Molecular phylogeny and historical biogeography of Apicotermitinae (Blattodea: Termitidae)

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Abstract

Soil-feeding termites are abundant in tropical regions and play an important role in soil bioturbation and in the organic matter cycle. The Apicotermitinae are arguably the most diverse lineage of soil-feeding termites, but they are also the most understudied, probably because many species are soldierless, which makes identification difficult. Although the backbone of the termite phylogenetic tree is now well-resolved, the relationships among representatives of Apicotermitinae are still largely unknown. Here, we present phylogenetic trees inferred from 113 mitochondrial genomes of Apicotermitinae representative of the group diversity. Our analyses confirm the monophyly of the Apicotermitinae and the basal position of soldiered taxa, within which two lineages of soldierless species are nested. We describe two new monotypic genera, whose phylogenetic position appeared of special interest: *Koutabatermes* **gen. n.**, lies on a long branch among soldiered taxa, and *Apolemotermes* **gen. n.**, is sister to *Adaiphrotermes*. We

resolved, with high support, the position of Asian genera as sister group of a clade comprising the monophyletic neotropical *Anoplotermes*-group and the small African clade including *Adaiphrotermes* and *Apolemotermes* **gen. n.**. Our trees cast light on the intergeneric and interspecific relationships within Apicotermitinae and reveal the polyphyly of several genera, including *Ruptitermes*, *Astalotermes* and *Anoplotermes*. Biogeographic reconstructions revealed two dispersal events out of Africa, one to the Oriental realm and one to the Neotropical realm. Overall, the timing of Apicotermitinae diversification and dispersal, following the Eocene-Oligocene boundary, matches that found for other groups of Neoisoptera. Nomenclatural acts are registered in ZooBank: http://zoobank.org/urn:lsid:zoobank.org:pub:CA1A21B6-573E-4855-8C88-372453C922F7.

KEYWORDS

humivorous, Isoptera, mitochondrial genome, molecular clock, systematics

Introduction

The Apicotermitinae is a subfamily of soil-feeding termites that play important roles in soil bioturbation and organic matter cycling in tropical rainforests and savannas (Jones & Eggleton, 2011; Bourguignon *et al.*, 2016b). This subfamily was originally erected by Grassé & Noirot (1955) for the African soldiered taxa related to *Apicotermes* Holmgren, which were previously placed in the Termitinae. At that time, the soldierless termites were classified in the Amitermitinae and were divided into two genera: *Anoplotermes* Müller, which included the African species and most Neotropical species, and *Speculitermes* Wasmann, which included the then-believed soldierless Asian species and some Neotropical species (Snyder, 1949). Soon afterwards, Roonwal and Sen-Sarma (in Roonwal, 1958) erected the Indotermitidae family for the soldiered genus *Indotermes* Roonwal & Sen-Sarma from South Asia, but Ahmad (1963) outlined the close relatedness between *Indotermes* and *Speculitermes* and moved both genera to the Amitermitinae. Soldiers were later discovered in one *Speculitermes* species (Roonwal & Chhotani, 1966), but remain unknown, and are possibly absent, in several Oriental species. In his revision of African soldierless termites, Sands (1972) expanded the Apicotermitinae subfamily and distinguished two 'branches': *(i)* the *Apicotermes*-branch, including *Apicotermes* and related African soldiered genera; and *(ii)* the *Anoplotermes*-branch, including the soldierless taxa, the soldiered genus *Firmitermes* Sjöstedt, and the Oriental genera *Speculitermes*, *Indotermes* and relatives. More recently, based on gut anatomy, Noirot (2001) recognized three 'groups': *(i)* the *Apicotermes*-group, including the soldiered African taxa and the soldierless genus *Skatitermes* Coaton; *(ii)* the *Speculitermes*-group, including all Asian Apicotermitinae; and *(iii)* the *Anoplotermes*-group, including the African and Neotropical soldierless species plus the soldiered genus *Firmitermes*.

Soldierless species are very abundant in Africa and the Neotropics, where they can locally make up more than 30% of the termite species diversity (Eggleton *et al.*, 1995, 2002; Bourguignon *et al.*, 2011, 2016b; Dahlsjö *et al.*, 2015, 2020; Nduwarugira *et al.*, 2017). This makes the Apicotermitinae one of the most diverse subfamilies of Termitidae. It is also the most understudied, probably because many species, being soldierless, can only be distinguished morphologically by tedious and difficult dissections of the worker digestive tract (Grassé & Noirot, 1955; Sands, 1972, 1998; Noirot, 2001; Bourguignon *et al.*, 2016b). The shape of the mesenteron-proctodeum junction forming a mixed segment and the morphologically variable enteric valve armature are the conventional characters used for the Apicotermitinae taxonomy (Grassé & Noirot, 1955; Sands, 1972, 1998; Noirot, 2001). The gizzard ornaments are also useful as taxonomic characters, particularly in the African soldierless species (Noirot, 2001; Romero Arias *et al.*, 2020). While some genera are characterized by conspicuous apomorphies, such as the hypertrophied sclerotized cushion 1 of the enteric valve in *Ateuchotermes* Sands, others mostly accommodate species that lack particular diagnostic features, such as *Astratotermes* Sands, whose enteric valve lacks distinctive characters (Sands, 1972).

Despite recent progress on the taxonomy of Neotropical Apicotermitinae (e.g. Bourguignon *et al.*, 2010, 2016a; Acioli & Constantino, 2015; Constantini *et al.*, 2018), the systematics of this subfamily remains largely unresolved. To date, 224 species and 52 genera of Apicotermitinae have been described, with diversity hotspots located in Africa and South America (Bourguignon *et al.*, 2016b; Constantino, 2020; Roisin, 2021). However, the actual diversity of the group is much larger, and many species, still awaiting formal description, have been informally labelled as morphospecies in ecological surveys (e.g. Eggleton *et al.*, 1995, 2002; Davies, 2002; Bourguignon *et al.*, 2011; Nduwarugira *et al.*, 2017).

The first comprehensive phylogenetic study of termites was based on a combination of morphological characters and genetic markers, including two mitochondrial genes (COII and 12S) and one nuclear gene (28S) (Inward *et al.*, 2007). This study supported the monophyly of Apicotermitinae, which were retrieved as the sister group of a clade composed of all other Termitidae except the fungus-growers (Macrotermitinae) and the two small subfamilies Sphaerotermitinae and Foraminitermitinae (Inward *et al.*, 2007). This phylogenetic position was later confirmed by molecular phylogenies inferred from complete mitochondrial genomes and transcriptomes (Bourguignon *et al.*, 2015, 2017; Bucek *et al.*, 2019). In addition, the phylogenetic tree of Inward *et al.* (2007) suggested that (1) the African soldiered taxa are paraphyletic to a clade composed of the soldierless lineages and the Asian (soldiered) Apicotermitinae; (2) the Oriental *Speculitermes*-group is monophyletic; (3) the Neotropical *Anoplotermes*-group is monophyletic; and (4) the Oriental *Speculitermes*-group, the Neotropical *Anoplotermes*-group and the African soldierless genus *Adaiphrotermes* Sands form a monophyletic group sister to all other African soldierless taxa. This tree topology implies two independent losses of soldiers in Apicotermitinae and two independent dispersal events between continents, with unclear directionality. Complete mitochondrial genome phylogenies confirmed that Asian and Neotropical taxa are closer to each other than most African soldierless genera (Bourguignon *et al.*, 2017). However, because of their insufficient sampling, poor characterization of some described genera (e.g. *Astalotermes* Sands, *Anenteotermes* Sands), and uncertain identifications, the history of Apicotermitinae remains unclear. In addition, most relationships among African and Neotropical soldierless taxa were unresolved by Inward *et al.* (2007), and several genera (e.g. *Aderitotermes* Sands, *Astalotermes* or *Anoplotermes*) appeared as polyphyletic in the phylogenetic trees of Bourguignon *et al.* (2017).

In this study, we used 113 mitochondrial genomes of Apicotermitinae species to reconstruct robust phylogenetic trees including most morphologically defined genera. Using these trees, we tested previous phylogenetic hypotheses regarding the relationships among major Apicotermitinae clades and provided a timeframe for their evolution. We also investigated the historical biogeography of Apicotermitinae and determined the number of independent losses of soldiers. Our analyses clarify the taxonomy of Apicotermitinae and pave the path to future taxonomic revisions of non-monophyletic genera, such as *Astalotermes* or *Anoplotermes*, and provide a framework to study the anatomical evolution of the subfamily.

Material and methods

Sampling

Termite sampling was conducted in Burundi (n = 7), Cameroon (n = 28), Ivory Coast (n = 18), Kenya (n = 1), French Guiana (n = 12) and Argentina (n = 1) (Table S1). For each sample, we collected specimens in RNA-later® or in 100% ethanol for genetic analyses, and in 80% ethanol for morphological analyses. Samples collected in RNA-later® and 100% ethanol were temporarily stored at a temperature ranging from -20°C to 4°C, and shipped to the Czech University of Life Sciences or to the Okinawa Institute of Science and Technology, where they were stored at -80°C until DNA extraction. Samples collected in 80% ethanol are stored at the Université Libre de Bruxelles and the Czech University of Life Sciences. In addition to the 67 samples collected in this study, we also obtained the full mitochondrial genome sequences of 43 samples of Apicotermitinae from GenBank (Bourguignon *et al.*, 2015, 2017) and reconstructed mitochondrial genomes from transcriptome sequences of three species (Bucek *et al.*, 2019) (Table S1).

Species identifications were based on morphological and anatomical characters, which included the worker digestive tube configuration, the shape of the gizzard and enteric valve armature, as described in Romero Arias *et al.* (2020). The taxon sampling attempted to maximize the number of species and their geographic distribution, including samples of *Astalotermes* and *Anoplotermes* groups in addition to new morphologically-characterized specimens. We also re-examined the voucher material of samples sequenced in previous studies and whose phylogenetic position appeared inconsistent. In a few cases, we found that the voucher samples contained a mixture of two species. We labelled these samples with both species names. Revised species identifications are detailed in Supplementary Appendix S1 (see also Table S1).

DNA extraction and sequencing

Whole genomic DNA was extracted from head and thorax of three to five workers using the DNeasy Blood & Tissue extraction kits (Qiagen). Because DNA extracts were sequenced at different periods of time, two different approaches were used. For the first approach, the complete mitochondrial genome was amplified in two long-PCR reactions with the TaKaRa LA Taq polymerase, using primers previously designed for termites (Bourguignon *et al.*, 2015). The concentration of both long PCR fragments was determined using a Qubit 3.0 fluorometer, and the two fragments were mixed in equimolar concentration. Libraries were prepared with the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs) and sequenced with Illumina MiSeq. For the second approach, whole genomic DNA libraries were directly prepared with the aforementioned NEB kit and sequenced using Illumina HiSeq4000.

Assembly and annotation of mitochondrial genomes

Paired-end reads were quality-assessed using FastQC v0.11.7 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and adapter sequences were removed with Trim Galore v0.4.5 (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) using default settings. Mitochondrial

reads were identified using the mitogenome of *Astalotermes murcus* Sands (accession no. KY224676) as a reference and assembled using GetOrganelle v1.5.1 (Jin *et al.*, 2020). Each resulting assembly graph was inspected with Bandage v0.8.1 (Wick *et al.*, 2015) and mitochondrial genome sequences were manually circularized when necessary. Control regions were discarded from the final assemblies as they provide limited phylogenetic information and are difficult to accurately assemble with short reads. We used the MITOS2 Webserver with the invertebrate genetic code and the protein prediction method of Donath *et al.* (2019) to annotate the two rRNA genes, 22 tRNA genes, and 13 protein-coding genes. Other parameters were set to default. Annotated genomes are deposited in GenBank (accession numbers in Table S1). In total, we generated 67 new mitochondrial genome sequences, mostly from African species (54). Fifty-nine mitochondrial genomes were complete, and eight mitochondrial genomes were nearly complete because of ambiguous circularization. The mitochondrial genomes from Bourguignon *et al.* (2015), which included 60 non-Apicotermitinae termites and eight non-termite polyneopteran insects, were used as outgroups (Table S2). Therefore, the final data set comprised 181 mitochondrial genomes, including 113 genomes of Apicotermitinae.

Sequence alignment

We aligned separately each of the two rRNA genes, 22 tRNA genes, and 13 protein-coding genes using MAFFT v7.300b (Katoh *et al.*, 2002; Katoh & Standley, 2013) with default settings. Protein-coding genes were aligned as protein sequences and back-translated into nucleotide sequences using PAL2NAL (Suyama *et al.*, 2006). rRNAs and tRNAs were aligned as DNA sequences. The 37 aligned genes were concatenated and partitioned into five partitions: one for each codon position of the combined protein-coding genes; one for the combined 12S and the 16S rRNA genes; and one for the combined tRNA genes. We found no clear evidence of mutational saturation for the third codon positions of the protein-coding genes (I_{SS}=0.572, $I_{SS.C}$Sym=0.809) using Xia's method implemented in DAMBE (Xia *et al.*, 2003; Xia & Lemey, 2009) and therefore retained the third codon positions in our phylogenetic analyses.

Phylogenetic inference

We used RAxML version 8.2.4 (Stamatakis, 2014) to reconstruct a maximum-likelihood phylogenetic tree, with the GTR+G model for each partition. Bootstrap values were estimated from 1000 replicates. We used MrBayes version 3.2 (Ronquist *et al.*, 2012) to reconstruct a Bayesian phylogenetic tree. The analysis was run with four chains (three hot and one cold), and we estimated posterior distributions using Markov chain Monte Carlo (MCMC) sampling, with samples drawn every 5000 generations. The chain was run for a total of 10 million generations, with the first 1 million generations discarded as burnin, as suggested by inspection of the trace files using Tracer v1.5 (Rambaut & Drummond, 2009). We used a GTR model with gamma-distributed rate variation across sites (GTR+G) for each partition. The analysis was run in triplicate to ensure convergence of the chains and check for consistency. The effective sample size was higher than 400 for every parameter of every run. Node support was estimated using Bayesian posterior probabilities.

Molecular dating

We estimated time-calibrated trees using BEAST2 version 2.4.4 (Bouckaert *et al.*, 2014). We performed the analyses with and without third codon positions to assess the influence of third codon positions on time estimates. The trees were reconstructed using an uncorrelated lognormal relaxed clock to model rate variation among branches, with single model for each partition, allowing different relative rates. A Yule speciation model was used as tree prior. We used a GTR+G model of nucleotide substitution for each partition. The chains were run for 500 million steps and were sampled every 10,000 generations to estimate the posterior distribution. We discarded the first 50 million steps as burn-in, as suggested by inspection of the trace files using Tracer v1.5 (Rambaut & Drummond, 2009). We used the 13 fossils used by Bucek *et al.* (2019) (see Table 1). The phylogenetic position and age of fossils were carefully assessed as recommended by Parham *et al.* (2012). Bucek *et al.* (2019) provided detailed justifications about the placement of these fossils, which we followed. The youngest fossil age estimates reported on the Fossilworks database (Alroy, 2016) were used as minimum age constraints. We determined soft upper bounds using phylogenetic bracketing (Ho & Phillips, 2009). Each calibration was implemented as an exponential prior of node time. The analyses were run in triplicate to ensure convergence of the chains and check for consistency.

Reconstruction of ancestral distribution

The ancestral distribution of Apicotermitinae was reconstructed using the ace function of the R package APE version 5.0 (Paradis & Schliep, 2018). We used the Maximum Likelihood model described by Pagel (1994) for discrete data. We tested three models available in APE: the equal-rates model (ER), the all-rate-different model (ARD) and the symmetrical model (SYM). The function was implemented as follow: "ace(distribution, tree, type="discrete", model="ER" or "ARD" or "SYM", CI=TRUE)". Other parameters were set on default values. We also used the Bayesian Binary mcmc analysis implemented in RASP4.2 (Yu *et al.,* 2015, 2020), with maximum number of areas set to 1 and 3. We ran separate analyses for the four possible combinations of state frequencies, set to fixed (JC) or estimated (F81), and among-site rate variation, set to equal or gamma (+G). Default values were used for other parameters. The seven analyses, implemented in APE and RASP4.2, were run on the four phylogenetic trees reconstructed in this study. Sampling locations were used to assign each tip to one biogeographic realm. Apicotermitinae are distributed across three biogeographic realms, as described by Holt *et al.* (2013): Afrotropical, Neotropical, and Oriental. We reconstructed ancestral distribution on the maximum-likelihood tree, the Bayesian tree, and the two time-calibrated trees.

Reconstruction of soldier loss

We used the ace function of the R package APE version 5.0 (Paradis & Schliep, 2018) to reconstruct the evolution of soldier loss in Apicotermitinae. As for the reconstruction of ancestral distribution, we used

three models, the ER model, the ARD model and the SYM model. Soldier presence, or absence, was determined for each tip of the phylogenetic trees based on literature data.

Results

Molecular phylogeny

Our phylogenetic trees fully supported the monophyly of Apicotermitinae (Figs 1 and S1-S3). African soldiered taxa (the *Apicotermes*-group) formed a paraphyletic assemblage, composed of two or three lineages, within which a clade composed of Asian genera and soldierless African and Neotropical taxa was nested (Figs 1 and S2-S3). This latter clade was divided into four lineages, fully supported in all analyses: (I) the African soldierless species, with the exclusion of *Adaiphrotermes* and *Apolemotermes* **gen. n.**, were retrieved as sister to the other three lineages, (II) the Asian *Speculitermes*-group was sister to the last two lineages, (III) the African genera *Adaiphrotermes* and *Apolemotermes* **gen. n.**, and their sister group, (IV) the Neotropical soldierless *Anoplotermes*-group.

Discrepancies among analyses were found for the position of soldiered lineages. More precisely, the position of the clade including *Koutabatermes* **gen. n.** + (*Hoplognathotermes* Silvestri + *Labidotermes* Deligne & Pasteels) was variable among analyses (Figs 1 and S1-S3). Similarly, the position of species within the *Astalotermes*-group and the *Anoplotermes*-group was variable. The relationships among taxa of *Astalotermes*-group were often weakly supported, and several genera were retrieved as polyphyletic, i.e. *Astalotermes*, *Anenteotermes* and *Astratotermes*. Among the monophyletic genera, we retrieved well-supported sister relationships between *Acholotermes* Sands and *Amicotermes* Sands and between *Ateuchotermes* and *Anaorotermes* Sands. Other well-supported monophyletic genera were *Alyscotermes* Sands, *Acidnotermes* Sands, *Aderitotermes*, and *Adaiphrotermes*. Within the neotropical *Anoplotermes*-group (clade IV), the relationships among genera were weakly supported, and many species, referred to as *Anoplotermes*-group sp., were found on long branches and belong to undescribed genera (Figs 1 and S1-S3). The genus *Ruptitermes* Mathews appeared to be polyphyletic, the arboreal *R. arboreus* Emerson being broadly separated from the other species of the genus.

Divergence time estimation

The time-tree reconstructed with third codon positions included yielded older age estimates (Fig. S1), up to 10.9 million years (My) older than the analysis with third codon positions excluded (Fig. 1). The ranges given hereafter encompass the results of both analyses, with and without third codon positions. We estimated that the most recent common ancestor of Apicotermitinae lived 39.5–48.6 million years ago (hereafter Ma) (95% HPD: 34.7–53.2 Ma), during the middle Eocene. The most recent common ancestor of the soldierless Apicotermitinae + *Speculitermes*-group was estimated at 34.9–44.2 Ma (95% HPD: 30.8–48.5 Ma). The split between the *Speculitermes*-group and their sister group was dated at 31.7–41.6 Ma (95% HPD: 27.7–45.9 Ma), during the early Oligocene. The Neotropical *Anoplotermes*-group diverged from its African sister lineage (*Adaiphrotermes* + *Apolemotermes* **gen. n.**) 28.0–38.0 Ma (95%

HPD: 24.4–42.0 Ma). The age estimates of cladogenesis for the current taxonomic groups are summarized in Table 2.

Ancestral distribution

We reconstructed the ancestral distribution of Apicotermitinae on the four phylogenetic trees generated in this study. For each tree, we tested three Maximum Likelihood models and four Bayesian Binary mcmc models. Bayesian models allowing multiple distribution areas yielded similar trends on ancestral distribution. Therefore, the results were congruent among analyses, and consequently, we only present here the results of the ER model that posits an equal rate of transition among states (Figs S4-S7). Our results indicate that the Apicotermitinae originated in the African realm, and dispersed from there twice: once to the Oriental realm, where they gave rise to the *Speculitermes*-group, and once to the Neotropical realm, where they gave rise to the *Anoplotermes*-group.

Reconstruction of soldier loss

We reconstructed the evolution of soldier loss in Apicotermitinae on the four phylogenetic trees generated in this study using three Maximum Likelihood models. The reconstructions of the four phylogenetic trees yielded almost identical results and therefore we only present here the results of the reconstructions on the phylogenetic tree reconstructed with MrBayes. The ARD model clearly supported independent loss of soldiers in the African soldierless Apicotermitinae and in the Neotropical soldierless *Anoplotermes*-group (Fig. S8). In contrast, the ER model and the SYM model provided more ambiguous results, with the scenario of a dual loss of soldiers in the African soldierless Apicotermitinae and in the Neotropical soldierless *Anoplotermes*-group being equally probable to another scenario implying a loss of soldiers in the ancestor of non-*Apicotermes*-group Apicotermitinae followed by a secondary reacquisition of soldiers in the *Speculitermes*-group (Figs S9-10).

Taxonomy

Apicotermitinae Grassé and Noirot, 1955

Type genus: *Apicotermes* Holmgren, 1912

***Koutabatermes* Romero Arias & Roisin, gen. n.**

http://zoobank.org/urn:lsid:zoobank.org:act:EB5EED27-355F-40F5-9FAD-D7C812466395.

(Figs 2, 3, 4A)

Type species: *Koutabatermes spinifer* Romero Arias & Roisin, **sp. n**., described below. This genus is presently monotypic.

*Diagnosis***.** The general configuration of the worker digestive tube (Fig. 2), characterized by the absence of a mixed segment and a rather short P1, matches that of other genera of the *Apicotermes*-group (Noirot, 2001). *Koutabatermes* workers possess a singular enteric valve armature, comprising six

subequal membraneous cushions covered with polygonal scales in their basal part, bearing numerous tiny spines in their middle part, and distally dome-shaped, bearing a variable number (5-25) of sharp spines about 20 µm in length, penetrating into the valve seating (Fig. 3C,D). Species of the *Labidotermes* subgroup (see Noirot, 2001) also possess six subequal scaly cushions, but they bear a stout spine basally and no apical armature entering the paunch. In the clade comprising *Apicotermes* and *Coxotermes*, the armature is always heavily asymmetrically sclerotized in its apical part and of very diverse shape resembling spiny, club- or feather-shaped expansions, as it enters the paunch. *Eburnitermes* and *Machadotermes* possess elongated spiny pads internally, prolonged in the seating by long plates bearing spines or pectinated scales. In *Phoxotermes*, *Jugositermes* and *Trichotermes*, the apical part of the pads is heavily sclerotized and bears spines of various shapes.

Description.

Imago. Unknown.

Soldier. Unknown, but the phylogenetic position of *Koutabatermes* among soldiered taxa suggests that this caste may exist.

Worker. Head, thorax, abdomen and legs covered with numerous setae. Antennae with 14 articles. Postclypeus moderately inflated. Fore coxa without prominent ridge or projection, fore tibia slender, not inflated. Tibial spurs 3, 2, 2. Crop voluminous, gizzard armature reduced to six pulvilli, one of them (in position 4 according to Romero Arias *et al.*, 2020) much larger than the other five. All pulvilli bear a group of 25-50 pointed spines, about 10 µm long (Fig. 3A,B). Mesenteron forming almost complete loop; M–P1 junction circular, without mixed segment; P1 short, about 3 times as long as wide (Fig. 2). Enteric valve armature comprising six subequal bulky membraneous cushions within lumen of P2, extending distally to emerge within P3, where each cushion is capped by irregularly-oriented curved spines in variable number (5–25), about 20 µm long. No distinct lobes at P2–P3 junction. First section of the paunch slightly narrower and about half the length of P1, internally-garnished with tiny spicules but without conspicuous sclerotized armature as present in *Gastrotermes* (Scheffrahn, 2020).

Etymology. From Koutaba, a town in Cameroon, and Latin *termes*, termite. Gender: masculine.

***Koutabatermes spinifer* Romero Arias and Roisin, sp. n.**
http://zoobank.org/urn:lsid:zoobank.org:act:46DC8A42-06E0-47B7-86F4-27BA95970EB7.

(Figs 2, 3, 4A)

Type material. Holotype: worker. Paratypes: workers, from the same series (most probably from the same colony) as the holotype. The whole series comprises about 60 workers. Cameroon, Région Ouest, near the town of Koutaba, Koumaja village (N 05.6244°, E 10.7542°), elevation (GPS) 1203 m, collected on 29 May 2017. Collected in soil under an old nest of *Pseudacanthotermes* sp. at the edge of a cultivated field (J. Romero Arias, Y. Roisin, accession CMRT074).

Type repository. AfricaMuseum (formerly known as Royal Museum of Central Africa), Tervuren, Belgium. Part of the type series is stored in the ULB (Université Libre de Bruxelles) collection.

Diagnosis. As for the genus (*vide supra*).

Description.

Worker. Monomorphic, rather large for the subfamily. Head, thorax, abdomen and legs covered with numerous, rather long (13–20 μm) setae. Head capsule and antennae pale brownish-yellow, remainder of the body whitish. Fore tibia with three spurs, the external one about half the length of the other two. Left mandible with posterior edge of apical tooth about 1.5 times as long as anterior edge of first marginal tooth; premolar tooth (as per Deligne, 1999; "subsidiary marginal" of Sands, 1972) as long as third marginal, visible in front of molar prominence (Fig. 4A). Right mandible with posterior edge of apical tooth about 1.8 times as long as anterior edge of first marginal (Fig. 4A).

Measurements (in mm) of 10 workers: mean (range), holotype.

Head length to front margin of postclypeus: 1.216 (1.176–1.275), 1.218; postclypeus length: 0.312 (0.291–0.336), 0.294; head maximum width: 1.323 (1.277–1.359), 1.344; pronotum maximum width: 0.852 (0.830–0.883), 0.852; fore tibia length: 1.003 (0.968–1.040), 1.023; fore tibia width: 0.137 (0.121–0.151), 0.126; hind tibia length: 1.392 (1.366–1.423), 1.396; fore tibia length/width ratio: 7.343 (6.628–8.125), 8.119.

Measurements (in mm) of mandibles from 5 workers: mean (range).

Left mandible. Apical tooth to first marginal (L_A in Sands, 1972): 0.147 (0.143–0.155); first to third marginal (L_1): 0.190 (0.185–0.195); third marginal to molar (L_m): 0.061 (0.054-0.069). Right mandible. Apical to first marginal (R_A): 0.155 (0.148–0.177); first to second marginal (R_1): 0.125 (0.120–0.128); second marginal to molar (R_m): 0.083 (0.076–0.088).

This species was called "Cameroon Genus C" in Romero Arias *et al.* (2020), and illustrated in Figs A7C (gizzard and enteric valve) and A8C (gizzard).

Etymology: Latin *spinifer*, bearing spines, referring to the enteric valve armature of the worker.

Genus *Apolemotermes* Romero Arias & Roisin, gen. n.
http://zoobank.org/urn:lsid:zoobank.org:act:4747BA58-8470-42CB-8209-87CDE0F050E9.

(Figs 4B,C, 5, 6)

Type species: *Apolemotermes fodiens* Romero Arias & Roisin, **sp. n.**, described below. This genus is presently monotypic.

Diagnosis. The general configuration of the worker digestive tube (Fig. 5), characterized by a mixed segment with the mesenteric part on the inner side of the gut loop and a long P1, corresponds to the usual pattern of soldierless Apicotermitinae. The mixed segment is rather long, about twice the width of the mesenteron, slightly thickened but without distinct bulge at the end of its mesenteric part. The gizzard features five weakly developed pulvilli (Fig. 6A). The enteric valve armature comprises six subequal broadly reticulated pads with almost parallel sides, ending rather abruptly at the entrance of P3. They show a faint crescent-shaped thickening at their base (Fig. 6B).

Adaiphrotermes species also possess an elongated mixed segment, but it terminates in a distinct mesenteric bulge (Sands, 1972). Pulvilli of the gizzard are more prominent in *Adaiphrotermes* species and bear distinct pectinated scales (Fig. 7A). The enteric valve armature of *Adaiphrotermes* species (Figs 7B,C) consists of parallel pads extending through the length of the narrow tubular section of P2, variable according to the species. As in *Apolemotermes* **gen. n.**, their distal end is almost straight, rectangular. Pads are covered with imbricated (fish-like) scales, more prominent basally.

Anenteotermes and *Aderitotermes* are the other African genera possessing a long mixed segment (Sands, 1972). *Anenteotermes* comprises some tiny species with enteric valve armatures harbouring very distinctive sclerotized ornamentations (Sands, 1972; Scheffrahn & Roisin, 2018). Other species possess scaly pads, with an ovoid or fusiform shape (not distally rectangular). *Aderitotermes* includes much larger species, with distinctive enteric valves (Sands, 1972).

Among African Apicotermitinae, *Adaiphrotermes* and *Apolemotermes* **gen. n.** stand out for their highly inflated fore tibiae, but *Apolemotermes* **gen. n.** (Fig. 4C), with a length/width ratio below 3, appears more extreme than *Adaiphrotermes* in this respect.

Description.

Imago. Unknown.

Soldier. Unknown. *Apolemotermes* **gen. n.** is the sister genus of *Adaiphrotermes*. Together, they form the sister group of the Neotropical Apicotermitinae, all of which are soldierless, strongly suggesting that soldiers are absent.

Worker. Head and body covered by numerous short, erect setae. Antennae with 14 articles. Postclypeus only slightly raised, in line with frons. Fore coxa without prominent ridge or projection, fore tibia strongly inflated (Fig. 4C). Tibial spurs 2, 2, 2. Crop moderately developed, gizzard armature reduced to five weakly raised pulvilli bearing only a faint projection (Fig. 6A). Mesenteron forming almost complete loop; distinct mixed segment, about as long as twice the mesenteric diameter. Mesenteric part of mixed segment slightly enlarged distally, but not forming a distinct terminal bulge as found in *Adaiphrotermes* species (Sands, 1972). P1 long and of constant diameter, enteric valve situated on the right side at the back of the paunch. Enteric valve seating without distinct lobes. Enteric valve armature comprising six subequal, reticulated pads, with a small thickening at their base (Fig. 6B).

Etymology. Ancient Greek ἀπόλεμος, peaceful, and Latin *termes*, termite. This name evokes the absence of soldiers, in the line of names created by Sands (1972) for 16 new African soldierless genera. Gender: masculine.

Apolemotermes fodiens **Romero Arias & Roisin, sp. n.**

http://zoobank.org/urn:lsid:zoobank.org:act:3001D19F-6EB8-410F-9287-F7BC7C6026B8.

(Figs 4B,C, 5, 6)

Type material. Holotype: worker. Paratypes: workers, from the same series (most probably from the same colony) as the holotype. Whole series comprises about 60 workers. Cameroon, Région Centre, near town of Mbalmayo, Feikele village (N 03.4807°, E 11.5772°), elevation (GPS) 688 m, 06 June 2017. Collected in soil in forest fragment (J. Romero Arias, Y. Roisin, accession CMRT159).

Type repository. AfricaMuseum, Tervuren, Belgium. Part of the type series in the ULB collection.

Diagnosis. As for the genus (*vide supra*).

Description.

Imago. Unknown.

Soldier. Unknown and probably absent.

Worker. Monomorphic, very small, with elongated body. Head and body covered by numerous short, erect setae, about 0.04 mm long. Head capsule pale yellow, remainder of the body and appendages whitish. Fore tibia with two developed spurs, external one vestigial. Enteric valve pads subequal —the shortest ones about ¾ the length of the longest ones— with almost parallel sides, distal edge slightly convex. Pads broadly reticulated, with faint crescent-shaped thickening at base, more visible on shortest pads (Fig. 6B). Left mandible with posterior edge of apical tooth about 1.6 times as long as anterior edge of first marginal; premolar tooth clearly shorter than third marginal, under edge of molar prominence. Right mandible with posterior edge of apical tooth about twice as long as anterior edge of first marginal (Fig. 4B).

Measurements (in mm) of 10 workers: mean (range), holotype.

Head length to front margin of postclypeus: 0.498 (0.474–0.519), 0.482; postclypeus length: 0.135 (0.121–0.151), 0.135; head maximum width: 0.506 (0.496–0.519), 0.501; pronotum maximum width: 0.308 (0.296–0.320), 0.299; fore tibia length: 0.359 (0.346–0.373), 0.351; fore tibia width: 0.138 (0.128–0.145), 0.140; hind tibia length: 0.364 (0.346–0.380), 0.360; fore tibia length/width ratio: 2.604 (2.483–2.710), 2.507.

Measurements (in mm) of mandibles from 5 workers: mean (range).

Left mandible. Apical tooth to first marginal (L_A in Sands, 1972): 0.044 (0.042–0.046); first to third marginal (L_1): 0.085 (0.084–0.086); third marginal to molar (L_m): 0.026 (0.024-0.027). Right mandible. Apical to first marginal (R_A): 0.046 (0.042–0.049); first to second marginal (R_1): 0.062 (0.060–0.066); second marginal to molar (R_m): 0.042 (0.040–0.043).

Etymology. Latin *fodiens*, digging.

Discussion

Phylogenetic relationships and systematics

Our findings are in partial agreement with those of Inward *et al.* (2007) and Bourguignon *et al.* (2017). For instance, we confirm the paraphyly of the *Apicotermes*-group, which is composed of several basal lineages, closely matching the subgroups proposed by Noirot (2001) on the basis of digestive anatomy: the *Labidotermes* subgroup, also comprising *Hoplognathotermes* (+ *Acutidentitermes* Emerson, not sequenced), is characterized by a simple enteric valve armature wholly enclosed within the P2 section of the hindgut, which probably represents an ancestral condition; the *Apicotermes* subgroup, including also *Allognathotermes* Silvestri + *Duplidentitermes* Emerson and *Coxotermes* Grassé & Noirot + *Heimitermes* Grassé & Noirot, possesses very sophisticated enteric valve armatures protruding into the paunch; and the *Trichotermes* subgroup, including *Jugositermes* Emerson and *Phoxotermes* Collins (+ *Rostrotermes* Grassé, not sequenced), displays an enteric valve with six sclerotized plates bearing numerous, variously developed spines, which also penetrate into the paunch. Noirot (2001) made a fourth subgroup for *Eburnitermes* Noirot and *Machadotermes* Weidner, which were not sequenced in this study. These two genera possibly constitute another basal lineage. Finally, some new taxa are known from the worker only, although their anatomy places them in the *Apicotermes*-group: this is the case of the new *Koutabatermes* **gen. n.**, whose mitochondrial genome confirmed distant affinities with *Labidotermes* and *Hoplognathotermes*. Another such taxon is the new genus labelled "*Kaktotermes*" (*nomen nudum*) by Donovan (2002), which still awaits sequencing.

The phylogeny of Inward *et al.* (2007) featured a large clade comprising, on the one hand, the *Astalotermes*-group (including all African soldierless taxa except *Adaiphrotermes*, without deeper resolution), and on the other hand, an unresolved clade including *Adaiphrotermes*, which appeared paraphyletic, the Asian taxa (with soldiers known in all genera, but often very rare) and the Neotropical taxa (all soldierless, not further resolved). Here, we confirm the monophyly of the *Astalotermes*-group and determine its sister clade with a strong support: Asian genera (*Indotermes* + *Euhamitermes* Holmgren) now branch out first, as sister group to a soldierless clade including the Neotropical taxa, confirmed as monophyletic (= the *Anoplotermes*-group clade), and an African branch composed of *Adaiphrotermes* plus a distinctive new African genus here called *Apolemotermes* **gen. n.** (*Adaiphrotermes*-group clade).

Inward *et al.* (2007) suggested that the loss of the soldier caste occurred only once in the evolution of the Apicotermitinae, but in view of the best supported phylogeny, this hypothesis cannot account for the presence of soldiers in Asian taxa. Unless soldiers have been reacquired in Asian taxa, their loss must have occurred at least twice: once at the origin of the *Astalotermes*-group (clade I on Fig. 1), and once at the origin of the clade composed of the *Adaiphrotermes*-group and the *Anoplotermes*-group (clades III+IV on Fig. 1). The rarity of soldiers in species of the *Speculitermes*-group suggests that soldier abundance gradually dwindled to the point of disappearing completely in some species of the *Speculitermes*-group. We posit that a similar scenario likely took place in the ancestor of the *Astalotermes*-group and in the ancestor of *Adaiphrotermes*-group + *Anoplotermes*-group. Future phylogenetic trees, inferred from more comprehensive sampling, might also uncover additional loss events. For example, according to Noirot (2001), the soldiered genus *Firmitermes* possesses a digestive anatomy reminiscent of soldierless species, and the soldierless genus *Skatitermes* anatomically matches the *Apicotermes*-group, raising the possibility of additional soldier losses not represented in our phylogenetic reconstructions.

Thus far, most generic descriptions of Apicotermitinae have been written in the absence of a solid phylogenetic background. For instance, as Sands (1972: 51) himself admitted, *Astalotermes* was difficult to define because this genus "occupies a transitional position between others with more primitive and more specialized characters". Unsurprisingly, this genus came out of our study as polyphyletic. Likewise, *Astratotermes*, which differs from *Astalotermes* by the enteric valve scales ending in tiny points, was defined based on characters of poor phylogenetic significance and is polyphyletic. The situation is even more accentuated in the Neotropics, where the genus *Anoplotermes* lumps all soldierless species that have not been considered distinctive enough to deserve a transfer to another genus. All those genera are now in need of an in-depth revision. The present phylogeny will constitute a useful framework to revise the whole subfamily and identify characters of phylogenetic interest.

This work also yielded less intuitive results. For instance, the tiny *Anenteotermes nanus* Sjöstedt now appears distant from the equally tiny *An. polyscolus* Sands and other species with a bilateral enteric valve armature —*An. cnaphorus* Sands, *An.* sp. A (CIVT120), and probably *An. cherubimi* Scheffrahn, recently described (Scheffrahn & Roisin, 2018) and awaiting sequencing. In the Neotropics, the arboreal open-air forager *Ruptitermes arboreus* was known to be slightly different, on anatomical grounds, from other species of the genus which are ground-dwelling litter feeders (Acioli & Constantino, 2015). Our results now show that *R. arboreus* has been wrongly assigned to *Ruptitermes*, being closer to *Tetimatermes* Fontes than to other *Ruptitermes* species.

Time frame of Apicotermitinae evolution

As suggested by Inward *et al.* (2007) and Bourguignon *et al.* (2017), our results support the African origin of Apicotermitinae. The molecular dating analyses with and without third codon positions yield age estimates diverging by up to 10.9 My. This difference is likely caused by the high base compositional

heterogeneity at third codon positions which can influence the estimation of divergence times (Zheng *et al.*, 2011). However, time estimates of our tree with the third codon position excluded are similar to those of other phylogenetic trees (Bourguignon *et al.*, 2015, 2017; Bucek *et al.*, 2019). For instance, our estimation of the most recent ancestor of Apicotermitinae diverged by less than 5 My from those time-trees (Bourguignon *et al.*, 2015, 2017; Bucek *et al.*, 2019). According to both molecular clocks, Apicotermitinae cladogenesis was initiated during the Eocene 39.5–48.6 Ma (95% HPD: 34.7–53.2 Ma) when rainforests were more widely distributed than nowadays. Nevertheless, most clades originated after the Eocene-Oligocene transition (about 34 Ma), coinciding with sharp drops in atmospheric concentration of carbon dioxide (Pagani *et al.*, 2005) and global temperatures, and with retraction of the megathermal rainforests to low latitudes (Morley, 2011). Thus, there is the possibility that climate cooling led to species extinctions and created refuges in relicts of equatorial forests that could have driven speciation events. The age estimates of our molecular clock analysis without the third codon positions match with past climatic transitions and forest distributions that may have led to the diversification of the Apicotermitinae lineage.

According to our results, migratory movements of the ancestors of the *Speculitermes* and *Anoplotermes* groups occurred in two separate occasions out of the Afrotropical realm. The first dispersal event occurred 21.0–31.7 Ma (95% HPD: 16.2–35.9 Ma, without 3rd codon position) or 29.6–41.6 (95% HPD: 23.6–45.9 Ma, with 3rd codon position) and gave rise to the Oriental soldiered species while the sister lineage remained in the Afrotropical realm. The second dispersal event occurred 24.0–28.1 Ma (95% HPD: 20.8–31.9 Ma, without 3rd codon position) or 34.2–38.0 (95% HPD: 30.8–41.9 Ma, with 3rd codon position) and gave rise to the Neotropical *Anoplotermes* soldierless species, sister of the African *Adaiphrotermes*-group. The timing of these dispersal events coincides with a generalized trend of species dispersion and diversification in other insects and plants following the extensive extinction during the Eocene–Oligocene transition (Maley, 1996; Engel, 2001; Morley, 2011). As suggested by Bourguignon *et al.* (2015), we confirm the single dispersion to the Neotropical region. Since no land connection between the Neotropics and the Afrotropics existed during the Tertiary, the ancestor of the *Anoplotermes*-group must have reached the Neotropics over the ocean, possibly by rafting in wood (Bourguignon *et al.*, 2017). However, soil-feeding behavior was recently suggested as the ancestral state in the major clade of Termitidae, after the Macrotermitinae and Sphaerotermitinae branched off (Bucek *et al.*, 2019), and certainly at the origin of the Apicotermitinae. Soil-feeding termites are especially poor dispersers, but a possible explanation for their overseas dispersal is through nests attached to floating logs (Bourguignon *et al.*, 2017). Another possibility is the colonization by a soil-wood interface feeder settled in a decayed part of a log. The closest African living relative of the Neotropical *Anoplotermes*-group, the *Adaiphrotermes*-group, is not known to build cohesive nests, but *Adaiphrotermes* species have been found on wood baits (Sands, 1972). Nowadays, several neotropical species are known to build nests, often attached to tree trunks or roots, and some commonly occur under bark or in decayed logs (Bourguignon *et al.*, 2016b).

The large number of species used in this study sheds new light on the evolution of a poorly-known lineage, the Apicotermitinae, and highlights the need for efficient taxon sampling. Recently, many new species of Apicotermitinae have been described, in particular in the Neotropics. However, the systematics of this termite group requires an integrative approach combining morphological taxonomy and modern molecular techniques. In this study, we followed an integrative taxonomic approach to describe *Apolemotermes* **gen. n.** and *Koutabatermes* **gen. n.** and provide a comprehensive phylogenetic tree that will make future species descriptions easier. However, a denser sampling of individual subclades in *Astalotermes* and *Anoplotermes* groups is necessary to resolve intra-group relationships and understand their diversification processes.

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Author contributions

Johanna Romero Arias: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing-original draft, Writing-review & editing. **Arthur Boom:** Data curation, Formal analysis, Methodology, Validation, Writing-original draft. **Menglin Wang**: Data curation, Investigation. **Crystal Clitheroe:** Investigation. **Jan Šobotník:** Resources. **Petr Stiblik:** Resources. **Thomas Bourguignon:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Resources, Writing-original draft, Writing - review & editing. **Yves Roisin:** Conceptualization, Data curation, Funding acquisition, Resources, Writing-original draft, Writing-review & editing, Supervision.

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736 Table 1. Fossils used as calibration for estimating divergence times among Apicotermitinae.

Species	Minimum age constraint (MY)	Calibration group	Soft maximum bound (97.5% probability)	Reference	Note on maximum bound
Juramantis initialis	145.5	Dictyoptera	315.2	Vršanský (2002)	First cockroach fossils
Valditermes brenanae	130	Isoptera + *Cryptocercus*	235	Krishna *et al*. (2013) and references therein	*Triassoblatta argentina*, earliest fossil of Mesoblattinidae (Martins-Neto *et al.* 2005)
Archeorhinotermes rossi	94.3	Kalotermitidae + Rhinotermitidae + Serriteritidae + Termitidae	235	Krishna & Grimaldi (2003).	*Triassoblatta argentina*, earliest fossil of Mesoblattinidae (Martins-Neto *et al.* 2005)
Dolichorhinotermes dominicanus	16	*Dolichorhinotermes* + *Schedorhinotermes*	94.3	Schlemmermeyer & Cancello (2000)	Oldest fossil of Rhinotermitinae
Reticulitermes antiquus	33.9	*Reticulitermes* + *Coptotermes* + *Heterotermes*	94.3	Engel *et al.* (2007)	Oldest fossil of Rhinotermitinae
Coptotermes sucineus	16	*Coptotermes* + *Heterotermes*	33.9	Emerson (1971)	Oldest *Heterotermes* fossil
Constrictotermes electroconstrictus	13.8	*Constrictotermes* + *Caetetermes* + *Nasutitermes*	47.8	Krishna (1996)	Oldest fossil of Termitidae
Anoplotermes sensu lato	13.8	South American *Anoplotermes*-group + *Adaiphrotermes* + Genus F	47.8	Krishna & Grimaldi (2009)	Oldest fossil of Termitidae
Microcerotermes insularis	13.8	*Microcerotermes* + Syntermitinae	47.8	Krishna & Grimaldi (2009)	Oldest fossil of Termitidae
Termes primitivus	13.8	*Cavitermes* + *Macrognathotermes* + Cubitermitinae	47.8	Krishna & Grimaldi (2009)	Oldest fossil of Termitidae
Nanotermes	47.8	Termitidae + *Coptotermes* + *Heterotermes* + *Reticulitermes*	94.3	Engel *et al.* (2011)	Oldest fossil of Rhinotermitinae
Amitermes lucidus	13.8	*Drepanotermes* + *Sinocapritermes* + *Pericapritermes*	47.8	Krishna & Grimaldi (2009)	Oldest fossil of Termitidae
Macrotermes pristinus	16	*Macrotermes* + *Synacanthotermes* + *Odontotermes*	47.8	Charpentier (1843)	Oldest fossil of Termitidae

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Table 2. Estimation dates for the major and basal Apicotermitinae clades (Ma) with all sites included and without third codon positions. The differences (**δ**) of node ages are included.

crown clade	description	without Third codon positions		with Third codon positions		δ node ages
		node ages	95% HPD	node ages	95% HPD	
I	*Astalotermes*-group	30.7	27.0-34.8	40.5	36.8-44.8	9.8
II	*Speculitermes*-group	21.0	16.2-25.6	29.6	23.6-34.8	8.6
III	*Adaiphrotermes* + *Apolemotermes* **gen. n.**	23.0	18.4-27.7	32.2	27.5-37.3	9.2
IV	*Anoplotermes*-group	24.0	20.8-27.2	34.2	30.8-37.9	10.2

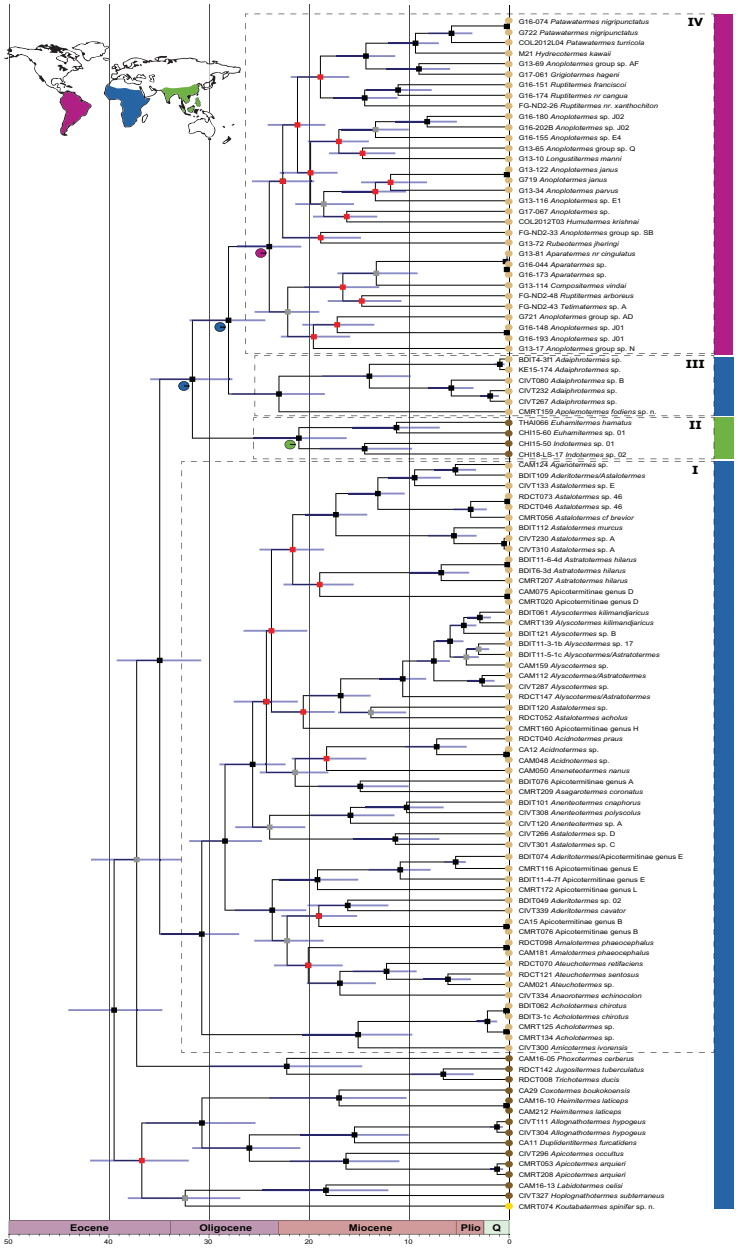


Fig. 1. Bayesian phylogenetic chronogram of Apicotermitinae inferred from mitochondrial genomes, with third codon positions excluded. The scale bar is given in millions of years. Node bars represent the 95% HPD intervals for the ages. Nodes are labelled with symbols representing posterior probabilities and bootstrap support for all analyses (1/100% = black; <1/100%= gray) and with red squares when the topology differs among analyses. Pie charts close to the nodes show the inferred relevant ancestral shifts of biogeographic distributions on the map: Afrotropical, Oriental and Neotropical realms. Wide bars indicate current distribution of species. Dotted boxes with roman numbers indicate the crown clades: I *Astalotermes*-group, II *Speculitermes*-group, III *Adaiphrotermes* + *Apolemotermes* **gen. n.** and IV *Anoplotermes*-group. Tip circles represent soldiered (dark brown), soldierless (light brown) species and unknown soldier caste presence (yellow). Names of species include colony code and scientific name, respectively.

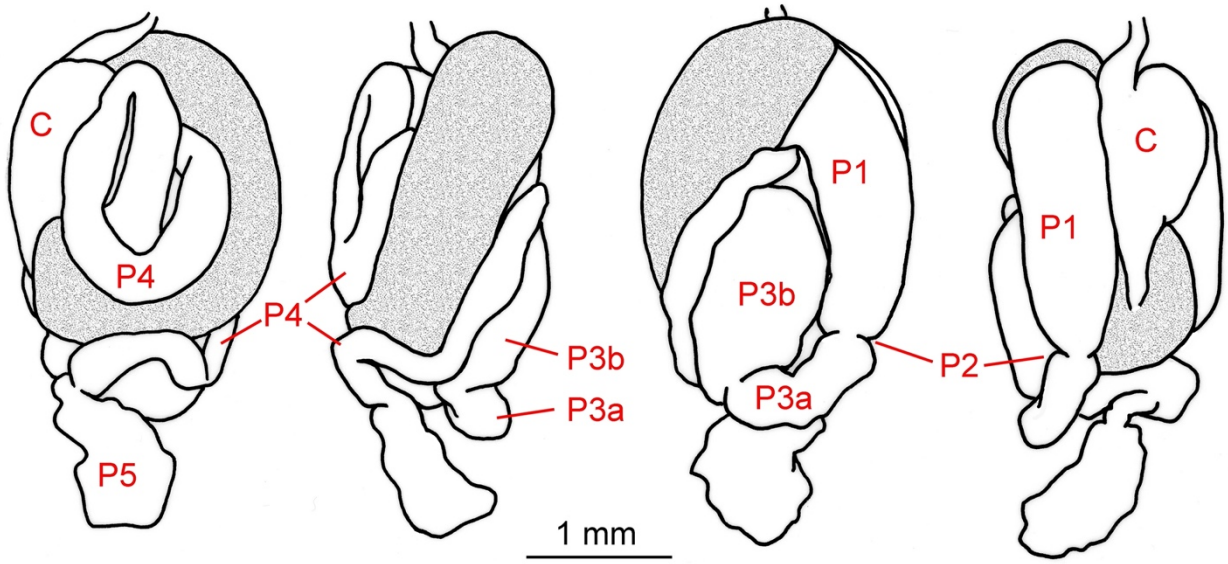
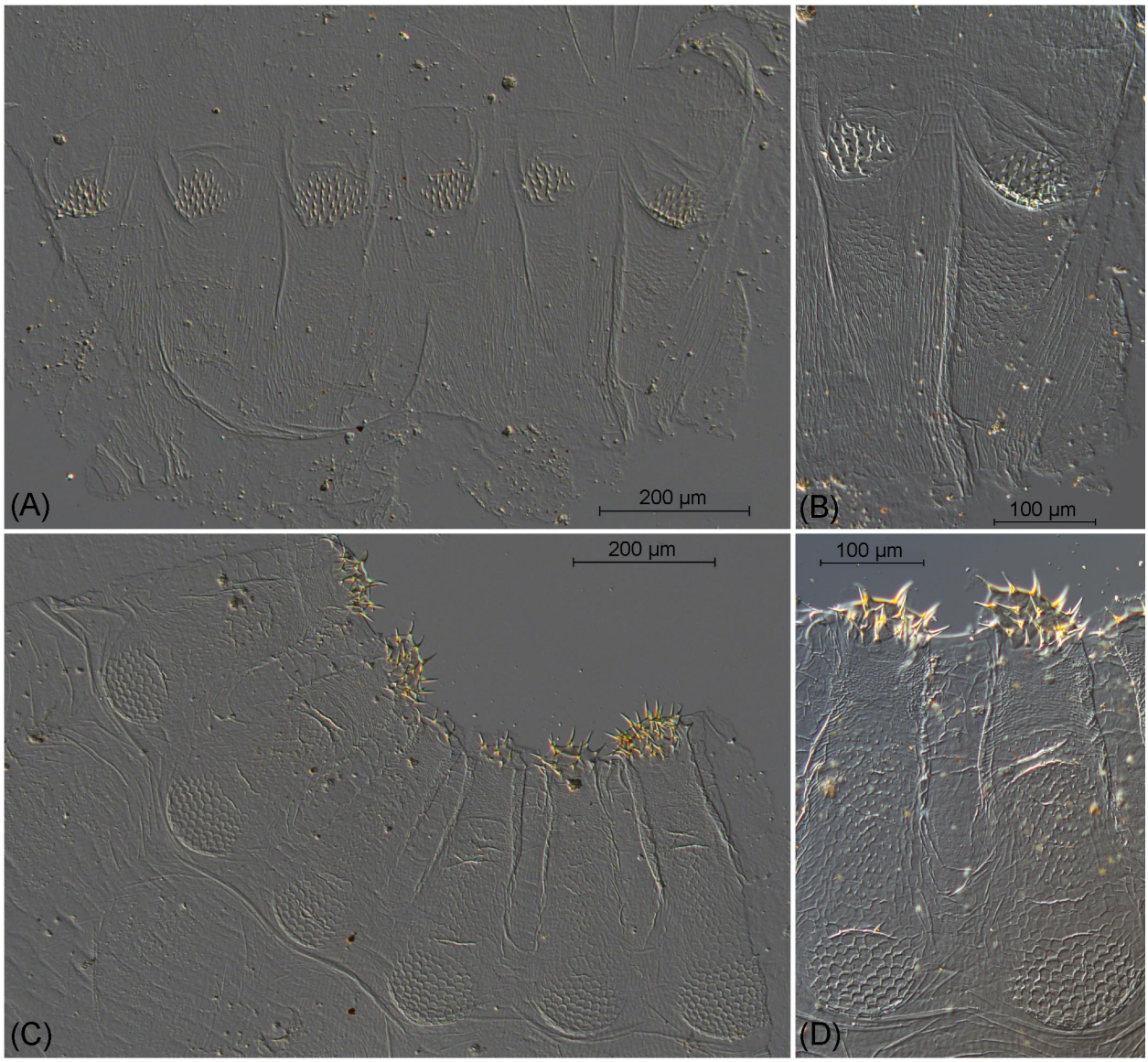


Fig. 2. Camera lucida drawings of digestive tube of *Koutabatermes spinifer* **sp. n.** worker. From left to right, viewed from above, right, below, left. Successive sections are: crop (C), mesenteron (*stippled*), first section of the paunch (P1), enteric valve (P2), third section of the paunch, subdivided into enteric valve seating (P3a) and main section (P3b), colon (P4) and rectal pouch (P5).



762

763 **Fig. 3.** Internal armature of digestive tube in worker of *Koutabatermes spinifer* **sp. n.** Organs splayed on
764 slide. (A) Gizzard, showing six spiny mats corresponding to pulvilli, one of which (far right) is more
765 developed than the others and bears tiny spicules on its upper surface. Direction of flow downwards. (B)
766 Details of pulvilli showing scaly surface. (C) Enteric valve, showing six cushions bearing polygonal
767 scales basally, tiny spicules in their middle part and longer, strong spines on their distal end, penetrating
768 into the paunch. (D) Detail of two enteric valve cushions. Direction of flow upwards.

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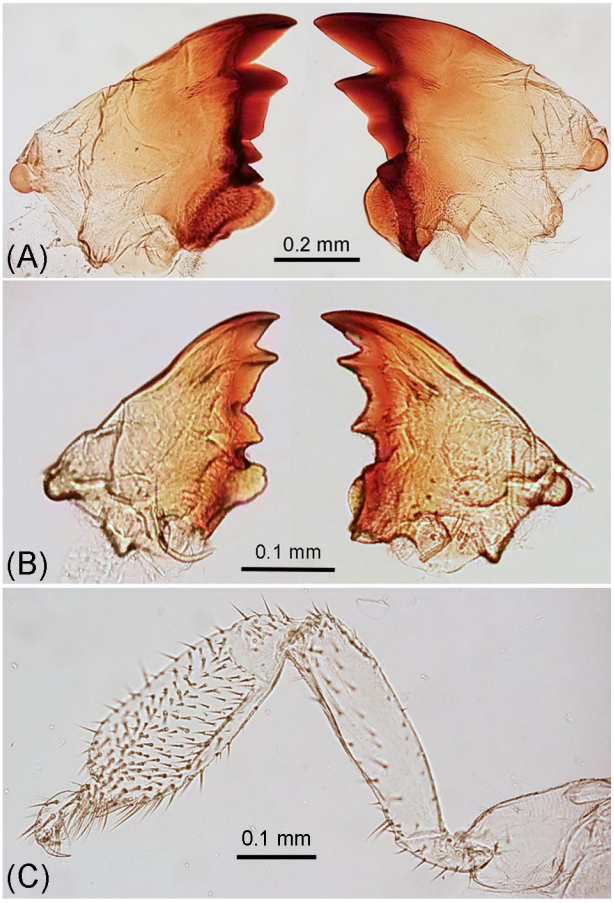
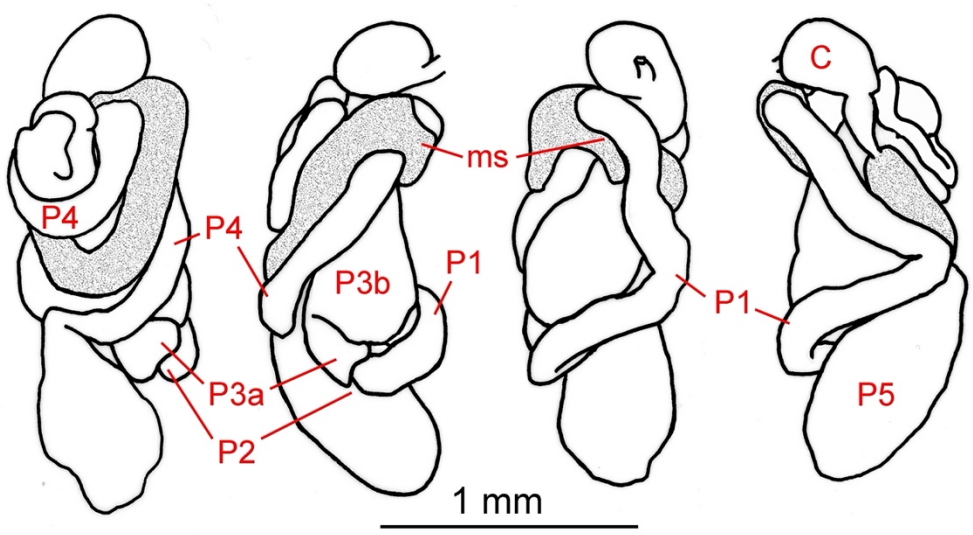


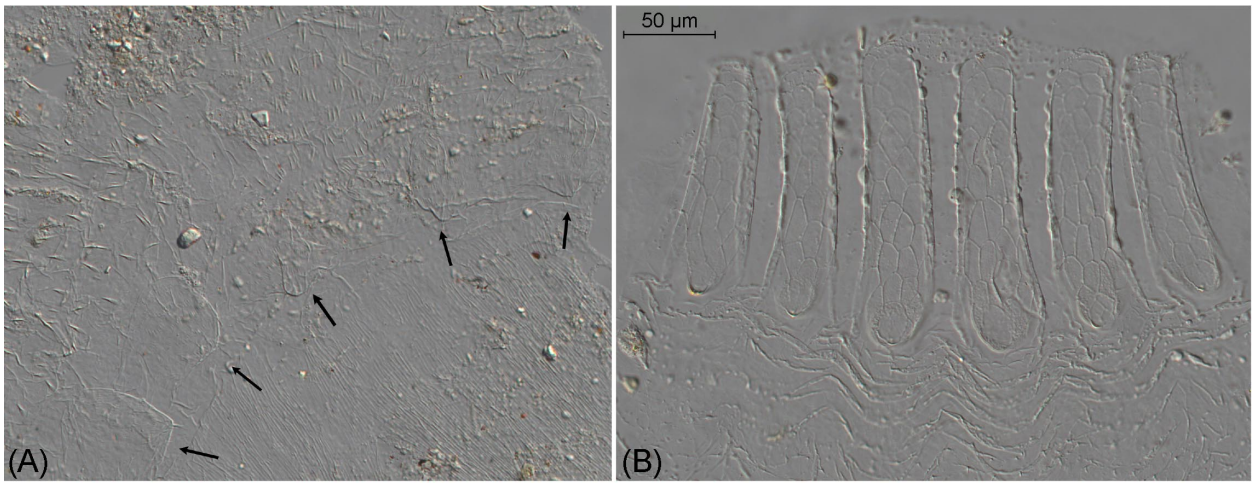
Fig. 4. Worker mandibles and fore leg. (A) Mandibles of *Koutabatermes spinifer* **sp. n.** (B) Mandibles of *Apolemotermes fodiens* **sp. n.** (C) Fore leg of *Apolemotermes fodiens* **sp. n.**, showing inflated tibia.



774

775 **Fig. 5.** Camera lucida drawings of digestive tube of *Apolemotermes fodiens* **sp. n.** worker. From left to
776 right, viewed from above, right, below, left. Successive sections are: crop (C), mesenteron (*stippled*),
777 mixed segment (ms), first section of the paunch (P1), enteric valve (P2), third section of the paunch,
778 subdivided into enteric valve seating (P3a) and main section (P3b), colon (P4) and rectal pouch (P5).

779

780 

781 **Fig. 6.** Internal armature of digestive tube in worker of *Apolemotermes fodiens* **sp. n.** Organs splayed on
782 slide. (A) Gizzard, showing five weakly developed pulvillar prominences (*arrows*). Direction of flow
783 downwards. (B) Enteric valve, showing six subequal, reticulated cushions bearing a very slight thickening
784 basally. Direction of flow upwards.

785

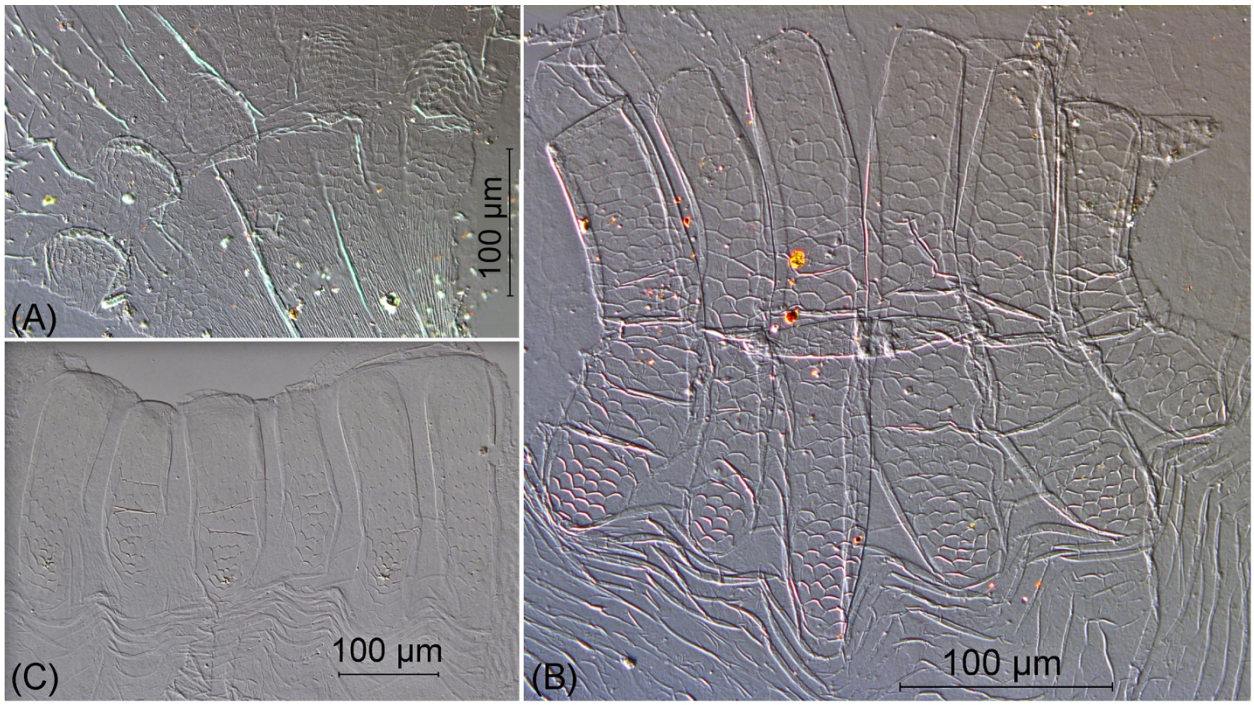


Fig. 7. Internal armature of digestive tube in worker of two *Adaiphrotermes* species. (A) Gizzard, CIVT232. Note five swollen pulvilli bearing pectinated scales. Direction of flow downwards. (B) Enteric valve, CIVT232. The armature consists of six scaly pads originating in the funnel-like end of P1 and proceeding through a curved, narrow tubular section to the paunch entrance. Direction of flow upwards. (C) Enteric valve, BDIT4-3f1. Scaly pads bear tiny spines and follow a much shorter tube to the paunch than in the preceding species. Direction of flow upwards.

Supporting Information

Appendix S1. Revised identifications.

Table S1. Apicotermitinae samples used in this study.

Table S2. Species included as outgroups in this study.

Figure S1. Bayesian phylogenetic chronogram of Apicotermitinae inferred from mitochondrial genomes, with third codon positions included.

Figure S2. Bayesian phylogenetic tree of Apicotermitinae inferred from mitochondrial genomes.

Figure S3. Maximum-likelihood phylogenetic tree of Apicotermitinae inferred from mitochondrial genomes.

Figure S4. Ancestral biogeographic reconstruction for the Bayesian phylogenetic time-calibrated tree with third codon positions excluded.

Figure S5. Ancestral biogeographic reconstruction for the Bayesian phylogenetic time-calibrated tree with third codon positions included.

Figure S6. Ancestral biogeographic reconstruction for the Bayesian phylogenetic tree of Apicotermitinae.

Figure S7. Ancestral biogeographic reconstruction for the Maximum likelihood phylogenetic tree of Apicotermitinae.

Figure S8. Ancestral state reconstruction of soldier presence for the Bayesian phylogenetic tree of Apicotermitinae using the Maximum Likelihood all-rate-different model.

Figure S9. Ancestral state reconstruction of soldier presence for the Bayesian phylogenetic tree of Apicotermitinae using the Maximum Likelihood equal-rate model.

Figure S10. Ancestral state reconstruction of soldier presence for the Bayesian phylogenetic tree of Apicotermitinae using the Maximum Likelihood symmetrical model.