PROCEEDINGS B

rspb.royalsocietypublishing.org

Research

Cite this article: Bourguignon T, Lo N, Šobotník J, Sillam-Dussès 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. B* **283**: 20160179. http://dx.doi.org/10.1098/rspb.2016.0179

Received: 26 January 2016
Accepted: 3 March 2016

Subject Areas:
evolution, taxonomy and systematics, ecology

Keywords:
Isoptera, long distance dispersal, molecular clock, Rhinotermitidae

Author for correspondence:
Thomas Bourguignon
e-mail: thomas.bourgui@gmail.com

Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2016.0179 or via http://rspb.royalsocietypublishing.org.

THE ROYAL SOCIETY
PUBLISHING

Oceanic dispersal, vicariance and human introduction shaped the modern distribution of the termites *Reticulitermes*, *Heterotermes* and *Coptotermes*

Thomas Bourguignon[1,2,3], Nathan Lo[3], Jan Šobotník[2], David Sillam-Dussès[4,5], Yves Roisin[6] and Theodore A. Evans[1,7]

[1]Department of Biological Sciences, National University of Singapore, Singapore 117543, Singapore
[2]Faculty of Forestry and Wood Sciences, Czech University of Life Sciences, Prague, Czech Republic
[3]School of Biological Sciences, University of Sydney, Sydney, New South Wales 2006, Australia
[4]Institut de Recherche pour le Développement, Sorbonne Universités, iEES-Paris, Bondy U 242, France
[5]Université Paris 13, Sorbonne Paris Cité, LEEC, Villetaneuse EA 4443, France
[6]Evolutionary Biology and Ecology, Université Libre de Bruxelles (ULB), Brussels, Belgium
[7]School of Animal Biology, University of Western Australia, Perth, Western Australia 6009, Australia

Reticulitermes, *Heterotermes* and *Coptotermes* form a small termite clade with partly overlapping distributions. Although native species occur across all continents, the factors influencing their distribution are poorly known. Here, we reconstructed the historical biogeography of these termites using mitochondrial genomes of species collected on six continents. Our analyses showed that *Reticulitermes* split from *Heterotermes* + *Coptotermes* at 59.5 Ma (49.9–69.5 Ma 95% CI), yet the oldest split within *Reticulitermes* (Eurasia and North America) is 16.1 Ma (13.4–19.5 Ma) and the oldest split within *Heterotermes* + *Coptotermes* is 36.0 Ma (33.9–40.5 Ma). We detected 14 disjunctions between biogeographical realms, all of which occurred within the last 34 Ma, not only after the break-up of Pangaea, but also with the continents in similar to current positions. Land dispersal over land bridges explained four disjunctions, oceanic dispersal by wood rafting explained eight disjunctions, and human introduction was the source of two recent disjunctions. These wood-eating termites, therefore, appear to have acquired their modern worldwide distribution through multiple dispersal processes, with oceanic dispersal and human introduction favoured by the ecological traits of nesting in wood and producing replacement reproductives.

1. Introduction

Termites are a small insect clade comprising about 3000 described species [1], yet they are important beyond their modest diversity, due to their ability to digest lignocellulose, the most abundant biomolecule on the Earth [2,3]. Consequently, they are hugely abundant and important ecologically, due to their essential role in decomposition and nutrient recycling [4–6]. Termites are also economically important, as they are significant pests of trees in agriculture, timber in forestry and human construction [7–9].

Pest species are concentrated in a few clades of termites. There are 97 species that are considered to be major pests of trees in agriculture, forestry and/or human-made wooden structures [1]. Of these major pests, 39 species are found in the clade comprising *Reticulitermes*, *Heterotermes* and *Coptotermes* [1,10]. Although native species in these three genera are found around the world, about half of the 28 species of invasive termites belong to these genera, compounding their international economic significance [11,12].

Despite their enormous economic importance, the factors influencing the distribution of *Reticulitermes*, *Heterotermes* and *Coptotermes* species around the world have not been investigated in detail. *Reticulitermes* has a temperate,

© 2016 The Author(s) Published by the Royal Society. All rights reserved.

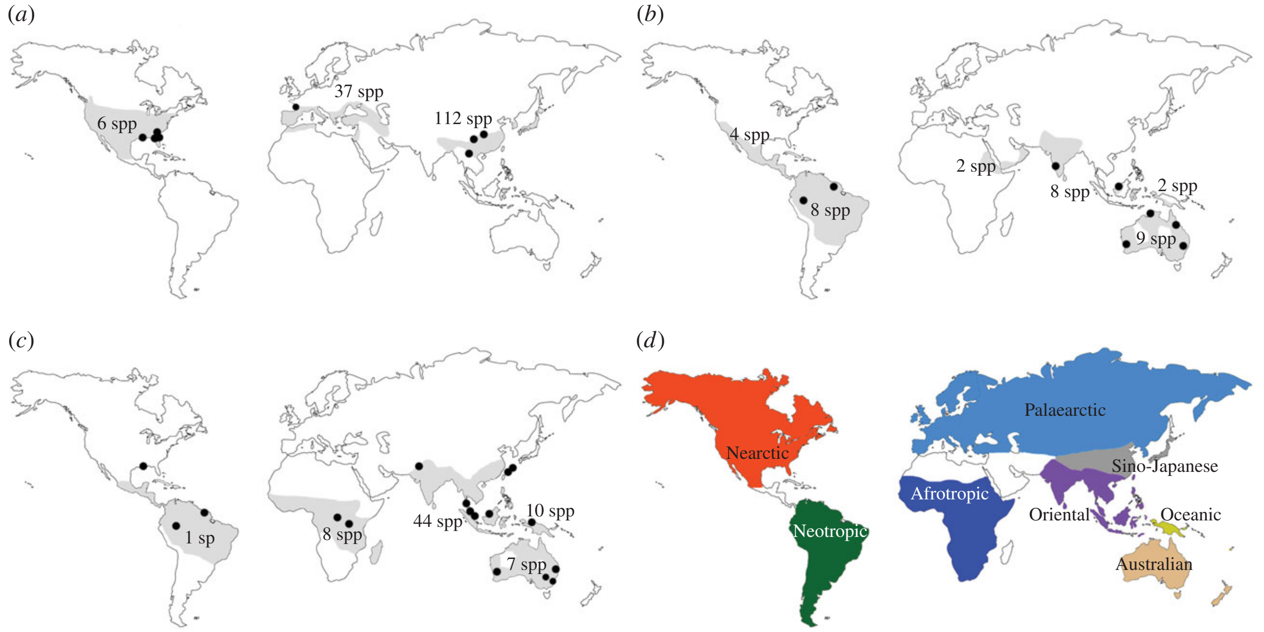

Figure 1. Distribution map of (*a*) *Reticulitermes*, (*b*) *Heterotermes* and (*c*) *Coptotermes*. Light grey areas are the native range of each genus, black circles represent the sampling locations of the specimens used in this study, and species numbers are given as per Krishna *et al*. [1] and Scheffrahn *et al*. [16]. As *C. formosanus* was sampled from introduced populations, a few black circles occur outside the native range of *Coptotermes*. (*d*) Map of the main zoogeographic realms considered in this study [17]. White areas are regions from which no samples were analysed in this study.

holarctic distribution, although it is absent from the drier areas of north Asia east of the Caspian Sea [13,14], with the highest diversity found in China and then around the Mediterranean (although many species are likely synonyms [1,15]). *Heterotermes* and *Coptotermes* are pantropical, although *Heterotermes* is absent from Africa except a small area around Ethiopia (figure 1) [13,14]. The diversity of *Heterotermes* is about the same in the Australian, Oriental and Neotropical regions, and that of *Coptotermes* is highest in Oriental Asia then Papua (although many species are likely synonyms [1,18]). The oldest known fossils are not found in the same locations as the highest diversity. Those of *Reticulitermes* are from Europe (Baltic amber), dated from the Late Eocene, approximately 34 Ma [13], those of *Heterotermes* from Mexico and those of *Coptotermes* from the Dominican Republic (Dominican amber), dated from the Late Oligocene to Early Miocene, 14–26 Ma [13,19].

There are three mechanisms that may explain these distributions and lack of correlation with fossil ages: vicariance and long distance dispersal either by oceanic rafting or by human transport. Vicariance would split groups, due to splitting of landmasses or the raising of mountains. Long distance dispersal could occur over land and sea by natural means in the past, or by human introduction recently. Land dispersal may be through natural flight of winged alates; this would require considerable time, as alates typically fly less than 1 km [20,21], and generation times may be years [22]. Oceanic rafting occurs when floating wood carries termite occupants across otherwise non-traversable space [23]. The floating wood can become a propagule when it contains whole colonies, or even groups of foragers, so long as they can produce replacement reproductives from wingless individuals [24]. Human transport mimics these conditions, just more quickly, and is responsible for the spread of invasive species [11,12].

It is possible to use the timing of splits between species to identify the most likely mechanism behind distribution patterns: vicariance is old and concurs with past geophysical events, human transport young and ocean rafting sometime between. Vicariance may explain some aspects of relatively separate distributions among the genera: molecular-clock analyses estimated that *Reticulitermes* + *Heterotermes* + *Coptotermes* split from Termitidae about 67 Ma, that *Reticulitermes* split up from *Coptotermes* + *Heterotermes* about 50–56 Ma, and that the most recent common ancestor of *Coptotermes* and *Heterotermes* dated back to 27–29 Ma [10,25]. Note that *Reticulitermes* occupies a relatively separate distribution from *Heterotermes* + *Coptotermes* (figure 1*d*), which may be explained by its adaptation to a temperate climate.

The dispersal mechanisms that shaped the distribution within genera are unclear, with the best information for *Reticulitermes*. Intriguingly, although *Reticulitermes* is an old lineage, modern species of *Reticulitermes* shared a common ancestor relatively recently, about 18 Ma [10,15,25]. The modern distribution of *Reticulitermes* can be explained by dispersal using land bridges, possibly over the Pacific via Beringia, as is the case for other insects [26–29]. Alternatively, they may have dispersed between the two biogeographic realms through rafting. By contrast, the pantropical distribution of *Heterotermes* and *Coptotermes* must be mostly the result of ocean dispersal by wood rafting or human transportation, as there has been just one land bridge (between Africa and Asia) since they evolved. Unfortunately, data found in existing studies are insufficient to make conclusions, due to low sampling of species and geographical regions, or use of relatively small DNA regions, thus preventing any conclusions to be drawn [30–35].

Here, we aimed to identify the mechanisms explaining the worldwide distribution of *Reticulitermes*, *Heterotermes* and *Coptotermes*. We did so by determining the full mitochondrial genome sequences of 31 *Coptotermes*, 14 *Heterotermes* and 13 *Reticulitermes* samples, collected along their entire distribution range (figure 1). Termite mitochondrial genomes are about 16 kb, of which 15 kb is phylogenetically informative, encoding 13 protein-coding genes, two ribosomal RNA genes and 22 transfer RNA genes [10,36–38]. Mitochondrial genomes are now becoming a common marker for phylogenetic studies, thanks to the progress of next generation sequencing and their resulting phylogenies have successfully resolved the relationships of several insect groups [38]. With this information, we built a phylogeny based on the mitochondrial genome data and calculated dates for evolutionary splits based on fossils of *Coptotermes*, *Heterotermes* and *Reticulitermes*. We used this phylogeny to resolve the relationships between species of the group and to investigate the origin and timing of the disjunctions in their distribution across biogeographic realms. We then discuss the relative role of vicariance, oceanic rafting and human transport in shaping the modern global distribution pattern of subterranean termites.

2. Material and methods

(a) Mitochondrial genome sequencing

We collected 44 samples of termites of the genera *Coptotermes*, *Heterotermes* and *Reticulitermes* around the world, and used 14 samples from these genera sequenced in previous studies [10,36,37,39–41], for a total of 37 species (electronic supplementary material, table S1). We stored all samples in RNA-later® at −80°C until DNA extraction. We extracted whole genomic DNA using the phenol–chloroform extraction procedure, from about five individual specimens per sample, after removing the digestive tract. We amplified the complete mitochondrial genomes with TaKaRa LA Taq in two long PCR reactions using primers that we specifically designed for termites (electronic supplementary material, table S2). We determined the concentration of both long PCR fragments using QUBIT v. 3.0 fluorometer, then mixed them in equimolar concentration and then multiplexed and paired-end sequenced them with Illumina HiSeq2000.

We assembled separately 88 bp paired-end reads using the CLC suite of programs, as described in Bourguignon *et al.* [10]. In all cases of polymorphic bases, we selected the base with the highest representation. We omitted control regions of the mitochondrial genomes from the final matrix, as they present repetitive patterns that are generally poorly assembled with short reads, and thus provide no useful information. We annotated the 22 tRNAs, the 13 protein-coding genes and the two ribosomal RNAs by eye, aided by previously published sequences that we aligned on each mitochondrial genome using the Muscle algorithm [42] implemented in MEGA v. 5.2.1 [43].

(b) Alignment

We carried out alignments for the 45 mitochondrial genomes sequenced in this study with those from an additional 66 termite species, whose mitochondrial genome sequences have been deposited in GenBank [10,36,37,39–41]. Among these species, there were nine *Reticulitermes*, four *Coptotermes* and one *Heterotermes* species (see electronic supplementary material, table S1). Additionally, we included the sequences of five other polyneopteran insect outgroups whose mitochondrial genomes have been deposited in GenBank: two cockroaches, *Periplaneta fuliginosa* and *Cryptocercus relictus*; a mantis, *Tamolanica tamolana*; a phasmid, *Megacrania alpheus*; and a locust *Locusta migratoria*. We pruned all tips but those of the 13 *Reticulitermes*, 31 *Coptotermes* and 14 *Heterotermes* after phylogenetic analyses for depicting tree topologies. We aligned each gene individually using the Muscle algorithm [42] implemented in MEGA 5.2 [43]. We aligned protein-coding genes as codons, and tRNA and ribosomal RNA genes as DNA, then we concatenated the resulting alignments with SequenceMatrix [44].

(c) Phylogenetic analyses and molecular dating

We determined the partitioning scheme with PartitionFinder [45]. We used a GTR model with gamma-distributed rate variation across sites for all partitions. We found between-species heterogeneity in base composition for the third codon position of *Coptotermes*, *Heterotermes* and *Reticulitermes* ($\chi^2 = 181.18$; d.f. $= 54$; $p < 10^{-6}$). To determine the effect of third codon position on the tree topology and timing, phylogenetic analyses were carried out twice independently, once with the third codon position included in the analysis (hereafter PF3+) and once without the third codon position (PF3−). PF3+ comprised 21 partitions and PF3− comprised 13 partitions (electronic supplementary material, table S3).

We analysed the concatenated DNA sequence alignment with a relaxed molecular-clock model using the Bayesian phylogenetic software BEAST v. 1.8.0 [46] (see electronic supplementary material, datasets S1 and S2). Rate variation was modelled among branches using uncorrelated lognormal relaxed clocks [46,47], with a single model for all genes, that allows for a different relative rate for each partition. We used a Yule speciation process for the tree prior [48] and estimated posterior distributions of parameters, including the tree, using MCMC sampling. We performed two replicate MCMC runs, with the tree and parameter values sampled every 10 000 steps over a total of 100 million generations. We obtained a maximum clade credibility tree using Tree Annotator within the BEAST software package with a burn-in of the first 10 millions generations as determined with TRACER v. 1.5 [49]. We checked acceptable sample sizes and convergence to the stationary distribution using Tracer v. 1.5.

We calibrated the molecular clock using 13 minimum age constraints (electronic supplementary material, table S4). Minimum age constrains were determined based on the fossil record, and we systematically selected the youngest possible age for each fossil as mentioned in the Paleobiology Database (PaleoBioDB, www.paleobiodb.org). We implemented fossil calibrations as exponential priors on node times (electronic supplementary material, table S4). Some of these dates differ from previous calibrations we used [10,25], which were based on dates reported in Emerson [13]. This is because the ages of certain periods, such as the Oligocene, have been revised over time.

We used the RAxML v. 7.7.1 (black-box webserver; http://embnet.vital-it.ch/raxml-bb/) [50] in order to test the effect of phylogenetic method on tree topology. We carried out two analyses, one on PF3+ and one of PF3−, and we used a Gamma model of rate heterogeneity. Bootstrap supports were computed using 100 replications.

We used mitochondrial genomes and phylogenetic analyses to explain the biogeographic patterns of *Reticulitermes*, *Heterotermes* and *Coptotermes*. Although mitochondrial genomes are large compared with regular PCR-amplified markers, all their genes are linked and transmitted as a single package maternally. Mitogenome phylogenies can potentially be discordant to species phylogenies, in the case of hybridization-introgression or lineage sorting. However, because alleles coalesce with time, discordances generally occur only between closely related species and deep nodes are generally adequately inferred [51]. The rare cases of discordance in deep nodes are associated with ancient rapid radiations, which are characterized by very short branches with low posterior probabilities, and require enormous datasets to be resolved [52]. We can, therefore, reasonably conclude that nodes recovered with 100% Bayesian posterior probabilities and 100% bootstrap in all our analyses adequately reflect the relationships among taxa.

(d) Biogeographic analyses

We reconstructed the evolution of termite geographical ranges using a Bayesian binary model implemented in the RASP v. 2.1 software [53,54]. We used two models of state frequencies, fixed (JC) and estimated (F81); and two models of among-site rate variation, equal and gamma (+G), 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. Sample locations were used to give each tip one biogeographic area. We distinguished eight biogeographic realms as determined in Holt *et al*. [17]: Australian, Afrotropical, Oriental, Nearctic, Neotropical, Oceanian (New Guinea), Palaearctic and Sino-Japanese.

3. Results

(a) Tree topology

Our Bayesian analyses supported the monophyly of *Reticulitermes* and *Coptotermes*, but *Heterotermes* was paraphyletic with respect to *Coptotermes* (figure 2). The tree topology was unaffected by exclusion of the third codon position, except for the branching patterns of Australian species of *Heterotermes*. Likelihood analyses yielded the same topology as the Bayesian analyses with three exceptions: (i) for the PF3+ partition, *Reticulitermes* formed a polytomy divided into three groups: *R. grassei*, *R.* sp. A + *R. aculabialis* + *R. chinensis*, and the Nearctic *Reticulitermes*; (ii) *C. elisae* was the sister group of *C. formosanus* + *C. kalshoveni* + *C. remotus* + *C. sepengensis* in PF3−. (iii) The branching patterns of Australian species of *Heterotermes* were variable among analyses.

(b) Molecular dating

The two chronograms recovered very similar divergence date estimates, with a maximum difference of 1.9 Ma between analyses, therefore, we display the results of PF3+ only (figure 2). With PF3+, the divergence between *Reticulitermes* and *Heterotermes* + *Coptotermes* was estimated at 59.5 Ma (49.9–69.5 Ma 95% CI) and the most recent common ancestor of *Reticulitermes* at 16.1 Ma (13.4–19.5 Ma 95% CI). The most recent common ancestor of *Heterotermes* + *Coptotermes* was dated at 36.0 Ma (33.9–40.5 Ma 95% CI) and the most recent common ancestor of *Coptotermes* at 24.5 Ma (21.0–27.8 Ma 95% CI; figure 2).

(c) Biogeographic reconstruction

We reconstructed the ancestral range distribution on all trees independently in order to assess the effect of uncertainties in phylogenetic reconstruction. The Bayesian tree computed with the PF3+ partition scheme was illustrative of the variation in ancestral state reconstruction (figure 2). The Bayesian binary models of state frequencies and site variation only marginally differ, so we present the results of the analysis with fixed (JC) state frequencies and equal among-site rate variation only. Overall, our analyses retrieved 14 disjunctions between biogeographic areas (as determined by Holt *et al*. [17]).

We inferred four disjunctions for the genus *Reticulitermes*, including one of human origin. *Reticulitermes* was characterized by an early split between Nearctic and Old World species estimated at 12.6–16.1 Ma (9.8–19.5 Ma 95% CI). Old World *Reticulitermes* species were monophyletic but the origin of the distribution patterns between Palaearctic, Sino-Japanese and Oriental species was unresolved. The Palaearctic (France) *R. santonensis*, synonymized with *R. flavipes* [55], was nested within the Nearctic clade, supporting its recent human introduction.

We inferred three dispersal events for the genus *Heterotermes*. The most recent common ancestor of modern *Heterotermes* species was inferred to have existed in the Oriental realm, with an origin around 36.0 Ma (33.9–40.5 Ma 95% CI). *Heterotermes* appears to have dispersed from the Oriental realm twice, once to the Neotropical realm around 28.3–32.7 Ma (24.5–37.2 Ma 95% CI) and once to the Australian realm around 14.3 Ma (19.7 Ma 95% upper bound). There was a second dispersal to Australia from the Neotropics around 12.9–21.0 Ma (10.3–25.1 Ma 95% CI), based on the nested position of the larger Australian *Heterotermes* clade (figure 2).

We inferred seven dispersal events for the genus *Coptotermes*, but these events may be construed in two different scenarios. In the first scenario, the ancestral *Coptotermes* was from the Oriental realm, with an origin around 24.5 Ma (21.0–27.8 Ma 95% CI). *Coptotermes* dispersed five times from there, to the Afrotropics around 16.0–24.5 Ma (11.3–27.8 Ma 95% CI), to the Neotropics (ambiguous support) around 1.7–21.7 Ma (1.1–27.8 Ma 95% CI), to Australia around 13.3–16.0 Ma (11.1–18.5 Ma 95% CI), and twice independently to New Guinea, once less than 4.3 Ma (5.7 Ma 95% upper bound) and once less than 11.6 Ma (14.1 Ma 95% upper bound). In the second scenario, the ancestral *Coptotermes* evolved in the Afrotropics, and then dispersed to the Neotropics then the Oriental realm independently, and from the Oriental realm to Australia and New Guinea independently (figure 2). The ancestor of the major invasive pest *C. formosanus* was found to have either a New Guinean or Oriental origin, although the modern *C. formosanus* is known to be native to the Sino-Japanese realm, and was introduced to the Nearctic realm from there [56].

4. Discussion

In this study, we confirmed the relationship pattern and dating of the three genera as found in earlier molecular studies [10,15,25,37]. We found that the split between *Reticulitermes* and *Heterotermes* + *Coptotermes* occurred around 59 Ma (49.9–69.5 Ma 95% CI), the most recent common ancestor of the clade *Heterotermes* + *Coptotermes* arose 36 Ma (33.9–40.5 Ma 95% CI), and *Coptotermes* split from sister *Heterotermes* species around 24.5 Ma (21.0–27.8 Ma 95% CI). Therefore, the entire clade *Reticulitermes* + *Heterotermes* + *Coptotermes* originated after the break-up of Pangaea, and after the partial break-up of Gondwana. Consequently, the current native distribution of modern taxa cannot be explained by the past distribution of their ancestors spread across the earlier super continent. All three genera must have dispersed over oceans, either by land bridges or by rafting in wood.

Our results cannot identify the centre of origin of *Reticulitermes*, but we found a split between Old World and New World species dating to around 16.1 Ma (13.4–19.5 Ma 95% CI). The low nodal support for the early branching of *Reticulitermes*, combined with low sampling of species from the Palaearctic, Sino-Japanese and Oriental realms, prevent us from drawing a definitive scenario of the historical

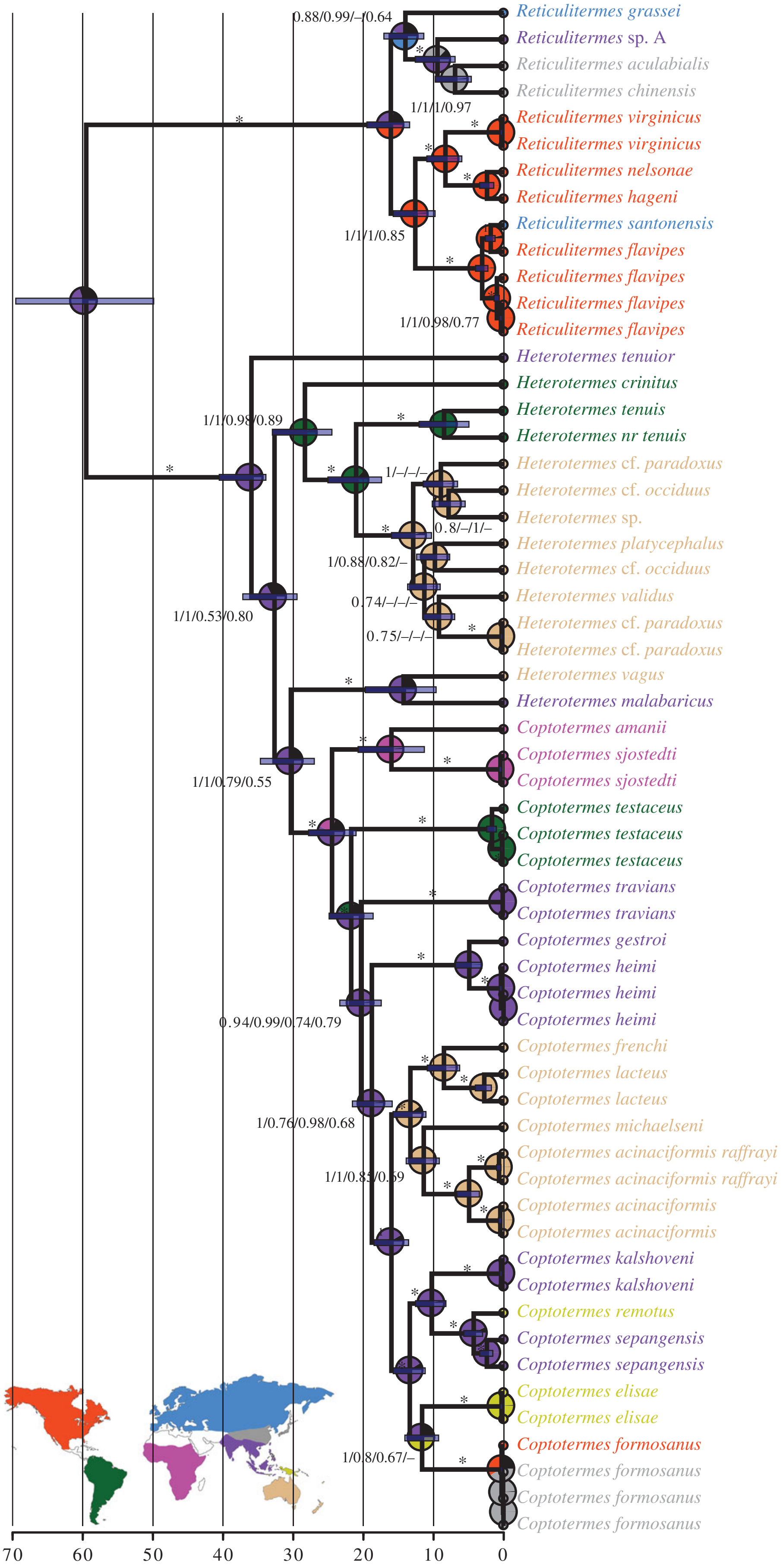

Figure 2. Phylogenetic chronogram of termites based on the full mitochondrial genome, with third codon position included (PF3+), reconstructed using BEAST. Scale bar estimates age in millions of years. Node bars represent 95% CIs. Branch labels are support for the four analyses in the following order: Bayesian posterior probabilities of the Bayesian trees with PF3+ and PF3− partition schemes, bootstrap supports of the maximum-likelihood trees with PF3+ and PF3− partition schemes; dashes (–) indicate that the node is absent for a given analysis; asterisks (*) indicate 100% support for all four analyses. Node pie charts are the inferred ancestral distribution reconstructed using a Bayesian binary model. The map shows the zoogeographic realms considered in the analyses [17].

biogeography of the genus. However, one scenario consistent with our results is that the native distribution is relictual from a past Holarctic distribution, followed by a gradual retraction as the Earth cooled down since the Middle Miocene, 14 Ma [57]. The Palaearctic, Sino-Japanese and most of the Oriental realms are part of a single continental mass on one tectonic plate. *Reticulitermes* is distributed in two distinct areas on this continent: an area roughly including South Europe and Western Asia (Palaearctic); and an area including the Sino-Japanese realm and the Northernmost Oriental realm (figure 1*a*). The most recent common ancestor of European and Sino-Japanese species of *Reticulitermes* was dated to the Middle Miocene, 14.0 Ma (11.4–17.1 Ma 95% CI), concurring with previous estimations [15], when temperate forests stretched from Europe to Far East Asia [58,59], therefore, we consider it likely that *Reticulitermes* declined in central Asia as the climate became cooler and dryer [60].

The other major disjunction in the distribution of *Reticulitermes* is between the Old World and the New World species, whose most recent common ancestor was dated to the Early Miocene, 16.1 Ma (13.4–19.5 Ma 95% CI), coinciding with the end of a global climatic warming 25–15 Ma [57]. Dedeine *et al*. [15] also dated the origin of extant *Reticulitermes* species during the Early Miocene, 18.4 Ma. The Bering Land Bridge was covered by coniferous forests similar to the present taiga [29], through which cold-adapted insects, such as bumblebees [26], may have dispersed between the Nearctic and Palaearctic realms during that period of time. In this scenario, the modern distribution of *Reticulitermes* was shaped by vicariance from a Holarctic distribution which gradually retracted southward as forests were replaced by grassland during the second half of the Miocene [29]. However, modern species of *Reticulitermes* are not adapted to taiga, suggesting that *Reticulitermes* dispersed between the Nearctic and the Palaearctic realms by rafting across ocean, or using the land bridge across Iceland that possibly existed during the Miocene [61]. Our ancestral range reconstruction concurred with previous studies that showed that the French *R. santonensis* is a synonym of the American *R. flavipes* and was introduced into France by humans [55,62].

The most recent common ancestor of *Heterotermes* may have appeared in the Orient around 36.0 Ma (33.9–40.6 Ma 95% CI) (in the Eocene), after the initial collision of the Indian and Eurasian plates [63]. The oldest fossil species of *Heterotermes*, *H. eocenicus*, lived in Europe around 34 Ma, suggesting that *Heterotermes* was more widely distributed in the past and went extinct in Europe, and possibly in other nowadays cooler realms. The spread from the Orient to Africa may have been over land, however, the spread to the Neotropics and Australia must have been over oceans. Sampling of additional species of *Heterotermes* from India, Ethiopia and from the Neotropics could improve the reconstruction of the genus' ancestral range.

The direction of more recent dispersal events received stronger support, and was consistently recovered in all our analyses. Our ancestral state reconstruction showed that *Heterotermes* colonized Australia at least once from the Neotropical realm 12.9–21.0 Ma (10.3–25.1 Ma 95% CI), and possibly from the Oriental realm less than 14.3 Ma (19.7 Ma 95% CI upper bound). Southeast Asia and Australia have never been connected by land bridges, although chains of volcanic island arcs may have existed, over which termites might have island hopped [64]. Additionally, the collision of the Australian and Asian plates closed deep water passages between the Pacific and Indian oceans about 20 Ma, causing major changes in oceanic currents [65], and possibly favouring the colonization of Australia by Neotropical *Heterotermes* in the same time period.

The most recent common ancestor of modern *Coptotermes* species may have evolved in either Africa or the Orient around 24.5 Ma (21.0–27.8 Ma 95% CI) (in the Oligocene), and the movement between Africa and Asia may have been over the ocean before the land bridge connecting Africa and Eurasia formed, around 18 Ma, or over land afterwards [66]. However, the dispersal events to the Neotropics, Australia and New Guinea were all over water, considering that these regions were isolated at the time dispersals took place.

For modern *Coptotermes*, our analyses inferred an Oriental origin, with subsequent dispersal to the Afrotropics, Neotropics, Australia and New Guinea. However, support for this scenario was not strong, and we cannot exclude an African origin. Additional sampling within the sister groups of *Coptotermes* may help, as it did for Asian and Australian *Coptotermes* alone (cf. [25] and [33]). However, additional sampling within basal *Coptotermes* species is unlikely to provide resolution for either the node including all *Coptotermes* or the node including all non-African *Coptotermes*, because there are few additional extant species of *Coptotermes* in Africa and none in South America.

Coptotermes species diversity may have changed markedly in Africa and South America over time. Three *Coptotermes* species were described from Dominican amber [19], but only *C. testaceus* occurs today in the Neotropics [16]. Although the fossil record from the Afrotropics is non-existent for *Coptotermes*, only three described modern species have solid evidence for validity [18], two of which were included in this study. *Coptotermes*, therefore, appears to have experienced relatively high extinction rates in the Neotropics, and possibly in the Afrotropics, that blurred the signal and left too few modern species to resolve the early dispersal events of the genus based on phylogenetic methods.

The direction of more recent dispersal events received stronger support and was consistently recovered in all our analyses. We found that *Coptotermes* colonized Australia once from the Oriental realm 13.3–16.0 Ma (11.1–18.5 Ma 95% CI), which matched the estimate of Lee *et al*. [25].

We found that the New Guinean species of *Coptotermes* considered in this study have the most recent origin, also from the Orient, concurring with Bourguignon & Roisin [67], who proposed that the termite fauna from New Guinea originated from the Orient. This may be due to the reappearance of New Guinea and the creation of the high mountains from the collision of the Australian plate with the Asian plate in the Miocene [65]. Finally, our results also placed *C. formosanus* as the sister group of the New Guinean *C. elisae*, both genera being nested within Oriental *Coptotermes*. *C. elisae* shares many morphological similarities with the Oriental *C. curvignathus* (T Bourguignon 2013, personal observation) supporting the notion that *C. elisae* originated from the Oriental realm. *C. formosanus* is currently believed to be native to southern China [56] and our results show it is descended from an Oriental ancestor.

For all three genera, the dispersal over oceans was probably by rafting, i.e. termites living in floating dead wood. Lower termites, including those in the Rhinotermitidae, often nest in dead wood [25,68,69]. Such wood may be washed into rivers and thence into oceans. These wood rafts can become functional propagules with a complete colony (that is, one containing the reproductive king and queen), or without, as secondary reproductives can easily be generated in these genera [70]. The relative amount of over ocean dispersal may be related to the types of food targeted by the different genera. *Reticulitermes* and *Heterotermes* target relatively small pieces of dead, fallen wood, whereas *Coptotermes* targets entire trees, often nesting inside the tree trunk [25,68,69]. One tree floating on sea would probably contain enough food for a termite colony to survive for many months, allowing longer distance colonization events. Such rafting is usually used to explain the origin of the biota of oceanic islands [71] and was inferred many times from dated phylogenies [72–74].

The process of crossing an ocean in a wooden raft is analogous to human introduction. Termites introduced by humans are all wood-eating and wood-nesting species. Some of the worst invaders are species of *Reticulitermes*, *Heterotermes* and *Coptotermes*. Humans simply assist dispersal over oceans and make it more efficient [11,12]. Interestingly, various *Coptotermes* species have invaded more locations than either *Reticulitermes* or *Heterotermes*, mimicking the pattern from natural oceanic crossings.

Our sampling covered most of the realms in which each genus occurs, and is therefore likely to have identified most disjunctions arising without the influence of humans. However, additional sampling will possibly reveal further examples. Multispecies sampling within a biogeographical realm can reveal multiple colonization events, such as in the case of *Heterotermes*, which appears to have colonized Australia twice. More dispersal events, therefore, cannot be excluded, especially in the genera *Heterotermes* and *Coptotermes* in view of their high dispersal ability. In addition, more future dispersal events assisted by humans are likely.

Data accessibility. Mitochondrial genome sequences have been deposited in GenBank (see electronic supplementary material, table S1 for accession numbers).

Authors' contributions. T.B. and T.A.E. conceived the study, T.B. designed the study, T.B., J.S., D.S.D., Y.R. and T.A.E. collected the material, and T.B. and N.L. analysed the data. All authors contributed significantly to the text of the manuscript and approved the final version.

Competing interests. We declare we have no competing interests.

Funding. This work was supported by the LHK fund of the National University of Singapore; by the Singapore–MIT Alliance for Research and Technology; the Alliance National University of Singapore, Université Sorbonne Paris Cité, the Internal Grant Agency of Faculty of Forestry and Wood Sciences, CULS (IGA B03/15) and the Czech Science Foundation (project no. 15–07015Y). T.B. was supported by the University of Sydney through a postdoctoral fellowship.

Acknowledgements. We thank Brian Forschler, Naeem Iqbal and Tamara Hartke for providing specimens, Barbora Křižková, Maria Lee An and Martin Jendryka for laboratory assistance, Jan Křeček for help with species identification and Simon Ho for assistance with analyses.

References

1. Krishna K, Grimaldi DA, Engel MS. 2013 Treatise on the Isoptera of the world, vol. 1. *Bull. Am. Mus. Nat. Hist*. **377**, 1–196. (doi:10.1206/377.1)
2. Béguin P, Aubert JP. 1994 The biological degradation of cellulose. *FEMS Microbiol. Rev*. **13**, 25–58. (doi:10.1111/j.1574-6976.1994.tb00033.x)
3. Dixon RK, Solomon AM, Brown S, Houghton RAM, Trexier C, Wisniewski J. 1994 Carbon pools and flux of global forest ecosystems. *Science* **263**, 185–190. (doi:10.1126/science.263.5144.185)
4. Bignell DE. 2006 Termites as soil engineers and soil processors. In *Intestinal microorganisms of soil invertebrates* (eds H König, A Varma), pp. 183–220. Berlin, Germany: Springer.
5. Evans TA, Dawes TZ, Ward PR, Lo N. 2011 Ants and termites increase crop yield in a dry climate. *Nat. Commun*. **2**, 1257. (doi:10.1038/ncomms1257)
6. Jouquet P, Traoré S, Choosai C, Hartmann C, Bignell DE. 2011 Influence of termites on ecosystem functioning. Ecosystem services provided by termites. *Eur. J. Soil Biol*. **47**, 215–222. (doi:10.1016/j.ejsobi.2011.05.005)
7. Hickin NE. 1971 *Termites, a world problem*. London, UK: The Rentokil Library, Hutchinson.
8. Su NY, Scheffrahn RH. 2000 Termites as pests of buildings. In *Termites: evolution, sociality, symbioses, ecology* (eds T Abe, DE Bignell, M Higashi), pp. 437–453. Dordrecht, The Netherlands: Kluwer.
9. Rouland-Lefèvre C. 2011 Termites as pests of agriculture. In *Biology of termites: a modern synthesis* (eds DE Bignell, Y Roisin, N Lo), pp. 499–517. Dordrecht, The Netherlands: Springer.
10. Bourguignon T *et al.* 2015 The evolutionary history of termites as inferred from 66 mitochondrial genomes. *Mol. Biol. Evol*. **32**, 406–421. (doi:10.1093/molbev/msu308)
11. Evans TA. 2011 Invasive termites. In *Biology of termites: a modern synthesis* (eds DE Bignell, Y Roisin, N Lo), pp. 519–562. Dordrecht, The Netherlands: Springer.
12. Evans TA, Forschler BT, Grace JK. 2013 Biology of invasive termites: a worldwide review. *Annu. Rev. Entomol*. **58**, 455–474. (doi:10.1146/annurev-ento-120811-153554)
13. 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.
14. Eggleton P. 2000 Global patterns of termite diversity. In *Termites: evolution, sociality, symbioses, ecology* (eds T Abe, DE Bignell, M Higashi), pp. 25–51. Dordrecht, The Netherlands: Kluwer Academic Publishers.
15. Dedeine F, Dupont S, Guyot S, Matsuura K, Wang C, Habibpour B, Bagnères AG, Mantovani B, Luchetti A. 2016 Historical biogeography of *Reticulitermes* termites (Isoptera: Rhinotermitidae) inferred from analyses of mitochondrial and nuclear loci. *Mol. Phylogenet. Evol*. **94**, 778–790. (doi:10.1016/j.ympev.2015.10.020)
16. Scheffrahn RH, Carrijo TF, Křeček J, Su NY, Szalanski AL, Austin JW, Chase JA, Mangold JR. 2015 A single endemic and three exotic species of the termite genus *Coptotermes* (Isoptera, Rhinotermitidae) in the New World. *Arthropod. Syst. Phylogenet*. **73**, 333–348.
17. Holt BG *et al.* 2013 An update of Wallace's zoogeographic regions of the world. *Science* **339**, 74–79. (doi:10.1126/science.1228282)
18. Chouvenc T *et al.* 2016 Revisiting *Coptotermes* (Isoptera: Rhinotermitidae): a global taxonomic roadmap for species validity and distribution of an economically important subterranean termite genus. *Syst. Entomol*. **41**, 299–306. (doi:10.1111/syen.12157)
19. Krishna K, Grimaldi D. 2009 Diverse Rhinotermitidae and Termitidae (Isoptera) in Dominican Amber. *Am. Mus. Novit*. **3640**, 1–48. (doi:10.1206/633.1)
20. Messenger MT, Mullins AJ. 2005 New flight distance recorded for *Coptotermes formosanus* (Isoptera: Rhinotermitidae). *Fla Entomol*. **88**, 99–100. (doi:10.1653/0015-4040(2005)088[0099:NFDRFC]2.0.CO;2)
21. Hu J, Zhong JH, Guo MF. 2007 Alate dispersal distances of the black-winged subterranean termite *Odontotermes formosanus* (Isoptera: Termitidae) in southern China. *Sociobiology* **50**, 1–8.
22. Keller L. 1998 Queen lifespan and colony characteristics in ants and termites. *Insect. Soc*. **45**, 235–246. (doi:10.1007/s000400050084)
23. Thiel M, Haye PA. 2006 The ecology of rafting in the marine environment. III. Biogeographical and evolutionary consequences. *Oceanogr. Mar. Biol. An Annu. Rev*. **43**, 323–429. (doi:10.1201/9781420006391.ch7)

24. Bourguignon T, Chisholm RA, Evans TA. 2016 The termite worker phenotype evolved as dispersal strategy for fertile wingless individuals before eusociality. *Am. Nat*. **187**, 372–387. (doi:10.1086/684838)
25. Lee TRC, Cameron SL, Evans TA, Ho SYW, Lo N. 2015 The origins and radiation of Australian *Coptotermes* termites: from rainforest to desert dwellers. *Mol. Phylogenet. Evol*. **82**, 234–244. (doi:10.1016/j.ympev.2014.09.026)
26. Hines HM. 2008 Historical biogeography, divergence times, and diversification patterns of bumble bees (Hymenoptera: Apidae: *Bombus*). *Syst. Biol*. **57**, 58–75. (doi:10.1080/10635150801898912)
27. Vila R *et al*. 2011 Phylogeny and palaeoecology of *Polyommatus* blue butterflies show Beringia was a climate-regulated gateway to the New World. *Proc. R. Soc. B* **278**, 2737–2744. (doi:10.1098/rspb.2010.2213)
28. Meseguer AS, Coeur d'acier A, Genson G, Jousselin E. 2015 Unravelling the historical biogeography and diversification dynamics of a highly diverse conifer-feeding aphid genus. *J. Biogeogr*. **88**, 1482–1492. (doi:10.1111/jbi.12531)
29. Sanmartín I. 2001 Patterns of animal dispersal, vicariance and diversification in the Holarctic. *Biol. J. Linn. Soc*. **73**, 345–390. (doi:10.1111/j.1095-8312.2001.tb01368.x)
30. Austin JW, Szalanski AL, Uva P, Bagnères AG, Kence A. 2002 A comparative genetic analysis of the subterranean termite genus *Reticulitermes* (Isoptera: Rhinotermitidae). *Ann. Entomol. Soc. Am*. **95**, 753–760. (doi:10.1603/0013-8746(2002)095[0753:ACGAOT]2.0.CO;2)
31. Austin JW, Szalanski AL, Cabrera BJ. 2004 Phylogenetic analysis of the subterranean termite family Rhinotermitidae (Isoptera) by using the mitochondrial cytochrome oxidase II gene. *Ann. Entomol. Soc. Am*. **97**, 548–555. (doi:10.1603/0013-8746(2004)097[0548:PAOTST]2.0.CO;2)
32. Luchetti A, Trenta M, Mantovani B, Marini M. 2004 Taxonomy and phylogeny of north mediterranean *Reticulitermes* termites (Isoptera, Rhinotermitidae): a new insight. *Insect. Soc*. **51**, 117–122. (doi:10.1007/s00040-003-0715-z)
33. Lo N, Eldridge RH, Lenz M. 2006 Phylogeny of Australian *Coptotermes* (Isoptera: Rhinotermitidae) species inferred from mitochondrial COII sequences. *Bull. Entomol. Res*. **96**, 433–437.
34. Yeap BK, Othman AS, Lee CY. 2009 Molecular systematics of *Coptotermes* (Isoptera: Rhinotermitidae) from East Asia and Australia. *Ann. Entomol. Soc. Am*. **102**, 1077–1090. (doi:10.1603/008.102.0616)
35. Velonà A, Ghesini S, Luchetti A, Marini M, Mantovani B. 2010 Starting from Crete, a phylogenetic re-analysis of the genus *Reticulitermes* in the Mediterranean area. *Mol. Phylogenet. Evol*. **56**, 1051–1058. (doi:10.1016/j.ympev.2010.04.037)
36. Cameron SL, Whiting MF. 2007 Mitochondrial genomic comparisons of the subterranean termites from the genus *Reticulitermes* (Insecta: Isoptera: Rhinotermitidae). *Genome* **202**, 188–202.
37. 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. (doi:10.1016/j.ympev.2012.05.034)
38. Cameron SL. 2014 Insect mitochondrial genomics: implications for evolution and phylogeny. *Annu. Rev. Entomol*. **59**, 95–117. (doi:10.1146/annurev-ento-011613-162007)
39. Tokuda G, Isagawa H, Sugio K. 2011 The complete mitogenome of the Formosan termite, *Coptotermes formosanus* Shiraki. *Insect. Soc*. **59**, 17–24. (doi:10.1007/s00040-011-0182-x)
40. Chen Q, Wang K, Tan Y, Xing L. 2016 The complete mitochondrial genome of the subterranean termite, *Reticulitermes chinensis* Snyder (Isoptera: Rhinotermitidae). *Mitochondrial DNA* **27**, 1428–1429. (doi:10.3109/19401736.2014.953077)
41. Kai W, Xiao-Hui G, Chun-Hua D, Lian-Xi X, Jiang-Li T, Xiao-Hong S. In press. Complete mitochondrial genome of a parthenogenetic subterranean termite, *Reticulitermes aculabialis* Tsai et Hwang (Isoptera: Rhinotermitidae). *Mitochondrial DNA*.
42. Edgar RC. 2004 MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinform*. **5**, 113. (doi:10.1186/1471-2105-5-113)
43. 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. (doi:10.1093/molbev/msr121)
44. Vaidya G, Lohman DJ, Meier R. 2011 SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* **27**, 171–180. (doi:10.1111/j.1096-0031.2010.00329.x)
45. 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. (doi:10.1093/molbev/mss020)
46. Drummond AJ, Rambaut A. 2007 BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol*. **7**, 214. (doi:10.1186/1471-2148-7-214)
47. Drummond AJ, Ho SYW, Phillips MJ, Rambaut A. 2006 Relaxed phylogenetics and dating with confidence. *PLoS Biol*. **4**, e88. (doi:10.1371/journal.pbio.0040088)
48. Gernhard T. 2008 The conditioned reconstructed process. *J. Theor. Biol*. **253**, 769–778. (doi:10.1016/j.jtbi.2008.04.005)
49. Rambaut A, Drummond AJ. 2007 *Tracer*. http://tree.bio.ed.ac.uk/software/tracer/.
50. Stamatakis A, Hoover P, Rougemont J. 2008 A rapid bootstrap algorithm for the RAxML Web servers. *Syst. Biol*. **57**, 758–771. (doi:10.1080/10635150802429642)
51. Degnan JH, Rosenberg NA. 2009 Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends Ecol. Evol*. **24**, 332–340. (doi:10.1016/j.tree.2009.01.009)
52. Whitfield JB, Lockhart PJ. 2007 Deciphering ancient rapid radiations. *Trends Ecol. Evol*. **22**, 258–265. (doi:10.1016/j.tree.2007.01.012)
53. 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. (doi:10.1016/j.ympev.2015.03.008)
54. Ronquist F, Huelsenbeck JP. 2003 MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574. (doi:10.1093/bioinformatics/btg180)
55. Austin J, Szalanski AL, Scheffrahn RH, Messenger MT, Dronnet P, Bagnères AG. 2005 Genetic evidence for the synonymy of two *Reticulitermes* species: *Reticulitermes flavipes* and *Reticulitermes santonensis*. *Ann. Entomol. Soc. Am*. **98**, 395–401. (doi:10.1603/0013-8746(2005)098[0395:GEFTSO]2.0.CO;2)
56. Husseneder C, Simms DM, Delatte JR, Wang C, Grace JK, Vargo EL. 2011 Genetic diversity and colony breeding structure in native and introduced ranges of the Formosan subterranean termite, *Coptotermes formosanus*. *Biol. Invasions* **14**, 419–437. (doi:10.1007/s10530-011-0087-7)
57. Zachos J, Pagani M, Sloan L, Thomas E, Billups K. 2001 Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* **292**, 686–694. (doi:10.1126/science.1059412)
58. Zhilin SG. 2001 Structure of the Turgayan flora in the Oligocene and Miocene and its palaeoclimatic features. *Acta Palaeobot*. **41**, 141–146.
59. Tang ZH, Ding ZL. 2013 A palynological insight into the Miocene aridification in the Eurasian interior. *Palaeoworld* **22**, 77–85. (doi:10.1016/j.palwor.2013.05.001)
60. Miao Y, Herrmann M, Wu F, Yan X, Yang S. 2012 What controlled Mid–Late Miocene long-term aridification in Central Asia?—global cooling or Tibetan Plateau uplift: a review. *Earth Sci. Rev*. **112**, 155–172. (doi:10.1016/j.earscirev.2012.02.003)
61. Denk T, Grímsson F, Zetter R. 2010 Episodic migration of oaks to Iceland: evidence for a North Atlantic 'land bridge' in the latest Miocene. *Am. J. Bot*. **97**, 276–287. (doi:10.3732/ajb.0900195)
62. Perdereau E, Bagnères AG, Bankhead-Dronnet S, Dupont S, Zimmermann M, Vargo EL, Dedeine F. 2013 Global genetic analysis reveals the putative native source of the invasive termite, *Reticulitermes flavipes*, in France. *Mol. Ecol*. **22**, 1105–1119. (doi:10.1111/mec.12140)
63. van Hinsbergen DJJ, Lippert PC, Dupont-Nivet G, McQuarrie N, Doubrovine PV, Spakman W, Torsvik TH. 2012 Greater India Basin hypothesis and a two-stage Cenozoic collision between India and Asia. *Proc. Natl Acad. Sci. USA* **109**, 7659–7664. (doi:10.1073/pnas.1117262109)
64. Moss SJ, Wilson MEJ. 1998 Biogeographic implications of the Tertiary palaeogeographic evolution of Sulawesi and Borneo. In *Biogeography and geological evolution of SE Asia* (eds R Hall,

JD Holloway), pp. 133–163. Leiden, The Netherlands: Backhuys Publishers.

65. Hall R. 1998 The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. In *Biogeography and geological evolution of SE Asia* (eds R Hall, JD Holloway), pp. 99–131. Leiden, The Netherlands: Backhuys Publishers.
66. Rögl F. 1999 Mediterranean and Paratethys. Facts and hypotheses of an Oligocene to Miocene paleogeography (short review). *Geol. Carpathica* **50**, 339–349.
67. Bourguignon T, Roisin Y. 2011 Revision of the termite family Rhinotermitidae (Isoptera) in New Guinea. *ZooKeys* **148**, 55–103. (doi:10.3897/zookeys.148.1826)
68. Noirot C. 1970 The nests of termites. In *Biology of termites*, vol. II (eds K Krishna, FM Weesner), pp. 311–350. New York, NY: Academic Press.
69. Noirot C, Darlington JPEC. 2000 Termite nests: architecture, regulation and defence. In *Termites: evolution, sociality, symbioses, ecology* (eds T Abe, DE Bignell, M Higashi), pp. 121–139. Dordrecht, The Netherlands: Kluwer Academic Publishing.
70. Myles TG. 1999 Review of secondary reproduction in termites (Insecta: Isoptera) with comments on its role in termite ecology and social evolution. *Sociobiology* **33**, 1–88.
71. de Queiroz A. 2005 The resurrection of oceanic dispersal in historical biogeography. *Trends Ecol. Evol.* **20**, 68–73. (doi:10.1016/j.tree.2004.11.006)
72. Ward PS, Brady SG, Fisher BL, Schultz TR. 2015 The evolution of myrmicine ants: phylogeny and biogeography of a hyperdiverse ant clade (Hymenoptera: Formicidae). *Syst. Entomol*. **40**, 61–81. (doi:10.1111/syen.12090)
73. 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. B* **280**, 20131733. (doi:10.1098/rspb.2013.1733)
74. Antoine PO *et al.* 2012 Middle Eocene rodents from Peruvian Amazonia reveal the pattern and timing of caviomorph origins and biogeography. *Proc. R. Soc. B* **279**, 1319–1326. (doi:10.1098/rspb.2011.1732)