OKINAWA INSTITUTE OF SCIENCE AND TECHNOLOGY GRADUATE UNIVERSITY
沖縄科学技術大学院大学

Transoceanic Dispersal and Plate Tectonics Shaped Global Cockroach Distributions: Evidence from Mitochondrial Phylogenomics

Article: Discoveries "CFP-Tree"

Transoceanic dispersal and plate tectonics shaped global cockroach distributions: evidence from mitochondrial phylogenomics

Thomas Bourguignon[1,2,3]*†, Tang Qian[4]†, Simon Y. W. Ho[1], Frantisek Juna[2], Zongqing Wang[5], Daej A. Arab[1], Stephen L. Cameron[6], James Walker[7], David Rentz[8], Theodore A. Evans[9], Nathan Lo[1]

[1]School of Life and Environmental Sciences, University of Sydney, Sydney, NSW 2006, Australia

[2]Okinawa Institute of Science & Technology Graduate University, 1919–1 Tancha, Onna-son, Okinawa, 904–0495, Japan

[3]Faculty of Forestry and Wood Sciences, Czech University of Life Sciences, Prague, Czech Republic

[4]Department of Biological Sciences, National University of Singapore, 117543 Singapore, Singapore

[5]College of Plant Protection, Southwest University, Beibei, Chongqing, China

[6]Department of Entomology, Purdue University, West Lafayette, IN, 47907, United States of America

[7]Department of Agriculture and Water Resources, Box 96 Cairns International Airport, Cairns 4870, Australia

[8]School of Marine and Tropical Biology, James Cook University, Townsville, Australia

[9]School of Animal Biology, University of Western Australia, Perth WA 6009, Australia.

*Corresponding author: E-mail: thomas.bourguignon@oist.jp

†These authors contributed equally to the work

1

Abstract

Following the acceptance of plate tectonics theory in the latter half of the 20th century, vicariance became the dominant explanation for the distributions of many plant and animal groups. In recent years, however, molecular-clock analyses have challenged a number of well-accepted hypotheses of vicariance. As a widespread group of insects with a fossil record dating back 300 million years, cockroaches provide an ideal model for testing hypotheses of vicariance through plate tectonics *vs* transoceanic dispersal. However, their evolutionary history remains poorly understood, in part due to unresolved relationships among the nine recognized families. Here we present a phylogenetic estimate of all extant cockroach families, as well as a timescale for their evolution, based on the complete mitochondrial genomes of 119 cockroach species. Divergence dating analyses indicated that the last common ancestor of all extant cockroaches appeared ~235 million years ago, approximately 95 million years prior to the appearance of fossils that can be assigned to extant families, and before the breakup of Pangaea began. We reconstructed the geographic ranges of ancestral cockroaches and found tentative support for vicariance through plate tectonics within and between several major lineages. We also found evidence of transoceanic dispersal in lineages found across the Australian, Indo-Malayan, African, and Madagascan regions. Our analyses provide evidence that both vicariance and dispersal have played important roles in shaping the distribution and diversity of these insects.

Introduction

The processes that explain the distributions of taxa across the globe have long been a central theme in evolutionary biology. Darwin (1859), among others, proposed that the disjunct distribution of related taxa on different continents came about through occasional transoceanic dispersal. This hypothesis was supported by the presence of diverse taxa on islands without any previous continental connections. In the 1960s and 1970s, the validation of plate tectonics theory (postulated half a century earlier by Wegener (1912)), along with the spread of cladistics thinking, led to a revolution in biogeography. It became widely accepted that vicariance, rather than dispersal, provided a more convincing explanation for the distributions of a wide array of plant and animal groups.

Recent decades have seen a number of well-accepted vicariance hypotheses called into question by date estimates based on molecular clocks. These include textbook examples of taxa found across multiple continents, such as the Antarctic beech *Nothofagus* (Cook and Crisp 2005; Knapp et al. 2005), ratite birds (Phillips et al. 2014), and cichlid fishes (Friedman et al. 2013). On the basis of robust phylogenetic and molecular dating analyses, the distributions of these organisms now appear to have been strongly influenced by post-Gondwanan transoceanic dispersal. The field of biogeography has thus undergone a second paradigm-shift, in which long-distance dispersal has risen to prominence as a primary explanation of the global patterns of organismal distributions.

The antiquity, diversity, and widespread distribution of insects make them excellent models for testing hypotheses of vicariance and dispersal. Among the most ancient groups of winged insects are the cockroaches, which have a fossil record stretching back to the Carboniferous. Fossil 'roachoid' insects first appeared ~315–318 Ma (Garwood and Sutton 2010; Zhang et al. 2013), and are generally considered to represent the stem group of the superorder Dictyoptera, which, along with extant cockroaches, comprises termites and mantids. Although numerous cockroach-like insect fossils are found from the Carboniferous through to the Jurassic, the first unambiguous fossil representatives of extant cockroach families are from the beginning of the Cretaceous, ~140 Ma (Labandeira 1994; Vršanský 1997; Grimaldi and Engel 2005). The oldest fossils of termites and mantises are also from this period (Thorne et al. 2000; Vršanský 2002; Grimaldi 2003; Krishna et al. 2013), which

suggests that the modern lineages of Dictyoptera emerged during the Jurassic (Grimaldi and Engel 2005). This view is further supported by recent molecular-clock analyses which suggest that cockroaches, mantises and termites descend from a common ancestor that appeared sometime between 192 and 307 Ma (Misof et al. 2014; Djernæs et al. 2015; Tong et al. 2016; Ware et al 2010; Wang et al. 2017). The wide interval of estimates from different studies warrants further investigation.

The fossil record of cockroaches as well as the results from molecular clock analysis suggest that most extant families evolved during or prior the breakup of Pangaea (which began ~200 Ma) and prior to the beginning of continental separation within Gondwana (~135 Ma) (Scotese 2004a). Cockroaches are generally considered to have limited flight capacity and to lack the ability for long-distance flight and dispersal from their natural environments (Peck and Roth 1992; Bell et al. 2007). Their global distribution patterns are thus likely to have been influenced by plate tectonics. However, recent studies of termites, which are derived from cockroaches (Klass 1997; Lo et al. 2000; Inward et al. 2007) and are expected to have similarly poor dispersal capabilities, showed that their global distributions have in fact been shaped by multiple transoceanic dispersal events (Bourguignon et al. 2016, 2017).

Numerous studies have investigated the relationships among the main dictyopteran and cockroach families, and some of these estimated the timescale of evolution of these taxa (Kambhampati 1995; Grandcolas 1996; Lo et al. 2000, 2003, 2007; Svenson and Whiting 2004, 2009; Klass and Meier 2006; Ware et al. 2008; Murienne 2009; Djernæs et al. 2012, 2015; Legendre et al. 2015, 2017; Wang et al. 2017). Although previous studies have investigated the biogeography of selected cockroach families or subfamilies (Maekawa et al. 2003; Che et al. 2016; Lo et al. 2016), to our knowledge none has examined the global historical biogeography of cockroaches as a whole.

In this study, we examine the evidence for vicariance versus dispersal across the cockroach tree. We analyse mitochondrial genome sequences of 119 cockroaches, 13 termite species, seven mantis species, and multiple outgroups. Our phylogenetic reconstructions increase the amount of molecular data per sample by approximately threefold, and include representatives of all nine cockroach families, and 20 of the 27 subfamilies composing the Blaberidae, Blattidae, Corydiidae, and Ectobiidae. These data substantially improve our understanding of the relationships among most of the major cockroach lineages. A secondary aim is to investigate the large differences in

divergence-time estimates from recent studies of cockroach evolution (Ware et al. 2010; Misof et al. 2014; Djernæs et al. 2015; Tong et al. 2016). We carefully assess the cockroach fossil record and select 13 fossil calibrations following the recommendations of Parham et al. (2012). We also test the influence of three additional fossil placements on the estimation of node ages, examine potentially questionable calibrations, and propose a revised timeframe for cockroach evolution.

Results

Tree Topology

We obtained complete or near-complete sequences of mitochondrial genomes of 113 species of cockroaches (supplementary table S1, Supplementary Material online). Our data matrix comprised sequences from 153 species, including 119 species of cockroaches, 13 species of termites, and seven species of mantises. The data set was partitioned into four subsets, each of which was assigned an independent model of nucleotide substitution. Phylogenetic analyses were performed using Bayesian inference in MrBayes 3.2 (Ronquist et al. 2012) and BEAST 1.8.4 (Drummond and Rambaut 2007), and using maximum likelihood in RAxML 8.2.4 (Stamatakis 2014). These methods yielded highly similar estimates of the phylogeny with respect to interfamily relationships, with the exception of a relatively small number of nodes that had low support (fig. 1, supplementary figs. S1–S3, Supplementary Material online). In all analyses, we found strong support for the monophyly of the Dictyoptera and of cockroaches (including termites).

In all analyses, each cockroach family other than the Ectobiidae was found to be monophyletic with strong support, although only 1–2 taxa were examined for some families (Lamproblattidae, Anaplectidae, Nocticolidae, and Tryonicidae). Termites and the Cryptocercidae were consistently recovered as sister taxa, and together these groups were most closely related to a clade containing the Blattidae and Tryonicidae (figs S1-S2, S4-S9). Termites, Cryptocercidae, Blattidae+Tryonicidae, and Anaplectidae+Lamproblattidae formed a monophyletic group in all analyses. Blaberidae was consistently placed within the Ectobiidae clade. In all analyses the cave cockroach *Nocticola* sp. was found to be the sister group of Corydiidae, and Nocticolidae+Corydiidae was recovered as the sister group of Ectobiidae+Blaberidae,

although these two groupings did not have consistently high support.

To determine the influence of the long branch leading to *Nocticola* sp. on phylogenetic inference, we performed additional analyses excluding this taxon. These resulted in phylogenetic estimates that were almost identical to those based on the complete data set (supplementary Figs. S4, S7, Supplementary Material online). Additional analyses in which the alignment was partitioned into 28 subsets instead of four also yielded phylogenetic estimates that were very similar to those from the complete data set (see supplementary figs. S5–S6, Supplementary Material online).

In our Bayesian analysis using BEAST, we found some unexpected relationships among more recently diverged taxa (fig. 1), for example the relationship (*Drepanotermes*,(*Nasutitermes+Macrognathotermes*)). The analyses using MrBayes recovered the expected relationship among these taxa (*Nasutitermes*,(*Macrognathotermes+Drepanotermes*)), except when the data were partitioned into 28 subsets.

Divergence Dating Analyses

We inferred the evolutionary timescale of cockroaches with BEAST, using calibrations for 15 internal nodes (fig. 1). Our analysis was based on the data set with third codon positions removed, in order to reduce the level of saturation in the sequence data. The best-fitting model of rate variation for our data was an uncorrelated lognormal relaxed clock (Drummond et al. 2006), whereas a Yule model provided a best-fitting tree prior. The marginal log likelihood of this combination of models was -440,461.5, whereas the marginal log likelihoods of other tested combinations, including a strict-clock model and birth-death-process tree prior, were between -440,488.9 and -442,144.6.

The divergence between the lineages leading to Dictyoptera and its sister clade (containing stick insects and grylloblattids) was inferred to have occurred 319.4 Ma (95% credibility interval 315.0–333.6 Ma). The lineages leading to mantids and cockroaches+termites subsequently diverged 263.4 Ma (95% CI 236.3–291.5 Ma), with the last common ancestor of cockroaches+termites appearing 235.2 Ma (95% CI 209.5–263.2 Ma).

To examine the influence of the roachoid fossil *Mylacris* on the estimated age of the Dictyoptera, we performed an additional analysis in which we excluded this

fossil calibration (fig. 2, supplementary figs. S8–S12, Supplementary Material online). This resulted in the inferred age of modern Dictyoptera being reduced to 216.0 Ma (95% CI 188.5–246.9 Ma) (fig. 2). We also compared the effect of including the controversial 279.5-million-year-old fossil *Homocladus*, thought by some to represent the most primitive mantis taxon, with more conservative approaches using *Juramantis* or no calibration at the node representing the divergence between mantids and other dictyopterans. Inclusion of *Homocladus* increased the estimated appearance of the Dictyoptera to 283.1 Ma (95% CI 279.5–292.5 Ma). Finally, when excluding both *Mylacris* and *Homocladus*, the estimated appearance of the Dictyoptera dropped to 228.0 Ma (95% CI 196.8–259.4 Ma). In analyses in which *Nocticola* sp. was excluded, we used the *Mylacris* and *Juramantis* calibrations and estimated the appearance of the Dictyoptera at 213.5 Ma (95% CI 195.3–231.6 Ma). The analysis with the alignment partitioned into 28 subsets yielded results similar to that with four partitions (supplementary figs. S4–S7, Supplementary Material online).

Biogeographic Analyses

We reconstructed the native geographic ranges of ancestral cockroach lineages to shed light on their historical biogeography (fig. 3). Within the Blaberidae, two sister genera found only in South America (*Epilampra* and *Galiblatta*) were found to have diverged from *Paranauphoeta* (from the Australian and Indomalayan regions) 98.0 Ma (95% CI 81.8–116.2 Ma) (fig. 3, numbered circle 2). The monophyletic group composed of the Neotropical Blaberinae genera *Blaberus, Archimandrita, Blaptica, Byrsotra,* and *Eublaberus* was found to have diverged from its sister lineage, the Afrotropical Gyninae, 104.9 Ma (95% CI 90.4–118.7 Ma) (fig. 3, numbered circle 1). *Diploptera*, an Indomalayan genus, was found to have diverged from a lineage containing African and Madagascan taxa 115.7 Ma (95% CI 99.9–132.0 Ma) (fig. 3, numbered circle 3).

Within the Ectobiidae, the Neotropical genus *Ischnoptera* diverged from its Australian sister lineage (comprising *Beybienkoa* and *Carbrunneria*) 105.4 Ma (95% CI 84.9–125.8 Ma) (fig. 3, numbered circle 4). The Neotropical *Megaloblatta* diverged from its sister group, composed of Blaberidae and a collection of Ectobiidae genera, 168.6 Ma (95% CI 147.8–187.9 Ma) (fig. 3, numbered circle 5). The Neotropical lineage composed of *Euphyllodromia* and *Amazonina* diverged from the

lineage comprising *Allacta*, *Balta*, and *Ellipsidion* 152.8 Ma (95% CI 129.7–177.5 Ma) (fig. 3, numbered circle 6). The Australian *Ectoneura* diverged from *Ectobius+Phyllodromica*, a group distributed across the African, Palearctic, and Indo-Malayan regions, 118.2 Ma (95% CI 83.0–150.6 Ma) (fig. 3, numbered circle 7).

Within the blattid subfamily, Polyzosteriinae, the mostly Australian lineage composed of *Melanozosteria*, *Platyzosteria*, *Polyzosteria*, *Cosmozosteria*, and *Methana* diverged from the Neotropical *Eurycotis* 75.5 Ma (95% CI 63.3–91.5 Ma) (fig. 3, numbered circle 9). *Tryonicus*, from the strictly Australian family Tryonicidae, was found to have diverged 144.0 Ma (95% CI 125.0–172.7 Ma) from the entire Blattidae family (fig. 3, numbered circle 10). Similarly, *Lamproblatta*, from the strictly Neotropical family Lamproblattidae, diverged 179.0 Ma (95% CI 147.1–209.9 Ma) from the more widespread *Anaplecta* (fig. 3, numbered circle 11). Within the Corydiidae, the Nearctic genus *Arenivaga* split from taxa present in Gondwanan continents 168.7 Ma (95% CI 136.0–202.6 Ma) (fig. 3, numbered circle 8).

A number of groups, distributed across more than one biogeographic area, arose after the breakup of Gondwana (fig. 3). These groups, which include the genera *Panesthia*, *Rhabdoblatta*, *Periplaneta*, *Neostylopyga*, and *Melanozosteria*, are distributed across the Indo-Malayan and Australian biogeographic areas. The strictly Madagascan Gromphadorhini, which includes *Gromphadorhina*, *Aeluropoda*, and *Elliptorhina*, diverged from their Afrotropical relatives 68.3 Ma (95% CI 55.4–82.7 Ma).

Discussion

Resolution of Relationships Among Major Cockroach Lineages

Previous studies have demonstrated that mitochondrial genomes are suitable markers for resolving phylogenetic relationships among families within various insect orders (Cameron et al. 2012; Cameron 2014a; Bourguignon et al. 2015, 2016, 2017). Our results confirm the value of the mitochondrial genome for resolving ancient divergences among insects, in this case among the lineages leading to extant families of cockroaches.

Our analyses support a monophyletic Dictyoptera, with mantises as the sister

group to cockroaches and termites, as found in several previous studies (Lo et al. 2000; Svenson and Whiting 2004; Inward et al. 2007; Djernæs et al. 2012; Legendre et al. 2015). We confirmed the monophyly of all families, with the exception of Ectobiidae, which was paraphyletic with respect to Blaberidae. Our analyses improve our understanding of relationships among the key families Blattidae, Lamproblattidae, Tryonicidae, and Anaplectidae, and the clade Cryptocercidae+termites, which together formed a well-supported clade. We found support for sister group relationships between the Lamproblattidae and Anaplectidae, and between Tryonicidae and Blattidae. Knowledge of the sister group of the Cryptocercidae+termites clade has the potential to shed light on how social behaviour, and the key acquisition of parabasalid and oxymonad flagellates, evolved in the ancestors of termites and *Cryptocercus*. Previous studies (Djernæs et al. 2015; Wang et al. 2017) inferred trees that grouped either Tryonicidae, Anaplectidae, or a combination of these two taxa with *Cryptocercus*+termites, although without strong support. We found support for Blattidae+Tryonicidae being the sister group to Cryptocercidae and termites, except in the MrBayes analysis implemented with a GTR+G+I model, that placed Lamproblattidae+Anaplectidae as the sister group to this clade. These results would appear to rule out tryonicids and anaplectids as potential model transitional forms in the evolution of social behaviour and the acquisition of flagellate protozoa in the Cryptocercidae+termites clade (Djernæs et al. 2015).

Nocticolidae was the sister group of the Corydiidae in most analyses, and together these two taxa were grouped with the Ectobiidae+Blaberidae. However, the position of Nocticolidae generally received weak support, and in one analysis it was positioned within the Ectobiidae. The phylogenetic position of Nocticolidae has been variable in a number of other phylogenetic studies of cockroaches (Inward et al. 2007; Lo et al. 2007; Djernæs et al. 2015; Legendre et al. 2015; Wang et al. 2017). Djernæs et al. (2015) found that the inferred phylogenetic position of Nocticolidae varied between analyses; the most extreme scenario was a polyphyletic Nocticolidae, with one lineage placed as the sister lineage to mantises and a second lineage nested within Corydiidae, although support for these groupings was low.

One reason for the difficulty in resolving the position of *Nocticola* might be the extreme length of the branch on which it is placed. This has been a common feature in all studies that have included members of this genus (Inward et al. 2007; Lo

et al. 2007; Djernæs et al. 2012, 2015; Legendre et al. 2015). The Nocticolidae is a group of peculiar and poorly studied cockroaches that comprises nine described genera and 34 described species. The present-day habitats in which Nocticolidae exist have relatively high humidity and stable temperatures. They are mostly found in caves, and occasionally as inquilines in the nests of social insects (Roth and Mcgavin 1994), under rotting logs, or in the nests of wood-feeding or soil-burrowing cockroaches, such as the specimen of *Nocticola* sp. examined in this study. The ancestral habitat of extant Nocticolidae is not known. Although the population sizes of cave-dwelling Nocticolidae are not well understood, only small numbers of individuals have been found on baits within the Australian caves that have been sampled (N. Lo and J. Walker, pers. obs.). These populations might have been subjected to repeated genetic bottlenecks over time. The lineages present in caves might also have been subject to relaxed selection, as has been found in studies of blind cave fish (Calderoni et al. 2016). The biology of Nocticolidae found in the nests of other insects is not well characterized (Bell et al. 2007), but it is possible that a number of traits present in their non-inquiline ancestors were also subject to relaxed selection following their transition to inquilinism.

Timescale of cockroach evolution

Previous estimates of the age of Dictyoptera, as retrieved from the TimeTree database (Hedges et al. 2006), varied between 137 Ma and 307 Ma. Older estimates of 192 to 307 Ma have been obtained in recent molecular-clock studies that have used relaxed-clock models. Misof et al. (2014) analysed a large nuclear genomic data set using 37 fossil calibrations (none of which was included in our study) and found that the last common ancestor of Dictyoptera appeared 197 Ma (95% CI 159–243 Ma). Tong et al. (2016) reanalysed this data set, adding the roachoid fossil *Mylacris*, and produced a date estimate of 236 Ma (95% CI 215–273 Ma) for this node. The latter 95% credibility interval overlaps substantially with the age estimates from our analyses (216–283 Ma), despite the fact that a completely different data set and different fossil calibrations were used (with the exception of *Mylacris*, which was common to both analyses).

The oldest age estimate of Dictyoptera in our study (283 Ma; 95% CI 280–292 Ma; fig. 2) was obtained in the analyses that included the 280-million-year-old

Homocladus fossil calibration. This estimate is similar to that of 273 Ma (95% CI 258–288 Ma) reported by Djernæs et al. (2015), who used three fossil calibrations, including *Homocladus*. One problem with the inclusion of *Homocladus* is that its taxonomic affinity is not clearly understood. It was originally assigned to Protorthoptera (Carpenter 1966), and was only recently suggested to belong to stem mantises (Béthoux and Wieland 2009). This reassignment was entirely based on some reinterpretations of wing venation (Béthoux and Wieland 2009; Béthoux et al. 2010), and is opposed by several authors (e.g., Gorochov 2013; Prokop et al. 2014). Prokop et al. (2014) suggested instead that the Paoliida, to which *Homocladus* belongs, is the sister group of modern Dictyoptera. The controversial status of *Homocladus* should preclude its use as a reliable calibration for analyses of modern Dictyoptera, because incorrect fossil calibrations can introduce large errors in molecular date estimates.

The numerous roachoid fossils that date from the late Carboniferous are widely recognized as stem dictyopterans (Grimaldi and Engel 2005). Our analyses without these fossil calibrations date the origins of Dictyoptera to 197-207 Ma, consistent with the 197 Ma (95% CI 159–243 Ma) age estimate of Misof et al. (2015). However, without inclusion of Carboniferous roachoid fossils, molecular-clock analyses will potentially underestimate the age of Dictyoptera and its sister groups (Tong et al. 2016). Our analyses that included *Mylacris* suggest that the last common ancestor of cockroaches and termites appeared significantly earlier (i.e., 235 Ma; 95% CI 210–263 Ma) than the first undisputed fossils of modern cockroaches dating from the Cretaceous, ~140 Ma (Vršanský 1997; Nalepa and Bandi 2000; Lo et al. 2003). Cockroach-like fossils are common from deposits representing all epochs from the late Carboniferous to the late Jurassic, with a general trend of reduction in size of the ovipositor over time, until the Cretaceous, by which time ovipositors are absent from cockroach fossils (which instead resemble extant lineages). One explanation for the absence of fossils resembling extant cockroach families at their inferred origin of ~235 Ma is that these ancestors are actually represented by fossil taxa with ovipositors. In this case, ovipositors in the ancestors of modern cockroaches would have been lost independently in multiple lineages by the Cretaceous. An alternative explanation is that modern cockroaches were not common in the Jurassic and are not represented in the fossil record.

Our analyses with *Mylacris* are also consistent with the fossil record of other insect orders (note that fossil calibrations from these orders were not used in our

study). For example, among the polyneoptera orders represented in our tree, the split between Phasmatodea and Mantophasmatodea was dated at 292 Ma (95% CI 264–314 Ma), and the split between Grylloblattodea and Phasmatodea+Mantophasmatodea was dated at 307 Ma (95% CI 283–326 Ma). These dates are somewhat earlier than the oldest known fossils of Phasmatodea (272.5–279.5 Ma) (Aristov and Rasnitsyn 2015; but see Bradler and Buckley 2011, who propose that the oldest crown phasmid fossils are from the Eocene) and Grylloblattodea (290–295 Ma) (Kutalova 1964, but see Prokop et al. 2014).

Impacts of Plate Tectonics on Cockroach Evolution

Our results indicate that extant cockroach families have evolved over periods of up to ~180 million years. Through reconstructions of the ancestral distribution of cockroaches using the known distributions of extant genera sampled in this study, we found evidence that continental breakup has had important impacts on cockroach biogeography. Evidence for ancient vicariance was found in multiple comparisons within and between families.

The split between Africa and South America is believed to have commenced ~140 Ma, with separation complete by 100 Ma (Cracraft 2001). The split ~105 Ma (95% CI 90–119 Ma) between the South and Central American blaberid genera (*Blaberus*, *Archimandrita*, *Blaptica*, *Byrsotra*, and *Eublaberus*) and their African sister group *Gyna* is consistent with this timeframe.

South America and Australia are thought to have remained in contact through a landbridge over Antarctica until ~60–70 Ma (Scotese 2004a). The severing of the connection between these two continents can explain the splits occurring between *Ischnoptera* and *Cabrunneria+Beybienkoa* at 105 Ma (95% CI 85–126 Ma)*,* between *Eurycotis* and the Australian Polyzosteriinae at 76 Ma (95% CI 63–92 Ma), and between *Epilampra+Galiblatta* and the Australian/Indomalayan *Paranauphoeta* at 98.0 Ma (95% CI 81.8–116.2 Ma).

South America is believed to have become isolated from all other continents 60–100 Ma (Cracraft 2001, Scotese 2004a). We found that some lineages endemic to South America diverged from lineages found in other regions prior to this timeframe, including *Megaloblatta,* which diverged from its sister group at 168.6 Ma (95% CI 147.8–187.9 Ma), and the pseudophyllodromiine taxa *Euphyllodromia* and

Amazonina, which diverged from their sister group 152.8 Ma (95% CI 129.7–177.5 Ma).

The last connection between Africa and the remaining Gondwanan continents is thought to have occurred ~100 Ma (Cracraft 2001). This potentially explains the divergence between the Indomalayan *Diploptera* and the clade containing African *Nauphoeta* and *Rhyparobia* 116 Ma (95% CI 100–132 Ma).

The last connection between Australia and the Old World (African, Palearctic, and Indo-Malayan regions) was through Antarctica and South America ~100 Ma (Cracraft 2001, Scotese 2004a). The Australian *Ectoneura* diverged from *Ectobius+Phyllodromica*, a group distributed across the African, Palearctic, and Indo-Malayan regions, 118.2 Ma (95% CI 83.0–150.6 Ma). Plate tectonics may therefore explain the distribution of this group, which possibly went extinct in South America.

Previous work has shown that the ancestor of Asian and North American *Cryptocercus* most likely inhabited the temperate deciduous forests in the late Cretaceous to early Paleogene in the northern regions of the globe (Che et al. 2016). A general cooling trend began in the mid-Eocene and is thought to have forced ancestral *Cryptocercus* lineages to move south into Asia and North America (MacGinitie 1958). The boreotropical flora is thought to have spread between Eurasia and the Americas during the early Eocene (~55 Ma) via early connections through Beringia. Boreotropical forests are thought to have gradually retracted towards the equator until the Eocene-Oligocene boundary ~35 Ma (Morley 2011). Within the Ectobiidae, the Japanese *Asiablatta* is nested within Nearctic *Parcoblatta*, from which it diverged ~37 Ma (95% CI 27–50 Ma). These two genera may therefore have been influenced by Beringian land connections between Asia and North America (SanMartin et al. 2001).

A number of other divergences suggest the influence of ancient vicariance caused by Pangean or Gondwanan breakup. These include the very deep splits between the Lamproblattidae (found only in the Neotropics) and the Anaplectidae (cosmopolitan) ~179 Ma (95% CI 147–210 Ma), the Nearctic *Arenivaga* and the remaining Corydiidae at 169 Ma (95% CI 136–203 Ma), and the Australian Tryonicidae from their sister group 144 Ma (95% CI 125–173 Ma).

Overall, the multiple hypotheses for vicariant divergence proposed above are somewhat preliminary, in view of the absence of many important taxa. Taken together, however, we believe that our results point to an important role for vicariance in

13

determining the global distributions of cockroaches. The ages of cockroach fossils that we did not use as calibrations in our study are consistent with this hypothesis. Some fossils of modern cockroach genera, including *Morphna* and *Ectobius*, are dated at 50 Ma (Vršanský et al. 2013, 2014), and possibly as old as 90 Ma (Anisyutkin et al. 2008), revealing the antiquity of many extant genera. To our knowledge, no known cockroach fossils significantly extend the geographic distribution of the groups considered above, suggesting that the distributions of modern cockroach genera have been stable over long periods of time. Our results provide an important framework for future investigations of cockroach biogeography, adding to a number of other molecular-clock studies of insect and other invertebrate taxa that indicate an important role for ancient plate tectonics in shaping modern distributions (Cranston et al. 2012; Giribet et al. 2012; Murienne et al. 2014; Wood et al. 2013).

Our analyses also indicate a series of disjunctions that are best explained by dispersal. For example, our ancestral state reconstructions show that the Madagascan Oxyhaloinae, containing the genera *Gromphadorhina*, *Aeluropoda*, and *Elliptorhina*, diverged from the African *Rhyparobia* ~68 Ma (95% CI 55–83 Ma). Madagascar started separating from Africa, together with India, ~160 Ma and is believed to have been completely separated by sea channels ~140 Ma (Scotese 2004a; Seward et al. 2004). Therefore, the African Oxyhaloinae appear to have colonized Madagascar by dispersal, as hypothesized for many other animal and plant groups (Yoder and Nowak 2006). Other evidence of dispersal across sea gaps comes from several genera distributed across the Australian and Indo-Malayan regions. These include *Panesthia*, *Rhabdoblatta*, *Periplaneta*, *Neostylopyga*, and *Melanozosteria*, all of which originated after the breakup of Gondwana, and must have acquired their distribution through dispersal.

Conclusions

Our study has increased by approximately threefold the amount of molecular data available for inferring cockroach phylogenetic relationships. We found strong support for the clades Blattidae+Tryonicidae and Lamproblattidae+Anaplectidae, and some support for Blattidae+Tryonicidae being the sister group of termites+*Cryptocercus*. We were not able to resolve the positions of the Corydiidae and Nocticolidae. The use

of nuclear genomic or *Blattabacterium* endyosymbiont genomic data may help to resolve these and other uncertainties.

Our estimate of ~235 Ma for the age of the clade containing extant cockroaches and termites substantially predates the oldest known fossils of modern cockroaches from the early Cretaceous (~140 Ma). Our examination of the geographic distributions of taxa, in the light of their phylogenetic relationships and inferred divergence times, provides preliminary support for a key role for both vicariance and dispersal in determining the global distribution of cockroaches. Plate tectonics appear to have shaped the distribution of early cockroach lineages through vicariance, whereas the occurrence of dispersals is supported by the distribution of younger genera across several biogeographic areas, especially between the Australian and Indo-Malayan areas. However, further taxon sampling is required to determine the roles of vicariance and dispersal in finer detail. Our study provides a framework for a greater understanding of the evolution of this ecologically and economically important group of insects.

Materials and Methods

Mitochondrial Genome Sequencing

We used specimens from 113 non-termite cockroach species (see supplementary table S1, Supplementary Material online). All specimens were preserved in RNA-later® or in 100% Ethanol and kept at -80 °C until DNA extraction. All specimens are stored at the Okinawa Institute of Science and Technology, Japan, and are available for examination upon request. Because genomes were sequenced over a period of five years, we used three different strategies, reflecting improvements in sequencing technologies: (i) long-range PCR followed by primer walking (for a general description of the method, see Cameron 2014b); (ii) long-range PCR followed by high-throughput DNA sequencing; and (iii) whole-genome shotgun sequencing.

For the first strategy, thoracic or leg muscle tissue was extracted using DNeasy Blood & Tissue extraction kits (Qiagen). Long PCRs were performed with Elongase (Invitrogen), and then Sanger sequenced with the ABI Big Dye ver3 chemistry on an ABI 3770 automated sequencer. Amplification and sequencing primers are listed in supplementary tables S2–S7 (Supplementary Material online).

For the second sequencing strategy, whole genomic DNA was extracted from cockroach muscles with the phenol-chloroform procedure and the complete mitochondrial genome amplified with TaKaRa LA Taq in two long PCRs. Long PCRs used previously published primers or cockroach-specific primers designed in this study (supplementary table S8, Supplementary Material online). The concentration of both long PCR fragments was determined using a Qubit 3.0 fluorometer and mixed in equimolar concentration. We then prepared one library with unique barcode for each sample separately, pooled 96 samples together and paired-end sequenced them in one lane of Illumina HiSeq2000.

For the third strategy, whole-genome shotgun sequencing, we extracted DNA from cockroach fat bodies with the DNeasy® Blood & Tissue Kit (Qiagen). Libraries were prepared for each sample separately, using unique barcodes. Forty-eight libraries were then pooled together and paired-end sequenced in one lane of Illumina HiSeq4000. The resulting data included cockroach genomic and mitochondrial reads, as well as reads from cockroach-associated bacteria. Despite the multiple origins of the DNA used for assembling, the resulting mitochondrial genomes were typical of those of cockroaches, and careful examinations reveal no contamination with bacterial sequences.

Mitochondrial genomes sequenced with the first strategy, by primer-walking, were assembled in Sequencher 4 (GeneCodes Corp., Ann Arbor, MI, USA). Mitochondrial genomes sequenced with the second and third strategies, using high-throughput methods, were assembled using the CLC suite of programs, as described by Bourguignon et al. (2015). Briefly, we used de novo assembling to determine a consensus sequence for each species separately. We then mapped the original reads on each consensus sequence and corrected the mistakes that occurred during the initial assembling step, therefore generating a new consensus sequence. This procedure was repeated until we reached stability, with no inconsistencies detected. For polymorphic bases, we selected the base with the highest representation. We omitted the control regions of the mitochondrial genomes from subsequent phylogenetic data sets, because they include repetitive DNA regions that are generally poorly assembled from short reads. We annotated the 22 tRNAs, 13 protein-coding genes, and two ribosomal RNAs using the MITOS Webserver with the invertebrate genetic code and default settings (Bernt et al. 2013), with quality control checks against published cockroach mitochondrial genomes.

Data Set

We carried out phylogenetic analyses on the 119 species of cockroaches, combined with sequences from 20 species of Dictyoptera from GenBank (table 1), including 13 termites and seven mantises. Additionally, we included as outgroups the sequences of 14 polyneopteran insect species from GenBank, including one grasshopper, one stonefly, one grylloblattid, and 11 stick insects. Therefore, the final data set included the mitochondrial genomes of 153 species. We aligned each gene individually using the Muscle algorithm (Edgar 2004), with default settings, implemented in MEGA 5.2 (Tamura et al. 2011). We aligned protein-coding genes as codons.

We partitioned the concatenated alignment into four subsets: (i) first codon positions of protein-coding genes; (ii) second codon positions of protein-coding genes; (iii) 12S and 16S rRNA genes; and (iv) tRNA genes. In addition, we tested an alternative partitioning scheme in which the concatenated alignment was split into 28 subsets. This scheme divided the data into the first and second codon positions of each gene (26 subsets), 12S rRNA, 16S rRNA, and the combined tRNA genes. In all cases, we excluded the third codon positions of the protein-coding genes because of the high level of mutational saturation at these sites. Using Xia's method as implemented in DAMBE (Xia et al. 2003; Xia and Lemey 2009), we found that the third codon position (I_{SS}=0.682) was much more saturated than the first codon position (I_{SS}=0.248) and second codon position (I_{SS}=0.127). Although it was significant, the I_{SS} score for the third codon positions is close to the critical value ($I_{SS.C}$Asym=0.799; based on 32-taxon simulations), indicating that these data are less suitable for analysing deep divergences in the cockroach phylogeny.

Phylogenetic Analyses

We conducted Bayesian phylogenetic analyses in MrBayes 3.2 (Ronquist et al. 2012) with an independent substitution model assigned to each data subset. Posterior distributions were estimated using Markov chain Monte Carlo (MCMC) sampling with four chains (three hot and one cold). Samples were drawn every 2000 steps over a total of MCMC 5×10^6 steps. A burnin of 2×10^6 steps was discarded, based on inspection of the trace files using Tracer v1.5 (Rambaut and Drummond 2007). We

used the Bayesian information criterion in PartitionFinder (Lanfear et al. 2012) to select the best-fitting model of nucleotide substitution, which for all data subsets was a GTR model with gamma-distributed rate variation across sites and a proportion of invariable sites (GTR+G+I).

We also ran the analysis using a GTR model with gamma-distributed rate variation across sites (GTR+G) to examine the impact of allowing a proportion of invariable sites. We ran this analysis for 2×10^7 MCMC steps, with samples drawn every 5000 steps. These analyses were only run on the alignment partitioned into four subsets. For the alignment partitioned into 28 subsets, we ran a separate analysis using a GTR+G model for 10^7 MCMC steps, with samples drawn every 5000 steps and with a discarded burn-in of 10^6 steps as determined with Tracer. We performed maximum-likelihood analyses with the GTR+G model in RAxML 8.2.4 (Stamatakis 2014). We used 1000 bootstrap replicates to estimate node support. The RAxML analysis was only run using the alignment partitioned into four subsets. All analyses were carried out in duplicate and the results were checked for consistency.

Molecular Dating

To estimate the evolutionary timescale of cockroaches, we analysed the concatenated sequence alignment using the Bayesian phylogenetic software BEAST 1.8.4 (Drummond and Rambaut 2007). This analysis was performed using a partitioning scheme that divided the data into four subsets. As a first step, we determined the model and speciation process that provided the best fit to our dataset. We compared two models of rate variation across branches: a strict-clock model and an uncorrelated lognormal relaxed clock model (Drummond et al. 2006). A Yule process and a birth-death process were compared for the tree prior (Gernhard 2008). Therefore, we performed four analyses in total, one for each possible combination of clock model and tree prior. For each analysis, posterior distributions of parameters, including the tree, were estimated using MCMC sampling. We performed two replicate MCMC runs, with the tree and parameter values sampled every 5000 steps over a total of 10^8 generations. A burn-in of 10^7 steps was discarded. A maximum-clade-credibility tree was obtained using TreeAnnotator in the BEAST software package. Acceptable sample sizes and convergence to the stationary distribution were checked using Tracer.

As a second step, we examined the influence of selected node calibrations on our molecular dating analyses. We calibrated the molecular clock using minimum age constraints based on the fossil record and implemented these as exponential priors on node ages. Soft maximum bounds were determined using phylogenetic bracketing (Ho and Phillips 2009). Altogether, we used 16 fossils to calibrate 15 nodes of our tree (table 1). We selected fossils following the suggested criteria for justifying fossil calibrations described by Parham et al. (2012). This procedure led us to exclude several fossils, such as those of *Piniblattella sharingolensis* and *Blattella lengleti*, which we could not assign to a node with enough confidence. We also used *Mylacris* and *Homocladus*, two fossil calibrations that did completely meet all the requirements, and for which we tested the effects of their placements. The first of these was the node representing the split between Dictyoptera and their sister group, represented by Phasmatodea+Grylloblattodea in our taxon sampling (see Misof et al. 2014), on which we placed a minimum age constraint of 315 Ma based on the primitive roachoid *Mylacris*. The second node represented the split between mantises and other dictyopterans, on which we tested two minimum age constraints: one of 279.5 Ma based on *Homocladus*, and the other of 145 Ma based on *Juramantis* (table 1).

We tested all possible combinations of the calibrations mentioned above, which involved six molecular-clock analyses to test their influence on our date estimates. We ran two replicates of the analysis with *Mylacris* and *Juramantis* calibrations in BEAST using an uncorrelated lognormal relaxed clock and a Yule tree prior. Other settings were as described above. The other five analyses were run in BEAST using the same parameters, but with the topology fixed to that obtained from the analysis using the *Mylacris* and *Juramantis* calibrations. This approach allowed us to test the influence of different node calibrations on our estimates of divergence times while excluding any impacts of differences in the inferred tree topology.

As a third step, we examined the influence of the partitioning scheme on the date estimates. We repeated our analysis using a partitioning scheme in which we divided the data into 28 subsets. The data were analysed using BEAST, with all fossil calibrations included (table 1). The analysis was performed using an uncorrelated lognormal relaxed clock and a Yule tree prior.

Testing the Influence of *Noctisola* on the Phylogenetic Estimate

In our trees estimated using maximum likelihood in RAxML and Bayesian inference in MrBayes, the branch leading to *Noctisola* was extremely long. Therefore, we suspected that *Noctisola*, and its erratic placement, might have been responsible for some inconsistency in the position of Corydiidae between the trees inferred using BEAST and using other phylogenetic methods. To test the influence of *Noctisola* on our phylogenetic estimates, we carried out one BEAST analysis and one MrBayes analysis without *Noctisola*. These analyses were based on a partitioning scheme with four data subsets. The MrBayes analysis used a GTR+G substitution model, and was run for 10^7 MCMC steps with samples drawn every 5000 steps, as described above. For the BEAST analysis, we used all fossil calibrations including *Mylacris* and *Juramantis* (table 1), and used an uncorrelated lognormal relaxed clock and a Yule tree prior.

Biogeographic Analyses

We reconstructed the evolution of the geographic ranges of cockroaches using the maximum-clade-credibility tree from BEAST (fig. 1), pruned to keep one representative for each genus. In the case of polyphyletic and paraphyletic genera, we kept one representative from each lineage. We chose this approach because the sampling of cockroach diversity was too incomplete to use species distribution ranges as input. We also only kept one termite representative, as the biogeography of this group has been thoroughly investigated elsewhere (Bourguignon et al. 2016, 2017). Geographic ranges were obtained from the Blattodea Species File (Beccaloni et al 2014) and mapped onto the tree using a Bayesian binary model implemented in the RASP 2.1 software (Yu et al. 2015). We used the F81 model with estimated state frequencies and gamma-distributed rate variation among sites (F81+G), with the default chain parameters for the Bayesian analysis (50,000 steps, sampling every 100 steps, 10 chains, and a temperature of 0.1). Using the JC model did not change the results. The maximum number of areas for each node was set to 1. The known native distribution of each genus was used to give each tip one or more biogeographic areas. We distinguished seven biogeographic areas: Australian, Afrotropical, Indo-Malayan, Madagascan, Nearctic, Neotropical, and Palearctic.

Acknowledgments

References

Anisyutkin LN, Rasnitsyn AP, Vršanský P. 2008. Cockroaches and mantises. Orders Blattodea (= Blattida) and Mantodea (= Mantida) descriptions. In: Krassilov V, Rasnitsyn A, editors. Plant-Arthopod interactions in the early angiosperm history: evidence from the Cretaceous of Israel. Sofia–Moscow & Leiden–Boston: Pensoft Publishers. p. 199–209.

Aristov DS, Rasnitsyn AP. 2015. Insects in the Paleozoic: milestones. *Priroda* 5:64-67.

Beccaloni GW. 2014. Blattodea Species File Online. Last accessed on May 2017. Available from: http://cockroach.speciesfile.org/HomePage/Cockroach/HomePage.aspx

Bell WJ, Roth LM, Nalepa CA. 2007. Cockroaches: ecology, behavior, and natural history. New York: John Hopkins University Press.

Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: Improved de novo metazoan mitochondrial genome annotation. *Mol Phylogenet Evol.* 69:313–319.

Béthoux O, Beckemeyer RJ, Engel MS, Hall JD. 2010. New data on *Homocladus grandis*, a Permian stem-mantodean (Polyneoptera: Dictyoptera). *J Paleontol.* 84:746–753.

Béthoux O, Wieland F. 2009. Evidence for Carboniferous origin of the order

Mantodea (Insecta: Dictyoptera) gained from forewing morphology. *Zool J Linn Soc.* 156:79–113.

Bourguignon T, Lo N, Cameron SL, Šobotník J, Hayashi Y, Shigenobu S, Watanabe D, Roisin Y, Miura T, Evans TA. 2015. The evolutionary history of termites as inferred from 66 mitochondrial genomes. *Mol Biol Evol.* 32:406–421.

Bourguignon T, Lo N, Šobotník J, Sillam-Dusses D, Roisin Y, Evans TA. 2016. Oceanic dispersal, vicariance and human introduction shaped the modern distribution of the termites *Reticulitermes*, *Heterotermes* and *Coptotermes*. *Proc R Soc Lond B.* 283:20160179.

Bourguignon T, Lo N, Šobotník J, Ho SYW, Iqbal N, Coissac E, Lee M, Jendryka MM, Sillam-Dussès D, Křížková B, et al. 2017. Mitochondrial phylogenomics resolves the global spread of higher termites, ecosystem engineers of the tropics. *Mol Biol Evol.* 34:589–597.

Bradler S, Buckley TR. 2011. Stick insect on unsafe ground: Does a fossil from the early Eocene of France really link Mesozoic taxa with the extant crown group of Phasmatodea? *Syst. Entomol.* 36:218–222.

Calderoni L, Rota-Stabelli O, Frigato E, Panziera A, Kirchner S, Foulkes NS, Kruckenhauser L, Bertolucci C, Fuselli S. 2016. Relaxed selective constraints drove functional modifications in peripheral photoreception of the cavefish *P. andruzzii* and provide insight into the time of cave colonization. *Heredity.* 117:383–392.

Cameron SL. 2014a. Insect mitochondrial genomics: Implications for evolution and phylogeny. *Annu Rev Entomol.* 59:95–117.

Cameron SL. 2014b. How to sequence and annotate insect mitochondrial genomes for systematic and comparative genomics research. *Syst Entomol.* 39:400–411.

Cameron SL, Lo N, Bourguignon T, Svenson GJ, Evans TA. 2012. A mitochondrial genome phylogeny of termites (Blattodea: Termitoidae): Robust support for interfamilial relationships and molecular synapomorphies define major clades. *Mol Phylogenet Evol.* 65:163–173.

Carpenter FM. 1966. The Lowe Permian Insects of Kansas. Part 11. The Orders Protorthoptera and Orthoptera. *Psyche.* 73:46–88.

Che Y, Wang D, Shi Y, Du X, Zhao Y, Lo N, Wang Z. 2016. A global molecular phylogeny and timescale of evolution for *Cryptocercus* woodroaches. *Mol Phylogenet Evol.* 98:201–209.

Cockerell TDA. 1920. Fossil arthropods in the British Museum - I. *Ann Mag Nat Hist 9th ser.* 5:273–279.

Cook LG, Crisp MD. 2005. Not so ancient: the extant crown group of *Nothofagus* represents a post-Gondwanan radiation. *Proc R Soc Lond B.* 272:2535–2544.

Cracraft J. 2001. Avian evolution, Gondwana biogeography and the Cretaceous-Tertiary mass extinction event. *Proc R Soc Lond B.* 268:459–469.

Cranston PS, Hardy NB, Morse GE. 2012. A dated molecular phylogeny for the Chironomidae (Diptera). *Syst Entomol.* 37:172–188.

Darwin C. 1859. On the origin of the species by means of natural selection. London: John Murray.

Djernæs M, Klass KD, Eggleton P. 2015. Identifying possible sister groups of Cryptocercidae + Isoptera: A combined molecular and morphological phylogeny of Dictyoptera. *Mol Phylogenet Evol.* 84:284–303.

Djernæs M, Klass KD, Picker MD, Damgaard J. 2012. Phylogeny of cockroaches (Insecta, Dictyoptera, Blattodea), with placement of aberrant taxa and exploration of out-group sampling. *Syst Entomol.* 37:65–83.

Drummond AJ, Ho SYW, Phillips MJ, Rambaut A. 2006. Relaxed phylogenetics and dating with confidence. *PLOS Biol.* 4:699–710.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol.* 7:214.

Edgar RC. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5:113.

Emerson AE. 1971. Tertiary fossil species of the Rhinotermitidae (Isoptera), phylogeny of genera, and reciprocal phylogeny of associated *Flagellata* (Protozoa) and the Staphylinidae (Coleoptera). *Bull Am Mus Nat His* 146:243–304.

Engel M, David AG. 2004. New light shed on the oldest insect. *Nature* 427:627–630.

Engel MS, Grimaldi DA, Krishna K. 2007. Primitive termites from the Early Cretaceous. *Stuttgarter Beitr Naturk.* 371:1–32.

Engel M, Grimaldi D, Nascimbene P, Singh H. 2011. The termites of Early Eocene Cambay amber, with the earliest record of the Termitidae (Isoptera). *ZooKeys* 148:105–123.

Friedman M, Keck BP, Dornburg A, Eytan RI, Martin CH, Hulsey CD, Wainwright

PC, Near TJ. 2013. Molecular and fossil evidence place the origin of cichlid fishes long after Gondwanan rifting. *Proc R Soc Lond B*. 280:20131733–20131733.

Garwood R, Sutton M. 2010. X-ray micro-tomography of Carboniferous stem-Dictyoptera: new insights into early insects. *Biol Lett.* 6:699–702.

Gernhard T. 2008. The conditioned reconstructed process. *J Theor Biol.* 253:769–778.

Giribet G, Sharma PP, Benavides LR, Boyer SL, Clouse RM, de Bivort BL, Dimitrov D, Kawauchi GY, Murienne J, Schwendinger PJ. 2012. Evolutionary and biogeographical history of an ancient and global group of arachnids (Arachnida: Opiliones: Cyphophthalmi) with a new taxonomic arrangement. *Biol J Linn Soc.* 105:92–130.

Gorochov AV. 2013. Taxonomy of the katydids (Orthoptera: Tettigoniidae) from East Asia and adjacent islands. Communication 7. *Far East Entomol.* 266:1–24.

Grandcolas P. 1996. The phylogeny of cockroach families: a cladistic appraisal of morpho-anatomical data. *Can J Zool.* 74:508–527.

Grimaldi D. 2003. A revision of Cretaceous mantises and their relationships, including new taxa (Insecta: Dictyoptera: Mantodea). *Am Museum Novit.* 3412:1–47.

Grimaldi D, Engel MS. 2005. Evolution of the Insects. Cambridge: Cambridge University Press

Grimaldi DA, Engel MS, Krishna K. 2008. The species of Isoptera (Insecta) from the Early Cretaceous Crato formation: a revision. *Am Museum Novit.* 3626:1–30.

Hedges SB, Dudley J, Kumar S. 2006. TimeTree: A public knowledge-base of divergence times among organisms. *Bioinformatics* 22:2971–2972.

Ho SYW, Phillips MJ. 2009. Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. *Syst Biol.* 58:367–380.

Inward D, Beccaloni G, Eggleton P. 2007. Death of an order: a comprehensive molecular phylogenetic study confirms that termites are eusocial cockroaches. *Biol Lett.* 3:331–335.

Kambhampati S. 1995. A phylogeny of cockroaches and related insects based on DNA sequence of mitochondrial ribosomal RNA genes. *Proc. Natl. Acad. Sci. USA.* 92:2017–2020.

Klass KD, Meier R. 2006. A phylogenetic analysis of Dictyoptera (Insecta) based on morphological characters. *Entomol Abhandlungen.* 63:3–50.

Klass KD. 1997. The External Male Genitalia and the Phylogeny of Blattaria and Mantodea. *Bonn Zool Monogr.* 42.

Knapp M, Stöckler K, Havell D, Delsuc F, Sebastiani F, Lockhart PJ. 2005. Relaxed molecular clock provides evidence for long-distance dispersal of Nothofagus (southern beech). *PLOS Biol.* 3:e14.

Krishna K, Grimaldi DA, Krishna V, Engel MS. 2013. Treatise on the Isoptera of the World: Volume 1 Introduction. *Bull Am Mus Nat Hist.* 377:1–200.

Kukalová J. 1964. Permian insects of Moravia. Part 2. Liomopteridae. Sborník geologických véd: *Paleontologie.* 3:39–118.

Labandeira CC. 1994. A compendium of fossil insect families. In: Watkins R, editor. Contributions in Biology and Geology, Vol. 88. Wisconsin:Milwaukee Public Museum.

Lanfear R, Calcott B, Ho SYW, Guindon S. 2012. PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol Biol Evol.* 29:1695–1701.

Legendre F, Grandcolas P, Thouzé F. 2017. Molecular phylogeny of Blaberidae (Dictyoptera, Blattodea) with implications for taxonomy and evolutionary studies. *Eur J Taxon.* 291:1-13

Legendre F, Nel A, Svenson GJ, Robillard T, Pellens R, Grandcolas P. 2015. Phylogeny of dictyoptera: Dating the origin of cockroaches, praying mantises and termites with molecular data and controlled fossil evidence. *PLOS ONE.* 10:1–27.

Lin Q. 1980. Fossil insects from the Mesozoic of Zhejiang and Anhui provinces. In: Division and Correlation of the Mesozoic Volcano-Sedimentary Strata in Zhejiang and Anhui Provinces. Science Press Beijing. p. 211–234.

Lo N, Bandi C, Watanabe H, Nalepa C, Beninati T. 2003. Evidence for cocladogenesis between diverse dictyopteran lineages and their intracellular endosymbionts. *Mol Biol Evol.* 20:907–913.

Lo N, Beninati T, Stone F, Walker J, Sacchi L. 2007. Cockroaches that lack Blattabacterium endosymbionts: the phylogenetically divergent genus *Nocticola*. *Biol Lett.* 3:327–330.

Lo N, Tokuda G, Watanabe H, Rose H, Slaytor M, Maekawa K, Bandi C, Noda H. 2000. Evidence from multiple gene seqeunces indicates that termites evolved from wood-feeding cockroaches. *Curr Biol.* 10:801–804.

Lo N, Tong KJ, Rose HA, Ho SYW, Beninati T, Low DLT, Matsumoto T, Maekawa
  K. 2016. Multiple evolutionary origins of Australian soil-burrowing cockroaches
  driven by climate change in the Neogene. *Proc R Soc Lond B*. 283: 20152869.

MacGinitie HD. 1958. Climate since the late Cretaceous. In: Hubbs CL, editor.
  Symposium on zoogeography. Washington DC: American Association for the
  Advancement of Science. p. 61–80.

Maekawa K, Lo N, Rose HA, Matsumoto T. 2003. The evolution of soil-burrowing
  cockroaches (Blattaria: Blaberidae) from wood-burrowing ancestors following
  an invasion of the latter from Asia into Australia. *Proc R Soc Lond B*. 270:1301–
  1307.

Martins-Neto RG, Mancuso A, Gallego OF. 2005. The Triassic insect fauna from
  Argentina. Blattoptera from the Los Rastros formation (Bermejo Basin), La
  Rioja Province. *Ameghiniana.* 42:705–723.

Misof B, Liu S, Meusemann K, Peters RS, Flouri T, Beutel RG, Niehuis O, Petersen
  M. 2014. Phylogenomics resolves the timing and pattern of insect evolution.
  Science. 346:763–768.

Morley RJ. 2011. Cretaceous and Tertiary climate change and the past distribution of
  megathermal rainforests. In: Bush MB, Flenley J, Gosling WD, Morley RJ,
  editors. Tropical rainforest responses to climatic change. Berlin: Springer. p. 1–
  34.

Murienne J. 2009. Molecular data confirm family status for the *Tryonicus-
  Lauraesilpha* group (Insecta: Blattodea: Tryonicidae). *Org Divers Evol.* 9:44–51.

Murienne J, Daniels SR, Buckley TR, Mayer G, Giribet G. 2014. A living fossil tale
  of Pangaean biogeography. *Proc R Soc Lond B*. 281:20132648.

Nalepa CA, Bandi C. 2000. Characterizing the ancestors: paedomorphosis and termite
  evolution. In: Abe T, Bignell DE, Higashi M, editors. Termites: evolution,
  sociality, symbioses, ecology. Dordrecht: Springer. p. 53–75.

Parham J, Donoghue PCJ, Bell CJ, Calway TD, Head JJ, Holroyd PA, Inoue JG, Irmis
  RB, Joyce WG, Ksepka DT, et al. 2012. Best practices for justifying fossil
  calibrations. Syst. Biol. 61:346–359.

Peck SB, Roth LM. 1992. Cockroaches of the Galápagos Islands, Ecuador, with
  descriptions of three new species (Insecta: Blattodea). *Can J Zool.* 70:2202–2217.

Phillips MJ, Gibb GC, Crimp EA, Penny D. 2010. Tinamous and moa flock together: Mitochondrial genome sequence analysis reveals independent losses of flight among ratites. *Syst Biol.* 59:90–107.

Piton LE. 1940. Paléontologie du gisement Éocène de Menat. Paris: Lechevalier.

Prokop J, Krzeminski W, Krzeminska E, Hörnschemeyer T, Ilger JM, Brauckmann C, Grandcolas P, Nel A. 2014. Late Palaeozoic Paoliida is the sister group of Dictyoptera (Insecta: Neoptera). *J Syst Palaeontol.* 12:601–622.

Rambaut A, Drummond AJ. 2007. Tracer, version 1.5. Last accessed on February 2017. Available from: http://beast.bio.ed.ac.uk/Tracer.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Syst Biol.* 61:539–542.

Roth LM, Mcgavin GC. 1994. Two new species of Nocticolidae (Dictyoptera: Blattaria) and a rediagnosis of the cavernicolous genus *Spelaeoblatta* Bolívar. *J Nat Hist.* 28:1319–1326.

SanMartin I, Enghoff H, Ronquist F. 2001. Patterns of animal dispersal, vicariance and diversification in the Holarctic. *Biol J Linn Soc.* 73:345–390.

Shelford R. 1910. On a collection of Blattidae preserved in amber, from Prussia. *J. Linn Soc Lond Zool.* 30:336-355.

Scotese CR. 2004a. Cenozoic and Mesozoic Paleogeography: Changing terrestrial biogeographic pathways. In: Lomolino MV, Heaney LR, editors. Frontiers of biogeography: new directions in the geography of nature. Sunderland: Sinauer Associates. p. 9-26.

Scotese CR. 2004b. A continental drift flipbook. *J Geol.* 112:729–741.

Scudder SH. 1868. Supplement to descriptions of articulates. Descriptions of fossil insects found on Mazon Creek, and near Morris, Grundy Co. Ill. *Geological Survey of Illinois* **3**:566-572

Sendi H, & Azar D. 2017. New aposematic and presumably repellent bark cockroach from Lebanese amber. *Cret Res*. 72:13–17.

Seward D, Grujic D, Schreurs G. 2004. An insight into the breakup of Gondwana: Identifying events through low-temperature thermochronology from the basement rocks of Madagascar. *Tectonics* 23:C3007.

Stamatakis A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-

analysis of large phylogenies. *Bioinformatics.* 30:1312–1313.

Svenson GJ, Whiting MF. 2004. Phylogeny of Mantodea based on molecular data: Evolution of a charismatic predator. *Syst Entomol.* 29:359–370.

Svenson GJ, Whiting MF. 2009. Reconstructing the origins of praying mantises (Dictyoptera, Mantodea): The roles of Gondwanan vicariance and morphological convergence. *Cladistics.* 25:468–514.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol.* 28:2731–2739.

Thorne BL, Grimaldi DA, Krishna K. 2000. Early Fossil History of the Termites. In Termite: evolution, sociality, symbioses, ecology. Dordrecht:Springer. p.77–93.

Tong KJ, Duchene S, Ho SYW, Lo N. 2015. Comment on "Phylogenomics resolves the timing and pattern of insect evolution." *Science* 349:487b.

Vršanský P. 1997. *Piniblattella* gen. nov. — the most ancient genus of the family Blattellidae (Blattodea) from the Lower Cretaceous of Siberia. *Entomol Probl.* 28:67–79.

Vršanský P. 2002. Origin and the early evolution of mantises. *Amba Proj.* 6:1–16.

Vršanský P. 2005. Lower Cretaceous cockroaches and mantids (Insecta: Blattaria, Mantodea) from the Sharin-Gol in Mongolia. Entomological Problems 35:163–167.

Vršanský P, Vidlička L, Barna P, Bugdaeva E, Markevich V. 2013. Paleocene origin of the cockroach families blaberidae and corydiidae: Evidence from amur river region of Russia. *Zootaxa* 3635:117–126.

Vršanský P, Oružinský R, Barna P, Vidlička L, Labandeira CC. 2014. Native *Ectobius* (Blattaria: Ectobiidae) from the Early Eocene Green River formation of Colorado and its reintroduction to North America 49 Million years later. Ann. Entomol. *Soc. Am.* 107:28–36.

Vršanský PV, Šmídová L, Valaška D, Barna P, Vidlička L, Takáč P, Pavlik L, Kúdelová T, Karim TS, Zelagin D, Smith D. 2016. Origin of origami cockroach reveals long-lasting (11 Ma) phenotype instability following viviparity. *Sci Nat.* 103:78.

Wang ZQ, Shi Y, Qiu Z, Che Y, Lo N. 2017. Reconstructing the phylogeny of Blattodea: robust support for interfamilial relationships and major clades. *Sci*

Rep. 7:3903.

Ware JL, Grimaldi DA, Engel MS. 2010. The effects of fossil placement and calibration on divergence times and rates: An example from the termites (Insecta: Isoptera). *Arthropod Struct Dev.* 39:204–219.

Ware JL, Litman J, Klass KD, Spearman LA. 2008. Relationships among the major lineages of Dictyoptera: The effect of outgroup selection on dictyopteran tree topology. *Syst Entomol.* 33:429–450.

Wegener A. 1912. Die entstehung der kontinente. *Geol Rundschau.* 3:276–292.

Wood HM, Matzke NJ, Gillespie RG, Griswold CE. 2013. Treating fossils as terminal taxa in divergence time estimation reveals ancient vicariance patterns in the palpimanoid spiders. *Syst Biol.* 62:264–284.

Xia X, Lemey P. 2009. Assessing substitution saturation with DAMBE. In: Lemey P, Salemi M, Vadamme A, editors. The phylogenetic handbook: a practical approach to DNA protein phylogeny. United Kingdom: Cambridge University Press. p.615–630.

Xia X, Xie Z, Salemi M, Chen L, Wang Y. 2003. An index of substitution saturation and its application. *Mol Phylogenet Evol.* 26:1–7.

Yoder AD, Nowak MD. 2006. Has vicariance or dispersal been the predominant biogeographic force in Madagascar? Only time will tell. *Annu Rev Ecol Evol Syst.* 37:405–431.

Yu Y, Harris AJ, Blair C, He X. 2015. RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. *Mol Phylogenet Evol.* 87:46–49.

Zhang Z, Schneider JW, Hong Y. 2013. The most ancient roach (Blattodea): a new genus and species from the earliest Late Carboniferous (Namurian) of China, with a discussion of the phylomorphogeny of early blattids. *J Syst Palaeontol.* 11:27–40.

Species	Age (Ma) / min. age constraint for group	Calibration group	Soft max. bound (97.5% probability)	Reference	Comments on soft max. bound
Mylacris anthracophila	315	Dictyoptera + Phasmatodea + Grylloblattodea	407	Scudder, 1868	First insect fossil (Engel and Grimaldi 2004)
Homocladus grandis	279.5	Dictyoptera	315.2	Carpenter, 1966	First cockroach-like fossils
Juramantis initialis	145	Dictyoptera	315.2	Vrsansky, 2002	First cockroach-like fossils
Valditermes brenanae	130	Hodotermitidae + other Isoptera, excluding *Mastotermes*	235	Krishna et al. 2013 and refs therein	*Triassoblatta argentina*, first fossil of Mesoblattinidae (Martins-Neto and Gallego 2005)
Cratokalotermes santanensis	112	Kalotermitidae + Rhinotermitidae + Termitidae	145	Grimaldi et al. 2008	First fossil of termites
Reticulitermes antiquus	33.9	*Reticulitermes + Coptotermes + Heterotermes*	94.3	Engel and Krishna 2007b	First fossil of Rhinotermitinae
Coptotermes sucineus	16	*Coptotermes + Heterotermes*	33.9	Emerson 1971	First *Heterotermes* fossil
Nanotermes	47.8	Termitidae + *Coptotermes + Heterotermes + Reticulitermes*	94.3	Engel et al. 2011	First fossil of Rhinotermitinae
Balatronis libanensis	125	Blattidae + Tryonicidae	235	Sendi and Azar 2017	*Triassoblatta argentina*, first fossil of Mesoblattinidae (Martins-Neto and Gallego 2005*)*
Ergaula stonebut	61.7	*Ergaula + Therea*	145	Vrsansky et al. 2013	First modern cockroach: Zhujiblatta (Lin 1980)
Periplaneta houlberti	56	*Periplaneta + Shelfordella + Blatta + Neostylopyga + Deropeltis*	145	Piton 1940	First modern cockroach: *Zhujiblatta* (Lin 1980)
Gyna obesa	56	Gyninae + Panchlorinae + Blaberinae	145	Piton 1940	First modern cockroach: *Zhujiblatta* (Lin 1980)
Diploptera	56	Diplopterinae + Oxyhaloinae	145	Vrsansky et al. 2016	First modern cockroach: *Zhujiblatta* (Lin 1980)
Pycnoscelus gardneri	41.3	Panesthiinae + Perisphaerinae + Pycnoscelinae (+*Rhabdoblatta*)	145	Cockerell 1920	First modern cockroach: *Zhujiblatta* (Lin 1980)
Ischnoptera gedanensis	33.9	*Ischnoptera* + sister	145	Scheffold 1910	First modern cockroach: *Zhujiblatta* (Lin 1980)
Epilampra	41.3	*Epilampra + Galiblatta*	145	Beccaloni 2014	First modern cockroach: *Zhujiblatta* (Lin 1980)

Table 1. Fossils used to calibrate the estimates of divergence times of major cockroach clades (see fig. 1). Several molecular dating analyses were run with and

without *Mylacris anthracophila*, *Homocladus grandis*, and *Juramantis initialis* to test the influence of these fossils on cockroach age estimates (see fig. 2).
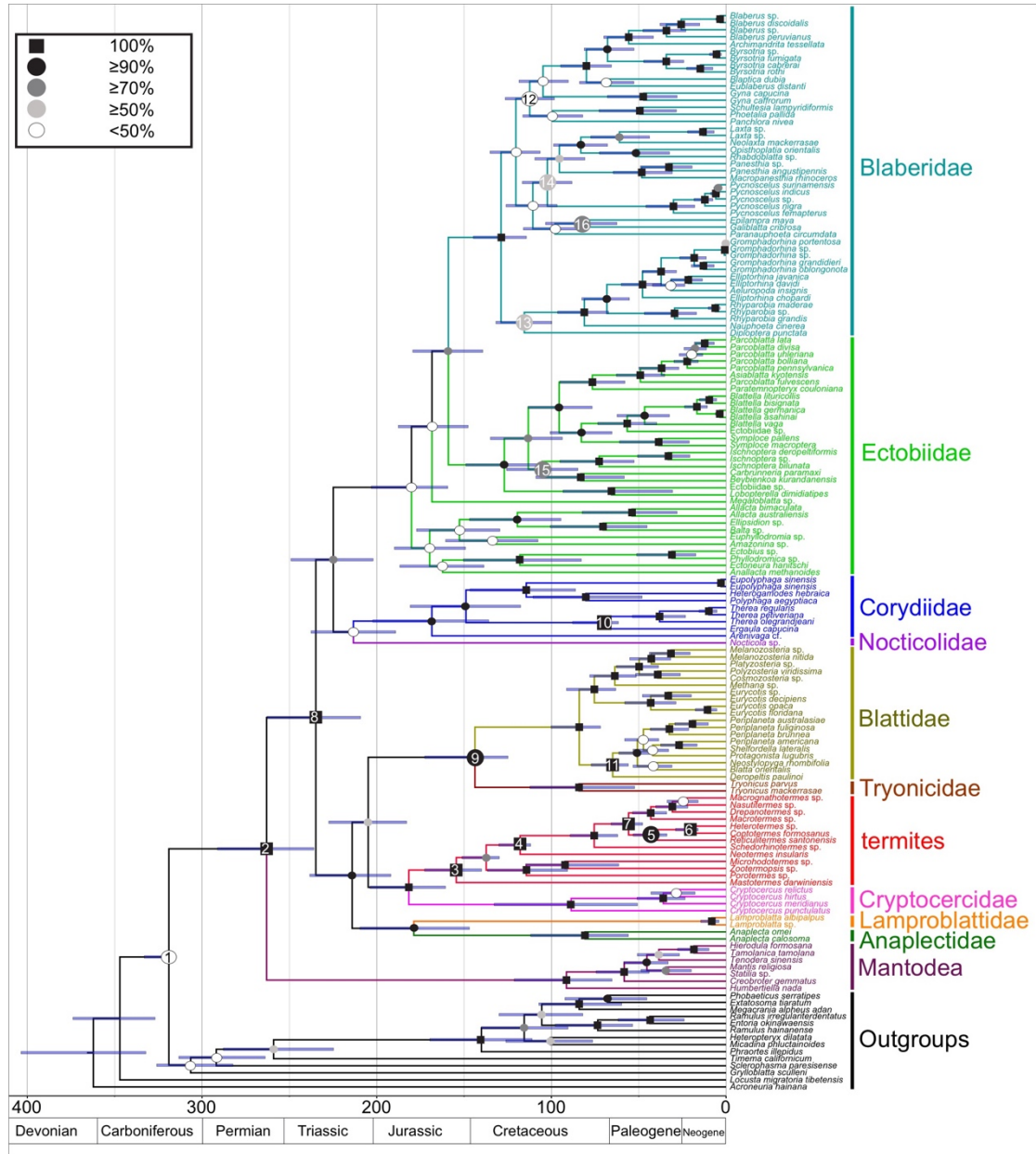

Fig. 1. Bayesian time-tree of cockroaches inferred from complete mitochondrial genomes, with third codon positions excluded. The time-tree was calibrated with 15 fossils, including *Mylacris* and *Juramantis*. Numbers are labels for calibrated nodes: 1. *Mylacris anthracophila*, 2. *Juramantis initialis*, 3. *Valditermes brenanae*, 4. *Cratokalotermes santanensis*, 5. *Reticulitermes antiquus*, 6. *Coptotermes sucineus*, 7. *Nanotermes*, 8. *Balatronis libanensis*, 9. *Ergaula stonebut*, 10. *Periplaneta houlberti*, 11. *Gyna obesa*, 12. *Diploptera*, 13. *Pycnoscelus gardneri*, 14. *Ischnoptera gedanensis*, 15. *Epilampra*. The scale bar is given in millions of years. Grey bars at internal nodes represent the 95% credibility intervals of age estimates. Branches are

31

labelled with symbols representing the minimal support in three analyses: posterior probabilities inferred with BEAST, MrBayes under a GTR+G substitution model, and bootstrap support inferred with RAxML.
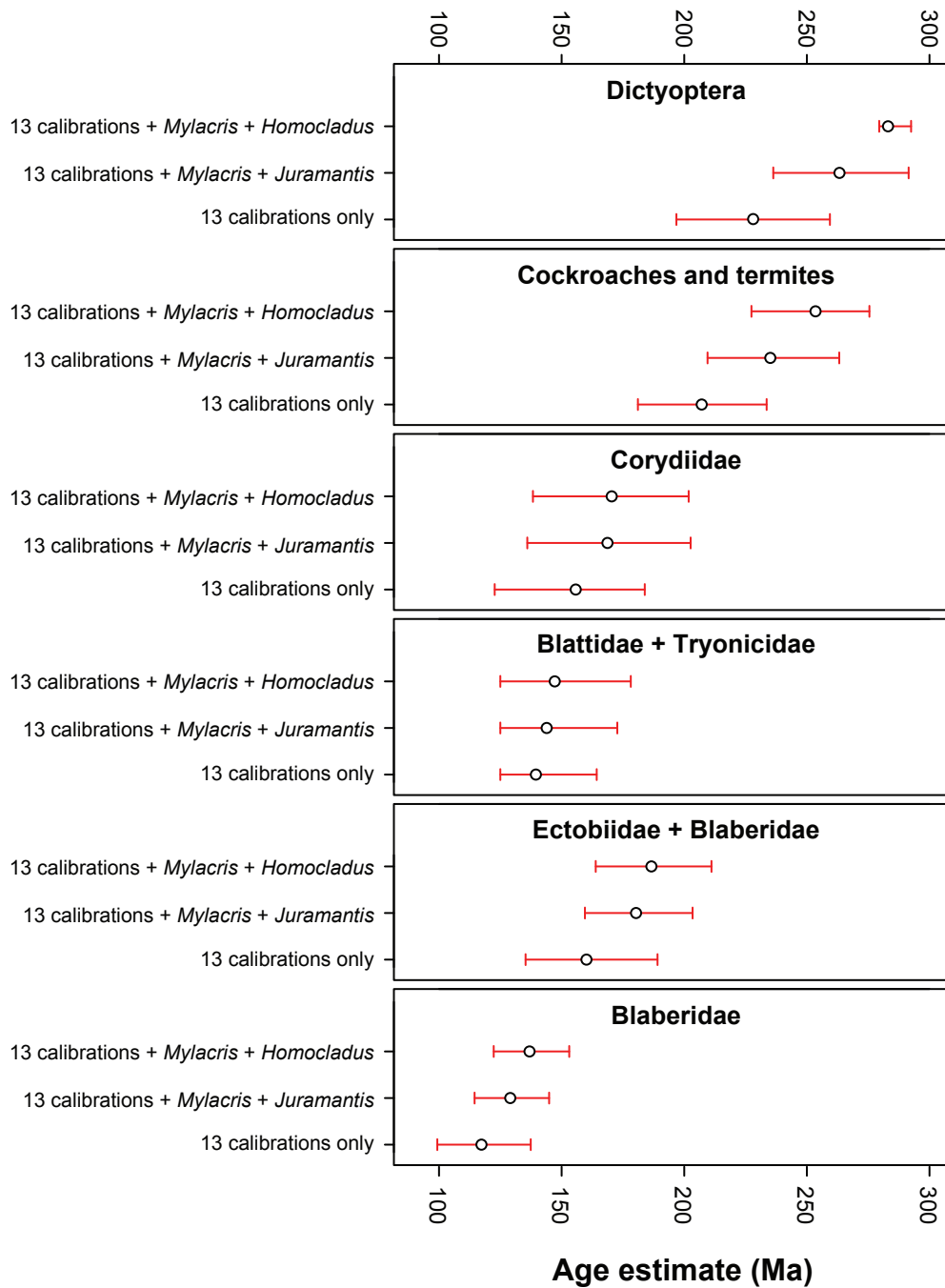
FIG. 2. Bayesian phylogenetic estimates for the ages of major cockroach clades, using a core set of 13 fossil calibrations and up to two additional fossil calibrations.
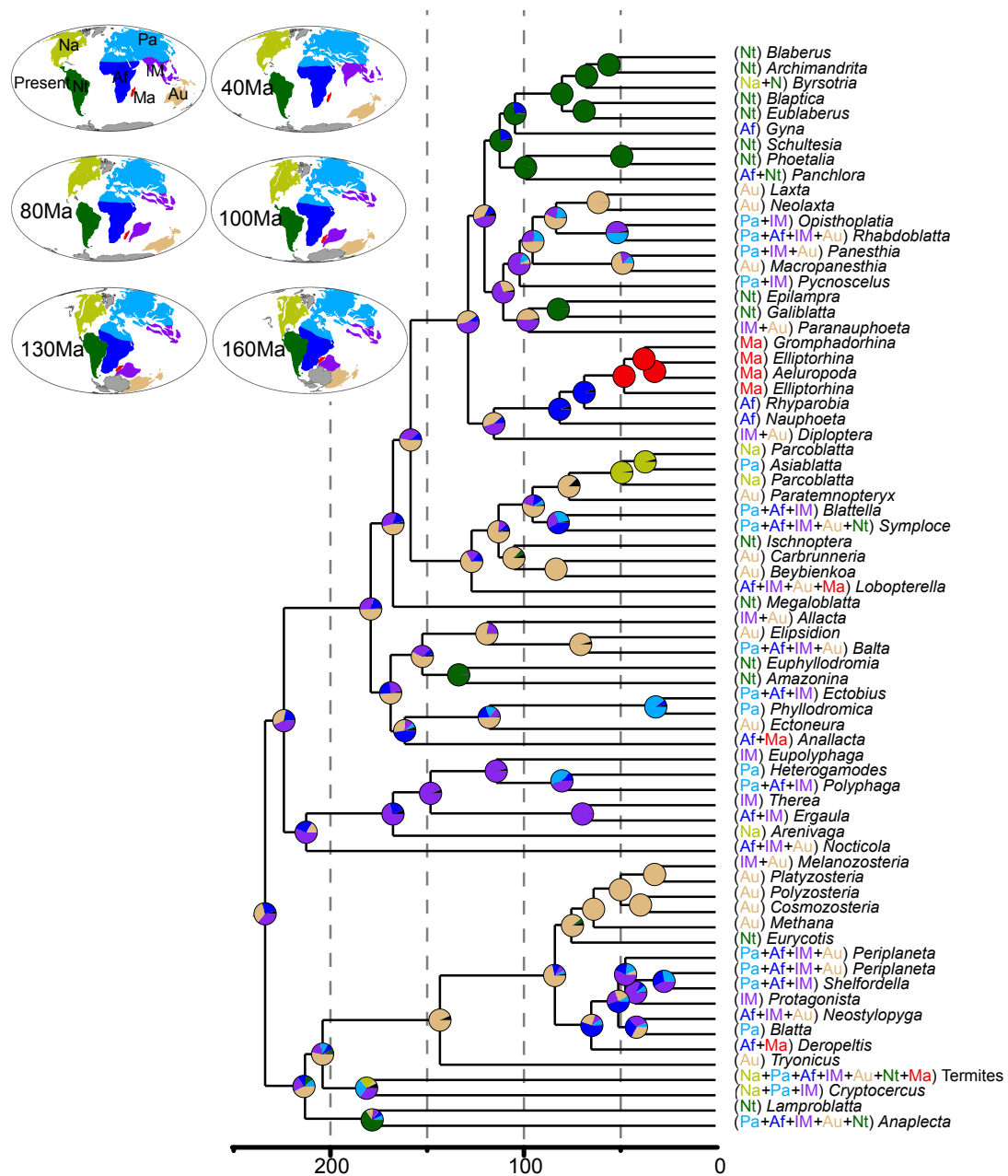
FIG. 3. Reconstruction of ancestral distributions of cockroaches using a Bayesian binary model implemented in RASP (Yu et al. 2015). We used the time-tree from figure 1, with branches from congeneric species collapsed. Node pie charts show the reconstructed ancestral states. Maps show the evolution of Earth during the last 160 million years (Scotese 2004b), and the position of each biogeographic area considered in the analysis (Udvarty 1975): Au, Australian; Af, Afrotropical; IM, Indo-Malayan; Ma, Madagascan; Na, Nearctic; Nt, Neotropical; Pa, Palaearctic. Briefly, India and Madagascar became separated from Africa 140 Ma, and India severed from Madagascar 85–95 Ma (Scotese 2004a, Seward et al. 2004); Africa was last connected to the rest of Gondwana through South America 100 Ma (Cracraft 2001); and South

America and Australia maintained connections through Antarctica until 60–70 Ma (Scotese 2004a). Numbered grey circles indicate divergences that are consistent with vicariance through plate tectonics.