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Characterization of the complete mitochondrial genome of *Brentisentis yangtzensis* Yu & Wu, 1989 (Acanthocephala, Illiosentidae)

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Abstract

The mitogenome of *Brentisentis yangtzensis* is 13,864 bp in length and has the circular structure typical of metazoans. It contains 36 genes: 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs) and 12 protein-encoding genes (PCGs). All genes are transcribed from the same strand. Thirteen overlapping regions were found in the mitochondrial genome. The overall A+T content of *B. yangtzensis* is 68.3% versus 31.7% of G+C content (A = 27.8%, T = 40.5%, C = 9.0%, G = 22.7%). *B. yangtzensis* (Illiosentidae) and *Leptorhynchoides thecatus* (Rhadinorhynchidae) form a sister clade, showing the relatively close relationship between the Illiosentidae and the Rhadinorhynchidae. The mitochondrial gene arrangements of acanthocephalan species are relatively conserved, with only a few translocations of tRNAs (trnS1, trnS2, trnV, and trnK) detected. An identical gene order was found both in a sister clade (*Centrorhynchus aluconis* and *Plagiorhynchus transversus*) and across different classes (*B. yangtzensis* (Palaeacanthocephala), *Acanthosentis cheni* (Eoacanthocephala) and *Macracanthorhynchus hirudinaceus*(Archiacanthocephala), *Oncicola luehei* and *L. thecatus* (Palaeacanthocephala)). More studies and more sequences of acanthocephalan species are needed to gain a clear understanding of the phylogenetic relationships.

Keywords

Echinorhynchida, gene order, molecular phylogeny

Introduction

Members of the Acanthocephala are obligate endoparasites which utilize arthropods as intermediate hosts and vertebrates as definitive hosts. This phylum contains approximately 1300 documented species, and is classified into three classes (Archiacanthocephala, Palaeacanthocephala and Eoacanthocephala). The Palaeacanthocephala has the highest species richness with 65% of the total acanthocephalan species, and comprises three orders: Echinorhynchida, 472 species; Polymorphida, 372 species; and Heteramorphida, one species (Amin 1987, 2013). The classifications proposed by Golvan (1960) and Amin (1987) have been challenged by recent phylogenetic studies, which indicated that the genus *Leptorhynchoides* (Rhadinorhynchidae Kostylew, 1924) is more closely related to the genera of the Illiosentidae Golvan, 1960 rather than those of the Rhadinorhynchidae in morphological (Monks 2001) and the molecular phylogenies (García-Varela and Nadler 2005; García-Varela and González-Oliver 2008). Intriguingly, *Illiosentis* Van Cleave & Lincicome, 1939 was first placed in the Rhadinorhynchidae (Van Cleave and Lincicome 1939), but Golvan (1960) decided that a new family was required to accommodate the genus, and so erected the Illiosentidae.

This debate continues, and molecular markers carrying stronger phylogenetic signals are needed to resolve the phylogenetic relationships with a higher resolution. The mitogenome is a good candidate, being approximately ten times larger than commonly used single-gene molecular markers (ITS, 18S, and 28S) (Zhang et al. 2018), and considered to provide the best interrelationship estimate for the Cestoda (Waeschenbach et al. 2012). Mitochondrial genome sequences are becoming prevalent and are increasingly used in population genetics (Yin et al. 2015), phylogenetics (Gazi et al. 2012, 2015, 2016; Weber et al. 2013; Li et al. 2017; Pan and Nie 2013) and the diagnostics (Huyse et al. 2008; Jia et al. 2010) of metazoans. However, many groups of parasitic organisms are unrepresented, and the resolving power of mitochondrial genomics is still limited by the small number of acanthocephalan mitogenomes (only 13 species available), with many taxonomic categories, e.g. the Illiosentidae, poorly represented or unrepresented.

The complete mitogenome of an illiosentid species has not previously been published. In order to fill this knowledge gap, we have sequenced and annotated the complete mitogenome of *Brentisentis yangtzensis* Yu & Wu, 1989 (Palaeacanthocephala, Illiosentidae), a parasite from the intestines of many freshwater fish species in the middle reaches of the Yangtze River (Yi and Wu 1989). Previous studies on this parasite have focused on its morphology and population ecology (Fang 1999, Fang and Dai 2000; Fang et al. 2004); molecular data have not previously been reported.

Materials and methods

Specimen collection and DNA extraction

The acanthocephalans were collected on 24 September 2018 from the intestine of 36 bullhead catfish *Tachysurus fulvidraco* (Richardson, 1846) from east Dongting Lake in

Yueyang (29°22'N, 113°06'E), Hunan Province, China. *Brentisentis yangtzensis* was identified by morphology (e.g., Yu 1989) using a stereomicroscope and a light microscope. The parasites were preserved in 100% ethanol and stored at 4 °C. The total genomic DNA was extracted from an entire acanthocephalan using a TIANamp Micro DNA Kit (Tiangen Biotech, Beijing, China) according to manufacturer's recommended protocol, and stored at -20 °C. Eleven acanthocephalans were collected in total.

PCR and DNA sequencing

Partial sequences of rrnL, cytb, nad1, and rrnS genes were amplified via a polymerase chain reaction (PCR) using four primer pairs. Based on these fragments, we designed specific primers for subsequent PCR amplification (Suppl. material 1). PCR reactions were conducted in a 50 ml reaction mixture, containing 18.5 ml double-distilled water (dd H_2O), 25 ml 2×PCR buffer (Mg^{2+}, dNTP plus, Takara, China), 1.5 ml of each primer, 1 ml rTaq polymerase (250U, Takara, China) and 2.5 ml DNA template. Amplification was performed under the following conditions: initial denaturation at 98 °C for 2 min, followed by 40 cycles at 98 °C for 10 s, 48–60 °C for 15 s, 68 °C for 1 min/kb, and a final extension at 68 °C for 10 min. PCR products were sequenced bidirectionally at Sangon Biotech (Shanghai) Co., Ltd. (China) using the primer walking strategy.

Sequence annotation analyses

The mitogenome of *B. yangtzensis* were assembled manually in a stepwise manner with the help of the DNAstar v7.1 program (Burland 2000), after quality-proofing of the obtained fragment. The mitogenome was annotated mainly following the procedures described previously (Zou et al. 2017; Zhang et al. 2017a; Li et al. 2017). In detail, protein-coding genes (PCGs) were inferred with the help of BLAST and ORF Finder tools (both available from the National Center for Biotechnology Information (NCBI)), employing the invertebrate mitochondrial code (Codon Table 5), and checking the nucleotide alignments against the reference genomes in acanthocephalan *Leptorhynchoides thecatus* (Linton, 1891) Kostylew, 1924 (NC_006892). A majority of the tRNAs were identified using the results of ARWEN (Laslett and Canback 2008) and MITOS web server (Bernt et al. 2013), the rest were found by alignment with other acanthocephalans (Suppl. material 2). Two ribosomal RNA genes (rrnL and rrnS) were found by alignment with other published acanthocephalan mitogenomes, and their ends were assumed to extend to the boundaries of their flanking genes. Codon usage and relative synonymous codon usage (RSCU) for 12 protein-encoding genes (PCGs) of the *B. yangtzensis* and *L. thecatus* (NC_006892) were computed and sorted using PhyloSuite (Zhang et al. 2018), and finally the RSCU figure drawn using ggplot2 plugin (Wickham 2016). The circular map of *B. yangtzensis* mitogenome was drawn with the mitochondrial visualization tool MTVIZ (http://pacosy.informatik.uni-leipzig.de/mtviz/).

Phylogenetic analyses

Phylogenetic analyses were carried out on the newly sequenced mitogenome of *B. yangtzensis* and the 12 acanthocephalan mitogenomes available in GenBank (Suppl. Table S2). Two species of the Bdelloidea, *Rotaria rotatoria* (Pallas, 1766) (NC013568.1) and *Philodina citrina* Ehrenberg, 1832 (FR856884.1), were used as outgroups. Fasta files with the amino acid sequences for all 12 PCGs were extracted from the GenBank files using PhyloSuite. All the genes were aligned in batches with MAFFT (Katoh et al. 2002) integrated in PhyloSuite, using normal-alignment mode. PhyloSuite was then used to concatenate these alignments into a single alignment and generate phylip and nexus format files for the phylogenetic analyses, conducted using maximum likelihood (ML) and Bayesian inference (BI) methods. The selection of the best-fit partition strategy and models was carried out using PartitionFinder2 (Lanfear et al. 2017). ML analysis was performed using IQ-TREE (Nguyen et al. 2015) with 50, 000 Ultrafast bootstraps (Minh et al. 2013). BI analysis was performed in MrBayes 3.2.6 (Ronquist et al. 2012) with the default settings, and 3×10^6 metropolis-coupled Markov Chain Monte Carlo generations.

Results and discussion

Genome organization and base composition

The circular duplex molecule mitogenome of *B. yangtzensis* is 13,864bp in size (GenBank accession number MK651258) and contains all 36 of the typical metazoan genes: 22 tRNA genes, 2 rRNA genes and 12 protein-encoding genes (PCGs) (lacking atp8) (Fig. 1). All genes are transcribed from the same strand, and 13 overlapping regions were found in the genome (Table 1). The lack of the gene atp8 is common in acanthocephalans (Gazi et al. 2016; Song et al. 2016) with one exception; in *L. thecatus* two putative atp8 genes have been suggested (Steinauer et al. 2005).

Protein-coding genes and codon usage

The total length of the concatenated 12 protein-coding genes is 10,355 bp, with the average A+T content of 68.0%, ranging from 65.9% (nad3) to 69.5% (atp6 and nad4) (Suppl. material 3). ATG (for 6 PCGs) is the most commonly used start codon, whereas nad6, nad4, cox1 and cox2 used GTG, nad1 and nad3 used TTG and ATA, respectively. The most frequent terminal codons are TAG (for 7 PCGs), followed by T (4 PCGs) (Table 1).

Codon usage, relative synonymous codon usage (RSCU) and codon family proportion (corresponding to the amino acids usage) of *B. yangtzensis* and *L. thecatus* (NC_006892) is presented (Suppl. material 4). Leucine (16.28%), valine (11.92%)

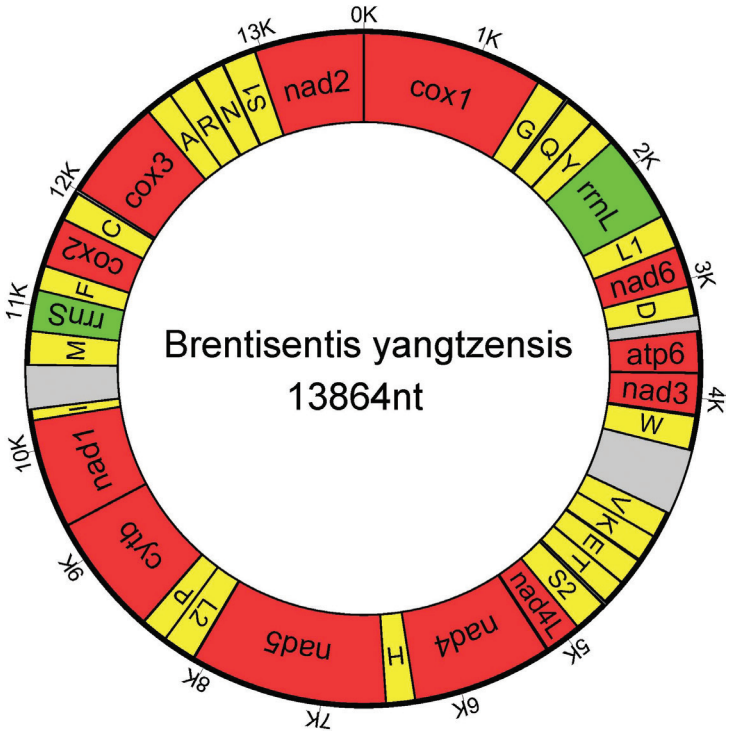

Figure 1. Map of the complete mitochondrial genome of *Brentisentis yangtzensis.*

and serine (10.94%) are the most frequent amino acids in the PCGs of *B. yangtzensis*, whereas glutamine (0.81%), arginine (1.36%) and histidine (1.44%) are relatively scarce. A higher T content (42.2%) in 12 PCGs corresponds to a relatively high frequency of T-rich codons: TTA for leucine (9.9%), TTT for phenylalanine (7.2%), ATT for isoleucine (6.1%) and GTT for valine (5.0%).

Transfer and ribosomal RNA genes

All 22 commonly found tRNAs are present in the mitogenome of *B. yangtzensis*, ranging from 50 bp (trnS2) to 70 bp (trnT) in size, with a concatenated length of 1,247 bps (Table 1).

The genes rrnL and rrnS are 912 bp and 574 bp in size, with 71.8% and 69.8% A+T content, respectively (Suppl. Material 3). The location of rrnL is between trnY and trnL1, and rrnS is located between trnM and trnF; this is the same arrangement reported for other acanthocephalans (Fig. 2), except for *Hebesoma violentum* (Van Cleave, 1928) Salgado-Maldonado, 1978 in which rrnS is located between trnS1 and trnF (Pan and Nie 2014).

Table 1. Annotated mitochondrial genome of *Brentisentis yangtzensis*.

Gene	Position		Size	Intergenic nucleotides	Codon		Anti-codon
	From	To			Start	Stop	
cox1	1	1531	1531	–	GTG	T	–
trnG	1532	1585	54	–	–	–	TCC
trnQ	1565	1630	66	-21	–	–	TTG
trnY	1626	1678	53	-5	–	–	GTA
rrnL	1679	2590	912	–	–	–	–
trnL1	2591	2644	54	–	–	–	TAG
nad6	2645	3080	436	–	GTG	T	–
trnD	3081	3135	55	–	–	–	GTC
atp6	3240	3797	558	104	ATG	TAG	–
nad3	3794	4147	354	-4	ATA	TAG	–
trnW	4138	4197	60	-10	–	–	TCA
trnV	4635	4694	60	437	–	–	TAC
trnK	4695	4755	61	–	–	–	CTT
trnE	4747	4800	54	-9	–	–	TTC
trnT	4803	4872	70	2	–	–	TGT
trnS2	4851	4900	50	-22	–	–	TGA
nad4L	4901	5149	249	–	ATG	TAA	–
nad4	5159	6413	1255	9	GTG	T	–
trnH	6414	6466	53	–	–	–	GTG
nad5	6467	8110	1644	–	ATG	TAG	–
trnL2	8106	8159	54	-5	–	–	TAA
trnP	8160	8211	52	–	–	–	TGG
cytb	8215	9346	1132	3	ATG	T	–
nad1	9345	10242	898	-2	TTG	T	–
trnI	10243	10301	59	–	–	–	GAT
trnM	10595	10651	57	293	–	–	CAT
rrnS	10652	11225	574	–	–	–	–
trnF	11226	11281	56	–	–	–	GAA
cox2	11281	11931	651	-1	GTG	TAG	–
trnC	11931	11983	53	-1	–	–	GCA
cox3	12005	12736	732	21	ATG	TAG	–
trnA	12736	12791	56	-1	–	–	TGC
trnR	12793	12854	62	1	–	–	TCG
trnN	12846	12900	55	-9	–	–	GTT
trnS1	12894	12946	53	-7	–	–	ACT
nad2	12949	13863	915	2	ATG	TAG	–

Phylogeny

BI and ML yielded phylograms with identical topology and strong statistical support for all nodes (BP ≥ 75, BPP ≥ 0.96) (except the branch of *Centrorhynchus aluconis* (Müller, 1780) Lühe 1911 and *Southwellina hispida* (Van Cleave, 1925) Witenberg, 1932 52/0.96). Since both phylograms have an identical topology, only the latter was shown (Fig. 2). The resulting combined phylogenetic tree depicted almost the same results found in previous mitogenomic studies (Gazi et al. 2016), except for the position of *B. yangtzensis* (Palaea-

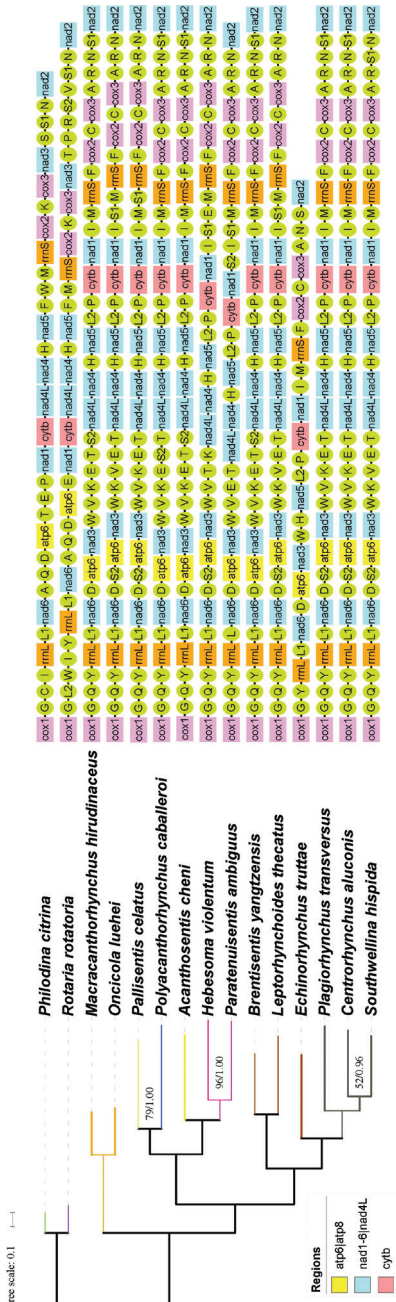

Figure 2. Phylogenetic tree of acanthocephalans inferred from maximum likelihood analysis with concatenated nucleotide sequence of all 36 genes (12 PCGs, 2 rRNAs, and 22 tRNAs). Bootstrap (BS)/Bayesian posterior probability (BPP) support values are shown above the nodes, only BS < 100 and BPP < 1 are displayed.

canthocephala, Illiosentidae), as this was included for the first time in the present study. Within the Acanthocephala, tree topology indicates the existence of two major clades: class Archiacanthocephala (monophyletic and the most basal clade) and the other two classes (Eoacanthocephala + Palaeacanthocephala), resulting in the three monophyletic clades corresponding to the three classes (Archiacanthocephala, Eoacanthocephala, and Palaeacanthocephala) in the most widely accepted classification of the Acanthocephala.

The Echinorhynchida is paraphyletic, with three species separated into two clades: *Echinorhynchus truttae* Schrank, 1788 (Echinorhynchidae) formed a sister clade with species of the Polymorphida and *B. yangtzensis* (Illiosentidae) formed a sister clade with *L. thecatus* (Rhadinorhynchidae). The result shows the relatively close relationship between the Illiosentidae and the Rhadinorhynchidae; however, as each family of the Echinorhynchida was represented by a single species in our study, this topology should be interpreted with some caution. Previous studies have shown the close relationship between *Leptorhynchoides* (Rhadinorhynchidae) and a genus of the Illiosentidae (Monks 2001; García-Varela and Nadler 2005; García-Varela and González-Oliver 2008). Two species of the Gyracanthocephala Van Cleave, 1936, one (*Pallisentis celatus* (Van Cleave, 1928) Baylis, 1933) formed a sister clade with *Polyacanthorhynchus caballeroi* Diaz-Ungria et Rodrigo, 1960 (Polyacanthorhynchida Amin, 1987), and another (*Acanthosentis cheni* Amin, 2005) nested with species of the Neoechinorhynchida. This result suggests paraphyly of the Gyracanthocephala and corresponds with previous phylogenetic analyses (Song et al. 2016). As the lack of the mitochondrial genome information on acanthocephalan species, more studies and sequences of acanthocephalan (Illiosentidae and Rhadinorhynchidae) species are needed to gain a clear understanding of the phylogenetic relationships of these acanthocephalans.

Gene order

The mitochondrial gene arrangements of acanthocephalan species are relatively conserved (Fig. 2). Besides the incomplete mitochondrial genome of *E. truttae*, the gene arrangement of 12 protein coding genes and two rRNA genes are highly conserved, with only a few translocations of tRNAs (trnS1, trnS2, trnV, and trnK) detected. In mitochondrial genomes, conserved gene arrangement is considered to be a typical characteristic (Boore 2000; Li et al. 2012), but the arrangement of tRNAs shows more variability, and there are examples of extensive gene rearrangement (Hyman et al. 2011; Zhang et al. 2017b; Wang et al. 2017). Transfer RNA genes are more often translocated than other genes, probably because of their small size (Kilpert and Podsiadlowski 2006).

In many cases, the gene order of the mitochondrial genome can form useful information in inferring phylogenetic relationships of metazoans (Littlewood et al. 2006; Luo et al. 2008; Wang et al. 2017; Zhang et al. 2018); however, for the taxa in our study, a phylogeny inferred from gene order is incompatible with that based on amino acid sequence (Fig. 2). The translocations of four tRNAs (trnS2, trnV, trnk, trnS1) were detected between *B. yangtzensis* and *L. thecatus* (Echinorhynchida), which formed

a monophyletic clade in the phylogenetic tree. However, only 1–4 translocations of tRNAs were detected among all of the acanthocephalan species. And these four tRNA (trnS2, trnV, trnk, trnS1) translocations were also detected within the Archiacanthocephala clade. Moreover, identical gene order was found both in a sister clade (*C. aluconis* and *Plagiorhynchus transversus* (Rudolphi, 1819) Travassos, 1926) and across different classes (*B. yangtzenensis* (Palaeacanthocephala), *A. cheni* (Eoacanthocephala), and *Macracanthorhynchus hirudinaceus* (Pallas, 1781) Travassos, 1917 (Archiacanthocephala); and *Oncicola luehei* (Travassos, 1917) Schmidt, 1972 (Archiacanthocephala) and *L. thecatus* (Palaeacanthocephala)).

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References

Amin OM (1987) Key to the families and subfamilies of Acanthocephala, with the erection of a new class (Polyacanthocephala) and a new order (Polyacanthorhynchida). Journal of Parasitology 73(6): 1216–1219. https://doi.org/10.2307/3282307

Amin OM (2013) Classification of the Acanthocephala. Folia Parasitologica 60(4): 273–305. https://doi.org/10.14411/fp.2013.031

Bernt M, Donath A, Juhling F, Externbrink F, Florentz C, Fritzsch G, Putz J, Middendorf M, Stadler PF (2013). MITOS: Improved de novo metazoan mitochondrial genome annotation. Molecular Phylogenetics and Evolution 69(2): 313–319. https://doi.org/10.1016/j.ympev.2012.08.023

Boore JL (2000) The duplication/random loss model for gene rearrangement exemplified by mitochondrial genomes of deuterostome animals. In: Sankoff D, Nadeau JH (Eds) Comparative Genomics: Kluwer Academic Publishers, Dordrecht, 133–147. https://doi.org/10.1007/978-94-011-4309-7_13

Boore JL, Fuerstenberg SI (2008) Beyond linear sequence comparisons: the use of genome-level characters for phylogenetic reconstruction. Philosophical Transactions of the Royal Society B- Biological Sciences 363(1496): 1445–1451. https://doi.org/10.1098/rstb.2007.2234

Burland TG (2000) DNASTAR's lasergene sequence analysis software. Methods in Molecular Biology 132: 71–91. https://doi.org/10.1385/1-59259-192-2:71

Dougherty EC (1951) Evolution of zooparasitic groups in the phylum Nematoda, with special reference to host-distribution. The Journal of Parasitology 37(4): 353–378. https://doi.org/10.2307/3273571

Fang JP (1999) Studies on the population ecology of *Brentisentis yangtzensis*. Chinese Journal of Ecology 18(6): 16–19.

Fang JP, Dai BS (2000) Studies on the site distributions of acanthocephalan worms in the intestine of *Pseudobaguas fulvidraco*. Chinese Journal of Zoology 35(5): 9–12.

Fang JP, Zhan HY, Lei GL (2004) An ecological study on acanthocephalans parasitic in the intestine of *Pseudobagrus eupogon* in Huangzhou section of the Yangtze River. Acta Hydrobiologica Sinica 28(3): 279–283.

García-Varela M, Nadler SA (2005) Phylogenetic relationships of Palaeacanthocephala (Acanthocephala) inferred from SSU and LSU rDNA gene sequences. Journal of Parasitology 91(6): 1401–1409. https://doi.org/10.1645/GE-523R.1

García-Varela M, González-Oliver A (2008) The systematic position of *Leptorhynchoides* (Kostylew, 1924) and *Pseudoleptorhynchoides* (Salgado-Maldonado, 1976), inferred from nuclear and mitochondrial DNA gene sequences. Journal of Parasitology 94(4): 959–962. https://doi.org/10.1645/GE-1420.1

Gazi M, Kim J, Park JK (2015) The complete mitochondrial genome sequence of *Southwellina hispida* supports monophyly of Palaeacanthocephala (Acanthocephala: Polymorphida). Parasitology International 64(4): 64–68. https://doi.org/10.1016/j.parint.2015.01.009

Gazi M, Kim J, García-Varela M, Park C, Littlewood DTJ, Park JK (2016) Mitogenomic phylogeny of Acanthocephala reveals novel class relationships. Zoologica Scripta 45(4): 437–454. https://doi.org/10.1111/zsc.12160

Gazi M, Sultana T, Min GS, Park YC, García-Varela M, Nadler SA, Park JK (2012) The complete mitochondrial genome sequence of *Oncicola luehei* (Acanthocephala: Archiacanthocephala) and its phylogenetic position within Syndermata. Parasitology International 61(2): 307–316. https://doi.org/10.1016/j.parint.2011.12.001

Ginestet C (2011) ggplot2: elegant graphics for data analysis. Journal of The Royal Statistical Society Series A- Statistics in Society 174: 245–245. https://doi.org/10.1111/j.1467-985X.2010.00676_9.x

Golvan YJ (1960) The phylum of Acanthocephala. Third note. The class of Palaeacanthocephala (Meyer 1931). Annales de Parasitologie Humaine et Comparée 35: 138–165. https://doi.org/10.1051/parasite/1960351138

Huyse T, Buchmann K, Littlewood DT (2008) The mitochondrial genome of *Gyrodactylus derjavinoides* (Platyhelminthes: Monogenea) – a mitogenomic approach for *Gyrodactylus* species and strain identification. Gene 417(1–2): 27–34. https://doi.org/10.1016/j.gene.2008.03.008

Hyman BC, Lewis SC, Tang S, Wu Z (2011) Rampant gene rearrangement and haplotype hypervariation among nematode mitochondrial genomes. Genetica 139(5): 611–615. https://doi.org/10.1007/s10709-010-9531-3

Jia WZ, Yan HB, Guo AJ, Zhu XQ, Wang YC, Shi WG, Chen HT, Zhan F, Zhang SH, Fu BQ, Littlewood DTL, Cai XP (2010) Complete mitochondrial genomes of *Taenia multiceps, T. hydatigena* and *T. pisiformis*: additional molecular markers for a tapeworm genus of human and animal health significance. BMC Genomics 11: 447. https://doi.org/10.1186/1471-2164-11-447

Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30: 3059–3066. https://doi.org/10.1093/nar/gkf436

Keane TM, Creevey CJ, Pentony MM, Naughton TJ, Mclnerney JO (2006) Assessment of methods for amino acid matrix selection and their use on empirical data shows that ad hoc assumptions for choice of matrix are not justified. BMC Evolutionary Biology 6:29. https://doi.org/10.1186/1471-2148-6-29

Kilpert F, Podsiadlowski L (2006) The complete mitochondrial genome of the common sea slater, *Ligia oceanica* (Crustacea, Isopoda) bears a novel gene order and unusual control region features. BMC Genomics 7: 241. https://doi.org/10.1186/1471-2164-7-241

Laslett D, Canback B (2008) ARWEN: a program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. Bioinformatics 24(2): 172–175. https://doi.org/10.1093/bioinformatics/btm573

Le TH, Blair D, McManus DP (2002) Mitochondrial genomes of parasitic flatworms. Trends in Parasitology 18(5): 206–213. https://doi.org/10.1016/S1471-4922(02)02252-3

Li H, Liu H, Shi AM, Stys P, Zhou XG, Cai WZ (2012) The complete mitochondrial genome and novel gene arrangement of the unique-headed bug *Stenopirates* sp. (Hemiptera: Enicocephalidae). Plos ONE 7: e29419. https://doi.org/10.1371/journal.pone.0029419

Littlewood DTJ, Lockyer AE, Webster BL, Johnston DA, Le TH (2006) The complete mitochondrial genomes of *Schistosoma haematobium* and *Schistosoma spindale* and the evolutionary history of mitochondrial genome changes among parasitic flatworms. Molecular Phylogenetics and Evolution 39(2): 452–467. https://doi.org/10.1016/j.ympev.2005.12.012

Li WX, Zhang D, Boyce K, Xi BW, Zou H, Wu SG, Li M, Wang GT (2017) The complete mitochondrial DNA of three monozoic tapeworms in the Caryophyllidea: a mitogenomic perspective on the phylogeny of eucestodes. Parasites & Vectors 10(1): 314. https://doi.org/10.1186/s13071-017-2245-y

Luo HW, Shi J, Arndt W, Tang JJ, Friedman R (2008) Gene order phylogeny of the genus Prochlorococcus. Plos One 3(12): e3837. https://doi.org/10.1371/journal.pone.0003837

Monks S (2001) Phylogeny of the Acanthocephala based on morphological characters. Systematic Parasitology 48(2): 81–115. https://doi.org/10.1023/A:1006400207434

Pan TS, Nie P (2013) The complete mitochondrial genome of *Pallisentis celatus* (Acanthocephala) with phylogenetic analysis of acanthocephalans and rotifers. Folia Parasitologica 60(3): 181–191. https://doi.org/10.14411/fp.2013.021

Pan TS, Nie P (2014) The cloning of the mitochondrial genome of *Hebesoma violentum* (Acanthocephala) and the phylogenetic analysis of acanthocephalans. Acta Hydrobiologica Sinica 38(2): 351–361.

Park JK, Sultana T, Lee SH, Kang, S, Kim HK, Min GS, Eom KS, Nadler SA (2011) Monophyly of clade III nematodes is not supported by phylogenetic analysis of complete mitochondrial genome sequences. BMC Genomics 12(1): 392. https://doi.org/10.1186/1471-2164-12-392

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. Organisms Diversity & Evolution 12(4): 335–337. https://doi.org/10.1007/s13127-011-0056-0

Song R, Zhang D, Deng S M, Ding DM, Liao FC, Liu LS (2016) The complete mitochondrial genome of *Acanthosentis cheni* (Acanthocephala: Quadrigyridae). Mitochondrial DNA Part B 1(1): 797–798. https://doi.org/10.1080/23802359.2016.1197076

Steinauer ML, Nickol BB, Broughton R, Ortí G (2005) First sequenced mitochondrial genome from the phylum Acanthocephala (*Leptorhynchoides thecatus*) and its phylogenetic position within Metazoa. Journal of Molecular Evolution 60(6): 706–715. https://doi.org/10.1007/s00239-004-0159-8

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28(10): 2731–2739. https://doi.org/10.1093/molbev/msr121

Van Cleave HJ, Lincicome DR (1939) On a new genus and species of Rhadinorhynchidae (Acanthocephala). Parasitology 31(4): 413–416. https://doi.org/10.1017/S0031182000012919

Waeschenbach A, Webster BL, Littlewood DTJ (2012) Adding resolution to ordinal level relationships of tapeworms (Platyhelminthes: Cestoda) with large fragments of mtDNA. Molecular Phylogenetics and Evolution 63(3): 834–847. https://doi.org/10.1016/j.ympev.2012.02.020

Wang JG, Zhang D, Jakovlić I, Wang WM (2017) Sequencing of the complete mitochondrial genomes of eight freshwater snail species exposes pervasive paraphyly within the Viviparidae family (Caenogastropoda). Plos One 12(7): e0181699. https://doi.org/10.1371/journal.pone.0181699

Weber M, Wey-Fabrizius AR, Podsiadlowski L, Witek A, Schill RO, Sugár L, Herlyn H, Hankeln T (2013) Phylogenetic analyses of endoparasitic Acanthocephala based on mitochondrial genomes suggest secondary loss of sensory organs. Molecular Phylogenetics and Evolution 66(1): 182–189. https://doi.org/10.1016/j.ympev.2012.09.017

Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. ISBN 978-3-319-24277-4. https://doi.org/10.1002/wics.147

Yin MB, Zheng HX, Su J, Feng Z, McManus DP, Zhou XN, Jin L, Hu W (2015) Co-dispersal of the blood fluke *Schistosoma japonicum* and *Homo sapiens* in the Neolithic Age. Scientific Reports 5: 18058. https://doi.org/10.1038/srep18058

Yi Y, Wu H (1989) Studies on the fauna of Acanthocephala of fishes from middle reaches of the Changjiang (Yangtze) River. Acta Hydrobiologica Sinica 13(1): 38–50.

Zhang D, Zou H, Wu SG, Li M, Jakovlić I, Zhang J, Chen R, Wang GT, Li WX (2017a) Sequencing, characterization and phylogenomics of the complete mitochondrial genome of *Dactylogyrus lamellatus* (Monogenea: Dactylogyridae). Journal of Helminthology 92(4): 455–466. https://doi.org/10.1017/S0022149X17000578

Zhang D, Zou H, Wu SG, Li M, Jakovlić I, Zhang J, Chen R, Wang GT, Li WX (2017b) Sequencing of the complete mitochondrial genome of a fish-parasitic flatworm *Paratetraonchoides inermis* (Platyhelminthes: Monogenea): tRNA gene arrangement reshuffling and implications for phylogeny. Parasites & Vectors 10: 462. https://doi.org/10.1186/s13071-017-2404-1

Zhang D, Li WX, Zou H, Wu SG, Li M, Jakovlic I, Zhang J, Chen R, Wang GT (2018) Mitochondrial genomes of two diplectanids (Platyhelminthes: Monogenea) expose paraphyly

of the order Dactylogyridea and extensive tRNA gene rearrangements. Parasites & Vectors 11: 601. https://doi.org/10.1186/s13071-018-3144-6

Zhang YJ, Sun J, Rouse GW, Wiklund H, Pleijel F, Watanabe HK, Chen C, Qian PY, Qiu JW (2018) Phylogeny, evolution and mitochondrial gene order rearrangement in scale worms (Aphroditiformia, Annelida). Molecular Phylogenetics and Evolution 125: 220–231. https://doi.org/10.1016/j.ympev.2018.04.002

Zou H, Jakovlić I, Chen R, Zhang D, Zhang J, Li WX, Wang GT (2017) The complete mitochondrial genome of parasitic nematode *Camallanus cotti*: extreme discontinuity in the rate of mitogenomic architecture evolution within the Chromadorea class. BMC Genomics 18(1): 840. https://doi.org/10.1186/s12864-017-4237-x

Zuker M (2003) Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Research 31(13): 3406–3415. https://doi.org/10.1093/nar/gkg595

Supplementary material 1

Table S1. Primers used to amplify and sequence the mitochondrial genomes of *Brentisentis yangtzensis*

Authors: Rui Song, Dong Zhang, Jin-Wei Gao, Xiao-Fei Cheng, Min Xie, Hong Li, Yuan-An Wu

Data type: molecular data

Link: https://doi.org/10.3897/zookeys.861.34809.suppl1

Supplementary material 2

Table S2. The list of species of the Acanthocephala and the outgroups used for comparative mitogenomic and phylogenetic analyses

Authors: Rui Song, Dong Zhang, Jin-Wei Gao, Xiao-Fei Cheng, Min Xie, Hong Li, Yuan-An Wu

Data type: species data

Link: https://doi.org/10.3897/zookeys.861.34809.suppl2

Supplementary material 3

Table S3, Nucleotide composition and skewness of different elements of the studied mitochondrial genome

Authors: Rui Song, Dong Zhang, Jin-Wei Gao, Xiao-Fei Cheng, Min Xie, Hong Li, Yuan-An Wu

Data type: molecular data

Link: https://doi.org/10.3897/zookeys.861.34809.suppl3

Supplementary material 4

Figure S1. Relative Synonymous Codon Usage (RSCU) of *Brentisentis yangtzensis*

Authors: Rui Song, Dong Zhang, Jin-Wei Gao, Xiao-Fei Cheng, Min Xie, Hong Li, Yuan-An Wu

Data type: molecular data

Link: https://doi.org/10.3897/zookeys.861.34809.suppl4

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Sarothrogammarus yiiruae, a new species of Amphipoda (Gammaridae) from China

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Abstract

A relic amphipod of the Tethys, *Sarothrogammarus yiiruae* **sp. nov.**, is described from Xinjiang, China. The new species is characterized by the absence of eyes; having the palm of the propodus without a mid-palmar spine on gnathopods I–II; a weakly concave coxal plate IV; narrow bases of pereopods V–VII; a peduncle of uropod I without a basofacial spine; uropod III longer than uropods I–II, a scale-like inner ramus, and a biarticulate outer ramus with distinct second article. Detailed morphological comparisons with related species are discussed. Genetic distances of the new and related species are provided as proof of species identification.

Keywords

genetic distance, sarothrogammarid amphipods, taxonomy, Tethys

Introduction

Sarothrogammarid amphipods are considered relics of the Tethys fauna (Stock 1971, 1995), consisting of a freshwater *Sarothrogammarus*-group in Pamir and a brackish genus, *Rhipidogammarus* Stock, 1971 along the Mediterranean coast (Hou et al. 2011). This disjunct distribution is related to the Late Eocene retreat of the Tethys

from Pamir (Hou et al. 2014). The freshwater *Sarothrogammarus*-group is characterized by having filtrative setae on pereopod III and urosomites with reduced spines or setae. The group contains the genera *Sarothrogammarus* Martynov, 1935, *Comatogammarus* Stock, 1971, and *Barnardiorum* Iwan & Löbl, 2007 (=*Tadzhikistania* Barnard & Barnard, 1983). The genus *Comatogammarus* is distinguished from *Sarothrogammarus* in having filtrative setae on pereopod IV; while the genus *Barnardiorum* differs from *Sarothrogammarus* in having a vestigial second article on uropod III. Up to now, there are eight species recorded in Pamir: *Comatogammarus ferghanensis* (Martynov & Behning, 1948), *Barnardiorum ruffoi* (Karaman, 1971), *B. shadini* (Birstein, 1948), *Sarothrogammarus asiaticus* Martynov, 1935, *S. multipennatus* Karaman, 1969, *S. lindbergi* Karaman, 1969, *S. afghanus* (Ruffo, 1958), and *S. trichiatus* Stock, 1971. They were all reported from Afghanistan or Tadzhikistan.

To explore the retreat route of the Tethys, an expedition was organized along the south side of the Tian Shan, China in 2014. A new species of *Sarothrogammarus yiiruae* sp. nov. was found from the east margin of Pamir. In the current paper, the new species is described and illustrated. The genetic distances between the new species and known species are calculated to confirm the species delimitation.

Materials and methods

Morphological observations

The specimens were collected with a fine-meshed hand net. Samples were preserved in 95% ethanol in the field and deposited in a -20 °C refrigerator for long term preservation. The body length was recorded by holding the specimen straight and measuring the distance along the dorsal side of the body from the base of the first antenna to the base of the telson. All dissected appendages were mounted on slides and were drawn using a Leica DM2500 compound microscope equipped with a drawing tube. Terminology and taxonomic descriptions follow the literature (Zheng and Hou 2017). The terms "setae" and "spines" are used to distinguish between thin or fine and more robust setal structures. All types and other materials are lodged in the Institute of Zoology, Chinese Academy of Sciences (IZCAS), Beijing.

Molecular methods

Partial fragments of mitochondrial cytochrome oxidase subunit (COI) and nuclear 28S rRNA were amplified to confirm identifications. Genomic DNA extraction, amplification and sequencing procedures were performed as in Hou et al. (2007). Uncorrected pairwise distances were calculated using MEGA 7.0.16 (Kumar et al. 2016). The new sequences were deposited in GenBank and the accession numbers are provided in Tables 1, 2.

Table 1. GenBank accession numbers and uncorrected pairwise distances of the COI partial sequences between species in this text.

	Species	Genbank accession number	1	2	3	4
1	*Comatogammarus ferghanensis*	JF965996	–			
2	*Barnardiorum shadini*	JF965994	0.1853	–		
3	*Barnardiorum* sp.	JF965995	0.1654	0.1838	–	
4	*Sarothrogammarus yiiruae* sp. nov.	MK770173	0.2009	0.2224	0.2193	–

Table 2. GenBank accession numbers and uncorrected pairwise distances of the 28S partial sequences between species in this text.

	Species	Genbank accession number	1	2	3	4
1	*Comatogammarus ferghanensis*	JF965828	–			
2	*Barnardiorum shadini*	JF965826	0.0369	–		
3	*Barnardiorum* sp.	JF965827	0.0497	0.0466	–	
4	*Sarothrogammarus yiiruae* sp. nov.	MK770174	0.0347	0.0395	0.0436	–

Taxonomy

Family Gammaridae Leach, 1814

Genus *Sarothrogammarus* Martynov, 1935

Type species. *Sarothrogammarus asiaticus* Martynov, 1935

Sarothrogammarus yiiruae Hou & Li, sp. nov.

http://zoobank.org/6218CB7A-B50B-430C-9F8D-5AC2D3764082
Figs 1–8

Material examined. ***Holotype*** ♂, 8.0 mm; CHINA, Xinjiang Uygur Autonomous Region, Kizilsu Kirghiz Autonomous Prefecture, Wuqia County, Jigen Town; 39.82N, 74.10E; 2729 m a.s.l.; 26 July 2014; K Meng, YC Lin leg.; IZCAS-I-A1636-1.

Paratype. 1♀, 7.0 mm; same data as for preceding; IZCAS-I-A1636-2.

Other materials. Four males, two females, and two juveniles, same data as for preceding. Two males were used for molecular analysis, but no variation was found between them. Sequences were submitted to GenBank (MK770173 for COI and MK770174 for 28S).

Diagnosis. Eyes absent; antenna II calceoli absent; gnathopods I–II without midpalmar spine; coxal plate IV weakly concave; bases of pereopods V–VII narrow; uropod I normal, without basofacial spine; uropod III longer than uropods I–II, peduncle about 1/3 length of outer ramus, inner ramus scale-like, with one spine on distal margin, outer ramus biarticulate, first article with four groups of spines on both margins, second article distinct.

Description of male holotype (IZCAS-I-A1636-1), 8.0 mm.

Head (Fig. 3A): eyes absent.

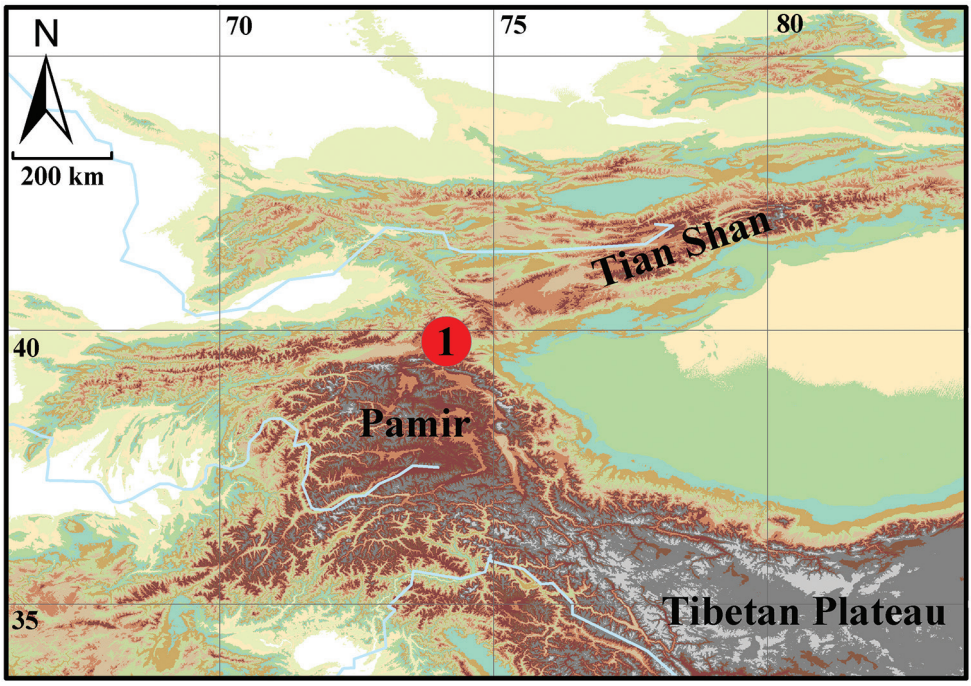

Figure 1. Locality of *Sarothrogammarus yiiruae* sp. nov. in Xinjiang, China.

Antenna I (Fig. 3B): longer than antenna II. Peduncle ratio of articles I–III 1.0: 0.6: 0.3, with distal setae; flagellum with 20 articles; accessory flagellum with three articles; both primary and accessory flagellum with short distal setae.

Antenna II (Fig. 3C): peduncle ratio of articles III–V 1.0: 3.0: 2.2, article III with two distal setae, article IV slightly longer than article V, both with setae along anterior and posterior margins; flagellum with ten articles, each article with distal setae; calceoli absent.

Upper lip (Fig. 3D): ventral margin rounded, bearing minute setae.

Mandible (Fig. 3E, F): asymmetrical. Right mandible incisor with four teeth; lacinia mobilis bifurcate; palp composed of three articles, second article with eight setae on inner margin, third article with two B-setae on outer margin, four E-setae on apical margin. Incisor of left mandible with four teeth, lacinia mobilis with three teeth.

Lower lip (Fig. 3G): inner lobe lacking, covered with thin setae.

Maxilla I (Fig. 3H, K): outer plate with nine apical spines, including simple (naked) spines, and spines bearing one, two or multiple dentitions; left palp biarticulate, distal article with eight stiff setae. Distal article of right palp with five stout spines and two setae.

Maxilla II (Fig. 3I): inner plate with plumose and four simple setae medially, nine simple setae apically; outer plate with 12 simple setae apically.

Maxilliped (Fig. 3J): inner plate with nine plumose setae apically; outer plate with spines and setae; palp with four articles, terminal article hooked.

Pereon. Gnathopod I (Fig. 4A, B): coxal plate with two setae on anterior margin, one seta on lower margin; basis sub-linear, with two long setae on anterior margin, 13 unequal setae on posterior margin; merus with eight long setae on posterior margin;

Figure 2. *Sarothrogammarus yiiruae* sp. nov. from Xinjiang, China. Photo showing habitus of male holotype (8.0 mm).

carpus with five long setae on posterior margin, tapered distolateral lobe; propodus 1.78 times as long as wide, palmar margin crenellated only in its proximal (angular) part, with 13 setae and defined by three stout spines; dactylus reaching approx. 68% length of propodus, posterior margin arc-shaped, with one seta.

Gnathopod II (Fig. 4C, D): coxal plate with three setae on anterior margin, one seta on lower margin; basis with one long seta and six setae on anterior margin, posterior margin with a row of long setae; merus with two long setae and two short setae on posterior margin; carpus with long setae on posterior margin; propodus 1.8 times as long as wide, palmar margin crenellated only in its proximal (angular) part, with one stout spine and 12 setae, posterior margin with a row of setae extending on proximolateral margin; dactylus reaching approx. 71% length of propodus.

Pereopods III–IV (Fig. 5A, B, O, P): similar to each other, pereopod III slightly longer than pereopod IV. Coxal plate III with three setae on anterior margin; coxal plate IV weakly concave, with two setae on anterior margin, one seta on posterior margin and three distal setae; bases sub-linear, bearing unequal spines on both margins; merus to carpus with spines but without long setae on both margins; dactyli with one seta at hinge of unguis.

Pereopods V–VII (Fig. 5C–E, Q–S): similar in shape, pereopod V shorter than pereopods VI and VII. Coxal plates V–VII small, without any spine and setae on both

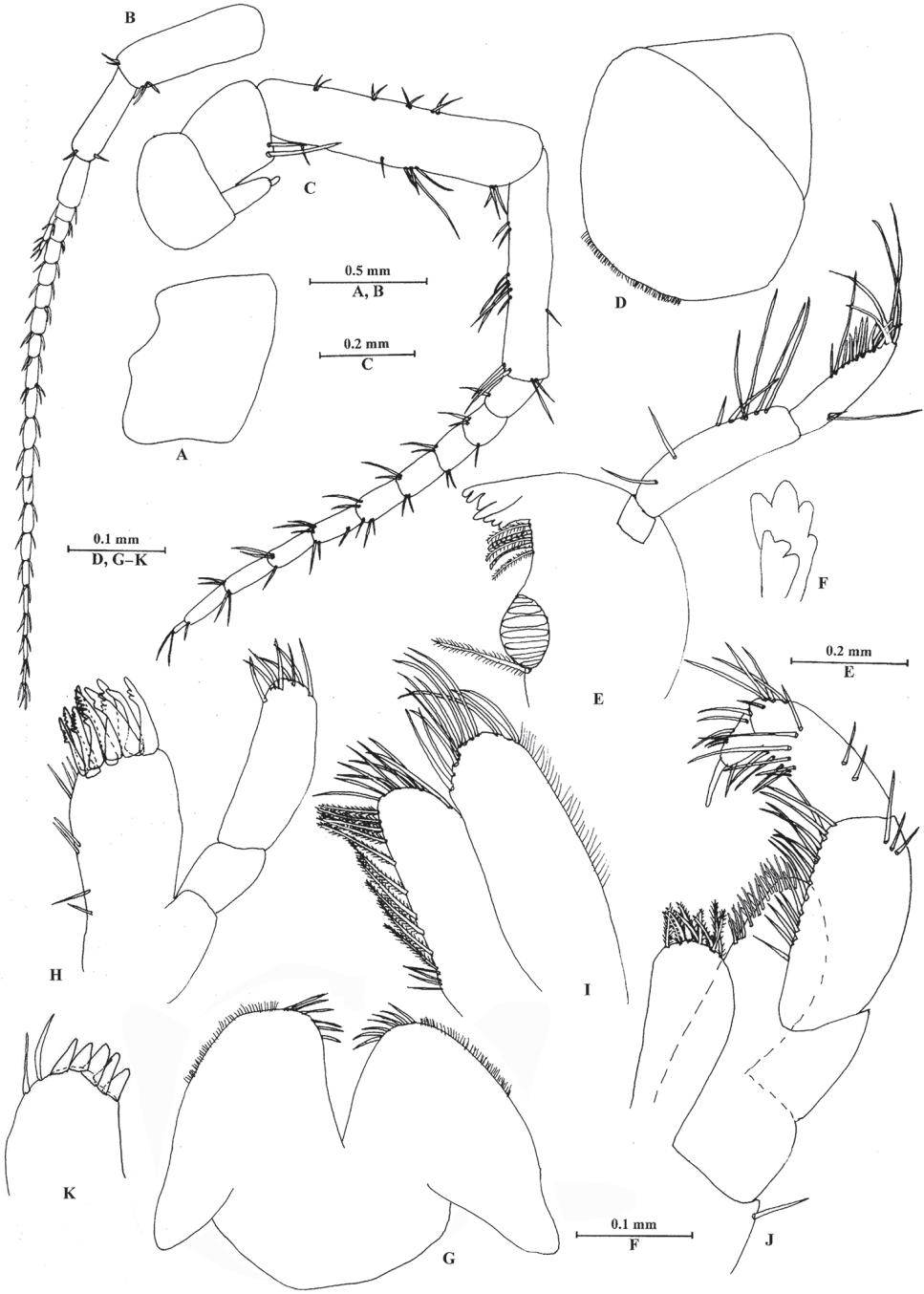

Figure 3. *Sarothrogammarus yiiruae* sp. nov. male holotype, from Xinjiang, China. **A** Head **B** antenna I **C** antenna II **D** upper lip **E** right mandible **F** incisor of left mandible **G** lower lip **H** maxilla I **I** maxilla II **J** maxilliped **K** right palp of maxilla I.

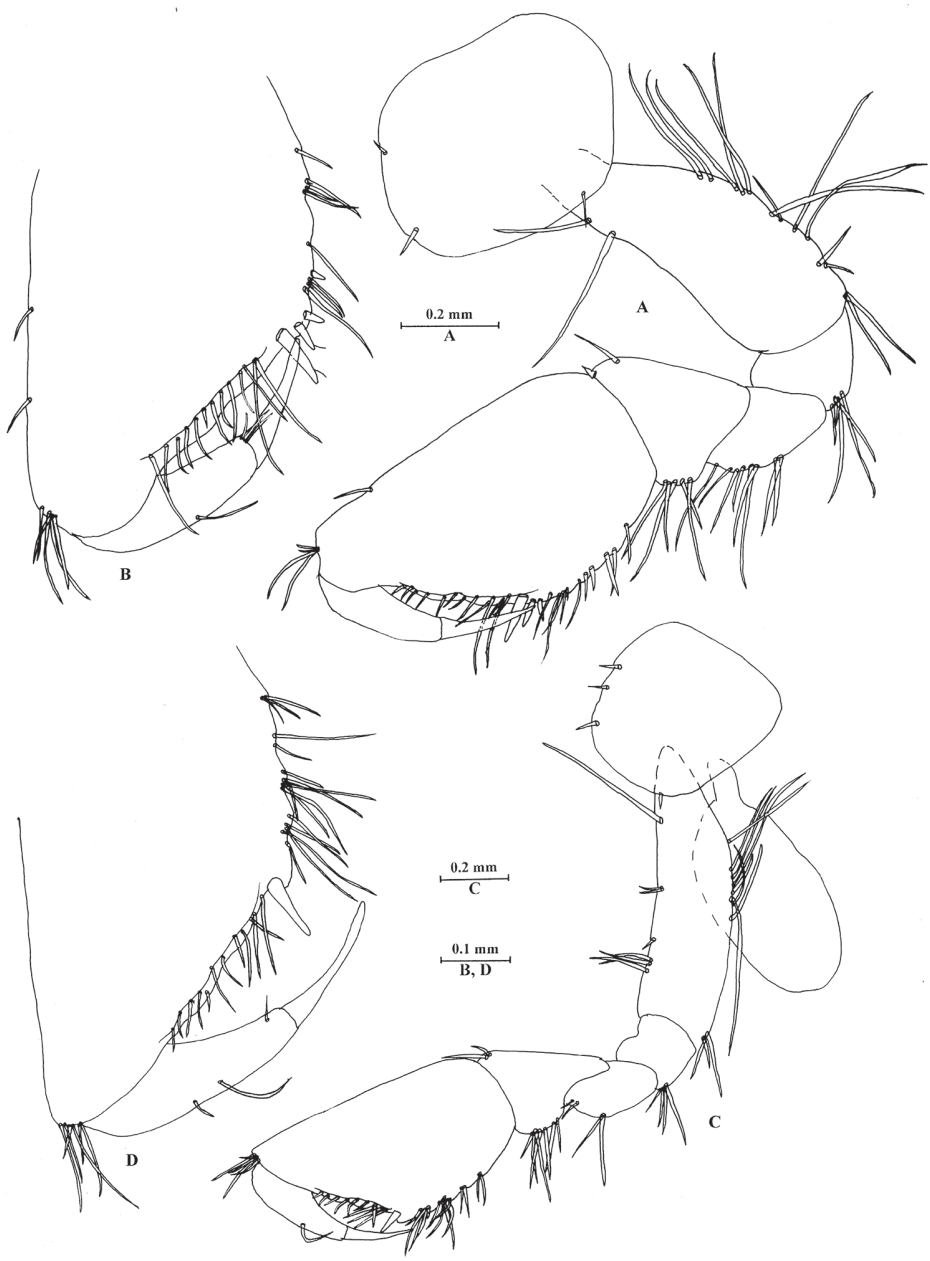

Figure 4. *Sarothrogammarus yiiruae* sp. nov. male holotype, from Xinjiang, China. **A** Gnathopod I **B** propodus of gnathopod I **C** gnathopod II **D** propodus of gnathopod II.

margins; bases narrow, with spines on anterior and posterior margins; dactyli with one seta at hinge of unguis.

Coxal gills present on gnathopod II and pereopods III–VI.

Figure 5. *Sarothrogammarus yiiruae* sp. nov. male holotype, from Xinjiang, China. **A** Pereopod III **B** pereopod IV **C** pereopod V **D** pereopod VI **E** pereopod VII **F** pleopod I **G** uropod I **H** uropod II **I** uropod III **J** telson **K** epimeral plate I **L** epimeral plate II **M** epimeral plate III **N** urosomites (dorsal view) **O** dactylus of pereopod III **P** dactylus of pereopod IV **Q** dactylus of pereopod V **R** dactylus of pereopod VI **S** dactylus of pereopod VII.

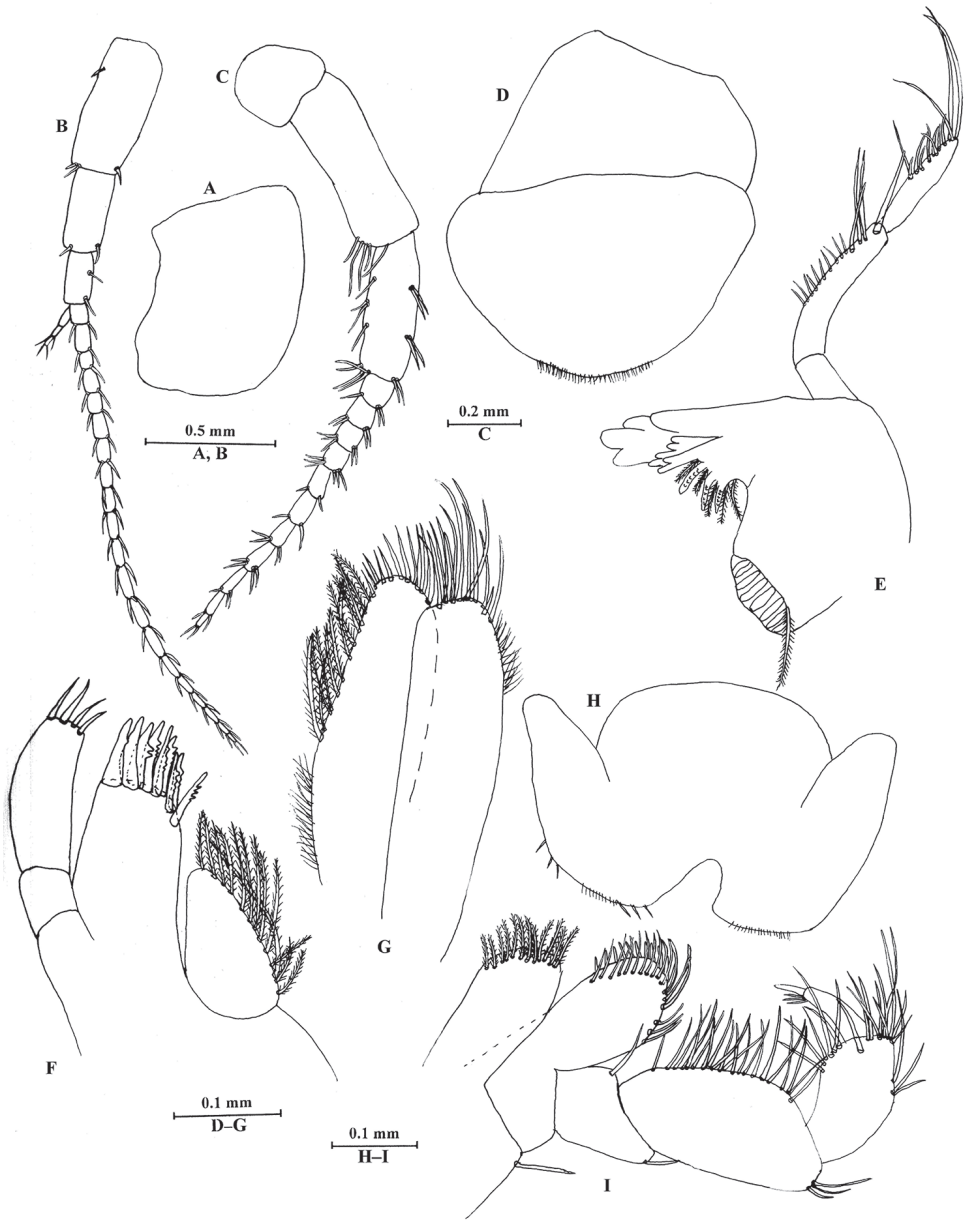

Figure 6. *Sarothrogammarus yiiruae* sp. nov. female paratype, from Xinjiang, China. **A** Head **B** antenna I **C** antenna II **D** upper lip **E** right mandible **F** maxilla I **G** maxilla II **H** lower lip **I** maxilliped.

Pleon. Epimeral plates (Fig. 5K–M): plate I ventrally rounded, with one seta on posterior margin; plate II posterior corner blunt, with two setae on ventral margin and two setae on posterior margin; plate III with three setae on ventral margin and one seta on posterior margin.

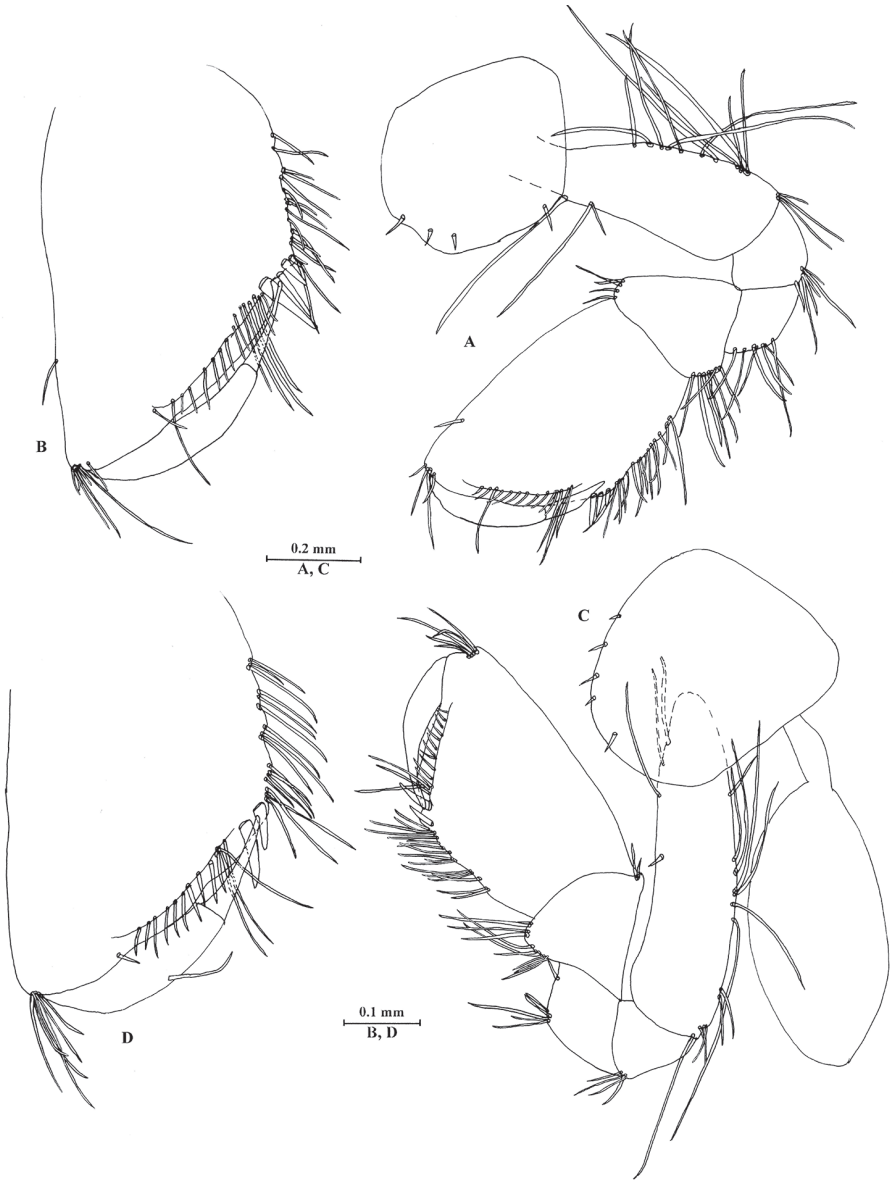

Figure 7. *Sarothrogammarus yiiruae* sp. nov. female paratype, from Xinjiang, China. **A** Gnathopod I **B** propodus of gnathopod I **C** gnathopod II **D** propodus of gnathopod II.

Pleopods I–III (Fig. 5F): similar to each other. Both rami subequal, with plumose setae.

Urosome. Urosomites I–III (Fig. 5N): with one, two, and one seta on dorsodistal margins, respectively.

Uropod I (Fig. 5G): normal, without basofacial spine; inner ramus slightly shorter than outer ramus, bearing one spine on inner margin; outer ramus with one spine on inner margin; both rami with five terminal spines. Uropod II (Fig. 5H): smaller than

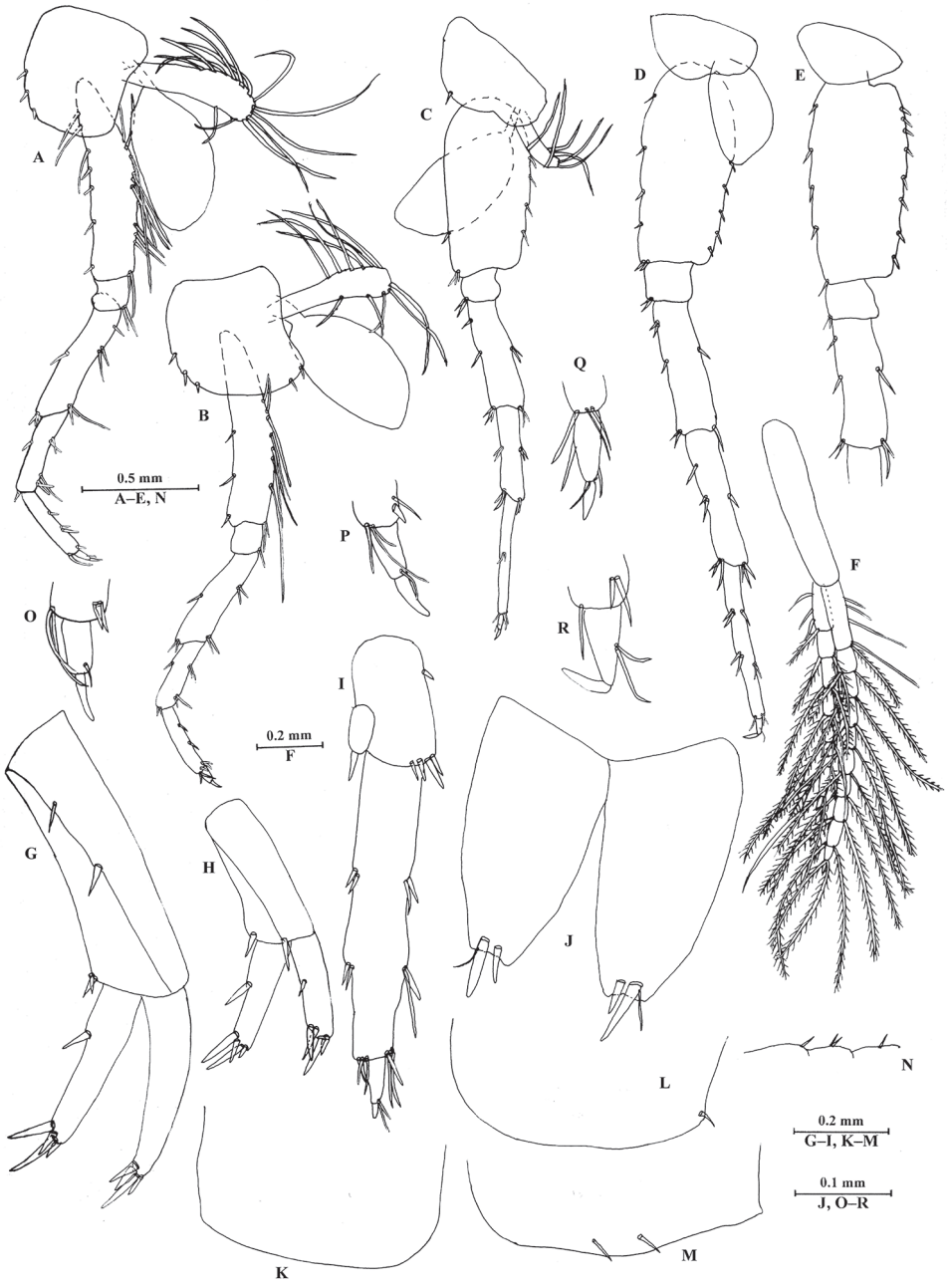

Figure 8. *Sarothrogammarus yiiruae* sp. nov. female paratype, from Xinjiang, China. **A** Pereopod III **B** pereopod IV **C** pereopod V **D** pereopod VI **E** pereopod VII **F** pleopod I **G** uropod I **H** uropod II **I** uropod III **J** telson **K** epimeral plate I **L** epimeral plate II **M** epimeral plate III **N** urosomites (dorsal view) **O** dactylus of pereopod III **P** dactylus of pereopod IV **Q** dactylus of pereopod V **R** dactylus of pereopod VI.

uropod I, peduncle with one spine on inner and outer margins, respectively; inner ramus with one spine on outer margin; outer ramus with one spine on outer margin; both rami with four terminal spines. Uropod III (Fig. 5I): longer than uropods I and II, peduncle about 1/3 the length of outer ramus, with one spine on anterior margin, three spines on posterior margin and three spines on distal margin; inner ramus reduced, scale-like, with one spine on distal margin; outer ramus with two articles, first article with four groups of spines on both margins, second article distinct.

Telson (Fig. 5J): deeply cleft, each lobe with two distal spines accompanied by setae.

Description of paratype female (IZCAS-I-A1636-2), 7.0 mm.

Head. Antennae and mouthparts (Fig. 6A–I) similar to those of male.

Pereon. Gnathopod I (Fig. 7A, B): Coxal plate with three setae on anterior margin, one seta on distal margin; basis sub-linear; carpus with tapered projection; propodus 1.53 times as long as wide, palmar margin crenellated only in its proximal (angular) part, with four stout spines and 16 setae, posterior margin with a row of setae extending on proximolateral margin; dactylus reaching approx. 68% length of propodus.

Gnathopod II (Fig. 7C, D): Coxal plate with four setae on anterior margin and one distal seta; propodus 1.56 times as long as wide, palmar margin crenellated only in its proximal (angular) part, with two stout spines and 14 setae, posterior margin with a row of setae extending on proximolateral margin; dactylus reaching approx. 67% length of propodus, with one seta on posterior margin.

Pleon. Epimeral plates I–III (Fig. 8K–M): plates I–III with no, one and two setae on ventral margins, respectively.

Urosome. Uropod I (Fig. 8G): normal, without basofacial spine; inner ramus with one spine on inner margin; both rami with five terminal spines. Uropod II (Fig. 8H): peduncle with one spine on inner and outer margins, respectively; both rami with four terminal spines. Uropod III (Fig. 8I): peduncle shorter than outer ramus; inner ramus reduced, scale-like, with one spine on distal margin; outer ramus with two articles, first article with two groups of spines on both margins, second article distinct.

Etymology. The specific name is named in honor of Ms Menghe Yiiru, lovely daughter of the collector Meng Kaibayier, for their kind support of Amphipoda research in Xinjiang; noun (name) in genitive case.

Habitat. This species was collected from a stream, rising in snow-capped mountains.

Remarks. The new species is assigned to the genus *Sarothrogammarus* according to the scale-like inner ramus of uropod III and the narrow bases of pereopods V–VII. It is not a member of the genus *Comatogammarus* because pereopod V lacks filtrative setae. It is not assigned to the genus *Barnardiorum* because the second article of the outer ramus in uropod III is distinct rather than vestigial.

Sarothrogammarus yiiruae sp. nov. is most similar to *S. trichiatus* Stock, 1971 in having urosomites I–III with setae on the dorsal margin and the shape of uropods I and II. *Sarothrogammarus yiiruae* sp. nov. differs from *S. trichiatus* (character states for *S. trichiatus* in parentheses) in the following: the absence of eyes (the presence of eyes); gnathopod II without mid-palmar spine (with mid-palmar spine); pereopod III without filtrative long setae on merus and carpus (pereopod III with filtrative setae); second

article of outer ramus in uropod III distinct, longer than adjacent spines (rudimentary, shorter than adjacent spines). The comparison between the species of sarothrogammarids from the Pamir region is presented in the following key.

We downloaded COI and 28S sequences of sarothrogammarids from Pamir (Hou et al. 2014), including *Comatogammarus ferghanensis* (voucher number SLOCHN266), *Barnardiorum shadini* (voucher number SLOCHN263), and *Barnardiorum* sp. (voucher numbers SLOCHN265, 264, 267). Molecular analyses showed high interspecific divergence (Tables 1, 2). The uncorrected distances between *S. yiiruae* sp. nov. and its congeners are higher than 20% for COI, which is larger than the accepted threshold (16%) for crustacean species delimitation (Lefébure et al. 2006). The genetic distances between the new species and its congeners are 3.5–4.4% for 28S, which is comparable to interspecific differentiation for amphipods (Hou et al. 2014). Therefore, morphological and molecular data support *S. yiiruae* sp. nov. being a new species.

Key to the species of sarothrogammarids from the Pamir region

1 Pereopod IV with filtrative setae..... ***Comatogammarus ferghanensis***
– Pereopod IV with no filtrative setae..... **2**
2 Second article of outer ramus in uropod III vestigial..... **3**
– Second article of outer ramus in uropod III distinct..... **4**
3 Outer ramus of uropod III slender..... ***Barnardiorum ruffoi***
– Outer ramus of uropod III extended..... ***Barnardiorum shadini***
4 Eyes absent..... ***Sarothrogammarus yiiruae* sp. nov.**
– Eyes present..... **5**
5 Antenna I peduncle with long setae on ventral margin..... ***S. trichiatus***
– Antenna I peduncle with short setae on ventral margin..... **6**
6 Antenna I accessory flagellum with three or four segments..... **7**
– Antenna I accessory flagellum with two segments..... **8**
7 Both male and female pereopod III with rows of long setae..... ***S. multipennatus***
– Female pereopod III without long setae..... ***S. lindbergi***
8 Gnathopod II palm very oblique..... ***S. afghanus***
– Gnathopod II palm not oblique..... ***S. asiaticus***

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References

Barnard JL, Barnard CM (1983) Freshwater Amphipoda of the World I and II. Hayfield Associates, Mt. Vernon, Virginia.

Birstein JA (1948) Amphipoda of Tadzjikistan. Prof. Zernow Memorial Volume: 263–273.

Hou Z, Fu J, Li S (2007) A molecular phylogeny of the genus *Gammarus* (Crustacea: Amphipoda) based on mitochondrial and nuclear gene sequences. Molecular Phylogenetics and Evolution 45: 596–611. https://doi.org/10.1016/j.ympev.2007.06.006

Hou Z, Sket B, Fiser C, Li S (2011) Eocene habitat shift from saline to freshwater promoted Tethyan amphipod diversification. Proceedings of the National Academy of Sciences of the United States of America 108: 14533–14538. https://doi.org/10.1073/pnas.1104636108

Hou Z, Sket B, Li S (2014) Phylogenetic analyses of Gammaridae crustacean reveal different diversification patterns among sister lineages in the Tethyan region. Cladistics 30: 352–365. https://doi.org/10.1111/cla.12055

Karaman GS (1969) XXII. Beitrag zur Kenntnis der Amphipoden. Über einige neue Formen des Genus *Sarathrogammarus* (Gammaridae) aus Afghanistan. Acta Musei Macedonici Scientiarum Naturalium 6: 195–208.

Karaman GS (1971) Eine neue Art des Gattung *Sarothrogammarus* (Gammaridae) aus Afghanistan, *S. ruffoi* n. sp. Crustaceana 20: 199–207. https://doi.org/10.1163/156854069X00222

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. Molecular Phylogenetics and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Iwan D, Löbl I (2007) Nomenclatural notes on tenebrionid beetls of the Palaearctic region (Insecta: Coleoptera). Annales Zoologici (Warszawa) 57: 733–739.

Leach WE (1814) Crustaceology. The Edinburgh Encyclopaedia **7**: 402–403.

Lefébure T, Douady CJ, Gouy M, Gibert J (2006) Relationship between morphological taxonomy and molecular divergence within Crustacea: proposal of a molecular threshold to help species delimitation. Molecular Phylogenetics and Evolution 40: 435–447. https://doi.org/10.1016/j.ympev.2006.03.014

Martynov A (1935) K poznaniju Amphipoda tekucih vod Turkestana. Travaux de l'Institut de zoologique de l'Äcadémie des sciences de l'U.R.S.S. 2: 411–508.

Martynov AW, Behning AL (1948) Some centra-asiatic gammarids. Akademiia Nauk SSSR, Trudy Zoologicheskogo Instituta 7: 171–176.

Ruffo S (1958) Due nuove specie di Anfipodi delle acque sotterranee dell' Afghanistan. Memorie del Museo civico di storia naturale di Verona 6: 389–403.

Stock JH (1971) A revision of the *Sarothrogammarus*-group (Crustacea, Amphipoda). Bijdragen tot de Djerkunde 41: 96–129. https://doi.org/10.1163/26660644-04102002

Stock JH (1995) The genus *Sarothrogammarus* Martynov, 1935 (Crustacea, Amphipoda) in Madeira. Boletim do Museu Municipal do Funchal 45: 29–40.

Zheng Y, Hou Z (2017) *Myanmarorchestia victoria* sp. nov. (Crustacea, Amphipoda, Talitridae), a new species of landhopper from the high altitude forests in Myanmar. Zoological Research 38: 1–11.

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RESEARCH ARTICLE

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Two new species of the tribe Hemisphaeriini (Hemiptera, Fulgoromorpha, Issidae) from southwestern China

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Abstract

Two new species of the tribe Hemisphaeriini: *Ceratogergithus brachyspinus* Yang & Chen, **sp. nov.** (Yunnan) and *Neohemisphaerius clavatus* Yang & Chen, **sp. nov.** (Guizhou) are described and illustrated. A checklist to Hemisphaeriini genera is provided. The generic characteristics of the genera *Ceratogergithus* Gnezdilov, 2017 and *Neohemisphaerius* Chen, Zhang & Chang, 2014 are redefined. Checklists and keys to the species of each genus are given.

Keywords

Fulgoroidea, morphology, Oriental region, planthoppers, taxonomy

Introduction

Hemisphaeriini Melichar, 1906 is the second largest tribe of the planthopper family Issidae with currently 25 genera and 181 species known (Bourgoin 2018). It was erected by Melichar (1906) as family Hemisphaeridae but more recently Gnezdilov (2003, 2013a) downgraded it to the tribe level. Sun et al. (2015) raised the group again to the

subfamily level based on partial sequences of the nuclear *Wingless* (*Wg*) and *18S rDNA* genes and Wang et al. (2016) enlarged the subfamily based on 18S, 28S, COXI and Cytb genes to include four tribes (Kodaianellini, Sarimini, Parahiraciini, Hemisphaeriini). However, here we prefer to follow Gnezdilov (2013a) and treat the group as a tribe of the subfamily Issinae which was also followed by Meng et al (2017).

Hemisphaeriini are characterized as follows: body hemispherical; vertex with anterior margin approximately transverse or triangularly elongate; pronotum with convex anterior margin; forewings thick and convex, claval suture present or absent, venation reticulate; hindwings single-lobed, being either well developed, i.e., longer than half length of forewings, venation reticulate, or rudimentary, shorter than half length of forewings, venation simple.

The tribe Hemisphaeriini is divided into two groups based on the presence or absence of the forewing claval suture. The genera *Neohemisphaerius* and *Paramongoliana* both have the forewing claval suture developed. The genus *Neohemisphaerius* was erected by Chen et al. (2014) for three species (*N. wugangensis*, *N. yangi* and *N. signifer* Walker, 1851) having a forewing with distinct claval suture. Recently Zhang et al. (2016) reviewed *Neohemisphaerius*, transferred species *N. signifer* Walker, 1851 to *Hemisphaerius* Schaum, 1850 and described species *N. guangxiensis* Zhang, Chang & Chen, 2016. The genus *Ceratogergithus* was erected by Gnezdilov (2017) for three species (*C. chelates*, *C. pseudotessellatus* and *C. spinosus*) having a forewing without a claval suture and pygofer with a large horn-shaped process on posterior margin. In this paper, we describe and illustrate two new species of the tribe Hemisphaeriini, give a checklist to Hemisphaeriini genera, redefine the generic characteristics and provided checklists and keys to the species of these two genera.

Hemisphaeriini are usually collected in broad-leaved forest, although some species are also found on Poaceae in open areas (Gnezdilov 2013b). The species *Neohemisphaerius clavatus* Yang & Chen, sp. nov. was captured on *Bambusa emeiensis*.It maybe the second species that feeds exclusively on bamboos (host plant *Bambusa emeiensis*; Fig. 36), the other species is *Rotundiforma nigrimaculata*, Meng, Wang & Qin, 2013, whose host plants may be *Gigantochloa ligulata* Gamble and *Dendrocalamus* sp. (Meng, Wang & Qin, 2013).

Materials and methods

The morphological terminology follows Chan and Yang (1994) and Bourgoin et al. (2015), except those for male genitalia following Gnezdilov (2003). Dry specimens were observed by stereoscopic microscope Leica M125 for illustration and description. All measurements are in millimeters (mm). The genital segments were separated and macerated in 10% NaOH, transferred to glycerine for observing and drawing. Illustrations of the specimens were made with a Leica MZ 12.5 stereomicroscope. Photographs of the types were taken by KEYENCE VHX-1000C.

The type specimens are deposited in the Institute of Entomology, Guizhou University, Guiyang, China (GUGC) and one paratype of *Neohemisphaerius clavatus* Yang & Chen, sp. nov. in the Natural History Museum, London (BMNH).

Checklist of genera of Hemisphaeriini

Bolbosphaerius Gnezdilov, 2013; Brunei, Vietnam.
Bruneastrum Gnezdilov, 2015; Borneo.
Ceratogergithus Gnezdilov, 2017; China: Hainan, Yunnan.
Choutagus Zhang, Wang & Che, 2006; China: Guangxi, Hainan.
Clypeosmilus Gnezdilov & A. Soulier-Perkins, 2017; Northern Vietnam.
Euxaldar Fennah, 1978; Vietnam.
Epyhemisphaerius Chan & Yang, 1994; China: Taiwan.
Euhemisphaerius Chan & Yang, 1994; China: Taiwan.
Gergithus Stål, 1870; India, Indonesia, Malaysia, Myanmar, Sri Lanka, Southern China, Thailand.
Gergithoides Schumacher, 1915; Japan, Southern China, Vietnam.
Gnezdilovius Meng, Webb & Wang, 2017; Southern China, Vietnam, Japan.
Hemisphaerius Schaum, 1850; China, India, Indonesia, Japan, Malaysia, Myanmar, New Guinea, Philippines, Sri Lanka, Thailand, Vietnam.
Hemisphaeroides Melichar, 1903; Sri Lanka.
Hemiphile Metcalf, 1952; Indonesia.
Hysteropterissus Melichar, 1906; New Guinea.
Hysterosphaerius Melichar, 1906; Singapore.
Ishiharanus Hori, 1969; Vietnam.
Macrodaruma Fennah, 1978; Southern China, Vietnam.
Maculergithus Constant & Pham, 2016; Northern Vietnam, Southern China.
Mongoliana Distant, 1906; Japan, Southern China.
Neogergithoides Sun, Meng & Wang, 2012; China: Guangxi, Guangdong, Hainan, Yunnan, Vienam.
Neohemisphaerius Chen, Zhang & Chang, 2014; Southern China.
Ophthalmosphaerius Gnezdilov, 2017; Southern China: Yunnan.
Paramongoliana Chen, Zhang & Chang, 2014; China: Guizhou.
Rotundiforma Meng, Wang & Qin, 2013; China: Yunnan.

Taxonomy

Family Issidae Spinola, 1839
Subfamily Issinae Spinola, 1839
Tribe Hemisphaerini Melichar, 1906

Genus *Ceratogergithus* Gnezdilov, 2017

Type species. *Ceratgergithus spinosus* (Che, Zhang & Wang, 2007).

Diagnosis. Vertex subsquare or transverse. Metope wide, without median carinae. Postclypeus with distinct median carinae, elevated above the level surface of base of the frons (Figs 6, 7) or without carinae (Chen et al. 2014: figs 2–14D–E, 2–15D–E). Forewings without claval suture and shoulder-like projections (Chen et al. 2014: figs 2–14A–C, 2–15A–C) or with claval suture developed through its whole length, basally depressed (Figs 1, 2). Hindwing one lobed, longer than half length of forewing. Pygofer of male symmetrical (in lateral view), posterior margin with a large horn-shaped process in upper half. Anal tube of male apically enlarged (in dorsal view).

Distribution. China: Hainan, Yunnan.

Discussion. This genus is similar to *Gergithus* and *Neohemisphaerius*, but can be clearly separated from *Gergithus* by the posterior margin of the pygofer with a large horn-shaped process (Fig. 12) and the aedeagus without pair of short ventral directed toward its apex. It differs from the genus *Neohemisphaerius* by having a frons without a median carina, with colored marking, a hindwing well developed and longer than half the length of the forewing, and venation reticulate.

List of *Ceratogergithus* species

Ceratogergithus chelates (Che, Zhang & Wang, 2007); China: Hainan.
Ceratogergithus pseudotessellatus (Che, Zhang & Wang, 2007); China: Hainan.
Ceratogergithus spinosus (Che, Zhang & Wang, 2007); China: Hainan.
Ceratogergithus brachyspinus Yang & Chen, sp. nov.; China: Yunnan.

Key to species of the genus *Ceratogergithus* (male)

1 Clypeus with distinct median carina. Forewing with claval suture developed (Figs 6–8) ..... ***C. brachyspinus* Yang & Chen, sp. nov.**

– Clypeus without median carina. Forewing without claval suture..... **2**

2 Forewing with four pale green transverse fasciae. Anal tube with apical margins strongly convex (in dorsal view) (Che et al. 2007: figs 26, 28) .... ***C. chelates* (Che, Zhang & Wang)**

– Forewing and anal tube not as above..... **3**

3 Forewing yellowish hazel. Anal tube with apical margins slightly concave (Che et al. 2007: figs 16, 18) ..... ***C. spinosus* (Che, Zhang & Wang)**

– Forewing dark with 3 large elongate spots in basal half, with 6 or 7 smaller elongate spots at apical margin in apical half. Anal tube margin nearly truncate (Che et al. 2007: figs 53, 55) .... ***C. pseudotessellatus* (Che, Zhang & Wang)**

***Ceratogergithus brachyspinus* Yang & Chen, sp. nov.**
http://zoobank.org/CA131906-B935-4958-8B37-BEB4B11CE542
Figs 1, 2, 5–18

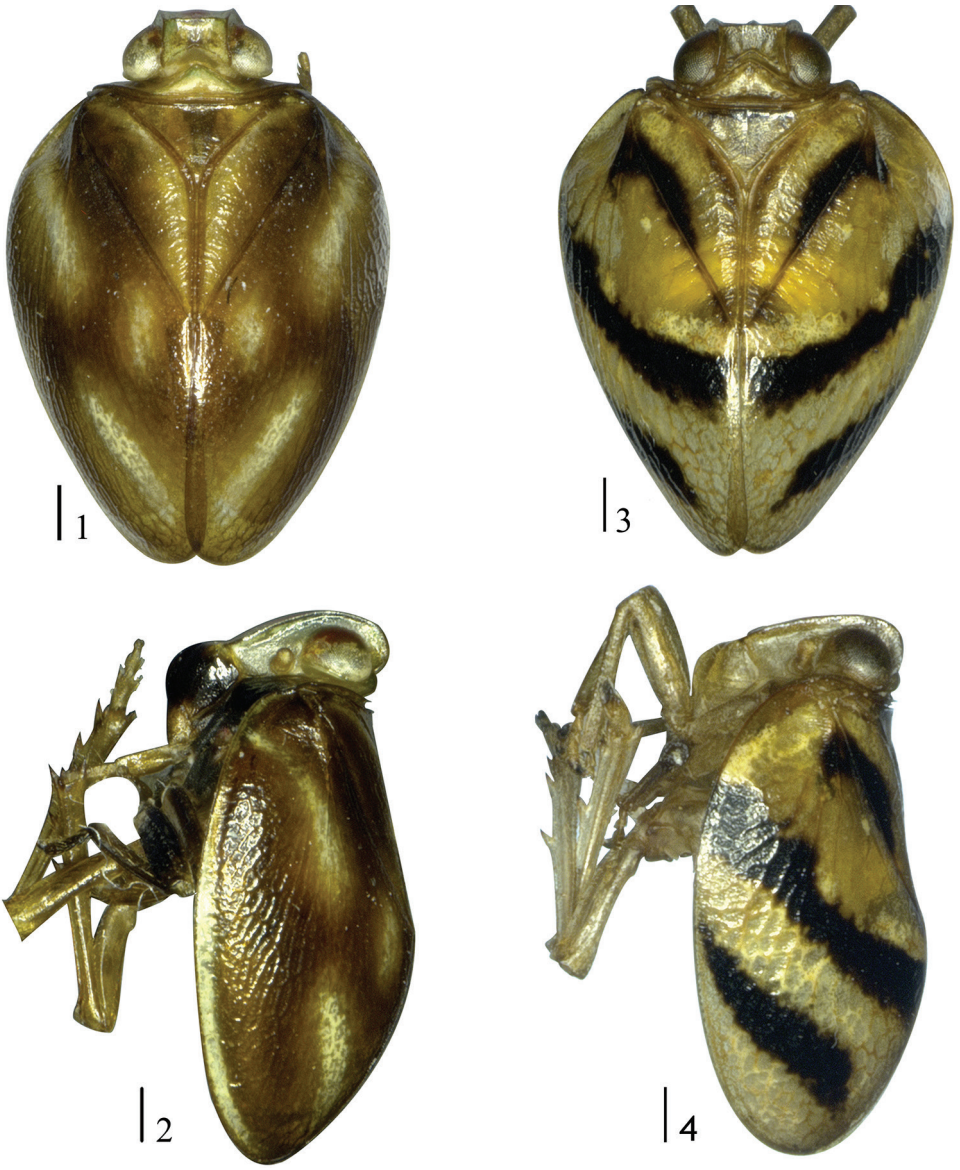
Type material. Holotype: ♂, China: **Yunnan**, Daweishan National Nature Reserve (103°20'E, 23°07'N), 8 May 2016, L.-J. Yang. Paratypes: 1♂, same data as holotype; 1♂, same data as holotype, except 19 August, 2017, Y.-J. Sui. All in GUGC.

Description. Male body length (from apex of vertex to tip of forewing): 5.16–5.31 mm (n = 3); male forewing 4.43–4.58 mm (n = 3); male hindwing 3.30–3.47 mm (n = 3).

Coloration (Figs 1, 2, 5–7). Vertex straw-yellow to pale green, all margins brownish (Fig. 5). Frons with brick-red markings, margins brownish (Fig. 7). Clypeus dark brown. Eyes reddish brown to greenish-brown (Figs 6, 7). Pronotum straw-yellow, margins brown (Fig. 5). Mesonotum (Fig. 5) fulvous, with fuscous subtriangular marking. Forewing fulvous, with three white markings irregular, costal margin white from middle to subapical part (Figs 1, 2, 8). Hindwing brownish and hyaline.

Head and thorax (Figs 5–9). Vertex shorter in middle than width at base (0.41: 1.00), transverse, anterior margin weakly convex, posterior margin angularly concave, disc depressed and all margins elevated (Figs 1, 5). Frons longer along midline than maximal width (1.53: 1.00) (Fig. 7), smooth, without median carina or pustules, apical margin nearly straight, margins carinate, disc slightly elevated (in frontal view) (Fig. 7) and arcuate (in lateral view) (Fig. 6). Clypeus with median carina obvious, postclypeus distinctly elevated (Figs 6, 7). Ocelli absent. Pronotum longer than vertex (1.56: 1.00), slightly depressed, margins elevated (Fig. 5). Mesonotum subtriangular, longer than pronotum (3.23:1.00) (Fig. 5), without median and lateral carinae, anterior margin nearly transverse (Fig. 5). Forewings about 2 times longer than maximal width (Figs 1, 2), with claval suture developed through its whole length, without "shoulder" basally, venation obscure. Hindwing 0.70 times as long as forewings (Figs 8, 9), reaching pygofer; venation reticulate (Fig. 9). Hind tibiae with two lateral teeth. Metatibiotarsal formula: 7–8–2.

Male genitalia (Figs 10–19). Anal tube 1.35 times as long as wide (in dorsal view) (Fig. 10), enlarged apically, apical margin deeply notched medially, bent ventrad (in lateral view) (Fig. 11). Pygofer symmetrical, posterior margin with large horn-shaped process in apical fourth (Fig. 12). Genital style subquadrate (in lateral view), moderately long, depressed in base near ventral margin, caudo-ventral angle rounded (Fig. 12). Capitulum with neck and small lateral tooth directed cephalad and big lateral tooth on posterior margin, directed laterad (Figs 12, 13). Connective cup-shaped (Figs 14, 15). Penis twisted medially (Figs 16, 17). Phallobase asymmetrical, with basal tooth process directed caudad (Figs 16, 17a), with pair of short lateral hooks in basal third, directed basad (Figs 16, 17b, e); dorso-lateral lobes of phallobase membranous in apical two-fifth (Figs 16, 19), with two differently shaped processes of different length directed apically: one process slender and short, arising in apical fourth (Figs 16–19c), other one arising in basal third, extended ventrad, with subapical process horn-shaped (Figs 17–19d). Ventral lobe of phallobase apically convex (in ventral view), shorter than dorso-lateral lobes (Fig. 18).



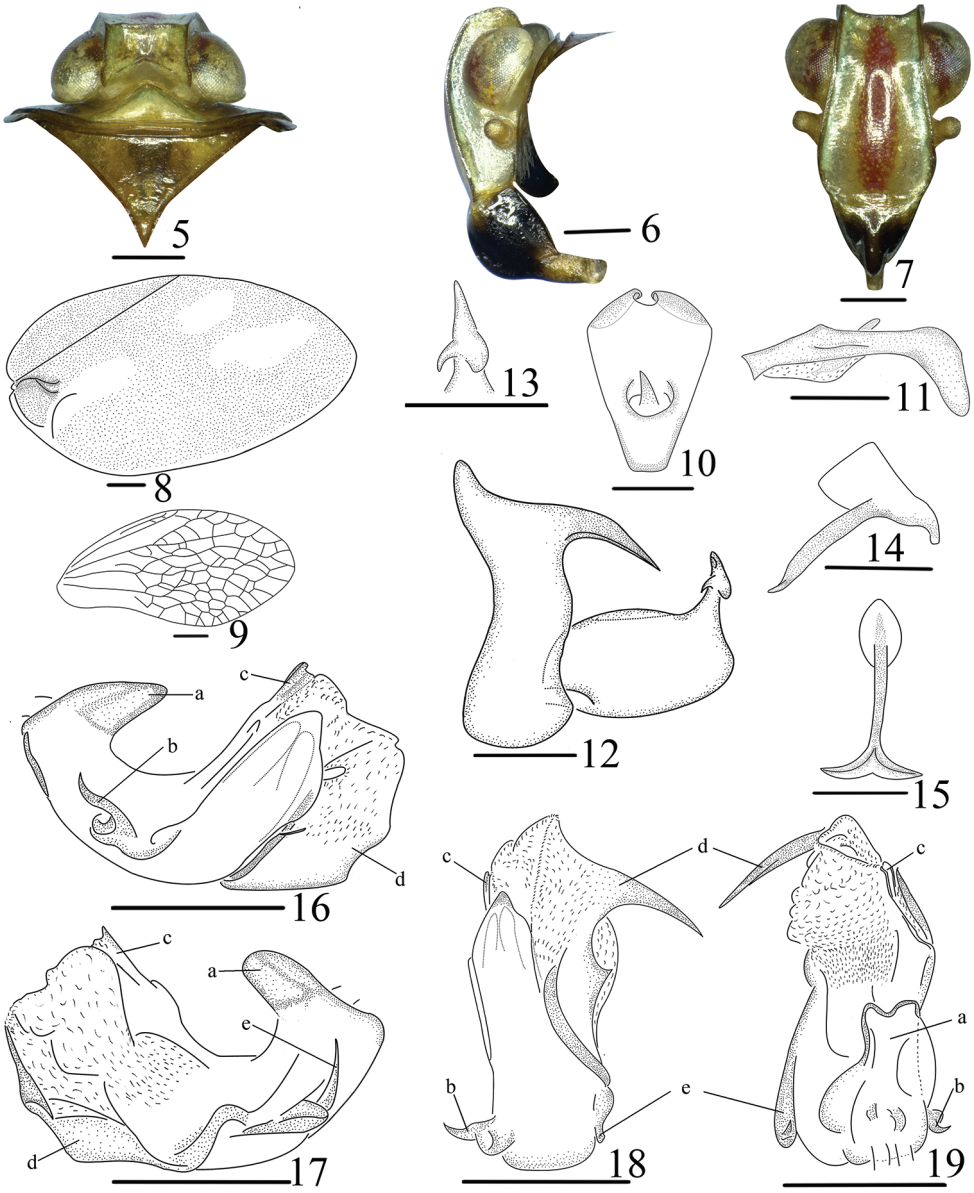
Figures 1–4. Dorsal and lateral habitus of two new species adult (male), **1, 2** *Ceratogergithus brachyspinus* Yang & Chen, sp. nov. **3, 4** *Neohemisphaerius clavatus* Yang & Chen, sp. nov. Scale bars: 0.5 mm.

Etymology. The specific name is derived from the Latin words "*brachys*"and "*spina*", referring to the short lateral hooks on the basal third of the phallobase.

Host plant. Unknown.

Distribution. Southwestern China (Yunnan).

Remarks. This species can be distinguished from all the other species of genus *Ceratogergithus* by the following characteristics: Frons with brick-red markings (Fig. 7);



Figures 5–19. *Ceratogergithus brachyspinus* Yang & Chen, sp. nov. adult (male), **5** head and thorax, dorsal view **6** head and thorax, lateral view **7** face, front view **8** fore wing **9** hindwings **10** anal tube, dorsal view **11** anal tube, lateral view **12** pygofer and genital style, lateral view **13** capitulum of gonostylus, dorsal view **14** connective, lateral view **15** connective, caudal view **16** penis, right lateral view **17** penis, left lateral view **18** penis, ventral view **19** penis, dorsal view. Scale bars: 0.5 mm.

clypeus with distinct median carina, postclypeus distinctly elevated (Figs 6, 7); forewing fulvous, with three white irregular markings, with claval suture developed, basally depressed (Figs 1, 2).

Genus *Neohemisphaerius* Chen, Zhang & Chang, 2014

Type species. *Neohemisphaerius wugangensis* Chen, Zhang & Chang, 2014.

Diagnosis. Body hemispherical. Vertex about 2.5–3.9 times as wider than long along midline, anterior margin straight, posterior margin angulately excavated. Frons elongate, with median carina, lateral margins elevated. Clypeus with median carina moderately convex, median carinae with or without a tubercle process in middle. Pronotum depressed, edges elevated. Mesonotum subtriangular, anterior margin approximately straight. Forewings hemispherical, claval suture developed, without shoulder-like projections basally. Hindwing rudimentary, shorter than half length of forewing, venation indistinct and simple. Hind tibiae with 2 lateral teeth. Metatibiotarsal formula: (9, 10)–(4, 5)–2. Anal tube of male wide and short. Phallobase with pair of ventral hooks directed basad.

Distribution. China (Guangdong, Guangxi, Hunan, Guizhou).

Discussion. *Neohemisphaerius* is similar to Hemisphaerius Schaum, 1850 and Gergithus Stål, 1870, but it differs from the two genera by having a frons with a median carina, and forewings with a claval suture developed. The genus *Neohemisphaerius* runs close to *Paramongoliana* in the key by Meng et al. (2017). It differs from *Paramongoliana* in: frons with median carinae, without a row of pustules along the lateral margins; clypeus distinctly convex on disc in midline; forewings with irregular markings; phallobase with pair of ventral hooks directed basad.

List of *Neohemisphaerius* species

Neohemisphaerius clavatus Yang & Chen, sp. nov.; China: Guizhou.
Neohemisphaerius guangxiensis Zhang, Chang & Chen, 2016; China: Guangxi.
Neohemisphaerius wugangensis Chen, Zhang & Chang, 2014; China: Hunan.
Neohemisphaerius yangi Chen, Zhang & Chang, 2014; China: Guangdong.

Key to species of the genus *Neohemisphaerius* (males; modified from Zhang et al. 2016)

1 Frons with disc rugose (Fig. 22); clypeus with distinct median carinae (Fig. 22); forewings with three subparallel dark stripes, slanted caudad (Figs 3, 4); anal tube (in dorsal view) with apical margin concave medially (Fig. 25); Phallobase asymmetrical (Figs 33–35).....***N. clavatus* Yang & Chen, sp. nov.**

– Frons with disc smooth (Zhang et al. 2016: figs 3, 16, 20); clypeus with a hump-shaped median carinae (Zhang et al. 2016: figs 2, 16, 19); anal tube not as above; phallobase symmetric.....**2**

2 Forewings pale brown, with two black patches at costal margin (Zhang et al. 2016: figs 1, 2, 4–5); anal tube with apical margin medially convex (in dorsal view) (Zhang et al. 2016: fig. 12) ..... ***N. guangxiensis***

– Forewings black brown, with 4 or 5 light yellow patches (Zhang et al. 2016: figs 13, 14); anal tube not as above **3**

3 Frons with obscurely short median carinae; anal tube with apical margin round (Chen et al. 2014: figs 2–36: H); a pair of ventral hooks of phallobase longer than half length of aedeagus ..... ***N. yangi***

– Frons with distinctly long median carina; apical margin of anal tube sinuate (in dorsal view) (Chen et al. 2014: fig. 2–35: H); a pair of ventral hooks of phallobase shorter than fifth length of aedeagus (Chen et al. 2014: figs 2–35: M, K) ..... ***N. wugangensis***

***Neohemisphaerius clavatus* Yang & Chen, sp. nov.**

http://zoobank.org/DE9C89F6-24C8-4E2F-9EF1-E4354252141F

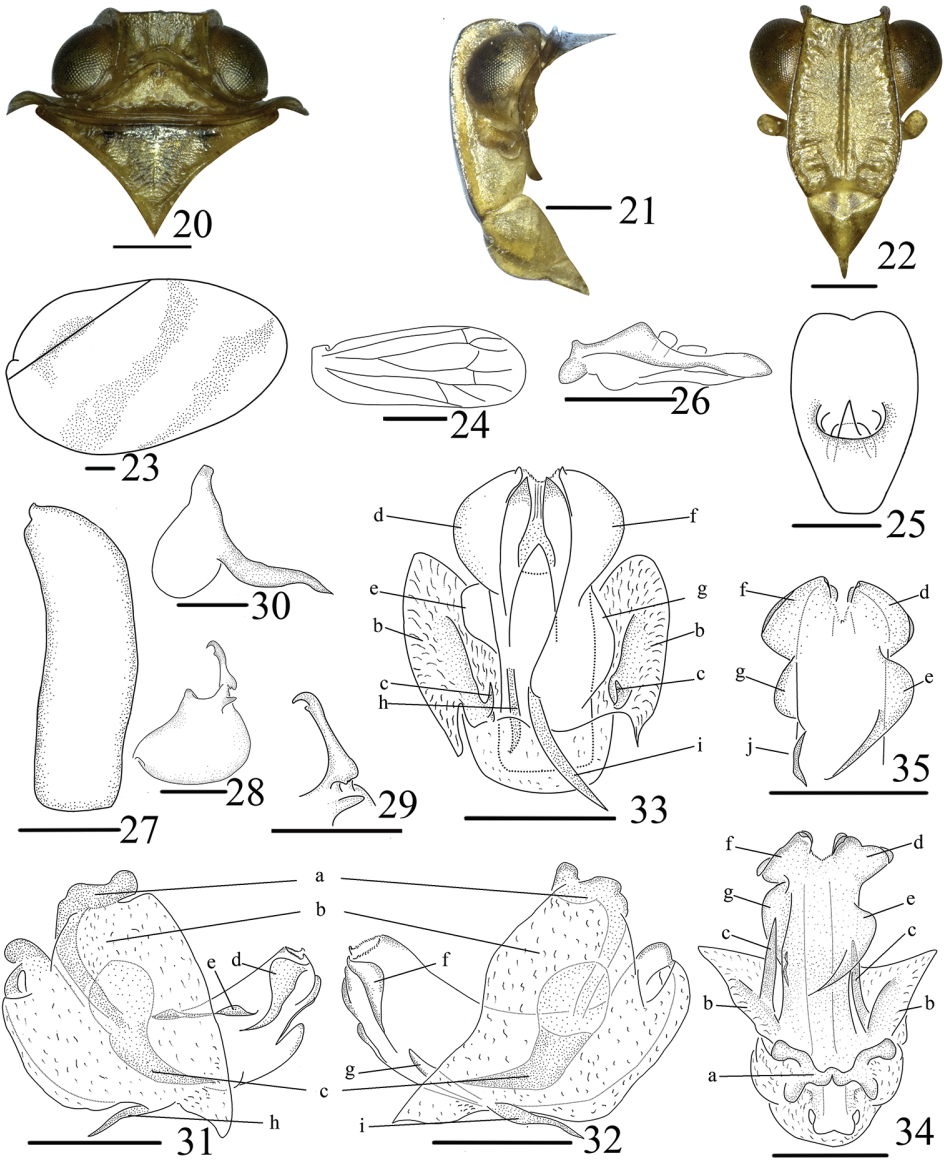
Figs 3, 4, 20–36

Type material. Holotype: ♂, China: **Guizhou**, Duyun, Doupengshan (107°07'E, 25°51'N), L.-J. Yang, 19 August 2017; paratypes 2♂♂, same data as holotype; 3♂♂, same data as holotype except J.-K. Long, 8 August 2016. GUGC and one paratype in BMNH.

Description. Male body length: 4.53–4.76 mm (n = 5); male forewings 4.23–4.38 mm (n = 5); male hindwing 1.17–1.42 (n = 5).

Coloration (Figs 3, 4, 20–22). Head fulvous, margins of vertex and frons brown (Figs 20, 22). Clypeus with dark brown strip on each side of median carinae (Figs 21, 22). Rostrum brown (Figs 21, 22). Eyes dark brown, antennae brown (Fig. 21). Pronotum and mesonotum yellow brown, mesonotum with anterior margin dark brown in the middle (Fig. 20). Forewings yellowish and slightly pellucid, with three dark brown irregular stripes subparallel, slanted caudad, venation mostly fulvous (Figs 3, 4). Hindwing brownish hyaline. Legs brown. Abdomen yellowish.

Head and thorax (Figs 5–9). Vertex longer in middle than maximal width (0.37:1.00), quadrangular, anterior margin nearly straight, posterior margin angularly concave, margins elevated (Fig. 20). Frons rough, basally narrow, longer than maximal width in basal third (1.45:1.00), with median carinae, margins elevated (Figs 21–22). Clypeus with median carinae moderately convex, arcuate in lateral view (Figs 21–22). Pronotum longer than vertex in midline (1.63:1.00), slightly depressed, without carinae and pustules (Fig. 20). Mesonotum subtriangular, about 3 times longer than pronotum, anterior margin approximately straight (Fig. 20). Forewings about 1.70 times longer than maximal width, with claval suture developed through its whole, venation obscurely reticulate (Figs 3, 23). Hindwings rudimentary, shorter than half length of forewing, venation simple (Fig. 24). Hind tibiae with 2 lateral teeth. Metatibiotarsal formula of hind leg: 10–4–2.



Figures 20–35. *Neohemisphaerius clavatus* Yang & Chen, sp. nov. adult (male), **20** head and thorax, dorsal view **21** head and thorax, lateral view **22** head and thorax, front view **23** forewing **24** hindwing **25** anal tube, dorsal view **26** anal tube, lateral view **27** pygofer, lateral view **28** genital styles, lateral view **29** capitulum of gonostylus, dorsal view **30** connective, lateral view **31** penis left lateral view **32** penis, right lateral view **33** penis, ventral view **34** apical penis, dorsal view **35** penis, dorsal view. Scale bars: 0.5 mm.

Male genitalia (Figs 25–35). Anal tube pyriform, midline longer than broad (in dorsal view) (Fig. 25). Pygofer nearly rectangular (in lateral view), narrow, anterior and posterior margin subparallel (Fig. 27). Genital styles subtriangular (in lateral view), dorsal margin with triangular process, disc with fingerlike process below capitulum

Figure 36. Host plant of *Neohemisphaerius clavatus* Yang & Chen, sp. nov. in Doupengshan, Duyun (Guizhou, China). Photograph by L.-J. Yang.

(Fig. 28). Capitulum with subapical tooth and lateral tooth (Figs 28, 29). Connective short and thick (Fig. 30). Phallobase asymmetrical, with process clavate, arched in basal third (in lateral view), directed basad, H-shaped (in dorsal view) (Figs 31, 32, 34a), process apically and phallobase basally with transparently membranous process with pair of strong hooks directed caudad (Figs 31–34b, c). Ventral lobe with pair of hooks asymmetrical in apical third, directed cephalad (Figs 31–34h, i); Lateral lobe bifurcate. Dorsal lobe with apical margin slightly notched medially (in dorsal view), with four differently sheet-shaped subapical processes (Figs 34–35d, e, f, g), the smallest near the left middle (Figs 34, 35g), with a short carinae left dorsally near its middle (Fig. 35j).

Etymology. The name of new species is derived from the Latin words "clavate", referring to the club-shaped process of the aedeagus in basal third (in lateral view).

Host plant. *Bambusa emeiensis*.

Distribution. Southwestern China (Guizhou).

Remarks. This species resembles *N. wugangensis*, *N. yangi* and *N.guangxiensis*, but can be distinguished by the following characteristics: Frons rough (Fig. 22), disc flat, slightly depressed; clypeus with median carinae without a tubercles process in middle

(Fig. 22); forewings yellowish brown, with three dark stripes subparallel (Figs 3, 4); anal tube with apical margin concave medially (in dorsal view) (Fig. 25); Phallobase asymmetrical, with process clavate in basal third (in lateral view), process directed basad, H-shaped (in dorsal view) (Figs 31, 32a, 34a).

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We are grateful to Dr. Mick Webb (Department of Entomology, The Natural History Museum, U. K.) for proofreading and advice. This work was supported by the National Natural Science Foundation of China (No. 31472033, 31601886), the Program of Science and Technology Innovation Talents Team, Guizhou Province (No. 20144001), the Program of Excellent Innovation Talents, Guizhou Province (No. 20154021), the International Cooperation Base for Insect Evolutionary Biology and Pest Control (No. 20165802), the Project Funded by China Postdoctoral Science Foundation (No. 2017M613002), the Science and Technology Project of Guiyang (No. 2017525), the Academic New Cultivation and Innovation Exploration Special Project of Guizhou University(No. 20175788) and the Science and Technology Program in Guizhou Province (No. 20177267, 20181032).

References

Bourgoin T (2018) FLOW (Fulgoromorpha Lists on the Web): a world knowledge base dedicated to Fulgoromorpha. Version 8, updated 30 September 2018. http://hemiptera-databases.org/flow/

Bourgoin T, Wang RR, Asche M, Hoch H, Soulier-Perkins A, Stroiński A., Yap S, Szwedo J (2015) From micropterism to hyperpterism: recognition strategy and standardized homology-driven terminology of the Forewing venation patterns in planthoppers (Hemiptera: Fulgoromorpha). Zoomorphology 134(1): 63–77. https://doi.org/10.1007/s00435-014-0243-6

Chan ML, Yang CT (1994) Issidae of Taiwan (Homoptera: Fulgoroidea). Chen Chung Book, Taichung, 188 pp.

Chen XS, Zhang ZG, Chang ZM (2014) Issidae and Caliscelidae (Hemiptera: Fulgoroidea) from China. Guizhou Science and Technology Publishing House, Guiyang, 242 pp.

Gnezdilov VM (2003) Review of the family Issidae (Homoptera, Cicadina) of the European fauna, with notes on the structure of ovipositor in planthoppers. Chteniya pamyati N.A. Kholodkovskogo (Meetings in memory of N.A. Cholodkovsky), St. Petersburg 56(1): 1–145.

Gnezdilov VM (2013a) Modern classification and the distribution of the family Issidae Spinola (Homoptera, Auchenorrhyncha, Fulgoroidea). Entomologicheskoe Obozrenie 92(4): 724–738.

Gnezdilov VM (2013b) Notes on planthoppers of the tribe Hemisphaeriini (Homoptera, Fulgoroidea, Issidae) from Vietnam with description of a new genus and new species. Zoologichesky Zhurnal 92(6): 659–663. [English translation published in Entomological Review (2013), 93(8): 1024–1028] https://doi.org/10.1134/S0013873813080095

Gnezdilov VM (2017) Addenda to the revisions of the genera Gergithus Stål and Hemisphaerius Schaum (Hemiptera, Auchenorrhyncha, Fulgoroidea, Issidae). Entomological Review 97(9): 1338–1352. https://doi.org/10.1134/S0013873817090123

Melichar DL (1906) Monographie der Issiden (Homoptera). Abhandlungen der k. k. Zoologisch-botanischen Gesellschaft in Wien 3: 1–327.

Meng R, Wang YL, Qin DZ (2013) A new genus of the tribe Hemisphaeriini (Hemiptera: Fulgoromorpha: Issidae) from China. Zootaxa 3691(2): 283–290. https://doi.org/10.11646/zootaxa.3691.2.7

Meng R, Webb MD, Wang YL (2017) Nomenclatural changes in the planthopper tribe Hemisphaeriini (Hemiptera: Fulgoromorpha: Issidae), with the description of a new genus and a new species. European Journal of Taxonomy 298: 1–25. https://doi.org/10.5852/ejt.2017.298

Sun YC, Meng R, Wang YL (2015) Molecular systematics of the Issidae (Hemiptera: Fulgoroidea) from China based on wingless and 18S rDNA sequence data. Entomotaxonomia 37(1): 15–26.

Wang ML, Zhang YL, Bourgoin T (2016) Planthopper family Issidae (Insecta: Hemiptera: Fulgoromorpha): linking molecular phylogeny with classification. Molecular Phylogenetics and Evolution 105: 224–234. https://doi.org/10.1016/j.ympev.2016.08.012

Zhang ZG, Chang ZM, Chen XS (2016) Review of the planthopper genus *Neohemisphaerius* (Hemiptera, Fulgoroidea, Issidae) with description of one new species from China. Zookeys 568: 13–21. https://doi.org/10.3897/zookeys.568.6700

ZooKeys 861: 43–52 (2019)
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http://zookeys.pensoft.net

RESEARCH ARTICLE

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Taxonomic study of the genus *Malaxa* Melichar, with descriptions of two new species from China (Hemiptera, Fulgoroidea, Delphacidae)

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Abstract

Two new species of the delphacid genus *Malaxa* Melichar, 1914, *M. hamuliferum* **sp. nov.** and *M. tricuspis* **sp. nov.**, are described and illustrated from southwest China (Yunnan and Hainan), providing the genus with eleven species in total. A key is provided to distinguish the seven Chinese species in the genus.

Keywords

Bamboo planthopper, Fulgoromorpha, morphology, oriental region, taxonomy

Introduction

The genus *Malaxa* Melichar, 1914 (Hemiptera, Auchenorrhyncha, Fulgoroidea, Delphacidae) falls within the tribe Tropidocephalini in the subfamily Delphacinae and is easily recognized from other members in this tribe by the very long antennae, and by the tegmina often with blackish brown markings (Chen et al. 2006). The Chinese species of *Malaxa* have been reviewed by Chen et al. (2006) and Hou et al. (2013). Recently, the New World species attributed to the genus was reviewed, *Malaxa occidentalis* Muir, 1926 and *Malaxa gracilis* Fennah, 1945 were transferred to *Lamaxa*

Bartlett & Kennedy, 2018 (type species: *Malaxa occidentalis* Muir, 1926), *Malaxa microstyla* Muir, 1930 was transferred to *Xalama* Bartlett & Kennedy, 2018 (type species: *Malaxa microstyla* Muir, 1930), and the type species *M. acutipennis* Melichar, 1914 was redescribed by Bartlett and Kennedy (2018). This genus is known to occur in the Oriental region. So far, nine species of *Malaxa* are described, including from China (five species: *M. bispinata* Muir, 1926, *M. delicata* Ding & Yang, 1986, *M. fusca* Yang & Yang, 1986, *M. hunanensis* Chen, 2006, and *M. semifusca* Yang & Yang, 1986) (Yang and Yang 1986; Ding et al. 1986; Chen et al. 2006; Ding 2006; Hou et al. 2013), Philippines (two species: *M. acutipennis* Melichar, 1914 and *M. nigra* Muir, 1919) (Melichar 1914; Muir 1919), Indonesia (two species: *M. bispinata* Muir, 1926 and *M. javanensis* Muir, 1919) (Muir 1919, 1926), Malaysia (one species: *M. obtusipennis* Muir, 1919) (Muir 1919).

Species of *Malaxa* from China with reported plant associations feed on bamboo. Specimens have been collected on leaves of bamboo in several genera, including *Bambusa*, *Indocalamus*, *Fargesia* and *Phyllostachys* (Yang and Yang 1986; Chen et al. 2006; Hou et al. 2013).

Herein, two new species: *Malaxa hamuliferum* sp. nov. and *M. tricuspis* sp. nov. are described and illustrated from Hainan and Yunnan province, China. A key to species of *Malaxa* from China is provided.

Materials and methods

The morphological terminology and measurements follow Hou et al. (2013). Body length was measured from apex of vertex to tip of tegmina. Dry male specimens were used for the description and illustration. External morphology was observed under a stereoscopic microscope and characters were measured with an ocular micrometer. Color pictures for adult habitus were obtained by the KEYENCE VHX-1000 system. The genital segments of the examined specimens were macerated in 10% KOH and drawn from preparations in glycerin jelly using a Leica MZ 12.5 stereomicroscope. Illustrations were scanned with a Canon CanoScan LiDE 200 and imported into Adobe Photoshop 6.0 for labeling and plate composition.

The type specimens of the new species are deposited in the Institute of Entomology, Guizhou University, Guiyang, China (**IEGU**).

Taxonomy

Malaxa Melichar, 1914

Malaxa Melichar, 1914: 275; Muir 1926: 7; Metcalf 1943: 103; Fennah 1945: 429; Yang and Yang 1986: 56; Ding et al. 1999: 443; Chen et al. 2006: 160; Ding 2006: 150; Bartlett 2009: 387; Hou et al. 2013: 864; Bartlett and Kennedy 2018: 514.

Type species. *Malaxa acutipennis* Melichar, 1914.

Diagnosis. Description from Hou et al. (2013: 286–287) "Body slender and elongate, length (from apex of vertex to tip of tegmina): male 3.7–4.8 mm, female 4.3–5.1 mm, often with blackish brown markings. Head with eyes narrower than pronotum. Vertex longer or slightly shorter in middle than broad at base (0.95–1.24: 1), apex projected in front of eyes. Submedian carinae uniting before apex, greatest length of basal compartment shorter than wide at base of vertex (0.48–0.81: 1). Frons relatively long, longer in middle line than wide at widest part (~ 2.73–3.00: 1), widest at middle or apex. Rostrum reaching mesothoracic trochanters. Antennae cylindrical, very long, surpassing apex of clypeus, basal segment longer in middle than wide at apex (3.67–5.22: 1), shorter than frons in middle line (0.49–0.74: 1), shorter than the second segment (0.40–0.56: 1). Pronotum shorter than vertex in middle line (0.58–0.96: 1), lateral carinae attaining hind margin. Mesonotum longer in middle line than vertex and pronotum together (1.33–2.05: 1). Tegmina elongate, longer in middle line than wide at widest part (1.76–3.16: 1), much longer than abdomen, hyaline, cross vein deposited medially, apical margin acutely rounded. Spinal formula of hind tibia 5-6-4. Post-tibial spur large and thick, concave on inner surface, without teeth along the hind margin, with an apical tooth. Anal segment of male short, ring-like, left lateroapical angle produced into process. Pygofer with two broad lamellate medioventral processes, between of them with a V-like emargination. Genital styles broad in basal half, forked or with process at apex. Aedeagus with or without phallobase, phallus tubular, curved C-like and directed segmental venter."

Key to species (males) of *Malaxa* from China *(revised from Hou et al. 2013)*

1 Postclypeus yellow; tegmina with apical veins Cu_1 and M_3 diverging apically, posterior half of apical tegmina dark brown (see Chen et al. 2006: figs 2, 3) .....***M. semifusca***

– Postclypeus with basal half blackish brown; tegmina with apical veins Cu_1 and M_3 fused, first and second apical cells hyaline **2**

2 Anal segment of male without process; aedeagus with phallobase..... **3**

– Anal segment of male with a long process; aedeagus without phallobase **4**

3 Genae with basal half dark brown, apical half yellowish white; tegmina mostly hyaline; pygofer with 3 medioventral processes very distinct; genital styles with inner margin without process (see Hou et al. 2013: figs 2, 3, 8–11)..... .....***M. bispinata***

– Genae all dark brown; tegmina with basal half most yellow, apical half most dark brown; pygofer with medioventral processes not distinct; genital styles with inner margin with large process medially, hook-like (Figs 7–9, 11, 13). ..... ***M. hamuliferum* sp. nov.**

4 Genae dark brown; in posterior view, process of anal segment of male situated in middle of ventral margin (Chen et al. 2006: figs 21, 23, 25)..... ***M. hunanensis***

– Genae mostly dark brown but apical with small part yellow; in posterior view, process of anal segment of male situated on left side of ventral margin..... **5**

5 Genital styles with apex not forked; aedeagus with three processes (Figs 22–24) ..... ***M. tricuspis* sp. nov.**
– Genital styles with apex forked; aedeagus with two processes **6**
6 Area between lateral carinae of pronotum dark brown; two branches of outer apical angle of genital styles subequal; aedeagus with a small spine situated near basal third, directed caudally (see Chen et al. 2006: figs 30, 37, 38) ..... ***M. delicata***
– Area between lateral carinae of pronotum mostly yellow; two branches of outer apical angle of genital styles unequal; aedeagus with a small tooth situated near middle, directed right (see Chen et al. 2006: figs 11, 18, 19) ..... ***M. fusca***

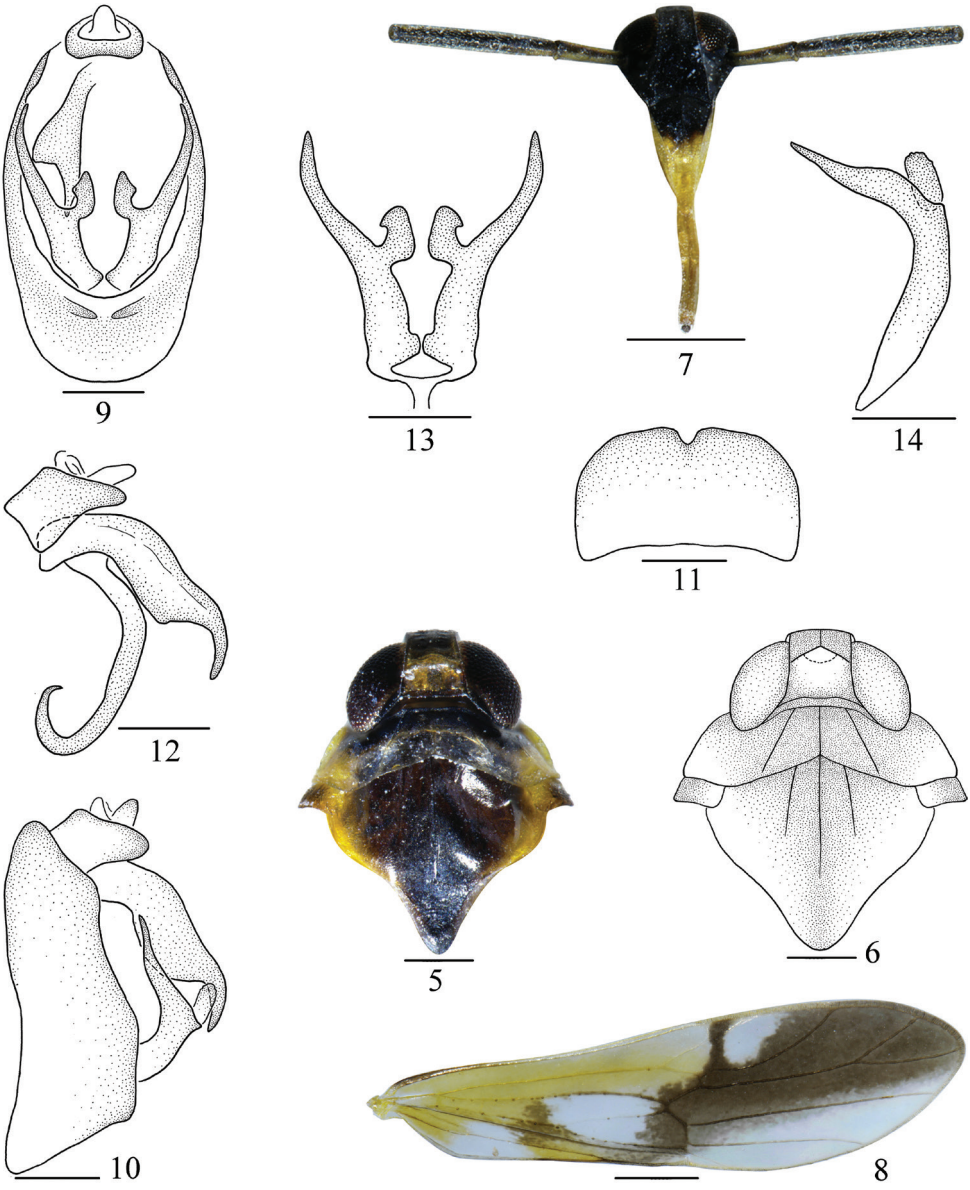
***Malaxa hamuliferum* sp. nov.**
http://zoobank.org/590A3548-6436-40FB-91F0-3A1CB1CD0237
Figs 1, 2, 5–14

Type material. Holotype: ♂, **China:** Yunnan, Yingjiang County (24°44'N, 97°33'E), on bamboo, 17 August 2018, Hong-Xing Li; paratypes, 5♂♂, 10♀♀, same data as holotype, Hong-Xing Li and Qiang Luo.

Etymology. The specific name is derived from the Latin word "*hamulus*" and the postfix "*-ferus*", referring to the middle of genital styles with large process, hook-like.

Measurements. Body length including tegmina: male 3.9–4.1 mm (N = 10); female 4.8–5.0 mm (N = 5); tegmen length: male 3.4–3.6 mm (N = 10); female 4.0–4.4 mm (N = 5).

Figures 1–4. *Malaxa hamuliferum* sp. nov. **1** male habitus, dorsal view **2** same, lateral view **3–4** *Malaxa tricuspis* sp. nov. **3** male habitus, dorsal view **4** same, lateral view. Scale bars: 0.5 mm.



Figures 5–14. *Malaxa hamuliferum* sp. nov. **5** head and thorax, dorsal view **6** same **7** face **8** tegmen **9** male genitalia, posterior view **10** same, lateral view **11** pygofer, ventral view **12** anal segment and aedeagus, lateral view **13** genital style, posterior view **14** same, left lateral view. Scale bars: 0.5 mm (**5–8**); 0.2 mm (**9–14**).

Diagnosis. The salient features of the new species include the following: aedeagus with phallobase broad basally, apical third narrowing abruptly, and genital styles with large process at middle, hook-like.

Description. *Coloration*. General color pale yellowish brown, with dark brown to black markings, shiny (Figs 1, 2). Vertex with basal half yellowish brown, apical half

pale black. Pronotum and mesonotum brown to black except each lateral side yellow (Figs 5, 6). Frons and genae black. Clypeus with basal half black, rest yellow. Rostrum yellow except apex pale brown (Fig. 7). Eyes and ocelli reddish brown. First segment of antennae with dorsal side pale yellow, with ventral side brown, second segment dark brown. Tegmina with basal half yellow except areas around apex of Cu_1, after bifurcation of IA and IIA hyaline, at apical half, along Sc_1, sc-r and area between R_1 and M_2 dark brown (Fig. 8). Wings hyaline, veins brown. Abdomen with dorsal side black, with ventral side yellow white. Genitalia dark brown.

Head and thorax. Vertex (Figs 5, 6) longer submedially than wide at base ~ 0.98: 1, at base longer than at apex ~ 1.4: 1, submedian carinae uniting slightly beyond middle, apex produced in front of eyes, apical margin straight, greatest length of basal compartment shorter than wide at base of vertex ~ 0.53: 1. Frons (Fig. 7) longer in middle line than wide at widest part ~ 1.47: 1, widest at apex. Postclypeus wide at base as wide as frons at apex. Antennae very long, cylindrical, surpassing apex of clypeus, first segment longer than wide ~ 4.06: 1, shorter than frons in middle line ~ 0.79: 1, shorter than the second segment ~ 0.43: 1 (Fig. 7). Pronotum with lateral carinae not attaining hind margin, shorter than vertex ~ 0.69: 1. Mesonotum with lateral carinae not attaining hind margin, longer in middle line than vertex and pronotum together ~ 1.76: 1 (Figs 5, 6). Tegmina narrow, longer than widest part ~ 4.12: 1 (Fig. 8).

Male genitalia. Anal segment of male small, ring-like (Fig. 9). Pygofer in profile tapering to dorsad (Fig. 10), in posterior view with opening longer than wide (Fig. 9), in ventral view medioventral margin V-like (Fig. 11). Aedeagus with phallus slender, tubular, acute at apex, apical third curved C-like, phallobase in profile broad basally, apical third narrowing abruptly (Fig. 12). Genital styles long and slender, tapering to apex, inner margin with large process at middle, hook-like (Fig. 13).

Host plant. Bamboo.

Distribution. Southwest China (Yunnan).

Remarks. This species is similar to *Malaxa semifusca* Yang & Yang, 1986 but differs from it by: (1) frons and genae black, clypeus with basal half black (frons with apical third, genae with ventral half and clypeus yellow in *M. semifusca*); (2) anal segment of male without process (anal segment with left lateroapical process small and obtuse in *M. semifusca*); (3) aedeagus with phallobase without tooth at apex (aedeagus with phallobase incomplete, apex membraneous, with several teeth along margin and around apex in *M. semifusca*).

***Malaxa tricuspis* sp. nov.**

http://zoobank.org/D71D3BB4-F1DC-4030-926A-1270252C7532

Figs 3, 4, 15–24

Type material. Holotype: ♂, **China:** Hainan, Wanning County (18°55'N, 110°20'E), on bamboo, 6 May 2017, Hong-Xing Li; paratypes, 6♂♂, 8♀♀, same data as holotype.

Etymology. The specific name is derived from the Latin word "*tricuspis*", referring to aedeagus with three small processes.

Measurements. Body length including tegmina: male 3.5–3.7 mm (*N* = 7); female 4.1–4.3 mm (*N* = 8); tegmen length: male 3.0–3.2 mm (*N* = 7); female 3.5–3.8 mm (*N* = 8).

Diagnosis. The salient features of the new species include the following: left lateroapical process of anal segment stout and twisted, tapering apically; aedeagus with three small processes.

Description. *Coloration*. General color pale yellowish brown, with dark brown to black markings, shiny (Figs 3, 4). Vertex, pronotum and mesonotum pale black except each lateral side yellow (Figs 15, 16). Frons and genae black except small area at apex yellow. Clypeus with basal half black, rest yellow (Fig. 17). Eyes and ocelli reddish brown. Antennae with dorsal side pale yellow, with ventral side brown. Tegmina with basal half pale yellow except areas around Sc+R, apex of Cu_1, after bifurcation of IA and IIA hyaline, at apical half, along Sc_1, sc-r, and area between R_1 and M_2 dark brown (Fig. 18). Wings hyaline, veins brown. Abdomen with dorsal side black, with ventral side yellow. Genitalia brown.

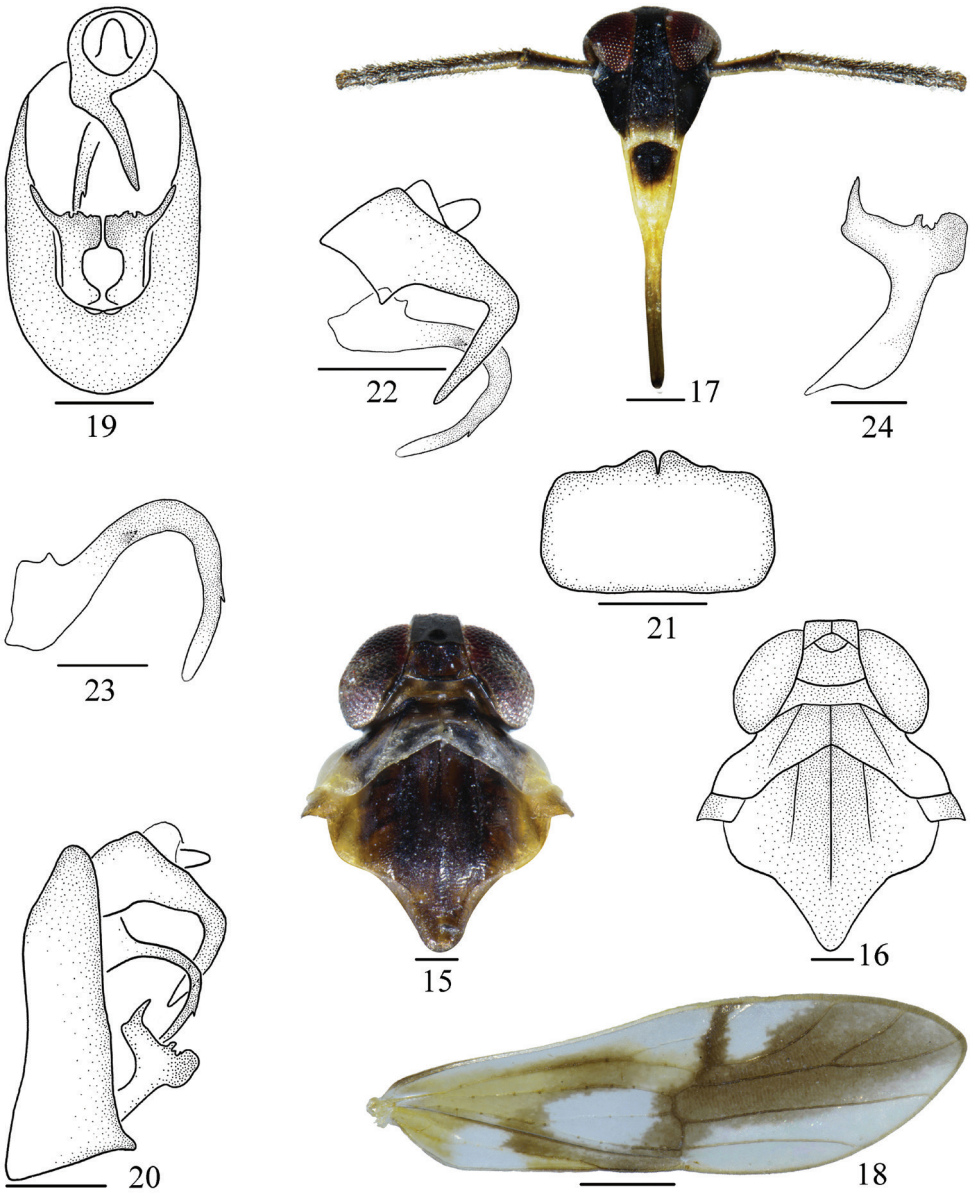
Head and thorax. Vertex (Figs 15, 16) longer submedially than wide at base ~ 0.91: 1, at base longer than at apex ~ 1.65: 1, forming a circular cell, submedian carinae uniting slightly beyond middle, apex produced in front of eyes, apical margin straight, greatest length of basal compartment shorter than wide at base of vertex ~ 0.55: 1. Frons (Fig. 17) longer in middle line than wide at widest part ~ 2.83: 1, widest at near apex. Postclypeus wide at base as wide as frons at apex. Antennae very long, cylindrical, surpassing apex of clypeus, first segment longer than wide ~ 4.44: 1, shorter than frons in middle line ~ 0.57: 1, shorter than the second segment ~ 0.41: 1 (Fig. 17). Pronotum with lateral carinae not attaining hind margin, shorter than vertex ~ 0.55: 1. Mesonotum with lateral carinae not attaining hind margin, longer in middle line than vertex and pronotum together ~ 2.06: 1 (Figs 15, 16). Tegmina narrow, longer than widest part ~ 3.28: 1 (Fig. 18).

Male genitalia. Anal segment of male small, ring like, left lateroapical process stout and twisted, tapering to apex (Fig. 19). Pygofer in profile tapering to dorsad, ventral angles strongly produced (Fig. 20), in posterior view with opening longer than wide (Fig. 19), in ventral view medioventral processes wide, concave medially (Fig. 21). Aedeagus simple, tubular, broad basally then tapering to apex, with stout process at base, a spine at basal third and with small tooth at apical third (Figs 22, 23). Genital styles long, broad basally, apical half narrowing abruptly, inner margin with several teeth at middle (Figs 19, 24).

Host plant. Bamboo.

Distribution. Southwest China (Hainan).

Remarks. This species is similar to *Malaxa fusca* Yang & Yang, 1986 but differs from it by: (1) anal segment of male with left lateroapical process twisted but not S-like, not swelled subapically (anal segment with left lateroapical process twisted, S-like, swelled subapically in *M. fusca*); (2) aedeagus with stout process at base, a spine at basal third and with small tooth at apical third (aedeagus with small process at base and with a spine near middle in *M. fusca*); (3) genital styles with apical half narrowing abruptly, not forked at apex (genital styles with outer angle forked at apex, inner branch longer than outer one in *M. fusca*).



Figures 15–24. *Malaxa tricuspis* sp. nov. **15** head and thorax, dorsal view **16** same **17** face **18** tegmen **19** male genitalia, posterior view **20** same, lateral view **21** pygofer, ventral view **22** anal segment and aedeagus, lateral view **23** aedeagus **24** genital style, left lateral view. Scale bars: 0.5 mm (**15–18**); 0.2 mm (**19–22**); 0.1 mm (**23, 24**).

This species is also similar to *M. delicata* Ding & Yang, 1986 but differs from it by: (1) anal segment of male with left lateroapical process twisted near base (anal segment with left lateroapical process twisted near apex in *M. delicata*) (2) aedeagus with stout process at base, a spine at basal third and with small tooth at apical third (aedeagus

with process at base and with small spine at basal third in *M. delicata*); (3) genital styles with outer angle not forked at apex (genital styles with outer angle forked at apex, two branches subequally long in *M. delicata*).

Discussion

Melichar (1914) established the genus *Malaxa* with the type species *M. acutipennis* Melichar, 1914 from Philippines. This genus is only known to occur in the Oriental region, with highest species density occurring in China. Bartlett and Kennedy (2018: 515–516) noted "Several differences can be observed between *Malaxa acutipennis* and the Chinese species in that genus. The most salient of these are that *M. acutipennis* has an apically pointed forewing (rounded in all other *Malaxa*) with the leading margin arced (giving the wing a spatulate appearance; parallel-sided in all other *Malaxa*); the more elongate pronotum with the carinae clearly reaching the hind margin (most other *Malaxa* with a relatively shorter pronotum with lateral carinae not reaching); and the genitalia with a simple ventral margin of the pygofer opening (vs. having projections on the opening of the pygofer); and the simple anal tube (most Chinese *Malaxa* bear a single, large, asymmetrical projection on the anal tube)."

The *Malaxa* species distributed in China with common type characters: body slender and elongate, often with blackish brown markings; antennae cylindrical, very long, surpassing apex of clypeus, basal segment shorter than the second segment (0.40–0.56: 1); tegmina apically rounded, leading margin straight; anal tube of male either simple or with 1–2 processes; opening of pygofer usually bearing two broad lamellate medioventral processes, between them a V-like emargination; genital styles broad in basal half, forked or with process at apex; aedeagus with or without phallobase, phallus tubular, curved C-like and directed segmental venter. Based mainly on the characters of the morphological and male genitalia, we also found obvious differences between the Chinese *Malaxa* and the type species *M. acutipennis*, which agrees Bartlett and Kennedy's (2018) description. Therefore, the genus level composition of *Malaxa* may require reconsideration, with the general concern that the Chinese species may not be congeneric with the type species from the Philippines. However, *M. obtusipennis* Muir, 1919 (from Malaysia: Sabah) was described from three females. The features of the male terminalia of *M. obtusipennis* are not available for consideration, which limits our ability to place this species. Therefore, in this paper, we provisionally place the two new species in the genus *Malaxa*, but the genus level composition of *Malaxa* is required to reconsideration and more taxon samples or molecular data are still required to confirm the relationships within *Malaxa* in the future.

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References

Bartlett CR (2009) A new genus of New World Tropidocephalini (Hemiptera: Delphacidae: Delphacinae), with the description of two new species. Entomological News 120(4): 387–396. https://doi.org/10.3157/021.120.0407

Bartlett CR, Kennedy AC (2018) A review of New World *Malaxa* (Hemiptera: Fulgoroidea: Delphacidae). Zootaxa 4441 (3): 511–528. https://doi.org/10.11646/zootaxa.4441.3.5

Chen XS, Li XF, Liang AP, Yang L (2006) A review of the bamboo delphacid genus *Malaxa* (Hemiptera: Fulgoroidea: Delphacidae) from China. Annales Zoologici 56(1): 159–166.

Ding JH, Yang LF, Hu CL (1986) Descriptions of new genera and species of Delphacidae attacking bamboo from Yunnan Province, China. Acta Entomologische Sinica 29(4): 415–425.

Ding JH (2006) Fauna Sinica. Insecta Vol. 45. Homoptera, Delphacidae. Science Press, Beijing, 150–154.

Ding JH, Zhuo WX, Huang BK (1999) Delphacidae of Fujian (Homoptera: Fulgoroidea). In: Huang BK (Ed.) Fauna of Insects in Fujian Province of China. Vol. 2. Fujian Science and Technology Press, Fuzhou, 432–464.

Fennah RG (1945) New lanternflies (Fulgoroidea) from South America. Proceedings of the United States National Museum 96(3189): 95–106. https://doi.org/10.5479/si.00963801.96-3189.95

Hou XH, Yang L, Chen XS (2013) A checklist of the genus *Malaxa* (Hemiptera: Fulgoromorpha: Delphacidae) with descriptions and illustrations of *Malaxa bispinata* newly recorded in China and the fifth instar of *Malaxa delicata*. Florida Entomologist 96(3): 864–870. https://doi.org/10.1653/024.096.0321

Melichar L (1914) Neue Fulgoriden von den Philippinen: I. Theil. Philippine Journal of Science 9(3): 269–283.

Metcalf ZP (1943) General Catalogue of the Hemiptera. Fascicle IV, Fulgoroidea, Part 3, Araeopidae (Delphacidae). Smith College, Northhampton, Massachusetts, 551 pp.

Muir F (1919) Some Malayan Delphacidae (Homoptera). Philippine Journal of Science 15(6): 521–531.

Muir F (1926) Contributions to our knowledge of South American Fulgoroidea (Homoptera). Part I. The Family Delphacidae. Experiment Station of the Hawaiian Sugar Planters' Association, Entomological Series, Bulletin 18: 1–51.

Muir F (1930) On some South American Delphacidae (Homoptera, Fulgoroidea). Entomologisk Tidskrift 51: 207–215.

Yang JT, Yang CT (1986) Delphacidae of Taiwan (1) Asiracinae and the tribe Tropidocephalini (Homoptera: Fulgoroidea). Taiwan Museum Special Publication Series 6: 1–79.

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RESEARCH ARTICLE

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A key to the bamboo-feeding genus *Bambusana* Anufriev (Hemiptera, Cicadellidae, Deltocephalinae, Athysanini), with description of one new species from China

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Abstract

A new species of the bamboo-feeding leafhopper genus *Bambusana* Anufriev, 1969, *B. longispina* Luo & Chen, **sp. nov.** is described and illustrated from China (Yunnan Province). A checklist and key to known species of this genus are provided. Figures are also provided for *B. bambusae*, *B. biflaka*, *B. fopingensis* and *B. multidentata*.

Keywords

Homoptera, morphology, new species, taxonomy

Introduction

The leafhopper genus *Bambusana* (Deltocephalinae, Athysanini) was established by Anufriev (1969) with two species: *B. bambusae* (Matsumura, 1914) (type species) and *B. jenjouristi* Anufriev, 1969 from Japan. Later, Anufriev and Emeljanov (1988) reported *B. bambusae* from the Soviet Far East. Dai and Zhang (2006) first recorded this genus from China and described two new species: *B. fopingensis* and *B. multidentata*, and reported *B. bambusae* from China. Recently, Li (in Li et al. 2011) described two new species

from China: *B. biflaka* and *B. nigrimaculata*, and recognized *B. fopingensis* as a junior synonym of *B. multidentata* in their study but did not provide any justification for the synonymy. We here still recognize *B. fopingensis* as a valid species based on the pygofer with a strong ventro-caudal process which is significantly different from *B. multidentata.*

In this paper, a new species, *B. longispina* sp. nov. is described and illustrated from Yunnan Province, China bringing the total in the genus to seven (six from China); see key.

Material and methods

The terminology of morphological and genital characters follows Li et al. (2011) and Zahniser and Dietrich (2013). Male specimens were used for the descriptions and illustrations. External morphology was observed under a stereoscopic microscope and characters were measured with an ocular micrometer. Color pictures for adult habitus were obtained by using the KEYENCE VHX-1000 system. The genital segments of the examined specimens were macerated in 10% NaOH and drawn from preparations in glycerin jelly using a Leica MZ 12.5 stereomicroscope. Illustrations were scanned with a Canon CanoScan LiDE 200 and imported into Adobe Photoshop CS8 for labeling and plate composition.

The type specimens of the new species are deposited in the Institute of Entomology, Guizhou University, Guiyang, China (IEGU).

Taxonomy

Genus *Bambusana* Anufriev, 1969
Figs 1–51

Bambusana Anufriev, 1969: 403; Dai and Zhang 2006: 63; Li et al. 2011: 40.

Type species. *Thamnotettix bambusae* Matsumura, 1914, by original designation.

Diagnosis. This genus can be differentiated from other genera of Athysanini by the follow characters: relatively elongate leafhoppers with crown slightly longer medially than next to eyes; male pygofer side elongate, with one or two well sclerotized processes on ventral margin; subgenital plate usually elongate, triangular; aedeagus with basal apodeme usually present, shaft with or without small distal processes; gonopore apical or apical on ventral surface.

Description. Body elongate. Head including eyes subequal to or slightly wider than pronotum (Figs 1, 21, 24, 27, 30, 33). Crown with anterior margin roundly produced anteriorly, distinctly shorter medially than width between eyes (Figs 1, 21, 24, 27, 30, 33). Transition of crown to face rounded (Figs 5, 22, 25, 28, 31, 34); ocellus situated on or near frontal lateral margin of crown, less than 1/3 distant from eye to crown apex (Figs 1, 21, 24, 27, 30, 33). Clypellus widening apically, relatively flat (Figs 6, 23, 26, 29, 32, 35). Pronotum with anterior margin strongly and roundly

Figures 1–6. *Bambusana longispina* sp. nov. **1** Male habitus, dorsal view **2** male habitus, dorsal and lateral view **3** male habitus, lateral view **4** head and thorax, dorsal view **5** head and thorax, lateral view **6** face. Scale bars: 1.0 mm (**1–3**); 0.5 mm (**4–6**).

produced, posterior margin slightly concave. Scutellum subequal to or slightly shorter than pronotum (Figs 1, 4, 22, 25, 28, 31, 34). Forewing elongate and rounded apically, considerably longer than abdomen, with four apical cells; appendix well developed (Figs 1–3, 21, 22, 24, 25, 27, 28, 30, 31, 33, 34).

Male genitalia with pygofer elongate in profile, with one or two sclerotized processes on ventral margin or ventral margin dentate; several macrosetae posteriorly (Figs 7, 8, 36, 37, 40, 41, 44, 45, 48, 49). Valve narrowly triangular, subequal to or shorter than length of subgenital plate (Fig. 15). Subgenital plate elongate, triangular, a uniseriate row of macrosetae along ventrolateral margin (Fig. 15). Connective Y-shaped, shaft subequal to or distinctly longer than arms (Fig. 13). Styles elongate, apical process short to long, tapered to acute apex; lateral lobe weakly or well developed, with a few fine setae (Figs 12, 14). Aedeagus with basal apodeme usually present, shaft elongate, cylindrical,

with or without small process near apex, gonopore small, apical or apical on ventral surface; with short preatrium sometimes present (Figs 9–11, 38, 39, 42, 43, 46, 47, 50, 51).

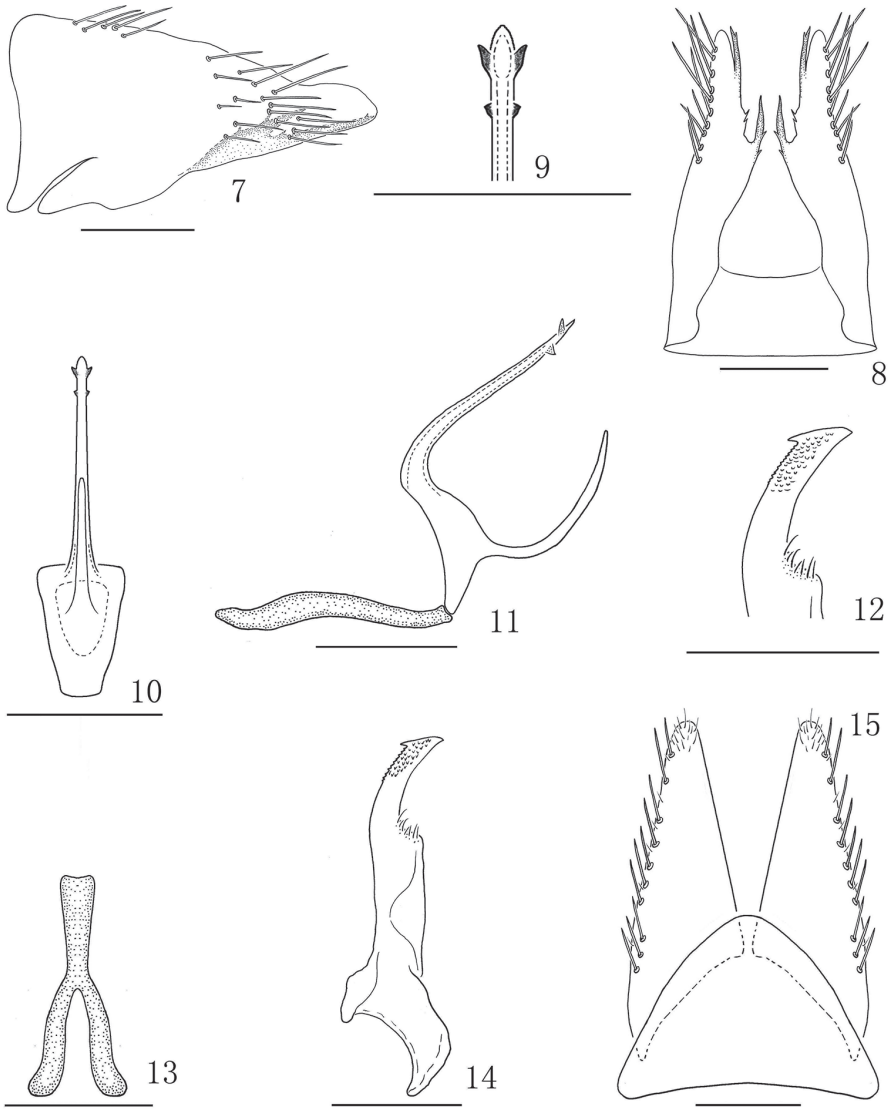
Distribution. China; Japan; Russia.

Checklist and distributions of species of *Bambusana* Anufriev, 1969

B. bambusae (Matsumura, 1914), Anufriev 1969: figs 1–6; Dai and Zhang 2006: figs 14–19; Li et al 2011: figs 5–29(1–6). China (Guizhou, Henan, Gansu); Japan (Hokkaido, Honshu, Shikoku); Russia
B. biflaka Li, 2011: figs 5–31(1–7), II-1. China (Sichuan, Hainan)
B. fopingensis Dai & Zhang, 2006: figs 1–8. China (Shaanxi, Guizhou)
B. jenjouristi Anufriev, 1969: figs 7–11. Japan (Honshu, Kobe)
B. longispina Luo & Chen, sp. nov. China (Yunnan)
B. multidentata Dai & Zhang, 2006: figs 9–13. China (Guizhou, Shaanxi)
B. nigrimaculata Li, 2011: figs 5–32(1–7), II-2. China (Yunnan)

Key to species of *Bambusana* (males only)

1 Pygofer with two sclerotized processes on ventral margin **2**
– Pygofer with one sclerotized process on ventral margin or ventral margin dentate **3**
2 Aedeagus with shaft with a tooth-like ventro-basal process, directed ventrally, without small processes subapically (Figs 38, 39) ..... ***B. bambusae***
– Aedeagus with a subbasal medial process from ventral margin, directed dorsally; shaft with a pair of subapical processes on each side (Figs 9–11)..... ***B. longispina* sp. nov.**
3 Pygofer with a long process arising from base of ventral margin, shaft with one pair of small processes near apex..... **4**
– Pygofer with one sclerotized process or ventral margin dentate, shaft without process near apex **5**
4 Aedeagal shaft nearly straight in lateral view (Fig. 42) ..... ***B. biflaka***
– Aedeagal shaft sinuate in lateral view (Li et al. 2011: fig. 5-32-5)..... ***B. nigrimaculata***
5 Pygofer with a process arising from middle of ventral margin; aedeagal shaft with a tooth-like process subbasally (Anufriev 1969: figs 7, 10, 11) ..... ***B. jenjouristi***
– Pygofer with a strong ventro-caudal process or ventral margin dentate; aedeagal shaft without process subbasally **6**
6 Pygofer with ventral margin dentate; style with lateral lobe well developed (Figs 48, 49, Dai and Zhang 2006: figs 9–11) ..... ***B. multidentata***
– Pygofer with a strong ventro-caudal process, style with lateral lobe weakly developed (Figs 44, 45, Dai and Zhang 2006: figs 4, 5)..... ***B. fopingensis***



Figures 7–15. *Bambusana longispina* sp. nov. **7** Male pygofer, lateral view **8** male pygofer, ventral view **9** apex of shaft, ventral view **10** aedeagus, caudal view **11** aedeagus and connective, lateral view **12** apex of style, dorsal view **13** connective, dorsal view **14** style, dorsal view **15** valve and subgenital plate, ventral view. Scale bars: 0.2 mm (**7–15**).

***Bambusana longispina* Luo & Chen, sp. nov.**
http://zoobank.org/141DEC67-E295-4678-B247-35D9AFC29B39
Figs 1–20

Description. *Measurements.* Body length (including forewing): male 6.76 mm (1 specimen); female 6.80 (1 specimen); forewing length: male 5.60 mm (1 specimen); female 5.72 (1 specimen).

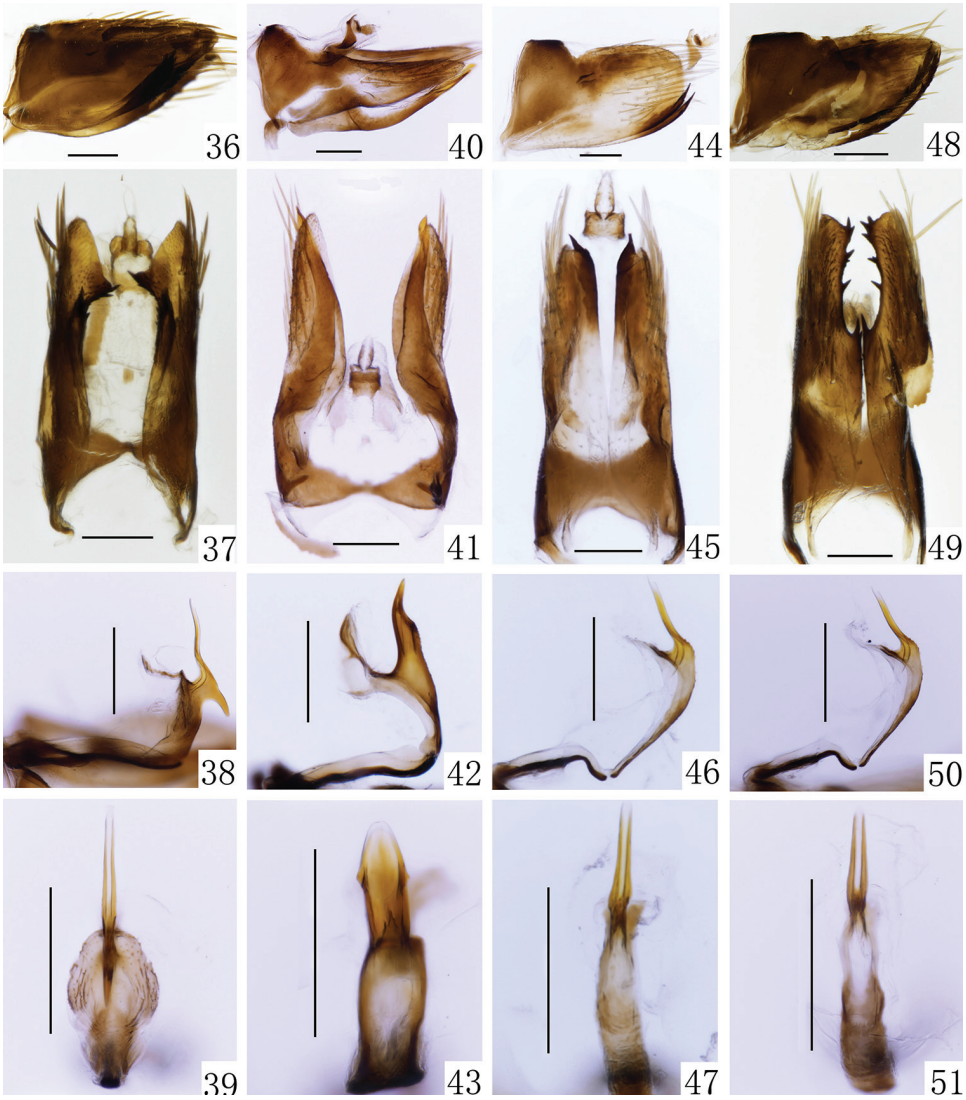
Figures 16–20. *Bambusana longispina* sp. nov. **16** Female sternite VII, ventral view **17** first valvula, lateral view **18** apex of first valvula, lateral view **19** second valvula, lateral view **20** apex of second valvula, lateral view. Scale bars: 0.5 mm (**16–17, 19**); 0.2 mm (**18, 20**).

Coloration. Generally yellowish brown (Figs 1–3). Crown and pronotum yellowish brown to brown (Figs 4, 5). Face dark brown (Fig. 6). Legs with dark spots (Fig. 3). Forewing with yellow veins (Figs 1–3).

Head and thorax. Head including eyes as long as width of pronotum. Crown with fore margin arc-shaped, median length shorter than width between eyes (0.4:1) (Figs 1, 4). Coronal suture visible at basal one-third to one-half of crown (Figs 1, 4). Ocelli about 1/3 distant from eye to crown apex (Figs 1–4). Face with frontoclypeus longer than wide; anteclypeus slightly expanded apically; lorum broad (Fig. 6); antennae arising near lower corner of eye (Fig. 6). Scutellum slightly shorter than pronotum (0.8:1) (Fig. 4). Forewing elongate, with four apical cells; appendix wide (Figs 1–3).

Male genitalia. Pygofer elongate in profile, ventral margin with two elongate acute processes at distal one-third and subapically (Figs 7, 8), with a few fine teeth-like processes along ventroposterior margin (Fig. 8). Valve triangular, basal width slightly longer than median length (1.42:1) (Fig. 15). Subgenital plate elongate, triangular; with uniseriate row of ventral macrosetae along lateral margin; apical margin rounded with very short fine setae (Fig. 15). Connective Y-shape, shaft robust, similar length to arms (Fig. 13). Styles (Figs 10, 13) elongate, with apophysis relatively long and stout with small subapical tooth-like process from inner margin (Figs 12, 13). Aedeagus with basal apodeme absent; shaft elongate, cylindrical, tapering to acute apex, with two pairs of small triangle processes near apex (Figs 9–11); with subbasal elongate medial process from ventral margin, directed dorsally; with short preatrium.

Figures 21–35. 21–23 *B. bambusae* **21** male habitus, dorsal view **22** male habitus, lateral view **23** face **24–26** *B. biflaka* **24** male habitus, dorsal view **25** male habitus, lateral view **26** face **27–29** *B. fopingensis* **27** male habitus, dorsal view **28** male habitus, lateral view **29** face **30–32** *B. multidentata* **30** male habitus, dorsal view **31** male habitus, lateral view **32** face **33–35** *B. nigrimaculata* **33** male habitus, dorsal view **34** male habitus, lateral view **35** face. Scale bars: 1.0 mm (**21, 22, 24, 25, 27, 28, 30, 31, 33, 34**); 0.5 mm (**23, 26, 29, 32, 35**).



Figures 36–51. 36–39 *B. bambusae* **36** male pygofer, lateral view **37** male pygofer, ventral view **38** aedeagus and connective, lateral view **39** aedeagus, caudal view **40–43** *B. biflaka* **40** male pygofer, lateral view **41** male pygofer, ventral view **42** aedeagus and connective, lateral view **43** aedeagus, caudal view **44–47** *B. fopingensis* **44** male pygofer, lateral view **45** male pygofer, ventral view **46** aedeagus and connective, lateral view **47** aedeagus, caudal view **48–51** *B. multidentata* **48** male pygofer, lateral view **49** male pygofer, ventral view **50** aedeagus and connective, lateral view **51** aedeagus, caudal view. Scale bars: 0.2 mm.

Female genitalia. Sternite VII (Fig. 16) with anterior margin nearly straight and posterior margin strongly convex with blunt median tooth. First valvula (Figs 17, 18) curved, tapering apically with strigate sculpture extended to dorsal margin. Second valvula (Figs 19, 20) broad, gradually tapered to acute apex; dorsal margin with numerous small triangular teeth; with dorsal sclerotized and hyaline region.

Type material. ***Holotype:*** ♂, **China**: Yunnan Province, Maguan County, 23. XI. 2016, Ya-Lin Yao. ***Paratype:*** 1♀, same as holotype.

Host plants. Bamboo.

Distribution. Southwest China (Yunnan Province).

Remarks. This new species is similar to *B. bambusae*, but can be distinguished from the latter by: aedeagus with a long medial process subbasally from ventral margin, directed dorsally (Figs 10, 11) (aedeagus with a tooth-like ventro-basal process, directed ventrally in *bambusae*); shaft long and tapered to apex, with two pairs of small triangle processes near apex (Figs 9–11) (shaft without process subapically in *bambusae*).

Etymology. The name is derived from the Latin words "longus" and "spina", referring to the aedeagus with a long spinous process near base (Fig. 11).

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References

Anufriev GA (1969) Description of a new genus of leafhoppers (Homoptera, Auchenorrhyncha) from Japan. Bulletin de l'Academie Polanaise des Sciences. Serie des Sciences Biologiques 17(6): 403–405.

Anufriev GA, Emeljanov AF (1988) Suborder Cicadinea (Auchenorrhyncha). Keys to the identification of insects of the Soviet Far East. Vol. 2: Homoptera & Heteroptera. Nauka, Leningrad, 972 pp.

Dai W, Zhang YL (2006) Taxonomic study on new-record genus *Bambusana* Anufriev (Hemiptera: Cicadellidae: Deltocephalinae) in China. Zootaxa 1187: 61–68. https://doi.org/10.11646/zootaxa.1187.1.4

Li ZZ, Dai RH, Xing JC (2011) Deltocephalinae from China (Hemiptera: Cicadellidae). Popular Science Press, Beijing, 40–44. [in Chinese with English summary]

Matsumura S (1914) Die Jassinen und einige neue Acocephalinen Japans. The Journal of the Sapporo Agricultural College (Sapporo, Japan) 5(7): 165–240.

Zahniser JN, Dietrich CH (2013) A review of the tribes of Deltocephalinae (Hemiptera: Auchenorrhyncha: Cicadellidae). European Journal of Taxonomy 45: 1–211. https://doi.org/10.5852/ejt.2013.45

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RESEARCH ARTICLE

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Revision of the Neotropical *Neuratelia* Rondani (Diptera, Mycetophilidae, Sciophilinae): two new species, a new combination, and a new synonym

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Abstract

We describe two new Neotropical species of *Neuratelia* Rondani from the high Central Andes of Colombia, *N. altoandina* **sp. nov.** and *N. colombiana* **sp. nov.** The holotype of *Eudicrana elegans* Lane actually is a species of *Neuratelia* and a new combination is proposed. Our examination of the holotype of *Neuratelia sapaici* Lane from southeastern Brazil shows this species to be a synonym of *N. elegans* (Lane), which is formally proposed here. *Neuratelia sapaici* is redescribed. The position of these three species within the genus is discussed. A key for the Neotropical species of *Neuratelia* is provided.

Keywords

Andean ecosystem, biogeography, Neotropical diversity, taxonomy

Introduction

Neuratelia Rondani, 1856 is a clearly monophyletic genus which currently includes 31 species (16 Palaearctic, 13 Nearctic, one Oriental, and one Neotropical) (Kurina et al. 2015). In Borkent and Wheeler's (2013) broad phylogenetic analysis of the Sciophilinae, *Neuratelia* was resolved as sister to a large clade that included all genera of

the subfamily except *Acomoptera* Vockeroth, *Drepanocercus* Vockeroth, *Loicia* Vockeroth, *Paratinia* Mik, *Taxicnemis* Tonnoir and Edwards, *Aneura* Marshall, and *Phthinia* Winnertz. Søli's (1997) mycetophilid phylogeny, which included 13 taxa of Sciophlinae, showed *Neuratelia* as basal to the clade including all other Sciophilinae except *Anaclileia* and *Phthinia*. The only divergence between both studies concerns *Anaclileia* Meunier, which in Borkent and Wheeler's (2013) tree is placed inside the large clade sister to *Neuratelia*.

Søli et al. (2000) and Borkent and Wheeler (2013) characterized *Neuratelia* as a genus with a setose laterotergite, vein sc-r placed before the origin of Rs, C not produced beyond the apex of R_5, R_5 sinuous, presence of both medial and cubital forks, stem of M_{1+2} shorter than fork, M_1 incomplete basally, and the origin of the anterior fork beyond that of the posterior fork.

Neuratelia is one of the rarest genera of Mycetophilidae in the Neotropical region. The catalogue of the family (Oliveira and Amorim 2014) includes a single species for the genus, *N. sapaici*, which is known only from the male holotype collected in 1947 at the Estação Biológica de Boracéia in the Atlantic Forest of the state of São Paulo, southern Brazil (Lane 1952). Despite intensive collecting of insects in this area over many decades, no other specimen of *Neuratelia* has been found so far.

Recent extensive collecting in the temperate environments of Colombia, mainly in the paramos of the high-Andean ecosystems, revealed a number of mycetophilid genera previously unknown for Colombia. This includes representatives of genera with Holarctic distributions, such as *Docosia* Winnertz and *Cordyla* Meigen (Oliveira and Amorim 2011; Kurina and Oliveira 2015), genera with southern temperate distribution, such as *Paraleia* Tonnoir and *Duretophragma* Borkent (shared mostly between Chile, Andean Argentina, and southern Brazil, sometimes with species in Australia), and the special case of the genus *Eumanota* Edwards (Manotinae), previously known only from the Oriental region (Amorim et al. 2018). Examining the holotype of *Eudicrana elegans* Lane (Lane 1948) we realize that it is a species of *Neuratelia* and a synonym of *Neuratelia sapaici* Lane (Lane 1952).

In this paper, we describe two new species of *Neuratelia* from the paramo of the Central Andes of Colombia. We also propose a new combination for *Eudicrana elegans*, the synonymy of *N. sapaici* with *E. elegans*, and redescribe the species. A key for Neotropical species of *Neuratelia* is provided, and their taxonomic relationships are discussed.

Materials and methods

The Colombian specimens examined in this study are deposited in the Diptera collection of the Colección Entomológica Universidad de Antioquia (CEUA), at the Departmento de Antioquia, Medellin, Colombia. The specimens were collected between 2011 and 2014 with Malaise traps in the Central Andes in the Department of Antioquia, Colombia (Fig. 1A–D). The materials were originally preserved in 96%

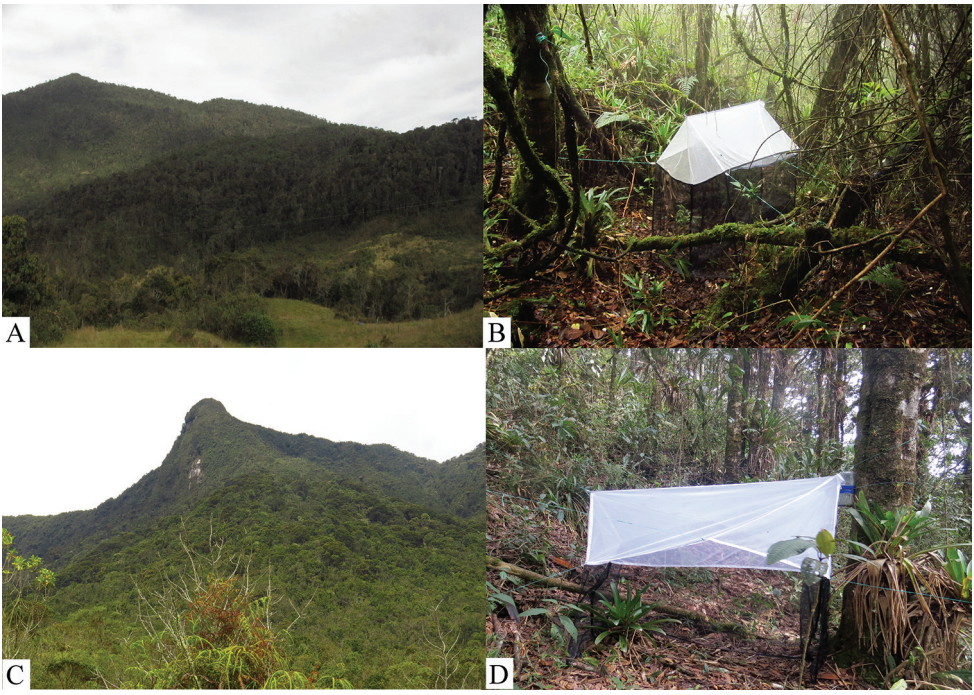

Figure 1. A Type locality of *Neuratelia altoandina* sp. nov. (Holotype), landscape municipality of San José de la Montaña, paramo El Congo, Colombia **B** Malaise trap **C** type locality of *Neuratelia colombiana* sp. nov. (Holotype), landscape municipality of Sonsón, Norí mountain, paramo of Sonsón, Colombia **D** Malaise trap.

ethanol. One wing and the terminalia were separated from the rest of the body. Wings were mounted in permanent slides in Euparal. After removal, the terminalia were kept in KOH 10% for 12 h and then heated for 10 min, neutralized in acetic acid for 10 min, dehydrated in ethanol 70–90%, and preserved in a microvial in glycerine. The holotype of *N. elegans* is mounted on a pin, deposited at the Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo, Brazil.

Photographs of the Colombian types were taken using a Moticam 3.0 megapixel DFC500 digital camera attached to an Olympus SZX7 stereomicroscope; the type of the Brazilian species was photographed with a Leica DC 500 camera coupled to a Leica M16 stereomicroscope. Photo stacking was performed using Helicon Focus v. 6.7.2 and edited with Adobe Photoshop CC 2017; photographs and illustrations of the terminalia were preparing using the U-DA Olympus drawing tube attached to an Olympus BX40 compound microscope, then vectorized with Illustrator CC 2017. Morphological terminology for head, thorax, pleural sclerites, and terminalia follows Søli (1997), Amorim and Oliveira (2008), and Kurina et al. (2015), while terminology of the wing venation follows Cumming and Wood (2017). For easy comparisons with other papers, we used Kurina et al.'s (2015) abbreviation system.

Results

Genus *Neuratelia* Rondani

Neuratelia **Rondani 1856: 195.**

Type species. *Mycetophila nemoralis* Meigen (original designation).

Diagnosis. (modified from Matile 1974; Søli et al. 2000; Sasakawa 2004; Borkent and Wheeler 2013). Vein R_5 sinuous, sc-r placed basal to origin of Rs, C not produced beyond apex of R_5, stem of M_{1+2} shorter than medial fork, base of M_1 absent, origin of posterior fork basal to origin of anterior fork. Tibiae with distinct setae. Laterotergite and mediotergite setose.

Key to Neotropical species of *Neuratelia*

1 Scutum with a pair of dark lateral stripes and a pair of slender dorsocentral stripes (Fig. 3H); CuA gradually curving on apical third, reaching wing margin at an acute angle; CuP short, extending only half the length of CuA (Fig. 4B); terminalia with digitiform cerci (Figs 5F, 6D) (northeastern Colombia) ..... ***N. colombiana* sp. nov.**

– Scutum more or less homogeneously brown (Figs 3G, I); CuA strongly curved on apical third, reaching wing margin at angle of ~90°; CuP long, reaching the distal third of CuA (Figs 4A, C); terminalia with lobular cerci (Figs 5C, I, 6B, F) **2**

2 Syngonocoxite fully covering entire ventral surface of terminalia (Figs 5A, 6A); dorsal branch of gonostylus digitiform; gonostylus with ventral branch digitiform (Figs 5B, 6B) (northwestern Colombia)..... ***N. altoandina* sp. nov.**

– Syngonocoxite covering only anterior half terminalialia ventrally (Fig. 5G); gonostylus triangular, with a flat dorsal projection; gonostylus without ventral branch digitiform (Figs 5H, 6E) (southeastern Brazil) ..... ***N. elegans* (Lane)**

Neuratelia altoandina sp. nov.

http://zoobank.org/4918CB5F-E452-41BE-AB33-518E539E90F4
Figs 2A, 3A, D, G, 4A, 5A–C, 6A, B

Type locality. Colombia, department of Antioquia, San José de la Montaña municipality, El Congo municipal rural settlement, paramo El Congo locality, 6°46.5651'N, 75°42.5701'W, alt. 3000 m a.s.l.; forest, L. Rios leg.

Type specimen. Holotype male, wing mounted in Euparal on microscope slide, rest of body in alcohol 96%, genitalia in glycerine microvial. Original label: " Co-

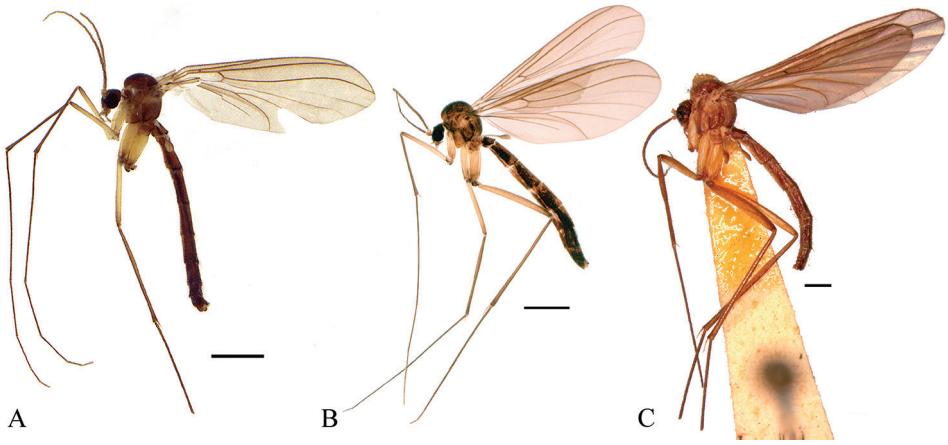

Figure 2. A Male habitus of *Neuratelia altoandina* sp. nov. (holotype) **B** male habitus of *Neuratelia colombiana* sp. nov. (holotype) **C** male habitus of *Neuratelia elegans* (Lane) (holotype of *N. sapaici*). Scale bars: 1 mm.

lombia, Antioquia, San José de la Montaña, Vda. El Congo, páramo El Congo; 6°46'33.91"N, 75°43'34.21"W, 3000 m a.s.l.; forest, Malaise trap; 10–15 Sept. 2011; L. Ríos col.; CEUA 94078".

Material examined. Holotype ♂, Colombia, Department of Antioquia, San José de la Montaña municipality, El Congo municipal rural settlement, paramo El Congo locality; 6°46.551'N, 75°42.5701'W; alt. 3000 m a.s.l.; Malaise trap forest, 10–15 Sept. 2011; L. Rios leg., CEUA 94078.

Diagnosis. Thorax brown, scutum with a pair of lighter longitudinal stripes medially. CuA with strong apical curve, reaching wing margin at an angle of about 90°, CuP long, ending at distal third of CuA. Syngonocoxites wide, fused medially, extending posteriorly almost to level of apical end of gonocoxites. Gonocoxite with a wide dorsoposterior lobular projection. Dorsal gonostylus shape like clamps, tapering apically.

Description. Male (Fig. 2A). Body length, 5.8 mm. ***Head*** (Fig. 5A). Width 0.57 mm, height 0.35 mm. Vertex brown, with abundant brownish-yellow short setae. Three ocelli, mid ocellus smaller; lateral ocelli separated from eye margin by less than their diameter. Occiput chestnut brown. Ommatrichia abundant, short, yellowish. Scape, pedicel brownish yellow, cylindrical, scape slightly longer than pedicel, both with small brownish-yellow setae; 14 flagellomeres, mostly light brown, with scattered small dark setae; first flagellomere almost twice as long as second. Frons, clypeus brown, longer than wide, subtriangular; palpus with five palpomeres, light brown, apical palpomere twice as long as fourth. ***Thorax*** (Figs 3D, G). Mostly brown. Scutum with medial, light brown, triangular area, wide at anterior margin narrowing towards scutellum. A row of stronger setae present above wing; a single row of differentiated dorsocentrals. Scutellum with scattered smaller setae over disc, some longer setae along margin. Pleural sclerites mostly chestnut brown, katepisternum and laterotergite dark brown ventrally. Pleural membrane yellowish brown. Antepronotum with nine setae, proespisternum with three setae of different size. Proepimeron, anepisternum, katepisternum, mesepimeron, and

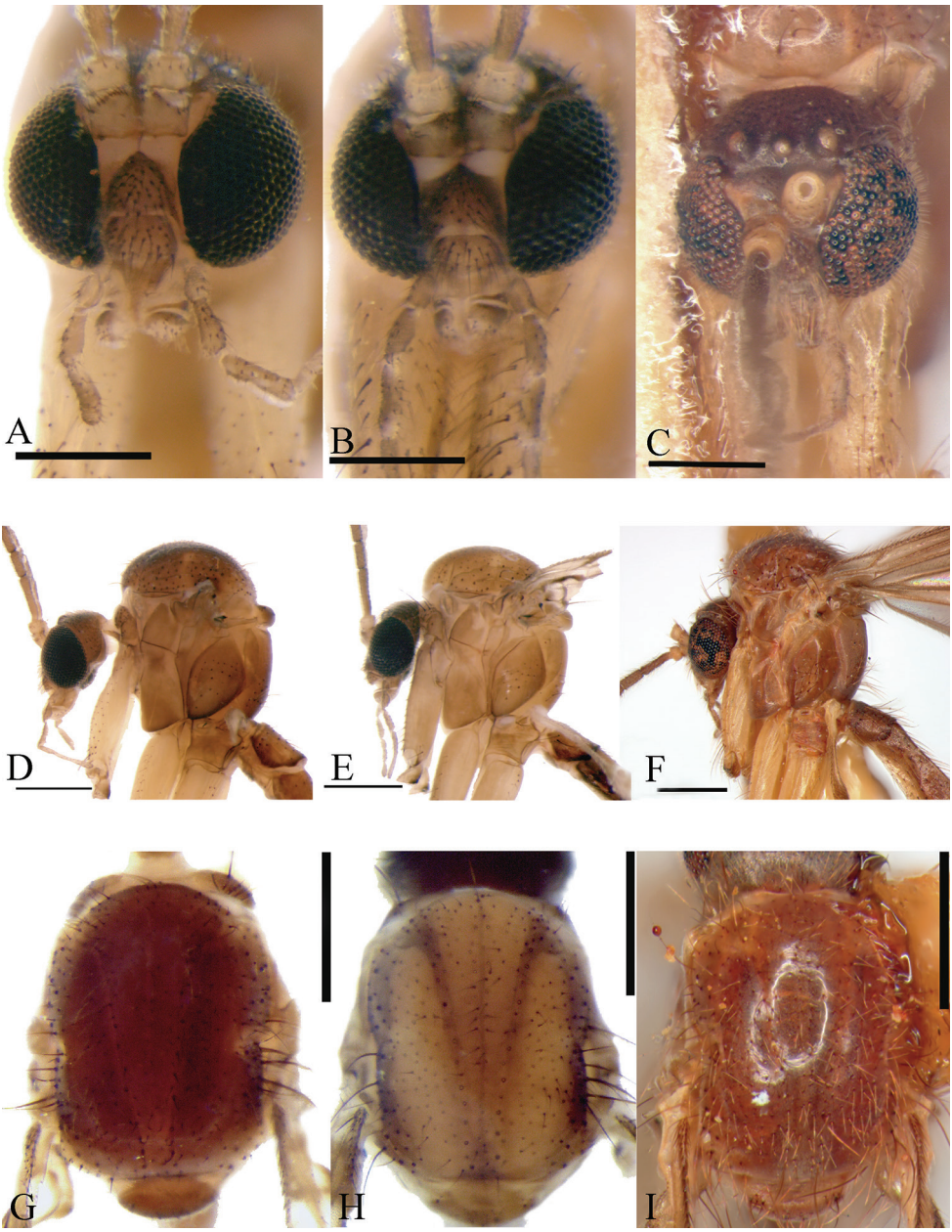

Figure 3. A Frontal head *Neuratelia altoandina* sp. nov. (holotype) **B** frontal head *Neuratelia colombiana* sp. nov. (holotype) **C** frontal head *Neuratelia elegans* (Lane) (holotype of *N. sapaici*) **D** lateral thorax *Neuratelia altoandina* sp. nov. (holotype) **E** lateral thorax *Neuratelia colombiana* sp. nov. (holotype) **F** lateral thorax *Neuratelia elegans* (Lane) (holotype of *N. sapaici*) **G** dorsal thorax *Neuratelia altoandina* sp. nov. (holotype) **H** dorsal thorax *Neuratelia colombiana* sp. nov. (holotype) **I** dorsal thorax *Neuratelia elegans* (Lane) (holotype of *N. sapaici*). Scale bars: 0.25 mm.

metepisternum bare, laterotergite with about 20 dark large setae, mediotergite with 9 or 10 dark long setae laterally on the basal area. Halter pedicel yellowish, knob chestnut brown, setose. ***Legs.*** Coxae, femora yellow, tibia, tarsi brown. Foreleg tibia with ventral oval depression distally with abundant and irregularly distributed trichia; first tarsomere 1.5 times tibia length. Tibiae and tarsi with dark, short erect setae along their whole length. Tibial spurs 1:2:2, light brown, spurs as long as tibia apical width. Tarsal claws with large apical tooth, smaller basal tooth. ***Wing*** (Fig. 4A). Length, 5.0 mm, width, 2.0 mm. Membrane light brown, densely covered with macrotrichia, decumbent on all cells; veins brown. Sc complete, setose ventrally, reaching C well beyond base of Rs, almost at mid of the wing; sc-r present, bare, basal to the mid of Sc. C ending at apex of R_5. R_1 long, reaching C beyond apical fifth of wing. First sector of Rs oblique, setose ventrally, slightly longer than r-m; R_5 sinuous, reaching C at wing apex; r-m bare, oblique. Medial and cubital veins complete, reaching wing margin. M_{1+2} stem shorter than anterior fork. M_1 weak, obsolete basally. CuA strongly curved towards wing margin for apical third, reaching margin at an angle of about 90°. CuP long, reaching level of apical third of CuA. ***Abdomen.*** Segments chestnut brown, cylindrical, slender, brownish long setae covering tergites, sternites. Sternite 8 longer than wide, projecting medially, tergite 8 wider than long, also projecting medially. ***Terminalia*** (Figs 5A–C, 6A, B). Slightly wider than longer, gonocoxite ventral surfaces almost fused medially, forming a syngonocoxite with a ventral deep medial cleft, extending nearly to level of ventroapical margin of gonocoxite; gonocoxites dorsally with apical large, setose, lobular projections. Gonostylus small, dorsal branch digitiform, tapering towards apex, with scattered small setae. Tergite 9 weakly sclerotized, wide, short, restricted to basal portion of terminalia. Parameres projecting slightly beyond gonocoxite apical margin. Aedeagus short. Cerci typically well developed, lobular, setose, projecting beyond distal margin of gonocoxites.

Female. Unknown.

Etymology. The specific epithet of this species combines the Latin word *altus* (nominative, adjective masculine or neutre) for "high", with the name *andina* (nominative, adjective feminine) for the South American mountain chain system, referring to the presence of this species in higher elevations in the Andean ecosystems.

***Neuratelia colombiana* sp. nov.**
http://zoobank.org/5A4A2D1B-CB31-4591-A8FF-075C58A0E2ED
Figs 2B, 3B,E, H, 4B, 5D–F, 6C, D

Type locality. Colombia, department of Antioquia, Sonsón municipality, Norí municipal rural settlement, Norí mountain, paramo of Sonsón locality, 5°48.716'N, 75°16.1066'W, alt. 3045 m a.s.l. paramo, A. Cardona and D. Cardona leg.

Type specimen. Holotype male, wing mounted in Euparal on microscope slide, rest of body in alcohol 96%. Original label: "Colombia, Antioquia, Sonsón, Vda. Norí, cerro Norí, páramo de Sonsón, 5°48'46.3"N, 75°16'6.398"W, páramo, 3045 m,

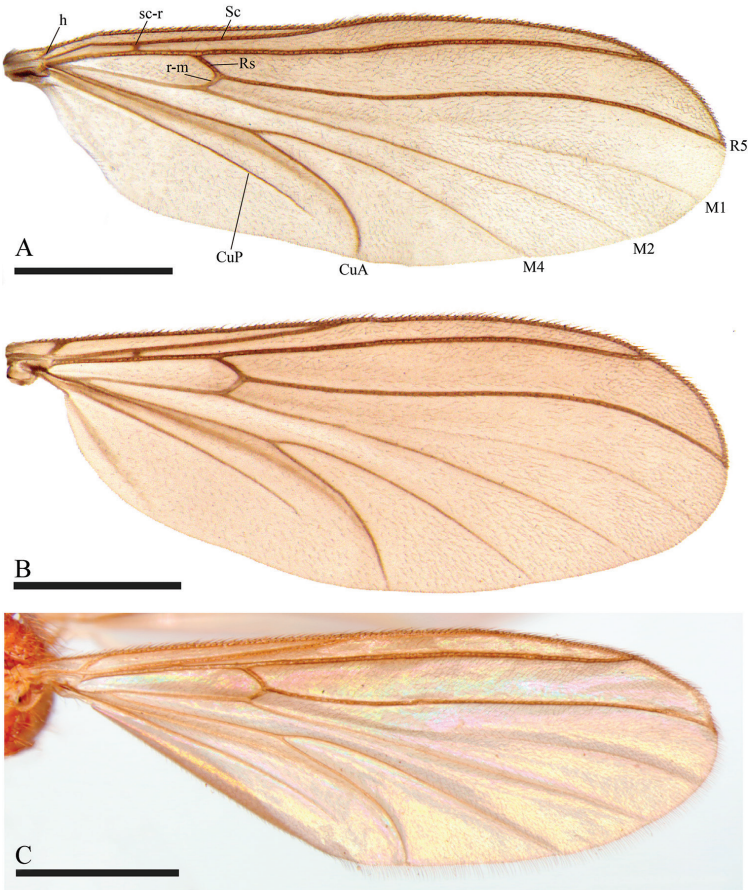

Figure 4. A Wing of *Neuratelia altoandina* sp. nov. (holotype) **B** wing of *Neuratelia colombiana* sp. nov. (holotype) **C** Wing of *Neuratelia elegans* (Lane) (holotype of *N. sapaici*). Scale bars: 1 mm.

1–12 Sept. 2018. Proyecto Moscas de las flores. M. Salinas, A.M. Echeverry and A.L. Montoya cols. CEUA 94079".

Material examined. Holotype Colombia ♂, department of Antioquia, Sonsón municipality, Norí locality, Norí mountain, paramo of Sonsón; 5°48.7716'N, 75°16.1966'W, alt. 3045 m a.s.l.; 1–12 Sept. 2018. Proyecto Moscas de las flores. M. Salinas and A.L. Montoya leg.; paramo; Malaise trap; CEUA 94079. **Paratype** 1♂, Colombia, same data as holotype but differ on: 5°48.5751'N, 75°16.1178'W; alt. 2888 m a.s.l.; 7–9 May 2014; A. Cardona and D. Cardona leg.; forest; Malaise trap; CEUA 94076.

Diagnosis. Thorax light brown, scutum with a pair of dark slender dorsocentral stripes. Vein CuA with gentle distal curve, CuP short, ending around mid of CuA. Syngonocoxite not extending to distal margin of terminalia; gonocoxites without an inner dorsal projection, inner apical projections small, slender, acute. Gonostylus ventral branch digitiform, dorsal branch short, pointed.

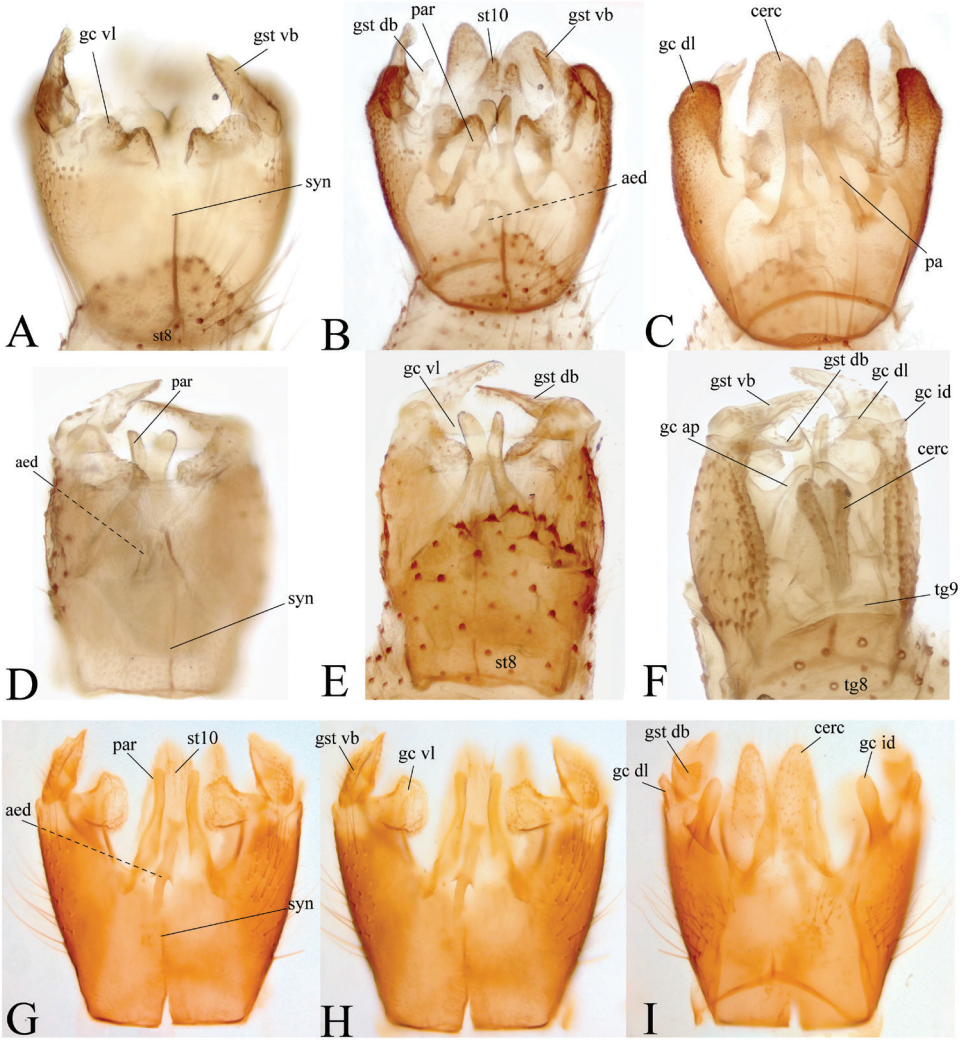

Figure 5. A Syngocoxite ventral view of the male terminalia of *Neuratelia altoandina* sp. nov. (holotype) **B** ventral view **C** dorsal view **D** syngocoxite ventral view of male terminalia of *Neuratelia colombiana* sp. nov. (holotype) **E** ventral **F** dorsal view **G** syngocoxite ventral view of male terminalia of *Neuratelia elegans* (Lane) (holotype of *N. sapaici*) **H** ventral view **I** dorsal view.

Description. Male (Fig. 2B). Body length, 5.2 mm. ***Head*** (Fig. 3B). Width, 0.54mm, height, 0.49mm. Vertex brown, with abundant brownish short setae. Three ocelli, mid ocellus smaller; lateral ocelli separated from eye margin by less than their diameter. Occiput brown. Ommatotrichia abundant, short, yellowish. Scape and pedicel brownish yellow, cylindrical, scape longer than pedicel, both with small brownish-yellow setae; 14 flagellomeres, mostly light brown, with scattered dark small setae; first flagellomere almost twice as long as second. Frons and clypeus

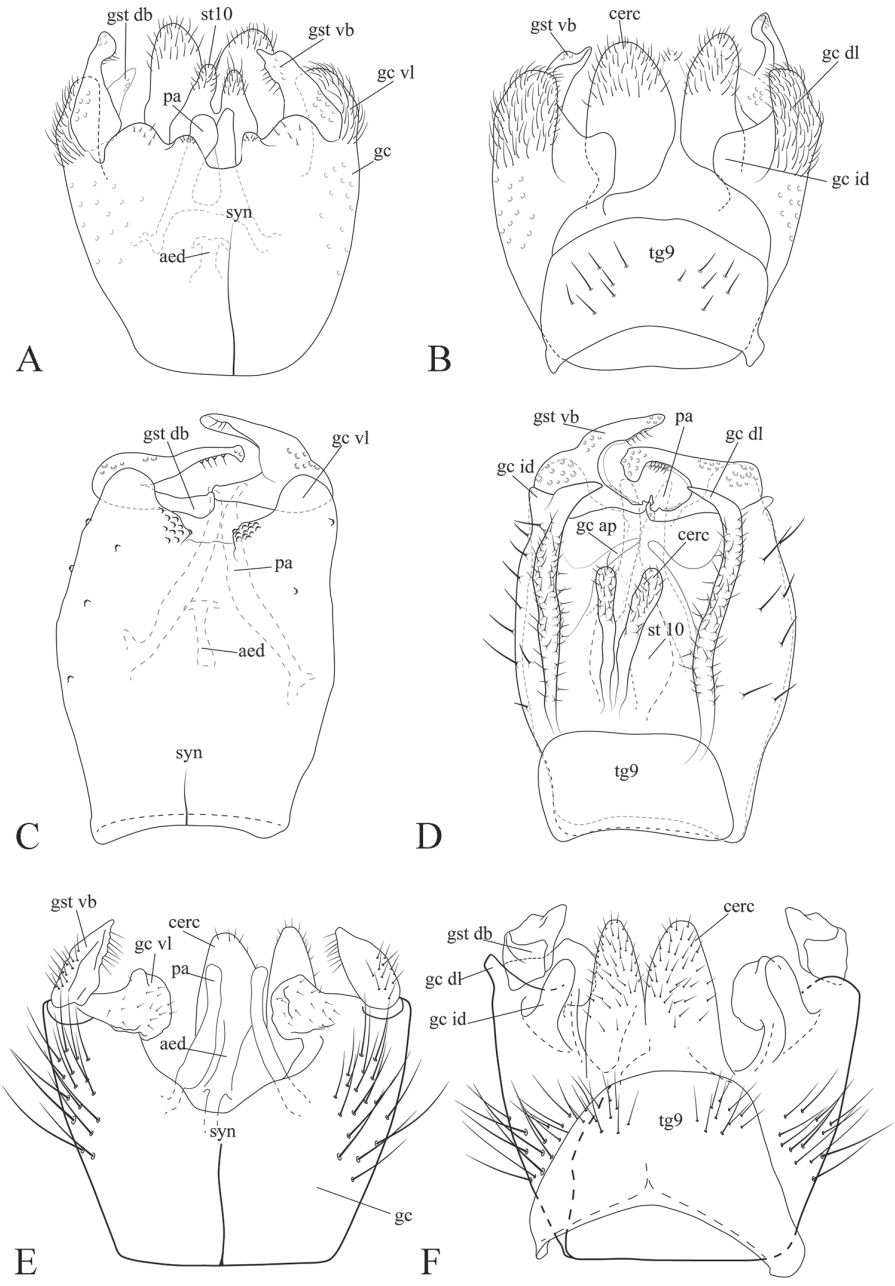

Figure 6. A Male terminalia illustrations of *Neuratelia altoandina* sp. nov. (holotype) **B** ventral view **C** dorsal view **D** male terminalia illustrations of *Neuratelia colombiana* sp. nov. (holotype) **E** ventral view **F** dorsal view **G** male terminalia illustrations of *Neuratelia elegans* (Lane) (holotype of *N. sapaici*) **H** ventral view I dorsal view. Abbreviations: **aed** = aedeagus; **cerc** = cercus; **gc** = gonocoxite; **gc ap** = gonocoxal apodeme; **gc id** = gonocoxite inner dorsal projection; **gc dl** = gonocoxite dorso-apical lobe; **gc vl** = gonocoxite ventral lobe; **gst** = gonostylus, **gst db** = dorsal branch of gonostylus; **gst vb** = ventral branch of gonostylus; **syn** = syngocoxite; **par** = paremeres.

light brown, subrectangular, covered with dark setae; palpus with five light brown palpomeres, lighter towards apex, last palpomere about twice as long as fourth. ***Thorax*** (Figs 3E, H). Scutum light brown, with a pair of dark brown dorsocentral stripes and a weak medial dark stripe, all three connected at posterior margin of scutum, a pair of dark brown lateral longitudinal stripes above wings. A row of stronger setae above wing and a single row of differentiated dorsocentrals. Scutellum dark brown, with a lateral pair of scutellars on each side and three medial setae on posterior margin. Pleural sclerites dark brown, with some light areas on dorsal half of katepisternum and at dorsal end of mesepisternum. Pleural membrane yellowish brown. Antepronotum with nine long setae, proepisternum with four setae. Proepimeron, anepisternum, katepisternum, mesepimeron, and metepisternum bare. Laterotergite with 18 long dark setae, mediotergite with a row of dark setae on ventral half. Halter pedicel yellowish, knob light brown, both setose. ***Legs.*** Coxae and femora yellowish brown, tibiae and tarsi light brown. Foreleg tibiae with ventral oval depression with abundant irregular trichia; first tarsomere about 1.5 times as long as tibiae. Tibiae and tarsi with erect, short, dark setae along their entire length. Tibial spurs 1:2:2, brown, slightly longer than tibia width at apex. Tarsal claws with a large apical tooth and a smaller basal one. ***Wing*** (Fig. 4B). Length 4.0 mm, width 1.8 mm. Membrane light brown, densely covered with decumbent macrotrichia on all cells, veins brownish. Sc complete, setose ventrally, reaching C well beyond base of Rs, almost at mid length of wing; sc-r present, bare, placed basal to middle of Sc. C ending at apex of R_5. R_1 long, reaching C beyond distal fifth of wing. First sector of Rs oblique, setose ventrally, slightly longer than r-m. R_5 sinuous, reaching C at wing apex, r-m bare, oblique. M_{1+2} stem shorter than medial fork. M_1 weak, obsolete basally. M_4 complete. CuA complete, gently curving on distal third. CuP short, reaching around level of mid CuA. ***Abdomen.*** Segments chestnut brown, cylindrical, slender, with long brownish setae on tergites and sternites. Sternite 8 not projecting posteriorly beyond basal half of gonocoxite. Tergite 8 well developed, with short medial projection covering less than basal half of tergite 9, almost rectangular, occupying anterior fourth of terminalia. ***Terminalia*** (Figs 5D–F, 6C, D). Light brown. Longer than wide. Gonocoxite ventral surface almost fused medially, forming a syngonocoxite with a deep ventral medial cleft, extending close to half the level of gonocoxite. Gonocoxites elongate, with a short inner dorsal projection, apically truncated, and a slender, dorsal-apical projection. Gonocoxal apodem conspicuous, with acute apex. Dorsal branch of gonostylus wider, tapering towards apex, ventral branch digitiform, with scattered setae; parameres curved, approaching each other medially and then diverging, slightly projecting beyond apex of gonocoxites. Aedeagus very short, not reaching apex of cerci. Cerci typically slender, digitiform, with abundant short strong setae, not projecting beyond distal margin of gonocoxites.

Female. Unknown.

Etymology. The specific epithet *colombiana* (nominative, adjective feminine) of this species refers to Colombia, the country in which the type materials were collected.

***Neuratelia elegans* (Lane, 1948), comb. nov.**
Figs 2E, 3C, F, I, 4C, 5G–I, 6E, F

Eudicrana elegans Lane 1948: 251, fig. 8 (gonocoxite and gonostyle), 9 ("mesosome"), 10 (tergite 9). **Type.** Holotype male, pinned, genitalia in permanent Canada balsam microslide mounting pinned with specimen. Original label: "Brazil, São Paulo, Salesópolis, Estação Biológica de Boraceia, xi.1947, F. Travassos and E. Rabello leg. MZUSP-07105".

Neuratelia sapaici Lane 1952: 135, fig. 3 (male terminalia), syn. nov. **Type.** Holotype male, pinned, genitalia in permanent Canada balsam microslide mounting pinned with specimen. Original label: "Brazil, São Paulo, Salesópolis, Estação Biológica de Boraceia, 14 viii. 1947, E. Rabello, F. Travassos and J. Lane leg. MZUSP-04030".

Type locality. Brazil, state of São Paulo, Salesópolis municipality, Boraceia Biological Station [23°41.4378'S, 45°49.4288'W].

Material examined. Holotype ♂ Brazil; State of São Paulo, Salesópolis municipality, Boraceia Biological Station; [23°41.4378'S, 45°49.4288'W]; Nov. 1947; F. Travassos and E. Rabello leg. MZUSP–07105. **Paratype** 1♂; Brazil, same data as holotype but differ on: 14 Aug.1947; E. Rabello, F. Travassos and J. Lane leg. MZUSP–04030.

Diagnosis. Thorax brown, scutum without longitudinal stripes. Vein CuA with strong curve on distal third, CuP long, ending at about level of distal third of CuA. Gonocoxite fused medially only on basal half, with a ventral inward lobular distal projection and a digitiform laminar projection at dorsal surface of terminalia; gonostylus short, triangular in ventral view, with a flat basal projection dorsally.

Redescription. Male (Fig. 2C). Body length 6.8 mm. ***Head*** (Fig. 3D) Width 0.51mm, length 0.54mm. Vertex dark brown, with abundant brownish setae. Three ocelli, mid ocellus smaller; lateral ocelli separated from eye margin by less than their diameter. Occiput dark brown. Ommatotrichia abundant, short, yellowish. Scape and pedicel yellowish brown, cylindrical, scape longer than pedicel, slightly darker, both with short setae; with 14 dark brown flagellomeres, with scattered yellowish setae, first flagellomere almost twice as long as second flagellomere. Frons and clypeus brown, subrectangular, densely covered with yellowish setae; labella caramel brown, with five light brown palpomeres, last palpomere about twice length of fourth. ***Thorax*** (Figs 3F, I). Scutum, scutellum, and pleura brownish, scutum with two darker stripes connected medially at posterior end and reaching scutellum, with scattered yellowish setae, a single row of differentiated dorsocentrals, and no clear row of acrostichals; prealars and postalars strong. Scutellum brownish, with two stronger setae laterally and two medially, and scattered, smaller, marginal setae. Pleural sclerites mostly brown, ventral half of katepisternum and mediotergite dark brown; pleural membrane yellowish brown. Proepimeron, anepisternum, katepisternum, mesepimeron, and metepisternum bare, antepronotum and proepisternum setose, laterotergite with 25 well developed brown

setae, mediotergite with a row of around 25 well-developed setae across ventral margin. Halter setose, pedicel yellowish, knob light brown. ***Legs.*** Coxae yellowish brown, darker apically, femora light brown, tibiae and tarsi brown. Fore femora, tibiae, and tarsi missing in the holotype; mid tibia with dorsal and ventral irregular rows of slightly longer dark setae, hind tibia with a regular row of dark setae posteriorly; mid tibia spurs subequal, almost twice apical width of tibia, hind tibia outer spur longer than inner spur. Mid and hind first tarsomeres very long (distal tarsomeres of mid and hind tarsi missing in holotype). ***Wing*** (Fig. 4C). Length, 4.6 mm, width, 1.5 mm. Membrane light brown, without dark maculae, membrane densely covered with microtrichia and decumbent macrotrichia on all cells. Anterior veins brown, medial and cubital veins yellowish brown; Sc complete, reaching C well beyond base of Rs, almost at the middle of anterior margin of wing, setose; sc- r present basally, well before origin of Rs, bare. C ending at apex of R_5. R_1 long, reaching C beyond distal fifth of wing. First sector of Rs oblique, bare, only slightly longer than r-m. R_5 slightly sinuous, reaching C near wing apex. Vein r-m oblique, bare. M_{1+2} stem more than twice length of r-m. M_1 obsolete basally. Medial and cubital veins complete, reaching wing margin. CuA with a strong curve towards base at distal third. CuP well sclerotized, extending to level of distal third of CuA. ***Abdomen.*** Brown, cylindrical, slender, with long dark setae covering tergites and sternites. Tergite 8 longer than wide, projected medially, sternite 8 wider than long, with a medial projection apically. ***Terminalia*** (Figs 5G–I, 6E, F). Syngonocoxite extending medially slightly beyond half the length of the gonocoxite; gonocoxites with a pair of inward lobes ventrally, with setulae, and a dorsoapical lobe slightly projecting distally beyond the base of the gonostyli; gonocoxites with a flat digitiform inner projection dorsally. Gonostylus relatively simple, covered only with setulae, without spines or long setae, ventral branch triangular in ventral view, dorsal branch short, flat. Tergite 9 weakly sclerotized, restricted to basal half of terminalia, with a group of short setae at each side. Parameres straight (not curved as in many species of the genus), reaching level of gonocoxite apex distally. Aedeagus elongate, straight. Sternite 10 present as a pair of elongated sclerotized stripes with setae. Cerci typicallywell-developed, lobular, extending distally almost as far as the apex of gonostylus, touching each other medially, but without evidence of fusion.

Female. Unknown.

Comments. The holotypes of *Eudicrana elegans* and *Neuratelia sapaici* are males and originally had their terminalia slide-mounted between cover slips, pinned with the respective specimens. The terminalia of both species are identical in every aspect and as well as the general colour of the specimens. Lane's (1948, fig. 8) illustration of the gonocoxite and the gonostyle of *E. elegans* makes clear its identity with Lane's (1952, fig. 3) illustration of the male terminalia of *N. sapaici* (Figs 5G–I, 6E,F). Along the original description of *Eudicrana elegans*, Lane (1948: 252) mentioned the interrupted M_1, but did not comment on the lack of R_4, which would immediately raise suspicious of the generic position of the species. We propose a new combination of the *Eudicrana elegans* and the synonymy of *Neuratelia sapaici*.

Discussion

The distinction between the three known Neotropical species of *Neuratelia* is very straightforward based on thorax coloration, specifically the patterns of stripes over the scutum, but the wing venation, morphology of clypeus, and the male terminalia are also distinctive. *Neuratelia altoandina* shares with *N. elegans*, some distinctive characters, such as the length of CuP, while in *N. colombiana* CuP is shorter, extending beyond mid of CuA length. Body coloration of *N. colombiana* is lighter, with yellowish brown and brown tones; and a the scutum has a darker V-shaped brown mark over a lighter, ochre-brown background color, while *N. altoandina* and *N. elegans*, have a more homogeneously brown scutum. *Neuratelia altoandina* and *N. elegans* have a rather strong curve on the distal third of CuA, reaching the wing margin at an angle of about 90°, while *N. colombiana* has a gentle distal curve, reaching the wing margin at an acute angle. Also, *N. altoandina* and *N. elegans* have a slightly longer CuP than *N. colombiana*. *N. colombiana* and *N. elegans* share a subrectangular shape of the clypeus, while the clypeus in *N. altoandina* is subtriangular.

The male terminalia is also distinct between the three species. In *N. altoandina* and *N. elegans* the male terminalia is wider than long, while in *N. colombiana* it is longer than wide. The ventral branch of the gonostylus is nearly digitiform in *N. altoandina* and *N. colombiana*, while in *N. elegans* it is triangular in ventral view. In *N. elegans*, the gonostylus dorsal branch is a flat, short lobe. The distal margin of the syngonocoxite in *N. altoandina* extends medially almost to the level of the distal end of the gonocoxite, while in *N. colombiana* it is slightly shorter, and in *N. elegans* the syngonocoxite extends to only half the length of the gonocoxites. The gonocoxites in *N. altoandina* have lobular dorsal projections distally, while in *N. colombiana* there is a slender acute projection and in *N. elegans* a digitiform flat projection. In *N. elegans*, the gonocoxites have an additional ventral lobe distally, which projects medially. Finally, *N. altoandina* and *N. elegans* have a pair of very characteristic lobular cerci, different from the slender, much short cerci in *N. colombiana*.

All *Neuratelia* species have a relatively short tergite 9, restricted to the anterodorsal portion of the male terminalia, in such a way that the gonocoxites project well beyond the distal margin of tergite 9 (e.g. Matile 1974: figs 2–7; Zaitzev 1994: figs 5, 6; Sasakawa 2004: figs 1–5; Kurina et al. 2015: figs 7, 21, 22). In some species, tergite 9 extends only half way the extension of the gonocoxite, but in other species it is restricted to an even more anterior position. The syngonocoxite medial extension, in most cases, reaches two-thirds of the length of the gonocoxite medially. Thus, the condition in *N. altoandina* is quite extreme, with the syngonocoxites covering the entire ventral surface of the terminalia. The shape of the gonostylus is variable between the species and in some cases very complex. Some *Neuratelia* species have a simpler gonostylus, as in *N. spinosa* Matile (Matile 1974: fig. 4), but it can also be very complex, as in *N. microdigitata* Sasakawa (Sasakawa 2004: fig. 4), *N. jabalmoussae* Kurina et al., *N. caucasica* Zaitzev, and *N. minor* (Lundström) (Kurina et al. 2015: figs 9, 11, 13). The parameres are usually curved, close to each other midway to apex and then strongly diverging distally.

Publications on *Neuratelia* do not provide a formal discussion on species groups or on the relationships within the genus. The only exception is Kurina et al. (2015), who indicated two pairs of species, *N. jabalmoussae*/*N. caucasica* and *N. nemoralis*/*N. salmelai*, based on a study with morphological and molecular data.

It is not possible to discuss the placement of the Neotropical species of *Neuratelia* within the genus without a broader phylogenetic study involving all species of the genus. It is clear, however, that the three Neotropical species share a clearly apomorphic feature: the gonocoxite internal dorsal projection. This feature is absent in all other species of the genus illustrated in the literature and suggests that this Neotropical group may be a monophyletic clade within the genus. *Neuratelia colombiana* and *N. elegans* might form together a clade, as the wing features (particularly the strong distal curve on vein CuP, quite unique in the genus) clearly suggest. The Nearctic and Palaearctic species of the genus with wing illustrations (e.g. *N. sayi* in Vockeroth 1981: fig. 36; *N. nemoralis* Meigen in Matile 1974: fig. 1) show a more strongly sinuous R_5, a condition not so distinct in the Neotropical species. In this sense, the relatively simple gonostylus and the less sinuous R_5 in the Neotropical species of *Neuratelia* may correspond to plesiomorphic conditions, indicating that the set of characters present in the Nearctic and Palearctic species may reflect the existence of a large Holarctic clade in the genus.

Neuratelia is currently unknown from Chile, but it is likely that the genus is also present there. This would confirm another case of a southern temperate South America group (with representatives in Chile, southern Argentina, and southern Brazil) that extends its distribution the north of the Andes, reaching of Colombia. This pattern is also known, within the Mycetophilidae, in the genera *Paraleia* (Oliveira and Amorim 2012), *Duretophragma* Borkent, *Eudicrana* Loew, and others, and in the Rangomaramidae clade with the genera *Chiletricha* and *Eratomyia* (Amorim and Rindal 2007; Amorim and Falaschi 2010).

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References

Amorim DS, Rindal E (2007) A phylogenetic study of the Mycetophiliformia, with creation of the subfamilies Heterotrichinae, Ohakuneinae, and Chiletrichinae for the Rangomaramidae (Diptera, Bibionomorpha). Zootaxa 1535: 1–92. https://doi.org/10.11646/zootaxa.1535.1.1

Amorim DS, Oliveira SS (2008) Eleven new species of the genus *Cluzobra* Edwards (Diptera, Mycetophilidae, Sciophilinae) from the Atlantic Forest of Brazil. Zootaxa 1920: 1–28. https://doi.org/10.11646/zootaxa.1920.1.1

Amorim DS, Falaschi RL (2010) Second known species of *Eratomyia* Amorim & Rindal (Diptera, Rangomaramidae, Chiletrichinae), from Colombia. Zootaxa 2641: 55–61. https://doi.org/10.11646/zootaxa.2641.1.6

Amorim DS, Oliveira SS, Henao-Sepúlveda AC (2018) A new species of *Eumanota* Edwards (Diptera, Mycetophilidae, Maotinae) from Colombia: evidence for a pseudogondwanan pattern. American Museum Novitates 3915: 1–19. https://doi.org/10.1206/3915.1

Borkent CJ, Wheeler TA (2013) Phylogeny of the tribe Sciophilini (Diptera: Mycetophilidae, Sciophilinae). Systematic Entomology 38: 407–427. https://doi.org/10.1111/syen.12002

Cumming JW, Wood DM (2017) Adult morphology and terminology. In: Kirk-Spriggs AH, Sinclair BJ (Eds) Manual of Afrotropical Diptera, Volume 1. Introductory chapters and keys to Diptera families. Suricata 4. South African National Biodiversity Institute, Pretoria, 89–133.

Kurina O, Oliveira SS (2015) A new case of an Holarctic element in the Colombia Andes: first record of *Cordyla* Meigen (Mycetophilidae) from the Neotropical region. Zookeys 510: 87–108. https://doi.org/10.3897/zookeys.342.6045

Kurina O, Őunap E, Põldmaa K (2015) Two new Neuratelia Rondani (Diptera, Mycetophilidae) species from Western Palaeartic: a case of limited congruence between morphology and DNA sequence data. Zookeys 496: 105–129. https://doi.org/10.3897/zookeys.496.9315

Lane J (1948) Mycetophilidae do Brasil (Diptera, Nemocera). Revista Brasileira de Biologia 8: 247–254.

Lane J (1952) Some New Brazilian Mycetophilidae (Diptera, Nematocera). Dusenia 3(2): 131–140.

Matile L (1974) Notes sur les Mycetophilidae (Diptera) de la faune de France. III Le genre *Neuratelia*. L'Entomologiste 3: 28–33.

Oliveira SS, Amorim DS (2011) *Docosia adusta* sp. n. (Diptera, Mycetophilidae) from the Colombian Andes: a Holarctic element in northwestern South America. Canadian Entomologist 143: 688–696. https://doi.org/10.3897/zookeys.520.6142

Oliveira SS, Amorim DS (2012) Seven new species of *Paraleia* Tonnoir (Diptera, Mycetophilidae): amphinotic elements at the northern range of the Andes. Zootaxa 3186: 1–24. https://doi.org/10.11646/zootaxa.3186.1.1

Oliveira SS, Amorim DS (2014) Catalogue of Neotropical Diptera. Mycetophilidae. Neotropical Diptera 25: 1–87.

Sasakawa M (2004) Fungus gnats of the genus *Neuratelia* Rondani in Japan (Diptera, Mycetophilidae). Esakia 44: 67–79.

Søli GEE (1997) The adult morphology of Mycetophilidae (sensu stricto), with a tentative phylogeny of the family (Diptera, Sciaroidea). Entomologica Scandinava Supplement 50: 5–55.

Søli GEE, Vockeroth JR, Matile L (2000) Families of Sciaroidea. In: Papp L, Darvas B (Eds) Contributions to a Manual of Palaearctic Diptera. Science Herald, Budapest, 49–92.

Rondani C (1856) Dipterologiae Italicae prodromus: Genera Italica ordinis dipterorum ordinatim disposita et distincta et in familias stirpes aggregata. Parmae, Volume 1, Parma, Italy, 228 pp. https://doi.org/10.5962/bhl.title.8160

Vockeroth JR (1981) Mycetophilidae. In: McAlpine JF, Peterson BV, Shewell GE, Teskey HJ, Vockeroth JR, Wood DM (Eds) Manual of Nearctic Diptera. Volume 1. Research Branch Agriculture Canada. Monograph 27. Ottawa, Ontario, 223–255.

Zaitzev AI (1994) [Fungus gnats of the fauna of Russia and adjacent regions]. Part 1. Nauka, Moscow, 288 pp. [In Russian]

Supplementary material 1

Neuratelia Neotropical species list

Authors: Carolina Henao-Sepúlveda, Marta Wolff, Dalton de Souza Amorim

Data type: species data

Link: https://doi.org/10.3897/zookeys.861.32835.suppl1

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Two new species of the tribe Synanthedonini (Lepidoptera, Sesiidae), with new hostplant associations from Taiwan

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Abstract

Two new species of the tribe Synanthedonini are described from Taiwan: *Synanthedon auritinctaoidis* **sp. nov.** and *Paranthrenella helvola* **sp. nov.** Diagnostic characters for the two species are presented using body color, wing pattern, and genitalia. New records of the relationships between host plants and the immatures are also provided. *S. auritinctaoidis* feeds in the trunk of *Cinnamomum camphora* (Lauraceae) and *P. helvola* in callus tissue of *Helicia formosana* (Proteaceae) or *Prunus campanulata* (Rosaceae).

Keywords

host plants, *Paranthrenella*, *Synanthedon*

Introduction

Clearwing moths (Sesiidae) are a family of small to medium-sized moths that are well-known for their striking mimicry of various Hymenoptera. The Synanthedonini forms the largest tribe in the family Sesiidae Boisduval, 1828 (Špatenka et al. 1999), with 737 species in 38 genera described worldwide (Pühringer and Kallies 2017). The most diverse fauna of clearwing moths, both in species numbers and higher-level diversity, can be found in the tropics and subtropics of the Oriental and Afrotropical regions (Agassiz and Kallies 2018). However, the Synanthedonini fauna of these regions is only incompletely known and many more taxa await discovery or formal description.

Collecting of clearwing moths during the last decades largely relied on artificial pheromone lures (Gorbunov and Arita 1999, 2000; Arita and Gorbunov 2001, 2002; Kallies et al. 2014). However, these lures were produced on the basis of pheromone components identified for a relatively small number of Palaearctic and Nearctic species, and many taxa that occur in the tropical regions do not respond to these lures. Thus, discovery of the host plants and the rearing of larvae are still critical when assessing Sesiidae distribution and diversity. Most clearwing moths are host-specific, as they utilize only one or few related plants as larval hosts (Špatenka et al. 1999; Ahmad et al. 2001; Robinson et al. 2001, 2002). Liang and Hsu (2015) undertook a survey of the family by exploring potential larval host plants, resulting in the discovery of six new species in the tribe Synanthedonini of Taiwan. We continued to employ this investigation strategy, and subsequently two previously unknown Synanthedonini species were discovered in Taiwan, one in the genus *Synanthedon* Hübner, 1819 and the other in *Paranthrenella* Strand, 1916, based on diagnostic characters in body size, wing pattern, and genitalia.

The present article provides the taxonomic treatments and documents host associations, and immature biology for these two species.

Materials and methods

Adults were collected from flowers or vegetation in the field. Immatures were collected from host plants and reared using an artificial diet. The ingredients of the artificial diet were modified from Liang and Hsu (2015) by adding wood powder of their specific host plant. Forewing length is defined as the distance between the base of the forewing and forewing apex. Genitalia slides were prepared following procedures of Common (1990). Terminology of genitalia follows Klots (1970) and Kristensen (2003), that of wing pattern and venation Špatenka et al. (1999). The names of hostplants follow Boufford et al. (2003). Holotypes will be deposited in the Natural History Museum, London. Additional type series or vouchers are deposited in the following collections abbreviated in the text as follows:

NHMUK Department of Entomology, The Natural History Museum, London;
NTNU Department of Life Science, National Taiwan Normal University, Taipei, Taiwan.

Taxonomic accounts

***Synanthedon auritinctaoidis* sp. nov.**
http://zoobank.org/FA037243-D2EE-4094-98AB-03EC7A78EA9A
Figs 1, 2, 5, 7, 9–11

Type material. Holotype: ♂, HUALIEN: Ruisui, Fuyuan National Forest Recreation Area, 410 m, 18 Feb 2018, reared from *Helicia formosana*, emg. 8 Mar 2014, J.Y. Liang

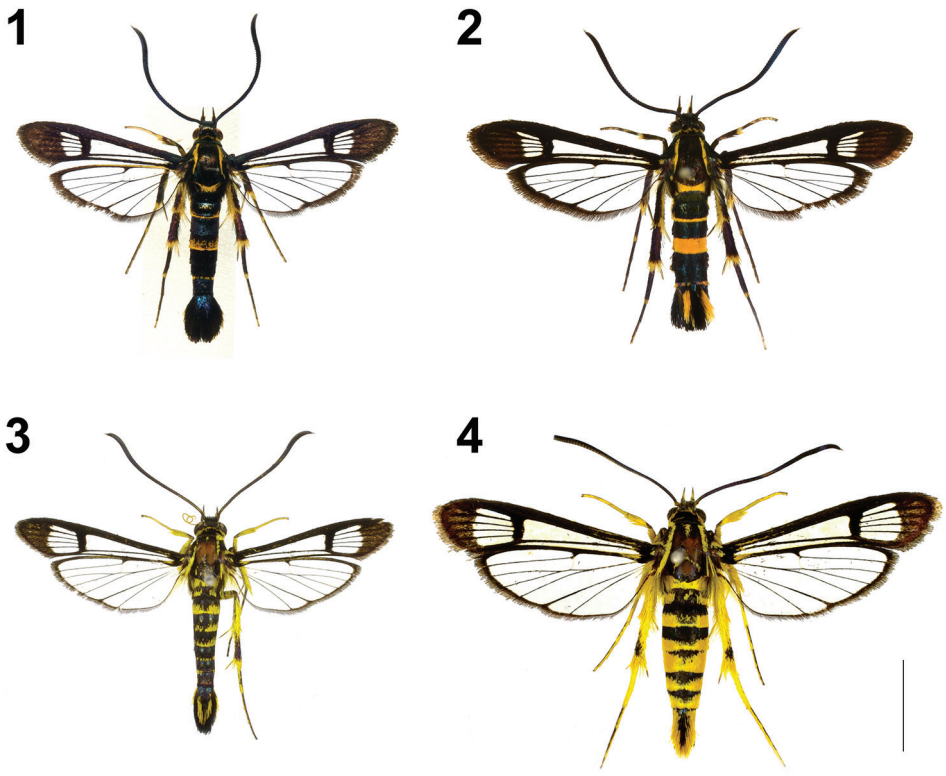
Coll. (NHMUK). Paratypes: 5♀, same locality and date as holotype, emg. 12–24 Mar 2014, J.Y. Liang Coll. (1♀ Gen. Prep. JYL-303) (NTNU); 1♀, same locality, 6 Feb 2016, reared from *H. formosana*, emg. 11 Mar 2016, HSUM 16B82M, J.Y. Liang Coll. (NTNU); 1♂, 4♀, NEW TAIPEI CITY: Shenkeng, Houshanyue, 470 m, 16 Nov 2014, reared from *Prunus campanulata*, emg. 27 Dec 2014–4 Jan 2015, HSUM 14L07M, J.Y. Liang Coll. (1♂ Gen. Prep. JYL-302) (2♀ Gen. Prep. JYL-271 and JYL-306) (NTNU).

Description. Male (Fig. 1): Antenna length 6.9–7.8 mm (n = 2); forewing length 8.4–9.2 mm (n = 2); body length 10.7–12.1 mm (n = 2). Head: antenna black with blue-violet sheen; frons white; labial palpus black, yellow ventrally; vertex black with purplish sheen; pericephalic scales yellow with a few black scales dorsally. Thorax: patagia black with violet sheen; tegula black, bronzed-blue sheen with a yellow dorsal line; mesothorax black with blue sheen; metathorax yellow; thorax laterally yellow with a few black scales. Legs: fore coxa externally black, internally yellow; fore femur black, with violet sheen; fore tibia dark brown to black, with admixture of yellow scales distally; fore tarsus dorsally dark brown to black, ventrally entirely yellow; mid coxa and femur black, with violet sheen; mid tibia dark brown to black, base-ventrally with a large yellow spot, base of spurs yellow; spurs yellow with black distally; mid tarsus dorsally dark brown to black, with admixture of yellow scales distally, ventrally yellow; hind leg similar. Abdomen: black with blue sheen; tergites 2 and 6 with a narrow yellow stripe distally; tergite 4 with a broad yellow stripe; abdominal tuft black with bronzed-blue sheen, lateral margins with some yellow-orange scales. Forewing: basally black; costal margin dark brown to black; discal spot and veins within exterior transparent area dark brown to black; apical area dark brown with admixture of brown scales; discal spot broad; exterior transparent area large divided into four cells, level to M2 about 1.5×as broad as discal spot and 0.6× as broad as apical area; posterior transparent area reaching discal spot; cilia dark brown. Hindwing: transparent; veins, discal spot and outer margin dark brown to black with bronzed sheen; discal spot small, cuneiform, reaching to vein M2; cilia dark brown, pale yellow anally.

Female (Fig. 2): Antenna length 5.1–6.2mm (n = 9); forewing length 7.9–8.8 mm (n = 9); body length 8.5–10.8 mm (n = 9). Tergite 4 throughout yellow; anal tuft yellow laterally. Other characters identical to those of male.

Male genitalia (Gen. Prep. JYL-302, NTNU, Fig. 5): Tegumen-uncus complex broad; socii well-developed with scopula androconialis, long, about as short as tegumen-uncus complex; uncus with a small narrow wing ventrally; crista gnathi medialis broad, with distal margin divided in two narrow wings; crista gnathi lateralis consisting of a rather broad, subcordiform, distal part and a narrow, crescent-shaped, proximal part; valva elongated, trapeziform, slightly turned down ventro-caudally; crista sacculi well-developed, large, divided into two pocket-shaped parts; dorsal part larger and armed at distal margin with strong, short, slightly bifurcate distally setae; ventral part of crista sacculi narrow, without setae; saccus rounded basally; phallus thin, about 0.8× as short as valva; vesica without cornuti.

Female genitalia (Gen. Prep. JYL-306, NTNU, Fig. 7): 8th tergite relatively large and broad with a few setae at distal margin; posterior apophysis long, about 2× as long as anterior apophysis; ostium bursae opening near anterior margin of 8th sternite;



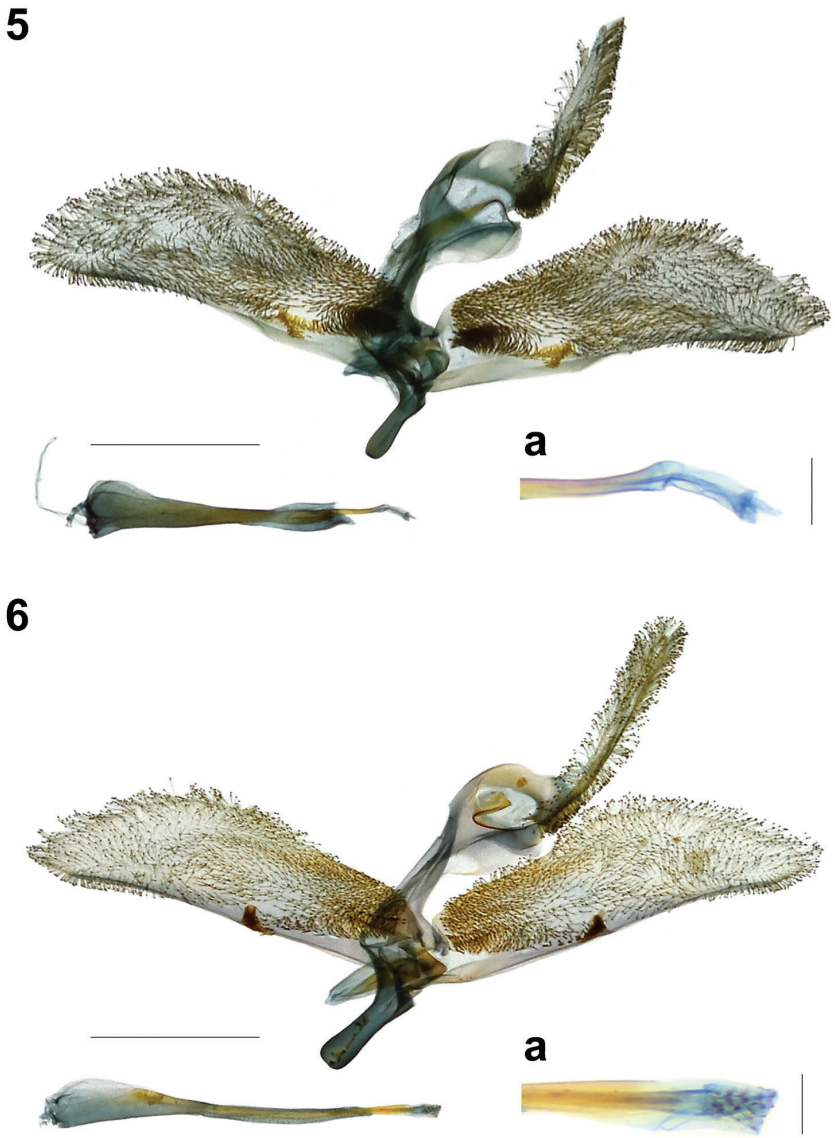
Figures 1–4. Synanthedonini adults **1, 2** *Synanthedon auritinctaoidis* sp. nov. **1** ♂, holotype, Taiwan: Hualien (NHMUK). **2** ♀, paratype, Taiwan: Hualien (NTNU). **3, 4** *Paranthrenella helvola* sp. nov. **3** ♂, holotype, Taiwan: Nantou (NHMUK) **4** ♀, paratype, Taiwan: Nantou (NTNU). Scale bar: 10 mm.

antrum broad ring, well-sclerotized; ductus bursae narrow, long, membranous; corpus bursae membranous, ovoid, without signum.

Diagnosis. *Synanthedon auritinctaoidis* sp. nov. is similar to *S. auritincta* (Wileman & South, 1918) in markings of body and wing, but may be distinguished by the following genitalia characters: saccus base rounded in *S. auritinctaoidis*, but emarginate in *S. auritincta*; phallus without tooth in *S. auritinctaoidis*, but *S. auritincta* with a small, strong tooth ventro distally; ostium bursae opening near anterior margin of 8th sternite in *S. auritinctaoidis*, but middle of 8th sternite in *S. auritincta*; antrum broad ring in *S. auritinctaoidis*, but funnel-shaped in *S. auritincta*.

Etymology. This species is named *auritinctaoidis*, an adjective formed by adding the suffix –oides to *auritincta*, because of its superficial resemblance with *S. auritincta* (Wileman & South, 1918).

Biology. The larva bores into burls of 5–20 cm in diameter on the trunk or branch of *Helicia formosana* Hemsl. (Proteaceae) (Figs 9, 11) or *Prunus campanulata* (Maxim.)



Figures 5, 6. Male genitalia of Synanthedonini. **5** *Synanthedon auritinctaoidis* sp. nov. paratype (NTNU) **5a** distal part of phallus. **6** *Paranthrenella helvola* sp. nov. holotype (NHMUK) **6a** distal part of phallus. Scale bars: 1 mm; 0.1 mm (**5a, 6a**).

Koidz. (Rosaceae) (Fig. 10), and feeds on callus tissue around the hole, which is covered with silk, debris, and frass.

Distribution. Known only from Taiwan.

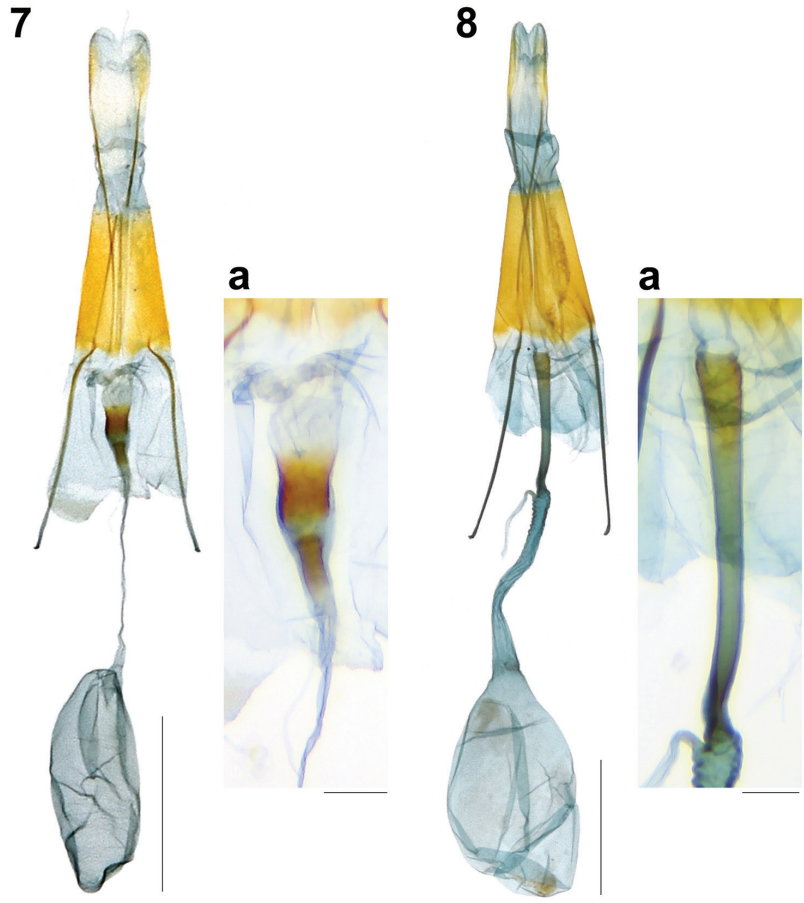
***Paranthrenella helvola* sp. nov.**
http://zoobank.org/996688CE-0504-45CC-B319-3F08ED4114A1
Figs 3, 4, 6, 8, 12

Type material. Holotype: ♂, NANTOU: Renai, Lushan, 1150 m, 23 Jun 2016, on flower of *Ampelopsis brevipedunculata*, J.Y. Liang Coll. (Gen. Prep. JYL-301, NHMUK). Paratypes: 1♀, NANTOU: Renai, Aowanda National Forest Recreation Area, 1240 m, 15 Jul 2016, J.Y. Liang Coll. (NYNU); 1♀, TAOYUAN: Fuxing, Lian, 790 m, 22 May 2018, on trunk of *Cinnamomum camphora*, Y.F. Hsu Coll. (Gen. Prep. JYL-300, NTNU).

Description. Male (Fig. 3): Antenna length 7.2 mm; forewing length 10.3 mm; body length 12.5 mm. Head: antenna black with blue sheen, dorsally and light brown with a few yellow scales ventrally; frons white; labial palpus yellow with black apically; vertex black; pericephalic scales yellow with a few yellow-orange scales. Thorax: patagia dark brown to black, with a small yellow spot laterally; tegula black with a yellow dorsal line, narrowly bordered with yellow scales; thorax laterally yellow with small black scales; mesothorax black with a few yellow scales anteriorly; metathorax yellow, with a few black scales; posteriorly metepimcron and metameron with brown and yellow hair-like scales. Legs: fore coxa yellow with golden sheen, with a broad dark brown to black stripe; fore femur externally dark brown, internally yellow; fore tibia yellow with golden sheen, with admixture of dark brown scales; fore tarsus yellow; mid coxa dark brown to black, with a few yellow scales; mid femur externally dark brown to black, internally yellow; mid tibia yellow, spurs yellow; mid tarsus exterior-dorsally dark brown to black, interior-ventrally entirely yellow; hind leg similar; hind tibia yellow, with a dark brown to black spot distally. Abdomen: dorsally dark brown to black with blue sheen; tergites each with a broad, broadened laterally, yellow stripe distally; abdominal tuft black, dorsal with yellow V shape spot, lateral margins with yellow-orange scales. Forewing: basally black; costal margin dark brown to black, with a narrow yellow stripe between vein Sc and R-stem; discal spot and veins within exterior transparent area dark brown to black; apical area brown with admixture of orange scales; discal spot broad; exterior transparent area large divided into four cells, level to M2 about 3.2× as broad as discal spot and 0.8× as broad as apical area; posterior transparent area reaching distal margin of discal spot; cilia brown. Hindwing: transparent; veins, discal spot black, small, cuneiform, reaching to vein M2; cilia dark brown, yellow anally.

Female (Fig. 4): Antenna length 8.3–8.7 mm (n = 2); forewing length 12.1–12.5 mm (n = 2); body length 13.8–14.1 mm (n = 2). Body and legs with more numerous yellow scales; anal tuft yellow-orange laterally. Color patterns otherwise as in male.

Male genitalia (Gen. Prep. JYL-301, NTNU, Fig. 6): Tegumen-uncus complex narrow; scopula androconialis well-developed, long, about as long as tegumen-uncus complex; crista gnathi medialis relatively long with sinusoid margin; crista gnathi lateralis shorter and narrower than crista gnathi medialis; valva trapeziform-oval, covered with apically bifurcate setae; ventral crista small, covered with triangular flat-topped setae; phallus thin, slightly shorter than valva; vesica with numerous small cornuti.



Figures 7, 8. Female genitalia of Synanthedonini. **7** *Synanthedon auritinctaoidis* Liang & Hsu, sp. nov. paratype (NTNU) **7a** antrum. **8** *Paranthrenella helvola* Liang & Hsu, sp. nov. paratype (NTNU) **8a** antrum. Scale bars: 1 mm; 0.1 mm (**7a, 8a**).

Female genitalia (Gen. Prep. JYL-300, NTNU, Fig. 8): Apophysis posterioris about as long as apophysis anterioris; ostium bursae opening somewhat anteriorly of 8th tergite; antrum narrow, long, about twice shorter than anterior apophysis, well-sclerotized; ductus seminalis just from anterior margin of antrum; ductus bursae membranous, narrow, slightly longer than antrum, gradually broadened towards corpus bursae; corpus bursae globose to ovoid, without signum.

Diagnosis. *Paranthrenella helvola* sp. nov. is similar to *P. similis* Gorbunov & Arita, 2000 which was described from Vietnam. *P. helvola* may be distinguished from *P. similis* by forewing without a transparent area of a cell between veins R4 and R5, with a narrow yellow stripe between vein Sc and R-stem; abdominal tuft with yellow V shape spot of male. *P. helvola* possesses wing pattern similar to *P. albipuncta* Gorbunov

Figures 9–12. Biology. **9** Galls induced by infection of a *Synanthedon auritinctaoidis*. Caterpillar on burls of *Helicia formosana* (Hualien Prefecture, Ruisui Township, Fuyuan National Forest Recreation Area) **10** galls induced by infection of a *S. auritinctaoidis* caterpillar on burls of *Prunus campanulata* (New Taipei City, Shenkeng District, Houshanyue) **11** caterpillar of *S. auritinctaoidis* sp. nov. in burls of *H. formosana* (Hualien Prefecture, Ruisui Township, Fuyuan National Forest Recreation Area) **12** female adult of *Paranthrenella helvola* sp. nov. ovipositing on bark of *Cinnamomum camphora* (Taoyuan City, Fuxing District, Lian).

& Arita, 2000 from Vietnam. *P. helvola* can be distinguished by the coloration of the antenna black, with a large yellow spot in *P. albipuncta*.

Etymology. The name of this new species is the feminine form of the Latin adjective *helvolus,* meaning yellowish, because of the overall yellow coloration of the body trunk and legs of the moth.

Biology. Females lay eggs in the crack of bark (Fig. 12). The larva bores into the trunks of *Cinnamomum camphora* (L.) (Lauraceae) and feeds on callus tissue around the hole, which is covered with silk, debris, and frass. The adults have been observed taking nectar from flowers of *Ampelopsis brevipedunculata* (Maxim.) Traut. (Vitaceae) during day time.

Distribution. Known only from Taiwan.

Remarks. The male genitalia of *P. helvola* sp. nov. are similar to those of *P. albipuncta* Gorbunov & Arita, 2000, but the color of the antenna of these two species differs considerably.

Discussion

Although many plant families are known to be utilized as larval hostplants by sesiid moths, Lauraceae are rarely used, with merely two species recorded: *Paranthrenella weiyui* Liang & Hsu, 2015 and *Ichneumenoptera gryphus* Liang & Hsu, 2015 (Liang and Hsu 2015). *Paranthrenella helvola*, sp. nov. represents the third known species associated with Lauraceae.

Although most clearwing moths are host-specific, utilizing only one or few related plants as larval hosts, their host range seems to increase with some particular modes of feeding. A wider host range seems also to occur in other groups of insects with particular feeding habits (Nyman et al. 2010). For instance, *Synanthedon scitula* (Harris, 1839), a species feeding exclusively on callus, is known to use 17 genera in nine plant families as larval hosts (Robinson et al. 2002). Larvae of *Synanthedon auritinctaoidis* sp. nov. were found feeding on callus tissue of plants in both Proteaceae and Rosaceae, suggesting more plant families may serve as larval diet for this species.

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References

Agassiz D, Kallies A (2018) A new genus and species of myrmecophile clearwing moth (Lepidoptera: Sesiidae) from East Africa. Zootaxa 4392(3): 588–594. https://doi.org/10.11646/zootaxa.4392.3.8

Ahmad M, Mishra R, Ahmad J (2001) Insect pest spectrum of Poplar in India. Indian Forester 127(12): 1353–1366.

Arita Y, Gorbunov O (2001) Sesiidae of Taiwan. I. The Tribes Tinthiini, Similipepsini, Paraglosseciini, Pennisetiini, Paranthrenini and Cissuvorini. Japanese Journal of Systematic Entomology 7(2): 131–188.

Arita Y, Gorbunov O (2002) Sesiidae of Taiwan. II. The tribes Osminiini, Melittiini and Sesiini. Japanese Journal of Systematic Entomology 8(2): 199–241.

Boufford DE, Ohashi H, Huang TC, Hsieh CF, Tsai JL, Yang KC, Peng CI, Kuoh CS, and Hsiao A (2003) A checklist of the vascular plants of Taiwan. In: Editorial Committee of the Flora of Taiwan, Second Edition (Eds) Flora of Taiwan, second edition. Volume six, Department of Botany, National Taiwan University, 15–139.

Common IFB (1990) Moths of Australia. Melbourne University Press, Carlton, Victoria, 535 pp. https://doi.org/10.1071/9780643101227

Gorbunov O, Arita Y (1999) New taxa of the clearwing moths (Lepidoptera, Sesiidae) from Nepal. Tinea 16(2): 106–143.

Gorbunov O, Arita Y (2000) Study on the Synanthedonini (Lepidoptera, Sesiidae) of Vietnam. Japanese Journal of Systematic Entomology 6(1): 85–113.

Kallies A, Arita Y, Owada M, Wu G-Y, Wang M (2014) The Paranthrenini of mainland China (Lepidoptera, Sesiidae). Zootaxa 3811(2): 185–206. https://doi.org/10.11646/zootaxa.3811.2.2

Klots AB (1970) Lepidoptera. In: Tuxen SL (Ed.) Taxonomist's glossary of genitalia in insects. Munksgaard, Copenhagen, 115–130.

Kristensen NP (2003) Skeleton and muscles: adults. In: Kristensen NP (Ed.) Lepidoptera, Moths and Butterflies, 2 Morphology, physiology and development. Handbook of Zoology 4 (36), De Gruyter, Berlin, New York, 39–131. https://doi.org/10.1515/9783110893724.39

Liang JY, Hsu YF (2015) A review of clearwing moths in the tribe Synanthedonini, with descriptions of six new species from Taiwan (Lepidoptera: Sesiidae). Zootaxa 4044(4): 535–555. https://doi.org/10.11646/zootaxa.4044.4.4

Nyman T, Vikberg V, Smith D, Boevé J-L (2010) How common is ecological speciation in plant-feeding insects? A 'Higher' Nematinae perspective. BMC Evolutionary Biology 10: 266. https://doi.org/10.1186/1471-2148-10-266

Pühringer F, Kallies A (2017) Provisional checklist of the Sesiidae of the world (Lepidoptera Ditrysia). http://www.sesiidae.net/Checklst.htm [Last modified January 28, 2017; accessed March 8, 2019]

Robinson GS, Ackery PR, Kitching IJ, Beccaloni GW, Hernández LM (2001) Hostplants of the moth and butterfly caterpillars of the Oriental Region. Southdene Sdn Bhd, Kuala Lumpur, 744 pp.

Robinson GS, Ackery PR, Kitching IJ, Beccaloni GW, Hernández LM (2002) Hostplants of the moth and butterfly caterpillars of America north of Mexico. American Entomological Institute 69: 1–824.

Špatenka K, Gorbunov O, Laštůvka Z, Toševski I, Arita Y (1999) Sesiidae, Clearwing Moths. In: Naumann CM (Ed.) Handbook of Palaearctic Macrolepidoptera. Gem Publishing Company, Wallingford, England, 569 pp.

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RESEARCH ARTICLE

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Three new subfamilies of skipper butterflies (Lepidoptera, Hesperiidae)

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Abstract

We obtained and analyzed whole genome data for more than 160 representatives of skipper butterflies (family Hesperiidae) from all known subfamilies, tribes and most distinctive genera. We found that two genera, *Katreus* Watson, 1893 and *Ortholexis* Karsch, 1895, which are sisters, are well-separated from all other major phylogenetic lineages and originate near the base of the Hesperiidae tree, prior to the origin of some subfamilies. Due to this ancient origin compared to other subfamilies, this group is described as Katreinae Grishin, **subfam. n.** DNA sequencing of primary type specimens reveals that *Ortholexis melichroptera* Karsch, 1895 is not a female of *Ortholexis holocausta* Mabille, 1891, but instead a female of *Ortholexis dimidia* Holland, 1896. This finding establishes *O. dimidia* as a junior subjective synonym of *O. melichroptera*. Furthermore, we see that *Chamunda* Evans, 1949 does not originate within Pyrginae Burmeister, 1878, but, unexpectedly, forms an ancient lineage of its own at the subfamily rank: Chamundinae Grishin, **subfam. n**. Finally, a group of two sister genera, *Barca* de Nicéville, 1902 and *Apostictopterus* Leech, [1893], originates around the time Hesperiinae Latreille, 1809 have split from their sister clade. A new subfamily Barcinae Grishin, **subfam. n.** sets them apart from all other Hesperiidae.

Keywords

Africa, Asia, genomics, higher classification, phylogeny

Introduction

New methods bring new discoveries. While careful expert-driven morphological analysis can be insightful in revealing synapomorphies and predicting evolutionary relationships between animals, DNA sequences offer additional insights. Phylogenetic analysis at the genomic scale is expected to give an unprecedented resolution and clarify many questions, providing a firm basis for the best taxonomic classification. Butterflies are attracting attention with a number of large scale phylogeny studies published recently (Espeland et al. 2015; Cao et al. 2016; Espeland et al. 2018; Seraphim et al. 2018; Toussaint et al. 2018; Li et al. 2019;). The butterfly family Hesperiidae (skippers), which includes butterflies with stout bodies, large heads and rapid wing beats, is still comparatively less known. Groundbreaking DNA analysis by Warren et al. (2008, 2009) based on several genes revealed many new phylogenetic relationships compared to the last comprehensive morphological treatment (Evans 1937, 1949, 1951, 1952, 1953, 1955) and offered an updated classification of Hesperiidae. Additional insights came from follow-up studies (Sahoo et al. 2016; Sahoo et al. 2017; Espeland et al. 2018; Toussaint et al. 2018; Li et al. 2019; Zhang et al. 2019) posing questions about phylogenetic and taxonomic placement of genera such as *Ortholexis* Karsch, 1895, *Barca* de Nicéville, 1902 and *Apostictopterus* Leech, 1893 (Fig. 1).

Here, we tackle the questions about deep phylogeny of Hesperiidae using whole genome shotgun analysis. We selected 160 representative species of skippers that cover all known subfamilies and tribes, including some genera that we thought would be interesting to analyze at the genomic scale. To make this work taxonomically sound, we used type genera and their type species where possible, and for some species used their primary type specimens. Our genomic methods break the time barrier and allow us to work with specimens more than a century old from museum collections. We find that while the backbone of the current classification of Hesperiidae stands the test of genomic data (Li et al. 2019), unexpected deep divergence of some groups awards them the status of subfamilies that are described here.

Materials and methods

Bodies of freshly collected specimens were stored in RNAlater, and their wings and genitalia dried and kept in envelopes to address possible misidentification issues. DNA was extracted from a piece of tissue of these specimens. For specimens in museum collections, DNA was extracted either from the abdomen or from a leg. The abdomen was gently pushed from above and below (while watching for the legs not to be damaged) until it cracked off, and placed in a DNA extraction buffer. After extraction (see below), the abdomen was transferred to 10% KOH solution and genitalia were dissected in a standard manner. A leg was used for primary type specimens. A leg was removed from a specimen using fine forceps and placed in a plastic tube. The forceps were wiped with clean paper tissue after each sample was taken.

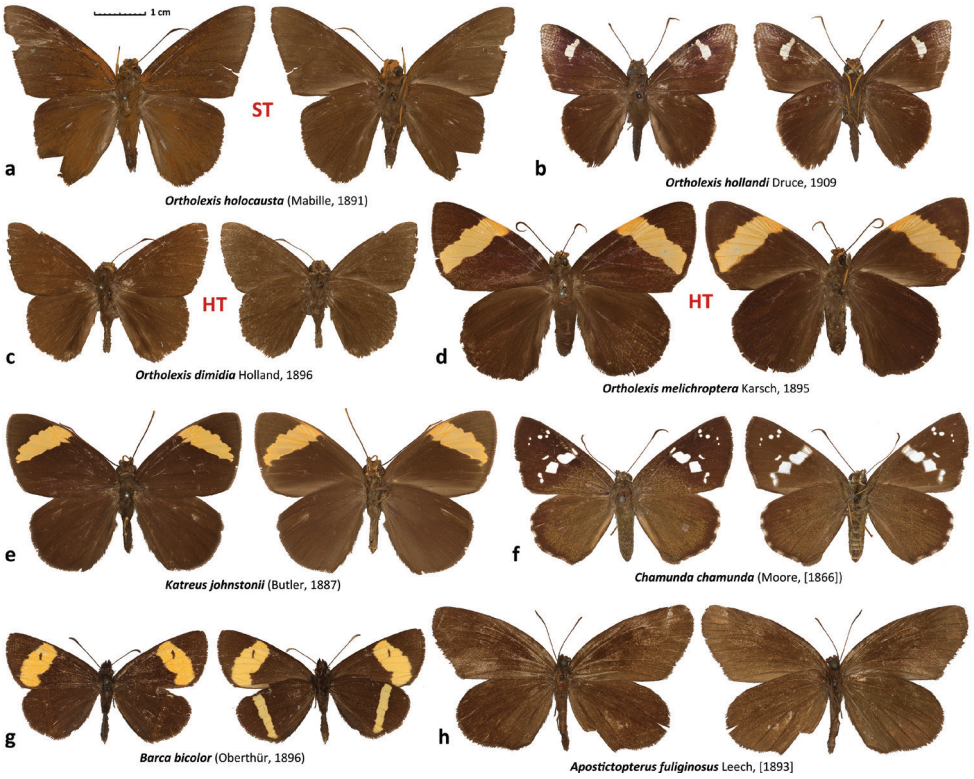

Figure 1. Sequenced specimens from the new Hesperiidae subfamilies. DNA sample numbers are given for each specimen, additional data are in the Suppl. material 1: Table S1 **a** *Ortholexis holocausta* syntype, NVG-18053C02 **b** *Ortholexis hollandi*, NVG-18082A08 **c** *Ortholexis melichroptera*, holotype of *Acallopistes dimidia* Holland, 1896; NVG-18053C05 **d** *Ortholexis melichroptera*, holotype, NVG-18053A06 **e** *Katreus johnstonii*, NVG-18053B05 **f** *Chamunda chamunda*, NVG-18086E02 **g** *Barca bicolor*, NVG-17069C10 **h** *Apostictopterus fuliginosus*, NVG-17069C12.

DNA was extracted from legs (and abdomens) non-destructively using Macherey-Nagel (MN) reagents. 70 µl buffer T1 and 10 µl protK were added to the tube without crushing the leg, and the mixture was incubated at 57 °C for 24 hours. Then, 80 µl buffer B3 was added and incubation continued for 2 hours, after which 85 µl of absolute EtOH was added and thoroughly mixed. The resulting liquid was transferred to a different tube and DNA extraction continued according to MN protocol (https://www.MN-net.com/Portals/8/attachments/Redakteure_Bio/Protocols/Genomic%20DNA/UM_gDNATissueXS.pdf), leaving the leg intact. Mate-pair libraries were constructed according to our published protocols (Cong et al. 2015; Cong et al. 2017; Li et al. 2019).

The libraries were sequenced for 150 bp from both ends targeting 4 to 6 Gbp of data (depending on the expected genome size) on Illumina HiSeq x10 at GENEWIZ. The resulting reads were matched using Diamond (Buchfink et al. 2015) to the exons of the reference genome of *Cecropterus lyciades* (Shen et al. 2017), which we

obtained previously, and the exons assembled and aligned to other Hesperiidae genomes obtained using the same methods. Coding regions of the mitochondrial genome (including the COI barcode) were assembled similarly. Exons expected to be from the Z-chromosome were predicted assuming similar syntenic arrangement with *Heliconius* (Heliconius Genome Consortium 2012). Phylogenetic trees were generated from three sets of exons: whole nuclear genome, whole mitochondrial genome and Z-chromosome using RAxML-NG (Kozlov et al. 2018) with default parameters (-m GTRGAMMA). Further details of experimental and computational protocols can be found in the "SI Appendix" to Li et al. (2019) (available at https://www.pnas.org/content/pnas/suppl/2019/03/15/1821304116.DCSupplemental/pnas.1821304116.sapp.pdf).

Diagnostic DNA characters were identified in nuclear genomic sequences using our recently published procedure (see SI Appendix to Li et al. 2019). Namely, the positions in exons were found that are most likely synapomorphic to the clade defined as a subfamily. For the clades where we had several species sequenced, positions that were invariant in all species and had a base pair different from the (mostly invariant) base pair in the outgroups were found, and those with the smallest number of species with missing data were selected. If the subfamily had only one species sequenced, we frequently looked for synapomorphic characters for its sister, noting the base pair as the character state, and uniting these with synapomorphic characters for the clade that leads to the common ancestor of this subfamily and its sister clade. Such a treatment increased the chances that the character found is not a random, non-conserved change or a sequencing error. The number of sequence reads covering this position was taken into account in choosing the characters, and those positions with higher coverage were given priority. The character states are given in diagnoses below as abbreviations. For example, aly728.44.1:G672C means position 672 in exon 1 of gene 44 from scaffold 728 of *Cecropterus* [formerly *Achalarus*] *lyciades* (aly) reference genome (Shen et al. 2017) is C, changed from G in the ancestor. When characters were found for the sister clade of the diagnosed taxon, the following statement was used: aly5294.20.2:A548A (not C), which means that position 547 in exon 2 of gene 20 on scaffold 5294 is occupied by the ancestral base pair A, which was changed to C in the sister clade (so it is not C in the diagnosed taxon). 169A, means position 169 is A, but the ancestral state is unclear. The sequences of exons from the reference genome with the positions used as character states highlighted in green are given in the Suppl. material 1. The distribution of these sequences together with this publication ensures that the numbers given in the diagnoses can be easily associated with actual sequences. Notations like A79T or 59C, without scaffold. gene.exon prefix separated by colon, refer to positions in the standard COI barcode region of 658 positions as defined previously (Ratnasingham and Hebert 2007). The sequences reported in this paper have been deposited in the NCBI Sequence Read Archive with accession PRJNA544364.

Results and discussion

Genomic phylogeny of Hesperiidae

We obtained whole genome shotgun sequence reads for 160 Hesperiidae specimen of representative species. The lengths of resulting genomic regions were: nuclear total 11,835,126 +/-3,035,464, Z-chromosome 99,237 +/-24,462, mitogenomes 12,144 +/-958. We considered Z-chromosome separately. Butterfly males carry two copies of Z, and females possess Z and W. In Z, recombination is reduced to half of that in autosomes, and sexual selection acts differently on genes encoded by it. Thus, the analysis of genes encoded by the Z-chromosome may provide additional information about species evolution. Phylogenetic trees were constructed from coding regions of nuclear genome, Z-chromosome and mitogenome. The trees were rooted with the genomic sequence of *Pterourus glaucus* that we obtained previously (Cong et al. 2015). Comparison of these trees yielded the same conclusions.

Several conclusions confirmed previous findings (Warren et al. 2008; 2009; Sahoo et al. 2016; Sahoo et al. 2017; Zhang et al. 2017; Espeland et al. 2018; Toussaint et al. 2018; Li et al. 2019). (1) The subfamily Coeliadinae Evans, 1937 is sister to all other Hesperiidae; (2) Euschemonidae Kirby, 1897 branches off next; (3) Eudaminae is sister to Pyrginae; (4) Heteropterinae is sister to Trapezitinae with Hesperiinae; and (5) Groupings into tribes mostly agree with what is known about Hesperiidae. However, several findings were new and some were unexpected. Three cases were particularly interesting and were analyzed in detail, as follows.

The *Katreus* and *Ortholexis* clade is a new subfamily

Unexpected placement of *Ortholexis holocausta* (Mabille, 1891) (Fig. 1a) as a sister of Pyrrhopygini Mabille, 1877 in a recently published phylogeny of Hesperiidae based on several genes (Sahoo et al. 2017) peaked our interest about this taxon and its relatives. The genome-based phylogeny we obtained (Fig. 2) confidently (>99% bootstrap) places it (Fig. 1a–d), together with its sister genus *Katreus* Watson, 1893 (Fig. 1e), near the base of the Hesperiidae tree, dating prior to divergence between Eudaminae and Pyrginae (Fig. 2) and suggesting a rank of subfamily for these skippers.

Katreinae Grishin, subfam. n.
http://zoobank.org/EFD73E63-A0FE-4AB3-B6F2-318977EF7F83

Type genus. *Katreus* Watson, 1893.

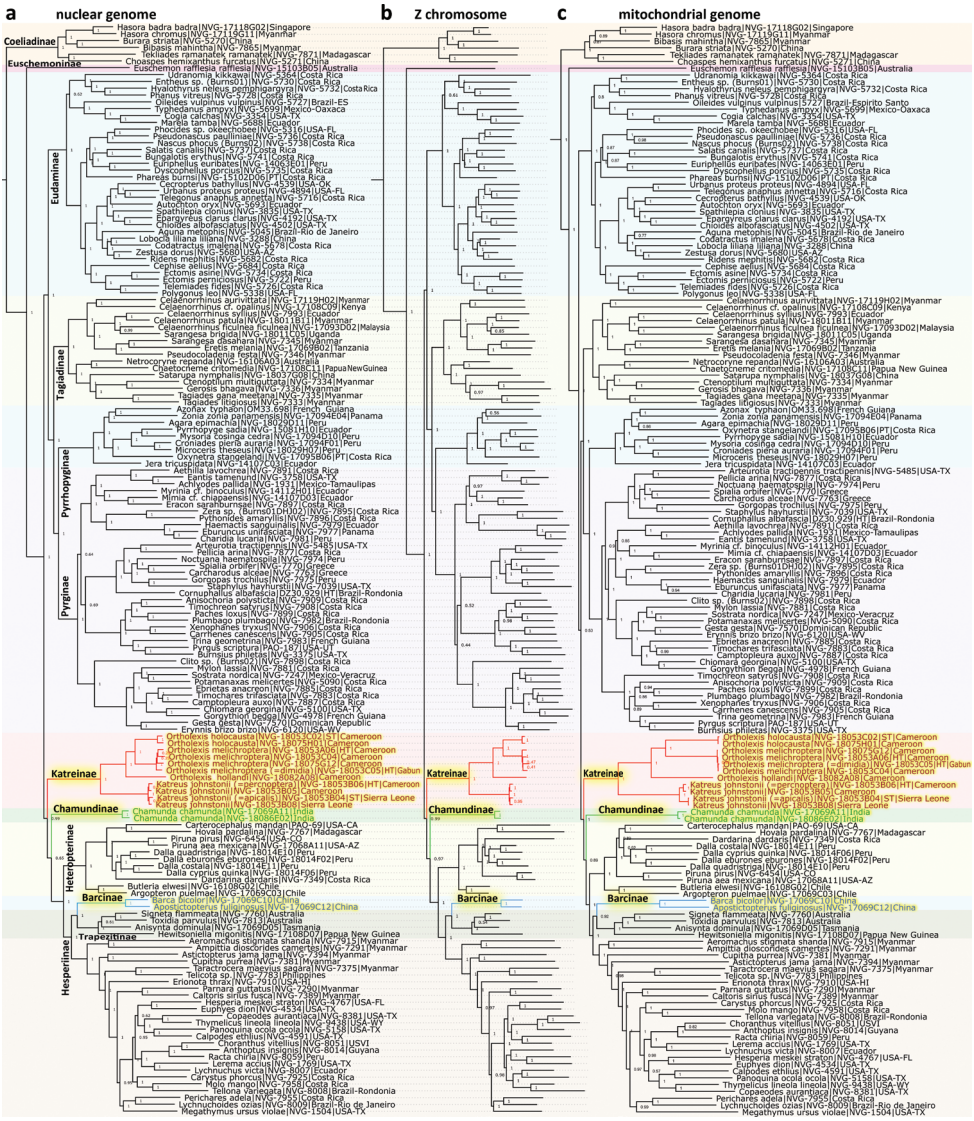

Figure 2. Phylogenetic trees. The trees are constructed from protein-coding regions of **a** nuclear genome **b** Z-chromosome, and **c** mitochondrial genome. The trees are rooted with *Pterourus glaucus* (NVG-1670). Specimen names are not shown in the Z-chromosome tree and can be deduced from the nuclear tree by corresponding dotted lines. Details about specimens are in Suppl. material 1: Table S1. Sections of the tree corresponding to different subfamilies are highlighted in different colors. Names of new subfamilies and specimens in them are highlighted yellow. Names of other subfamilies are shown by their clades in the nuclear tree.

Diagnosis. In appearance, most similar to *Celaenorrhinus* Hübner, [1819] and its relatives (Evans 1937), and was placed in Celaenorrhinini Swinhoe, 1912 by Warren et al. (2008, 2009) but differs by longer apiculus of antennae and hindwing produced at vein 1A+2A. Morphologically, distinguished from all Hesperiidae by the

combination of the following characters. Abdomen short, shorter than inner margin of hindwing. Antennal club arcuate, bent in the middle, apiculus long, pointed. Second segment of palpi protrudes partly forward and partly upward (at an angle between the axis of the body and the axis perpendicular to it, =sub-erect). Males with hair pencil on hind tibiae, without stigmas or brands on wings. Forewing discal cell long, about 2/3 of the costa; vein M_2 originates about midway between or closer to M_1 than to M_3 and vein CuA_2 originates closer to the base of wing than to the end of discal cell. Hindwing produced at vein 1A+2A, vein 3A much shorter than vein CuA_2. Male genitalia with a well-developed gnathos, which is not smaller than uncus, uncus bulging dorsad in lateral view, with small or tiny arms distant from each other, tegumen robust, extends caudad for the length of uncus, harpe longer than sacculus. See Larsen (2005: 469-471) for illustrations of all representative species in this subfamily. In DNA, a combination of the following base pairs in the nuclear genome is diagnostic: aly528.10.2:G940C, aly925.27.5:A3610T, aly84.77.5:T1651G, aly595.14.2:G184C, aly2284.22.2:G967C, and in COI barcode region: C235T, A335T, C347T, and T349A.

Genera included. *Katreus* with its invalid synonym *Choristoneura* Mabille, 1889 (junior homonym of *Choristoneura* Lederer 1859 in Lepidoptera: Tortricidae) and subjective synonyms *Loxolexis* Karsch, 1895 and *Daratus* Lindsey, 1925 (replacement name for *Choristoneura*) (Fig. 1e); and *Ortholexis* Karsch, 1895 with its subjective synonym *Acallopistes* Holland, 1896 (Fig. 1a-d).

Comments. Taxonomy of these skippers has been confusing until it was resolved by Cock and Congdon (2011). For the most part, they were all placed in the genus *Katreus*, until Larsen emphasized the differences in genitalia of those species placed in *Ortholexis* from true *Katreus* (Larsen 2005). Indeed, the two genera are quite distinct in our genomic analysis. A recent study based on several genes placed this group (only *Ortholexis holocausta* (Mabille, 1891) was included in that study) as a sister of Pyrrhopygini Mabille, 1877 (Sahoo et al. 2017), probably due to an insufficient number of genes included. In their study, *Euschemon* Doubleday, 1846 grouped with Eudaminae instead of being sister to all other Hesperiidae with exclusion of Coeliadinae Evans, 1937 (Warren et al. 2009; Zhang et al. 2017; Toussaint et al. 2018); such problems are expected from smaller datasets. We find (Fig. 2) that the Katreinae subfam. n. is an ancient and unique Afrotropical lineage that diverged from other Hesperiidae at the time when the family was diversifying into subfamilies.

Acallopistes dimidia Holland, 1896 is a new subjective synonym of *Ortholexis melichroptera* Karsch, 1895

We sequenced a syntype of *Erionota holocausta* Mabille, 1891 (Fig. 1a, judging from the original description (Mabille 1891) the type series of this species almost certainly consisted of this single syntype), and the holotypes of *Acallopistes dimidia* Holland, 1896 (Fig. 1c) and *Ortholexis melichroptera* Karsch, 1895 (Fig. 1d), which

are in the Museum für Naturkunde, Berlin, Germany. The phylogenetic trees (Fig. 2) revealed that *O. melichroptera* is not a female of *O. holocausta* as it has been assumed (Evans 1937), but instead a female of *O. dimidia*. This association of sexes is supported by both nuclear (protein-coding genes of autosomes and of Z-chromosome) and mitochondrial (all genes) DNA trees (Fig. 2). COI barcodes of the *O. holocausta* syntype and *O. melichroptera* holotype differ by 8.8% (58 bp), but barcodes of *O. melichroptera* and *O. dimidia* are essentially identical (1 bp difference). Thus, we conclude that *O. dimidia* syn. n. is a junior subjective synonym of *O. melichroptera*.

Unexpected uniqueness of *Chamunda*

The next find was particularly unexpected and was not likely to happen in the absence of DNA sequences. Nearly as ancient as Katreinae subfam. n., is the lineage consisting of a single genus *Chamunda* Evans, 1949, which is sister to the group collectively known as "grass skippers": subfamilies Heteropterinae Aurivillius, 1925, Trapezitinae Waterhouse & Lyell, 1914 and Hesperiinae Latreille, 1809 (Fig. 2), whose caterpillars feed mostly on monocots. The surprise comes due to the fact that *Chamunda* looks like an ordinary skipper, quite similar to several others in wing patterns: brown with forewing white spots forming a typical arrangement for dicot-feeding skippers (Fig. 1f). Nevertheless, its ancient origin suggests a subfamily rank, as described below.

Chamundinae Grishin, subfam. n.

http://zoobank.org/4FE1725C-4BF1-4D1A-B4A7-4BD409AA154A

Type genus. *Chamunda* Evans, 1949.

Diagnosis. Keys to C.10 in Evans (1949: 14). In appearance similar to Pyrginae, such as *Celaenorrhinus* Hübner, [1819] and its relatives, from which it is distinguished by the second segment of palpi protruding forward (in line with the body, =porrect) and not pointing dorsad (perpendicular to the body line, =erect); and Eudaminae, such as *Lobocla* Moore, 1884, from which it differs by narrower hindwing without tornal lobe (concave outer margin near tornus) and the lack of costal fold in males. Morphologically, distinguished from all Hesperiidae by a combination of the following characters. Body robust, abdomen stout, shorter than the inner margin of hindwing. Palpi porrect, 3rd segment stout, pointing forward, set at the outer edge of the second segment (not in the middle). Antennae longer than half of costa, with thin arcuate (not hooked) club and apiculus tapered to a sharp point, nudum of about 20 segments. Males with hair pencil on hind tibiae, without stigmas or brands on wings. Females with anal tuft of scales. Forewing discal cell long, about 2/3 of the wing; vein M_2 origin slightly closer to M_1 than to M_3. Five subapical spots in a S-shaped curve on right forewing. Hindwing inner margin shorter than costal margin; vein M_2 straight

and oblique: closer to M_3 at the outer margin, but closer to M_1 at its origin from the discal cell (not curved toward M_3); the angle formed by the median and discocellular veins acute, discocellular vein directed at tornus and outer margin, and not at the inner margin. In male genitalia, uncus elongated, undivided, uniquely shaped like a narrow mushroom at the tip; valva simple, without processes, spines or elaborations, lanceolate, with a small harpe only narrowly separated from the ampulla. In DNA, a combination of the following base pairs in the nuclear genome is diagnostic: aly528.10.2:A631C, aly3277.11.2:A1726G, aly4523.3.2:T143C, aly499.37.1:G77G (not A), aly363.14.5:A76A (not C), aly2700.1.4:T70T (not G), and in COI barcode region: G38A, A81C, A307G, C347T, T349A, A430T, A604C.

Genera included. Only *Chamunda*, a monotypic genus for *Plesioneura chamunda* Moore, 1866 (Fig. 1f).

Comments. The subfamily-worthy uniqueness of this butterfly from southwestern Asia, dubbed "Olive" or "Crescent Spotted Flat", is perhaps the largest surprise of our study. *Chamunda* is not clearly distinct in appearance, it is similar to *Lobocla* (Eudaminae) and *Celaenorrhinus* (Tagiadinae) in the spotting of the forewing. Uniqueness of *Chamunda* was not noticed before Evans, who established a new monotypic genus for this skipper (Evans 1949). Nevertheless, Evans placed it with Pyrginae according to its appearance, among genera currently in the tribe Tagiadini Mabille, 1878. We take the next step and establish a subfamily for it. It is unlikely that its subfamily status would have become apparent without genomic sequences placing this skipper far from all others with strong statistical support.

The *Barca* and *Apostictopterus* clade originates near Trapezitinae and Hesperiina

These two genera that are apparently each other's closest relatives have been enigmatic for decades (Evans 1949) (Fig. 1g, h). Their mitogenomes have recently been sequenced (Han et al. 2018) and revealed that among species with known mitogenomes (which did not include any Trapezitinae), they are sister to Hesperiinae and not Heteropterinae. Ironically, our study suggests that Trapezitinae may be sister to the group formed by these two genera (Fig. 2). However, no apparent morphological synapomorphies unify the group of the two genera with Trapezitinae, and their morphology is quite different, so we award them a subfamily rank:

Barcinae Grishin, subfam. n.
http://zoobank.org/A3512E6F-78AF-4AB5-9562-43C160BFA2D7

Type genus. *Barca* de Nicéville, 1902.

Diagnosis. Keys to F.4a in Evans (1949: 23). The synapomorphy of the subfamily is likely to be the bow-like shape of the forewing vein A_1+A_2. In appearance similar to Heteropterinae (slender body and characteristic relatively broad for monocot-feeding

Hesperiidae but rounded wing shape), from which it is distinguished by this bowed vein and not flattened antennal club with obtuse apiculus. Morphologically, distinguished from all Hesperiidae, by the following combination of additional characters. Body slender, abdomen not longer than inner margin of hindwing. Second segment of palpi protruding forward (in line with the body, =porrect) and not pointing dorsad (perpendicular to the body line, =erect). Apiculus of antennae blunt, with black nudum of 10 segments, more than in Heteropterinae (6-9) but fewer than Trapezitinae (12-26). Mid tibiae without spines and hind tibiae with 2 pairs of short spurs. No secondary sexual characters. Forewing discal cell about 2/3 of costa in length, apex rounded. Hindwing with a rounded tornus, costal margin longer than inner margin; discal cell not shorter than half of the wing; discocellular vein points toward tornus, not inner margin. Male genitalia with extended, undivided uncus (Evans 1949: plate 29 F.4, F.5) more similar to Heteropterinae, but valva broader and more robust and reminiscent of that in Trapezitinae: expanded and modified costa-ampulla, harpe prominent, with serrated edge. In DNA, a combination of the following base pairs in the nuclear genome is diagnostic: aly525.83.3:A682T, aly525.83.3:G683C, aly1139.27.4:G112T, aly1139.27.4:G113C, aly23605.15.15:G49A, and in COI barcode region: G101A, A166G, and 474C.

Genera included. *Barca* de Nicéville, 1902 with its invalid synonym *Dejeania* Oberthür, 1896 (junior homonym of *Dejeania* Robineau-Desvoidy, 1830 in Diptera) (Fig. 1g) and *Apostictopterus* Leech, [1893] with its subjective synonym *Tecupa* Swinhoe, 1917 (Fig. 1h). Both valid genera are monotypic.

Comments. These two genera from southwestern China were (with disclaimers) placed in Heteropterinae by Evans (Evans 1949) and transferred to Hesperiinae by Warren et al. (2009), owing to different from Heteropterinae genitalia. Mitochondrial genomes for both genera were determined recently, and they confirmed the lack of affinity to Heteropterinae (Han et al. 2018). However, in the absence of Trapezitinae mitogenome, the two genera remained in Hesperiinae. Our phylogenies place the two genera as sister to Trapezitinae, thus they may not belong to Hesperiinae. This placement is unexpected because there are no obvious morphological features than unify Trapezitinae and the two genera. Therefore, we decided on the level of a subfamily for these two unusual skippers. They form an ancient phylogenetic group, and placing them within Trapezitinae seems unfitting due to the lack of morphological affinities.

Phylogeny and classification

While classification relies on phylogeny, it does not require phylogeny to be fully resolved. Good classification only requires a clade itself to be well supported and distinct from other clades of the same rank. However, the exact position of that clade in the tree, which reflects the order in time when these clades originated, does not need to be fully resolved. Thus, accurate classification is a simpler task than phylogenetic inference. These considerations are relevant to our treatment of Chamundinae subfam. n. While in the Z-chromosome tree (Fig. 2b), the node at which Chamundinae have split

from its sister is well supported (97% bootstrap), both nuclear genome and mitogenome trees (Fig. 2a, c) reveal weaker support: 65% and 89% respectively. It is likely that the weak support is a consequence of rapid radiation at the time of divergence between Katreinae subfam. n., Chamundinae and the sister of these taxa (the monocot feeding clade: Heteropterinae plus their sister clade). Possible incomplete lineage sorting and introgression obscured phylogenetic signal and leave the exact position of Chamundinae clade weakly resolved.

Nevertheless, the decision to treat Chamundinae as a subfamily is supported by the following reasons. We consider three clades in the trees (Fig. 2): Katreinae (colored red), Chamundinae (colored green), and the clade of monocot feeders (Heteropterinae plus their sister clade that includes Barcinae subfam. n., Trapezitinae and Hesperiinae). The clade of monocot feeders is well supported in all three trees (Fig. 2, bootstrap 100%), therefore Chamundinae does not belong to this clade. The clade of Katreinae plus their sister (Chamundinae, Heteropterinae, Barcinae, Trapezitinae, and Hesperiinae) is also strongly supported in all three trees (bootstrap >99%), therefore Chamundinae belong to this clade. The placement of the three subclades in this clade (Katreinae, Chamundinae and the monocot feeder) is poorly resolved. I.e., it is possible that: (1) Chamundinae are the sister to the clade consisting of the two others, or (2) Katreinae are the sister to the clade consisting of the two others (as in the trees in Fig. 2), or (3) Katreinae and Chamundinae are sister taxa. In all three scenarios, Chamundinae get the subfamily rank. In (1) & (2), Chamundinae originated prior to the split of their sister into subfamilies, so they should be a subfamily. If the scenario (3) is true, it would be conceivable to unify Katreinae and Chamundinae in a single subfamily, but the monophyly of this putative subfamily would be poorly supported (the same 65% bootstrap in nuclear genome tree). Therefore, because of this weak support, Chamundinae should receive the subfamily rank. Moreover, in the scenario (3), Katreinae and Chamundinae would have diverged from each other prior to divergence of the monocot-feeding clade into subfamilies, so each clade is more consistent with the subfamily rank.

Conclusions

Genomics analysis has been instrumental in revealing the ancient origins of several groups of Hesperiidae that have not been understood before. Moreover, previous studies based on smaller DNA datasets, such as several genes (Sahoo et al. 2016; Sahoo et al. 2017) or mitochondrial genomes (Han et al. 2018) remained inconclusive. Whole genome shotgun reads assembled into protein-coding genes strongly support the uniqueness of the three groups of skippers dealt with in this study and indicate that these groups diverged from other Hesperiidae very early in the evolution of the family. Divergence times of Katreinae subfam. n. and Chamundinae subfam. n. from other Hesperiidae are earlier than the split of the ancestors of subfamilies Heteropterinae and Trapezitinae. Deep divergence times argue for the subfamily status of these groups. Subfamily Barcinae subfam. n. unexpectedly emerges as a possible sister of Trapezitinae,

but is morphologically quite different from them. Whole genome shotgun sequencing was instrumental for this study. Notably, our methods are equally applicable to specimens kept in collections for more than a century. Sequencing of the primary type specimens collected over 120 years ago establishes sex association for the species with extreme sexual dimorphism. As a result, a new synonymy is introduced, and the species known before as *Ortholexis dimidia* should be referred to as *Ortholexis melichroptera*.

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References

Buchfink B, Xie C, Huson DH (2015) Fast and sensitive protein alignment using DIAMOND. Nature Methods 12: 59–60. https://doi.org/10.1038/nmeth.3176

Cao Y, Hao JS, Sun XY, Zheng B, Yang Q (2016) Molecular phylogenetic and dating analysis of pierid butterfly species using complete mitochondrial genomes. Genetics and Molecular Research 15. https://doi.org/10.4238/gmr15049196

Cock MJ, Congdon TC (2011) Observations on the biology of Afrotropical Hesperiidae (Lepidoptera) principally from Kenya. Part 3. Pyrginae: Celaenorrhinini. Zootaxa 3033: 1–67. https://doi.org/10.11646/zootaxa.3033.1.1

Cong Q, Borek D, Otwinowski Z, Grishin NV (2015) Tiger Swallowtail Genome Reveals Mechanisms for Speciation and Caterpillar Chemical Defense. Cell Reports 10: 910–919. https://doi.org/10.1016/j.celrep.2015.01.026

Cong Q, Shen J, Borek D, Robbins RK, Opler PA, Otwinowski Z, Grishin NV (2017) When COI barcodes deceive: complete genomes reveal introgression in hairstreaks. Proceedings of the Royal Society B: Biological Sciences 284(1848): 20161735. https://doi.org/10.1098/rspb.2016.1735

Espeland M, Breinholt J, Willmott KR, Warren AD, Vila R, Toussaint EFA, Maunsell SC, Aduse-Poku K, Talavera G, Eastwood R, Jarzyna MA, Guralnick R, Lohman DJ, Pierce NE, Kawahara AY (2018) A Comprehensive and Dated Phylogenomic Analysis of Butterflies. Current Biology 28: 770–778. https://doi.org/10.1016/j.cub.2018.01.061

Espeland M, Hall JP, DeVries PJ, Lees DC, Cornwall M, Hsu YF, Wu LW, Campbell DL, Talavera G, Vila R, Salzman S, Ruehr S, Lohman DJ, Pierce NE (2015) Ancient Neotropical origin and recent recolonisation: Phylogeny, biogeography and diversification of the Riodinidae (Lepidoptera: Papilionoidea). Molecular Phylogenetics and Evolution 93: 296–306. https://doi.org/10.1016/j.ympev.2015.08.006

Evans WH (1937) A Catalogue of the African Hesperiidae indicating the classification and nomenclature adopted in the British Museum. British Museum (Natural History), London, 212 pp. [30 pls]

Evans WH (1949) A Catalogue of the Hesperiidae from Europe, Asia, and Australia in the British Museum (Natural History). British Museum (Natural History), London, 502 pp. [53 pls] https://doi.org/10.5962/bhl.title.105941

Evans WH (1951) A catalogue of the American Hesperiidae indicating the classification and nomenclature adopted in the British Museum (Natural History). Part I. Introduction and Group A Pyrrhopyginae. British Museum (Natural History), London, 92 pp. [pls 1–9]

Evans WH (1952) A catalogue of the American Hesperiidae indicating the classification and nomenclature adopted in the British Museum (Natural History). Part II. Pyrginae. Section I. British Museum (Natural History). London, 178 pp. [pls 10–25]

Evans WH (1953) A catalogue of the American Hesperiidae indicating the classification and nomenclature adopted in the British Museum (Natural History). Part III. Pyrginae. Section 2. British Museum (Natural History), London, 246 pp. [pls 26–53]

Evans WH (1955) A catalogue of the American Hesperiidae indicating the classification and nomenclature adopted in the British Museum (Natural History). Part IV. Hesperiinae and Megathyminae. British Museum (Natural History), London, 499 pp. [pls 54–88]

Han Y, Huang Z, Tang J, Chiba H, Fan X (2018) The complete mitochondrial genomes of two skipper genera (Lepidoptera: Hesperiidae) and their associated phylogenetic analysis. Scientific Reports 8: 15762. https://doi.org/10.1038/s41598-018-34107-1

Heliconius Genome Consortium (2012) Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. Nature 487: 94–98. https://doi.org/10.1038/nature11041

Kozlov A, Darriba D, Flouri T, Morel B, Stamatakis A (2018) RAxML-NG: A fast, scalable, and user-friendly tool for maximum likelihood phylogenetic inference. bioRxiv. https://doi.org/10.1101/447110

Larsen TB (2005) The Butterflies of West Africa. Apollo Books, 595 pp.

Li W, Cong Q, Shen J, Zhang J, Hallwachs W, Janzen DH, Grishin NV (2019) Genomes of skipper butterflies reveal extensive convergence of wing patterns. Proceedings of the National Academy of Sciences of the United States of America 116: 6232–6237. https://doi.org/10.1073/pnas.1821304116

Mabille P (1891) Description d'Hespérides nouvelles (Deuxième partie). Annales de la Société entomologique de Belgique 35: 106–121.

Ratnasingham S, Hebert PD (2007) bold: The Barcode of Life Data System (http://www.barcodinglife.org). Molecular Ecology Resources 7: 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Sahoo RK, Warren AD, Collins SC, Kodandaramaiah U (2017) Hostplant change and paleoclimatic events explain diversification shifts in skipper butterflies (Family: Hesperiidae). BMC Evolutionary Biology 17: 174. https://doi.org/10.1186/s12862-017-1016-x

Sahoo RK, Warren AD, Wahlberg N, Brower AV, Lukhtanov VA, Kodandaramaiah U (2016) Ten genes and two topologies: an exploration of higher relationships in skipper butterflies (Hesperiidae). PeerJ 4: e2653. https://doi.org/10.7717/peerj.2653

Seraphim N, Kaminski LA, Devries PJ, Penz C, Callaghan C, Wahlberg N, Silva-Brandão KL, Freitas AVl (2018) Molecular phylogeny and higher systematics of the metalmark butterflies (Lepidoptera: Riodinidae). Systematic Entomology 43: 407–425. https://doi.org/10.1111/syen.12282

Shen J, Cong Q, Borek D, Otwinowski Z, Grishin NV (2017) Complete Genome of Achalarus lyciades, The First Representative of the Eudaminae Subfamily of Skippers. Current Genomics 18: 366–374. https://doi.org/10.2174/1389202918666170426113315

Toussaint EFA, Breinholt JW, Earl C, Warren AD, Brower AVZ, Yago M, Dexter KM, Espeland M, Pierce NE, Lohman DJ, Kawahara AY (2018) Anchored phylogenomics illuminates the skipper butterfly tree of life. BMC Evolutionary Biology 18: 101. https://doi.org/10.1186/s12862-018-1216-z

Warren AD, Ogawa JR, Brower AVZ (2008) Phylogenetic relationships of subfamilies and circumscription of tribes in the family Hesperiidae (Lepidoptera: Hesperioidea). Cladistics 24: 642–676. https://doi.org/10.1111/j.1096-0031.2008.00218.x

Warren AD, Ogawa JR, Brower AVZ (2009) Revised classification of the family Hesperiidae (Lepidoptera: Hesperioidea) based on combined molecular and morphological data. Systematic Entomology 34: 467–523. https://doi.org/10.1111/j.1365-3113.2008.00463.x

Zhang J, Cong Q, Shen J, Brockmann E, Grishin NV (2019) Genomes reveal drastic and recurrent phenotypic divergence in firetip skipper butterflies (Hesperiidae: Pyrrhopyginae). Proceedings of the Royal Society B: Biological Sciences 286(1903): 20190609. https://doi.org/10.1098/rspb.2019.0609

Zhang J, Cong Q, Shen J, Fan XL, Wang M, Grishin NV (2017) The complete mitogenome of Euschemon rafflesia (Lepidoptera: Hesperiidae). Mitochondrial DNA Part B: Resources 2: 136–138 https://doi.org/10.1080/23802359.2017.1292478

Supplementary material 1

Table S1. Specimen data and DNA sequences

Authors: Jing Zhang, Qian Cong, Jinhui Shen, Ernst Brockmann, Nick V. Grishin

Data type: table, text and DNA sequences

Explanation note: Table S1 with data for 160 sequenced specimens and diagnostic nucleotide characters of the new subfamilies mapped to the reference genome of *Cecropterus lyciades*.

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RESEARCH ARTICLE

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First record of the dotted grouper *Epinephelus epistictus* (Temminck & Schlegel, 1843) (Perciformes, Serranidae) in Malaysia

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Abstract

Five specimens of *Epinephelus epistictus* (Temminck & Schlegel, 1843) were collected from a major landing site located on the west coast of Peninsula Malaysia during a fish faunal survey on 23 August 2017. The present study extends the distribution range of *E. epistictus* southwards from Andaman Sea to the Strait of Malacca. Species identification was confirmed by colour pattern and DNA barcoding (567 bp of cytochrome C oxidase I) of all *E. epistictus* specimens and nine closely related *Epinephelus* species. The interspecies genetic distance ranged from 0.002–0.245. This study also presents, for the first time for Malaysia, data on length-weight relationships and otolith measurements. It contributes to a better understanding of taxonomy, and phylogenetic and genetic diversity of *E. epistictus*.

Keywords

DNA barcoding, new record, otolith aspect ratio, phylogenetic and genetic diversity, taxonomy

Introduction

Groupers (subfamily Epinephelinae of the family Serranidae) are a commercially valuable taxa globally and in Malaysia, in particular (Craig et al. 2011; Bray 2018; Froese and Pauly 2018). To date, a total of approximately 65 species in 15 genera have been reported in Malaysia (Department of Fisheries Malaysia 2009; Chong et al. 2010; Matsunuma et al. 2011; Ambak et al. 2012; Yusri et al. 2015).

The dotted grouper *Epinephelus epistictus* (Temminck & Schlegel, 1843) is in the Least Concern (LC) category (Leung and Sadovy 2018). It is a demersal species, which inhabits the continental shelf over soft or rocky bottom at a considerable depth (71–800 m); however little else is known about this species (Paxton et al. 1989; Heemstra and Randall 1993; Goldshmidt et al. 1996; Sommer et al. 1996; To and Pollard 2008). This is a widely distributed species in the Indo-West Pacific, occurring off South Africa, in the Red Sea (Randall and Adam 1983), off Iraq (Mustafa et al. 2011), Oman (Krupp et al. 2000), on the west coast of India, Korea, Japan, in the South China Sea (Randall and Lim 2000), off Taiwan, Hong Kong, Indonesia (Limmon et al. 2017; Mous and Pet 2018), Papua New Guinea (Froese and Pauly 2018), and northern Australia (Bray 2018; Dianne 2019). It can be identified by a pale brown body with irregular rows of small dark spots on the back and sides of the body. Some specimens have a broad dark band from the eye to the gill cover, and two narrower bands running diagonally across the cheek (Bray 2018).

Epinephelus epistictus is a medium-sized grouper, with a maximum of 80 cm total length, and may be misidentified as *Epinephelus magniscuttis* Postel, Fourmanoir & Guézé, 1963 or *Epinephelus heniochus* Fowler, 1904 (To and Pollard 2008; Froese and Pauly 2018; Leung and Sadovy 2018). *E. epistictus* and *E. heniochus* differ from *E. magniscuttis* by having fewer and smaller dark spots on head and body and dark spots arranged in three longitudinal rows on body of juveniles (Heemstra and Randall 1993). However, *E. heniochus* and *E. epistictus* share the following characters: distinctly enlarged serrae at the corner of the preopercle, 14 or 15 dorsal-fin rays, interspinous dorsal-fin membranes distinctly incised, midlateral part of lower jaw with two rows of teeth, similar morphometric features and colour pattern (Heemstra and Randall 1993). These similarities might have contributed to *E. epistictus* misidentifications in the past. Though a number of barcoding (CO1) studies of groupers from Malaysia have been conducted (Chu et al. 2011; Nurnadia et al. 2016; Rahim et al. 2016), the species occurrence remained undetected. A recent ichthyofaunal survey found specimens of *E. epistictus* in a commonly surveyed major landing site on the west coast of Peninsula Malaysia and the present study reports on size and genetic data that confirm its identification as well as some aspects of its biology and phylogeny.

Materials and methods

Five specimens of *E. epistictus* were collected from a major fish landing site in Hutan Melintang, northeastern Peninsula Malaysia during a fish faunal survey on 23 August 2017 (Fig. 1). These specimens were caught using trawl nets operating in the Straits of

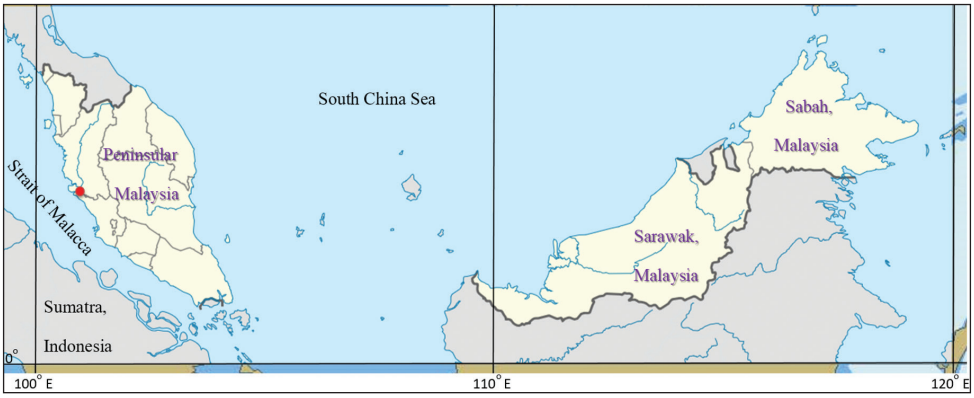

Figure 1. The map of Hutan Melintang (red dot) showing the location of the landing site.

Malacca. The tissue samples were preserved in 95% ethanol solution and deposited in the Institute of Ocean and Earth Sciences (IOES), University of Malaya (UM), Kuala Lumpur. Preliminary species identification was made based on the morphology of the whole fish specimens using the species identification keys and diagnostic features reported in Heemstra and Randall (1993). Morphological measurements taken included total length (TL, mm), standard length (SL, mm), and total weight (Wt, g). Otoliths were extracted and various measurements were made, namely the sagitta otolith length (O_L, mm), the longest distance between the most anterior and posterior points, otolith width (O_w, mm), the longest distance between the ventral and dorsal edges, and the weight of the sagittal (O_{wt}, g). Otolith aspect ratio (O_{AS}) was calculated by dividing O_L by O_W for the left otolith (Table 1).

Molecular DNA sequencing was used to confirm the species identification. Total DNA extractions were performed on the collected tissue samples using the G-spin™ Total DNA Extraction Kit (iNtRON Biotechnology, Inc., Korea) following the manufacturer's instructions. The primers, including combinations of the forward (FishF1 or FishF2) and the reverse (FishR1 or FishR2) primer pairs, followed Ward et al. (2005). A 20 µl PCR reaction mixture was prepared in a 1.5 ml tube containing 13.25 µl double distilled water (ddH_2O), 2 µl 10x *i*-Taq plus PCR buffer, 1 µl of deoxynucleotide triphosphate (dNTP), 1 µl of each primer used, 0.25 µl *i*-Taq plus DNA polymerase and 1.5 µl of total genomic DNA. The Eppendorf thermal cycler was used to run the following thermal cycle profile: initial denaturation at 94 °C for 5 minutes; 35–40 cycles of denaturation at 94 °C for 30s, annealing at 44–50 °C for 30s, extension at 72 °C for 1 minute; followed by a final extension at 72 °C for 5 minutes. The PCR products were stained with loading dye, and loaded on to the wells of 1.0% agarose gel before conducting gel electrophoresis. Successfully amplified PCR products were sent to 1st BASE Laboratories (Malaysia) with the same primers used for PCR reactions for sequencing.

For systematic relationships with congeners, the raw sequences were first assembled and edited via ChromasPro ver 1.42 (Technelysium Pty Ltd), subsequently aligned using Clustal X v. 2.0.8 (Larkin et al. 2007) and then manually adjusted with Bioedit v. 7.0.9.0 (Hall 1999). The COI gene of the five *E. epistictus* specimens, and nine

Table 1. Morphometric measurements for *Epinephelus epistictus*.

Measurements	DOS339	DOS340	DOS341	DOS342	DOS370
TL (mm)	180	164	158	150	145
SL (mm)	154	135	130	122	127
Wt (g)	70.75	59.25	49.30	41.55	36.44
Otolith morphometrics					
Left					
O_L (mm)	8.17	8.57	7.43	7.59	8.12
O_W (mm)	3.98	3.84	3.78	3.81	3.69
O_{Wt} (g)	0.0284	0.0292	0.0238	0.0241	0.0228
Right					
O_L (mm)	7.09+	8.42	7.36	7.57	8.07
O_W (mm)	3.87+	3.85	3.64	3.78	3.61
O_{Wt} (g)	0.0272+	0.0291	0.0235	0.0238	0.0225

Note: + broken; total lenght (TL), standard length (SL), total weight (Wt); otolith length (O_L), Otolith width (O_W), weight of the otolith (O_{Wt}).

closely related species (*Epinephelus bleekeri* (Vaillant, 1878), *Epinephelus fuscoguttatus* (Forsskål, 1775), *Epinephelus latifasciatus* (Temminck & Schlegel, 1843), *Epinephelus quoyanus* (Valenciennes, 1830), *Epinephelus areolatus* (Forsskål, 1775), *Epinephelus coioides* (Hamilton, 1822), *Epinephelus erythrurus* (Valenciennes, 1828), *E. heniochus*, and *Epinephelus sexfasciatus* (Valenciennes, 1828)) sampled from Malaysian waters were also sequenced. Other sequences available in GenBank of *E. epistictus*, *E. bleekeri*, *E. fuscoguttatus*, *E. heniochus*, *E. latifasciatus* and *E. quoyanus* were also added to the analysis using the slender grouper, *Anyperodon leucogrammicus* (Valenciennes, 1828) (GQ131336) as outgroup (Table 2). The maximum likelihood (ML) tree was reconstructed based on the best evolutionary model, namely the General Time Reversible model (GTR) with the Gamma distributed (G) distance and invariable sites (I), which was selected using the lowest bias-corrected Akaike Information Criterion (AICc) value in model test, with 1000 replications for bootstrap analysis. Both tree construction and model test were completed in MEGA (Molecular Evolution Genetic Analysis) version 7.0 (Kumar et al. 2016). Similarly, a neighbor joining (NJ) tree was constructed based on the pairwise genetic distance using the Kimura 2-parameter (K2P) model with 1000 bootstrap resampling. Genetic distances of the sequences were calculated with the K2P model (Kimura 1980) using MEGA 7.0.

Results

The colour pattern of the Malaysian specimens identified as *E. epistictus* was similar to the species' description in Bray (2018) (Fig. 2). The five specimens were 122–154 mm SL (145–180 mm TL) and 36.44–70.75 g total weight. Estimates of the length-weight parameters were -2.0542 for log *a* (95% CI = -2.1378, -2.0353). The length-weight relationship based on total length and total weight showed positive allometric growth (*b*

Table 2. Accession numbers of sequences used in the analysis and voucher catalogue numbers.

Species	Location	Accession number	Reference
Epinephelus areolatus	Malaysia (PK 011)	JN208570	This study (Rizman-Idid et al.)
	Malaysia (PK 017)	JN208571	This study (Rizman-Idid et al.)
E. bleekeri	USA	JN021297	Shen and Ishida (2016)
	Philippines	KU668653	Cabana (2017)
	Malaysia (DOS 361)	MK118153	This study
E. coioides	Malaysia (LK 021)	JN208587	This study (Rizman-Idid et al.)
	Malaysia (LK 033)	JN208589	This study (Rizman-Idid et al.)
E. epistictus	Saudi Arabia	KU499627	Rabaoui et al. (2016)
	India	KM226255	Vineesh et al. (2014)
	Malaysia (DOS 339)	KM118148	This study
	Malaysia (DOS 340)	KM118149	This study
	Malaysia (DOS 341)	KM118150	This study
	Malaysia (DOS 342)	MK118151	This study
	Malaysia (DOS 370)	MK118152	This study
E. erythrurus	Malaysia (LK 039)	JN208608	This study (Rizman-Idid et al.)
	Malaysia (LK 073)	JN208609	This study (Rizman-Idid et al.)
E. fuscoguttatus	Malaysia (PG 016)	JN208615	This study (Rizman-Idid et al.)
	Andaman, India	JX674997	Sachithananda et al. (2012)
E. heniochus	China	MF185518	Qu et al. (2018)
	Malaysia	KY371468	Hou et al. (2017)
	Malaysia (DOS 343)	MK118155	This study
	Malaysia (DOS 344)	MK118156	This study
E. latifasciatus	China	KC480177	Lai et al. (2013)
	China	MF185521	Qu et al. (2018)
	Malaysia (DOS 369)	MK118154	This study
E. quoyanus	Malaysia (LK 058)	JN208619	This study (Rizman-Idid et al.)
	China	MF185570	Qu et al. (2018)
E. sexfasciatus	Malaysia (LK 035)	JN208565	This study (Rizman-Idid et al.)
	Malaysia (LK 053)	JN208566	This study (Rizman-Idid et al.)
Anyperodon leucogrammicus	China	GQ131336	Lin et al. (2016)

Figure 2. *Epinephelus epistictus* (DOS339), 180 mm TL, 70.75 g.

= 3.1245) and the coefficient of determination (r^2) of the regression was 0.9725. Otolith measurements were presented using mean (standard deviation): O_L 7.98 (0.46) mm; O_w 3.82 (0.11) mm; and $O_W t$ 0.0257 (0.0029) g (Table 1). The otolith aspect ratio (O_{AS}) averaged 2.09 (0.12) for the left otolith (Fig. 3).

The 19 grouper specimens were classified as ten species based on external morphology, which was consistent with the species names in both the GenBank BLAST and BOLD-IDS. DNA sequences from the above said ten species were submitted to GenBank (PubMed) and their accession number were given in Table 2. There was a total of 181/567 bp of variable sites and 174/567 bp of parsimony-informative sites after aligning the sequences. The average base composition obtained was 25.0% A bases, 27.7% of C bases, 16.4% of G bases and 30.9% of T bases.

Both ML and NJ trees showed two major groups: one group containing of *E. epistictus*, *E. heniochus* and the other comprising other *Epinephelus* species. All species are monophyletic with bootstrap values range of 63–100%. All five *E. epistictus* samples were clustered together with the *E. epistictus* reference sequences from GenBank (KM226255, KU499627) with high bootstrap values (63–99% in ML tree, 62–100% in NJ tree) (Fig. 4).

Genetic distances between grouper species based on the 567 bp COI consensus sequences and the respective reference sequences with the K2P model were given in Table 3. The intraspecific nucleotide distances for *E. epistictus* were low, ranging from 0.000–0.005. The interspecific differences between other grouper species of the genus *Epinephelus* ranged from 0.002–0.245. The largest difference was found between *E. heniochus* and *E. sexfasciatus* (0.245).

Discussion

Our study confirmed a new record of *E. epistictus* in the waters of Malaysia (in the Strait of Malacca). Although the strait is one of the busiest shipping channels in the world, our discovery of this commercially important grouper species suggests that much work remains to be done with documenting the local fish diversity. While the strait itself is considerably shallow in the south (close to Singapore) with average minimum depth of 25 m, the northern part of the Strait connecting to the Andaman Sea is up to 200 m deep; this depth profile is consistent with the depth range at which the species reportedly occurs. An undergraduate thesis reported the use of this species at an aquaculture farm in the Sabahan Borneo (Chen 2015), however, examination of the photos included in the thesis revealed that the species was erroneously identified as *E. epistictus*. A morphometric comparison and characters distinguishing *E. epistictus* from *E. bleekeri*, *E. heniochus* and *E. latifasciatus* were compared with existing literature (Sommer et al. 1996; Krupp et al. 2000; Froese and Pauly 2018) in Appendix 1.

Our study demonstrated evidence to support fine-scale monophyly for the subset of *Epinephelus* species examined in this region based on COI sequence data. Despite

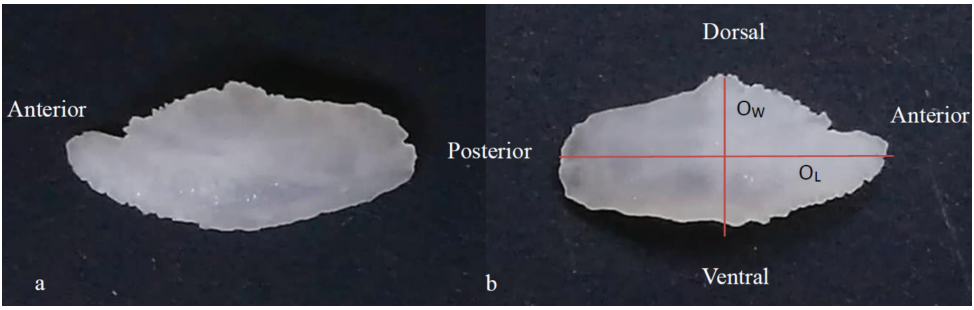

Figure 3. Sagittae of *Epinephelus epistictus* DOS370 **a** right otolith **b** left otolith, the positioning of otolith morphometrics measured (in mm), O_L (otolith length) the longest distance between the most anterior and posterior points; O_w (Otolith width) the longest distance between the ventral and dorsal edges.

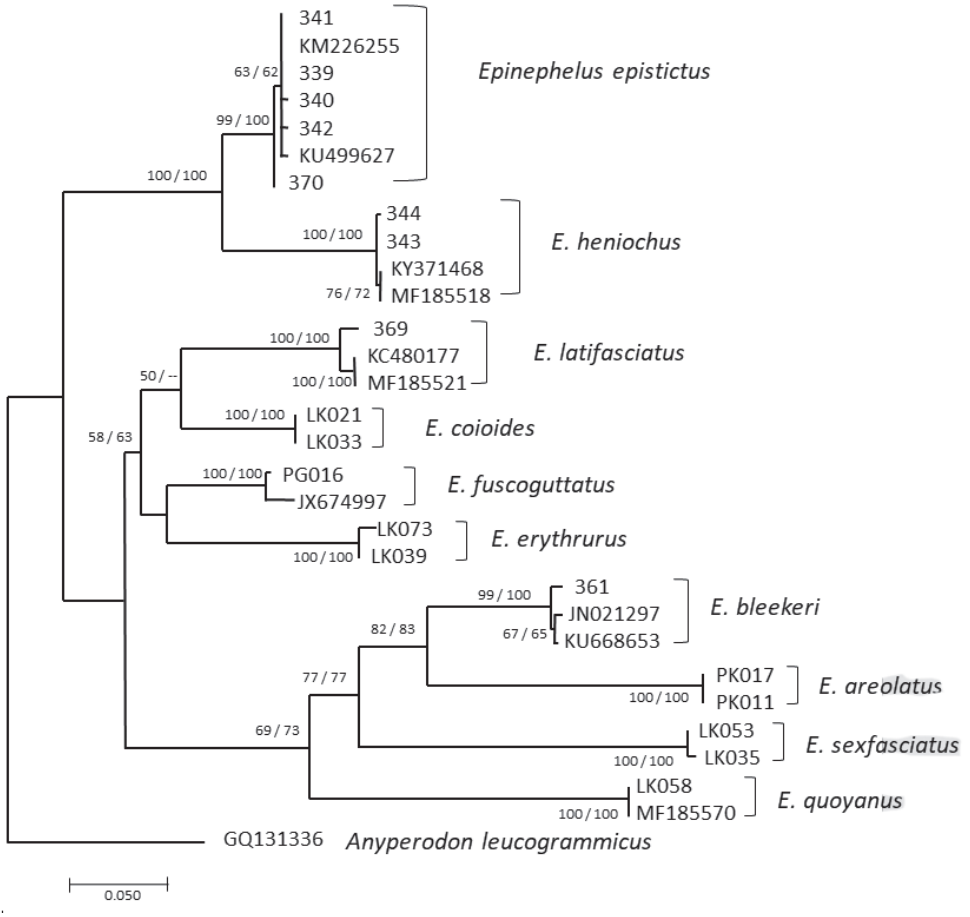

Figure 4. Phylogenetic inferred based on COI gene sequences (567 bp) for the ten *Epinephelus* species. The bootstrap values higher than 50% are shown at the branching points, methods ML/ NJ.

Table 3. Pairwise comparisons of genetic distances (*d*) within all the grouper samples.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1																													
2	0.002																												
3	0.000	0.002																											
4	0.002	0.004	0.002																										
5	0.004	0.005	0.004	0.005																									
6	0.002	0.004	0.002	0.004	0.005																								
7	0.000	0.002	0.000	0.002	0.004	0.002																							
8	0.082	0.084	0.082	0.084	0.082	0.082	0.082																						
9	0.084	0.087	0.084	0.087	0.084	0.084	0.084	0.002																					
10	0.084	0.087	0.084	0.087	0.084	0.084	0.084	0.002	0.004																				
11	0.084	0.087	0.084	0.087	0.084	0.084	0.084	0.002	0.004	0.000																			
12	0.174	0.174	0.174	0.177	0.168	0.174	0.174	0.189	0.186	0.192	0.192																		
13	0.180	0.180	0.180	0.183	0.174	0.180	0.180	0.186	0.189	0.189	0.189	0.016																	
14	0.180	0.180	0.180	0.183	0.174	0.180	0.180	0.186	0.189	0.189	0.189	0.016	0.000																
15	0.204	0.200	0.204	0.207	0.197	0.204	0.204	0.194	0.198	0.198	0.198	0.190	0.187	0.187															
16	0.204	0.200	0.204	0.207	0.197	0.204	0.204	0.198	0.201	0.201	0.201	0.181	0.178	0.178	0.009														
17	0.204	0.200	0.204	0.207	0.197	0.204	0.204	0.198	0.201	0.201	0.201	0.184	0.181	0.181	0.011	0.005													
18	0.187	0.190	0.187	0.187	0.187	0.190	0.187	0.209	0.212	0.212	0.212	0.200	0.200	0.200	0.130	0.132	0.130												
19	0.187	0.190	0.187	0.187	0.187	0.190	0.187	0.209	0.212	0.212	0.212	0.200	0.200	0.200	0.130	0.132	0.130	0.000											
20	0.138	0.138	0.138	0.136	0.138	0.138	0.138	0.158	0.155	0.161	0.161	0.117	0.109	0.109	0.180	0.174	0.174	0.200	0.200										
21	0.138	0.138	0.138	0.136	0.138	0.138	0.138	0.158	0.155	0.161	0.161	0.117	0.109	0.109	0.180	0.174	0.174	0.200	0.200	0.000									
22	0.172	0.172	0.172	0.175	0.178	0.172	0.172	0.188	0.185	0.191	0.191	0.135	0.138	0.138	0.175	0.166	0.166	0.180	0.180	0.142	0.142								
23	0.169	0.169	0.169	0.172	0.169	0.169	0.169	0.185	0.181	0.188	0.188	0.133	0.135	0.135	0.169	0.160	0.160	0.177	0.177	0.139	0.139	0.009							
24	0.144	0.144	0.144	0.146	0.138	0.144	0.144	0.167	0.170	0.170	0.170	0.127	0.125	0.125	0.145	0.142	0.142	0.198	0.198	0.117	0.117	0.123	0.115						
25	0.143	0.143	0.143	0.146	0.138	0.143	0.143	0.172	0.175	0.175	0.175	0.134	0.131	0.131	0.161	0.158	0.158	0.197	0.197	0.124	0.124	0.122	0.119	0.014					
26	0.204	0.207	0.204	0.207	0.197	0.200	0.204	0.242	0.238	0.245	0.245	0.192	0.199	0.199	0.167	0.183	0.179	0.174	0.174	0.190	0.190	0.188	0.175	0.178	0.192				
27	0.200	0.204	0.200	0.204	0.194	0.197	0.200	0.238	0.234	0.242	0.242	0.196	0.196	0.196	0.164	0.179	0.176	0.171	0.171	0.187	0.187	0.184	0.172	0.175	0.189	0.002			
28	0.185	0.189	0.185	0.182	0.185	0.185	0.185	0.188	0.191	0.191	0.191	0.242	0.232	0.232	0.173	0.167	0.167	0.199	0.199	0.194	0.194	0.190	0.190	0.183	0.194	0.203	0.200		
29	0.185	0.189	0.185	0.182	0.185	0.185	0.185	0.188	0.191	0.191	0.191	0.242	0.232	0.232	0.173	0.167	0.167	0.199	0.199	0.194	0.194	0.190	0.190	0.183	0.194	0.203	0.200	0.000	
30	0.156	0.156	0.156	0.153	0.156	0.156	0.156	0.182	0.179	0.185	0.185	0.160	0.181	0.181	0.188	0.188	0.188	0.213	0.213	0.155	0.155	0.200	0.190	0.140	0.156	0.198	0.194	0.188	0.188

Genetic distances were calculated to Kimura 2-parameter (K2P) model. 1= DOS339, 2= DOS340, 3= DOS341, 4= DOS342, 5= DOS370, 6= KU499627, 7= KM226255, 8= DOS343, 9= DOS344, 10= MF185518, 11= KY371468, 12= DOS369, 13= MF185521, 14= KC480177, 15= DOS361, 16= KU668653, 17= JN021297, 18= PK011, 19=PK017, 20=LK033, 21=LK021, 22=LK073, 23=LK039, 24=PG016, 25= JX674997, 26=LK035, 27=LK053, 28=LK058, 29=MF185570, 30=GQ131336.

comparison of specimens from various distant locations, namely India, Saudi Arabia and Malaysia, the intraspecific nucleotide distances of *E. epistictus* was relatively low (0.000–0.005). The interspecific differences between ten grouper species examined ranged from 0.002 to 0.245, and 0.213 from the closest outgroup *Anyperodon leucogrammicus*. This species was included by some (Craig and Hastings 2007, Ma and Craig 2018) in the genus *Epinephelus* but others have kept it in the monotypic genus (Rhodes 2018) until further phylogenetic study is done. A recent study placed groupers in the family Epinephelidae sensu Smith and Craig (2007) and supports the idea of assigning both *E. epistictus* and *E. heniochus* to *Mycteroperca* (Ma and Craig 2018). At the species level, the status of *E. epistictus* has not been questioned.

The findings of this study contribute to better understanding on the taxonomy, biology, phylogenetic and genetic diversity of *E. epistictus*, which is important for sustainable management of the species in Malaysia.

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References

Ambak MA, Mansor MI, Zakaria MZ, Mazlan AG (2012) Fishes of Malaysia, 2nd edition, Penerbit UMT, Universiti Malaysia Terengganu, Malaysia, 301 pp.

Bray DJ (2018) *Epinephelus epistictus*. Fishes of Australia. http://fishesofaustralia.net.au/home/species/4668 [accessed 16 Mar 2018]

Chen SM (2015) Accumulation of selected heavy metals in *Epinephelus epistictus* (grouper) and water from Terayung aquaculture farm in Tuaran, Sabah. MsD Thesis, University of Sabah,94 pp.

Chong VC, Lee PK, Lau CM (2010) Diversity, extinction risk and conservation of Malaysian fishes. Journal of Fish Biology 76(9): 2009–2066. https://doi.org/10.1111/j.1095-8649.2010.02685.x

Chu C, Rizman-Idid M, Chong VC (2011) The relationship of groupers (subfamily: Epinephelinae) from Peninsular Malaysia based on COI. IOC/WESTPAC 8th International Scientific Symposium, 28–31 March 2011, Busan, Republic of Korea. [Poster abstract]

Cites U (2017) The Checklist of CITES Species Website. Appendices I, II and III valid from 04 April 2017. CITES Secretariat, Geneva, Switzerland. Compiled by UNEP-WCMC, Cambridge. https://www.cites.org/eng/app/appendices.php [01/08/2017]

Craig MT, Hastings PA (2007) A molecular phylogeny of the groupers of the subfamily Epinephelinae (Serranidae) with a revised classification of the Epinephelini. Ichthyological Research 54(1): 1–17. https://doi.org/10.1007/s10228-006-0367-x

Craig MT, Sadovy YJ, Heemstra PC (2011) Groupers of the world: a field and market guide. NISC, Grahamstown.

Department of Fisheries Malaysia (2009) Valid local name of Malaysian marine fishes. Department of Fisheries Malaysia. Ministry of Agriculture and Agro-based Industry, 180 pp.

Dianne JB (2019) *Epinephelus epistictus*. Fishes of Australia. https://fishesofaustralia.net.au/home/species/4668 [03 Jan 2019]

Froese R (2006) Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. Journal of Applied Ichthyology 22: 241–253. https://doi.org/10.1111/j.1439-0426.2006.00805.x

Froese R, Pauly D (2018) FishBase. https:// www.fishbase.org [Jun 2018]

Goldshmidt O, Galil B, Golani D, Lazar B, Erez J, Baranes A (1996) Food selection and habitat preferences in deep-sea fishes of the northern Red Sea. In: Uiblein F, Ott J, Stachowtisch M (Eds) Deep-sea and extreme shallow-water habitat: affinities and adaptations. Biosystematics and Ecology Series 11: 271–298.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Heemstra PC, Randall JE (1993) FAO Species Catalogue. Vol. 16. Groupers of the world (family Serranidae, subfamily Epinephelinae). An annotated and illustrated catalogue of the grouper, rockcod, hind, coral grouper and lyretail species known to date. FAO Fisheries Synopsis 125(16): 1–382.

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120. https://doi.org/10.1007/BF01731581

Krupp F, Almarri M, Zajonz U, Carpenter K, Almatar S, Zetzsche H (2000) Twelve new records of fishes from the Gulf. Fauna of Arabia 18: 323–335.

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution 33(7): 1870–1874. https://doi.org/10.1093/molbev/msw054

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947–2948. https://doi.org/10.1093/bioinformatics/btm404

Leung Lap Boon B, Sadovy Y (2018) *Epinephelus epistictus*. The IUCN Red List of Threatened Species 2018: e.T132824A46631320. http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T132824A46631320.en [03 January 2019]

Limmon G, Ruoly F, Khouw AS, Manuputty GD, Pattikawa JA (2017) The diversity of grouper (Epinephelinae) in Ambon Island, Maluku, Indonesia. Kagoshima University Research Center for the Pacific Islands Occasional Papers 58: 23–29.

Ma KY, Craig MT (2018) An inconvenient monophyly: an update on the taxonomy of the groupers (Epinephelidae). Copeia 106(3): 443–456. https://doi.org/10.1643/CI-18-055

Matsunuma M, Motomura H, Matsuura K, Shazili NAM, Ambak MA (2011) Fishes of Terengganu: East Coast of Malay Peninsula, Malaysia. National Museum of Nature and Science, Universiti Malaysia Terengganu and Kagoshima Universiti Museum, Malaysia, 251 pp.

Mous PJ, Pet JS (2018) Length-Based Assessment of data-poor multi-species deep grouper fisheries in fisheries management areas (WPP) 573, 712, 713, 714, 715 & 718 in Indonesia. TNC-IFCP Technical Paper. The Nature Conservancy Indonesia Fisheries Conservation Program, Bali.

Mustafa AA, Laith AJ, Abbas JA, Mustafa F (2011) Dotted Grouper, *Epinephelus epistictus* (Temminck & Schlegel, 1842) (Osteichthyes: Serranidae), recorded from the marine waters of Iraq, Zoology in the Middle East 54(1):136–138. https://doi.org/10.1080/09397140.2011.10648887

Nurnadia MAA, Yuzine E, Aziz A (2016) DNA Barcoding and phylogenetic analysis of Malaysian groupers (Subfamily: Epinephelinae) using mitochondrial cytochrome c oxidase I (COI) Gene. Journal of Environmental Biology 37(4): 725–733.

Paxton JR, Hoese DF, Allen GR, Hanley JE (1989) Pisces. Petromyzontidae to Carangidae. Zoological catalogue of Australia. Vol. 7. Canberra: Australian Government Publishing Service, 665 pp.

Peng ZQ, Huang Y, Chen J, Lai TH, Wu LZ (2014) Complete mitochondrial genome of dotted grouper *Epinephelus epistictus* (Serranidae: Epinephelinae), Mitochondrial DNA 25(3): 190–191. https://doi.org/10.3109/19401736.2013.792070

Rahim N, Syakina N, Yuzine E, Aziz A (2016) Molecular phylogeny of some Malaysian groupers (subfamily: Epinephelinae, family: serranidae) inferred from mitochondrial and nuclear gene sequences. Journal of Sustainability Science and Management 11(2): 1–10.

Randall JE, Adam BT (1983) A review of the groupers (Pisces: Serranidae: Epinephelinae) of the Red Sea, with description of a new species *Cephalopholis*. Bulletin of Marine Science 33(2): 373–426.

Randall JE, Lim Kelvin KP (2000) A checklist of the fishes of the South China Sea. The Raffles Bulletin of Zoology Supplement 8: 569–667.

Rhodes K (2018) *Anyperodon leucogrammicus*. The IUCN Red List of Threatened Species 2018: e.T132787A46630137. http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T132787A46630137.en [08 Jan 2019]

Smith WL, Craig MT (2007) Casting the percomorph net widely: the importance of broad taxonomic sampling in the search for the placement of serranid and percid fishes. Copeia 35–55. https://doi.org/10.1643/0045-8511(2007)7[35:CTPNWT]2.0.CO;2

Sommer C, Schneider W, Poutiers JM (1996) FAO species identification field guide for fishery purposes. The living marine resources of Somalia. FAO, Rome, 376 pp.

Temminck CJ, Schlegel H (1843) Pisces – Fauna Japonica, sive descriptio animalium quae in itinere per Japoniam suscepto annis 1823-30 collegit, notis observationibus et adumbrationibus illustravit P. F. de Siebold. Lugduni Batavorum [Leiden] (A. Arnz et soc.), Part 1: 1–20.

To AWL, Pollard D (2008) *Epinephelus epistictus*. The IUCN Red List of Threatened Species 2008: e.T132824A3461503. Downloaded on 27 February 2018. http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T132824A3461503.en

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PD (2005) DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B 360: 1847–1857. https://doi.org/10.1098/rstb.2005.1716

Yusri A, Jaafar H, Abdul, MAM (2015) Ikan Laut Malaysia: Glosari Nama Sahih Spesis Ikan (2nd edn). Dewan Bahasa dan Pustaka, Kuala Lumpur, 290 pp.

Appendix 1

Morphometric comparison and characteristics distinguishing *Epinephelus epistictus* from *E. bleekeri*, *E. heniochus* and *E. latifasciatus* (References: Sommer et al. 1996, Krupp et al. 2000, Cites 2017, Froese and Pauly 2018).

	***E. epistictus* (Temminck & Schlegel, 1843)**	***E. bleekeri* (Vaillant, 1878)**	***E. heniochus* Fowler, 1904**	***E. latifasciatus* (Temminck & Schlegel, 1843)**
Commen name	Dotted grouper	Duskytail grouper	Bridled grouper	Striped grouper
Type locality	Japan	Indonesia	Indonesia	Japan
Holotype	RMNH D88 (stuffed)	MNHN 0000-7401	ANSP 27558	Lectotype: RMNH D21 (stuffed)
Dorsal fin	XI	XI	XI	XI
Dorsal rays	14–15	16–18	14–15	12–14
Anal spins	III	III	III	III
Anal rays	8	8-9.	8	8
Pectoral rays	18	17–19	16–18	17–19
Gill rakers	8+16	25–28 (9–11+16–18)	7–9+14–16	8–11+15–18
Lateral line scales	60	49–53	54–60	56–65
Lateral scale series	108	99–104	80–100	91–106
in SL				
Head length	2.4	2.4–2.7	2.2–2.4	2.3–2.6
Body depth	3.4	3.0–3.5	2.7–3.2	2.9–3.4
Colour	Light brownish on flanks with very small dark brown spots on upper sides of body; dorsal fins, pectoral fins, anal fin, caudal-fin membranes yellow	Head and body brownish, reddish brown or purplish grey, covered (except ventrally) with numerous reddish orange, gold, or yellow spots; dorsal fin and upper third of caudal fin with spots like those on body; lower two-thirds of caudal fin dusky	Head and body pale brown dorsally, shading to whitish or pale pink ventrally; faint dark brown stripe from eye to end of operculum; pectoral fins hyaline greyish yellow; margin of interspinous dorsal-fin membranes yellow	2 black-edged white longitudinal bands, upper band extending from above the eye to the anterior dorsal-fin rays, lower band from below the eye to the lower caudal-fin rays; black spots and streaks on dorsal and caudal fins; head and body of large adults, uniformly grey
Geographical distribution	Indo-West Pacific species	Indo-West Pacific species	West Pacific species	Indo-West Pacific species
Habitat	Rocky and trawlable bottoms	shallow rocky banks	mud or silty-sand bottom	Rocky, silty-sand and mud bottom
Depth	71–800 m	30–104 m	40–235 m	20–230 m
IUCN category	Least Concern (LC)	Near threatened (NT)	Least Concern (LC)	Least Concern (LC)

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Scolopsis lacrima, a new species of monocle bream (Teleostei, Perciformes, Nemipteridae) from New Caledonia

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Abstract

The new monocle bream *Scolopsis lacrima* **sp. nov.** is described from a single specimen (213.6 mm standard length) collected from Grande-Terre Island, New Caledonia. The new species closely resembles *S. meridiana*, both species having the upper part of the pectoral-fin base with reddish blotch when fresh, two bands across the top of the snout, a dorsal scaled area on the head reaching anteriorly to between the anterior margin of the eye and anterior nostril, a similar number of lateral-line scales, and absence of a small antrorse spine below the eye. However, *S. lacrima* **sp. nov.** is distinguished from *S. meridiana* by having diagonal lines on the body absent (vs. 18–20 diagonal lines in the latter), a dark longitudinal band below the lateral line (vs. longitudinal lines absent), the caudal fin central area not patterned (vs. with several dark horizontal lines), a narrower body and shallower caudal peduncle.

Keywords

Grande-Terre Island, morphology, *Scolopsis meridiana*, taxonomy

Introduction

The monocle bream genus *Scolopsis* Cuvier, 1814 is widespread throughout shallow Indo-West Pacific tropical and subtropical waters, some species being marketed in Southeast Asia (Russell 1990, 2001). The genus was reviewed by Russell (1990), who recognised 16 valid species; it was characterised by a distinct posteriorly-directed suborbital spine, the suborbital area without scales, the posterior margin of the preopercle coarsely denticulate or serrate, and jaws without canine teeth. Subsequently, *Scolopsis igcarensis* Mishra, Biswas, Russell, Satpathy & Selvanayagam, 2013 and *Scolopsis meridiana* Nakamura, Russell, Moore & Motomura, 2018 were described, and *Scolopsis torquata* (Cuvier, 1830) recognised as a valid species by Psomadakis et al. (2015). Accordingly, 19 valid species are currently recognised in the genus (Russell 1990, Mishra et al. 2013, Psomadakis et al. 2015, Nakamura et al. 2018).

During a taxonomic study of *Scolopsis*, a single specimen from New Caledonia, having a distinctively elongate body and unique colouration, was examined. It is described herein as a new species of *Scolopsis*.

Materials and methods

Counts and proportional measurements followed Nakamura et al. (2018). All measurements were made with calipers to the nearest 0.1 mm. Standard length is abbreviated as SL. Institutional codes follow Sabaj (2016), with the following addition: Département d'Archéologie du Service des Musées de Nouméa, New Caledonia (**DASMN**). Examined specimens of *S. meridiana* and *Scolopsis taenioptera* (Cuvier, 1830) are listed in Nakamura et al. (2018).

***Scolopsis lacrima* sp. nov.**
http://zoobank.org/5AF65765-E484-41A9-ABB2-E88A93034AE5
Figures 1–3, 5a, 6, 7
New English name: Teary Monocle Bream

Scolopsis taeniopterus (non Cuvier): Béarez 2003: 62, fig. 1 (Nouméa, Grande-Terre Island, New Caledonia).
Scolopsis taenioptera (non Cuvier): Fricke et al. 2011: 401 (New Caledonia).

Holotype. MNHN 2002–2930, 213.6 mm SL, Nouméa, Grande-Terre Island, New Caledonia, 1 Aug 2002, purchased at market by P. Béarez.

Diagnosis. A species of *Scolopsis* with the following combination of characters: pectoral-fin rays 17; lateral-line scales 47; no antrorse spine below eye; dorsal scaled area on head reaching anteriorly to between anterior margin of eye and anterior nostril; bony opercular ridge and lower limb of preopercle without scales; 3rd anal-fin spine

Figure 1. Holotype of *Scolopsis lacrima* sp. nov., MNHN 2002-2930, 213.6 mm SL, Grande-Terre Island, New Caledonia **a** fresh condition (photo by P. Béarez) **b** preserved condition.

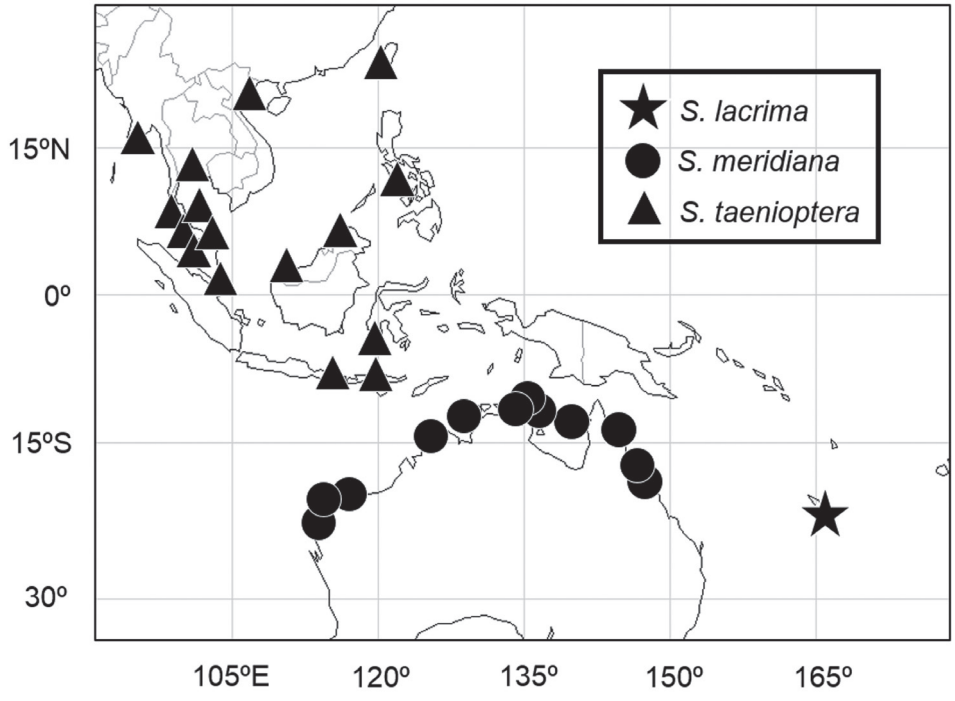

Figure 2. Distribution records of *Scolopsis lacrima* sp. nov. (star), *S. meridiana* (circles), and *S. taenioptera* (triangles), based on specimens examined in Nakamura et al. (2018) and this study.

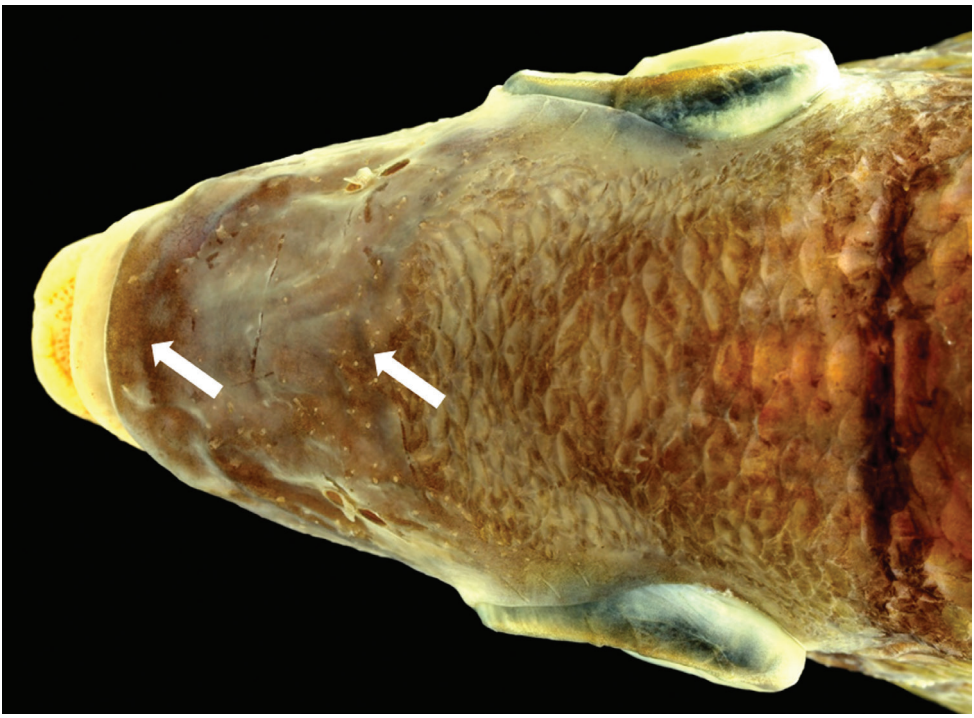

Figure 3. Dorsal view of snout of *Scolopsis lacrima* sp. nov. (MNHN 2002-2930, holotype, 213.6 mm SL).

longer than 2nd anal-fin spine; narrow body, its depth at dorsal, pelvic, and anal fin origins 29.2, 29.5 and 26.6% of SL, respectively; caudal-peduncle depth 10.4% of SL; head length 29.9% of SL; upper part of pectoral-fin base with reddish blotch when fresh; two dark bands across dorsum of snout; body below lateral line with a dark longitudinal band, without diagonal lines; no blotches or lines on central area of caudal fin.

Description. Dorsal-fin rays X, 9; anal-fin rays III, 7; pectoral-fin rays (left / right) 17 / 17; pored lateral-line scales 47; pelvic-fin rays I, 5; scale rows above lateral line 5; scale rows below lateral line 10; gill rakers (upper / lower) 5 / 8; preopercle scale rows (behind eye) 3; preopercle scale rows (below eye) 4. The following morphometrics are expressed as percentages of SL: body depth at dorsal-fin origin 29.2; body depth at pelvic-fin origin 29.5; body depth at anal-fin origin 26.6; body depth at posterior margin of orbit 23.7; body depth at anterior margin of orbit 17.5; pre-dorsal-fin length 32.7; pre-pelvic-fin length 37.9; pectoral-pelvic length 15.4; pre-anus length 61.4; head length 29.9; snout length 11.4; posterior nostril (horizontal) 0.8; posterior nostril (vertical) 0.8; upper-jaw length 10.7; orbit diameter 7.8; interorbital width 10.0; suborbital depth 5.7; caudal-peduncle length 22.8; caudal-peduncle depth 10.4; dorsal-fin base length 54.1; 1st dorsal-fin spine length 6.2; 2nd dorsal-fin spine length 8.5; 3rd dorsal-fin spine length 10.4; 4th dorsal-fin spine length 10.8; 5th dorsal-fin spine length 11.0; 6th dorsal-fin spine length 11.0; 7th dorsal-fin spine length 10.9; 8th dorsal-fin

Figure 4. *Scolopsis meridiana* **a** CSIRO H 4029–01, holotype, 194.8 mm SL, Western Australia, Australia **b** AMS I.21957-013, paratype, 94.9 mm SL, Northern Territory, Australia.

spine length 11.0; 9th dorsal-fin spine length 10.7; 10th dorsal-fin spine length 10.4; longest dorsal-fin soft ray length 16.0; 1st anal-fin spine length 3.9; 2nd anal-fin spine length 7.4; 3rd anal-fin spine length 8.0; anal-fin base length 15.0; pectoral-fin length 21.3; pelvic-fin spine length (measured on right side because the left side damaged) 13.8; longest pelvic-fin soft ray length 24.0.

Body oblong, rather compressed, deepest at pelvic-fin origin. Dorsal profile rising from snout tip to dorsal-fin origin, lowering slightly between origins of 1st to 10th dorsal-fin spines, thereafter more steeply to caudal peduncle. Ventral profile of body lowering from lower-jaw tip to anus, thereafter rising to caudal peduncle. Dorsal-fin origin just above posteriormost point of opercle, base extending posterior to posteriormost point of anal-fin base. First to 5th dorsal-fin spines gradually lengthening, 5th to 8th spine lengths similar, 8th to 10th spines gradually shortening. Seventh dorsal-fin soft ray longest. All dorsal-fin soft rays non-filamentous. Uppermost point of pectoral-fin base slightly posterior to posteriormost point of opercle. Lowermost point of pectoral-fin base anterior to pelvic-fin origin. Posterior tip of pectoral fin pointed, reaching to vertical through 7th dorsal-fin spine origin. Pelvic-fin origin posterior to dorsal-fin origin. Posterior tip of depressed pelvic fin reaching anus, not reaching anal-fin origin. Anal-fin origin below 1st dorsal-fin ray origin, ending below 6th dorsal-fin ray origin.

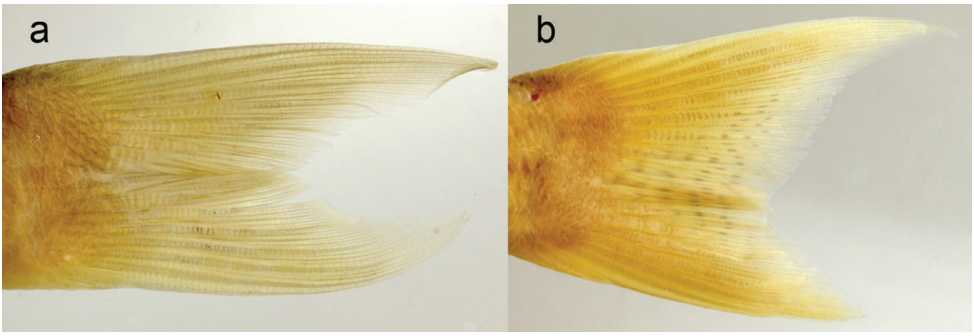

Figure 5. Caudal fin **a** *Scolopsis lacrima* sp. nov. (MNHN 2002-2930, holotype, 213.6 mm SL, flip horizontal) **b** *S. meridiana* (CSIRO H 4029–01, holotype, 194.8 mm SL).

First anal-fin spine shortest, 3rd spine longest. Caudal-fin forked, upper lobe longer than lower lobe. Posterior tip of both lobes of caudal fin pointed, non-filamentous. Anus oblong, anterior to anal-fin origin. Eye and pupil round. Lower margin of eye above a line from snout tip to uppermost part of pectoral-fin base. Nostrils round, paired, positioned close together anterior to orbit, anterior nostril with small dermal flap. Snout pointed. Posterior tip of maxilla not reaching to vertical through anterior margin of eye. Distinct suborbital spine posteriorly directed. Small antrorse spine below eye absent. Posterior margins of suborbital and preopercle serrated. Scales ctenoid; both lips, snout, area around eye, and bony opercular ridge and lower limb of preopercle scaleless. Lateral line complete, originating above opercle, extending to central part of caudal-fin base. Both jaws with small conical teeth, forming dense bands. Canine teeth absent. Gill rakers long, slender.

Colour when fresh. Based on colour photograph of holotype (MNHN 2002–2930; Fig. 1a). Head and body reddish-brown dorsally, silver-white ventrally. Upper lip blue. Two brown bands across dorsum of snout, connecting eyes. Upper band above posterior nostril, lower band below anterior nostril. Blue band on suborbital from anteroventral margin of orbit to just short of upper lip. Gill membrane yellow. A dark longitudinal band below lateral line from behind posterior margin of opercle to caudal peduncle. No diagonal lines on body. Distinct reddish blotch on upper end of pectoral-fin base. Pectoral fin pale yellow. Dorsal-fin membrane yellowish, semi-transparent, with yellow outer margin. Pelvic and anal fins white. Several indistinct yellowish longitudinal stripes on caudal peduncle. Upper base of caudal fin with blue blotch. Caudal fin red with yellowish upper margin. Central area of caudal fin without blotches or lines.

Colour in alcohol. (Fig. 1b) Head, body, and caudal fin uniformly pale brown. Three dark bands radiating from orbit. A dark brown longitudinal band below lateral-line. Dorsal, pectoral, pelvic, and anal fins pale yellow.

Distribution. Currently known only from New Caledonia (Fig. 2).

Etymology. The specific name *lacrima* is derived from Latin meaning a tear, in reference to the distinct blue band below the eye of the species.

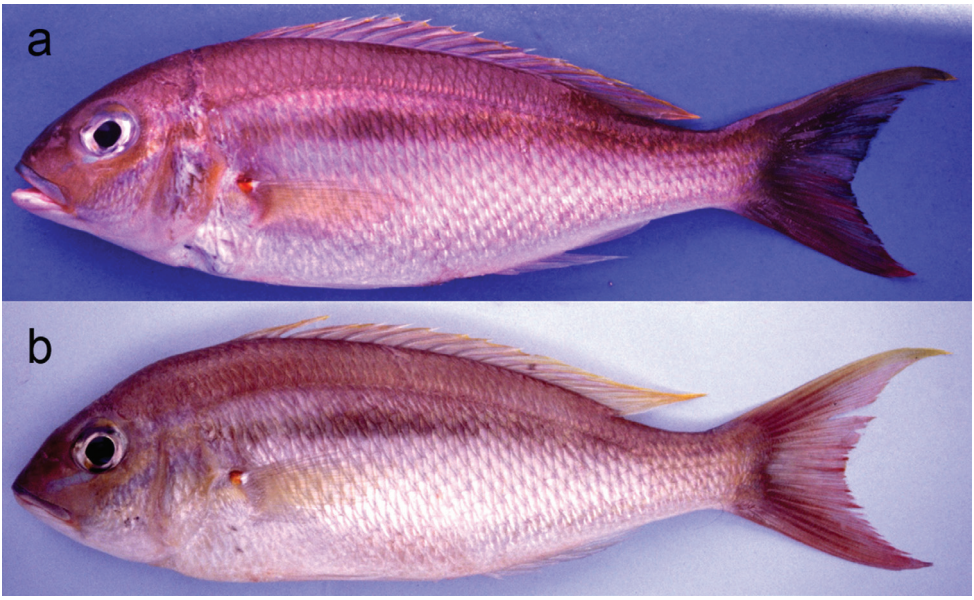

Figure 6. Colour photographs of *Scolopsis lacrima* sp. nov., Grande-Terre Island, New Caledonia (photos by P. Béarez) **a** DASMN-52, 187 mm SL **b** MNHN-ICOS-00437, 192 mm SL.

Remarks. The new species is assignable to the genus *Scolopsis*, defined by Russell (1990, 2001), due to its distinct posteriorly-directed suborbital spine, the suborbital area without scales, the posterior margin of the preopercle coarsely denticulate or serrate, and jaws without canine teeth. *Scolopsis lacrima* (Fig. 1) is similar to *S. meridiana* (Fig. 4) and *S. taenioptera*, the three species uniquely sharing the upper pectoral-fin base with a reddish blotch when fresh (Fig. 1a) (vs. reddish blotch absent in all other congeners). *Scolopsis lacrima* and *S. meridiana* are easily distinguished from *S. taenioptera* by having two bands across the top of the snout (Fig. 3) [vs. single band; Nakamura et al. (2018: fig. 4)]; detailed comparisons of *S. meridiana* with *S. taenioptera* were given in Nakamura et al. (2018). *Scolopsis lacrima* is distinguished from *S. meridiana* by the lack of diagonal lines on the body below the lateral line (Fig. 1) [vs. 18–20 brown diagonal lines in preserved specimens in *S. meridiana* (Fig. 4)], presence of a dark longitudinal band on the body below the lateral line (Fig. 1) [vs. longitudinal band absent (Fig. 4a), although young individuals (< 108.9 mm SL) may rarely have an indistinct dark longitudinal band (Fig. 4b)], and lack of blotches centrally on the caudal fin (Fig. 5a) [vs. several small poorly-defined blotches (Fig. 5b), although young individuals (< 108.9 mm SL) may rarely lack blotches (Fig. 4b)]. Moreover, body depths at the origins of the dorsal (29.2% of SL vs. 30.8–35.0% in *S. meridiana*, Fig. 7a), pelvic (29.5% vs. 31.6–38.4%, Fig. 7b), and anal fins (29.6% vs. 28.1–32.5%, Fig. 7c) are narrower in *S. lacrima*, and the caudal-peduncle depth (10.4% of SL vs. 11.4–13.1%, Fig. 7d) and head length (29.9% vs. 30.1–32.7%) less.

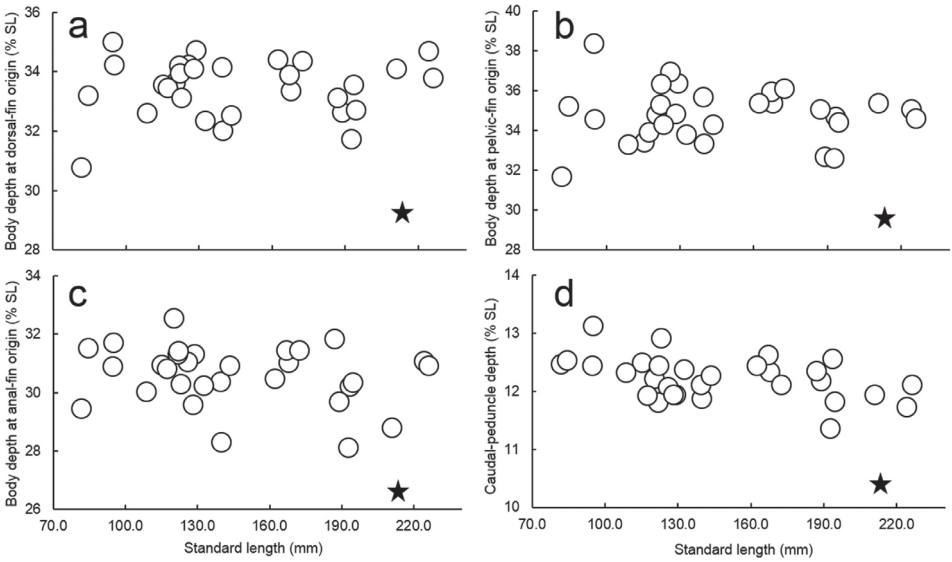

Figure 7. Relationships of (**a**) body depth at dorsal-fin origin, (**b**) body depth at pelvic-fin origin, (**c**) body depth at anal-fin origin, and (**d**) caudal-peduncle depth % of SL to SL in *Scolopsis lacrima* sp. nov. (diamond) and *S. meridiana* (circles).

Béarez (2003) reported three specimens [MNHN 2002–2930 (designated here as the holotype of *S. lacrima*; Fig. 1), MNHN-ICOS-00437, and DASMN-52] as *Scolopsis taeniopterus* (Cuvier, 1830) from New Caledonia. The latter two specimens have been reduced to bones and otoliths only. However, fresh colour photographs of both specimens prior to dissection (Fig. 6) support their identification here as *S. lacrima*.

Scolopsis meridiana and *S. taenioptera* are restricted to northern Australia and Southeast Asia, respectively (Fig. 2), probably not occurring in New Caledonia, suggesting that the three species of *Scolopsis* with a reddish blotch on the upper part of the pectoral-fin base are allopatrically distributed in the Indo-West Pacific.

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Conservation in the Satsunan Islands" project of Kagoshima University adopted by the Ministry of Education, Culture, Sports, Science and Technology, Japan; and the "Island Research" project of Kagoshima University.

References

Béarez P (2003) Premier signalement de *Scolopsis taeniopterus* (Nemipteridae) et de *Gymnocranius elongatus* (Lethrinidae) en Nouvelle-Calédonie. Cybium 27: 61–62.

Fricke R, Kulbicki M, Wantiez L (2011) Checklist of the fishes of New Caledonia, and their distribution in the Southwest Pacific Ocean (Pisces). Stuttgarter Beiträge zur Naturkunde A, Neue Serie 4: 341–463.

Nakamura J, Russell BC, Moore GI, Motomura H (2018) *Scolopsis meridiana*, a new species of monocle bream (Perciformes: Nemipteridae) from northern Australia. Zootaxa 4500(2): 222–234. https://doi.org/10.11646/zootaxa.4500.2.4

Mishra SS, Biswas S, Russell BC, Satpathy KK, Selvanayagam M (2013) A new species of the genus *Scolopsis* Cuvier, 1830 (Perciformes: Nemipteridae) from southern India and Sri Lanka. Zootaxa 3609(4): 443–449. https://doi.org/10.11646/zootaxa.3609.4.7

Psomadakis PN, Osmany HB, Moazzam M (2015) Field identification guide to the living marine resources of Pakistan. FAO species identification guide for fishery purposes. FAO, Rome, 386 pp. [42 pls]

Russell BC (1990) FAO species catalogue. Nemipterid fishes of the world (thread fin breams, whiptail breams, monocle breams, dwarf monocle breams and coral breams). Family Nemipteridae. An annotated and illustrated catalogue of the nemipterid species known to date. FAO Fisheries Synopsis, No. 125, 149 pp. [pls 1–8]

Russell BC (2001) Nemipteridae, threadfin breams (also whiptail breams, monocle breams, dwarf monocle breams, and coral breams). In: Carpenter KE, Niem VH (Eds) FAO species identification guide for fishery purposes. The living marine resources of the western central Pacific. Vol. 5. Bony fishes part 3 (Menidae to Pomacentridae). FAO, Rome, 3051–3089.

Sabaj MH (2016) Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an online reference. Version 6.5 (16 August 2016). http://www.asih.org/resources/standard-symbolic-codes-institutionalresource-collections-herpetology-ichthyology [Electronic version accessed 12 March 2019]

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Two new camaenid land snails (Eupulmonata) from Central China

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Abstract

Two new camaenid land snails are reported from Central China. The new genus, represented by *Sinochloritis lii* Wu & Chen, **gen. & sp. nov.**, the type of the genus from Sichuan, is close to *Yakuchloritis* Habe, *Nipponochloritis* Habe, *Neochloritis* Minato and *Trichochloritis* Pilsbry, but is well characterized by the smooth adult shell, highly developed epiphallic papilla, absence of penial caecum, and the presence of an epiphallus-binding muscle that binds the proximal epiphallus to the distal penis. A new species *Bradybaena linjun* Wu & Chen, **sp. nov.** is described from Hubei Province and is characterized by having two shell bands, a spoon-shaped love dart and the proportionally shortest mucous glands among Chinese congeners.

Chinese abstract

摘 要 从华中地区报道了两种坚螺科陆生软体动物的新物种。由新种李氏华砾螺*Sinochloritis lii* Wu & Chen, **gen. & sp. nov.** 为属模式种的华砾螺属*Sinochloritis* Wu & Chen, **gen. nov.** 与*Yakuchloritis* Habe, *Nipponochloritis* Habe, *Neochloritis* Minato及毛蜗牛属*Trichochloritis* Pilsbry接近，但因华砾螺属的成体贝壳光滑无毛，成荚器乳突高度发达，具成荚器绑结肌以及交接器盲囊阙如而与上述4属区别。李氏华砾螺记录于四川。另一新种廪君巴蜗牛*Bradybaena linjun* Wu & Chen **sp. nov.** 记录于湖北，它以两条色带，呈中空的勺形恋矢及具有相对最短的粘液腺等特征与所有其它中国巴蜗牛属物种相区别。

Keywords

Bradybaeninae, Camaeninae, Hubei, Sichuan, taxonomy

Introduction

Trichochloritis Pilsbry, 1891 (type species *Helix breviseta* Pfeiffer, 1862, original designation) was established as a subgenus of *Chloritis* Beck, 1837 to accommodate species that featured a "shell depressed, rather thin, the spire low-convex or plane, last whorl not carinated, but usually obtusely angled around the umbilicus; but little deflexed in front; epidermis not deciduous; apex, as well as the whole shell, hirsute or marked by hair-scars arranged in regular lines. Lip narrowly expanded or reflexed" (Pilsbry 1891). *Trichochloritis* is now recognized as a distinct genus (Schileyko 2007, 2011), which ranges from South China to the Philippines and in Japan (Pilsbry 1891, Azuma 1995, Schileyko 2011). Based on conchological and anatomical features (Azuma 1995), species of the Japanese region that were previously included in *Trichochloritis* have been treated as three separate genera, namely *Yakuchloritis* Habe, 1955, *Neochloritis* Minato, 1982 and *Nipponochloritis* Habe, 1955, and were assigned to the family Bradybaenidae (=Bradybaeninae sensu Bouchet et al. 2017) by Schileyko (2004). Based on the genital morphology of *Trichochloritis brevidens* (Sowerby, 1841) (Schileyko 2007; Table 1), *Trichochloritis* is unambiguously distinct from the Japanese genera.

Ten Chinese species and subspecies have been assigned to *Trichochloritis* (Table 2). In 1882, Heude described the first species *Helix percussa* from Wudangshan Mountain, Hubei. Möllendorff (1884) assigned it to *Hadra* Albers, 1850. Pilsbry (1890) placed *H. percussa* in his new genus *Euhadra*. Schmacker and Böttger (1894) followed this arrangement and listed a series of specimens that were considered to be similar to *H. percussa* from Tchen k'eou (=Chengkou, Chongqing), Kaochahien and Patung (=Badong, Hubei). Möllendorff (1884) reported a new hairy-shelled species *Helix hungerfordiana* Nevill, 1884 from Taiwan, with the subspecies *H. hungerfordiana rufopila* Möllendorff from Hong Kong and assigned it to ?*Trichia* Hartmann, 1840 (=*Trochulus* Chemnitz, 1786, Hygromiidae). Tryon (1887) followed his arrangement. In 1885, Heude described *H. mola* from Ta-kouan (=Daguan, Zhaotong, Yunnan). In 1887, Gredler described *H. franciscanorum* (Peishan, Hunan), which was assigned to subspecies of *Trichochloritis hungerfordiana* by Yen (1939) and was later listed as *Chloritis* (*Trichochloritis*) *hungerfordiana franciscanorum* by Zilch (1974; not "1886" in Zilch 1974). In 1888, Möllendorff described a new species *Helix herziana* from Hoihow (=Haikou, Hainan) and pointed out that it is close to *H. puberula* Heude, 1885, *H. hungerfordiana* and *H. franciscanorum*. Two years later, Heude published *H. molina* from Pa-tong (=Badong, Hubei). In 1891, Pilsbry included *H. herziana*, *H. puberula*, *H. franciscanorum* and *H. hungerfordiana* in his newly established genus *Trichochloritis*. In 1894, Gredler described *H.* (*Fruticicola*) *adaequata* (Secusan, W Hubei), which was assigned to *Chloritis* (*Trichochloritis*) by Zilch (1974). Yen assigned *T. submissa* (Deshayes, 1873), *T. diploblepharis* (Möllendorff, 1899), *T. hungerfordiana* (Yen 1939; 1940), *T. mola*, *T. percussa*, *T. herziana*, *T. molina*, *T. hunanensis* Yen, 1939 (Yen 1939), *Helix patungana* Gredler, 1887 (not "*patungensis*" in Yen 1942; not "Gredler, 1888" in Richardson 1983) and *Helix epixantha* Pfeiffer, 1850 (Yen 1942) to

Table 1. Comparison of *Sinochloritis* Wu & Chen, gen. nov. to *Trichochloritis* Pilsbry, 1891 and the other genera previously listed as *Trichochloritis* (Habe 1955, Minato 1982, Azuma 1995, Schileyko 2004, Schileyko 2007, this work). EBM – epihallus-binding muscle, the muscle binding proximal epiphallus to distal end of penis; Ep – epiphallus; EpP – epiphallic papilla; Fl – flagellum; PC – penial caecum; PS – penis sheath.

Groups	Spire	Hair	PS	Ep	EpP	EBM	PC	Fl
Trichochloritis Pilsbry, 1891	lower	thin	+	–	–	–	N/A	–
Yakuchloritis Habe, 1955	lower	thick	–	+	?	–	–	++
Nipponochloritis Habe, 1955	lower	thin	–	+	?	–	+	++/+
Neochloritis Minato, 1982	higher	thin	–	+	?	–	–	+
Sinochloritis Wu & Chen gen. nov.	higher	N/A	–	+	++	+	–	+

++ developed, + present, –absent, N/A not applicable.

Table 2. Comparison among *Sinochloritis lii* Wu & Chen, gen. & sp. nov. and the Chinese species once placed in *Trichochloritis* Pilsbry, 1891.

	Diameter major (mm)	Height (mm)	Whorls	Hairy	Distribution
T. adaequata (Gredler, 1894)	12	7	$4^{1/2}$	No	W Hubei,
T. herziana (Möllendorff, 1888)	14.5–17	10.5	5	No	Hainan
T. hunanensis Yen, 1939	11	7.2	$4^{1/2}$	No	Hunan
T. hungerfordiana (Nevill, 1884)	14.5–18	10.5	5	Yes	Taiwan, Hongkong, Guangdong,
T. hung. rufopila (Möllendorff, 1884)	15	9.25	5	Yes	Hongkong
T. hung. franciscanorum (Gredler, 1887)	18–22	9–12	$5^{2/3}$–6	No	S Hunan
T. mola (Heude, 1885)	30–31	15	4.5 **	No	Yunnan
T. molina (Heude, 1890)	14–17	10	4	No	Hubei
T. percussa (Heude, 1882)*	26–30	19	$5^{1/4}$	No	Hubei
T. puberula (Heude, 1885)	15–18	9	5	Yes	Chongqing
Sinochloritis lii Wu & Chen, gen. & sp. nov.	25.0–30.6	16.0–17.1	$4^{3/4}$–$4^{7/8}$	No	Sichuan

* The specimens studied by Schmacker and Böttger (1894) were excluded because they are conchologically different forms and might represent species other than *H. percussa*.
** Counted from fig. 5 (Heude 1885: pl. 29).

Trichochloritis. Among them, *T. submissa* is now the type species of *Trichobradybaena* Wu & Guo, 2003 (Bradybaeninae), and *T. diploblepharis* was assigned to *Plectotropis* Martens, 1860 (subfamily Bradybaeninae sensu Bouchet et al. 2017) by Möllendorff (1899), *H. patungana* was treated by Richardson (1983) as a *Plectotropis* species, and *H. epixantha* was sunk as a synonym as *Bradybaena similaris* (Rang, 1831) by Tryon (1887). Chang (1990) moved *Trichochloritis hungerfordianus* to *Yakuchloritis* based on genital anatomy.

Until now we have little idea if the species previously placed in *Trichochloritis* form a monophyletic group, although some recent work suggests that *Trichochloritis* as currently understood, consists of species from the Bradybaenidae (=Bradybaeninae sensu Bouchet et al. 2017) and from the Camaenidae (=Camaeninae sensu Bouchet et al. 2017) (Schileyko 2003, 2004, 2007). Here, we report a new species

from Sichuan that is conchologically most similar to *T. percussa* but shows marked differences from *Trichochloritis*, *Yakuchloritis*, *Neochloritis* and *Nipponochloritis*. In addition, we describe a new *Bradybaena* species discovered during our recent field work in Hubei Province.

Methods

Living specimens were relaxed by drowning in water before being transferred to 70% ethanol for fixation, which was replaced with ethanol of the same concentration after three days. The shell and genitalia were measured with digital vernier calipers and from photographs to the nearest 0.1 mm. Whorl number was recorded as described by Kerney and Cameron (1979), with 0.125 whorl accuracy. Soft parts were measured after the specimens were sufficiently fixed in 70% ethanol. Directions used in descriptions: proximal, toward the genital atrium; distal, away from the genital atrium.

Abbreviations: **At** – atrium; **BC** – bursa copulatrix; **BCD** – bursa copulatrix duct; **DS** – dart sac; **DVM** – membranous sac surrounding terminal genitalia; **EBM** – epiphallus-binding muscle, the muscle binding proximal epiphallus to distal end of penis; **Ep** – epiphallus; **EpP** – epiphallic papilla; **Fl** – flagellum; **fma** – fully mature animal; **fms** – empty fully mature shell; **FO** – free oviduct; **HBUMM** – mollusc collection of the Museum of Hebei University, Baoding, China; **MG** – mucous glands; **P** – penis; **PC** – penial caecum; **PR** – penial retractor muscle; **PP** – penial pilaster; **PS** – penis sheath; **Va** – vagina; **VD** – vas deferens.

Systematics

Helicoidea Rafinesque, 1815
Camaenidae Pilsbry, 1895
Bradybaeninae Pilsbry, 1898

***Sinochloritis* Wu & Chen, gen. nov.**
http://zoobank.org/926FDE79-21D2-4464-835D-972E04CF300E

Type species. *Sinochloritis lii* Wu & Chen, gen. & sp. nov.

Diagnosis. Adult shell smooth. Shell evenly covered with fine granules throughout. Dart sac apparatus absent. Penis sheath absent. Highly developed epiphallic papilla present. Penial caecum absent. Epiphallus-binding muscle connecting proximal epiphallus to distal end of penis. Flagellum present.

Description. Shell depressed. Whorls convex. Suture rather impressed. Protoconch and teleoconch densely and evenly covered with fine granules. Adult shell not hairy or scaly. Peristome abruptly angulated at top; narrowly and uniformly reflexed. Shell glossy; uniformly colored; not banded.

Figure 1. Distribution map. **1** *Sinochloritis lii* Wu & Chen, gen. & sp. nov.; **2** *Bradybaena linjun* Wu & Chen, sp. nov.

Genitalia. Penis sheath absent. Penis externally simple; internally with several pilasters. Epiphallus internally with a large epiphallic papilla that enters penis; externally with proximal part connected with distal end of penis by strong muscles (epiphallus-binding muscles). Flagellum present. Vas deferens uniformly thin.

Etymology. This new genus is named after "sino" (=China) and "chloritis" (the genus used to include many Chinese *Trichochloritis* species).

Distribution. Sichuan Province.

Remarks. Compared to *Trichochloritis*, *Yakuchloritis*, *Neochloritis* and *Nipponochloritis* (Table 1), the new genus exhibits distinct genital features that justify recognition of a new generic rank. Many Chinese species mentioned above, i.e., the species in *Trichochloritis*, possess general similarity in shell morphology but placement within genera requires evidence from either, or both, reproductive morphology and molecular data.

***Sinochloritis lii* Wu & Chen, gen. & sp. nov.**

http://zoobank.org/FCC544B8-3DD5-4785-A1C0-5A2E2240C55E

Figs 1–6

Type material. Holotype, fully matured animal (HBUMM08294). Sichuan Province, Dujiangyan, Qingchenghoushan, 30°56'39.38"N, 103°28'47.21"E, 1500 m a.

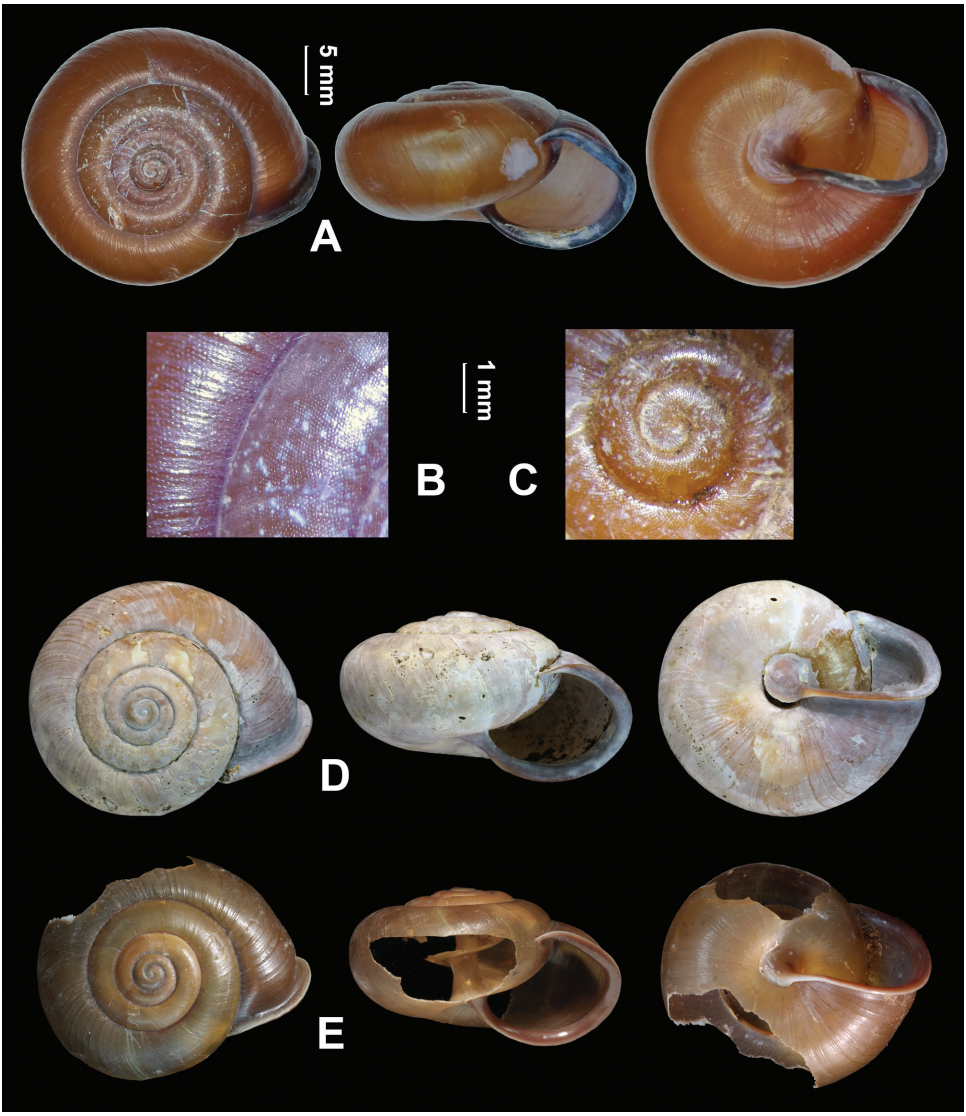

Figure 2. *Sinochloritis lii* Wu & Chen, gen. & sp. nov., holotype, HBUMM08294. **A** shell **B** magnified surface of teleoconch **C** magnified embryonic shell **D** paratype HBUMM10008 **E** paratype, HBUMM10009.

s. l., 2018-XI-8, coll. Li, Chenliang & Zhu, Xiaoran. A sample of foot muscle tissue was preserved in 99.7% ethanol at –20 °C (HBUMM08295). **Paratypes**, 1 old fms (HBUMM10008), Sichuan, Dujiangyan, Qinchenghoushan, 1500 m a. s. l., 2018-V, coll. Liu, Zhengping; 1 broken fully matured shell (HBUMM10009), Sichuan, Dujiangyan, Qinchenghoushan, 1500 m a. s. l., 2017-X, coll. Liu, Zhengping.

Description. Shell (Fig. 2). Depressed; thick and solid; dextral. Whorls convex. Suture rather impressed. Umbilicus closed by reflexed columellar lip. Columella oblique. Proto-

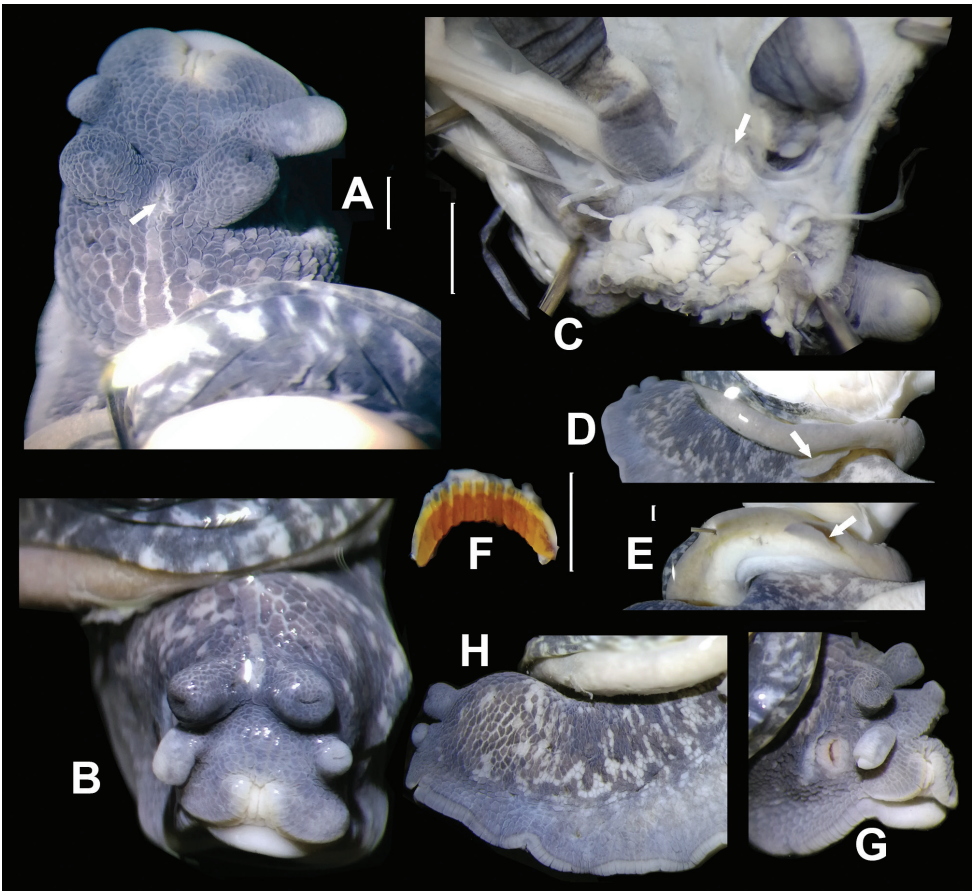

Figure 3. *Sinochloritis lii* Wu & Chen, gen. & sp. nov., holotype, HBUMM08294. **A** anterior part of animal, dorsal view, showing the pore (arrowed) of head gland between ommatophore tentacles **B** head of the animal **C** internal body wall of head, showing the head gland (arrowed) between the ommatophore tentacles **D, E** the leaf-shaped appendage (arrowed) on the left margin of mantle, in two views **F** left side of animal, showing coloration and skin pattern **G** right side of head. Scale bars: 1 mm.

conch and teleoconch densely and uniformly covered with fine granules, without spiral furrows. Aperture oblique; not sinuate at peristome. Body whorl not descending behind aperture. Shell surface without ribs. Growth lines fine. Adult shell not hairy or scaly. Adult body whorl rounded at periphery; basally convex. Ring-like thickening within aperture absent. Peristome thin; abruptly angled at top; narrowly and uniformly reflexed; brownish purple. Callus thin and transparent. Shell glossy; uniformly reddish brown. Measurements (type material): shell height 16.0–17.1 (16.5 ± 0.55) mm, shell breadth 25.0–30.6 (27.0 ± 3.10) mm, aperture height 11.5–12.5 (11.9 ± 0.51) mm, aperture width 13.4–16.8 (14.9 ± 1.72) mm, embryonic shell whorls 1.375–1.500 (1.458 ± 0.072), whorls 4.750–4.875 (4.833 ± 0.072), shell height/ breadth ratio 0.56–0.65 (0.62 ± 0.049).

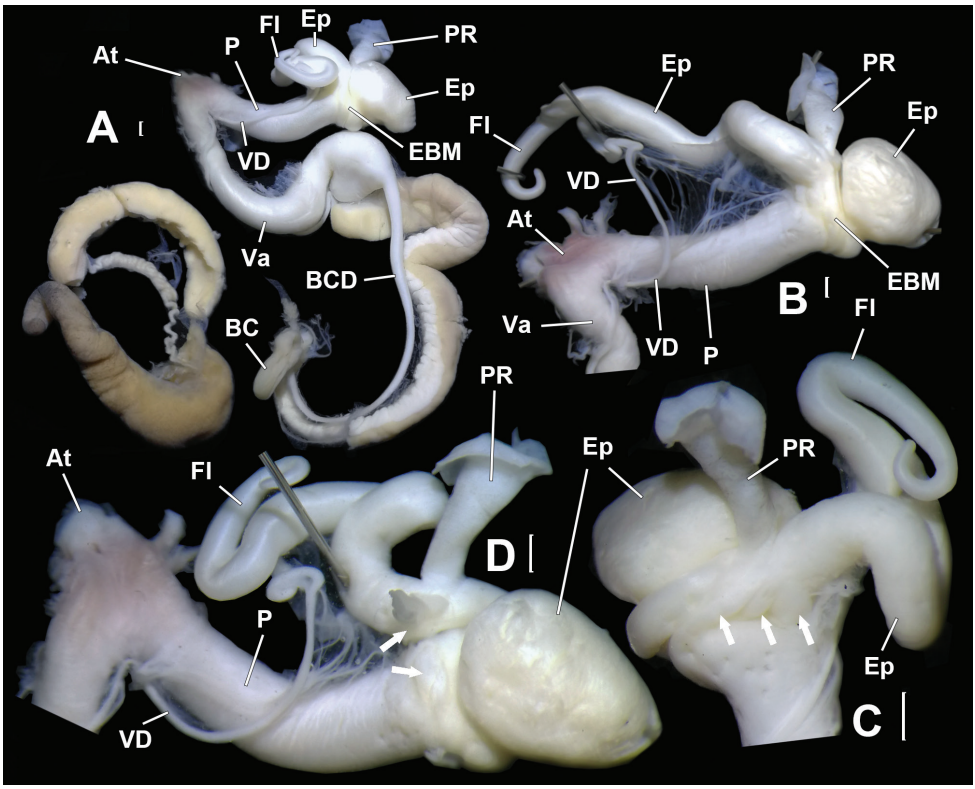

Figure 4. *Sinochloritis lii* Wu & Chen, gen. & sp. nov., holotype, HBUMM08294. **A** genitalia, general view, distal part opened **B** male part, intact **C** male part, showing the muscle (arrowed) connecting epiphallus and penis **D** male part, with the muscle connecting epiphallus and penis partially severed (arrowed). Scale bars: 1 mm. At – atrium; BC – bursa copulatrix; BCD – bursa copulatrix duct; EBM – epihallus-binding muscle, the muscle binding the proximal epiphallus to the distal end of penis; Ep – epiphallus; Fl – flagellum; P – penis; PR – penial retractor muscle; Va – vagina; VD – vas deferens.

General anatomy (Fig. 3). A heart-shaped head gland between ommatophore insertions present on inner body wall (Fig. 3C, arrowed), externally with a visible gland pore (Fig. 3A, arrowed). On internal body wall, at the base of ommatophore with two groups of glands each consisted of numerous small sacs (Fig. 3C). On left side of mantle edge, a leaf-shaped appendage present (Fig. 3D, E). Body blueish purple with scattered lighter spots (Fig. 3H). Sole dirty white. Jaw arcuate; with twelve more or less projecting ribs (Fig. 3F).

Genitalia (Figs 4, 5). Penis sheath absent. Penis thick; externally simple; internally with five thick and high plicae/pilasters (Fig. 5D). Epiphallus longer than penis; with section between penial retractor muscle and epiphallic papilla one-third thickness of penis; section between penial retractor muscle and vas deferens insertion much thicker than proximal part but thinner than penis (Fig. 5A); internally with a large peach-shaped epiphallic papilla (approximate size 3.5×4.0×6.0 mm^3) entering penis (Fig. 5A,

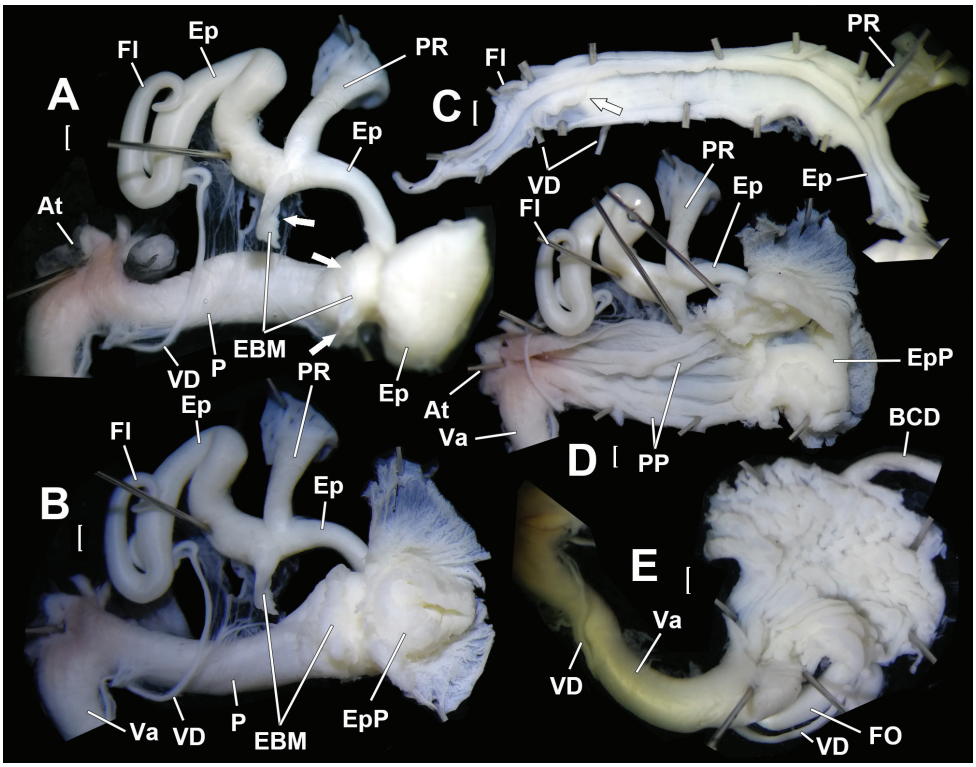

Figure 5. *Sinochloritis lii* Wu & Chen, gen. & sp. nov., holotype, HBUMM08294. **A** male part, with the muscle (arrowed) connecting epiphallus and penis completely severed **B** exposed part containing epiphallic papilla **C** exposed epiphallus and flagellum. Arrow indicates insertion of vas deferens **D** opened penis, showing penial pilasters **E** basal part of bursa copulatrix duct, exposed. Scale bars: 1 mm. At – atrium; BCD – bursa copulatrix duct; EBM – epihallus-binding muscle, the muscle binding proximal epiphallus to distal end of penis; Ep – epiphallus; EpP – epiphallic papilla; Fl – flagellum; FO – free oviduct; P – penis; PR – penial retractor muscle; PP – penial pilaster; Va – vagina; VD – vas deferens.

B); externally, partially connected with distal penis by strong muscles that insert on penis just opposite to penial retractor muscle (Figs 4C, 4D, 5A, arrowed). Flagellum cylindrical; tapering. Inside flagellum and epiphallus, a long pilaster running from tip of flagellum to epiphallic papilla and a much shorter wavy pilaster running from tip of flagellum to vas deferens insertion (Fig. 5C). Vas deferens thin; of even thickness (Fig. 4A). Vagina subequal to penis in length (Fig. 4A). Base of bursa copulatrix duct expanded and ball-shaped (Fig. 4A); internal wall strongly corrugated (Fig. 5E). Measurement of holotype: P–13.6 mm; Ep–19.3 mm; Fl–8.6 mm; VD–31.4 mm; PR–4.6 mm; Va–18.8 mm; FO–5.4 mm; BC plus BCD–37.9 mm.

Etymology. This species is named in honor of Dr. Chenliang Li who collected and sent us the holotype (HBUMM08294).

Distribution. Sichuan (Qingchengshan), only known from the type locality (Fig. 1).

Ecology. This species was found living in the well-developed forest (Fig. 6).

Figure 6. *Sinochloritis lii* Wu & Chen, gen. & sp. nov., holotype, HBUMM08294. Habitat, photographer Chenliang Li.

Taxonomic remarks. The new species has a closed umbilicus, otherwise is very close to *Trichochloritis percussa* in shell size (Table 2), the general shape and the microsculpture of shell. *Camaena hemiclista* (Schmacker & Böttger, 1894) known only from Hubei (Lytschouan=Lichuan), which has a closed umbilicus and is bluntly shouldered (but not visible in Yen 1939: pl. 12, fig. 42) resembles the new species; however, the new species has fewer whorls and a clearly rounded periphery.

Bradybaena Beck, 1837

Type species. *Bradybaena similaris* (Rang, 1831); original designation.

Bradybaena linjun Wu & Chen, sp. nov.

http://zoobank.org/834A1B9F-7272-4188-9FD5-2121A9D26443
Figs 1, 7–10

Material examined. Holotype, fma (HBUMM08241-specimen 1, Fig. 7A). Hubei Province, Yichang, Changyang Tujia Autonomous Prefecture, Longzhoupin; 31°28'9"N, 111°11'14"E, 103 m a. s. l.; 2018-VII; coll. Chen, Zheyu. **Paratype**, 1 fma (HBUMM08241-specimen 2, Fig. 7B), the same collection information as holotype. Foot muscle was cut off and preserved in 99.7% alcohol at –20 °C (HBUMM08242).

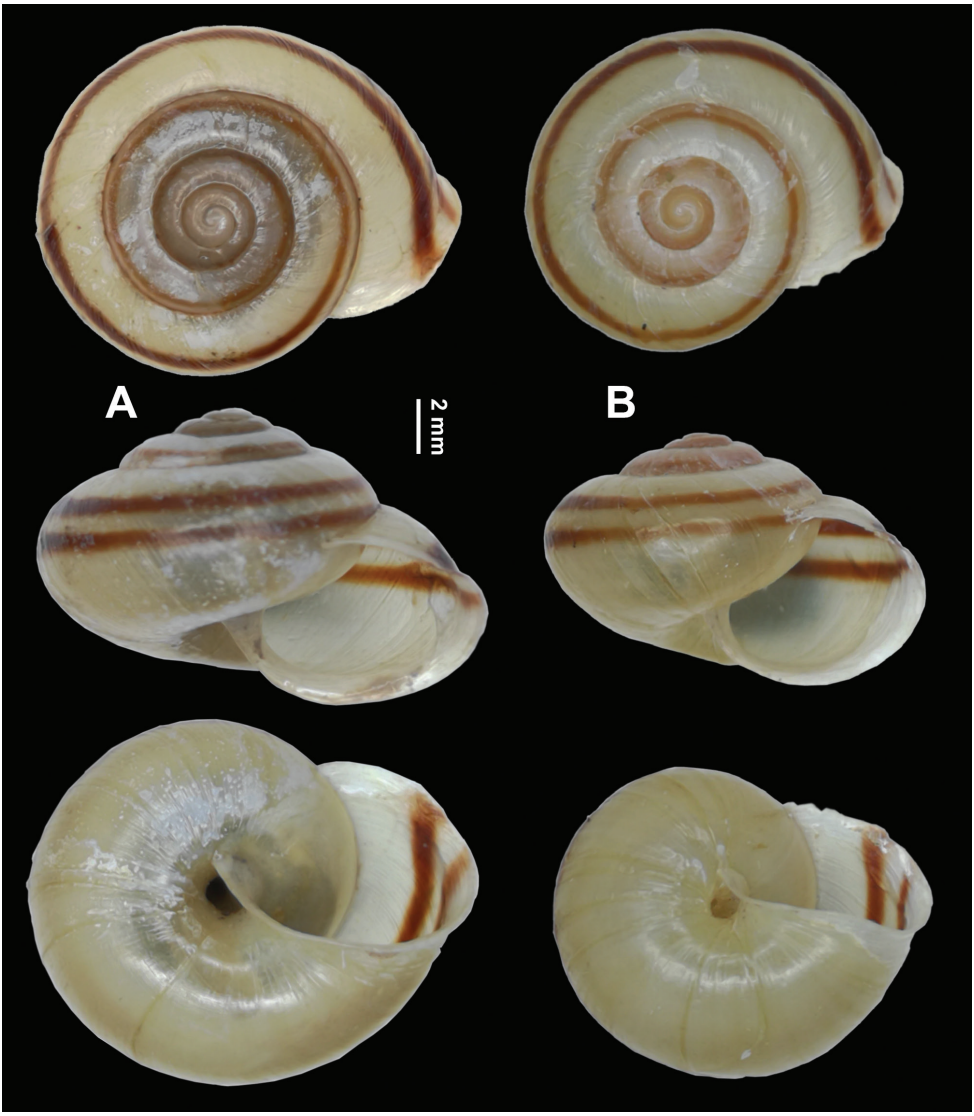

Figure 7. *Bradybaena linjun* Wu & Chen, sp. nov. **A** holotype, HBUMM08241-specimen 1 **B** paratype, HBUMM08241-specimen 2.

Diagnosis. Shell depressed; dextral. Columella oblique. Periphery rounded. A peripheral and a supraperipheral chestnut band present. Penis internally with numerous crossing pilasters of equal thickness that form a network. Love dart hollow and C-shaped in cross section. Accessory sac externally invisible. Mucous glands two, very short thyrsiform (not branched) tubes; entering accessory sac through simple pore. Shell about 4.5 whorls, breadth 13–17 mm.

Description. Shell (Fig. 7). Depressed; thin; dextral. Whorls convex. Suture impressed. Half umbilicus covered by reflexed columellar lip. Columella oblique. Proto-

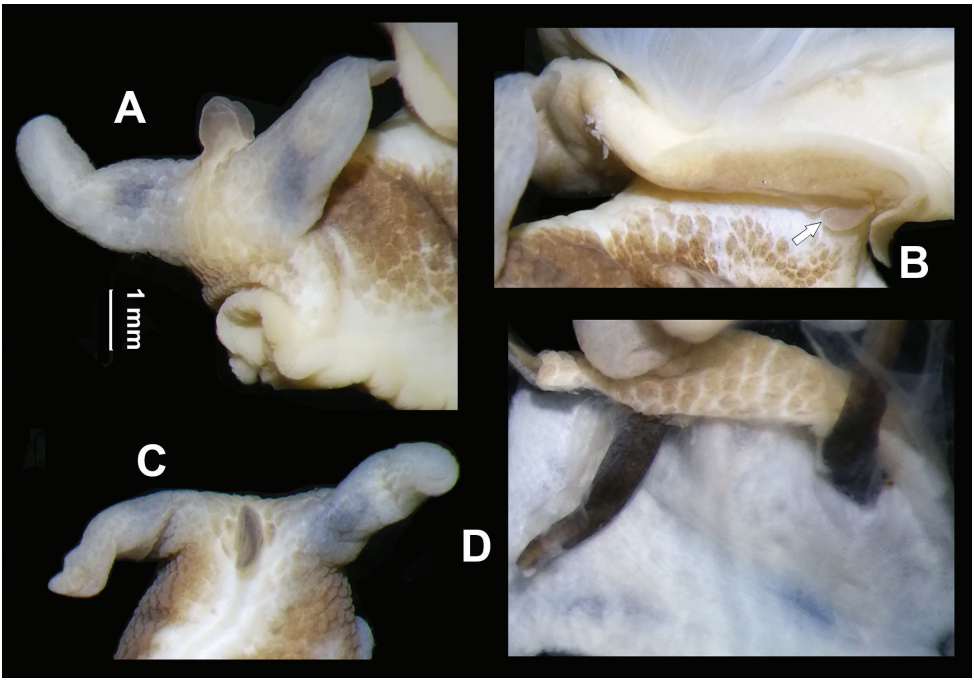

Figure 8. *Bradybaena linjun* Wu & Chen, sp. nov., holotype, HBUMM08241-specimen 1. **A, C** showing head wart **B** leaf-shaped appendage (arrowed) on left margin of mantle **D** internal body wall between ommatophore insertions, showing no gland.

conch not granulate, smooth. Teleoconch with dense spiral furrows. Aperture oblique; not sinuate at peristome. Body whorl slightly descending behind aperture. Shell surface without ribs. Growth lines fine. Adult shell not hairy or scaly. Adult body whorl rounded at periphery; basally convex. Ring-like thickening within aperture absent. Peristome thin; slightly reflexed. Callus thin and transparent. Shell glossy; uniformly brownish yellow; with a peripheral and a supraperipheral chestnut bands. Measurements (holotype is larger in size): shell height 8.8–10.7 mm, shell breadth 13.2–16.6 mm, aperture height 5.9–6.0 mm, aperture width 7.0–9.7 mm, embryonic shell whorls 1.625, whorls 4.250–4.625, shell height/ breadth ratio 0.64–0.67.

General anatomy (Figs 8, 9F). A high head wart between ommatophores present (Fig. 8A, C). On corresponding internal body wall no particular structure present (Fig. 8D). On left side of mantle edge, a leaf-shaped appendage present (Fig. 8B, arrowed). Body light brown; with whitish striae posterior to wart. Sole creamy white. Jaw arcuate; with about thirteen more or less projecting ribs (Fig. 9F).

Genitalia (Fig. 9). Membranous sac surrounding terminal genitalia present (Fig. 9A, B). Penis sheath about 1/3 penis length. Penis very thick; externally simple. Penial retractor muscle inserting on epiphallus. Epiphallus slightly thicker than vas deferens. Flagellum absent. Epiphallic papilla absent (Fig. 9D). Penis internally with numerous crossing pilasters of equal thickness that form a network (Fig. 9D). Dart sac

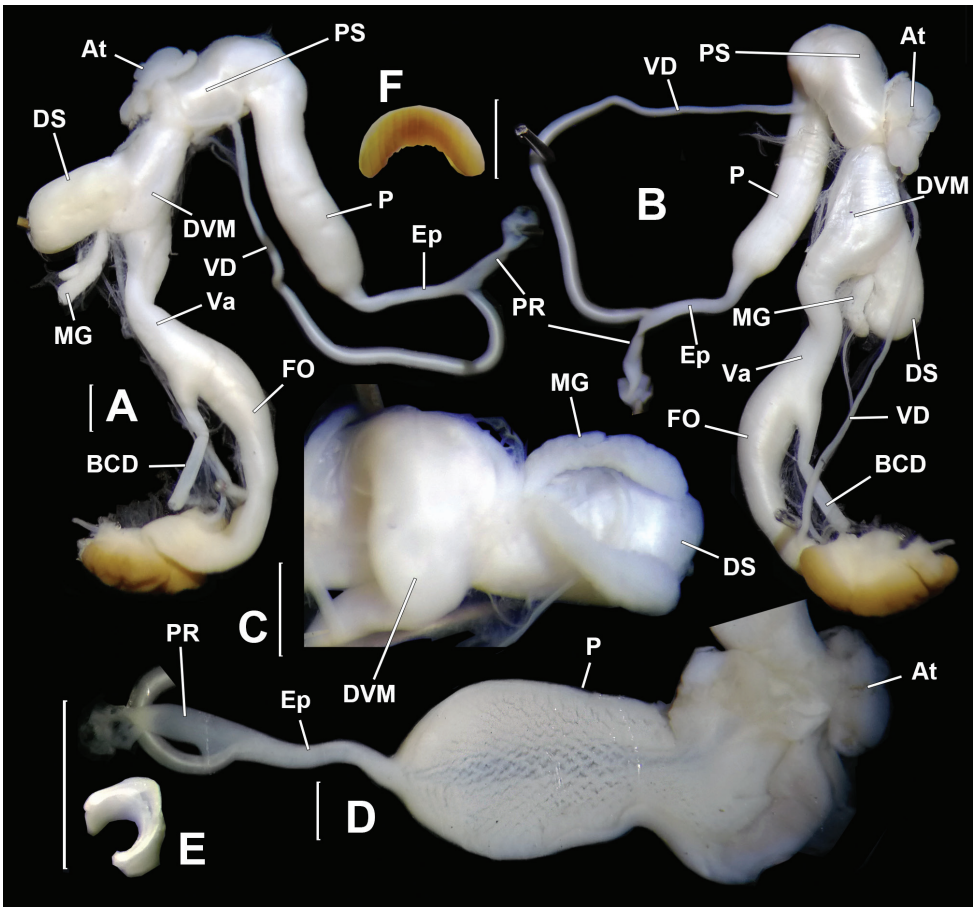

Figure 9. *Bradybaena linjun* Wu & Chen, sp. nov., holotype, HBUMM08241-specimen 1. **A, B** both sides of genitalia, general view **C** bottom of dart apparatus, showing mucous glands insertion **D** penis, exposed **E** love dart in cross section **F** jaw. Scale bars: 1 mm. At – atrium; BCD – bursa copulatrix duct; DS – dart sac; DVM – membranous sac surrounding terminal genitalia; Ep – epiphallus; FO – free oviduct; MG – mucous glands; P – penis; PR – penial retractor muscle; PS – penis sheath; Va – vagina; VD – vas deferens.

present. Love dart spoon-shaped, hollow and C-shaped in cross section (observed in holotype, Fig. 9E). Accessory sac invisible externally (Fig. 9C). Poly-layered structure present between mucous gland insertion and vagina. Mucous glands two tubes; much shorter than dart sac in length; each thyrsiform rather than branched (Fig. 9A, C); entering accessory sac through simple pore. Vagina about half of penis in length. Measurement of holotype: DS–4.6 mm long, 1.4 mm broad; MG–1.7 mm; PS–1.2 mm; P–7.1 mm; Ep–2.6 mm; VD–21.5 mm; PR–1.7 mm; Va–4.5 mm; FO–2.8 mm.

Etymology. The new species is named after the legendary tribal leader "Lin-Jun (廩君)" of the Tujiazu people who live at the type locality.

Distribution. Hubei (Changyang), only known from the type locality.

Figure 10. *Bradybaena linjun* Wu & Chen, sp. nov., holotype, HBUMM08241-specimen 1. Habitat, photographer Zheyu Chen.

Ecology. This species was found living in a well-established secondary forest, on limestone cliffs, often in cracks (Fig. 10). A large number of broken shells, presumably caused by bird predation, were observed at the type locality.

Taxonomic remarks. The new species is assigned to *Bradybaena* because of the presence of a smooth protoconch, membranous sac surrounding terminal genitalia, poly-layered structure in dart apparatus, two mucous glands and the absence of a flagellum; characters that are consistent with the type of the genus *B. similaris* (Wu 2004).

On the left side of the mantle edge, this species possesses a leaf-shaped appendage (Fig. 8B). The existence of this structure in other bradybaenine genera is not known except in *Sinochloritis lii* Wu & Chen, gen. & sp. nov. described here (Fig. 3D, E). In our other work on *Bradybaena* this structure is observed (*Bradybaena* sp., HBUMM06125, Wuyuan, Jiangxi Province, 147 m, 29°22'18.8"N, 118°02'45.2"E, 2007-V-26; unpublished data).

Only a few Chinese species in the subfamily Bradybaeninae have double bands. The double-banded shells occur more frequently in *Cathaica* Möllendorff, 1884 than in *Bradybaena* where only four species exhibit double bands, namely *B. billiana* (Heude, 1882), *B. mimicula* (Heude, 1888), *B. diplodesma* (Möllendorff, 1899), *B. sueshanensis* Pilsbry, 1934 (Heude 1882, 1888; Möllendorff 1899; Pilsbry 1934). Although the new species has double bands, in aspect of shell morphology it most resembles *B. qixiaensis* Wu & Asami, 2017. However, the new species has very short mucous glands which are proportionally the shortest in the subfamily Bradybaeninae, the thyrsiform mucous gland duct, and the spoon-shaped love dart, which distinguish this species from all Chinese *Bradybaena* species with known genital anatomy.

Acknowledgments

We want to thank Zhengping Liu and Chenliang Li for the field work. We are grateful to the Biodiversity Heritage Library (www.biodiversitylibrary.org) for access to precious literatures. The efforts of Fred Naggs and Barna Páll-Gergely to review this manuscript are acknowledged. Their constructive comments have helped to improve this work.

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References

Azuma M (1995) Colored Illustrations of the Land Snails of Japan. Hoikusha, Osaka, 343 pp. [80 pls.]

Bachmann O, Gredler V (1894) Zur Conchylien-Fauna von China. XVIII. Stück. Annalen des K. K. Naturhistorischen Hofmuseums 9: 415–429.

Bouchet P, Rocroi J-P, Hausdorf B, Kaim A, Kano Y, Nützel A, Parkhaev P, Schrödl M, Strong EE (2017) Revised classification, nomenclator and typification of gastropod and monoplacophoran families. Malacologia 61(1–2): 1–526. https://doi.org/10.4002/040.061.0201

Chang KM (1990) Systematics on *Trichochloritis hungerfordianus* from Taiwan. Bulletin of Malacology ROC 15: 35–41.

Gredler V (1887) Zur Conchylien-Fauna von China. IX. Stück. Malakozoologische Blätter, 1–20.

Habe T (1955) Anatomical studies on Japanese land snails (3). Venus 18(4): 221–234.

Heude PM (1882–1890) Notes sur les mollusques terrestres de la vallée du Fleuve Bleu. Mémoires concernant l'histoire naturelle de l'Empire chinois 1882: 1–84; 1885: 89–132; 1890: 125–188.

Heude PM (1888) Diagnoses Molluscorum novorum, in Sinis collectorum (3). Journal de Conchyliologie 36: 235–243.

Kerney MP, Cameron RAD (1979) A Field Guide to the Land Snails of Britain and North-West Europe. Collins, London, 288 pp. [24 pls.]

Minato H (1982) Land shell fauna of Ujigunto and Kusakakigunto Islets, the Southwestern Kyushu, Japan, with the descriptions of a new genus and six new species. Venus 41(2): 124–140.

Möllendorff OF (1884) Materialien zur Fauna von China. Jahrbücher der Deutschen Malakozoologischen Gesellschaft 11: 307–390. [pls 7–9.]

Möllendorff OF (1888) Diagnoses specicrum novarum sinensium. Nachrichtsblatt der Deutschen Malakozoologischen Gesellschaft, 38–44.

Möllendorff OF (1899) Binnen-Mollusken aus Westchina und Centralasien. I. Annuaire du Musée Zoologique de l'Académie Impériale des St.-Petersburg 4: 46–144. [pls 2–8.] https://doi.org/10.5962/bhl.title.13125

Pilsbry HA (1890–1891) In: Tryon GW, Pilsbry HA (Eds) Manual of Conchology (2)6: 5–324. [pls 1, 39–69.]

Pilsbry HA (1934) Zoological results of the Dolan West China expedition of 1931, Part II, Mollusks. Proceeding of the Academy of natural Sciences of Philadelphia 86: 5–28. [6 pls]

Richardson L (1983) Bradybaenidae: Catalog of species. Miscellaneous Publications of the Department of Malacology of the Academy of Natural Sciences of Philadelphia 9: 1–207.

Schileyko AA (2003) Treatise on recent terrestrial pulmonate molluscs. Part 11. Trigonochlamydidae, Papillodermidae, Vitrinidae, Limacidae, Bielziidae, Agriolimacidae, Boettgerillidae, Camaenidae. Ruthenica Supplement 2: 1467–1626.

Schileyko AA (2004) Treatise on recent terrestrial pulmonate molluscs. Part 12. Bradybaenidae, Xanthonychidae, Epiphragmophoridae, Helminthoglyptidae, Elonidae, Sphincterochilidae, Cochlicellidae. Ruthenica Supplement 2: 1627–1763.

Schileyko AA (2007) Treatise on recent terrestrial pulmonate molluscs. Part 15. Oopeltidae, Anadenidae, Arionidae, Philomycidae, Succineidae, Athoracophoridae. Additions and corrections. Indexes. Ruthenica Supplement 2: 2049–2209.

Schileyko AA (2011) Check-list of land pulmonate molluscs of Vietnam (Gastropoda: Stylommatophora). Ruthenica 21(1): 1–68.

Schmacker B, Böttger O (1894) Description of some Chinese land-shells. Proceedings of the Malacologial Society 1: 169–174. https://doi.org/10.1093/oxfordjournals.mollus.a064109

Tryon GW (1887) Manual of Conchology (2)3: 1–313. [pls 1–63.]

Wu M, Guo J (2003) Contribution to the knowledge of Chinese terrestrial malacofauna (Helicoidea): description of a new bradybaenid genus with three species. The Veliger 46(3): 239–251.

Wu M (2004) Preliminary phylogenetic study of Bradybaenidae (Gastropoda: Stylommatophora: Helicoidea). Malacologia 46(1): 79–125.

Wu M, Asami T (2017) Taxonomical notes on Chinese camaenids with description of three species (Gastropoda: Pulmonata). Molluscan Research, https://doi.org/10.1080/13235818.2017.1380145

Yen TC (1939) Die chinesischen Land- und Süßwasser-Gastropoden des Natur-Museums Senckenberg. Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft 444: 1–234. [16 pls.]

Yen TC (1942) A Review of Chinese Gastropods in the British Museum. Proceedings of the Malacological Society of London 24: 170–288. [pls 11–28.]

Zilch A (1974) Vinzenz Gredler und die Erforschung der Weichtiere Chinas durch Franziskaner aus Tirol. Archiv für Molluskenkunde 104(4/6): 171–228.

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SHORT COMMUNICATION

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Developing a standardized list of entomological collection methods for use in databases

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Abstract

The current natural history specimen databasing paradigm focuses on standardizing occurrence data: where and when a specimen was collected. In order to gather more information about a particular species, researchers also must know how to encounter, and possibly collect, the species. For entomological specimens, collection method terminology written on labels has not been standardized, and perhaps should not be; however, use of a broad-scale collection method framework may aid in communication among researchers especially within the context of public databases. Three main categories of collection methods are proposed: active human collecting; active specimen orientation; and passive specimen collection and/or concentration. General categories contain more specific sub-categories and so on. A bibliography of useful works describing entomological collection and curation methods (e.g., "How to make an insect collection") is provided.

Keywords

curation, entomology, insect, museum

Introduction

For many invertebrates, distressingly little is known about their distribution, phenology, and natural history. Learning more about any aspect of a particular species requires three pieces of information: where to find it, when to go looking, and how to encounter it. For a select few species a meaningful encounter can take place without

* Illustrator

collecting the specimen, many dragonflies, bees, and butterflies can be sight identified, but for most species an accurate identification can be made only once a specimen is captured, after which it may be retained or released.

Recent technological advances and environmental regressions have precipitated a desire to create databases of natural history collections. To that end, Biodiversity Information Standards (TDWG) (www.tdwg.org), was created to "establish international collaboration among biological database projects" (https://www.tdwg.org/about/). One result was creation of the Darwin Core standard (http://rs.tdwg.org/dwc/), a list of agreed upon terms used when reporting the occurrence of taxa, the when and where.

However, little standardization has been applied to collection method (see "dwc:sampling Protocol" http://terms.tdwg.org/wiki/dwc:samplingProtocol. Examples include: "UV light trap", "mist net", and "Penguins from space: faecal stains reveal the location of emperor penguin colonies"). As more material is added to databases and more databases are available online, standardization of the collection method field will become important, otherwise useful collection methods may become lost within a fog of overly vague or overly specific individualized terminology resulting in reduced searchability and loss of ability to collate records based on collection method. Additionally, a better understanding of collection method possibilities may help expand methods used when attempting to control or eradicate pests, study endangered species, conduct comprehensive surveys, or develop efficient surveys. While Darwin Core's terminology concerning collection method may or may not change, the entomological, and wider natural history community, should consider adopting a more standardized list of collection method terms and/or concepts to use when databasing physical specimens.

The universe is complicated and diverse. Often, to ease communication or reduce overwhelming complexity, humans will create a small set of "boxes" or categories that, together, hold the majority of the entities within a particular system. For example, the life stages of an insect can be separated into four general "boxes": egg, larva, pupa, and adult. Despite a wide variety of exceptions (ametabolous and hemimetabolous orders, subimagos, paedomorphic females, hypermetamorphosis, prepupae, etc.) these are useful and meaningful designations. It is important to remember that these categories are for human convenience, may or may not follow the natural world closely, and may be more (or less) useful in different forms to different users. Therefore, attempts to refine concepts to create one system that works equally well in all situations may be foolhardy (like the search for a single species-concept, or the definition of "life").

A general outline of collection methods is presented below with examples (Whitman et al. 2019 independently started a very similar list). Referencing each method would become cumbersome, therefore a bibliography of some popular, useful, and/or interesting sources on collecting terrestrial and aquatic arthropods is provided. Several qualifications should be interjected here: 1) the list may not be complete and can/will be added to over time, the design allows for easy expansion; 2) equally valid alternative arrangements of the general concepts may exist; 3) the list as presented is biased for finer divisions among popular methods (lights: UV, MV, LED), and more generalized for less widely-used methods (thermal lure); 4) "sampling," a *type* of collecting, is used to capture a subset of a population often for statistical analysis and not dealt with in

this scheme; 5) laboratory colonies are not listed below, presumably the progenitors were originally collected in one of the following manners.

Collection methods

All insect collecting falls into three broad categories: 1) active human collecting; 2) active specimen orientation; and 3) passive collection and/or concentration (Fig. 1).

A. Active human collecting

Active human collecting falls within three different categories: immediate collection of a specimen from the environment; filtration of the environment to concentrate specimens; and agitation of specimens from their present location. In each case the collector is present, specimens are removed from a location where they (more or less) would naturally occur, and the collector has some knowledge of when and where a specimen was collected.

1. Immediate collection of specimens with hands, aspirator, vial, etc. While a specialized tool may be used to collect the specimens (e.g., aspirator), here, specimens are recognized as such, individually targeted, and often directly removed from their native substrate by the collector (contrast with "sifting" below which lacks many of these elements). The method provides the highest accuracy and precision when reporting the location or activities of the specimen (host plant/animal, substrate, etc.).
   1.1 Hand/Direct.
2. Sequestration and concentration of specimens using a net/strainer/filter. A net, sensu lato, is a filtering device. The meaningful difference among nets is not design (aquatic, aerial) but the size of the holes, as that determines the smallest organism likely to be captured. Entomologists rarely consider mesh size except in aquatic situations. However, the interplay of substrate sampled (which carries ecological information) and net design creates the categories most often used to define net type.
   2.1 Net (general).
      2.1.1 Net – Aquatic (from water).
      2.1.2 Net – Aerial (collection of flying specimens).
      2.1.3 Net – Sweeping (collection of specimens that were attached to a substrate).
   2.2 Vacuum collector (hand held or backpack device that gathers and filters substrate, may be air or liquid based).
3. Separation of specimens from a substrate or matrix, collector induced (not sequestered or concentrated, see below).
   3.1 Agitation (direct or indirect stimulus used to induce movement that increases likelihood of capture: flooding, soil flotation, waving hand over the trunk of a tree, sifting litter into a pan and immediately collecting, etc.).
   3.2 Beating (mechanical removal from substrate).
   3.3 Fogging (chemical used to cause agitation on, or removal from, substrate).

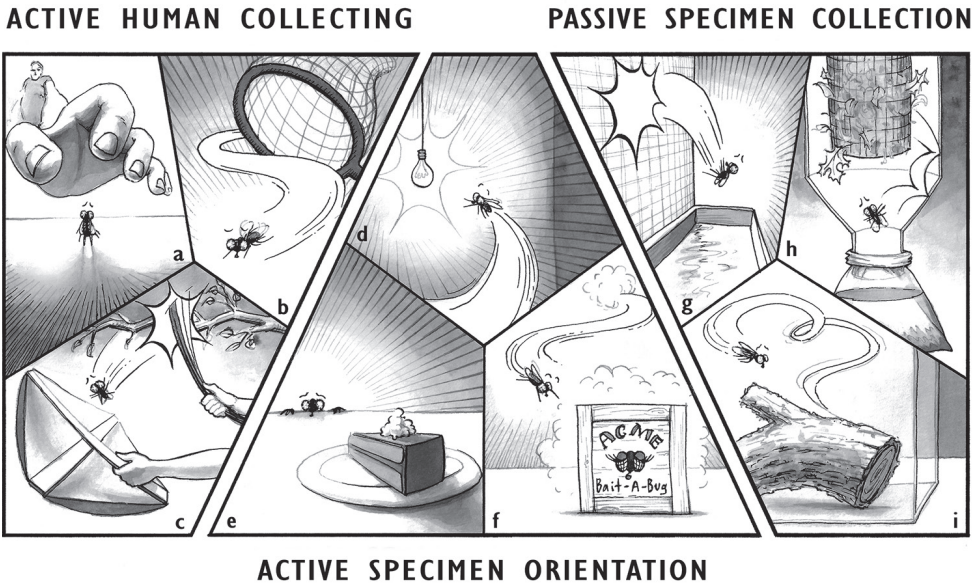

Figure 1. Examples of the three general collecting technique categories. Active human collecting: **a** hand collecting **b** netting **c** using a beat sheet; active specimen orientation **d** light trapping **e** baiting **f** pheromone attractant; passive specimen collection **g** flight intercept trap **h** Winkler Sampler **i** emergence trap.

B. Active specimen orientation

Specimens may be collected by altering the environment in such a way that the specimen actively moves toward the collection device or arena. Five general methods are employed: lights; chemicals; vibrations; heat; and ecological cues (often complex and utilize a combination of the preceding categories). Sections one through four below represent "attractants," the factor that causes the specimen to reorient. They are often paired with a device from Category C when in use, for example a carrion baited (B.2.1) pitfall (C.1.1) trap. Often traps in section five are designed to combine an attractive feature and a passive collection method, for example a Lindgren funnel (B.5.2) incorporates an element of a flight intercept trap (C.1.2).

1. Positive phototaxis – specimen actively moves toward a light contrasted with darkness – night, caves, deep water. In general, this method is poorly known and little studied. The wavelengths of light differ among bulb type and are meaningful when attempting to recollect specimens and or understand specimen response to stimuli. Additional sub-categories could include specific wavelengths, wattages, etc. of each bulb type.
   1.1 Light (general, including standard visible-spectrum bulbs).
      1.1.1 Light – UV (general ultra-violet, typically fluorescent bulb).
      1.1.2 Light – MV (mercury vapor).
      1.1.3 Light – Metal halides.
      1.1.4 Light – LED (light emitting diode, presumably specific wavelengths/ranges could be reported).

2. Chemical bait/lure – use of a medium that emits chemicals to attract specimens. A natural division of this category would be autochthonous (self-originating) and allochthonous (other-originating). Mimicry of chemicals with synthetics, etc. would fall within the category of the chemical being mimicked.
  2.1 Bait – animal matter (carrion, dung, CO_2) (allochthonous).
  2.2 Bait – plant matter (sugar, ethanol) (allochthonous).
  2.3 Bait – Pheromones, etc. (autochthonous) synthetic or natural, e.g. collection of Cupedidae with bleach or male Strepsiptera by using a live female as bait.

3. Vibrational lure – use of vibrations to attract specimens.
  3.1 Sound – air or substrate born vibrations (used for mosquitoes, Asian citrus psyllid).

4. Thermal lure – use of differences in temperature to attract specimens.
  4.1 Heat – used to attract mosquitoes and ticks.

5. Ecological cues – structural alteration of the environment that passively mimics or creates potential habitat or resources that could be used by the specimen. Often multiple attributes, such as color, shape, and size are required to create an effective lure/trap. Two equally valid ways of subdividing ecological cue collection methods are: 1) design (color, structure, etc.); or 2) resource being mimicked (food source, habitat, etc.). Generally, collection with ecological cues will be specific to taxa and an enormous variety of sub-sub-sub (e.g., 5.1.1) categories potentially exist. Plants excel in utilization of this category for pollination and seed dispersal. Often inclusion of chemicals, sounds, heat, etc. may render the trap more effective.
  5.1 Food resource trap – specimens orient toward the trap because it appears to represent a possible food source (yellow pan traps, trap crop, etc.).
  5.2 Habitat resource trap – specimens orient toward the trap because it appears to represent appropriate habitat (Lindgren funnel, bee "hotel", purple panel trap, etc.).

  Alternative 5.1:
  Color trap – specimens actively orient towards the trap because of its color (yellow pan trap, purple panel trap, etc.).

  Alternative 5.2:
  Structure trap – creation of a seemingly suitable habitat (carpenter bee trap, Lindgren funnel, bee "hotel", trap crop, etc.).

C. Passive specimen collection and/or concentration

A structure is introduced into the environment, or an arena is created, where specimens, by virtue of their general movement, concentrate themselves in space and/or time.

1. Passive Trap – alteration of the environment in such a way that specimens unwittingly place themselves in a situation from which they cannot escape.
  1.1 Pitfall, ramp trap – gravity is used to collect and keep specimens in a location, essentially a "walking intercept trap". The trap may be augmented with a barrier.

1.2 Flight Intercept Trap (FIT), window trap – the path of flying insects is obstructed by a barrier. Specimen reaction to the barrier is often taxon specific, therefore placement of the collection device is meaningful. Height of the trap (ground based, canopy, etc.) may also be important.
- **1.2.1** Malaise trap – specimens collected at top.
- **1.2.2** Ground based FIT – specimens collected at bottom.
- **1.2.3** Canopy Trap – elevated, often collection at top and bottom (Sante Trap).

1.3 Flow intercept trap – insects in flowing water are obstructed by a barrier and waylaid or cannot escape.

1.4 Suction trap – air flow is used to collect and keep specimens in a location.

1.5 Glue – "sticky" material used to hold specimens in place (Tanglefoot® placed on a log or standing dead tree).

2. "Sifting" – Short term concentration and manipulation of inhabited material in such a way as to induce specimens to migrate to a collection point. The method is generally defined by the way the material is manipulated.
- **2.1** Berlese/Tullgren funnel – habitat manipulation through heat (Berlese) or heat and light (Tullgren).
- **2.2** Winkler sampler – habitat manipulation through increased surface area and drying.

3. Emergence – long term sequestration of inhabited material with the aim of allowing immature specimens to mature before collection. (As opposed to rearing. Rearing a specimen requires that you have already collected it, then provide it with resources and time to continue its life cycle.) Often the arena is designed so that emergent specimens concentrate themselves in a pitfall- or Malaise-type apparatus.
- **3.1** Emergence chamber – material removed from original site, often fully enclosed.
- **3.2** Emergence trap – full or partial enclosure of material in the field.

Discussion

The above scheme allows for standardization of collection methods within and among databases. The ability to designate a collection method using general standard sets rather than specific, often regional, terms will aid in present and future communication. For example: "treading" and "flooding" are both A.3.1; "Brown sampler" and "vacuum benthos sampler" are both A.2.2; and "freshet," "rejectamenta," and "flood rubbish" are different terms for C.1.3 that occurs during a flood. For some methods separation of attractant used and method of capture will be important.

Additionally, researchers can build a better picture of which general collection methods are best at collecting specific taxa, the highest biodiversity, or even members within a particular guild. However, verbal descriptions can and should be maintained. For example, descriptions such as, "sifting pine duff," "oak leaf litter" "Berlese Hemlock," and "Berl. oak stump" all fall within the same collecting method, C.2, but all contain additional ecological information as well.

The creation and implementation of any standardized collection methods will require an extended discussion by the entomological and biodiversity databasing communities. The above list is provided to begin that discussion. Once approved, those standard methods can be provided as a drop-down list within databases. The numbering scheme can be retained to maintain organization and to illustrate relatedness of techniques, and verbal descriptions should be available to offer guidance.

The following is one possible implementation schema. Collection methods recorded on labels and within notes-sections of databases will continue to be as individualized, general, or specific as collectors wish, similar to the verbal locality on labels (e.g. 24 km SE Cole Camp). Within the database itself, a specific field would accept numbered methods. A drop-down list and descriptions can be provided. Multiple collection methods can be selected, similar to key-words for a manuscript. For example, specimens collected using an emergence chamber would get "C.3.1"; a CDC trap that emits CO_2 and uses a UV light would be "B.1.1.1, B.2.1". If the databasing community is so inclined, additional fields related to collection method can be added, such as killing agent, preservative, minimum and maximum time between specimen death and retrieval, etc.

While compiling the list above it became apparent that no single source contained descriptions of all collection methods known, even those which attempted to be as comprehensive as possible (e.g., Schauff 2001). Therefore, a bibliography is provided containing popular, useful, and/or interesting English language sources on collecting and preserving terrestrial and aquatic arthropods (see Bibliography and References, Suppl. material 1). Many of these works have gone through multiple editions, even though a single edition is cited. Some methods of collection and preservation have fallen out of favor or have been lost to the ages but are just as useful today as in the past, thus perusal of older literature is highly recommended.

However, two general areas need to be updated: chemical usage; and pinned insect labeling. The combination of the two sections below to the materials in the bibliography will go a long way toward elevating those references to a modern standard.

Chemicals: Many chemicals recommended in the past to kill or preserve specimens are now known to be deadly dangerous to humans, and more importantly, can damage the specimen DNA. Authors of new material, especially targeted at younger collectors, have removed dangerous chemicals from their works (modern 4-H manuals (Hall 1998) no longer recommend cyanide (Jones 1940)) and modern workers are more consciousness of killing and preservation methods. When utilizing, citing, or recommending older works, care should be taken to point out some chemical recommendations should not be followed. Consult Whitman et al. (2019) for a discussion on modern views of chemicals used for killing and preserving insects.

Labeling: Recent sources continue to repeat labeling recommendations that were created in a pre-personal printing era (e.g., Gibb and Oseto 2012). Those "systems" spread information across multiple labels (1: locality and date, 2: collector, 3: disposition of specimen) while today, all that information can be contained on a single label. Wheeler et al. (2001) provide a good description of label content and format (but not spacing). Modern specimen labeling should emphasize: 1) making sure that labels are positioned

correctly laterally to reduce the specimen footprint and vertically to leave space for additional labels below; 2) keeping labels small and rectangular; and 3) making sure the label does not get bent or the hole enlarged. Correct labeling takes no more time than incorrect labeling and saves curators and workers an immense amount of work and frustration.

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References

The works cited below consist of, or contain, a section on insect collection and curation methods. A few additional sources specific to particular methods that also provide literature reviews are included.

Arnett Jr RH (2000) American Insects: A Handbook of the Insects of America North of Mexico (2nd edn). CRC Press, NY, 1003 pp. https://doi.org/10.1201/9781482273892

Arnett Jr RH, Jacques Jr RL (1985) Insect Life: A Field Entomology Manual for the Amateur Naturalist. Prentice-Hall, Englewood Cliffs, NJ, 354 pp.

Banks N (1909) Direction for collecting and preserving insects. United States National Museum Bulletin 67: 1–135. https://doi.org/10.5479/si.03629236.67.i

Beirne BP (1955) Collecting, Preparing and Preserving Insects. Canada Department of Agriculture. Science Service, Entomology Division, Publication 932, 133 pp.

Bioquip Products, Inc (2003) How to make an insect collection 3rd ed. Rancho Dominguez, CA, 32 pp.

Borror DJ, White RE (1970) A Field Guide to the Insects of America North of Mexico. Peterson Field Guide Series. Houghton Mifflin, Boston, 404 pp.

Catanach TA (2012) Invertebrate sampling methods for use in wildlife studies. In: Silvy NJ (Ed.) The Wildlife Techniques Manual Research Volume 1 (7th edn). The Johns Hopkins University Press, Baltimore, MD, 336–348.

Chu HF (1949) How to Know the Immature Insects. Pictured Key Nature Series. Wm. C. Brown Company, Dubuque, IA, 234 pp.

Comstock AB (1923) Directions for collecting and preserving insects. Cornell Extension Bulletin 59: 1–12.

Comstock AB (1947) Handbook of Nature-Study. Comstock Publishing Company, Ithaca, NY, 937 pp.

Comstock JH (1914) Insect Life. An Introduction to Nature-Study and a Guide for Teachers, Students, and Others Interested in the Out-of-Door Life. D Appleton and Co., NY, 349 pp.

Dindal DL (1990) Soil Biology Guide. John Wiley and Sons, NY, 1349 pp.

Eymann J, Degreef J, Häuser Ch, Monje JC, Samyn Y, VandenSpiegel D (2010) Manual on Field Recording Techniques and Protocols for All Taxa Biodiversity Inventories and Monitoring 8(1–2): 1–653.

Ferro ML, Carlton CE (2011) A practical emergence chamber for collecting Coleoptera from rotting wood, with a review of emergence chamber designs to collect saproxylic insects. The Coleopterists Bulletin 65: 115–124. https://doi.org/10.1649/072.065.0202

Fox RC (1968) Collecting, Fixing, Preserving, and Shipping of Soft-Bodied Arthropods. South Carolina Agricultural Experiment Station, Miscellaneous Station Circular, Clemson University, Clemson, SC, 7 pp.

Gibb TJ, Oseto CY (2012) How to Make an Awesome Insect Collection! A Beginners Guide to Finding, Collecting, Mounting, Identifying and Displaying Insects. 2nd edition. Perdue Extension, ID-401, Perdue University, 216 pp.

Hall M (1998) 4-H Project Book. Entomology: Insect Study (7th–9th Grade Level). Louisiana Cooperative Extension Service Publication 2609, 47 pp.

Hienton TE (1974) Summary of investigations of electric insect traps. United States Department of Agriculture Technical Bulletin 1498: 1–136.

Hopkins JD, Studebaker G, Lorenz GM III, Loftin KM, Akin S (2007) Making an insect collection. University of Arkansas Division of Agriculture [MP83-PD-3-13RWC]: 1–35.

Howard LO (1923) The Insect Book: A Popular Account of the Bees, Wasps, Ants, Grasshoppers, Flies and other North American Insects Exclusive of the Butterflies, Moths and Beetles, with Full Life Histories, Tables and Bibliographies. Doubleday, Page & Company, Garden City, NY, 429 pp.

Jaques HE (1947) How to Know the Insects. Pictured Key Nature Series. Wm. C. Brown Company, Dubuque, IA, 205 pp.

Jones MP (1940) 4-H club insect manual. United States Department of Agriculture Miscellaneous publication No. 318: 1–63. https://doi.org/10.5962/bhl.title.65490

Kaston BJ (1972) How to Know the Spiders. 2nd edition. WM. C. Brown Company Publishers, Dubuque, IA, 289 pp.

Klots AB (1932) Directions for Collecting and Preserving Insects. Ward's Natural Science Establishment. Rochester, NY, 29 pp.

Knudsen JW (1966) Biological Techniques. Collecting, Preserving, and Illustrating Plants and Animals. Harper and Row, NY, 525 pp. https://doi.org/10.1086/405509

Lutz FE (1917) How to collect and preserve insects. American Museum of Natural History Guide Leaflet Series 39: 1–22.

Lutz FE (1948) Field Book of Insects of the United States and Canada, Aiming to Answer Common Questions. 3rd edition. G. P. Putnam's Sons, NY, 510 pp.

Mansuy MC (1929) Collection and Preservation of Insects for Use in the Study of Agriculture. U.S. Department of Agriculture Farmer's Bulletin No. 1601, 20 pp.

Marshall SA (2006) Insects their Natural History and Diversity. With a Photographic Guide to Insects of Eastern North America. Firefly Books, Buffalo, NY, 718 pp.

Martin JEH (1977) The Insects and Arachnids of Canada Part 1. Collecting, Preparing, and Preserving Insects, Mites, and Spiders. Publication no. 1643, Canada Department of Agriculture, Québec, 182 pp.

McDunnough MC (1919) Directions for collecting and preserving insects. Dominion of Canada Department of Agriculture Entomological Branch Circular 12: 1–14. https://doi.org/10.5962/bhl.title.65610

Merritt RM, Cummins KW, Resh VH, Batzer DP (2008) Chapter 3. Sampling aquatic insects: collection devices, statistical considerations, and rearing procedures. In: Merritt RW, Cummins KW, Berg MB (Eds) An Introduction to the Aquatic Insects of North America, 4th ed. Kendall/Hunt, Dubuque, IA, 15–37.

Millar IM, Uys VM, Urban RP (2000) Collecting and Preserving Insects and Arachnids. A Manual for Entomology and Arachnology. Ultra Litho (Pty) Ltd., Johannesburg, South Africa, 105 pp.

Miller D (2014) Adventures with Insects. 4-H Entomology Project Book. Virginia Cooperative Extension. Publication 444–408, 20 pp.

Nageleisen LM, Bouget C (2009) Forest insect studies: methods and techniques. Key considerations for standardization. An overview of the reflections of the "Environmental Forest Inventories" working group (Inv. Ent. For.). Office National des Forêts, Les Dossiers Forestiers 19: 1–144. https://doi.org/10.1007/978-90-481-3233-1_2

Needham JG (1943) Introducing Insects: A Book for Beginners. The Jaques Cattell Press, Lancaster, PA, 129 pp.

Oldroyd H (1958) Collecting, Preserving and Studying Insects. Hutchinson Scientific and Technical, London, 327 pp.

Oman PW, Cushman AD (1960) Collection and preservation of insects. U. S. Department of Agriculture Miscellaneous Publication No. 601: 1–42.

Oman PW (1952) How to collect and preserve insects for study. In: Stefferud A (Ed.) Insects: The Yearbook of Agriculture 1952. United States Department of Agriculture, U.S. Government Printing Office, 65–78.

Owens BE, Carlton CE (2015) "Berlese vs. Winkler": comparison of two forest litter Coleoptera litter extraction methods and the ECOLI (extraction of Coleoptera in litter) protocol. The Coleopterists Bulletin 69(4): 645–661. https://doi.org/10.1649/0010-065X-69.4.645

Packard Jr AS (1873) Directions for collecting and preserving insects. Smithsonian Miscellaneous Collections 261: 1–55.

Packard AS (1890) Entomology for Beginners. For the Use of Young Folks, Fruit-Growers, Farmers, and Gardeners. Henry Holt and Company, NY, 367 pp.

Peterson A (1937) A Manual of Entomological Equipment and Methods. Part I. Edwards Brothers, Inc., Ann Arbor, MI, 185 pp. [not consecutively paginated]

Peterson A (1937) A Manual of Entomological Equipment and Methods. Part II. Edwards Brothers, Inc., Ann Arbor, MI, 334 pp.

Post RL, Aarhus DG, Perkins HF, Thomasson GL (1969) Insect Collecting Manual. Department of Entomology Agricultural Experiment Station North Dakota State University. North Dakota Insects 8: 1–36.

Riley CV (1892) Directions for collecting and preserving insects. Bulletin of the United States National Museum 39 (Part F): 1–147. https://doi.org/10.5962/bhl.title.2506

Ross HH (1934) How to Collect and Preserve Insects. Illinois State Natural History Survey Circular 25: 1–27. https://doi.org/10.5962/bhl.title.46167

Ross HH (1941) How to Collect and Preserve Insects. Natural History Survey Division Circular 39: 1–48.

Samouelle G (1826) General Directions for Collecting and Preserving Exotic Insects and Crustacea: Designed for the use of Residents in Foreign Countries, Travelers, and Gentlemen going Abroad. With Illustrative Plates. Longman, Rees, Orme, Brown, and Green, London, 72 pp. https://doi.org/10.5962/bhl.title.31086

Sanders DP, Lehker GE, Matthew DL (1980) How to Collect, Preserve and Identify Insects. Extension Circular 509. Cooperative Extension Service, Purdue University, Lafayette, IN, 51 pp.

Sanderson DW, Jackson CF (1912) Elementary Entomology. Ginn and Co., NY, 372 pp. https://doi.org/10.5962/bhl.title.29213

Schauff ME (2001) Collecting and preserving insects and mites: techniques and tools. [Updated and modified WWW version of Steyskal GC, Murphy WL, Hoover EH (Eds) (1986) Insects and mites: techniques for collection and preservation.] Agricultural Research Service, USDA, Miscellaneous Publication 1443: 1–103. http://www.ars.usda.gov/SP2UserFiles/ad_hoc/12754100CollectingandPreservingInsectsandMites/collpres.pdf

Skvarla MJ, Larson JL, Dowling APG (2014) Pitfalls and preservatives: a review. Journal of the Entomological Society of Ontario 145: 15–43.

Smithers C (1981) Handbook of Insect Collecting: Collection, Preparation, Preservation and Storage. David and Charles, London, 120 pp.

Southwood TRE (1975) Ecological Methods. With Particular Reference to the Study of Insect Populations. Chapman and Hall, London, 391 pp.

Southwood TRE, Henderson PA (2000) Ecological Methods. Third Edition. Blackwell Science, London, 575 pp.

Stoltz RL, Homan HW, Craig MJ (2002) Preparing insect specimens for exhibit: for 4-H entomology projects. University of Idaho Extension #82358: 1–9.

Swain RB (1948) The Insect Guide: Orders and Major Families of North American Insects. Doubleday Nature Guide Series. Doubleday and Company, Garden City, NY, 261 pp.

Triplehorn CA, Johnson NA (2005) Borror and Delong's Introduction to the Study of Insects. 7th ed. Brooks/Cole, Belmont, CA, 864 pp.

Ubick D, Paquin P, Cushing PE, Roth V (2005) Spiders of North America: An Identification Manual. American Arachnological Society, Keen, NH, 377 pp.

Uprety DR, Kapoor VC (1979) Collection, Preservation and Identification of Insects. Tribhuvan University Press, Kathmandu, Nepal, 94 pp.

Upton MS (1991) Methods for collecting, preserving, and studying insects and allied forms. Fourth edition. The Australian Entomological Society, Miscellaneous Publication 3: 1–86.

Urquhart FA (1949) Introducing the Insect. Henry Holt and Company, NY, 287 pp.

Wards (1945) How to Make an Insect Collection. Ward's Natural Science Establishment, Inc. Rochester, NY, 32 pp.

Wards (1958) How to Make an Insect Collection: A Beginner's Guide to the Methods and Materials of Insect Collecting, Revised and Expanded. Ward's Natural Science Establishment, Inc. Rochester, NY, 68 pp.

Wards (1967) How to Make an Insect Collection. A Beginner's Guide to the Methods and Materials of Insect Collecting, Revised and Expanded. Ward's Natural Science Establishment, Inc. Rochester, NY, 66 pp.

Wheeler TA, Huber JT, Currie DC (2001) Label data standards for terrestrial arthropods. Biological Survey of Canada (Terrestrial Arthropods), Document Series No. 8: 1–19.

Whitman JD, Yanega D, Watson CBG, Strode VW (2019) Chapter 17: Collection and preservation of terrestrial arthropods. In: Yong WH (Ed.) Biobanking: Methods and Protocols. Methods in Molecular Biology, Vol. 1897. Humana Press, NY, 163–189. https://doi.org/10.1007/978-1-4939-8935-5_17

Wilcox EM (1901) Directions for collecting and preserving insects and plants. Oklahoma Agricultural Experiment Station Circular of Information 3: 1–15.

Williamson EB (1916) Directions for collecting and preserving specimens of dragonflies for museum purposes. University of Michigan Museum of Zoology Miscellaneous Publications 1: 1–15.

Supplementary material 1

Bibliography

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