
B		1	0.25	0.25	0.25	0.75	0.25	0.1	0	0	0.01	0.01
C			1	0.25	0.25	0.75	0.75	0.1	0	0	0.01	0.01
D				1	0.25	0.25	0.25	0.25	0	0	0.1	0.01
E					1	0.25	0.25	0.1	0	0	0.01	0.01
F						1	0.25	0.25	0	0	0.01	0.01
G							1	0.1	0	0	0.01	0.01
H								1	0	0	0.25	0.01
I									0	0	0	0.01
J										0	0	0.01
K											1	0.1
L												1
time slice 3: 15 Ma to present												
	A	B	C	D	E	F	G	H	I	J	K	L
A	1	1	0.25	0.1	0.25	0.25	0.1	0.1	0.01	0.01	0.01	0.01
B		1	0.25	0.1	0.25	0.25	0.25	0.1	0.1	0.01	0.01	0.01
C			1	0.25	0.25	0.25	0.75	0.1	0.25	0.01	0.01	0.01
D				1	0.25	0.25	0.25	0.25	0.25	0.75	0.25	0.01
E					1	0.25	0.25	0.1	0.25	0.1	0.01	0.01
F						1	0.25	0.25	0.25	0.1	0.01	0.01
G							1	0.1	0.25	0.1	0.01	0.01
H								1	0.25	0.25	0.25	0.01
I									1	0.25	0.25	0.01
J										1	0.25	0.25
K											1	0.25
L												1

Simplified set of dispersal multipliers 2 (0, 0.1, 0.5, 1.0) – probabilities of 0.25, 0.5 and 0.75 in the base matrix are given a probability of 0.5.

time slice 1: 45-25 Ma												
	A	B	C	D	E	F	G	H	I	J	K	L
A	1	1	0.5	0.5	1	0.5	0.5	0.1	0	0	0	0.01
B		1	1	0.5	0.5	0.5	0.5	0.1	0	0	0	0.01
C			1	0.5	0.5	1	1	0.1	0	0	0	0.01
D				1	0.1	0.5	0.5	0.1	0	0	0	0.01
E					1	0.5	0.5	0.1	0	0	0	0.01
F						1	1	0.5	0	0	0	0.01
G							1	0.5	0	0	0	0.01
H								1	0	0	0	0.01
I									0	0	0	0
J										0	0	0
K											0	0
L												1
time slice 2: 25-15 Ma												
	A	B	C	D	E	F	G	H	I	J	K	L
A	1	1	0.5	0.1	0.5	0.5	0.5	0.1	0	0	0.01	0.01
B		1	0.5	0.5	0.5	0.5	0.5	0.1	0	0	0.01	0.01
C			1	0.5	0.5	0.5	0.5	0.1	0	0	0.01	0.01
D				1	0.5	0.5	0.5	0.5	0	0	0.1	0.01
E					1	0.5	0.5	0.1	0	0	0.01	0.01
F						1	0.5	0.5	0	0	0.01	0.01
G							1	0.1	0	0	0.01	0.01
H								1	0	0	0.5	0.01
I									0	0	0	0.01
J										0	0	0.01
K											1	0.1
L												1
time slice 3: 15 Ma to present												
	A	B	C	D	E	F	G	H	I	J	K	L
A	1	1	0.5	0.1	0.5	0.5	0.1	0.1	0.01	0.01	0.01	0.01
B		1	0.5	0.1	0.5	0.5	0.5	0.1	0.1	0.01	0.01	0.01
C			1	0.5	0.5	0.5	0.5	0.1	0.5	0.01	0.01	0.01
D				1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.01
E					1	0.5	0.5	0.1	0.5	0.1	0.01	0.01
F						1	0.5	0.5	0.5	0.1	0.01	0.01
G							1	0.1	0.5	0.1	0.01	0.01
H								1	0.5	0.5	0.5	0.01
I									1	0.5	0.5	0.01
J										1	0.5	0.5
K											1	0.5
L												1

Simplified set of dispersal multipliers 3 (0, 0.1, 0.75, 1.0) – probabilities of 0.25, 0.5 and 0.75 in the base matrix are given a probability of 0.75.

time slice 1: 45-25 Ma												
	A	B	C	D	E	F	G	H	I	J	K	L
A	1	1	0.75	0.75	1	0.75	0.75	0.1	0	0	0	0.01
B		1	1	0.75	0.75	0.75	0.75	0.1	0	0	0	0.01
C			1	0.75	0.75	1	1	0.1	0	0	0	0.01
D				1	0.1	0.75	0.75	0.1	0	0	0	0.01
E					1	0.75	0.75	0.1	0	0	0	0.01
F						1	1	0.75	0	0	0	0.01
G							1	0.75	0	0	0	0.01
H								1	0	0	0	0.01
I									0	0	0	0
J										0	0	0
K											0	0
L												1
time slice 2: 25-15 Ma												
	A	B	C	D	E	F	G	H	I	J	K	L
A	1	1	0.75	0.1	0.75	0.75	0.75	0.1	0	0	0.01	0.01
B		1	0.75	0.75	0.75	0.75	0.75	0.1	0	0	0.01	0.01
C			1	0.75	0.75	0.75	0.75	0.1	0	0	0.01	0.01
D				1	0.75	0.75	0.75	0.75	0	0	0.1	0.01
E					1	0.75	0.75	0.1	0	0	0.01	0.01
F						1	0.75	0.75	0	0	0.01	0.01
G							1	0.1	0	0	0.01	0.01
H								1	0	0	0.75	0.01
I									0	0	0	0.01
J										0	0	0.01
K											1	0.1
L												1
time slice 3: 15 Ma to present												
	A	B	C	D	E	F	G	H	I	J	K	L
A	1	1	0.75	0.1	0.75	0.75	0.1	0.1	0.01	0.01	0.01	0.01
B		1	0.75	0.1	0.75	0.75	0.75	0.1	0.1	0.01	0.01	0.01
C			1	0.75	0.75	0.75	0.75	0.1	0.75	0.01	0.01	0.01
D				1	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.01
E					1	0.75	0.75	0.1	0.75	0.1	0.01	0.01
F						1	0.75	0.75	0.75	0.1	0.01	0.01
G							1	0.1	0.75	0.1	0.01	0.01
H								1	0.75	0.75	0.75	0.01
I									1	0.75	0.75	0.01
J										1	0.75	0.75
K											1	0.75
L												1

References

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Appendix S3. Summary of ancestral area estimation with different models and dispersal rate multipliers. The gray line indicates the most supported model.

Multipliers sets	max_range_size	Speciation model	LnL	Parameter estimates		
				d	e	j
(0.01,0.1,0.25,0.05,0.75,1.0)	2	DEC	- 128.05	0.024	0.0175	0.0000
		DEC+j	- 113.82	0.009	0.0072	0.1632
	3	DEC	- 131.32	0.0163	0.0162	0.0000
		DEC+j	- 115.63	0.0067	0.007	0.1166
	4	DEC	- 131.55	0.0151	0.0154	0.0000
		DEC+j	- 115.26	0.0073	0.0069	0.1670
(0.01, 0.1,0.25, 1.0)	2	DEC	- 128.99	0.0383	0.0168	0.0000
		DEC+j	- 114.02	0.0145	0.0068	0.2302
(0.01, 0.1, 0.05, 1.0)	2	DEC	- 130.83	0.0217	0.0172	0.0000
		DEC+j	- 116.57	0.0083	0.0072	0.1500
(0.01, 0.1, 0.75, 1.0)	2	DEC	- 132.14	0.0152	0.0175	0.0000
		DEC+j	- 118.42	0.0059	0.0075	0.1059

Appendix S4. Sampling of species groups in BMM analysis.

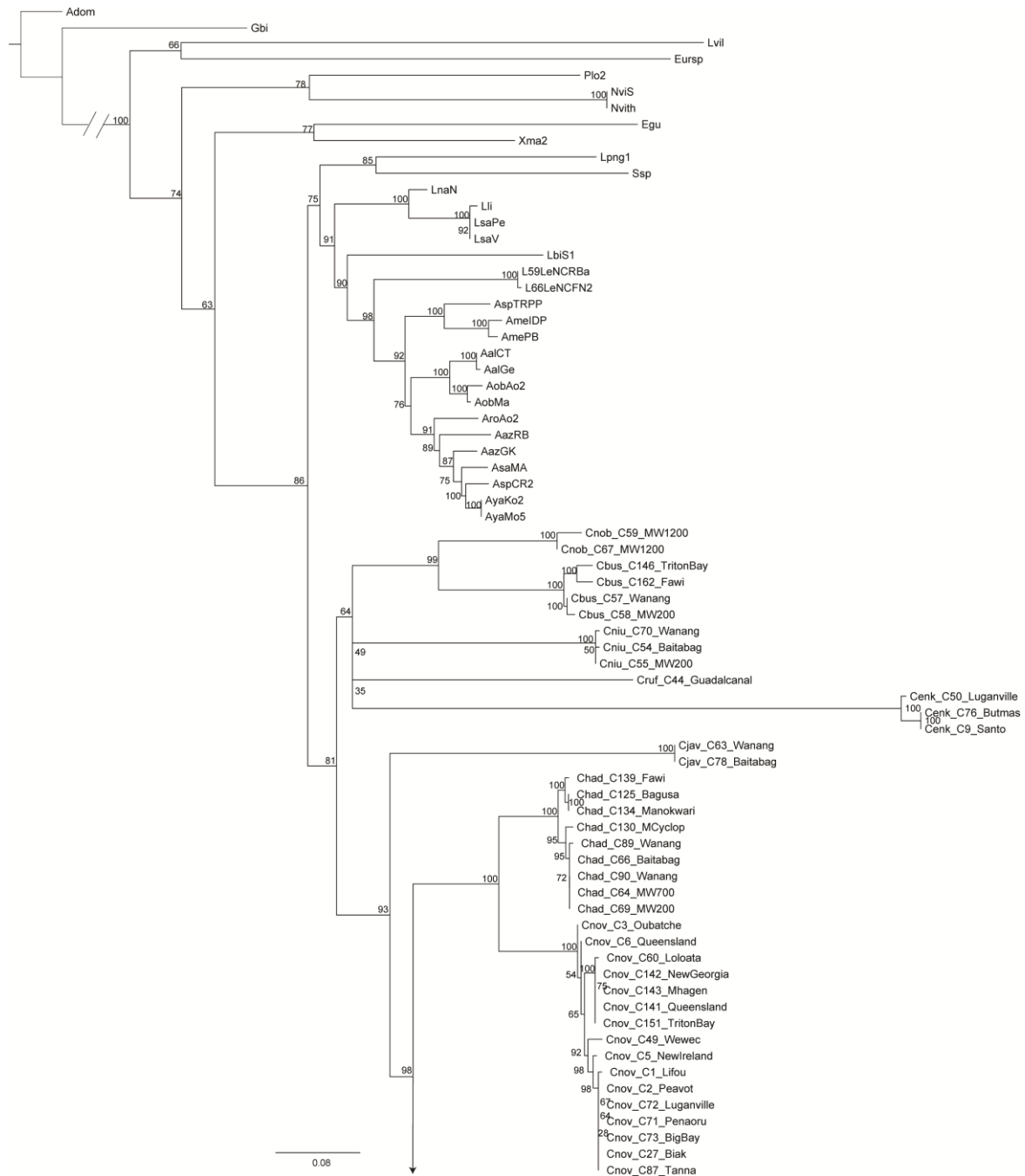
Because *C. javarere* and *C. kolombangara* show intermediate characters between *Novaeguineae* and *Efordi* species groups (Dong & Robillard, 2016), these two species form the third species group, called here *Javarere* species group. Therefore, there are 60 species in *Novaeguineae* species group, 20 in *Efordi* species group and two in *Javarere* species group. In total, there are 35 species of the *Novaeguineae* species group and one of the *Efordi* species group in SEA. The number of species in each species group and the sampling fraction in BMM analysis were given in the table below:

Species group	Total numbers	Numbers in SEA	Sampling fraction
<i>Novaeguineae</i>	60/35	35/28	0.58
<i>Efordi</i>	20/5	1/0	0.25
<i>Javarere</i>	2/1	0/0	0.5

Note: Numbers (*/*) in the column named "Total numbers" correspond to the total numbers in each species group (left of sign /) and the species numbers in this study (right of sign /), respectively. Numbers (*/*) in the column named "Numbers in SEA" correspond to the total numbers in each species group distributed in SEA (left of sign /) and the species numbers in this study in SEA (right of sign /), respectively. "Sampling fraction" corresponds to the proportion of the number of species sampled in this study vs total numbers of each species group.

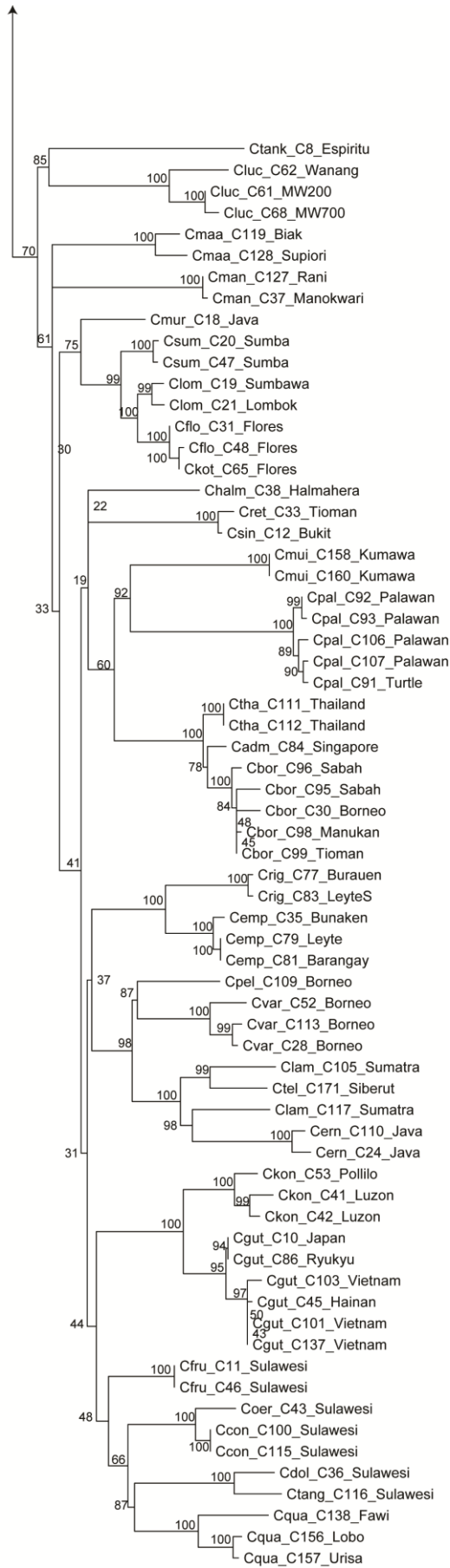
Appendix S5. Results of preliminary maximum likelihood (ML) analyses for each marker: 16S (a), 12S (b), COI (c), COII (d), Cytb (e), 18S (f), 28S (g), EF1a (h), H3 (i).

16S (a):

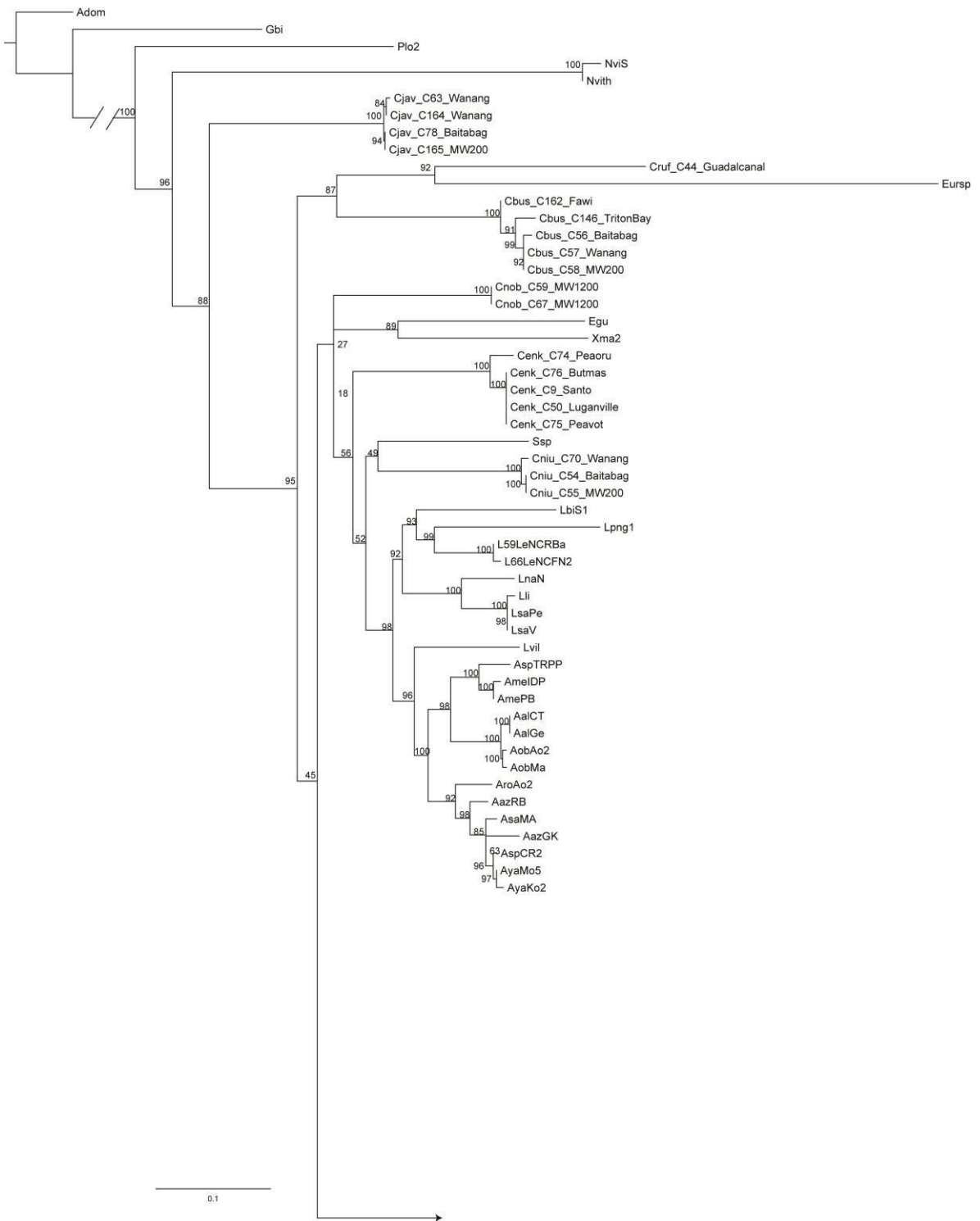


(continued)

16S (a): continued

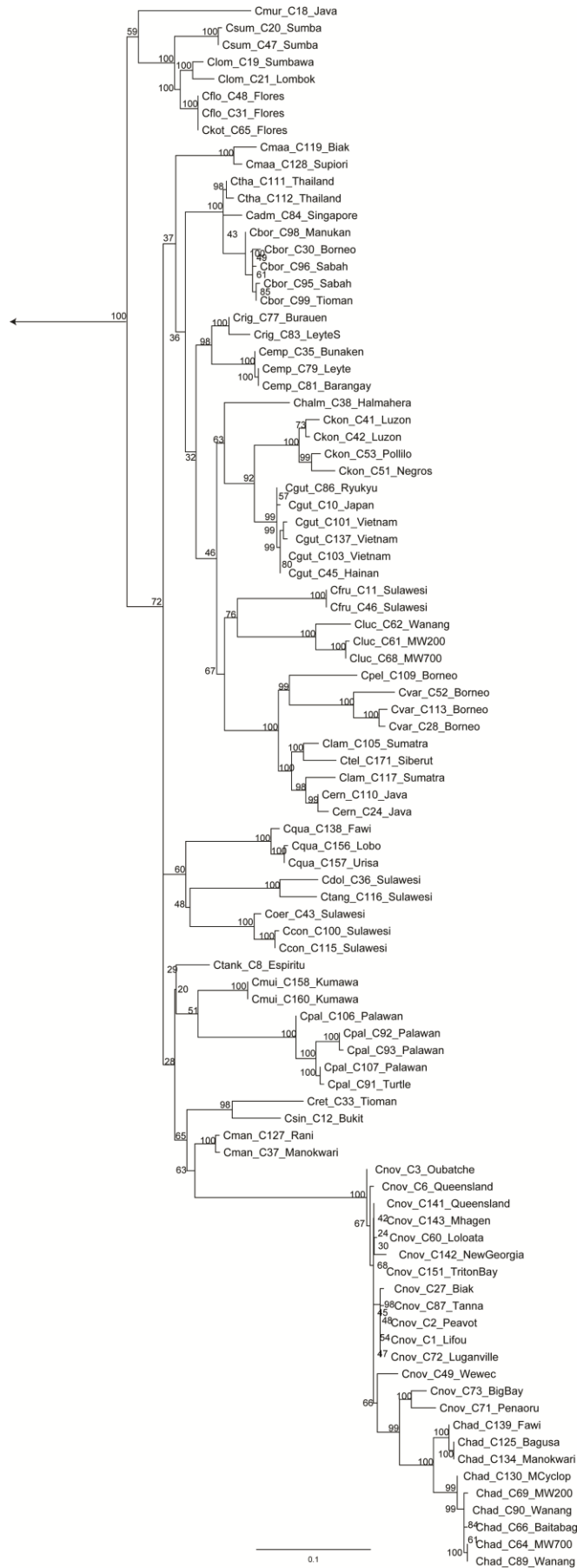


12S (b):

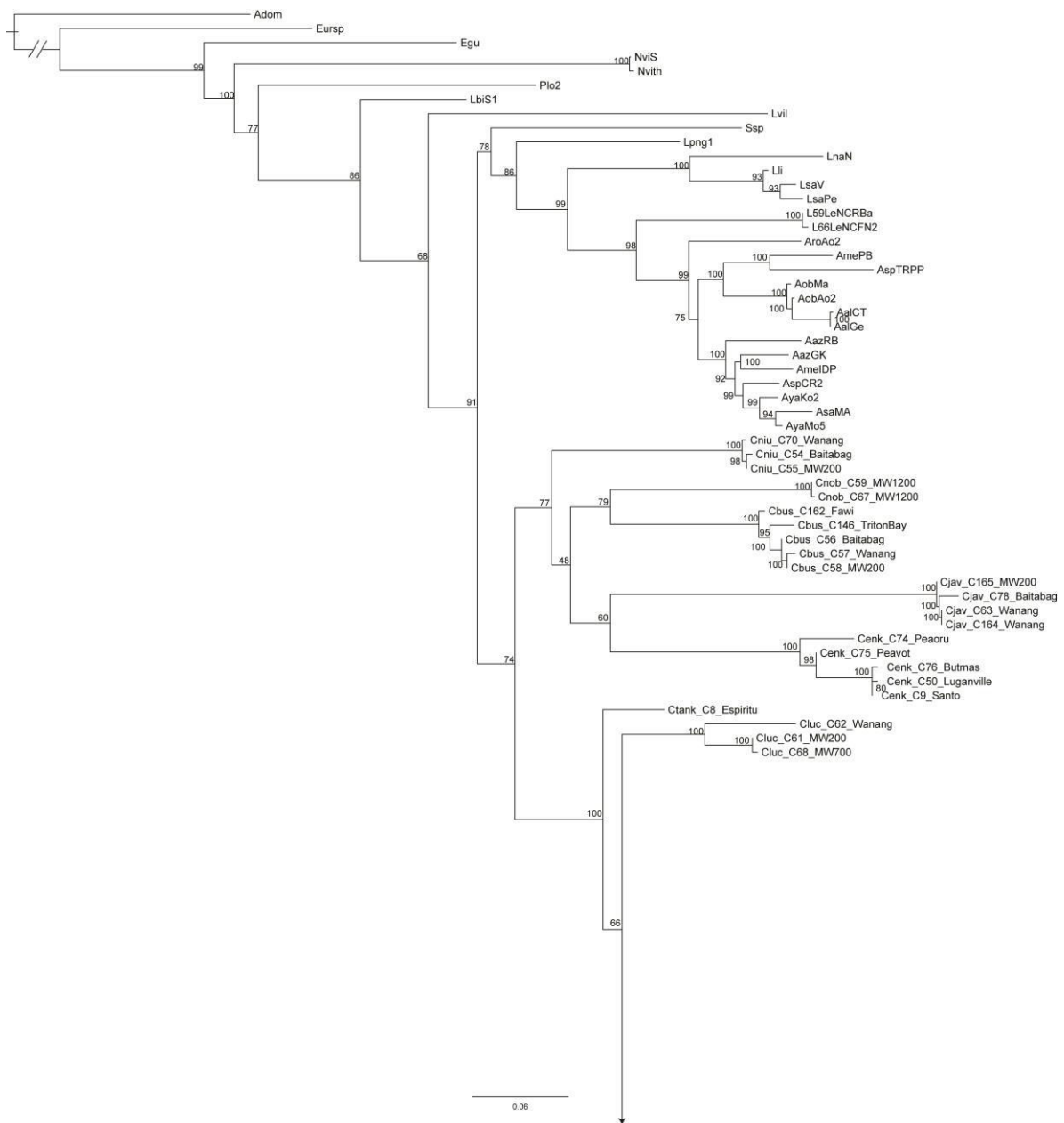


(continued)

12S (b): continued

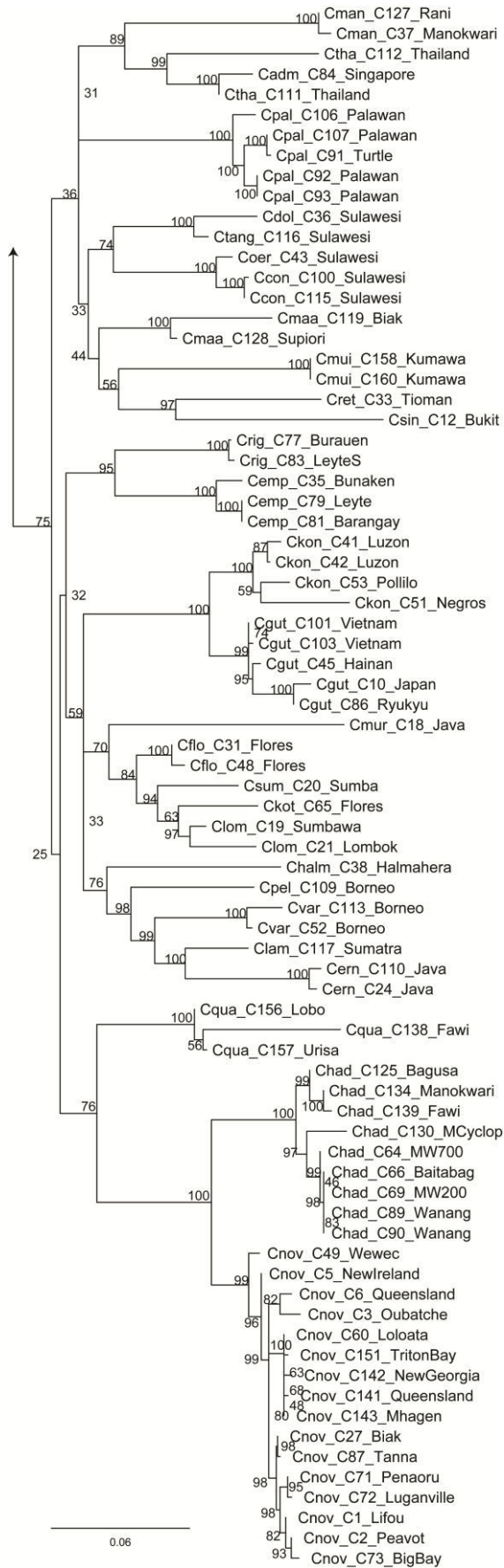


COI (c):

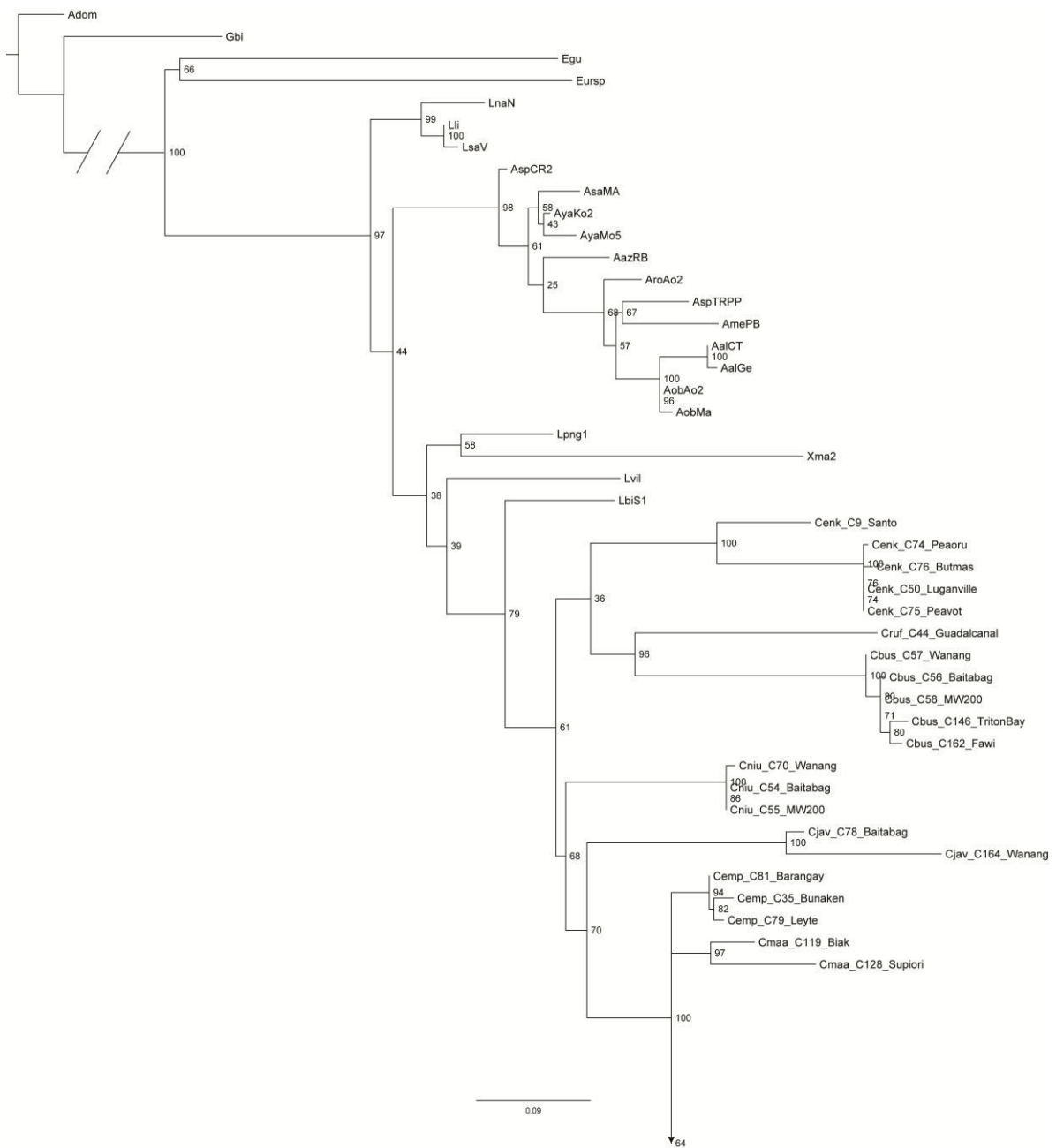


(continued)

COI (c): continued

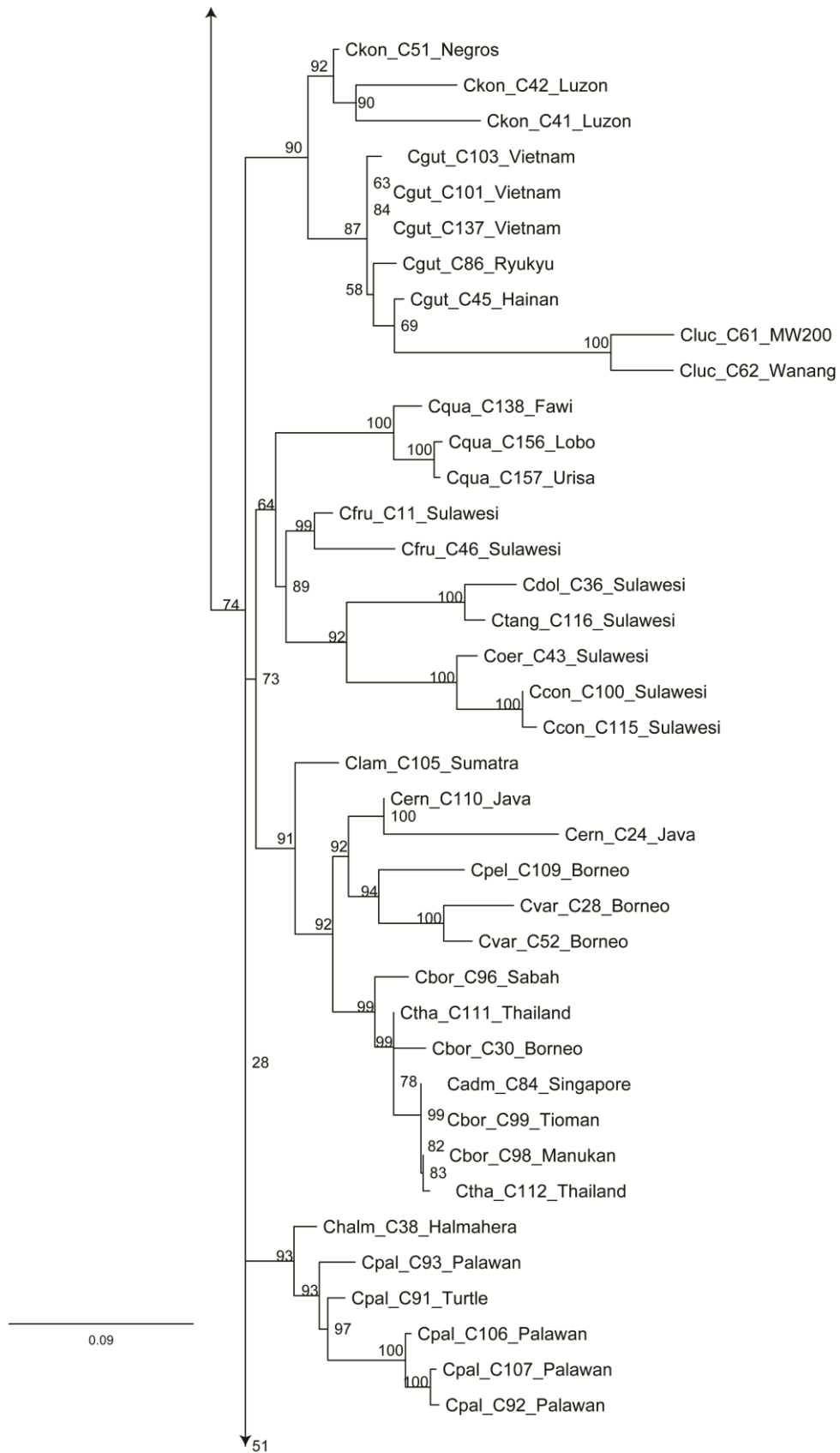


COII (d):



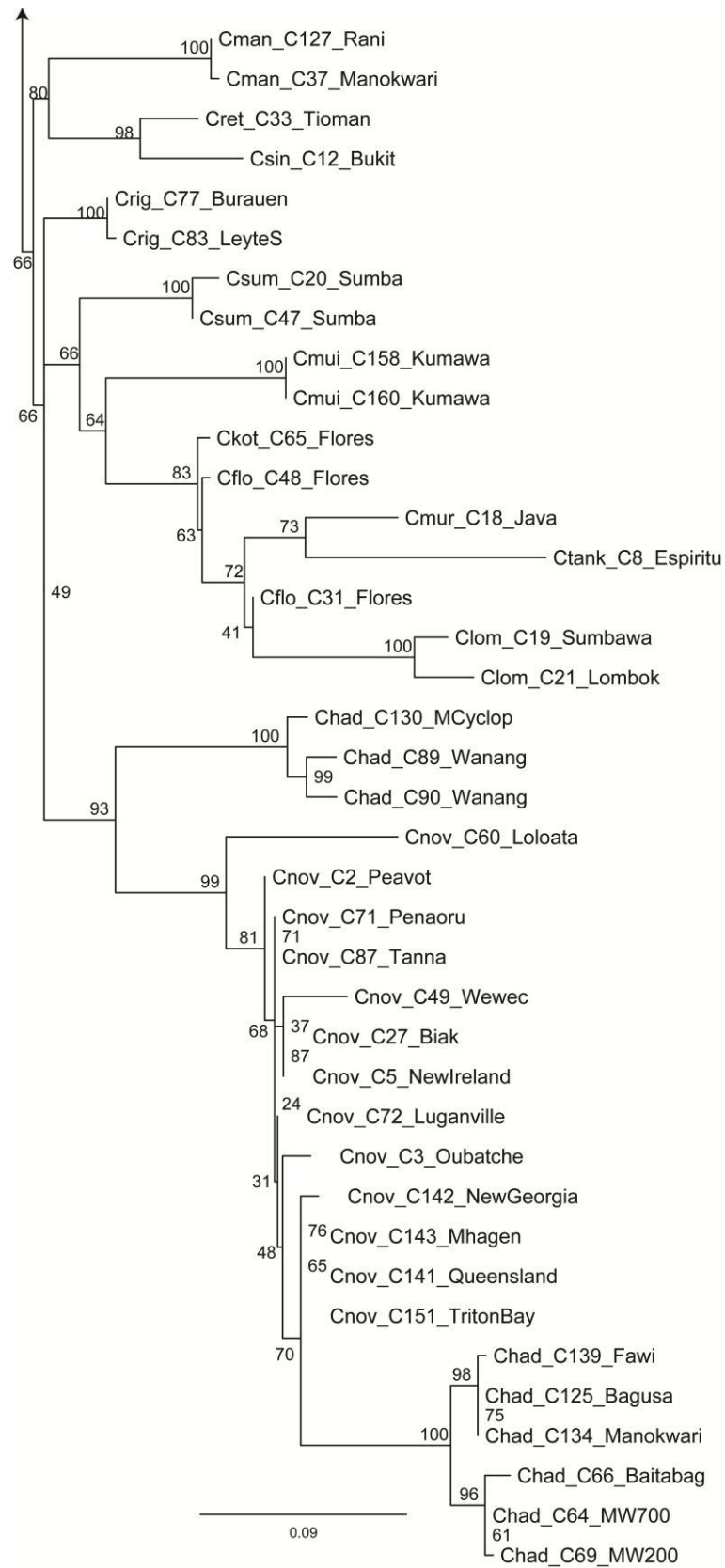
(continued)

COII (d): continued

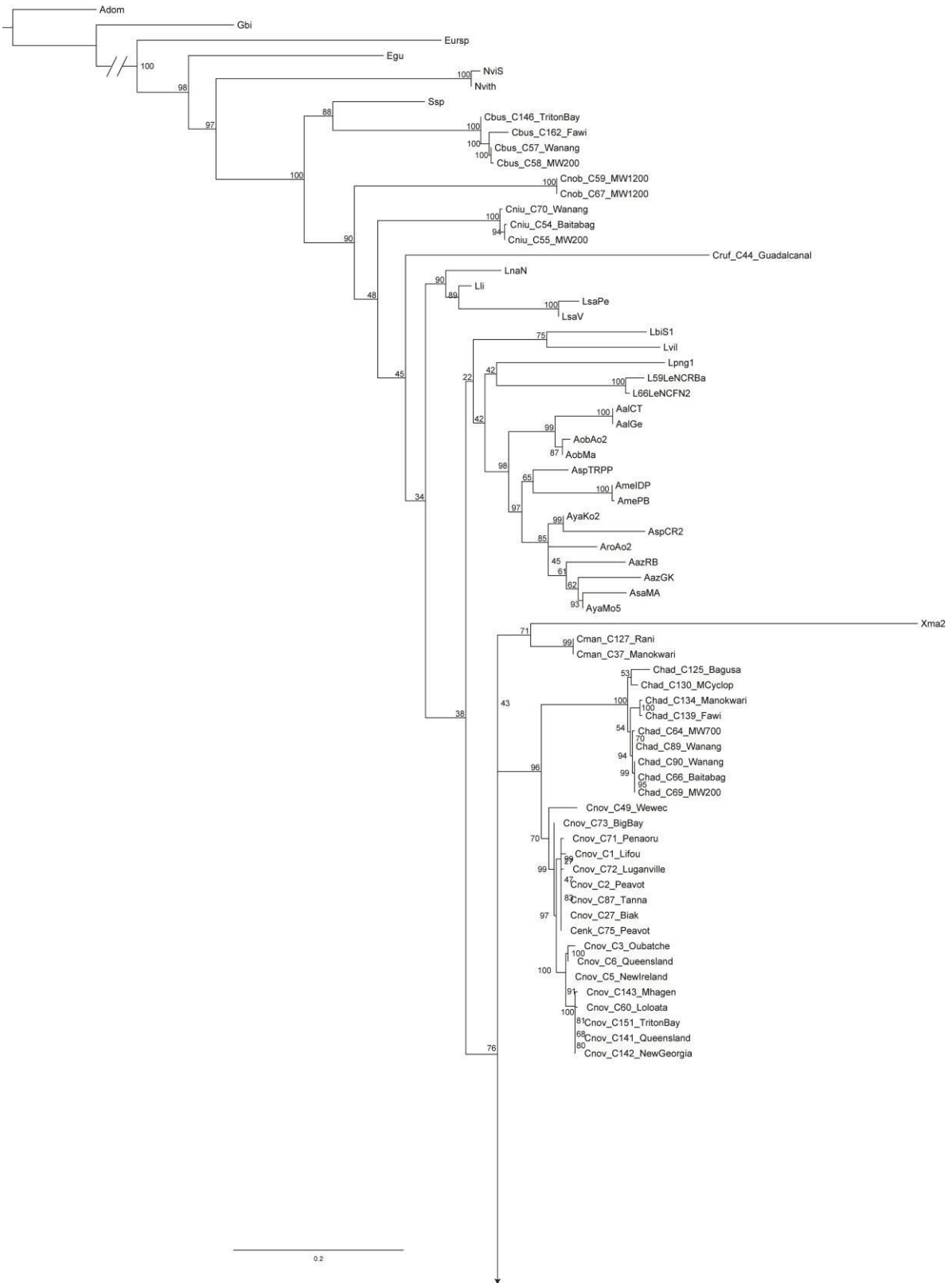


(continued)

COII (d): continued

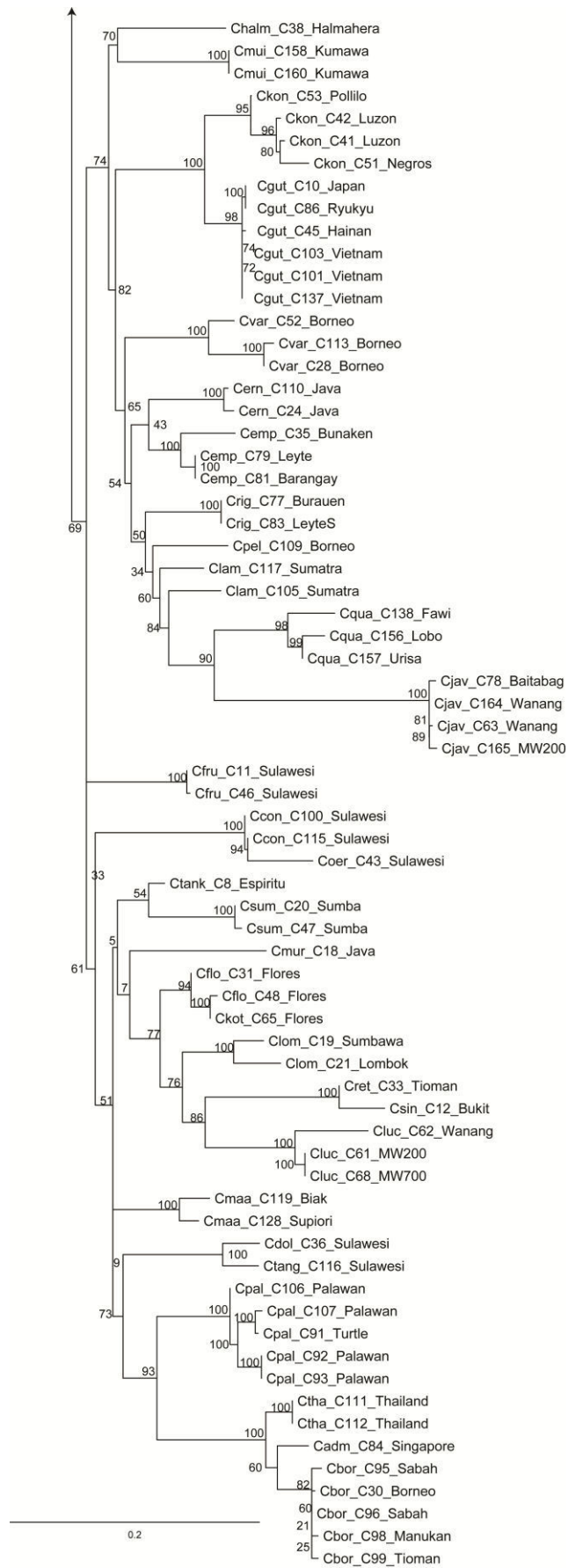


Cytb (e):

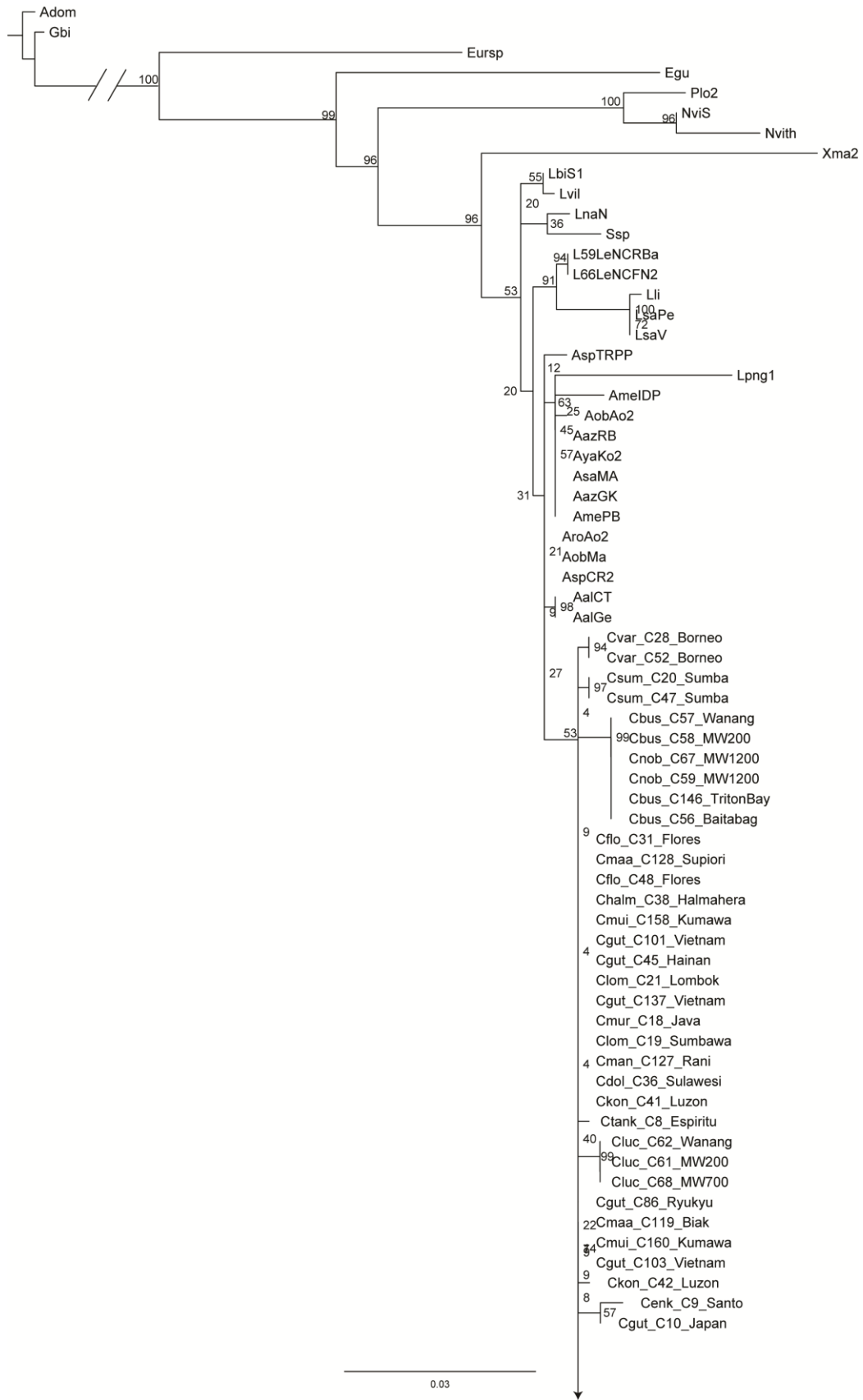


(continued)

Cytb (e): continued

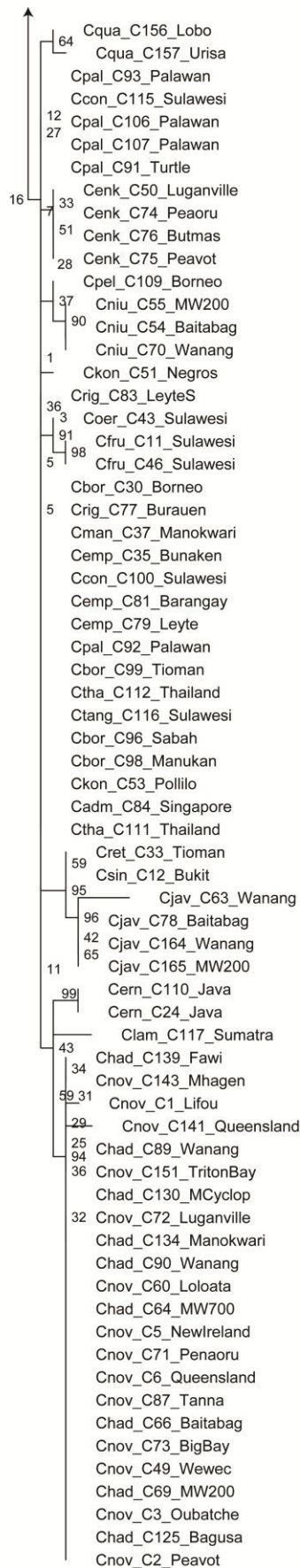


18S (f):

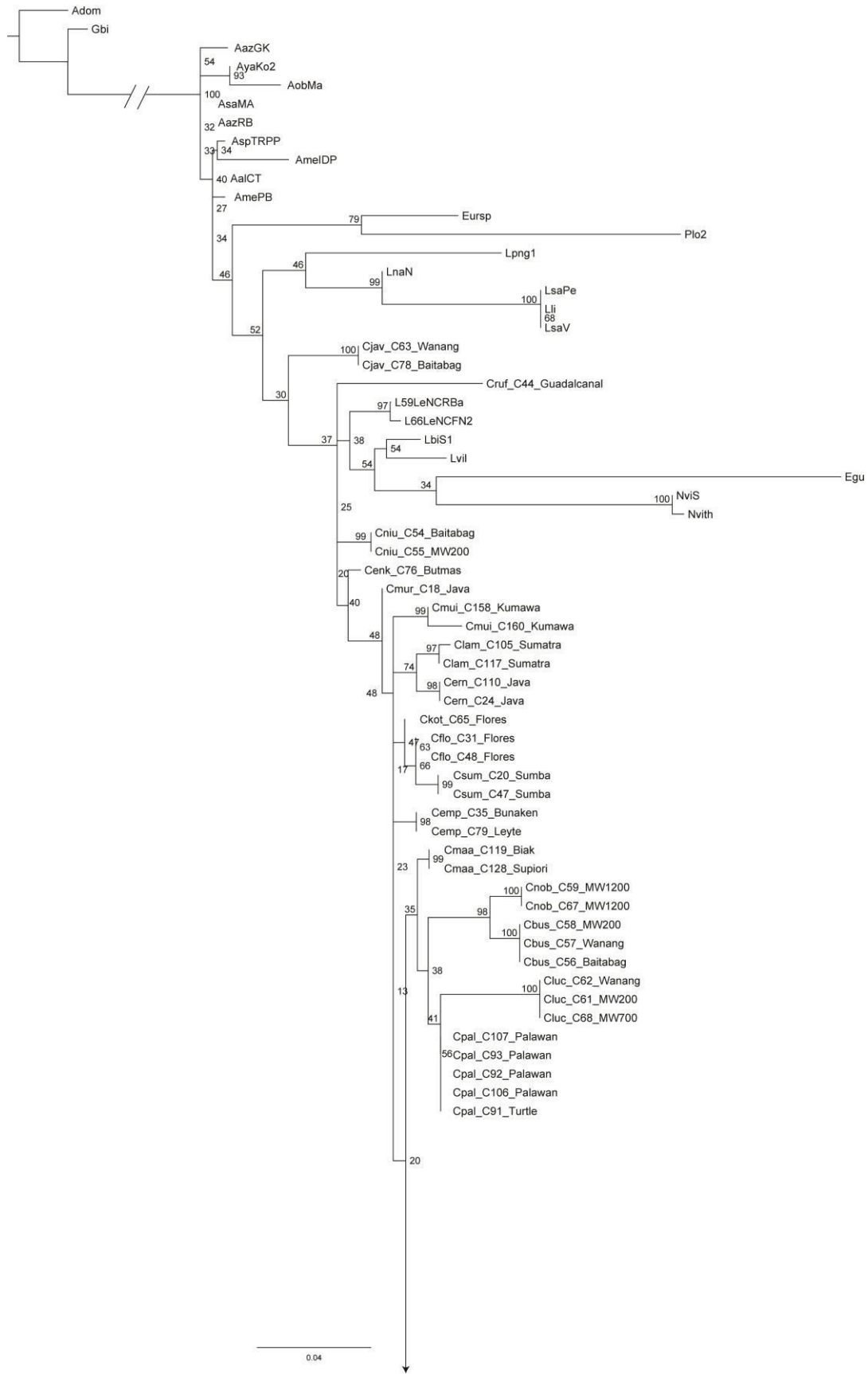


(continued)

18S (f): continued

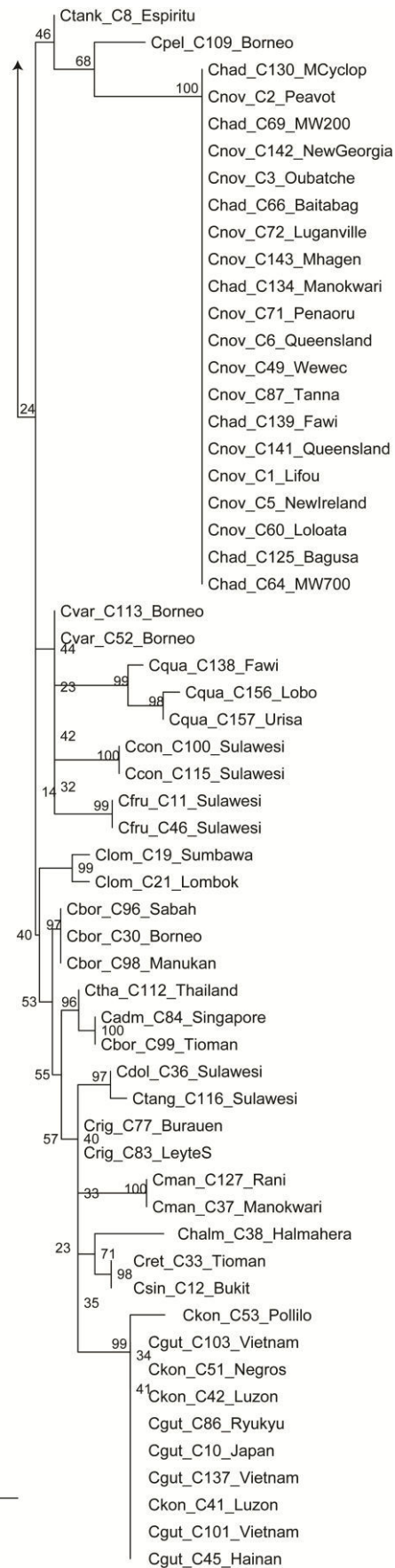


28S (g):

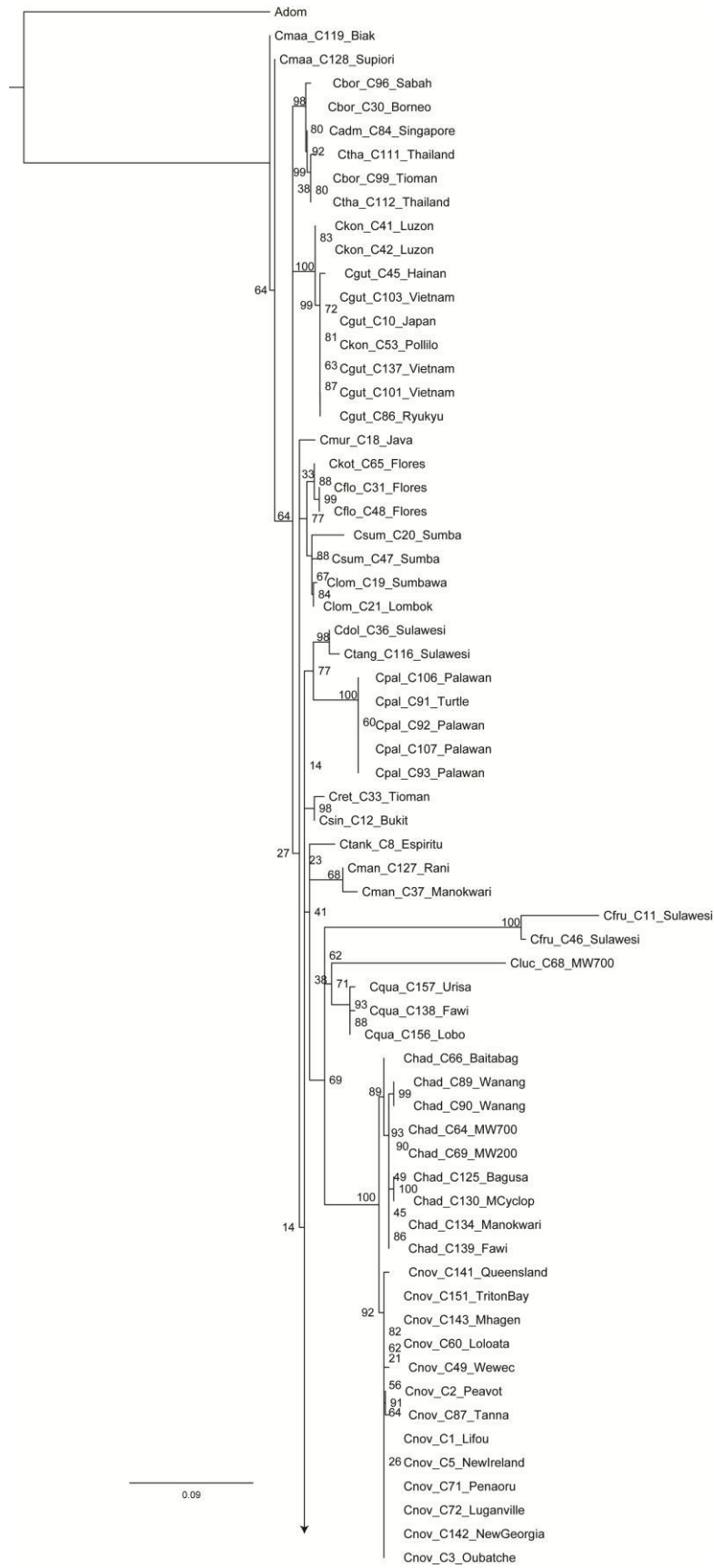


(continued)

28S (g): continued

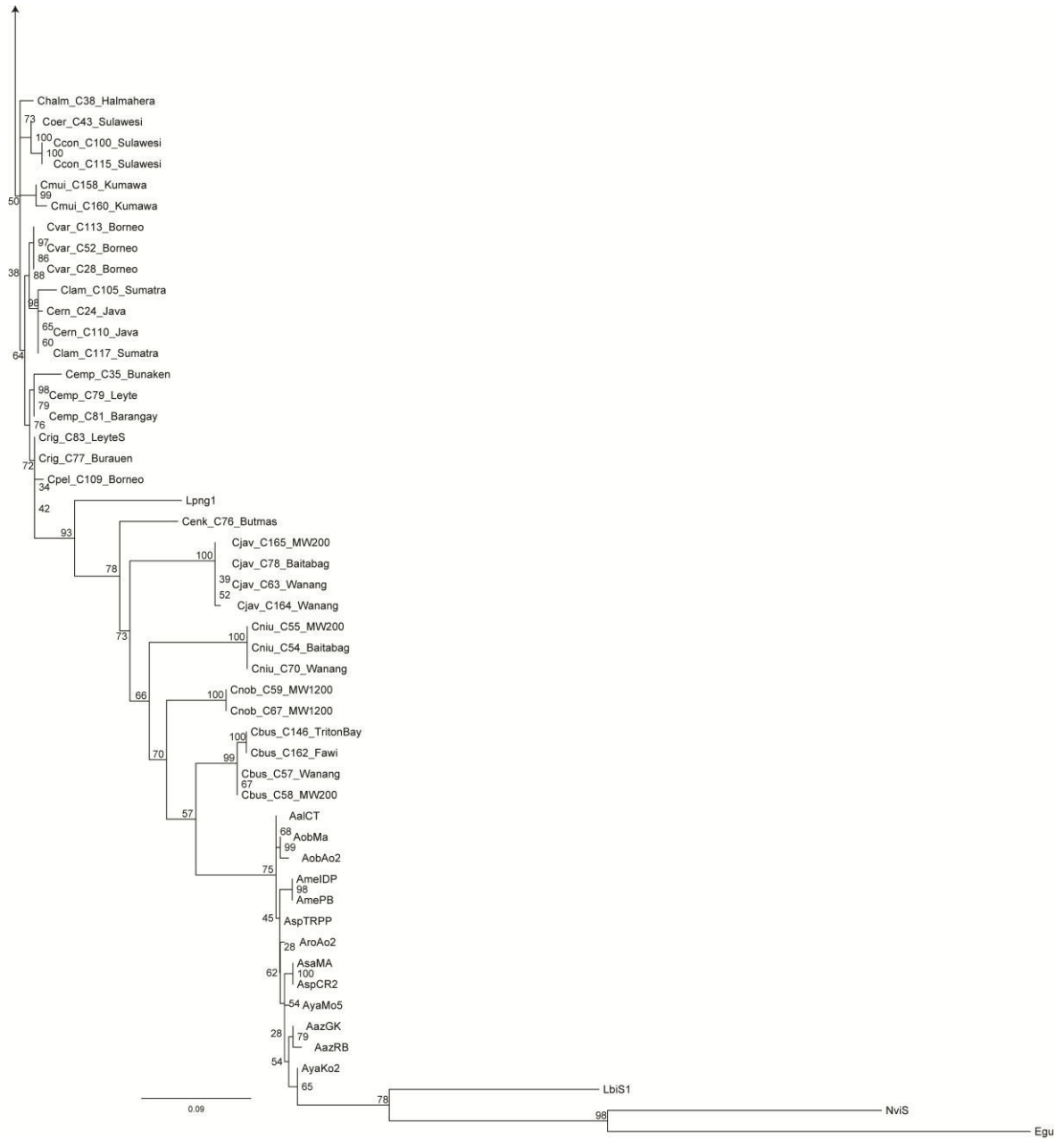


EF1a (h):



(continued)

EF1a (h): continued

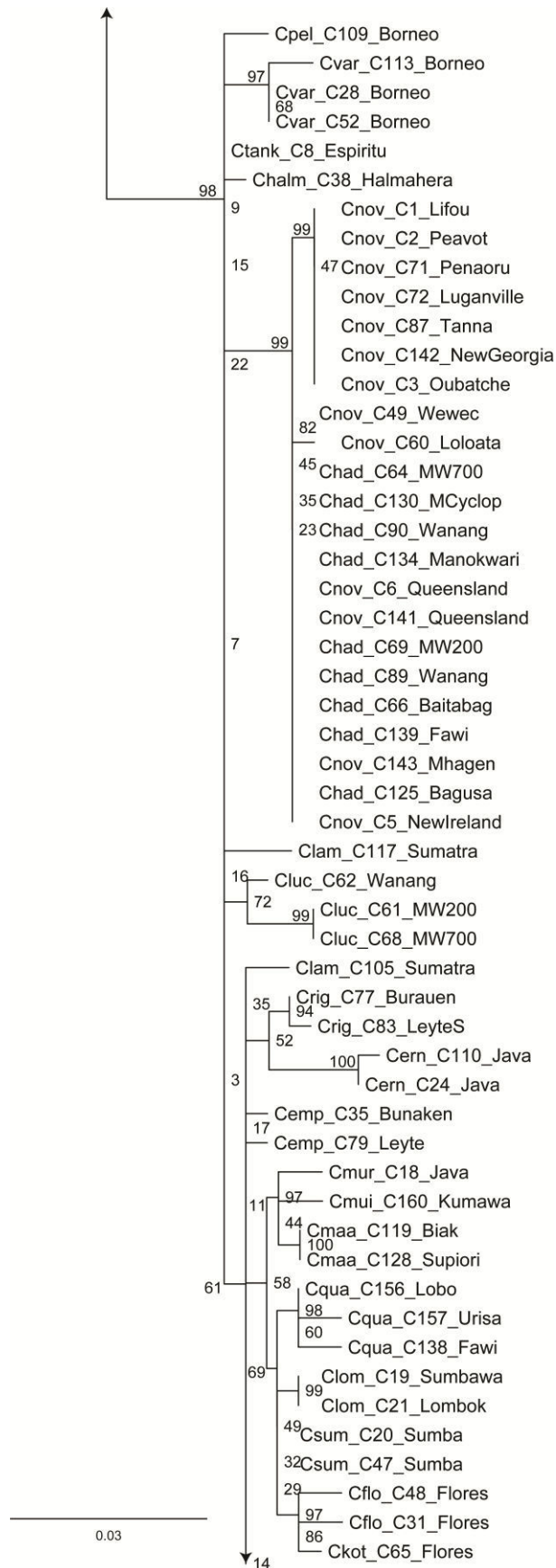


H3 (i):



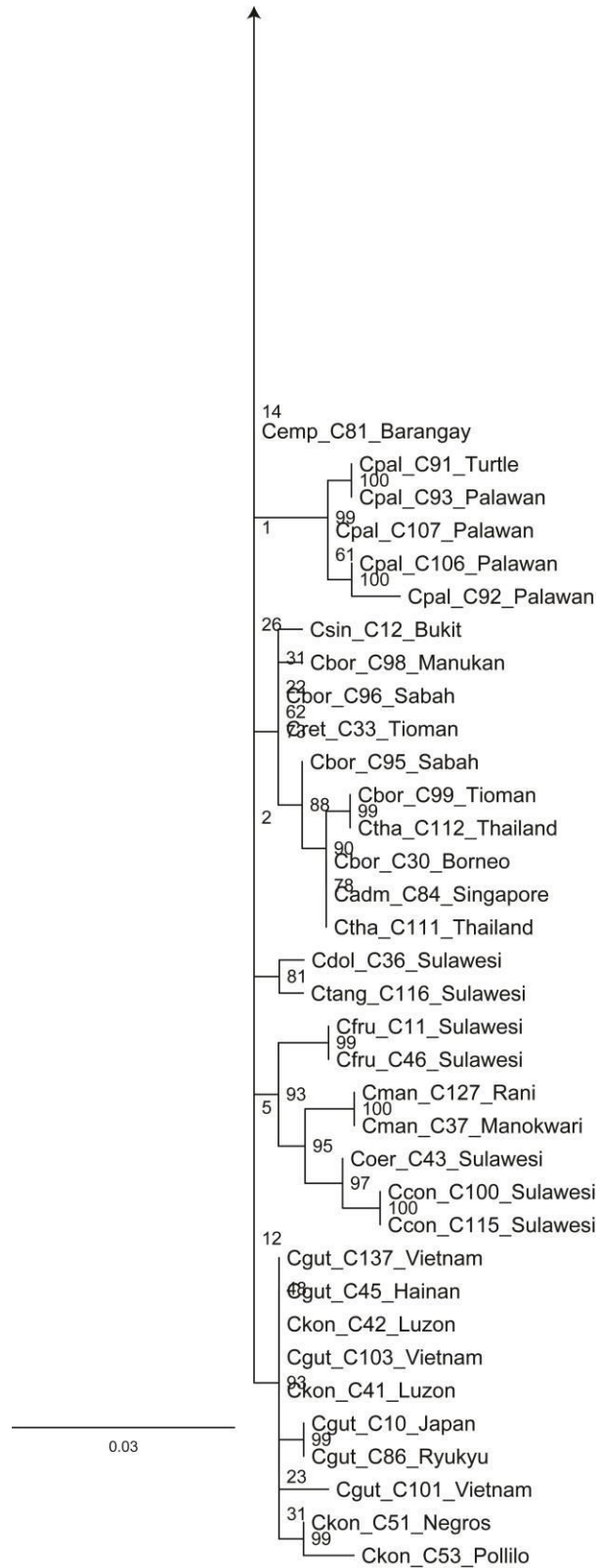
(continued)

H3 (i): continued



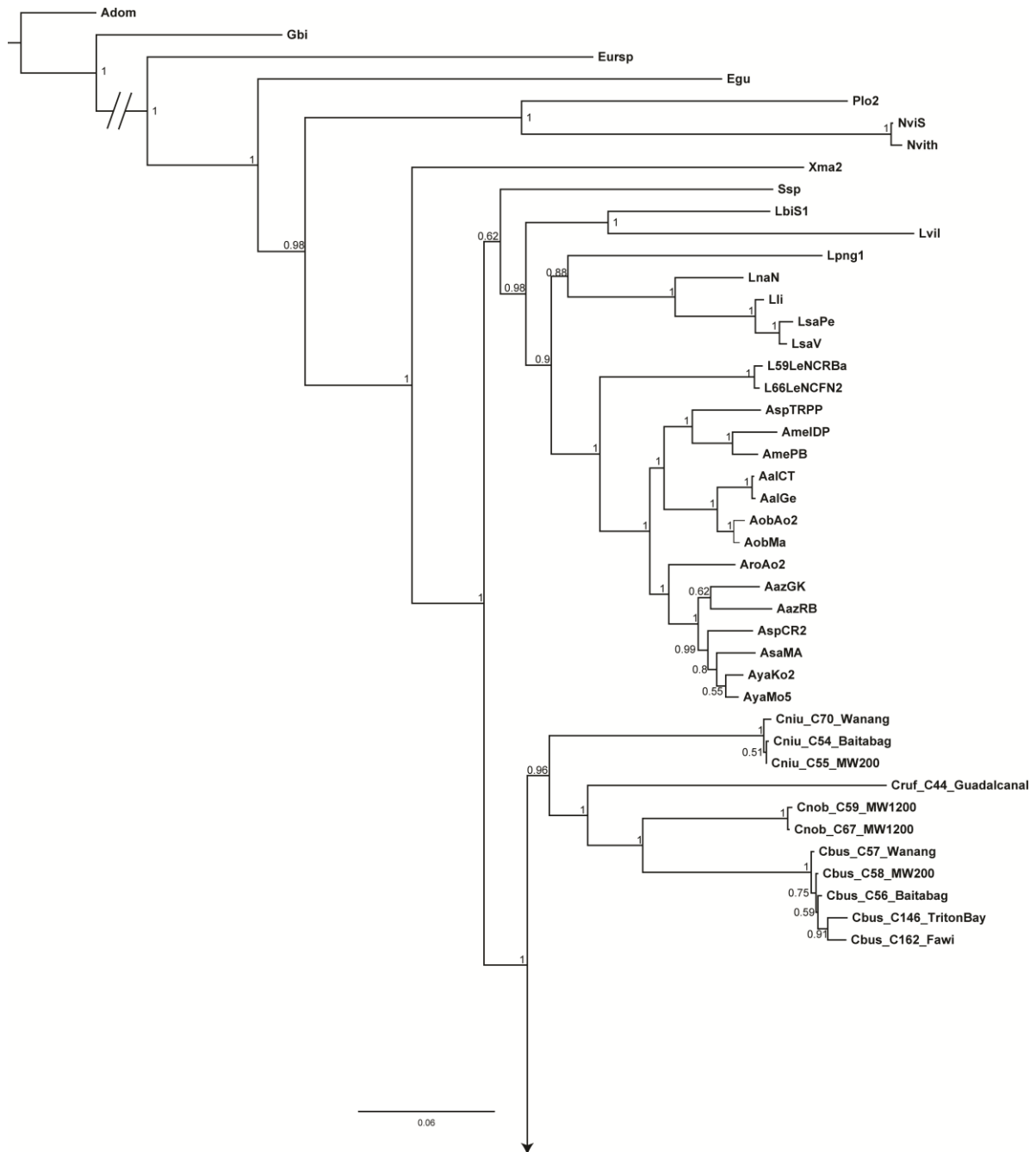
(continued)

H3 (i): continued

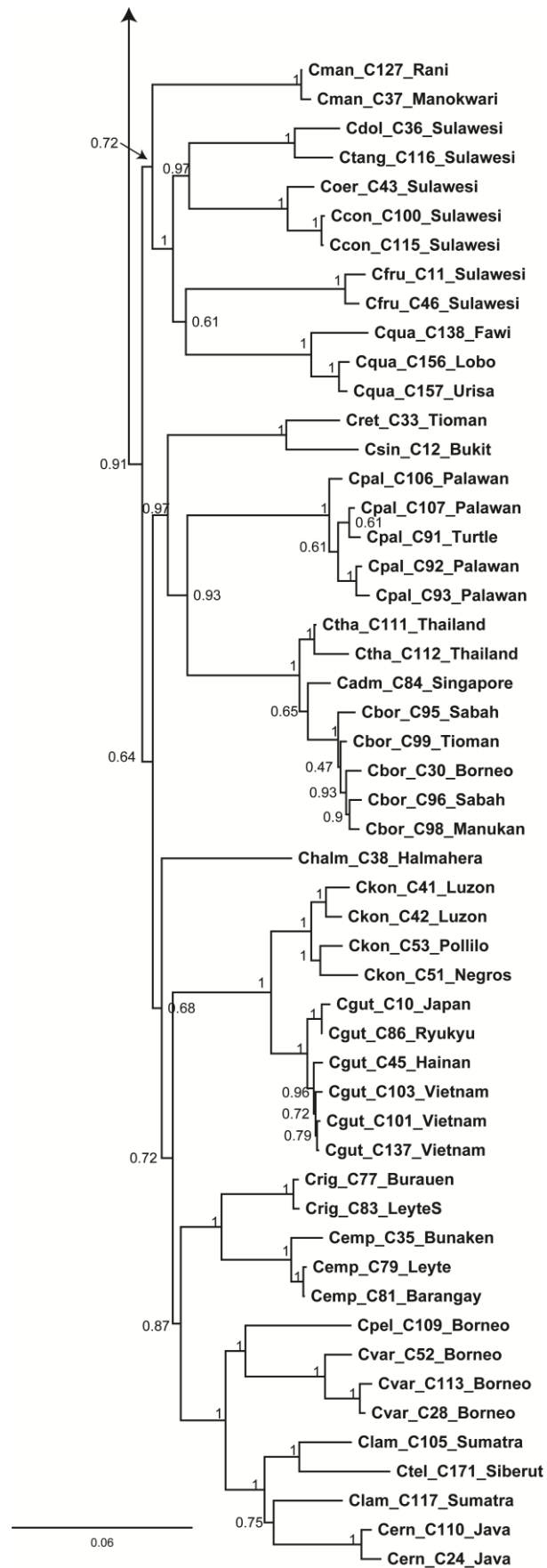


Appendix S6. Original output results of Bayesian inference (BI) and maximum likelihood (ML) analyses for concatenated dataset.

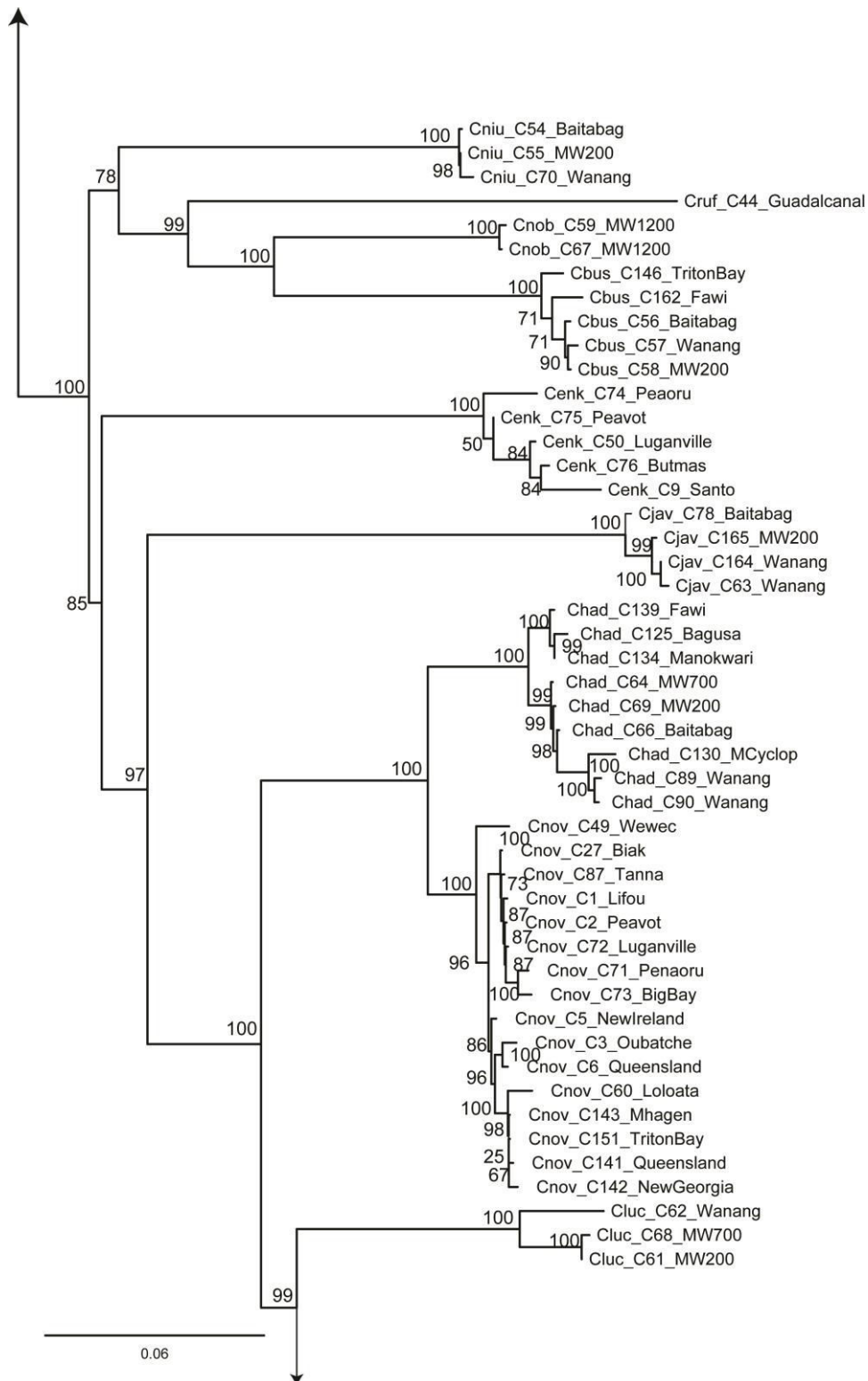
BI tree (concatenated dataset):



BI tree (concatenated dataset): continued

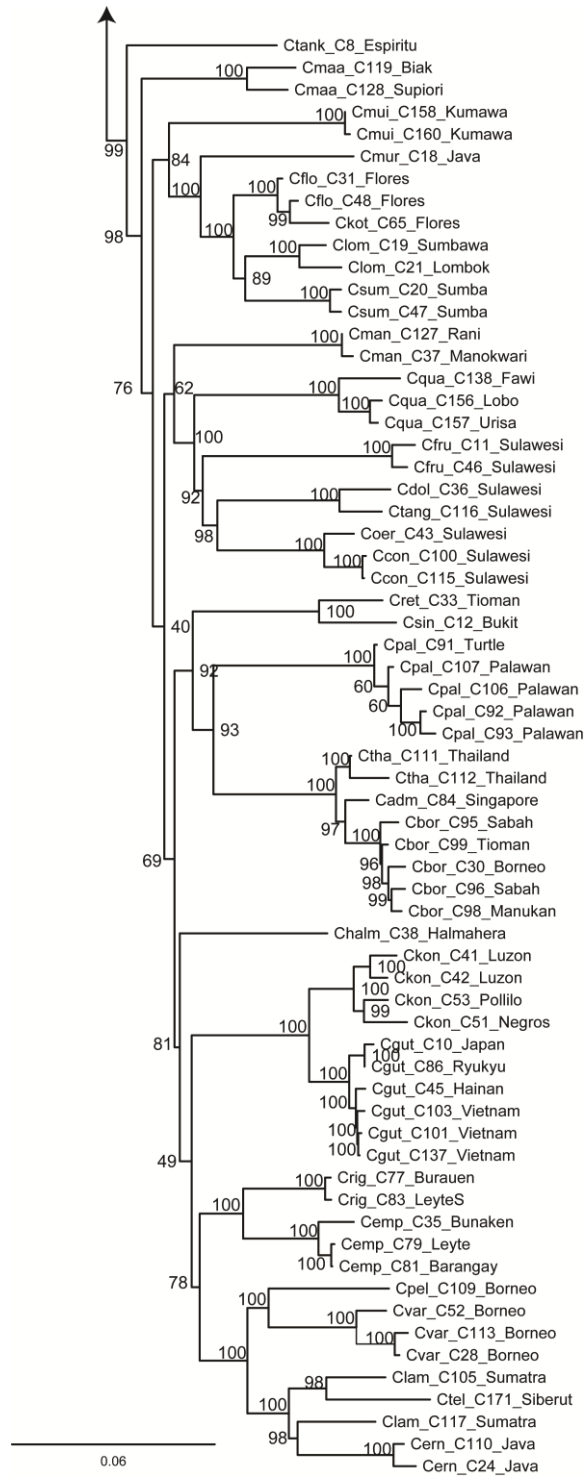


ML tree (concatenated dataset): continued

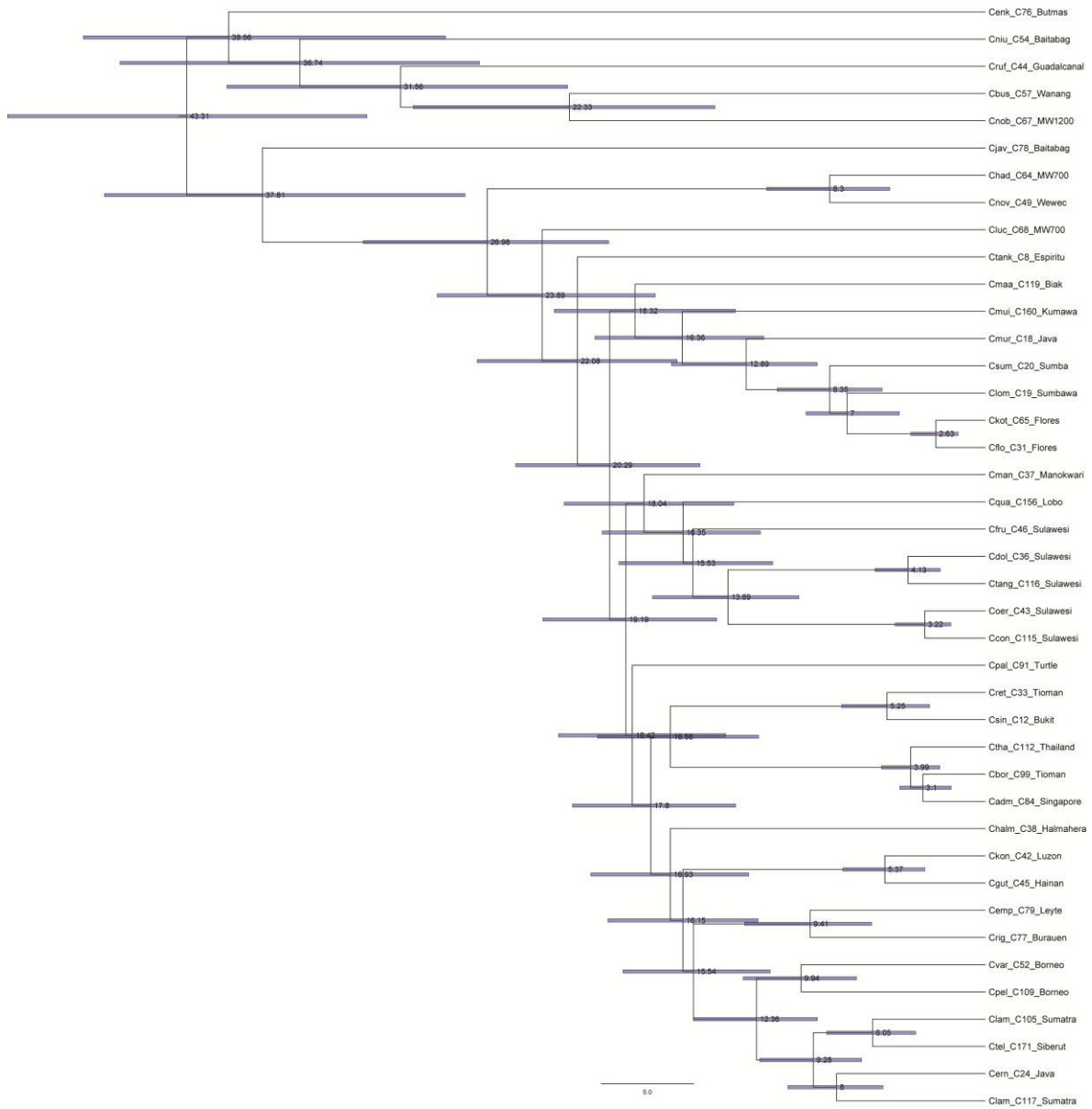


(continued)

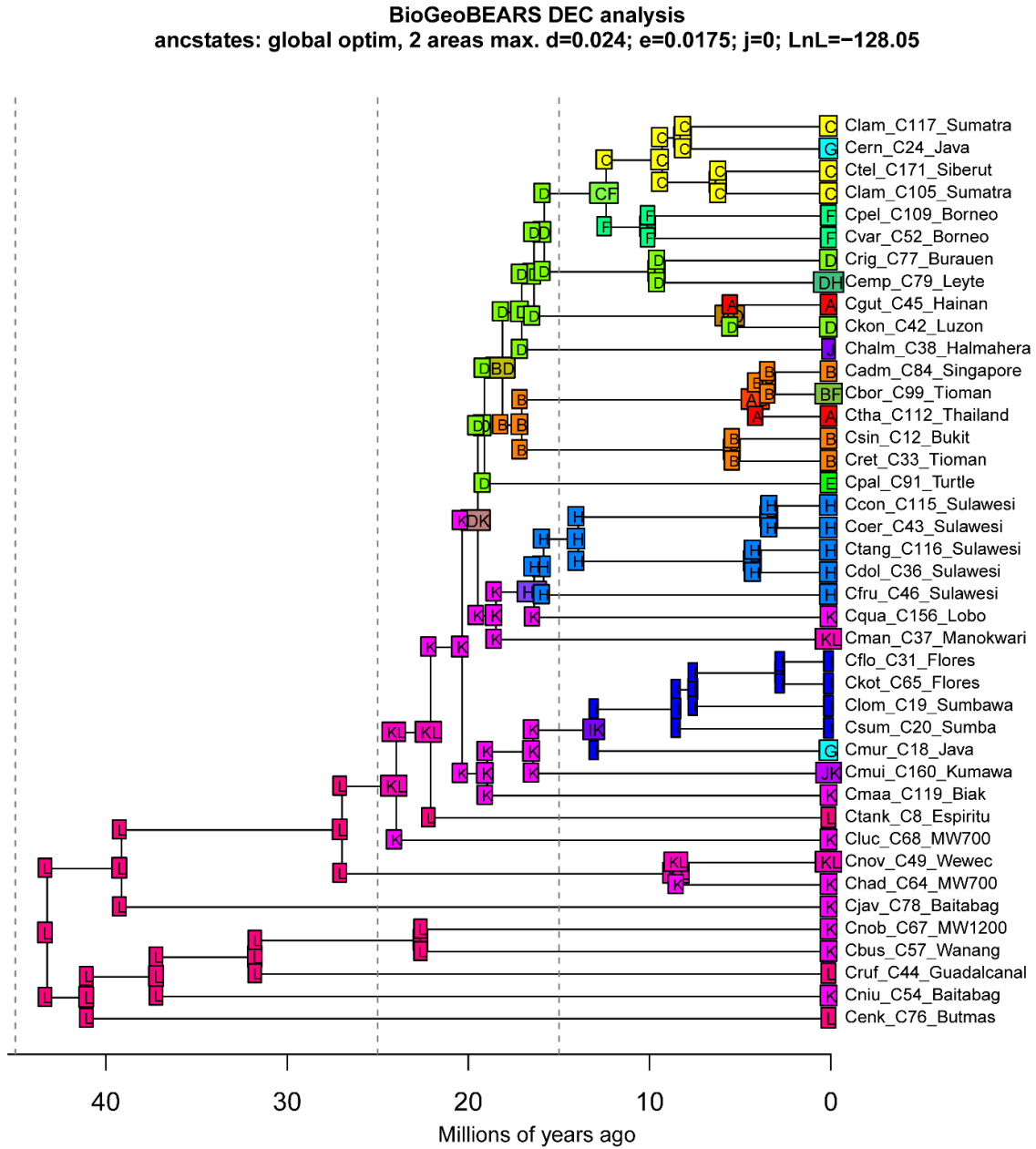
ML tree (concatenated dataset): continued



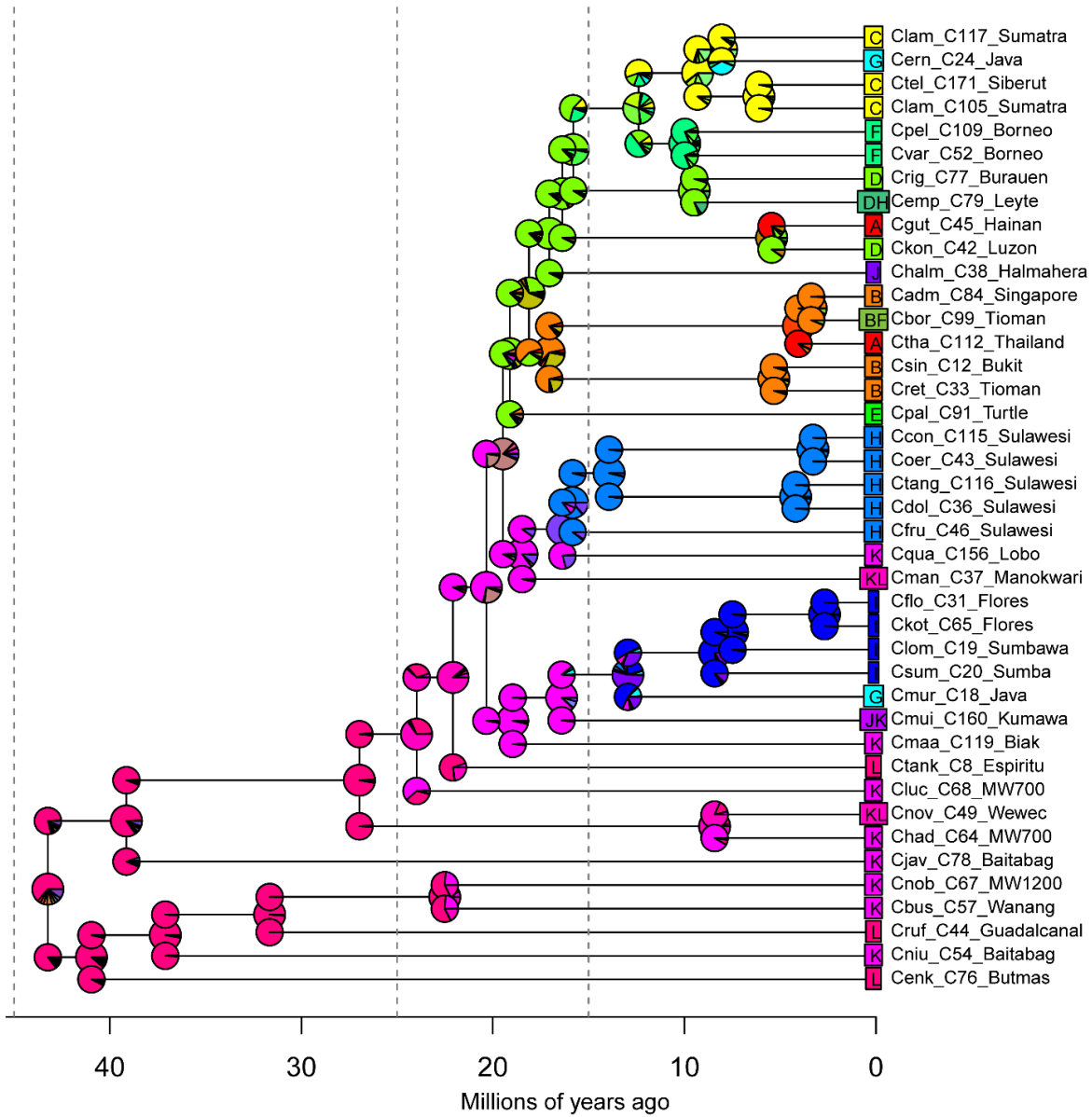
Appendix S7. Original output result of divergence time estimation.



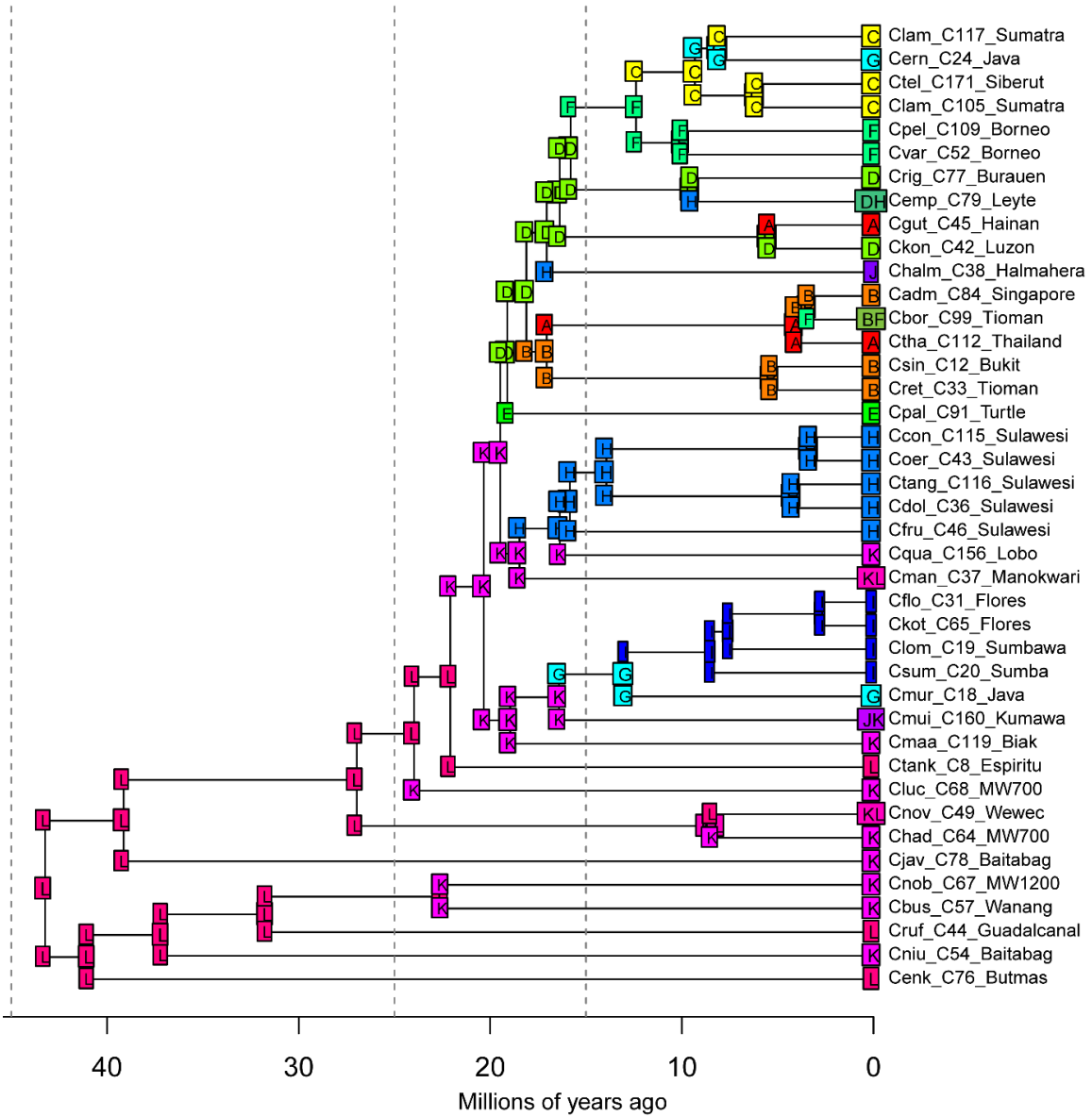
Appendix S8. Results of ancestral area estimation in BioGeoBEARs based on the most complex set of dispersal rate multipliers with the models DEC and DEC+j.



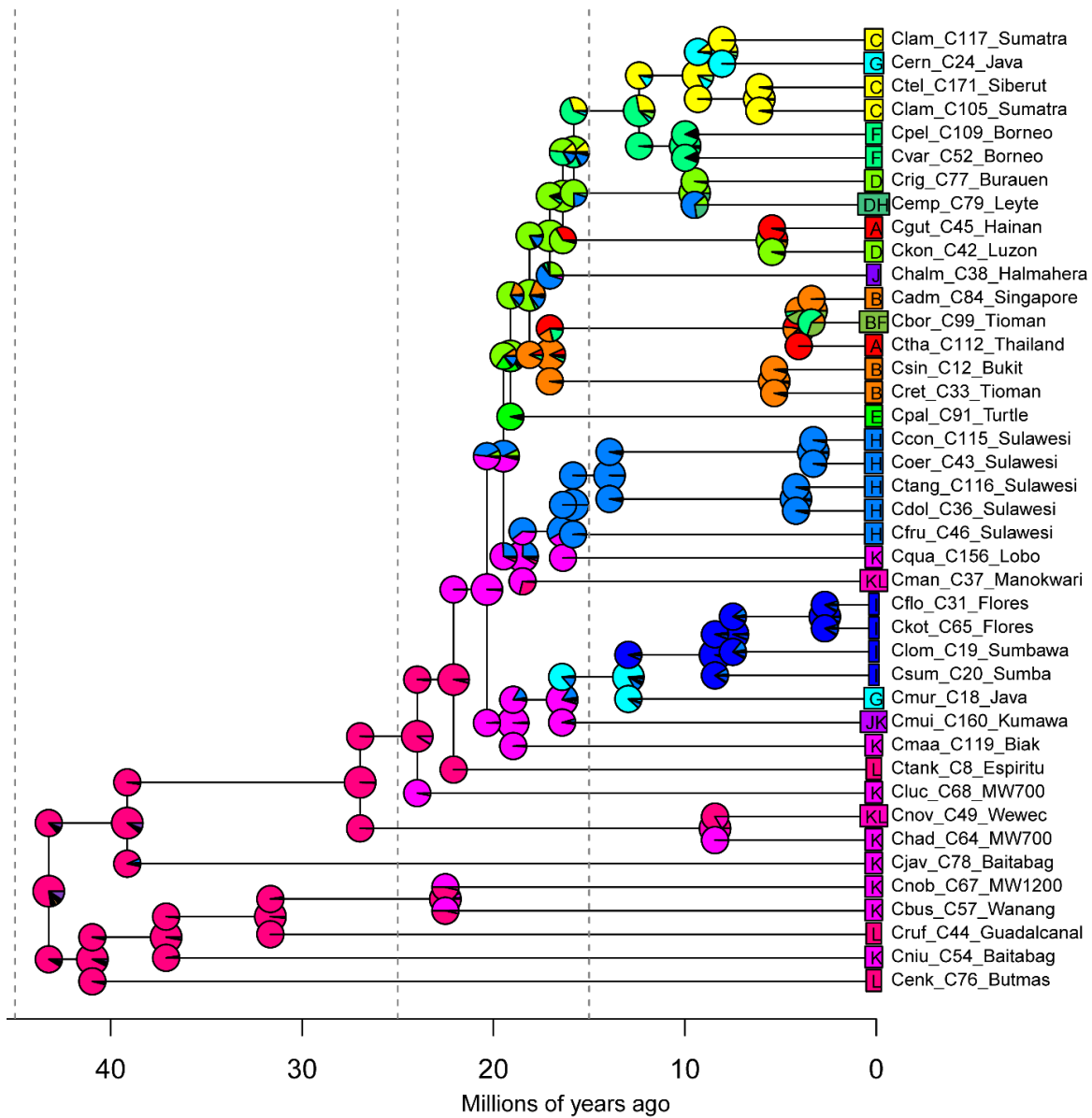
BioGeoBEARS DEC analysis
 ancstates: global optim, 2 areas max. d=0.024; e=0.0175; j=0; LnL=-128.05



BioGeoBEARS DEC+J analysis
 ancstates: global optim, 2 areas max. d=0.009; e=0.0072; j=0.1632; LnL=-113.82



BioGeoBEARS DEC+J analysis
 ancstates: global optim, 2 areas max. d=0.009; e=0.0072; j=0.1632; LnL=-113.82



Annexes II - Supporting Information for Chapter III

TABLE S1. Genetic distance for complete mitogenome, all PCGs and two ribosomal genes showed by the percentage of pairwise identical bases (% identity). The average genetic distances and their standard deviation between two subfamilies (Eneopterinae and Gryllinae) and within the subfamily Gryllinae are also shown in the table. The abbreviations of species as following: *C. muiri* (C), *L. equestris* (L), *T. emma* (T) and *V. hemelytrus* (V).

	Percentage of pairwise identical bases between subfamilies (%)			Percentage of pairwise identical bases within Gryllinae (%)				Mean ± SD
	C / L	C / T	C / V	Mean ± SD	L / T	L / V	T / V	
Mitogenome	71.80	72.01	71.80	71.87±0.12	74.82	73.76	78.62	75.73±2.56
nd2	66.76	66.08	67.36	66.73±0.52	68.44	69.35	74.40	70.73±3.21
cox1	83.1	83.63	83.27	83.34±0.22	84.41	82.65	87.15	84.74±2.27
cox2	78.96	82.01	79.94	80.30±1.27	81.04	79.70	81.42	80.72±0.90
atp8	59.88	64.15	57.86	60.63±2.62	64.15	64.15	69.93	66.08±3.34
atp6	76.51	77.09	76.36	76.65±0.31	80.47	75.77	0.816	79.29±3.11
cox3	79.77	80.15	80.03	79.98±0.16	80.03	81.17	83.21	81.47±1.61
nd3	75.21	74.93	76.35	75.50±0.61	73.50	72.65	76.64	74.26±2.10
nd5	73.73	75.60	76.42	75.25±1.13	77.51	77.21	81.09	78.60±2.16
nd4	75.02	76.88	75.39	75.76±0.80	78.82	76.96	84.04	79.94±3.67
nd4l	72.11	72.45	75.17	73.24±1.37	79.25	79.93	82.99	80.72±1.99
nd6	68.01	66.29	67.82	67.37±0.77	69.19	64.72	69.19	67.70±2.58
cytb	77.90	77.28	76.83	77.34±0.44	79.54	79.89	80.16	79.86±0.31
nd1	76.19	73.55	78.70	76.15±2.10	74.22	78.60	0.798	77.55±2.95
rrnL	71.73	73.13	69.90	71.59±1.32	75.78	72.06	78.71	75.52±3.33
rrnS	69.50	71.66	69.67	70.28±0.98	76.28	74.04	81.04	77.12±3.57

TABLE S2. Comparison of Ka/Ks ratios for all PCGs. The average Ka/Ks ratio and their standard deviation between subfamilies (Eneopterinae and Gryllinae) within the subfamily Gryllinae are also shown in the table. The abbreviations of species as following: C. muiri (C), L. equestris (L), T. emma (T) and V. hemelytrus (V).

Gene	Ka/Ks ratio pairwise comparison between subfamilies				Ka/Ks ratio pairwise comparison within Gryllinae			
	C / L	C / T	C / V	Mean ± SD	L / T	L / V	T / V	Mean ± SD
nd2	0.47	0.79	0.57	0.61±0.17	1.01	0.97	1.05	1.01±0.04
cox1	1.00	0.91	0.95	0.95±0.05	1.01	1.25	0.73	1.00±0.26
cox2	1.02	0.70	1.24	0.99±0.27	1.10	1.41	1.32	1.28±0.16
atp8	0.07	0.15	0.34	0.19±0.13	0.77	0.32	0.75	0.61±0.25
atp6	0.67	0.81	0.76	0.75±0.07	0.71	1.18	0.90	0.93±0.23
cox3	0.72	0.79	0.74	0.75±0.04	1.11	0.88	0.91	0.97±0.13
nd3	0.57	0.70	0.73	0.67±0.09	1.27	1.28	1.28	1.27±0.00
nd5	0.56	0.41	0.33	0.43±0.12	0.66	0.65	0.61	0.64±0.03
nd4	0.50	0.42	0.49	0.47±0.04	0.64	0.84	0.42	0.63±0.21
nd4l	0.67	0.61	0.28	0.52±0.21	0.96	0.71	0.63	0.77±0.17
nd6	0.68	0.72	0.61	0.67±0.05	0.76	1.35	0.81	0.97±0.32
cytb	0.75	0.99	1.13	0.96±0.19	0.93	0.83	1.26	1.01±0.22
nd1	0.73	0.81	0.56	0.70±0.13	0.66	0.66	0.58	0.63±0.05