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

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30

31 **Abstract**

32 Termites are social cockroaches distributed throughout warm temperate and tropical ecosystems.

33 The ancestor of modern termites roamed the earth during the early Cretaceous, suggesting that

34 both vicariance and overseas dispersal may have shaped the distribution of early diverging termites.

35 We reconstruct the historical biogeography of three early diverging termite families –

36 Stolotermitidae, Hodotermitidae, and Archotermopsidae (clade Teletisoptera)– using the nuclear

37 rRNA genes and mitochondrial genomes of 27 samples. Our analyses confirm the monophyly of

38 Teletisoptera, with Stolotermitidae diverging from Hodotermitidae + Archotermopsidae

39 approximately 100 Ma. While Hodotermitidae is monophyletic, our results demonstrate the

40 paraphyly of Archotermopsidae. Phylogenetic analyses indicate that the timing of divergence

41 among the main lineages of Hodotermitidae + Archotermopsidae are compatible with vicariance.

42 In the Stolotermitidae, however, the common ancestors of modern *Porotermes* Hagen and

43 *Stolotermes* Hagen are roughly as old as 20 and 35 Ma respectively, indicating that the presence

44 of these genera in South America, Africa, and Australia involved over-water dispersals. Overall,

45 our results suggest that early diverging termite lineages acquired their current distribution through

46 a combination of over-water dispersals and dispersal via land bridges. We clarify the classification

47 by resolving the paraphyly of Archotermopsidae, restricting the family to *Archotermopsis* Desneux

48 and *Zootermopsis* Emerson and elevating Hodotermopsinae (*Hodotermopsis* Holmgren) as

49 Hodotermopsidae (*status novum*).

50

1 Introduction

Termites are a clade of social cockroaches having a sister relationship with the wood-feeding cockroach genus *Cryptocercus* Scudder (Lo *et al.*, 2000; Grimaldi & Engel 2005; Inward *et al.*, 2007a, 2007b). The fossil record of termites dates back to the Early Cretaceous, ~130 Ma (Thorne *et al.* 2000; Engel *et al.* 2016), and time-calibrated phylogenies suggest that the first termites appeared 140–150 million years ago (Ma) (Engel *et al.*, 2009; Legendre *et al.*, 2015; Bourguignon *et al.*, 2015; Engel *et al.*, 2016; Bucek *et al.*, 2019). 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 amongst modern termites is that of Mastotermitidae and Euisoptera, the clade composed of all non-mastotermitid termites, 140–150 Ma (Inward *et al.*, 2007a; Engel *et al.*, 2009; Bourguignon *et al.*, 2015; Bucek *et al.*, 2019). While 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 (Krishna & Emerson 1983; Krishna & Grimaldi 1991; Wappler & Engel 2006; Krishna *et al.*, 2013; Vršanský & Aristov 2014; Engel *et al.*, 2015; Zhao *et al.*, 2020; Bezerra *et al.*, 2020). 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

81 fossils, putatively stem groups to this clade, are known from the mid-Cretaceous (*e.g.*,

82 Arceotermitidae and Krishnatermitidae at 99 Ma: Jiang *et al.*, 2021). A comprehensive phylogeny

83 including samples collected across the range of these three early diverging termite families could

84 help determine whether their modern distribution was shaped primarily by dispersal, vicariance,

85 or a combination of these two phenomena.

86 Extant Stolotermitidae are found in Australia, South Africa, South America, and New Zealand, a

87 distribution often interpreted as relict and reflecting an ancient occurrence across Gondwana prior

88 to its initial breakup approximately 100 Ma (Krishna *et al.*, 2013). Modern Hodotermitidae are

89 distributed across the deserts of Africa, the Middle East, and South Asia. This distribution was

90 possibly acquired as arid biomes gradually expanded during the Oligocene and Miocene (Edwards

91 *et al.*, 2010). Finally, the Archotermopsidae have a disjunct distribution across the Northern

92 Hemisphere, with *Archotermopsis* Desneux living at the foothills of the Himalayan region and in

93 mountains of Vietnam; *Hodotermopsis* Holmgren living in Vietnam, South China, and Japan; and

94 *Zootermopsis* Emerson native to the western part of the Nearctic region (Krishna *et al.*, 2013) and

95 introduced to Japan (Yashiro *et al.*, 2018). While the fossil record of the three families is more

96 fragmentary than that of Mastotermitidae, most of these fossils indicate that the families once

97 enjoyed a broader distribution. For example, the genus *Chilgatermes* Engel, Pan & Jacobs from

98 Oligocene deposits of Ethiopia is a relative of Porotermitinae (Stolotermitidae) (Engel *et al.*, 2013),

99 while *Termopsis* Heer (of the extinct family Termopsidae) is found in middle Eocene Baltic amber

100 (Engel *et al.*, 2007; Krishna *et al.*, 2013). Similarly, the extinct archotermopsid genus *Gyatermes*

101 Engel & Gross is known from a variety of fossil deposits in Europe and Asia (Engel & Gross 2009;

102 Krishna *et al.*, 2013; Engel & Tanaka 2015). Additionally, various extinct genera from the

103 Cretaceous are putatively stem groups to the Teletisoptera, such as *Arceotermes* Engel & Jiang

104 and *Cosmotermes* Zhao, Yin, Shih & Ren from the 99 Ma Kachin amber (Arceotermitidae: Jiang

105 *et al.*, 2021, Zhao *et al.*, 2020) and *Cretatermes* Emerson from 95 Ma deposits in Labrador

106 (Emerson 1967). Thus, the historical biogeography of Teletisoptera may be more intricate than

107 previously acknowledged.

108 The classification of the lineages composing Teletisoptera has changed considerably over the last

109 century (Table 1). The classification was stable for the 60 years following the elevation of the so-

110 called dampwood termites to family rank as Termopsidae and as more formally distinct from the

111 harvesters of the Hodotermitidae s.str. (= Hodotermitinae sensu Emerson, 1942) by Grassé (1949).

112 However, morphological and paleontological phylogeny prompted Engel et al. (2009) to
113 reconsider the classification of Teletisoptera. In their analysis, *Termopsis* was recovered as
114 unrelated to the modern genera of "Termopsidae", necessitating the removal of the extant diversity
115 to the Archotermopsidae and Stolotermitidae, while most recently Jiang *et al.* (2021) separated
116 *Hodotermopsis* into a monogeneric subfamily, Hodotermopsinae.

117 While the historical biogeography of Neoisoptera and Kalotermitidae has been studied in detail
118 (Bourguignon *et al.*, 2016, 2017; Wang *et al.*, 2019; Romero Arias *et al.*, 2021; Bucek *et al.*, 2021),
119 only a few species of Stolotermitidae, Hodotermitidae, and Archotermopsidae have been included
120 in previous termite phylogenies. In this paper, we carried out a representative sampling of species
121 belonging to these three families. We obtained the nuclear ribosomal RNA genes (5S, 5.8S, 18S,
122 28S) and mitochondrial genomes of 27 samples collected across the distribution of the group. We
123 used this dataset to reconstruct time-calibrated phylogenies, clarify the classification, and shed
124 light on the historical biogeography of these early diverging termite lineages.

125

126 **2 Materials and Methods**

127 **2.1 Sampling and sequencing**

128 We sequenced five samples of Stolotermitidae, five samples of Archotermopsidae, and six samples
129 of Hodotermitidae. In addition to these 16 samples, we also sequenced 32 termite species
130 belonging to other families that we used as outgroups, including 15 species of Termitidae, 10
131 species of Rhinotermitidae, seven species of Kalotermitidae. We combined these sequences with
132 previously published mitochondrial genomes of four species of Stolotermitidae, five species of
133 Archotermopsidae, two species of Hodotermitidae, two samples of *M. darwiniensis*, two species
134 of Termitidae, and one species of Cryptocercidae. Our final dataset comprised sequence data for
135 64 termite species and one non-termite cockroach species, *Cryptocercus kyebangensis* Grandcolas
136 (Table S1).

137 Termites samples used in this study were collected during the last 30 years by the authors and Prof.
138 Rudolph Scheffrahn from the University of Florida. Voucher samples are stored in the University
139 of Florida or in one of the institutions of the authors (Table S1). Subsamples were shipped to the
140 Okinawa Institute of Science and Technology and stored at -20 ˚C until DNA extraction. Whole

141   genomic DNA was extracted with the DNeasy Blood & Tissue kit using complete individuals,

142   including guts. The concentration of DNA was measured with Qubit 3.0 fluorometer and adjusted

143   to a concentration of 0.5 ng/μl. The library of each sample was prepared separately with the

144   NEBNext® Ultra ™ II FS DNA Library Preparation Kit and the Unique Dual Indexing kit (New

145   England Biolabs), with reagent volumes reduced to one-fifteenth of that advised by the

146   manufacturer. We retained the enzymatic fragmentation step during library preparation for the few

147   samples collected for genomic analyzes and preserved in RNA-later® at -20°C or -80°C until

148   DNA extraction. However, most samples were collected over the past decades in alcohol and

149   stored at room temperature for taxonomic purposes. Because the DNA of these samples was

150   typically highly fragmented, we prepared libraries without the enzymatic fragmentation step using

151   NEBNext® Ultra ™ II End Repair/dA-Tailing kit. Libraries were pooled together and paired-end

152   sequenced with the Illumina sequencing platform at a read length of 150 bp.

153

154   **2.2 Assembly and Alignment**

155   We checked read quality using Fastp v0.20.1 (Chen *et al.*, 2018). Read adapters and poly-G tails

156   at the end of the reads were trimmed. Filtered reads were assembled using MetaSPAdes v3.13.0

157   (Nurk *et al.*, 2017). The Nuclear ribosomal RNA genes (5S, 5.8S, 18S, and 28S) were predicted

158   from assemblies using Barrnap v0.9 (Seemann 2013). Mitochondrial genomes were retrieved and

159   annotated using MitoFinder v1.4 (Allio *et al.*, 2020). All genes were aligned separately using Mafft

160   v7.305 (Katoh & Standley 2013). We obtained the 13 mitochondrial protein-coding genes using

161   the transeq command of the EMBOSS v6.6.0 suite of programs (Rice *et al.*, 2000) and carried out

162   sequence alignment on the amino acid sequences. Amino acid sequence alignments were converted

163   into DNA sequence alignments using PAL2NAL v14 (Suyama *et al.*, 2006). Individual gene

164   alignments were concatenated using FASconCAT-G (Kück & Longo 2014). The 22 mitochondrial

165   transfer RNA genes, two ribosomal RNA genes (12S and 16S), and the six ribosomal RNA genes

166   (mitochondrial 12S and 16S and nuclear 5S, 5.8S, 18S, and 28S) were aligned as DNA sequences,

167   separately.

168

169   **2.3 Phylogenetic analyses**

170   All phylogenetic analyses were performed with and without the third codon positions of protein-
171   coding genes. We reconstructed Maximum Likelihood phylogenetic trees using IQ-TREE 1.6.12
172   (Minh *et al.*, 2020). The best-fit partition scheme and nucleotide substitution model was
173   determined with ModelFinder (Kalyaanamoorthy *et al.*, 2017) implemented in IQ-TREE v1.6.12
174   (Table S3). Branch supports were calculated using 1,000 bootstrap replicates (Hoang *et al.*, 2018).
175   Bayesian phylogenetic trees were inferred with MrBayes v3.2.3 using the GTR+G model of
176   nucleotide substitution (Ronquist *et al.*, 2012). Substitution models were unlinked during all the
177   analyses. The Markov chain Monte Carlo (MCMC) chains were run for 20 million generations for
178   the datasets with or without the third codon positions of protein-coding genes. In all analyses, the
179   MCMC chains were sampled every 5,000 generations to estimate the posterior distribution. The
180   first 10% of sampled trees were excluded as burn-in. Visual inspection of the trace files with Tracer
181   v1.7.1 confirmed that all analyses converged (Rambaut *et al.*, 2018). The effective sample size
182   was higher than 220 for every parameter of every run. The MCMC chains were run four times in
183   parallel for both datasets.

184

185   **2.4 Divergence time estimation**

186   We reconstructed Bayesian time-calibrated phylogenies using BEAST v2.6.2 (Bouckaert *et al.*,
187   2019). Bayesian analyses were performed with and without the third codon positions of protein-
188   coding genes. We used an uncorrelated lognormal relaxed clock to model rate variation among
189   branches. A Yule model was used as tree prior. A GTR+G model of nucleotide substitution was
190   applied to each partition. The MCMC analyses were run for 250 million generations for the
191   analyses without and with third codon positions. The chains were sampled every 5,000 generations.
192   We checked the convergence of the MCMC runs with Tracer v1.7.1 and consequently discarded
193   the first 20% of generations as burn-in. We used ten fossils as time constraints (Table S2). Each
194   calibration was implemented as an exponential prior on node time. The use of these calibrations
195   has been thoroughly justified previously (Bucek *et al.*, 2019, 2021). We used TreeAnnotator
196   implemented in the BEAST2 suite of programs to generate a consensus tree. Tree topology and
197   95% height posterior density (HPD) were visualized with FigTree v 1.4.4 (Rambaut 2018).

198

199   **3 Results**

3.1 Phylogenetic reconstructions

The phylogenetic trees obtained using Maximum Likelihood and Bayesian analyses received high nodal support values and possessed almost identical topologies (Fig. 1). Our analyses retrieved Mastotermitidae as sister group to Euisoptera, which comprised all non-mastotermitid 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* Sjostedt. Each of the eight genera studied here were monophyletic.

3.2 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 four million years for each node. For this reason, we only provide the results of the analysis with the third codon position excluded (Fig. 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

230   (72.0–92.7 Ma, 95% HPD). Within the Hodotermitidae, *Hodotermes* + *Microhodotermes* diverged

231   from *Anacanthotermes* 31.4 Ma (22.5–41.3 Ma, 95% HPD). *Hodotermes* and *Microhodotermes*

232   split 18.7 Ma (12.0–25.6 Ma, 95% HPD).

233

4 Discussion

235   In this study, we present a comprehensive phylogenetic reconstruction of the early diverging

236   termite families Stolotermitidae, Archotermopsidae, and Hodotermitidae (Figs 1, S1). We used

237   three phylogenetic reconstruction methods and repeated the analyses on datasets with and without

238   third codon positions of protein-coding genes. The topology of the phylogenetic trees of

239   Teletisoptera was identical across methods and datasets. Our phylogenies were also congruent with

240   previous estimates based on mitochondrial genomes and transcriptomes (Cameron *et al.*, 2012;

241   Bourguignon *et al.*, 2015; Bucek *et al.*, 2019). *Mastotermes* was found to be the sister group of

242   Euisoptera and Teletisoptera was sister to Kalotermitidae + Neoisoptera (Icoisoptera). Our

243   analyses supported the monophyly of Stolotermitidae, which was found to be sister

244   Archotermopsidae + Hodotermitidae, the former paraphyletic to the latter. The paraphyly of

245   Archotermopsidae was already indicated by previous phylogenies based on full mitochondrial

246   genomes (Bourguignon *et al.*, 2015). It is clear that a simple augmentation of the current

247   classification by removing *Hodotermopsis* from Archotermopsidae resolves this paraphyly, while

248   simultaneously maximizing nomenclatural stability with the literature of the last 70 years (*i.e.*,

249   maintaining Grassé's distinction between a family of harvesters and dampwood termites).

250   Accordingly, we restrict Archotermopsidae to *Archotermopsis* and *Zootermopsis* (*i.e.*,

251   Archotermopsinae sensu Jiang *et al.* (2021) elevated as Archotermopsidae Engel et al., ***status***

252   ***novum***), and elevate Hodotermopsinae to familial rank (*i.e.*, Hodotermopsidae Engel, ***status***

253   ***novum***). While this system is finely split, it is preferable to obscuring the biological differences

254   and confusing the historical literature that has deployed these names, particularly Hodotermitidae,

255   in such a context since Grassé (1949). The alteratives would be 1) recognizing all of the

256   aforementioned families as subfamilies of Hodotermitidae (semantically equivalent to the multi-

257   family system), or 2) to recognize two families, Stolotermitidae and Hodotermitidae, the former

258   with Stolotermitinae and Porotermitinae, and the latter with Hodotermitinae, Archotermopsinae,

259   and Hodotermopsinae. Neither of these alternatives maximize nomenclatural stability in the sense

260 of the ICZN (1999), nor do they provide any greater clarity regarding relationships. Accordingly,

261 the system we adopt (Table 1) emphasizes the ecological differences between the taxonomic units,

262 with all Archotermopsidae and Hodotermopsidae feeding on damp wood (usually coniferous),

263 while all Hodotermitidae are desert harvester termites feeding predominantly on dry grasses

264 (Krishna *et al.*, 2013). In the remainder of the discussion we shall refer to the families in this new

265 context.

266 The time-calibrated trees estimated with and without third codon positions of protein-coding genes

267 yielded similar time estimates. Our time estimates of the branching among early diverging termite

268 families were largely congruent with the estimates obtained with time-calibrated trees

269 reconstructed using node-dating approaches. For example, we estimated the most recent common

270 ancestor of termites at 143.2 Ma (125.5–163.3 Ma, 95% HPD), while previous studies estimated

271 crown termites at 149 Ma (136–170 Ma, 95% HPD) (Bourguignon *et al.*, 2015), 151.3 Ma (149.3–

272 153.7 Ma, 95% HPD) (Legendre *et al.*, 2015), and 140.6 Ma (112.6–170.5 Ma, 95% HPD) (Bucek

273 *et al.*, 2019). In contrast, tip-dating analyses estimated crown termites at 205 Ma (171–234 Ma,

274 95% HPD) (Jouault et al. 2021) and Ware et al. (2010) carried out multiple analyses yielding age

275 estimates in excess of 200 Ma for crown termites. Differences among studies in terms of fossil

276 calibrations, fossil age estimations, taxonomic sampling, methods, and models used for the

277 reconstruction of time-calibrated trees may be the causes of this variation. In any case, the absence

278 of termite fossils older than ~130 Ma suggest that tip-dating approaches overestimate the age of

279 termites.

280 We did not attempt to reconstruct the ancestral range of Stolotermitidae + Hodotermopsidae +

281 Archotermopsidae + Hodotermitidae, particularly given that the many fossils occurring well

282 outside of modern distributions would render meaningless such an estimate based solely on extant

283 taxa. Ancestral range reconstructions have been performed previously for Neoisoptera and

284 Kalotermitidae (Bourguignon *et al.*, 2016, 2017; Wang *et al.*, 2019; Romero Arias *et al.*, 2021;

285 Bucek *et al.*, 2021). However, compared to Teletisoptera, Neoisoptera and Kalotermitidae are

286 diverse and widespread, comprising many extant species whose distribution and phylogenetic

287 relationships can inform on past vicariance and dispersal events, and with most fossils nested

288 within those distributions (Krishna *et al.*, 2013). Stolotermitidae, Hodotermopsidae,

289 Archotermopsidae, and Hodotermitidae are species-poor families, with limited modern

290 distributions, relict of past wider distributions as evidenced from the fossil record (Fig. 2) (Krishna

291 *et al.*, 2013; Engel *et al.*, 2013, 2016; Jiang *et al.*, 2021). Most geographic lineages of Teletisoptera
292 inhabit regions hosting few other termites and may have been competitively excluded from regions
293 where termitids and other Neoisoptera became dominant during the Oligocene and Miocene (Engel
294 *et al.*, 2009; Bourguignon *et al.*, 2017). Teletisoptera inhabit regions generally devoid of other
295 members of the group, preventing a meaningful reconstruction of its historical biogeography.

296 While the low diversity of teletisopteran families hamper meaningful ancestral range
297 reconstructions, our time-calibrated trees permit the identification of several biogeographic
298 disjunctions. The two modern stolotermitid genera, *Porotermes* and *Stolotermes*, have a
299 Gondwanan distribution (Emerson 1942, 1955; Gay & Calaby 1969; Kaulfuss *et al.*, 2010; Krishna
300 *et al.*, 2013). However, our time-calibrated phylogeny indicated that all species of *Porotermes*
301 share a common ancestor 20.2 Ma (14.1–27.1 Ma, 95% HPD) and the common ancestor of the
302 species of *Stolotermes* sequenced in this study lived 35.0 Ma (25.5–45.2 Ma, 95% HPD), both
303 considerably younger than the breakup of Gondwana. Although we could not sequence
304 *Stolotermes africanus* Emerson, the only species of *Stolotermes* found in Africa, our time-
305 calibrated trees showed that *Stolotermes* diverged from *Porotermes* 70.0 Ma (53.8–85.5 Ma, 95%
306 HPD), after the breakup of Gondwana. Interestingly, an extinct genus allied to *Porotermes* is
307 known from the Oligocene of Ethiopia (Engel *et al.*, 2013), predating the divergence of crown-
308 group *Porotermes* but postdating the divergence of the lineages comprising Porotermitinae and
309 Solotermitinae. Collectively, these results imply that the presence of *Stolotermes* in South Africa,
310 eastern Australia as well as New Zealand, and the presence of *Porotermes* in southern Australia,
311 southern Africa, and South America is not the result of vicariance during the breakup of Gondwana,
312 as hypothesized previously (Krishna *et al.*, 2013; Bourguignon *et al.*, 2015). Instead, *Porotermes*
313 and *Stolotermes* acquired their modern distribution through long-distance oversea dispersal events.

314 The biogeographic disjunctions among modern genera of Hodotermopsidae + Archotermopsidae
315 + Hodotermitidae may be explained by land bridges. Indeed, we estimated that Hodotermopsidae
316 + Archotermopsidae + Hodotermitidae shared a common ancestor around 90.1 Ma (82.1–100.5
317 Ma, 95% HPD), indicating vicariance through continental drift may explain the distribution of
318 early diverging members of this clade. The Palearctic region remained connected to North America
319 through Greenland until about 50 Ma (Scotese 2004), possibly explaining the disjunction between
320 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), that 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.

351

Conflict of Interest

The authors declare no conflict of interest.

354

Data Availability statement

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

358

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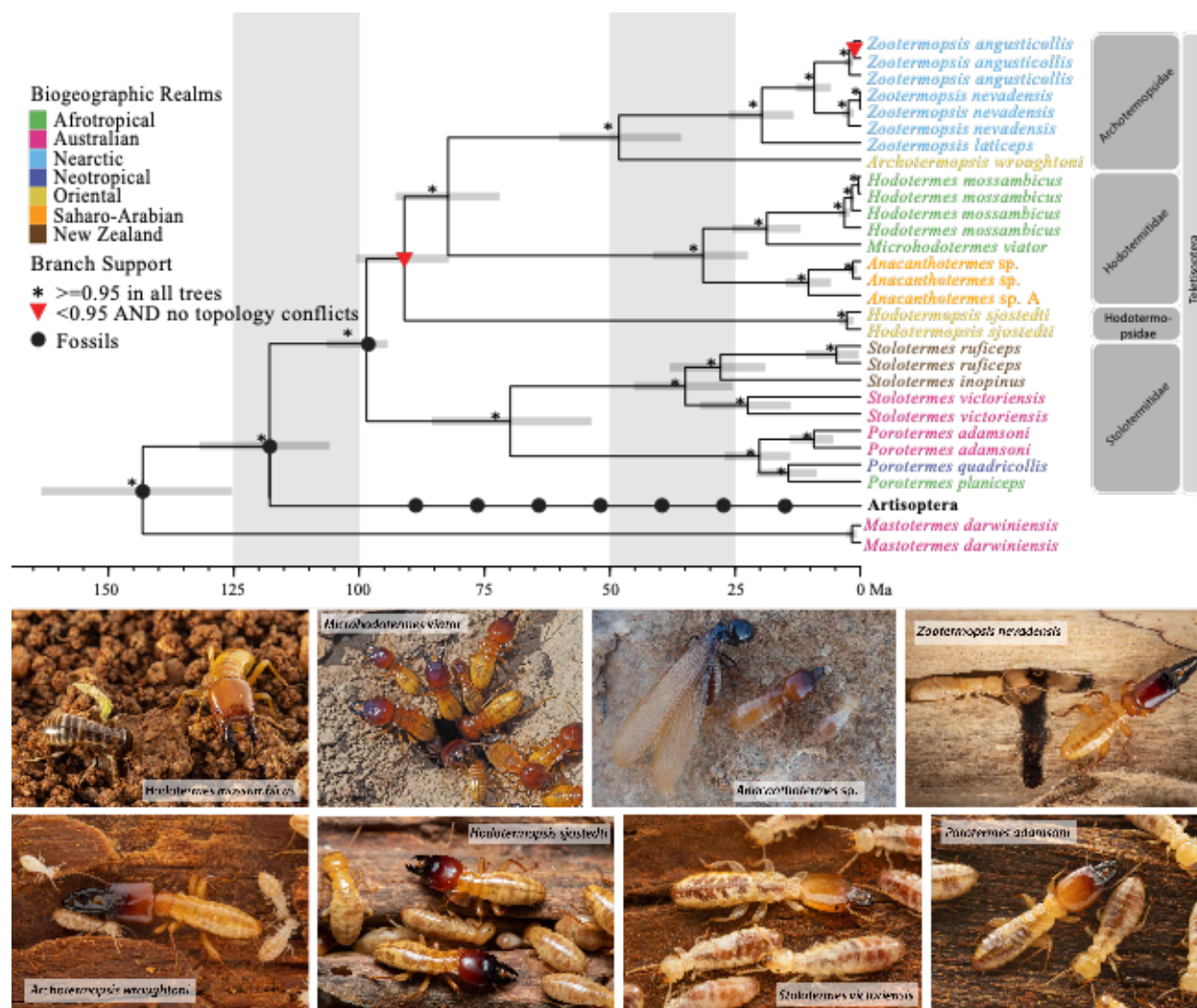537   *Entomology*, **44**, 612–623.

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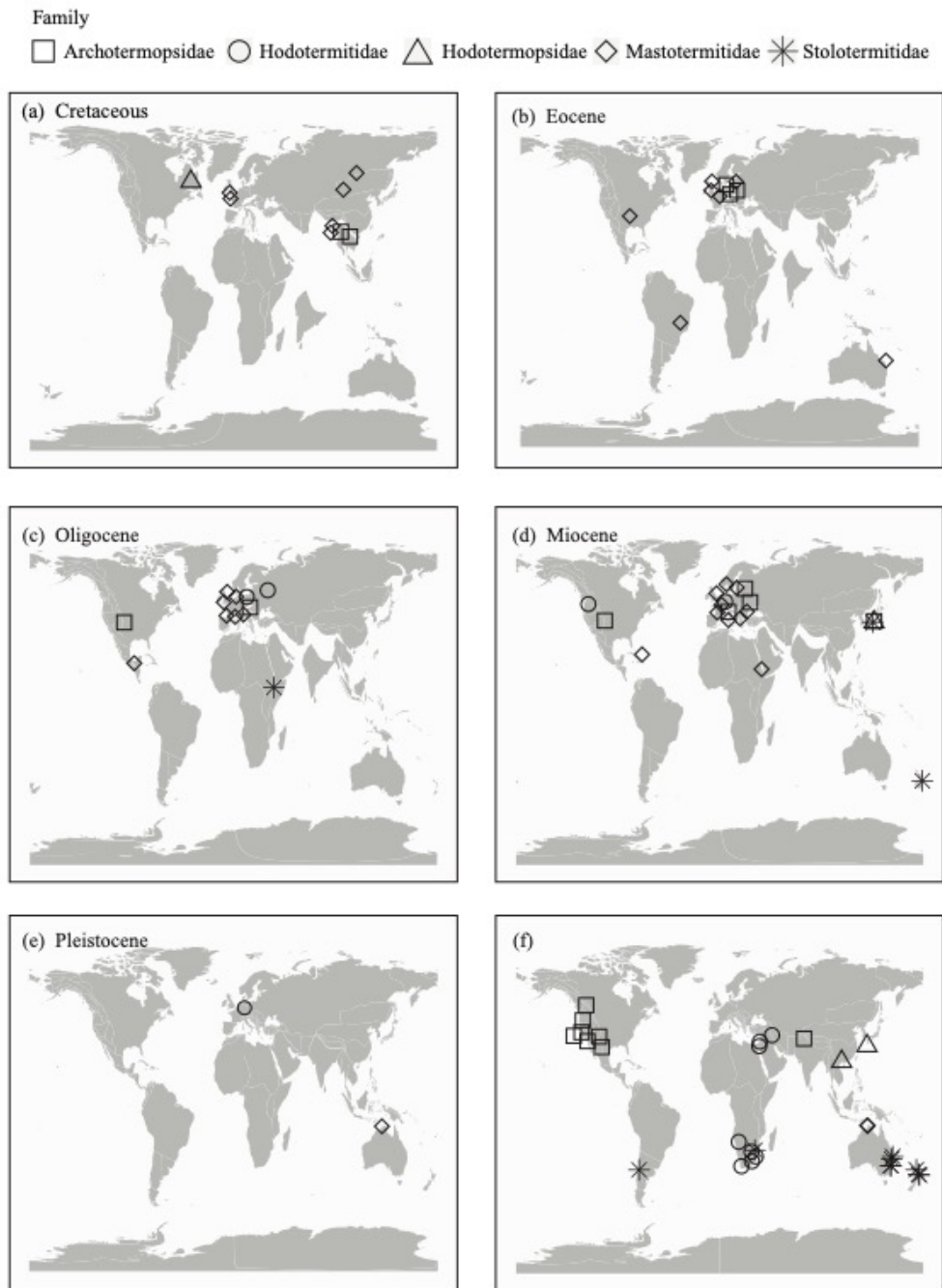| Holmgren (1911)	Emerson (1942), Snyder (1949), Krishna (1970)	Grassé (1949), Weidner (1955), Engel & Krishna (2004)	Engel *et al*. (2009, 2016), Krishna *et al*. (2013)	Jiang *et al*. (2021)	Herein
PROTERMITIDAE[1]	**HODOTERMITIDAE**	**HODOTERMITIDAE**	**HODOTERMITIDAE**	**HODOTERMITIDAE**	**HODOTERMITIDAE**
Hodotermitinae	Hodotermitinae	*Anacanthotermes*	*Anacanthotermes*	*Anacanthotermes*	*Anacanthotermes*
Archotermopsis	*Anacanthotermes*	*Microhodotermes*	*Microhodotermes*	*Microhodotermes*	*Microhodotermes*
Hodotermes[2]	*Microhodotermes*	*Hodotermes*	*Hodotermes*	*Hodotermes*	*Hodotermes*
Stolotermitinae	*Hodotermes*	**TERMOPSIDAE**	**ARCHOTERMOPSIDAE**	**ARCHOTERMOPSIDAE**	**HODOTERMOPSIDAE**
Stolotermes	Termopsinae	*Hodotermopsis*	*Hodotermopsis*	Hodotermopsinae	*Hodotermopsis*
Calotermitinae[3]	*Hodotermopsis*	*Archotermopsis*	*Archotermopsis*	*Hodotermopsis*	**ARCHOTERMOPSIDAE**
Porotermes	*Archotermopsis*	*Zootermopsis*	*Zootermopsis*	Archotermopsinae	*Archotermopsis*
	Zootermopsis	Porotermitinae	**STOLOTERMITIDAE**	*Archotermopsis*	*Zootermopsis*
	Porotermitinae	*Porotermes*	Porotermitinae	*Zootermopsis*	**STOLOTERMITIDAE**
	Porotermes	Stolotermitinae	*Porotermes*	**STOLOTERMITIDAE**	Porotermitinae
	Stolotermitinae	*Stolotermes*	Stolotermitinae	Porotermitinae	*Porotermes*
	Stolotermes		*Stolotermes*	*Porotermes*	Stolotermitinae
				Stolotermitinae	*Stolotermes*
				Stolotermes	

544 [1] Holmgren's (1911) Protermitidae also included Mastotermitinae, not covered herein.
545 [2] Holmgren (1911) included *Anacanthotermes* as a subgenus of *Hodotermes*.
546 [3] Holmgren (1911) also included in this subfamily *Calotermes* (= *Kalotermes s.l.*, or what today is recognized as Kalotermitidae).
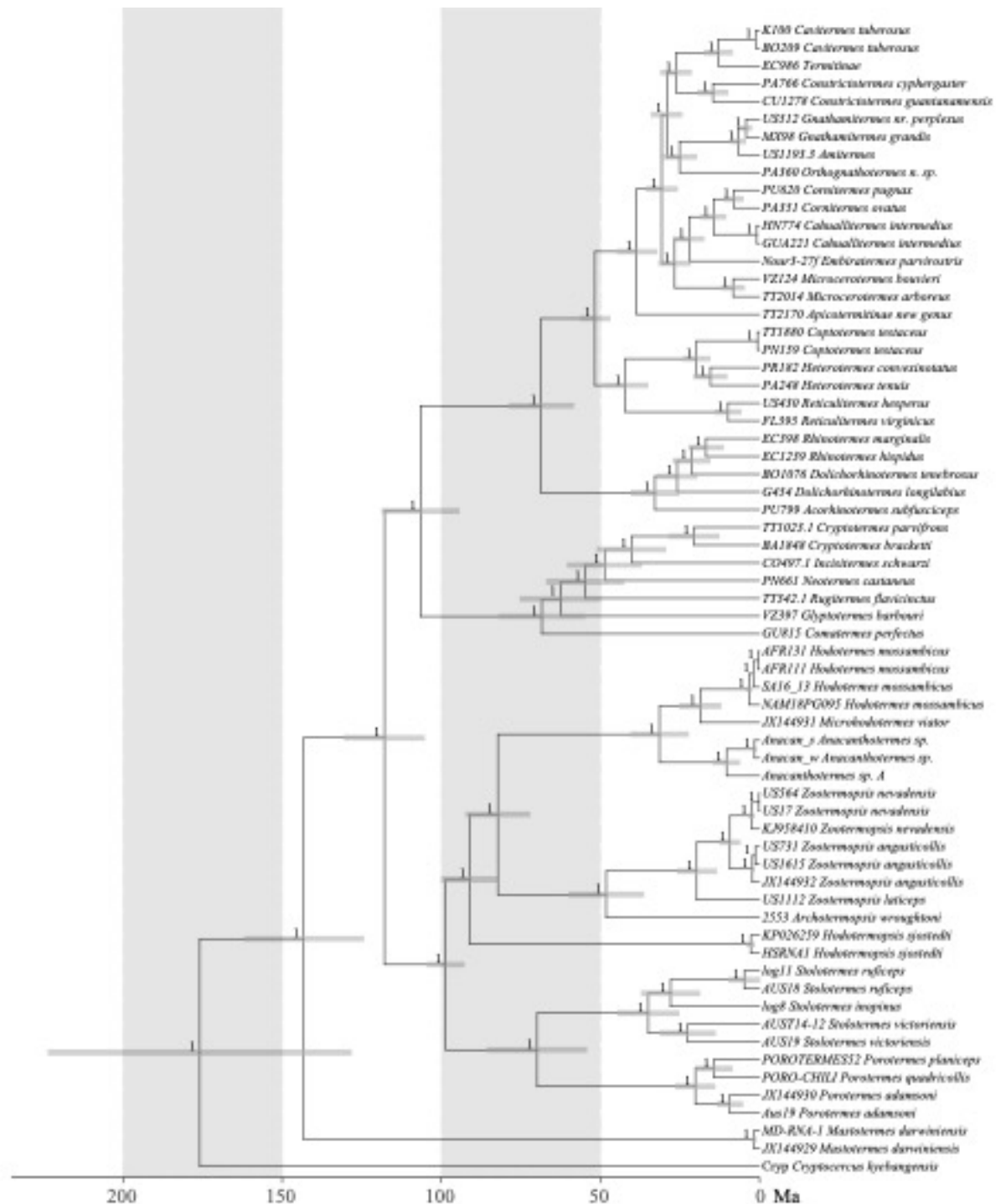
Fig. 1. Chronogram of early-diverged termite lineages. Time-calibrated phylogenetic tree of Stolotermitidae, Archotermopsidae, Hodotermopsidae, and Hodotermitidae based on full mitochondrial genomes and 5S, 5.8S, 18S, and 28S rRNA genes. The tree was reconstructed without third codon positions of protein-coding genes with BEAST2. The map shows the sampling locations of Stolotermitidae, Archotermopsidae, Hodotermopsidae, and Hodotermitidae. Node symbols (asterisk and red triangle) represent the bootstrap support and posterior probability values obtained with IQTREE, MrBayes, and BEAST2 on the dataset with and without third codon positions of protein-coding genes. Node bars indicate 95% Height Posterior Density intervals of age estimates. Biogeographic realms are given and based on the descriptions in Holt et al. 2013. Tip colors coincide with collect localities. The photographs depict one species of each genus included in this study. Photographs of *Microhodotermes* and *Anacanthotermes* were provided by Felix Riegel and Omer Theodore, respectively.

Fig. 2. Maps showing the distribution of known fossils of 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".

565
566 **Fig. S1.** Time-calibrated phylogenetic tree of 64 termite samples reconstructed with BEAST2
567 using mitochondrial genomes without third codon positions and 5S, 5.8S, 18S, and 28S rRNA
568 genes. Node bars indicate the 95% Height Posterior Density intervals. Branch labels represent
569 posterior probabilities.

570

571

572   **Table S1.** Samples used in this study with corresponding collection details and accession numbers.
573
574   **Table S2.** Fossils used for time calibration in this study.
575
576   **Table S3.** Substitution models used for phylogenetic reconstruction in this study.
577