Novel gene rearrangement pattern in mitochondrial genome of *Ooencyrtus plautus* Huang & Noyes, 1994: new gene order in Encyrtidae (Hymenoptera, Chalcidoidea)

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Abstract

Studies of mitochondrial genomes have a wide range of applications in phylogeny, population genetics, and evolutionary biology. In this study, we sequenced and analyzed the mitochondrial genome of *Ooencyrtus plautus* Huang & Noyes, 1994 (Hymenoptera, Encyrtidae). The nearly complete mitogenome of *O. plautus* was 15,730 bp in size, including 13 PCGs (protein-coding genes), 22 tRNAs, 2 rRNAs, and a nearly complete control region. The nucleotide composition was significantly biased toward adenine and thymine, with an A + T content of 84.6%. We used the reference sequence of *Chouioia cunea* and calculated the Ka/Ks ratio for each set of PCGs. The highest value of the Ka/Ks ratio within 13 PCGs was found in *nad2* with 1.1, suggesting that they were subjected to positive selection. This phenomenon was first discovered in Encyrtidae. Compared with other encyrtid mitogenomes, a translocation of *trnW* was found in *O. plautus*, which was the first of its kind to be reported in Encyrtidae. Comparing with ancestral arrangement pattern, wasps reflect extensive gene rearrangements. Although these insects have a high frequency of gene rearrangement, species from the same family and genus tend to have similar gene
sequences. As the number of sequenced mitochondrial genomes in Chalcidoidea increases, we summarize some of the rules of gene rearrangement in Chalcidoidea, that is four gene clusters with frequent gene rearrangements. Ten mitogenomes were included to reconstruct the phylogenetic trees of Encyrtidae based on both 13 PCGs (nucleotides of protein coding genes) and AA matrix (amino acids of protein coding genes) using the maximum likelihood and Bayesian inference methods. The phylogenetic tree reconstructed by Bayesian inference based on AA data set showed that *Aenasius arizonensis* and *Metaphycus eriococci* formed a clade representing Tetracneminae. The remaining six species formed a monophyletic clade representing Encyrtinae. In Encyrtinae, *Encyrtus* forms a monophyletic clade as a sister group to the clade formed by *O. plautus* and *Diaphorencyrtus aligarhensis*. *Encyrtus asakii* and *Encyrtus rhodooccisi* were most closely related species in this monophyletic clade. In addition, gene rearrangements can provide a valuable information for molecular phylogenetic reconstruction. These results enhance our understanding of phylogenetic relationships among Encyrtidae.

**Keywords**
Encyrtinae, mitogenome, pairwise breakpoint distance, phylogenetic tree, Tetracneminae

**Introduction**

The mitochondrial genome is a standard circular molecule, mostly range between 15 kb and 18 kb in size, with 37 genes, including 13 protein-coding genes, three of which are oxidative phosphorylation complexes, 2 rRNAs, and 22 tRNAs and a major non-coding region which mainly regulates replication and transcription (Fig. 1) (Boore 1999; Cameron 2014). The mitochondrial (mt) genome has the characteristics of gene recombination, maternal inheritance, conservation of gene components, and high AT content, and it is considered to be an ideal molecular marker for species identification and phylogenetic or evolutionary studies (Boore and Brown 1998; Curole and Kocher 1999). In addition, the gene content, genome size, and RNA secondary structure of the mt genome can also provide useful information for phylogeny (Boore and Brown 1998; Gissi et al. 2008; Cameron 2014). Gene rearrangement is one of the most frequently studied features in animal mitochondrial genomes (Boore and Brown 1998; Cameron 2014; Li et al. 2016). It is usually conserved in major lineages but may be rearranged in some groups (Boore and Brown 1998; Boore 1999; Dowton et al. 2002a). The rearrangement processes of mitochondrial genes can be described as transposition, inversion, reverse transposition, and TDRL (tandem-duplication-random-loss) (Dowton et al. 2002b; Cameron 2014). The movement of TDRL is to describe the duplication of multiple consecutive genes and the successive random loss of one of the two copies (Bernt et al. 2007). Previous works by Sankoff et al. (1992) and Boore et al. (1998) showed that the gene sequence of the mitochondrial genome contains phylogenetic signals. Studying gene rearrangements in lower taxonomic lineages of insects can provide some evidence for the evolution of these groups (Mao et al. 2014; Li et al. 2016).

Extensive mitochondrial genome data indicates that, compared to other orders in the hexapoda, Hymenoptera (Dowton and Austin 1999; Dowton et al. 2009; Wei et al. 2014) and Hemiptera (Shao and Barker 2003; Shao et al. 2003; Johnson et al. 2004)
have highly accelerated and independent gene arrangement events. Chalcidoidea is one of the most diverse groups of insects in the order Hymenoptera. Parasitic chalcidoids are widely used as biological control of various agricultural pests (Heraty 2017). At the same time, diverse morphology, body sizes, lifestyles, and different types of parasitism, which is also reflected in their amazing genetic arrangement (Austin et al. 1998; Heraty et al. 2013). The mitochondrial genome of Chalcidoidea has been confirmed to have a large number of gene rearrangements (Oliveira et al. 2008; Xiao et al. 2011). A total of 40 species of mitochondrial genomes have been reported in the Chalcidoidea, including 11 families and all sequenced mitochondrial genomes have rearrangements compared with putative ancestor (Fig. 2) (up to date: 15 March 2022). The parasitic life history, body size, and developmental period in Hymenoptera are hypothesized to be related to their gene rearrangements (Dowton and Austin 1995; Shao et al. 2001), and the information of these gene rearrangements may be valuable for the phylogenetic reconstruction of specific lineages (Yuan et al. 2015; Liu et al. 2017).

**Figure 1.** Circular map of the *Ooencyrtus plautus* mitochondrial genome.
Table 1. Mitochondrial genome organization in Chalcidoidea. Colored boxes indicate rearranged gene clusters, and gray boxes indicate conserved gene blocks.

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agaonidae</td>
<td>Pristomeris longicornis</td>
</tr>
<tr>
<td>Apheleidae</td>
<td>Apheleidae</td>
</tr>
<tr>
<td>Chalcidinae</td>
<td>Chalcidinae</td>
</tr>
<tr>
<td>Eurytidae</td>
<td>Eurytidae</td>
</tr>
<tr>
<td>Eupelmidae</td>
<td>Eupelmidae</td>
</tr>
<tr>
<td>Eurytomidae</td>
<td>Eurytomidae</td>
</tr>
<tr>
<td>Mymaridae</td>
<td>Mymaridae</td>
</tr>
<tr>
<td>Pieromalidae</td>
<td>Pieromalidae</td>
</tr>
<tr>
<td>Torymidae</td>
<td>Torymidae</td>
</tr>
<tr>
<td>Trichogrammatidae</td>
<td>Trichogrammatidae</td>
</tr>
</tbody>
</table>

Figure 2. Mitochondrial genome organization in Chalcidoidea. Colored boxes indicate rearranged gene clusters, and gray boxes indicate conserved gene blocks.
Mitochondrial genome of *Ooencyrtus plautus* is a kind of parasitoid in the family Encyrtidae, which has the characteristics of high parasitism rate and strong reproduction ability. In the Encyrtidae, seven species of complete mitochondrial genes and one species of partial mitochondrial genes have been reported (Table 1). In this study, the nearly complete mitochondrial genome of *O. plautus* was measured, sequenced, and annotated. The characteristics of the mitogenomes are described in terms of genome structure, nucleotide content, frequency of usage of start and stop codons, and gene rearrangement. It reported a new gene rearrangement which was found in the mitochondrial genome of *O. plautus* (The long-distance transposition of *trnW* and the reverse transposition of gene cluster *trnN-trnS1-trnY*) and conducted phylogenetic analyses using the current mitogenome along with those previously reported from Encyrtidae.

**Table 1.** List of species investigated and their related information.

<table>
<thead>
<tr>
<th>Family</th>
<th>Subfamily</th>
<th>Taxa</th>
<th>GenBank Accession No.</th>
<th>Location/Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aphelinidae</td>
<td><em>Encarsia formosa</em></td>
<td>MG813797.1</td>
<td>(Zhu et al. 2018)</td>
</tr>
<tr>
<td>2</td>
<td>Encyrtidae</td>
<td><em>Encarsia obtusiclava</em></td>
<td>MG813798.1</td>
<td>(Zhu et al. 2018)</td>
</tr>
<tr>
<td>3</td>
<td>Tetracneminae</td>
<td><em>Aenasius arizonensis</em></td>
<td>MK630013</td>
<td>(Ma et al. 2019)</td>
</tr>
<tr>
<td>4</td>
<td>Tetracneminae</td>
<td><em>Metaphycus eriococci</em></td>
<td>MW255970</td>
<td>Direct Submission</td>
</tr>
<tr>
<td>5</td>
<td>Encyrtinae</td>
<td><em>Diaphorencyrtus algarhensis</em></td>
<td>MN274569</td>
<td>(Du et al. 2019)</td>
</tr>
<tr>
<td>6</td>
<td>Encyrtinae</td>
<td><em>Encyrtus eulecaniumiae</em></td>
<td>NC_051459</td>
<td>Direct Submission</td>
</tr>
<tr>
<td>7</td>
<td>Encyrtinae</td>
<td><em>Encyrtus infelix</em></td>
<td>MH729198</td>
<td>(Xiong et al. 2019)</td>
</tr>
<tr>
<td>8</td>
<td>Encyrtinae</td>
<td><em>Encyrtus rhodooccisae</em></td>
<td>NC_051460</td>
<td>Direct Submission</td>
</tr>
<tr>
<td>9</td>
<td>Encyrtinae</td>
<td><em>Encyrtus sasakii</em></td>
<td>NC_051458</td>
<td>Direct Submission</td>
</tr>
<tr>
<td>10</td>
<td>Encyrtinae</td>
<td><em>Ooencyrtus plautus</em></td>
<td>OP442361</td>
<td>This study</td>
</tr>
</tbody>
</table>

**Materials and methods**

**Sample collection and DNA extraction**

Specimens of *Ooencyrtus plautus* were collected from Fuzhou city, Fujian province, in September 2020. They were reared in the laboratory, then processed for DNA extraction. Total genomic DNA was extracted using the cetyltrimethyl ammonium bromide (CTAB) method (Shahjahan et al. 1995).

**Sequencing and assembly**

Sequencing was performed using a whole genome shotgun (WGS) strategy on the Illumina Novaseq platform. The quality of data was checked using FastQC (Andrews 2013). Raw data were filtered into high-quality data after processing with AdapterRemoval v. 2. Then, Single Nucleotide Polymorphism (SNP), Insertion and Deletion (InDel), Copy Number Variation (CNV), and Structural Variation (SV) analysis were conducted to ensure the reliability of nucleotides. The assembly of the mitochondrial genome was accomplished with Novoplyasy v. 2.7 (Dierckxsens et al. 2017).
Mitochondrial genome annotation

Gene annotation of mitochondrial genome was performed using MitoZ and 13 protein-coding genes as well as 22 tRNA genes are annotated (Meng et al. 2019). Twenty-two tRNA genes were verified with the use of MITOS WebServer, setting the parameters with the Invertebrate Mito genetic code (Minh et al. 2013). Every sequence of tRNA genes was manually checked separately. According to the secondary structure of tRNA predicted by MITOS, it was drawn manually using Adobe Illustrator. Annotation of the 13 protein-coding genes were refined manually by identifying the corresponding open reading frames using the invertebrate mitochondrial code. The mitogenome maps were produced using Organellar Genome DRAW (OGDRAW) (Marc et al. 2013).

Comparative analysis

Geneious v. 11.0.2 was used to examine all genes in the mitochondrial genome (Kearse et al. 2012). MEGA X (Sudhir et al. 2018) was used to analyze base composition and relative synonymous codon usage (RSCU). To calculate the number of synonymous substitutions for each synonymous site (Ks) and the number of non-synonymous substitutions for each non-synonymous site (Ka) through DnaSP 5. AT/GC skewness is calculated as AT skewness = (A − T) / (A + T) and GC-skew = (G − C) / (G + C) (Rozas et al. 2003). To measure the relative composition of different bases was measured based on GC and AT skew.

Phylogenetic analysis

In this study, a total of 10 species of mitogenomes were analyzed, of which Encarsia formosa and Encarsia obtusiclava of family Aphelinidae were selected as outgroups (Table 1). MAFFT v. 7.3.1 was used to align the PCGs (Kazutaka and Standley 2016). Alignments of individual genes were concatenated to generate two kind of data sets: 1) the PCG matrix, including all three codon positions of protein-coding genes; 2) the AA matrix, translated 13 PCG into amino acids.

Both ML (maximum likelihood) and BI (Bayesian inference) analyses were performed on the concatenated data set used for phylogenetic reconstruction. In W-IQtree (Trifinopoulos et al. 2016), the best-fit model was used for maximum likelihood analysis. The site-heterogeneous mixture model (CAR+GTR) was used in PhyloBayes analysis. The trees were sampled every 1000 generations (Huelsenbeck 2012). FigTree v. 1.3.1 was used to view the generated tree.

Results

Genome structure and organization

The nearly complete mitogenome of O. plautus was 15,730 bp in size, including 13 PCGs, 22 tRNAs, 2 rRNAs, and a nearly complete control region. In Ooencyrtus plautus,
27 genes (15 tRNAs, 2 rRNAs, and 10 PCGs) were encoded by the majority-strand (J-strand), and 10 genes (3 PCGs and 7 tRNAs) were encoded by the minority-strand (N-strand). The values of AT skew and GC skew are often used to reveal the nucleotide composition of the mitochondrial genome (Alexandre et al. 2005). In this study, the nucleotide compositions of eight complete or nearly complete mitogenomes in Encyrtidae were investigated by calculating the percentages of AT-skew and GC-skew (Fig. 3). The results of the nucleotide skew statistics showed that the AT-skews in PCGs, tRNAs, and rRNAs of encyrtid mitogenomes were almost all positive, while the GC-skews were almost all obviously negative.

The nucleotide composition *O. plautus* was significantly biased toward adenine and thymine, with an A + T content of 84.6% (Table 2). The PCGs on the N strand had a T skew and slight G skew. However, the PCGs on the J strand exhibited a C skew. The tRNAs was negative for AT skews, besides the tRNAs on the N strand, which had an equal G and C distribution. Two rRNA genes (lrRNA and srRNA) were encoded on the J strand and exhibit an A skew and slight C skew.

Intergenic spacers and overlapping genes are very common in arthropod mitochondrial genomes (Ma et al. 2015; Chen et al. 2018). In the mitochondrial genome of *O. plautus*, a total of 166 bp of intergenic spacers ranging from 1 to 47 bp were found in eight locations. The minimum intergenic spacers (1 bp) located at *trnT-trnP, trnQ-trnW*, *nad6-cob,*

![Figure 3. Nucleotide composition of various data sets of mitogenomes. Hierarchical clustering of Encyrtidae species based on the AT-skew and GC-skew.](image-url)
and trnS2-nd1. The maximum (47 bp) gene spacer was located at trnW-trnC. There were 18 overlapping gene regions, ranging from 2 bp to 7 bp in length in the nine mitogenomes. The longest overlapping sequence (7 bp) was between atp6-atp8 and nad4-nad4L.

**Protein-coding genes (PCGs)**

In the mitochondrial genome of *O. plautus*, the total length of protein-coding genes was 11,072 bp, accounting for 70.39% of the entire genome. Most of them were encoded on the J strand, and only nad2, nad6, and cob were encoded on the N strand. The average A + T content of the 13 protein-coding genes was 82%, ranging from 73.7% (cox1) to 93.80% (atp8) for individual genes.

To investigate further this high A and T content, and the frequency of synonymous codon usage, we calculated relative synonymous codon usage (RSCU) values. The relative synonymous codon usages (RSCU) of the *O. plautus* are shown in Fig. 4. Taken together, the most frequently used codons are UUA (Leu2), AGA (Ser1), and CGA (Arg), whereas those ending in G or C, CUG, CUC, CAG, and GGC were the less frequently used codons. The codons ending with A or T are predominant, which at least partly leads to the bias towards A and T.

The predicted initiation codons are ATN as in most other insect mitochondrial genomes (Dowton and Austin 1999). There were six genes (cox1, cob, nad4, nad6, atp6, and cox3) starting with ATG and seven genes (nad1, nad3, nad6, nad4L, nad5, cox2, and atp8) starting with ATT. All protein-coding genes terminated at the most common stop codon TAA, except for nad1 and nad5, which stopped with single T (Table 3).

Previous work (Zhang et al. 2020) showed that Eulophidae and Encyrtidae have close relationships, so we chose Eulophidae species as the reference sequence. Using Chouioia cunea as a reference sequence, we calculated the non-synonymous substitution rate (Ka), synonymous substitution (Ks), and Ka/Ks ratio of each PCG for *O. plautus* (Fig. 5). Ka and Ks are non-synonymous and synonymous substitution rates, respectively. They are controlled by functionally related sequence contexts, such as encoding amino acids and participating in exon splicing (Parmley and Hurst 2007). The ratio of the two parameters Ka/Ks (a measure of the strength of selection) is defined as

| Table 2. Nucleotide composition and skewing of the *Ooencyrtus plautus* mitogenome. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | size            | T    | C    | A    | G    | AT-skew | GC-skew |
| whole genome    | 15730           | 0.401| 0.085| 0.445| 0.069| 0.052   | -0.104 |
| PCGs            | 11072           | 0.381| 0.097| 0.439| 0.083| 0.071   | -0.078 |
| PCGs(J)         | 8402            | 0.345| 0.086| 0.467| 0.102| 0.150   | 0.085  |
| PCGs(N)         | 2670            | 0.494| 0.081| 0.352| 0.073| -0.168  | -0.052 |
| tRNA            | 1467            | 0.455| 0.066| 0.437| 0.042| -0.020  | -0.222 |
| tRNA(J)         | 994             | 0.454| 0.036| 0.437| 0.073| -0.019  | 0.339  |
| tRNA(N)         | 473             | 0.457| 0.053| 0.438| 0.053| -0.021  | 0.000  |
| tRNA            | 2103            | 0.437| 0.068| 0.453| 0.042| 0.018   | -0.236 |
Figure 4. Relative synonymous codon usage (RSCU) of the mitochondrial genomes of *Ooencyrtus plautus*.

Figure 5. Evolutionary rates of mitochondrial genomes in *Ooencyrtus plautus*. The numbers of nonsynonymous substitutions per nonsynonymous site (Ka), the number of substitutions per synonymous site (Ks), and the ratio of Ka/Ks for each gene are given, using *Chouioia cunea* as the reference sequence.
the degree of evolutionary change (Wang et al. 2011). A value of Ka/Ks greater than 1 means positive selection exists, indicating that non-synonymous mutations are more favored by Darwinian selection, and they will be retained at a rate greater than synonymous mutations. In *O. plautus*, the Ka/Ks ratio of *nad2* was greater than 1, indicating that the *nad2* gene had a positive selection. This phenomenon was first discovered in Encyrtidae. In addition, the Ka/Ks of *atp8* is as high as 0.9389, which is the highest except for *nad2*. The high Ka/Ks phenomenon of *nad2* and *atp8* is also found in other species (Jia et al. 2020; Guo et al. 2021; Xu et al. 2021). The reason for this phenomenon may be that the evolution speed of a gene is related to its function (Wang et al. 2011).

**Table 3.** Mitogenomic organization of *Ooencyrtus plautus*.

<table>
<thead>
<tr>
<th>Name</th>
<th>Start</th>
<th>Stop</th>
<th>Strand</th>
<th>Length</th>
<th>Codons</th>
</tr>
</thead>
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<td>1</td>
<td>68</td>
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<td>68</td>
<td></td>
</tr>
<tr>
<td>nad2</td>
<td>1064</td>
<td>75</td>
<td>-</td>
<td>870</td>
<td>ATT/TAA</td>
</tr>
<tr>
<td>trnR</td>
<td>1146</td>
<td>1082</td>
<td>-</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>trnQ</td>
<td>1227</td>
<td>1160</td>
<td>-</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>trnN</td>
<td>1229</td>
<td>1296</td>
<td>+</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>trnW</td>
<td>1299</td>
<td>1367</td>
<td>+</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>trnC</td>
<td>1481</td>
<td>1415</td>
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<td></td>
</tr>
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<tr>
<td>nad3</td>
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<tr>
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<td>atp6</td>
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<td>3502</td>
<td>+</td>
<td>666</td>
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<tr>
<td>nad5</td>
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<td>7898</td>
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<td>1419</td>
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<td>7966</td>
<td>+</td>
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</tr>
<tr>
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<td>9588</td>
<td>+</td>
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<td>9725</td>
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<td>9744</td>
<td>-</td>
<td>501</td>
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</tr>
<tr>
<td>cob</td>
<td>11424</td>
<td>10291</td>
<td>-</td>
<td>1098</td>
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</tr>
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<td>13791</td>
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</tr>
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Transfer and ribosomal RNA genes

In total, 22 transfer RNA genes were found, ranging in size from 59 bp \( (\text{trnS1}) \) to 71 bp \( (\text{trnT}) \). Most of the tRNAs were encoded on the J strand and only 7 tRNAs \( (\text{trnT}, \text{trnE}, \text{trnK}, \text{trnC}, \text{trnQ}, \text{trnR}, \text{and trnS2}) \) were encoded on the N strand. The average nucleotide composition of these tRNAs was A: 43.7%, T: 45.5%, C: 6.6%, and G: 4.2%, with a total average A + T content of 89.2%. All tRNA sequences can be folded into the canonical cloverleaf secondary structure, except for \text{trnS1} \) which lacked the dihydrouridine (DHU) arm. A lack of the DHU arm in \text{trnS1} \) was found in the mitochondrial genomes of most insects (Dowton et al. 2002b). Changes in the length of the DHU and TΨC arms lead to differences in the size of the tRNA sequence (Shao et al. 2001). In addition, some mismatches (G-U in \text{trnD}, \text{trnA}, and \text{trnV}, U-U in \text{trnS1}, and two U-U in \text{trnA}) were found in \text{O. plautus}.

The two rRNA genes, the larger ribosomal gene \( (\text{rrnL}) \) and the smaller ribosomal gene \( (\text{rrnS}) \), were located between \text{trnA} and \text{trnL1}, and \text{trnV} and \text{trnA}, respectively. The average of the total size of two rRNAs was 2,103 bp and the average A + T content was 89%.

Gene arrangement patterns

Gene rearrangement in the mitochondrial genome is a relatively rare event in the evolutionary history of insects (Cameron 2014). However, in the Hymenoptera lineage, more and more species have mitochondrial gene rearrangements (Shao et al. 2003). Gene rearrangements are a common phenomenon found in almost all the sequenced Chalcidoidea mt genomes (Fig. 2). In the Chalcidoidea mitochondrial genomes studies, most of the tRNA genes are rearranged (Tang et al. 2018). In the Hymenoptera, numerous rearrangements of protein-coding genes have been identified in several groups (Xiao et al. 2011; Wei et al. 2014). Compared with the putative ancestral pattern of the insect mitochondrial genome, dramatic gene rearrangements, not only in tRNA genes but also in protein-coding genes, were found in \text{O. plautus} mitochondrial genomes. In the mitogenome of \text{O. plautus}, a total of 17 genes, including six PCGs, 10 tRNAs, and one rRNA gene are rearranged (Fig. 7).

In Encyrtidae, the gene order of all species of \text{Encytus} are identical. We used their gene order as a template to analyze the gene rearrangement of Encyrtidae. It can be summarized into four obvious rearrangements. They are the rearrangement of gene clusters \text{trnF-nad2-trnW}, \text{trnY-trnS1-trnN-trnC-trnR}, and \text{trnA-trnQ-rrns-trnV-trnM}. In addition, there are inversions of \text{trnE-trnF} and \text{trnP-trnT}.

Firstly, the \text{trnW-trnI-nad2} gene cluster in these four species \( (\text{Aenasius arizonensis}, \text{Diaphorencyrtus aligarhensis}, \text{Metaphycus eriococci}, \text{and Platencytus parkeri}) \) was rearranged as \text{trnI-nad2-trnW}, most species in Chalcidoidea of which were in this order. However, in \text{O. plautus}, \text{trnW} was translocated, causing the gene cluster \text{trnI-nad2-trnW} to be divided into \text{trnI-nad2} and \text{trnW}, which is the first of its kind to be reported in Chalcidoidea. Besides, the \text{trnI-nad2} gene cluster was translocated to upstream. Secondly, the
Figure 6. Predicted secondary cloverleaf structure for the tRNAs of *Ooencyrtus plautus.*
Mitochondrial genome of *Ooencyrtus plautus*

The mitochondrial genome of *Ooencyrtus plautus* has undergone disorderly rearrangements, including the trnN-trnS1-trnY-trnC-trnR gene clusters in four species (*A. arizonensis*, *D. aligarhensis*, *M. eriococci*, and *O. plautus*). Thirdly, the trnE-trnF inversion occurred in both *A. arizonensis* and *D. aligarhensis*. In addition, the trnP-trnT inversion also occurred in *D. aligarhensis*. Finally, in *D. aligarhensis* and *O. plautus*, trnQ in the gene cluster trnA-trnQ-rrnS-trnV-trnM has a long-distance transposition. In *M. eriococci*, trnM and trnQ were transposed in the gene cluster trnA-trnQ-rrnS-trnV-trnM.

**Phylogenetic analyses**

Phylogenetic relationships were analyzed using the concatenated nucleotides and amino acids sequences of 13 PCGs from eight encyrtid species and two outgroups. Four topologies were constructed using both ML and BI methods and two different data sets. The topological structures of these two phylogenetic trees reconstructed by ML analysis were identical with the phylogenetic tree reconstructed by BI based on PCG (Fig. 8). While Bayesian tree reconstructed on the AA data set showed different phylogenetic relationships in the clade consist of *Encyrtus rhodococcusiae*, *E. eulecaniumiae* and *E. sasakii* which was *E. eulecaniumiae* + (*E. rhodococcusiae* + *E. sasakii*) rather than *E. rhodococcusiae* + (*E. eulecaniumiae* + *E. sasakii*). The deviation and rate heterogeneity of the nucleotide composition of the mitogenomes are the basis of the rapid evolution of Chalcidoidea, which leads to inconsistent topologies based on different data types and analysis strategies (Wu et al. 2020). Currently, the CAT + GTR model implemented in PhyloBayes software was found to be the best fitting model for all data sets. In addition, the AA data set is considered as the best fit matrix for reconstructing phylogenetic trees (Li et al. 2015, 2017).
Figure 8. Phylogenetic tree produced by maximum likelihood and Bayesian inference analyses based on the 13 PCG and the AA data set A phylogenetic tree produced by Bayesian inference analyses based on 13 PCG data set B phylogenetic tree produced by maximum likelihood analyses based on the AA data set C phylogenetic tree produced by maximum likelihood analyses based on the 13 PCG data set.

In the BI topology based on the AA data set, *A. arizonensis* and *M. eriococci* formed a clade representing Tetracneminae. The remaining six species formed a monophyletic clade representing Encyrtinae. In Encyrtinae, *Encyrtus* formed a monophyletic clade as a sister group to the clade formed by *O. plautus* and *D. aligarhensis*. *Encyrtus sasakii* and *E. rhodooccisiae* are most closely related in this monophyletic clade. In the BI topology based on the PCG data set, *E. eulecaniumiae* and *E. sasakii* form a sister group then to *E. rhodooccisiae*. *Encyrtus infelix* was the first diverged in *Encyrtus*. 
Pairwise breakpoint distances (PBD) between the mitochondrial genomes of each species in Encyrtidae were calculated using the web server CREx, and heatmaps were constructed (Fig. 9). In the BI topology based on the AA data set, *E. infelix* was the first divergence branch in *Encyrtus*, which is consistent with the structure of the rest phylogenetic trees. The value of pairwise breakpoint distances between *E. infelix* and *D. aligarhensis* was 14, and the value of *E. infelix* and *O. plautus* was 10. Lower values of PBD indicated closer relationships which was consistent with the topology among *E. infelix*, *O. plautus*, and *D. aligarhensis* on the phylogenetic tree.

**Discussion**

In Encyrtidae, the gene rearrangement processes can be summarized into four rearrangement mechanisms, which can also be applied to Chalcidoidea (Fig. 2). First, the rearrangement between *trnI-nad2-trnW* includes the inversion and translocation. The gene cluster *trnI-nad2-trnW* presents three gene orders, one is *trnI-nad2-trnW*, another is *trnW-trnI-nad2*, and the last is one of them has a translocation. For example, in Pteromalidae, *nad2* in *Sycobia* sp. was translocated, but in other species, the order of *trnI-nad2-trnW* has not changed. The second is the rearrangement in gene cluster *trnN-trnS1-trnY-trnC-trnR* where gene rearrangements occurred most frequently. The main rearrangements were inversions and long-distance translocations (mainly *trnR*). In *Encyrtus*, translocation of *trnR* were occurred in all these four species, and this phenomenon was also found in Agaonidae. The final mechanism is the inversion of *trnE-trnF* and *trnP-trnT*. The *trnE-trnF* inversion occurred in *A. arizonensis*, *D. aligarhensis*, *Tamarixia radiata*, and *Aisopteromalus clanda*ae. And the *trnP-trnT* inversion occurred in *Ceratosolen fusiceps*, *Eupelmus* sp. and *A. arizonensis*. 

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**Figure 9.** Mitogenomic phylogeny of eight Encyrtidae species and two outgroups based on the AA data set using the Bayesian inference. Heatmap of PBD values among encyrtid species is nested within the phylogeny, and the PBD values are listed.
These four gene rearrangements can be applied to most of Chalcidoidea. When these tRNA gene rearrangement patterns were mapped on the estimated phylogenetic tree, the gene order of mitochondrial genome may resolve some contentious evolutionary questions (Shen et al. 2019). Based on the gene rearrangements occurring in Encyrtidae and the pairwise breakpoint distances heatmap, the hypotheses can be verified: the closely related species tend to have similar mitochondrial gene orders (Wu et al. 2020). For example, the gene order of all species of *Encytus* are identical. Correspondingly, the value of pairwise breakpoint distances among them are zero. Besides, the long-distance translocation of *trnQ* occurred in *D. aligarhensis* and *O. plautus*. In the topology of the BI tree based on AA matrix, *D. aligarhensis* and *O. plautus* are closely related and sister to the clade formed by genus *Encytus*. The PBD value between *O. plautus* and *Encytus* is 10, and the PBD value between *D. aligarhensis* and *Encytus* is 14. Lower PBD values indicates that *O. plautus* is more closely related to *Encytus*, which is in accordance with the phylogenetic tree. This phenomenon verified that PBD values could be used for inferring phylogenetic relationships. In summary, the gene rearrangement of mitogenome can provide a valuable source of characteristics for the reconstruction of molecular phylogeny (Wu et al. 2020).

As the sampling diversity of Encyrtidae is limited, it cannot completely solve the main classification question. Considering the limited research on taxonomic relationships inference based on molecular data, a comprehensive comparison of species morphology and genetic characteristics is needed to better understand the phylogenetic relationship of Encyrtidae. If more mitochondrial genomes are sequenced, the accuracy of phylogenetic relationships could be improved. We hope that future studies will combine morphology with more data sets from the mitochondrial genome to provide sufficient evidence for the phylogenetic relationship of the Encyrtidae.

**Conclusions**

In this study, we sequenced the nearly complete mitogenome of *O. plautus* that contains 37 genes and one control region. The nucleotide composition, codon usage, RNA structures, and protein-coding genes evolution were analyzed. The mitogenome genome of *O. plautus* reveals the phylogenetic relationship of Encyrtidae for the first time. In addition, the regularity of gene rearrangement within Encyrtidae is discussed. The results of this study will contribute to further studies on evolutionary relationships within Encyrtidae.

**Funding**

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Mitochondrial genome of Ooencyrtus plautus

ince (grant no. 202003a06020009), the National Science and Technology Fundamental Resources Investigation Program of China (grant no. 2019FY101800) and the Natural Science Foundation of Anhui Normal University (grant no. 2020XJ19).

References


Mitochondrial genome of *Ooencyrtus plautus*


Mitochondrial genome of *Ooencyrtus plautus* 


Hidden in the jungle of Vietnam: a new species of *Quasipaa* (Amphibia, Anura, Dicroglossidae) from Ngoc Linh Mountain

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Abstract

A new species of *Quasipaa* is described from Ngoc Linh Mountain of the Kon Tum Massif in central Vietnam. The new species is morphologically distinguishable from its congeners on the basis of a combination of the following diagnostic characters: SVL 79.6–84.3 mm in males and 64.6–69.9 mm in females; head broader than long; vomerine teeth present; external vocal sacs absent; tympanum slightly visible; dorsum with lines of thick ridges and small round tubercles; flanks covered by oval and round tubercles; supratympanic fold present; dorsolateral fold absent; ventrolateral sides, ventral surface of arms, and all fingers with spines in males; the absence of spines on chest and belly in males; toes fully webbed to distal portion of terminal phalanx; in life, dorsum dark brown, chest and belly immaculate white. Phylogenetic analyses found that the genetic divergence of the new species and its congeners ranged from 4.2–5.1% (compared with *Quasipaa boulengeri*) to 7.6–8.1% (compared with *Q. shini*) in the 16S gene.

Keywords

Kon Tum Province, molecular phylogeny, *Quasipaa taoi* sp. nov., taxonomy
Introduction


During our recent fieldwork in the Central Highlands of Vietnam, specimens of *Quasipaa* were collected in the evergreen forests of Ngoc Linh Mountain, Kon Tum Province. These specimens were identified as members of the “*Quasipaa sensu stricto*” species group (Group II-2) (Che et al. 2010) and *Quasipaa* sp. 1 (Yan et al. 2021) based on molecular data. Closer morphological examination showed that the population from Ngoc Linh Mountain in the Central Highlands of Vietnam could be clearly distinguished from other *Quasipaa* species by a combination of morphological features. Also, in phylogenetic analyses, this taxon was clearly separated from its congeners. Therefore, we describe here the unnamed taxon from the Central Highlands of Vietnam, based on our integrative taxonomical analyses, as a new species.

Materials and methods

Sampling

A field survey was conducted in March 2019 in Ngoc Linh Nature Reserve, Dak Giei District, Kon Tum Province. Frogs were collected between 19:00 and 23:00. After taking photographs of living specimens, they were anaesthetized and euthanized in a closed vessel with a piece of cotton wool containing ethyl acetate (Simmons 2002), fixed in 80% ethanol for 5 h, and later transferred to 70% ethanol for permanent storage. Tissue samples were preserved separately in 70% ethanol prior to fixation. Voucher specimens referred to in this paper were deposited in the collections of the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam.

Molecular data and phylogenetic analyses

In this study, 15 samples of five species of *Quasipaa* were used for molecular analysis (Table 1). Tissue samples were extracted using PureLink™ RNA Micro Scale Kit (Thermo Fisher Scientific company), following the manufacturer’s instructions. DNA was amplified using PCR Applied Biosystems. PCR volume consisted of 25 μl, including 12 μl of Mastermix, 6 μl of water, 1 μl of each primer at concentration of 10 pmol/μl, and 5 μl
A new species of *Quasipaa* from Vietnam

of DNA. A fragment of the mitochondrial gene (16S) with ~570 base pairs length was amplified using the primer pair LR-N-13398 (5´-CGCCTGTTTTACCCCAAAACAT-3´; forward) and LR-J 12887 (5´-CCGGTGAACTCAGATCAGT-3´; reverse) (Simon et al. 1994). PCR conditions: 94 °C for 5 min of initial denaturation; with 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 45 s; and the final extension at 72 °C for 7 min. PCR products were sent to Apical Scientific company for sequencing (https://apicalscientific.com).

In addition, we used 11 available sequences of 16S rRNA of nine species of the genus *Quasipaa* in GenBank for phylogenetic analyses (Che et al. 2009; Zhang et al. 2018). A sequence of *Fejervarya limnocharis* was included in the analysis as the outgroup (Che et al. 2009). For locality information and accession numbers for all sequences used in this study, see Table 1.

Phylogenetic trees were constructed by using maximum likelihood (ML) and Bayesian inference (BI). Chromas Pro software (Technelysium Pty Ltd., Tewantin, Australia) was used to edit the sequences, which were aligned using the ClustalW (Thompson et al. 1997) option in MEGA X (Kumar et al. 2018) with default parameters and subsequently optimized manually in BioEdit v. 7.0.5.2 (Hall 1999). We then checked the initial alignments by eye and adjusted slightly. Prior to ML and Bayesian phylogenetic analyses, we chose the optimum substitution models for entire sequences using Kakusan 4 (Tanabe

Table 1. GenBank accession numbers and associated samples that used in this study.

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<td>25 Q. delacouri</td>
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<td>26 Q. delacouri</td>
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Outgroup

*Fejervarya limnocharis*  Vinh Phuc, Vietnam  EU979847  Che et al. (2009)
2011) based on the Akaike information criterion (AIC). The BI was performed in MrBayes v. 3.2 (Ronquist et al. 2012). The BI summarized two independent runs of four Markov Chains for 10 million generations. A tree was sampled every 100 generations and a consensus topology was calculated for 70 000 trees after discarding the first 30 001 trees (burn in = 3 000 000) (Nguyen et al. 2017). We checked parameter estimates and convergence using Tracer v. 1.7.1 (Rambaut et al. 2018). The strength of nodal support in the ML tree was analyzed using non-parametric bootstrapping (MLBS) with 1000 replicates. We regarded tree nodes in the ML tree with bootstrap values of 75% or greater as sufficiently resolved (Hillis and Bull 1993; Huelsenbeck and Hillis 1993), and nodes with a BPP of 95% or greater as significant in the BI analysis (Leaché and Reeder 2002).

**Morphological characters**

Measurements were taken with digital calipers to the nearest 0.1 mm. The following abbreviations were used:

- **SVL**: snout–vent length;
- **HL**: head length (measured as a parallel line with the vertebral column from posterior margin of mandible to tip of snout);
- **HW**: maximum head width (at rictus);
- **RL**: rostral length (from anterior corner of orbit to tip of snout);
- **NS**: distance from nostril to tip of snout;
- **EN**: distance from anterior corner of orbit to nostril;
- **IND**: internarial distance;
- **IOD**: interorbital distance;
- **ED**: eye diameter;
- **UEW**: maximum width of upper eyelid;
- **DAE**: distance between anterior margins of orbits;
- **DPE**: distance between posterior margins of orbits;
- **MN**: posterior margin of mandible to nostril;
- **MFE**: posterior margin of mandible to anterior margin of orbit;
- **MBE**: posterior margin of mandible to posterior margin of eye;
- **TD**: tympanum diameter;
- **TYE**: distance from anterior margin of tympanum to posterior corner of orbit;
- **UAL**: upper arm length (from axilla to elbow);
- **FAL**: forearm length (from elbow to tip of third finger);
- **FL1–4**: finger length I–IV (from inner to outer);
- **NPL**: nuptial pad length - finger I;
- **FeL**: femur length (from vent to knee);
- **TbL**: tibia length (from knee to tarsus);
- **TbW**: maximum tibia width;
- **FoL**: foot length (from tarsus to tip of fourth toe);
- **TL**: 1–5 toe length I–V;
- **IMT**: inner metatarsal tubercle length.
A new species of *Quasipaa* from Vietnam

For webbing formula, we followed Glaw and Vences (2007). Sex was determined by gonadal inspection.

Morphological data were obtained by comparison of the new species with specimens of other members of the genus *Quasipaa* (see Appendix 1) and from literature (e.g., Angel 1928; Bourret 1937, 1942; Liu 1950; Inger 1970; Liu and Hu 1975; Huang and Liu 1985; Wu and Zhao 1995; Inger et al. 1999; Chen et al. 2002; Ohler and Dubois 2006; Dubois and Ohler 2009; Fei et al. 2009, 2012).

**Principal component analysis (PCA)**

Measurements were used to compare the morphometric difference between the new species from Kon Tum Province (three males and three females) vs *Quasipaa boulengeri* from Cao Bang Province (six males and five females). All statistical analyses were performed using PAST v. 2.17b software (Hammer et al. 2001).

**Results**

**Phylogenetic analyses**

The combined matrix contained 495 aligned characters. Of those, 416 sites were conserved, and 78 sites were variable, of which 62 were found to be potentially parsimony informative. The estimated Transition/Transversion bias (R) is 3.86. Substitution pattern and rates were estimated under the Tamura (1992) model. The nucleotide frequencies are A = 26.91%, T/U = 26.91%, C = 23.09%, and G = 23.09%. In terms of pairwise genetic distance, interspecific uncorrected *p*-distance of the *Quasipaa* species ranged from 1.4% (between *Quasipaa* sp. from Laos and *Q. delacouri*), 1.6–1.9% (between *Q. boulengeri* and *Q. robertingeri*) to 7.6–8.1% (between *Q. shini* and the new form) (Table 2). The genetic divergence of the new form and its congeners ranged from 4.2–5.1% (*Q.boulengeri*) to 7.6–8.1% (*Q. shini*), which was greater than genetic distances between *Q. boulengeri* and *Q. robertingeri* (1.6–1.9%); between *Q. boulengeri* and *Q. jiulongensis* (3.8–4.0%); and between *Q. boulengeri* and *Q. delacouri* (4.0–4.5%) (Table 2).

The ML and BI analyses produced topologies with $-\ln L = 1672.0337$ and 1729.0216, respectively, with a gamma shape parameter (G: 0.1363 in ML and 0.1767 in BI). Phylogenetic analyses employing ML and BI methods were nearly identical, with most well-supported nodes on the ML tree also well-supported on the BI tree, and only the BI tree is presented in Fig. 1. In both analyses, the newly collected *Quasipaa* specimens from Kon Tum Province were recovered as a separate branch from the *Q. boulengeri* group (*Q. boulengeri*, *Q. robertingeri*, and *Q. verrucospinosa*), the *Q. shini* group (*Q. shini* and *Q. yei*), and the *Q. delacouri* group (*Q. delacouri* and *Quasipaa* sp.).

Our phylogenetic results were in general agreement with those supported by analyses in Che et al. (2009). Although, unlike the topology supported by Zhang et al. (2018), the clade containing the *Q. acanthophora* (haplotypes from Lang Son Province,
Table 2. Uncorrected $p$-distance matrix showing percentage pairwise genetic divergences (%) for the 16SrRNA gene between members of the genus *Quasipaa*.

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Figure 1. Bayesian phylogram based on a partial 16S mitochondrial fragment. Numbers above and below branches are MP/ML bootstrap values and Bayesian posterior probabilities (>50%), respectively. Hyphen denotes < 50% value. Bold text highlights new samples collected within this study.
A new species of *Quasipaa* from Vietnam was recovered as a sister clade to *Q. exilispinosa* with rather strong nodal support from both analyses (0.71/80.4); the clade containing the *Q. delacouri* (haplotypes from Tuyen Quang Province, Vietnam) was recovered as a sister clade to *Quasipaa* sp. from Laos with strong nodal support from both analyses (0.98/98.6) (Fig. 1).

In the following, based on the distinct molecular divergence in concert with diagnostic morphological differences compared to congeners, we describe the *Quasipaa* population from Ngoc Linh based on our integrative taxonomic analysis, as new species to science.

**Taxonomic account**

*Quasipaa taoi* sp. nov.

https://zoobank.org/EEE47B08-108A-49F0-B89E-3512EF353BB1

Figs 2–4

**Holotype.** IEBR A.4997, adult male, collected by T. Q. Phan and T. D. Tran on 6 March 2019 (15°05’23.3”N, 107°51’17.5”E, at an elevation of 1,560 m asl.) in the evergreen forest of Ngoc Linh Natural Reserve, Xop Commune, Dak Glei District, Kon Tum Province, Vietnam.

**Paratypes.** IEBR A.4998, adult male; IEBR A.4999, adult male; IEBR A.5000, adult female; IEBR A.5037, adult female; IEBR A.5038, adult female, the same data as the holotype.

**Diagnosis.** Both morphological characters (body very stout, skin rough with dermal ridges and tubercles, forelimbs of males strongly enlarged, with inner side of arms or fingers or chest and belly with black spines (see Fei et al. 2009) and molecular data revealed the new species to be nested within *Quasipaa*. *Quasipaa taoi* sp. nov. is distinguishable from its congeners by a combination of the following morphological characters: (1) SVL 79.6–84.3 mm in males, 64.6–69.9 mm in females; (2) head broader than long (HL/HW 0.90 in males, 0.92 in females); (3) vomerine teeth present; (4) external vocal sacs absent; (5) tympanum slightly visible; (6) dorsum with lines of thick ridges and small round tubercles; (7) flanks covered by oval and round tubercles; (8) supratympanic fold present; (9) dorsolateral fold absent; (10) ventrolateral sides, ventral surface of arms, and all fingers with spines in males; (11) the absence of spines on chest and belly in males; (12) toes fully webbed to distal end of terminal phalanx; (13) in life, dorsum dark brown, chest and belly immaculate white.

**Description of holotype.** A large frog (SVL 84.3 mm); habitus robust with enlarged head (HL/SVL 0.40, HW/SVL 0.43); head broader than long (HL 33.5 mm, HW 36.3 mm); snout round anteriorly in dorsal view, projecting beyond lower jaw; nostril lateral, closer to eye than to the tip of snout (NS 7.6 mm, EN 5.5 mm); canthus rostralis indistinct; loreal region oblique and slightly concave; rostral length greater than eye diameter (RL 13.1 mm, ED 9.7 mm); internarial distance wider than interorbital distance and upper eyelid width (IND 8.6 mm, IOD 6.2 mm, UEW 7.7 mm); tympanum slightly visible (TYD 4.1 mm) smaller than the distance from tympanum to eye (TYE 4.9 mm); vomerine teeth in two oblique ridges; tongue cordiform, notched posteriorly; external vocal sac absent.
Figure 2. *Quasipaa taoi* sp. nov., holotype (IEBR A.4997, male) in life **A** dorsolateral view **B** ventral view.

Forelimbs: arms short; upper arm length (UAL) 17.1 mm, forearm length (FAL) 41.5 mm; relative finger lengths: II < I < IV < III; fingers free of webbing; dermal ridge on sides of fingers present on fingers I, II, III; tips of fingers swollen, not expanded; subarticular tubercles prominent, round, formula 1, 1, 2, 2; inner metatarsal tubercle round; outer metatarsal tubercle elongate; finger I with nuptial pad.

Hindlimbs: tibia length longer than thigh length (FeL 44.2 mm, TbL 49.7 mm), approximately 3.4 times longer than wide (TbW 14.5 mm); tips of toes swollen, slightly round; relative length of toes: I < II < V < III < IV; toes fully webbed to distal end of terminal phalanx; dermal ridge present on outer sides of toes I and V; subarticular tubercles prominent, elongate, formula 1, 1, 2, 3, 2; inner metatarsal tubercle elongate; outer metatarsal tubercle absent; tibio-tarsal articulation reaching to tip of snout.

Figure 3. *Quasipaa taoi* sp. nov., holotype (IEBR A.4997, male) in preservative **A** dorsolateral view **B** ventral view.
A new species of *Quasipaa* from Vietnam

Skin texture in life: dorsal surface of head with oval and round tubercles, dorsum with six lines of thick ridges intermixed with small round tubercles; flanks covered by oval and round tubercles; supratympanic fold distinct, extending from eye to angle of jaw; dorsolateral fold absent; dorsal surface of forelimbs and hindlimbs with small tubercles; belly and ventral surface of thighs smooth.

Nuptial spines: body of males with spines except for chest, belly, and ventral surface of hindlimbs; dense spines on dorsum, flanks, ventral surface of forelimbs, ventrolateral sides, and fingers I, II; spines present on throat, dorsal surface of fore- and hindlimbs, and fingers III, IV, small and scattered.

Coloration in life: iris dark copper; dorsum and upper part of flanks dark brown; lower part of flanks whitish brown with white tubercles and black spines on top; supratympanic fold dark brown; dorsal surface of limbs yellowish brown with dark cross-bars; ventral surface of limbs light yellow with brown markings; throat white with brown markings; chest and belly immaculate white; toe webbing dark brown.

Coloration in preservative: coloration in preservative is the same in life but somewhat faded.

**Sexual dimorphism.** Measurements and morphological characters of the type series are provided in Table 3. Males are larger than females (SVL 82.7 ± 2.69 mm, *n* = 3 males vs 67.6 ± 2.7 mm, *n* = 3 females). The male specimens have a nuptial pad on finger I and dark spines on flanks, ventral surface of forelimbs, ventrolateral sides, and all fingers. The females contained yellowish-cream eggs of varying sizes.

**Etymology.** The new species is named in honor of our colleague and friend, Assoc. Prof. Dr. Tao Thien Nguyen from the Institute of Genome Research, Vietnam Academy of Science and Technology, in recognition of his numerous scientific contributions towards a better understanding of the amphibians of Vietnam. We recommend “Tao's
Spiny Frog” as the common English name of the new species and the common name in Vietnamese as “Éch gai sân tao”.

**Ecological notes.** Specimens were found between 19:00 and 23:00 in the headwaters of rocky streams (Fig. 5B). They were found in the water or on the ground of stream banks at an elevation of above 1,500 m a.s.l. The surrounding habitat was secondary forest of large, medium-sized, and small hardwoods mixed with shrubs and vines (Fig. 5A). Air temperatures at the sites ranged from 18.5–22.5 °C and relative humidity was 68–85%. Male advertisement calls and tadpoles of the species have not been recorded during our field surveys. Other amphibian species found at the sites included *Leptobrachella* sp., *Limnonectes kiziriani* Pham, Le, Ngo, Ziegler & Nguyen, 2018, *Amolops spinapectoralis* Inger, Orlov & Darevsky, 1999, *Odorrana khalam* (Stuart, Orlov & Chan-ard, 2005), *O. morafkai* (Bain, Lathrop, Murphy, Orlov & Ho, 2003), *Kurixalus cf. banaensis* (Bourret, 1939), and *Rhacophorus annamensis* (Smith, 1924).

**Distribution.** *Quasipaa taoi* sp. nov. is currently known from Ngoc Linh Mountain of the Central Highlands in Vietnam (Fig. 6). Data obtained from GenBank show that this species was also recorded from Xekong Province, Lao PDR (Yan et al. 2021; see Discussion below).

**Comparisons.** We compared the new species with its congeners. *Quasipaa taoi* sp. nov. differs from *Q. boulengeri* by having a smaller size, SVL 79.6–84.3 mm, $n = 3$ in males, 64.6–69.9 mm, $n = 3$ in females (vs 87.8–101.7 mm, $n = 6$ in males, 82.5–105.5 mm, $n = 5$ in females), dorsum with thick ridges and round tubercles (vs elongate ridges), males with nuptial spines on all fingers (vs absent on finger IV); males with nuptial spines on throat and ventral surface of arms (vs absent), and the absence of nuptial spines on chest and belly in males (vs present). In the PCA analysis, the first two principal component axes could separate *Quasipaa taoi* sp. nov. from *Q. boulengeri* by 24 characters (Fig. 7), mainly based on limb and head measurements, namely: SVL, HW, HL, MN, MFE, MBE, RL, ED, UEW, IND, IOD, DAE, DPE, NS, EN, TD, TYE, UAL, FAL, FeL, TbL, TbW, FoL, and IMT (Tables 3, 4). In males, the PCA extracted three principal component axes with eigenvalues greater than 0.002 and, of

**Figure 5.** Habitat of *Quasipaa taoi* sp. nov. in Ngoc Linh Nature Reserve, Kon Tum Province, Viet Nam. **A** evergreen forest **B** microhabitat.
A new species of Quasipaa from Vietnam

Figure 6. Map showing the type locality (circle) of Quasipaa taoi sp. nov. in Kon Tum Province, Vietnam.
these, the first two component axes accounted for 85.50% of the variation (Table 4). Species with a larger and positive score on PC1 reflected shorter SVL including all traits. The PC2 with positive scores were associated with species having greater measurements of RL, ED, UEW, IND, IOD, DAE, DPE, NS, EN, TYE, UAL, FeL, TbL, and FoL, while negative scores with species having smaller measurements of SVL, HW, HL, MN, MFE, MBE, TD, FAL, TbW, and IMT (Table 4). In females, the PCA extracted three principal component axes with eigenvalues greater than 0.01 and of these, the first two component axes accounted for 85.98% of the variation (Table 4). Species with a higher and positive score on PC1 reflected having shorter measurements of SVL, HW, HL, MN, MFE, MBE, RL, ED, UEW, IND, IOD, DAE, NS, EN, TD, TYE, UAL, FAL, FeL, TbL, TbW, FoL, and IMT, while a negative score with species having smaller DPE. The PC2 with positive scores were associated with species having greater measurements of SVL, HW, HL, MN, MFE, MBE, RL, ED, UEW, IND, IOD, DAE, DPE, NS, EN, TD, TYE, UAL, FAL, FeL, TbL, TbW, and FoL, while a negative score with species having smaller measurements of UEW, IND, and IMT (Table 4). *Quasipaa taoi* sp. nov. differs from *Q. acanthophora* by having the dorsum with thick ridges and round tubercles (vs small tubercles), males with nuptial spines on ventrolateral sides and ventral surface of arms (vs absent), males with nuptial spines on all fingers (vs absent on finger IV), and the absence of spines on chest of males (vs present). *Quasipaa taoi* sp. nov. differs from *Q. courtoisi* by having a smaller size in males, SVL 79.6–84.3 mm, \( n = 3 \) (vs 126 mm, \( n = 1 \)); males with nuptial spines on throat and ventral surface of arms (vs absent); and the absence of nuptial spines on chest in males (vs present). *Quasipaa taoi* sp. nov. differs from *Q. delacouri* by having a smaller size, SVL 79.6–84.3 mm, \( n = 3 \), in males and 64.6–69.9 mm, \( n = 3 \), in females (vs 92.9–115.5 mm, \( n = 4 \), in males and 94.5–117.5 mm, \( n = 3 \), in females); a greater ratio of TD/ED, \( 0.44 \pm 0.02 \), \( n = 3 \), in males and \( 0.49 \pm 0.01 \), \( n = 3 \), in females (vs 0.26 in males and 0.24 in females);

![Figure 7](image-url) Plots of the first principal component (PC1) versus the second (PC2) for the males and the females of *Quasipaa taoi* sp. nov. (red +) and *Q. boulengeri* (blue □).
Table 3. Measurements (in mm) and proportions of the type series of *Quasipaa boulenegeri* and *Quasipaa taoi* sp. nov. (H = holotype, P = paratype, SD = standard deviation; for other abbreviations see Material and methods).

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A new species of *Quasipaa* from Vietnam

Table 4. Variable loadings for principal components with eigenvalue greater than 0.01, from morphometric characters corrected by SVL. All measurements were given in millimeter (mm).

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dorsum with thick ridges and round tubercles (vs smooth); males with nuptial pad on finger I (vs absent in males); and males with nuptial spines (vs absent). The new species differs from *Q. exilispinosa* by having a larger size in males (SVL 79.6–84.3 mm, *n* = 3, in males and 64.6–69.9 mm, *n* = 3, in females (vs 61.2 mm, *n* = 20, in males and 57.1 mm, *n* = 20, in females); dorsum with thick ridges and round tubercles (vs small tubercles); males with nuptial spines on ventrolateral sides and ventral surface of arms (vs absent); males with nuptial spines on all fingers (vs absent on finger IV); and absence of spines on chest in males (vs present). *Quasipaa taoi* sp. nov. differs from *Q. fasciculispina* by having a smaller size, SVL 79.6–84.3 mm, *n* = 3 in males and 64.6–69.9 mm, *n* = 3 in females (vs 106 mm, *n* = 1 in males and 104 mm, *n* = 1 in females); a smaller ratio of TYE/TD (1.11 ± 0.16, *n* = 3, in males and 1.2 ± 0.16, *n* = 3, in females (vs 2.0 in male and 1.75 in female); the absence of nuptial spines on chest in males (vs circular whitish tubercles each bearing 5–10 strong black spines). *Quasipaa taoi* sp. nov. differs from *Q. jiulongensis* by having dorsum with thick ridges and round tubercles (vs small tubercles), males with nuptial spines on ventrolateral sides and ventral surface of arms of males (vs absent), males with nuptial spines on all fingers (vs absent on fingers III and IV); the absence of light-colored longitudinal stripes on upper jaw edge (vs
present); and the absence of 4 or 5 yellow dorsal dots arranged in longitudinal rows (vs present). *Quasipaa taoi* sp. nov. differs from *Q. robertingeri* by having dorsum with thick ridges and round tubercles (vs elongate ridges), males with nuptial spines on all fingers (vs absent on finger IV); males with nuptial spines on throat and ventral surface of arms (vs absent), and the absence of nuptial spines on chest and belly of males (vs present). *Quasipaa taoi* sp. nov. differs from *Q. shini* by its smaller size, SVL 79.6–84.3 mm, *n* = 3, in males and 64.6–69.9 mm, *n* = 3, in females (vs 98.6 mm, *n* = 9, in males and 94.9 mm, *n* = 10, in females); dorsum with thick ridges and round tubercles (vs elongate ridges), males with nuptial spines on all fingers (vs absent on finger IV); males with nuptial spines on throat and ventral surface of arms (vs absent), and the absence of nuptial spines on chest and belly of males (vs present). *Quasipaa taoi* sp. nov. differs from *Q. spinosa* by its smaller size, SVL 79.6–84.3 mm, *n* = 3, in males and 64.6–69.9 mm, *n* = 3, in females (vs 106.0–142.0 mm, *n* = 20, in males and 115.0–152.5 mm, *n* = 10, in females); dorsum with short, thick ridges and round tubercles (vs small tubercles); the absence of light colored longitudinal stripes on upper jaw edge (vs present); and the absence of nuptial spines on chest of males (vs small and dense spines on chest of males). *Quasipaa taoi* sp. nov. differs from *Q. verrucospinosa* by its smaller size (SVL 79.6–84.3 mm, *n* = 3, in males and 64.6–69.9 mm, *n* = 3, in females (vs 90.0–117.0, *n* = 8, in males, 83.2–113.9 mm, *n* = 9, in females); males with nuptial spines on all fingers (vs absent on fingers III and IV); males with nuptial spines on ventral surface of arms (vs absent), and the absence of nuptial spines on chest and belly in males (vs present). *Quasipaa taoi* sp. nov. differs from *Q. yei* by its larger size in males (SVL 79.6–84.3 mm, *n* = 3, in males and 64.6–69.9 mm, *n* = 3, in females (vs 49.7–64.0 mm, *n* = 25); males with nuptial spines on ventrolateral sides and ventral surface of arms (vs absent); and males with nuptial spines on all fingers (vs absent); absence of nuptial spines around and inside vent (vs present).

**Discussion**

Mount Ngoc Linh, on the northwestern border of the Kon Tum Massif, is the highest peak in central Vietnam at 2,598 m (Sterling et al. 2006). Ngoc Linh is the type locality of several new species of amphibians, namely *Leptobrachium ngoclinhense* (Orlov, 2005), *Thelederma nebulosum* Rowley, Le, Hoang, Dau & Cao, 2011, *Leptobrachella firthi* (Rowley, Hoang, Dau, Le & Cao, 2012); *Gracixalus lumarius* Rowley, Le, Dau, Hoang & Cao, 2014, *G. trieng* Rowley, Le, Hoang, Cao & Dau, 2020 (Orlov 2005, Rowley et al. 2011, 2012, 2014, 2020). Most recently, Krzikowski et al. (2022) highlighted the extraordinary endemism rate of amphibians in the Central Highlands of Vietnam and, thus, the special role in amphibian diversification and evolution. A number of amphibian species are currently known only from this region, namely *Leptobrachium crocea* (Rowley, Hoang, Le, Dau & Cao, 2010), *Leptobrachium ngoclinhense; Microhyla darevskii* Poyarkov, Vassilieva, Orlov, Galoyan, Tran, Le, Kretova, & Geissler, 2014, *Gracixalus lumarius, G. trieng, and Thelederma nebulosum* (Frost 2022; Krzikowski et al. 2022). *Quasipaa taoi* represents the thirteenth known species of *Quasipaa* and the sixth
A new species of Quasipaa from Vietnam

known species of this genus from Vietnam (Frost 2022; this study). Further studies, as a result, will likely uncover more cryptic species in this poorly known group of frogs.

The new species has a restricted distribution in central Vietnam and Xekong Province, Lao PDR. A major threat to the new species in the area is habitat loss by agricultural extension for medicinal trees (e.g. Panax vietnamensis), illegal timber logging, and tourism development. In addition, the species Q. taoi is collected by local people for food. We suggest assessment of this species as Near Threatened in the IUCN Red List of Threatened Species because the continued survival of this species is largely dependent on the protection and rigorous management provided by local authorities of the protected areas in both countries.

In this study, we first uploaded to GenBank the 16S gene sequence of Quasipaa acanthophora from the type locality (Mau Son Mountain) in Lang Son Province, northern Vietnam. We confirm that Q. acanthophora is currently known only from Vietnam and does not correspond to a population of Q. spinosa according to Yan et al. (2021). Based on morphological comparisons, we also provided the 16S gene sequences of true Quasipaa delacouri from Tuyen Quang Province, near the type locality in Bac Kan Province, Vietnam. This will assist in clarifying the taxonomy of species in the genus Quasipaa in the future.

Acknowledgements

We are grateful to the directorates of the Ngoc Linh Nature Reserve, Forest Protection Department of Kon Tum Province for support of our field work. For the fruitful cooperation within joint research projects, we cordially thank S.V. Nguyen (IEBR, Hanoi), as well as T. Pagel and C. Landsberg (Cologne Zoo). We thank T.D. Tran (IEBR, Hanoi) for assistance in the field. We thank T.A. Tran (IEBR, Hanoi) for providing the map. We thank Annemarie Ohler and an anonymous reviewer for their helpful comments. This research is funded by the National Foundation for Science and Technology Development (NAFOSTED) under grant number 106.05-2020.02.

References


A new species of *Quasipaa* from Vietnam


Tanabe AS (2011) Kakusan 4 and Aminosan: Two programs for comparing nonpartitioned, pro-portional and separate models for combined molecular phylogenetic analyses of


Appendix I

Specimens examined


First records of *Trichina* Meigen, *Euthyneura* Macquart and *Oedalea* Meigen (Diptera, Hybotidae) from North Africa, with descriptions of two new species

Laila Zouhair¹, Patrick Grootaert², Kawtar Kettani¹

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Abstract

*Trichina* Meigen, 1830, *Euthyneura* Macquart, 1836 and *Oedalea* Meigen, 1820 are reported for the first time in North Africa from northern Morocco, with seven species including two ones new to science, based on material collected at nine sites located in the Moroccan sector of the Mediterranean Intercontinental Biosphere reserve (MIBR). These new records represent also the first evidence of the occurrence of Trichininae and Oedaleinae subfamilies throughout North Africa and bring the total of Moroccan hybotid fauna to 51 species. The new species are described and illustrated. A key to Moroccan *Trichina* species is provided.

Keywords

Hybotidae, Morocco, new records, new species, North Africa, Oedaleinae, Trichininae

Introduction

*Trichina* Meigen, *Euthyneura* Macquart and *Oedalea* Meigen genera were previously included within the Trichiniini and Oedaleini tribes, which were included for a long time beside the Ocydromiini tribe in Ocydromiinae subfamily in Chvála’s (1983) classification. More recent classifications, such as Sinclair and Cumming (2006) and Wahlberg and Johanson (2018), now treat these three tribes as separate subfamilies within the Hybotidae.
The genus *Trichina* attributed to the subfamily Trichininae consists of small black species (2.5–3 mm), with small mouthparts, large wing stigma extending to tip of R2+3, and hind tibiae dilated towards tip, and whose adults are known as predaceous in both sexes and occur for a long period during the summer in somewhat shaded humid biotopes (Chvála 1983). They are often found in the low grasses in deciduous forests, and are also observed at the tips of branches or along forest margins from approximately sunset until dusk (Chvála 1983). Larvae of *Trichina* occur probably in the soil (Smith 1989). The genera *Euthyneura* and *Oedalea* are both assigned to the Oedaleinae subfamily historically considered as Oedaleini tribe, which includes the most abundant of all flower-visiting empidoids in the Holarctic region (Marshall 2012). Species of *Euthyneura* are small black flies (2–3 mm) with a relatively long proboscis directed forward and simple legs devoid of long bristles; they are usually found on flowers and low herbage, but also on conifers (Chvála 1983). Among the representatives of the family Hybotidae, this genus represents one of the few groups whose adults feed only on nectar and pollen and are entirely flower visitors (Chvála 1983; Chandler 1992; Shamshev and Kustov 2012), while their larvae develop in rotting wood (Chvála 1983). The *Oedalea* species are more robust than those of *Euthyneura*. These are predaceous flies with conspicuously long antennae, shiny black thorax and raptorial hind legs (Chvála 1983; Shamshev 2020) and whose adults can usually be swept in low numbers from leaves of trees and shrubs, while the larvae can be bred from dead wood (Beuk 1992).

Various studies have been recently carried out on the aforementioned genera, mainly in Europe in the west Palaearctic region (Kanavalová et al. 2021). Thus, *Euthyneura* is known from the Palaearctic region with seven species (Shamshev and Kustov 2012) whereas data for *Trichina* and *Oedalea* in this region remain very poor and do not exceed seven species for *Trichina* and fourteen for *Oedalea* known to date from Europe (Barták and Kubík 2009; De Jong et al. 2014; Evenhuis and Pape 2021). As for North Africa, studies on hybotids are even scarcer and rather fragmentary, as is the case for Morocco where only 44 species of Hybotidae are recorded so far (Kettani et al. 2022), reflecting the lack of studies on such an important family of Diptera in the region.

In the present paper, we report the first record of *Trichina*, *Euthyneura* and *Oedalea* genera from the whole of North Africa, thus representing the first evidence of the occurrence of Trichininae and Oedaleiniae subfamilies in North Africa, knowing that all species found so far in the west Palaearctic region of the genera of these subfamilies have only been recorded in Europe (De Jong et al. 2014), and no records have been reported until now in North Africa. Importantly, the genus *Euthyneura* is recorded here for the first time in the entire Mediterranean region.

In addition to these first records, we provide evenly here diagnosis and descriptions of two species described as new to science, belonging to the genus *Trichina*: *Trichina azizi* Zouhair & Grootaert sp. nov. and *Trichina rifensis* Zouhair & Grootaert sp. nov.
Materials and methods

The studied specimens originate from the entomological field surveys undertaken by the first author (LZ) over the years 2020 and 2021, and by the third author (KK) between 2017 and 2019. Specimens are preserved in 70° alcohol and housed in the private collection of the first author (PCLZ) at the University Abdelmalek Essaadi (Tetouan, Morocco) and in the private collection of the second author (PCPG). Most of the collected specimens were sampled using sweep net, and some with Malaise trap.

The specimens examined were mainly collected in mixed forests and riparian areas at nine sites located in the northern part of Morocco (Table 1, Fig. 1). With the exception of two forest sites situated within unprotected areas (S8, S9), the remaining sampling sites belong to the National Park of Talassemtane (NPTL) and the Project of Natural Park of Bouhachem (PNPB), which are considered the most important protected areas in terms of conservation in Morocco. The nine sites are located in the Rif, which consists of a mountainous chain located in the northernmost part of Morocco. This chain represents, along with the Atlas mountain region, the most important endemic areas of the country. It is important to highlight that all the studied sites belong also to the Mediterranean Intercontinental Biosphere Reserve (MIBR) which encompasses southern Spain and northern Morocco, and is known as a reservoir of biodiversity with regard to the Moroccan part (Rosas et al. 2017; Bachar et al. 2021).

In the Rif, the climate is of the Mediterranean type and is composed of two distinct periods, a hot and dry summer and the other a relatively cold and rainy winter (Al Karkouri 2017). The bioclimate is primarily wet with high humidity which allows the growth

Table 1. Coordinates, altitudes and localities of the studied sites.

<table>
<thead>
<tr>
<th>Code</th>
<th>Site</th>
<th>Protected area, locality</th>
<th>Province</th>
<th>Altitude</th>
<th>Geographical coordinates</th>
<th>Habitat</th>
<th>Collecting tool</th>
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<tr>
<td>S1</td>
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<td>PNPB, Hammadess</td>
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<td>PNPB, Moulay Abdessalam</td>
<td>Tetouan</td>
<td>1267 m</td>
<td>35.2657°N, 5.483595°W</td>
<td>River bank</td>
<td>Sweep net</td>
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<tr>
<td>S4</td>
<td>Lemtahane</td>
<td>PNPB, Dar Abdessalam</td>
<td>Tetouan</td>
<td>964 m</td>
<td>35.2708°N, 5.434864°W</td>
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<td>Malaise trap</td>
</tr>
<tr>
<td>S5</td>
<td>Sefihat telj Bouslimane</td>
<td>NPTL, Talembote Chefchaouen</td>
<td>Chefchaouen</td>
<td>1745 m</td>
<td>35.1852°N, 5.211176°W</td>
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<td>Sweep net</td>
</tr>
<tr>
<td>S6</td>
<td>Oued El Ferda Bouslimane</td>
<td>NPTL, Jbel Boulimane</td>
<td>Chefchaouen</td>
<td>1350 m</td>
<td>35.0971°N, 5.14505°W</td>
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<td>Sweep net</td>
</tr>
<tr>
<td>S7</td>
<td>Aïn Lahec</td>
<td>Unprotected area, Aïn Lahec</td>
<td>Tetouan</td>
<td>316 m</td>
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<tr>
<td>S8</td>
<td>Fifi</td>
<td>Unprotected area, Bab Taza</td>
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<td>1332 m</td>
<td>34.9803°N, 5.2266°W</td>
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<td>Sweep net</td>
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</table>
Laila Zouhair et al. / ZooKeys 1124: 43–58 (2022)

of a rich flora, including nearly all of the Moroccan forest species such as deciduous groves, the endemic fir forests of Morocco (*Abies marocana*), the Atlas Cedar tree (*Cedrus atlantica*), Pine forests (*Pinus pinaster*), Cork Oak (*Quercus suber*), Olives, Thuja articulata (*Tetraclinis articulata*), Kermes Oak (*Quercus coccifera*), Holm Oak (*Quercus ilex*), and the *coccifera* which have formed extensive forests in the western part of the Rif (Taiqui 1997).

Most species were recognized using a stereomicroscope, but for the new ones it was necessary to make a preparation of the male genitalia for their accurate identification. The male terminalia were removed from the insect body, macerated in the 10% KOH for 24h in order to dissolving the tissues. When all darkly sclerotized structures were transparent

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**Figure 1.** Location of the nine studied sites in the north of Morocco.
null
Descriptions of new species

*Trichina azizi* Zouhair & Grootaert, sp. nov.

https://zoobank.org/E397A731-4350-4B07-8EA1-16E81947642E

Figs 3A–D, 7D

**Material examined.** *Holotype*. 1♂. Morocco, Rif, Bouslimane, NPTL, 1350 m, 28.iv.2019, sweep net, leg. K. Kettani, PCLZ.

**Habitat.** (S6: Bouslimane) (Fig. 2): The holotype was swept from the foothills of Bouslimane mountain located at the south of the national park of Talassetane. The habitat consists of a fir forest (*Abies marocana*) dominated by a humid bioclimate as part of the mesomediterranean zone. This fir formation grows on brown fersiallitic soil.

**Differential diagnosis.** *Trichina azizi* sp. nov. has 3rd antennal segment considerably long and slender like in *T. elongata* according to the specimen we have and to the description in Chvála (1983), but it differs in length since it is less than 3.4 times as long as broad in *T. elongata* (Barták and Kubík 2009) whereas it is more than 3.6 times as long as broad in the new species. Both species differ also in the length of the stylus, which in the new species is little longer than half-length of 3rd segment; while in *T. elongata*, the stylus is less than half the length of the 3rd segment. The bare terminal part of antennae is shorter than the pubescent thicker basal part which is the same in both. The new species has only two pairs of scutellar setae, like in *T. elongata* and also in *T. clavipes* and *T. bilobata*. In addition, it can be recognized by yellowish hind trochanter, femora, tibiae and metatarsi, and by the lack of a ventral spine on hind trochanters, like in *T. opaca*. Male terminalia with the hypandrial projection is rather long, like in *T. elongata*, but in the latter it is more slender than in the new species. The left surstylus is similar in structure to that of *T. elongata*, but it is not sharpened apically like in *T. elongata*. The right surstylus is spiny apically and sub-apically, which is the most important diagnostic character in this new species. Cerci are long and well developed but the left cercus is longer than the right one.

**Etymology.** This species is dedicated to the father (Aziz Zouhair) of the first author.

**Description.** *Male*. Small brown species (2.8 mm) (Fig. 7D).

**Head.** Brown in ground colour. Ommatidia bicoloured. Antennae brown, with postpedicel considerably long and slender with 0.22 mm long over 0.06 mm wide, stylus is 0.134 mm long (0.6 × the length of postpedicel hence a little longer than half the length), its bare terminal part shorter than the pubescent thicker basal part. Palpus small, brownish, with a brownish bristle. Proboscis paler. Face narrow, less than width of scape.

**Thorax.** Entirely brown, (unfortunately, all thoracic setae are broken, but according to setae follicles, 2 pairs of scutellar setae). Mesonotum shining black except for the extreme anterior border that bears some sparse microtrichia, the notopleural depression with some denser microtrichia, but is still subshining black. Wings very faintly brown yellowish infuscated, veins pale brown, pterostigma yellowish brown and indistinct, costa running to tip of M1, squamae including fringes brown, halteres brown yellowish. Legs almost uniformly brownish leaving hind trochanter, femora, tibia, and hind metatarsi yellowish. Fore femur slender with paler hairs; mid femur with 2 ventral
First records of *Trichina*, *Oedalea* and *Euthyneura* from North Africa

rows of dark and thick bristles; hind femur longer than mid and fore femora, with a row of thick and long spine-like bristles. No spine on the hind trochanter. Fore tibia more slender at base, covered with small paler hairs; mid tibia very slender than fore tibia and covered mostly with dark hairs; hind tibia much longer than fore and mid tibiae, laterally compressed and dilated towards tip.

**Abdomen.** Brown, tergites and sternites covered with scattered black setulae. Terminalia somewhat large and blackish brown, with spiny right surstylus in apical and sub-apical parts (Fig. 3B, D), left surstylus longer and slender in comparison with right surstylus, hypandrial projection moderately long, slender (Fig. 3D). Cerci longer than wider, the left cercus longer than the right cercus (Fig. 3B).

**Figure 2.** Type habitat of *Trichina azizi* Zouhair & Grootaert, sp. nov. in the fir forest at Bouslimane locality (Photo: K. Kettani, 28.04.2019).
Female. (Unknown).
Abbreviations: ae: aedeagus (phallus); cer: cercus; hyp: hypandrium; hypp: hypandrial projection; lel: left epandrial lamella; ls: left surstylus; rel: right epandrial lamella; rs: right surstylus.

**Trichina rifensis** Zouhair & Grootaert, sp. nov.
https://zoobank.org/FB3A6D61-8C16-4AAC-A20D-E7D7EA783F7A
Figs 5A–D, 7E


**Habitat.** (S4: Lemtahane) (Fig. 4): The Malaise trap was set up in a scrubland composed of *Pinus pinaster* and some fruit trees growing on siliceous soil. The bioclimatic is subhumid and favours thermo-Mediterranean vegetation.

**Differential diagnosis.** The newly described species *Trichina rifensis* sp. nov., is very similar to *T. opaca* as it is described in Chvála (1983) and according to the key of *Trichina* in Barták and Kubík (2009) and to our *T. opaca* specimen, in: the absence of the ventral spine in hind trochanters, and in the colour of legs which are extensively blackish to blackish brown, with the knees and extreme base of tibiae often paler. It is similar to *T. opaca* also in having the same number of scutellar pairs setae (3 pairs). The wings are conspicuously darkened brown, veins blackish, squamae including fringes and halters deep black in both species. However, both species differ in the pruinosity of the

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**Figure 3.** *Trichina azizi* Zouhair & Grootaert sp. nov., Holotype male, terminalia **A** right surstylus, lateral view **B** epandrium, dorsal view **C** left surstylus, lateral view **D** epandrium, ventral view. Scale: 0.1 mm. (Drawn by Patrick Grootaert).
mesonotum: in the new species, the mesonotum is shining black but anteriorly there is a very narrow stripe with weak microtrichia and the notopleural depression is more distinctly set with microtrichia (not grey dusted), while in *T. opaca* the mesonotum is entirely thinly brownish pollinose and consequently rather dulled, and differ also in the length of the 3d antennal segment that is less than 3.2 times as long as broad in *T. opaca*, while it is more than 3.2 times (4 times) as long as broad in *T. rifensis* sp. nov.

The male terminalia in the new species is with the hypandrial projection much longer than in *T. opaca*, the structure of the left surstylus is similar in the two species so that is C-shaped in both. The right surstylus of the new species is spiny in the apical and sub-apical parts contrary to *T. opaca*, which forms an important differential diagnosis character, cerci are equal in length and shape in *T. opaca*, while in the new species, the left cercus is narrower and longer than the right cercus which is shorter and conical in shape.

**Etymology.** The new species is named *rifensis*, after the Moroccan Rif region where it was found.
Description. **Male.** Black species with body small (3 mm) Fig. 7E.

**Head.** Black in ground colour. Eyes meeting on frons for a long distance. A distinct prominent ocellar tubercle with 2 pairs of ocellar bristles, anterior pair as long as posterior one, occiput and vertex finely greyish pollinose, covered with black and distinct hairs. Face linear, narrow less than width of scape. Antennae entirely black, inserted at middle of head in profile with postpedicel is 0.24 mm long over 0.06 mm wide, stylus (apical naked part missing) has the basal part and the thickened second segment 0.079 mm long. Palpus short with a very long black apical bristle, 1.5 times as long as palpus and a subapical bristle half as long as apical one. Proboscis yellowish, pointing obliquely forward and covered with several brownish hairs.

**Thorax.** Polished black with all hairs black and long. Acr biserial, dc uniserial, ending in 1 pair of very long prescutellars, 1 humeral, 1 notopleural, 1 postalar bristles, 3 scutellar pairs. Mesonotum covered with microtrichia. Mesonotum shining black but anterior part with a very narrow stripe with weak microtrichia, notopleural depression more distinctly set with microtrichia (not grey dusted). Wings conspicuously brownish, stigma blackish brown extending to tip of vein R2+3, veins blackish brown, costa reaching vein M1, squamae, including fringes, and halters black. Legs mostly covered with hairs, extensively blackish leaving base of hind tibia and knees paler. No spine on hind trochanter. Fore femur rather slender, covered with paler hairs and bearing 6–8 black bristles apically; mid femur with 2 rows of paler bristles and 2–4 black bristles apically; hind femur much longer than fore and mid femora with ventral and dorsal paler bristles. Fore tibia rather slender, with black hairs; mid tibia with distinct a pair of black and
strong bristles; hind tibia much longer than fore and mid tibiae, laterally compressed and dilated towards tip, with 2 rows of black bristles, with one dorsal black bristle.

**Abdomen.** Tergites and sternites blackish, covered with scattered black setulae. Terminalia black, with right surstylus somewhat robust and spiny in sub-apical and apical parts (Fig. 5B, D), left surstylus slender and hypandrial projection long and slender. Right cercus shorter, conical, left cercus longer and narrower (Fig. 5B).

**Female.** (Unknown)

Abbreviations: ae: aedeagus (phallus); cer: cercus; hyp: hypandrium; hypp: hypandrial projection; lel: left epandrial lamella; ls: left surstylus; rel: right epandrial lamella. rs: right surstylus.

**General comments.** Despite the male terminalia of the two new species described above being very similar in structure, it is easy to distinguish between the both species: *Trichina azizi* sp. nov. has yellowish hind trochanters, femora tibiae and metatarsi, so it is very distinct from *Trichina rifensis* sp. nov., which has entirely black hind trochanters, femora, tibiae (except at base) and tarsi, as may be seen in Fig. 7D, E.

Compared to other species of *Trichina* described so far, *Trichina rifensis* sp. nov., is very close to *Trichina opaca* in most morphological characters (colour of legs and wings, shape of antennae, number of scutellar pairs, absence of hind trochanters spine(s)) but as we previously noted in the differential diagnosis to this new species, it differs clearly in the male terminalia. As to *Trichina azizi* sp. nov., is similar to *T. elongata* in the shape of the antennae, number of scutellar pairs and absence of the trochanter spine, but it is easy to differentiate these species by the male terminalia as we have described in the differential diagnosis above. The common character between the right surstylus of the both new species is that they are spiny, which is a character not described in *Trichina* at all.

**Key to the Moroccan species of Trichina Meigen (males)**

This key is compiled by referring to the key of *Trichina* in Barták & Kubík (2009).

1. The whole of mesoscutum microtrichose. 3d antennal segment usually less than 3.2 times as long as broad ........................................... *T. opaca* Loew, 1864
   – The central parts of mesoscutum without microtrichiae. 3d antennal segment more than 3.4 times as long as broad ............................................... 2

2. Face broader ........................................................................................................................................................................... 4
   – Face narrow .................................................................................................................................................................................. 3

3. Hind trochanter with posterior to ventral spine(s)-like setae. Legs uniformly brownish. Right surstylus not spiny in apical and sub-apical parts .............  ................................................................. *T. elongata* Haliday, 1833
   – Hind trochanter without posterior to ventral spine(s)-like setae. Hind trochanter, femora, tibia and metatarsi yellowish (*T. azizi* sp. nov.) or at least knees and base of hind tibia paler (*T. rifensis* sp. nov.). Right surstylus spiny in apical and sub-apical parts .................................................................... 4

4. 3 pairs of scutellar setae.......................................................................................................................... *T. rifensis* sp. nov.
   – 2 pairs of scutellar setae.................................................................................................................. *T. azizi* sp. nov.
Subfamily OEDALEINAE Chvála, 1983

Genus Euthyneura Macquart, 1836

Euthyneura myrtilli Macquart, 1836

Fig. 7F


Distribution. Common in northern and central Europe, absent in the south (Chvála and Vonicka 2008); also European part and Western Siberia of Russia (Shamshev 2016). First record for Morocco.

Genus Oedalea Meigen, 1820

Oedalea portugalica Barták & Grootaert, 2021

Figs 6A–E, 7G

Material examined. 1 ♀. Morocco, Rif, PNPB, Tissegris, 505 m, 20.iv.2021, sweep net, leg. L. Zouhair, PCLZ.

Distribution. Known up to present only from the type locality in Portugal. First record for Morocco.

Remarks. Oedalea portugalica was described by Barták & Grootaert (2021) in Kanavalová et al. (2021) from Portugal. The male terminalia were not illustrated or described, and are provided herein for the first time.

Terminalia (Fig. 6) small. Cerci (Fig. 6B) digitiform, covered toward tip with long setae, right cercus (Fig. 6B) slightly longer and more narrowed apically than left cercus. Epandrium covered with long setae (Fig. 6A, C), right epandrial lamella somewhat longer, broader and more elongated towards apex (Fig. 6A) than left epandrial lamella (Fig. 6C).

In Kanavalová et al. (2021), O. portugalica was compared with O. stigmatella. In the terminalia of O. stigmatella (figs 401–404 in Chvála 1983), the shape of the right and left epandrial lamellae is different, so that in O. portugalica there is a deep medial apical incision in the hypandrium (it is forked Fig. 6D), while in O. stigmatella the fork is shallow and broader (fig. 403 in Chvála 1983). The tip of the left postgonite is very characteristic and different. A sharp protrusion is apical in O. stigmatella (fig. 404 in Chvála 1983) while it is lateral (subapical) in O. portugalica (Fig. 6E seen from the right, while on Fig. 6A it is below the right epandrial lamella seen from the right side).

Abbreviations: ae: aedeagus (phallus); cer: cercus; hyp: hypandrium; lep: left epandrial lamella; lpgt: left postgonite; rep: right epandrial lamella; rpgt: right postgonite.
First records of *Trichina*, *Oedalea* and *Euthyneura* from North Africa

Discussion

The current study contributes significantly to the enrichment of the faunistic database of the Hybotidae fauna of Morocco in particular and that of North Africa in general. Seven species belonging to three genera (*Trichina*, *Euthyneura* and *Oedalea*) which were unprecedentedly reported in North Africa as well as their respective subfamilies (*Trichininae* and *Oedaleinae*) are newly recorded in the region, increasing the total of the known hybotid species of Morocco from 44 species to 51. The description of two new species belonging to the *Trichina* genus provides also an important contribution to the hybotid fauna. Our results show that the distribution of the three mentioned genera has expanded beyond Europe where they were originally recorded.

These findings highlight the richness of the Moroccan biodiversity, particularly in the Western Rif, which is considered a biodiversity hotspot, where the new species were found, and which constitutes the only part of Morocco included in the Mediterranean Intercontinental Biosphere Reserve (MIBR) due to the great specific richness and the
Figure 7. A Trichina elongata Haliday, 1833 B Trichina opaca Loew, 1864 C Trichina unilobata Chvála, 1981 D Trichina azizi Zouhair & Grootaert sp. nov. E Trichina rifensis Zouhair & Grootaert sp. nov. (Left antennal style missing) F Euthynena myrtilli Macquart, 1836 G Oedalea portugalica Barták & Grootaert, 2021.
high rate of endemism recorded there (Bachar et al. 2021). Indeed, of the 51 species of Hybotidae occurring in Morocco, 25 of them are cited exclusively in the Rif.

As noted by Stark (2008) regarding the weak diversity of the subfamilies Trichininae and Oedaleinae compared to the Tachydromiinae, our results exhibit the same pattern following our study of ancient and recent hybotid samples collected from various habitats in Morocco by the first and the third authors, where Tachydromiinae subfamily was remarkably always dominant. However, this can also be explained by the fact that the Moroccan Hybotidae fauna is strongly under-collected and there is still at least as much to discover there. There is no doubt that the number of species of Hybotidae occurring in Morocco as in North Africa will increase when collecting is more intensified.

References


Taxonomic study on Mysmenidae spiders (Mysmenidae, Araneae) from Xishuangbanna of Yunnan, China

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Abstract
Thirteen spider species belonging to the family Mysmenidae Petrunkevitch, 1928 are reported from Xishuangbanna Tropical Botanical Garden (XTBG), Menglun Township, Mengla County, Yunnan Province of China. One genus and five species are documented as new to science: Mengmena banna gen. nov. et sp. nov. (♂♀), Mengmena yulin sp. nov. (♀), Mosu heguomu sp. nov. (♂♀), Mysmena luosuo sp. nov. (♂♀), and Mysmena dai sp. nov. (♀). One species is proposed as a new combination: Mosu zhengi (Lin & Li, 2008) comb. nov. (♂♀, ex Mysmena Simon, 1894). The females of Microdipoena menglunensis (Lin & Li, 2008), Mysmena arcilonga Lin & Li, 2008, Mysmena furca Lin & Li, 2008, and Mysmena rostella Lin & Li, 2008 are described for the first time. Three known species are re-examined and photographed: Gaoligonga taeniata Lin & Li, 2014, Mysmena biangulata (Lin & Li, 2008), and Mysmena cornigera (Lin & Li, 2008). Morphological diagnoses and illustrations are provided for these thirteen mysmenid species.

Keywords
Diagnoses, discovery, minute clasping weavers, rainforest, types
Introduction

Xishuangbanna is a key biogeographic area and a biodiversity hotspot in China (Wang et al. 2020; Li et al. 2021a; Yao et al. 2021; Hong et al. 2022; Zhu et al. 2022). It shares a border with Myanmar in the southwest and Laos in the southeast and harbours more species diversity than typical tropical rain forests of Southeast Asia (Zhu et al. 2006). Implementing an “All Species Inventory” of spiders in Xishuangbanna Tropical Botanical Garden (XTBG, 1125-hectare area in total) has increased the spider species from fewer than 50 before 2006 to about 800 by the end of 2020 (Li 2020). The fifteen times increase in XTBG spider species during the past 15 years provides a striking example of high species richness within a small area.

Mysmenidae Petrunkevitch, 1928 is a small family of minute araneoids. Although widely distributed (except in the northern Holarctic realm, arid regions and Antarctica), Mysmenidae is still a poorly studied spider group in terms of faunal investigation and species diversity. These spiders live in cryptic habitats of moist leaf litter, mosses, and even dark caves in tropical and subtropical regions. Currently, 158 described species of 14 genera have been recorded worldwide (WSC 2022), of which nearly half of species have been discovered in the past two decades, including 38 species in eight genera from China. Lin and Li (2008) reported that 11 mysmenid species in China, of which eight came from XTBG. This is the only report of Mysmenidae spiders from this area so far, and most species were only described based on male specimens.

In the present paper, 13 species classified in five genera of mysmenid spiders from XTBG are recorded and illustrated, including five new species and one new genus. The goal of this paper is to provide detailed descriptions of these new taxa, to provide descriptions of the females of three known species for the first time, and to propose a new combination.

Materials and methods

The inventory for this study included more than 800 spider specimens from XTBG belonging to the family Mysmenidae. Specimens were examined and measured in a 75% ethanol solution under a Leica M205 C stereomicroscope and photographed with a Canon EOS 60D wide zoom digital camera (8.5 megapixels) mounted on an Olympus BX 43 compound microscope. The digital photos were montaged using Helicon Focus 3.10 (Khmelik et al. 2006) image stacking software. Male palps and epigyna were examined and photographed after dissection. The left palp was photographed and described (if missing, the right was used). Epigyna were treated with lactic acid before being embedded in Hoyer’s gum and placed on an ultrathin slide to take photos of both sides of the vulva. All measurements are in millimetres. Leg measurements are given as follows: total length (femur, patella, tibia, metatarsus, and tarsus).

Abbreviations used in the text or figures are given in Table 1. References to figures in the cited papers are in lowercase (fig. or figs), figures in this paper are noted with an initial capital (Fig. or Figs). Apart from the type specimens of previously described species kept in IZCAS, all other examined morphological material is deposited in the NHMSU and IZCAS.
Table 1. List of abbreviations used in the figures or text.

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Somatic morphology

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**Taxonomy**

**Mysmenidae Petrunkevitch, 1928**

**Gaoligonga Miller, Griswold & Yin, 2009**

*Gaoligonga taeniata* Lin & Li, 2014

Figs 1, 2


**Type material.** Holotype ♂ (IZCAS) and paratypes 1♂ 5♀ (IZCAS), Vietnam: Ninh Binh, Cuc Phuong National Park, natural forest (20.410°N, 105.624°E; 436 m), by sieving leaf litter, 8.X.2007, D. Pham leg. Examined.

Figure 1. *Gaoligonga taeniata* A–C male habitus D–F female habitus G male prosoma H epigyne I, J vulva A, D, J dorsal B, E, H, I ventral C, F lateral G anterolateral. Abbreviations: CD = copulatory duct; CS = cheliceral spines on male; EH = epigynal hood; FD = fertilization duct; S = spermatheca; Sp = scape. Scale bars: 0.50 mm (A–E); 0.20 mm (G); 0.10 mm (H–J).
**Diagnosis.** This species can be distinguished from *G. changya* Miller, Griswold & Yin, 2009 (Miller et al. 2009: 48, figs 38A–E, 39A, B, 40A–F, 41A, B, 43A, B) and *G. zhusun* Miller, Griswold & Yin, 2009 (Miller et al. 2009: 50, figs 43D, E, 44A–E, 45A, B, 46A–D, 47D) by the male having three frontal spines near the base of each chelicera (Fig. 1G vs. fig. 38A, 40E, 44A, 46E, Miller et al. 2009: 119, 421, 125, 127), the palp having a “S”-shaped cymbium and median keel on cymbium, lacking basal keel and tooth on cymbium (Fig. 2B, D vs. fig. 39A–B, 40A–C, 45A–B, 46A–C, Miller et al. 2009: 120, 121, 126, 127), and the strong, anticlockwise spiral embolus with a tortuous end (Fig. 2A, C vs. fig. 39A–B, 45A–B, Miller et al. 2009: 120, 126). Females can be distinguished by the large, long central knob-shaped scape (Fig. 1H vs. fig. 43B, E, Miller et al. 2009: 124), having a saccular epigynal hood, the nearly transversely clubbed spermathecae, and the membranous, broad and complicated copulatory ducts (Fig. 1J vs. fig. 43B, E, Miller et al. 2009: 124).

**Description.** See Lin and Li (2014: 178), and Figs 1, 2.

**Distribution.** China (Yunnan), Vietnam.

**Remark.** This species is newly recorded in XTBG, China.

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**Mengmena Lin & Li, gen. nov.**

https://zoobank.org/91BD56B2-545A-4D61-86DF-19D0ED9671BF

**Type species.** *Mengmena banna* Lin & Li, sp. nov.

**Etymology.** The generic name is a combination of the first four letters of Menglun (type locality of type species) and the latter half of *Mysmena*. The gender is feminine.

**Diagnosis.** The *Mengmena* gen. nov. can be easy distinguished from other mysmenids, except *Mysmeniola* Thaler, 1995, by lacking anterior median eyes in both sexes (Figs 3A, D, 6A). It resembles *Mysmeniola* in having six eyes (anterior median eyes absent), a submesial mating clasper on metatarsus I of males, and a long filiform embolus extending to the distal tip of the cymbium, but differs from *Mysmeniola* in lacking a cluster of strong spines at the base of the male clypeus (*Mysmeniola*, Thaler 1995: figs 1, 2), and lacking a prolateral apical process on male palpal tibia (Thaler 1995: fig. 5). In addition, the male can be distinguished from other mysmenids by the complex structure of the apical part of cymbium (Figs 5A–C). The cymbium tip specialized as a triangular cymbial conductor (Figs 4A–B, 5A–C), and the retrolateral base of cymbial conductor present a distal lobe (Figs 4B, 5A), the cymbial fold originated from the base of cymbial conductor and the cymbial fold distal end extended anteriorly form a sclerotized cymbial process (Figs 4D, 5A–C); the absence of cymbial spur (or cymbial tooth) and paracymbium (Figs 4A–D, 5C). The female can be distinguished by the widely separated spermathecae (at least 4× their width, 2–3× in other mysmenids), and copulatory opening situated at the union of copulatory ducts (Figs 3H, I, 6E, F).

**Description.** Body bicolour, dorsally grey, ventrally yellow or pale yellow (Figs 3A–F, 6A, B). Anterior median eyes absent (Figs 3A, 3D, 6A). Abdomen without posterior
Figure 2. *Gaoligongga taeniata*  

**A** bulbus, **B** cymbium, **C, D** male palp.  
**A–C** prolateral, **D** retrolateral.  

Abbreviations:  
- Cy = cymbium;  
- CyF = cymbial fold;  
- CyFs = setae on cymbial fold;  
- CyP = cymbial process;  
- DL = distal lobe on cymbium;  
- E = embolus;  
- MK = median keel on cymbium;  
- PC = paracymbium;  
- SD = spermatic duct;  
- Ti = palpal tibia.  

Scale bars: 0.10 mm (**A**); 0.20 mm (**B–D**).
tubercle (Figs 3A–D, 6A–C). Male cephalic area moderately elevated, tibia I without prolateral macrosetae (Fig. 3C). Femoral spots present on leg I of the males and legs I–II of the females (Figs 3B, 3E, 6B).

**Male palp:** cymbium oriented ventrally on the palp (Fig. 4A, B). Cymbial spur and paracymbium absent (Figs 4A–D, 5C). Cymbial process arising from the cymbial fold at apex, strongly sclerotized (Figs 4D, 5A–C). Cymbial conductor wide (Fig. 5A–C). Cymbial fold long and sclerotized, from the base of cymbial conductor (Fig. 5C). Distal lobe on retrolateral tip of cymbium (Fig. 4A, B). Embolus threadlike, coiled with at least two loops (Figs 4A–D, 5D–E).

**Epigyne:** weakly sclerotized (Figs 3G, 6D). Scape absent (Figs 3G–I, 6D–F). Spermathecae ovate or slightly twisted, separated by at least four times their width. Copulatory ducts wide, shape convoluted. Copulatory opening small hole-shaped or arc shape, situated at the union of copulatory ducts (Figs 3H, I, 6E, F).

**Composition.** *Mengmena banna* sp. nov. and *M. yulin* sp. nov.

**Distribution.** China (Yunnan).

*Mengmena banna* Lin & Li, sp. nov.
https://zoobank.org/66FB56DD-4BF3-487E-8412-9F95DF392C58
Figs 3–5

**Type material.** **Holotype** ♂ (IZCAS) and **paratypes** 3♂ 4♀ (IZCAS), **China:** Yunnan: Mengla County, Menglun Town, Xishuangbanna National Nature Reserve, the primary tropical seasonal rain forest (21.957°N, 101.217°E; 744±15 m), 16–31.I.2007, by pitfall trapping, G. Zheng leg.; 6♂ 25♀ (NHMSU), **China:** Yunnan: Mengla County, Menglun Town, XTBG, in the plantation of Paramichelia baillonii (about 20 yr.) (21.903°N, 101.282°E; 608±11 m), 5–12.IX.2006, by searching, G. Zheng leg.

**Other material examined:** 11♂ 33♀ (IZCAS), **China:** Yunnan, Mengla County, Menglun Town, Xishuangbanna National Nature Reserve, the plantation of Paramichelia baillonii (21.956°N, 101.523°E; 608±11 m), 5–12.XI.2006, by search collecting, G. Zheng leg.

**Etymology.** The specific name derives from the type locality; noun in apposition.

**Diagnosis.** *Mengmena banna* sp. nov. can be distinguished from its congener *M. yulin* sp. nov. by both sides of the copulatory duct being fused at the midline position and forming a V-shaped structure, and the copulatory opening situated below the bottom of the V-shaped structure (Fig. 3H, I vs. Fig. 6E, F).

**Description. Male. Measurements:** total length 0.58. Prosoma 0.30 long, 0.28 wide, 0.29 high. Abdomen 0.34 long, 0.31 wide, 0.38 high. Clypeus 0.07 high. Sternum 0.20 long, 0.19 wide. Length of legs: I 0.91 (0.31, 0.12, 0.21, 0.11, 0.16); II 0.80 (0.25, 0.11, 0.18, 0.10, 0.16); III 0.62 (0.18, 0.09, 0.11, 0.11, 0.13); IV 0.72 (0.22, 0.10, 0.15, 0.12, 0.13).
Figure 3. *Mengmena banna* sp. nov. A–C male habitus D–F female habitus G epigyne H, I vulva A, D, I dorsal B, E, G, H ventral C, F lateral. Abbreviations: CD = copulatory duct; CO = copulatory opening; FD = fertilization duct; FS = femoral spot; MC = Metatarsal claspign spine; S = spermatheca. Scale bars: 0.50 mm (A–F); 0.10 mm (G–I).
**Somatic characters** (Fig. 3A–C). **Coloration:** prosoma light-yellow centrally, deep yellow marginally. Ocular base black. Chelicera yellow, endites and labium yellow, the sternum light-yellow. Abdomen silver grey dorsally, yellow with a “U”-shaped white and a “U”-shaped brown stripe ventrally. Legs yellow. **Prosome:** carapace near round.
Figure 5. *Mengmena banna* sp. nov. A, B cymbial terminals C palpal cymbium and tibia D, E bulbus. A prodorsal B retroventral C retrodorsal D ventral E dorsal. Abbreviations: Cy = cymbium; CyC = cymbial conductor; CyF = cymbial fold; CyFs = setae on cymbial fold; CyP = cymbial process; DL = distal lobe on cymbium; E = embolus; SD = spermatic duct; Te = tegulum; Ti = palpal tibia. Scale bars: 0.05 mm (A, B); 0.20 mm (C); 0.10 mm (D, E).
Mysmenidae from Xishuangbanna

Cephalic part slightly elevated. Ocular area at apex. AME absent, six eyes in two rows. ALE and PLE contiguous. PER slightly recurved. Sternum scutiform, plump, covered with sparse setae. **Legs:** covered with setae and bristles. The leg I with a mating clasper on distal 1/3 position of metatarsus and a subdistal sclerotized femoral spot present at surface of ventral femur. **Abdomen:** near round in dorsum.

**Palp** (Figs 4–5): weakly sclerotized. The tibia cup-shaped, covered with long setae along distal brim (Fig. 4A, B). Cymbium membranous, with a distal lobe on cymbium (Figs 4A, B, 5A–C). Cymbial conductor triangular, distal end hook-shaped (Figs 4A–D, 5A–C). Cymbial process straight but the tip recurved, situated on dorsal cymbial conductor (Figs 4D, 5A–C). Cymbial fold sclerotized, derived from the base of cymbial process, and bears a row of ordered setae (Fig. 5A, C). The absence of paracymbium (Fig. 5C). Tegulum smooth, translucent; spermatic duct visible from the tegulum (Figs 4B, D, 5D). Embolus threadlike, coiled into two crossed loops (Figs 4A–D, 5D, E).

**Female. Measurements:** total length 0.64. Prosoma 0.29 long, 0.27 wide, 0.27 high. Abdomen 0.42 long, 0.40 wide, 0.46 high. Clypeus 0.06 high. Sternum 0.20 long, 0.19 wide. Length of legs: I 0.87 (0.27, 0.12, 0.18, 0.14, 0.16); II 0.75 (0.22, 0.11, 0.15, 0.12, 0.15); III 0.61 (0.17, 0.09, 0.11, 0.11, 0.13); IV 0.72 (0.21, 0.10, 0.14, 0.13, 0.14).

**Somatic characters** (Fig. 3D–F). **Coloration:** prosoma light-brown centrally, deep brown marginally. Ocular base black. Chelicera, endites, labium and sternum light-brown. Abdomen silver grey, with a “U”-shaped white stripe. Legs brown. **Prosoma:** carapace pear-shaped. Ocular pattern as in male. AME absent, six eyes in two rows, the PER slightly recurved, the ALE and PLE contiguous. Sternum scutiform, plump, covered with sparse setae. **Legs:** covered with setae and bristles, a sclerotized femoral spot present at apical ventral surface of leg I and II. **Abdomen:** same as in male.

**Epigyne** (Fig. 3G–I): weakly sclerotized, covered with sparse short setae along ventral brim (Fig. 3G, H). Internal structures indistinctly visible from translucent cuticle (Fig. 3G). Spermathecae small, ovate, widely separated by at least four times their width (Fig. 3H, I). Fertilization ducts short, winding, arising from ventral of the spermathecae (Fig. 3H, I). Copulatory ducts wide, both sides of copulatory duct fused at the midline position and formed a V-shaped structure; the copulatory opening situated below the bottom of the V-shaped structure (Fig. 3H, I).

**Distribution.** Known only from the type locality.

*Mengmena yulin* Lin & Li, sp. nov.
https://zoobank.org/5EE0208C-62D5-46A6-83FE-F9FD50A9A5EF
Fig. 6

Figure 6. Mengmena yulin sp. nov. A–C female habitus D epigyne E, F vulva. A, F dorsal B, D, E ventral C lateral. Abbreviations: CD = copulatory duct; FD = fertilization duct; FS = femoral spot; S = spermatheca. Scale bars: 0.50 mm (A–C); 0.10 mm (D–F).
Etymology. The specific name derives from the Chinese pinyin for rainforest (yǔ lín), refers to it living in rainforest habitats. The epithet is a noun in apposition.

Diagnosis. The new species is similar to *Mengmena banna* sp. nov. but can be distinguished by having a straight and smooth posterior brim formed by the fusion of the copulatory ducts, and the copulatory opening situated above this brim (Fig. 6E, F vs. Fig. 3H, I).

Description. Female. Measurement: total length 0.78. Prosoma 0.32 long, 0.29 wide, 0.28 high. Abdomen 0.49 long, 0.48 wide, 0.53 high. Clypeus 0.05 high. Sternum 0.21 long, 0.20 wide. Length of legs [total length (femur, patella, tibia, metatarsus, tarsus)]: I 0.92 (0.30, 0.11 0.19, 0.15, 0.17); II 0.85 (0.28, 0.10, 0.17, 0.13, 0.17); III 0.66 (0.19, 0.09, 0.12, 0.11, 0.15); IV 0.79 (0.25, 0.10, 0.16, 0.12, 0.16).


Epigyne (Fig. 6D–F): spermathecae small, ovate, widely separated by at least four times their width (Figs 6E, F). Fertilization ducts short, derived from inner side of the spermathecae and bent anteriorly to form an arc (Fig. 6F). Copulatory ducts arising from ventral of spermathecae, both sides of copulatory duct fused at the midline position and forming two symmetrical peak shapes (the anterior brim of the fused copulatory ducts broad arc shape, the posterior brim of the fused copulatory ducts near straight) (Fig. 6E, F). Copulatory opening inconspicuous, situated above the straight brim (Fig. 6E, F).

Male. Unknown.

Distribution. Known only from the type locality.

*Microdipoena* Banks, 1895

*Microdipoena menglunensis* (Lin & Li, 2008)
Figs 7–9


*Microdipoena menglunensis* Lopardo and Hormiga 2015: 783.

Type material. Holotype ♂ (IZCAS), CHINA: Yunnan, Mengla, XTBG (21.913°N, 101.267°E; 556±11 m), by pitfall trapping, 18.VII.2007, Guo Zheng leg.; paratypes 1♂ (IZCAS), same site as for preceding, Rubber plantation (21.908°N, 101.266°E;
Figure 7. *Microdiponea menglunensis* A–C male habitus D–F female habitus. A, E dorsal B, F ventral C, D lateral. Abbreviations: FS = femoral spot; MC = Metatarsal clasping spine; TS = tibial spine on male leg I. Scale bars: 0.50 mm.


**Diagnosis.** This species is similar to *Microdipoena jobi* (Kraus, 1967) and *Microdipoena samoensis* (Marple, 1955), but can be distinguished by the detailed structures of the embolus; this species has a distal lobe on the cymbium apex and has a sclerotized cymbial fold bore a row of ordered setae (Fig. 8A, C–F vs. fig. 132D–F, Lopardo and Hormiga 2015: 675). The female distinguished by the semicircular spermathecae separated by 2.5 times their diameter, near globular in *M. jobi* and *M. samoensis* (Fig. 9C, D vs. fig. 129E, F, Lopardo and Hormiga 2015: 672).

**Description.** **Male.** See Fig. 7A–C and Lin and Li (2008): 506.

**Palp** (Fig. 8A–F): orange; tibia small, cup-shaped, except for retrolateral region, a row of long setae almost encircling the distal brim (Fig. 8E, F). Cymbium nearly transparent, with a large cymbial tooth at the ventral median, a distal lobe and a cymbium process on the cymbium apex; the cymbial fold slightly sclerotized, bore a row of ordered setae (Fig. 8A, C–F). Paracymbium wider, tongue-shaped, with long setae (Fig. 8E). The bulb is embedded in a translucent membranous tegulum. Embolus very long, coiled into two crossed loops; the apical structure of the embolus considerably complicated (Fig. 8C–F).

**New morphological data.** **Female. Measurements:** total length 0.81. Prosoma 0.24 long, 0.20 wide, 0.20 high. Abdomen 0.57 long, 0.57 wide, 0.62 high. Clypeus 0.06 high. Sternum 0.18 long, 0.16 wide. Length of legs: I 0.92 (0.30, 0.12, 0.28, 0.12, 0.10); II 0.90 (0.28, 0.12, 0.26, 0.14, 0.10); III 0.70 (0.26, 0.08, 0.16, 0.10, 0.10); IV 0.94 (0.32, 0.12, 0.28, 0.12, 0.10).

**Somatic characters** (Fig. 7D–F). **Coloration:** same as in male. **Prosoma:** carapace long, nearly pear-shape. Cephalic part lower than in male. Ocular pattern as in male. Chelicerae, endites, labium and sternum as in male. **Legs:** covered with setae and bristles, a sclerotized subdistal-ventral femoral spot present on surface of legs I and II. **Abdomen:** same as in male.

**Epigyne** (Fig. 9A–D): the structure can be seen through the cuticle (Fig. 9A–D). Scape long, curved, with narrow folds (Fig. 9B–D). Spermathecae large, semicircular, separated by 2.5 times their diameter (Fig. 9C, D). Fertilization ducts short, bending anteriorly, arising from lower edge of spermathecae (Fig. 9C, D). Copulatory ducts membranous, slightly sclerotized, coiled posterior of spermathecae, connected above the spermathecae (Fig. 9C, D).

**Distribution.** Southwestern China (Yunnan).

**Remarks.** The female of *M. menglunensis* is described for the first time.
Figure 8. Microdiponea menglunensis A, E, F male palp B conductor C, D embolus and cymbial terminal A apical B dorsal C, E prolateral D, F retrolateral. Abbreviations: C = conductor; CT = cymbial tooth; Cy = cymbium; CyF = cymbial fold; CyFs = setae on cymbial fold; CyP = cymbial process; DL = distal lobe on cymbium; E = embolus; PC = paracymbium; SD = spermatic duct; Te = tegulum; Ti = palpal tibia. Scale bars: 0.10 mm (A, C–F); 0.05 mm (B).
**Figure 9.** *Microdiponea menglunensis* A, B epigyne C, D vulva. A, C ventral B lateral D dorsal. Abbreviations: CD = copulatory duct; FD = fertilization duct; S = spermatheca; Sp = scape. Scale bars: 0.10 mm.

**Mosu Miller, Griswold & Yin, 2009**

**Mosu heguomu Lin & Li, sp. nov.**

https://zoobank.org/8A572298-31C4-4011-B966-B8D26E9283CA

Figs 10–12


**Etymology.** The specific name derives from a Chinese Pinyin “hé guǒ mù”, referring to the Chinese name of *Paramichelia baillonii*. 
Diagnosis. Male differed from other congeners by the male palp with a cymbial serrula, a distal lobe and median keel on the cymbium, and cymbial tooth on the median cymbium (Fig. 11C). Female can be distinguished by the sclerotized and expanded copulatory opening at the tip of scape (Fig. 11C, D).

Description. Male. Measurements: total length 1.06. Prosoma 0.34 long, 0.5 wide, 0.5 high. Abdomen 0.7 long, 0.76 wide, 0.7 high. Clypeus 0.08 high. Sternum 0.3 long, 0.32 wide. Length of legs: I 1.46 (0.50, 0.16, 0.40, 0.20, 0.20); II 1.32 (0.40, 0.20, 0.30, 0.20, 0.22); III 0.88 (0.30, 0.10, 0.20, 0.16, 0.12); IV 1.04 (0.36, 0.10, 0.24, 0.16, 0.18).


Palp (Fig. 11A–C, E, F): orange; tibia small, about 1/5 volume of the bulb, with a row of long setae almost encircling the brim (Fig. 11E, F). Cymbium nearly transparent, “right angle”-shaped, with a cymbial tooth at the ventral median, a list of cymbial serrula, a distal lobe and a median keel on cymbium; the cymbial fold slightly sclerotized, with a row of setae; the tip of cymbium specialized as cymbial conductor (Fig. 11A, C, E). Bulb oblate, embedded in a translucent membranous tegulum. Embolus filiform, length of embolus coiled into two loops in tegulum, the apical of embolus coiled on the bulb (Fig. 11A, B, E, F).

Female. Measurements: total length 1.14. Prosoma 0.4 long, 0.48 wide, 0.4 high. Abdomen 0.7 long, 0.7 wide, 0.9 high. Clypeus 0.06 high. Sternum 0.3 long, 0.28 wide. Length of legs: I 1.16 (0.50, 0.12, 0.14, 0.22, 0.18); II 1.06 (0.44, 0.12, 0.14, 0.20, 0.16); III 0.92 (0.28, 0.08, 0.20, 0.16, 0.20); IV 1.22 (0.40, 0.10, 0.30, 0.24, 0.18).


Epigyne (Fig. 12A–D): scape long, the tip with a sclerotized and expanded copulatory opening (Fig. 12A–D). Spermathecae oval, inclined at 45 degrees. Fertilization ducts short, derived from anterior border of spermathecae. Copulatory ducts around the spermathecae, coiled into multiple loops below the spermathecae (Fig. 12C, D).
Figure 10. *Mosu heguomu* sp. nov. A–C male habitus D–F female habitus A, E dorsal B, F ventra C, D lateral. Abbreviations: FS = femoral spot; MC = Metatarsal clasping spine. Scale bars: 0.50 mm.
Figure 11. *Mosu beguomu* sp. nov. A, B, E, F male palp C cymbium D male left metatarsus I A, C ventral B apical D, E prolateral F retrolateral. Abbreviations: CT = cymbial tooth; Cy = cymbium; CyC = cymbial conductor; CyF = cymbial fold; CyFs = setae on cymbial fold; CyS = cymbial serrula; DL = distal lobe on cymbium; E = embolus; MC = Metatarsal clasping spine; MK = median keel on cymbium; SD = spermatic duct; Te = tegulum; Ti = palpal tibia. Scale bars: 0.20 mm (A–C, E, F); 0.10 mm (D).
Distribution. Southwestern China (Yunnan).

Remarks. The genus Mosu established by Miller et al. (2009) based on only known females of two species (*M. nujiang* and *M. huogou*), the common characteristics of the genus: kidney-shaped spermathecae, sclerotized fertilization ducts, the copulatory duct membranous and convoluted, sclerotized at end of path near spermathecae. Lin et al. (2013) supplemented male morphological characters, which distinguish the male palps: cymbial process present; bulb nearly globose; tegulum plump, lacks movable sclerites; embolus long, filiform, coiling into two loops under tegulum and reaching to the distal end of cymbium. This new species conforms to this generic characters, but can be clearly distinguished from *M. dayan* Lin & Li, 2013, *M. huogou* Miller, Griswold & Yin, 2009, *M. nujiang* Miller, Griswold & Yin, 2009, *M. tanjia* Lin & Li, 2013, we propose it as a new species.

Figure 12. *Mosu heguomu* sp. nov. **A, B** epigyne **C, D** vulva **A, C** ventral **B** lateral **D** dorsal. Abbreviations: CD = copulatory duct; CO = copulatory opening; FD = fertilization duct; S = spermatheca; Sp = scape. Scale bars: 0.10 mm.
**Mosu zhengi** (Lin & Li, 2008) comb. nov.
Figs 13–15


**Type material.** Holotype ♀ (IZCAS) and paratypes 6♂ 6♀, China: Yunnan, Mengla, Menglun, Primary tropical seasonal rainforest in XTBG (21.917°N, 101.275°E; 558±17 m), by pitfall trapping, 22.VII.2007, G. Zheng leg. Examined.


**Diagnosis.** This species is similar to *M. tanjia* Lin & Li, 2013, but can be distinguished by the male and female each with a short abdominal protuberance, the male with sclerotized femoral spot present on the surface of ventral femur I, the female with a femoral spot present on the surfaces of femur I and II (Fig. 13A–F vs. fig. 7A–F, Lin et al. 2013: 458). The palp can be distinguished by the cymbial tooth located in the cymbial center (Fig. 14B–C vs. figs 8A, C, 10C, Lin et al. 2013: 459). The female can be distinguished by the margin inferior to the epigyne incrassate, the reniform spermathecae, and the copulatory ducts without a curve and sclerotized parts above the spermathecae (Fig. 15A–C vs. figs 9A, B, 12A, B, Lin et al. 2013: 460, 463).

**Description.** Male. **Measurements:** total length 1.45. Prosoma 0.55 long, 0.55 wide, 0.43 high. Abdomen 0.90 long, 0.75 wide, 0.75 high. Clypeus 0.08 high. Sternum 0.38 long, 0.35 wide. Length of legs: I 1.44 (0.43, 0.18, 0.38, 0.20, 0.25); II 1.19 (0.38, 0.13, 0.25, 0.18, 0.25); III 0.69 (0.25, 0.10, 0.10, 0.12, 0.12); IV 0.98 (0.33, 0.10, 0.30, 0.15, 0.10).

**Somatic characters** (Fig. 13A–C). **Coloration:** prosoma deep yellow dorsally, yellow ventrally, ocular base black. Abdomen brown, with multiple yellow spots. Legs brown-yellow. **Prosoma:** carapace near round in dorsal and peak-shaped in lateral, marginally smooth. Cephalic area sharply elevated. Ocular region projecting, eight eyes in two rows. All eyes round, AER and PER recurved in dorsal view, ALE and PLE contiguous. Labium rectangle. Sternum scutiform, smooth surface. **Legs:** leg I with a mating clasper on metatarsus, a subdistal sclerotized femoral spot present at surface of ventral femur, the two spines on tibia. Legs covered with setae and bristles. **Abdomen:** near ladle-shaped in dorsum, covered with pale short setae.

**Palp** (Fig. 14A–D): orange; tibia cup-shaped, except for retrolateral region, a row of long setae almost encircled the distal brim (Fig. 14C, D). Cymbium transparent, nearly slant parallelogram, with a thorn-shaped cymbial tooth, cymbial fold long and sclerotized, bears a row of ordered setae (Fig. 14A, B); Cymbial conductor wide, arc (Fig. 14B). Paracymbium with long setae (Fig. 14B). Bulb near round, embedded in a translucent membranous tegulum. Embolus long, coiled into 2 loops (Fig. 14A, C, D).
Figure 13. *Mosu zhengi* comb. nov. A–C male habitus D–F female habitus A, E dorsal B, F ventral C, D lateral. Abbreviations: AP = abdominal protuberance; FS = femoral spot; MC = Metatarsal claspers spine; TS = tibial spine on male leg I. Scale bars: 0.50 mm.
Figure 14. *Mosu zhengi* comb. nov. **A, C, D** male palp **B** cymbium. **A** apical **B** proventral **C** prolateral **D** retrolateral. Abbreviations: Abbreviations: CT = cymbial tooth; Cy = cymbium; CyC = cymbial conductor; CyF = cymbial fold; CyFs = setae on cymbial fold; E = embolus; PC = paracymbium; SD = spermatic duct; Te = tegulum; Ti = palpal tibia. Scale bars: 0.10 mm (**A, B**); 0.20 mm (**C, D**).

**Female. Measurements**: total length 1.58. Prosoma 0.45 long, 0.45 wide, 0.30 high. Abdomen 1.13 long, 0.85 wide, 0.80 high. Clypeus 0.08 high. Sternum 0.38 long, 0.32 wide. Length of legs: I 1.10 (0.35, 0.10, 0.25, 0.20, 0.20); II 1.01 (0.25, 0.10, 0.24, 0.22, 0.20); III 0.76 (0.23, 0.08, 0.20, 0.15, 0.10); IV 0.92 (0.20, 0.15, 0.25, 0.16 0.16).
Somatic characters (Fig. 13D–F). Coloration: prosoma deep yellow dorsally, yellow ventrally, ocular base black. Abdomen brown-yellow, with multiple white spots. Legs brown-yellow. Prosoma: carapace long, nearly pear-shaped. Cephalic part lower than in male, flatted on top. Eight eyes in three rows. AER and PER straight in dorsal view. Chelicerae, endites as in male, labium triangle, and sternum scutiform. Legs: covered with setae and bristles, a sclerotized subdistal-ventral femoral spot present at surface of leg I and II. Abdomen: same as in male.

Epigyne (Fig. 15A–C): spermathecae big, reniform (Fig. 15B–C). Fertilization ducts short, derived from anterior border of spermathecae. Copulatory ducts membranous, slightly sclerotized, around the spermathecae; the part of below the spermathecae coiled into two loops (Fig. 15C).
Distribution. Southwestern China (Yunnan).

Remarks. Miller et al. (2009) established the genus Mosu based on only known females of two species (M. jujiang and M. huogou), while studying the symphytognathoid spiders of the Gaoligongshan Mountain. They thought that Mysmena zhengi Lin & Li, 2008 may also belongs to this genus (The species consistent with the common characteristics of the genus: reniform and sclerotized spermathecae, sclerotized fertilization ducts, the copulatory duct membranous and convoluted, sclerotized at end of path near spermathecae). In this paper, we formally proposed transferring this species to Mosu as a new combination, based on a similar configuration of the vulva.

Mysmena Simon, 1894

Mysmena arcilonga Lin & Li, 2008
Figs 16–18

Mysmena arcilongus Lin and Li 2008: 497, fig. 7A–I (♂).


Diagnosis. This species can be distinguished from other congeners except for M. furca, M. luosuo sp. nov., and M. rostella by the presence of modified cheliceral spines on males, a row of cymbial serrula on the cymbium, a long, bow-shaped embolus spans retrorlaterally to the entire bulbus, and the partial swollen copulatory ducts larger than the spermathecae (cf. Figs 16C, 17A–D, 18B–C). Its males differed from that of Mysmena furca, M. luosuo sp. nov., and M. rostella by having a long, bow-shaped embolus and a serrated cymbial conductor (CyC, Fig. 17B, C), but short embolus in M. furca (Fig. 23C), twisted embolus and absence of a serrated CyC in M. luosuo sp. nov. (Fig. 25B, E), long hooked embolus and CyC with a distal keel in M. rostella (Fig. 28A, C). Females by the curved, rod-shaped spermathecae and the long fertilization ducts (Fig. 18C), but transverse ovoid spermathecae and short fertilization ducts in M. furca and M. luosuo sp. nov. (Figs 23F, 26C), reniform spermathecae in M. rostella (Fig. 29C).

Description. Male. See Fig. 16A–D and Lin and Li (2008): 497.

Palp (Fig. 17A–D): Orange, the tibia comparatively small, about one-quarter the volume of the bulb; except for retrorlaterial region, a row of long setae almost encircled the distal brim of tibia (Fig. 17A–D). Cymbium nearly transparent, the tip specialized as a wide cymbial conductor; a row of cymbial serrula on the cymbium; there is a distal lobe on cymbium and a median keel on the middle of the cymbium (Fig. 17B–D). Paracymbium big, with long setae (Fig. 17B–C). Tegulum translucent membranous,
Figure 16. *Mysmena arcilonga* A, B, D male habitus C male prosoma E–G female habitus A, F dorsal B, G ventral C anterolateral D, E lateral. Abbreviations: CS = cheliceral spines on male; FS = femoral spot; MC = Metatarsal clasping spine. Scale bars: 0.50 mm (A, B, D–G); 0.20 mm (C).
Figure 17. *Mysmena arcilonga* A–D male palp A dorsal B ventral C prolateral D retrolateral. Abbreviations: AA = apical apophysis on tegulum; Cy = cymbium; CyC = cymbial conductor; CyS = cymbial serrula; DL = distal lobe on cymbium; E = embolus; MK = median keel on cymbium; PC = paracymbium; SD = spermatic duct; Te = tegulum; Ti = palpal tibia. Scale bars: 0.10 mm.
with apical apophysis. Embolus long, with two ends, one end extends to cymbial conductor, the other end extends upon the tegulum (Fig. 17A–D).

**New morphological data. Female. Measurements:** total length 0.64 Prosoma 0.25 long, 0.27 wide, 0.16 high. Abdomen 0.39 long, 0.39 wide, 0.32 high. Clypeus 0.05 high. Sternum 0.23 long, 0.18 wide. Length of legs: I 0.70 (0.19, 0.08, 0.16, 0.13, 0.14); II 0.67 (0.13, 0.08, 0.18, 0.14, 0.14); III 0.46 (0.11, 0.07, 0.12, 0.08, 0.08); IV 0.53 (0.16, 0.10, 0.13, 0.08 0.06).

**Somatic characters** (Fig. 16E–G). **Coloration:** same as in male. **Prosoma:** carapace nearly peach-shaped. Ocular region projecting, eight eyes in two rows, ALE and
PLE contiguous. Chelicerae, endites and labium as in male, the sternum scutiform, covers with short setae. **Legs:** covered with setae and bristles, a sclerotized subdistal-ventral femoral spot present at surface of leg I. **Abdomen:** same as in male.

**Epigyne** (Fig. 18A–C): the scape short, surface with sparse fold (Fig. 18B). Spermathecae small, irregular (Fig. 18B–C). Fertilization ducts long, derived from anterior border of spermathecae and extended posteriorly. Copulatory ducts long and membranous, the other part slightly sclerotized, extending anteriorly to form an oval (Fig. 18C).

**Distribution.** Southwestern China (Yunnan).

**Remarks.** The female of *M. arcilonga* is reported for the first time.

*Mysmena biangulata* (Lin & Li, 2008)
Figs 19–20


*Mysmena biangulata* Lopardo and Hormiga 2015: 784.

**Type material.** **Holotype** ♂ (IZCAS) and **paratypes** 10♂ 7♀ (IZCAS), **China:** Yunnan, Mengla, XTBG, secondary tropical seasonal rainforest (21.924°N, 101.274°E; 598±17 m), by pitfall trapping, 22.VII.2007, G. Zheng leg. Examined.


**Diagnosis.** This species can be distinguished from other species except for *M. awari* (Baert, 1984), *M. marijkeae* (Baert, 1982), *M. vangoethemi* (Baert, 1982) and *M. nubiai* (Baert, 1984) by the elongate palpal bulbus, the cymbial process (CyP) juxtaposed with cymbial conductor (CyC) and both curved (cf. Fig. 20B, figs 11–12 in Baert 1982, and figs 9–10, 12–13 in Baert 1984), and the twisted, widely spaced spermathecae (cf. Fig. 20E, fig. 9H in Lin and Li 2008). *Mysmena biangulata* distinguished from those four species by CyP near same length as CyC at *M. biangulata*, shorter in four species (Fig. 20A, B vs. fig. 133D–F, Lopardo & Hormiga, 2015, 676, figs 11, 12, Baert, 1982, 306, figs 9–11, 12–13 in Baert 1984), and the twisted, widely spaced spermathecae (cf. Fig. 20E, fig. 9H in Lin and Li 2008). *Mysmena biangulata* distinguished from those four species by CyP near same length as CyC at *M. biangulata*, shorter in four species (Fig. 20A, B vs. fig. 133D–F, Lopardo & Hormiga, 2015, 676, figs 11, 12, Baert, 1982, 306, figs 9–11, 12–13 in Baert 1984), and the twisted, widely spaced spermathecae (cf. Fig. 20E, fig. 9H in Lin and Li 2008). *Mysmena biangulata* distinguished from those four species by CyP near same length as CyC at *M. biangulata*, shorter in four species (Fig. 20A, B vs. fig. 133D–F, Lopardo & Hormiga, 2015, 676, figs 11, 12, Baert, 1982, 306, figs 9–11, 12–13 in Baert 1984), and the twisted, widely spaced spermathecae (cf. Fig. 20E, fig. 9H in Lin and Li 2008). *Mysmena biangulata* distinguished from those four species by CyP near same length as CyC at *M. biangulata*, shorter in four species (Fig. 20A, B vs. fig. 133D–F, Lopardo & Hormiga, 2015, 676, figs 11, 12, Baert, 1982, 306, figs 9–11, 12–13 in Baert 1984), and the twisted, widely spaced spermathecae (cf. Fig. 20E, fig. 9H in Lin and Li 2008). *Mysmena biangulata* distinguished from those four species by CyP near same length as CyC at *M. biangulata*, shorter in four species (Fig. 20A, B vs. fig. 133D–F, Lopardo & Hormiga, 2015, 676, figs 11, 12, Baert, 1982, 306, figs 9–11, 12–13 in Baert 1984), and the twisted, widely spaced spermathecae (cf. Fig. 20E, fig. 9H in Lin and Li 2008). *Mysmena biangulata* distinguished from those four species by CyP near same length as CyC at *M. biangulata*, shorter in four species (Fig. 20A, B vs. fig. 133D–F, Lopardo & Hormiga, 2015, 676, figs 11, 12, Baert, 1982, 306, figs 9–11, 12–13 in Baert 1984), and the twisted, widely spaced spermathecae (cf. Fig. 20E, fig. 9H in Lin and Li 2008).

**Description.** See Fig. 19A–F and Lin and Li 2008: 499.

**Male palp** (Fig. 20A, B): light-yellow; tibia big, about 2/3 volume of the bulb, cup-shaped; Except for retrolateral region, a row of long setae almost encircling the distal brim (Fig. 20B). Cymbium nearly transparent; the cymbial conductor lateral
Figure 19. *Mysmena biangulata* A–C male habitus D–F female habitus A, E dorsal B, F ventral C, D lateral. Abbreviations: AP = abdominal protuberance; FS = femoral spot; MC = Metatarsal clasping spine. Scale bars: 0.50 mm.
Figure 20. *Mysmena biangulata* A, B male palp C epigyne D, E vulva. A prolateral B retrolateral C, D ventral E dorsal. Abbreviations: CD = copulatory duct; Cy = cymbium; CyC = cymbial conductor; CyF = cymbial fold; CyFs = setae on cymbial fold; CyP = cymbial process; E = embolus; FD = fertilization duct; S = spermatheca; SD = spermatic duct; Sp = scape; Te = tegulum; Ti = palpal tibia. Scale bars: 0.20 mm (A, B); 0.10 mm (C–E).
bending, parallel to the cymbial process; the cymbial fold long and sclerotized, bears a row of ordered setae (Fig. 20A, B); Embolus threadlike, coiled into 2 loops in tegulum. Spermatic ducts can be seen through tegulum (Fig. 20A, B).

**Epigyne** (Fig. 20C–E). The scape stubby, surface smooth (Fig. 20C–E). Spermaticae small, the diameter same as the copulatory ducts (Fig. 20D). Fertilization ducts and copulatory ducts slightly sclerotized, coiling around each other; the fertilization ducts opening to both edges of epigyne; anterior copulatory ducts sclerotized, flow-shaped; two openings converge toward the centre of epigyne (Fig. 20D, E).

**Distribution.** Southwestern China (Yunnan).

*Mysmena cornigera* (Lin & Li, 2008)

Fig. 21

*Calodipoena cornigera* Lin and Li 2008: 501, fig. 10A–J (♂).

*Mysmena cornigera* Lopardo and Hormiga 2015: 784.


**Diagnosis.** This species seems close to *M. caribbaea* (Gertsch, 1960) and *M. stathamae* (Gertsch, 1960) in the shape of palpal bulbus, the earlobe-shaped paracymbium and the simple distal part of cymbium (cf. Fig. 21D–F, and figs 30–31, 35–36 in Gertsch 1960), but can be distinguished by lacking a posterior abdominal tubercle, having a cymbial tooth and a distal process (CyP), (Fig. 21A, D, F and fig. 10A, B, G in Lin and Li 2008), with abdominal tubercle and lacking cymbial tooth and CyP in *M. caribbaea* and *M. stathamae* (figs 24, 27, 30–31, 35–36 in Gertsch, 1960).

**Description.** Male. See Fig. 21A–C and Lin and Li (2008): 501.

**Palp** (Fig. 21D–G): light-orange, comparatively large; tibia cup-shaped, except for retrolateral region, a row of long setae almost encircling the distal brim (Fig. 21F, G). Cymbium nearly transparent, the tip specialized as the cymbial conductor; cymbium with a process and a tooth-shaped cymbial tooth; cymbial fold long and slightly sclerotized, bore a row of ordered setae; paracymbium large, with long setae (Fig. 21D). Embolus threadlike, coiled into 1.5 loops in tegulum. Tegulum nearly transparent. Spermatic ducts can be seen through tegulum (Fig. 21F, G).

**Female.** Unknown.

**Distribution.** Southwestern China (Yunnan).
Figure 21. *Mysmena cornigera* A–C male habitus D cymbium E bulbus F, G male palp A dorsal B ventral C lateral D, E apical F prolateral G retrolateral. Abbreviations: CT = cymbial tooth; Cy = cymbium; CyC = cymbial conductor; CyF = cymbial fold; CyFs = setae on cymbial fold; CyP = cymbial process; E = embolus; FS = femoral spot; MC = Metatarsal clasping spine; PC = paracymbium; SD = spermatic duct; Te = tegulum; Ti = palpal tibia. Scale bars: 0.50 mm (A–C); 0.10 mm (D, E); 0.20 mm (F, G).
Mysmenidae from Xishuangbanna

*Mysmena furca* Lin & Li, 2008
Fig. 22, 23

*Mysmena furca* Lin & Li, 2008: 495, fig. 6A–G (♂).


**Diagnosis.** This species is similar to *M. arcilonga* but can be distinguished by the presence of four pairs of cheliceral spines (Fig. 22C vs. Fig. 16C), the palp presence of the cymbial fold, the cymbial process on the tip of cymbium; absence of distal lobe, a paracymbium and a cymbial conductor (Fig. 23A–C vs. Fig. 17A–D). The female can be distinguished by the spermathecae situated at the posterior of vulva, the diameter of copulatory ducts same as spermathecae, fertilization ducts shorter and extended to anterior of spermathecae (Fig. 23E, F vs. Fig. 18B, C).

**Description. Male.** See Fig. 22A–D and Lin and Li (2008): 495.

**Palp** (Fig. 23A–C): the tibia comparatively large, about the two-thirds volume of the bulb, except for retrolateral region, a row of long setae almost encircled the distal brim of tibia (Fig. 23A–C). Cymbium translucent, with a median keel and a row of cymbial serrula on the cymbium, the tip extended to be a cymbial process, and long cymbial fold slightly sclerotized, bears a row of short setae (Fig. 23A–C). The tegulum with apical apophysis, the embolus short, extended to cymbial conductor and the spermathecae can be seen through tegulum (Fig. 23A–C).

**New morphological data. Female.** Measurements: total length 0.70 Prosoma 0.26 long, 0.27 wide, 0.21 high. Abdomen 0.44 long, 0.44 wide, 0.38 high. Clypeus 0.06 high. Sternum 0.21 long, 0.13 wide. Length of legs: I 0.70 (0.24, 0.08, 0.20, 0.07, 0.11); II 0.64 (0.17, 0.08, 0.18, 0.10, 0.11); III 0.52 (0.16, 0.08, 0.12, 0.07, 0.09); IV 0.61 (0.21, 0.08, 0.12, 0.10 0.10).

**Somatic characters** (Fig. 22E–G). **Coloration:** same as in male. **Prosoma:** carapace nearly peach-shaped. Ocular region projecting, eight eyes in two rows, ALE and PLE contiguous. Chelicerae, endites as in male, labium triangle, and sternum scutiform, covers with short setae. **Legs:** covered with setae and bristles. A sclerotized sub-distal-ventral femoral spot present at surface of leg I and II. **Abdomen:** same as in male.

**Epigyne** (Fig. 23D–F): The scape short, transparent, tip thin (Fig. 23F). Spermathecae small, nearly round. Fertilization ducts short, derived from dorsal of spermathecae, and extended to anterior of spermathecae. Copulatory ducts sclerotized, the diameter of copulatory ducts same as spermathecae, connected to the lateral of spermathecae (Fig. 23E, F).

**Distribution.** Southwestern China (Yunnan).

**Remarks.** The female description of *M. furca* is provided for the first time.
Figure 22. *Mysmena furca* A, B, D male habitus C male prosoma E–G female habitus A, F dorsal B, G ventral C anterolateral D, E lateral. Abbreviations: CS = cheliceral spines on male; FS = femoral spot; MC = Metatarsal clasping spine. Scale bars: 0.50 mm (A, B, D–G); 0.20 mm (C).
Figure 23. Mysmena furca A–C male palp D epigyne E, F vulva. A prolateral B, D, E ventral C retro-lateral F dorsal. Abbreviations: AA = apical apophysis on tegulum; CD = copulatory duct; Cy = cymbium; CyC = cymbial conductor; CyF = cymbial fold; CyFs = setae on cymbial fold; CyP = cymbial process; CyS = cymbial serrula; E = embolus; FD = fertilization duct; MK = median keel on cymbium; S = spermatheca; SD = spermatic duct; Sp = scape; Te = tegulum; Ti = palpal tibia. Scale bars: 0.10 mm.
Mysmena luosuo Lin & Li, sp. nov.
https://zoobank.org/05B79993-3BFA-4F2A-9D10-DC195EED80B6
Figs 24–26

Type material. Holotype ♂ (IZCAS) and paratypes 10 ♂ 3 ♀ (IZCAS), CHINA: Yunnan, Mengla, XTBG, secondary tropical seasonal moist forest (21.916°N, 101.283°E; 656±15 m), by pitfall trapping, 1–24.X.2007, G. Zheng leg.


Etymology. The specific name derives from the Luosuo River, which is a main river in the type locality; noun in apposition.

Diagnosis. Mysmena luosuo sp. nov. seems similar to M. furca and M. rostella by the presence of modified cheliceral spines on the male (cf. Figs 22C, 24C, and 27C), the shape of the male palps (cf. Figs 25A, B, D, E and 28A–D), and the configuration of the vulvae (cf. Figs 26B, C, 23E, F). It can be distinguished from males of M. furca by lacking a serrated cymbial process (CyP), present in M. furca (Fig. 25C–E vs. Fig. 23A) and by a longer coiled embolus, shorter in M. furca (Fig. 25A, B vs. Fig. 23C); from M. rostella by the shorter embolus and lacking a cymbial process, but longer embolus and having cymbial process in M. rostella (Fig. 25C, D vs. Fig. 28A, B). Females can be distinguished from M. furca and M. rostella by the near globular spermathecae (Fig. 26C), ovoid in M. furca (Fig. 23F) and reniform in M. rostella (Fig. 29C).

Description. Male. Measurements: total length 0.57, Prosoma 0.21 long, 0.26 wide, 0.26 high. Abdomen 0.36 long, 0.37 wide, 0.40 high. Clypeus 0.06 high. Sternum 0.21 long, 0.20 wide. Length of legs: I 0.72 (0.21, 0.10, 0.18, 0.12, 0.11); II 0.68 (0.18, 0.10, 0.16, 0.10, 0.14); III 0.54 (0.12, 0.10, 0.12, 0.10, 0.10); IV 0.52 (0.14, 0.10, 0.12, 0.08, 0.08).


Palp (Fig. 25A–E): orange, the tibia comparatively large, about half the volume of the bulb. Except for retrolateral region, a row of long setae almost encircled the distal brim of tibia (Fig. 25D, E). Cymbium nearly transparent, with a cymbial conductor, distal lobe and median keel on cymbium, the paracymbium comparatively small, with long setae (Fig. 25C–E). Bulb irregular, embedded in a translucent membranous tegulum. Spermatic ducts can be seen through tegulum. Embolus wide, coiled into “S”-shaped, tip extended to cymbial conductor (Fig. 25A–E).

Female. Measurements: total length 0.64 Prosoma 0.26 long, 0.23 wide, 0.26 high. Abdomen 0.38 long, 0.35 wide, 0.38 high. Clypeus 0.05 high. Sternum 0.22 long, 0.20
Figure 24. *Mysmena luosuo* sp. nov. **A, B, D** male habitus **C** male prosoma **E–G** female habitus **A, F** dorsal **B, G** ventral **C** anterolateral **D, E** lateral. Abbreviations: CS = cheliceral spines on male; FS = femoral spot; MC = Metatarsal clasping spine. Scale bars: 0.50 mm (**A, B, D–G**); 0.20 mm (**C**).
Figure 25. *Mysmena luoso* sp. nov. A bulbus B male palp C cymbium D, E male palp A, B apical C retroventral D prolateral E retrolateral. Abbreviations: *Cy* = cymbium; *CyC* = cymbial conductor; *CyF* = cymbial fold; *CyFs* = setae on cymbial fold; *CyP* = cymbial process; *DL* = distal lobe on cymbium; *E* = embolus; *PC* = paracymbium; *MK* = median keel on cymbium; *SD* = spermatic duct; *Te* = tegulum; *Ti* = palpal tibia. Scale bars: 0.10 mm (A–C); 0.20 mm (D, E).
Figure 26. *Mysmena luosuo* sp. nov. A epigyne B–C vulva A, B ventral C dorsal. Abbreviations: CD = copulatory duct; FD = fertilization duct; S = spermatheca; Sp = scape. Scale bars: 0.20 mm (A); 0.10 mm (B–C).

Somatic characters (Fig. 24E–G). Coloration: prosoma brown dorsally, yellow ventrally with two brown strips, ocular base black. Abdomen brown dorsally, yellow ventrally with multiple arc brown strips and spots. Legs brown-yellow. **Prosoma:** carapace nearly pear-shaped. The eight eyes in two rows, AER and PER recurved in dorsal view. ALE and PLE contiguous. Chelicerae, endites as in male, labium rectangle, and sternum scutiform, covers with short setae. **Legs:** covered with setae and bristles. A sclerotized subdistal-ventral femoral spot present at surface of leg I and II. **Abdomen:** nearly round in dorsum, covered with short brown setae.

**Epigyne** (Fig. 26A–C): The scape short, transparent (Fig. 26C). The spermathecae globular, situated at the middle of vulva. Fertilization ducts short, derived from dorsal of the spermathecae and coiled to anterior of spermathecae. Copulatory ducts sclerotized and wider, coiled around the spermathecae, the posterior part expanded to a globular, connected to the ventral of spermathecae. (Fig. 26B, C).

**Distribution.** Southwestern China (Yunnan).

**Remarks.** The diagnostic features of *Mysmena luosuo* sp. nov. are also largely broad Myssmeninae (Lopardo and Hormiga 2015), but shape of the male palps and the config-
uration of the vulvae are similar to other species of the same genus (cf. *M. furca* and *M. rostella*), without share features of other genera. Therefore, we propose it as a new species.

**Mysmena rostella** Lin & Li, 2008  
Figs 27–29

*Mysmena rostella* Lin & Li, 2008: 492, fig. 5A–I (♂).

**Type material.** *Holotype* ♂ (IZCAS), **China**: Yunnan, Mengla, XTBG, secondary tropical montane evergreen broad-leaved forest (21.963°N, 101.200°E; 895±10 m), by searching, 6.VIII.2007, G. Zheng leg. Examined.


**Diagnosis.** *Mysmena rostella* is similar to *M. luosuo* sp. nov. in the shape of male palp and the configuration of vulva (cf. Figs 28C, D, 29B, C and Figs 25D, E, 26B, C), but males can be distinguished by having five pairs of modified spines on the chelicerae, three pairs in *M. luosuo* (Fig. 27C vs. Fig. 24C), and by longer embolus extending prolaterally, shorter embolus coils only at the top of bulbus in *M. luosuo* (Fig. 28A, C vs. Fig. 25A, B, D). Females distinguished from *M. luosuo* by the reniform spermathecae, but near globular in *M. luosuo* (Fig. 29C vs. Fig. 26C).

**Description. Male.** See Fig. 27A–D and Lin and Li (2008): 492, 495.

*Palp* (Fig. 28A–D): orange, comparatively large. Except for retrolateral region, a row of long setae almost encircled the distal brim of tibia (Fig. 28A–D). Cymbium nearly transparent, tip specialized as cymbial conductor, a distal keel on outer wall of cymbium conductor, the cymbial process tip shape, parallel to the cymbial conductor (Fig. 28A–D). Paracymbium big, with long setae (Fig. 28B). Bulb ball shape, embedded in a translucent membranous tegulum. Tegulum with apical apophysis. Embolus long and winding, the tip interacts with cymbial conductor. Spermatic ducts can be seen through tegulum (Fig. 28D).

**New morphological data. Female.** **Measurements:** total length 0.57  
Prosoma 0.18 long, 0.21 wide, 0.18 high. Abdomen 0.39 long, 0.38 wide, 0.36 high. Clypeus 0.05 high. Sternum 0.16 long, 0.14 wide. Length of legs: I 0.61 (0.17, 0.07, 0.15, 0.12, 0.10); II 0.52 (0.18, 0.08, 0.12, 0.08, 0.06); III 0.52 (0.16, 0.08, 0.12, 0.07, 0.09); IV 0.50 (0.14, 0.08, 0.12, 0.10 0.06).

**Somatic characters** (Fig. 27E–G). **Coloration:** prosoma brown-yellow, endites brown, labium white, sternum brown with four yellow spots, ocular base black. Abdomen yellow dorsally, brown ventrally, with white and yellow spots. Legs brown-yellow. **Prosoma:** carapace nearly pear-shaped. The eight eyes in two rows, AER and PER straight in dorsal view. Chelicerae, endites and labium rectangle, and sternum scutiform, covered with short setae. **Legs:** number of setae and bristles same as in male, a sclerotized subdistal-ventral femoral spot present at surface of leg I and II. **Abdomen:** as in male.
Figure 27. *Mysmena rostella* A, B, D male habitus C male prosoma E–G female habitus A, F dorsal B, G ventral C anterolateral D, E lateral. Abbreviations: CS = cheliceral spines on male; FS = femoral spot; MC = Metatarsal clasping spine. Scale bars: 0.50 mm (A, B, D–G); 0.20 mm (C).
Figure 28. *Mysmena rostella* A–D male palp A dorsal B ventral C prolateral D retrolateral. Abbreviations: AA = apical apophysis on tegulum; Cy = cymbium; CyC = cymbial conductor; CyP = cymbial process; DK = distal keel on cymbium; E = embolus; SD = spermatic duct; Te = tegulum; Ti = palpal tibia. Scale bars: 0.10 mm (A, B); 0.20 mm (C, D).
Epigyne (Fig. 29A–C): the scape short and thick, and the surface with fine folds (Fig. 29B). Spermathecae small, nearly semicircle. Fertilization ducts short, derived from lateral of spermathecae, twisted anteriorly and then extended to the anterior of spermathecae. Copulatory ducts slightly sclerotized, coiled around the spermathecae, hooklike symmetrically, connected to the ventral of spermathecae (Fig. 29B, C).

Distribution. Southwestern China (Yunnan).

Remarks. The female description of *M. rostella* is provided for the first time.

**Mysmena dai** Lin & Li, sp. nov.
https://zoobank.org/7897FEFB-88B4-418D-BBA0-850F18F33A1F
Fig. 30

Figure 30. *Mysmena dai* sp. nov. A–C female habitus D epigyne E, F vulva A, F dorsal B, D, E ventral C lateral. Abbreviations: CD = copulatory duct; FD = fertilization duct; S = spermatheca; Sp = scape. Scale bars: 0.50 mm (A–C); 0.20 mm (D–F).
Etymology. The new species is named after the Dai people, an ethnic minority living in Xishuangbanna of Yunnan Province; noun in apposition.

Diagnosis. Females of this new species seem most similar to *M. leucoplagiata* (Simon, 1880) and *M. mooatae* (Baert, 1988) in the configuration of vulva and the rugose long scape, but can be distinguished by the globular spermathecae and the distal end of the descending fertilization ducts, while twisted, ovoid spermathecae, and ascending fertilization ducts in *M. leucoplagiata* (Fig. 30F vs. fig. 11 in Kraus, 1967), semicircle spermathecae in *M. mooatae* (Fig. 30F vs. fig. 24 in Baert, 1988).

Description. Female (holotype). Measurements: total length 0.56 Prosoma 0.18 long, 0.23 wide, 0.20 high. Abdomen 0.38 long, 0.30 wide, 0.36 high. Clypeus 0.05 high. Sternum 0.17 long, 0.13 wide. Length of legs: I 0.64 (0.16, 0.08, 0.16, 0.12, 0.12); II 0.51 (0.12, 0.10, 0.15, 0.08, 0.06); III 0.35 (0.10, 0.05, 0.10, 0.06, 0.04); IV 0.47 (0.20, 0.05, 0.10, 0.08 0.04).


Epigyne (Fig. 30D–F): the posterior brim with sparse short setae, internal structures visible via translucent cuticle (Fig. 30D). Scape long, with narrow folds (Fig. 30E, F). Spermathecae small, nearly globose, separated by 4× diameter. Fertilization ducts short, derived from lateral of spermathecae vertical posteriorly, curved to the middle distally. Copulatory ducts membranous, connected to lateral margin of spermathecae, fused at the midline position of lower edge of vulva (Fig. 30E, F).

Male. Unknown.

Distribution. Southwestern China (Yunnan).

Remarks. The vulva configuration of this species similar to type species of this genus (*M. leucoplagiata* (Simon, 1880)): the presence of scape, the same deriving of fertilization ducts, and same trajectory and extension of copulatory ducts. Therefore, we propose it as a new species.

Conclusions

The study on spiders in XTBG were mainly on the following representative families: Araneidae (ex. Mi and Li 2021a, 2021b), Clubionidae (ex. Yu and Li 2019a, 2019b; Zhang et al. 2021a, 2021b), Linyphiidae (ex. Zhao and Li 2014), Pholcidae (ex. Yao et al. 2018; Yao and Li 2018), Theridiidae (ex. Gao and Li 2014), Thomisidae (ex. Tang and Li 2010), and Salticidae (ex. Cao et al. 2016). The investigation about small-size, cryptic symphytognathoid spiders is obviously inadequate. So far, only two anpid species (Lin and Li 2012; Zhang and Lin 2018), five symphytognathid species (Lin et al.
2013; Li et al. 2020, 2021b), four theridiosomid species (Song and Zhu 1994; Zhao and Li 2012) and eight mysmenid species (Lin and Li 2008) were reported.

The current paper draws a general situation of the species composition of the family Mysmenidae in XTBG, and expands the cognition of its mysmenid species diversity. The decision of these new taxa in this study was based on morphological evidences. The next stage of our research will be to verify them by phylogenetic analysis based on molecular evidences.

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Nine new species of *Trigonopterus* Fauvel (Coleoptera, Curculionidae) from Sundaland

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**Abstract**

The DNA of *Trigonopterus* specimens from the Sundaland region stored between ten and 32 years in museums could be used for next-generation sequencing. The availability of their *cox1* sequence allowed the description of the following nine new species: *Trigonopterus grimmi* sp. nov., *T. johorensis* sp. nov., *T. lambirensis* sp. nov., *T. linauensis* sp. nov., *T. microreticulatus* Riedel, Trnka & Wahab sp. nov., *T. mulensis* sp. nov., *T. sarawakensis* sp. nov., *T. siamensis* sp. nov., and *T. singaporensis* sp. nov. The alternative original spelling of the name *T. tounensis* Narakusumo & Riedel is chosen to prevail over *T. tounaensis* Narakusumo & Riedel. The new species represent the first country records of *Trigonopterus* for Brunei, Singapore, and Thailand. Thus, the genus’ known area of distribution in the Sundaland region is significantly extended. A key and a catalogue are provided to the *Trigonopterus* species from Borneo, W-Malaysia, Singapore, and Thailand.

**Keywords**

Ancient DNA, Cryptorhynchinae, DNA barcoding, endemism, hyperdiverse, integrative taxonomy, morphology, turbo-taxonomy

**Introduction**

*Trigonopterus* is a hyperdiverse genus of flightless weevils (Curculionidae, Cryptorhynchinae) ranging over the Indo-Australian-Melanesian archipelago. It originated in northern Australia and rapidly diversified in New Guinea (Toussaint et al. 2017). After colonizing Sulawesi, this island acted as a hub for the further dispersal to Borneo, Java, and the Lesser Sunda Islands (Tänzler et al. 2016; Letsch et al. 2020). Currently, there are 489
described species (Riedel et al. 2013b, 2014; Riedel and Tänzler 2016; Narakusumo et al. 2019; Riedel and Narakusumo 2019; Narakusumo and Riedel 2021, and herein), yet a much larger number of undescribed species are at hand. In the following, I report nine new species from the Sundaland region, i.e., from Borneo, Singapore, the Malaysian Peninsula, and Ko Chang Island off the coast of Thailand. These new records significantly expand the genus’ known area of distribution to the west and northwest (Fig. 10). The specimens sequenced for this study were stored ten to 32 years in dry museum collections. Thus, their DNA was somewhat degraded and not suitable for the usual approach of PCR and subsequent Sanger sequencing (Tänzler et al. 2012). However, shotgun sequencing could be used successfully to assemble large portions of the mitochondrial genome (Staats et al. 2013; Yeates et al. 2016). Under the self-imposed premise of describing new species only if some diagnostic DNA sequence data are available (Riedel et al. 2013a) it is now possible to provide names to these species. In doing so, the first records of Trigonopterus for the countries Brunei, Singapore, and Thailand are here presented.

Materials and methods

This study is based on 46 museum specimens from the Sundaland region. Holotypes were selected from ten specimens for which the cox1 gene had been sequenced. DNA was extracted nondestructively as described by Riedel et al. (2010) but eluted in only 30 μl of TE buffer. This relatively concentrated template was fully used for library preparation. Genitalia of most specimens did not require extra maceration. They could be directly stained with a 0.01% alcoholic Chlorazol Black solution and stored in glycerol in microvials attached to the pin of the specimens. Illustrations of habitus and genitalia were prepared from holotypes. Type series were supplemented with additional specimens wherever possible. Type depositories are cited using the following codens:

ANIC  Australian National Insect Collection, Canberra, Australia;
SMNK  Staatliches Museum für Naturkunde, Karlsruhe, Germany;
SMNS  Staatliches Museum für Naturkunde, Stuttgart, Germany;
UBDC  Universiti Brunei Darussalam, Brunei;
UPOL  Palacky University, Olomouc, Czech Republic.

The methods applied for DNA sequencing differ from our earlier publications as the fragmented DNA of collection vouchers was not suitable for amplifying longer fragments by PCR. Instead, sequencing libraries were prepared with the NEBnext ultra II kit (New England Biolabs, Ipswich, Massachusetts, USA) and tagged with universal dual indexes. Procedures were followed the manufacturer’s protocol except that only half of the recommended volumes were used, i.e., starting with 25 μl of genomic DNA template containing 1.73–18.33 ng DNA. Resulting libraries were quantified using a Qubit 3.0 Fluorometer with the dsDNA HS assay kit (Thermo Fisher Scientific, Waltham, MA, USA). Fragment distribution of libraries was examined using a Fragment Analyzer dsDNA 910 kit (Agilent Technologies, Santa Clara, CA,
USA) for a range of 35 to 1,500 bp. Based on the concentration and the average size distribution, the molar concentration was calculated for each sample. The ten samples of this project were pooled in equimolar amounts together with other samples and submitted to Novogene Inc. (Cambridge, U.K.) for sequencing. Libraries of samples ARC1453 (*T. linataensis* sp. nov.), ARC5226 (*T. johorensis* sp. nov.), ARC5227 (*T. singaporensis* sp. nov.) were sequenced using an Illumina Hiseq X 10, while samples ARC7266 (*T. siamensis* sp. nov.), ARC7267 (*T. sarawakensis* sp. nov.), ARC7268 (*T. microreticulatus* Riedel, Trnka & Wahab sp. nov.), ARC7269 (*T. lambirensis* sp. nov.), ARC7270 (*T. grimmi* sp. nov.), ARC7272 (*T. mulensis* sp. nov.) were sequenced on an Illumina Novaseq, in each case for 2 × 150 bp. Reads were processed, assembled and annotated as described earlier (Narakusumo et al. 2020). The *cox1* gene was extracted, used for further analysis, and submitted to GenBank of NCBI (National Center for Biotechnology Information). The accession numbers are provided under each species, e.g., as “(GenBank # OP078703)”. The remaining sequence data generated will be analyzed in future in a wider context. The closest relatives of the species described herein were identified by creating a limited alignment of 23 *cox1* sequences representing 22 species from Sundaland and generating a maximum likelihood reconstruction using the program IQTREE (Nguyen et al. 2015). The uncorrected p-distance was calculated in Geneious Prime 2019.1.3 (Biomatters Ltd, Auckland, New Zealand).

Morphological descriptions are limited to major diagnostic characters as outlined by Riedel et al. (2013a, b). Negative character states (i.e., the absence of a character) are only mentioned explicitly where it appears appropriate. In groups comprising hundreds of species enumerating the absence of rare character states leads to inflated descriptions that distract the reader from the important information, i.e., the diagnostic characters present in a given species.

Morphological terminology follows Beutel and Leschen (2005) and Leschen et al. (2009), i.e., the terms “mesoventrite” / “metaventrite” are used instead of “mesosternite” / “metasternite” and “mesanepisternum” / “metanepisternum” instead of “mesepisternum” / “metepisternum”; “penis” is used instead of “aedeagus” as the tegmen is usually without useful characters in *Trigonopterus* and therefore omitted from species descriptions. Specimens were examined with a Leica M205 C dissecting microscope and a fluorescent desk lamp for illumination. Measurements were taken with the help of an ocular grid. The length of the body was measured in dorsal aspect from the elytral apex to the front of the pronotum. Legs were described in an idealized laterally extended position; there is a dorsal / ventral and an anterior / posterior surface. Habitus illustrations were compiled using a DFC5400 camera adapted to a Z6 APO (all from Leica Microsystems). Photographic illustrations of genitalia were made using a DFC450 camera with L.A.S. 4.8.0 software adapted to an Axio Imager M2 microscope (Carl Zeiss Microscopy), with 5×, respectively 10× A-Plan lenses. Resulting image stacks were compiled using the Helicon Focus 8.1.0 Pro software (Helicon Soft Ltd). For photography genitalia were temporarily embedded in glycerol gelatin as described by Riedel (2005), with their longitudinal axis somewhat lifted caudally, to adequately illustrate structures of the curved down apex. All photographs were enhanced using the program Adobe Photoshop CS6. However, care was taken not to obscure or alter any features of the specimens illustrated.
Results

_Trigonopterus_ Fauvel, 1862

Type species. _Trigonopterus insignis_ Fauvel, 1862, by monotypy.

**Diagnosis.** Fully apterous genus of Cryptorhynchinae. Length 1.5–6.0 mm. Rostrum in repose not reaching center of mesocoxa. Scutellar shield completely absent externally. Mesothoracic receptacle deep, posteriorly closed. Metanepisternum completely absent externally. Elytra with nine striae (sometimes superficially effaced). Tarsal claws minute. Usually body largely unclothed, without dense vestiture. For additional information, see http://species-id.net/wiki/Trigonopterus.

Descriptions of the species

1. _Trigonopterus grimmi_ sp. nov.
https://zoobank.org/21C702AF-9376-45F0-A3AA-9E6D436467A7
Figs 1, 10

**Material examined. Holotype (SMNS):** ARC7270 (GenBank # OP078711), E-Malaysia, Sarawak, Gn. Gading NP, 50–300 m, 20-23-II-2012. **Paratypes (SMNK, SMNS):** 2 exx, ARC7271, E-Malaysia, Sarawak, Gn. Gading NP, 50–300 m, 08-10-XII-2010.

**Diagnostic description.** Holotype, male (Fig. 1a). Length 2.90 mm. Color black except antennae light ferruginous, legs dark ferruginous. Body in dorsal aspect subrhomboïd, with marked constriction between pronotum and elytron; profile dorsally convex. Rostrum with median and pair of submedian ridges; intervening furrows with sparse rows of erect, clavate scales; epistome with transverse, angulate ridge; forehead in profile with subangulate knob. Pronotum with indistinct subapical constriction; disk densely punctate, interspaces weakly microreticulate; each puncture containing small recumbent seta. Elytra with humeri markedly swollen, laterally subangularly projecting; striae marked by fine lines and rows of small punctures; stria 8 and 9 along humerus each with four to five large punctures; intervals flat, microreticulate; sutural interval with row of minute punctures. Metathorax with dorsoangular edge denticulate; subapically with stridulatory patch. Dorsal edge of tibiae subbasally dentate. Abdominal ventrites 1–2 forming common cavity; at middle flat, subglabrous, with sparse erect scales; laterally with distinct rim; lateral rim of abdominal ventrite 2 in profile projecting dentiform; abdominal ventrite 5 flat, coarsely punctate-foveate, with sparse erect scales. Penis (Fig. 1b) with sides of body subparallel; apex subangulate; transfer apparatus flagelliform, ca. 4.3 × as long as body; apodemes 3.5 × as long as body; ductus ejaculatorius without bulbus. **Intraspecific variation.** Length 2.70–2.90 mm. Female rostrum dorsally medially subglabrous, sublaterally punctate-rugose; epistome simple. Female elytra with humeri less prominent, convex.

**Distribution.** Sarawak (Gn. Gading NP). Elevation: ca. 50–300 m.

**Etymology.** The species is named for the late darkling beetle expert Roland Grimm, who collected the type series of this species. The epithet is a noun in the genitive case.
Notes. *Trigonopterus grimmi* sp. nov. is coded as “*Trigonopterus* sp. 1247”. This species belongs to the *T. trigonopterus* group. It is closely related to *T. trigonopterus* Riedel, 2014, from which it can be distinguished by its flat elytral intervals and 17.3% p-distance of its *cox1* sequence.

2. *Trigonopterus johorensis* sp. nov.
https://zoobank.org/494AD9FE-E5EE-439B-BB4C-11E85A24EF23
Figs 2, 11

**Material examined. Holotype** (ANIC): ARC5226 (GenBank # OP078705), MALAYSIA, Johor, Kotta Tingi Falls, 01°50’N, 103°50’E, 100 m, 22-XI-1988, sifted litter.

**Diagnostic description.** Holotype, male (Fig. 2a). Length 2.88 mm. Color ferruginous. Body in dorsal aspect subovate, with weak constriction between pronotum and elytron; in profile dorsally convex. Rostrum with median and pair of submedian ridges; intervening furrows with punctures and rows of sparse suberect scales; epistome
with indistinct subangulate ridge. Pronotum with disk densely coarsely punctate, reticulate; each puncture containing small recumbent scale. Elytra with striae distinct, with small punctures and rows of small suberect scales; intervals costate, subglabrous; elytral apex subtruncate, intervals 1–3 subapically swollen. Femora with crenate anteroventral ridge. Metafemur dorsally scabrous; subapically with stridulatory patch. Dorsal edge of tibiae subbasally dentate. Abdominal ventrites 1–2 forming common cavity, at middle flat, glabrous, laterally and posteriorly with distinct rim; lateral rim of ventrites 1 and 2 in profile projecting dentiform; abdominal ventrite 5 concave, basally with large punctures, apically with smaller punctures. Penis (Fig. 2b) with sides of body slightly diverging; with large anchor-shaped sclerites in apical half; apex bisinuate, with median incision; transfer apparatus small, dentiform; apodemes 1.7 × as long as body; ductus ejaculatorius without distinct bulbus.

**Distribution.** Malaysia (Johor). Elevation: 100 m.

**Etymology.** This epithet is a Latinized adjective based on the name of the Malaysian state Johor.

**Notes.** *Trigonopterus johorensis* sp. nov. is coded as “*Trigonopterus* sp. 1112”. This species belongs to the *T. attenboroughi* group. It is closely related to *T. attenboroughi* Riedel, 2014, *T. mulensis* sp. nov., and *T. sarawakensis* sp. nov., from which it can be distinguished by the subtruncate elytral apex and 13.7–15.3% p-distance of its *cox1* sequence.
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3. *Trigonopterus lambirensis* sp. nov.

https://zoobank.org/6D8EAEB1-A045-4309-90FD-0A88D2B1F184

Figs 3, 11


**Diagnostic description.** Holotype, male (Fig. 3a). Length 2.55 mm. Color of antennae light ferruginous; legs and elytra dark ferruginous; remainder almost black. Body in dorsal aspect subrotund, with constriction between pronotum and elytron; in profile dorsally convex. Rostrum with median ridge and pair of submedian ridges; median ridge in basal half distinct, terminating at level of antennal insertion; intervening furrows with rows of punctures and erect subclavate scales; epistome with subangulate ridge, at middle with denticle. Pronotum with disk densely coarsely punctate, interspaces reticulate; each puncture containing thin, recumbent seta. Elytra with striae distinct; punctures containing short recumbent seta hardly visible; basal margin bordered by transverse row; intervals flat; sutural interval with row of punctures, coarse near base, minute near apex; other intervals basally with interspersed punctures; stria 8 along humerus with regular punctures. Femora with simple anteroventral ridge. Metafemur with

![Figure 3. *Trigonopterus lambirensis* sp. nov., holotype a habitus b penis.](image-url)
dorsoposterior edge denticulate; subapically with stridulatory patch. Dorsal edge of tibiae subbasally dentate, denticle acute in pro- and mesotibia, blunt in metatibia. Abdominal ventrites 1–2 forming deep common cavity, at middle concave, subglabrous, with sparse erect scales; laterally and posteriorly with distinct rim; abdominal ventrite 5 flat, with coarse punctures, with sparse erect scales. Penis (Fig. 3b) with sides of body subparallel; apex rounded, with sparse setae; transfer apparatus flagelliform, ca. 2.0 × as long as body, coiled, with supporting sclerites; apodemes 1.9 × as long as body; ductus ejaculatorius without distinct bulbus. **Intraspecific variation.** Length 1.95–2.55 mm.

**Distribution.** Sarawak (Lambir-Hills NP). Elevation: 200 m.

**Etymology.** This epithet is a Latinized adjective based on Lambir-Hills NP.

**Notes.** *Trigonopterus lambirensis* sp. nov. is coded as “*Trigonopterus* sp. 1246”. Morphologically it appears related to *T. microreticulatus* Riedel, Trnka & Wahab sp. nov., from which it can be distinguished by the rather polished elytral intervals, the morphology of the penis and 20.9% p-distance of its *cox1* sequence. A comprehensive molecular analysis will need to determine its phylogenetic position.

4. **Trigonopterus linauensis** sp. nov.
https://zoobank.org/4C75E8B6-D47C-4120-8A67-536E38664CE5
Figs 4, 11

**Material examined.** **Holotype** (SMNK): ARC1453 (GenBank # OP078704), E-MALAYSIA, Sarawak, Belaga, Long Linau, logging camp, ca. 02°45’N, 113°46’E, ca. 400 m, 19-III-1990. **Paratypes** (ARC in SMNK): 2 exx, same data as holotype.

**Diagnostic description.** Holotype, male (Fig. 4a). Length 2.28 mm. Color ferrugineous, pronotum somewhat darker. Body in dorsal aspect subrhomboid, with weak constriction between pronotum and elytron; in profile dorsally convex, calli at elytral base weakly projecting from outline. Rostrum with median and pair of submedian ridges; intervening furrows with rows of punctures and sparse suberect scales; epistome with indistinct subangulate ridge. Pronotum with disk densely coarsely punctate, reticulate; each puncture containing suberect seta. Elytra with striae marked by hairlines and rows of punctures each containing minute seta; intervals flat, subglabrous, with interspersed punctures, weakly coriaceous; sutural interval at base with glabrous callus. Femora with weakly crenate anteroventral ridge, ending in apical third with small blunt tooth in pro- and metafemur, with minute acute tooth in mesofemur. Profemur in basal third with denticulate posteroventral ridge. Metafemur subapically with stridulatory patch. Dorsal edge of tibiae subbasally dentate, in pro- and mesotibia denticle acute, in metatibia blunt. Abdominal ventrites 1–2 forming deep common cavity, at middle concave, subglabrous, with sparse erect clavate scales; laterally and posteriorly with distinct rim; lateral rim of ventrites 1 and 2 in profile projecting dentiform; abdominal ventrite 5 medially with marked longitudinal impression delimited by distinct ridges, laterally punctate; median depression glabrous, midline with indistinct ridge. Penis (Fig. 4b) with sides of body subparallel, in apical third with narrow lateral flanges; with complex endophallic sclerites; apex with shallow median incision, with sparse setae; transfer apparatus spiniform,
fitted into supporting sclerites; apodemes 1.9 × as long as body; ductus ejaculatorius with indistinct bulbus. **Intraspecific variation.** Length 2.15–2.28 mm. Female unknown.

**Distribution.** Sarawak (Belaga). Elevation: ca. 400 m.

**Etymology.** This epithet is a Latinized adjective based on the type locality Long Linau.

**Notes.** *Trigonopterus linauensis* sp. nov. is coded as “*Trigonopterus* sp. 1123”. This was the first species of *Trigonopterus* that I personally collected. At the time, I did not fully appreciate the scientific value of the specimens. This species belongs to the *T. attenboroughi* group. It is closely related to *T. sepuluh* Riedel, 2014 and *T. singkawangensis* Riedel, 2014, from which it can be distinguished by the more slender elytral apex, the morphology of the penis and 18.6–19.2% p-distance of its *cox1* sequence.

**5. Trigonopterus microreticulatus** Riedel, Trnka & Wahab, sp. nov.
https://zoobank.org/2D9BCEB3-3E8D-4001-9AD9-0E37F8577777
Figs 5, 11

**Material examined.** **Holotype** (SMNS): ARC7268 (GenBank # OP078709), E-MALAYSIA, Sarawak, Mulu NP, 100 km SEE Miri, 200 m, 19-24-VIII-2003, sifted.

**Diagnostic description.** Holotype, male (Fig. 5a). Length 2.48 mm. Color of antennae light ferruginous; legs and elytra dark ferruginous; remainder almost black. Body in dorsal aspect subrotund, with constriction between pronotum and elytron; in profile dorsally convex. Rostrum with median and pair of submedian ridges, ending before apex; intervening furrows with rows of punctures and sparse erect scales; epistome with subangulate ridge. Pronotum with disk densely coarsely punctate, interspaces reticulate, microreticulate; each puncture containing small seta. Elytra with striae distinct; punctures each containing short recumbent seta; intervals flat, markedly microreticulate, dull; sutural interval with row of punctures from base to apex; intervals 3–6 with row of punctures in basal half; stria 8 along humerus with three large punctures and four smaller ones. Femora with simple anteroventral ridge. Metatarsus with denticulate, subapically with stridulatory patch. Dorsal edge of tibiae subbasally dentate, denticle acute. Abdominal ventrites 1–2 forming deep common cavity, at middle flat, subglabrous, with sparse erect scales; laterally and posteriorly with distinct rim; abdominal ventrite 5 flat, with coarse punctures, basally with sparse erect scales. Penis (Fig. 5b) with sides of body subparallel; apex rounded, with sparse setae; transfer apparatus complex; apodemes 2.3 × as long as body; ductus ejaculatorius without distinct bulbous. **Intraspecific variation.** Length 2.00–2.63 mm. Coloration from light ferruginous to dark ferruginous. **Distribution.** Sarawak (Mulu NP); Brunei (Ulu Temburong NP). Elevation: 100–200 m.

**Etymology.** This epithet is an adjective formed as a compound of the Greek *mikros* (small) plus the Latin *reticulatus* (netted) and refers to the elytral microsculpture.

**Notes.** *Trigonopterus microreticulatus* Riedel, Trnka & Wahab sp. nov. is coded as “*Trigonopterus* sp. 1245”. Morphologically it appears related to *T. lambirensis* sp. nov., from which it can be distinguished by the microreticulate elytra, the morphology of the
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penis and 20.9% p-distance of its *cox1* sequence. A comprehensive molecular analysis will need to determine its phylogenetic position. It is described under joint authorship with Rodzay Abdul Wahab (Universiti Brunei Darussalam, Tungku, Brunei) and Filip Trnka (Palacky University, Olomouc, Czech Republic).

6. *Trigonopterus mulensis* sp. nov.
https://zoobank.org/E34DD151-EC3D-48DE-9330-FD0DEB7BBF5F
Figs 6, 11

**Material examined.** *Holotype* (SMNS): ARC7272 (GenBank # OP078712), E-MALAYSIA, Sarawak, Mulu NP, 100 km SEE Miri, 200 m, 19-24-VIII-2003, sifted. *Paratype* (SMNK): 1 ex, same data as holotype.

**Diagnostic description.** Holotype, male (Fig. 6a). Length 2.75 mm. Color of antennae light ferruginous; legs and elytra dark ferruginous; remainder black. Body in dorsal aspect subovate, with weak constriction between pronotum and elytron; in profile dorsally convex. Rostrum with median and pair of submedian ridges; intervening furrows with rows of sparse suberect scales; epistome with median ridge. Pronotum with disk densely coarsely punctate, reticulate; each puncture

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*Figure 5. Trigonopterus microreticulatus* Riedel, Trnka & Wahab sp. nov., holotype a habitus b penis.
containing small upcurved scale. Elytra with striae distinct, with small punctures and rows of small suberect scales; intervals costate, subglabrous, coriaceous, sutural interval with row of minute punctures. Femora with simple anteroventral ridge. Metafemur dorsally coarsely punctate; subapically with stridulatory patch. Dorsal edge of tibiae subbasally dentate, in pro- and mesotibia denticle acute, in metatibia blunt; metatibia posteriorly with dense white subclavate scales. Abdominal ventrites 1–2 forming deep common cavity, at middle weakly concave, glabrous, laterally and posteriorly with distinct rim; lateral rim of ventrite 2 in profile projecting dentiform; abdominal ventrite 5 at middle with round subglabrous concavity, laterally swollen, punctate. Penis (Fig. 6b) with sides of body subparallel; with large π-shaped sclerite in apical half; apex bisinuate, with median incision; transfer apparatus small, dentiform; apodemes 1.8 X as long as body; ductus ejaculatorius without distinct bulbus.

**Distribution.** Sarawak (Mulu NP). Elevation: 200 m.

**Etymology.** This epithet is a Latinized adjective based on Mulu NP.

**Notes.** *Trigonopterus mulensis* sp. nov. is coded as “Trigonopterus sp. 1248”. This species belongs to the *T. attenboroughi* group. It is closely related to *T. attenboroughi* Riedel, 2014 and *T. sarawakensis* sp. nov., from which it can be distinguished by its dense white scales of the metatibia and 9.2–10.3% p-distance of its *cox1* sequence.
Nine new *Trigonopterus* from Sundaland

7. *Trigonopterus sarawakensis* sp. nov.
https://zoobank.org/F03675AE-22DF-406B-BE14-9C814586378F
Figs 7, 11

**Material examined.** *Holotype* (SMNS): ARC7267 (GenBank # OP078708), E-Malaysia, Sarawak, Mt. Santubong, 17 km N Kuching, 200–400 m, 17-VIII-2003.  
*Paratypes*: 1 ex, same data as holotype (SMNK); 2 exx (ARC5228, very low DNA concentration, not sequenced), 11 mi SW Kuching, Semengoh Forest Reserve, leafmould berlesate RWT-68.197, 28-31-V-1968 (ANIC, SMNK).

**Diagnostic description.** Holotype. Male (Fig. 7a). Length 2.55 mm. Color of antennae light ferruginous; legs and elytra dark ferruginous; remainder black. Body in dorsal aspect subovate, with weak constriction between pronotum and elytron; in profile dorsally convex. Rostrum with median and pair of submedian ridges; intervening furrows with rows of punctures and sparse suberect scales; epistome with indistinct subangulate ridge, at middle with minute denticle. Pronotum with disk densely coarsely punctate, reticulate; each puncture containing small suberect scale. Elytra with striae distinct, with coarse punctures and rows of small suberect clavate scales; intervals weakly costate, subglabrous. Femora with weakly crenate anterodorsal ridge. Profemur

![Figure 7. *Trigonopterus sarawakensis* sp. nov., holotype a habitus b penis.](image-url)
in basal third with weakly denticulate posteroventral ridge. Metafemur subapically with stridulatory patch. Dorsal edge of tibiae with subbasal, blunt angulation. Abdominal ventrites 1–2 forming deep common cavity, at middle weakly concave, glabrous, laterally and posteriorly with distinct rim; lateral rim of ventrite 2 in profile projecting dentiform; abdominal ventrite 5 medially concave, microreticulate, with sparse punctures, laterally with distinct ridges. Penis (Fig. 7b) with sides of body subparallel; with rhombiform orifical sclerite, medially with pincer-shaped sclerites; apex subangulate; transfer apparatus spiniform; apodemes 2.5 × as long as body; ductus ejaculatorius with distinct bulbous. **Intraspecific variation.** Length 2.38–2.55 mm. Female unknown. **Distribution.** Sarawak (Mt. Santubong). Elevation: ca. 200–400 m. **Etymology.** This epithet is a Latinized adjective based on Sarawak. **Notes.** *Trigonopterus sarawakensis* sp. nov. is coded as “*Trigonopterus* sp. 1244”. This species belongs to the *T. attenboroughi* group. It is closely related to *T. attenboroughi* Riedel, 2014 and *T. mulensis* sp. nov., from which it can be distinguished by the subangulate apex of the penis and 8.2–9.2% p-distance of its *cox1* sequence.

8. **Trigonopterus siamensis** sp. nov. https://zoobank.org/56CE721D-4D2E-446A-B2D9-26034C2082B7 Figs 8, 10

**Material examined.** Holotype (SMNS): ARC7266 (GenBank # OP078707), Thailand, Ko Chang, Westseite, 1999. **Diagnostic description.** Holotype, male (Fig. 8a). Length 3.19 mm. Color black except antennae light ferruginous, legs dark ferruginous. Body in dorsal aspect subrhomboid, with marked constriction between pronotum and elytron; profile dorsally convex. Rostrum with median and pair of submedian ridges; intervening furrows with sparse rows of suberect, clavate scales; epistome with transverse, angulate ridge; forehead in profile with weak subangulate knob. Pronotum with indistinct subapical constriction; disk densely coarsely punctate, interspaces reticulate, weakly microreticulate; each puncture containing small seta. Elytra with humeri swollen, laterally subangularly projecting, coarsely punctate; striae 1–5 distinct, deeply impressed, each with row of coarse punctures and sparse row of suberect scales; intervals costate, microreticulate; sutural interval with row of small punctures; interval 2 almost impunctate; intervals 3–5 with rows of coarse punctures; lateral coarse punctuation confused. Metafemur with dorsoposterior edge denticulate; subapically with stridulatory patch. Abdominal ventrites 1–2 forming common cavity, at middle flat, subglabrous, with sparse erect scales; laterally with distinct rim; lateral rim of abdominal ventrite 2 in profile projecting dentiform; abdominal ventrite 5 flat, coarsely punctate-foveate, with sparse erect scales. Penis (Fig. 8b) with sides of body subparallel; apex subangulate; transfer apparatus flagelliform, ca. 3.2 × as long as body; apodemes 3.9 × as long as body; ductus ejaculatorius without bulbous. **Distribution.** Thailand (Ko Chang Is.). **Etymology.** This epithet is a Latinized adjective based on Siam, the former name of Thailand.
Nine new *Trigonopterus* from Sundaland

Notes. *Trigonopterus siamensis* sp. nov. is coded as “*Trigonopterus* sp. 1243”. This species belongs to the *T. trigonopterus* group. It is related to *T. singaporensis* sp. nov., from which it can be distinguished by its elytral intervals 3–5 each with a row of coarse punctures and 18.1% p-distance of its cox1 sequence. It would be important to confirm the locality of this species by additional records.

9. *Trigonopterus singaporensis* sp. nov.
https://zoobank.org/38C610DD-5D33-49BD-A057-CE819E967437
Figs 9, 10


Diagnostic description. Holotype, male (Fig. 9a). Length 3.20 mm. Color black except antennae light ferruginous, legs dark ferruginous. Body in dorsal aspect subovate, with constriction between pronotum and elytron; profile dorsally convex. Rostrum with median and pair of submedian ridges; intervening furrows with sparse rows of erect, clavate scales; epistome with transverse, angulate ridge;
forehead in profile with subangulate knob. Pronotum with indistinct subapical constriction; disk densely coarsely punctate, interspaces reticulate, weakly microreticulate; each puncture containing small recumbent seta. Elytra with humeri swollen, drawn ventrad, in dorsal aspect hardly projecting, rounded; striae distinct, deeply impressed, each with row of small punctures and sparse row of suberect scales; stria 8 along humerus with five large punctures; intervals costate-carinate, microreticulate; sutural interval with denser punctures. Metafemur subapically with stridulatory patch. Dorsal edge of pro- and mesatibia subbasally dentate, metatibia with small denticle. Abdominal ventrites 1–2 forming common cavity, at middle flat, subglabrous, with sparse erect scales; laterally with distinct rim; lateral rim of abdominal ventrite 2 in profile projecting dentiform; abdominal ventrite 5 coarsely punctate, with sparse erect scales, in apical half concave. Penis (Fig. 9b) with body relatively small, sides membranous, appearing weakly concave; apex angulate; transfer apparatus flagelliform, ca. 5.5 × as long as body; apodemes 4.4 × as long as body; ductus ejaculatorius without bulbus. **Intraspecific variation.** Length 3.16–3.20 mm.

**Distribution.** Singapore (Bukit Timah). Elevation: 100 m.

**Etymology.** This epithet is a Latinized adjective based on the island of Singapore.
Nine new *Trigonopterus* from Sundaland

*Notes.* *Trigonopterus singaporensis* sp. nov. is coded as “*Trigonopterus* sp. 742”. This species belongs to the *T. trigonopterus* group. It is related to *T. siamensis* sp. nov., from which it can be distinguished by its costate-carinate elytral intervals never having rows of coarse punctures and 18.1% p-distance of its *cox1* sequence.
Remark on *T. tounensis* Narakusumo & Riedel

In the abstract of Narakusumo and Riedel (2021) *T. tounensis* Narakusumo & Riedel was spelled as “*T. tounaensis*”, while in the description and the following figure headings the name *T. tounensis* was used, presenting two alternative spellings. As the first reviser according to article 24.2.1 of the ICZN (International Commission on Zoological Nomenclature, 1999) I decide that the name *T. tounensis* Narakusumo & Riedel should prevail, while “*T. tounaensis*” is to be considered an incorrect alternative spelling.

Key to the *Trigonopterus* species from Borneo, west Malaysia, Singapore, and Thailand

1. Body small, 1.52–1.79 mm. Epistome with dorsoposteriadi directed horn and rostrum at middle with dorsal protrusion..........................*T. wallacei* Riedel, 2014
   - Body larger, 1.95–3.20 mm. Epistome at most with minute denticate; rostrum with longitudinal ridges but without dorsal protrusions..........................2
   - Pronotum anteriorly simple. Anteroventral ridge of meso- and metafemur simple or crenate, but not denticate ........................................3
3. Elytra with humeri swollen, more or less projecting laterad between mid- and hind leg. Penis with very long apodemes and long flagelliform transfer apparatus ......4
   - Elytra with humeri simple, not swollen and projecting between mid- and hind leg. Penis with dentiform transfer apparatus (except *T. lambirensis* sp. nov.), usually with additional endophallic sclerites ...........................................7
4. Elytral striae marked by lines and rows of small punctures, not deeply impressed; elytral intervals flat ..................................................*T. grimmi* sp. nov.
   - Elytral striae deeply impressed; intervals costate or weakly carinate .................5
5. Elytra ferruginous, humeri markedly projecting....*T. trigonopterus* Riedel, 2014
   - Elytra black, humeri less projecting ........................................................................
6. Elytral intervals 3–5 costate, each with row of coarse punctures; punctation of humeri coarse, confused ..................................................*T. siamensis* sp. nov.
   - Elytral intervals costate-carinate, with few small punctures; humeri with regular punctation ..................................................*T. singaporensis* sp. nov.
7. Body in dorsal aspect subrotund ..........................................................8
   - Body in dorsal aspect subovate ........................................................................
   - Elytra with interspaces between punctures subglabrous. Penis with flagelliform transfer apparatus ..................................................*T. lambirensis* sp. nov.
9. Elytral apex with suture distinctly incised. Abdominal ventrite 2 in profile simple .........................................................................................10
   - Elytral apex with suture simple, not incised. Abdominal ventrite 2 in profile projecting dentiform ...........................................................................11
Discussion

Specimens of *Trigonopterus* found in two museum collections represented not only new species, but also very notable distribution records. While the hitherto known range of the genus reached its western limit in Lampung province of East Sumatra, the new records from the Malay Peninsula and from a small island off the coast of Thailand extend it considerably to the west and northwest respectively. Since the record from Thailand is represented by a unique specimen and the collector visited Borneo on other trips, there is a small chance of confounded samples. A successful search for additional specimens at the type locality could settle these doubts. However, the biogeographic picture of the *T. trigonopterus* group is one of numerous, genetically divergent species spread over the area of the Sunda shelf. A clade ranging from Borneo to Singapore and the coast of Thailand
is not out of the ordinary. Since most of the species from Java and Sumatra (Riedel et al. 2014) were discovered during dedicated field-work focused on the known area of distribution, there is a high likelihood of additional species to be found in north and central Sumatra, the Malay Peninsula, and other parts of Sundaland, areas that have not been searched specifically for these weevils. This emphasizes the value of general museum collections (Fontaine et al. 2012), as they can provide an unbiased overview of larger areas. The past problem precluding their use for molecular analysis was their usually highly fragmented DNA. This could be overcome with next generation sequencing methods (Staats et al. 2013; Yeates et al. 2016). The preparation of the sequencing library and the sequencing itself comes with a cost (currently ~ € 60–70 per sample), but it generates a large amount of sequence data. It is also much cheaper than any dedicated fieldwork to retrieve fresh material. Naturally, not all specimens will work equally well. However, the measurement of the DNA concentration after a non-destructive DNA extraction can quickly clarify the suitability of the material. Library preparation usually works well with as little as 1 ng of input DNA. There is no formal requirement that descriptions of new species need to include sequence information according to the ICZN. However, in a hyperdiverse genus comprising hundreds of similar species, DNA barcodes are of utmost importance to quickly sort and identify species and to avoid producing synonyms. So, under our self-imposed standards that require a fragment of the cox1 gene for a new description (Riedel et al. 2013a) it was now possible to formally name these interesting new records. The consequent application of this method of generating sequence data from museum specimens will undoubtedly solve many taxonomic issues in future.

Provisional catalogue of species groups of *Trigonopterus* in the Sundaland region (except Sumatra and Java)


*T. trigonopterus* group: *T. grimmi* sp. nov., *T. trigonopterus* Riedel, *T. siamensis* sp. nov., *T. singaporensis* sp. nov.

*T. wallacei* group: *T. wallacei* Riedel.

Incertae sedis: *T. lambirensis* sp. nov., *T. microreticulatus* Riedel, Trnka & Wähab sp. nov.

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Nine new *Trigonopterus* from Sundaland

References


Two new species of *Diphya* Nicolet, 1849 (Araneae, Tetragnathidae) from Southwest China

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Abstract

Two new species of tetragnathid spiders from Guizhou and Sichuang provinces of China are described: *Diphya guiyang* J. Zhang & H. Yu, *sp. nov.* (♂♀) and *Diphya weimiani* J. Zhang & H. Yu, *sp. nov.* (♀). Detailed descriptions, diagnoses, and photographs are provided for these two species, as well as a key and a distribution map for Chinese *Diphya* species. DNA barcodes (a partial fragment of the mitochondrial cytochrome oxidase subunit I gene, COI) of both new species were obtained for species delimitation, matching of different sexes, and future use in molecular studies.

Keywords

DNA barcoding, identification key, morphology, taxonomy, tetragnathid

Introduction

*Diphya* Nicolet, 1849 is a small spider genus with an unusual distribution, it is disjunctively distributed in South America, southern Africa, and East Asia (Marusik et al. 2017; Omelko et al. 2020; World Spider Catalog 2022). *Diphya* currently includes 18 described species, with seven species recorded from Asia, six of which are known from China (Omelko et al. 2020; World Spider Catalog 2022).
The genus has been revised both regionally and on a worldwide scale (Tanikawa 1995; Álvarez-Padilla and Hormiga 2011; Marusik 2017; Omelko et al. 2020). However, debate is ongoing on the genus’s limit and subfamily placement (Álvarez-Padilla and Hormiga 2011; Marusik et al. 2017; Omelko et al. 2020). Marusik et al. (2017) have expressed doubts about the monophyly of the genus and thought that African, Asian, and South Neotropical species may in the future be considered to belong to separate genera. Despite of the dispute about the limits of this genus, most Diphya species have been well studied, especially several new species described in recent years. These species have been described in detail, alongside high-quality illustrations, to allow easy species recognition (Álvarez-Padilla and Hormiga 2011; Yu et al. 2014; Marusik 2017; Marusik and Omelko 2017a, b; Omelko et al. 2020).

While examining spiders recently collected from Guizhou and Sichuan provinces, southwestern China, we have found some Diphya specimens that belong to two undescribed species. With that, the total number of Diphya species in China reaches nine species, five known by both sexes. This makes China the country with the most Diphya species. The goal of this paper is to provide detailed descriptions, illustrations, and diagnosis of these two new species: D. guiyang J. Zhang & H. Yu, sp. nov. and D. weimi-ani J. Zhang & H. Yu, sp. nov. The DNA barcodes of these two species were obtained to confirm matching of the sexes (for D. guiyang sp. nov.) and future use in molecular studies. Additionally, an identification key and a distribution map for Chinese Diphya species are given.

Materials and methods

Specimens in this study were collected by beating vegetation. The type specimens are deposited in the Museum of Guizhou Education University, Guiyang, China (MGEU; Hao Yu curator). Specimens were preserved in 95% alcohol and examined using an Olympus SZX7 stereomicroscope. Left male palps were examined and illustrated after dissection. Epigynes were removed and cleared in a warm 10% potassium hydroxide (KOH) solution. The vulvae were imaged after being embedded in Arabic gum. Images were captured with a Canon EOS 70D digital camera (20.2 megapixels) mounted on an Olympus CX41 compound microscope and assembled using Helicon Focus v. 6.80 image-stacking software. All measurements were obtained using an Olympus SZX7 stereomicroscope and are given in millimetres. Eye diameters were measured at the widest part. The total body length does not include the chelicerae or spinnerets. Leg lengths are given as total length (femur, patella+tibia, metatarsus, tarsus). The terminology used in the text and figure legends follows Marusik (2017), Marusik et al. (2017), and Omelko et al. (2020).

The abbreviations used in the text are: A = atrium; AER = anterior eye row; ALE = anterior lateral eye; AME = anterior median eye; C = conductor; Cd = copulatory duct; Co = copulatory opening; Cy = cymbium; Dp = dorsal process; Em B = basal portion of embolus; Em T = terminal portion of embolus; Fd = fertilisation duct;
Ip = intermediate process; Lp = lateral pocket; MOQ = median ocular quadrangle; MOQA = MOQ anterior width; MOQL = length of MOQ; MOQP = MOQ posterior width; Pc = paracymbium; PLE = posterior lateral eye; PME = posterior median eye; RER = posterior eye row; R = receptacle; Ra = anterior chamber of receptacle; Rp = posterior chamber of receptacle; Sb = septal base; Se = septum; Ss = septal stem; St = subtegulum; Te = tegulum; Vp = ventral process.

The distribution map was generated with ArcGIS v. 10.5 (Environmental Systems Research Institute, Inc.). Due to lack of locality coordinates in previous publications, locality coordinates for all known species are derived from ArcGIS, except for D. qianica and D. tanasevitchi, which were copied from the original publications (see Zhu et al. 2003: 57; Zhang et al. 2003: 407).

To obtain the DNA barcodes, a partial fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified and sequenced for four specimens, using the primers LCO1490 (5′-GGTCAACAAATCATCATAA-GATATTGG-3′) and C1-N-2776 (5′-GGATAATCAGAATANCGNCGAGG-3′). For additional information on extraction, amplification, and sequencing procedures, see Wheeler et al. (2016). Sequences were trimmed to 651 bp. All sequences were analysed using BLAST and are deposited in GenBank. The accession numbers are provided in Table 1.

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**Taxonomy**

**Family** Tetragnathidae Menge, 1866

**Subfamily** Diphyainae Simon, 1894

**Genus** Diphya Nicolet, 1849

**Type species.** Diphya macrophthalmalma Nicolet, 1849.

**Diagnosis.** For details see Álvarez-Padilla and Hormiga (2011) and Marusik et al. (2017).

**Description.** The genus is well described by Tanikawa (1995) and Álvarez-Padilla and Hormiga (2011).

**Composition and distribution.** For details see WSC (2022).

**Comments.** Although the debate on the limit of this genus remains open, a review of the genus Diphya is not within the scope of this work. Consequently, the present study follows WSC (2022) and Omelko et al. (2020) and temporarily places both new species in Diphya sensu lato for the lack of a better solution.
Key to *Diphy a* species occurring in China

1 Males ........................................................................................................................................2
   – Females .....................................................................................................................................5
2 Paracymbium simple and unbranched; embolus slender, distinctly longer than tegulum width, whip-shaped ........................................................................................................................................3
   – Paracymbium complex, with at least 3 processes (or outgrowths); embolus short and stout, shorter than tegulum width, embolar tip C-shaped, laminar or blade-shaped (Fig. 1A, B, D) ........................................................................................................................................4
3 Paracymbium thumb-like, slightly longer than wide; the middle part of embolus close to tegulum ........................................................................... *D. okumae*
   – Paracymbium distinctly longer than wide, \(\triangleright\)-shaped; the middle part of embolus well separated from tegulum ........................................................................... *D. tanasevitchi*
4 Paracymbium with 4 processes; embolar tip C-shaped, thick and heavily sclerotized, apex relatively sharp ........................................................................... *D. wulingensis*
   – Paracymbium with 3 processes (Fig. 1A, B, D); embolar tip blade-shape, hyaline, apex relatively wide (Fig. 2B–E) ........................................................................... *D. guiyang* sp. nov.
5 Epigynal atrium (or called fovea) distinct, lack of septum (Fig. 5A, C, E) ...........................................................................6
   – Epigynal atrium indistinct, divided or covered by septum (Fig. 3A, C, E) .................................................................7
6 Epigynal atrium located at anterior part of epigynal plate; copulatory ducts short and simple, not longer than epigyne length, not convoluted ................................................................. *D. okumae*
   – Epigynal atrium located posteriorly; copulatory ducts long, longer than epigyne length, strongly convoluted (Fig. 5A–E) ........................................................................... *D. weimiani* sp. nov.
7 Septal stem narrow, less than 1/2 of septal base ...........................................................................8
   – Septal stem relatively wide, not less than 1/2 of septal base (Fig. 3A, C, E) ...........................................................................9
8 Receptacles not subdivided ........................................................................... *D. tanasevitchi*
   – Receptacles subdivided in 2 chambers ...............................................................................................9
9 Receptacles separated by 4 diameters ........................................................................... *D. wulingensis*
   – Receptacles separated by no more than 1 diameter ........................................................................... *D. qianica*
10 Epigynal plate anteriorly with a V-shaped depression, septal base narrower than septal stem ........................................................................... *D. songi*
   – Epigynal plate anteriorly without depression, septal base wider than septal stem (Fig. 3A, C, E) ...........................................................................11
11 Septum \(T\) shaped, with a wide head (anterior part of septum); septal base short, about 1/3 of septum length (Fig. 3A, C, E); abdomen dorsally with 5 pairs of irregularly shaped black marks (Fig. 4D) ........................................................................... *D. guiyang* sp. nov.
   – Septum shaped like outline of a vase, lack head; septal base large, about 4/5 of septum length abdomen dorsally only with 2 pairs of muscular depressions ........................................................................... *D. taiwanica*
**Diphya guiyang** J. Zhang & H. Yu, sp. nov.

https://zoobank.org/D5FB012F-6152-4ADC-8619-1A3F2B874600

Figs 1–4, 7

**Material examined. Holotype.** ♂ (MGEU-TET-21-001, YHTET001), China: Guizhou Province: Guiyang City: Nanning District, Guiyang Forest Park, 26.55°N, 106.75°E, ca 1165 m, 10 August 2021, H. Yu et al. leg., hand picking on shrubs. **Paratypes:** 1♂ 1♀ (MGEU-TET-21-002–003, YHTET002–003), same data as holotype.

**Other material examined.** 1♂ 2♀, same data as holotype.

**Diagnosis.** The male of *D. guiyang* sp. nov. resembles that of *D. wulingensis* Yu, Zhang & Omelko, 2014 in having a similar complex paracymbium with several processes (other species have simple unbranched paracymbium and cymbial process) but can be distinguished from it by the different shape, locations, and number of paracymbial processes and by the different shape and degree of sclerotization of the embolus. In *D. guiyang* sp. nov., the paracymbium has 3 processes (vs 4), the intermediate process (Ip) is thumb-like and originates from the distal end of the paracymbium, close to tibia (Fig. 1A, B, D) (vs papilliform and located at the proximal margin of the paracymbium, well-separated from tibia); the embolar tip (Em T) is blade-shaped, hyaline, and with a relatively wide apex (Fig. 2B–E). (vs C-shaped, thick, heavily sclerotized, and with the apex relatively sharp; Yu et al. 2014: 31, figs 5, 10, 12; Marusik et al. 2017: 143, figs 13–15, 17). The female of *D. guiyang* sp. nov. also resembles that of *D. wulingensis* in having a similarly shaped vulva, but can be separated by having the septal base (Sb) relatively narrow (less than 1/3 of the epigynal plate width) (vs wide, about ½ of the plate width) (cf. Fig. 3A, C, E and Marusik et al. 2017: 143, figs 10, 11), and by the kidney-shaped posterior chamber of receptacle (Rp), which is distinctly larger than the anterior chamber (Ap) (vs both Ap and Rp nearly globular and Ap slightly larger than Rp) (cf. Fig. 3B, D and Yu et al. 2014: 31, figs 4, 9). In addition, the two species can be reliably separated by the abdominal pattern: dorsum of the abdomen centrally with a distinct symmetrical pattern in *D. guiyang* sp. nov. (Fig. 4A, D), vs without pattern centrally and only with black marks on both sides (Yu et al. 2014: 31, figs 1, 2; Marusik et al. 2017: 143, figs 1, 2, 5).

**Etymology.** The species name is derived from the type locality; noun in apposition.

**Description. Male.** Holotype (Figs 3F, 4A–C): total length 4.18; carapace 2.04 long, 1.48 wide; abdomen 2.14 long, 1.46 wide. **Carapace** dark brown, slightly lighter between PER and cervical groove. Clypeus dark brown, distinctly higher than AME diameter. Eye sizes and interdistances: AME 0.07, ALE 0.14, PME 0.15, PLE 0.14, AME–AME 0.09, AME–ALE 0.08, PME–PME 0.16, PME–PLE 0.20, MOQL 0.66, MOQA 0.22, MOQP 0.48. Chelicerae light brown, with 3 promarginal and 4 retromarginal teeth. Sternum coloured the same as carapace, 0.76 long, 0.85 wide.
Figure 1. Male palp of the holotype of *Diphyga guiyang* sp. nov. **A** ventral view **B** dorsal view **C** prolateral view **D** retrolateral view. Abbreviations: C = conductor; Cy = cymbium; Dp = dorsal process; Em B = basal portion of embolus; Em T = terminal portion of embolus; Ip = intermediate process; Pc = paracymbium; St = subtegulum; Te = tegulum; Vp = ventral process. Scale bars: 0.2 mm.
Figure 2. Male palpal bulb of the holotype of *Diphya guiyang* sp. nov. A ventral view B dorsal view C prolateral view D retrolateral view E anterior view. Abbreviations: C = conductor; Em B = basal portion of embolus; Em T = terminal portion of embolus; St = subtegulum; Te = tegulum. Scale bars: 0.2 mm.
Figure 3. *Diphya guiyang* sp. nov. **A–E** female paratype and male holotype, epigyne **A, B** macerated epigyne, ventral and dorsal **C, D** epigyne, macerated and embedded in Arabic gum, ventral and dorsal **E** intact epigyne **F, G** ventral view frontal view of prosoma **F** male **G** female. Abbreviations: Cd = copulatory duct; Fd = fertilisation duct; Ra = anterior chamber of receptacle; Rp = posterior chamber of receptacle; Sb = septal base; Se = septum (dashed line in C showing margin of septum); Sh = septal head; Ss = septal stem. Scale bars: 0.2 mm (**A–E**); 1 mm (**F, G**).
Figure 4. *Diphyia guiyang* sp. nov. A–C habitus of the male holotype D–F female paratype A, D dorsal view B, E ventral view C, F lateral view. Scale bars: 1 mm.
**Abdomen** dorsally dark with 5 pairs of spots (anterior pair circular, 2nd pair comma-shaped and largest, posterior 3 pairs represented by 6 short transverse bands), surrounded by line consisting of small white spots. Lateral sides whitish. Ventrally with irregularly shaped black pattern.

**Legs** uniformly yellowish. Leg measurements: I 8.90 (2.31, 2.62, 2.80, 1.17), II 7.56 (2.12, 2.30, 2.19, 0.95), III 6.70 (1.99, 2.06, 1.78, 0.87), IV – (1.87, 1.83, –, –).

**Palp** (Figs 1A–D, 2A–E): paracymbium (Pc) complex, with 3 processes: both ventral process (Vp) and intermediate process (Ip) large, thumb-like, dorsal process (Dp) relatively small, tooth-shaped; Vp originating from 1/3 to 1/4 proximal part of cymbium, slightly curved, apex pointing distally; Ip originating from base of cymbium, apex pointing ventrally; Dp originating from ca 2/5 proximal part of cymbium, slightly curved, apex pointing retrolaterally. Cymbium concave prolaterally. Subtegulum (St) large, hiding tegulum in retrolateral view; tegulum (Te) circular; sperm duct indistinct in ventral view. Conductor (C) laminar and hyaline, slightly smaller than tegulum, originating from dorsal-retrolateral portion of tegulum. Embolus (Em) slightly shorter than conductor, twisted around axis; embolar base (Em B) relatively sclerotized; embolar tip (Em T) blade-shaped, apex as wide as Em B and pointing ventrally.

**Female** (paratype: MGEU-TET-21-002) (Figs 3G, 4D–F). Total length 4.48; carapace 1.99 long, 1.53 wide; abdomen 2.49 long, 1.55 wide. **Carapace** uniformly red-brown, cervical groove and radial grooves distinct. Clypeus orange, distinctly higher than AME diameter. Eye sizes and inter-distances: AME 0.09, ALE 0.24, PME 0.26, PLE 0.19; AME–AME 0.09, AME–ALE 0.08, PME–PME 0.08, PME–PLE 0.06. MOQL 0.81, MOQA 0.24, MOQP 0.58. Chelicerae light orange, with 3 promarginal and 4 retromarginal teeth. Sternum 0.88 long, 0.87 wide, slightly darker than carapace. **Abdomen** basically yellowish white, dorsum centrally with indistinct, broken lengthwise band, reaching posterior half; with 2 pairs of muscular depressions located at two sides of lengthwise band; with 5 pairs of irregularly shaped black marks (frontal pair of marks largest), running longitudinally extending ca 4/5 of abdomen length. Lateral sides whitish. Ventrally yellowish white, without distinct pattern.

**Legs** uniformly yellowish. Measurements of legs: I 8.06 (2.11, 2.60 2.25, 1.10), II 7.22 (2.09, 2.24, 1.93, 0.96), III 4.62 (1.42, 1.44, 1.14, 0.62), IV 6.15 (1.93, 1.99, 1.56, 0.67).

**Epigyne** (Fig. 3A–E). Plate distinctly wider than long. Septum (Se) T-shaped, consisting of a transverse head (Sh), a narrow stem (Ss) and nose-shaped base (Sb); septal head wide, about 2/3 of the epigynal plate width; septal stem (Ss) slightly narrower than septal base, about twice longer that septal base length; septal base (Sb) shaped like a nose, nearly as wide as long. Copulatory openings indistinct, located in rebordered groove of lateral margins of septum. Copulatory ducts (Cd) diverging posteriorly, running along with lateral margin of septum. Receptacle subdivided in 2 chambers; anterior chamber (Ra) globular, relatively small, widely separated by ca 2.7 diameters; posterior chamber (Rp) kidney-shaped, distinctly larger than anterior chamber, 1.5 times longer than wide, separated by ca 1.3 widths. Fertilization ducts (Fd) acicular, membranous, located on posterior-interlateral surface of Rp.

**Distribution.** Known only from the Guiyang City, Guizhou Province, China (Fig. 7).
A new *Diphya* from Southwest China

*Diphya weimiani* J. Zhang & H. Yu, sp. nov.
https://zoobank.org/F79B3587-2128-4D61-BA1F-9AD6BA99A094
Figs 5–7


**Diagnosis.** The new species is easily distinguished from other congeners except *D. albula* (Paik, 1983) (Seo 2005: 49, figs 1, 2), *D. macrophthalma* Nicolet, 1849 (Marusik and Omelko 2017b: 25, 26, 30), and *D. okumae* Tanikawa, 1995 (Tanikawa 1995: 102, fig. 12; Zhu et al. 2003: 56, fig. 22) by the atrium distinct, and by lack of septum (vs atrium indistinct, divided or covered by septum; septum with variable shapes but distinct in all other *Diphya* species, such as *D. guiyang* sp. nov.; Fig. 3A, C, E), but differ from the latter three by the atrium located posteriorly (Fig. 5A, C, E) (vs located anteriorly), the copulatory ducts strongly entwined (Fig. 5B, D) (vs not entwined), and by the receptacles not subdivided (Fig. 5B, D) (vs receptacles subdivided in 2 chambers).

**Etymology.** The specific name is a patronym after Mian Wei (Chengdu City, China), the collector of the type material.

**Description. Female.** Holotype (Figs 5F, 6A–C): total length 3.79; carapace 1.57 long, 1.55 wide; abdomen 2.22 long, 1.55 wide.

**Carapace** red-brown, marginally slightly darker. Clypeus light orange, distinctly higher than AME diameter. Eye sizes and interdistances: AME 0.08, ALE 0.16, PME 0.15, PLE 0.16, AME–AME 0.06, AME–ALE 0.07, PME–PME 0.12, PME–PLE 0.16, MOQL 0.57, MOQA 0.20, MOQP 0.45. Chelicerae light orange, with 3 promarginal and 4 retromarginal teeth. Sternum coloured as carapace, 0.80 long, 0.68 wide.

**Abdomen** dorsally uniformly yellowish white, dorsum with two pairs of inconspicuous muscle depressions; laterally with lengthwise reticular pattern; ventrally white with no distinct pattern.

**Legs** uniformly red-brown. Leg measurements: I 5.58 (1.47, 1.85, 1.46, 0.80), II 5.10 (1.43, 1.65, 1.31, 0.71), III 3.36 (1.03, 1.03, 0.81, 0.49), IV 1.38 (1.44, 1.27, 1.14, 0.53).

**Epigyne** (Fig. 5A–E). Plate distinctly wider than long, with an atrium located posteriorly, receptacles and copulatory ducts indistinctly visible through integument. Atrium (A) shaped like an equilateral triangle, with reordered margin, about 1/2 epigyne length and 1/3 epigyne width. Lateral pocket (Lp) located anteriorly to atrium, more or less comma-shaped, heavily sclerotized. Copulatory openings (Co) indistinct, located at basolateral atrial borders. Copulatory ducts (Cd) strongly entwined, loop twice before connecting to receptacles. Receptacles (R) oval or balloon-shaped, not subdivided, relatively large, ca 1.3 times longer than wide, surface smooth; two receptacles close together. Fertilization ducts (Fd) acicular, membranous, located on posterior surface of receptacles.
Figure 5. *Diphya weimiani* sp. nov., female holotype, epigyne (A–E) and frontal view of prosoma (F).  

**A, B** macerated epigyne, ventral and dorsal  

**C, D** epigyne, macerated and embedded in Arabic gum, ventral and dorsal  

**E** intact epigyne, ventral view  

**F** female. Abbreviations: A = atrium; Cd = copulatory duct (dashed line in Fig. 5D showing schematic course of copulatory duct, dorsal); Co = copulatory opening; Fd = fertilisation duct; Lp = lateral pocket; R = receptacle. Scale bars: 0.2 mm (A–E); 1 mm (F).
A new *Diphyta* from Southwest China

Figure 6. Habitus of the female holotype of *Diphyta weimiani* sp. nov. **A** dorsal view **B** ventral view **C** lateral view. Scale bars: 1 mm.

Male. Unknown.

Comments. According to WSC (2022), only two species of *Diphya* are known only from males: *D. bicolor* Vellard, 1926 from Brazil, and *D. leroyorum* Omelko, Marusik & Lyle, 2020 from South Africa. However, neither could be matched with *D. weimiani* sp. nov. due to the long distance between their type localities (China is tens of thousands of kilometres from Brazil and South Africa).

Distribution. Known from the Mount Longmen Mountain (Sichuan Province), and Mount Leigong Mountain (Guizhou Province), China (Fig. 7).

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References


A new Diphya from Southwest China


A review of the spider genus Chthonopes (Araneae, Theridiosomatidae), with descriptions of two new species from China

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Abstract
The genus Chthonopes Wunderlich, 2011 is reviewed in this paper. The type species Chthonopes jaegeri Wunderlich, 2011 was illustrated based on new material from the type locality and the new distribution records (Bolikhamsay and Ban Kouanphavang Khammouane, Laos). Two new species are described from Yunnan, China: C. bifidum Yu & Lin, sp. nov. (♂♀) and C. jimudeng Yu & Lin, sp. nov. (♀). A key is provided for the genus, as well as species diagnoses, and a distribution map for all five species of Chthonopes.

Keywords
Araneoidea, Asia, key, revision, theridiosomatid, Yunnan

Introduction
Theridiosomatidae Simon, 1881 is a small-sized spider family with 20 genera and 135 valid species distributed worldwide, with 11 genera and 28 species recorded from China (World Spider Catalog 2022).

The genus Chthonopes was originally erected by Wunderlich (2011) based on two species from Laos, and C. jaegeri Wunderlich, 2011 was chosen as the genotype. Chthonopes is a relatively small theridiosomatid genus that is distributed exclusively...

While studying material from Yunnan Province, China, we recognized several specimens belonging to Theridiosomatidae. Detailed study of these specimens reveals that they belong to two undescribed species of *Chthonopes*, a genus previously unknown in China. The goals of this paper are 1) to describe the two new species under the names of *C. bifidum* Yu & Lin sp. nov. and *C. jimudeng* Yu & Lin sp. nov.; 2) to re-illustrate *C. jaegeri* based on new material from Laos, and give supplementary micrographs; and 3) to conduct a comprehensive review of the genus *Chthonopes*, including an identification key and a distribution map for all species.

**Materials and methods**

Specimens were examined and measured with a Leica M205 C stereomicroscope. Further details were studied with an Olympus BX43 compound microscope. Male and female copulatory organs were examined after they were dissected and detached from the bodies. Epigyne were removed and treated with lactic acid before photographed. All specimens were preserved in 95% ethanol. Photos were taken with a Canon EOS 60D wide zoom digital camera (8.5 megapixels) mounted on an Olympus BX43 stereomicroscope. The images were montaged using Helicon Focus ver.3.10 (Khmelik et al. 2006) image stacking software. All measurements in the paper are in millimetres. Leg measurements are given in the following sequence: total length (femur, patella, tibia, metatarsus, and tarsus).

The distribution map was generated with ArcGis ver.10.5 (Environmental Systems Research Institute, Inc.). Locality coordinates for all species are copied from the original publications (see Wunderlich 2011; Lin et al. 2014).

Abbreviations used in the text and figures are as follows:

- **Asp** accessory spermathecae;
- **CD** copulatory duct;
- **CL** cymbial lobe;
- **Co** conductor;
- **DA** distal apophysis on tegulum;
- **DH** distal horn on median apophysis;
- **ED** embolic distal end;
- **Em** embolus;
- **FD** fertilization duct;
- **MA** median apophysis;
- **Pc** paracymbium;
- **Sc** scape;
- **Sp** spermathecae;
St subtegulum;  
Te tegulum;  
TTr tibial trichobothrium.

All examined materials are deposited in the Natural History Museum of Sichuan University in Chengdu (NHMSU), China.

**Taxonomy**

**Family** Theridiosomatidae Simon, 1881

**Genus** *Chthonopes* Wunderlich, 2011

**Type species.** *Chthonopes jaegeri* Wunderlich, 2011 from Bolikhansay, Laos, by original designation.

**Diagnosis.** *Chthonopes* species can be recognised by the copulatory organs: In males, the cymbium apically-ventrally bearing several setae or hairs; median apophysis large and flat, located at the basal or subbasal portion of the tegulum, distally bearing a horn; bulb with an erect distal apophysis located on the apical part of the tegulum; embolus long, accompanied by a tubular conductor, embolic distal end forked. In females, the epigynal plate possesses a scape; vulval center with a V-shaped medial structure; copulatory ducts long, proximally thin but thick-walled, extending anteriorly along flanks of the V-shaped structure, the latter half wide and forming two egg-shaped bursae, surface membranous, wrinkled and ribbed, then connecting with main spermathecae at the central axis of the vulva; main spermathecae small, strongly sclerotized, globular or reniform, separated by about 0.1 – 1.2× their width; hyaline accessory spermathecae located laterally or anterolaterally to main spermathecae, usually claviform or tubular.

**Description.** See Wunderlich (2011).

**Composition and distribution.** *Chthonopes cavernicola* Wunderlich, 2011 (♂), *C. jaegeri* Wunderlich, 2011 (♂ ♀) and *C. thakekensis* Lin, Li & Jäger, 2014 (♀) from Laos, *C. bifidum* sp. nov. (♂ ♀) and *C. jimudeng* sp. nov. (♀) endemic to China.

**Key to *Chthonopes* species**

1 Males ..........................................................................................................................................................2
   – Females.................................................................................................................................................4

2 Anterior eye row with 6 eyes, posterior eye row with 2 eyes; cymbium apically bearing four tiny hairs which are not situated on a hump (Wunderlich 2011: 433, figs 8–10, 16) .................................................................................................................................C. cavernicola
   – Both anterior and posterior eye rows with 4 eyes; cymbium apically-ventrally with a pair of long and bristle-shaped hairs on a hump (Figs 1A, 2C, 3A, 4C) ..................................................................................................................................................3
3 Distal horn on median apophysis (DH) bifurcate (Fig. 2C); distal apophysis on tegulum (DA) partly membranous or hyaline, whisker-shaped (Fig. 2B, C); paracymbium (Pc) with a spine-like tip (Fig. 2D) .... **C. bifidum sp. nov.**

- Distal horn on median apophysis (DH) represented by a small needle or spine, not forked, (Fig. 4A–C); distal apophysis on tegulum (DA) relatively sclerotized, lamina-shaped (Fig. 4A–C); paracymbium (Pc) without spine-like tip (Fig. 4D) ........................................................................... **C. jaegeri**

4 Scape (Sc) long, more than 1/2 of epigyne length, rugose (Fig. 3E–G; Lin et al. 2014: 98, figs 17B–E, 18A–C) ........................................................................... 5

- Scape (Sc) short, about 1/5 of epigyne length, not rugose (Fig. 1E–G; Fig. 5C–E) .................................................................................................. 6

5 Main spermathecae (Sp) semi-circular, separated by about 1.2× their width; accessory spermathecae (Asp) claviform; fertilization ducts (FD) membranous (Lin et al. 2014: 98, figs 17C, E, 18C) .......................... **C. thakekensis**

- Main spermathecae (Sp) circular, separated by about 1/3 of their diameter; accessory spermathecae (Asp) consisting of tubular stalk and globular head; FD strongly sclerotized (Fig. 3G) ............................................. **C. jaegeri**

6 Scape (Sc) triangular, translucent, extending from posterior margin of epigynal plate; accessory spermathecae (Asp) located anterolaterally to main spermathecae, nearly claviform or tubular (Fig. 1G) .......... **C. bifidum sp. nov.**

- Scape (Sc) digitiform, relatively sclerotized, originating from dorsal side of posterior margin of epigynal plate; accessory spermathecae (Asp) located laterally to main spermathecae, consisting of tubular stalk and globular head (Fig. 5E) ............................................................................. **C. jimudeng sp. nov.**

**Chthonopes bifidum** Yu & Lin, sp. nov.
https://zoobank.org/7F648B7E-A751-4201-B5F4-2F9C2616BDAA
Figs 1, 2, 6

**Type material.** **Holotype:** ♂, CHINA: Yunnan Province: Xishuangbanna: Mengla County: Menglun Town: Shenmi cave, 21.97°N, 101.24°E, elevation 776 m, 3.X.2017, Y. Lin and Y. Li leg. **Paratypes:** 15♀♀11juv., same data as holotype.

**Other material examined.** 1♂ 24♀♀1juv., CHINA: Yunnan Province: Dehong: Luxi City: Mangliu village: Xianfo cave, 24.33°N, 98.52°E, elevation 1081 m, 25.VIII.2010, C. Wang leg.

**Etymology.** The species epithet is taken from the Latin adjective “bifidus” and refers to the forked distal horn of median apophysis.

**Diagnosis.** The males of *C. bifidum* sp. nov. easily differentiated from those of all other congeners by the bifurcate distal horn of median apophysis, the partly membranous or hyaline, whisker-shaped distal apophysis of tegulum, and by the paracymbium with a spine-like tip, vs. distal horn on median apophysis represented by a small needle or spine, not forked, distal apophysis on tegulum relatively sclerotized, lamina-shaped,
Figure 1. *Chthonopes bifidum* sp. nov., male holotype and female paratype, male habitus (A, B), female habitus (C, D) and epigyne (E–G) A dorsal view B ventral view C dorsal view D ventral view E intact, ventral view F cleared, ventral view G cleared, dorsal view. Abbreviations: Asp = accessory spermathecae; CD = copulatory duct; FD = fertilization duct; Sc = scape; SP = spermatheca. Scale bars: 0.5 mm (A–D); 0.2 mm (E, F, G).
Figure 2. Male palp of the holotype of *Chthonopes bifidum* sp. nov. **A** prolateral view **B** retrolateral view **C** ventral view **D** dorsal view. Abbreviations: Co = conductor; CL = cymbial lobe; DA = distal apophysis on tegulum; DH = distal horn on median apophysis; ED = embolic distal end; Em = embolus; MA = median apophysis; Pc = paracymbium; St = subtegulum; Te = tegulum; TTr = tibial trichobothium. Scale bars: 0.2 mm (**A–D**).
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paracymbium without spine-like tip in *C. jaegeri* and *C. cavernicola* (cf. Fig. 2A–D and Wunderlich 2011: 433, figs 13–17, 18b, Fig. 4A–D). The females of *C. bifidum* sp. nov. can be easily distinguished from other congeners except *C. jimudeng* sp. nov. by the short and smooth scape, about 1/5 of epigyne length (Figs 1E–G, 5C–E) (vs. scape rugose, longer than 1/2 of epigyne length in all other congeners, including *C. jaegeri* and *C. thakekensis*; Fig. 3E–G; Lin et al. 2014: figs 17B–E, 18A–C), but differ from the latter by the: (1) scape triangular, translucent, extending from posterior margin of epigynal plate (Fig. 1E–G) (vs. digitiform, relatively sclerotized, originating from dorsal side of posterior margin of epigynal plate; Fig. 5C–E); (2) accessory spermathecae located anterolaterally to main spermathecae, nearly claviform or tubular (Fig. 1G) (vs. located laterally to main spermathecae, consisting of tubular stalk and globular head; Fig. 5E).

**Description. Male** (holotype) (Fig. 1A, B): Carapace nearly pyriform, yellowish brown, without distinct pattern, slightly darker marginally. Anterior eye row recurved, posterior eye row distinctly procurved. Sternum heart-shaped, dark brown, with sparse setae. Mouthparts coloured as sternum. Legs uniformly brown, femora slightly darker. Abdomen round, dorsum centrally beige, marginally coffee coloured with sparse long hairs, weakly ossified at hair base; venter slightly darker than dorsum, posteriorly clothed with short setae. Measurements: Total length 2.1. Carapace 0.8 long, 0.9 wide. Clypeus 0.2. High. Sternum 0.5 long, 0.5 wide. Abdomen 1.4 long, 1.3 wide. Length of legs: I 4.0 (1.3, 0.4, 1.0, 0.9, 0.4); II 3.0 (0.9, 0.3, 0.8, 0.6, 0.4); III 2.4 (0.6, 0.2, 0.7, 0.6, 0.3); IV 3.0 (0.9, 0.3, 0.8, 0.7, 0.3).

**Palp** (Fig. 2A–D): Tibia small, about 1/5–1/6 length of cymbium, dorsally bears a short trichobothrium (TTr). Cymbium narrow, about 2.6 × longer than width, with long setae. Paracymbium (Pc) small, about 1/5–1/6 length of cymbium, with a nearly triangular base and spine-like tip. Tegulum (Te) capacious, 1.5 × longer than wide; sperm duct distinct in ventral view, running a V-shaped course along posterior part of the tegulum. Median apophysis (MA) originating from subbasal portion of tegulum, consisting of broad base and biforked distal horn (DH); base nearly triangular; distal horn heavily sclerotized, tip curved and bifurcate, lateral ramus short claw-shaped, mesal ramus filiform and ca. 2 × longer than lateral ramus. Distal apophysis (DA) located at distal-retrolateral position of tegulum, base partly membranous, and tip hyaline with a truncated apex. Embolus (Em) long and thick, hidden behind conductor, arising at approximately the 9–10 o’clock position, terminating at ca. 2 o’clock position, embolic distal end forked. Conductor (Co) tubular, covering almost whole embolus, apex translucent and pointing retrolaterally.

**Female** (one paratype). Somatic features as in Fig. 1C, D and coloration slightly lighter than in male. Measurements: Total length 2.5. Carapace 0.8 long, 1.1 wide. Clypeus 0.2 high. Sternum 0.6 long, 0.5 wide. Abdomen 1.9 long, 1.3 wide. Length of legs: I 4.0 (1.4, 0.4, 1.1, 0.7, 0.4); II 3.5 (1.0, 0.4, 1.0, 0.7, 0.4); III 2.6 (0.7, 0.3, 0.7, 0.6, 0.3); IV 3.1 (0.9, 0.3, 0.9, 0.7, 0.4).

**Epigyne** (Fig. 1C–E). Epigynal plate large, slightly wider than long, with long setae in midline, the arrangement of the various parts of the vulva are indistinctly visible through the tegument; scape (Sc) short, triangular, translucent, extending from
posterior margin of epigynal plate, less than 1/5 of epigyne length, apex blunt. The
distal part of copulatory ducts (CD) wide, forming two egg-shaped bursae, then
connecting with later margin of main spermathecae; the two bursae base closely spaced
but anterior surface widely separated by ca. 2x of bursae width. Main spermathecae
(Sp) small, reniform, strongly sclerotized, separated by about 1/10 of their width; ac-
cessory spermathecae (Asp) located anterolaterally to main spermathecae, translucent,
early claviform or tubular, about 1/2 of epigyne length. Fertilization ducts (FD),
short, ribbon-shaped, strongly sclerotized, located on dorsal-basal surface of main sper-
mathecae; apical parts separated by about 2.5× of FD width, apex curved and sharp.

**Distribution.** Known from Mengla County and Luxi City, Yunnan, China (Fig. 6).

*Chthonopes cavernicola* Wunderlich, 2011

*Chthonopes cavernicolus* Wunderlich, 2011: 433, figs 8–18 (♂).

**Material examined.** Not examined.

**Diagnosis.** See diagnosis for *C. jaegeri*.

**Description.** See Wunderlich (2011).

**Distribution.** Laos (Fig. 6).

*Chthonopes jaegeri* Wunderlich, 2011

Figs 3, 4, 6

*Chthonopes jaegeri* Wunderlich, 2011: 435, fig. 18a–f (♀♀).

**Material examined.** 2♀♀, Laos: Khammouan Province: Thakek area, Ban Phôngam-
Mai, 17.55°N, 104.81°E, elevation 495 m, 25.XI.2012, S. Li leg; 7♀♀, Bolikhamxay
Province: Khamkeut area, 18.22°N, 104.81°E, elevation 495 m, 27.XI.2012, Z. Yao
leg; 1♂ 2♀♀, Bolikhamxay Province: Lak Sao, 17.22°N, 104.81°E, elevation 501 m,
3.III.2010, H. Steiner leg.

**Diagnosis.** The male of *C. jaegeri* resembles those of *C. cavernicola* (Wunderlich,
2011: 433, figs 8–18) in having a large and flat, laminar median apophysis which bears
a tiny needle-shaped distal horn (Fig. 4A–C) (vs. median apophysis relatively small,
consisting of triangular base and biforked distal horn in *C. bifidum* sp. nov.; Fig. 2C),
but differs in the combination of genitalic and somatic characters: distal apophysis of
the tegulum is erect, apex relatively sharp, pointing distally (Fig. 4C; Wunderlich 2011:
435, fig. 18b) (vs. curved, apex truncated, pointing proximally; Wunderlich 2011: 433,
fig. 17); cymbium apically-ventrally with a pair of long and bristle-shaped hairs on a
hump (Fig. 4C; Wunderlich 2011: 435, fig. 18b) (vs. cymbium bearing apically four
tiny hairs which are not situated on a hump; Wunderlich 2011: 433, fig. 17); both
anterior and posterior eye rows with 4 eyes (Fig. 3A) (vs. anterior eye row with 6 eyes,
Figure 3. Chthonopes jaegeri, male habitus (A, B), female habitus (C, D) and epigyne (E–G) A dorsal view B ventral view C dorsal view D ventral view E intact, ventral view F cleared, ventral view G cleared, dorsal view. Abbreviations: Asp = accessory spermathecae; CD = copulatory duct; FD = fertilization duct; Sc = scape; SP = spermatheca. Scale bars: 0.5 mm (A–D); 0.2 mm (E, F, G).
Figure 4. Male palp of Chthonopes jaegeri A prolateral view B retrolateral view C ventral view D dorsal view. Abbreviations: Co = conductor; CL = cymbial lobe; DA = distal apophysis on tegulum; DH = distal horn on median apophysis; ED = embolic distal end; Em = embolus; MA = median apophysis; Pc = paracymbium; St = subtegulum; Te = tegulum. Scale bars: 0.2 mm (A–D).
posterior eye row with 2 eyes; Wunderlich 2011: 433, figs 8–10). Females of *C. jaegeri* are similar to those of *C. thakekensis* (Lin et al. 2014: 98, figs 17A–E, 18A–C) by the epigynal plate with a long, rugose scape, and by the similar configurations of vulva, but they can be differentiated by the circular main spermathecae separated by about 1/3 of their diameter (Fig. 3G) (vs. semi-circular main spermathecae separated by about 1.2 × their width; Lin et al. 2014: 98, figs 17C, E, 18C), the accessory spermathecae consisting of a tubular stalk and globular head (vs. accessory spermathecae claviform, not subdivided; Lin et al. 2014: 98, figs 17C, E, 18C), and by the strongly sclerotized fertilization ducts (Fig. 3G) (vs. membranous FDs; Lin et al. 2014: 98, figs 17C, E, 18C).

**Description.** See Wunderlich (2011). Habitus as in Fig. 3A–D, male palp as in Fig. 4A–D, epigyne as in Fig. 3E–G.

**Distribution.** Laos (Fig. 6).

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**Chthonopes jimudeng** Yu & Lin, sp. nov.

https://zoobank.org/27320301-272A-497C-B51C-DB133DDC9DF0

Fig. 5, 6

**Type material.** *Holotype:* ♀, CHINA: Yunnan Province: Gongshan County: Dulongjiang Town: Jimudeng village, 27.79°N, 98.33°E, elevation 1410 m, 15.VIII.2018, Y. Lin and Y. Li leg. *Paratypes:* 1 ♀ 2juv., same data as holotype.

**Other material examined.** 6juv., CHINA: Yunnan Province: Gongshan County: Dulongjiang Town: Maku village, 27.68°N, 98.30°E, elevation 1939 m, 14.VIII.2018, Y. Lin and Y. Li leg.

**Etymology.** This specific name is taken from type locality; noun in apposition.

**Diagnosis.** The new species is similar to *C. bifidum* sp. nov. (Fig. 1E–G) in the general appearance of the epigyne (also see above diagnosis for *C. bifidum* sp. nov.). From *C. bifidum* sp. nov., the female of *C. jimudeng* sp. nov. can be easily distinguished by the shape of the scape and accessory spermathecae, as well as the arrangement of the various parts of the vulva: (1) scape digitiform, relatively sclerotized (Fig. 5C–E) (vs. triangular, translucent; Fig. 1E–G); and (2) accessory spermathecae located laterally to the main spermathecae, consisting of a tubular stalk and globular head (Fig. 5C–E) (vs. located anterolaterally to the main spermathecae, nearly claviform or tubular; Fig. 1E–G).

**Description.** *Female* (holotype) (Fig. 5A, B): Carapace nearly triangular, marginally dark, with a dark, wide V-shaped paramedian stripe starting from behind PLE, almost reaching the indistinct cervical groove. Anterior eye row recurved, posterior eye row almost straight in dorsal view. Sternum heart-shaped, centrally dark brown, marginally dark, with sparse setae. Mouthparts coloured as sternum. Legs dark brown, all legs with conspicuous dark annuli in the distal parts of femur, and patella. Abdomen spherical, covered with sparse long setae, setal base sclerotized; dorsum basically black, centrally with three longitudinal white bands, the medial band relatively long, about 1/2 of abdomen length, the bilateral bands short, about 1/2 length of medial band;
Figure 5. Holotype female of *Chthonopes jimudeng* sp. nov., habitus (A, B) and epigyne (C–E) A dorsal view B ventral view C intact, ventral view D cleared, ventral view E cleared, dorsal view. Abbreviations: Asp = accessory spermathecae; CD = copulatory duct; FD = fertilization duct; Sc = scape; SP = spermatheca. Scale bars: 0.5 mm (A, B); 0.2 mm (C, F, G).
venter black, without pattern. **Measurements:** total length 2.3. Carapace 1.0 long, 0.9 wide. Clypeus 0.1 high. Sternum 0.4 long, 0.5 wide. Abdomen 1.6 long, 1.2 wide. Length of legs: I 3.1 (1.0, 0.3, 0.9, 0.6, 0.3); II 2.7 (0.9, 0.3, 0.6, 0.6, 0.3); III 1.8 (0.6, 0.2, 0.4, 0.4, 0.2); IV 2.4 (0.7, 0.3, 0.6, 0.5, 0.3).

**Epigyne** (Fig. 5C–E). Epigynal plate large, about 1.35× wider than long, with long setae in midline, through which spermathecae and copulatory ducts are indistinctly apparent; scape (Sc) distinctly short, about 1/5 of epigyne length, digitiform, relatively...
sclerotized, originating from dorsal side of posterior margin of epigynal plate, its tip slightly overpasses the posterior margin, apex blunt. The distal part of copulatory ducts (CD) wide, forming two egg-shaped bursae, then connecting with posterolateral surface of main spermathecae; the two bursae widely separated by one width. Main spermathecae (Sp) small, oval, strongly sclerotized, separated by about 1/3 of their diameter; accessory spermathecae (Asp) located laterally to main spermathecae, consisting of tubular stalk and globular head, translucent, about 1/4 of epigyne length. Fertilization ducts (FD) short, ribbon-shaped or lamellar, heavily sclerotized, located on posterior surface of main spermathecae, curved tips separated by about 3.6× FD width.

**Male.** Unknown.

**Distribution.** Known only from Gongshan County, Yunnan, China (Fig. 6).

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*Cthonopes thakekensis* Lin, Li & Jäger, 2014


**Material examined.** None.

**Diagnosis.** See diagnosis for *C. jaegeri*.

**Description.** See Lin et al. (2014).

**Distribution.** Laos (Fig. 6).

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**References**


New species and illustrated key of Macraspis (Scarabaeidae, Rutelinae, Rutelini) from the Amazon biome of Brazil

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Abstract

The phytophagous scarab genus Macraspis MacLeay (Scarabaeidae, Rutelinae, Rutelini) is reviewed from the Brazilian Amazon region. Three new species are described and illustrated from the states of Amazonas, Pará, and Rondônia: M. buehrnheimi sp. nov., M. opala sp. nov., and M. phallocardia sp. nov. Two species, Macraspis fernandezi Neita-Moreno and M. oblonga Burmeister, are recorded for the first time in Brazil (new country records). Macraspis maculata crosarai Soula is a new synonym of Macraspis maculata Burmeister; hence this species no longer includes subspecies. Furthermore, Macraspis cincta parensis Soula, 2005 is deemed unavailable under the provisions of ICZN Articles 16.4.1 and 16.4.2. An illustrated key to 15 species and subspecies of Macraspis from the Brazilian Amazon enables identification of this speciose leaf chafer genus.

Keywords

Chafers, identification, morphology, Neotropical Region, taxonomy

Introduction

The Neotropical genus Macraspis MacLeay, 1819 (Scarabaeidae, Rutelinae, Rutelini) is a distinctive and widely distributed leaf chafer group that occurs from Mexico to Argentina (Soula 1998). This genus is diagnosed by (1) mandibles with outer margin
bidentate (Fig. 2C), (2) pronotum evenly trisinuated posteriorly and with a concave posteromedial emargination (Fig. 3C), (3) scutellar shield as long as the elytral suture (Fig. 3C), (4) mesometaventral process well developed (Fig. 1A, D), and (5) femur-abdominal stridulatory apparatus present (Fig. 1K) (Ohaus 1903; Soula 1998).

We examined the *Macraspis* species and subspecies with distributions in the Brazilian Amazon biome (defined as northern Brazil including the states of Acre, Amapá, Amazonas, Pará, Rondônia, Roraima, as well as small portions of Mato Grosso, Tocantins, and Maranhão states) (IBGE 2004) (Fig. 12) and included them in the identification key. Species from the Atlantic Forest, Caatinga, Pampa, Pantanal, or Cerrado biomes in Brazil are not part of our research focus.

*Macraspis* currently includes 68 species and 21 subspecies (Soula 1998, 2002, 2005, 2006, 2010; Moore et al. 2014; Neita-Moreno 2014; Bento and Grossi 2021). As a result of our work, 15 species and subspecies are known from the Brazilian Amazon. The study of the taxa in Brazil led to the discovery of three undescribed species of *Macraspis* from two different Amazonian interfluvial areas, as well as two species that are new country records in Brazil: *M. fernandezi* Neita-Moreno, 2014 and *M. oblonga* Burmeister, 1844. Based on comparative examinations of type and non-type specimens, we synonymize *M. maculata crosarai* Soula, 1998 with *Macraspis maculata* Burmeister, 1844. Under the provisions of ICZN (1999) Articles 16.4.1 and 16.4.2, *M. cincta parensis* Soula, 2005 is an unavailable name. As a result of these changes, the genus *Macraspis* now includes 71 species and 19 subspecies. We present an illustrated key to all species and subspecies from Brazilian Amazon biome, and this key provides a foundation for broader identification of this speciose leaf chafer genus.

**Methods**

The type material and additional specimens used for descriptions, comparisons, and key are deposited in the following institutions (acronym and curators parenthesized): Seção de Entomologia da Coleção Zoológica da Universidade Federal do Mato Grosso, Cuiabá, Brazil (CEMT; Fernando Zagury Vaz-de-Mello); Coleção Entomológica do Instituto Oswaldo Cruz, Rio de Janeiro, Brazil (CEIOC; Márcio Felix, Claudia Leal Rodrigues); Musée des Confluences, Lyon, France (CCECL; Cédric Audibert); Coleção Zoológica Prof. Paulo Bührnheim, Universidade Federal do Amazonas, Manaus, Brazil (CZPB; Fábio Siqueira Pitaluga de Godoi); Coleção Entomológica Pe. Jesus Santiago Moure, Universidade Federal do Paraná, Curitiba, Brazil (DZUP; Lúcia Massutti de Almeida); Colección Entomológica del Instituto Alexander von Humboldt, Bogotá, Colombia (IAVH; Jhon César Neita-Moreno); Coleção de Invertebrados do Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (INPA; Márcio de Oliveira); Naturhistorisches Museum Wien, Vienna, Austria (NHMW; Matthias Seidel); Mary Liz Jameson Collection, Wichita, Kansas, USA (MLJC); Martin-Luther-Universität, Zentrlmagazin Naturwissenschaftlicher Sammlungen, Zoologische Sammlung, Halle, Germany (MLUH; Hendrik Müller); Muséum national d’Histoire Naturelle, Paris, France (MNHN; Antoine Mantilleri); Coleção Entomológica ‘Prof. J.M.F. Camargo’,
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Body length was measured from the apex of the clypeus to the apex of the elytra; body width was measured across the humeri. Regarding punctures, surfaces were considered punctostriate when punctures were confluent and elongated, densely punctate if punctures were nearly confluent to less than two puncture diameters apart, moderately punctate if 2–6 punctures diameters apart, and sparsely punctate if punctures were separated by more than six puncture diameters. The punctures were considered small when less than 0.019 mm in diameter, moderate when 0.02–0.049 mm, and large if larger than 0.05 mm. Setae were defined short if between 0.1–0.19 mm in length, moderately long if between 0.2–0.39 mm, and long if longer than 0.4 mm. Regarding density of setae, surfaces were considered densely setose when many setae completely covered the surface, moderately setose when the surface visible and with many setae, and sparsely setose when the surface was visible and with only a few setae.

Morphological terms follow Beutel and Lawrence (2005) for the general morphology and the male and female genitalia (with the adoption of the term tectum for the distal portion of the phallobase [non-apodeme]), and d’Hotman and Scholtz (1990) and Coca-Abia and Martín-Piera (1991) for endophallus structures.

Like many other *Macraspis* species, identification of the two new species described here requires examination of male genitalia. Female external genitalia of these new species showed conspicuous differences in the proximal and distal gonocoxites. However, caution must be taken concerning the identification of females based only on external genital characters because the whole range of intraspecific variation is not known for these structures. Accordingly, females of these species can be more reliably identified when collected with associated males. Therefore, females were not included in the diagnoses and key.

For purposes of the identification key, we include species known to occur only within the Brazilian Amazon biome (Soula 1998, 2002). The key as presented here does not include females of *M. lateralis* (Olivier, 1789) because of scarce specimens as well as the lack of associated males. Further advances must await additional female specimens and more morphological studies including type material to diagnose the female of *M. lateralis* and identify the hitherto unknown female of *M. fernandezi*.

Images were taken using a Leica DFC295 camera attached to a Leica M165C stereomicroscope and were processed using the Leica Application Suite (LAS) v. 4.1 and Helicon Focus (HeliconSoft) software. The photographic illumination follows Kawada and Buffington (2016).

The verbatim label data from type specimens are transcribed in quotation marks, with “/” used to separate lines on the same label, and “//” to separate labels. Label data from non-type specimens are provided as follows: country, state or province, locality, date, collector or old collection (quantity, sex symbol, collection acronym).

The geographic coordinates were obtained with Google Maps and the georeferenced points were plotted on the distribution map generated by the web software Simplemappr (Shorthouse 2010).
Results

Based on examination of specimens in 12 collections, we recorded seven species as occurring in the Brazilian Amazon: *M. buehrnheimi* sp. nov., *M. fernandezi* Neita-Moreno, *M. lateralis* (Olivier), *M. martinezi auzerali* Soula, 1998, *M. oblonga* Burmeister, *M. opala* sp. nov., and *M. phallocardia* sp. nov. We provide species treatments and a distribution map for all species including *M. maculata* Burmeister, 1844 (Fig. 12). Eight additional species are known to occur in the Amazon biome: *M. chloraspis chloraspis* Laporte, 1840, *M. festiva* Burmeister, 1844, *M. lepiouffi* Soula, 1998, *M. morio* Burmeister, 1844, *M. olivieri* (Waterhouse, 1881), *M. peruviana* Ohaus, 1898, *M. pseudochrysis pseudochrysis* Landin, 1965, *M. xanthosticta* Burmeister, 1844. As a result of our research, *M. maculata crosarai* Soula is synonymized with *M. maculata* Burmeister. The taxon *sensu* Soula (1998, 2003) is considered endemic to the Brazilian Cerrado and Atlantic Forest rather than the Brazilian Amazon. For this reason, this species was excluded from the key. All species and subspecies recorded from the Brazilian Amazon region are included in the identification key to allow for identification and comparison with other *Macraspis*.

*Macraspis cincta parensis* Soula, 2005 was described based on two specimens with the doubtful locality of Obidos, Pará state, Brazil. In the original description Soula (2005) stated: “I dare to hope that it is not a labeling error on the locality” (translated from French). Indeed, Obidos is a historically highly frequented collecting site, and *Macraspis cincta* was never reported from that location. Additionally, all *Macraspis cincta* subspecies occur exclusively in the Atlantic Forest from the Brazilian states of Rio de Janeiro, São Paulo, Espírito Santo, and Santa Catarina. Furthermore, the original description violates the ICZN (1999) Articles 16.4.1 and 16.4.2 stating that every new specific and subspecific name published after 1999 must be accompanied in the original publication “by the explicit fixation of a holotype, or syntypes, for the nominal taxon” and “where the holotype or syntypes are extant specimens, by a statement of intent that they will be (or are) deposited in a collection and a statement indicating the name and location of that collection”. Because Soula (2005) did not provide a type designation and collection information, *M. c. parensis* is unavailable.

Key to Brazilian Amazon species and subspecies of *Macraspis*

Lacking females of *M. buehrnheimi* sp. nov., *M. fernandezi*, *M. lateralis*, *M. opala* sp. nov., *M. phallocardia* sp. nov.

1 Protarsomere V enlarged; anterior protarsal claw thickened and unequally cleft; pygidium strongly convex, with posterior margin ventrally positioned, male............................................................................................................2

2 Protarsomere V not enlarged; anterior protarsal claw not thickened and equally cleft; pygidium weakly convex to plano-convex, with posterior margin dorsoapically positioned, female........................................................................................................16
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2 Mesometaventral process thickened and oblique in relation to the longitudinal axis of the body (Fig. 1A), with apex expanded and bluntly rounded in ventral view (Fig. 1D); apical margin of metatibia strongly projected externally (Fig. 2L); inner metatibial spur distinctly longer than metatarsomeres I and II combined (Fig. 2L) ........................................... M. morio Burmeister

3 Large body size (length 14–23 mm); head without supraorbital strigae (Fig. 2A, B); pronotum strongly emarginated posterolaterally (Fig. 2D); anterior bead of pronotum medially effaced or barely defined (Fig. 1C); mesotibia somewhat arcuate and as long as mesofemur, with an internoapical lobe weakly to strongly developed (Fig. 1G–I); mesepimeron ventrally concave and with a posterolateral carina (Fig. 2D) ...........................................................................

4 Mesotibia medially wider than half the mesofemur width (Fig. 1I); anterior meso- and metatarsal claw thick and narrowly cleft, and in flexed position distinctly longer than tarsomere V (Fig. 1M) .............................................. M. pseudochrysis pseudochrysis Landin

5 Mesotibia medially narrower than half the mesofemur width (Fig. 1G, H); anterior meso- and metatarsal claw flat and broadly cleft, and in flexed position subequal or shorter than tarsomere V (Fig. 1N) ................................... M. lepiouffi Soula

6 Dorsum unicolored, with elytra uniformly green and without metallic maculation ........................................... M. peruviana Ohaus

7 Pronotum entirely green (lateral margins same color as disc) ........................................... M. festiva Burmeister

8 Pronotum with disc green and lateral margins yellow
8 Clypeus rugopunctate at apex, with anterior margin strongly raised and subtruncated (Fig. 2A); scutellar shield shorter than elytral suture; posterior border of mesocoxal cavity narrow, with postcoxal line somewhat straight (Fig. 1H); internaopical lobe of mesotibia weakly developed and barely defined (Fig. 1H) ..............................................................................................................**M. oblonga** Burmeister

– Clypeus densely punctate at apex, with anterior margin weakly raised and rounded (Fig. 2B); scutellar shield longer than elytral suture; posterior border of mesocoxal cavity wide, with postcoxal line curved (Fig. 1G); internaopical lobe of mesotibia strongly developed and well defined (Fig. 1G) ..........................................................**M. olivieri** (Waterhouse)

9 Mesotibia medially narrower than mesofemur at middle (Fig. 1J); mesotarsomere IV with ventroapical process pointed and straight (Fig. 1N); mesotarsomere V ventrally untoothed and distinctly shorter than mesotarsomeres I–IV combined (Fig. 1N); anterior meso- and metatarsal claw broadly cleft and in flexed position shorter than metatarsomere V (Fig. 1N) .............................................................................................................................................**M. chloraspis chloraspis** Laporte

– Mesotibia medially as wide as mesofemur at middle (Fig. 2E); mesotarsomere IV with ventroapical process thick and ventrally swollen (Fig. 1O); mesotarsomere V with a ventromedial tooth and as long as mesotarsomeres I–IV combined (Fig. 1O); anterior meso- and metatarsal claw narrowly cleft and in flexed position shorter than metatarsomere V (Fig. 1O) .................................................................................................................................................................**M. fernandezi** Neita-Moreno

10 Paramera apically dilated (Fig. 1E–J) ...............................................................................................................

– Paramera apically narrowed (Figs 3E–L, 4E–J) .................................................................................................**M. buehrnheimi** Bento, Jameson & Seidel, sp. nov.

11 Lateral articular areas of tectum truncated and weakly projected distally (Fig. 3H–J); paramera in dorsal view with middle portion strongly constricted and narrower than half the apical portion (Fig. 3H) ...............................................................................................................................................................**M. xanthosticta** Burmeister

– Lateral articular areas of tectum pointed and strongly projected distally (Fig. 3E–G); paramera in dorsal view with middle portion slightly constricted and distinctly wider than half the apical portion (Fig. 3E) ..................................................................................................................................................................................**M. opala** Bento, Jameson & Seidel, sp. nov.

12 Apex of pygidium smooth (Fig. 9C); paramera laterally excavated (Fig. 9G) . .................................................................................................................................................................................................................................................................................................**M. xanthosticta** Burmeister

– Apex of pygidium with strong, concentric sculpturing (Fig. 9M); paramera laterally not excavated (Fig. 10G, J) ........................................................................................................................................................................................................................................................................................................13

13 Smaller specimens (length 7–9 mm); anterior margin of clypeus weakly notched medially (more evident in females) (Fig. 2C); scutellar shield slightly constricted basolaterally; paramera with apex acute ........................................................................................................................................................................................................................................................................................................14

– Larger specimens (length 10–11.7 mm); anterior margin of clypeus not notched; scutellar shield strongly constricted basolaterally; paramera with apex narrowly rounded or parabolic..............................................................................................................................................................................14
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14 Elytron metallic green with two median, yellow maculae or a single transverse yellow band ......................................................... *M. martinezi auzerali* Soula

– Elytron usually metallic green and without yellow maculae or band........15

15 Lateral articular areas of tectum thickened and deflected outward (Fig. 10E); paramera in caudal view rounded-oval, with sides not declivous and rounded to slightly constricted apically (Fig. 10F) .................................................................

......................................................... *M. phallocardia* Bento, Jameson, Seidel, sp. nov

– Lateral articular areas of tectum compressed and straight (Fig. 10H); paramera in caudal view laterobasally projected backward, with sides slightly declivous and narrowly constricted apically (Fig. 10I).............. *M. lateralis* (Olivier)

16 Mesometaventral process thickened and oblique to the body’s longitudinal axis (Fig. 1A), with apex expanded and bluntly rounded in ventral view (Fig. 1D); apical margin of metatibia strongly projected externally (Fig. 2L); inner metatibial spur distinctly longer than metatarsomeres I and II combined (Fig. 2L); posterior margin of pygidium medially truncated or slightly bidentate (Fig. 2I); ventrite 6 posteromedially emarginated (Fig. 2M) ........ *M. morio* Burmeister

– Mesometaventral process weakly flattened and parallel to body longitudinal axis (Fig. 1B), with apex abruptly narrowed in ventral view (Fig. 1E); apical margin of metatibia weakly projected externally (Fig. 2O); inner metatibial spur subequal or shorter than metatarsomeres I and II combined (Fig. 2O); posterior margin of pygidium medially rounded (Fig. 2G, H); ventrite 6 not emarginated (Fig. 2N) ..............................................................................17

17 Large specimens (length 14–23 mm); head without supraorbital strigae (Fig. 2A, B); pronotum strongly emarginated posterolaterally (Fig. 2D); anterior bead of pronotum medially effaced or barely defined (Fig. 1C); mesotibia somewhat arcuate and as long as mesofemur, with an internoapical lobe inconspicuous to strongly developed (Fig. 1G–I); mesepimeron ventrally concave and with a posterolateral carina (Fig. 2D) .........................................................18

– Small specimens (length 7–14 mm); head with supraorbital strigae (Fig. 2C); pronotum weakly or not emarginated posterolaterally (Fig. 2E, F); anterior bead of pronotum complete and well defined (Fig. 1F); mesotibia straight and distinctly shorter than mesofemur, with no internoapical lobe (Fig. 1J); mesepimeron slightly convex ventrally and without carina (Fig. 2E, F) ..... 23

18 Mesotibia medially wider than half the mesofemur width (Fig. 1I) ...............19

– Mesotibia medially narrower than half the mesofemur width (Fig. 1G, H) .... 20

19 Body non-metallic green; pygidium apically obtuse in dorsal view (Fig. 2J); posterior border of mesocoxal cavity narrow (Fig. 1I); mesotibia with internoapical lobe inconspicuous (Fig. 1I); mesepimeron barely concave ventrally .............................................................................. *M. pseudochrysis pseudochrysis* Landin

– Body metallic green; pygidium apically acuminate in dorsal view (Fig. 2K); posterior border of mesocoxal cavity wide (Fig. 1G); mesotibia with internoapical lobe strongly developed (Fig. 1G); mesepimeron distinctly concave ventrally .............................................................................. *M. lepiouffi* Soula
20 Dorsum unicolored, with elytra uniformly green and without metallic maculation........................................................................... *M. peruviana* Ohaus

– Dorsum bicolored, with elytra yellow with or without metallic green maculation........................................................................................................... 21

21 Pronotum entirely green (lateral margins same color as disc)..........................

........................................................................................................................................... *M. festiva* Burmeister

– Pronotum with disc green and lateral margins yellow..............................

22 Clypeus rugopunctate at apex, with anterior margin strongly raised and subtruncated (Fig. 2A); scutellar shield shorter than elytral suture; internoapical lobe of mesotibia weakly developed and barely defined (Fig. 1H); posterior margin of pygidium narrowly rounded at middle (Fig. 2G)........................

........................................................................................................................................... *M. oblonga* Burmeister

– Clypeus densely punctate at apex, with anterior margin weakly raised and rounded (Fig. 2B); scutellar shield longer than elytral suture; internoapical lobe of mesotibia strongly developed and well defined (Fig. 1G); posterior margin of pygidium evenly rounded (Fig. 2H)..... *M. olivieri* (Waterhouse)

23 Inner metatibial spur evenly and strongly curved (Fig. 1K)....................

........................................................................................................................................... *M. chloraspis chloraspis* Laporte

– Inner metatibial spur straight or barely curved (Fig. 1L)...........................

24 Smaller specimens (length 7–9 mm); anterior margin of clypeus weakly notched medially (Fig. 2C); scutellar shield slightly constricted basolaterally ........................

........................................................................................................................................... *M. xanthosticta* Burmeister

– Larger specimens (length 10–11.7 mm); anterior margin of clypeus not notched; scutellar shield more strongly constricted basolaterally........................

........................................................................................................................................... *M. martinezi auzerali* Soula

Species treatments

*M. buehrnheimi* Bento, Jameson & Seidel, sp. nov.
https://zoobank.org/EFC64A56-50AF-4C02-84AD-E6D05ECF3871
Figs 3A–G, 4A–D, 5A–C, 12


Diagnosis. Male genitalia are required for identification: lateral articular areas of tectum pointed and strongly projected distally (Fig. 3E); paramera in dorsal view slightly constricted medially, with middle portion almost as wide as apical portion (Fig. 3E); apex of paramera in caudal view obtusely triangulate, with a strong median tooth (Fig. 3F, G).
New species of *Macraspis* from the Amazon biome of Brazil

Figure 1. *Macraspis* spp. (A–O). Lateral and ventral views of pro- and mesothorax of A, B *M.* *morio* and D, E *M.* *p.* *pseudochrysis*. Dorsal view of head and anterior portion of pronotum of C *M.* *festiva* and F *M.* *c.* *chloraspis*. Anterior view of mesothoracic legs of G *M.* *festiva* H *M.* *oblonga* I *M.* *p.* *pseudochrysis* and J *M.* *c.* *chloraspis*. Female metatibial spurs of K *M.* *c.* *chloraspis* and L *M.* *phallocardia* sp. nov. Anterior view of mesotarsus of M *M.* *p.* *pseudochrysis* N *M.* *c.* *chloraspis* and O *M.* *fernandezi*. Scale bars: 1 mm.
**Figure 2.** *Macraspis* spp. (A–O). Dorsal view of head of A. *M. oblonga* B. *M. olivieri* and C. *M. xanthosticta*. Lateral view of mesothorax of D. *M. festiva* E. *M. maculata* and F. *M. c. chloraspis*. Female pygidium of G. *M. oblonga* H. *M. olivieri* and I. *M. morio*. Dorsal view of female abdomen of J. *M. p. pseudochrysis* and K. *M. lepiouffi* paratype. Metatarsus of L. *M. morio* and O. *M. olivieri*. Female last abdominal ventrites of M. *M. morio* and N. *M. p. pseudochrysis*. Scale bars: 0.5 mm (A–C), 1 mm (D–O).

**Description.** **Holotype male** (Fig. 3A, B, E–G). Length 10.6 mm, width 5.9 mm. Body rounded-oval. **Coloration.** Head, elytra, and scutellar shield shiny green, with brownish reflections. Pronotum shiny green, anterolateral areas with brownish reflection, and posterolateral areas with yellow maculae laterally extending to anterior margins. Pygidium and venter shiny green with strong brownish reflections. **Head.** Vertex sparsely
punctate on disc, laterally punctostriate. Frons with slight V-shaped depression, moderately punctate, punctures moderate and deep. Intercocular width 3.7 times wider than transverse eye diameter. Clypeus confluent punctate, with anterior margin subtrapezoidal, slightly raised medially. Mandible with outer teeth strongly raised, outer margin slightly curved near base. **Pronotum** shallowly and sparsely punctate on disc, punctures small and shallow; anterolaterally punctostriate, punctures large and deep. **Scutellar shield** moderately punctate, longer than elytral suture. **Elytra** 2 times longer than midwidth, moderately punctate, punctuations large and shallow. Posthumeral depression well developed laterally. Apical umbone wide and poorly defined. **Pygidium** strongly convex, with weak and concentric sculpturing, slightly effaced posteriorly. **Venter** glabrous, moderately punctate. Mesometaventral process anteriorly directed between procoxae, ventrally flat, with apex abruptly acute in anterocentral view. Mesepimera partially exposed in dorsal view, strongly convex and transversally ridged. **Legs**. Protibia externally tridentate, with proximal tooth well defined and acute. Protarsomere V longer than protarsomeres I–IV combined. Anterior protarsal claw enlarged, unequally bifid and obliquely truncated. Mesotibia with internal margin straight, with inner apex not dilated. Mesotarsomere IV with ventroapical projection well developed, thickened and ventrally swollen. **Abdomen** with ventrite 6 broadly and slightly emarginated posteriorly. **Aedeagus** (Fig. 3E–G). Tectum abruptly narrowed towards the apical edge, with lateral articular areas pointed and strongly projected distally. Paramera in dorsal view slightly constricted medially, with middle portion almost as wide as apical portion; apex in caudal view triangulated with a strong median tooth, strongly deflected ventrally. **Endophallus** (Fig. 4A–D) divided into three portions: one narrow, tube-shaped basal portion; one wide, sac-shaped medial portion; and one hairy, slender apical portion (partially lost in Fig. 4A, B, D). Proximal portion distally hairy; V-shaped sclerite with thin, long arms; and temones large, fused into a single sclerite with a mediolongitudinal carina. Medial portion with a broad ventral rasula and a small dorsomedial rasula bearing moderately dense, thin-walled asperites; a dorsodistal rasula bearing multiple, irregular, and dense rows of thick-walled asperites; and a large, triangular lateral sclerite, with distal edge thick and slightly raised.

**Paratype (1 female)** (Fig. 3C, D). Length 11.2 mm. Width 6.3 mm. The female differs from male by the more robust and more convex body; interocular width 4.2 times wider than transverse eye diameter; clypeus longer, with anterior margin narrower and more raised; pygidium plano-convex; protibia with outer teeth stronger and apically rounded; Mesotarsomere IV with a short ventroapical projection straight and pointed; and abdominal ventrite 6 not emarginated. **External genitalia** (Fig. 5A–C). Gonocoxites dark brown, strongly sclerotized and moderately setose apically, setae moderately long. Proximal gonocoxites rugostriate and large, as long as wide, overlapping the distal gonocoxites; inner margin abruptly deflected to apex narrow. Distal gonocoxites with inner margin curved and apex narrowly rounded.

**Etymology.** This species is named after the Brazilian zoologist Paulo Friederich Bührnheim (1937–2001), who greatly contributed to education and research in Amazonas state, Brazil. In addition, he founded the insect collection at the Universidade Federal do Amazonas (UFAM) and collected the type series of this species.

**Distribution** (Fig. 12). Brazil (2). Amazonas: Coari.
Remarks. *Macraspis buehrnhei* sp. nov. has the same color pattern as *M. lateralis* (Olivier, 1789), *M. fernandezi* Neita-Moreno, 2014, and *M. phallocardia* sp. nov. These species are only separated by careful comparison of male genitalia. The male aedeagus of *M. buehrnhei* is most similar to that of *M. fernandezi* (unknown female) in that both have paramera apically dilated. Other characters that serve to separate *M. buehrnhei* and *M. fernandezi* are (characters of *M. fernandezi* given in parenthesis): tectum abruptly narrowed towards the apical edge, with lateral articular areas pointed and strongly projected distally (tectum evenly narrowed towards the apical edge, with lateral articular areas truncated and weakly projected distally (Fig. 3H)); paramera in dorsal view slightly constricted medially, with middle portion almost as wide as apical portion (paramera in dorsal view strongly constricted medially, with middle portion narrower than half the apical portion (Fig. 3H, I)); apex of paramera in caudal view triangulated, with a strong median tooth (apex in caudal view oblong-oval, with a weak median tooth (Fig. 3I)).
Figure 4. Comparison of male endophalli of *Macraspis buehrnheimi* sp. nov. and *Macraspis fernandezi* Neita-Moreno, 2014. *Macraspis buehrnheimi* sp. nov. (A–D) in A dorsal B lateral and D lateral views, with C detail showing lateral sclerite. *Macraspis fernandezi* Neita-Moreno, 2014 (E–H) in E dorsal F lateral and H ventral views, with G detail showing lateral sclerite. Scale bars: 0.5 mm.
There are no reliable means to distinguish the female of *M. buehrnheimi* sp. nov. from that of *M. phallocardia* sp. nov. based on external morphology. Analysis of the external genitalia showed conspicuous differences in the proximal and distal portions of the gonocoxites of these species (compare Fig. 5A, B to Fig. 5D, E). However, the

**Figure 5.** Comparison of female external genitalia of *Macraspis buehrnheimi* sp. nov. (A–C) and *Macraspis phallocardia* sp. nov (D–G). *Macraspis buehrnheimi* sp. nov. in A ventral B lateral and C dorsal views. *Macraspis phallocardia* sp. nov. in D ventral E lateral and F dorsal views. Abbreviations: dgx = distal gonocoxite; pgx = proximal gonocoxite; pct = proctiger; ppt = paraproct. Scale bars: 0.3 mm.
scarce number of specimens prevented us from assessing intraspecific variation in these structures, which need further morphological examination within the genus. Females of these species should be reliably identified when collected with associated males.

**Macraspis fernandezi** Neita-Moreno, 2014
Figs 1O, 3H–J, 4E–G, 12

**Type material examined.** Holotype male deposited at IAVH, labeled: “COLOMBIA, Meta, PNN / La Macarena San Juan de / Arama 03°20’47”N, 73°53’22”W Caño La Curia / 580 m Bos. Galeria 13.iii.1986 / F. Fernández leg.” (white, printed; duplicated label) // “Macraspis fernandezi / Neita-Moreno 2014” (white, printed) // “HOLOTIPO / Macraspis fernandezi / Neita-Moreno, 2014” (red, printed) // “Instituto Humboldt / Colombia / IAvH-E-88479” (white, printed with added QR-code).


**Distribution (Fig. 12).** Colombia (1). Meta (Neita-Moreno 2014). Brazil (2). Roraima, Amazonas.

**Remarks.** This species was described based on a single male specimen from Colombia (Neita-Moreno 2014). Herein, two additional specimens of *M. fernandezi* from Roraima and Amazonas states are recorded for the first time from Brazil (new country record). Further comparison to the holotype male showed that the paramera of Brazilian specimens are slightly narrower at middle, but they do not differ in the lateral articular areas of the tectum. The male endophallus (Fig. 4E–H) of the two Brazilian specimens were inflated and showed no conspicuous intraspecific differences. Female specimens of this species have not been described.

**Macraspis lateralis** (Olivier, 1789)
Figs 6A–D, 7A–E, 10H–J, 11A–D, 12

*Cetonia lateralis* Olivier, 1789: 80.  


**Type material examined.** Neotype male (Fig. 6) deposited at MNHN, labeled: “Guyane franç. / Passoura / E.LeMoult 1905.6 // Muséum Paris / ex Coll. / R. Oberthür / 1952 // NEOTYPE / Cetonia / lateralis Ol. / M. SOULA det // MNHN / EC1215” (http://coldb.
mnhn.fr/catalognumber/mnhn/ec/ec1215). Images of the male aedeagus provided by Jhon César Neita Moreno and Julián Clavijo-Bustos (IAVH). **Holotype** female of *Cetonia virens* (Fig. 7) deposited at ZMUK, labeled: “Essequibo. / Smidt. / Mus. J: Lund. / Lateralis / Cetonia Oliv. / virens. F” (white, handwritten) // “TYPE” (red, printed) // “ZMUKFabricius / 002261” (white, printed with QR code) // “zmuc / 00031432” (white, printed).

**Non-type material examined** (2 males, 2 females). Brazil, Amazonas, Manaus, Reserva Ducke, 21.II.1978, Jorge Arias (leg.) (♀, INPA); idem, but no date and collector (♂, INPA); idem, but campus UA, 07.III.2005, Herbert & Marcos Pantoja (legs.) (♂, INPA); Brazil, Pará, Santarém, I.1922, J. F. Zikán Coll. (♀, CEIOC).


**Remarks.** Fabricius (1801) described *Cetonia virens* from “America Meridionali” (= South America). This species was synonymized under *M. lateralis* by Burmeister (1844), and the synonymy was maintained by Soula (2003) without any additional statements. One of us (MS) located and examined the female holotype of *C. virens* at ZMUK (Fig. 7A–E). Inspection of the label, which was never been completely transcribed, revealed additional, important information. The word “Essequibo” (Guiana), which was omitted from the original description and all treatments of this species, allowed us to ascertain that the type locality was from Guiana. The female holotype is not associated with male specimens so it cannot be reliably identified. However, because the distribution of *C. virens* lies within the known range of *M. lateralis* in the Guiana Shield, we maintain the synonymy.

**Macraspis maculata** Burmeister, 1844

Figs 2E, 8A–D, 9I–M, 12


New species of *Macraspis* from the Amazon biome of Brazil

**Figure 6.** Neotype male of *Cetonia lateralis* Olivier, 1789 (=*Macraspis lateralis* (Olivier)): A dorsal view B labels C aedeagus in frontal view D aedeagus in lateral view. A, B from MNHN website http://coldb.mnhn.fr/catalognumber/mnhn/ec/ec1215, accessed on 2022-07-23; C, D provided by Jhon César Neita-Moreno.
Additional material examined (18 males, 13 females). Brazil, Bahia, Prado, 05.III.1971, C. Elias (leg.) (♀, DZUP); Brazil, Espírito Santo, Conceição da Barra, 04.X.1969, C. T. & C. Elias (legs.) (♂, ♀, DZUP); idem, but 26.IX.1968 (2♂, ♀, DZUP); idem, but 19–25.X.II.1968 (♂, ♀, DZUP); Brazil, Espírito Santo, Linhares, X.1965 (♂, DZUP); idem, but XI.1965 (♀, DZUP); idem, but no date (♂, DZUP); idem, but XI.1966, A. Maller (leg.) (♂, ♀, DZUP); idem, but Rio Itabapoana, 26.X.1906, J. F.

Figure 7. Holotype female of Cetonia virens Fabricius, 1801 (=Macraspis lateralis Olivier): A dorsal view B ventral view C lateral view D head and pronotum in dorsal view E labels. Scale bars: 2 mm.
New species of *Macraspis* from the Amazon biome of Brazil

**Distribution (Fig. 12).** Brazil. São Paulo (Irisanga = Oriçanga; Burmeister 1844), Rio de Janeiro, Goiás (Soula 1998, 2003), Bahia, Espírito Santo, Minas Gerais (new state records).

**Remarks.** *Macraspis maculata* has been recorded from the Brazilian Amazon (Ohaus 1918, 1934; Machatschke 1972; Brûlé et al. 2014) but similarities with other species have posed problems with identification. In his treatment for *M. maculata*, Soula (1998) considered the species to be widely distributed, and he justified the creation of related taxa because: “… some populations that are more geographically isolated are already genetically isolated enough to describe subspecies (or even new species ...)” (translated from French). Soula (1998, 2003) restricted *M. maculata maculata* to Brazilian Atlantic Forest and described *M. maculata crosarai* from Brazilian Cerrado (distinguished from the nominotypical subspecies by means of coloration). Influenced by Antonio Martínez’s identification labels, Soula (1998) described *M. martinezi* and compared it with *M. maculata* (perhaps constituting one of Soula’s “genetically isolated” populations of *M. maculata*). He included three subspecies, *M. martinezi auzerali*, *M. martinezi curoei* Soula, 1998, and *M. martinezi colombica* Soula, 1998, all of which are distributed in the Amazon biome (only the first subspecies is known from the Brazilian Amazon) and similar to *M. maculata* in coloration, form, and genitalic form. The validity of *M. martinezi* and its subspecies-complex requires evaluation in future studies. Examination of the primary types of *M. maculata maculata* (Fig. 8A–D) and *M. maculata crosarai* (Fig. 8E–H) revealed no conspicuous differences between these taxa. Based on this examination, we synonymize *M. maculata crosarai* with the nominotypical *M. maculata*. Now, *M. maculata* no longer includes subspecies. Therefore, although the taxon has been recorded to the Brazilian Amazon and French Guiana by past authors (Ohaus 1918, 1934; Machatschke 1972; Brûlé et al. 2014), *M. maculata* is treated *sensu* Soula (1998, 2003) in this paper and considered to be distributed in the Brazilian Cerrado and Atlantic Forest rather than the Brazilian Amazon region. For this reason, this species was excluded from the key provided here.

*Macraspis oblonga* Burmeister, 1844

Figs 1H, 2A, G, 12


**Material examined (4 females).** Brazil, Amazonas, Manaus, Fazenda Esteio, 30.VIII.1984, R. C. Klein (leg.) (♀, INPA); Brazil, Amazonas, BR-174 Km 18, 11.VIII.1980, C. Fonseca & E. Bindá (legs.) (♀, INPA); Brazil, Amazonas, Manaus,

**Distribution.** Suriname (Hielkema and Hielkema 2019). French Guiana (Soula 1998). Brazil (Fig. 12). Amazonas (present paper).

**Remarks.** This species is sympatric with *M. olivieri*, and both have a very similar color pattern. However, *M. oblonga* is easily distinguished from *M. olivieri* by (characters of *M. olivieri* given in parenthesis): clypeal apex rugopunctate, with anterior margin strongly raised and subtruncated (Fig. 2A) (clypeal apex densely punctate, with anterior margin weakly raised and rounded (Fig. 2B)); scutellar shield shorter than elytral suture (longer); internoapical lobe of mesotibia weakly developed and

![Figure 8. *Macraspis maculata* Burmeister, 1844, lectotype male (A–D) A dorsal view B ventral view C aedeagus D lectotype label. *M. maculata crosarai* Soula, 1998, holotype male (E–H) E dorsal view F ventral view G aedeagus H labels.](image-url)
barely defined (Fig. 1H) (strongly developed and well defined); posterior margin of pygidium narrowly rounded at middle (Fig. 2G) (evenly rounded). This is the first record of *M. oblonga* in Brazil (new country record) based on female specimens collected in Amazonas state.

**Macraspis opala** Bento, Jameson, Seidel, sp. nov.
https://zoobank.org/CB4B582C-5434-46BB-A747-51EDD90D4EB1
Figs 9A–H, 12


**Diagnosis.** Apex of pygidium smooth (Fig. 9C); metatarsomere IV with ventroapical projection short and apex acute; apical edge of tectum wider than base of paramera, with lateral articular areas horizontally rotated (Fig. 9E–H); paramera strongly declivous laterally, with sides inconspicuous in caudal view and strongly excavated medially (Fig. 9F–H).

**Description.** **Holotype male (Fig. 9A–G).** Length 9.7 mm, width 5.6 mm. Body rounded-oval. **Coloration.** General color copper with green or red reflections. Head, pronotum, and scutellar shield with strong green reflections. Pronotum with yellow posterolateral maculae effaced. Elytra brownish copper, with two median yellow maculae somewhat effaced. Pygidium and venter with color more diffuse than dorsal surface. **Head.** Vertex sparsely punctate at disc, laterally punctostriate. Frons with slight V-shaped depression, densely punctate, punctures large. Interocular width 4.2 times wider than transverse eye diameter. Clypeus confluentely punctate, with anterior margin subtrapezoidal, slightly raised medially. **Pronotum** shallowly and sparsely punctate at disc, with slight anterolateral depression densely punctate at lateral corner, punctures moderate and deep. **Scutellar shield** moderately punctate, longer than elytral suture. **Elytra** 1.8 times longer than mid-width, moderately punctate, punctuations moderate and shallow. Posthumeral depression weak. Apical umbone wide and well defined. **Pygidium** (Fig. 9C) strongly convex and apically smooth. **Venter** glabrous, moderately punctate. Mesometaventral process anteriorly directed between procoxae, ventrally flat, with apex abruptly acute in anteroventral view. Mesepimera partially exposed in dorsal view, slightly convex and transversally ridged. **Legs.** Protibia strongly tridentate externally, with proximal tooth well defined and pointed. Protarsomere V longer than protarsomeres I–IV combined. Anterior protarsal claw enlarged, unequally cleft and obliquely truncated. Mesotibia with internal margin straight, with inner apex not dilated. Mesotarsomere IV with ventroapical projection well developed, thickened and ventrally curved. Metatarsomere IV with spine-like ventroapical projection short
and pointed. **Abdomen** with ventrite 6 broadly and slightly emarginated posteriorly. **Spiculum gastrale** (Fig. 9D) Y-shaped with proximal arms short and distal stem slender, about 2.8 times longer than arm length. **Aedeagus** (Fig. 9E–G). Tectum broadly curved laterally, not narrowed towards the apical third; apical edge wider than base of paramera, with lateral articular areas horizontally rotated. Paramera strongly declivous laterally, with sides inconspicuous in caudal view and strongly excavated medially to form a laterolongitudinal carina.

**Female.** Unknown.

**Paratype** (1 male). The male paratype differs from holotype by the general coloration darker with stronger green reflections and elytra without yellow maculae; paramera slightly narrower, with lateral margins parallel and apex slightly more rounded (Fig. 9H).

**Etymology.** The specific epithet is derived from the Latin word ‘opalus’ (= precious stone) in reference to opal gemstone, alluding to the metallic, multicolor cuticular surface.

**Distribution** (Fig. 12). Brazil (2). Pará: Itaituba, Rondônia: Candeias do Jamari. The two male specimens composing the type series were collected within the Madeira-Tapajós interfluvium.

**Remarks.** This species is quite similar to *Macraspis maculata* Burmeister, 1844. However, *M. opala* sp. nov. has a slightly smaller body size and is distinguished from *M. maculata* by (characters of *M. maculata* given in parenthesis): apex of pygidium smooth, without sculpturing (apex of pygidium with strong and concentric sculpturing (Fig. 9M)); metatarsomere IV with ventroapical projection short and pointed (metatarsomere IV with ventroapical projection thickened and truncated); tectum broadly curved laterally, apical edge wider than base of paramera, with lateral articular areas horizontally rotated (tectum laterally narrowed towards the apical third, apical edge as narrow as base of paramera, with lateral articular area vertically positioned (Fig. 9J–L)); paramera strongly declivous laterally, with sides inconspicuous in caudal view and strongly excavated medially to form a laterolongitudinal carina (paramera slightly declivous laterally, with sides conspicuous in caudal view and weakly excavated apically (Fig. 9K, L)).

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*Macraspis phallocardia* Bento, Jameson, Seidel, sp. nov.
https://zoobank.org/315D05D9-1487-4BEC-8F7D-730DF26DC6FB
Figs 1L, 5D–G, 10A–G, 11E–H, 12

New species of *Macraspis* from the Amazon biome of Brazil

**Diagnosis.** Male: lateral articular areas of tectum thickened and deflected outward (Fig. 10E); paramera in caudal view rounded-oval, with apex rounded to slightly constricted and sides not declivous (Fig. 10F). Female: Females of this species are diagnosed in association with male specimens.

**Description.** Male holotype (Fig. 10A, B, E–G). Length 11.3 mm, width 6.1 mm. Body rounded-oval. **Coloration.** General color shiny green with brownish reflections. Pronotum with posterolateral yellow maculae laterally extending to anterior margins. **Head.** Vertex sparsely punctate at disc, laterally punctostriate. Frons with slight V-shaped depression, densely punctate, punctures moderate and deep. Interocular width 4 times wider than transverse eye diameter. Clypeus conflually punctate, with anterior margin subtrapezoidal, slightly raised medially. **Pronotum** shallowly and
sparsely punctate at disc and moderately punctate anterolaterally, punctures moderate and deep. **Scutellar shield** moderately punctate, longer than elytral suture. **Elytra** 2.3 times longer than wide, moderately punctate, punctures large. Posthumeral depression weak. Apical umbone wide and poorly defined. **Pygidium** strongly convex, with concentric sculpturing and moderately punctate posteriorly. **Venter** glabrous, moderately punctate. Mesometaventral process anteriorly directed between procoxae, ventrally flat, with apex abruptly acute in anteroventral view. Mesepimera partially exposed in dorsal view, slightly convex and transversally ridged. **Legs.** Protibia externally tridentate, with proximal tooth well defined. Protarsomere V enlarged, longer than protarsomeres I–IV combined. Anterior protarsal claw enlarged, unequally cleft and obliquely truncated. Mesotibia with internal margin straight, with inner apex not dilated. Mesotarsomere IV with ventroapical projection well developed, thickened and ventrally curved. **Abdomen** with ventrite 6 broadly and slightly emarginated posteriorly. **Aedeagus** (Fig. 10E–G).

Figure 10. *Macraspis phallocardia* sp. nov. (A–G) and *Macraspis lateralis* (Olivier, 1789) (H–J). *Macraspis phallocardia* sp. nov. **A** holotype male dorsal view **B** holotype male ventral view **C** paratype female dorsal view **D** paratype female ventral view **E–G** aedeagus of holotype in **E** dorsal view **F** caudal view **G** lateral view. *Macraspis lateralis* (Olivier, 1789) from Manaus, Amazonas state, Brazil **H** aedeagus in dorsal view **I** caudal view **J** lateral view. Scale bars: 2 mm (A–D); 0.5 mm (E, G, H, J); 0.2 mm (F, I).
New species of *Macraspis* from the Amazon biome of Brazil

Figure 11. Comparison of male endophalli of *Macraspis lateralis* (Olivier, 1789) (A–D) and *Macraspis phallocardia* sp. nov. (E–H): *Macraspis lateralis* (Olivier, 1789) in A dorsal view B lateral view D lateral view and C showing detail of lateral sclerite. *Macraspis phallocardia* sp. nov. in E dorsal view F lateral view H ventral view and G showing detail of lateral sclerite. Scale bars: 0.5 mm (A, B, D, E, F, H); 0.2 mm (C, G).
Tectum moderately narrowed towards the apical edge, with lateral articular areas thickened and deflected outward. Paramera in caudal view rounded-oval, with apex parabolaic and sides not declivous. **Endophallus** (Fig. 11E–H) divided into three portions: one narrow, tube-shaped basal portion; one wide, sac-shaped medial portion; and one hairy, slender apical portion. Medial portion with a large ventral raspula and a small dorsoproximal raspula bearing dense, thin-walled asperites; a dorsodistal raspula bearing an irregular, dense multiple rows of thick-walled asperites; and a large and cultrate lateral sclerite, with proximal and distal edges thick and slightly raised.

**Paratypes** (3 males, 14 females): male paratypes differ from holotype in length (10.7–11.7 mm), width (5.8–6.7 mm), and form of the apex of paramera (more round to slightly narrowed). Female paratypes (Fig. 10C, D). Length 10.8–11.8 mm, width 6–6.4 mm. The females differ from males by the clypeus longer, anteriorly narrower and more raised; pygidium plano-convex; protibia with outer teeth stronger and apically rounded; protarsomere V simple, with anterior claw unenlarged and equally cleft; inner metatibial spur apically rounded; Mesotarsomere IV with a ventroapical projection short and straight; and abdominal ventrite 6 not emarginated. **External genitalia** (Fig. 5D–F). Gonocoxites light brown, slightly sclerotized and moderately setose apically, setae moderately long. Proximal gonocoxites short and semicircular, wider than long, barely overlapping the distal gonocoxites; surface moderately punctate. Distal gonocoxites broadly rounded apically, with inner margin almost straight.

**Figure 12.** Distribution of Brazilian species of *Macraspis* treated in this publication. The Brazilian Amazon biome is colored lime green. Brazilian states are abbreviated as follows: AC = Acre, AM = Amazonas, AP = Amapá, BA = Bahia, ES = Espírito Santo, GO = Goiás, MA = Maranhão, MG = Minas Gerais, MT = Mato Grosso = PA = Piauí, RO = Rondônia, RJ = Rio de Janeiro, RR = Roraima, SP = São Paulo, TO = Tocantins.
New species of *Macraspis* from the Amazon biome of Brazil

**Etymology.** The specific epithet is Greek for ‘*phallos* (= penis) and ‘*kardia*’ (= heart), refers to the heart-shaped male paramera of this species.

**Distribution (Fig. 12).** Brazil (10). Rondônia: Ouro Preto d’Oeste.

**Remarks.** This species has the same color pattern as *M. buehrnheimi* sp. nov., *M. fernandezi*, and *M. lateralis*. The male genitalia of *M. phallocardia* sp. nov. is more similar to that of *M. lateralis*, but these species are differentiated by (characters of *M. lateralis* given in parenthesis): tectum with lateral articular areas thickened and deflected outward (tectum with lateral articular areas compressed and straight; Fig. 10H); paramera in caudal view rounded-oval, with apex rounded to slightly constricted and sides not declivous (paramera in caudal view laterobasally projected backward, with sides slightly declivous and narrowly constricted apically; Fig. 10I); medial portion of endophallus with ventral and dorsoproximal raspulae (Fig. 11E–H), a dorsodistal raspula bearing dense and multiple rows of asperites (Fig. 11E), and a lateral sclerite with proximal and distal edges thin and slightly raised (Fig. 11F, G) (medial portion of endophallus without ventral rasulae, Fig. 11D; with a dorsodistal raspula bearing a sparse and simple row of asperites, Fig. 11A; and a lateral sclerite with medial edge raised and distal edge thickened and roundly protruding, Fig. 11B, C). *Macraspis phallocardia* sp. nov. is apparently sympatric with *M. opala* sp. nov., the type series of which was also collected in the Madeira-Tapajós interfluviun.

**Acknowledgements**

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**References**


New species of *Macraspis* from the Amazon biome of Brazil
Complete mitochondrial genomes of Boiga kraepelini and Hebius craspedogaster (Reptilia, Squamata, Colubridae) and their phylogenetic implications

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Abstract
The complete sequence of the mitochondrial genome is a powerful tool for studying phylogenetic relationships and molecular evolution in various species. In this work, the mitogenomes of Boiga kraepelini and Hebius craspedogaster were sequenced and characterized for the first time. The lengths of the B. kraepelini and H. craspedogaster mitogenomes were 17,124 bp and 17,120 bp, respectively, and both included 13 protein-coding genes, 22 tRNAs, two rRNAs and two control regions. The arrangements of these mitochondrial genes were the same in B. kraepelini and H. craspedogaster. In addition, both genome compositions showed A+T bias (59.03%, 60.93%) and had positive AT skews (0.179, 0.117) and negative GC skews (-0.397, -0.348). The phylogenetic results illustrated a close relationship between B. kraepelini and the genus Lycodon. Moreover, H. craspedogaster was clustered with other Hebius snakes and closely related to other Natricinae species. These results will provide references for further research on the phylogeny of Colubridae.

Keywords
Colubrinae, mitogenomes, Natricinae, phylogenetic analysis, protein-coding genes

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Introduction

Colubridae is a family with high species diversity in the suborder Serpentes, which is distributed on almost all continents (Pough et al. 2004). The hierarchical classification of Colubridae can be divided into eight subfamilies (Ahaetuliinae, Calimariinae, Colubrinae, Dipsadinae, Grayiinae, Natricinae, Pseudoxenodontinae, and Sibynophiinae) based on molecular markers and morphological characters (Figueroa et al. 2016; Zaher et al. 2019). However, the relationships among these subfamilies and the relationships among genera in a specific subfamily are still unclear since varied genes have been applied in phylogenetic statistics (Lawson et al. 2005; Pyron et al. 2013a, b; Figueroa et al. 2016; Zheng and Wiens 2016; Zaher et al. 2019). Boiga kraepelini Stejneger, 1902 and other Boiga species are arboreal snakes distributed in Asia, Australia and Pacific islands (Weinell et al. 2021). As a genus belonging to Colubridae, Boiga species share the characteristics of rapid movement with other colubrid species, with the exception of posterior groove teeth and low toxicity. Species listed in the genus Hebius are mainly distributed in the eastern, southern and southeastern regions of Asia (Guo et al. 2012). They are usually small- to medium-sized snakes and considered innocuous (Zhao 2006). More evidence should be obtained to understand their phylogenetic position since Hebius is a relatively new genus split from the genus Amphiesma in recent years (Guo et al. 2014).

The mitochondrial genomes of snakes are circular molecules that contain 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, 2 ribosomal RNA (rRNA) genes, and one or two duplicate control regions. Due to the advantages of small size, matrilineal inheritance, relatively stable genetic structure, easy amplification and high evolutionary rate, partial or full sequences of the mitogenome have been extensively used in molecular evolution, comparative and evolutionary genomics, phylogenetics and population genetics research in various animal species (Kim et al. 2018; Huang et al. 2019). With the development of sequencing technology, a large number of animal mitochondrial genomes have been sequenced and sequences are becoming more accessible (Zhou et al. 2016; Wang et al. 2019). As an informative molecular marker, phylogenetic relationships based on the mitogenome often result in better resolution, reliability and robustness than those of other molecular markers (Madsen et al. 2001). A previous study showed that B. kraepelini was the sister lineage to all 23 other Boiga species (Weinell et al. 2021) and that Hebius is a monophyletic genus (Guo et al. 2014) based on a few gene fragments. Here, the complete mitogenomes of B. kraepelini and H. craspedogaster Boulenger, 1899 were sequenced, annotated and characterized for the first time. To better understand the relationships among Colubridae, complete sequences of 13 mitochondrial PCGs from 38 species of Colubridae and two outgroup species were used to construct a comprehensive phylogenetic tree.
Materials and methods

Sampling and DNA extraction

Specimens of *B. kraepelini* and *H. craspedogaster* were collected from Jinhua, China (29°12’N, 119°37’E). Total genomic DNA (gDNA) was extracted from tail muscle using a Rapid Animal Genomic DNA Isolation Kit (Sangon Biotech, China) according to the manufacturer’s instructions.

PCR amplification and sequencing

Conventional polymerase chain reaction (PCR) assays were conducted to amplify the complete mitogenomes of *B. kraepelini* and *H. craspedogaster*. The specific primers were designed based on the known nucleotide sequences (Suppl. material 1: Table S1) (Guo et al. 2012; Li et al. 2020; Weinell et al. 2021). Amplification was performed in a total volume of 50 μL, which contained 25 μL of 2× Es Taq MasterMix (CW BIO, China) of 3.0 mM MgCl₂, each dNTP at 0.40 mM and 1.0 U of Taq DNA polymerase per μL, 2 μL each of forward and reverse primers (10 μM), 2 μL template DNA and 19 μL of sterilized water. The thermal cycling procedure was applied as follows: an initial pre-denaturation step at 95 °C for 3 min, followed by 35 cycles at 95 °C for 30 s, 60 °C for 45 s, and 72 °C elongation for 1–4 min (depending on the size of fragments), with a final extension at 72 °C for 10 min. The PCR products were recycled and purified using 1.5% agarose gel electrophoresis and genotyped using Sanger sequencing by Sangon Biotech (Shanghai) Co., Ltd., China.

Sequence assembly and gene annotation

The obtained sequences were identified using the Basic Local Alignment Search Tool (BLAST) from NCBI and were assembled using SeqMan software (DNAStar Inc., USA). The complete mitochondrial sequences were annotated by the MITOS web server (http://mitos.bioinf.uni-leipzig.de/index.py) (Bernt et al. 2013) and corrected manually. Transfer RNA (tRNA) genes were identified and predicted in the tRNAscan-SE search server (http://lowelab.ucsc.edu/tRNAscan-SE/) (Lowe and Chan 2016) using the vertebrate genetic code, and their secondary structures were visualized in the Forna web server (http://rna.tbi.univie.ac.at/forna/forna.html) (Kerpedjiev et al. 2015). The base composition of the mitogenome and the relative synonymous codon usage (RSCU) of PCGs were determined using MEGA X (Kumar et al. 2018). The skewness of nucleotide composition was measured according to the following formulas: AT-skew = (A – T) / (A + T) and GC-skew = (G – C) / (G + C) (Perra and Kocher 1995). Graphical maps of the complete mitochondrial genomes were drawn using the online visualization tool mtviz (http://pacosy.informatik.uni-leipzig.de/mtviz).
Phylogenetic analyses

To understand the phylogenetic positions of *B. kraepelini* and *H. craspedogaster*, the complete mitochondrial sequences of 13 PCGs in 38 previously available species of Colubridae and two outgroups (*Naja atra* and *Hypsiscopus plumbea*) were obtained from GenBank (Table 1). Since nucleotide sequences with substitution saturation has previously plagued phylogenetic analyses, the suitability for phylogenetic tree construction from the dataset was tested first using DAMBE7 software (Xia 2018). The nucleotide sequences were aligned through the MAFFT v.7.475 program with default settings (Katoh et al. 2002). Sequence gaps and poorly aligned regions were removed using Gblocks v.0.91 (Castresana 2000). The best-fit substitution model for the dataset was GTR + I + G by jModelTest v.2.1.10 (Darriba et al. 2012) based on Akaike Information Criterion (AIC). Phylogenetic analyses were performed using Bayesian inference (BI) and maximum likelihood (ML) methods by MrBayes v.3.2.7 (Ronquist and Huelsenbeck 2003) and IQ-TREE v.2.1.2 (Minh et al. 2020), respectively. Four independent runs were conducted using the default settings for 5,000,000 generations with a sampling frequency of 1000 and a burn-in of 25% of samples with Bayesian analyses. Only when the average standard deviation of the split frequencies was less than 0.01 and the effective sampling size greater than 200 were the Markov chain Monte Carlo (MCMC) chains considered convergent. All parameters were assessed by Tracer v.1.7.1 (Rambaut et al. 2018). In the ML analyses, branch support was estimated by 1000 ultrafast bootstrap replicates. The resultant trees were visualized using FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Results and discussion

Genome content and organization

The complete mitogenomes of *B. kraepelini* and *H. craspedogaster* (GenBank accession numbers: MW699848 and MW699847, respectively) were closed double stranded DNA molecules 17,124 bp and 17,120 bp in length, respectively (Fig. 1). Both contained 37 typical mitochondrial genes, including 13 PCGs, 22 tRNA genes, two rRNA genes (*rrnS* and *rrnL*), two putative control regions (CRs) and one origin of light-strand replication (*OL*). Among these genes, 28 were encoded on the heavy strand, while the remaining nine genes, including one PCG (*nad6*) and eight tRNAs (*trnQ, trnA, trnN, trnC, trnY, trnS2, trnE* and *trnP*), were located on the light strand (Fig. 1, Table 2). The arrangement of genes in these two species was consistent with other species of snakes (Dong and Kumazawa 2005; Li 2014; Qian 2018). The nucleotide composition of *B. kraepelini* was 34.81% A, 24.22% T, 28.61% C and 12.36% G, and that of *H. craspedogaster* was 34.04% A, 26.89% T, 26.34% C and 12.73% G. Both species showed a significant bias toward A + T (59.03% for *B. kraepelini* and 60.93% for *H. craspedogaster*). In addition, the positive AT skew (0.179 and 0.117) and negative GC skew (-0.397 and -0.348) for *B. kraepelini* and *H. craspedogaster*, respectively,
Mitogenomes of *Boiga kraepelini* and *Hebius craspedogaster*

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| Elapidae       | *Naja atra*                 | EU913475      |
| Homalopsidae   | *Hypsiscopus plumbea*       | DQ343650      |

indicated higher frequencies of A and C than of T and G present in the whole mitogenome (Table 3). The biased A+T content and skewness in nucleotide composition of *B. kraepelini* and *H. craspedogaster* were highly similar to those of other Colubridae species (He et al. 2010; Sun et al. 2017; Wang et al. 2019).
Protein-coding genes and codon usage

The lengths of 13 PCGs of *B. kraepelini* and *H. craspedogaster* varied from 159 bp (*atp8*) to 1764 bp (*nad5*) and from 165 bp (*atp8*) to 1782 bp (*nad5*), respectively (Table 2). The A+T content, AT skew and GC skew of the 13 PCGs in *B. kraepelini* and *H. craspedogaster* were 59.01% and 61.92%, 0.203 and 0.122, and -0.463 and -0.415, respectively (Table 3). Excluding terminal codons, a total of 3751 codons were used to encode proteins of *B. kraepelini*, while a total of 3759 codons were used to encode proteins of *H. craspedogaster*. All PCGs started with a standard ATN codon (ATA, ATT or ATG) and ended with the stop codon TAA, AGG, AGA or a single T in both species (Table 2). The incomplete stop codon T was frequently found in both species and in other animal mitogenomes (Ojala et al. 1981; Ki et al. 2010; Tang et al. 2020), which might be the result of post-transcriptional polyadenylation (Donath et al. 2019). Relative synonymous codon usage (RSCU), as a key parameter, was used to evaluate the bias of the synonymous codon, and the values obtained reflecting codon usage preference directly in certain gene samples (Table 4). For *B. kraepelini* and *H. craspedogaster*, the RSCU showed bias toward AT rather than GC at the third codon position. Twenty-five out of all 60 codons were regarded as abundant since these synonymous codons had positive codon usage bias (RSCU value > 1.0). However, the remaining codons, except for the UCU codon (RSCU value = 1.0) in *H. craspedogaster*, had negative codon usage bias (RSCU value < 1.0), and they were considered less abundant codons (Li et al. 2018). Furthermore, threonine, leucine 1, and isoleucine were the most common amino acids, while cysteine, serine 1, and aspartic acid were the least common amino acids in these two species.
Table 2. Summary of the mitogenomes of *Boiga kraepelini* and *Hebius craspedogaster*.

<table>
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<th>Hebius craspedogaster</th>
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</tr>
<tr>
<td>trnS1</td>
<td>H</td>
<td>12402–12458</td>
<td>57</td>
<td>–</td>
</tr>
<tr>
<td>trnL1</td>
<td>H</td>
<td>12456–12526</td>
<td>71</td>
<td>–</td>
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<tr>
<td>nad5</td>
<td>H</td>
<td>12527–14290</td>
<td>1764</td>
<td>ATG/TAA</td>
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<tr>
<td>nad6</td>
<td>L</td>
<td>14286–14798</td>
<td>513</td>
<td>ATG/AGGG</td>
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<tr>
<td>trnE</td>
<td>L</td>
<td>14799–14860</td>
<td>62</td>
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<tr>
<td>cob</td>
<td>H</td>
<td>14861–15977</td>
<td>1117</td>
<td>ATG/T</td>
</tr>
<tr>
<td>trnT</td>
<td>H</td>
<td>15978–16043</td>
<td>66</td>
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Transfer RNA, ribosomal RNA genes and the A + T-rich region

Similar to other snakes, 22 tRNA genes were recovered from the mitogenomes of *B. kraepelini* and *H. craspedogaster*. The tRNA lengths of these two species ranged from 57 bp (*trnS1*) to 73 bp (*trnL2*) (Table 2). The AT content of *B. kraepelini* and
H. craspedogaster were between 43.94% (trnI) and 66.20% (trnQ) and 43.75% (trnK) and 66.67% (trnI), respectively (Suppl. material 1: Table S2). In addition, the tRNA genes of B. kraepelini and H. craspedogaster had a positive AT skew (0.16 and 0.13, respectively) and a negative GC skew (-0.21 and -0.16, respectively) (Table 3). All tRNA genes, except trnS1 and trnC, showed typical cloverleaf secondary structures (Figs 2, 3). The trnS1 gene lacked a dihydroxyuridine arm (D arm), and the trnC gene lacked the TΨC loop. Deletions of the D arm and/or TΨC loop in tRNA genes of the mitogenome are known to occur in other Colubridae species (Li 2014). tRNA genes may lack the D arm or the T arm may exhibit lower amounts of peptide production or lower levels of aminoacylation and EF-Tu binding abilities (Watanabe et al. 2014). No pseudogene trnP was found between mitochondrial genes trnI and CR2 in either species, although it was present in some snakes (Kumazawa et al. 1998; Dong and Kumazawa 2005; Jiang et al. 2007). Species without pseudogene trnP were considered primitive snakes (Wang et al. 2009). Different from the typical arrangement of the mitogenome in vertebrates, here trnL (UUR) translocated from its original position between rrnL and nad1 to the position between CR2 and trnQ. The rearrangement of the trnL (UUR) gene is common in Alethinophidia (Dong and Kumazawa 2005; Yan et al. 2008; Chen and Zhao 2009).

As shown in Table 2, the gene rrnS in B. kraepelini was 917 bp in length and located between trnF and trnV, while the gene rrnL was 1456 bp in length and located between trnV and nad1. The rrnS and rrnL genes in H. craspedogaster were 8 bp longer and 11 bp shorter, respectively, than in B. kraepelini. These two rRNA genes were AT biased; the A+T content of rrnS genes was 55.29% in B. kraepelini and 57.08% in H. craspedogaster, and the A+T content of rrnL genes was 61.13% in B. kraepelini and 60.90% in H. craspedogaster (Table 3). Both rrnS and rrnL in the two species showed the same nucleotide composition of the mitogenome: A > C > T > G.

Additionally, similar to some snakes, there were two control regions in both species mitogenomes, in which CR1 was located between trnP and trnF, and CR2 was located between trnI and trnL (UUR). The nucleotide composition and length of the two control regions in the same species were almost identical. The AT skews and GC skews of the two CRs in B. kraepelini and H. craspedogaster were negative, indicating that T and C were more numerous than A and G (Table 3).

### Table 3. Nucleotide composition of Boiga kraepelini and Hebius craspedogaster mitogenomes; the values for B. kraepelini are shown before the slash (/) and of H. craspedogaster are listed after the slash.

<table>
<thead>
<tr>
<th></th>
<th>A %</th>
<th>T %</th>
<th>G %</th>
<th>C %</th>
<th>A+T %</th>
<th>AT-skew</th>
<th>GC-skew</th>
</tr>
</thead>
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<tr>
<td>Mitogenome</td>
<td>34.81 / 34.04</td>
<td>24.22 / 26.89</td>
<td>12.36 / 12.73</td>
<td>28.61 / 26.34</td>
<td>59.03 / 60.93</td>
<td>0.18 / 0.12</td>
<td>-0.40 / -0.35</td>
</tr>
<tr>
<td>PCGs</td>
<td>35.48 / 34.72</td>
<td>23.53 / 27.19</td>
<td>11.00 / 11.15</td>
<td>29.99 / 26.94</td>
<td>59.01 / 61.92</td>
<td>0.20 / 0.12</td>
<td>-0.46 / -0.42</td>
</tr>
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<td>tRNAs</td>
<td>33.38 / 32.82</td>
<td>24.39 / 25.32</td>
<td>16.80 / 17.60</td>
<td>25.44 / 24.26</td>
<td>57.77 / 58.13</td>
<td>0.16 / 0.13</td>
<td>-0.21 / -0.16</td>
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<td>rrnS</td>
<td>36.75 / 36.97</td>
<td>18.54 / 20.11</td>
<td>17.78 / 17.84</td>
<td>26.94 / 25.08</td>
<td>55.29 / 57.08</td>
<td>0.33 / 0.30</td>
<td>-0.21 / -0.17</td>
</tr>
<tr>
<td>rrnL</td>
<td>40.80 / 39.65</td>
<td>20.33 / 21.25</td>
<td>15.38 / 16.40</td>
<td>23.49 / 22.70</td>
<td>61.13 / 60.90</td>
<td>0.34 / 0.30</td>
<td>-0.21 / -0.16</td>
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<tr>
<td>rRNAs</td>
<td>39.23 / 38.61</td>
<td>19.64 / 20.80</td>
<td>16.31 / 16.96</td>
<td>24.82 / 23.63</td>
<td>58.87 / 59.41</td>
<td>0.33 / 0.30</td>
<td>-0.21 / -0.16</td>
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<tr>
<td>CR1</td>
<td>27.67 / 26.37</td>
<td>33.17 / 33.43</td>
<td>11.68 / 12.64</td>
<td>27.48 / 27.56</td>
<td>60.84 / 59.80</td>
<td>-0.09 / -0.12</td>
<td>-0.40 / -0.37</td>
</tr>
<tr>
<td>CR2</td>
<td>27.17 / 26.24</td>
<td>33.20 / 33.00</td>
<td>11.86 / 12.92</td>
<td>27.77 / 27.85</td>
<td>60.38 / 59.23</td>
<td>-0.10 / -0.11</td>
<td>-0.40 / -0.37</td>
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<tr>
<td>CRs</td>
<td>27.42 / 26.30</td>
<td>33.19 / 32.22</td>
<td>11.77 / 12.78</td>
<td>27.62 / 27.71</td>
<td>60.61 / 59.52</td>
<td>-0.10 / -0.12</td>
<td>-0.40 / -0.37</td>
</tr>
</tbody>
</table>
Mitogenomes of *Boiga kraepelini* and *Hebius craspedogaster*

Phylogenetic analyses

Phylogenetic trees were constructed based on nucleotide sequences of 13 PCGs in 38 Colubridae species and two outgroups from the families Elapidae and Homalopsidae (Fig. 4). An identical topological structure was produced using both BI and ML methods. Five monophyletic clades that represented five subfamilies, Colubrinae, Natricinae, Sibynophiinae, Dipsadinae and Pseudoxenodontinae, were identified in the family Colubridae. The tree showed a close relationship (BI posterior probabilities [PP] = 1; ML bootstrap [BP] = 67) between Natricinae and Sibynophiinae, and the subfamily Colubrinae was a sister clade of the clade containing Natricinae and Sibynophiinae. These results were consistent with the findings from previous phylogenetic studies (Figueroa et al. 2016; Zaher et al. 2019). In terms of species, *B. kraepelini* was well supported as most closely related to the genus *Lycodon* in the subfamily Colubrinae. In addition, both Figueroa et al. (2016) and Weinell et al. (2021) reported that the genus *Boiga* was the sister group of the genus *Lycodon* based on multiple mitochondrial segments and nuclear genes. *Hebius craspedogaster* was clustered with other *Hebius* species and formed a monophyletic clade. The monophyly of the genus *Hebius* was also supported by multi-locus (Deepak et al. 2021) and morphological (Hou et al. 2021) phylogenetic analyses.
Both *Boiga* and *Hebius* are species-rich genera in the family Colubridae, with more than 30 species each (Uetz et al. 2022). The phylogenetic relationships within each genus are still unresolved since there are still some species with uncertain systematic positions (Pyron et al. 2013a, 2013b; Deepak et al. 2021). The first mitogenome sequence of *Boiga* and the complete mitochondrial sequence of *H. craspedogaster* from this study will provide more molecular evidence to clarify their taxonomic status and understand potential unknown evolutionary relationships.

**Conclusions**

In this study, we sequenced and characterized the complete mitochondrial genomes of *B. kraepelini* and *H. craspedogaster* for the first time. The mitogenomes of *B. kraepelini* and *H. craspedogaster* were 17,124 bp and 17,120 bp in size, respectively, including 13 PCGs, 22 tRNAs, two rRNAs and two control regions. Both (*B. kraepelini* and *H. craspedogaster*) genome compositions were A+T biased (59.03% and 60.93%, respectively) and showed positive AT skews (0.179 and 0.117, respectively) and negative GC skews (-0.397 and -0.348, respectively). All of the tRNA genes could be
Figure 4. Phylogenetic tree inferred from the nucleotide sequences of 13 mitogenome protein-coding genes using the Bayesian inference (BI) and maximum likelihood (ML) methods. Values on branches separated by slash (/) indicate posterior probability (BI, left) and bootstrap (ML, right).

Table 4. Amino acid composition and relative synonymous codon usage (RSCU) in the mitogenome of Boiga kraepelini and Hebius craspedogaster. RSCU values of B. kraepelini are shown before the slash (/) and of H. craspedogaster are listed after the slash.
folded into typical cloverleaf secondary structures, with the exception of \textit{trnS1}, which lacks the D arm, and \textit{trnC}, which lacks the T \(\Psi\) C loop. Phylogenetic analyses were performed with 38 other species from the family Colubridae and two outgroup species. Five clades that represent five subfamilies, Colubrinae, Natricinae, Sibynophiinae, Dipsadinae and Pseudoxenodontinae, were identified. The genus \textit{Boiga} was closely related to the genus \textit{Lycodon}, and both genera belong to the subfamily Colubrinae. \textit{Hebius craspedogaster} was clustered with the other two \textit{Hebius} species and closely related to other Natricinae species. This work will be helpful for understanding the evolutionary relationships within the family Colubridae and will provide basic data for the molecular identification of these two species.

**Acknowledgements**

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**References**


Supplementary material I

Table S1, S2

Authors: Shuangshuang Shan, Yu Wang
Data type: docx file

Explanation note: Table S1. Primers used for mitogenome amplification of Boiga kraepelini and Hebius craspedogaster. Table S2. Nucleotide composition of each tRNA of Boiga kraepelini and Hebius craspedogaster.

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