

Some new viewpoints revealed by the phylogeny of Blaberidae (Blattaria) based on seven gene fragment markers

Jiawei ZHANG¹, Zengzeng SHI¹, Xiudan WANG², Wenwen YAO¹, Qianqian LI¹, Yanli CHE¹, Zongqing WANG¹①

1. College of Plant Protection, Southwest University, Chongqing 400715, China

2. College of Life Science and Technology, Central South University of Forestry & Technology, Changsha, Hunan 410004, China

Abstract: Despite consistent support from previous studies for the monophyly of the Blaberidae family, the phylogenetic relationship among its subfamilies remain unresolved. To establish a robust phylogenetic framework for Blaberidae, we analyzed 139 blaberid taxa (including the rarely sampled genus *Rhabdoblattella*) and 35 outgroup taxa based on seven gene fragments, using maximum likelihood (ML) and Bayesian inference (BI) methods. The results indicate that, after excluding *Epilampra taira* and *Galiblatla cribrata*, the Epilamprinae is monophyletic and sistergroup to *Calolamprodes beybienkoi*. Panesthiinae and Geoscapheinae together form a monophyletic group, but *Panesthia* and four genera within Geoscapheinae (*Neogeoscapheus*, *Parapanesthia*, *Geoscapheus* and *Macropanesthia*) are polyphyletic. Our phylogenetic analyses support the establishment of the subfamilies Rhabdoblattellinae and Calolamprodinae, as well as the classification of Geoscapheinae as a synonym under Panesthiinae, and propose that the four genera within Geoscapheinae should be synonymized with *Panesthia*. Furthermore, based on phylogenetic evidence and similarities in the right phallomere (the R2 moderately rounded, R3 fused with R5, enlarged caudally), we transfer *Rhabdoblattella monticola* to the genus *Opisthoplatia*.

Key words: Epilamprinae; Panesthiinae; Geoscapheinae; *Rhabdoblattella*; synonym

基于七个基因片段的硕蠊科系统发育所揭示的新观点（蜚蠊目）

张佳伟¹, 时增增¹, 王秀丹², 姚文文¹, 李前前¹, 车艳丽¹, 王宗庆¹①

1. 西南大学植物保护学院, 重庆 400715; 2. 中南林业科技大学生命科学与技术学院, 湖南 长沙 410004

摘要: 尽管已有研究一致支持硕蠊科为单系群, 但其内部亚科间的系统发育关系仍存在争议。为构建 1 个可靠的硕蠊科系统发育框架, 本研究基于 7 个基因片段, 选择 139 个硕蠊科分类单元 (包括近年来采样较少的棒光蠊属 *Rhabdoblattella*) 及 35 个外群分类单元, 通过最大似然法和贝叶斯推断法进行系统发育分析。结果表明排除 *Epilampra taira* 和 *Galiblatla cribrata* 后, 光蠊亚科为单系群, 并与丽光蠊 *Calolamprodes beybienkoi* 互为姐妹群; 弯翅蠊亚科 Panesthiinae 与掘蠊亚科 Geoscapheinae 共同构成单系群, 但弯翅蠊属 *Panesthia* 及掘蠊亚科下 4 属 (新掘蠊属 *Neogeoscapheus*、拟弯翅蠊属 *Parapanesthia*、

Accepted 27 March 2025. Published online 10 December 2025.

① Corresponding author, E-mail: zqwang2006@126.com

巨弯翅蠊属 *Macropanesthia*) 均为多系群。我们的系统发育分析支持棒光蠊亚科 Rhabdoblattellinae 和丽光蠊亚科 Calolamprodiinae 的建立, 同时支持掘蠊亚科 Geoscapheinae 为弯翅蠊亚科 Panesthiinae 的异名, 并建议将掘蠊亚科下 4 属作为弯翅蠊属 *Panesthia* 异名。此外, 基于系统发育分析及右阳茎骨片的相似性 (R2 钝圆, R3 与 R5 相融合且显著扩大), 本研究将丘大光蠊 *Rhabdoblatta monticola* 归入水蠊属 *Opisthoplatia*。

关键词: 光蠊亚科; 弯翅蠊亚科; 掘蠊亚科; 棒光蠊属; 异名

Introduction

Blaberidae is globally distributed and exhibits remarkable species diversity (Beccaloni 2014; Djernæs 2018). Among its members are several fascinating and unique species, such as the singing *Gromphadorhina portentosa* (Schaum, 1853), the giant cockroach *Macropanesthia rhinoceros* (Saussure, 1895), the ball-rolling species of *Perisphaerus*, and the beetle-mimicking viviparous *Diploptera punctata* (Eschscholtz, 1822). As a model species for endocrinological studies (Marchal *et al.* 2013), *D. punctata* provides nutrients to its nymphs through milk produced by the mother (Bell *et al.* 2007). Despite its diversity, the phylogenetic relationships and evolutionary history of Blaberidae remain poorly understood.

Blaberidae comprises multiple subfamilies, with its taxonomic system formalized into twelve subfamilies following the establishment of Paranauphoetinae by Anisytukin (2003). However, the taxonomic status of many subfamilies remains unresolved. For instance, the widely used classification system proposed by Roth (2003), based on the frameworks of McKittrick (1964) and Grandcolas (1996), did not recognize the subfamily Geoscapheinae established by Rugg and Rose (1984b), instead placing it within Panesthiinae. On the other hand, the monophyly of Blaberidae itself has been consistently supported by numerous studies, both morphological (McKittrick 1964; Grandcolas 1996; Roth 2003; Klass & Meier, 2006) and molecular (Inward *et al.* 2007; Djernæs *et al.* 2012, 2015; Legendre *et al.* 2014, 2015, 2017; Wang *et al.* 2017a; Bourguignon *et al.* 2018; Evangelista *et al.* 2019, 2021; Arab *et al.* 2020), yet, many subfamilies have been found to be non-monophyletic.

Epilamprinae is one of the largest subfamilies within Blaberidae, primarily distributed across the Palearctic, Oriental, Australasian, Neotropical and African realms. A total of 48 genera are recorded in this subfamily (Beccaloni 2014), most of which are terrestrial, although some are amphibious, living near the water but feeding on decaying plants on land (Bell *et al.* 2007). Previous studies have identified Epilamprinae as a polyphyletic group (Legendre *et al.* 2014, 2017; Bourguignon *et al.* 2018; Djernæs *et al.* 2020; Evangelista *et al.* 2021). However, geographic congruence has been observed in certain clades of Epilamprinae (Legendre *et al.* 2017; Evangelista *et al.* 2021). At the genus level, several unresolved issues remain. *Rhabdoblatta*, one of the largest genera within Epilamprinae, is particularly challenging to define and delimit due to its wide distribution and high diversity. Anisytukin (2014) noted that no specific diagnostic characters could be identified as synapomorphy, suggesting that this genus is likely polyphyletic, a conclusion supported by Legendre *et al.* (2017), Evangelista *et al.* (2021) and Wang *et al.* (2023). Furthermore, many genera, aside from a few well-studied ones, have not been thoroughly studied. For example, *Rhabdoblattella* has only been

examined in Wang *et al.* (2023), while *Calolamprodes* has been sampled in Wang *et al.* (2023) and Liu *et al.* (2023).

Panesthiinae and Geoscapheinae exhibit significant morphological similarities and are phylogenetically related (Humphrey *et al.*, 1998; Maekawa *et al.* 2003; Legendre *et al.* 2014, 2017; Lo *et al.* 2016; Djernæs *et al.* 2020; Beasley-Hall *et al.* 2021). Members of Panesthiinae inhabit wood and feed on decaying wood, whereas Geoscapheinae dwell in caves and consume fallen leaves. Morphologically, these two subfamilies can be distinguished by differences in the tegmina and wings, genital phallomere and abdominal tergites (Rose *et al.* 2014). However, the taxonomic status of Geoscapheinae always remains contentious. Roth (1977, 1979a, 1979b, 1982) classified Geoscapheinae as a tribe within Panesthiinae following a systematic revision. In contrast, Rugg and Rose (1984c) elevated Geoscapheinae to subfamily status based on its soil-burrowing habitat and reproductive mode, a classification not accepted by Roth (2003). Recent molecular studies have confirmed that Panesthiinae and Geoscapheinae form a monophyletic group, but they also question the validity of Geoscapheinae as a distinct subfamily (Maekawa *et al.* 2003; Lo *et al.* 2016; Legendre *et al.* 2017; Djernæs *et al.* 2020; Beasley-Hall *et al.* 2021). Lo *et al.* (2016) and Beasley-Hall *et al.* (2021) investigated the evolutionary history of Panesthiinae and Geoscapheinae, and suggested that soil-burrowing cockroaches independently evolved from wood-feeding ancestors. Their studies have significantly advanced our understanding of the evolution of Panesthiinae and Geoscapheinae, although the limited sampling of Asian Panesthiinae remains a constraint. Additionally, the relationship among genera within each subfamily remains problematic, as highlighted in previous studies (Lo *et al.* 2016; Beasley-Hall *et al.* 2021). Furthermore, the increasing number of species has revealed previously unexamined relationships, offering new insights for taxonomic revision. For instance, *Panesthia birmanica* (Brunner von Wattenwyl, 1893) and *Miopanesthia* share morphological similarities (Hanitsch 1933), but their relationship has neither been analyzed nor clearly defined (Hanitsch 1933; Roth 1979b).

In this study, we analyzed 139 Blaberidae samples to infer the phylogenetic relationships of Blaberidae using seven gene fragment markers. Our sampling focused on expanding coverage of Asian Panesthiinae, *Rhabdoblattella* and *Calolamprodes*. Although it remains impossible to sample all taxa, we included several rarely studied taxa to improve the phylogenetic resolution of Epilamprinae, Panesthiinae and Geoscapheinae. Furthermore, we integrated morphological characters with our phylogenetic results to emphasize the polyphyly of Epilamprinae and refine the taxonomic assignments of Panesthiinae and Geoscapheinae.

Material and methods

Sampling and DNA sequencing

We obtained 46 samples of four subfamilies of Blaberidae in this study (Table S1): 10 samples of Panesthiinae, 34 samples of Epilamprinae, 1 sample of Rhabdoblattellinae and 1 sample of Calolamprodinae. We adopted the morphological term of McKittrick (1964) and Klass (1997). We sequenced seven gene fragment markers of these samples: *12S rRNA*, *16S rRNA*, *COI* and *COII* of mitochondrial genes, and *18S rRNA*, *28S rRNA* and *H3* of nuclear

genes. All specimens were preserved in 100% ethanol at $-20\text{ }^{\circ}\text{C}$, and deposited in the College of Plant Protection, Southwest University (SWU).

Total DNA was extracted from leg tissue of samples using the DNA Mini Kit (Beijing Liuhe BGI Technology Co., LTD, China). All fragments were amplified using PCR; primers for amplifications are given in Table 1. Reactions were carried out in volumes of $25\text{ }\mu\text{L}$, containing $22\text{ }\mu\text{L}$ of 1×1 T3 supper mix (Tsingke Biological Technology, Beijing, China), $1\text{ }\mu\text{L}$ of each primer and $1\text{ }\mu\text{L}$ of DNA template. The amplification conditions were: initial denaturation at $95\text{ }^{\circ}\text{C}$ for 3 min, followed by 35 cycles for 30s at $95\text{ }^{\circ}\text{C}$, 45s at $46\text{--}57\text{ }^{\circ}\text{C}$ ($50\text{ }^{\circ}\text{C}$ for *12S* and *COII*, $53\text{ }^{\circ}\text{C}$ for *16S*, $57\text{ }^{\circ}\text{C}$ for *18S*, $52\text{ }^{\circ}\text{C}$ for *28S* and *COI*, $46\text{ }^{\circ}\text{C}$ for *H3*), and 1 min at $72\text{ }^{\circ}\text{C}$, with a final extension of 10 min at $72\text{ }^{\circ}\text{C}$. PCR products were separated by electrophoresis on a 1% agarose gel. DNA purification and sequencing were carried out by Beijing Liuhe BGI Technology Co., LTD, China using the aforementioned primers (Table 1). We obtained seven fragments from these samples, with a total of 311 sequences deposited in GenBank (Table S1).

Table 1. Primers used for sequences

Genes	Forward/Reverse	Sequence (5'-3')	References
<i>12S</i>	F	ATCTATGTTACGACTTAT	Inward <i>et al.</i> (2007)
	R	AAACTAGGATTAGATACCC	Inward <i>et al.</i> (2007)
<i>16S</i>	F	CGCCTGTTTAACAAAAACAT	Simon <i>et al.</i> (1994)
	R	TTTAATCCAACATCGAGG	Cognato and Vogler (2001)
<i>COI</i>	F	CAACYAATCATAAAGANATTGGAAC	Here designated
	R	TAAACTTCAGGGTGACCAAAAAATCA	Here designated
<i>COII</i>	F	ATGGCAGATTAGTGCAATGG	Maekawa <i>et al.</i> (1999)
	R	GTTTAAGAGACCAGTACTTG	Maekawa <i>et al.</i> (1999)
<i>18S</i>	F	CTTATCAACTGTCGATGGTAGG	Here designated
	R	CCTTCCGTC AATTCTTTAAG	Here designated
<i>28S</i>	F	ACACGGACCAAGGAGTCTAAC	Here designated
	R	GTCCTGCTGTCTTAAGCAACC	Here designated
<i>H3</i>	F	ATGGCTCGTACCAAGCAGACVGC	Inward <i>et al.</i> (2007)
	R	ATATCCTTRGGCATRATRGTGAC	Inward <i>et al.</i> (2007)

Sequence alignment and data set

We combined these newly sequenced samples (46) with the sequences of 93 samples of Blaberidae from GenBank (Table S1). Meanwhile, we added 10 samples from Blattellidae, 14 from Blattodea, 4 from Corydioidea and 7 from non-Blattodea as outgroup taxa (Table S1). Our data set contained all subfamilies of Blaberidae: Oxyhaloinae (9 samples), Blaberinae (10), Panchlorinae (3), Perisphaerinae (4), Diplopterinae (1), Paranauphoetinae (2), Gyninae (3), Pycnoscelinae (2), Zetoborinae (6), Panesthiinae (35), Geoscapheinae (22), Epilamprinae (40), Rhabdoblattellinae (1), Calolamprodiniae (1).

We aligned protein-coding genes (PCGs) and RNA genes using MAFFT v7 (Katoh *et al.* 2019) with the G-INS-i and Q-INS-i strategies, respectively. Using MEGA-X (Kumar *et al.* 2018), we manually corrected alignments of PCG sequences by translation into amino acids,

visually inspected alignments of the RNA sequences and removed a small number of poorly aligned sites within the intergenic region. We performed substitution saturation tests on the first and second codon position (pos12) and third codon position (pos3) of protein-coding genes on DAMBE v7.3.11 (Xia 2018). The result showed that the pos3 (Iss: 0.68 for *COI*, 0.694 for *COII*, 0.554 for *H3*) was much more saturated than the pos12 (Iss: 0.085 for *COI*, 0.182 for *COII*, 0.061 for *H3*), indicating that third codon position of protein-coding genes were less suitable for the next phylogenetic analysis. Therefore, we removed the third codon of the protein-coding genes in our data set that comprised 4218 nucleotide sites from *12S rRNA*, *16S rRNA*, *18S rRNA*, *28S rRNA*, *COI_pos12*, *COII_pos12* and *H3_pos12*.

Phylogenetic analyses

We used PartitionFinder v2.1.1 (Lanfear *et al.* 2017) to select the best-fitting models for the data subsets. We performed model selection on the Phylosuite v1.2.2 (Zhang *et al.* 2020), the search of models was set to be “all” and “AICc” (the corrected Akaike Information Criterion), using greedy algorithm, and the best-fitting models were SYM+I+G for *COI_pos12*, and GTR+I+G for the rest partitions.

Phylogenetic analyses were constructed using maximum likelihood (ML) and Bayesian inference (BI) methods. The ML analysis was constructed in IQ-TREE v1.6.8 (Nguyen *et al.* 2015). Node and branch supports were assessed by 10000 ultrafast bootstrap (UFBoot) replicates (Minh *et al.* 2013; Hoang *et al.* 2018) and 5000 SH-like approximate likelihood ratio (SH-aLRT) tests (Guindon *et al.* 2010). The BI analysis was performed in MrBayes v3.2.5 (Ronquist *et al.* 2012). We ran two independent Markov chains, each run with one cold and three heated chains. Each chain was run for 10^7 generations, with samples drawn every 1000 generations and burnin was set as 1000. Convergence was assessed with effective sample size (ESS = 1954) of parameters ≥ 200 in Tracer v1.6.0 (Rambaut *et al.* 2018).

Result

The two trees from our ML and BI (Figs 1, 2) were largely identical in deep relationships, although they did not provide high support values. The differences between the trees focused on the unstable placements of a few taxa, for example, Paranauphoetinae, Pycnoscelinae, *Rhabdoblattella disparis*, *Thanatophyllum akinetum*, *Laxta*, *Parasphaeria boleiriana* and *Pronauphoeta*. The monophyly of the largest principal clade, Blaberidae, was consistently obtained and strongly supported in both ML and BI analysis.

In our ML analysis (Fig. 1), the subfamily Panchlorinae and Oxyhaloinae (except *Pronauphoeta* sp.) were sister to each other, together as the sister group of the other Blaberidae with high support values (SH-aLRT/UFBoot = 100/100); but in our BI analysis (Fig. 2), Paranauphoetinae was the sister group to the other Blaberidae with high support (BPP = 1).



Figure 1. ML tree derived from analysis of combined data *12S rRNA*, *16S rRNA*, *COI*, *COII*, *28S rRNA* and *H3* gene fragment markers. Branch labels indicating SH–aLRT/UFBoot respectively.

In our study, Epilamprinae was a polyphyletic group, including *Epilampra taira*, *Galiblatia cribrosa*, and others. The clade, *E. taira* + (*G. cribrosa* + *T. akinetum*) was recovered as sister to Blaberinae and Zetoborinae in the ML analysis (Fig. 1), but *E. taira* + *G. cribrosa* and Diplopterinae as sister groups in the BI analysis (Fig. 2). *R. disparis* and Pycnoscelinae are sister groups, and then together formed the clade with *P. boleiriana* and *Pronauphoeta* sp. (the phylogenetic relationship is (*Pronauphoeta viridula* + (*P. boleiriana* + (*R. disparis* + Pycnoscelinae))), in subsequent analysis, we named it Clade PPPRH). The other genera of Epilamprinae were grouped together to form the largest clade, including seven genera *Anisolampra*, *Brepballus*, *Cyrtanotula*, *Stictolampra*, *Opisthoptlatia*, *Rhabdoblatta* and *Pseudophoraspis*. *C. beybienkoi* as the sister group with other taxa of this clade. *Rhabdoblatta* was recognized as a polyphyletic group with respect to *Opisthoptlatia*, *Pseudophoraspis*, *Rhichnoda* and *Stictolampra* in our two analyses: *Opisthoptlatia ecarinata*, *Rhabdoblatta monticola* and *Opisthoptlatia saussurei* form a clade with *Opisthoptlatia orientalis*; *Acutirhabdoblatta densimaculata* forms a clade with *Pseudophoraspis* and *Rhichnoda*; a sample of *Stictolampra* is embedded in *Rhabdoblatta*.

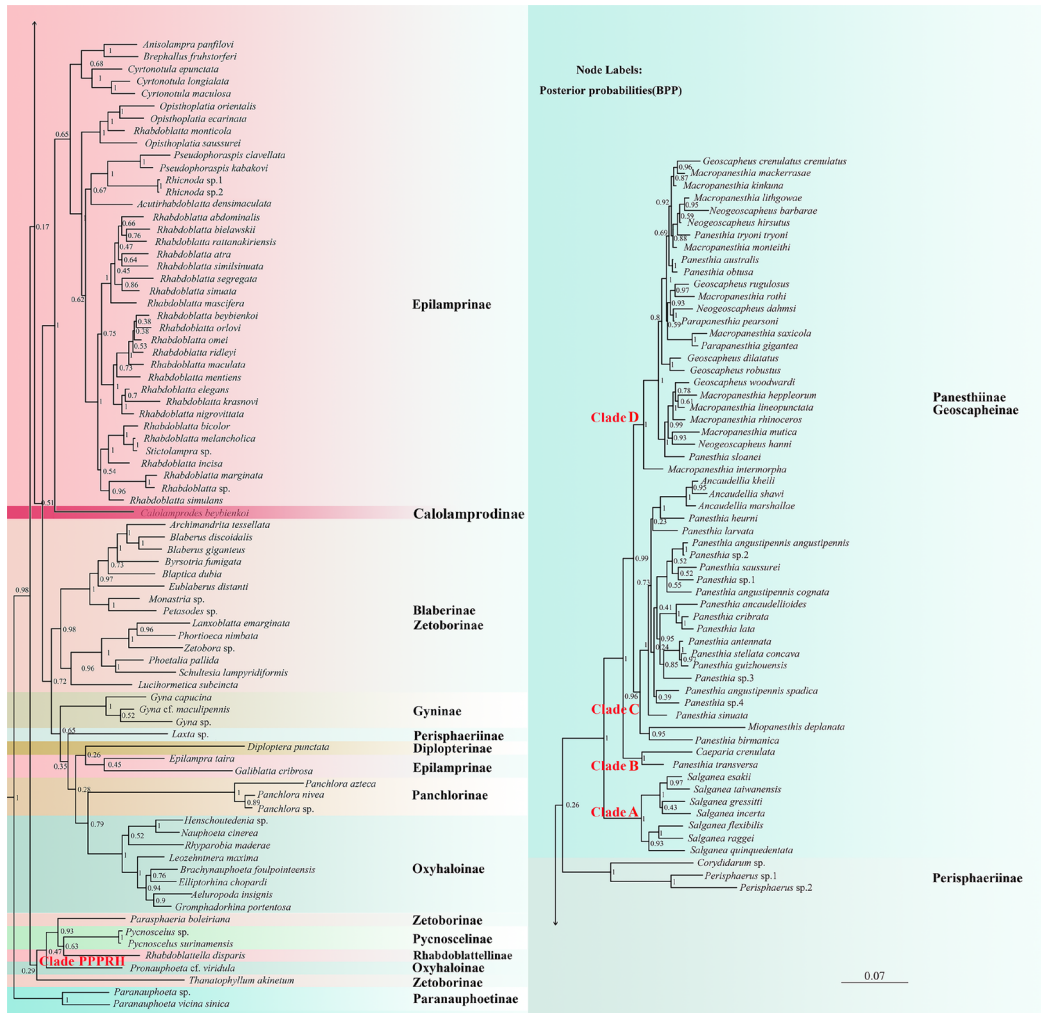


Figure 2. BI tree derived from combined data *12S rRNA*, *16S rRNA*, *COI*, *COII*, *28S rRNA* and *H3* gene fragment markers. Numbers above branches represent Posterior probabilities (BPP).

Blaberinae and Zetoborinae form a monophyletic group with the exception of *T. akinetum* (Zetoborinae), which was clustered with Neotropical Epilamprinae (ML) or Clade PPPRH (BI), and *P. boleiriana* (Zetoborinae) as the sister group to *R. disparis* + Pycnoscelinae. *Phoetalia pallida* (Blaberinae), was identified as the sister group with *Schultesia lampyridiformis* (Zetoborinae). *Lucihormetica subcincta* (Blaberinae) was recovered as the sister group with other of Zetoborinae (Figs 1, 2). In our results, Oxyhaloinae was recognized as non-monophyletic, with *Pronauphoeta* sp. in Clade PPPRH both in ML and BI analysis. Perisphaerinae was found to be a paraphyletic group owing to *Laxta* far away from the other Perisphaerinae. Although the position of *Laxta* was unstable in our ML and BI analysis, it represented a separate clade, sister to Blaberidae except Panchlorinae and Oxyhaloinae (with the exception of *Pronauphoeta* sp.) in ML analyses (Fig. 1) and ((Panchlorinae and Oxyhaloinae) + ((*E. taira* + *G. cribrosa*) + Diplopterinae)) in BI analyses

(Fig. 2).

Panesthiinae and Geoscapheinae formed a monophyletic group with high support values (SH–aLRT/UFBoot = 100/100, BPP = 1), and as the sister group to Perisphaerinae (except *Laxta*) both in ML and BI analysis. Our results strongly supported the non-monophyly of both Panesthiinae and Geoscapheinae, among them, *Panesthia*, *Neogeoscapheus*, *Parapanesthia*, *Geoscapheus* and *Macropanesthia* were recovered as polyphyletic. Panesthiinae and Geoscapheinae fell into four clades: *Salganea* as the sister group with other taxa of Panesthiinae and Geoscapheinae (Clade A); *Caeparia crenulata* and *Panesthia transversa* (Clade B); *Panesthia*, *Ancaudellia*, and *Miopanesthia* (Clade C); Australian species including wood-feeding and soil-burrowing cockroaches (Clade D). The phylogenetic relationship of these four clades was: Clade A + (Clade B + (Clade C + Clade D)) (Figs 1, 2).

Taxonomic revision

Genus *Opisthoplatia* Brunner von Wattenwyl, 1865.

Type species. *Opisthoplatia orientalis* (Burmeister, 1838).

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

Distribution. Oriental Region.

Remarks. Based on our phylogenetic results, *Opisthoplatia ecarinata*, *Opisthoplatia saussurei*, and *Rhabdoblatta monticola* formed a clade with *Opisthoplatia orientalis* in both ML and BI analyses, with high support values (SH–aLRT/UFBoot = 99/97, BPP = 1). Additionally, these species exhibit a high degree of similarity in the shape of the right phallomere (the R2 moderately rounded, R3 fused with R5, enlarged caudally, and R4 separated from other sclerites) (Figs 3G–J).

Discussion

Focus issues within Epilamprinae

Based on the hypandrial median tooth (Fig. 3B) and right phallomere morphology (divided R1T sclerite, plate-like R5, and R2–R5 connectivity; Fig. 3C) (Wang *et al.* 2017b), Anisyutkin (2000) established *Rhabdoblattella* and classified it under Epilamprinae. *Rhabdoblattella* is distributed in Oriental region with eight species totally (Anisyutkin & Yushkova 2017; Wang *et al.* 2017b), but it has rarely been included in molecular analysis. Wang *et al.* (2023) sampled *Rhabdoblattella* for the first time and found it as the sister group of the other Blaberidae. In our study, *R. disparis* was recovered as the sister group to Pycnoscelinae in both ML and BI analysis. Considering its unexpected phylogenetic position, small body size, and unique characteristics, Wang *et al.* (2023) established the subfamily Rhabdoblattellinae to accommodate this genus. Our results also support the establishment of Rhabdoblattellinae.

In our study, *Anisolampra*, *Brephallus*, *Cyrtonotula*, *Stictolampra*, *Opisthoplatia*, *Rhabdoblatta*, and *Pseudophoraspis* formed the largest lineage within Epilamprinae, with *Calolamprodes* as the sister group to the other genera in ML and BI analysis (Figs 1, 2). The

basal segment of the hind metatarsus in *Calolamprodes* is longer than the remaining segments, and its ventral margin had either one row of small spines or two rows of irregular spines. Additionally, the right phallomere of *Calolamprodes* exhibits unique morphological structures (e.g., the caudal part of sclerite R1T strongly reduced)(Anisyutkin 2006; Anisyutkin & Yushkova 2017) (Fig. 3F). Based on phylogenetic analysis and morphological characteristics, our results support the establishment of Calolamprodiinae, as proposed by Wang *et al.* (2023).

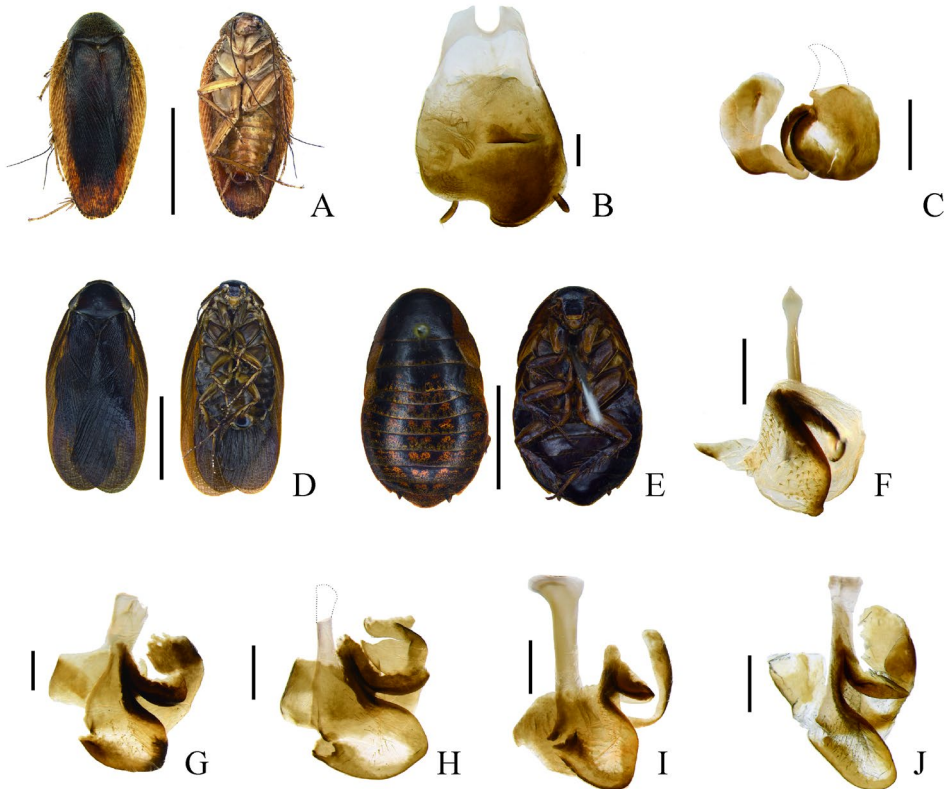


Figure 3. A–C. *Rhabdoblattella disparis*. A. Male, dorsal and ventral view; B. Hypandrium; C. Sclerite of right phallomere. D–F. *Calolamprodes beybienkoi*. D. Male, dorsal and ventral view; E. Female, dorsal and ventral view; F. Sclerite of right phallomere. G–J. Sclerite of right phallomere: G. *Opisthoptlatia orientalis*; H. *Opisthoptlatia ecarinata*; I. *Rhabdoblatta monticola*; J. *Opisthoptlatia saussurei*. Scale bars = 10 mm (A, D, E); 0.5 mm (B, C, F–J). * The dashed line indicates the sclerite fractured there.

Rhabdoblatta, the most diverse genus within Epilamprinae, was recovered to be a polyphyletic group in previous studies (Legendre *et al.* 2017; Evangelista *et al.* 2021; Wang *et al.* 2023) and in our study. According to our results, *Opisthoptlatia ecarinata*, *Opisthoptlatia saussurei* and *Rhabdoblatta monticola* formed a clade with *Opisthoptlatia orientalis* both in ML and BI analysis with high support values (SH–aLRT/UFBboot = 99/97, BPP = 1). The right phallomere of these species was highly similar in structure (Figs 3G–J): the caudal R2 moderately rounded, R3 fused with R5, enlarged caudally, and R4 separated from other

sclerites. The phylogenetic results, combined with the morphological similarity of the right phallomere, suggest that these three species do not belong to *Rhabdoblatta*. Wang *et al.* (2023) transferred *Rhabdoblatta ecarinata* and *Rhabdoblatta saussurei* to *Opisthoplatia*. Based on our findings, we propose transferring *Rhabdoblatta monticola* to *Opisthoplatia* as well. Additionally, our results showed that *Acutirhabdoblatta densimaculata* was grouped together with *Pseudophoraspis* and *Rhcnoda* (Figs 1, 2), which supports the establishment of the genus *Acutirhabdoblatta* by Wang *et al.* (2023).

Resolution of relationships within Panesthiinae and Geoscapheinae

Cockroaches that burrow in wood or soil exhibit remarkable morphological convergence, leading to Panesthiinae, Geoscapheinae and Cryptocercidae being originally grouped into the same family (Wheeler 1904). Cryptocercidae had been recognized as the sister of termites, whereas Panesthiinae and Geoscapheinae are classified under Blaberidae. Geoscapheinae, a group of soil-burrowing cockroaches, constructs curved permanent burrows in sandy soil and feed on fallen leaf litter within these burrows (Rugg & Rose 1984a). Members of this subfamily exhibited adaptation to subterranean life, such as reduced eyes and ocellar spots, shovel-like tibiotarsi and aptery (Roth 1977; Beasley-Hall *et al.* 2018). However, the subfamily status of Geoscapheinae within Blaberidae has been questioned in multiple studies (Maekawa *et al.* 2003; Lo *et al.* 2016; Djernæs *et al.* 2020; Beasley-Hall *et al.* 2021), as also observed in our study. Lo *et al.* (2016) and Beasley-Hall *et al.* (2021) both suggested that soil-burrowing cockroaches independently evolved from wood-feeding ancestors. Recent studies have shown that soil-burrowing cockroaches should be classified within Panesthiinae (Maekawa *et al.* 2003; Lo *et al.* 2016; Legendre *et al.* 2017; Djernæs *et al.* 2020; Beasley-Hall *et al.* 2021). Based on our findings, we also suggest that Geoscapheinae should not be treated as subfamily and instead be synonymized with Panesthiinae.

Our study uncovered a new sister-group relationship between *P. birmanica* and *Miopanesthia deplanata* (SH-aLRT/UFBboot = 82/99, BPP = 0.95), which together form a strongly supported clade sister to other members within Clade C (Figs 1, 2). *P. birmanica* is small in size with the pronotum almost flat, resembling *Miopanesthia* in appearance. Hanitsch (1933) considered that *P. birmanica* closely related to *Miopanesthia*, but Princis (1953) and Roth (1979b) argued that they could be distinguished by L2d (fully developed in *P. birmanica* but typically absent in *Miopanesthia*). Besides, the hind tarsus structure of *P. birmanica* is characteristic of *Panesthia* (Roth 1979b; Wang *et al.* 2014). Roth (1979b) also suggested that *P. birmanica* strongly resembles *Miopanesthia* in habitus and may represent an intermediate form between the two genera. Our molecular analysis is the first to confirm the close relationship between *P. birmanica* and *Miopanesthia*, supporting the hypothesis that *P. birmanica* represents a transitional form. We propose establishing a new genus with *P. birmanica* as the type species.

Our results (Figs 1, 2) were consistent with those of previous studies (Lo *et al.* 2016; Legendre *et al.* 2017; Beasley-Hall *et al.* 2021), which found *C. crenulata* and *Ancaudellia* embedded within *Panesthia*. Beasley-Hall *et al.* (2021) believed that *Caeparia* and *Ancaudellia* should likely be subsumed within *Panesthia*. In our ML and BI inferences (Figs 1, 2), *P. transversa* and *C. crenulata* were recovered as a sister group. Morphological evidence

supports this relationship: all species of *Caeparia* are macropterous with bicoloured tegmina, L2d fully developed and toothed along the posterior margin, L1 reduced, R2 reduced and not hook-shaped (Roth 1979a); *P. transversa* also macropterous with bicoloured tegmina, L2d fully developed, L1 developed, R2 slightly reduced and hook-shaped (Roth 1979b). These shared characteristics suggest a close relationship between *P. transversa* and *Caeparia*. Furthermore, some *Panesthia* species (*Panesthia flavipennis*, *Panesthia necrophoroides*, *Panesthia regails*, etc.) also exhibit bicoloured tegmina and similar male genitalia to *P. transversa* (Roth 1979b). However, these species have not been included in molecular studies. To ensure the accuracy of Blaberidae classification, we do not propose any taxonomic changes until additional samples are analyzed to clarify the phylogenetic relationship between *Caeparia* and *Panesthia*.

Regarding *Ancaudellia*, this genus is distinguished from *Panesthia* by the following characteristics: the lateral margin of abdominal segment 7 weakly indented to strongly notched in front of the laterocaudal angle (Roth 1977, 1982), and the presence of holes in the anterolateral corners of abdominal tergites 6 and 7, and segment 7, which were often accompanied by dense patches of setae. But in our study and previous molecular studies (Lo *et al.* 2016; Legendre *et al.* 2017; Beasley-Hall *et al.* 2021), *Ancaudellia* was consistently sister to *Panesthia heurni*, embedded within the *Panesthia* (Figs 1, 2). This suggests that these three *Ancaudellia* species may actually belong to *Panesthia*. Similarities in male genitalia between *Ancaudellia* and certain *Panesthia* species (Roth 1977, 1982) further support the transference of these three *Ancaudellia* species.

Clade D contained all soil-burrowing cockroaches and four wood-feeding cockroaches from Australia: *Panesthia tryoni tryoni* (Kroombit Tops), *Panesthia sloanei*, *Panesthia australis* and *Panesthia obtusa*. These wood-feeding species are embedded in the soil-burrowing cockroaches. The geoscapheine-like species, *P. tryoni tryoni* and *P. sloanei*, were both non-monophyletic in Beasley-Hall *et al.* (2021) with distinct geographic lineages, which might imply that they are distinct species. *P. australis* and *P. obtusa* were clustered together as the sister to the soil-burrowing cockroaches, as observed in Beasley-Hall *et al.* (2021). These two species were morphologically typical of the genus *Panesthia*. However, both our study and previous research (Lo *et al.* 2016; Beasley-Hall *et al.* 2021) showed a close phylogenetic relationship between these species and the soil-burrowing cockroaches. This situation indicates the common ancestral origin of these species.

Neogeoscapheus, *Parapanesthia*, *Geoscapheus* and *Macropanesthia* are polyphyletic in our and other studies (Lo *et al.* 2016; Beasley-Hall *et al.* 2021). These four genera are primarily distinguished by the characteristics of the margin of the abdominal tergites 6 (Rose *et al.*, 2014). Such tergite-based classifications have been widely used for defining genera and species within Panesthiinae (Roth 1977, 1979a, b, 1982). However, the strong inconsistency between taxonomic assignments and phylogenetic results suggests that tergite-based classifications are insufficient. Considering the pronounced morphological convergence, we propose synonymizing *Neogeoscapheus*, *Parapanesthia*, and *Macropanesthia* with *Panesthia*. Future revisions of Panesthiinae will require comprehensive sampling and detailed morphological observations.

The phylogeny within Blaberinae and Zetoborinae

Our results showed that Blaberinae and Zetoborinae together formed a monophyletic group, with the exception of *T. akinetum* and *P. boleiriana* from Zetoborinae (Figs 1, 2). However, *Lucihormetica subcincta* and *Phoetalia pallida* did not cluster with other groups of Blaberinae. Instead, they were closely related to the Zetoborinae group in both ML (SH-aLRT/UFBboot = 69/93, 98/100) and BI (BPP = 0.96, 1) analysis. Similar results were also reported in previous studies (Legendre *et al.* 2015, 2017; Djernæs *et al.* 2020). These findings highlight a clear mismatch between the phylogenetic results and the current taxonomic assignments of these species. *Lucihormetica* and *Phoetalia* belong to the tribe Brachycolini. McKittrick (1964) noted that Zetoborinae was closely related to Brachycolini, a relationship further supported by the similarity in the L2d shape of some Brachycolini species (particularly *Phoetalia*) to that of many males in Zetoborinae (Roth 1970). Djernæs *et al.* (2020) proposed transferring *Hormetica*, *Lucihormetica*, and *Phoetalia* (all three genera belonging to the tribe Brachycolini) into the Zetoborinae. Based on our findings, we also recommend reassigning *Lucihormetica* and *Phoetalia* to Zetoborinae.

P. boleiriana and *T. akinetum* were distantly separated from the rest of Zetoborinae in our analyses. *P. boleiriana* clustered within Clade PPPRH, which was sister to Pycnoscelinae + *Rhabdoblattella* (Rhabdoblattellinae) (Figs 1, 2). Historically, *Parasphaeria* was initially placed in Epilamprinae (Princis 1964; Roth 1973b) but was later moved to Zetoborinae as the sister group to *Schultesia* (Grandcolas 1991, 1993). However, *P. boleiriana* and *Schultesia* did not show a close phylogenetic relationship. Instead, they appeared as sister groups to other taxa of Blaberinae and Zetoborinae (Legendre *et al.* 2014, 2015; Djernæs *et al.* 2020), or as sister groups to Epilamprinae (South America) (Legendre *et al.* 2017). As for *T. akinetum*, its phylogenetic position was uncertain. In the ML analysis (Fig. 1), it was recovered as the sister to Neotropical Epilamprinae, but to Clade PPPRH in BI analysis (Fig. 2). A similar pattern was observed in Legendre *et al.* (2017), whose results were largely consistent with our ML analysis. These findings suggest that the taxonomic placement of *P. boleiriana* and *T. akinetum* requires further investigation.

The nonmonophyly of Oxyhaloinae and Perisphaerinae

Oxyhaloinae was found to be nonmonophyletic in our analyses due to the placement of *Pronauphoeta* sp. in Clade PPPRH both in ML (SH-aLRT/UFBboot = 90/92) and BI analysis (BPP = 0.47). And other oxyhaloinae species were recovered as the sister group to Panchlorinae (Figs 1, 2), consistent with previous findings (Legendre *et al.* 2017). However, when using different datasets, *Pronauphoeta* grouped with some taxa of Epilamprinae and Perisphaerinae, suggesting that the taxonomic status of *Pronauphoeta* remains uncertain. Historically, *Pronauphoeta* was considered an intermediate group between *Panchlorina* (Panchlorinae) and *Nauphoeta* (Oxyhaloinae) (Shelford 1909). Besides, Rehn (1932) and Kumar (1975) proposed that *Pronauphoeta* and Oxyhaloinae were closely related. However, our results, which included more species, showed that *Pronauphoeta* did not exhibit a closer phylogenetic relationship with Oxyhaloinae and Panchlorinae. Instead, it was recovered as the sister group to other taxa in Clade PPPRH (Figs 1, 2), conflicting with its morphology-based classification. We believe that this conflict arises from the morphological convergence

between *Pronauphoeta* and some members of Oxyhaloinae and Panchlorinae. Our findings, along with partial results from Legendre *et al.* (2017), challenge the historical taxonomic placement of *Pronauphoeta*. Therefore, we propose that *Pronauphoeta* may not belong to Oxyhaloinae. Its phylogenetic position requires future investigation, and the taxonomic status of this genus remains uncertain.

Recent phylogenetic studies (Legendre *et al.* 2014, 2017; Djernæs *et al.* 2020; Evangelista *et al.* 2021) have shown that Perisphaerinae is a paraphyletic group, with clades exhibiting geographic distribution patterns. In our study, the phylogenetic position of *Laxta* remained unresolved, and it was distantly separated from other Perisphaerinae taxa. *Laxta* was primarily distributed in Australia, and Roth (1992) provided a detailed diagnostic characteristics of *Laxta*, suggesting that *Laxta* should belong to Epilamprinae, a view also supported by McKittrick (1964). However, Grandcolas (1997) redefined the subfamily Perisphaerinae using five characters (two in male genitalia, two in female genitalia, and one in head) and assigned *Laxta* to Perisphaerinae. Two of male characters were refuted by Anisyutkin (2003), and the two female characters were also deemed questionable (Li *et al.* 2018). Given the geographic distribution patterns observed in *Perisphaerinae* and the limited sampling of its taxa in our study, we refrain from making taxonomic proposals regarding *Laxta*. We therefore recommend that future studies conduct extensively sample across Perisphaerinae to improve our understanding of its taxonomy and phylogeny.

Taxonomic status of Paranauphoetinae and Pycnoscelinae

Djernæs *et al.* (2020) suggested that Paranauphoetinae and Pycnoscelinae might not retain their status as subfamilies and could potentially be downranked to tribes within Perisphaerinae. In our analysis, Pycnoscelinae was found to be the sister to *Rhabdoblattella* (*Rhabdoblattellinae*) (Figs 1, 2). The male genitalia of Pycnoscelinae exhibited several distinguishing characteristics: the L1 is well developed, with an upward cleft and heavily sclerotized edges (except *Proscratea*); L2d is separated from the L2vm; the outer lower curved portion of the L2d is specular, and the prepuce below is densely hairy (Roth 1973a). Additionally, the subgenital plate of *Pycnoscelus* was unique: the right posterior border is concave with the end distinctly ossified and sharply convex; the left posterior corner is convex or not; the left styli is reduced or absent, while the right styli is enlarged (Roth 1998). Although no consensus has been reached regarding the sister group of Pycnoscelinae (Legendre *et al.* 2015, 2017; Wang *et al.* 2017a; Bourguignon *et al.* 2018; Arab *et al.*, 2020; Djernæs *et al.* 2020; Evangelista *et al.* 2021), considering its unique characteristics and the results of phylogenetic studies, we proposed that Pycnoscelinae should retain its status as a subfamily rather than being downgraded to a tribe within Perisphaerinae.

In our ML analysis (Fig. 1), Paranauphoetinae was recovered as the sister group to Perisphaerinae (excluding *Laxta*) + (Panesthiinae and Geoscaphinae), while in BI analysis (Fig. 2), it was recovered as the sister group to the remaining Blaberidae. Our ML results were consistent with Anisyutkin (2003), who erected Paranauphoetinae on the basis of morphological evidence, an elongate process at the apex of L2d, and the hollow junction of R2 and R5 of the right phallomere. These right phallomere characters clearly distinguish it from other subfamilies. However, *Paranauphoetinae* has been underrepresented in recent

phylogenetic studies, and Djernæs *et al.* (2020) did not include it in their sampling. Two recent studies (Arab *et al.* 2020; Evangelista *et al.* 2021) indicated that Paranauphoetinae is more closely related to Panesthiinae than to Perisphaerinae. Based on the combined evidence from phylogenetic studies and morphological characteristics, we argue that the subfamily status of Paranauphoetinae should be maintained, although further research is needed to clarify its sister group relationships.

Reproductive Strategies and Phylogenetic Position of Diplopterinae

D. punctata is the most extensively studied species within Diplopterinae and produces only about a dozen eggs per reproduction cycle (Roth & Hahn 1964). Most nymphal development in *D. punctata* occurs during the embryonic stage, with the necessary nutrition coming from the mother's food intake. Consequently, starvation of the mother results in embryo death (Bell *et al.* 2007; Jennings *et al.* 2020). *D. punctata* has fully developed wings and strong flight capabilities, allowing it to secure sufficient food resources to meet its nutritional needs and ensure a high offspring survival rate. When born, the nymphs of *D. punctata* could reach 6mm in length, and then develop into adults after three molts (four times for females) (Marchal *et al.* 2013) In contrast, the nymphs of *Perisphaerus* sp. measure only about 2 mm at hatching (Zhang JW, personal observation) and require a longer developmental period to reach maturity. The unique reproductive strategy of *D. punctata*, which is characterized by a short life cycle, high survival rate and fewer eggs, has enabled its success in natural environments. Despite its prominence as a model organism for endocrinology research, the evolutionary origins of viviparous reproduction in *D. punctata* remain poorly understood.

The subfamily Diplopterinae currently contains 8 species (Li *et al.* 2017), of which, only *D. punctata* had been widely studied, and other species were limited to morphological descriptions. The sister group of Diplopterinae remains unresolved, with various studies suggesting Oxyhaloinae (Legendre *et al.* 2014; Bourguignon *et al.* 2018; Evangelista *et al.* 2021), Epilamprinae (Wang *et al.* 2017a), Panchlorinae (Legendre *et al.* 2017), and *T. akinetum* (Zetoborinae) (Djernæs *et al.* 2020) as possible candidates. In our study, the sister group of Diplopterinae was also inconclusive, with Gyninae recovered in ML analysis (Fig. 1) while *E. taira* (Epilamprinae) + *G. cribrata* (Epilamprinae) was recovered in BI analysis (Fig. 2). The distinct coleopterous appearance of Diplopterinae, the high similarity of male genitalia to some members of Perisphaerinae (Roth 1973a) and the viviparous reproduction (Li *et al.* 2017) highlight the importance of this group in understanding cockroach evolution. Further research on Diplopterinae and its sister groups is urgently required to clarify the origin of viviparous reproduction.

Conclusions

We conducted a phylogenetic analysis of the family Blaberidae using ML and BI based on seven gene fragment markers. Our results provide new insights into the phylogenetic relationships among the subfamilies of Blaberidae. Notably, we included *Rhabdoblattella* and *Calolamprodes*, which have been rarely sampled in the recent studies. Based on their

unexpected phylogenetic positions and unique characteristics, we support the establishment of Rhabdoblattellinae and Calolamprodinae. Additionally, we propose that Geoscaphinae should no longer be recognized as a distinct subfamily, and its four constituent genera should be reassigned to Panesthiinae. The current taxonomic classifications, which rely on the characteristics of abdominal tergites, are inconsistent with existing phylogenetic evidence. Therefore, we recommend treating *Neogeoscaphus*, *Parapanesthia*, and *Macropanesthia* as synonyms of *Panesthia*, in accordance with our phylogenetic findings. Future research should focus on a more detailed and systematic revision of Panesthiinae, including the identification of new diagnostic characters to refine its taxonomy.

Acknowledgements

We extend our gratitude to those who collected and identified specimens for this study, and to Drs. Yishu WANG and Wenbo DENG for their valuable comments on the manuscript. This research was supported by the National Natural Science Foundation of China (31872271; 32170458).

References

- Anisyutkin LN. 2000. New cockroaches of the subfamily Epilamprinae, with description of a new genus *Rhabdoblattella* (Dictyoptera: Blaberidae). *Zoosystematica Rossica*, 8: 251–255.
- Anisyutkin LN. 2003. Contribution to knowledge of the cockroach subfamilies Paranauphoetinae (stat. n.), Perisphaeriinae and Panesthiinae (Dictyoptera: Blaberidae). *Zoosystematica Rossica*, 12: 55–77.
- Anisyutkin LN. 2006. Notes on the genus *Calolamprodes* Bey-Bienko, with descriptions of four new species (Dictyoptera: Blaberidae: Epilamprinae). *Cockroach Studies*, 1: 3–14.
- Anisyutkin LN. 2014. On cockroaches of the subfamily Epilamprinae (Dictyoptera: Blaberidae) from South India and Sri Lanka, with descriptions of new taxa. *Zootaxa*, 3847: 301–332.
- Anisyutkin LN and Yushkova OV. 2017. New data on cockroaches of the subfamily Epilamprinae (Dictyoptera: Blaberidae) from India and Sri Lanka, with descriptions of new species and the genital complex of *Aptera fusca* (Thunberg, 1784). *Zootaxa*, 4236: 41–64.
- Arab DA, Bourguignon T, Wang Z, Ho SYW & Lo N. 2020. Evolutionary rates are correlated between cockroach symbionts and mitochondrial genomes. *Biology Letters*, 16: 1–6.
- Beasley-Hall PG, Lee TRC, Rose HA & Lo N. 2018. Multiple abiotic factors correlate with parallel evolution in Australian soil burrowing cockroaches. *Journal of Biogeography*, 45: 1515–1528.
- Beasley-Hall PG, Rose HA, Walker J, Kinjo Y, Bourguignon T & Lo N. 2021. Digging deep: a revised phylogeny of Australian burrowing cockroaches (Blaberidae: Panesthiinae, Geoscaphinae) confirms extensive nonmonophyly and provides insights into biogeography and evolution of burrowing. *Systematic Entomology*, 46: 767–783.
- Beccaloni GW. 2014. Cockroach Species File Online. Version 5.0/5.0. Available from: <http://cockroach.speciesfile.org/> (accessed 20 Oct. 2022)
- Bell WJ, Roth LM & Nalepa CA. 2007. Cockroaches: ecology, behavior, and natural history. The Johns Hopkins University Press, Baltimore, 230 pp.
- Bourguignon T, Tang Q, Ho SYW, Frantisek J, Wang Z, Arab DA, Cameron SL, James W, David R, Evans TA

- & Lo N. 2018. Transoceanic Dispersal and Plate Tectonics Shaped Global Cockroach Distributions: Evidence from Mitochondrial Phylogenomics. *Molecular Biology and Evolution*, 35: 970–983.
- Cognato AI & Vogler AP. 2001. Exploring Data Interaction and Nucleotide Alignment in a Multiple Gene Analysis of Ips (Coleoptera: Scolytinae). *Systematic Biology*, 50: 758–780.
- Djernæs M. 2018. Biodiversity of Blattodea – the Cockroaches and Termites: Science and Society. *Insect Biodiversity: Science and Society*, 2: 359–387.
- Djernæs M, Klass KD, Picker MD & Damgaard J. 2012. Phylogeny of cockroaches (Insecta, Dictyoptera, Blattodea), with placement of aberrant taxa and exploration of out - group sampling. *Systematic Entomology*, 37: 65–83.
- Djernæs M, Klass KD & Eggleton P. 2015. Identifying possible sister groups of Cryptocercidae+Isoptera: a combined molecular and morphological phylogeny of Dictyoptera. *Molecular Phylogenetics & Evolution*, 84: 284–303.
- Djernæs M, Varadinová ZK, Kotyk M, Eulitz U & Klass KD. 2020. Phylogeny and life history evolution of Blaberoidea (Blattodea). *Arthropod Systematics and Phylogeny*, 78: 29–67.
- Evangelista DA, Wipfler B, Béthoux O, Donath A, Fujita M, Kohli MK, Legendre F, Liu S, Machida R & Misof B. 2019. An integrative phylogenomic approach illuminates the evolutionary history of cockroaches and termites (Blattodea). *Proceedings of the Royal Society B: Biological Sciences*, 286: 1–9.
- Evangelista D, Simon S, Wilson MM, Kawahara AY, Kohli MK, Ware JL, Wipfler B, Béthoux O, Grandcolas P & Legendre R. 2021. Assessing support for Blaberoidea phylogeny suggests optimal locus quality. *Systematic Entomology*, 46: 157–171.
- Grandcolas P. 1991. Descriptions de nouvelles Zetoborinae guyanaises avec quelques remarques sur la sous-famille (Dict. Blattaria Blaberidae). *Bulletin de la Société Entomologique de France*, 95: 241–246.
- Grandcolas P. 1993. Monophylie et structure phylogénétique des [Blaberinae + Zetoborinae + Gyninae + Diplopterinae] (Dictyoptera: Blaberidae). *Annales–Société Entomologique de France*, 29: 195–222.
- Grandcolas P. 1996. The phylogeny of cockroach families: a cladistic appraisal of morpho-anatomical data. *Canadian Journal of Zoology*, 74: 508–527.
- Grandcolas P. 1997. The monophyly of the subfamily Perisphaeriinae (Dictyoptera: Blattaria: Blaberidae). *Systematic Entomology*, 22: 123–130.
- Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W & Gascuel O. 2010. New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. *Systematic Biology*, 59: 307–321.
- Hanitsch R. 1933. The Blattidae of Mount Kinabalu, British North Borneo. *Journal of The Federated Malay States Museums*, 17: 297–337.
- Hoang DT, Chernomor O, Von Haeseler A, Minh BQ & Vinh LS. 2018. UFBoot2: Improving the Ultrafast Bootstrap Approximation. *Molecular Biology and Evolution*, 35: 518–522.
- Humphrey N, Rose HA & Colgan DJ. 1998. Electrophoretic studies of cockroaches of the Australian endemic subfamily Geoscapheinae. *Zoological Journal of the Linnean Society*, 124: 209–234.
- Inward D, Beccaloni G & Eggleton P. 2007. Death of an order: a comprehensive molecular phylogenetic study confirms that termites are eusocial cockroaches. *Biology Letters*, 3: 331–335.
- Jennings EC, Korthauer MW, Hendershot JM, Bailey ST, Weirauch MT, Ribeiro JMC & Benoit JB. 2020. Molecular mechanisms underlying milk production and viviparity in the cockroach, *Diploptera punctata*. *Insect Biochemistry and Molecular Biology*, 120: 103333
- Katoh K, Rozewicki J & Yamada KD. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief Bioinform*, 20: 1160–1166.

- Klass KD. 1997. The external male genitalia and the phylogeny of Blattaria and Mantodea. *Zoologisches Forschungsinstitut*, 42: 341
- Klass KD & Meier R. 2006. A phylogenetic analysis of Dictyoptera (Insecta) based on morphological characters. *Entomologische Abhandlungen*, 63: 3–50.
- Kumar R. 1975. A review of the cockroaches of West Africa and the Congo Basin (Dictyoptera: Blattaria). *Bulletin de l'Institut fondamental d'Afrique noire (Sciences naturelles)*, 37: 27–121.
- Kumar S, Stecher G, Li M, Knyaz C & Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*, 35: 1547–1549.
- Lanfear R, Frandsen PB, Wright AM, Senfeld T & Calcott B. 2017. PartitionFinder 2: New Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological Phylogenetic Analyses. *Molecular Biology and Evolution*, 34: 772–773.
- Legendre F, D'haese CA, Deleporte P, Pellens R, Whiting MF, Schliep K & Grandcolas P. 2014. The evolution of social behaviour in Blaberid cockroaches with diverse habitats and social systems: phylogenetic analysis of behavioural sequences. *Biological Journal of the Linnean Society*, 111: 58–77.
- Legendre F, Nel A, Svenson GJ, Robillard T, Pellens R & Grandcolas P. 2015. Phylogeny of Dictyoptera: Dating the Origin of Cockroaches, Praying Mantises and Termites with Molecular Data and Controlled Fossil Evidence. *Plos One*, 10: e0130127.
- Legendre F, Grandcolas P & Thouze F. 2017. Molecular phylogeny of Blaberidae (Dictyoptera, Blattodea), with implications for taxonomy and evolutionary scenarios. *European Journal of Taxonomy*, 291: 1–13.
- Li XR, Li M & Wang ZQ. 2017. Preliminary molecular phylogeny of beetle cockroaches (*Diploptera*) and notes on male and female genitalia (Blattodea: Blaberidae: Diplopterinae). *Zootaxa*, 4320: 523–534.
- Li XR, Wang LL & Wang ZQ. 2018. Rediscovered and new perisphaerine cockroaches from SW China with a review of subfamilial diagnosis (Blattodea: Blaberidae). *Zootaxa*, 4410: 251–290.
- Liu JL, Zhang JW, Han W, Wang YS, He S & Wang ZQ. 2023. Advances in the understanding of Blattodea evolution: insights from Phylotranscriptomics and Spermathecae. *Molecular Phylogenetics and Evolution*, 182: 107753.
- Lo N, Tong KJ, Rose HA, Ho SYW, Beninati T, Low DLT, Matsumoto T & Maekawa K. 2016. Multiple evolutionary origins of Australian soil-burrowing cockroaches driven by climate change in the Neogene. *Proceedings of the Royal Society B*, 283: 1–8.
- Maekawa K, Lo N, Kitade O, Miura T & Matsumoto T. 1999. Molecular Phylogeny and Geographic Distribution of Wood-Feeding Cockroaches in East Asian Islands. *Molecular Phylogenetics and Evolution*, 13: 360–376.
- Maekawa K, Lo N, Rose HA & Matsumoto T. 2003. The evolution of soil-burrowing cockroaches (Blattaria : Blaberidae) from wood-burrowing ancestors following an invasion of the latter from Asia into Australia. *Proceedings of the Royal Society B—Biological Sciences*, 270: 1301–1307.
- Marchal E, Hult EF, Huang J, Stay B & Tobe SS. 2013. *Diploptera punctata* as a model for studying the endocrinology of arthropod reproduction and development. *General and Comparative Endocrinology*, 188: 85–93.
- Mckittrick FA. 1964. Evolutionary studies of cockroaches. *Memoirs of the Cornell University Agricultural Experiment Station*, 389: 1–197.
- Minh BQ, Nguyen MaT & Von Haeseler A. 2013. Ultrafast Approximation for Phylogenetic Bootstrap. *Molecular Biology and Evolution*, 30: 1188–1195.
- Nguyen LT, Schmidt HA, Von Haeseler A & Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, 32: 268–74.

- Princis K. 1953. Kleine Beiträge zur Kenntnis der Blotterien und ihrer Verbreitung. V. *Opuscula Entomologica*, 18: 53–58.
- Princis K. 1964. Blattariae: Subordo Blaberoidea: Fam.: Panchloridae, Gynopeltidae, Derocalymmidae, Perisphaeriidae, Pycnoscelidae. In: Beier M (Ed.), *Orthopterorum Catalogus* 6. W. Junk, The Hague, pp. 173–281.
- Rambaut A, Drummond AJ, Xie D, Baele G & Suchard MA. 2018. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Systematic Biology*, 67: 901–904.
- Rehn JaG. 1932. African and Malagasy Blattidae (Orthoptera), part II. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 84: 405–511.
- Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA & Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61: 539–42.
- Rose HA, Walker J & Woodward JB. 2014. Five new species of soil burrowing cockroaches from Queensland (Blattodea: Blaberidae: Geoscapheinae). *Memoirs of the Queensland museum*, 59: 11–23.
- Roth LM. 1970. The Male Genitalia of Blattaria. IV. Blaberidae Blaberinae. *Psyche*, 77: 308–342.
- Roth LM. 1973a. The Male Genitalia of Blattaria. X. Blaberidae. *Pycnoscelus*, *Stilpnoblatta*, *Proscratea* (Pycnoscelinae), and *Diploptera* (Diplopterinae). *Psyche*, 80: 249–264.
- Roth LM. 1973b. *Paramuzoa* (Nyctiborinae), a New Cockroach Genus Previously Confused With *Parasphaeria* (Epilamprinae). *Psyche*, 80: 063172.
- Roth LM. 1977. A Taxonomic Revision of the Panesthiinae of the World I. The Panesthiinae of Australia (Dictyoptera : Blattaria : Blaberidae). *Australian Journal of Zoology*, 48: 1–112.
- Roth LM. 1979a. A Taxonomic Revision of the Panesthiinae of the World II. The Genera *Salganea* Stål *Microdina* Kirby and *Caeparia* Stål (Dictyoptera : Blattaria : Blaberidae). *Australian Journal of Zoology*, 69: 1–70.
- Roth LM. 1979b. A Taxonomic Revision of the Panesthiinae of the World III. The Genera *Panesthia* Serville and *Miopanesthia* Serville (Dictyoptera : Blattaria : Blaberidae). *Australian Journal of Zoology*, 74: 1–276.
- Roth LM. 1982. A Taxonomic Revision of the Panesthiinae of the World IV. The Genus *Ancaudellia* Shaw, with Additions to Parts I–III, and a General Discussion of Distribution and Relationships of the Components of the Subfamily (Dictyoptera : Blattaria : Blaberidae). *Australian Journal of Zoology*, 82: 1–142.
- Roth LM. 1992. The Australian cockroach genus *Laxta* Walker (Dictyoptera: Blattaria: Blaberidae). *Invertebrate Systematics*, 6: 389–435.
- Roth LM. 1998. The cockroach genus *Pycnoscelus* Scudder, with a description of *Pycnoscelus femapterus*, sp. nov. (Blattaria: Blaberidae: Pycnoscelinae). *Oriental Insects*, 32: 93–130.
- Roth LM. 2003. Systematics and phylogeny of cockroaches (Dictyoptera: Blattaria). *Oriental Insects*, 37: 1–186.
- Roth LM & Hahn W. 1964. Size of new-born larvae of cockroaches incubating eggs internally. *Journal of Insect Physiology*, 10: 65–72.
- Rugg D & Rose HA. 1984a. Reproductive biology of some Australian cockroaches (Blattodea: Blaberidae). *Austral Entomology*, 23: 113–117.
- Rugg D & Rose HA. 1984b. The taxonomic significance of reproductive-behavior in some Australian cockroaches (Blattodea, Blaberidae). *Journal of the Australian Entomological Society*, 23: 118–118.
- Shelford RWC. 1909. Descriptions of some new genera and species of Blattidae. *Deutsche entomologische Zeitschrift*, 1909: 611–624.

- Simon C, Frati F, Beckenbach A, Crespi B, Liu H & Flook P. 1994. Evolution, Weighting, and Phylogenetic Utility of Mitochondrial Gene Sequences and a Compilation of Conserved Polymerase Chain Reaction Primers. *Annals of the Entomological Society of America*, 87: 651–701.
- Wang XD, Wang ZQ & Che YL. 2014. A taxonomic study of the genus *Panesthia* (Blattodea, Blaberidae, Panesthiinae) from China with descriptions of one new species, one new subspecies and the male of *Panesthiaantennata*. *Zookeys*, 466: 53–75.
- Wang YS, Zhang JW, Lo N, Bourguignon T, Guo L, Li BL, Che YL & Wang ZQ. 2023. Phylogenetic analysis of Blaberoidea reveals non-monophyly of taxa and supports creation of multiple new subfamilies. *Cladistics*, 39: 198–214.
- Wang ZQ, Shi Y, Qiu ZW, Che YL & Lo N. 2017a. Reconstructing the phylogeny of Blattodea: robust support for interfamilial relationships and major clades. *Scientific Reports*, 7: 3903.
- Wang ZZ, Yang R & Wang ZQ. 2017b. First record of *Rhabdoblattella* (Blaberidae, Epilamprinae) from China with descriptions of two new species. *Zootaxa*, 4294: 381–388.
- Wheeler WM. 1904. The phylogeny of the termites. *The Biological Bulletin*, 8: 29–37.
- Xia X. 2018. DAMBE7: New and Improved Tools for Data Analysis in Molecular Biology and Evolution. *Molecular Biology and Evolution*, 35: 1550–1552.
- Zhang D, Gao FL, Jakovlić I, Zou H, Zhang J, Li WX & Wang GT. 2020. PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resources*, 20: 348–355.

Table_S1 access: https://github.com/CQSZZ/Blaberidae/blob/main/Table_S1.xlsx