

# Phylogenetic analysis of Blaberoidea reveals non-monophyly of taxa and supports the creation of multiple new subfamilies

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## Abstract

The superfamily Blaberoidea is a highly species-rich group of cockroaches. High-level blaberoidean phylogenetics are still under debate owing to variable taxon sampling and incongruence between mitochondrial and nuclear evolution, as well as different methods used in various phylogenetic studies. We here present a phylogenetic analysis of Blaberoidea based on a dataset combining the mitochondrial genome with two nuclear markers from representatives of all recognized families within the superfamily. Our results support the monophyly of Blaberoidea, which includes Ectobiidae s.s. (=Ectobiinae), Pseudophyllodromiidae, Nyctiboridae, Blattellidae s.s. (=Blattellinae) and Blaberidae. Ectobiidae s.s. was recovered as sister to the remaining Blaberoidea in all inferences. Pseudophyllodromiidae was paraphyletic with respect to *Anaplectoidea* + *Malaccina*. Blattellidae s.s. excluding *Anaplectoidea* + *Malaccina* formed a monophyletic group that was sister to Blaberidae. Based on our results, we propose a revised classification for Blaberoidea: Anaplectoidinae subfam.nov. and *Episorineuchora* gen.nov., and two new combinations at species level within Pseudophyllodromiidae; Rhabdoblattellinae subfam.nov., Calolamprodiinae subfam.nov., *Acutirhabdoblatta* gen.nov., as well as new combinations for three species within Blaberidae. Ancestral state reconstructions based on four morphological characters allow us to infer that the common ancestor of blaberoidean cockroaches is likely to be a species with characteristics similar to those found in Ectobiidae, that is, front femur Type B, arolium present, abdomen with a visible gland and male genital hook on the left side.

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## Introduction

Blaberoidea (Fig. 1) is a large Blattodea group that comprises nearly half of the species of cockroaches (Djernæs, 2018). Previously, only two families were assigned into this group, viz. Blattellidae Brunner von Wattenwyl and Blaberidae Saussure (Roth, 2003; Beccaloni, 2014). Nevertheless, the paraphyletic nature of Blattellidae has been revealed by several studies (Grandcolas, 1996; Klass

and Meier, 2006; Inward et al., 2007). Especially over the last few years, some studies have resulted in changes in the arrangement of blaberoidean groups (Djernæs et al., 2015; Legendre et al., 2015; Evangelista et al., 2019, 2021; Djernæs et al., 2020).

Based on the result of Djernæs et al. (2015) and Wang et al. (2017a), the genus *Anaplecta* was transferred from Blaberoidea to Blattoidea. Djernæs et al. (2020) revised Blaberoidea into five families: Ectobiidae (Ectobiinae), Pseudophyllodromiidae (Pseudophyllodromiinae), Nyctiboridae (Nyctiborinae), Blattellidae (Blattellinae) and Blaberidae, based on mitochondrial genes paired with nuclear genes. This

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Fig. 1. Examples of taxa from Blaberoidea. (a) *Rhabdoblattella disparis* (Blaberidae); (b) *Calolamprodes beybienkoi* (Blaberidae); (c) *Opisthoplattia orientalis* (Blaberidae); (d) *Acutirhabdoblatta densimaculata* (Blaberidae); (e) *Caeparia donskoffi* (Blaberidae); (f) *Paranauphoeta nigra* (Blaberidae); (g) *Pseudoglomeris magnifica* (Blaberidae); (h) *Chorisoserrata biceps* (Pseudophyllodromiidae); (i) *Allacta transversa* (Pseudophyllodromiidae); (j) *Anaplectoidea varia* (Pseudophyllodromiidae); (k) *Episymphloe sinensis* (Blattellidae); (l) *Hemithyrsocera vittata* (Blattellidae). Taxonomy following changes introduced herein. All species were photographed from China by Lu Qiu (a, b, d, f, g, k), Dong-Dong Wang (c, h, i), Zhi-Wei Dong (e), Xin-Ran Li (j) and Jin Chen (l).

revision was also generally supported by Evangelista et al. (2021) on the basis of 265 genomic loci. However, phylogenetic relationships among these five families remain incompletely resolved. Evangelista et al. (2021) proposed a two-subfamily system for Pseudophyllodromiidae by considering *Anallacta* (which previously was a member of Blattellidae) as a new subfamily within it, implying that one of the key criteria used to delimit Pseudophyllodromiidae and Blattellidae, that is, the location of the hook-like phallosomere, is no longer considered reliable.

The genera *Anaplectoidea* and *Malaccina* possess notable differences compared with other blaberoids, including their small size and the reflexed appendicular field of their hind wings (Roth, 1996). Recent phylogenetic work has reported that these two genera are more closely related to Pseudophyllodromiinae than other blaberoid lineages (Jin, 2021). The systematic status of *Anaplectoidea* and *Malaccina* therefore requires further attention.

The sister group of the speciose family Blaberidae has remained ambiguous, with a number of candidates



proposed, including Pseudophyllodromiidae (Grandcolas, 1996), Ectobiidae + Pseudophyllodromiidae (Djernæs et al., 2020), Blattellinae (Bourguignon et al., 2018; Li, 2022), Nyctiborinae (Klass, 1997; Wang et al., 2017a) and Nyctiboridae + Blattellidae (Evangelista et al., 2019, 2021). At present, there are 12 subfamilies within Blaberidae, including 10 subfamilies recorded by Roth (2003), Paranauphoetinae elevated by Anisyutkin (2003) and Geoscapheinae proposed by Rugg and Rose (1984). Previous contributions on Blaberidae phylogeny have also revealed discordant relationships among most subfamilies, particularly Epilamprinae and Perisphaerinae (Djernæs et al., 2012; Legendre et al., 2014; Legendre et al., 2017; Bourguignon et al., 2018; Evangelista et al., 2019, 2021; Arab et al., 2020).

Epilamprinae is one of the most diverse groups in Blaberidae. Although there has yet to be a study that has specifically focused on this subfamily, some studies have recovered it as polyphyletic and with ambiguous generic-level relationships (Legendre et al., 2017; Wang, 2018; Djernæs et al., 2020). Unlike many other Epilamprinae genera, *Rhabdoblattella* and *Calolampyprodes* have an unusual sclerite R of the male genitalia. Their phylogenetic position within the family is unclear. The understanding of relationships within Perisphaerinae is also inadequate. From the morphological standpoint, this subfamily remains to be clearly defined because four out of five synapomorphies proposed by Grandcolas (1997) have been refuted by subsequent studies (Anisyutkin, 2003; Li et al., 2018). McKittrick (1964) suggested that Perisphaerinae and Epilamprinae were closely related, while a close relationship between Perisphaerinae and Panesthiinae was proposed by Anisyutkin (2003). However, based on molecular phylogenies, Perisphaerinae was not monophyletic and its placement within Blaberidae was uncertain (Legendre et al., 2017; Djernæs et al., 2020).

In recent decades, a number of studies have employed both mitochondrial and nuclear data to reconstruct phylogenetic relationships within Blattodea. However, most of these studies have used a small number of mitochondrial and nuclear gene sequences (Pellens et al., 2007; Djernæs et al., 2012; Legendre et al., 2014; Djernæs et al., 2015; Legendre et al., 2017; Wang et al., 2017a; Djernæs et al., 2020). Owing to high mutation rates and more phylogenetic signal, whole mitochondrial genomes have been used to infer the phylogeny of Blattodea (Bourguignon et al., 2018; Deng et al., 2023). On the other hand, compared with mitochondrial sequences, nuclear genes have high homogeneity of among-site rate variation and a slow rate of evolution, and thus are more suitable to resolve relationships at deep taxonomic levels (Lin and Danforth, 2004). More recently, Beasley-Hall et al. (2021) and Che et al. (2022) have conducted molecular

analyses of specific Blattodea groups based on whole mitogenomes and nuclear markers. This approach could help shed light on internal relationships of Blaberoidea at both high and deep levels.

To date, approximately 80 complete mitochondrial genomes of Blaberoidea have been published in the NCBI GenBank database (<https://www.ncbi.nlm.nih.gov/>). In this study, we combined the whole mitochondrial genomes with nuclear data to present the most species-rich phylogenetic analysis of Blaberoidea, of which 93 species were newly sequenced. Further, we conducted ancestral state reconstructions based on the phylogenetic framework to outline the morphological evolution of Blaberoidea. Based on our results, we proposed a revised classification for Blaberoidea and improved the knowledge of the blaberoidean evolutionary history.

## Material and methods

### Material examined

Direct observations and dissections were made with a Motic K400 stereomicroscope or a Leica M205A stereomicroscope. Photographs were taken with a Leica DFC digital microscope camera attached to a Leica M205A stereomicroscope. All photos and images were processed with Adobe Photoshop CS6. The terminology for sclerites in male genitalia follows McKittrick (1964) and Klass (1997).

### Sampling, DNA sequencing and mitochondrial genome annotation

Ninety-three new mitochondrial genomes were generated in this study, including those from 38 Blaberidae species, 31 Pseudophyllodromiidae species and 24 Blattellidae species (Table S1). All specimens were preserved in 100% ethanol at  $-80^{\circ}\text{C}$  until DNA extraction, and deposited in the Institute of Entomology, College of Plant Protection, Southwest University.

As these sequences were acquired over a period of four years, mitogenomes were sequenced using the whole-genome shotgun sequencing either at Personal Biotechnology Co. Ltd, Shanghai, China or at Genesky Biotechnologies Inc. Shanghai, China. The former company directly provided assembled sequences, while the latter provided raw data with DNA sequencing done using a Illumina NovaSeq (150 bp paired-end reads), then quality trimming was conducted using AdapterRemoval (Schubert et al., 2016) and SOApec (Luo et al., 2012). We assembled mitogenomes using Geneious Prime 2020.2.2 (Biomatters Ltd, Auckland, New Zealand). Finally, for each mitogenome, annotation of tRNA genes was performed using MITOS webservers (Bernt et al., 2013) with the invertebrate genetic code and default settings. Protein-coding genes and rRNA genes were identified by homologous alignment using MegAlign (DNASTAR Lasergene Inc.). For nuclear data, the *18S* sequences of some species were obtained from Sanger sequencing followed by primer walking (forward, 5'-ATGGTTGCAAAGCTGAAAC-3'; reverse, 5'-TGCTTTRAGCACTCTAA-3'). Total DNA was purified from muscle tissues using a Hipure Tissue DNA Mini Kit; the amplification reaction was performed following the protocols described in Wang et al. (2021) and sequencing was carried out by BGI Technology Solutions Co. Ltd (BGI-Tech, Beijing, China). The remaining *18S*

and 28S sequences were assembled in Geneious Prime (Biomatters Ltd) from raw data based on the homology alignment to related species. More detailed information was provided in Table S1. All sequences were deposited in GenBank with GenBank accession numbers OQ736902-OQ736999 and OQ737943-OQ738194.

### Sequence alignment and dataset

The final dataset included 216 mitochondrial genomes, 161 18S and 164 28S sequences, representing 172 taxa of Blaberoidea. These blaberoidean samples belonged to 67 genera of all five recognized families (Djernæs et al., 2020). Nine taxa of Mantodea, Phasmatoidea, Mantophasmatodea, Orthoptera, Plecoptera and Dermaptera from GenBank were selected as outgroups (Table S1).

All sequences were aligned in online MAFFT version 7 (Katoh et al., 2019) using the G-INS-i algorithm. The alignments of protein-coding and tRNAs sequences were then manually corrected in MEGA 7 (Kumar et al., 2016), and protein-coding sequences were aligned as nucleotides and translated to amino acids to check for internal stop codons; 12S, 16S, 18S and 28S were assessed with BMGE v1.12 (Block Mapping and Gathering with Entropy; Criscuolo and Grimaldo, 2010). A substitution saturation test in DAMBE 7 (Xia, 2018) showed that the Iss value for the third codon position of the protein-coding genes (Iss = 0.703) was significantly larger than those of the first and second positions (Iss = 0.245) and much closer to the critical value (Iss.cSym = 0.808), which meant that it was much more saturated. As a consequence, we constructed two concatenated datasets with the third codon positions of the protein-coding genes entirely removed: (i) Dataset 1—first and second codon positions of 13 protein-coding genes, 22 tRNA genes, 12S rRNA, 16S rRNA, 18S rRNA and 28S rRNA; and (ii) Dataset 2—the genes of Dataset 1 with the exclusion of tRNA genes. The total length of the aligned Dataset 1 is 16 290 bp, with 22 tRNAs 1468 bp, *COI* 1022 bp, *COII* 442 bp, *COIII* 524 bp, *CYTB* 754 bp, *ATP6* 452 bp, *ATP8* 102 bp, *ND1* 626 bp, *ND2* 684 bp, *ND3* 234 bp, *ND4* 892 bp, *ND4L* 190 bp, *ND5* 1152 bp, *ND6* 330 bp, *12S* 667 bp, *16S* 1017 bp, *18S* 1678 bp and *28S* 4056 bp. The total length of the aligned Dataset 2 is 14 822 bp. The heterogeneous sequence divergence of the two datasets was analysed using AliGROOVE (Kück et al., 2014) with the default sliding window size. Considering that the AliGROOVE analysis of Dataset 1 received relatively higher scores than that of Dataset 2 (Appendix S4), below we only discuss the results from the analyses of Dataset 1.

### Phylogenetic analyses and topology test

We inferred phylogenetic analyses of Blaberoidea utilizing maximum likelihood (ML) and maximum parsimony (MP). Each dataset was analysed with PartitionFinder v.2.1.1 (Lanfear et al., 2017) to determine the best-fitting partitioning scheme and substitution models and the settings were as follows: branchlengths = linked, models = all, model\_selection = AICc (the corrected Akaike information criterion) and search = greedy. The best-fitting substitution model for 18S was a SYM model with gamma-distributed rate variation across sites and a proportion of invariable sites (SYM + I + G), and the best model for the remaining partitions was a GTR + I + G model.

The ML reconstruction was performed using IQ-TREE v.1.6.12 (Nguyen et al., 2015) under the best model of sequence evolution. Branch support was assessed by 10 000 ultrafast bootstrap replicates (Minh et al., 2013; Hoang et al., 2018) and the SH-like approximate likelihood ratio (SH-aLRT) test with 5000 replicates (Guindon et al., 2010). The maximum parsimony inference was performed using PAUP\* v.4.0a169 (Swofford, 2003), and we ran heuristic searches with 100 random sequence addition replicates using a tree-bisection-reconnection branch-swapping algorithm. Support values

were calculated using 1000 bootstrap replicates. Bremer support values (BrS) for each node (Bremer, 1994) were additionally calculated using the script bremer.tcl in conjunction with PAUP\* (Göker et al., 2009).

Additionally, we conducted the approximately unbiased test (AU test) (Shimodaira, 2002) for possible alternative topologies with respect to the placement of Ectobiidae, *Anaplectoidea* + *Malaccina* and *Sorineuchora formosana*. These constrained trees were compared with the unconstrained one (the ML tree from Dataset 1) by computing log-likelihood with the -zb 10 000 -au parameters in IQ-TREE (see Table S2 and Appendix S1 for details).

### Ancestral state reconstructions

To study the evolution of morphological characters of Blaberoidea, we performed ancestral state reconstructions (ASR) on the ML framework of Dataset 1. Five discrete characters were selected as follows: (i) the position of the male genital hook (two states)—left and right; (ii) the spination on the anteroventral edge of the front femur (four states)—large proximal spines succeeded by progressively shorter spines and the distal spines larger (Type A, Fig. 6g), proximal spines succeeded by a row of piliform spinules (Type B, Fig. 6h), proximal spines absent, with a row of piliform spinules (Type C, Fig. 6i), and proximal spines and spinules absent (Type D; see Roth, 2003); (iii) tergal modification (two states)—hidden and visible; (iv) location of tergal glands (nine states)—absent, gland on the first tergite (T1), gland on T2, gland on T7, gland on T8, glands on T1 and T7, gland on T7 and T8, gland on T1 & T2 and gland on T1–T7; and (v) arolia between the claws (two states)—present and absent (or much reduced). These characters were gathered from literature and our direct observation. Unknown features were recorded as “N.A.” (more details are provided in Table S3).

Stochastic character mapping was implemented in the function make.simmmap of R package phytools v.1.0-1 (Bollback, 2006; Revell, 2012). We used the fitMk function in ape v.4.1 package (Lewis, 2001) to select the best-fitting model for each character, of which, the one with the lowest AIC (the Akaike information criterion) values was chosen (Posada and Buckley, 2004).

## Results

### The phylogeny of different datasets

We present ML and MP results based on two datasets (Datasets 1 and 2) in Fig. 2 and Figs S1–S3 (more details for Bremer support values are provided in Appendix S3). Regardless of the method and dataset used, Blattoidea, Corydioidea and Blaberoidea were consistently recovered as monophyletic groups with robust support. Within Blaberoidea, our ML trees (Figs 2 and S1) using Datasets 1 and 2 produced similar topologies with respect to backbone relationships, with both supporting the relationship of Ectobiidae as a sister to the remaining Blaberoidea (SH-aLRT/MLBS: 100/100, 100/100). MP trees (Figs S2 and S3) using Datasets 1 and 2 yielded largely congruent topologies, with the exception of Ectobiidae and Anallactinae. All analyses recovered the monophyly of Ectobiidae, Nyctiboridae and Blaberidae with strong support; Pseudophyllodromiidae was retrieved as a paraphyletic with respect to *Anaplectoidea*

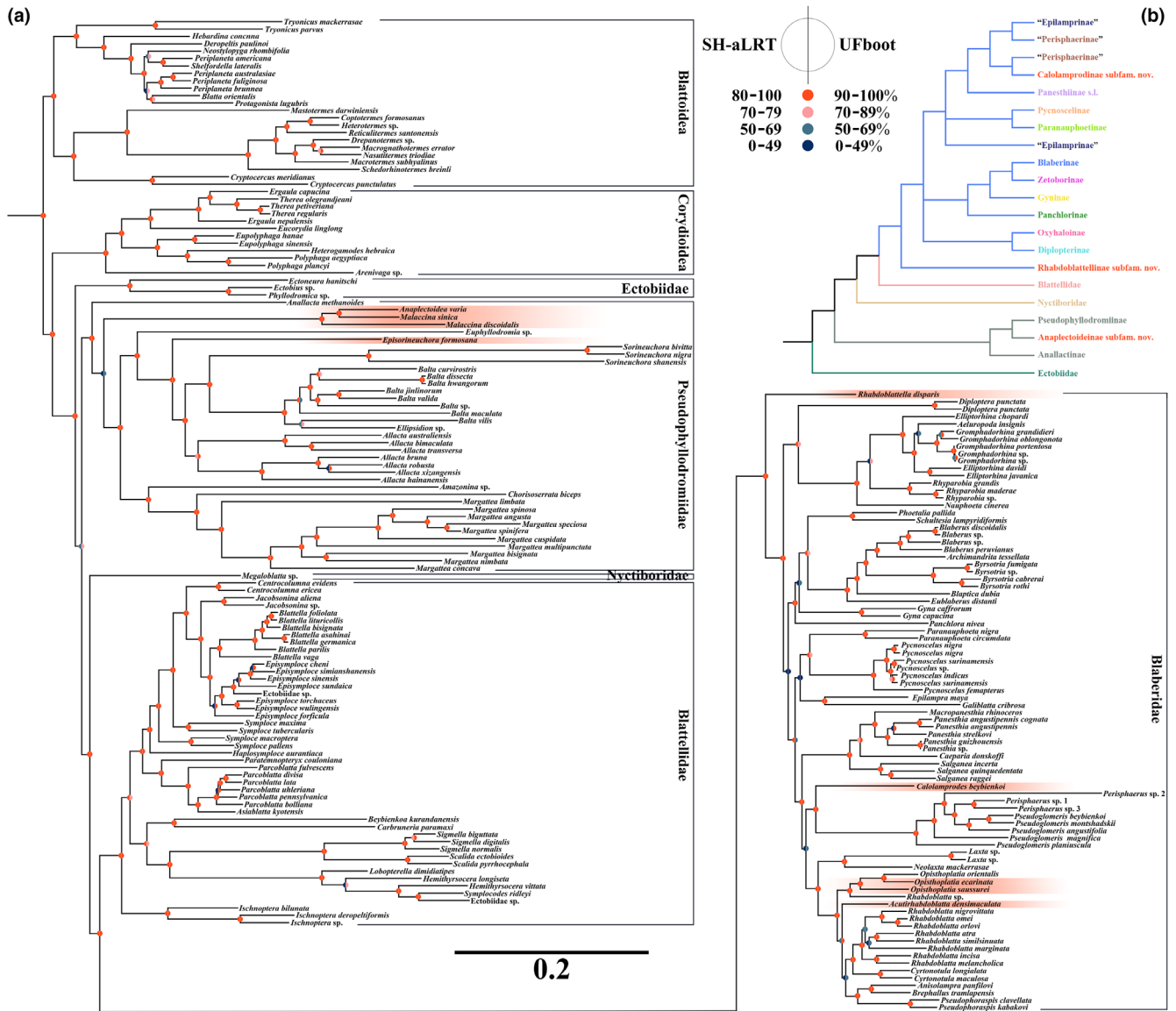


Fig. 2. Phylogeny of Blaberoidea. (a) Topology generated by maximum likelihood (ML) analysis of Dataset 1, with pie charts at nodes representing support values (left, SH-aLRT; right, UFboot). (b) Represented by a simplified tree, nodes are collapsed when UFboot support is <50. The new classification proposed in this paper is used to annotate the tree with polyphyletic groups marked by double quotes.

and *Malaccina*, with the remaining Blattellidae forming a monophyletic group that was the sister group of Blaberidae with strong support. The relationship of Nyctiboridae with the remaining Blattellidae and Blaberidae was stable in all analyses, namely, Nyctiboridae + (Blattellidae + Blaberidae) (note that we only included one nyctiborid taxon).

Within Pseudophyllodromiidae, we recovered a close relationship between *Anaplectoidea* + *Malaccina*, Anallactinae and Pseudophyllodromiinae in our ML analyses (SH-aLRT/MLBS: 98.9/97). In the MP tree *Anaplectoidea* + *Malaccina* was nested within

Pseudophyllodromiinae (BrS/MPBS: –11/45) and together they were a sister to Anallactinae, then this combined clade was sister to Ectobiidae (BrS/MPBS: –4/22). *Margattea* was recovered as a monophyletic group in ML analysis with high support (SH-aLRT/MLBS: 93.6/100) but as a paraphyletic group in the MP tree. *Sorineuchora* was paraphyletic in ML analysis with high support values (SH-aLRT/MLBS: 100/100), in contrast to the monophyly of *Sorineuchora* in MP analysis (BrS/MPBS: 7/70).

Within Blaberidae, the monophyly of the subfamilies Oxyhaloinae, Diplopterinae, Panchlorinae, Gyninae,

Paranauphoetinae and Pycnoscelinae were always recovered in our study, as well as the clades comprising Panesthiinae s.l. (see Djernæs et al., 2020) and Blaberinae + Zetoborinae + Gyninae. Of these subfamilies, Paranauphoetinae formed a sister group with Pycnoscelinae in both analyses (SH-aLRT/MLBS: 90/80; BrS/MPBS: 7/27); Oxyhaloinae was placed as the sister group to Diplopterinae (SH-aLRT/MLBS: 100/87), or Diplopterinae + Panchlorinae (BrS/MPBS: 9/29). In addition, the polyphyly of Epilamprinae and Perisphaerinae was also consistently found, with terminal taxa of the two subfamilies split into four and two clades, respectively. These four clades of Epilamprinae were widely separated across the Blaberidae, with robust support for the monospecific genus *Rhabdoblattella* being the earliest branch within Blaberidae (SH-aLRT/MLBS: 100/100; BrS/MPBS: 85/100). *Calolampyris* was found as sister group to one of the clades comprising Perisphaerinae (*Perisphaerus* + *Pseudoglossa*) (SH-aLRT/MLBS: 96.9/91; BrS/MPBS: -4/28). The remaining epilamprines, consisting of *Anisilampyris*, *Brephallus*, *Cyrtonotula*, *Opisthoptilia*, *Pseudophraspis* and *Rhabdoblatta*, formed a well-supported monophyletic group that was sister to two other genera of Perisphaerinae (*Laxta* + *Neolaxta*) (SH-aLRT/MLBS: 99.7/98; BrS/MPBS: -3/48). However, *Rhabdoblatta* was recovered to be a polyphyletic group with respect to *R. densimaculata*, *R. ecarinata* and *R. saussurei*.

### Morphological evolution

The estimated ancestral states of selected characters are presented in Figs 3 and 4. The ancestral Blaberoidea was inferred to have a genital hook on the left side (100%), with a switch occurring at least twice in this superfamily. One shift occurred in the ancestor of Pseudophyllodromiinae (98.9%). The genital hook on the right side was recovered as the ancestor of Blaberidae (91.1%) and is present in all known species of this family (Fig. 3 and Appendixes S2, S5). In Ectobiidae and Blattellidae, the ancestral state has been retained with no reversal.

Our ASR analyses showed that the ancestor of Blaberoidea was most likely to have visible tergal modifications (100%), as did the common ancestors of each of its families (88.3–100%). This trait was independently lost three times in Pseudophyllodromiidae, once in Nyctiboridae and four times in Blattellidae. With regard to the location of the tergal glands, eight conditions of modified tergites were observed in 69 blaberoidean taxa in our study. The T7 was the most commonly specialized segment, which was found in all Ectobiidae. Four genera of Pseudophyllodromiidae with modified T7 were observed, and glands occurring on T7 and T8 independently emerged in *Margattea*. In

Blattellidae, there was a 43.2% probability that glands were originally located on the seventh segment, with glands present on either T7, T7 and T1, or T7 and T8 with the exception of *Parcoblatta*, *Asiablatta* and *Beybienkoa*, which have glands on T1, or T1 and T2. However, the loss of tergal glands has occurred multiple times across Blaberidae, and they were only retained in a few taxa within this family, for example, *Schultesia* and *Cyrtonotula* with T1 specialized, *Rhyparobia maderae* with T2 specialized, but *Rhabdoblattella disparis* with T1–T7 all specialized (Fig. 4 and Appendix S2).

Our results indicate that the common ancestor of Blaberoidea possessed arolia between the tarsal claws (100%). This trait has been retained in the majority of extant Blaberoidean species, but might have been independently lost once in Nyctiboridae, and three times across the Blaberidae, including once in Blaberinae (99.8%), once in Paranauphoetinae (99.8%) and once in Panesthiinae + Geoscapheinae (99%) (Fig. 3 and Appendixes S2, S5).

With regard to the front femur, our results suggested that Type B was the most likely ancestral state of Blaberoidea (73.7%), and was also the ancestral type of all its families (73.3–86.7%). Type A independently evolved several times in Blattellidae and once in Blaberidae (*Neolaxta*). This trait also had two independent origins in Pseudophyllodromiidae, once in *Anallacta* and once in the ancestor of *Malaccina sinica* + *Anaplectoidea varia* (99.1%). Type C probably originated three times in Pseudophyllodromiidae and evolved several times across Blaberidae (Fig. 3 and Appendix S2).

## Discussion

### Phylogeny of Blaberoidea

Within Blaberoidea, the monophyly of Blaberidae is undisputed in prior studies, regardless of whether morphological characters (e.g. McKittrick, 1964; Grandcolas, 1996; Klass and Meier, 2006), molecular data (e.g. Inward et al., 2007; Wang et al., 2017a; Bourguignon et al., 2018; Evangelista et al., 2019) or combined molecular and morphological data (Djernæs et al., 2015) are used to infer relationships. The sister group of Blaberidae has remained unclear. The molecular phylogeny presented herein provided strong support for the division of Blaberoidea into five principal lineages, as also found in previous studies: Ectobiidae (Ectobiinae), Pseudophyllodromiidae (Pseudophyllodromiinae), Nyctiboridae (Nyctiborinae), Blattellidae (Blattellinae) and Blaberidae (Djernæs et al., 2020; Evangelista et al., 2021). Our results confirmed that three of these families were monophyletic, and the



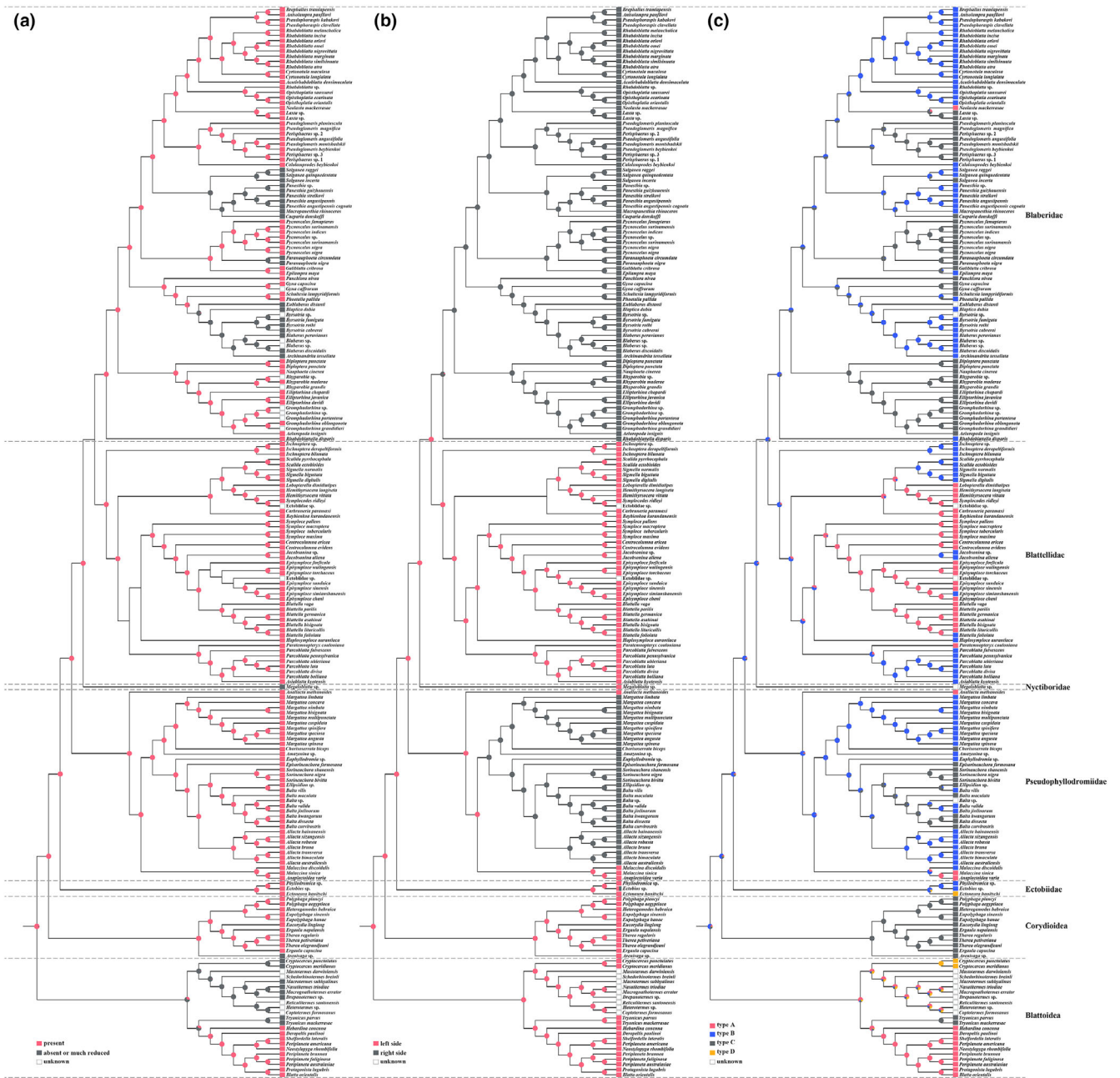


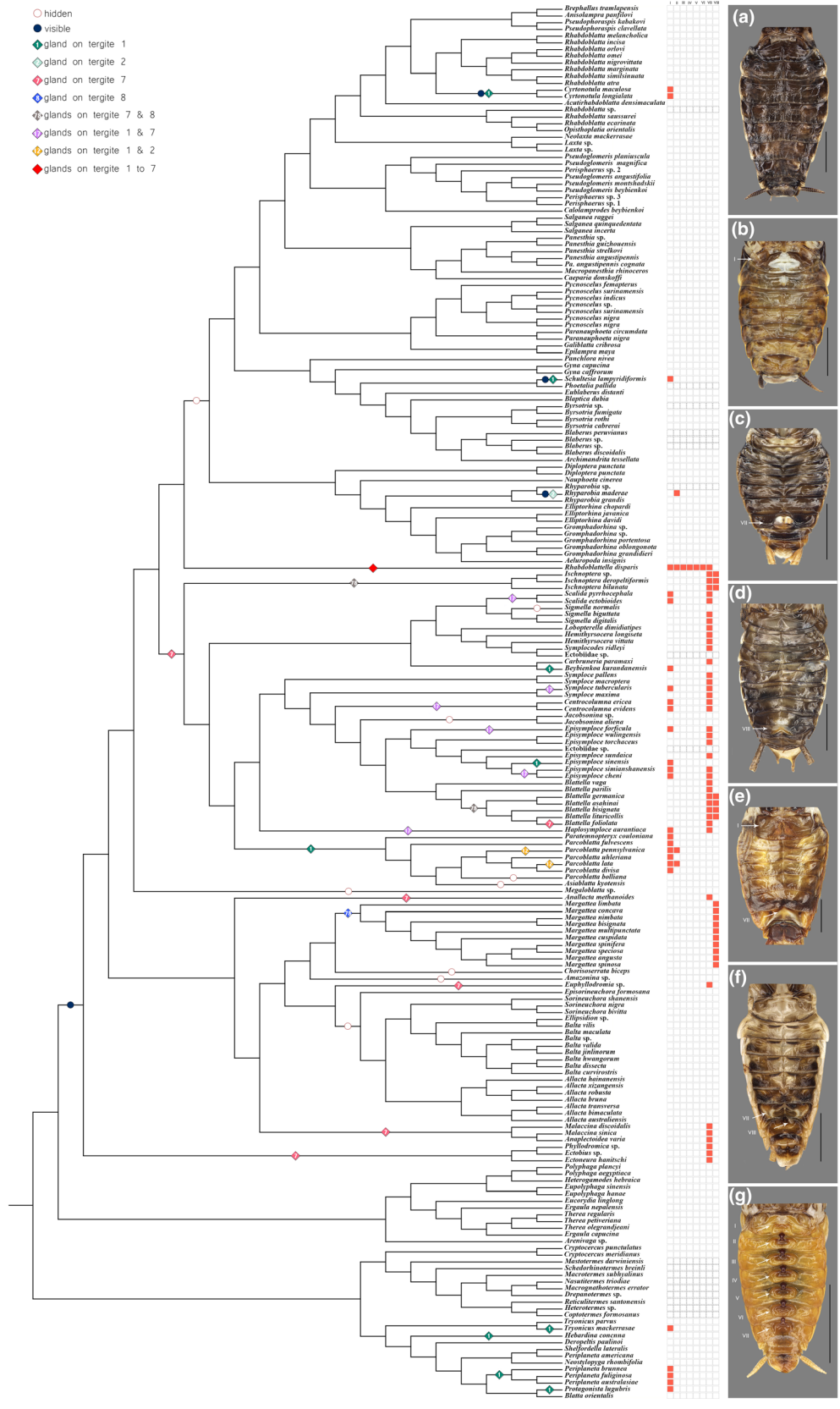
Fig. 3. Ancestral state reconstruction of selected characters of Blaberoidea. Topology generated by ML analysis of Dataset 1. (a) Arolium reconstructed under the ER model. (b) Position of male genitalia hook reconstructed under the ARD model. (c) Type of front femur reconstructed under the ER model. Pie charts at the internal nodes represent posterior probabilities of character states.

other two (Pseudophyllodromiidae and Blattellidae) were not.

Regarding interfamilial relationships, the inferred position of Ectobiidae varied among our analyses,

being well supported either as a sister to the remaining Blaberoidea in both ML trees (Fig. 2; Fig. S1), or as sister to the clade composed of *Anallacta*, *Anaplectoidea* + *Malaccina* and Pseudophyllodromiinae

Fig. 4. Reconstructed shifts of tergal glands on the phylogeny, based on the results of ancestral state reconstruction. Illustrations on the right represent the tergal morphology of Blaberoidea: (a) Epilamprinae—*Acutirhabdoblatta densimaculata*; (b) Epilamprinae—*Cyrtonotula longialata*; (c) Anaplectoidinae—*Anaplectoidea varia*; (d) Pseudophyllodromiinae—*Margattea concava*; (e) Blattellidae—*Centrocolumna ericea*; (f) Blattellidae—*Blattella bisignata*; and (g) Rhabdoblattellinae—*Rhabdoblattella disparis*. Scale bars: (a, b, g) 5 mm; (c–f) 2 mm.





(the MP tree of Dataset 1, Fig. S2), or grouped with *Anallacta* placed elsewhere (the MP tree of Dataset 2, Fig. S3). The former was consistent with that of Evangelista et al. (2019, 2021), which was inferred from transcriptome data or 256 loci with strong support. Nonetheless, in other recent molecular studies based on multiple gene markers, the sister relationship between Ectobiidae and Pseudophyllodromiidae was consistently found, but all with low support values (Inward et al., 2007; Legendre et al., 2015; Djernæs et al., 2020). The above indicated that Ectobiidae was more likely to be the sister of the other Blaberoidea, while the topology that constrained the monophyly of Ectobiidae and Pseudophyllodromiidae was not rejected in the present study (Table S2; Appendix S1). The phylogenetic position of Ectobiidae needs to be confirmed in future studies.

#### Redefinition of Blattellidae and Pseudophyllodromiidae

Based on our phylogenetic analyses, Blattellidae taxa failed to form a monophyletic lineage, with *Anallacta*, *Anaplectoidea* and *Malaccina* recovered at distant positions from this group. Most of our analyses showed that *Anallacta* was a sister to the clade containing *Anaplectoidea* + *Malaccina* and Pseudophyllodromiinae (Fig. 2; Figs S1 and S2). A close relationship between *Anallacta* and Ectobiidae was recovered in Bourguignon et al. (2018); while in Evangelista et al. (2019, 2021) and Bläser et al. (2020), the genus *Anallacta* was found as a sister group to Pseudophyllodromiinae. Regarding the status of *Anallacta*, Evangelista et al. (2019) proposed a new subfamily Anallactinae with *Anallacta* as the type genus (also included was another genus, *Lobopteromorpha*). Our results are in agreement with the proposal of Evangelista et al. (2021).

*Anaplectoidea* had previously been sampled in Wang et al. (2017a) and D.A. Evangelista (personal communication), the former inferred *Anaplectoidea* to be deeply embedded in Blattellinae and the latter recovered *Anaplectoidea* as sister to all other Blaberoidea. In this study, the genus *Malaccina* was introduced for the first time and grouped together with *Anaplectoidea* in all analyses (Fig. 2; Figs S1–S3), and then this clade was recovered either as a sister to Pseudophyllodromiinae (Fig. 2; Fig. S1) or within it (Figs S2 and S3). However, the alternative topologies recovered in both studies mentioned above and our MP analyses were all rejected in the AU test (Table S2, Appendix S1). Further, the spermatheca of *Malaccina* is similar to those of Pseudophyllodromiinae (one pair of secondary spermathecae and each with one ampulla; Liu et al., 2023: Fig. 4), but distinctly different from those of other non-blaberid Blaberoidea. Therefore, we considered the sister relationship of *Anaplectoidea* +

*Malaccina* and Pseudophyllodromiinae to be more reliable.

In the early taxonomic works, Hebard (1929) considered *Malaccina* Hebard as the intermediate group between *Anaplectella* Hanitsch and *Anaplectoidea* Shelford, and these three genera share some characters, including the seventh abdominal tergites usually specialized in males (Fig. 4c), hind wings with reflexed appendicular field (Fig. 5c–e), and the highly specialized tarsal claws (Fig. 6a). Roth (1996) indicated that these genera all belonged to Blattellinae based on McKittrick's (1964) system with respect to the side of the retractable hook. Unfortunately, we were not able to include *Anaplectella* in our study. Our phylogenetic analysis found that *Anaplectoidea* was nested within *Malaccina*, of which *A. varia* was clustered with *M. sinica*, and *M. discoidalis* was placed as the basal taxon to *Anaplectoidea* + *Malaccina*. It is noteworthy that *M. sinica* was transferred from *Anaplectoidea* by Roth (1996). The main differences between these two genera were the number of incomplete branches of the hind wing cubitus vein and the length of the appendicular field of hind wings. Based on the phylogenetic result and morphological similarities, *Anaplectoidea* and *Malaccina* should probably be recognized as one genus, as also hypothesized by Che et al. (2017). Future studies including a more extensive sampling for these genera will be necessary to resolve the current ambiguity in their relationships. Moreover, the sister-group relationship between *Anaplectoidea* + *Malaccina* and Pseudophyllodromiinae indicated that the establishment of one subfamily was needed to accommodate *Anaplectoidea* and *Malaccina* (Anaplectoidinae subfam. nov., more details in *Taxonomic revision*). Thus, Pseudophyllodromiidae was modified to include three monophyletic subfamilies herein.

In Pseudophyllodromiinae, our ML analyses strongly supported that *Sorineuchora formosana* was sister to the clade consisting of *Allacta*, *Ellipsoidion*, *Balta* and other *Sorineuchora* (Fig. 2; Fig. S1). Previously, the only molecular phylogenetic analysis of Blattodea to include *Sorineuchora* species was Wang et al. (2017a), with a sole species closely related to *Balta*. Morphological studies also proposed that there are several similar character states presented in both *Sorineuchora* and *Balta* (Li et al., 2017). Based on characteristics of the front femur, tarsal claw and unspecialized tergite, Roth (1998) transferred *S. formosana* and *S. lativitrea* from *Chorisoneura* to *Sorineuchora*. However, these two species are still distinguishable from other *Sorineuchora* species by their similar and cylindrical styles, the subgenital plate with medial incision and setae scattered on the margin, and the cubitus vein of hind wings with two or three cross veins (Fig. 5f–h). Thus, *S. formosana* and its related species *S. lativitrea* should be removed from

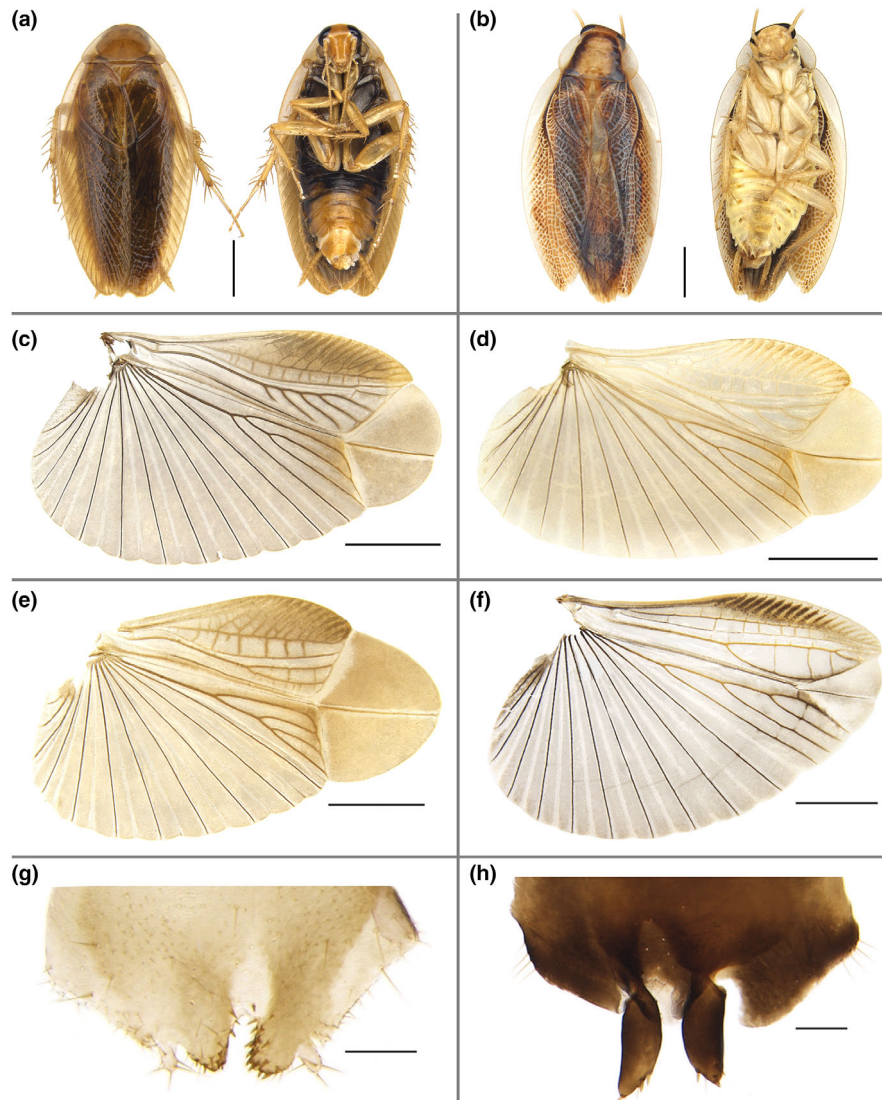


Fig. 5. Habitus, hind wings and subgenital plates of Anaplectoidinae, *Episorineuchora* and *Sorineuchora*. (a, c) *Anaplectoidea varia*. (b, f, g) *Episorineuchora formosana*. (d) *Malaccina sinica*. (e) *Anaplectella lompatensis*. (h) *Sorineuchora nigra*. Scale bars: (a) 1 mm; (b–f) 2 mm; (e, f) 0.2 mm.

*Sorineuchora*. Accordingly, we established *Episorineuchora* gen. nov. to accommodate these two species.

#### Phylogenetic relationships within Blaberidae

Our analyses confirmed Blaberidae as monophyletic with strong support. The relationships among subfamilies and genera in our study confirmed many relationships proposed previously, for example, the close relationship of Diplopterinae + Oxyhaloinae (Bourguignon et al., 2018; Evangelista et al., 2019, 2021). From the morphological standpoint, this sister group relationship is supported by four synapomorphies: the elongated and strongly curved genital hook, minute

undulating irregularities at the inner margin of the curved portion of the hook and the coleopteroid habitus, as well as the spermatophore shaped like a bowling pin (Graves, 1969; Roth, 1971, 1973; D.A. Evangelista, personal communication).

There is still no consensus on the phylogenetic position of Paranauphoetinae and Pycnoscelinae (Anisyutkin, 2003; Djernæs et al., 2012; Legendre et al., 2014; Legendre et al., 2015; Mavropulo et al., 2015; Legendre et al., 2017; Evangelista et al., 2018; Li et al., 2018). Most recently, Djernæs et al. (2020) considered that they should be relegated into tribes within Perisphaerinae. In our study, Paranauphoetinae and Pycnoscelinae were recovered as two distinct

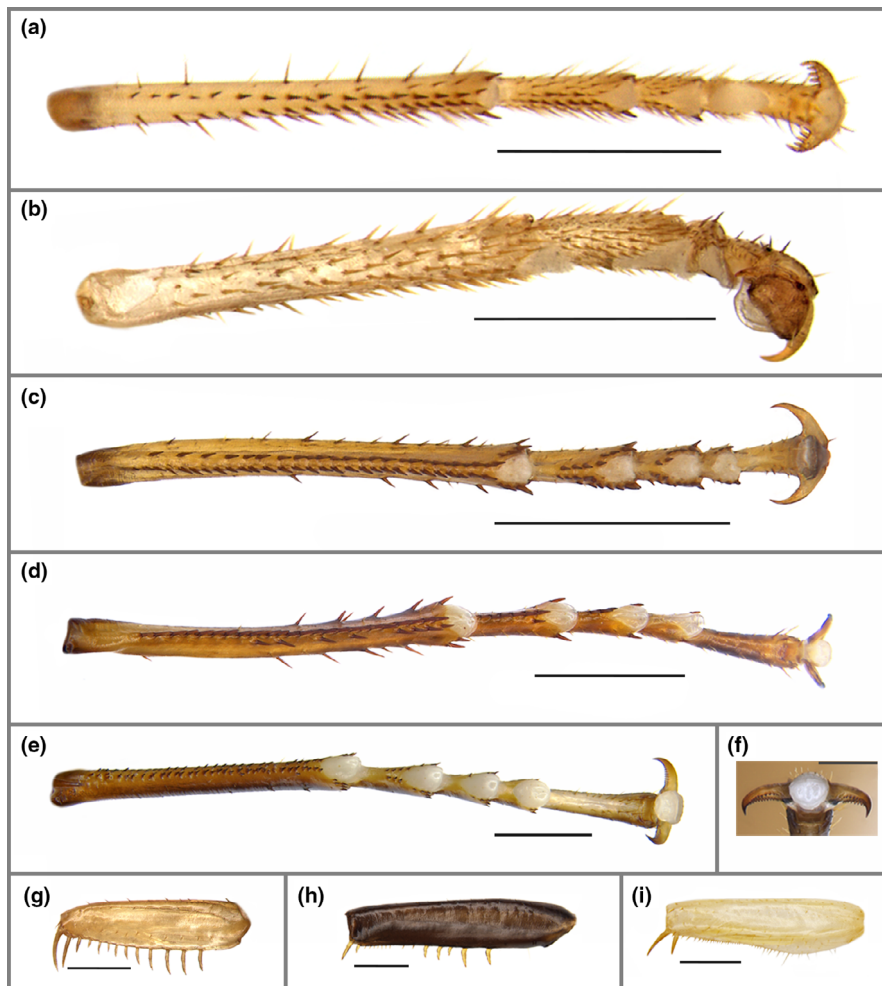


Fig. 6. Legs of newly proposed subfamilies and genera. (a–f) Hind tarsi; (g–i) Front femur. (a, g) *Anaplectoidea varia*. (b, i) *Episorineuchora formosana*. (c) *Rhabdoblattella disparis*. (d) *Calolamprodes beybienkoi*. (e, f, h) *Acutirhabdoblatta densimaculata*. Scale bars: (a, b, f, g, i) 0.5 mm; (c–e, h) 1 mm.

subfamilies and most analyses support their sister relationship (Fig. 2; Figs S2 and S3), as was recovered in Wang et al. (2017a). Furthermore, we also found some morphological characters to support our view: front femur Type C, the similar relative positions of the complex sclerite R, the subgenital plate with the right posterolateral region excavated and the posterior corners sharply produced. Representatives of Pycnoscelinae and Paranauphoetinae in the present paper were each sampled from the type genus (*Pycnoscelus* and *Paranauphoeta*, respectively). Moreover, a close relationship was also confirmed by the morphological similarity between *Proscratea* (another pycnosceline genus) and *Paranauphoeta* (Hebard, 1926; Rehn, 1932). Future phylogenetic studies including *Proscratea* will help to better understand the relationship between Pycnoscelinae and Paranauphoetinae.

The non-monophyly of Epilamprinae and Perisphaerinae has been found previously (e.g. Legendre

et al., 2014; Legendre et al., 2017; Bourguignon et al., 2018; Arab et al., 2020; Evangelista et al., 2021). Our study comprised representatives from 10 genera of Epilamprinae, among them *Rhabdoblattella* and *Calolamprodes* were sampled for the first time. All analyses in our study placed *Rhabdoblattella disparis* outside all Epilamprinae groups, and placed it as the earliest branching lineage within Blaberidae (Fig. 2; Figs S1–S3). The genus *Rhabdoblattella* (Fig. 1a) is characterized by the structure of sclerite R (Fig. 7k) and an asymmetrical subgenital plate with a median tooth on the caudal margin (Fig. 7q) (Anisyutkin, 2000; Anisyutkin and Yushkova, 2017; Wang et al., 2017b). The phylogenetic position and autapomorphic features of *Rhabdoblattella* led us to propose Rhabdoblattellinae subfam.nov. to accommodate this genus. Species of *Rhabdoblattella* are relatively small in size (ca. 20.2–22.7 mm, Wang et al., 2017b) and almost identical to the ancestral body length of both Blaberoidea and



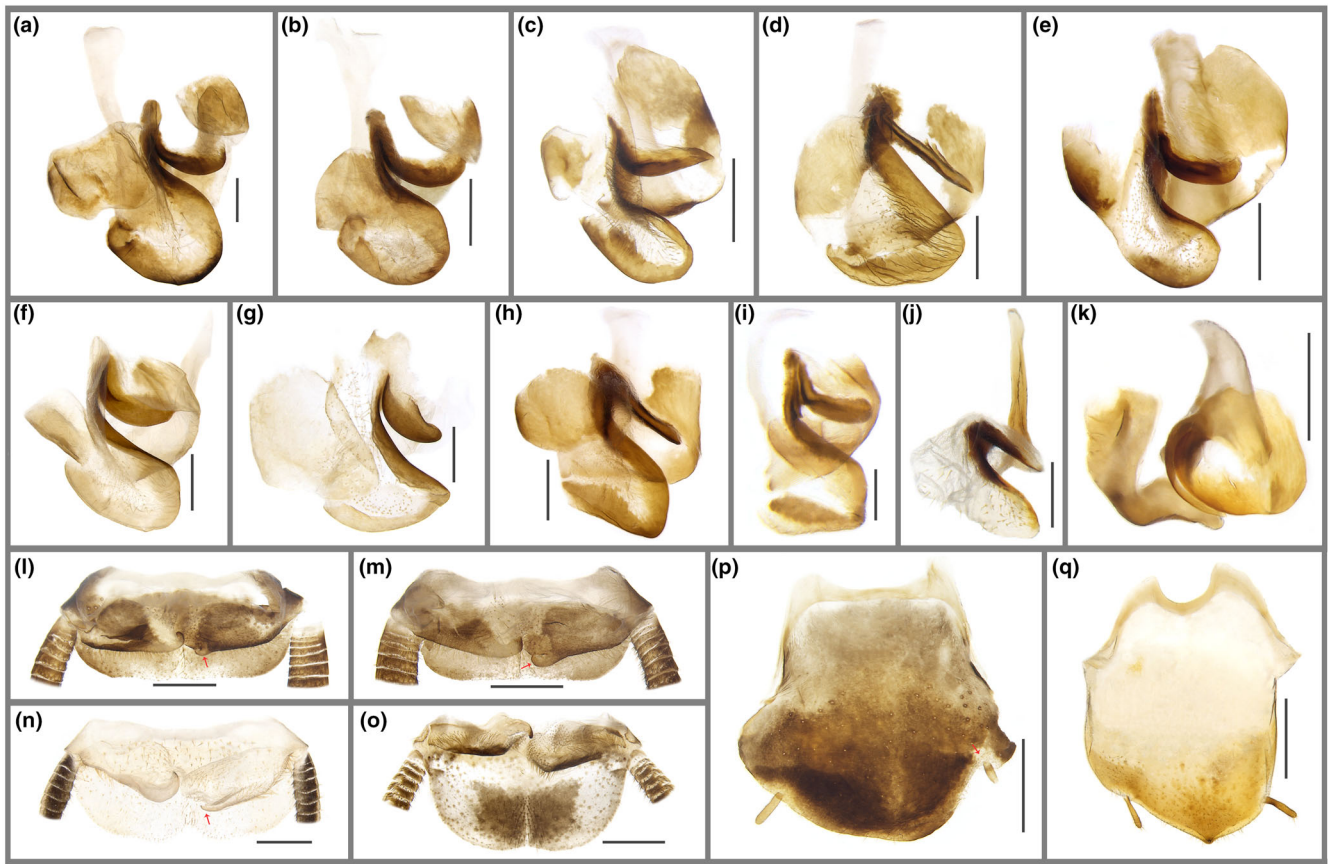


Fig. 7. Representation of external male genitalia of Epilamprinae, Calolamprodinae and Rhabdoblattellinae. (a–k) Sclerite R; (l–o) Supra-anal plate; (p, q) Subgenital plate. (a) *Opisthoptatia orientalis*. (b) *O. ecarinata*. (c, o) *O. saussurei*. (d, l, m) *Acutirhabdoblatta densimaculata*. (e) *Anisoptampra panfilovi*. (f) *Brepballus tramlapensis*. (g, n) *Pseudophoraspis kabakovi*. (h) *Rhabdoblatta marginata*. (i) *Cyrtototula maculosa*. (j, p) *Calolamprodes beybienkoi*. (k) *Rhabdoblattella disparis*. (q) *Rhabdoblattella hainanensis*. Scale bars: (a–k) 0.5 mm; (l–q) 1 mm.

Blaberidae (10–22.5 mm, Djernæs et al., 2020). Instead, the size is comparable with members of Blattellidae, suggesting that they have both retained the plesiomorphic body length. Furthermore, both *Rhabdoblattella* and some Blattellidae (e.g. *Episymphloe*) exhibit notably similar appearances in the male genitalia (Yi-Shu Wang, personal observation). A close relationship between Blattellidae and Blaberidae was strongly supported in this study. Rhabdoblattellinae could thus be considered as plesiomorphic for Blaberidae in comparison with Blattellidae and the sclerite R was possibly a plesiomorphy that underwent a series of evolutionary changes during the diversification of Blaberidae. Similarly, Evangelista et al. (2021) found that *Rhabdoblatta stipata*, an epilamprine cockroach, was also recovered in an early branching position within Blaberidae with high support. Inclusion of more epilamprine samples in subsequent studies would be helpful to elucidate the polyphyly of Epilamprinae.

In our analyses, we found that *Calolamprodes beybienkoi* (Fig. 1b) was always closely related to *Perisphaerus* + *Pseudoglomeris* (Perisphaerinae) (Fig. 2;

Figs S1–S3), which was supported by the similar structure of sclerite R of these taxa (Fig. 7p). In terms of both morphological and molecular data, *Calolamprodes* probably deserved a subfamily rank (Calolamprodinae subfam. nov.). The genus *Rhabdoblatta* was not recovered as a monophyletic group (Fig. 2; Figs S1–S3), which was consistent with the proposal of Anisyutkin (2014). Since the current classification of *Rhabdoblatta* is mostly based on a combination of homoplastic characters rather than autapomorphies, this genus needs further revision. We thus proposed various changes in *Rhabdoblatta* to better reflect the phylogenetic relationships recovered herein. *Rhabdoblatta densimaculata* was recovered as an independent lineage from *Rhabdoblatta*; the new genus *Acutirhabdoblatta* gen. nov. was consequently established to accommodate *R. densimaculata*, while *R. ecarinata* and *R. saussurei* always around *Opisthoptatia* resulted in the new combination *O. saussurei* comb. nov. and *O. ecarinata* comb. nov. (see section *Taxonomic revision* for details). Past studies have shown that the phylogenetically disjunct placements of Epilamprinae and Perisphaerinae are

congruent with their geographic distributions. The Australasian Perisphaerinae, the Asian Perisphaerinae, the African Epilamprinae and the Asian–Oceanian Epilamprinae were each retrieved as a monophyletic group (Legendre et al., 2017; Djernæs et al., 2020). The phylogeny with geographic consistency was also recovered in our study. Furthermore, we found that each clade showed uniqueness in the structure of sclerite R (Fig. 7a–k), with individuals of each clade showing a close resemblance in that structure especially those of the main Epilamprinae group. Sclerite R of this group was composed of five elements with only small variations in shape and relative positions (Fig. 7a–i). Nevertheless, we did not propose modifications to Perisphaerinae and the remaining Epilamprinae owing to the lack of sample examination. However, the systematic position of those doubtful genera should be scrutinized in the future, and a taxonomic revision and phylogenetic analyses including more different taxa might help to clarify the relationship among these two subfamilies.

#### *Evolution of selected morphological characters in Blaberoidea*

Since we considered Anallactinae and Anaplectoidinae as members of Pseudophyllodromiidae, the ancestral state of the genital hook in Pseudophyllodromiidae was recovered as on the left side (Fig. 3b), unlike previously hypothesized by (Bohn, 1987), who speculated “if in some Plectopterinae a re-reversal to the original state could have taken place”. On the other hand, our ASR analyses inferred that transition of the hook from left to right had at least two independent origins, which agreed somewhat with “Alternative A” in Bohn (1987); further, once the shift occurred, it would not be reversed. Also, although we found that no shift occurred in Ectobiidae (Fig. 3b), in fact, there should have once since the *Ectobius* species with both hook positions existed (Bohn, 1987).

In our ASR analyses, the presence of tergal glands was treated as a two-state character, and as a nine-state character for assessing variation in the location (Fig. 4 and Appendix S2). The extraordinary diversity of tergal glands in Blattodea was thought to have evolved in relation to sexual behaviour (Roth, 1969; Brossut and Roth, 1977). Roth (1969) suggested that there was a change in the location of tergal glands from a posterior to a more anterior position on the abdomen in the “Blattellidae” s.l. (including Ectobiidae, Nyctiboridae, Pseudophyllodromiidae and Blattellidae). A similar hypothesis emerged from our analysis: the glands’ position in Blattellidae either independently changed from T7 to T1 or from T1 and T2, or evolved to T7 and T1. Moreover, only a small number of blaberids evolved tergal specializations independently;

when it occurred in *Rhabdoblattella disparis*, to our knowledge, this is the first species in which modified tergites ranging from T1 to T7 (Fig. 4g). The maximum number of modified segments in Blaberoidea used to be five (T2–T4, T7 and T8 in *Pseudomops*, and T4–T8 in *Maretina*) (Roth, 1969). All of the above showed the uniqueness of *Rhabdoblattella disparis* and this trait when it occurred in Blaberidae was more likely inherited from the Blattellidae-like ancestor.

Although the presence of an arolium between the tarsal claws is a common feature in Blattodea, a few lineages were found without this structure in the present study, for example, Blaberinae, Paranauphoetinae and Panesthiinae + Geoscapheinae within Blaberoidea, *Arenivaga* (Corydioidea) and *Tryonicus* (Blattoidea) (Fig. 3a). A possible explanation for this loss is their nesting and feeding habitat: wood feeding or soil burrowing (Bell et al., 2007). Contrary to the lack of arolium in all Blaberinae species, the genus *Phoetalia* has the arolia as does *Schultesia*, which also provides evidence for the taxonomic transference of *Phoetalia* to Zetoborinae.

Overall, on the basis of these results we hypothesized the common ancestor of the blaberoid cockroach to probably be a species that had a visible gland on the abdomen, front femur Type B, arolia between tarsal claws and a genital hook on the left side in males. These characters were quite well matched with most species of Ectobiidae, which was indeed the first derived lineage of Blaberoidea in our inferred phylogeny.

#### *Taxonomic revision*

Following the phylogenetic relationship recovered in our analyses, we propose a revised classification for Blaberoidea, with the establishment of three new subfamilies (Anaplectoidinae subfam. nov., Rhabdoblattellinae subfam. nov., and Calolamprodinae subfam. nov.), two new genera (*Episorineuchora* gen. nov. and *Acutirhabdoblatta* gen. nov.), and five new combinations from the genera *Rhabdoblatta* (*Opisthoplatia saussurei* (Kirby), comb. nov. and *O. ecarinata* (Yang, Wang, Zhou, Wang & Che), comb. nov., and *Acutirhabdoblatta densimaculata* (Yang, Wang, Zhou, Wang & Che), comb. nov.) and *Sorineuchora* (*Episorineuchora formosana* (Matsamura), comb. nov., *E. lativitrea* (Walker), comb. nov.).

*Anaplectoidinae* Wang & Wang subfam. nov. <http://zoobank.org/urn:lsid:zoobank.org:act:F728A7FE-9E79-480A-97C5-EB21180C616C>.

Type genus: *Anaplectoidea* Shelford, 1906, present designation.

Taxa included: *Anaplectoidea* Shelford, 1906, *Malacina* Hebard, 1929, *Anaplectella* Hanitsch, 1928.

Distribution: Oriental Region.

Diagnosis: The new subfamily (Fig. 5a) can be readily distinguished from Pseudophyllodromiinae by the

genital hook on the left side (on the right side for Pseudophyllodromiinae); it differs from Anallactinae by the hindwings with well-developed appendix area (Fig. 5c–e) and the distinctly serrated tarsal claws (tarsal claws simple and unspecialized in Anallactinae; Princis, 1963; Fig. 6a). Anaplectoidinae are further characterized by the following combination of features: body small; cubitus and vein usually with pseudocomplete and incomplete branches; front femur Type A (Fig. 6g) or B; tarsal claws symmetrical; male usually with seventh tergite specialized in abdomen (Fig. 4c). Similar characters of hind wings and claws either or both also present in some genera of Pseudophyllodromiinae (e.g. *Chorisoneura* and *Chorisoserrata*).

*Episorineuchora* Wang & Wang gen. nov. <http://zoobank.org/urn:lsid:zoobank.org:act:CC339292-D10C-4B40-A81E-19766F5FFF50>.

Type species: *Sorineuchora formosana* (Matsumura, 1913), here designated.

Taxa included: *Episorineuchora formosana* (Matsumura, 1913), comb. nov., *Episorineuchora lativitrea* (Walker, 1868), comb. nov.

Distribution: Oriental Region.

Diagnosis: The new genus (Fig. 5b) has the following homoplastic characters shared with *Sorineuchora*: abdominal terga of male unspecialized, front femur Type C<sub>2</sub> (Fig. 6i), four proximal tarsomeres with pulvilli terminal and asymmetrical tarsal claws (Fig. 6b). However, this new genus differs from *Sorineuchora* by the cubitus vein of the hind wing with a curved complete branch and two or three cross veins (Fig. 5f), the two similar and cylindrical styli, and the interstyler margin bilobed with minute hairs (Fig. 5g). A similar condition of the cubitus vein is also present in some members of *Chorisoneurodes*.

Etymology: The generic epithet is a combination of the Greece prefix -epi-, and the genus name *Sorineuchora*, indicating these two genera are similar in some characteristics. The gender of the name is feminine.

Rhabdoblattellinae Wang & Wang subfam. nov. <http://zoobank.org/urn:lsid:zoobank.org:act:35D85C8D-CF2B-4AB2-BAEF-39F54A0F05D5>.

Type genus: *Rhabdoblattella* Anisutkin, 2000, present designation.

Taxa included: *Rhabdoblattella* Anisutkin, 2000.

Distribution: Oriental Region.

Diagnosis: Now only the genus *Rhabdoblattella* is included in Rhabdoblattellinae. Consequently, the diagnostic features cited for the subfamily are the same for the genus *Rhabdoblattella* (Fig. 1a). *Rhabdoblattella* shares a series of features with other Epilamprinae genera: front femur Type B; hind metatarsus longer than other tarsal segments combined, with spines in two equal rows along ventral margin, all pulvilli

present apically, tarsal claws symmetrical (Fig. 6c). However, this genus is well defined by two autapomorphies that differentiate it from other groups of Blaberidae: the right phallomere of the male genitalia with R4 absent and R2 fused with R5; a median tooth present on the caudal margin of subgenital plate (Fig. 7k,q).

Comments: Anisutkin (2000) erected *Rhabdoblattella* and placed it in Epilamprinae. Our phylogenetic results clearly indicated that this genus did not belong to any recognized subfamily of Blaberidae. As mentioned above, *Rhabdoblattella* had some unique morphological characters, which could not be found in any other known genera of Blaberidae. In consequence, we established the new subfamily Rhabdoblattellinae for this genus.

*Calolamprodinae* Wang & Wang subfam. nov. <http://zoobank.org/urn:lsid:zoobank.org:act:BD7FC3A8-F9DB-450E-A841-C6F310E087CD>.

Type genus: *Calolamprodes* Bey-Bienko, 1969, present designation.

Taxa included: *Calolamprodes* Bey-Bienko, 1969.

Distribution: Oriental Region.

Diagnosis: *Calolamprodinae* (Fig. 1b) is characterized by two autapomorphies: hind metatarsus with two distinctly unequal rows of spines along ventral margin (Fig. 6d), sclerite R4 of right phallomere substituted with membranous lobe and fused with caudal part of sclerite R1 and sclerite R5 of right phallomere usually absent (Fig. 7j). This subfamily also can be distinguished by the following unique combination of characters: sexual dimorphism (male with the tegmina and wings completely developed, female with tegmina reduced or absent); front femur Type B; subgenital plate asymmetrical with a small membranous area around right stylus (Fig. 7p).

*Acutirhabdoblatta* Wang & Wang gen. nov. <http://zoobank.org/urn:lsid:zoobank.org:act:FD2CCBD9-6FC4-451E-8D97-54348A48498A>.

Type species: *Rhabdoblatta densimaculata* Yang et al., 2019, present designation.

Taxa included: *Acutirhabdoblatta densimaculata* (Yang et al., 2019), comb. nov.

Distribution: Oriental Region.

Diagnosis: At present, *Acutirhabdoblatta* (Fig. 1d) is composed of only one species, *Acutirhabdoblatta densimaculata*. As originally established (Yang et al., 2019), *A. densimaculata* has the similar shape of pronotum, front femur spine type and structure of the male genitalia with other *Rhabdoblatta* species, but it can be separated from the latter by having tarsal claws with inner margins heavily toothed (Fig. 6e,f), each paraproct with a finger-like process bending backwards (Fig. 7l,m) rather than typical blaberid-type (Fig. 7o), and the right phallomere with RIT wrinkled (Fig. 7d). A similar condition of the paraprocts also presents in *Pseudophoraspis kabakovi* (Fig. 7n).



Etymology: The generic epithet is a combination of *acuti-* and the genus name *Rhabdoblatta*, referring to the sharp tooth present on inner margins of tarsal claws. The gender of the name is feminine.

Genus *Opisthoptatia* Brunner von Wattenwyl, 1865.

Type species: *Opisthoptatia orientalis* (Burmeister, 1838).

Taxa included: *Opisthoptatia orientalis* (Burmeister, 1838), *Opisthoptatia beybienkoi* Anisyutkin, 2005, *Opisthoptatia saussurei* (Kirby, 1903), comb. nov., *Opisthoptatia ecarinata* (Yang et al., 2019), comb. nov.

Distribution: Oriental Region.

Comments: Although *Rhabdoblatta saussurei* (Kirby, 1903) and *Rhabdoblatta ecarinata* (Yang et al., 2019) significantly differ from the type species of *Opisthoptatia* (*O. orientalis*, Fig. 1c) in the external appearance, our phylogenies strongly support them as a monophyletic group. Morphological similarities in the shape of the right phallomere, particularly those between *O. orientalis* and *O. ecarinata* (R1T well developed with caudal margin broadly round, R2 rounded, and R4 wide, Fig. 7a–c) could be the evidence to support the transference of *R. saussurei* and *R. ecarinata* to the genus *Opisthoptatia*.

## Conclusions

Our study is the first comprehensive phylogenetic analysis of Blaberoidea based on mitochondrial genomics and nuclear genes (*18S*, *28S*) sequences. The results considerably advance our understanding of the relationships within Blaberoidea, although there are still some unresolved deep nodes requiring further study. Our results support the five-family system of Blaberoidea, with the establishment of three new subfamilies and two new genera and the proposal of five new combinations. Despite the monophyly of Ectobiidae and the placement of Nyctiboridae being robustly supported based on different datasets using two analyses (maximum-likelihood and maximum-parsimony), inadequate sampling of these two families is undeniable, so the future research on Blaberoidea should focus on increasing taxa of these groups. In addition, the male genitalia showed a high taxonomic value for diagnosing subfamilies within Blaberidae. The characters observed from the genitalia in this study, particularly those of the right phallomere (sclerite R with five principal sclerites composed), present a strong phylogenetic signal, which points to the future research direction of Blaberidae.

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## Conflict of interest

None declared.

## Data availability statement

All data that support the findings of this study are available in the supplementary materials. Newly generated mitogenome and nuclear sequences have been uploaded to GenBank with accession numbers OQ736902–OQ736999 and OQ737943–738194.

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## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Fig. S1.** ML tree generated in IQ-TREE based on Dataset 2.

**Fig. S2.** MP trees generated in PAUP based on Dataset 1.

**Fig. S3.** MP trees generated in PAUP based on Dataset 2.

**Table S1.** List of taxa used in the present study with locality, identifiers and GenBank accession numbers.

**Table S2.** Results of alternative phylogenetic hypothesis testing using the approximately unbiased test (AU test).

**Table S3.** Character matrices for the ancestral state reconstructions used to study the evolution of morphological characters in Blaberoidea.

**Data S1.** The Nexus files and partitions of two datasets used in this study.

**Appendix S1.** Supplemental materials of AU tests.

**Appendix S2.** Results of the ancestral state reconstruction of selected morphological characters.

**Appendix S3.** Supplemental materials of Bremer support.

**Appendix S4.** Heterogeneity of sequence composition for Datasets 1 and 2.

**Appendix S5.** Supplemental materials of ancestral state reconstruction of selected characters of Blaberoidea (Fig. 3).