




Phylogeny, biogeography and classification of Teletisoptera (Blattaria: Isoptera)

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Abstract

Termites are social cockroaches distributed throughout warm temperate and tropical ecosystems. The ancestor of modern termites roamed the earth during the early Cretaceous, suggesting that both vicariance and overseas dispersal may have shaped the distribution of early diverging termites. We investigate the historical biogeography of three early diverging termite families – Stolotermitidae, Hodotermitidae and Archotermopsidae (clade Teletisoptera) – using the nuclear rRNA genes and mitochondrial genomes of 27 samples. Our analyses confirm the monophyly of Teletisoptera, with Stolotermitidae diverging from Hodotermitidae + Archotermopsidae approximately 100 Ma. Although Hodotermitidae are monophyletic, our results demonstrate the paraphyly of Archotermopsidae. Phylogenetic analyses indicate that the timing of divergence among the main lineages of Hodotermitidae + Archotermopsidae are compatible with vicariance. In the Stolotermitidae, however, the common ancestors of modern *Porotermes* Hagen and *Stolotermes* Hagen are roughly as old as 20 and 35 Ma, respectively, indicating that the presence of these genera in South America, Africa and Australia involved over-water dispersals. Overall, our results suggest that early diverging termite lineages acquired their current distribution through a combination of over-water dispersals and dispersal via land bridges. We clarify the classification by resolving the paraphyly of Archotermopsidae, restricting the family to *Archotermopsis* Desneux and *Zootermopsis* Emerson and elevating Hodotermitopsinae (*Hodotermitopsis* Holmgren) as Hodotermitopsidae (*status novum*).

KEYWORDS

Archotermopsidae, Gondwana, historical biogeography, Hodotermitidae, Hodotermitopsidae, insects, Stolotermitidae

INTRODUCTION

Termites are a clade of social cockroaches having a sister relationship with the wood-feeding cockroach genus *Cryptocercus* Scudder (Grimaldi & Engel, 2005; Inward et al., 2007a; Inward et al., 2007b; Lo et al., 2000). The fossil record of termites dates back to the Early Cretaceous, ~130 million years ago (Ma) (Engel et al., 2016; Thorne et al., 2000) and time-calibrated phylogenies suggest that the first termites appeared 140–150 Ma (Bourguignon et al., 2015; Bucek et al., 2019; Engel et al., 2009; Engel et al., 2016; Legendre et al., 2015). Therefore, the origin of termites predates the final stage of the breakup of Pangaea, and early diverging termite lineages may have a distribution based on vicariance through continental drift.

The first divergence among modern termites is that of Mastotermitidae and Euisoptera, the clade composed of all non-mastotermitid termites, 140–150 Ma (Bourguignon et al., 2015; Bucek et al., 2019; Engel et al., 2009; Inward et al., 2007a). Although the only extant species of Mastotermitidae, *Mastotermes darwiniensis* Froggatt, is native to northern Australia, fossils of *Mastotermes* Froggatt have been unearthed in Russia, Mexico, the Dominican Republic, Brazil, Europe, Ethiopia and Myanmar (Bezerra et al., 2020; Engel et al., 2015; Krishna & Emerson, 1983; Krishna & Grimaldi, 1991; Krishna et al., 2013; Vrřanský & Aristov, 2014; Wappler & Engel, 2006; Zhao et al., 2019). Because of the relict distribution of modern *Mastotermes*, molecular-based time-calibrated phylogenies cannot be used to investigate the historical biogeography of the genus. However, the method can be used to study the historical biogeography of representatives of other early diverging termite families with broader extant diversity.

The first divergence within the Euisoptera is the separation of Teletisoptera (Stolotermitidae + Hodotermitidae + Archotermopsidae) from Icoisoptera (Kalotermitidae + Neoisoptera), dated at 130–145 Ma (Bourguignon et al., 2015; Bucek et al., 2019). The most recent common ancestor of the former clade corresponds to the split between Stolotermitidae and Archotermopsidae + Hodotermitidae and was estimated at 80–115 Ma (Bourguignon et al., 2015; Bucek et al., 2019). Therefore, cladogenesis in Stolotermitidae + Hodotermitidae + Archotermopsidae was initiated before the final stage of the breakup of Pangaea, indicating that their current distribution may have been shaped by vicariance through continental drift (Bourguignon et al., 2015). Alternatively, Stolotermitidae + Hodotermitidae + Archotermopsidae may have acquired their modern distribution by dispersal, with extensive extinction of stem-group Teletisoptera. Indeed, several fossils, putatively stem groups to this clade, are known from the mid-Cretaceous (e.g., Arceotermitidae and Krishnatermitidae at 99 Ma; Jiang et al., 2021). A comprehensive phylogeny including samples collected across the range of these three early diverging termite families could help determine whether their modern distribution was shaped primarily by dispersal, vicariance or a combination of these two phenomena.

Extant Stolotermitidae are found in Australia, South Africa, South America and New Zealand, a distribution often interpreted as relict and reflecting an ancient occurrence across Gondwana prior to its initial breakup approximately 100 Ma (Krishna et al., 2013). Modern

Hodotermitidae are distributed across the deserts of Africa, the Middle East and South Asia. This distribution was possibly acquired as arid biomes gradually expanded during the Oligocene and Miocene (Edwards et al., 2010). Finally, the Archotermopsidae have a disjunct distribution across the Northern Hemisphere, with *Archotermopsis* Desneux living at the foothills of the Himalayan region and in the mountains of Vietnam; *Hodotermopsis* Holmgren living in Vietnam, South China and Japan; and *Zootermopsis* Emerson native to the western part of the Nearctic region (Krishna et al., 2013) and introduced to Japan (Yashiro et al., 2018). Although the fossil record of the three families is more fragmentary than that of Mastotermitidae, most of these fossils indicate that the families once enjoyed a broader distribution. For example, the genus *Chilgatermes* Engel, Pan & Jacobs from Oligocene deposits of Ethiopia is a relative of Porotermitinae (Stolotermitidae) (Engel et al., 2013), whereas *Termopsis* Heer (of the extinct family Termopsidae) is found in middle Eocene Baltic amber (Engel et al., 2007; Krishna et al., 2013). Similarly, the extinct Archotermopsid genus *Gyatermes* Engel & Gross is known from a variety of fossil deposits in Europe and Asia (Engel & Gross, 2009; Engel & Tanaka, 2015; Krishna et al., 2013). Additionally, various extinct genera from the Cretaceous are putatively stem groups to the Teletisoptera, such as *Arceotermes* Engel & Jiang and *Cosmotermes* Zhao, Yin, Shih & Ren from the 99 Ma Kachin amber (Arceotermitidae; Jiang et al., 2021; Zhao et al., 2019) and *Cretatermes* Emerson from 95 Ma deposits in Labrador (Emerson, 1967). Thus, the historical biogeography of Teletisoptera may be more intricate than previously acknowledged.

The classification of the lineages composing Teletisoptera has changed considerably over the last century (Table 1). The classification was stable for the 60 years following the elevation of the so-called dampwood termites to family rank as Termopsidae and as more formally distinct from the harvesters of the Hodotermitidae s.str. (= Hodotermitinae sensu Emerson, 1942) by Grassé (1949). However, morphological and paleontological phylogeny prompted Engel et al. (2009) to reconsider the classification of Teletisoptera. In their analysis, *Termopsis* was recovered as unrelated to the modern genera of “Termopsidae”, necessitating the removal of the extant diversity to the Archotermopsidae and Stolotermitidae, whereas most recently Jiang et al. (2021) separated *Hodotermopsis* into a monogeneric subfamily, Hodotermopsinae.

Although the historical biogeography of Neoisoptera and Kalotermitidae has been studied in detail (Bourguignon et al., 2016, 2017; Bucek et al., 2021; Romero Arias et al., 2021; Wang et al., 2019), only a few species of Stolotermitidae, Hodotermitidae and Archotermopsidae have been included in previous termite phylogenies. In this study, we carried out a representative sampling of species belonging to these three families. We obtained the nuclear ribosomal RNA genes (5S, 5.8S, 18S and 28S) and mitochondrial genomes of 27 samples collected across the distribution of the group. We used this dataset to reconstruct time-calibrated phylogenies, clarify the classification and shed light on the historical biogeography of these early diverging termite lineages.

TABLE 1 Comparison of different classifications of extant basal Euisoptera

Holmgren (1911)	Emerson (1942), Snyder (1949), Krishna (1970)	Grassé (1949), Weidner (1955), Engel and Krishna (2004)	Engel et al. (2009, 2016), Krishna et al. (2013)	Jiang et al. (2021)	Herein
PROTERMITIDAE ^a	HODOTERMITIDAE	HODOTERMITIDAE	HODOTERMITIDAE	HODOTERMITIDAE	HODOTERMITIDAE
Hodotermitinae	Hodotermitinae	<i>Anacanthotermes</i>	<i>Anacanthotermes</i>	<i>Anacanthotermes</i>	<i>Anacanthotermes</i>
<i>Archotermopsis</i>	<i>Anacanthotermes</i>	<i>Microhodotermes</i>	<i>Microhodotermes</i>	<i>Microhodotermes</i>	<i>Microhodotermes</i>
<i>Hodotermes</i> ^b	<i>Microhodotermes</i>	<i>Hodotermes</i>	<i>Hodotermes</i>	<i>Hodotermes</i>	<i>Hodotermes</i>
Stolotermitinae	<i>Hodotermes</i>	TERMOPSIDAE	ARCHOTERMOPSIDAE	ARCHOTERMOPSIDAE	HODOTERMOPSIDAE
<i>Stolotermes</i>	Termopsinae	<i>Hodotermopsis</i>	<i>Hodotermopsis</i>	Hodotermopsinae	<i>Hodotermopsis</i>
Calotermitinae ^c	<i>Hodotermopsis</i>	<i>Archotermopsis</i>	<i>Archotermopsis</i>	<i>Hodotermopsis</i>	ARCHOTERMOPSIDAE
<i>Porotermes</i>	<i>Archotermopsis</i>	<i>Zootermopsis</i>	<i>Zootermopsis</i>	Archotermopsinae	<i>Archotermopsis</i>
	<i>Zootermopsis</i>	Porotermitinae	STOLOTERMITIDAE	<i>Archotermopsis</i>	<i>Zootermopsis</i>
	Porotermitinae	<i>Porotermes</i>	Porotermitinae	<i>Zootermopsis</i>	STOLOTERMITIDAE
	<i>Porotermes</i>	Stolotermitinae	<i>Porotermes</i>	STOLOTERMITIDAE	Porotermitinae
	Stolotermitinae	<i>Stolotermes</i>	Stolotermitinae	Porotermitinae	<i>Porotermes</i>
	<i>Stolotermes</i>		<i>Stolotermes</i>	<i>Porotermes</i>	Stolotermitinae
				Stolotermitinae	<i>Stolotermes</i>
				<i>Stolotermes</i>	

Note: Fossil representatives are not covered here but are largely summarized by Krishna et al. (2013), Barden and Engel (2021) and Jiang et al. (2021). Families boldfaced in small caps, and genera colour coded by clades.

^aHolmgren's (1911) Protermitidae also included Mastotermitinae, not covered herein.

^bHolmgren (1911) included *Anacanthotermes* as a subgenus of *Hodotermes*.

^cHolmgren (1911) also included in this subfamily *Calotermes* (= *Kalotermes* s.l., or what today is recognized as Kalotermitidae).

MATERIALS AND METHODS

Sampling and sequencing

We sequenced five samples of Stolotermitidae, five samples of Archotermopsidae and six samples of Hodotermitidae. In addition to these 16 samples, we also sequenced 32 termite species belonging to other families that we used as outgroups, including 15 species of Termitidae, 10 species of Rhinotermitidae and seven species of Kalotermitidae. We combined these sequences with previously published mitochondrial genomes of four species of Stolotermitidae, five species of Archotermopsidae, two species of Hodotermitidae, two samples of *M. darwiniensis*, two species of Termitidae and one species of Cryptocercidae. Our final dataset comprised sequence data for 64 termite species and one nontermite cockroach species, *Cryptocercus kyebangensis* Grandcolas (Table S1).

Termites samples used in this study were collected during the last 30 years by the authors and Prof Rudolph Scheffrahn from the University of Florida. Voucher samples are stored in the University of Florida or in one of the institutions of the authors (Table S1). Subsamples were shipped to the Okinawa Institute of Science and Technology and stored at -20°C until DNA extraction. Whole genomic DNA was extracted with the DNeasy Blood & Tissue Kit using complete individuals, including guts. The concentration of DNA was measured with Qubit 3.0 fluorometer and adjusted to a concentration of $0.5\text{ ng}/\mu\text{l}$. The library of each sample was prepared separately with the NEBNext Ultra II FS

DNA Library Preparation Kit and the Unique Dual Indexing Kit (New England Biolabs, Ipswich, MA), with reagent volumes reduced to one-fifteenth of that advised by the manufacturer. We retained the enzymatic fragmentation step during library preparation for the few samples collected for genomic analyses and preserved them in RNA-later at -20 or -80°C until DNA extraction. However, most samples were collected over the past decades in alcohol and stored at room temperature for taxonomic purposes. Because the DNA of these samples was typically highly fragmented, we prepared libraries without the enzymatic fragmentation step using NEBNext Ultra II End Repair/dA-Tailing Kit. Libraries were pooled together and paired-end sequenced with the Illumina sequencing platform at a read length of 150 bp.

Assembly and alignment

We checked read quality using FASTP v0.20.1 (Chen et al., 2018). Read adapters and poly-G tails at the end of the reads were trimmed. Filtered reads were assembled using METASPADES v3.13.0 (Nurk et al., 2017). The Nuclear ribosomal RNA genes (5S, 5.8S, 18S and 28S) were predicted from assemblies using BARRNAP v0.9 (Seemann, 2013). Mitochondrial genomes were retrieved and annotated using MITOFINDER v1.4 (Allio et al., 2020). All genes were aligned separately using MAFFT v7.305 (Katoh & Standley, 2013). We obtained the 13 mitochondrial protein-coding genes using the transeq command of the EMBOSS v6.6.0 suite of programs (Rice et al., 2000) and carried out sequence alignment on

were excluded as burn-in. Visual inspection of the trace files with Tracer v1.7.1 confirmed that all analyses converged (Rambaut et al., 2018). The effective sample size was higher than 220 for every parameter of every run. The MCMC chains were run four times in parallel for both datasets.

Divergence time estimation

We reconstructed Bayesian time-calibrated phylogenies using BEAST v2.6.2 (Bouckaert et al., 2019). Bayesian analyses were performed with and without the third codon positions of protein-coding genes.

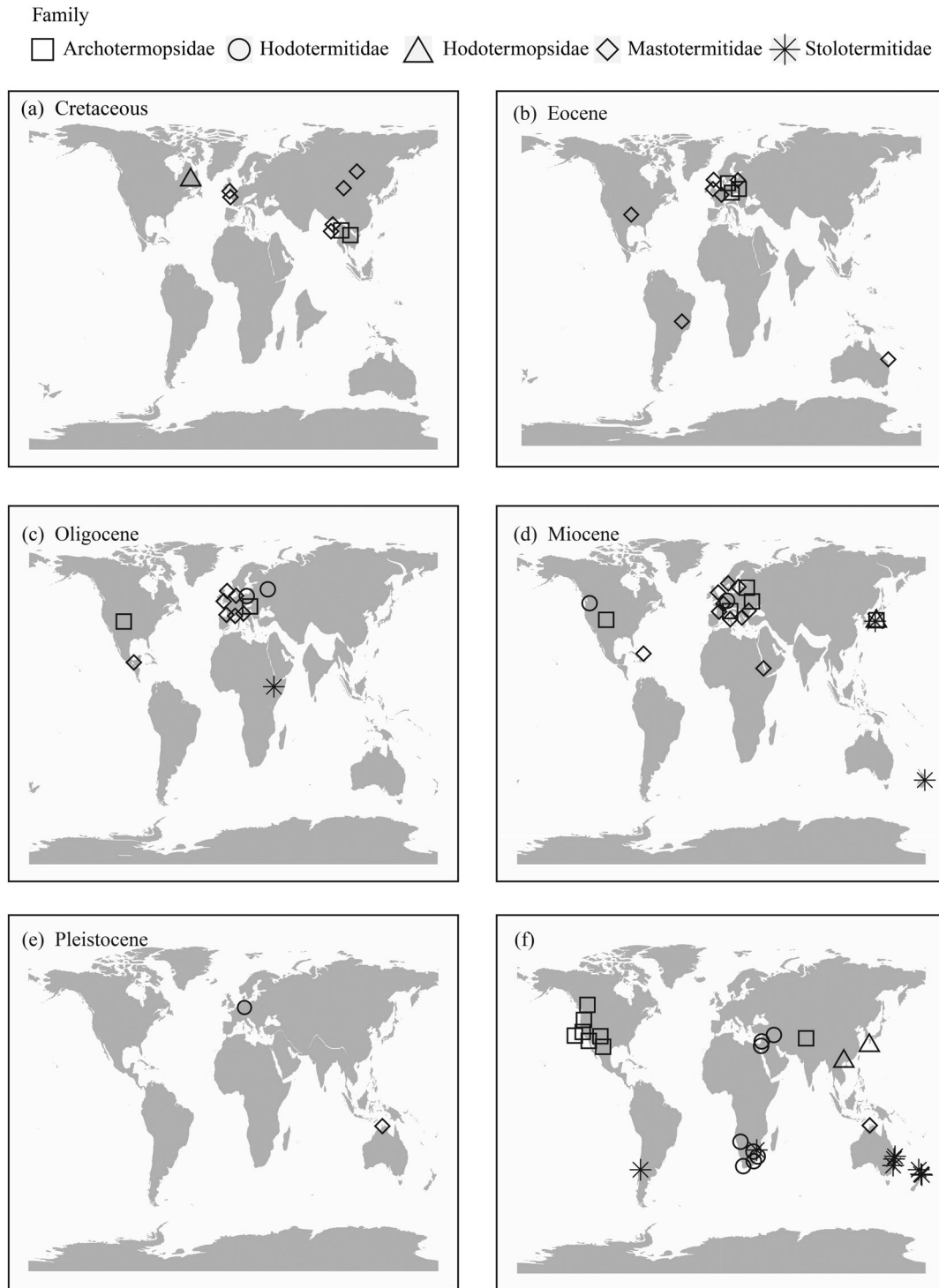


FIGURE 2 Maps showing the distribution of known fossils of Mastotermitidae, Hodotermitidae, Archotermopsidae, Hodotermopsidae and Stolotermitidae during the (a) Cretaceous, (b) Eocene, (c) Oligocene, (d) Miocene, (e) Pleistocene and (f) the sampling locations of the specimens used in this study. Paleogeographic maps were generated using the R package “mapast.”

We used an uncorrelated lognormal relaxed clock to model rate variation among branches. A Yule model was used as tree prior. A GTR + G model of nucleotide substitution was applied to each partition. The MCMC analyses were run for 250 million generations for the analyses with and without third codon positions. The chains were sampled every 5000 generations. We checked the convergence of the MCMC runs with TRACER v1.7.1 and consequently discarded the first 20% of generations as burn-in. We used 10 fossils as time constraints (Table S2). Each calibration was implemented as an exponential prior on node time. The use of these calibrations has been thoroughly justified previously (Bucek et al., 2019, 2021). We used TreeAnnotator implemented in the BEAST2 suite of programs to generate a consensus tree. Tree topology and 95% height posterior density (HPD) were visualized with FIGTREE v 1.4.4 (Rambaut, 2018).

RESULTS

Phylogenetic reconstructions

The phylogenetic trees obtained using Maximum Likelihood and Bayesian analyses received high nodal support values and possessed almost identical topologies (Figure 1). Our analyses retrieved Mastotermitidae as sister group to Euisoptera, which comprised all nonmastotermitid termites, and confirmed the monophyly of Stolotermitidae + Archotermopsidae + Hodotermitidae (Teletisoptera), which was retrieved as the sister group of Kalotermitidae + Neoisoptera (Icoisoptera). Stolotermitidae was found to be monophyletic and formed the sister group of Archotermopsidae + Hodotermitidae. The Archotermopsidae were retrieved as paraphyletic with respect to a monophyletic Hodotermitidae. Within the lineage composed of Archotermopsidae and Hodotermitidae, *Hodotermopsis* (Hodotermopsinae) was sister to the other five genera. *Zootermopsis* and *Archotermopsis* formed a monophyletic group sister to the three genera of Hodotermitidae (i.e., Archotermopsidae would be monophyletic with the removal of Hodotermopsinae). Within the Hodotermitidae, *Anacanthotermes* Jacobson was found to be sister to *Hodotermes* Hagen + *Microhodotermes* Sjöstedt. Each of the eight genera studied here were monophyletic.

Divergence dating

Time-calibrated phylogenies reconstructed with and without the third codon positions of protein-coding genes yielded similar time estimates, differing by less than 4 Ma for each node. For this reason, we only provide the results of the analysis with the third codon position excluded (Figure 1). The clade Teletisoptera diverged from other Euisoptera 117.9 Ma (106.0–131.8 Ma, 95% HPD). Stolotermitidae diverged from Hodotermitidae + Archotermopsidae 98.6 Ma (94.3–106.5 Ma, 95% HPD). The most recent common ancestor of Stolotermitidae occurred around 70.0 Ma (53.8–85.5 Ma, 95% HPD), and the most recent common ancestors of *Porotermes* Hagen and *Stolotermes* Hagen were estimated to have existed 20.2 Ma

(14.1–27.1 Ma, 95% HPD) and 35.0 Ma (25.5–45.2 Ma, 95% HPD), respectively. *Hodotermopsis* and other Archotermopsidae + Hodotermitidae diverged 90.1 Ma (82.1–100.5 Ma, 95% HPD). The divergence time of *Zootermopsis* and *Archotermopsis* was estimated to have occurred 48.2 Ma (35.8–60.1 Ma, 95% HPD), and the most recent common ancestor of *Zootermopsis* was estimated at 19.7 Ma (13.4–26.2 Ma, 95% HPD). Hodotermitidae diverged from *Zootermopsis* + *Archotermopsis* 82.3 Ma (72.0–92.7 Ma, 95% HPD). Within the Hodotermitidae, *Hodotermes* + *Microhodotermes* diverged from *Anacanthotermes* 31.4 Ma (22.5–41.3 Ma, 95% HPD). *Hodotermes* and *Microhodotermes* split 18.7 Ma (12.0–25.6 Ma, 95% HPD).

DISCUSSION

In this study, we present a comprehensive phylogenetic reconstruction of the early diverging termite families Stolotermitidae, Archotermopsidae and Hodotermitidae (Figures 1 and S1). We used three phylogenetic reconstruction methods and repeated the analyses on datasets with and without third codon positions of protein-coding genes. The topology of the phylogenetic trees of Teletisoptera was identical across methods and datasets. Our phylogenies were also congruent with previous estimates based on mitochondrial genomes and transcriptomes (Bourguignon et al., 2015; Bucek et al., 2019; Cameron et al., 2012). *Mastotermes* was found to be the sister group of Euisoptera and Teletisoptera was sister to Kalotermitidae + Neoisoptera (Icoisoptera). Our analyses supported the monophyly of Stolotermitidae, which was found to be sister to Archotermopsidae + Hodotermitidae, the former being paraphyletic to the latter. The paraphyly of Archotermopsidae was already indicated by previous phylogenies based on full mitochondrial genomes (Bourguignon et al., 2015). It is clear that a simple augmentation of the current classification by removing *Hodotermopsis* from Archotermopsidae resolves this paraphyly, although simultaneously maximizing nomenclatural stability with the literature of the last 70 years (i.e., maintaining Grassé's distinction between a family of harvesters and dampwood termites). Accordingly, we restrict Archotermopsidae to *Archotermopsis* and *Zootermopsis* (i.e., Archotermopsinae sensu Jiang et al. (2021) elevated as Archotermopsidae Engel et al., stat.n.), and elevate Hodotermopsinae to familial rank (i.e., Hodotermopsidae Engel, stat.n.). Although this system is finely split, it is preferable to obscuring the biological differences and confusing the historical literature that has deployed these names, particularly Hodotermitidae, in such a context since Grassé (1949). The alternatives would be (1) recognizing all of the aforementioned families as subfamilies of Hodotermitidae (semantically equivalent to the multi-family system), or (2) to recognize two families, Stolotermitidae and Hodotermitidae, the former with Stolotermitinae and Porotermitinae and the latter with Hodotermitinae, Archotermopsinae and Hodotermopsinae. Neither of these alternatives maximizes nomenclatural stability in the sense of the ICZN (1999), nor do they provide any greater clarity regarding relationships. Accordingly, the system we adopt (Table 1) emphasizes the ecological differences between the taxonomic units, with all Archotermopsidae and Hodotermopsidae feeding on damp wood (usually coniferous), although all Hodotermitidae are desert

harvester termites feeding predominantly on dry grasses (Krishna et al., 2013). In the remainder of this section, we shall refer to the families in this new context.

The time-calibrated trees estimated with and without third codon positions of protein-coding genes yielded similar time estimates. Our time estimates of the branching among early diverging termite families were largely congruent with the estimates obtained with time-calibrated trees reconstructed using node-dating approaches. For example, we estimated the most recent common ancestor of termites at 143.2 Ma (125.5–163.3 Ma, 95% HPD), whereas previous studies estimated crown termites at 149 Ma (136–170 Ma, 95% HPD) (Bourguignon et al., 2015), 151.3 Ma (149.3–153.7 Ma, 95% HPD) (Legendre et al., 2015) and 140.6 Ma (112.6–170.5 Ma, 95% HPD) (Bucek et al., 2019). In contrast, tip-dating analyses estimated crown termites at 205 Ma (171–234 Ma, 95% HPD) (Jouault et al., 2021) and Ware et al. (2010) carried out multiple analyses yielding age estimates in excess of 200 Ma for crown termites. Differences among studies in terms of fossil calibrations, fossil age estimations, taxonomic sampling, methods and models used for the reconstruction of time-calibrated trees may be the causes of this variation. In any case, the absence of termite fossils older than ~130 Ma suggest that tip-dating approaches overestimate the age of termites.

We did not attempt to reconstruct the ancestral range of Stolotermitidae + Hodotermopsidae + Archotermopsidae + Hodotermitidae, particularly given that the many fossils occurring well outside of modern distributions would render meaningless such an estimate based solely on extant taxa. Ancestral range reconstructions have been performed previously for Neoisoptera and Kalotermitidae (Bourguignon et al., 2016, 2017; Bucek et al., 2021; Romero Arias et al., 2021; Wang et al., 2019). However, compared to Teletisoptera, Neoisoptera and Kalotermitidae are diverse and widespread, comprising many extant species whose distribution and phylogenetic relationships can inform on past vicariance and dispersal events, and with most fossils nested within those distributions (Krishna et al., 2013). Stolotermitidae, Hodotermopsidae, Archotermopsidae and Hodotermitidae are species-poor families, with limited modern distributions, relict of past wider distributions as evidenced from the fossil record (Figure 2) (Engel et al., 2013, 2016; Jiang et al., 2021; Krishna et al., 2013). Most geographic lineages of Teletisoptera inhabit regions hosting few other termites and may have been competitively excluded from regions where termitids and other Neoisoptera became dominant during the Oligocene and Miocene (Bourguignon et al., 2017; Engel et al., 2009). Teletisoptera inhabit regions generally devoid of other Teletisoptera, preventing a meaningful reconstruction of its historical biogeography.

Although the low diversity of teletisopteran families hampers meaningful ancestral range reconstructions, our time-calibrated trees permit the identification of several biogeographic disjunctions. The two modern stolotermitid genera, *Porotermes* and *Stolotermes*, have a Gondwanan distribution (Emerson, 1942, 1955; Gay & Calaby, 1969; Kaulfuss et al., 2010; Krishna et al., 2013). However, our time-

calibrated phylogeny indicated that all species of *Porotermes* share a common ancestor 20.2 Ma (14.1–27.1 Ma, 95% HPD) and the common ancestor of the species of *Stolotermes* sequenced in this study lived 35.0 Ma (25.5–45.2 Ma, 95% HPD), both considerably younger than the breakup of Gondwana. Although we could not sequence *Stolotermes africanus* Emerson, the only species of *Stolotermes* found in Africa, our time-calibrated trees showed that *Stolotermes* diverged from *Porotermes* 70.0 Ma (53.8–85.5 Ma, 95% HPD), after the breakup of Gondwana. Interestingly, an extinct genus allied to *Porotermes* is known from the Oligocene of Ethiopia (Engel et al., 2013), predating the divergence of crown-group *Porotermes* but postdating the divergence of the lineages comprising Porotermitinae and Solotermitinae. Collectively, these results imply that the presence of *Stolotermes* in South Africa, eastern Australia as well as New Zealand and the presence of *Porotermes* in southern Australia, southern Africa and South America is not the result of vicariance during the breakup of Gondwana, as hypothesized previously (Bourguignon et al., 2015; Krishna et al., 2013). Instead, *Porotermes* and *Stolotermes* acquired their modern distribution through long-distance oversea dispersal events.

The biogeographic disjunctions among modern genera of Hodotermopsidae + Archotermopsidae + Hodotermitidae may be explained by land bridges. Indeed, we estimated that Hodotermopsidae + Archotermopsidae + Hodotermitidae shared a common ancestor around 90.1 Ma (82.1–100.5 Ma, 95% HPD), indicating vicariance through continental drift may explain the distribution of early diverging members of this clade. The Palearctic region remained connected to North America through Greenland until about 50 Ma (Scotese, 2004), possibly explaining the disjunction between the Palearctic *Archotermopsis* and the Nearctic *Zootermopsis*, the modern descendants of more widespread ancestors (Krishna et al., 2013). The African *Hodotermes* + *Microhodotermes* diverged from *Anacanthotermes*, a genus found in Africa, the Middle East, and South Asia, 31.4 Ma (22.5–41.3 Ma, 95% HPD) and the most recent common ancestors of *Hodotermes* + *Microhodotermes* and *Anacanthotermes* lived 19.0 Ma (12.0–25.6 Ma, 95% HPD) and 10.4 Ma (6.0–15.0 Ma, 95% HPD), respectively. The timing of the biogeographic disjunction between these two lineages may coincide with the existence of the *Gomphotherium* land bridge that connected Africa and Eurasia 18–20 Ma (Rögl, 1998, 1999). The sequencing of African *Anacanthotermes* in future studies is needed to confirm this scenario.

Our study showcases the importance of samples collected before the genomics era for future phylogenetic reconstructions. One limitation of many studies attempting to reconstruct the evolution of diverse taxa is the sampling of a representative set of specimens covering the diversity of the groups of interest. Because species of Stolotermitidae, Hodotermopsidae, Archotermopsidae and Hodotermitidae occur in regions where termite diversity is generally low, we made fewer attempts to collect them. Instead, this study is largely based on samples collected in ethanol during the last three decades for taxonomic purposes. In addition, we

sequenced a syntype of *Archotermopsis wroughtoni* (Desneux), which was collected in the Kashmir Valley. The systematic sequencing of type material, such as a syntype of *A. wroughtoni* sequenced in this study, holds the promise of clarifying the taxonomic literature and making available type-based species identification to the whole scientific community.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Information on the accessibility of samples and the accession numbers of sequences used in this study are provided in Table S1.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Figure S1 Time-calibrated phylogenetic tree of 64 termite samples reconstructed with BEAST2 using mitochondrial genomes without third codon positions and 5S, 5.8S, 18S and 28S rRNA genes. Node bars indicate the 95% height posterior density intervals. Branch labels represent posterior probabilities.

Table S1 Samples used in this study with corresponding collection details and accession numbers.

Table S2 Fossils used for time calibration in this study.

Table S3 Substitution models used for phylogenetic reconstruction in this study.

Appendix S1 Supporting Information

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