The Evolutionary History of Termites as Inferred from 66 Mitochondrial Genomes

Thomas Bourguignon,*[1,2,3] Nathan Lo,[4] Stephen L. Cameron,[5] Jan Šobotník,[3] Yoshinobu Hayashi,[2,4] Shuji Shigenobu,[6] Dai Watanabe,[2] Yves Roisin,[7] Toru Miura,[2] and Theodore A. Evans[1]

[1]Department of Biological Sciences, National University of Singapore, Singapore, Singapore

[2]Graduate School of Environmental Science, Hokkaido University, Sapporo, Hokkaido, Japan

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

[4]School of Biological Sciences, University of Sydney, Sydney, NSW, Australia

[5]Earth, Environmental and Biological Sciences, Science and Engineering Faculty, Queensland University of Technology, Brisbane, QLD, Australia

[6]Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences, Okazaki, Japan

[7]Evolutionary Biology and Ecology, Université Libre de Bruxelles, Bruxelles, Belgium

***Corresponding author:** E-mail: thomas.bourgui@gmail.com.

Associate editor: Nicolas Vidal

Abstract

Termites have colonized many habitats and are among the most abundant animals in tropical ecosystems, which they modify considerably through their actions. The timing of their rise in abundance and of the dispersal events that gave rise to modern termite lineages is not well understood. To shed light on termite origins and diversification, we sequenced the mitochondrial genome of 48 termite species and combined them with 18 previously sequenced termite mitochondrial genomes for phylogenetic and molecular clock analyses using multiple fossil calibrations. The 66 genomes represent most major clades of termites. Unlike previous phylogenetic studies based on fewer molecular data, our phylogenetic tree is fully resolved for the lower termites. The phylogenetic positions of Macrotermitinae and Apicotermitinae are also resolved as the basal groups in the higher termites, but in the crown termitid groups, including Termitinae + Syntermitinae + Nasutitermitinae + Cubitermitinae, the position of some nodes remains uncertain. Our molecular clock tree indicates that the lineages leading to termites and *Cryptocercus* roaches diverged 170 Ma (153–196 Ma 95% confidence interval [CI]), that modern Termitidae arose 54 Ma (46–66 Ma 95% CI), and that the crown termitid group arose 40 Ma (35–49 Ma 95% CI). This indicates that the distribution of basal termite clades was influenced by the final stages of the breakup of Pangaea. Our inference of ancestral geographic ranges shows that the Termitidae, which includes more than 75% of extant termite species, most likely originated in Africa or Asia, and acquired their pantropical distribution after a series of dispersal and subsequent diversification events.

Key words: **biogeography, Isoptera, molecular clock, molecular phylogeny.**

Introduction

Termites are important organisms for economic and ecological reasons. Termites are well known for their ability to damage human structures (Su and Scheffrahn 2000) and agricultural crops (Rouland-Lefèvre 2011); however, the vast majority of species are not pests (only 12%; for a comprehensive list, see table 9 in Krishna et al. 2013). Most species inhabit tropical and subtropical ecosystems, where they are particularly abundant in terms of biomass (Eggleton et al. 1996) and are the principal decomposers of organic matter (Holt and Lepage 2000; Sugimoto et al. 2000). Termites also have a strong influence on their habitat, modifying the topography, soil structural and chemical properties, and plant growth rates. These activities have led to their recognition as ecosystem engineers (e.g., Jouquet et al. 2006; Fox-Dobbs et al. 2010; Evans et al. 2011). The diet of termites primarily consists of wood in basal lineages, but it has diversified in the more advanced Termitidae where it includes several kinds of organic matter: Sound or decayed wood, litter, grasses, lichens, soil organic matter, and fungi that members of the subfamily Macrotermitinae cultivate in their nests (Abe 1979; Bourguignon et al. 2011).

The evolution of termites is of great interest, due to their diversity of diet, social structures, and phenotypes. Termites evolved from wood-feeding cockroaches, and form a sister group with the cockroach genus *Cryptocercus* (Lo et al. 2000). The precise timing of the appearance and subsequent diversification of termites is not well understood, although fossil records do provide a glimpse into the evolutionary history of these insects. The first undisputed termite fossils are from the early Cretaceous, 110–135 Ma, and all belong to Mastotermitidae, Hodotermitidae, Kalotermitidae or to extinct families (Krishna et al. 2013). The oldest known fossil of Rhinotermitidae is *Archeorhinotermes* from the mid-Cretaceous, 98 Ma (Krishna and Grimaldi 2003; Krishna et al. 2013). The first Termitidae fossil is much more recent

doi:10.1093/molbev/msu308 Advance Access publication November 10, 2014

and dated to the early Eocene, 50 Ma (Engel et al. 2011). The family then remains rare in the fossil record until it reappears as a well-diversified group in the Dominican amber, 18 Ma, and includes some modern genera such as *Constrictotermes*, *Nasutitermes*, and *Microcerotermes* (Krishna and Grimaldi 2009). Fossil records therefore suggest that termites probably diverged from cryptocercid roaches during the late Jurassic, and that Termitidae, which includes 75% of modern termite species, diversified over the last 50 Ma, becoming abundant more recently (Engel et al. 2009). Molecular clocks have also been used to estimate divergence dates of the genus *Macrotermes* (Brandl et al. 2007) and the extant termite families using supertree methods (Davis et al. 2009). Recently, Ware et al. (2010) carried out the most comprehensive analysis and tested different analytical methods to investigate termite dating and divergence rates. They estimated termites to have originated between 172 and 235 Ma, and that Termitidae originated between 44 and 132 Ma. Given the wide range of divergence dates currently available, more studies are needed to provide a more precise picture.

The two most comprehensive studies of termite phylogenetic relationships in terms of taxon sampling were performed by Inward et al. (2007) and Legendre et al. (2008). The former analyzed 40 morphological characters and sequenced three genetic markers for 231 species, whereas the latter used seven genetic markers and 40 species. Although congruence exists between both trees, their topologies differ in several respects. Legendre et al. (2013) reanalyzed their data set and reconciled part of their results with Inward et al. (2007) and Cameron et al. (2012) resolved some of the differences with a termite phylogeny inferred from the full mitochondrial genomes of 13 species. Mitochondrial genomes contain approximately 16,000 pairs of bases, which is approximately five times the number used in previous studies. Unlike previous studies, the mitochondrial genome based tree inferred by Cameron et al. (2012) recovered high nodal support across almost all nodes.

Termites have a typical metazoan mitochondrial genome (Gray 2012) of around 16 kb which encodes for 37 genes, including 22 transfer RNAs (tRNAs), 13 protein-coding genes, and 2 ribosomal RNA genes (Cameron et al. 2012). Phylogenetic information for resolving deeper relationships can be provided by mitochondrial DNA gene rearrangements (Boore 2006; Cameron 2014); however, gene order is conserved in termites and arranged as in the putative ancestral insect mitochondrial genome (Cameron and Whiting 2007; Cameron et al. 2012). Termite mitochondrial genomes do contain rare genomic changes consisting of a major hairpin loop located in the control region that they share with the related *Cryptocercus* and *Eupolyphaga* roaches (Cameron et al. 2012). The same region also includes a macrorepeat structure shared by all Neoisoptera that changed in structure at least twice, once in Heterotermitinae + Coptotermitinae + Termitidae and once in Nasutitermitinae + Termitinae (Cameron et al. 2012). Although the mitochondrial genome represents a single marker, maternally transmitted in a single package, mitochondrial genome sequences are very informative and recent molecular phylogenies inferred from the full mitochondrial genome complement shed light on the relationships between insect orders (Trautwein et al. 2012) and within orders at the family level or above (see Cameron 2014).

A number of open questions remain concerning the monophyly of several termite families and subfamilies, particularly in the clade comprising Serritermitidae, Rhinotermitidae, and Termitidae. In this study, we sequenced the mitochondrial genome of 48 termite species, which, with the 18 termite mitochondrial genomes already sequenced, are representative of termite diversity. Our aims were 3-fold:

1. To provide a robust phylogenetic tree that can be used to infer termite family and subfamily relationships and be the basis of future taxonomic changes needed to reflect termite cladistics.
2. To date the origin of the main termite clades.
3. Using our phylogenetic tree, to disentangle the distribution patterns of extant termites.

Results

Mitochondrial Genome Phylogeny

Complete mitochondrial genomes of the 48 termite species sequenced in this study were deposited in GenBank (table 1). Trees obtained from the six Bayesian phylogenies were almost identical and neither partitioning schemes nor third base deletions affected tree topologies, except in the crown termitid subfamilies Termitinae + Nasutitermitinae + Syntermitinae + Cubitermitinae, in which several nodes differed between analyses. These nodes also had low Bayesian posterior probabilities, which indicated that the position of these branches on the tree was uncertain. Other branches all had 100% Bayesian posterior probabilities (fig. 1).

As in previous molecular phylogenetic studies, the Isoptera were confirmed as a clade. Mastotermitidae was retrieved as the sister group of other termites (= the Euisoptera clade). Archotermopsidae + Hodotermitidae + Stolotermitidae formed a monophyletic group, with Hodotermitidae nested within a paraphyletic Archotermopsidae. Kalotermitidae were monophyletic and the sister group of Neoisoptera, which comprised Serritermitidae + Rhinotermitidae + Termitidae. Rhinotermitidae formed a polyphyletic assemblage with Serritermitidae recovered as the sister group of *Termitogeton*, which together form the sister group of *Prorhinotermes*. Termitidae was the sister group of Coptotermitinae + Heterotermitinae. Among Rhinotermitidae subfamilies, Rhinotermitinae was found to be monophyletic and formed the most basal clade of the group, whereas Coptotermitinae was nested within Heterotermitinae. All subfamilies of Termitidae were retrieved as monophyletic except the Termitinae which was polyphyletic. Macrotermitinae was the sister group of other Termitidae, followed by the monospecific Sphaerotermitinae. Among the remaining five Termitidae subfamilies included in this study, Apicotermitinae was retrieved as the sister group of the four others, Termitinae + Nasutitermitinae + Syntermitinae + Cubitermitinae, whose phylogenetic position was unresolved (fig. 1).

Table 1. Samples Used in This Study, Collection Data, and GenBank Accession Numbers.

Species	Family	Subfamily	Collecting Locality	Date	Accession Number
Locusta migratoria	Acrididae	Oedipodinae	GenBank—Flook et al. 1995		X80245
Megacrania alpheus	Phasmatidae	Platycraninae	GenBank—Kômoto et al. 2011		AB477471
Sclerophasma paresiense	Mantophasmatidae	NA	GenBank—Cameron et al. 2006		DQ241798
Tamolanica tamolana	Mantidae	Mantinae	GenBank—Cameron et al. 2006		DQ241797
Blattella germanica	Blattellidae	Blattellinae	GenBank—Xiao et al. 2012		EU854321
Cryptocercus relictus	Cryptocercidae	NA	GenBank—Cameron et al. 2012		JX144941
Eupolyphaga sinensis	Polyphagidae	NA	GenBank—Zhang et al. 2010		FJ830540
Periplaneta fuliginosa	Blattidae	Blattinae	GenBank—Yamauchi et al. 2004		AB126004
Mastotermes darwiniensis	Mastotermitidae	NA	GenBank—Cameron et al. 2012		JX144929
Hodotermopsis sjostedti	Archotermopsidae	NA	Yakushima Island, Japan	May 15, 2011	KP026259
Zootermopsis angusticollis	Archotermopsidae	NA	GenBank—Cameron et al. 2012		JX144932
Microhodotermes viator	Hodotermitidae	NA	GenBank—Cameron et al. 2012		JX144931
Porotermes adamsoni	Stolotermitidae	NA	GenBank—Cameron et al. 2012		JX144930
Cryptotermes secundus	Kalotermitidae	NA	Darwin, Australia	July 7, 2012	KP026283
Glyptotermes satsumensis	Kalotermitidae	NA	Yakushima Island, Japan	May 15, 2011	KP026257
Glyptotermes sp A	Kalotermitidae	NA	Sete Lagoas, Brazil	July 13, 2012	KP026263
Glyptotermes sp B	Kalotermitidae	NA	15 km of Kuala Belait, Brunei	February 2013	KP026301
Glyptotermes sp C	Kalotermitidae	NA	Singapore	December 2012	KP026300
Neotermes insularis	Kalotermitidae	NA	GenBank—Cameron et al. 2012		JX144933
Neotermes sp A	Kalotermitidae	NA	Singapore	December 2012	KP026299
Rugitermes sp. A	Kalotermitidae	NA	Petit Saut, French Guiana	January 19, 2012	KP026284
Glossotermes oculatus	Serritermitidae	NA	Petit Saut, French Guiana	January 28, 2012	KP026291
Serritermes serrifer	Serritermitidae	NA	Brasília, Brazil	August 7, 2012	KP026264
Coptotermes formosanus	Coptotermitinae	NA	GenBank—Tokuda et al. 2011		AB626145
Coptotermes lacteus	Coptotermitinae	NA	GenBank—Cameron et al. 2012		JX144934
Heterotermes sp.	Heterotermitinae	NA	GenBank—Cameron et al. 2012		JX144936
Reticulitermes flavipes	Heterotermitinae	NA	GenBank—Cameron and Whiting 2007		EF206314
Reticulitermes hageni	Heterotermitinae	NA	GenBank—Cameron and Whiting 2007		EF206320
Reticulitermes santonensis	Heterotermitinae	NA	GenBank—Cameron and Whiting 2007		EF206315
Reticulitermes virginicus	Heterotermitinae	NA	GenBank—Cameron and Whiting 2007		EF206319
Prorhinotermes canalifrons	Rhinotermitidae	Prorhinotermitinae	Réunion Island	2001	KP026256
Dolichorhinotermes longilabius	Rhinotermitidae	Rhinotermitinae	Petit Saut, French Guiana	February 2011	KP026258
Parrhinotermes browni	Rhinotermitidae	Rhinotermitinae	50 km from Nabire, West Papua, Indonesia	June 2011	KP026295
Schedorhinotermes breinli	Rhinotermitidae	Rhinotermitinae	GenBank—Cameron et al. 2012		JX144935
Termitogeton planus	Rhinotermitidae	Termitogetoninae	50 km from Nabire, West Papua, Indonesia	June 2011	KP026298
Acanthotermes acanthothorax	Termitidae	Macrotermitinae	Ebogo, Cameroon	November 25, 2011	KP026280
Ancistrotermes pakistanicus	Termitidae	Macrotermitinae	50 km from Bangkok, Thailand	March 2011	KP026267
Macrotermes barneyi	Termitidae	Macrotermitinae	Wei et al. 2012		JX050221
Macrotermes subhyalinus	Termitidae	Macrotermitinae	GenBank—Cameron et al. 2012		JX144937
Odontotermes formosanus	Termitidae	Macrotermitinae	Iriomote Island, Japan	November 25, 2010	KP026254
Synacanthotermes sp.	Termitidae	Macrotermitinae	Ebogo, Cameroon	November 23, 2011	KP026270
Sphaerotermes sphaerothorax	Termitidae	Sphaerotermitinae	Ebogo, Cameroon	November 25, 2011	KP026279
Aderitotermes sp.	Termitidae	Apicotermitinae	Korup, Cameroon	December 3, 2011	KP026282
Amalotermes phaeocephalus	Termitidae	Apicotermitinae	Ebogo, Cameroon	November 24, 2011	KP026275
Astalotermes sp.	Termitidae	Apicotermitinae	Ebogo, Cameroon	November 23, 2011	KP026272
Ateuchotermes sp.	Termitidae	Apicotermitinae	Ebogo, Cameroon	November 23, 2011	KP026274
Anoplotermes-group sp E1	Termitidae	Apicotermitinae	Petit Saut, French Guiana	February 2011	KP026287
Duplidentitermes sp.	Termitidae	Apicotermitinae	Ebogo, Cameroon	November 23, 2011	KP026271
Jugositermes tuberculatus	Termitidae	Apicotermitinae	Ebogo, Cameroon	November 23, 2011	KP026269
Basidentitermes aurivillii	Termitidae	Cubitermitinae	Ebogo, Cameroon	December 1, 2011	KP026281
Cubitermes fungifaber	Termitidae	Cubitermitinae	Mbalmayo, Cameroon	November 19, 2011	KP026265
Procubitermes arboricola	Termitidae	Cubitermitinae	Ebogo, Cameroon	November 23, 2011	KP026273
Cavitermes tuberosus	Termitidae	Termitinae	Petit Saut, French Guiana	January 21, 2012	KP026294
Cephalotermes rectangularis	Termitidae	Termitinae	Ebogo, Cameroon	November 24, 2011	KP026277

(continued)

Table 1. Continued

Species	Family	Subfamily	Collecting Locality	Date	Accession Number
Drepanotermes sp.	Termitidae	Termitinae	GenBank—Cameron et al. 2012		JX144938
Macrognathotermes errator	Termitidae	Termitinae	GenBank—Cameron et al. 2012		JX144939
Microcerotermes biroi	Termitidae	Termitinae	50 km from Nabire, West Papua, Indonesia	June 2011	KP026297
Neocapritermes araguaia	Termitidae	Termitinae	Petit Saut, French Guiana	January 20, 2012	KP026286
Orthognathotermes aduncus	Termitidae	Termitinae	Petit Saut, French Guiana	January 25, 2012	KP026289
Promirotermes redundans	Termitidae	Termitinae	Mbalmayo, Cameroon	November 19, 2011	KP026266
Pericapritermes nigerianus	Termitidae	Termitinae	Ebogo, Cameroon	November 24, 2011	KP026278
Sinocapritermes mushae	Termitidae	Termitinae	Iriomote Island, Japan	November 25, 2010	KP026255
Constrictotermes cavifrons	Termitidae	Nasutitermitinae	Petit Saut, French Guiana	January 27, 2012	KP026290
Leptomyxotermes doriae	Termitidae	Nasutitermitinae	Ebogo, Cameroon	November 24, 2011	KP026276
Nasutitermes bikpelanus	Termitidae	Nasutitermitinae	50 km from Nabire, West Papua, Indonesia	June 2011	KP026296
Nasutitermes takasagoensis	Termitidae	Nasutitermitinae	Iriomote Island, Japan	November 25, 2010	KP026260
Nasutitermes triodiae	Termitidae	Nasutitermitinae	GenBank—Cameron et al. 2012		JX144940
Nasutitermes nr. perparvus	Termitidae	Nasutitermitinae	50 km from Bangkok, Thailand	March 2011	KP026261
Postsubulitermes parviconstrictus	Termitidae	Nasutitermitinae	Ebogo, Cameroon	November 23, 2011	KP026268
Caetetermes taquarussu	Termitidae	Nasutitermitinae	Petit Saut, French Guiana	January 19, 2012	KP026285
Silvestritermes holmgreni	Termitidae	Syntermitinae	Petit Saut, French Guiana	January 23, 2012	KP026288
Embiratermes neotenicus	Termitidae	Syntermitinae	Petit Saut, French Guiana	2011	KP026262
Labiotermes labralis	Termitidae	Syntermitinae	Petit Saut, French Guiana	January 28, 2012	KP026292
Syntermes spinosus	Termitidae	Syntermitinae	Petit Saut, French Guiana	February 2, 2012	KP026293

Altogether, we calculated 18 phylogenetic trees: six Bayesian phylogenies and 12 maximum-likelihood (ML) phylogenies (six based on the CAT model and another six based on the GTRGAMMA model). Increasing the number of generations to 20 million did not significantly change the Bayesian phylogenetic tree topology with the best partition scheme and third codon positions excluded. All analyses retrieved the same tree topologies for non-Termitidae with two exceptions in two ML analyses with third codon positions included: *Neotermes* and *Glyptotermes* were sister groups when data were partitioned by genes and followed a GTRGAMMA model; and *Zootermopsis* + *Microhodotermes* formed the sister group of *Porotermes* + *Hodotermopsis* in the partition by codon and following a CAT model. Relationships within the Macrotermitinae, Sphaerotermitinae, and Apicotermitinae were consistent in all analyses, except the Apicotermitinae genera *Aderitotermes* + *Amalotermes* + *Ateuchotermes*, whose relationships were not well resolved and varied between analyses. All analyses supported the monophyly of Cubitermitinae, Nasutitermitinae and Syntermitinae, and nested them within Termitinae, which formed a polyphyletic assemblage. Finally, all analyses placed *Macrognathotermes* and *Cavitermes*, both having symmetric snapping soldiers, as the sister group of Cubitermitinae.

Divergence Date Analyses

A chronogram for termite diversification based on whole mitochondrial first and second codon positions is shown in figure 2. Analyses including third codon positions resulted in highly similar divergence dates and topologies to those shown in figure 2 (data not shown). The divergence of the lineages leading to termites and *Cryptocercus* was estimated to have occurred 170 Ma (153–196 Ma 95% confidence interval [CI]), and the most recent termite common ancestor at 149 Ma (136–170 Ma 95% CI) (fig. 2). The clade comprising Archotermopsidae + Hodotermitidae + Stolotermitidae diverged from Kalotermitidae + Neoisoptera 137 Ma (130–156 Ma 95% CI), and Kalotermitidae diverged from Neoisoptera 127 Ma (114–147 Ma 95% CI). The most recent common ancestor of Neoisoptera was estimated to have arisen 92 Ma (73–107 Ma 95% CI). Termitidae split up from Coptotermitinae + Heterotermitinae at the beginning of the Paleogene, 67 Ma (54–80 Ma 95% CI), and modern Termitidae arose 54 Ma (46–66 Ma 95% CI) (fig. 2).

Biogeographic Analyses

A phylogenetic tree showing the reconstruction of ancestral distribution ranges based on a Bayesian model is shown in figure 3. The reconstruction provides insight into the ancestral range of Neoisoptera but failed to reconstruct the ancestral distribution of more basal termite taxa. The group including Coptotermitinae + Heterotermitinae + Termitidae originated from the Afrotropical + Indomalayan region. The Termitidae were also found to be of Afrotropical + Indomalayan origin when distribution across two areas was permitted in the analysis, and of Afrotropical origin when a single area was permitted (data not shown). The Apicotermitinae, Cubitermitinae, and Nasutitermitinae originated from the Afrotropical region. The Macrotermitinae were found to be of Afrotropical + Indomalayan origin. Finally, the Termitinae were inferred to have originated from the Afrotropical region.

Discussion

Termite Phylogeny and Nomenclature

Mitochondrial genomes form a single large marker and their phylogenies can, sometimes, be discordant with species

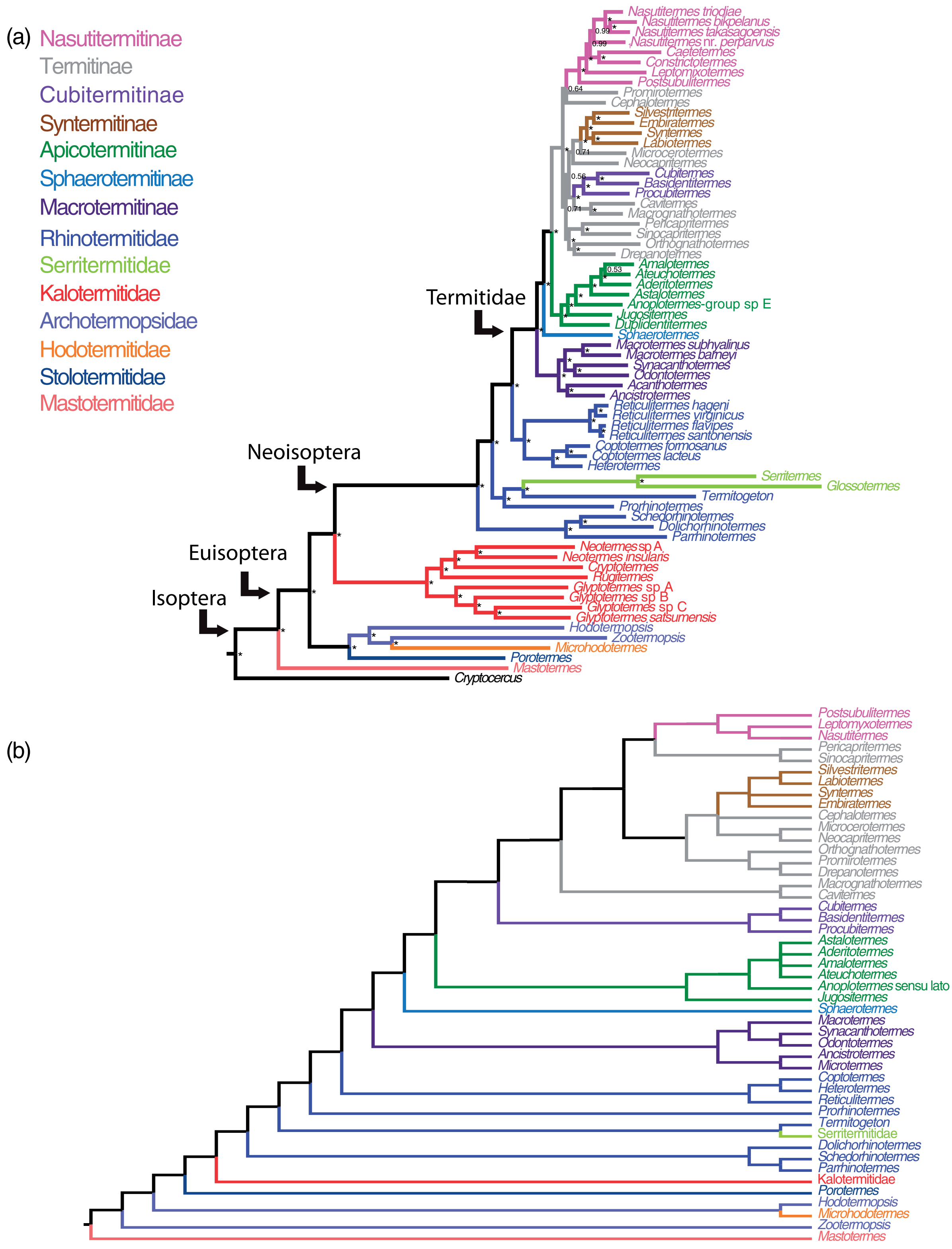

Fig. 1. Phylogenetic tree of termites: (*a*) Tree based on full mitochondrial genomes, reconstructed using Bayesian method and 20,000,000 generations. An optimal partitioning scheme was selected with PartitionFinder and third codon position was excluded from the analysis. Branch labels are the Bayesian posterior probabilities, with stars representing 100% support. (*b*) Molecular phylogenetic tree reproduced from Inward et al. (2007).

phylogenies (due to e.g., hybridization–introgression or incomplete lineage sorting). Caution is therefore required when interpreting single locus phylogenies, especially for phylogenies of closely related species for which the short divergence time is not always sufficient for alleles to coalesce (Degnan and Rosenberg 2009). Several lineages in the basal Termitinae-group have short internodes, characteristic of ancient rapid radiations, and for these few nodes we

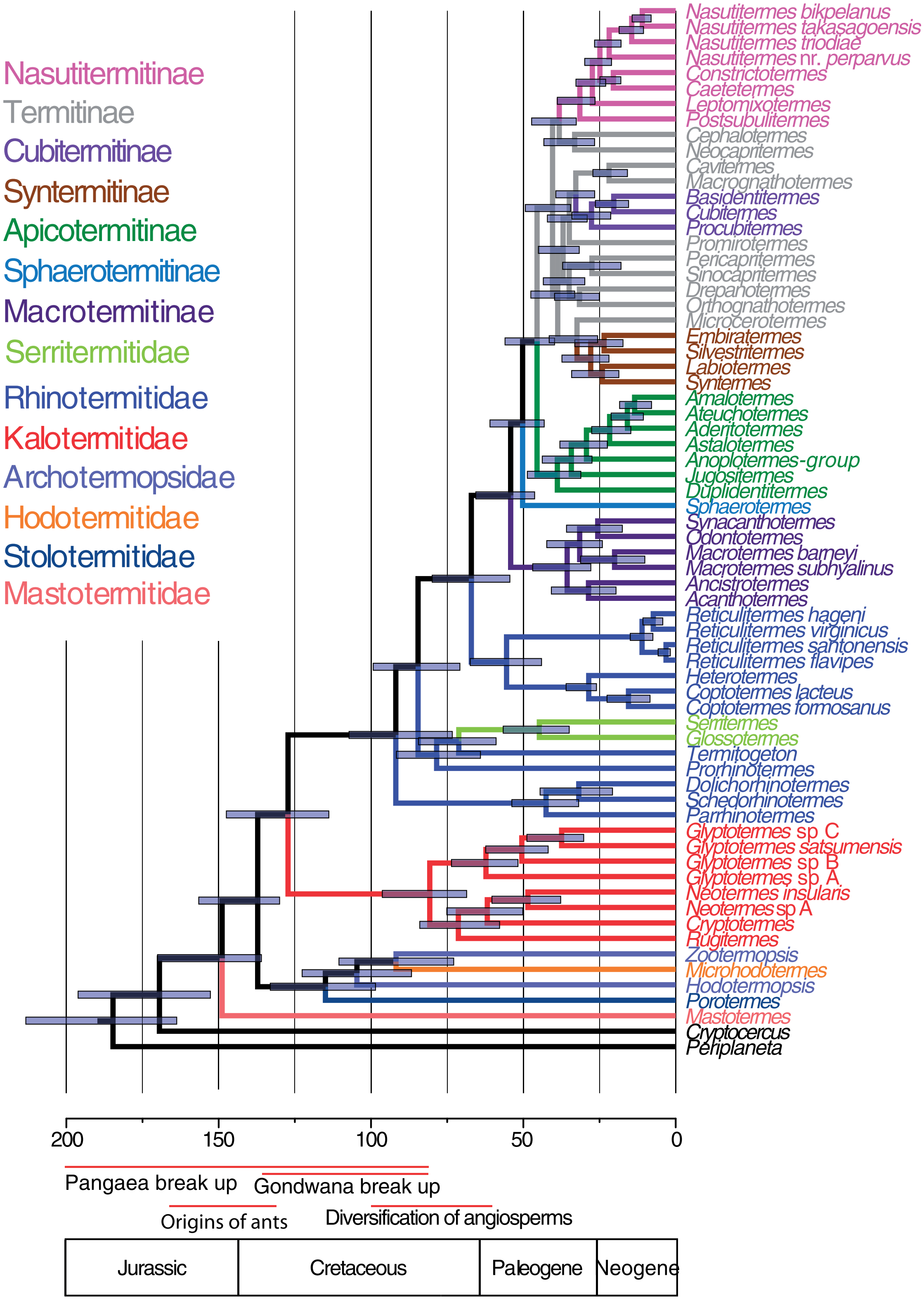

Fig. 2. Phylogenetic chronogram of termites based on the full mitochondrial genome, with third codon position deleted, reconstructed using BEAST. An optimal partitioning scheme was determined by PartitionFinder. Scale bar estimates age in millions of years. Node bar represents 95% CIs. Red bars show the estimated date range at which major biogeographic events occurred (Hay et al. 1999; McLoughlin 2001; Upchurch 2008).

cannot exclude incomplete lineage sorting (Whitfield and Lockhart 2007). Despite this potential drawback, previous studies have demonstrated that mitochondrial genomes, with the exclusion of the control region, accurately resolve phylogenetic relationships at various divergence levels within insects (see Cameron 2014). Our results confirm the utility of mitochondrial genomes to infer relationships between termite lineages, and with high nodal supports. With the

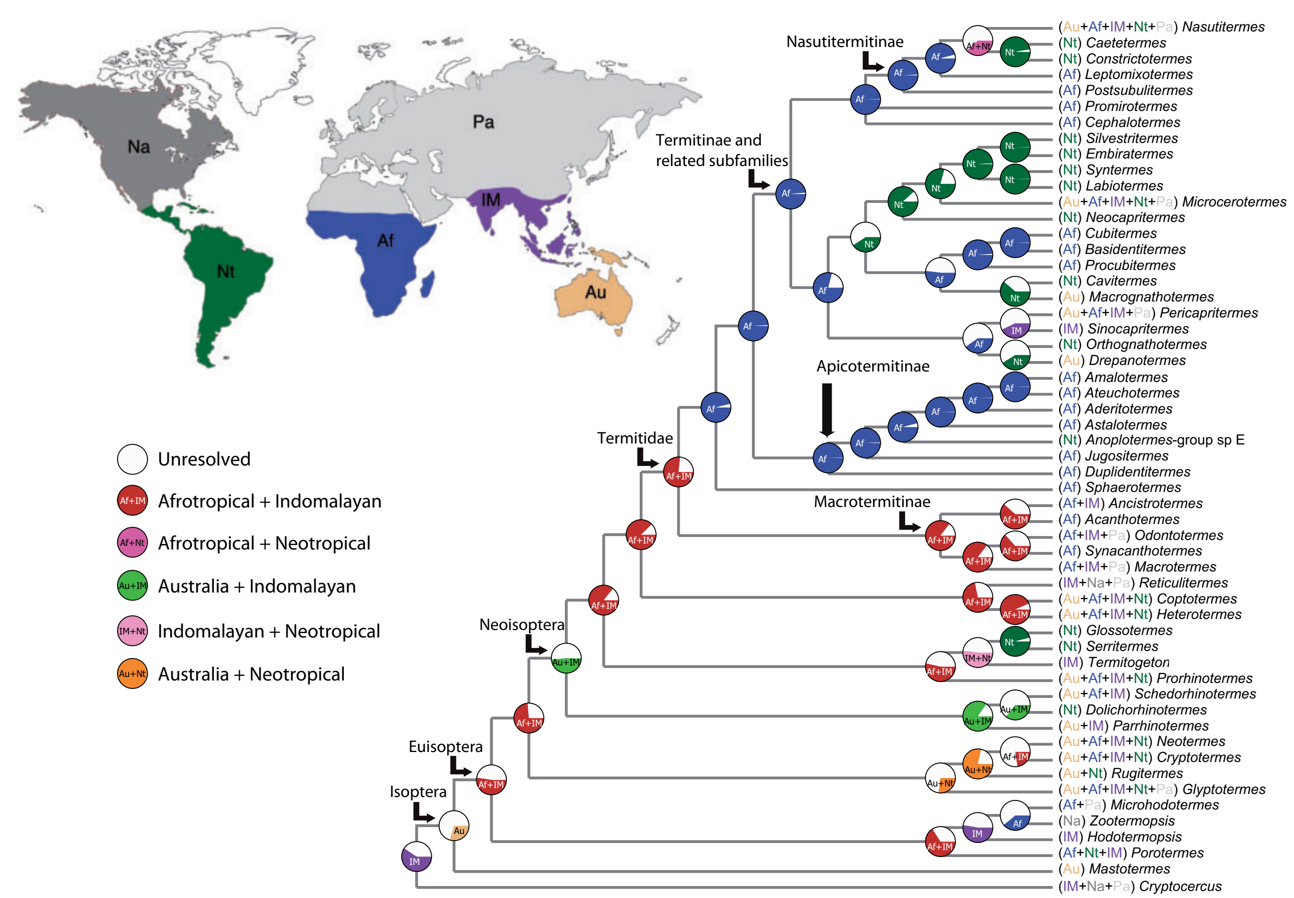

Fig. 3. Reconstruction of termite ancestral distribution using a Bayesian Binary model. The map shows the biogeographic areas considered in the analyses (Udvarty 1975), namely: Af, Afrotropical; Au, Australian; IM, Indomalayan; Na, Nearctic; Nt, Neotropical; Pa, Palearctic. The phylogenetic tree of termites was based on the full mitochondrial genome, with third codon position excluded, reconstructed using Bayesian method. Optimal partition scheme was determined by PartitionFinder.

exception of the termitid subfamilies Termitinae and Syntermitinae, nodes generally have 100% Bayesian posterior probabilities and are insensitive to reconstruction methods or deletion of the third codon position. ML bootstrap supports follow the same trend but are generally lower and some nodes remain unresolved.

To supplement previously sequenced mitochondrial genomes, we focused our sequencing efforts on Termitidae, although we also included 14 species of lower termites that were not previously sequenced. Our phylogenetic tree shows that Archotermopsidae form a paraphyletic group, with *Hodotermopsis* being the sister group of *Microhodotermes* (Hodotermitidae) + *Zootermopsis* (fig. 1). The newly proposed Archotermopsidae (Engel et al. 2009) was also polyphyletic in several other studies, although nodal support for these alternative relationships was low (Inward et al. 2007; Legendre et al. 2008; Ware et al. 2010). Our results suggest that the differing morphology and developmental patterns of Hodotermitidae (Watson 1973; Luamba 1980; Roisin 2000; Legendre et al. 2013) are likely derived and are adaptations to open-air foraging. Archotermopsidae is a nonmonophyletic group that should be synonymized with Hodotermitidae.

As in many other studies (e.g., Thompson et al. 2000; Inward et al. 2007; Engel et al. 2009; Ware et al. 2010; Cameron et al. 2012), we found that Kalotermitidae are monophyletic and form the sister group of Neoisoptera. Within the Neoisoptera, we were not able to include any member of Stylotermitidae but we did include a set of representative species of Serritermitidae, Rhinotermitidae, and Termitidae. Rhinotermitidae form a polyphyletic assemblage that can be split into three clades (fig. 1), as follows: 1) The Rhinotermitinae, which are monophyletic in our tree and basal to other Neoisoptera, as shown in previous studies (Lo et al. 2004; Inward et al. 2007; Cameron et al. 2012). They share several synapomorphies such as all-female workers and soldiers (Renoux 1976; Roisin 1988a) or soldiers with an elongated labrum ended by a brush that they use to apply defensive secretions from the frontal gland (Quennedey and Deligne 1975). 2) The clade composed of *Prorhinotermes*, *Termitogeton*, *Glossotermes* (Serritermitidae) and *Serritermes* (Serritermitidae), which are all single-piece nesters and have similar flexible development with pseudergates (Roisin 1988b; Parmentier and Roisin 2003; Bourguignon et al. 2009). The desert termite genus *Psammotermes* also shares developmental patterns and morphological features with *Prorhinotermes* (Bourguignon et al. 2012) and is likely part of this clade group (Lo et al. 2004). 3) The Heterotermitinae are paraphyletic to Coptotermitinae. Together, these two subfamilies form the sister group of Termitidae, a relationship recovered in some previous studies (e.g., Lo et al. 2004; Inward et al. 2007).

In this study, we included members of all the currently recognized Termitidae subfamilies except Foraminitermitinae. We retrieved the fungus-growing Macrotermitinae as the most basal subfamily, followed by Sphaerotermitinae and Apicotermitinae, which is consistent with the phylogenetic tree of Inward et al. (2007), but differs from several other studies (Legendre et al. 2008; Ware et al. 2010). Cubitermitinae, Nasutitermitinae, and Syntermitinae were all found to be monophyletic groups, nested within the Termitinae, that remains a highly polyphyletic assemblage. Inward et al. (2007), who sampled extensively within the Termitidae, also retrieved Termitinae as a polyphyletic group. Our phylogenetic tree suggests that the Cubitermitinae is nested within a group of termites with snapping soldiers, with *Cavitermes* and *Macrognathotermes* as their sister group. Snapping soldiers are already known to have evolved several times (Inward et al. 2007) and our phylogenetic tree shows that this character is likely to have reverted to its original state (biting type mandibles), at least three times, once in Cubitermitinae, once in *Drepanotermes* (+ *Amitermes* not represented here), and once in *Microcerotermes* + Syntermitinae. Alternatively, snapping mandibles could have evolved four times independently: In the *Termes*-group, in *Orthognathotermes* and related genera, in *Pericapritermes* + *Sinocapritermes* and in *Neocapritermes*. Finally, *Microcerotermes* and *Neocapritermes* are the sister genera of Syntermitinae, although the branching pattern of these taxa is unclear, varying between analyses. These results fit the distribution pattern of these clades, the latter two being strictly South American and the former having a worldwide distribution.

As is the case for Archotermopsidae, many Neoisopteran taxa are polyphyletic or paraphyletic and their classification should therefore be revised. The following changes will likely be necessary so that the classification of termites reflects their evolutionary history: 1) *Prorhinotermes*, *Termitogeton* and *Psammotermes* moved from Rhinotermitidae to Serritermitidae, although confirmation from the mitochondrial genome of *Psammotermes* is required to support this proposal; and 2) Coptotermitinae + Heterotermitinae elevated to family status (Heterotermitidae). These systematic changes would ensure the monophyly of the Rhinotermitidae family by restricting it to the current Rhinotermitinae. Similarly, Termitinae should either be split up into several subfamilies or encompass Nasutitermitinae, Syntermitinae and Cubitermitinae, which could be downgraded as tribes. Future phylogenetic reconstructions that include more representatives of these subfamilies are required to resolve these classification issues.

Dating of Termite Origin and Diversification

Our phylogenetic chronogram dates the most recent termite common ancestor to the early Cretaceous–late Jurassic, to 149 Ma (136–170 Ma 95% CI), and the split with the lineage leading to *Cryptocercus* roaches during the Jurassic, at 170 Ma (153–196 Ma 95% CI).

Our chronograms have smaller 95% CIs compared with those in previous studies (Brandl et al. 2007; Davis et al. 2009; Ware et al. 2010), possibly because we used several calibration points and based our analysis on the full mitochondrial genome, a comparatively larger data set. Our age estimation for Isoptera is congruent with the timeline for termite evolution presented by Engel et al. (2009) who estimated termite origins in the late Jurassic, 150 Ma. Other studies recovered an earlier origin of termites, generally in the late Triassic or early Jurassic, but are characterized by a high variation in their estimates. Our results do not directly contradict the results of these studies, as our dates are found in the lower range of their CIs (Brandl et al. 2007; Davis et al. 2009; Ware et al. 2010). A late Jurassic termite origin is congruent with the fossil record for termites (Thorne et al. 2000; Krishna et al. 2013).

Ware et al. (2010) carried out four analyses to infer the age of the termite clade (table 2). In their analysis B, they used fixed fossil ages and included fossils for which they had morphological data in their tree. This analysis provided older divergence times than the other three analyses and that presented in this study. In Ware et al.'s (2010) analyses A, C, and D, they used minimum and maximum hard bound constraints for 8 (A and C) or 19 (D) nodes and included fossils in analysis A but not in analyses C and D. Estimation dates reported in our tree fit the best with analysis A, although Neoisoptera and Isoptera are retrieved as significantly younger in our analyses (table 2). Other analyses from Ware et al. (2010) all retrieved consistently older ages than our analyses. A focus of Ware et al.'s (2010) study was dating methodology, and they tested the effect of using fossils with morphological data in combination with molecular sequences. As demonstrated by Ware et al. (2010), the inclusion of morphological data significantly affects the overall results (Ho and Phillips 2009).

Our results suggest that termites appear about 20 Ma before the age of oldest known termite fossils, which are dated from the beginning of the Cretaceous, 135 Ma (Krishna et al. 2013). This period is when the Mesozoic long-ovipositor roachoid families (Caloblattinidae, Mesoblattinidae, Raphidiomimidae, Umenocoleidae, and Blattulidae) declined and became extinct (Grimaldi and Engel 2005). Termite evolution may be connected to the rise of woody plants. The first "modern" conifers, such as the Cheirolepidiaceae (now extinct), Araucariaceae, and Podocarpaceae (both relictual, but still extant), evolved and became widespread during the late Jurassic and early Cretaceous (Meyen 1984). It is interesting to note that many of the wood-eating basal groups appear to prefer coniferous species. Grasses may be as old as 120 Ma (Prasad et al. 2011), but did not achieve diversity and abundance until 55 Ma, and the greatest abundance levels about 20 Ma (Strömberg 2011). This may coincide with the evolution of the Macrotermitinae (Aanen and Eggleton 2005; Engel et al. 2009), although the basal species use wood as a substrate for their fungus combs.

Ants may be important in the evolution of termites, as they are the main extant predators of termites (Prestwich 1984) and the two groups evolved at roughly the same time (Moreau et al. 2006). Many primitive ants are specialized

Table 2. Estimation Dates for the Major Termite Clades (Ma) as Estimated in This Study and in Ware et al. (2010).

	This Study		Ware et al. 2010			
	Without Third Codon	With Third Codon	Analysis A	Analysis B	Analysis C	Analysis D
Isoptera	146–165	141–154	172–191	223–235	191–211	213–228
Euisoptera	140–149	135–141	132–152	201–224	160–185	194–214
Kalotermitidae	—	—	100–100	133–181	100–100	107–149
Neoisoptera	73–97	67–95	100–100	167–201	105–142	144–179
Termitidae	43–55	49–63	44–54	98–132	45–63	72–120

termite predators (Prestwich 1984). Primitive termite colonies are likely to have contained a minimum of several dozen individuals (Lepage and Darlington 2000), potentially providing a rich food source for primitive ants. However, there is little evidence for a close association between primitive ants and primitive termites; instead primitive ants generally target higher termites, the origins of which postdate primitive ant origins by at least 50 Ma.

As is the case for ants (LaPolla et al. 2013), termite abundance was probably relatively low at its origin, increasing more recently, reaching 5–10% of insect fossils in the last 30 Ma, during the Oligocene and Miocene (Engel et al. 2009). Nowadays, termites are, with ants, among the most abundant animals in tropical and subtropical ecosystems (Eggleton et al. 1996) with members of the Termitidae largely dominant (e.g. Eggleton et al. 1996; Bourguignon et al. 2011). Our phylogenetic chronogram dated the most recent Termitidae common ancestor at the beginning of Eocene, 54 Ma (46–66 Ma 95% CI), and the origin of modern Termitidae subfamilies 10–25 Ma later, at the end of Eocene. The diversification of Termitidae postdates the split up of Gondwana. The pantropical distribution of several termitid genera and subfamilies is thus likely the result of dispersal events, which are indeed thought to be relatively frequent in wood-feeding species (Emerson 1955).

We suggest the major factor that drove modern termite diversity is the evolution of soil feeding at the end Eocene, about 45 Ma. The common ancestor of nonmacrotermitine and nonsphaerotermitine Termitidae was probably soil-feeding, and therefore wood feeding in the crown termitid subfamilies was secondarily reacquired (Inward et al. 2007). Colonization of soil opened up new niches and was likely the main driver of the increase in termite diversity. Upon evolution of soil feeding, termites might have experienced a rapid diversification in the early Miocene (~25 Ma) and became a well-diversified ecological guild dominant among tropical animals (Krishna and Grimaldi 2009). Additional sampling is required to test this hypothesis in a phylogenetic framework.

Termite Distribution Patterns

We reconstructed the ancestral distribution range of termites using our molecular phylogenetic tree and Bayesian biogeographic models. Our reconstruction of ancestral geographic range of termites was imprecise for termite stem groups, but sufficient information can be extracted for most Neoisoptera lineages.

The first termites likely appeared during the second half of the Jurassic, at the end of the breakup of Pangaea, and the beginning of Gondwana breakup (fig. 2). Modern species of *Cryptocercus* roaches, the sister group of termites, occur in the Indomalayan, Nearctic, and Palearctic regions. The most basal termite family, Mastotermitidae, includes a single extant member, *Mastotermes darwiniensis* that occurs in Australia only, but Mastotermitidae fossils have been found in the Nearctic, Neotropic, and Palearctic regions (Krishna et al. 2013). In our phylogenetic inference of ancestral geographic ranges of termites (fig. 3), we did not include fossil records and were unable to precisely determine the ancestral distribution of the termite common ancestor. Our present knowledge is too fragmentary to infer the distribution of early termite groups, which might have been once global and later shrank as advanced termites diversified.

The presence of extant and fossil mastotermitids across southern and northern hemisphere continents combined with the antiquity of the lineage suggests that the family may have evolved prior to the breakup of Pangaea, and acquired its distribution (including fossil members) through vicariance, although dispersal through rafting is also possible. Similarly, extant species of dampwood termites—the "archotermopsid" genera and the Stolotermitidae—occur in all continents (Krishna et al. 2013). Our analyses date the ancestor of these two groups to 115 Ma (98–133 Ma 95% CI), which suggests that it may have evolved prior to the final stages of the breakup of Pangaea, and acquired its current distribution through vicariance. The fact that each of the genera in this group has somewhat restricted distributions suggests that they may have limited dispersal ability, which would argue against long-distance travel through rafting. The presence of *Stolotermes* in the southern regions of Australia, Africa as well as New Zealand, and *Porotermes* in southern Australia, Africa and Southern America suggests vicariance of these genera during the breakup of Gondwana. However, we were not able to statistically confirm this hypothesis and additional species of *Stolotermes* and *Porotermes* are needed to draw clearer conclusions.

Extant and fossil Archotermopsidae are distributed in the northern hemisphere, and presumably evolved in this region. *Zootermopsis* occurs in western United States, *Hodotermopsis* in southern Japan, southern China and southern Vietnam and *Archotermopsis* in northern India and Pakistan, northern Thailand and Vietnam (Krishna et al. 2013). The common ancestor of *Zootermopsis* and *Microhodotermes* (found in

South Africa) was estimated to have existed 92 Ma (73–111 Ma 95% CI). The latter may therefore have diverged toward the end of the Pangaean breakup, enabling it to reach Africa from its northern hemispheric ancestors. Further analyses including additional members of the Archotermopsidae, Stolotermitidae, and Hodotermitidae are required to better resolve their biogeography. The modern distribution of these groups has likely been influenced by the rise of Termitidae that appear to have outcompeted them in areas where the latter are abundant.

We included only four genera of Kalotermitidae in our analyses, which is insufficient for an accurate dating of the last common ancestor of this family. Our phylogenetic chronogram estimates the splitting of Kalotermitidae and Neoisoptera to have occurred 127 Ma (114–147 Ma 95% CI), that of *Neotermes* and *Cryptotermes* 62 Ma (50–75 Ma 95% CI), and that of species of *Glyptotermes* 62 Ma (51–74 Ma 95% CI), postdating the breakup of Gondwana. These results suggest that the current distribution of *Neotermes*, *Glyptotermes*, and *Cryptotermes* results from dispersion only. Kalotermitidae are single piece nesters and can easily disperse through rafting, as evidenced by the presence of many species in coastal areas and on remote islands (Emerson 1955; Scheffrahn et al. 2006).

Among the Rhinotermitidae, the origin of Rhinotermitinae was estimated at 43 Ma (32–54 Ma 95% CI), which suggests that dispersion is the only possible mechanism responsible for their worldwide distribution. With an estimated origin of 56 Ma (44–67 Ma 95% CI), Heterotermitinae + Coptotermitinae follow a similar scenario. The rhinotermitids *Prorhinotermes* and *Termitogeton*, and the serritermitids *Glossotermes* and *Serritermes*, are the only genera whose origins could possibly fit with Gondwanan breakup. *Prorhinotermes* has an insular distribution and clearly has the potential of long-range dispersion by rafting, whereas *Termitogeton* and *Glossotermes* have more localized distributions. However, as they are wood-feeders, they could also disperse by rafting. *Serritermes* is the only obligate inquiline among "lower" termites (Emerson and Krishna 1975), but this mode of nesting almost certainly represents a recent specialization due the genus' close relationship to *Glossotermes* (Šobotník et al. 2010), whose diet is rotten wood (Bourguignon et al. 2009). The origin of these genera might therefore be linked with the breakup of Gondwana, although dispersion appears to have played a role, at least in *Prorhinotermes*.

Termitidae arose 54 Ma (46–66 Ma 95% CI) (fig. 2), which largely postdates the breakup of Gondwana, and therefore supports dispersal followed by diversification as the only mechanism responsible for the distribution of modern Termitidae species. Our data on ancestral distribution ranges suggest an Afrotropical and/or Indomalayan origin of Termitidae (fig. 3). Most of the basal Termitidae lineages occur in the Afrotropical region and Termitidae are the most diversified there (Eggleton 2000; Davies et al. 2003; Jones and Eggleton 2011). The Afrotropical region hosts the three most basal Termitidae lineages: Macrotermitinae, Foraminitermitinae (see Inward et al. 2007) and Sphaerotermitinae, which in the case of the former two groups also occur in the Indomalayan region. Aanen and Eggleton (2005) suggested that Macrotermitinae originated from African rainforest and later dispersed to Asia and colonized savanna. Although our tree topology differs in several respects, our biogeographic analysis supports this scenario and explains the absence of Macrotermitinae in South America and Australia. To digest cellulose, Macrotermitinae rely upon basidiomycete fungi *Termitomyces* that the first workers typically acquire from the environment as they emerge after colony foundation, making these species unable to disperse to new environments, or new continents, where spores of mutualistic fungi are absent (Nobre et al. 2010).

Apicotermitinae are well diversified in Africa and South America where they are among the most abundant soil-feeding termites (Eggleton 2000). Our biogeographic analysis shows that the group originated in the Afrotropics (fig. 3) and dispersed to the Neotropics probably between 29 and 34 Ma (22–44 Ma 95% CI) and were well-diversified 18 Ma in Dominican amber (Krishna and Grimaldi 2009). A monophyletic group of Neotropical soldierless Apicotermitinae nested within African apicotermitines was retrieved by Inward et al. (2007), suggesting that a single dispersal event occurred 29–34 Ma (22–44 Ma 95% CI), roughly matching the origin of New World monkey (Schrago and Russo 2003) and hystricognathous rodents (Sallam et al. 2009), among others, but this scenario needs confirmation by future studies. The last four subfamilies, Cubitermitinae, Nasutitermitinae, Syntermitinae, and Termitinae form a monophyletic group that diversified 40 Ma (25–49 Ma 95% CI). A clade including Nasutitermitinae and the Termitinae genera *Promirotermes* and *Cephalotermes* is weakly supported as the sister group of the clade containing the other groups (fig. 1). The Nasutitermitinae are of Afrotropical origin and subsequently dispersed across the world (fig. 3). The other clade, including Cubitermitinae, Syntermitinae, and most Termitinae, is most likely of Afrotropical origin (fig. 3). This clade also includes *Microcerotermes* and *Amitermes*, which are global. Our biogeographic analysis shows that African and South American termite fauna experienced several dispersal events, showing intricate distribution patterns (fig. 3). Sampling of additional species is needed to resolve this pattern.

Conclusions

Our phylogenetic tree based on mitochondrial genomes has improved our understanding of termite evolution, especially the timing of the appearance of families or subfamilies and their historical biogeography. The factors promoting termite evolution are elusive, as the timing does not appear to coincide with evolutionary origins of major food sources (woody plants and grasses), or predators (ants). Our phylogenetic tree has also helped to illuminate the global distribution pattern of termites; however a larger sampling of the termite diversity is needed to resolve the precise origins and date the dispersal events that gave rise to several clades, particularly in the polyphyletic Termitinae. Our study paves the way toward a sound understanding of the evolution of one of nature's most prevalent ecosystem engineers.

Materials and Methods

Mitochondrial Genome Sequencing

We sampled termites in various parts of the world and selected 48 species (see table 1), which, together with previously published termite mitochondrial genomes, are representative of termite diversity across all clades. We collected all specimens in RNA-later and kept them at −80 °C until DNA extraction. We extracted whole genomic DNA using TaKaRa DNA kit, from five to ten individual specimens, after we removed the digestive tract to avoid contaminants from the gut (symbionts and soil bacteria, food particles, soil minerals, etc.). We amplified the complete mitochondrial genomes with TaKaRa LA Taq in two long-polymerase chain reactions (PCR) using either previously published primers or primers specific to termites designed in this study (table 3). We determined the concentration of both long PCR fragments using Agilent 2100 bioanalyzer. We mixed both fragments in equimolar concentration, then multiplexed using Nextera XT DNA preparation kit following the manual's guidelines, and paired-end sequenced with Illumina HiSeq2000.

We sequenced the multiplexed libraries and produced about 31 million paired-end reads at a read length of 100 bp for the 48 samples. We assembled the sequences of each sample separately, using the CLC suite of programs. Briefly, after sample de novo assembling, we determined the consensus mitochondrial genome sequence for each species and used it as the frame to map all reads. Reads mapping allowed confirmation of the correctness of assembling and corrected mistakes if and when present. In all polymorphic base cases, we selected the base with the higher rate of representation. We cut control regions of the mitochondrial genomes from the final sequence, as they present repetitive patterns that are generally poorly assembled with short reads, and thus provide no useful information.

We conducted tRNA inference using tRNAscan-SE (Lowe and Eddy 1997), using invertebrate mitochondrial predictors and a cut off value of 1. When not inferred by tRNAScan-SE, we identified tRNAs by eye, through reference to secondary structure models for this gene from other termites. Similarly, we annotated the 13 protein-coding genes and the 2 ribosomal RNAs by eye, aided by previously published sequences we mapped on each mitochondrial genome using CLC.

Phylogenetic Analyses

We carried out alignments for the 48 species sequenced in this study with those from an additional 18 termite species, whose mitochondrial genome sequences have been deposited in GenBank (see table 1). Additionally, we included the sequences of seven other polyneopteran insect outgroups whose mitochondrial genomes have been deposited in GenBank: four roaches, *Periplaneta fuliginosa*, *Blattella germanica*, *Eupolyphaga sinensis*, and *Cryptocercus relictus*; a mantis, *Tamolanica tamolana*; a Mantophasmatodea, *Sclerophasma paresiense*; a phasmid, *Megacrania alpheus*; and the locust, *Locusta migratoria* (see table 1). We pruned outgroups after phylogenetic analyses for depicting tree topologies. We aligned each gene individually using Muscle algorithm implemented in MEGA 5.2 (Tamura et al. 2011). We aligned protein-coding genes as codon, and tRNA and ribosomal RNA genes as DNA, then we concatenated the resulting alignments with Mesquite (Maddison WP and Maddison DR 2010). We did not consider the secondary structure of ribosomal RNA genes for alignments, as the benefit of doing so is still unclear (Letsch et al. 2010, Letsch and Kjer 2011).

We computed phylogenies using Bayesian analyses and ML methods to compare the effect of analytical approaches on the tree topology (see table 4). For both analytical approaches, we used three types of partitioning: by genes, by codon position, and optimal partitioning as determined by PartitionFinder (Lanfear et al. 2012). PartitionFinder implements a method selecting the best-fit partitioning scheme and nucleotide substitution models (Leavitt et al. 2013). For our alignment, PartitionFinder selected a scheme with 25 partitions. Partitioning by genes resulted in 16 partitions: 13 protein-coding genes, 2 ribosomal RNA genes, and 1 for the combined tRNAs. Partitioning by codon position resulted in six partitions: one for each base of codons, two for each ribosomal RNA gene and one for the combined tRNAs. The three partitioning types were run twice independently, once with the third codon position included in the analysis and once without the third codon position, making a total of six distinct partition schemes. Note that for the partitioning by codon position and for the partitioning determined by PartitionFinder, analyses with the third codon position excluded resulted in 5 partitions and 14 partitions, respectively, whereas excluding the third codon position from the analysis did not change the number of partitions in the case of gene partitioning (see table 4).

We implemented Bayesian phylogenies in MrBayes version 3.2 (Ronquist et al. 2012) with unlinked partitions, each of four chains (three hot and one cold), with run length of 2 million generations and sampling every 1,000 generations. We examined the completed Bayesian analyses with Tracer v1.5 (Rambaut and Drummond 2007) to check mixing and asymptotic behavior of each parameter and of total tree likelihood; trees collected prior to this asymptotic point were treated as burn-in and discarded. Burn-in varied between 60,000 and 500,000 generations. Convergence was achieved by all analyses within 2 million generations. To make sure that our analyses were computationally intensive enough to retrieve consistent tree topology, we also carried out an extra analysis with run length of 20 million generations, sampling every 10,000 generations and a burn-in of 5 million generations. We only carried out this analysis with the best partition scheme, as determined by PartitionFinder (Lanfear et al. 2012), and third codon position excluded. We determined the best-fit nucleotide substitution model using jModelTest 2.1.3 (Darriba et al. 2012) and PartitionFinder (Lanfear et al. 2012), with a general time reversible (GTR) model with gamma-distributed rate variation across sites and a proportion of invariable sites selected for all partitions. We selected models based on Bayesian information criterion (BIC) in all cases (Sullivan and Joyce 2005). We implemented the ML

Table 3. Primers Used in This Study.

Primer	Gene	Sequence	Direction	Fragment Size	Source
16S13530F	16S	TWA AAC TCT ATA GGG TCT TCT CGT CCC A	Forward	6-kb fragment	This study
COII3810R	COII	TTT GCY CCR CAR ATT TCT GAG CAT TG	Reverse	6-kb fragment	This study
C2F2	COII	ATA CCT CGA CGW TAT TCA GA	Forward	10-kb fragment	Miura et al. 1998
Fleu	COII	TCT AAT ATG GCA GAT TAG TGC	Forward	10-kb fragment	Whiting 2002
12Sai	12S	AAA CTA GGA TTA GAT ACC CTA TTA T	Reverse	10-kb fragment	Simon et al. 1994
16SAr	16S	CGC CTG TTT ATC AAA AAC AT	Reverse	10-kb fragment	Simon et al. 1994

Table 4. Analytical Method and Partition Scheme Strategies Used in This Study.

Analytical Method	Partitioning Strategy	Number of Partitions	Partition Scheme
Bayesian, GTR + I + G	PartitionFinder	25	ATP6 1st + ND2 1st + ND3 1st, ATP6 2nd + ND2 2nd + ND3 2nd + ND6 2nd, ATP6 3rd + COII 3rd, ATP8 1st + ATP8 2nd, ATP8 3rd, COI 1st, COI 2nd + COIII 2nd, COI 3rd, COII 1st, COII 2nd + Cyt B 2nd, COIII 1st + Cyt B 1st, COIII 3rd, Cyt B 3rd, ND1 1st + ND4 1st + ND5 1st, ND1 2nd + ND4 2nd + ND4L 1st + ND4L 2nd + ND5 2nd, ND1 3rd, ND2 3rd, ND3 3rd, ND4 3rd + ND4L 3rd, ND5 3rd, ND6 1st, ND6 3rd, 12S, 16S, tRNAs
Bayesian, GTR + I + G	PartitionFinder—third codon	14	ATP6 1st + ND2 1st + ND3 1st, ATP6 2nd + ND2 2nd + ND3 2nd + ND6 2nd, ATP8 1st + ATP8 2nd, COI 1st, COI 2nd + COIII 2nd, COII 1st, COII 2nd + Cyt B 2nd, COIII 1st + Cyt B 1st, ND1 1st + ND4 1st + ND5 1st, ND1 2nd + ND4 2nd + ND4L 1st + ND4L 2nd + ND5 2nd, ND6 1st, 12S, 16S, tRNAs
Bayesian, GTR + I + G	Gene	16	ATP6, ATP8, COI, COII, COIII, Cyt B, ND1, ND2, ND3, ND4, ND4L, ND5, ND6, 12S, 16S, tRNAs
Bayesian, GTR + I + G	Gene—third codon	16	ATP6, ATP8, COI, COII, COIII, Cyt B, ND1, ND2, ND3, ND4, ND4L, ND5, ND6, 12S, 16S, tRNAs
Bayesian, GTR + I + G	Codon	6	1st codon, 2nd codon, 3rd codon, 12S, 16, tRNAs
Bayesian, GTR + I + G	Codon—third codon	5	1st codon, 2nd codon, 12S, 16, tRNAs
ML, GTRGAMMA	PartitionFinder	25	ATP6 1st + ND2 1st + ND3 1st, ATP6 2nd + ND2 2nd + ND3 2nd + ND6 2nd, ATP6 3rd + COII 3rd, ATP8 1st + ATP8 2nd, ATP8 3rd, COI 1st, COI 2nd + COIII 2nd, COI 3rd, COII 1st, COII 2nd + Cyt B 2nd, COIII 1st + Cyt B 1st, COIII 3rd, Cyt B 3rd, ND1 1st + ND4 1st + ND5 1st, ND1 2nd + ND4 2nd + ND4L 1st + ND4L 2nd + ND5 2nd, ND1 3rd, ND2 3rd, ND3 3rd, ND4 3rd + ND4L 3rd, ND5 3rd, ND6 1st, ND6 3rd, 12S, 16S, tRNAs
ML, GTRGAMMA	PartitionFinder—third codon	14	ATP6 1st + ND2 1st + ND3 1st, ATP6 2nd + ND2 2nd + ND3 2nd + ND6 2nd, ATP8 1st + ATP8 2nd, COI 1st, COI 2nd + COIII 2nd, COII 1st, COII 2nd + Cyt B 2nd, COIII 1st + Cyt B 1st, ND1 1st + ND4 1st + ND5 1st, ND1 2nd + ND4 2nd + ND4L 1st + ND4L 2nd + ND5 2nd, ND6 1st, 12S, 16S, tRNAs
ML, GTRGAMMA	Gene	16	ATP6, ATP8, COI, COII, COIII, Cyt B, ND1, ND2, ND3, ND4, ND4L, ND5, ND6, 12S, 16S, tRNAs
ML, GTRGAMMA	Gene—third codon	16	ATP6, ATP8, COI, COII, COIII, Cyt B, ND1, ND2, ND3, ND4, ND4L, ND5, ND6, 12S, 16S, tRNAs
ML, GTRGAMMA	Codon	6	1st codon, 2nd codon, 3rd codon, 12S, 16, tRNAs
ML, GTRGAMMA	Codon—third codon	5	1st codon, 2nd codon, 12S, 16, tRNAs
ML, CAT	PartitionFinder	25	ATP6 1st + ND2 1st + ND3 1st, ATP6 2nd + ND2 2nd + ND3 2nd + ND6 2nd, ATP6 3rd + COII 3rd, ATP8 1st + ATP8 2nd, ATP8 3rd, COI 1st, COI 2nd + COIII 2nd, COI 3rd, COII 1st, COII 2nd + Cyt B 2nd, COIII 1st + Cyt B 1st, COIII 3rd, Cyt B 3rd, ND1 1st + ND4 1st + ND5 1st, ND1 2nd + ND4 2nd + ND4L 1st + ND4L 2nd + ND5 2nd, ND1 3rd, ND2 3rd, ND3 3rd, ND4 3rd + ND4L 3rd, ND5 3rd, ND6 1st, ND6 3rd, 12S, 16S, tRNAs
ML, CAT	PartitionFinder—third codon	14	ATP6 1st + ND2 1st + ND3 1st, ATP6 2nd + ND2 2nd + ND3 2nd + ND6 2nd, ATP8 1st + ATP8 2nd, COI 1st, COI 2nd + COIII 2nd, COII 1st, COII 2nd + Cyt B 2nd, COIII 1st + Cyt B 1st, ND1 1st + ND4 1st + ND5 1st, ND1 2nd + ND4 2nd + ND4L 1st + ND4L 2nd + ND5 2nd, ND6 1st, 12S, 16S, tRNAs
ML, CAT	Gene	16	ATP6, ATP8, COI, COII, COIII, Cyt B, ND1, ND2, ND3, ND4, ND4L, ND5, ND6, 12S, 16S, tRNAs
ML, CAT	Gene—third codon	16	ATP6, ATP8, COI, COII, COIII, Cyt B, ND1, ND2, ND3, ND4, ND4L, ND5, ND6, 12S, 16S, tRNAs
ML, CAT	Codon	6	1st codon, 2nd codon, 3rd codon, 12S, 16, tRNAs
ML, CAT	Codon—third codon	5	1st codon, 2nd codon, 12S, 16, tRNAs

Table 5. Fossils Used in This Study for Estimation of Divergence Time of Major Clades.

Species	Age (Ma)/Minimum Age Constraint for Group	Calibration Group	Soft Maximum Bound (97.5% probability)	Reference
Baissatermes lapideus	137	*Cryptocercus* + Isoptera	250	Engel et al. 2007
Valditermes brenanae	130	Hodotermitidae + other Isoptera, excluding *Mastotermes*	250	Krishna et al. 2013 and references therein
Cratokalotermes santanensis	110	Kalotermitidae + Rhinotermitidae + Termitidae	200	Grimaldi et al. 2008
Glyptotermes grimaldii	18	*Glyptotermes* + *Neotermes* + *Rugitermes* + *Cryptotermes*	150	Engel and Krishna 2007a
Dolichorhinotermes dominicanus	18	*Dolichorhinotermes* + *Schedorhinotermes*	100	Schlemmermeyer and Cancello 2000
Reticulitermes antiquus	44	*Reticulitermes* + *Coptotermes* + *Heterotermes*	100	Engel and Krishna 2007b
Coptotermes priscus	26	*Coptotermes* + *Heterotermes*	70	Emerson 1971
Constrictotermes electroconstrictus	18	*Constrictotermes* + *Caetetermes*	70	Krishna 1996
Anoplotermes sensu lato	18	*Anoplotermes* + *Amalotermes* + *Ateuchotermes* + *Aderitotermes* + *Astalotermes*	70	Krishna and Grimaldi 2009
Microcerotermes insularis	18	*Microcerotermes* + *Embiratermes* + *Syntermes* + *Labiotermes* + *Armitermes*	70	Krishna and Grimaldi 2009
Additional calibrations				
All Dictyoptera[a]	140		311	Labandeira 1994
All cockroaches plus termites[a]	140		311	Labandeira 1994
Dictyoptera + Phasmida[a]	311		396	Labandeira 1994

[a] These groups were constrained to be monophyletic in BEAST analyses, in addition to the clade containing *Cryptocercus* + termites.

method in RAxML version 7.7.1 (black-box webserver; http://embnet.vital-it.ch/raxml-bb/, last accessed July 1, 2014) to generate phylogenetic trees (Stamatakis et al. 2008). Each partition scheme was run twice, once with the Gamma model of rate heterogeneity and once with the CAT model; the latter is faster but is an approximation of the best tree, although both methods generally provide the same tree topology (Stamatakis et al. 2008).

Molecular Dating

We analyzed the concatenated DNA sequence alignment with a relaxed molecular-clock model using the Bayesian phylogenetic software BEAST 1.8.0 (Drummond and Rambaut 2007). We carried out two analyses: One in which third codon positions were included and one in which third codon positions were excluded (to account for saturation among deeply diverged lineages). Partitioning of data and model selection was performed as described above, using PartitionFinder. We did not take into consideration the secondary structure of the two ribosomal RNA genes as this was shown to have minor effect on molecular clock tree computed with Bayesian Markov chain Monte Carlo (MCMC) (Dohrmann 2014). Rate variation was modeled among branches using uncorrelated lognormal relaxed clocks (Drummond and Rambaut 2007), with a single model for all genes. A Yule speciation process was used for the tree prior (Gernard 2008) and 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 1,000 steps over a total of 50 million generations. A maximum clade credibility tree was obtained using Tree Annotator within the BEAST software package with a burn-in of 10,000 trees. Acceptable sample sizes and convergence to the stationary distribution were checked using Tracer 1.5 (Rambaut and Drummond 2007). The molecular clock was calibrated using eight minimum age constraints as shown in table 5. Fossil calibrations were implemented as exponential priors on node times. Non-termite taxa included in this analysis were *Periplaneta fuliginosa*, *Cryptocercus relictus*, *Tamolanica tamolana*, *Megacrania alpheus* and *Locusta migratoria.*

Biogeographic Analyses

We reconstructed the evolution of termite geographic ranges using our Bayesian phylogenetic tree with third codon position excluded and with partition scheme as determined by PartitionFinder (Lanfear et al. 2012). We carried out a Bayesian binary model using the RASP 2.1 software (Yu et al. 2010, 2013). We used a fixed (JC) model and an Equal Among-Site rate variation with the default chain parameters (namely 50,000 cycles, 10 chains, with a sampling every 100 generations and a temperature of 0.1) for the Bayesian analysis. Root distribution was set to Null and the maximum number of areas for each node was set to 2. We considered the generic distribution as described by Krishna et al. (2013) and recognized six biogeographic areas: Australian (including New Guinea), Afrotropics, Nearctic, Neotropical, Indomalayan, and Palaearctic.

Acknowledgments

The authors are grateful to the Japan Society for Promotion of Science, for financial support through a postdoctoral fellowship to T.B. (No. 22-00393). This work was also supported by Grants-in-Aid for Young Scientists (No. 21677001) from the Ministry of Education, Culture, Sports, Science and Technology of Japan; by the LHK fund of the National University of Singapore; by the Singapore-MIT Alliance for Research and Technology; and by the Internal Grant Agency of Faculty of Forestry and Wood Sciences (Specific research of the Czech University of Life Sciences) (A08/14). S.L.C. (FT120100746) and N.L. (DP1097265) were supported by the Australian Research Council. Y.R. was supported by the Fonds de la Recherche Scientifique FNRS (Belgium) (FRFC 2.4594.12).

References

Aanen DK, Eggleton P. 2005. Fungus-growing termites originated in African rain forest. *Curr Biol.* 15:851–855.

Abe T. 1979. Studies on the distribution and ecological role of termites in a lowland rain forest of west Malaysia. 2. Food and feeding habits of termites in Pasoh Forest Reserve. *Jpn J Ecol.* 29:121–135.

Boore JL. 2006. The use of genome-level characters for phylogenetic reconstruction. *Trends Ecol Evol.* 21:439–446.

Bourguignon T, Leponce M, Roisin Y. 2011. Beta-diversity of termite assemblages among primary French Guiana rain forests. *Biotropica* 43:473–479.

Bourguignon T, Šobotník J, Hanus R, Roisin Y. 2009. Developmental pathways of *Glossotermes oculatus* (Isoptera, Serritermitidae): at the cross-roads of worker caste evolution in termites. *Evol Dev.* 668:659–668.

Bourguignon T, Šobotník J, Lepoint G, Martin JM, Hardy OJ, Dejean A, Roisin Y. 2011. Feeding ecology and phylogenetic structure of a complex neotropical termite assemblage, revealed by nitrogen stable isotope ratios. *Ecol Entomol.* 36:261–269.

Bourguignon T, Šobotník J, Sillam-Dussès D, Jiroš P, Hanus R, Roisin Y, Miura T. 2012. Developmental pathways of *Psammotermes hybostoma* (Isoptera: Rhinotermitidae): old pseudergates make up a new sterile caste. *PLoS One* 7:e44527.

Brandl R, Hyodo F, von Korff-Schmising M, Maekawa K, Miura T. 2007. Divergence times in the termite genus *Macrotermes* (Isoptera: Termitidae). *Mol Phylogenet Evol.* 45:239–250.

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

Cameron SL, Barker SC, Whiting MF. 2006. Mitochondrial genomics and the new insect order Mantophasmatodea. *Mol Phylogenet Evol.* 38: 274–279.

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.

Cameron SL, Whiting MF. 2007. Mitochondrial genomic comparisons of the subterranean termites from the genus *Reticulitermes* (Insecta: Isoptera: Rhinotermitidae). *Genome* 202:188–202.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods.* 9:772.

Davis R, Baldauf S, Mayhew P. 2009. Eusociality and the success of the termites: insights from a supertree of dictyopteran families. *J Evol Biol.* 22:1950–1761.

Davies RG, Eggleton P, Jones DT, Gathorne-Hardy FJ, Hernández LM. 2003. Evolution of termite functional diversity: analysis and synthesis of local ecological and regional influences on local species richness. *J Biogeogr.* 30:847–877.

Degnan JH, Rosenberg NA. 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends Ecol Evol.* 24: 332–340.

Dohrmann M. 2014. The influence of ignoring secondary structure on divergence time estimates from ribosomal RNA genes. *Mol Phylogenet Evol.* 71:214–223.

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

Eggleton P. 2000. Global patterns of termite diversity. In: Abe T, Bignell DE, Higashi M, editors. Termites: evolution, sociality, symbioses, ecology. Dordrecht (The Netherlands): Kluwer Academic Publishers. p. 25–51.

Eggleton P, Bignell DE, Sands WA, Mawdsley NA, Lawton JH, Wood TG, Bignell NC. 1996. The diversity, abundance and biomass of termites under differing levels of disturbance in the Mbalmayo Forest Reserve, southern Cameroon. *Philos Trans R Soc Lond B Biol Sci.* 351:51–68.

Emerson AE. 1955. Geographical origin and dispersions of termite genera. *Fieldiana Zool.* 37:465–521.

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 Hist.* 146:243–304.

Emerson AE, Krishna K. 1975. The termite family Serritermitidae (Isoptera). *Am Mus Novit.* 2570:1–31.

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

Engel MS, Grimaldi DA, Krishna K. 2009. Termites (Isoptera): their phylogeny, classification, and rise to ecological dominance. *Am Mus Novit.* 3650:1–27.

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

Engel MS, Krishna K. 2007a. Drywood termites in Dominican amber (Isoptera: Kalotermitidae). *Beitr Entomol.* 57:263–275.

Engel MS, Krishna K. 2007b. A synopsis of Baltic amber termites (Isoptera). *Stuttgarter Beitr Naturk.* 372:1–20.

Evans TA, Dawes TZ, Ward PR, Lo N. 2011. Ants and termites increase crop yield in a dry climate. *Nat Commun.* 2:262.

Flook PK, Rowell CHF, Gellissen G. 1995. The sequence, organization, and evolution of the *Locusta migratoria* mitochondrial genome. *J Mol Evol.* 41:928–941.

Fox-Dobbs K, Doak DF, Brody AK, Palmer TM. 2010. Termites create spatial structure and govern ecosystem function by affecting N_2 fixation in an East African savanna. *Ecology* 91:1296–1307.

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

Gray MW. 2012. Mitochondrial evolution. *Cold Spring Harb Perspect Biol.* 4:a011403.

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 Mus Novit.* 3626:1–30.

Hay WW, Deconto RM, Wold CN, Wilson KM, Voigt S, Schulz M, Wold RD, Dullo WC, Ronov AB, Söding E. 1999. Alternative global Cretaceous paleogeography. In: Barrera E, Johnson CC, editors. Evolution of the Cretaceous ocean-climate system. Boulder (CO): Geological Society of America Special Paper 332. p. 1–47.

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

Holt JA, Lepage M. 2000. Termites and soil properties. In: Abe T, Bignell DE, Higashi M, editors. Termites: evolution, sociality, symbioses, ecology. Dordrecht (The Netherlands): Kluwer Academic Publishers. p. 389–407.

Inward DJG, Vogler AP, Eggleton P. 2007. A comprehensive phylogenetic analysis of termites (Isoptera) illuminates key aspects of their evolutionary biology. *Mol Phylogenet Evol.* 44:953–967.

Jones DT, Eggleton P. 2011. Global biogeography of termites: a compilation of sources. In: Bignell DE, Roisin Y, Lo N, editors. Biology of termites: a modern synthesis. Dordrecht (The Netherlands): Springer. p. 477–498.

Jouquet P, Dauber J, Lagerlo J, Lavelle P, Lepage M. 2006. Soil invertebrates as ecosystem engineers: intended and accidental effects on soil and feedback loops. *Appl Soil Ecol.* 32:153–164.

Kômoto N, Yukuhiro K, Ueda K, Tomita S. 2011. Exploring the molecular phylogeny of phasmids with whole mitochondrial genome sequences. *Mol Phylogenet Evol.* 58:43–52.

Krishna K. 1996. New fossil species of termites of the subfamily Nasutitermitinae from Dominican and Mexican amber (Isoptera, Termitidae). *Am Mus Novit.* 3176:1–13.

Krishna K, Grimaldi DA. 2003. The first Cretaceous Rhinotermitidae (Isoptera): a new species, genus, and subfamily in Burmese amber. *Am Mus Novit.* 3390:1–10.

Krishna K, Grimaldi D. 2009. Diverse Rhinotermitidae and Termitidae (Isoptera) in Dominican amber. *Am Mus Novit.* 2640:1–48.

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

Labandeira CC. 1994. A compendium of fossil insect families. *Contrib Biol Geol.* 88:1–71.

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.

LaPolla JS, Dlussky GM, Perrichot V. 2013. Ants and the fossil record. *Annu Rev Entomol.* 58:609–630.

Leavitt JR, Hiatt KD, Whiting MF, Song H. 2013. Searching for the optimal data partitioning strategy in mitochondrial phylogenomics: a phylogeny of Acridoideae (Insecta: Orthoptera: Caelifera) as a case study. *Mol Phylogenet Evol.* 67:494–508.

Legendre F, Whiting MF, Bordereau C, Cancello EM, Evans TA, Grandcolas P. 2008. The phylogeny of termites (Dictyoptera: Isoptera) based on mitochondrial and nuclear markers: implications for the evolution of the worker and pseudergate castes, and foraging behaviors. *Mol Phylogenet Evol.* 48:615–627.

Legendre F, Whiting MF, Grandcolas P. 2013. Phylogenetic analyses of termite post-embryonic sequences illuminate caste and developmental pathway evolution. *Evol Dev.* 15:146–157.

Lepage M, Darlington JPEC. 2000. Population dynamics of termites. In: Abe T, Bignell DE, Higashi M, editors. Termites: evolution, sociality, symbioses, ecology. Dordrecht (The Netherlands): Kluwer Academic Publishers. p. 333–361.

Letsch HO, Kjer KM. 2011. Potential pitfalls of modelling ribosomal RNA data in phylogenetic tree reconstruction: evidence from case studies in the Metazoa. *BMC Evol Biol.* 11:146.

Letsch HO, Kück P, Stocsits RR, Misof B. 2010. The impact of rRNA secondary structure consideration in alignment and tree reconstruction: simulated data and a case study on the phylogeny of hexapods. *Mol Biol Evol.* 27:2507–2521.

Lo N, Kitade O, Miura T, Constantino R, Matsumoto T. 2004. Molecular phylogeny of the Rhinotermitidae. *Insectes Soc.* 51: 365–371.

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

Lowe TM, Eddy SR. 1997. TRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* 25:955–964.

Luamba JLN. 1980. Recherches sur le polymorphisme et aperçu sur l'influence de l'analogue de l'hormone juvénile sur le développement d'un termite, *Hodotermes mossambicus* (Isoptera, Hodotermitidae). *Biol Ecol Médit.* 7:169–171.

Maddison WP, Maddison DR. 2010. Mesquite: a modular system for evolutionary analysis. Version 2.73. [cited 2014 July 1]. Available from: http://mesquiteproject.org.

McLoughlin S. 2001. The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. *Aust J Bot.* 49: 271–300.

Meyen SV. 1984. Basic features of gymnosperm systematics and phylogeny as evidenced by fossil record. *Bot Rev.* 50:1–111.

Miura T, Maekawa K, Kitade O, Abe T, Matsumoto T. 1998. Phylogenetic relationships among subfamilies in higher termites (Isoptera: Termitidae) based on mitochondrial COII gene sequences. *Ann Entomol Soc Am.* 91:515–523.

Moreau CS, Bell CD, Vila R, Archibald SB, Pierce NE. 2006. Phylogeny of the ants: diversification in the age of angiosperms. *Science* 312: 101–104.

Nobre T, Eggleton P, Aanen DK. 2010. Vertical transmission as the key to the colonization of Madagascar by fungus-growing termites? *Proc R Soc Lond B Biol Sci.* 277 359–365.

Parmentier D, Roisin Y. 2003. Caste morphology and development in *Termitogeton nrplanus* (Insecta, Isoptera, Rhinotermitidae). *J Morphol.* 255:69–79.

Prasad V, Strömberg CA, Leaché AD, Samant B, Patnaik R, Tang L, Mohabey D, Ge S, Sahni A. 2011. Late Cretaceous origin of the rice tribe provides evidence for early diversification in Poaceae. *Nat Commun.* 2:480.

Prestwich GD. 1984. Defense mechanisms of termites. *Annu Rev Entomol.* 29:201–232.

Quennedey A, Deligne J. 1975. L'arme frontale des soldats de termites—I. Rhinotermitidae. *Insectes Soc.* 22:243–267.

Rambaut A, Drummond AJ. 2007. Tracer v. 1.5. [cited 2014 July 1]. Available from: http://www.beast.bio.ed.ac.uk/Tracer.

Renoux J. 1976. Le polymorphisme de *Schedorhinotermes lamanianus* (Sjöstedt) (Isoptera: Rhinotermitidae). *Insectes Soc.* 23:279–494.

Roisin Y. 1988a. The caste system of *Parrhinotermes browni* (Isoptera: Rhinotermitidae). *Sociobiology* 14:21–28.

Roisin Y. 1988b. Morphology, development and evolutionary significance of the working stages in the caste system of *Prorhinotermes* (Insecta, Isoptera). *Zoomorphology* 107:339–347.

Roisin Y. 2000. Diversity and evolution of caste patterns. In: Abe T, Bignell DE, Higashi M, editors. Termites: evolution, sociality, symbioses, ecology. Dordrecht (The Netherlands): Kluwer Academic Publishers. p. 95–119.

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.

Rouland-Lefèvre C. 2011. Termites as pests of agriculture. In: Bignell DE, Roisin Y, Lo N, editors. Biology of termites: a modern synthesis. Dordrecht (The Netherlands): Springer. p. 499–517.

Sallam HM, Seiffert ER, Steiper ME, Simons EL. 2009. Fossil and molecular evidence constrain scenarios for the early evolutionary and biogeographic history of hystricognathous rodents. *Proc Natl Acad Sci U S A.* 106:16722–16727.

Scheffrahn RH, Krecek J, Chase JA, Maharajh B, Mangol JR. 2006. Taxonomy, biogeography, and notes on termites (Isoptera: Kalotermitidae, Rhinotermitidae, Termitidae) of the Bahamas and Turks and Caicos Islands. *Ann Entomol Soc Am.* 99:463–486.

Schlemmermeyer T, Cancello EM. 2000. New fossil termite species: *Dolichorhinotermes dominicanus* from Dominican Amber (Isoptera, Rhinotermitidae, Rhinotermitinae). *Pap Avulsos Zool.* 41: 303–311.

Schrago CG, Russo CAM. 2003. Timing the origin of New World monkeys. *Mol Biol Evol.* 20:1620–1625.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann Entomol Soc Am.* 87:651–701.

Šobotník J, Bourguignon T, Hanus R, Weyda F, Roisin Y. 2010. Structure and function of defensive glands in soldiers of *Glossotermes oculatus* (Isoptera: Serritermitidae). *Biol J Linn Soc Lond.* 99:839–848.

Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML Web servers. *Syst Biol.* 57:758–771.

Strömberg CAE. 2011. Evolution of grasses and grassland ecosystems. *Annu Rev Earth Planet Sci.* 39:517–544.

Su NY, Scheffrahn RH. 2000. Termites as pests of buildings. In: Abe T, Bignell DE, Higashi M, editors. Termites: evolution, sociality, symbioses, ecology. Dordrecht (The Netherlands): Kluwer Academic Publishers. p. 437–453.

Sugimoto A, Bignell DE, Macdonald JA. 2000. Global impact of termites on the carbon cycle and atmospheric trace gases. In: Abe T, Bignell DE, Higashi M, editors. Termites: evolution, sociality, symbioses, ecology. Dordrecht (The Netherlands): Kluwer Academic Publishers. p. 409–435.

Sullivan J, Joyce P. 2005. Model selection in phylogenetics. *Annu Rev Ecol Evol Syst.* 36:445–466.

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.

Thompson GJ, Miller LR, Lenz M, Crozier RH. 2000. Phylogenetic analysis and trait evolution in Australian lineages of drywood termites (Isoptera, Kalotermitidae). *Mol Phylogenet Evol.* 17:419–429.

Thorne BL, Grimaldi DA, Krishna K. 2000. Early fossil history of termites. In: Abe T, Bignell DE, Higashi M, editors. Termites: evolution, sociality, symbioses, ecology. Dordrecht (The Netherlands): Kluwer Academic Publishers. p. 77–93.

Tokuda G, Isagawa H, Sugio K. 2011. The complete mitogenome of the formosan termite, *Coptotermes formosanus* Shiraki. *Insectes Soc.* 59:17–24.

Trautwein MD, Wiegmann BM, Beutel R, Kjer KM, Yeates DK. 2012. Advances in insect phylogeny at the dawn of the postgenomic era. *Annu Rev Entomol.* 57:449–468.

Udvarty MDF. 1975. A classification of the biogeographical provinces of the world. IUCN Occasional Paper No. 18. Morges (Switzerland): IUCN.

Upchurch P. 2008. Gondwanan break-up: legacies of a lost world? *Trends Ecol Evol.* 23:229–236.

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.

Watson JAL. 1973. The worker caste of the Hodotermitid harvester termites. *Insectes Soc.* 20:1–20.

Wei SJ, Ni JF, Yu ML, Shi BC. 2012. The complete mitochondrial genome of *Macrotermes barneyi* Light (Isoptera: Termitidae). *Mitochondrial DNA* 23:426–428.

Whitfield JB, Lockhart PJ. 2007. Deciphering ancient rapid radiations. *Trends Ecol Evol.* 22:258–265.

Whiting MF. 2002. Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. *Zool Scr.* 31:93–104.

Xiao B, Chen AH, Zhang YY, Jiang GF, Hu CC, Zhu CD. 2012. Complete mitochondrial genomes of two cockroaches, *Blattella germanica* and *Periplaneta americana*, and the phylogenetic position of termites. *Curr Genet.* 58:65–77.

Yamauchi MM, Miya MU, Nishida M. 2004. Use of a PCR-based approach for sequencing whole mitochondrial genomes of insects: two examples (cockroach and dragonfly) based on the method developed for decapod crustaceans. *Insect Mol Biol.* 13:435–442.

Yu Y, Harris AJ, He X. 2010. S-DIVA (Statistical Dispersal-Vicariance Analysis): a tool for inferring biogeographic histories. *Mol Phylogenet Evol.* 56:848–850.

Yu Y, Harris AJ, He XJ. 2013. RASP (Reconstruct Ancestral State in Phylogenies) 2.1 beta. [cited 2014 July 1]. Available from: http://mnh.scu.edu.cn/soft/blog/RASP.

Zhang YY, Xuan WJ, Zhao JL, Zhu CD, Jiang GF. 2010. The complete mitochondrial genome of the cockroach *Eupolyphaga sinensis* (Blattaria: Polyphagidae) and the phylogenetic relationships within the Dictyoptera. *Mol Biol Rep.* 37:3509–3516.