

Research Article

Taxonomic review of *Amemboa* Esaki, 1925 from China, with description of a new species (Hemiptera, Heteroptera, Gerridae)

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Abstract

The species of *Amemboa* Esaki, 1925 from China are reviewed. *Amemboa hainanica* **sp. nov.**, is described from Hainan Island, and *Amemboa burmensis* J. Polhemus & Andersen, 1984 is newly recorded from China. Additionally, diagnoses and new distribution records are provided for six species previously reported from China: *A. brevifasciata* Miyamoto, 1967, *A. cristata* J. Polhemus & Andersen, 1984, *A. esakii* J. Polhemus & Andersen, 1984, *A. fumi* Esaki, 1925, *A. riparia* J. Polhemus & Andersen, 1984, and *A. speciosa* J. Polhemus & Andersen, 1984. Photographs and line drawings of the habitus, the diagnostic characteristics of males, in-situ habitus, and their habitats are presented. A key and a distribution map are also provided for species of *Amemboa* occurring in China.

Key words: Amemboa, Eotrechinae, Hainan, new records, taxonomy



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Introduction

Amemboa Esaki, 1925 belongs to the water strider subfamily Eotrechinae. Members of this genus typically inhabit the edges of slow-flowing rivers or still ponds near rivers. The species of *Amemboa* are found in South and Southeast Asia, ranging from India to the Philippines, and exhibit a notable diversity in tropical regions (Polhemus and Andersen 1984; Zettel 1995, 1998, 2002; Zettel and Chen 1996, 1997; Zettel et al. 2007; Polhemus and Tran 2012; Basu et al. 2014; Polhemus 2017; Jehamalar et al. 2023). Jehamalar et al. (2023) classified *Amemboa* into 11 species groups based on the morphological characteristics of males. Prior to this study, *Amemboa* comprised a total of 28 described species (Jehamalar et al. 2023).

Six Amemboa species were recorded from China: Polhemus and Andersen (1984) described two species from Taiwan Island, namely A. esakii J. Polhemus & Andersen, 1984 and A. fumi Esaki, 1925. Cheng et al. (2006) reported the distribution of "A. lyra (Paiva, 1918)" in Yunnan. However, Zettel et al. (2007) re-examined the specimens of Cheng et al. (2006) and found that the profemora of males correspond to A. riparia J. Polhemus & Andersen, 1984. Amemboa riparia was recognized as a synonym of A. lyra by Zettel and Chen (1996) and subsequently re-established as a valid species by Polhemus and Tran (2012). Therefore, the species

reported by Cheng et al. (2006) was probably *A. riparia*, while *A. lyra* had no reliable distribution records from China. Additionally, Zettel et al. (2007) first reported the distribution of *A. brevifasciata* Miyamoto, 1967 and *A. speciosa* J. Polhemus & Andersen, 1984 in Hainan, and *A. cristata* Polhemus & Andersen, 1984 in Yunnan.

This study describes a new species, *Amemboa hainanica* sp. nov., from Hainan, China. The study also reports the distribution of *A. burmensis* J. Polhemus & Andersen, 1984 in China for the first time. New distribution data are provided for seven *Amemboa* species. Diagnoses, photographs of diagnostic characteristics, a distribution map, and a key are provided for all *Amemboa* species distributed in China. Line drawings, in-situ habitus photographs, and photographs of habitats are also included.

Materials and methods

All measurements are given in millimeters (mm), representing the average values of the measurements taken from the type specimens. Measurements, observations, and dissections were made using a Zeiss Discovery V8 stereo microscope. Male genitalia were macerated in 5% potassium hydroxide solution (KOH) at room temperature. Photographs of male genitalic structures (pygophore and proctiger) were taken by using a Canon 90D camera equipped with a micro lens. All other photographs except for the male genitalic structures were taken with a Nikon D500 camera equipped with a micro lens and a telephoto lens. The map was prepared using ArcMap v. 10.8 software.

Dried and alcohol-preserved specimens examined in this study have been deposited in Institute of Entomology, College of Life Sciences, Nankai University, Tianjin, China (**NKUM**).

Taxonomic accounts

Family Gerridae Leach, 1815 Subfamily Eotrechinae Matsuda, 1960 Genus *Amemboa* Esaki, 1925

Amemboa hainanica Leng, Jin & Ye, sp. nov.

https://zoobank.org/7D2BD0D7-CDAA-4523-90FB-0C54783AF425 Figs 1, 2A, B, 3A, 4A, B, E, F, 5, 6A, 7A, 8A, 9A, 10A, 11A, 12A, B, 15A, 16, 18



Figure 1. Habitus of *Amemboa hainanica* sp. nov., apterous form in dorsal view **A** holotype, male **B** paratype, female. Scale bar: 2 mm.



Figure 2. Photographs of bodies of *Amemboa* spp., apterous males in dorsal view **A**, **B** *A*. *hainanica* sp. nov **C** *A*. *brevifasciata* Miyamoto, 1967 **D** *A*. *burmensis* J. Polhemus & Andersen, 1984 **E** *A*. *cristata* Polhemus & Andersen, 1984 **F** *A*. *esakii* J. Polhemus & Andersen, 1984 **G** *A*. *fumi* Esaki, 1925 **H**, **I** *A*. *riparia* J. Polhemus & Andersen, 1984 **J** *A*. *speciosa* J. Polhemus & Andersen, 1984 **G** *A*. *fumi* Esaki, 1925 **H**, **I** *A*. *riparia* J. Polhemus & Andersen, 1984 **J** *A*. *speciosa* J. Polhemus & Andersen, 1984 **B** *A*. *speciosa B*. *b*.

19°11'11.1"N, 109°44'21.4"E; 631 m a.s.l.; 25 Jul. 2017; Zhen Ye leg. (NKUM) • 1 \bigcirc , 1 \bigcirc (apterous), Сніма, Hainan Province, Bai-sha County, Luo-shuai Village, Xian-nv-xi; 19°5'58.4"N, 109°32'46.2"E; 324 m a.s.l.; 26 Jul. 2017; Zhen Ye & Juanjuan Yuan leg. (NKUM) • 1 \bigcirc , 1 \bigcirc (apterous), Сніма, Hainan Province, Wan-ning City, Jian-feng-ling Nature Reserve, Yu-lin-gu; 18°42'30.9"N, 108°49'20.2"E; 1 Aug. 2017; Zhen Ye leg. (NKUM) • 1 \bigcirc , 1 \bigcirc (apterous), Сніма, Hainan Province, Qiongzhong County, Beng-ling Village; 18°46'42.7"N, 109°50'29.3"E; 253 m a.s.l.; 9 Aug.



Figure 3. Photographs of bodies of *Amemboa* spp., apterous males in ventral view **A** *A*. *hainanica* sp. nov. **B** *A*. *brevifasciata* Miyamoto, 1967 **C** *A*. *burmensis* J. Polhemus & Andersen, 1984 **D** *A*. *cristata* Polhemus & Andersen, 1984 **E** *A*. *esakii* J. Polhemus & Andersen, 1984 **F** *A*. *fumi* Esaki, 1925 **G**, **H** *A*. *riparia* J. Polhemus & Andersen, 1984 **I** *A*. *speciosa* J. Polhemus & Andersen, 1984. Scale bar: 1 mm.

2017; Zhen Ye leg. (NKUM) • 1 $\stackrel{\circ}{\supset}$, 1 $\stackrel{\circ}{\subsetneq}$ (apterous), Сніла, Hainan Province, Wanning City, Xing-long County, Ai-qing-gu; 18°47'54.3"N, 110°8'19.8"E; 148 m a.s.l.; 11 Aug. 2017; Juan-juan Yuan leg. (NKUM).

Non-type materials: 1 ♂ (apterous), Сніма, Hainan Province, Chang-jiang County, Ba-wang-ling Nature Reserve; 19°7′16.8″N, 109°9′38.2″E; 650 m a.s.l.; 6 Apr. 2008; Bo Cai leg. (NKUM) • 1 ♂ (apterous), Сніма, Hainan Province, Bao-ting County, Xian-an-shi-lin; 18°36′5.8″N, 109°25′59.5″E; 571 m a.s.l.; 7 Aug. 2017; Kun Jiang & Si-ying Fu leg. (NKUM) • 2 ♂♂, 1 ♀ (apterous), CHI-NA, Hainan Province, Bai-sha County, Shi-cai Village, Ying-ge-ling Nature Reserve, Nan-kai Branch Station; 19°4'23.0"N, 109°22'34.0"E; 310 m a.s.l.; 20 Jul. 2013; Yan-hui Wang leg. (NKUM) • 3 ♂♂ (apterous), CHINA, Hainan Province, Dan-zhou City, Lu-mu-wan Waterfall; 19°13'54.6"N, 109°41'16.5"E; 202 m a.s.l.; 22 Jul. 2017; Zhen Ye leg. (NKUM) • 3 ♂♂, 8 ♀♀ (apterous), CHINA, Hainan Province, Tun-chang County, Nan-Iv-ling; 19°13'49.0"N, 110°10'20.8"E; 172 m a.s.l.; 24 Jul. 2017; Zhen Ye leg. (NKUM) • 1 ♂ (apterous), CHINA, Hainan Province, Wu-zhi-shan City, Shui-man Township, Shi-ling-rui Bridge; 18°54'10.9"N, 109°39'23.7"E; 3 Jul. 2014; Qiang Xie leg. (NKUM) • 3 ♂♂, 6 ♀♀ (apterous), CHINA, Hainan Province, Bai-sha County, Ying-ge-ling Nature Reserve, Nan-kai Branch Station; 19°2'1.7"N, 109°24'14.5"E; 20 Jul. 2013; Haoyang Wu leg. (NKUM).

Diagnosis. Color pattern as shown in Figs 1, 2A, B, 3A, 4A, B, E, F, 5A–F, 12A, B. Males: profemur moderately incrassate (Figs 5G, 6A); ventral side of the profemur with two tufts of dark setae on apical 1/2 (Figs 5G, 6A); protibia slightly curved and with an indistinct tumescence on basal 1/3 (Figs 5G, 6A); abdominal segment VIII relatively short (Fig. 8A); pygophore posteriorly with a digitate median process, also with a pair of distinct blunt processes on both sides of the median process (Figs 5H, I, 8A, 9A); median process of pygophore relatively broad in lateral view (Figs 5J, K, 10A, 11A); lateral arm of proctiger relatively slender in ventral view (Figs 5H, 8A), with a distinct subapical process in lateral view, forming a distinct subapical notch on dorsal margin (Figs 5J, 10A).

Comparative notes. Amemboa hainanica sp. nov. is most similar to A. cambodiana D. Polhemus, 2017. In males of both species, the lateral arm of the proctiger in lateral view exhibits a distinct subapical notch on its dorsal margin, isolating a small, slightly recurved apical process, which gives the lateral arm a hooked appearance (Fig. 10A; also see Polhemus 2017: fig. 22). These are the only two known Amemboa species that share this morphological characteristic. Besides, the ventral side of the profemur bears two tufts of dark setae in the males of both species (Fig. 6A; also see Polhemus 2017: fig. 19). Some color patterns of both species are also similar (Figs 2A, 12A; also see Polhemus 2017: figs 14, 15). However, these two species can be distinguished by the following characteristics. The apical hook of lateral arm is more acutely pointed in A. cambodiana (see Polhemus 2017: fig. 22), while it is blunter in A. hainanica sp. nov. (Fig. 10A). The subapical process is weakly developed in A. cambodiana, while it is more distinct in A. hainanica sp. nov. (Fig. 10A; also see Polhemus 2017: fig. 22). The two species can also be separated by the shape of their pygophores, which is campanulate in A. cambodiana (see Polhemus 2017: fig. 21) and lacks the pair of lateral blunt processes seen in A. hainanica sp. nov. (Fig. 9A).

Amemboa hainanica sp. nov. is also similar to A. fumi Esaki, 1925. In males of both species, the ventral side of the profemur bears two tufts of dark setae on apical 1/2, and protibia exhibits an indistinct tumescence on the basal 1/3 (Fig. 6A, G); abdominal segment VIII relatively short (Fig. 7A, F); pygophore has a digitate median process posteromedially and a pair of distinct blunt processes to either side of the median process (Fig. 9A, F). However, A. hainanica sp. nov. differs from A. fumi in the following characteristics. In males of A. hainanica sp. nov., the lateral arm of proctiger bears a distinct subapical process in lateral view (Fig. 10A), while in A. fumi, the lateral arm of proctiger lacks a pro-



Figure 4. Photographs of bodies of *Amemboa* spp., apterous form in lateral view **A**, **B** *A*. *hainanica* sp. nov., males **C** *A*. *burmensis* J. Polhemus & Andersen, 1984, male **D** *A*. *fumi* Esaki, 1925, male **E**, **F** *A*. *hainanica* sp. nov., females **G** *A*. *burmensis*, female **H** *A*. *fumi*, female. Scale bar: 1 mm.

cess in lateral view (Fig. 10F). Additionally, although color characteristics are quite variable within *Amemboa* species, some generalizations can still be made based on the current specimens. In both sexes of *A. hainanica* sp. nov., the black marks and stripes on head, pronotum and mesonotum are more slender and weaker (Figs 2A, 4A, E, 5A, D, 12A), or nearly absent (Figs 2B, 4B, F, 12B), while they are broader and more prominent in *A. fumi* (Figs 2G, 12G). Moreover, the metanotum of *A. hainanica* sp. nov. exhibits brownish-yellow spots

(Figs 2A, 4A, E, 5A, D, 12A) or is predominantly brownish-yellow (Figs 2B, 4B, F, 12B), whereas it is entirely black in *A. fumi* (Figs 2G, 12G).

Description of apterous male. *Measurements.* Body length 3.68-3.96 (holotype 3.76), width 1.39-1.49 (holotype 1.39), head width 0.92-0.98, interocular width 0.43-0.44, eye length (in dorsal view) 0.39-0.43; lengths of antennal segments I-IV: 0.86-0.93: 0.78-0.82: 0.83-0.89: 1.32-1.51; pronotum: length 0.53-0.55, width 0.99-1.11; mesonotum: length 1.25-1.30, width 1.39-1.49; metanotum: length 0.44-0.48, width 1.11-1.17; lengths of leg segments (femur: tibia: tarsal segment I: tarsal segment II): fore leg: 1.24-1.30: 1.17-1.21: 0.28-0.29: 0.16-0.18, middle leg: 3.47-3.56: 2.19-2.25: 0.81-0.87: 0.40-0.43, hind leg: 3.05-3.28: 1.22-1.33: 0.48-0.53: 0.35-0.38.

Color. Body dorsally brownish yellow with black stripes (Figs 1A, 2A, B, 5A). Color pattern as shown in Figs 2A, B, 3A, 4A, B, 5A-C. Head dorsally with several tiny, discontinuous, black spots (Figs 2A, B, 5A); rostrum mainly yellow, with dorsomedian line and segment IV black (Fig. 4A, B). Pronotum with two pairs of black stripes in dorsal view (Figs 2A, 5A), sometimes the pair of lateral stripes nearly absent (Fig. 2B); proacetabulum with a pair of black or brown marks laterally, coxal cleft brown (Figs 3A, 5B). Mesonotum with broad, black stripes medially and slender, black stripes laterally (Figs 2A, 4A, 5B, C), sometimes stripes nearly absent (Figs 2B, 4B); mesopleuron with a relatively broad, black stripe medially on each side, covered by silvery setae (Figs 4A, B, 5C); mesacetabulum with broad, black mark dorsally, covered by silvery setae (Figs 2A, B, 4A, B), basal part of coxal cleft black, other part brownish-black (Figs 3A, 4A) or pale brown (Fig. 4B); mesosternum with a pair of elongate, black stripes anterolaterally, a broad, black stripe medially, and a pair of black spots posterolaterally (Figs 3A, 5B). Metanotum mainly black, with a pair of dark brown spots (Figs 2A, 5A) or mainly brownish-yellow (Fig. 2B); metapleuron dark brown or yellowish-brown (Fig. 4A, B); metacetabulum with broad, black mark dorsally, covered by silvery setae (Figs 2A, B, 4A, B); metasternum mainly black (Figs 3A, 5E). Abdominal tergites I-V black (Figs 2A, 5A) or brownish-yellow (Fig. 2B); mediotergites IV-VIII with brownish-yellow spots medially (Figs 2A, B, 5A); abdominal sterna II-VI completely black, sternite VII mainly black with a pair of yellowish-brown stripes on posterior margin (Figs 3A, 5B); abdominal segment VIII mainly yellow, dorsal part black (Figs 2A, 5A) or with brown spots (Fig. 2B), ventral part with a pair of black spots medially (Figs 3A, 5B).

Structure. Head: elongate, deflected anteriorly; antennal segment I slightly curved, stouter than other segments, with apical part slightly incrassate, antennal tubercles protruded, visible in dorsal view (Fig. 1A); compound eyes large, globular; rostrum stout, reaching anterior part of mesosternum (Fig. 2A, B). **Thorax:** prothorax and metathorax short, mesothorax prolonged; proacetabulum oblique, mesoacetabulum and metacetabulum almost horizontal (Figs 4A, B, 5C); profemur moderately incrassate (Figs 5G, 6A); ventral side of the profemur with two tufts of dark setae on apical 1/2 (Figs 5G, 6A); protibia slightly curved, with an indistinct tumescence on basal 1/3 (Figs 5G, 6A); protarsus stout, with large claws (Figs 5G, 6A); meso- and metafemora relatively long, stouter than meso- and metatibia (Fig. 1A); meso- and metatarsi slender (Fig. 1A); claws of middle and hind legs shorter than claw of fore leg (Fig. 1A). **Abdomen:** mediotergites declined, connexiva raised and convergent (Fig. 2A, B); abdominal segment VII short; abdominal segment VIII cylindriform, protruding from pregenital segments, relatively short



Figure 5. Morphological features of *Amemboa hainanica* sp. nov. **A** body of male, dorsal view **B** body of male, ventral view **C** body of male, lateral view **D** body of female, dorsal view **E** body of female, ventral view **F** body of female, lateral view **G** left foreleg of male, dorsal view **H** pygophore and proctiger of male, ventral view **I** pygophore of male, ventral view **J** pygophore and proctiger of male, lateral view **K** pygophore of male, lateral view. Scale bars: 1 mm (**A**–**F**); 0.2 mm (**G**); 0.1 mm (**H**–**K**).



Figure 6. Left forelegs of Amemboa spp. in dorsal view (males) **A** A. hainanica sp. nov. **B** A. brevifasciata Miyamoto, 1967 **C**, **D** A. burmensis J. Polhemus & Andersen, 1984 **E** A. cristata Polhemus & Andersen, 1984 **F** A. esakii J. Polhemus & Andersen, 1984 **G** A. fumi Esaki, 1925 **H**, **I** A. riparia J. Polhemus & Andersen, 1984 **J** A. speciosa J. Polhemus & Andersen, 1984. Scale bar: 0.2 mm.

and stout (Fig. 7A). *Genitalia*: pygophore posteriorly with a digitate median process (Figs 5H, I, 8A, 9A), also with a pair of distinct blunt processes on both sides of median process in ventral view (Figs 5H, I, 8A, 9A); median process of pygophore relatively broad in lateral view (Figs 5J, K, 10A, 11A); lateral arms of proctiger relatively slender in ventral view (Figs 5H, 8A), with a distinct subapical process in lateral view, forming a distinct subapical notch on the dorsal margin (Figs 5J, 10A).



Figure 7. Abdominal segment VIII of *Amemboa* spp. in dorsal view (males) **A** *A*. *hainanica* sp. nov. **B** *A*. *brevifasciata* Miyamoto, 1967 **C** *A*. *burmensis* J. Polhemus & Andersen, 1984 **D** *A*. *cristata* Polhemus & Andersen, 1984 **E** *A*. *esakii* J Polhemus & Andersen, 1984 **F** *A*. *fumi* Esaki, 1925 **G**, **H** *A*. *riparia* J. Polhemus & Andersen, 1984 **I** *A*. *speciosa* J. Polhemus & Andersen, 1984 **F** *A*. *fumi* Esaki, 1925 **G**, **H** *A*. *riparia* J. Polhemus & Andersen, 1984 **I** *A*. *speciosa* J. *p*. *speciosa* J. *p*. *speciosa A*. *speciosa B*. *specio*



Figure 8. Pygophore and proctiger of *Amemboa* spp. in ventral view **A** *A*. *hainanica* sp. nov. **B** *A*. *brevifasciata* Miyamoto, 1967 **C** *A*. *burmensis* J. Polhemus & Andersen, 1984 **D** *A*. *cristata* Polhemus & Andersen, 1984 **E** *A*. *esakii* J. Polhemus & Andersen, 1984 **F** *A*. *fumi* Esaki, 1925 **G**, **H** *A*. *riparia* J. Polhemus & Andersen, 1984 **I** *A*. *speciosa* J. Polhemus & Andersen, 1984. Abbreviation: la = lateral arm of proctiger. Scale bar: 0.2 mm.



Figure 9. Pygophore of *Amemboa* spp. in ventral view **A** *A*. *hainanica* sp. nov. **B** *A*. *brevifasciata* Miyamoto, 1967 **C** *A*. *burmensis* J. Polhemus & Andersen, 1984 **D** *A*. *cristata* Polhemus & Andersen, 1984 **E** *A*. *esakii* J. Polhemus & Andersen, 1984 **F** *A*. *fumi* Esaki, 1925 **G**, **H** *A*. *riparia* J. Polhemus & Andersen, 1984 **I** *A*. *speciosa* J. Polhemus & Andersen, 1984. Abbreviations: bp = blunt process, lp = lateral process, mp = median process. Scale bar: 0.2 mm.



Figure 10. Pygophore and proctiger of *Amemboa* spp. in lateral view **A** *A*. *hainanica* sp. nov. **B** *A*. *brevifasciata* Miyamoto, 1967 **C** *A*. *burmensis* J. Polhemus & Andersen, 1984 **D** *A*. *cristata* Polhemus & Andersen, 1984 **E** *A*. *esakii* J. Polhemus & Andersen, 1984 **F** *A*. *fumi* Esaki, 1925 **G**, **H** *A*. *riparia* J. Polhemus & Andersen, 1984 **I** *A*. *speciosa* J. Polhemus & Andersen, 1984. Abbreviation: la = lateral arm of proctiger. Scale bar: 0.2 mm.



Figure 11. Pygophore of *Amemboa* spp. in lateral view **A** *A*. *hainanica* sp. nov. **B** *A*. *brevifasciata* Miyamoto, 1967 **C** *A*. *burmensis* J. Polhemus & Andersen, 1984 **D** *A*. *cristata* Polhemus & Andersen, 1984 **E** *A*. *esakii* J. Polhemus & Andersen, 1984 **F** *A*. *fumi* Esaki, 1925 **G**, **H** *A*. *riparia* J. Polhemus & Andersen, 1984 **I** *A*. *speciosa* J. Polhemus & Andersen, 1984. Abbreviation: mp = median process. Scale bar: 0.2 mm.



Figure 12. Photographs of bodies of *Amemboa* spp., females in dorsal view, apterous form if not stated otherwise **A**, **B** *A*. *hainanica* sp. nov. **C** *A*. *brevifasciata* Miyamoto, 1967 **D** *A*. *burmensis* J. Polhemus & Andersen, 1984 **E** *A*. *cristata* Polhemus & Andersen, 1984 **F** *A*. *esakii* J. Polhemus & Andersen, 1984 **G** *A*. *fumi* Esaki, 1925 **H**, **I** *A*. *riparia* J. Polhemus & Andersen, 1984, macropterous form (**H**) and apterous form (**I**) **J** *A*. *speciosa* J. Polhemus & Andersen, 1984. Scale bar: 1 mm.

Description of apterous female. *Measurements.* Body length 4.19-4.52, width 1.76-1.78, head width 1.00-1.02, interocular width 0.45-0.49, eye length (dorsal view) 0.44-0.48; lengths of antennal segments I-IV: 0.93-0.99: 0.80-0.88: 0.91-1.00: 1.41-1.54; pronotum: length 0.50-0.55, width 1.01-1.24; mesonotum: length 1.52-1.58, width 1.75-1.78; metanotum: length 0.50-0.56, width 1.32-1.40; lengths of leg segments (femur: tibia: tarsal segment I: tarsal segment II): fore leg: 1.29-1.44: 1.33-1.38: 0.33-0.34: 0.20-0.21, middle leg: 3.88-4.10: 2.50-2.75: 1.02-1.06: 0.46-0.48, hind leg: 3.40-3.53: 1.48-1.56: 0.56-0.64: 0.43-0.46.

Color. Color pattern as shown in Figs 1B, 5D–F, 12A, B. Similar to apterous male with the following exceptions: mesosternum lacking a pair of black spots posterolaterally (Fig. 5E); metasternum mainly yellow with median part black

(Fig. 5E); metasternum yellow, with a median black mark (Fig. 5E); abdominal sternites II–VII mainly yellow with median black marks, the marks on sternites III–VII with brown spots in the center (Fig. 5E).

Structure. Similar to apterous male with some exceptions: body stouter than male; profemur slightly incrassate, without special modifications (Fig. 1B); protibia straight without special modifications (Fig. 1B). Abdominal segment VII relatively long, genital segments completely withdrawn into segment VII (Figs 5D–F, 12A, B). Proctiger simple, hind margin rounded.

Macropterous forms. Unknown.

Etymology. The specific epithet *hainanica* refers to the island of Hainan, China, the only area in which this species has so far been collected.

Distribution. China: Hainan (Fig. 18).

Habitats. Amemboa hainanica sp. nov. is found in rivers and pools below waterfalls (Fig. 15A), actively moving on the water surface (Fig. 16A, B) and resting at the edges of still water puddles (Fig. 16C).

Amemboa brevifasciata Miyamoto, 1967

Figs 2C, 3B, 6B, 7B, 8B, 9B, 10B, 11B, 12C, 18

Material examined. 1 \bigcirc , 2 \bigcirc \bigcirc (apterous), CHINA, Guangxi Province, Lai-bin City, Jin-xiu County; 24°8'40.2"N, 110°4'49.1"E; 565 m a.s.l.; 25 Jul. 2019; Zhen Ye leg. (NKUM) • 2 \bigcirc (apterous), CHINA, Guangxi Province, Fang-cheng-gang City, Shang-si County, Shi-wan-da-shan; 21°54'4.0"N, 107°54'22.0"E; 300 m a.s.l.; 13 Jul. 2019; Zhen Ye leg. (NKUM).

Diagnosis. Color pattern as shown in Figs 2C, 3B, 12C. Males: profemur moderately incrassate; ventral side of the profemur with two tufts of dark setae on apical 1/2 (Fig. 6B); protibia slightly curved and with an indistinct tumescence on basal 1/3 (Fig. 6B); abdominal segment VIII relatively short (Fig. 7B); pygophore posteriorly with a digitate median process in ventral view, without other special modifications (Figs 8B, 9B); median process of pygophore relatively broad in lateral view (Figs 10B, 11B); lateral arm of proctiger moderately curve and slender in ventral view (Fig. 8B), with a weakly developed subapical process in lateral view (Fig. 10B).

Comparative notes. *A. brevifasciata* Miyamoto, 1967 is most similar to *A. fumi* Esaki, 1925, see comparative notes in Polhemus and Andersen (1984).

Distribution. China: Hainan (Zettel et al. 2007), Guangxi (Fig. 18). Thailand; Vietnam; Peninsular Malaysia; Singapore; Indonesia: Sumatra (Polhemus and Andersen 1984).

Remarks. This species was previously recorded in Hainan (Zettel et al. 2007), but we have not found it in this **distribution.**

New record for China

Amemboa burmensis J. Polhemus & Andersen, 1984 Figs 2D, 3C, 4C, G, 6C, D, 7C, 8C, 9C, 10C, 11C, 12D, 13, 14, 15B, 18

Material examined. 5 ♂♂, 6 ♀♀ (apterous), CHINA, Yunnan Province, De-hong Autonomous Prefecture, Na-bang Village; 24°42'5.8"N, 97°34'25.0"E; 207 m a.s.l.; 15 Apr. 2023; Mu Qiao, Ze-zhong Jin and Zi-he Li leg. (NKUM).



Figure 13. Habitus of *Amemboa burmensis* J. Polhemus & Andersen, 1984, apterous form in dorsal view **A** male **B** female. Scale bar: 1 mm.



Figure 14. Morphological features of *Amemboa burmensis* J. Polhemus & Andersen, 1984 **A** body of male, dorsal view **B** body of male, ventral view **C** body of male, lateral view **D** body of female, dorsal view **E** body of female, ventral view **F** body of female, lateral view **G**, **H** left forelegs of different males, dorsal view **I** pygophore and proctiger of male, ventral view **J** pygophore of male, ventral view **K** pygophore and proctiger of male, lateral view. Scale bars: 1 mm (**A**–**F**); 0.2 mm (**G**, **H**); 0.1 mm (**I**–**L**).



Figure 15. Habitat of *Amemboa* spp. **A** habitat of *A. hainanica* sp. nov., a tranquil pool at the base of the waterfall **B** habitat of *A. burmensis* J. Polhemus & Andersen, 1984, a wide, rocky river **C** habitat of *A. riparia* J. Polhemus & Andersen, 1984, a muddy pool beside the river **D** habitat of *A. riparia*, a small stream in the jungle.

Diagnosis. Color pattern as shown in Figs 2D, 3C, 4C, G, 12D, 13, 14A–F. Males: profemur moderately incrassate; ventral side of the profemur with two tufts of dark setae on apical 1/2 and an additional elongate crest of dark setae on basal 1/2 (Figs 6C, 14G) (sometimes raised, as in Figs 6D, 14H); protibia slightly curved and with a tumescence on basal 1/3 (Figs 6C, 14G); abdominal segment VIII relatively long (Fig. 7C); pygophore posteriorly with a short knoblike median process, and with a pair of blunt processes on both sides of the median process in ventral view (Figs 8C, 9C, 14I, J); median process of pygophore relatively broad in lateral view (Figs 8C, 14I), distinctly broadened subapically in lateral view (Figs 10C, 14K), forming a blunt process (Figs 10C, 14K).

Comparative notes. Amemboa burmensis is most similar to A. kumari (Distant, 1910) and A. cambodiana D. Polhemus, 2017, see comparative notes in Polhemus and Andersen (1984) and Polhemus (2017).

Distribution. China: Yunnan (Fig. 18). Myanmar: Shigbwiyang (Polhemus and Andersen 1984).



Figure 16. *Amemboa hainanica* sp. nov., live habitus in situ **A** an apterous male moving on the water surface **B** an apterous male cleaning its forelegs **C** an apterous male and a nymph standing on the water surface (photographed by Fan Gao). Images not to scale.

Habitat. We found *A. burmensis* inhabiting stagnant pools at the edges of a wide river (Fig. 15B), located near the lower altitudes of the forest in Tong-biguan, Yunnan.

Remarks. In the original description of *A. burmensis*, Polhemus and Andersen (1984) described the profemur of males as follows: "Fore femur moderately incrassate in basal 1/2 (fig. 36), with an elongate patch of short dark hairs on

ventral side". However, among the specimens collected from China, this elongate tuft of setae is either more distinct than the original description (Fig. 6C) or nearly absent (Fig. 6D). We speculate that this may be caused by wear of the setae, or there may be two phenotypes of this species.

Amemboa cristata J. Polhemus & Andersen, 1984

Figs 2E, 3D, 6E, 7D, 8D, 9D, 10D, 11D, 12E, 18

Material examined. 3 33, 4 9 (apterous), THAILAND, Mae Hong Son Province, Pai, Thung Yao, Pam Bok Waterfall; 19°19'14.5"N, 98°24'18.6"E; 549 m a.s.l.; 26 Aug. 2018; Zhen Ye and Juan-juan Yuan leg. (NKUM).

Diagnosis. Color pattern as shown in Figs 2E, 3D, 12E. Males: profemur moderately incrassate (Fig. 6E); ventral side of the profemur with one elongate crest of dark setae on apical 1/2 and an additional elongate crest of dark setae on basal 1/2 (Fig. 6E); protibia slightly curved and with a tumescence on basal 1/3 (Fig. 6E); abdominal segment VIII relatively long (Fig. 7D); pygophore posteriorly with a bifid median process and a pair of strongly produced lateral processes in ventral view (Figs 8D, 9D); median process of pygophore relatively narrow in lateral view (Figs 10D, 11D); lateral arm of proctiger relatively curved and slender in ventral view (Fig. 8D), without subapical process in lateral view (Fig. 10D)

Comparative notes. Amemboa cristata is most similar to A. incurvata; see comparative notes in Polhemus and Andersen (1984)

Distribution. China: Yunnan (Zettel et al. 2007). Thailand; Vietnam; Peninsular Malaysia (Polhemus and Andersen 1984; Zettel and Chen 1996).

Remarks. Zettel et al. (2007) first reported the distribution of *A. cristata* in Xi-shuang-ban-na, Yunnan. In this study, all the examined specimens and those used in the Figures are from Thailand.

Amemboa esakii J. Polhemus & Andersen, 1984

Figs 2F, 3E, 6F, 7E, 8E, 9E, 10E, 11E, 12F, 18

Material examined. 2 33, 4 99 (apterous), CHINA, Taiwan Island, Gao-xiong City, Liu-gui District; 23°0'17.1"N, 120°39'32.7"E; 262 m a.s.l.; 12 Sep. 2017; Juan-juan Yuan leg. (NKUM) • 14 33, 12 99 (apterous), CHINA, Taiwan Island, Ping-dong County, Che-cheng Village, Si-chong-xi; 22°5'29.9"N, 120°45'44.9"E; 18 Nov. 2011; Wen-jun Bu leg. (NKUM) • 1 3, 4 99 (apterous), CHINA, Taiwan Island, Ping-dong County, Man-zhou Village, Lan-ren-xi; 22°2'31.5"N, 120°51'35.6"E; 9 Nov. 2016; Hua-xi Liu leg. (NKUM) • 13 33, 5 99 (apterous), CHINA, Taiwan Island, Ping-dong County, Mu-dan Village, Shou-ka-lin-dao; 22°14'55.3"N, 120°50'44.2"E; 15 Jun. 2013; Zhen Ye leg. (NKUM) • 7 33, 5 99 (apterous), CHINA, Taiwan Island, Ping-dong County, Shi-zi Village, Li-long-shan; 22°10'02.3"N, 120°44'32.5"E; 18 Jun. 2013; Zhen Ye leg. (NKUM) • 3 33, 6 99 (apterous), CHINA, Taiwan Island, Tai-dong County, Bei-nan Village; 22°46'51.9"N, 121°4'28.9"E; 14 Jun. 2013; Zhen Ye leg. (NKUM).

Diagnosis. Color pattern as shown in Figs 2F, 3E, 12F. Males: profemur moderately incrassate; ventral side of the profemur with two tufts of dark setae on apical 1/2 (Fig. 6F); protibia slightly curved and with an indistinct tumescence on basal 1/3 (Fig. 6F); abdominal segment VIII relatively long (Fig. 7E); pygophore posteriorly with a blunt T-shaped median process and a pair of right-angled lateral processes in ventral view (Figs 8E, 9E); median process of pygophore relatively narrow in lateral view (Figs 10E, 11E); posterior margin of pygophore nearly truncate ventrally (Figs 8E, 9E); lateral arm of proctiger relatively curved in ventral view (Fig. 8E), without subapical process in lateral view (Fig. 10E).

Comparative notes. Amemboa esakii is distinct from all congeners in having a T-shaped median process and a truncate posterior margin of pygophore in ventral view (Figs 8E, 9E).

Distribution. China: Taiwan (Fig. 18).

Amemboa fumi Esaki, 1925

Figs 2G, 3F, 4D, H, 6G, 7F, 8F, 9F, 10F, 11F, 12G, 18

Material examined. 3 333 (apterous), CHINA, Taiwan Island, Nan-tou County, Ren-ai Village, Nan-shan-xi; 24°1'36.9"N, 121°5'21.0"E; 6 Jun. 2013; Zhen Ye leg. (NKUM) • 6 333, 5 99 (apterous), CHINA, Taiwan Island, Xin-bei County, Wu-lai District, Xin-xian-bu-dao; 24°50'25.1"N, 121°32'15.0"E; 197 m a.s.l.; 6 Sep. 2017; Juan-juan Yuan leg. (NKUM) • 2 3333 (apterous), CHINA, Taiwan Island, Nan-tou County, Yu-chi Village; 23°50'58.9"N, 120°55'37.0"E; 6 Nov. 2016; Hua-xi Liu leg. (NKUM).

Diagnosis. Color pattern as shown in Figs 2G, 3F, 4D, H, 12G. Males: profemur moderately incrassate (Fig. 6G); ventral side of the profemur with two tufts of dark setae on the apical 1/2 (Fig. 6G); protibia slightly curved and with an indistinct tumescence on basal 1/3 (Fig. 6G); abdominal segment VIII relatively short (Fig. 7F); pygophore posteriorly with a digitate median process in ventral view, also with a pair of blunt processes at lateral sides of the median process, without distinct angular lateral process (Figs 8F, 9F); median process of pygophore relatively narrow in lateral view (Figs 10F, 11F); lateral arm of proctiger relatively curved and slender in ventral view (Fig. 8F), without subapical process in lateral view (Fig. 10F).

Comparative notes. See comparative notes under *A. hainanica* sp. nov. **Distribution.** China: Taiwan (Fig. 18).

Amemboa riparia J. Polhemus & Andersen, 1984

Figs 2H, I, 3G, H, 6H, I, 7G, H, 8G, H, 9G, H, 10G, H, 11G, H, 12H, I, 15C, D, 17, 18

Material examined. 1 ♂, 1 ♀ (apterous), CHINA, Yunnan Province, Xi-shuangban-na Autonomous Prefecture, Jing-hong City, Pu-wen Town; 22°30'36.5"N, 101°3'55.5"E; 880 m a.s.l.; 25 Apr. 2011; Zhen Ye leg. (NKUM) • 7 ♂♂, 6 ♀♀ (apterous), CHINA, Yunnan Province, Xi-shuang-ban-na Autonomous Prefecture, Jing-hong City, Man-dian Village, Na-ban-he Nature Reserve; 22°7'48.5"N, 100°39'46.1"E; 629 m a.s.l.; 28 Jul. 2016; Zhen Ye leg. (NKUM) • 1 ♂ (apterous), CHINA, Yunnan Province, Xi-shuang-ban-na Autonomous Prefecture, Jing-hong City, Meng-la County, Meng-lun Town, Ba-ka-xiao-zhai Village; 21°57'57.0"N, 101°12'16.2"E; 747 m a.s.l.; 5 Jul. 2018; Juan-juan Yuan and Yan-fei Li leg. (NKUM) • 1 ♂ (apterous), CHINA, Yunnan Province, Xi-shuang-ban-na Autonomous Prefecture, Jing-hong City, Meng-la County, Mo-han Town; 21°11'51.0"N, 101°41'55.8"E; 17 Aug. 2014; Zhen Ye leg. (NKUM) • 3 ♀♀ (apterous), CHI-NA, Yunnan Province, Xi-shuang-ban-na Autonomous Prefecture, Jing-hong City, Meng-la County, Yao-qu-yao-zu Township; 21°42'57.0"N, 101°32'32.2"E; 758 m a.s.l.; 26 Jul. 2016; Zhen Ye leg. (NKUM) • 4 \bigcirc \bigcirc , 3 \bigcirc (apterous), CHINA, Yunnan Province, Xi-shuang-ban-na Autonomous Prefecture, Jing-hong City, Man-dian Village, Man-dian-pu-bu; 22°7'45.1"N, 100°40'01.9"E; 660 m a.s.l.; 20 Apr. 2023; Ze-zhong Jin and Zi-he Li leg. (NKUM) • 3 \bigcirc \bigcirc , 2 \bigcirc (apterous), CHINA, Yunnan Province, Pu-er City, Meng-lian County, Meng-ma Town, Mangyun Village; 22°13'50.5"N, 99°21'30.1"E; 887 m a.s.l.; 20 Jul. 2016; Zhen Ye leg. (NKUM) • 2 \bigcirc \bigcirc , 1 \bigcirc (apterous), CHINA, Yunnan Province, Xi-shuang-ban-na Autonomous Prefecture, Jing-hong City, Pu-wen Town, Cai-yang-he; 22°2'43.2"N, 100°56'10.3"E; 25 Apr. 2011; Rui Wang leg. (NKUM) • 1 \bigcirc (apterous), CHI-NA, Yunnan Province, Pu-er City, Si-mao District, Nan-dao-he; 22°36'45.9"N, 100°59'46.6"E; 990 m a.s.l.; 15 Jul. 2018; Juan-juan Yuan leg. (NKUM) • 1 \bigcirc (apterous), 1 \bigcirc (macropterous), CHINA, Yunnan Province, Xi-shuang-ban-na Autonomous Prefecture, Jing-hong City, Nan-la County; 21°44'25.6"N, 101°18'8.0"E; 28 Apr. 2011; Rui Wang leg. (NKUM).

Diagnosis. Color pattern as shown in Figs 2H, I, 3G, H, 12H, I. Males: profemur incrassate (Fig. 6H, I); ventral side of the profemur with two indistinct tufts of short setae on the apical 1/2 and an additional large, elongate crest of dark setae on basal 1/2 (Fig. 6I), occasionally also with a tuft of short setae in the middle (Fig. 6H); protibia slightly curved and with a distinct tumescence on basal 1/2 (Fig. 6H, I); abdominal segment VIII relatively long (Fig. 7G, H); in ventral view, pygophore posteriorly with a short knob-like median process, a pair of distinct angular lateral processes, and a pair of indistinct blunt processes between the median process and lateral processes (Figs 8G, H, 9G; H); median process of pygophore relatively broad in lateral view (Figs 10G, H, 11G, H); lateral arm of proctiger with an angular process laterally and basally curved in ventral view (Fig. 8G, H), and in lateral view with a distinctly pointed subapical process (Fig. 10G, H).

Comparative notes. *A. riparia* is most similar to *A. lyra* (Paiva, 1918), see comparative notes in Polhemus and Tran (2012).

Distribution. China: Yunnan (Fig. 18). Thailand; Laos; Vietnam; Peninsular Malaysia; Singapore (Polhemus and Tran 2012).

Habitats. We observed *A. riparia* inhabiting the edges of streams with extremely slow water currents (Fig. 15D), as well as on completely still ponds (Figs 15C, 17A–C).

Remarks. Based on the reasons listed in the introduction, we believe that the "*A. lyra* (Paiva, 1918)" reported by Cheng et al. (2006) is actually *A. riparia*. Therefore, we conclude that *A. lyra* has not been recorded in China.

The differences between *A. riparia* and *A. lyra* have been extensively discussed by Polhemus and Tran (2012). Moreover, Polhemus and Tran (2012) discovered specimens of *A. riparia* with a small tuft of setae in the middle of the profemora. These specimens were collected from northern Thailand and northern Vietnam, exhibiting genitalic segments correspond to *A. riparia* (Polhemus and Tran 2012). Additionally, the middle setae tufts of profemur also slightly differed from those of *A. lyra* (Polhemus and Tran 2012).

We found several specimens with similar morphology characteristics in southern Yunnan (Figs 2H, 3G, 6G, 7G, 8G, 9G, 10G, 11G, 12H), including a couple from Jinghong City and a male from Pu'er City. These specimens also have a small tuft of setae in the middle of the male profemora. The lateral



Figure 17. *Amemboa riparia* J. Polhemus & Andersen, 1984, habitat and live habitus in situ **A** habitat of *A. riparia*, at the banks of a still pond next to the ravine rainforest **B** apterous male, dorsal view **C** apterous female, lateral view. Images not to scale.

process of the pygophore (Figs 8G, 9G) and the processes on the lateral arms of the proctiger (Fig. 10g) both aligned more closely with those of *A. riparia* (see Polhemus and Andersen 1984: figs 77, 78; also see Polhemus and Tran 2012: fig. 4) rather than *A. lyra* (see Polhemus and Andersen 1984: figs 74, 75; also see Polhemus and Tran 2012: fig. 2). Given that we only found a few individuals and not a large population, we are more inclined to believe that these specimens belong to *A. riparia*, with the male profemur differences resulting from occasional mutations.



Figure 18. Distribution map of Amemboa spp. of China.

Amemboa speciosa J. Polhemus & Andersen, 1984 Figs 2J, 3I, 6J, 7I, 8I, 9I, 10I, 11I, 12J, 18

Material examined. 5 ♂♂, 5 ♀♀ (apterous), CHINA, Hainan Province, Dan-zhou City, Lan-yang Town, Lian-hua-ling; 19°27'6.8"N, 109°38'48.5"E; 213 m a.s.l.; 21 Jul. 2017; Zhen Ye leg. (NKUM) • 5 ♂♂, 5 ♀♀ (apterous), CHINA, Hainan Province, Ling-shui County, Da-xing Waterfall; 18°43'54.9"N, 109°57'1.0"E; 170 m a.s.l.; 8 Aug. 2017; Zhen Ye leg. (NKUM) • 4 강강 (apterous), CHINA, Hainan Province, Qiong-zhong County, Chang-xing Village; 18°48'04.2"N, 110°4'38.2"E; 158 m a.s.l.; 11 Aug. 2017; Zhen Ye leg. (NKUM) • 1 ♂, 2 ♀♀ (apterous), CHINA, Hainan Province, Chang-jiang County, Ba-wang-ling Nature Reserve; 19°5'0.2"N, 109°13'34.0"E; 457 m a.s.l.; 25 Jul. 2017; Zhen Ye leg. (NKUM) • 1 👌 (apterous), CHINA, Hainan Province, Qiongzhong County, Beng-ling Village; 18°46'42.7"N, 109°50'29.3"E; 253 m a.s.l.; 8 Aug. 2017; Zhen Ye leg. (NKUM) • 2 ♂♂, 2 ♀♀ (apterous), CHINA, Yunnan Province, Hong-he Autonomous Prefecture, Lv-chun County, Huang-lian-shan; 22°53'7.0"N, 102°16'16.2"E; 1500-1800 m a.s.l.; 22 Apr. 2011; Rui Wang leg. (NKUM) • 6 공공, 7 ♀♀ (apterous), CHINA, Yunnan Province, Wen-shan Autonomous Prefecture, Ma-guan County, Bao-bao-zhai Village; 22°53'7.8"N, 104°10'59.9"E; 813 m a.s.l.; 8 Aug. 2020; Zhen Ye leg. (NKUM) • 4 ♂♂, 5 ♀♀ (apterous), CHINA, Yunnan Province, Lin-cang City, Cang-yuan County, Ban-hong Town, Nan-gun-he; 23°17'46.7"N, 99°6′18.6″E; 7 May 2011; Zhen Ye leg. (NKUM) • 8 ♂♂, 3 ♀♀ (apterous), CHINA, Yunnan Province, Xi-shuang-ban-na Autonomous Prefecture, Jing-hong City, Manhe-hui Waterfall; 21°53'49.5"N, 100°46'3.0"E; 603 m a.s.l.; 23 Nov. 2018; Zhen Ye leg. (NKUM).

Diagnosis. Color pattern as shown in Figs 2J, 3I, 12J. Males: profemur incrassate (Fig. 6J); ventral side of the profemur with two tufts of dark setae on the apical 1/2 and an additional large, elongate crest of dark setae on basal 1/2 (Fig. 6J); protibia slightly curved and with an indistinct tumescence medially (Fig. 6J); abdominal segment VIII relatively long (Fig. 7I); pygophore posteriorly with a median broad triangular process in ventral view, without other special modifications (Figs 8I, 9I); median process of pygophore relatively broad in lateral view (Figs 10I, 11I). Lateral arm of proctiger relatively simple, tapering towards narrow apex in ventral and lateral views (Figs 8I, 10I), relatively broad-ened proximally and without subapical process in lateral view (Fig. 10I).

Comparative notes. Amemboa speciosa J. Polhemus & Andersen, 1984 is most similar to *A. intermedia* Zettel & Chen, 1996; see comparative notes in Zettel and Chen (1996).

Distribution. China: Hainan, Yunnan (Fig. 18). Thailand (Zettel and Chen 1997); Laos (Zettel 1998); Vietnam (Polhemus and Andersen 1984).

Key to species of Amemboa Esaki, 1925 of China (Males)

- Profemur with 2 elongate crests of dark setae (Fig. 6E). Median process of pygophore posteriorly bifid (Fig. 9D).... A. cristata J. Polhemus & Andersen, 1984

- 3 Protibia with a relatively distinct process (Fig. 6H, I). Lateral process of pygophore angular and distinctly produced in ventral view (Fig. 9G, H). Lateral arm of proctiger with a distinct lateral process basally in ventral view (Fig. 8G, H), also with a distinctly pointed process in lateral view (Fig. 10G, H).....
- 4 Median process of pygophore knob-like in ventral view (Fig. 9C). Lateral arm of proctiger relatively wide in lateral view (Fig. 10C).....
- A. burmensis J. Polhemus & Andersen, 1984
 Median process of pygophore broadly triangular in ventral view (Fig. 9I). Lateral arm of proctiger relatively narrow in lateral view (Fig. 10I)
 A. speciosa J. Polhemus & Andersen, 1984
- 5 Protibia with a distinct angular process on basal part (Fig. 6F). Abdominal segment VIII relatively long (Fig. 7E). Pygophore posteriorly with a blunt T-shaped median process in ventral view (Fig. 9E). Posterior margin of pygophore truncated, not tapering towards the end in ventral view (Fig. 9E) A. esakii J. Polhemus & Andersen, 1984

- Lateral black stripes on mesonotum relatively slim (Figs 2A, 12A) or nearly lost (Figs 2B, 12B) in dorsal view. Lateral arm of proctiger with a distinct subapical process in lateral view (Fig. 10A). Endemic to Hainan Island
 A. hainanica sp. nov.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

The first author identified and dissected the specimens, took photographs, drawed line drawings, and wrote the manuscript. The second author participated in photographing the specimens. The third author contributed to the dissection of the specimens and the revision of the manuscript. The corresponding author was responsible for revising the manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

New records and revised distribution of tiger beetles in China (Coleoptera, Cicindelidae)

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Abstract

Based on the examination of specimens housed in several scientific collections, we expand the known tiger beetle fauna of China, and eight species are recorded from China for the first time. The occurrence of *Cicindela* (*Cicindela*) sachalinensis raddei Morawitz, 1863 in Shanxi Province, and *Neocollyris* (*Neocollyris*) saphyrina (Chaudoir, 1850) in China are re-established. We provide distribution maps and habitus photographs of examined specimens for the newly recorded and revised species. We also discuss potential research hotspots for future taxonomic studies of tiger beetles in China.

Key words: China, distribution, new records, taxonomy, tiger beetles



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Introduction

Tiger beetles (Coleoptera, Cicindelidae) are a globally distributed family that occupies almost all terrestrial ecosystems (Pearson and Vogler 2001). More than 2,850 species are currently known to science (Wiesner 2020), of which the richest diversity is found in the Oriental realm (Cassola and Pearson 2000; Pearson and Wiesner 2023). China spans both the Palearctic and Oriental realms, with significant climatic differences both between north and south as well as between west and east. In addition, altitudinal zones should be also considered. It features numerous examples of biogeographic isolation that can facilitate speciation. These biogeographic barriers include the Qinling Mountains–Huaihe River, Hengduan Mountains, Tianshan Mountains, Greater Khingan Mountains, Nanling Mountains, and so on. As a result, China has a unique and rich tiger beetle fauna, representing a mixture of Palearctic and Oriental species together with a high degree of endemism (Li et al. 2012).

There has been limited research on the overall distribution of tiger beetles in China (Shook and Wiesner 2006; Wu and Shook 2007; Wu 2011; Aston 2016, 2018). The distribution data that have been available was primarily sourced from publications, private specimen collections, and local museums. Consequently, the known distributional ranges of some species in China are patchy and the actual ranges of these species may extend far beyond the existing records. This article summarizes data from tiger beetle specimens in several universities and research institutes across China, along with those collected by the corresponding author's research team. Through these specimens, we update and expand the known distribution of some species of tiger beetle throughout China. Some provinces included here have lacked new records of tiger beetles for many years. The results expand our understanding of the tiger beetle fauna of China.

Materials and methods

In addition to these specimen data, other records were extracted from Wiesner (2020) and Lorenz (2021).

Photographs of the habitus of specimens were taken with a Canon EOS M6 Mark II. A Godox TT350c Flash was used as a light source. Helicon Focus v. 7 was used for image stacking and all images were further processed in Adobe Photoshop CS6.

The specimens reported below are deposited in the following collections:

EMCAU	Entomological Museum of China Agricultural University, Beijing,		
	China		
IZCAS	Institute of Zoology, Chinese Academy of Sciences, Beijing, China		
KIZCAS	Kunming Institute of Zoology, Chinese Academy of Sciences,		
	Kunming, China		
NEFU	Northeast Forestry University, Harbin, China		

Distribution maps were generated using ArcGIS v. 10.2. Map data were sourced online from http://xzqh.mca.gov.cn/map (Fig. 1). The English translations of Chinese provinces and regions were partially extracted from



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1.	Heilongjiang	2.	Jilin
3.	Inner Mongolia	4.	Xinjiang
5.	Liaoning	6.	Beijing
7.	Hebei	8.	Tianjin
9.	Gansu	10.	Shanxi
11.	Ningxia	12.	Shandong
13.	Qinghai	14.	Shaanxi
15.	Henan	16.	Xizang
17.	Jiangsu	18.	Anhui
19.	Shanghai	20.	Hubei
21.	Sichuan	22.	Chongqing
23.	Zhejiang	24.	Jiangxi
25.	Hunan	26.	Guizhou
27.	Fujian	28.	Yunnan
29.	Taiwan	30.	Guangxi
31.	Guangdong	32.	Hong Kong
33.	Масао	34.	Hainan

Table 1. Provinces, autonomous regions, and municipalities of China.

https://www.stats.gov.cn/sj/ndsj/2023/indexeh.htm (Table 1). Distribution maps of species were processed using Adobe Photoshop CS6.

Taxonomy

Family Cicindelidae Latreille, 1802 Tribe Cicindelini Latreille, 1802 Subtribe Cicindelina Latreille, 1802 Genus *Abroscelis* Hope, 1838

Abroscelis anchoralis anchoralis (Chevrolat, 1845) Figs 2A, 3A

Cicindela anchoralis: Chevrolat 1845: 7.

Abroscelis anchoralis anchoralis: Lin and Ho 2007: 180; Shook and Wiesner 2006: 7; Wu 2011: 23.

Published data. Liaoning (Shook and Wiesner 2006: 7; Wu 2011: 23), Beijing (Shook and Wiesner 2006: 7; Wu 2011: 23), Hebei (Shook and Wiesner 2006: 7), Shandong (Shook and Wiesner 2006: 7; Wu 2011: 23), Zhejiang (Shook and Wiesner 2006: 7; Wu 2011: 23), Hainan (Lin and Ho 2007: 180), Hong Kong (Shook and Wiesner 2006: 7; Wu 2011: 23), Macao (Chevrolat 1845: 7; Shook and Wiesner 2006: 7; Wu 2011: 23), Taiwan (Lin and Ho 2007: 180).

New records. Guangdong, Zhanjiang, Min'an Town, 20°57'28"N, 110°15'6"E, 1 m, leg. H.B. Liang and X.L. Huang, 28.vi.2014, 1 male (IZCAS).

Distribution. China (Guangdong, Liaoning, Beijing, Hebei, Shandong, ?Jiangsu, Zhejiang, ?Fujian, Hainan, Hong Kong, Macao, Taiwan).

Remarks. New provincial record for Guangdong.



Figure 2. Habitus photographs **A** *Abroscelis anchoralis anchoralis* (Chevrolat, 1845) **B** *Apterodela* (*Apterodela*) *bivirgulata bivirgulata* (Fairmaire, 1889) **C** *Callytron nivicinctum* (Chevrolat, 1845) **D** *Calomera chloris* (Hope, 1831) **E** *Calomera plumigera scoliographa* (Rivalier, 1953) **F** *Cicindela* (*Cicindela*) *campestris pontica* Fischer, 1828. Scale bars: 5 mm.

Genus Apterodela Rivalier, 1950 Subgenus Apterodela Rivalier, 1950

Apterodela (Apterodela) bivirgulata bivirgulata (Fairmaire, 1889) Figs 2B, 3B

Cicindela bivirgulata: Fairmaire 1889: 5. *Cylindera (Apterodela) lobipennis*: Shook and Wiesner 2006: 13; Wu 2011: 26. *Apterodela (Apterodela) bivirgulata*: Matalin et al. 2024: 311.

Published data. Shandong (Matalin et al. 2024: 311), Henan (Matalin et al. 2024: 311), Hubei (Matalin et al. 2024: 311), Anhui (Matalin et al. 2024: 311), Jiangxi (Matalin et al. 2024: 311), Jiangsu (Matalin et al. 2024: 311), Shang-



Figure 3. Distribution maps. Green indicates records with previously known distribution based on published data, blue indicates new records, red stars indicate the collection sites of the examined specimens **A** *Abroscelis anchoralis anchoralis* (Chevrolat, 1845) **B** *Apterodela* (*Apterodela*) *bivirgulata bivirgulata* (Fairmaire, 1889) **C** *Callytron nivicinctum* (Chevrolat, 1845) **D** *Calomera chloris* (Hope, 1831) **E** *Calomera plumigera scoliographa* (Rivalier, 1953) **F** *Cicindela* (*Cicindela*) *campestris pontica* Fischer, 1828.

hai (Matalin et al. 2024: 311), Chongqing (Matalin et al. 2024: 311), Zhejiang (Matalin et al. 2024: 311), Fujian (Matalin et al. 2024: 311).

Records. Jiangsu, leg. unknown, 18.iv.1923, 1 male (IZCAS).

Distribution. China (Shandong, Henan, Hubei, Anhui, Jiangxi, Jiangsu, Shanghai, Chongqing, Zhejiang, Fujian).

Remarks. Additional specimens contribute to a better understanding of the distribution and a more robust identification of *A*. (*A*.) *b. bivirgulata*.

Genus Callytron Gistl, 1848

Callytron nivicinctum (Chevrolat, 1845)

Figs 2C, 3C

Cicindela nivicincta: Chevrolat 1845: 98.

Callytron nivicinctum: Shook and Wiesner 2006: 8; Wu 2011: 23; Wiesner et al. 2017: 68.

Published data. Liaoning (Wu 2011: 23; Wiesner et al. 2017: 68), Jiangsu (Shook and Wiesner 2006: 8; Wu 2011: 23; Wiesner et al. 2017: 68), Zhejiang (Shook and Wiesner 2006: 8; Wu 2011: 23; Wiesner et al. 2017: 68), Shanghai (Shook and Wiesner 2006: 8; Wu 2011: 23; Wiesner et al. 2017: 68), Fujian (Shook and Wiesner 2006: 8; Wu 2011: 23; Wiesner et al. 2017: 68), Guangdong (Shook and Wiesner 2006: 8; Wu 2011: 23; Wiesner et al. 2017: 68), Hainan (Shook and Wiesner 2006: 8; Wu 2011: 23; Wiesner et al. 2017: 68), Hainan (Shook and Wiesner 2006: 8; Wu 2011: 23; Wiesner et al. 2017: 68), Hong Kong (Shook and Wiesner 2006: 8; Wu 2011: 23; Wiesner et al. 2017: 68), Macao (Shook and Wiesner 2006: 8; Wu 2011: 23; Wiesner et al. 2017: 68), Macao (Shook and Wiesner 2006: 8; Wu 2011: 23; Wiesner et al. 2017: 68), Macao (Shook and Wiesner 2006: 8; Wu 2011: 23; Wiesner et al. 2017: 68).

New records. Guangxi, Longzhou, Mount Daqing, leg. J.K. Yang, 15.v.1963, 1 female (EMCAU).

Distribution. China (Guangxi, Liaoning, Jiangsu, Zhejiang, Shanghai, Fujian, Guangdong, Hainan, Hong Kong, Macao), South Korea, Japan, Cambodia, Vietnam.

Remarks. New provincial record for Guangxi.

Genus Calomera Motschulsky, 1862

Calomera chloris (Hope, 1831) Figs 2D, 3D

Cicindela chloris: Hope 1831: 21.

New records. Xizang, Linzhi, Xiachayu, 28°29'59"N, 97°1'3"E, leg. J. Wu, 28.vii.2014, 1 female (IZCAS); Xizang, Bomi, Tongmai, 2250 m, leg. unknown, 31.viii.2005, 1 female (IZCAS).

Distribution. China (Xizang), Afghanistan, Pakistan, Nepal, Bhutan, India, Laos. **Remarks.** New state record for China and new provincial record for Xizang.

Calomera plumigera scoliographa (Rivalier, 1953) Figs 2E, 3E

193 22, 32

Calomera plumigera scoliographa: Shook and Wiesner 2006: 9; Shook and Wu 2006: 39; Wu 2011: 24.

Published data. Yunnan (Shook and Wiesner 2006: 9; Shook and Wu 2006: 39; Wu 2011: 24), Hainan (Shook and Wiesner 2006: 9; Wu 2011: 24).

New records. Guangxi, Jinxiu, Dazhang, leg. unknown, ?.v.1976, 1 male (IZCAS).

Distribution. China (Guangxi, Yunnan, Hainan), Vietnam, Laos, Cambodia, Malaysia, Thailand.

Remarks. New provincial record for Guangxi.

Genus Cicindela Linnaeus, 1758 Subgenus Cicindela Linnaeus, 1758

Cicindela (Cicindela) campestris pontica Fischer, 1828 Figs 2F, 3F

Cicindela campestris pontica: Gebert et al. 2021: 473.

New records. Xinjiang, Altai, Forest Region of Ashan Forest Management Bureau, leg. unknown, v.1981, 1 female (IZCAS).

Distribution. China (Xinjiang), Ukraine, Bulgaria, Turkey, Georgia, Azerbaijan, Kazakhstan, Russia.

Remarks. New state record for China and new provincial record for Xinjiang.

Cicindela (Cicindela) sachalinensis raddei Morawitz, 1863 Figs 4A, 5A

Cicindela raddei: Morawitz 1863: 237, 238.

Cicindela sachalinensis: Yu and Wang 2017:145; Di and Ren 2021: 146.

Cicindela sachalinensis raddei: Mandl 1981: 27.

Cicindela (*Cicindela*) *sachalinensis raddei*: Shook and Wiesner 2006: 11; Wu 2011: 25.

Published data. Heilongjiang (Shook and Wiesner 2006: 11; Wu 2011: 25), Gansu (Shook and Wiesner 2006: 11; Wu 2011: 25), Beijing (Huairou) (Yu and Wang 2017: 145), Hebei (Mount Xiaowutai) (Di and Ren 2021: 146), Qinghai (Shook and Wiesner 2006: 11; Wu 2011: 25), Shanxi (Wutaishan) (Mandl 1981: 27), Hubei (Shook and Wiesner 2006: 11; Wu 2011: 25), Sichuan (Shook and Wiesner 2006: 11; Wu 2011: 25).

Records. Shanxi, Mount Wutai, Dongtai, 2400 m, leg. T.S. Li, 13.vi.1964, 1 female (IZCAS).

Distribution. China (Shanxi, Heilongjiang, Gansu, Qinghai, Beijing, Hebei, Hubei, Sichuan), Russia, Mongolia.

Remarks. Mandl (1981) reported *C*. (*C*.) *s. raddei* from Chunshantu, Mount Wutai, Shanxi. This record was not accepted in previous checklists. We restore the provincial record of *C*. (*C*.) *s. raddei* for Shanxi.

Cicindela (Cicindela) transbaicalica hamifasciata Kolbe, 1886 Figs 4B, 5B

Cicindela japanensis hamifasciata: Kolbe 1886: 170. *Cicindela hybrida transbaicalica*: Wang et al. 2012: 15.



Figure 4. Habitus photographs **A** *Cicindela* (*Cicindela*) sachalinensis raddei Morawitz, 1863 **B** *Cicindela* (*Cicindela*) transbaicalica hamifasciata Kolbe, 1886 **C** *Cosmodela* separata (Fleutiaux, 1893) **D** *Cylindera* (*Cylindera*) obliquefasciata (Adams, 1817) **E** *Cylindera* (*Eriodera*) albopunctata (Chaudoir, 1852) **F** *Cylindera* (*Eugrapha*) contorta contorta (Fischer, 1828). Scale bars: 5 mm.

Cicindela (*Cicindela*) *transbaicalica hamifasciata*: Shook and Wiesner 2006: 11; Wu 2011: 25.

Published data. Heilongjiang (Wu 2011: 25), Jilin (Wu 2011: 25), Inner Mongolia (Wu 2011: 25), Liaoning (Wu 2011: 25; Wang et al. 2012: 15), Gansu (Shook and Wiesner 2006: 11; Wu 2011: 25), Xinjiang (Wu 2011: 25), Shaanxi (Shook and Wiesner 2006: 11; Wu 2011: 25), Shanxi (Wu 2011: 25), Anhui (Wu 2011: 25), Henan (Shook and Wiesner 2006: 11; Wu 2011: 25), Hebei (Shook and Wiesner 2006: 11; Wu 2011: 25), Jiangsu (Wu 2011: 25), Shandong (Shook and Wiesner 2006: 11; Wu 2011: 25), Sichuan (Shook and Wiesner 2006: 11; Wu 2011: 25), Sichuan (Shook and Wiesner 2006: 11; Wu 2011: 25), Fujian (Shook and Wiesner 2006: 11; Wu 2011: 25), Guangdong (Wu 2011: 25), Zhejiang (Wu 2011: 25), ? Yunnan (Wu 2011: 25).

New records. Beijing, Shunyi, leg. unknown, 13.v.1964, 2 males, 3 females (EMCAU); Jilin, Songhua River, Wukeshu, 44°27'43"N, 126°49'33"E, 200 m, leg. Y. Liu, 1.vi.2009, 1 male (IZCAS); Inner Mongolia, Chifeng, day collecting, leg. Y.


Figure 5. Distribution maps. Green indicates records with previously known distribution based on published data, blue indicates new records, red stars indicate the collection sites of the examined specimens **A** *Cicindela* (*Cicindela*) *sachalinensis raddei* Morawitz, 1863 **B** *Cicindela* (*Cicindela*) *transbaicalica hamifasciata* Kolbe, 1886 **C** *Cosmodela separata* (Fleutiaux, 1893) **D** *Cylindera* (*Cylindera*) *obliquefasciata* (Adams, 1817) **E** *Cylindera* (*Eriodera*) *albopunctata* (Chaudoir, 1852) **F** *Cylindera* (*Eugrapha*) *contorta contorta* (Fischer, 1828).

Wang and S.Y. Geng, 26–30.vii. 2009, 1 female (NEFU); Anhui, Yuexi, Yaoluoping, 30°59'01"N, 116°05'10", 1178 m, leg. unknown, 17.v.2021, 1 female (IZCAS). **Distribution.** China (Beijing, Heilongjiang, Jilin, Inner Mongolia, Liaoning, Gansu, Xinjiang, Shaanxi, Shanxi, Anhui, Henan, Hebei, Hubei, Jiangsu, Shandong, Sichuan, Qinghai, ?Fujian, ?Guangdong, ?Zhejiang, ?Yunnan), North Korea, South Korea, Russia.

Remarks. New provincial record for Beijing. Wu (2011) expanded the known distribution of *C*. (*C*.) *t. hamifasciata* Kolbe, 1886 in China by reviewing the Chinese literature, but Wu did not provide specimen data. We provide specimen data for this species from Jilin, Inner Mongolia and Anhui for the first time. Since *C*. (*C*.) *t. hamifasciata* Kolbe, 1886 is a typical Eastern Palearctic species, we believe previous records from Fujian, Zhejiang, Guangdong, and Yunnan require further confirmation.

Genus Cosmodela Rivalier, 1961

Cosmodela separata (Fleutiaux, 1893)

Figs 4C, 5C

Cicindela separata: Fleutiaux 1893: 491.

Cosmodela separata: Shook and Wiesner 2006: 13; Wu 2011: 26; Wiesner et al. 2017: 53; Tu et al. 2020: 68.

Published data. Shanxi (Shook and Wiesner 2006: 13; Wu 2011: 26; Wiesner et al. 2017: 53), Jiangsu (Shook and Wiesner 2006: 13; Wu 2011: 26; Wiesner et al. 2017: 53), Shanghai (Fleutiaux 1893: 491; Shook and Wiesner 2006: 13; Wu 2011: 26; Wiesner et al. 2017: 53), Zhejiang (Fleutiaux 1893: 491; Shook and Wiesner 2006: 13; Wu 2011: 26; Wiesner et al. 2017: 53), Henan (Shook and Wiesner 2006: 13; Wu 2011: 26; Wiesner et al. 2017: 53), Anhui (Shook and Wiesner 2006: 13; Wu 2011: 26; Wiesner et al. 2017: 53), Hubei (Wu 2011: 26; Wiesner et al. 2017: 53), Hubei (Wu 2011: 26; Wiesner et al. 2017: 53), Jiangxi (Tu et al. 2020: 68), Fujian (Shook and Wiesner 2006: 13; Wu 2011: 26; Wiesner et al. 2017: 53), Yunnan (Shook and Wiesner 2006: 13; Wu 2011: 26; Wiesner et al. 2017: 53), Yunnan (Shook and Wiesner 2006: 13; Wu 2011: 26; Wiesner et al. 2017: 53), Yunnan (Shook and Wiesner 2006: 13; Wu 2011: 26; Wiesner et al. 2017: 53), Yunnan (Shook and Wiesner 2006: 13; Wu 2011: 26; Wiesner et al. 2017: 53), Yunnan (Shook and Wiesner 2006: 13; Wu 2011: 26; Wiesner et al. 2017: 53), Yunnan (Shook and Wiesner 2006: 13; Wu 2011: 26; Wiesner et al. 2017: 53), Yunnan (Shook and Wiesner 2006: 13; Wu 2011: 26; Wiesner et al. 2017: 53).

New records. Guangxi, Jinxiu, Tongmu Town, leg. J.C. Huang, 4.vii.1981, 2 females (IZCAS); Guizhou, Shiqian, Mount Foding, 750 m, leg. X.K. Yang, 24.XI.1988, 1 female (IZCAS).

Distribution. China (Guangxi, Guizhou, Shanxi, Jiangsu, Shanghai, Zhejiang, Henan, Anhui, Hubei, Hunan, Jiangxi, Fujian, Yunnan), Vietnam.

Remarks. New provincial records for Guangxi and Guizhou.

Genus Cylindera Westwood, 1831 Subgenus Cylindera Westwood, 1831

Cylindera (Cylindera) obliquefasciata (Adams, 1817) Figs 4D, 5D

Cicindela obliquefasciata: Adams 1817: 280. *Cylindera (Cylindera) obliquefasciata obliquefasciata*: Shook and Wiesner 2006: 14; Wu 2011: 27.

Published data. Heilongjiang (Shook and Wiesner 2006: 14; Wu 2011: 27), Jilin (Wu 2011: 27), Inner Mongolia (Shook and Wiesner 2006: 14; Wu 2011: 27), Liaoning (Wu 2011: 27), Beijing (Wu 2011: 27), Hebei (Shook and Wiesner 2006: 14; Wu 2011: 27), Gansu (Shook and Wiesner 2006: 14; Wu 2011: 27), Qinghai

(Shook and Wiesner 2006: 14; Wu 2011: 27), Xinjiang (Shook and Wiesner 2006: 14; Wu 2011: 27), Shanxi (Wu 2011: 27), Shandong (Shook and Wiesner 2006: 14; Wu 2011: 27), Henan (Shook and Wiesner 2006: 14; Wu 2011: 27).

Records. Beijing, Haidian, Fragrant Hills Park, leg. unknown, 16.vii.1962, 2 males, 3 females (EMCAU); Shanxi, Taiyuan, Qingxu, leg. unknown, 20.vi.1960, 1 male (EMCAU).

Distribution. China (Heilongjiang, Jilin, Inner Mongolia, Liaoning, Beijing, Hebei, Gansu, Qinghai, Xinjiang, Shanxi, Shandong, Henan), Russia.

Remarks. Wu (2011) reported *C*. (*C*.) *obliquefasciata* (Adams, 1817) from Beijing and Shanxi but did not provide specific specimen data. We provide specimen data for this species from Beijing and Sichuan for the first time.

Subgenus Eriodera Rivalier, 1961

Cylindera (Eriodera) albopunctata (Chaudoir, 1852) Figs 4E, 5E

Cicindela albopunctata: Chaudoir 1852: 10. *Cylindera (Eriodera) albopunctata*: Shook and Wiesner 2006: 14.

Published data. Sichuan (Wu 2011: 27), Yunnan (Shook and Wiesner 2006: 14; Wu 2011: 27), Xizang (Shook and Wiesner 2006: 14; Wu 2011: 27).

Records. Sichuan, Yanyuan, Jinhe, 1200 m, leg. D.J. Liu, 29.vi.1984, 1 female (IZCAS).

Distribution. China (Sichuan, Yunnan, Xizang), Pakistan, Nepal, Bhutan, India, Vietnam.

Remarks. Wu (2011) reported *C*. (*E*.) *albopunctata* (Chaudoir, 1852) from Sichuan but did not provide specific specimen data. We provide specimen data for this species from Sichuan for the first time.

Subgenus Eugrapha Rivalier, 1950

Cylindera (Eugrapha) contorta contorta (Fischer, 1828) Figs 4F, 5F

Cylindera (Eugrapha) contorta contorta: Shook and Wiesner 2006: 14.

Published data. Gansu (Li and Chen 1993; Shook and Wiesner 2006: 14; Wu 2011: 27), Qinghai (Li and Chen 1993; Shook and Wiesner 2006: 14; Wu 2011: 27), Xinjiang (Li and Chen 1993; Shook and Wiesner 2006: 14; Wu 2011: 27), Inner Mongolia (Wu 2011: 27).

Records. Inner Mongolia, Alxa League, Alxa Right Banner, leg. Y.C. Lv, 12.vii.1986, 1 male, 1 female (IZCAS).

Distribution. China (Inner Mongolia, Gansu, Qinghai, Xinjiang), Ukraine, Romania, Moldova, Iran, Georgia, Azerbaijan, Kazakhstan, Uzbekistan, Turkmenistan, Tadzhikistan, Afghanistan, Russia, Mongolia.

Remarks. Wu (2011) reported *C*. (*E*.) *c*. *contorta* (Fischer, 1828) from Inner Mongolia but did not provide specific specimen data. We provide specimen data for this species from Inner Mongolia for the first time.

Cylindera (Eugrapha) elisae elisae (Motschulsky, 1859)

Figs 6A, 7A

Cicindela elisae: Motschulsky 1859: 487; Wang et al. 2012: 16. *Cylindera (Eugrapha) elisae elisae*: Shook and Wiesner 2006: 15; Wu 2011: 27; Bai 2016: 29.

Published data. Heilongjiang (Wu 2011: 27), Jilin (Wu 2011: 27), Inner Mongolia (Wu 2011: 27), Liaoning (Wang et al. 2012:16), Hebei (Shook and Wiesner 2006: 15, Wu 2011: 27), Shandong (Shook and Wiesner 2006: 15, Wu 2011: 27), Beijing (Shook and Wiesner 2006: 15, Wu 2011: 27), Shanxi (Shook and Wiesner 2006: 15, Wu 2011: 27), Gansu (Shook and Wiesner 2006: 15, Wu 2011: 27), Xinjiang (Wu 2011: 27), Anhui (Wu 2011: 27), Henan (Shook and Wiesner 2006: 15, Wu 2011: 27), Hubei (Shook and Wiesner 2006: 15, Wu 2011: 27), Hunan (Shook and Wiesner 2006: 15, Wu 2011: 27), Zhejiang (Shook and Wiesner 2006: 15, Wu 2011: 27), Guangdong (Shook and Wiesner 2006: 15, Wu 2011: 27), Hainan (Wu 2011: 27), Guangxi (Shook and Wiesner 2006: 15, Wu 2011: 27), Fujian (Shook and Wiesner 2006: 15), Shanghai (Shook and Wiesner 2006: 15, Wu 2011: 27), Jiangsu (Shook and Wiesner 2006: 15, Wu 2011: 27), Jiangxi (Shook and Wiesner 2006: 15, Wu 2011: 27), Qinghai (Wu 2011: 27, Bai 2016: 29), Sichuan (Shook and Wiesner 2006: 15, Wu 2011: 27), Yunnan (Shook and Wiesner 2006: 15, Shook and Wu 2006: 41, Wu 2011: 27), Xizang (Wu 2011: 27), Hong Kong (Shook and Wiesner 2006: 15), Taiwan (Shook and Wiesner 2006: 15, Wu 2011: 27).

New records. Heilongjiang, Harbin, Hadeng (哈灯, hand-written label), leg. unknown, 13.vii.1963, 2 males, 1 female (NEFU).

Distribution. China (Heilongjiang, Jilin, Inner Mongolia, Liaoning, Hebei, Shandong, Beijing, Shanxi, Gansu, Xinjiang, Anhui, Henan, Hubei, Hunan, Zhejiang, Guangdong, Hainan, Guangxi, Fujian, Shanghai, Jiangsu, Jiangxi, Qinghai, Sichuan, Yunnan, Xizang, Hong Kong, Taiwan), North Korea, South Korea, Vietnam, Russia, Mongolia.

Remarks. Wu (2011) and Wang et al. (2012) reported *C*. (*E*.) *elisae* from Heilongjiang without specimen record and detailed locality which makes it impossible to determine which subspecies was recorded. It is possible that *C*. (*E*.) *e. hulunbeierensis* is distributed in Heilongjiang as well. We hence provide the first record *C*. (*E*.) *elisae* for Heilongjiang.

Cylindera (Eugrapha) sublacerata vicaria (Semenov, 1895)

Figs 6B, 7B

Cicindela sublacerata: Mandl 1955: 321. *Cicindela (Eugrapha) sublacerata*: Acciavatti and Pearson 1989: 302. *Cylindera (Eugrapha) sublacerata vicaria*: Wu 2011: 27.

Published data. Xinjiang (Acciavatti and Pearson 1989: 303; Li and Chen 1993: 107; Wu 2011: 27).

Records. Xinjiang, Changji, Fukang Desert Ecological System Observatary, 44°17'31"N, 87°56'3"E, 474 m, light trap, leg. Y. Liu, 11.vi.2007, 1 female (IZCAS).



Figure 6. Habitus photographs A Cylindera (Eugrapha) elisae elisae (Motschulsky, 1859) B Cylindera (Eugrapha) sublacerata vicaria (Semenov, 1895) C Cylindera (Ifasina) decolorata (Horn, 1907) D Cylindera (Ifasina) lesnei (Babault, 1923) E Cylindera (Ifasina) sikhimensis (Mandl, 1982) F Lophyra (Spilodia) lineifrons (Chaudoir, 1865). Scale bars: 5 mm.

> **Distribution.** China (Xinjiang), Mongolia. **Remarks.** We provide additional specimen data for this species from Xinjiang.

Subgenus Ifasina Jeannel, 1946

Cylindera (Ifasina) decolorata (Horn, 1907) Figs 6C, 7C

Cicindela psilica decolorata: Horn 1907: 24. *Cylindera (Ifasina) decolorate*: Shook and Wiesner 2006: 15; Wu 2011: 27.

Published data. Fujian (Shook and Wiesner 2006: 15; Wu 2011: 27), Guangdong (Shook and Wiesner 2006: 15; Wu 2011: 27), Sichuan (Shook and Wiesner 2006: 15; Wu 2011: 27), Guizhou (Shook and Wiesner 2006: 15; Wu 2011: 27), Yunnan (Horn 1907: 24; Shook and Wiesner 2006: 15; Wu 2011: 27).

New records. Guangxi, Huaping Nature Reserve, Mount Tianping, leg. J.K. Yang, 5.vi.1963, 1 male, 1 female (EMCAU); Guangxi, Huaping Nature Reserve,



Figure 7. Distribution maps. Green indicates records with previously known distribution based on published data, blue indicates new records, red stars indicate the collection sites of the examined specimens **A** *Cylindera* (*Eugrapha*) *elisae elisae* (Motschulsky, 1859) **B** *Cylindera* (*Eugrapha*) *sublacerata vicaria* (Semenov, 1895) **C** *Cylindera* (*Ifasina*) *decolorata* (Horn, 1907) **D** *Cylindera* (*Ifasina*) *kaleea kaleea* (Bates, 1866) **E** *Cylindera* (*Ifasina*) *lesnei* (Babault, 1923) **F** *Cylindera* (*Ifasina*) *sikhimensis* (Mandl, 1982).

Dayan Station, 25°36'52"N, 109°52'33"E, 1061 m, leg. M.Y. Lin and Y.Y. Qin, 12.vii.2022, 5 males, 18 females (IZCAS).

Distribution. China (Guangxi, Fujian, Guangdong, Sichuan, Guizhou, Yunnan), Vietnam.

Remarks. New provincial record for Guangxi.

Cylindera (Ifasina) kaleea kaleea (Bates, 1866)

Fig. 7D

Cicindela kaleea: Bates 1866: 340, 341. *Cylindera (Ifasina) kaleea kaleea*: Shook and Wiesner 2006: 15, 16; Wu 2011: 28.

Published data. Beijing (Shook and Wiesner 2006: 15, 16; Wu 2011: 28), Hebei (Shook and Wiesner 2006: 15, 16; Wu 2011: 28), Shaanxi (Shook and Wiesner 2006: 15, 16; Wu 2011: 28), Gansu (Shook and Wiesner 2006: 15, 16; Wu 2011: 28), Shandong (Shook and Wiesner 2006: 15, 16; Wu 2011: 28), Jiangsu (Shook and Wiesner 2006: 15, 16; Wu 2011: 28), Shanghai (Shook and Wiesner 2006: 15, 16; Wu 2011: 28), Zhejiang (Shook and Wiesner 2006: 15, 16; Wu 2011: 28), Jiangxi (Shook and Wiesner 2006: 15, 16; Wu 2011: 28), Fujian (Shook and Wiesner 2006: 15, 16; Wu 2011: 28), Henan (Shook and Wiesner 2006: 15, 16; Wu 2011: 28), Hubei (Shook and Wiesner 2006: 15, 16; Wu 2011: 28), Hunan (Shook and Wiesner 2006: 15, 16; Wu 2011: 28), Guangdong (Shook and Wiesner 2006: 15, 16; Wu 2011: 28), Guangxi (Shook and Wiesner 2006: 15, 16; Wu 2011: 28), Sichuan (Shook and Wiesner 2006: 15, 16; Wu 2011: 28), Guizhou (Shook and Wiesner 2006: 15, 16; Wu 2011: 28), Yunnan (Shook and Wiesner 2006: 15, 16; Wu 2011: 28), Xizang (Shook and Wiesner 2006: 15, 16; Wu 2011: 28), Hong Kong (Shook and Wiesner 2006: 15, 16; Wu 2011: 28), Taiwan (Bates 1866: 340, 341; Shook and Wiesner 2006: 15, 16; Wu 2011: 28).

New records. Anhui, Tiantangzhai, Tudiling Bridge, 31°30'23"N, 116°09'07"E, 635 m, leg. unknown, 24.ix.2021, 1 male (IZCAS).

Distribution. China (Anhui, Beijing, Hebei, Shaanxi, Gansu, Shandong, Jiangsu, Shanghai, Zhejiang, Jiangxi, Fujian, Henan, Hubei, Hunan, Guangdong, Guangxi, Sichuan, Guizhou, Yunnan, Xizang, Hong Kong, Taiwan), India, Myanmar, Thailand, Laos, Vietnam.

Remarks. New provincial record for Anhui.

Cylindera (Ifasina) lesnei (Babault, 1923) Figs 6D, 7E

Cicindela lesnei: Babaule 1923: 7. *Cylindera (Ifasina) lesnei*: Shook and Wiesner 2006: 16; Wu 2011: 28.

Published data. Guangdong (Shook and Wiesner 2006: 16; Wu 2011: 28).

New records. Hunan, Huitong, Raochong Village, 26°51'23"N, 109°50'43"E, 650 m, leg. H.B. Liang, 21.vi.2015, 1 female (IZCAS).

Distribution. China (Hunan, Guangdong), Vietnam. **Remarks.** New provincial record for Hunan.

Cylindera (Ifasina) sikhimensis (Mandl, 1982) Figs 6E, 7F

Cicindela (Ifasina) discrete sikhimensis: Mandl 1982: 64, 65. Cylindera (Ifasina) sikhimensis: Jaskula 2008: 33. New records. Xizang, Motuo, 2 km SE of Beibeng, 29°14'8"N, 95°9'31"E, 843 m, light trap, leg. H.B. Liang, 29.vii.2012, 4 males, 9 females (IZCAS).
 Distribution. China (Xizang), India, Myanmar.
 Remarks. New state record for China and new provincial record for Xizang.

Genus *Lophyra* Motschulsky, 1859 Subgenus *Spilodia* Rivalier, 1961

Lophyra (Spilodia) lineifrons (Chaudoir, 1865) Figs 6F, 8A

Cicindela lineifrons: Chaudoir 1865: 39, 62. *Lophyra (Spilodia) lineifrons*: Shook and Wiesner 2006: 17; Wu 2011: 28.

Published data. Yunnan (Shook and Wiesner 2006: 17; Wu 2011: 28).

New records. Guangxi, Ningming, Longrui, 180 m, leg. F.S. Li, 18.v.1984, 1 female (EMCAU).

Distribution. China (Guangxi, Yunnan), Nepal, Bangladesh, India, Myanmar, Thailand, Cambodia, Laos, Vietnam, Malaysia.

Remarks. New provincial record for Guangxi.

Lophyra (Spilodia) striolata dorsolineolata (Chevrolat, 1845) Figs 8A, 9B

Cicindela dorsolineolata: Chevrolat 1845: 95, 96.

Lophyra (*Spilodia*) *striolata dorsolineolata*: Shook and Wiesner 2006: 17; Wu 2011: 28; Wiesner et al. 2017: 56.

Published data. Beijing (Shook and Wiesner 2006: 17; Wu 2011: 28; Wiesner et al. 2017: 56), Hebei (Wu 2011: 28; Wiesner et al. 2017: 56), Shandong (Shook and Wiesner 2006: 17; Wu 2011: 28; Wiesner et al. 2017: 56), Jiangsu (Shook and Wiesner 2006: 17; Wu 2011: 28; Wiesner et al. 2017: 56), Zhejiang (Shook and Wiesner 2006: 17; Wu 2011: 28; Wiesner et al. 2017: 56), Anhui (Wu 2011: 28, Wiesner et al. 2017: 56), Jiangxi (Shook and Wiesner 2006: 17; Wu 2011: 28; Wiesner et al. 2017: 56), Fujian (Shook and Wiesner 2006: 17; Wu 2011: 28; Wiesner et al. 2017: 56), Henan (Shook and Wiesner 2006: 17; Wu 2011: 28; Wiesner et al. 2017: 56), Hubei (Shook and Wiesner 2006: 17; Wu 2011: 28; Wiesner et al. 2017: 56), Hunan (Shook and Wiesner 2006: 17; Wu 2011: 28; Wiesner et al. 2017: 56), Guangdong (Shook and Wiesner 2006: 17; Wu 2011: 28; Wiesner et al. 2017: 56), Guangxi (Wu 2011: 28; Wiesner et al. 2017: 56), Hainan (Shook and Wiesner 2006: 17; Wu 2011: 28; Wiesner et al. 2017: 56), Yunnan (Shook and Wiesner 2006: 17; Wu 2011: 28; Wiesner et al. 2017: 56), Guizhou (Wu 2011: 28; Wiesner et al. 2017: 56), Sichuan (Wu 2011: 28; Wiesner et al. 2017: 56), Taiwan (Shook and Wiesner 2006: 17; Wu 2011: 28; Wiesner et al. 2017: 56).

New records. Xizang, Beibeng, 2 km from Highway Gelin, 29°14'56"N, 95°11'20"E, 1013 m, leg. J.W. Jiang, 29.vii.2019, 3 males, 5 females (IZCAS).



Figure 8. Habitus photographs **A** *Lophyra* (*Spilodia*) striolata dorsolineolata (Chevrolat, 1845) **B** *Heptodonta pulchella* (Hope, 1831) **C** *Pronyssiformia excoffieri* (Fairmaire, 1897) **D** *Therates biserratus* Tan, Mo & Liang, 1991 **E** *Therates guang-dongensis* Wiesner, 2016 **F** *Therates hunanensis* Matalin & Wiesner, 2023. Scale bars: 5 mm.

Distribution. China (Xizang, Beijing, Hebei, Shandong, Zhejiang, Jiangsu, Anhui, Jiangxi, Fujian, Henan, Hubei, Hunan, Guangdong, Guangxi, Hainan, Yunnan, Guizhou, Sichuan, Taiwan), Japan, Vietnam, Indonesia, Philippines. **Remarks.** New provincial record for Xizang.

Subtribe Dromicina Thomson, 1859 Genus *Heptodonta* Hope, 1838

Heptodonta pulchella (Hope, 1831) Figs 8B, 9C

Cicindela pulchella: Hope 1831: 21. *Heptodonta pulchella*: Wu 2011: 28; Wiesner and Geiser 2016: 82; Görn 2020: 48.



Figure 9. Distribution maps. Green indicates records with previously known distribution based on published data, blue indicates new records, red stars indicate the collection sites of the examined specimens **A** *Lophyra* (*Spilodia*) *lineifrons* (Chaudoir, 1865) **B** *Lophyra* (*Spilodia*) *striolata dorsolineolata* (Chevrolat, 1845) **C** *Heptodonta pulchella* (Hope, 1831) **D** *Pronyssiformia excoffieri* (Fairmaire, 1897) **E** *Therates biserratus* Tan, Mo & Liang, 1991 **F** *Therates guangdongensis* Wiesner, 2016.

Published data. Fujian (Wu 2011: 28; Wiesner and Geiser 2016: 82; Görn 2020:51), Guangdong (Görn 2020:51), Guangxi (Görn 2020:51), Yunnan (Wu 2011: 28; Wiesner and Geiser 2016: 82; Görn 2020:51), Sichuan (Görn 2020:51), Xizang (Wu 2011: 28; Wiesner and Geiser 2016: 82; Görn 2020:51), Macao (Wu 2011: 28; Wiesner and Geiser 2016: 82).

New records. Hainan, Jianfengling, Roadside of Tianchi, 18°43'44"N, 108°53'9"E, 1000 m, leg. H.B. Liang, 4.v.2007, 1 male, 2 females (IZCAS).

Distribution. China (Hainan, Fujian, Guangdong, Guangxi, Yunnan, Sichuan, Xizang, Macao), Nepal, India, Myanmar, Thailand, Laos, Vietnam. **Remarks.** New provincial record for Hainan.

Genus Pronyssiformia Horn, 1929

Pronyssiformia excoffieri (Fairmaire, 1897) Figs 8C, 9D

Cicindela excoffieri: Fairmaire 1897: 14. *Pronyssiformia excoffieri*: Horn 1929: 5; Shook and Wiesner 2006: 20; Wu 2011: 31.

Published data. Fujian (Shook and Wiesner 2006: 20; Wu 2011: 31), Hubei (Shook and Wiesner 2006: 20; Wu 2011: 31), Sichuan (Horn 1929: 6; Shook and Wiesner 2006: 20; Wu 2011: 31), Yunnan (Fairmaire 1897:14; Shook and Wiesner 2006; Wu 2011: 31).

New records. Guizhou, Mount Fanjing, Huguosi, 27°54'44"N, 108°38'37"E, 1350 m, leg. Q.Z. Song, 3.viii.2001, 1 female (IZCAS).

Distribution. China (Guizhou, Fujian, Hubei, Sichuan, Yunnan). **Remarks.** New provincial record for Guizhou.

Subtribe Theratina Horn, 1893 Genus *Therates* Latreille, 1816

Therates biserratus Tan, Mo & Liang, 1991 Figs 8D, 9E

Therates biserratus: Tan et al. 1991: 243; Matalin and Wiesner 2023: 414, 415.

Published data. Guizhou (Jiangkou; Yinjiang) (Tan et al. 1991: 243; Matalin and Wiesner 2023: 414, 415).

New records. Guangxi, Jinxiu, Luoxiang, 400 m, leg. D.C. Yuan, 14.v.1999, 1 male (IZCAS); Jiangxi, Longnan, Mount Jiulian, leg. Y.W. Zhang, 14.vi.1975, 1 female (IZCAS).

Distribution. China (Guangxi, Jiangxi, Guizhou).

Remarks. New provincial record for Guangxi and Jiangxi.

Therates guangdongensis Wiesner, 2016

Figs 8E, 9F

Therates guangdongensis: Wiesner 2016: 131, 132.

Published data. Guangdong (Yinnah Shan) (Wiesner 2016: 131,132).

New records. Hainan, Baisha County, Nanmaola, leg. X.L. Huang, 13.v.2009, 1 male (IZCAS).

Distribution. China (Guangdong, Hainan). **Remarks.** New provincial record for Hainan.

Therates hunanensis Matalin & Wiesner, 2023 Figs 8F, 11A

Therates hunanensis: Matalin and Wiesner 2023: 415.

Published data. Hunan (Dong'an; Chengbu) (Matalin and Wiesner 2023: 415).
New records. Chongqing, Mount Simian, 28°35–37'N, 106°23–24'E, 1190 m, leg. Z.H. Yang, 5.vii.2008, 1 male (IZCAS).
Distribution. China (Chongqing, Hunan).
Remarks. New provincial record for Chongqing.

Therates probsti Wiesner, 1988

Figs 10A, 11B

Therates probsti: Wiesner 1988:20.

New records. Xizang, Motuo, Yarang Hydropower Station, 29°15'54"N, 95°14'43"E, 850 m, leg. H.B. Liang, 1.viii.2019, 1 female (IZCAS).
 Distribution. China (Xizang), Laos, Vietnam.
 Remarks. New state record for China and new provincial record for Xizang.

Therates turnai Wiesner, 2015 Figs 10B, 11C

Therates turnai: Wiesner 2015: 44-47.

Published data. Guizhou (Xianheping) (Wiesner 2016: 44–47). New records. Sichuan, Mount Emei, Xixinsuo, 29°34'30"N, 103°22'26"E, 1300

m, day collecting, leg. H.B. Liang, 10–15.viii.2012, 1 male (IZCAS). Distribution. China (Guizhou, Sichuan). Remarks. New provincial record for Sichuan.

Tribe Collyridini Brullé, 1834 Subtribe Collyridini Brullé, 1834 Genus *Neocollyris* Horn, 1901 Subgenus *Isocollyris* Naviaux, 1994

Neocollyris (Isocollyris) grandivadosa (Horn, 1935) Figs 10C, 11D

Collyris aureofusca grandi-vadosa: Horn 1935: 50, 51. *Neocollyris (Isocollyris) grandivadosa*: Naviaux 2004: 74, 75, 76.

New records. Guangxi, Napo County, 23°43'33"N, 106°49'30"E, 111 m, hand collected, leg. Y. Wang, 16.v.2021, 1 male, 1 female (IZCAS).
 Distribution. China (Guangxi), Vietnam.
 Remarks. New state record for China and new provincial record for Guangxi.



Figure 10. Habitus photographs **A** *Therates probsti* Wiesner, 1988 **B** *Therates turnai* Wiesner, 2015 **C** *Neocollyris* (*Isocollyris*) grandivadosa (Horn, 1935) **D** *Neocollyris* (*Neocollyris*) saphyrina (Chaudoir, 1850) **E** *Neocollyris* (*Pachycollyris*) bicolor (Horn, 1902) **F** *Neocollyris* (*Pachycollyris*) mouhotii nagaii Naviaux & Sawada, 1992. Scale bars: 5 mm.

Subgenus Neocollyris Horn, 1901

Neocollyris (*Neocollyris*) *saphyrina* (Chaudoir, 1850) Figs 10D, 11E

Neocollyris (Neocollyris) saphyrina: Li and Chen 1993: 102.

Published data. ?Yunnan (Li and Chen 1993: 102), ?Sichuan (Xichang) (Li and Chen 1993: 102).

New records. Xizang, Shigatse, Gyirong, 2400 m, leg. F.S. Huang, 22.vii.1975, 1 female (IZCAS).



Figure 11. Distribution maps. Green indicates records with previously known distribution based on published data, blue indicates new records, red stars indicate the collection sites of the examined specimens **A** *Therates hunanensis* Matalin & Wiesner, 2023 **B** *Therates probsti* Wiesner, 1988 **C** *Therates turnai* Wiesner, 2015 **D** *Neocollyris* (*Isocollyris*) grandivadosa (Horn, 1935) **E** *Neocollyris* (*Neocollyris*) saphyrina (Chaudoir, 1850) **F** *Neocollyris* (*Pachycollyris*) bicolor (Horn, 1902).

Distribution. China (Xizang, ?Yunnan, ?Sichuan), India, Myanmar, Laos, Nepal, Bhutan, Bangladesh, Thailand, Indonesia.

Remarks. Gyirong borders Nepal, from where *N. saphyrina* was previously known. Shook and Wiesner (2006) indicated that records included by Li and Chen (1993) for *N. (N.) saphyrina* in Yunnan and Sichuan (Xichang) required confirmation. The location reported here, Gyirong, is far from Yunnan and Sichuan, and the closest confirmed distribution of *N. (N.) saphyrina* to Yunnan

and Sichuan is in Cambodia and Laos, but with some similar species present in this area, the records for Yunnan and Sichuan still need to be re-examined. We restore the record of *N*. (*N*.) saphyrina for China with a new provincial record for Xizang.

Subgenus Pachycollyris Naviaux, 1995

Neocollyris (Pachycollyris) bicolor (Horn, 1902)

Figs 10E, 11F

Collyris bicolor: Horn 1902: 70. *Neocollyris (Pachycollyris) bicolor*: Wu 2011: 30; Wiesner and Geiser 2016: 77.

Published data. Guangxi (Wu 2011: 30; Wiesner and Geiser 2016: 77), Yunnan (Wu 2011: 30; Wiesner and Geiser 2016: 77).

New records. Hainan, Wuzhishan, Fanyang Town, leg. G. Ros, 1.vii.1956, 1 female (IZCAS).

Distribution. China (Hainan, Guangxi, Yunnan), Laos, Vietnam. **Remarks.** New provincial record for Hainan.

Neocollyris (Pachycollyris) mouhotii nagaii Naviaux & Sawada, **1992** Figs 10F, 13A

Neocollyris mouhotii nagaii: Naviaux and Sawada 1992: 46, 47, 48. *Neocollyris (Pachycollyris) mouhotii nagaii*: Naviaux 1995: 267.

New records. Hainan, Baisha County, Yinggeling, 18°59'28"N, 109°20'18"E, leg. M.Y. Lin, 14.vi.2010, 1 female (IZCAS).

Distribution. China (Hainan), Vietnam.

Remarks. New state record for China and new provincial record for Hainan.

Neocollyris (Pachycollyris) sawadai Naviaux, 1991 Figs 12A, 13B

Neocollyris sawadai: Naviaux 1991a: 222.

New records. Yunnan, Pu'er, Simao, day collecting, leg. H.L. Han and M.J. Qi, 15–19.vii.2009, 1 female (NEFU).

Distribution. China (Yunnan), Vietnam. **Remarks.** New state record for China and new provincial record for Yunnan.

Neocollyris (Pachycollyris) strangulata Naviaux, 1991 Figs 12B, 13C

Neocollyris strangulata: Naviaux 1991b: 276, 277. Neocollyris (Pachycollyris) strangulata: Naviaux 1995: 263, 264.



Figure 12. Habitus photographs A Neocollyris (Pachycollyris) sawadai Naviaux, 1991 B Neocollyris (Pachycollyris) strangulata Naviaux, 1991 C Neocollyris (Pachycollyris) tricolor Naviaux, 1991 D Neocollyris (Pachycollyris) vitalisi (Horn, 1924). Scale bars: 5 mm.

New records. Guangxi, Jingxi City, Diding Nature Reserve, 23°6'47"N, 105°58'40"E, leg. S.Y. Zhou and J.H. Huang, 9.viii.2010, 1 female (IZCAS).
 Distribution. China (Guangxi), Laos, Vietnam.
 Remarks. New state record for China and new provincial record for Guangxi.

Neocollyris (Pachycollyris) tricolor Naviaux, 1991 Figs 12C, 13D

Neocollyris tricolor: Naviaux 1991c: 19, 20.











Figure 13. Distribution maps. Green indicates records with previously known distribution based on published data, blue indicates new records, red stars indicate the collection sites of the examined specimens **A** *Neocollyris* (*Pachycollyris*) *mouhotii nagaii* Naviaux & Sawada, 1992 **B** *Neocollyris* (*Pachycollyris*) *sawadai* Naviaux, 1991 **C** *Neocollyris* (*Pachycollyris*) *strangulata* Naviaux, 1991 **D** *Neocollyris* (*Pachycollyris*) *tricolor* Naviaux, 1991 **E** *Neocollyris* (*Pachycollyris*) *vitalisi* (Horn, 1924).

Neocollyris (Pachycollyris) tricolor: Wu and Shook 2007: 39; Wu 2011: 31.

Published data. Yunnan (Lincang) (Wu and Shook 2007:39; Wu 2011: 31).
New records. Xizang, Beibeng, 2 km from Highway Gelin, 29°14'56"N, 95°11'20"E, 1013 m, leg. J.W. Jiang, 29.vii.2019, 1 female (IZCAS).
Distribution. China (Xizang, Yunnan), Myanmar, Thailand, Laos, Vietnam.
Remarks. New provincial record for Xizang.

Neocollyris (Pachycollyris) vitalisi (Horn, 1924)

Figs 12D, 13E

Neocollyris (Pachycollyris) feai vitalisi: Naviaux 1995: 256, 257. Neocollyris (Pachycollyris) vitalisi: Cassola 2005: 15; Wiesner and Geiser 2016: 78.

Published data. Xizang (Wiesner and Geiser 2016: 78).

New records. Yunnan, Mount Huanglian, Beiluo, 22°44'8"N, 102°18'27"E, 1277 m, day collecting, leg. G.Z. Zhong and L.K. Zhang, 09–15.IX.2016, 1 female (KIZCAS).

Distribution. China (Yunnan, Xizang), Myanmar, Thailand, Laos, Vietnam. **Remarks.** New provincial record for Yunnan.

Discussion

During our investigation of Chinese collections, we encountered a single specimen of *Cicindela* (*Cicindela*) *japana* Motschulsky, 1858 [Anhui, China = CHINE, Prov. ANHWEI-printed label] (Fig. 14). *Cicindela* (*C.*) *japana* is only known from Japan, where it occurs on the four main islands and several smaller adjacent islands. One specimen deposited in a museum collection in Vladivostok was collected on Kunashir Island and supposedly blown there from Hokkaido by strong winds (A.V. Matalin pers. comm.). Previous records of *C.* (*C.*) *japana* from China and South Korea that could be examined by us all referred to misidentified specimens of *C.* (*C.*) *sachalinensis raddei*. Thus, it seems unlikely that *C.* (*C.*) *japana* occurs in China, especially in an inland province far from Japan. Given the age of this specimen, and the absence of labels indicating the collector and date, we suspect it may have been mislabeled.

With the new records presented here, the tiger beetle fauna of China now includes 208 species, 71 (34%) of which are endemic. Five of the new records reported here are from the Chayu and Motuo, regions in the southeast of Xizang. This area is located at the junction of the Hengduan Mountains and the Himalayas and is surrounded by many biogeographical barriers. These five species were previously known from adjacent regions and countries, including Yunan Province, Sichuan Province, Myanmar, and India, from which they may have dispersed. More research on the tiger beetle diversity in this region, the southeast of Xizang, is needed and has great potential to broaden our understanding of the biogeography of tiger beetles within China.

Recently, Matalin et al. (2024) restored *Apterodela* (*Apterodela*) *bivirgulata* (Fairmaire, 1889) as a separate species. Before the reclassification, *Apterodela* (*Apterodela*) *lobipennis* (Bates, 1888) was regarded as one of the common tiger beetles in China, with many specimens preserved in collections. All these specimens need to be re-examined to determine their exact classification. Therefore, additional specimens and photographic records will facilitate the progress of identification.

Furthermore, we report new provincial records of *Therates biserratus* Tan, Mo & Liang, 1991 and *Therates hunanensis* Matalin & Wiesner, 2023 in China. *Therates biserratus* has been included in the List of Key Protected Wild Animals





in China since 2003, although it has not been considered as a separate species for a long time. According to the known distribution, *T. biserratus* may indeed be widely distributed in Nan Ling Area (24°00'–26°30'N, 110°–116°E). This may ultimately lead to reconsideration of the taxonomic status between these two species. Due to the limited number of specimens included in this and other studies (Matalin and Wiesner 2023), more specimens are needed to adequately determine their status.

To date, taxonomic studies on tiger beetles from Guangxi and Hainan are relatively scarce (Naviaux 2010; Xiong and Wiesner 2022). Here, we report nine new records from Guangxi and four new records from Hainan. We believe that the diversity of tiger beetles in these two provinces far exceeds the currently known records and deserves more attention from taxonomists.

Many of the specimens reported here are old specimens that have not been previously identified. There are likely numerous unidentified tiger beetle specimens in other institutions and museums in China, which hold considerable yet untapped data. We encourage others to recognize and utilize this potential and are willing to help in identification.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: KYW. Formal analysis: JW, FAB. Funding acquisition: CDL. Investigation: KYW. Resources: CDL. Supervision: CDL. Visualization: KYW. Writing - original draft: KYW. Writing - review and editing: FAB, JW.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

Discovery of a new tarantula species from the Madrean Sky Islands and the first documented instance of syntopy between two montane endemics (Araneae, Theraphosidae, *Aphonopelma*): a case of prior mistaken identity

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Abstract

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The Chiricahua Mountains in southeastern Arizona are renowned for their exceptional biodiversity and high levels of endemism. Morphological, genomic, behavioral, and distributional data were used to report the discovery of a remarkable new tarantula species from this range. Aphonopelma jacobii sp. nov. inhabits high-elevation mixed conifer forests in these mountains, but also co-occurs and shares its breeding period with A. chiricahua-a related member of the Marxi species group-in mid-elevation Madrean evergreen oak and pine-oak woodlands. This marks the first documented case of syntopy between two montane endemics in the Madrean Archipelago and adds to our knowledge of this threatened region's unmatched tarantula diversity in the United States. An emended diagnosis and redescription for A. chiricahua are also provided based on several newly acquired and accurately identified specimens. Phylogenetic analyses of mitochondrial and genomic-scale data reveal that A. jacobii sp. nov. is more closely related to A. marxi, a species primarily distributed on the Colorado Plateau, than to A. chiricahua or the other Madrean Sky Island taxa. These data provide the evolutionary framework for better understanding the region's complex biogeographic history (e.g., biotic assembly of the Chiricahua Mountains) and conservation of these spiders.

Key words: Biodiversity hotspot, Chiricahua Mountains, conservation, cryptic species, molecular systematics, phylogenomics, spider taxonomy

Introduction

The mygalomorph spider genus *Aphonopelma* Pocock, 1901 is the most diverse group within the family Theraphosidae (tarantulas) and currently comprises 54 nominal species (World Spider Catalog 2024). The genus is widely distributed across two major biogeographic realms (i.e., the Nearctic and Neotropics) where it can be found throughout the southern third of the United States, ranging west of the Mississippi River to the Pacific Ocean, and south throughout

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Mexico and into Central America (but see Turner et al. 2018; Gabriel 2022). In the United States, these spiders are found across a wide range of physical and climatic conditions, from hot and arid desert valleys located near sea level to cool and mesic high-elevation forests (Hamilton et al. 2016).

Of particular interest are species occupying these high-elevation habitats, especially those found in the Madrean Archipelago (colloquially referred to as the Madrean "Sky Islands", hereafter MSI), a series of isolated mountain ranges that span the cordilleran gap between the Colorado Plateau and Rocky Mountains of the southwestern United States and the Sierra Madre Occidental of northwestern Mexico. Prior to our work on *Aphonopelma* (see Hamilton et al. 2011, 2014, 2016; Hendrixson et al. 2013, 2015; Hendrixson 2019), knowledge concerning the diversity and distribution of tarantulas from this region was largely nonexistent. An early revision of the genus (Smith 1995) did not include samples from any of these mountain ranges, and the only mention of tarantulas cocurring in the Madrean Archipelago was limited to two unpublished master's theses (Beatty 1961; Jung 1975).

Hendrixson et al. (2015) first investigated the diversity of MSI tarantulas based on samples collected from five mountain ranges in southeastern Arizona and southwestern New Mexico. The authors concluded there were three undescribed species that formed a clade: one endemic to the Santa Catalina Mountains, one endemic to the Peloncillo Mountains and surrounding grasslands, and one found more broadly in the Huachuca, Pajarito, and Santa Rita Mountains. Shortly thereafter, a newly collected male specimen from the Chiricahua Mountains turned out to represent yet another distinct species belonging to the group. Hamilton et al. (2016) subsequently described and named these four species Aphonopelma catalina Hamilton, Hendrixson & Bond, 2016, A. peloncillo Hamilton, Hendrixson & Bond, 2016, A. madera Hamilton, Hendrixson & Bond, 2016, and A. chiricahua Hamilton, Hendrixson & Bond, 2016, respectively, and placed them into the Marxi species group. Based on these patterns of diversity (i.e., high levels of MSI endemicity), Hendrixson et al. (2015) and Hamilton et al. (2016) hypothesized that the MSI should harbor many more undescribed species, especially in mountain ranges that have been poorly sampled or not sampled at all. Hendrixson (2019) confirmed this prediction with the discovery and description of A. bacadehuachi Hendrixson, 2019 from nearby pine-oak woodlands in northeastern Sonora, Mexico.

During a field trip to the Chiricahua Mountains in late October 2018, a series of tarantulas was observed in high-elevation (c. 2364 m) mixed conifer forest. These individuals were tentatively assigned to *A. chiricahua* based on their location (see Jacobi 2019), but preliminary phylogenetic analyses of DNA sequences gathered from the mitochondrial gene cytochrome *c* oxidase subunit I (hereafter COX1) suggested they were distinctly different from that species (i.e., these individuals were genetically divergent from *A. chiricahua* and were not recovered in the same clade; Fig. 1). The following year in late October, we (CAH, BEH) revisited the Chiricahua Mountains and successfully found more individuals from two additional populations. After analyzing genomic-scale data using Ultraconserved Elements (hereafter UCE) and reexamining the morphology of these spiders, we have confirmed that these tarantulas are indeed distinct from *A. chiricahua* and belong to a new species.

The purpose of this study is two-fold: (1) to report the discovery of this new *Marxi* group species and to document the first case of multiple short-range

endemic tarantula species inhabiting a single MSI range; and (2) to redescribe *A. chiricahua* based on newly acquired specimens because we have determined that all the material examined in the original description of *A. chiricahua* was misidentified (i.e., actually *A. jacobii* sp. nov.) except for the male holotype (see Hamilton et al. 2016).

Materials and methods

Museum repositories and comparative material examined

All voucher specimens examined as part of this study are deposited in the American Museum of Natural History, New York, New York (**AMNH**) and the University of Idaho William F. Barr Entomological Museum, Moscow, Idaho (**UIM**). Collection data for all comparative material examined as part of this study are provided in the Suppl. materials 1–3, 9, 10 and Hamilton et al. (2016). For newly examined samples, the accuracy (precision score) of each set of GPS coordinates (reported in decimal degrees) is annotated as a superscript in the material examined sections for each species using the scheme described in Hamilton et al. (2016), as modified from Murphey et al. (2004) and Bond (2012).

Measurements, characterization, and illustration of morphological features

All material was preserved in 80% ethanol and assigned a unique alphanumeric voucher number (APH-####) that can be used to cross-reference all images, measurements, and locality data. Abbreviations for all quantitative morphological characters follow Hamilton et al. (2016). Measurements are reported in millimeters and were made with a Leica M125C stereomicroscope using the Leica Application Suite software and a digital camera, or from a Mitutoyo 500-196-30 Advanced Onsite Sensor (AOS) Absolute Scale Digital Caliper. Unless otherwise stated, measurements were taken from left appendages. Lengths of leg segments were taken from the mid-proximal point of articulation to a distal point of the article, as detailed and shown in Hamilton et al. (2016). Quantitative measurements used for diagnosing and describing *Aphonopelma jacobii* sp. nov. were obtained from 14 mature male and 6 mature female specimens, whereas measurements used for re-diagnosing and redescribing *A. chiricahua* were gathered from six mature male and two mature female specimens.

Digital images for the morphological plates (Figs 4, 5, 9, Suppl. materials 6, 7, 11) were made using a Visionary Digital Imaging System (Visionary Digital[™], Richmond, VA) where images were recorded at multiple focal planes and then assembled into a single focused image using the computer program Zerene Stacker v. 1.04 (Zerene Systems LLC, Richland, WA). The female genital region was removed from the abdominal wall and the tissues were dissolved using trypsin (incubated overnight at 37 °C in a 1.5-ml microcentrifuge tube); spermathecae were examined and photographed in the manner described above. All images were cropped and toned in Adobe Photoshop (Adobe Systems, Inc.). All morphological measurements and high-resolution photographs are available in the Suppl. materials 9, 11.

Evaluation of quantitative morphological features for species diagnoses

Measurements and morphometric ratios that were determined to have non-overlapping ranges between members of the *Marxi* species group were used for establishing morphological diagnoses. Measurements for all previously described members of the *Marxi* species group were obtained from Hamilton et al. (2016) and Hendrixson (2019) and added to a dataset containing the newly generated measurements for *A. jacobii* sp. nov. and *A. chiricahua* (see Suppl. materials 5, 9). As in Hamilton et al. (2016), we investigated 153 ratio combinations for mature males and 135 for mature females. For all measurements and morphometric ratios, we acknowledge that by including additional specimens in the future, these boundaries could change. We have attempted to limit these effects by including specimens that span the breadth of size variation across each species' distribution.

For recently collected specimens of A. jacobii sp. nov. and A. chiricahua, species assignments were determined based on the results of the phylogenetic analyses (see Figs 1, 2) and verified with morphometrics. Specimens that grouped (without overlapping other species) with APH-3191-the male holotype of A. chiricahua-were assigned to that species, whereas specimens that did not group with APH-3191 were assigned to A. jacobii sp. nov. For older museum specimens previously identified as A. chiricahua (see Hamilton et al. 2016) that did not include genetic data, species assignments for these individuals were determined by performing a discriminant analysis in the statistical software program JMP ver. 17.2.0 (https://www.jmp.com). Separate models for males and females were trained using raw measurements from specimens whose species identities had been confirmed phylogenetically. These models were then used to classify (i.e., identify) the museum specimens as A. jacobii sp. nov. or A. chiricahua. To explore the extent of morphological variation found within and between species of the Marxi species group, we visually examined traditional PCA (principal component analysis) morphospace (see Suppl. material 4) and boxplots of morphometric ratios (see Suppl. material 5) using the R computing environment (https://www.r-project.org). Two-dimensional PCA morphospace was evaluated by plotting PC1 and PC2.

Molecular techniques, taxon sampling, and phylogenetic analyses

Legs were removed from all freshly collected material (generally from the R side) and preserved in \ge 95% ethanol or RNA*later*TM (Qiagen, Valencia, CA, USA) and stored at -80 °C. Genomic DNA was extracted from muscle tissues using the Qiagen DNeasy Tissue KitTM (Qiagen, Valencia, CA, USA) or OmniprepTM (G-Biosciences) and then qualitatively and quantitatively assessed using agarose gel electrophoresis and a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA), respectively.

All laboratory procedures for generating COX1 sequence data are described in Hendrixson et al. (2013). The dataset included 77 samples representing all described members of the *Marxi* species group. We obtained sequences from GenBank for the following outgroup taxa: *Ornithoctonus huwena* (= *Cyriopagopus schmidti*) (AY309259) and *Acanthoscurria* sp. (JX946011).



Figure 1. Maximum likelihood phylogeny of the *Marxi* species group based on the mitochondrial gene cytochrome oxidase c subunit I (COX1). The red clades highlight the diphyly of *Aphonopelma jacobii* sp. nov. Bootstrap node support values are indicated along branches of interest (*** indicates branches supported with values \geq 95).

Protocols for analyzing the COX1 dataset essentially follow Hendrixson (2019). Alignment of the dataset was straightforward, and sequences were translated and checked for unexpected stop codons in Mesquite v. 3.81 (Maddison



Figure 2. Maximum likelihood phylogeny of the *Marxi* species group based on Ultraconserved Element phylogenomics (UCE). The red clade highlights the monophyly/exclusivity of *Aphonopelma jacobii* sp. nov. Except where noted, all nodes are supported by bootstrap support values \geq 95. All samples included in this tree, except *A. jacobii* sp. nov. and *A. baca-dehuachi*, are the same samples used in the phylogeny from the US *Aphonopelma* revision (Hamilton et al. 2016).

and Maddison 2023). We performed a maximum likelihood (ML) phylogenetic analysis in IQ-TREE (Nguyen et al. 2015) through the W-IQ-TREE web server (Trifinopoulos et al. 2016) available at http://iqtree.cibiv.univie.ac.at. The substitution model was set to "Auto" which allows IQ-TREE to determine the best-fit substitution model for the data using ModelFinder (Kalyaanamoorthy et al. 2017); the FreeRate heterogeneity (+R) option was also selected. Clade support was estimated using 1,000 ultrafast bootstrap replicates (Hoang et al. 2018). The UCE dataset included 24 samples representing all described members of the *Marxi* species group, including two individuals whose species assignment has not yet been determined due to inadequate sampling from their respective areas (APH-0622, APH-0880). Except for *A. bacadehuachi*, each species was represented by at least two individuals—the same individuals used in the Anchored Hybrid Enrichment (AHE) phylogenomic tree from our previous revisionary work (Hamilton et al. 2016). We included six samples of *A. jacobii* sp. nov. covering its entire distribution in the Chiricahuas due to the curious placement of the Barfoot Park population in the COX1 phylogeny (see the Results and Discussion sections below). *Aphonopelma vorhiesi* (Chamberlin & Ivie, 1939) was used as an outgroup to root the phylogeny because our preliminary UCE phylogenomic analyses of the entire *Aphonopelma* genus consistently placed this species as sister to all other species in the *Marxi* species group with strong support (100 bs, unpublished).

To construct the UCE phylogenomic dataset, extracted DNA was sent to Rapid Genomics (Gainesville, FL) for library preparation, UCE hybridization, and high-throughput sequencing. Library preparation was performed for Illumina sequencing utilizing their high-throughput workflow with proprietary chemistry. DNA was sheared to a mean fragment length of ~ 500 bp, fragments were end-repaired and A-tailed, followed by incorporation of unique dual-indexed Illumina adaptors and PCR enrichment. Samples were pooled equimolar and sequenced on a SP flow cell (2 × 250 bp) or a S4 flow cell (2 × 150 bp). Data was assembled with SPAdes (Prjibelski et. al. 2020) and processed using Phyluce v. 1.7.1 (Faircloth 2016) and a combined arachnid-spider hybrid probe set (Starrett et al. 2017; Kulkarni et al. 2020), where match settings for minimum identity and minimum coverage of 95 and 95 (respectively) were used to create a dataset of 1311 loci. These loci were aligned using MUSCLE (Edgar 2022) then internally trimmed using GBlocks with b1, b2, b3, and b4, settings of 0.5, 0.6, 10, and 5, respectively, to remove poorly aligned blocks within the sequences. The data were then additionally cleaned using AMAS ver. 1.02 (Borowiec 2016) and Spruceup v. 2022.2.4 (Borowiec 2019), which removes poorly aligned sequence fragments from individual sequences within alignments. Visual examination of distance distribution plots identified a setting of 0.9 as optimal for trimming, with a 90% occupancy matrix then being generated for use in subsequent analyses. A maximum likelihood-based phylogeny was inferred using IQTree2 (Nguyen et al. 2015; Minh et al. 2020), with the ultrafast bootstrap flag and 1000 replicates for node support values. All analyses were performed on the University of Idaho Research Computing and Data Services (RCDS) high-performance computing cluster.

DNA sequence alignments, phylogenetic trees, and scripts have been deposited in the Suppl. material 8. A list of GenBank accession codes for all COX1 samples and the SRA BioProject number for all raw sequencing data used to generate the UCE loci in this study are provided in the Suppl. material 3.

Species concept

The species concept we employ follows the Unified Species Concept discussed by de Queiroz (2005). Where possible, we employ a combination of morphological, genomic, behavioral, and distributional evidence to identify independently evolving lineages.

Quantitative morphological landmarks (Hamilton et al. 2016: fig. 3)

- **CI** length of the carapace
- **Cw** width of the carapace
- LBI labial length
- LBw labial width
- **F1** femur I length (retrolateral aspect)
- F1w femur I width
- P1 patella I length
- T1 tibia I length
- M1 metatarsus I length
- A1 tarsus I length
- F3 femur III length (prolateral aspect)
- F3w femur III width
- P3 patella III length
- T3 tibia III length
- M3 metatarsus III length
- A3 tarsus III length
- F4 femur IV length (prolateral aspect)
- F4w femur IV width
- P4 patella IV length
- T4 tibia IV length
- M4 metatarsus IV length
- A4 tarsus IV length
- **PTI** palpal tibia length (retrolateral aspect)
- PTw palpal tibia width
- **SC3** ratio of the extent of metatarsus III scopulation (length of scopulation/ventral length of metatarsus III)
- **SC4** ratio of the extent of metatarsus IV scopulation (length of scopulation/ventral length of metatarsus IV)

Data resources

All specimens examined as part of this study are deposited in the William F. Barr Entomological Museum in the Department of Entomology, Plant Pathology and Nematology at the University of Idaho in Moscow, Idaho (**UIM**), and the American Museum of Natural History in New York City, New York (**AMNH**). All prior specimens deposited in the Auburn University Museum of Natural History, Auburn, Alabama (**AUMNH**) have been transferred to UIM. All data (molecular, morphological, geographic, and images) used to establish these species hypotheses can be found in the Suppl. materials. All UCE raw reads can be found on the Sequence Read Archive (SRA) (BioProject ID: PRJNA1099687). All specimen data can be found in the Suppl. materials. High resolution morphological images (Suppl. material 11) are stored on FigShare (doi:10.6084/m9.figshare.26133724). The data underpinning the analysis reported in this paper are deposited at GBIF, the Global Biodiversity Information Facility, and are available at https://doi.org/10.15468/9sf6jf.

Results

Discriminant analysis, morphometrics, and PCA

The discriminant analysis performed on both males and females accurately classified every individual of *A. jacobii* sp. nov. and *A. chiricahua* that was used to train the models. These models then classified each "unknown" museum specimen as *A. jacobii* sp. nov. with very high probability (≥ 0.99). Consequently, every older museum specimen previously identified as *A. chiricahua* in Hamilton et al. (2016) (i.e., APH-2097, APH-2101, APH-2102, APH-2105, APH-2480-A, APH-2480-B, and APH-2548) has been transferred to *A. jacobii* sp. nov.

Results of the PCA and comparative morphometric ratios (i.e., boxplots) can be viewed in the Suppl. materials 4, 5. Males of *A. chiricahua* (smaller individuals), *A. jacobii* sp. nov., *A. madera*, and *A. Marxi* separate from most other members of the *Marxi* species group along PC1. And while there is some overlap in PCA morphospace between various combinations of these taxa, it appears there is slight separation between *A. jacobii* sp. nov. and *A. marxi* (which are sister taxa, see below), and between *A. jacobii* sp. nov. and *A. chiricahua* (S4A). Females of *A. jacobii* sp. nov. clearly separate from all other members of the *Marxi* species group along PC1 (S4B). Additionally, there are a number of measurement ratios that can be used to separate species (i.e., they do not overlap) – some of these measurement ratios are used in the species diagnoses (below).

Phylogenetic and phylogenomic analyses

The aligned COX1 dataset comprised 906 sites (230 parsimony informative). ModelFinder determined that the best-fit model for the data (based on the Bayesian Information Criterion) was TIM2+F+I+G4. The ML tree topology (log-likelihood score = -4360.8489) (Fig. 1) shows strong support for a clade (97 bs) comprising the five previously described Madrean species (A. bacadehuachi, A. catalina, A. chiricahua, A. madera, and A. peloncillo). This group is sister to a weakly supported clade (65 bs) comprising A. vorhiesi and A. marxi + A. jacobii sp. nov. COX1 haplotypes for A. marxi and A. jacobii sp. nov. form a very strongly supported clade (100 bs), as do the haplotypes for A. catalina and A. chiricahua (99 bs). The most notable results are that (1) A. chiricahua and A. jacobii sp. nov. do not form a clade and are not the most closely related lineages to each other; and (2) COX1 haplotypes for A. jacobii sp. nov. are diphyletic (see red branches in Fig. 1), resulting in A. marxi paraphyly (i.e., A. marxi sample APH-1425 is sister to A. jacobii sp. nov. samples from the Barfoot Park locality). Individuals of A. jacobii sp. nov. from Cave Creek Canyon (APH-4006, APH-4020, APH-4022, APH-5079) and the type locality near Onion Saddle (APH-5001, APH-5002, APH-5003, APH-5082) form a very strongly supported clade (100 bs); each population is reciprocally monophyletic with strong support (94 bs and 99 bs, respectively). Individuals of A. jacobii sp. nov. from the high-elevation Barfoot Park locality (APH-4018, APH-4019, APH-4029, APH-5080, APH-5081) form a clade (90 bs) nested within A. marxi. Support for this internal nesting structure is moderately weak, however (56 bs, 61 bs, 77 bs, respectively, starting at the node that groups the Barfoot Park population with A. marxi (APH-1425)).

The Ultraconserved Elements (UCE) phylogeny was inferred from 1311 loci across 24 samples using a combined spider-arachnid probe set originally merged in Maddison et al. (2020). The total length of the concatenated alignment was 1,870,552 bp and mean loci length was 1331 bp (minimum and maximum length of loci were 207 and 2438 bp, respectively). ModelFinder determined the best-fit model for each partition (i.e., UCE locus). The UCE tree topology (Fig. 2) has very strong support (100 bs) throughout; only two nodes received bootstrap values below 95. Fig. 2 identifies strong support (100 bs) for a clade that includes six previously described Madrean species (A. marxi, A. bacadehuachi, A. chiricahua, A. catalina, A. madera, A. peloncillo), two undetermined species, and A. jacobii sp. nov. Within the UCE phylogeny, A. jacobii sp. nov. is sister to A. marxi, with A. bacadehuachi sister to those lineages. Aphonopelma chiricahua is sister to APH-0622 and A. catalina, with A. madera being sister to those lineages. Another undetermined species (APH-0880) is the sister lineage to these lineages, and A. peloncillo is sister to all other members of the Marxi species group except A. vorhiesi. Importantly, the UCE tree depicts A. jacobii sp. nov. as phylogenetically distant from its syntopic congener, A. chiricahua.

The primary differences between the UCE and COX1 phylogenies are the interrelationships between various clades and species (e.g., placement of *A. bacadehuachi* and *A. vorhiesi*) and whether *A. jacobii* sp. nov. and *A. marxi* are reciprocally monophyletic (Fig. 2) or not (Fig. 1). These phylogenies share two important similarities: (1) *A. marxi* and *A. jacobii* sp. nov. form a strongly supported clade (100 bs); and (2) *A. chiricahua* and *A. jacobii* sp. nov. do not form a clade and are not closely related to each other. Unlike COX1, individuals of *A. jacobii* sp. nov. from the high-elevation Barfoot Park (APH-4005, APH-4029) and Onion Saddle (APH-4024, APH-5001, APH-5003) populations in mixed conifer forest are reciprocally monophyletic (95 bs and 99 bs, respectively) and form a clade (95 bs) that is sister to the APH-5079 that was included from a lower-elevation site in Cave Creek Canyon.

Taxonomy

Family Theraphosidae Thorell, 1869 Subfamily Theraphosinae Thorell, 1870

Genus Aphonopelma Pocock, 1901

- Aphonopelma Pocock, 1901: 553 (type species by original designation *Eurypel-ma seemanni* Pickard-Cambridge, 1897). First synonymized with *Rhechostica* by Raven (1985: 149).
- *Rhechostica* Simon, 1892: 162 (type species by original designation *Homoeom-ma texense* Simon, 1891). Suppressed as a senior synonym of *Aphonopelma* by ICZN (1991: 166–167).
- *Delopelma* Petrunkevitch, 1939: 567 (type species by original designation *Eurypelma marxi* Simon, 1891) (considered a subgenus of *Aphonopelma* by Chamberlin, 1940: 26). First synonymized with *Rhechostica* by Raven (1985: 151).
- Gosipelma Chamberlin, 1940: 4 (type species by original designation Gosipelma angusi Chamberlin, 1940). Originally described as a subgenus of

Aphonopelma, but never elevated to full generic status. First synonymized with *Rhechostica* by Raven (1985: 153).

- *Chaunopelma* Chamberlin, 1940: 30 (type species by original designation *Delopelma radinum* Chamberlin & Ivie, 1939). First synonymized with *Rhechostica* by Raven (1985: 151).
- Apachepelma Smith, 1995: 45 (type species by original designation Aphonopelma paloma Prentice, 1992). First synonymized with Aphonopelma by Prentice (1997: 147).

Marxi species group (informally designated by Hamilton et al. 2016)

Aphonopelma bacadehuachi Hendrixson, 2019 Aphonopelma catalina Hamilton, Hendrixson & Bond, 2016 Aphonopelma chiricahua Hamilton, Hendrixson & Bond, 2016 Aphonopelma jacobii Hamilton & Hendrixson, 2024, sp. nov. Aphonopelma madera Hamilton, Hendrixson & Bond, 2016 Aphonopelma marxi (Simon, 1891) Aphonopelma peloncillo Hamilton, Hendrixson & Bond, 2016 Aphonopelma vorhiesi (Chamberlin & Ivie, 1939)

Aphonopelma jacobii Hamilton & Hendrixson, 2024, sp. nov.

https://zoobank.org/28ABD35B-FB05-4ADF-ABF0-622ED48DAC71 Figs 3-7, 11

Aphonopelma chiricahua, in part: Hamilton et al. 2016: 90, 91, 93–95, fig. 38 (APH-2097, misidentification); 95 (APH-2101, APH-2102, APH-2105, APH-2480-A, APH-2480-B, APH-2548, misidentifications).

Type material. *Holotype*. UNITED STATES • ♂; Arizona, Cochise County, Chiricahua Mountains, along Forest Road 42D above Onion Saddle; 31.92838°N, 109.26311°W ¹; 2364 m; 31 Oct. 2018; Brent E. Hendrixson & Michael A. Jacobi leg.; UIM; APH-5002.

Paratype. UNITED STATES • 1 ♀; same data as for holotype; UIM; APH-5001 • 1 ♂; same data as for holotype; AMNH; APH-5003.

Etymology. The specific epithet is a patronym in honor of our friend, Michael A. Jacobi, who facilitated many of our field trips into the Chiricahua Mountains in 2018 and 2019. In addition, he generously carried out field work in the MSI on our behalf and discovered many important specimens, including the first female burrows of *A. chiricahua* and this remarkable new species. His tireless work in the field and passion for natural history have immensely helped improve our knowledge of tarantula biology and biodiversity in the Chiricahua Mountains and surrounding areas.

Diagnosis. Aphonopelma jacobii sp. nov. is a member of the Marxi species group and can be distinguished by a combination of morphological, genomic, behavioral, and distributional features. This species is a mid- to late-fall breeder endemic to the Chiricahua Mountains in southeastern Arizona. Nuclear DNA identifies A. jacobii sp. nov. as a monophyletic lineage (Fig. 2) that is sister to A. marxi (distributed along the Colorado Plateau) and phylogenetically distinct from the



Figure 3. Live habitus of Aphonopelma jacobii sp. nov. a male holotype (APH-5002) b female paratype (APH-5001).


Figure 4. Morphology of *Aphonopelma jacobii* sp. nov. (male holotype, APH-5002) **a** carapace, dorsal view **b** coxa of leg I, prolateral view **c** femur of leg III, dorsal view **d** metatarsus and tarsus of leg III, ventral view **e** metatarsus and tarsus of leg IV, ventral view **f** pedipalp, prolateral view **g** palpal bulb, dorsal view **h** palpal bulb, retrolateral view **i** tibia of leg I showing mating clasper, prolateral view. Scale bars: 2 mm.

other tarantula species endemic to the Chiricahua Mountains (i.e., *A. chiricahua*). *Aphonopelma jacobii* sp. nov. is probably the only tarantula species encountered in the high-elevation mixed conifer forests of the Chiricahua Mountains, but its distribution overlaps with *A. chalcodes* Chamberlin, 1940, *A. chiricahua*, *A. gabeli* Smith, 1995, and *A. vorhiesi* at lower elevations in the oak and pine-oak woodlands.

Aphonopelma jacobii sp. nov. is readily distinguished from adult *A. chalcodes* and *A. gabeli* by coloration and size (Fig. 3; Hamilton et al. 2016: figs 30, 45). Males of the new species are similar in appearance to *A. vorhiesi* due to their shared coloration (i.e., general black body with bright orange or red setae on the abdomen, Fig. 3a; Hamilton et al. 2016: fig. 142), but are noticeably smaller (Cl 6.708–8.955 vs 10.018–13.980), possess a larger A3/M4 ratio (0.659–0.790 vs 0.469–0.566), and breed later in the fall (October–November vs July–October) (note: males of *A. vorhiesi* found in October are generally worn and faded whereas males of *A. jacobii* sp. nov. are lively and vibrant). Males of *A. jacobii* sp. nov. and *A. chiricahua* are very similar morphologically and behaviorally. They possess similar coloration (Figs 3a, 8d) and share a common breeding period, but the new species does separate slightly from *A. chiricahua* in PCA morphospace (S4A), is statistically smaller (Cl 7.679 ± 0.71 vs 9.864 ± 2.00, *t* (18) = 3.6964, *P* = 0.0017), possesses a slightly



Figure 5. Morphology of *Aphonopelma jacobii* sp. nov. (female paratype, APH-5001) **a** carapace, dorsal view **b** coxa of leg I, prolateral view **c** metatarsus and tarsus of leg III, ventral view **d** metatarsus and tarsus of leg IV, ventral view **e** pedipalp, prolateral view **f** spermathecae. Scale bars: 2 mm.

smaller T1/P4 ratio (2.175–2.545 vs 2.576–2.991), and has a proportionally shorter embolus relative to the palpal bulb (Fig. 4g, h; Hamilton et al. 2016: fig. 37g, h). Males of *A. jacobii* sp. nov. can be further distinguished from other members of the *Marxi* species group by the following important ratios and measurements: T1/P4 (2.175–2.545) is smaller than *A. catalina* (2.814–3.033); A3/M4 (0.659–0.790) is larger than *A. bacadehuachi* (0.495), *A. catalina* (0.477–0.520), *A. madera* (0.540–0.602), and *A. peloncillo* (0.457–0.581); and Bl_r (2.505–3.061) is smaller than *A. marxi* (3.194–3.781). Additional ratios that might be useful for separating males of *A. jacobii* sp. nov. from various other members of the *Marxi* species group include Cl/A3, Cl/M3, PTI/M3, PTI/M4, and T1/F3 (see Suppl. material 5).

Females of A. *jacobii* sp. nov. are noticeably smaller than A. *chiricahua* and A. *vorhiesi* (Cl7.621–9.018 v. 14.230–15.530 and 11.230–16.380, respectively), separate in PCA morphospace (S4B), and possess slightly different coloration (Figs 3b, 8a–c; Hamilton et al. 2016: fig. 142). Furthermore, females of the new species can be distinguished from other members of the *Marxi* species group by the following important ratio: M3/F4 (0.509–0.534) is smaller than A. *baca-dehuachi* (0.613), A. *catalina* (0.582–0.604), A. *madera* (0.550–0.616), A. *marxi* (0.550–0.598), A. *peloncillo* (0.598–0.655), and A. *vorhiesi* (0.587–0.657). Additional ratios that might be useful for separating females of A. *jacobii* sp. nov. from various other members of the *Marxi* species group include F1/T4, M1/A3, SC4, and T1/T4 (see Suppl. material 5).



Figure 6. Dorsal habitus of Aphonopelma jacobii sp. nov. a male (APH-4022) b female (APH-4018). Scale bar: 25 mm.

Description of male holotype (APH-5002: Figs 3a, 4). Specimen collected alive wandering on road, preserved in 80% ethanol; original coloration faded due to preservation (Fig. 3a). Left legs I, III, IV, and left pedipalp removed for measurements and photographs; stored in vial with specimen. Right leg III removed for DNA and stored at -80 °C at UIM. General coloration: black or faded black. Cephalothorax: Cl 6.708, Cw 6.509; densely clothed with black/faded black pubescence, appressed to surface; fringe covered in long setae not closely appressed to surface; foveal groove medium deep and slightly procurved; pars cephalica region rises gradually from foveal groove, gently arching anteriorly toward ocular area; AER procurved, PER slightly recurved; normal sized chelicerae; clypeus generally straight but extends forward on a slight curve near the eyes; LBI 0.942, LBw 1.596; sternum hirsute, clothed with short black/brown, densely packed setae. Abdomen: densely clothed in short black/brown pubescence with numerous longer, paler setae interspersed (generally red or orange in vita, Fig. 3a); dense dorsal patch of black Type I urticating setae (Cooke et al. 1972); ventral setae same as dorsal. Legs: Hirsute; densely clothed with short, similar length black/brown setae, and longer setae dorsally. Metatarsus I slightly curved. F1 7.478; F1w 1.836; P1 2.780; T1 6.482; M1 4.543; A1 4.251; L1 length 25.534; F3 5.765; F3w 1.823; P3 2.438; T3 4.445; M3 4.559; A3 4.475; L3 length 21.682; F4 7.082; F4w 1.554; P4 2.573; T4 6.199; M4 6.068; A4 4.980; L4 length 26.902; femur III is normal, not noticeably swollen or wider than other legs (Fig. 4c). All tarsi fully scopulate. Extent of metatarsal scopulation: leg III (SC3) = 48.8%, leg IV (SC4) = 29.3% (Fig. 4d, e). Six ventral spinose setae (megaspines), one prolateral spinose seta, and four ventral spinose setae at the apical edge on metatarsus III; nine ventral spinose setae (megaspines), two prolateral and one retrolateral spinose setae, and eight ventral spinose setae



Figure 7. Habitat images of *Aphonopelma jacobii* sp. nov. from the Chiricahua Mountains, Cochise County, Arizona **a, b** type locality along Forest Road 42D above Onion Saddle **c, d** Barfoot Park **e** along Forest Road 42A **f** open burrow at the type locality. Photographs of images **d** and **e** provided by Michael A. Jacobi.

at the apical edge on metatarsus IV; one prolateral megaspine and two ventral megaspines are present on the prolateral tibia of the mating clasper (tibia I); two megaspines, that project anteriorly, can be found at the apex of each tibial apophyses (Fig. 4i). Coxa I: prolateral surface a mix of fine hair-like and very thin tapered setae (Fig. 4b). Pedipalps: hirsute; densely clothed in the same setal color as the other legs, with numerous longer ventral setae; one spinose seta at the apical, prolateral femur and two spinose setae on the prolateral tibia (Fig. 4f); PTI 4.627, PTw 1.763. When extended, embolus tapers with a gentle curve to the retrolateral side near apex, embolus slender, no keels (Fig. 4g, h).

Male variation (*n* = 14). Cl 6.708-8.955 (7.679 ± 0.71), Cw 6.254-8.654 (7.467 ± 0.23), LBI 0.684-1.340 (0.930 ± 0.05), LBw 0.985-1.971 (1.513 ± 0.09), F1

7.145–9.585 (8.220 ± 0.19), F1w 1.821–2.596 (2.124 ± 0.06), P1 2.780–3.825 (3.225 ± 0.08), T1 5.851–7.851 (6.863 ± 0.54), M1 4.090–5.524 (4.807 ± 0.11), A1 3.572–4.975 (4.307 ± 0.10), L1 length 23.568–31.239 (27.422 ± 0.59), F3 5.591–7.285 (6.396 ± 0.14), F3w 1.688–2.478 (2.012 ± 0.06), P3 2.304–3.177 (2.620 ± 0.07), T3 4.162–5.726 (4.812 ± 0.12), M3 4.379–5.807 (4.916 ± 0.11), A3 3.955–5.389 (4.724 ± 0.09), L3 length 20.391–27.358 (23.468 ± 0.50), F4 6.648–9.006 (7.728 ± 0.18), F4w 1.554–2.349 (1.90 ± 0.06), P4 2.524–3.516 (2.861 ± 0.08), T4 5.784–7.380 (6.647 ± 0.13), M4 5.772–8.177 (6.762 ± 0.16), A4 4.944–6.379 (5.464 ± 0.11), L4 length 25.672–34.458 (29.463 ± 0.62), PTI 4.420–5.822 (5.113 ± 0.11), PTw 1.763–2.419 (2.041 ± 0.05), SC3 ratio 0.414–0.609 (0.533 ± 0.01), SC4 ratio 0.283–0.404 (0.351 ± 0.01), coxa I setae = fine/ very thin and tapered, femur III condition = normal, not noticeably swollen or wider than other legs.

Description of female paratype (APH-5001: Figs 3b, 5). Specimen collected alive from burrow, preserved in 80% ethanol; original coloration faded due to preservation (Fig. 5a). Left legs I, III, IV, and pedipalp removed for photographs and measurements; stored in vial with specimen. Right leg III removed for DNA and stored at -80 °C at UIM. Genital plate with spermathecae removed and cleared, stored in vial with specimen. General coloration: black or faded black and brown. Cephalothorax: Cl 9.018, Cw 8.908; hirsute, densely clothed with black/faded black, pubescence closely appressed to surface; fringe densely covered in longer setae; foveal groove medium deep and slightly procurved; pars cephalica region gently rises from thoracic furrow, arching anteriorly towards ocular area; AER procurved, PER slightly recurved; robust chelicerae, clypeus extends forward on a slight curve; LBI 1.403, LBw 2.110; sternum hirsute, clothed with shorter black/faded black setae. Abdomen: densely clothed dorsally in short black setae with numerous longer, paler setae interspersed (generally red or orange in vita, Fig. 3b); dense dorsal patch of black Type I urticating setae (Cooke et al. 1972); ventral setae same as dorsal. Spermathecae (Fig. 5f): paired and separated, tapering from wide bases (not fused) and slightly curving medially towards capitate bulbs. Legs: very hirsute, particularly ventrally; densely clothed in short and medium black/brown pubescence, with longer setae colored similarly as the long abdominal setae; F1 7.661; F1w 2.481; P1 3.568; T1 5.864; M1 3.553; A1 3.854; L1 length 24.500; F3 6.206; F3w 2.032; P3 2.628; T3 4.230; M3 4.191; A3 4.340; L3 length 21.595; F4 7.885; F4w 2.122; P4 2.969; T4 6.156; M4 6.079; A4 4.845; L4 length 27.904. All tarsi fully scopulate. Extent of metatarsal scopulation: leg III (SC3) = 50.4%, leg IV (SC4) = 27.3% (Fig. 5d). Two ventral spinose setae (megaspines) and six ventral spinose setae at the apical edge on metatarsus III; eight ventral spinose setae (megaspines), one prolateral spinose setae, and six ventral spinose setae at the apical edge on metatarsus IV. Coxa I: prolateral surface a mix of fine hairlike and very thin tapered setae (Fig. 5b). Pedipalps (Fig. 5e): Densely clothed in the same setal color as the other legs; two prolateral megaspines (one of these apical) and two apical ventral megaspines are present on the palpal tibia.

Female variation (n = 6). Cl 7.621–9.018 (8.320 ± 0.44), Cw 7.433–8.908 (8.171 ± 0.47), LBI 1.261–1.403 (1.332 ± 0.04), LBw 1.978–2.110 (2.044 ± 0.04), F1 6.692–7.661 (7.177 ± 0.31), F1w 2.131–2.481 (2.306 ± 0.11), P1 2.885–3.568 (3.227 ± 0.22), T1 4.952–5.864 (5.399 ± 0.32), M1 3.230–3.553 (3.392 ± 0.10), A1 3.530–3.854 (3.692 ± 0.10), L1 length 21.659–24.500 (23.080 ± 0.90), F3

5.383-6.206 (5.795 ± 0.26), F3w 1.808-2.032 (1.920 ± 0.07), P3 2.408-2.628 (2.518 ± 0.07), T3 3.847-4.230 (4.039 ± 0.12), M3 3.608-4.191 (3.900 ± 0.18), A3 4.104-4.340 (4.222 ± 0.07), L3 length 19.350-21.595 (20.473 ± 0.71), F4 6.867-7.855 (7.361 ± 0.31), F4w 1.968-2.122 (2.045 ± 0.05), P4 2.596-2.969 (2.783 ± 0.12), T4 5.630-6.156 (5.893 ± 0.17), M4 5.309-6.079 (5.694 ± 0.24), A4 4.741-4.845 (4.793 ± 0.03), L4 length 25.143-27.904 (26.524 ± 0.87), SC3 ratio 0.505-0.571 (0.538 ± 0.02), SC4 ratio 0.273-0.275 (0.274 ± 0.01), coxa I setae = fine/very thin and tapered. Spermathecae variation as in Fig. 5f, Suppl. material 6 (and Hamilton et al. 2016: fig. 38 for APH-2097).

Other material. UNITED STATES - Arizona · Cochise County · 12; Chiricahua Mountains, Southwest Research Station; 30 Nov. 1965; Jon Jenson leg.; AMNH; APH-2097 • 1♂; Chiricahua Mountains, Cave Creek Canyon; 30 Nov. 1963; V. Roth leg.; AMNH; APH-2101 • 13; Chiricahua Mountains, Upper Cave Creek Canyon; 1966; Marlene Posedly leg.; AMNH; APH-2102 • 13; Chiricahua Mountains, Southwest Research Station; 31 Oct. 1956; E. Ordway leg.; AMNH; APH-2105 • 13; Chiricahua Mountains, Sunny Flat; 30 Oct. 1971; V. Roth leg.; AMNH; APH-2480-A • 1 강; Chiricahua Mountains, Southwest Research Station; 20 Nov. 1971; V. Roth leg.; AMNH; APH-2480-B • 13; Chiricahua Mountains, Rustler and Long Park; 4 Nov. 1970; Joan Harper leg.; AMNH; APH-2548 • 1 imm.; Chiricahua Mountains, Barfoot Park Helispot; 31.91516°N, 109.28504°W 1; 2493 m; 27 Oct. 2019; Tom Patterson, Brent E. Hendrixson, Chris A. Hamilton, Michael A. Jacobi, Chad Campbell & Wyatt Mendez leg.; UIM; APH-4005 • 1 imm.; Chiricahua Mountains, along Forest Road 42D above Onion Saddle; 31.92838°N, 109.26311°W 1; 2364 m; 20 Oct. 2019; Wyatt Mendez leg.; UIM; APH-4024 · 1[°]; Chiricahua Mountains, on hillside along Forest Road 42A; 31.88062°N, 109.22087°W 1; 1717 m; 27 Oct. 2019; Chris A. Hamilton, Brent E. Hendrixson, Michael A. Jacobi, Wyatt Mendez, Chad Campbell & Tom Patterson leg.; UIM; APH-4006 • 2♀; Chiricahua Mountains, Barfoot Park Helispot; 31.91516°N, 109.28504°W 1; 2493 m; 27 Oct. 2019; Wyatt Mendez, Brent E. Hendrixson, Chris A. Hamilton, Michael A. Jacobi, Chad Campbell & Tom Patterson leg.; UIM; APH-4018; AMNH; APH-4019 · 13; Chiricahua Mountains, along Forest Road 42; 31.88139°N, 109.18732°W 1; 1593 m; 26 Oct. 2019; Chris A. Hamilton & Brent E. Hendrixson leg.; UIM; APH-4020 • 1∂; Chiricahua Mountains, 1 Pogo Hill; 31.88061°N, 109.20386°W 1; 1662 m; 27 Oct. 2019; Chris A. Hamilton, Brent E. Hendrixson & Wyatt Mendez leg.; UIM; APH-4022 • 13; Chiricahua Mountains, Barfoot Park Helispot; 31.91516°N, 109.28504°W ¹; 2493 m; 26 Oct. 2019; Chad Campbell, Michael A. Jacobi & Tom Patterson leg.; UIM; APH-4029 • 1²; Chiricahua Mountains, 1 Pogo Hill; 31.88061°N, 109.20386°W ¹; 1662 m; 9 Sept. 2019; Wyatt Mendez leg.; UIM; APH-5079 • 2∂; Chiricahua Mountains, Barfoot Park Helispot; 31.91516°N, 109.28504°W 1; 2493 m; 3 Nov. 2019; Wyatt Mendez leg.; UIM; APH-5080, APH-5081 • 1♂; Chiricahua Mountains, along Forest Road 42D above Onion Saddle; 31.92838°N, 109.26311°W 1; 2364 m; 8 Nov. 2019, Wyatt Mendez leg.; UIM; APH-5082.

Distribution and natural history. *Aphonopelma jacobii* sp. nov. is endemic to the Chiricahua Mountains (Figs 7, 11) in southeastern Arizona where it has been encountered in plant communities ranging from mid-elevation Madrean evergreen oak woodlands in Cave Creek Canyon (Fig. 7e) to high-elevation mixed conifer forests near Onion Saddle (Fig. 7a, b) and Barfoot Park (Fig. 7c, d). To our knowledge, it is the only tarantula in the Chiricahua Mountains encountered

above the pine-oak woodland zone. The highest confirmed elevation record for this species—as observed by us—is just below 2500 m at Barfoot Park, but other sightings suggest it is found perhaps as high as 2700 m near Rustler and Long Parks (see APH-2548, misidentified as *A. chiricahua* in Hamilton et al. 2016). In the United States, only *A. marxi* has been reported from higher elevations (~ 2830 m in the Chuska Mountains of northeastern New Mexico, APH-0452 in Hamilton et al. 2016), but still far below the elevation records for the remarkable neotropical tarantula genera *Antikuna* Kaderka, Ferretti, West, Lüddecke & Hüsser, 2021 (4689 m, Kaderka et al. 2021), *Hapalotremus* Simon, 1903 (4524 m, Ferretti et al. 2018), *Bistriopelma* Kaderka, 2015 (4398 m, Kaderka 2015), and *Euathlus* Ausserer, 1875 (4153 m, Quispe-Colca and Ferretti 2021) from the South American Andes.

Mature female and immature individuals of A. jacobii sp. nov. have only been extracted from burrows (i.e., specimens have not been observed beneath rocks or other surface debris). Burrows are generally located in meadows or exposed patches of soil with limited overstory structure. This perhaps allows their burrows to receive more direct sunlight to maintain higher temperatures in these otherwise cool habitats. Burrow entrances of mature females measure c. 15 mm in diameter and have been observed with (Fig. 7f) and without traces of silk along the perimeter. The breeding period for this species appears to be limited. Mature males are active during October and November, similar to other fall-breeding members of the Marxi species group in southeastern Arizona, including A. chiricahua (Hendrixson et al. 2015; Hamilton et al. 2016). In fact, males of A. jacobii sp. nov. and A. chiricahua (APH-4020 and APH-4023, respectively) were observed within 250 m of each other on consecutive days in late October 2019. Males are most frequently encountered during daylight hours, but one individual (APH-4022) was observed wandering on a mild evening (c. 20 °C) during early twilight. Two other males (APH-5002, APH-5003) were observed at the type locality on a breezy and cool morning ($\sim 5-10$ °C, ~ 1030 hrs).

The discovery of *A. jacobii* sp. nov. documents the first known case of syntopy between five species of *Aphonopelma* (i.e., the distributions of *A. jacobii* sp. nov., *A. chalcodes*, *A. chiricahua*, *A. gabeli*, and *A. vorhiesi* overlap in Cave Creek Canyon). As noted above, the breeding periods of *A. jacobii* sp. nov. and *A. chiricahua*—but not *A. chalcodes*, *A. gabeli*, or *A. vorhiesi*—coincide with each other. It is unknown how these two species maintain cohesion and reproductive isolation in the face of significant overlap between their distributions and breeding periods. Future studies should investigate the various factors that promote selection for prezygotic or postzygotic reproductive barriers and reduce potential hybridization between these synchronously breeding populations (see also Prentice 1997).

Aphonopelma chiricahua Hamilton, Hendrixson & Bond, 2016 Figs 8–11

Aphonopelma chiricahua Hamilton et al. 2016: 90–93, 95–98, figs 37, 39.

Type material. *Holotype.* UNITED STATES • ♂; Arizona, Cochise County, Chiricahua Mountains, Cave Creek Canyon, 1.6 km past the Cathedral Vista Trailhead along Forest Road 42 (toward the Southwest Research Station); 31.88133°N, 109.18797°W ⁴; 1600 m; 14 Nov. 2013; Helen Snyder leg.; UIM; APH-3191.



Figure 8. Live habitus of *Aphonopelma chiricahua* **a**, **b** in situ female (APH-5400) **c** female (APH-5050) **d** male (APH-5144). Photographs of images **a** and **b** provided by Leonardo Chávez; photograph of image **c** provided by Michael A. Jacobi.

Remarks. In the original description of A. chiricahua, Hamilton et al. (2016: 98) stated: "Of particular note is the size of the holotype male and paratype female; the two specimens probably represent opposite extremes on the size spectrum for what is possible in this species. The rather large holotype male was chosen because it was a fresh specimen and could be associated with molecular data that was unique from all other samples in the area, at the time. The female, though small, is sexually mature (based on spermathecal development)." Based on the body size, morphology, and collection data for the female paratype of A. chiricahua (APH-2097), we have determined that the specimen was misidentified and should now be considered A. jacobii sp. nov. Similarly, except for the male holotype (APH-3191), we have determined that the male specimens of A. chiricahua reported in Hamilton et al. (2016) were misidentified and should be considered A. jacobii sp. nov. too. Consequently, a redescription and emended diagnosis for A. chiricahua are necessary to reassess limits of morphological variation in the species. The following redescription of A. chiricahua is based on several newly acquired individuals (mature males and females) whose identities have been confirmed by com-



Figure 9. Morphology of *Aphonopelma chiricahua* (female, APH-5400) **a** carapace, dorsal view **b** coxa of leg I, prolateral view **c** metatarsus and tarsus of leg III, ventral view **d** metatarsus and tarsus of leg IV, ventral view **e** pedipalp, prolateral view **f** spermathecae. Scale bars: 2 mm.

paring their COX1 and UCE sequence data to the male holotype (Figs 1, 2; unpublished data).

Emended diagnosis. Aphonopelma chiricahua is a member of the Marxi species group and can be distinguished by a combination of morphological, genomic, behavioral, and distributional features. This species is a mid- to late-fall breeder endemic to the Chiricahua Mountains in southeastern Arizona. Mitochondrial and nuclear DNA identifies A. chiricahua as a monophyletic lineage (Figs 1, 2) that is sister to A. catalina (and an undetermined species) and phylogenetically distinct from the other tarantula species endemic to the Chiricahua Mountains (i.e., A. jacobii sp. nov.). Aphonopelma chiricahua is found in oak and pine-oak woodlands where its distribution overlaps with A. chalcodes, A. gabeli, A. jacobii sp. nov., and A. vorhiesi.

For features that can be used to distinguish *A. chiricahua* from *A. jacobii* sp. nov., refer to the diagnosis of the latter species provided above. When in doubt, the identity of both species (including immature specimens) can be readily confirmed with COX1 barcoding. *Aphonopelma chiricahua* is readily distinguished from *A. chalcodes* and *A. gabeli* by coloration (Fig. 8; Hamilton et al. 2016: figs 30, 45). Males of *A. chiricahua* are similar in appearance to *A. vorhiesi* due to their shared coloration (i.e., general black body with



Figure 10. Habitat images of *Aphonopelma chiricahua* from the Chiricahua Mountains, Cochise County, Arizona **a**, **b** Madrean pine-oak woodland near the Cathedral Vista Trail off Forest Road 42 **c** rocky and grassy microhabitat near the Cathedral Vista Trail **d** silk-covered burrow of a mature female (APH-5400). Photographs of images **b** and **d** provided by Leonardo Chávez.

bright orange or red setae on the abdomen, Fig. 8d; Hamilton et al. 2016: fig. 142), but possess a larger A3/M4 ratio (0.643–0.697 vs 0.469–0.566), and breed later in the fall (October–December vs July–October) (note: males of *A. vorhiesi* found in October are generally worn and faded whereas males of *A. chiricahua* are lively and vibrant). Males of *A. chiricahua* can be further distinguished from other members of the *Marxi* species group by the following important ratios: A3/M4 (0.643–0.697) is larger than *A. bacadehuachi* (0.495), *A. catalina* (0.477–0.520), *A. madera* (0.540–0.602), and *A. peloncillo* (0.457–0.581); and F1/T1 (1.118–1.196) is slightly smaller than *A. marxi* (1.199–1.297). Additional ratios that might be useful for separating males of *A. chiricahua* from various other members of the *Marxi* species group include CI/A3, CI/M3, F3/M4, PTI/M3, PTI/M4, PTI/w, and T1/F3 (see Suppl. material 5).

Females of *A. chiricahua* are similar in appearance to *A. vorhiesi* due to their overlapping body sizes (Cl 14.230–15.530 v. 11.230–16.380) but possess slightly different coloration (Fig. 8a–c; Hamilton et al. 2016: fig. 142) and a larger



Figure 11. Map showing the known distribution of *Aphonopelma jacobii* sp. nov. (red circles) and *A. chiricahua* (yellow circles) in the Chiricahua Mountains, Cochise County, Arizona.

T1/P4 ratio (2.217–2.311 vs 1.774–2.091). Furthermore, females of *A. chirica-hua* can be distinguished from other members of the *Marxi* species group by the following important ratio: T1/P4 (2.217–2.311) is larger than *A. bacade-huachi* (0.781), *A. catalina* (1.985–2.045), *A. madera* (1.854–2.097), *A. marxi* (1.909–2.108), and *A. peloncillo* (1.704–2.013). Additional ratios that might be useful for separating females of *A. chiricahua* from various other members of the *Marxi* species group include SC4 and M1/M4 (see Suppl. material 5).

Redescription of male holotype (APH-3191 Hamilton et al. 2016: figs 36, 37). Specimen collected alive wandering on road, preserved in 80% ethanol; original coloration faded due to preservation (Hamilton et al. 2016: fig. 37a). Left legs I, III, IV, and left pedipalp removed for measurements and photographs; stored in vial with specimen. Right legs III and IV removed for DNA and stored at -80 °C at UIM. General coloration: black or faded black. Cephalothorax: Cl 11.420, Cw 11.220; densely clothed with black/faded black pubescence, slightly appressed to surface and longer than lower elevation species, slight iridescence; fringe covered in long setae not closely appressed to surface; foveal groove medium deep and straight; pars cephalica region rises gradually from foveal groove, gently arching anteriorly toward ocular area; AER slightly procurved, PER very slightly recurved; normal sized chelicerae; clypeus slightly extends forward on a curve; LBI 1.37, LBw 1.61; sternum hirsute, clothed with medium black, densely packed setae. Abdomen: densely clothed in short black/ brown pubescence with numerous longer, paler setae interspersed (generally red or orange in vita, Hamilton et al. 2016: fig. 36), longer with a more hirsute

appearance than lower elevation species; dense dorsal patch of black Type I urticating setae (Cooke et al. 1972); ventral setae same as dorsal. Legs: hirsute; densely clothed with medium length black/brown setae, and longer setae ventrally. Metatarsus I slightly curved. F1 12.72; F1w 3.28; P1 4.95; T1 11.37; M1 7.61; A1 6.16; L1 length 42.812; F3 9.53; F3w 2.98; P3 4.11; T3 7.60; M3 7.79; A3 6.84; L3 length 35.878; F4 11.41; F4w 3.20; P4 4.41; T4 9.67; M4 10.28; A4 7.78; L4 length 43.559; femur III is normal, not noticeably swollen or wider than other legs (Hamilton et al. 2016: fig. 37c). All tarsi fully scopulate. Extent of metatarsal scopulation: leg III (SC3) = 65.5%, leg IV (SC4) = 37.9% (Hamilton et al. 2016: fig. 37d, e). Three ventral spinose setae (megaspines), one retrolateral spinose seta, and five ventral spinose setae at the apical edge on metatarsus III; nine ventral spinose setae (megaspines), one prolateral spinose setae, and three ventral spinose setae at the apical edge on metatarsus IV; two ventral megaspines are present on mating clasper (tibia I); three megaspines, that project anteriorly, can be found on the ventral tibial apophysis (Hamilton et al. 2016: fig. 37i). Coxa I: prolateral surface a mix of fine hair-like and thin/very thin tapered setae (Hamilton et al. 2016: fig. 37b). Pedipalps: hirsute; densely clothed in the same setal color as the other legs, with numerous longer ventral setae; one spinose seta at the apical, prolateral femur and four spinose setae on the prolateral tibia (Hamilton et al. 2016: fig. 37f); PTI 7.34, PTw 2.82. When extended, embolus tapers with a gentle curve to the retrolateral side near apex, embolus slender, no keels (Hamilton et al. 2016: fig. 37g, h).

Male variation (n = 6). Cl 7.673–12.230 (9.864 ± 2.00), Cw 6.968–11.620 (9.295±0.87), LBI 0.886–1.368 (1.147±0.08), LBw 1.609–2.019 (1.773±0.07), F1 8.560–13.229 (10.767±0.86), F1w 1.892–3.281 (2.465±0.22), P1 3.180–4.947 (3.965±0.31), T1 7.529–11.372 (9.396±0.72), M1 4.452–7.911 (6.307±0.61), A1 3.911–6.605 (5.279±0.49), L1 length 27.681–43.106 (35.713±2.96), F3 6.325–9.882 (8.167±0.70), F3w 1.673–3.038 (2.499±0.25), P3 2.397–4.112 (3.180±0.29), T3 4.605–7.673 (6.275±0.55), M3 4.603–7.919 (6.414±0.62), A3 4.452–6.952 (5.702±0.49), L3 length 22.517–35.878 (29.738±2.61), F4 7.650–12.048 (9.817±0.83), F4w 1.638–3.205 (2.348±0.24), P4 2.593–4.414 (3.368±0.29), T4 6.314–10.272 (8.129±0.66), M4 6.384–10.378 (8.616±0.76), A4 4.967–7.880 (6.415±0.51), L4 length 28.192–43.726 (36.345±2.98), PTI 4.885–7.529 (6.375±0.50), PTw 1.933–3.171 (2.536±0.20), SC3 ratio 0.542–0.656 (0.59±0.02), SC4 ratio 0.220–0.416 (0.324±0.03), coxa l setae = fine/very thin and tapered, femur III condition = normal, not noticeably swollen or wider than other legs.

Description of new female exemplar (APH-5400: Figs 8a, b, 9). Specimen collected live from burrow, preserved in 80% ethanol; original coloration faded due to preservation (Fig. 9a). Left legs I, III, IV, and pedipalp removed for photographs and measurements; stored in vial with specimen. Genital plate with spermathecae removed and cleared, stored in vial with specimen. General coloration: dark brown and faded black. Cephalothorax: Cl 15.530, Cw 14.350; hirsute, densely clothed with brown/black pubescence closely appressed to surface; fringe densely covered in longer setae; foveal groove medium deep and slightly procurved; pars cephalica region gently rises from thoracic furrow, arching anteriorly toward ocular area; carapace was cracked during specimen measurements; AER slightly procurved, PER recurved; robust chelicerae, clypeus is generally straight but extends forward on a slight curve in front of the

eyes; LBI 1.65, LBw 2.69; sternum hirsute, clothed with medium short brown setae. Abdomen: densely clothed dorsally in black/brown setae with numerous longer, paler setae interspersed (generally red or orange in vita, Fig. 8a, b); dense dorsal patch of black Type I urticating setae (Cooke et al. 1972); ventral setae shorter than dorsal. Spermathecae (Fig. 9f): paired and separated, tapering from wide bases (not fused) and slightly curving medially towards capitate bulbs. Legs: hirsute; densely clothed in short and medium black/brown pubescence; F1 12.486, F1w 4.127, P1 5.202, T1 9.772, M1 6.865, A1 6.037, L1 length 40.362, F3 10.323, F3w 3.768, P3 4.409, T3 6.94, M3 6.828, A3 7.108, L3 length 35.608, F4 11.915, F4w 3.634, P4 4.228, T4 9.58, M4 9.569, A4 6.976, L4 length 42.268. All tarsi fully scopulate. Extent of metatarsal scopulation: leg III (SC3) = 41.6%, leg IV (SC4) = 43.3% (Fig. 9c, d). Three ventral spinose setae (megaspines), one retrolateral spinose seta, and two ventral spinose setae at the apical edge on metatarsus III; four ventral spinose setae (megaspines), one prolateral spinose setae, and three ventral spinose setae at the apical edge on metatarsus IV. Coxa I: prolateral surface a mix of fine hair-like and thin tapered setae (Fig. 9b). Pedipalps (Fig. 9e): densely clothed in the same setal color as the other legs; one megaspine at the apical edge of the prolateral femur, five prolateral megaspines on the tibia (two on the apical edge), one ventral megaspine on the tibia.

Female variation (n = 2). Cl 14.230–15.530 (14.880 ± 0.65), Cw 12.960– 14.350 (13.655 ± 0.69), LBI 1.62–1.65 (1.635 ± 0.01), LBw 2.690–2.874 (2.782 ± 0.09), F1 11.362–12.486 (11.924 ± 0.56), F1w 4.058–4.127 (4.093 ± 0.03), P1 5.121–5.202 (5.162 ± 0.04), T1 9.599–9.772 (9.686 ± 0.09), M1 6.151– 6.865 (6.508 ± 0.36), A1 5.838–6.037 (5.938 ± 0.10), L1 length 38.071–40.362 (39.217 ± 1.15), F3 8.980–10.323 (9.652 ± 0.67), F3w 3.190–3.768 (3.479 ± 0.29), P3 3.737–4.409 (4.073 ± 0.34), T3 6.319–6.940 (6.630 ± 0.31), M3 6.761–6.828 (6.795 ± 0.03), A3 5.657–7.108 (6.383 ± 0.73), L3 length 31.454– 35.608 (33.531 ± 2.08), F4 11.749–11.915 (11.832 ± 0.08), F4w 3.418–3.634 (3.526 ± 0.11), P4 4.228–4.329 (4.279 ± 0.05), T4 9.439–9.580 (9.510 ± 0.07), M4 9.162–9.569 (9.366 ± 0.20), A4 6.697–6.976 (6.837 ± 0.14), L4 length 41.376–42.268 (41.822 ± 0.45), SC3 ratio 0.417–0.524 (0.47 ± 0.05), SC4 ratio 0.408–0.434 (0.421 ± 0.01), coxa I setae = fine/thin and tapered. Spermathecae variation as in Fig. 9f, Suppl. material 7.

Other material. UNITED STATES – Arizona • Cochise County • 1 imm.; Chiricahua Mountains, along Forest Road 42; 31.89129°N, 109.21079°W ¹; 1693 m; 26 Oct. 2019; Brent E. Hendrixson, Chris A. Hamilton, Michael A. Jacobi, Chad Campbell & Tom Patterson leg.; UIM; APH-4021 • 1♂; Chiricahua Mountains, along Forest Road 42; 31.88151°N, 109.18972°W ¹; 1608 m; 27 Oct. 2019; Wyatt Mendez leg.; UIM; APH-4023 • 1♂; Chiricahua Mountains, Cave Creek Canyon, along Forest Road 42; 31.90136°N, 109.15945°W ¹; 1501 m; 31 Oct. 2018; Brent E. Hendrixson & Michael A. Jacobi leg.; UIM; APH-5004 • 1♂; Chiricahua Mountains, Cave Creek Canyon, along Forest Road 42; 31.90136°N, 109.15945°W ¹; 1501 m; 31 Oct. 2018; Brent E. Hendrixson & Michael A. Jacobi leg., UIM; APH-5005 • 1♂; Chiricahua Mountains, 1 km from entrance to Chiricahua National Monument; 32.00918°N, 109.38123°W ¹; 1574 m; 30 Oct. 2018; Brent E. Hendrixson & Michael A. Jacobi leg.; UIM; APH-5006 • 1 imm.; Chiricahua Mountains, Cave Creek Visitor Information Center restroom; 31.89902°N, 109.16243°W ¹; 1512 m; 17 Oct. 2018; Michael A. Jacobi leg.; UIM; APH-5005 • 1♂; Chiricahua Mountains, Cave Creek Visitor Information Center restroom; 31.89902°N, 109.16243°W ¹; 1512 m; 17 Oct. 2018; Michael A. Jacobi leg.; UIM; APH-5006 • 1 imm.; Chiricahua Mountains, Cave Creek Visitor Information Center restroom; 31.89902°N, 109.16243°W ¹; 1512 m; 17 Oct. 2018; Michael A. Jacobi leg.; UIM; APH-5010 • 1♂; Chiricahua Mountains, Cave Creek Visitor Information Center restroom; 31.89902°N, 109.16243°W ¹; 1512 m; 17 Oct. 2018; Michael A. Jacobi leg.; UIM; APH-5010 • 1♂; Chiricahua Mountains, Cave Creek Visitor Information Center restroom; 31.89902°N, 109.16243°W ¹; 1512 m; 17 Oct. 2018; Michael A. Jacobi leg.; UIM; APH-5010 • 1♂; Chiricahua Mountains, Cave Creek

Canyon, along Forest Road 42 at horse corral; 31.89820°N, 109.16286°W ¹; 1515 m; 16 Nov. 2018; Michael A. Jacobi leg.; AMNH; APH-5049 • 1 \bigcirc ; Chiricahua Mountains, on hillside along Forest Road 42; 31.89057°N, 109.21072°W ¹; 1720 m; 21 June 2018; Michael A. Jacobi leg.; UIM; APH-5050 • 1 imm.; Chiricahua Mountains, Cave Creek Canyon, Cathedral Vista Point Trail; 31.88529°N, 109.17266°W ¹; 1567 m; 11 Nov. 2019; Wyatt Mendez & Walter Schoepfle leg.; UIM; APH-5078 • 1 $\stackrel{d}{\circ}$; Chiricahua Mountains, Cave Creek Visitor Information Center; 31.89916°N, 109.16204°W ⁴; 1506 m; 9 Nov. 2021; David Jasper leg.; UIM; APH-5126 • 1 $\stackrel{d}{\circ}$; Chiricahua Mountains, along Forest Road 42; 31.88151°N, 109.19304°W ⁴; 1618 m; 12 Oct. 2021; Wyatt Mendez leg.; UIM; APH-5144 • 1 \bigcirc ; Chiricahua Mountains, Cave Creek Canyon, Cathedral Vista Point area; 31.88434°N, 109.17143°W ¹; 1634 m; 30 Aug. 2023; Chris A. Hamilton, Leonardo Chávez & Wyatt Mendez leg.; UIM; APH-5400.

Distribution and natural history. Aphonopelma chiricahua is endemic to the Chiricahua Mountains (Figs 10, 11) in southeastern Arizona where it has been encountered in mid-elevation Madrean evergreen oak woodlands and pine-oak woodlands (Fig. 10a-c; ~ 1500-1720 m). Hamilton et al. (2016) noted that very little was known about the natural history of A. chiricahua. At the time, we had never observed this species in the field despite having spent hundreds of person-hours searching for it during the preceding decade. The lack of observations prompted us to hypothesize that "these spiders probably seek refuge under rocks and rarely place silk around their burrow entrances" (Hamilton et al. 2016: 95, 97). We now know that this is not entirely the case. The burrows of two mature females, one found in June 2018 (APH-5050) as reported by Jacobi (2018) and the other found in August 2023 (APH-5400; Fig. 10d), were indeed covered by a dense mat of silk. Both burrows were found in exposed grassy areas among scattered woodland vegetation. Nevertheless, mature females remain incredibly difficult to find. We are unsure whether this is because females are rare, if they are simply exceptionally good at concealing their burrow entrances, or both. Immature specimens have been found under rocks (APH-4021), inside small burrows located along vertical banks (APH-5078), and inside human-made structures (APH-5010). Mature males are diurnally active in October, November, and perhaps early December (see https://www.facebook. com/watch/?v=785835144804065 for an observation tentatively attributed to this species that was shared by the staff at Chiricahua National Monument).

Discussion

Non-monophyly of MSI endemics in the Chiricahua Mountains

The phylogenetic trees presented in Figs 1, 2 revealed two interesting results. First, the COX1 and UCE datasets both indicate that *A. jacobii* sp. nov. and *A. marxi* form a strongly supported clade. These results were unexpected because prior studies (Hendrixson et al. 2015; Hamilton et al. 2016; Hendrixson 2019) showed that the Madrean-affiliated taxa (i.e., *A. bacadehuachi*, *A. catalina*, *A. chiricahua*, *A. madera*, and *A. peloncillo*) belonged to a clade exclusive of *A. marxi*, a denizen of the Colorado Plateau (albeit weakly supported in Hamilton et al. 2016). Furthermore, monophyly of the Madrean species has been corroborated by samples we have analyzed from a handful of other MSI ranges in Arizona and Mexico (unpublished data), so it was expected that newly discovered endemic species from the MSI might follow a similar pattern and group with the Madrean taxa. In hindsight, however, the close relationship between *A. jacobii* sp. nov. and *A. marxi* is not surprising because biotic affinities between the Colorado Plateau and Chiricahua Mountains are well established, especially for taxa found at higher elevations (e.g., Linsley et al. 1961; Bennett et al. 1996). The rather "distant" relationship between *A. jacobii* sp. nov. and *A. chiricahua* is interesting because it suggests that *A. jacobii* sp. nov. and *A. chiricahua* have assimilated into the woodland and forest biota of the Chiricahua Mountains independently (see Agnarsson et al. 2016 for a similar result in Madagascan *Anelosimus* spiders), rather than having diverged in situ, as has been inferred for other invertebrate lineages with limited vagility that are endemic to specific MSI ranges (e.g., Weaver et al. 2010; Jochim et al. 2020).

The other intriguing result is that A. jacobii sp. nov. is diphyletic and A. marxi is paraphyletic in the COX1 analysis (Fig. 1). Mitochondrial haplotypes from the A. jacobii sp. nov. Barfoot Park population are embedded within a clade that includes all sampled haplotypes of A. marxi, whereas the Onion Saddle and Cave Creek Canyon populations of A. jacobii sp. nov. form a strongly supported clade that is sister to the A. marxi + Barfoot Park clade. This is in striking contrast to the UCE analysis (Fig. 2) which recovered both species as reciprocally monophyletic. These cases of "species-level polyphyly" and "paraphyly" (i.e., gene-tree/ species-tree incongruence) (see Funk and Omland 2003) are likely due to ancient admixture and asymmetric mitochondrial introgression (e.g., Horoiwa et al. 2021) between A. marxi and A. jacobii sp. nov., a phenomenon hypothesized to have occurred multiple times in Aphonopelma (Hamilton et al. 2016), including two other species from this region (A. vorhiesi and A. chalcodes). These two species are currently separated by c. 100 km of unsuitable habitat (i.e., arid valleys and hills consisting of semidesert grasslands and desert scrub), but cooler climates in the past may have permitted contact between them. Extensive coniferous woodlands persisted at lower elevations during the Pleistocene (Van Devender and Spaulding 1979) and could have provided habitat connectivity between the Chiricahua Mountains and the uplands of the Mogollon Rim and Colorado Plateau. Further sampling and population structure analyses will be necessary to address this matter (see Newton et al. 2020, 2023; Starrett et al. 2024).

Tarantula biodiversity of the Chiricahua Mountains

The Chiricahua Mountains are located at the intersection of the southern Colorado Plateau and Rocky Mountains, northern Sierra Madre Occidental, eastern Sonoran Desert, and western Chihuahuan Desert. As such, the massif's biota is extensively influenced by each of these ecoregions, resulting in a mosaic of diversity unlike that of any other region in the United States. Additionally, as part of the Madrean Archipelago, woodland and forest habitats in the Chiricahua Mountains are physically isolated from those of other MSI ranges, leading to the evolution of numerous short-range endemic species that are restricted to them. Taken together, these mountains are part of the Madrean pine-oak woodlands biodiversity hotspot (Mittermeier et al. 2004) and are among the most biodiverse regions in the United States (Coronado Planning Partnership 2008), so it is no surprise that tarantulas show similar patterns of high diversity.

The Chiricahua Mountains and surrounding foothills, canyons, and grasslands are home to the most diverse assemblage of tarantula species anywhere in the United States. Eight of the 30 (27%) described species have been documented from this region: two endemic species whose closest relatives have affinity to the Colorado Plateau and Sierra Madre Occidental (A. jacobii sp. nov. and A. chiricahua, respectively); one species with affinity to the Sonoran Desert (A. chalcodes); four species with affinity to the Chihuahuan Desert and associated grasslands (A. gabeli, A. hentzi (Girard, 1852), A. parvum Hamilton, Hendrixson & Bond, 2016, and A. peloncillo); and one species that is more broadly distributed throughout the Sonoran and Chihuahuan deserts (A. vorhiesi). Outside of the Madrean Archipelago, no more than three species of tarantulas inhabit any other region of comparable size in the United States (e.g., the Arizona Transition Zone near Payson; Hamilton et al. 2016). NOTE: Five species have been purportedly observed in the vicinity of Del Rio, Texas (Hamilton et al. 2016; also search https://www.inaturalist.org), but many of the records (in iNaturalist) need to be verified with physical and genetic vouchers.

Conservation of tarantulas in the Chiricahua Mountains

As is the case with other MSI species (see Hamilton et al. 2016), it is difficult to fully assess the distribution and abundance—and therefore the conservation status of *A. jacobii* sp. nov. and *A. chiricahua* because these tarantulas are challenging to find and many sections of the massif remain unsampled; the Chiricahua Mountains are the largest range in the U.S.-MSI region, and many locations are difficult to access due to their remoteness and rugged terrain. In addition, we lack basic life history, survivorship, and fecundity data for these spiders. However, despite being restricted to a single mountain range, which presents its own set of risks, these species are afforded some degree of protection due to the range's remoteness, and management by the U.S. Forest Service (Coronado National Forest, Douglas Ranger District) and U.S. National Park Service (Chiricahua National Monument).

Some important threats facing the Chiricahua Mountains and these tarantulas include exurban development in the San Simon Valley and Portal area, destructive recreational activities (e.g., offroad vehicles, degradation of undeveloped campsites), fire (natural and human-caused) due to fuel accumulation and fire suppression, invasive species, and climate change (i.e., increasing temperatures and decreasing precipitation amounts and patterns) (Coronado Planning Partnership 2008). A key advantage that A. jacobii sp. nov. may have compared to other MSI species is that it can be found in a variety of plant communities spanning at least 1100 m in elevation. However, the high-elevation population near Barfoot Park may be the most vulnerable. Warming temperatures and climate change have impacted the MSI biota in significant ways (see Brusca et al. 2013; Wiens et al. 2019), and these conditions are expected to continue "pushing" MSI species higher in elevation for the foreseeable future (Yanahan and Moore 2019). Graham et al. (2020) showed that tarantulas (A. marxi) have responded to past climate change by moving into suitable habitats as they became available, but if the Barfoot Park population is locally adapted to cooler mountain-top conditions, they can only continue moving higher in elevation until they are "pushed off" (i.e., local extinction) as their suitable habitats and temperature preferences disappear (see also Wiens et al. 2019).

There is also some concern that A. jacobii sp. nov. will be quickly introduced into the tarantula pet trade. We are aware that commercial field collectors will likely use the information contained in this article as a "treasure map" (see Stuart et al. 2006) for exploiting these spiders. Following the publication of our monograph documenting the diversity and distribution of Aphonopelma in the United States (Hamilton et al. 2016), each of the MSI species we described in that publication (i.e., A. catalina, A. chiricahua, A. madera, and A. peloncillo)-as well as several other species we reported GPS coordinates for-have since appeared on various pet trade lists. Market demand for A. jacobii sp. nov. may likewise be driven by its striking colors (Fig. 3), novelty, and docile disposition (Marshall et al. 2022; Fukushima et al. 2023). The Barfoot Park population is also found near a popular location for smuggling twin-spotted rattlesnakes (Prival 2007; Coronado Planning Partnership 2008), so the ease of access to these spiders' habitat by unethical collectors is concerning. Additional sampling and long-term monitoring across the Chiricahua Mountains are necessary to assess the impact of these various factors on A. jacobii sp. nov. and A. chiricahua.

Conclusions

The discovery of A. jacobii sp. nov. in the Chiricahua Mountains is exciting and noteworthy because it documents the first known case of multiple shortrange endemic tarantula species inhabiting a single MSI range. This species also adds to our knowledge of tarantulas distributed in the Madrean pine-oak woodlands biodiversity hotspot (Mittermeier et al. 2004) and shifts the way we must approach assessing this group's diversity moving forward. Our prior work on the Madrean-affiliated taxa suggested that only one species inhabited each MSI range (Hendrixson et al. 2015; Hamilton et al. 2016; unpublished data for other MSI ranges), similar to what has been observed in co-distributed scorpions (Bryson et al. 2013; Ayrey 2020; Jochim et al. 2020; Myers and Ayrey 2021), but we must reconsider this hypothesis in light of these new findings by revisiting previously sampled ranges and putting significant effort into ranges that have not yet been sampled. Future work on this group must address these sampling gaps, especially if we are to document this threatened ecoregion's tarantula biodiversity and gain further insight into the complex biogeographic history and conservation needs of these spiders. These results also further underscore the importance of applying integrative methods for identifying cryptic diversity and delimiting species in a group of spiders that has historically been referred to as a "nomenclatural and taxonomic nightmare" (Raven 1990: 126).

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Hamilton and Hendrixson are equal contributors to this research and manuscript. Silvestre Bringas worked on data gathering, analysis, and writing of the manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Comparative genetic vouchers examined

Authors: Chris A. Hamilton, Brent E. Hendrixson, Karina Silvestre Bringas Data type: docx

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Link: https://doi.org/10.3897/zookeys.1210.125318.suppl1

Supplementary material 2

A list of all morphological vouchers examined in this study

Authors: Chris A. Hamilton, Brent E. Hendrixson, Karina Silvestre Bringas Data type: xlsx

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Supplementary material 3

GenBank and SRA accession numbers for all genetic vouchers examined in this study

Authors: Chris A. Hamilton, Brent E. Hendrixson, Karina Silvestre Bringas Data type: xlsx

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Scatterplots of the first (PC1) and second (PC2) principal components based on measurements of mature male and female members of the *Marxi* species group

Authors: Chris A. Hamilton, Brent E. Hendrixson, Karina Silvestre Bringas Data type: pdf

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Supplementary material 5

Boxplots comparing various morphometric ratios based on measurements of mature male and female members of the *Marxi* species group

Authors: Chris A. Hamilton, Brent E. Hendrixson, Karina Silvestre Bringas Data type: zip

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Supplementary material 6

Variation in spermathecae of female Aphonopelma jacobii sp. nov.

Authors: Chris A. Hamilton, Brent E. Hendrixson, Karina Silvestre Bringas Data type: jpg

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Morphology of Aphonopelma chiricahua (female, APH-5050)

Authors: Chris A. Hamilton, Brent E. Hendrixson, Karina Silvestre Bringas Data type: jpg

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Link: https://doi.org/10.3897/zookeys.1210.125318.suppl7

Supplementary material 8

Phylogenomics files

Authors: Chris A. Hamilton, Brent E. Hendrixson, Karina Silvestre Bringas Data type: zip

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Supplementary material 9

Measurements for Aphonopelma chiricahua and A. jacobii specimens

Authors: Chris A. Hamilton, Brent E. Hendrixson, Karina Silvestre Bringas Data type: txt

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Specimen locality information for *Aphonopelma chiricahua* and *A. jacobii* specimens

Authors: Chris A. Hamilton, Brent E. Hendrixson, Karina Silvestre Bringas Data type: xlsx

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Research Article

Four new species of the spider genus *Liphistius* (Araneae, Mesothelae, Liphistiidae, Liphistiinae) from Myanmar

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Abstract

Four new species of *Liphistius* belonging to the *birmanicus* species group are described from Myanmar based on both sexes: *L. kalaw* Zhan & Xu, **sp. nov.** ($\mathscr{J} Q$), *L. kanpetlet* Zhan & Xu, **sp. nov.** ($\mathscr{J} Q$), *L. nawngau* Zhan & Xu, **sp. nov.** ($\mathscr{J} Q$) and *L. rostratus* Zhan & Xu, **sp. nov.** ($\mathscr{J} Q$). Currently, Myanmar stands as the westernmost country where *Liphistius* is distributed, with the new species *L. kanpetlet* **sp. nov.** being found in the westernmost region of Myanmar.

Key words: description, Liphistiomorphae, morphology, southeast Asia, taxonomy

Introduction

Currently, the spider family Liphistiidae includes eight genera divided into two subfamilies, the monotypic Liphistiinae Thorell, 1869 and Heptathelinae Kishida, 1923. *Liphistius* Schiödte, 1849, the sole genus of Liphistiinae, encompasses 77 valid species. Among these, six are known solely from females, while the remaining 71 species are described based on both sexes (WSC 2024). The geographical distribution of *Liphistius* spans from its northernmost point in China (Yunnan Province) to its southernmost in Indonesia (Sumatra), while its range extends from the easternmost areas of Laos, Thailand, and Peninsular Malaysia to the westernmost regions of Myanmar (WSC 2024). Until now, several regional revisions of *Liphistius* have been conducted, including those focused on Peninsular Malaysia, Myanmar, and Thailand (for more details, refer to Schwendinger 2017; Sivayyapram et al. 2017; Schwendinger et al. 2019, 2022).

Schwendinger (1990, 2017), Sivayyapram et al. 2017, and Schwendinger et al. 2022 split *Liphistius* into seven species groups based on the morphology of copulatory organs of both sexes: *batuensis*-group (e.g., *L. batuensis* Abraham, 1923), *birmanicus*-group (e.g., *L. birmanicus* Thorell, 1897), *bristowei*-group (e.g., *L. bristowei* Platnick & Sedgwick, 1984), *linang*-group (e.g., *L. linang* Schwendinger, 2017), *malayanus*-group (e.g., *L. malayanus* Abraham, 1923), *tioman*-group



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(e.g., *L. tioman* Platnick & Sedgwick, 1984), and *trang*-group (e.g., *L. trang* Platnick & Sedgwick, 1984). Currently, Myanmar hosts 11 known *Liphistius* species, including one species known only from females (Fig. 1; WSC 2024). These species belong to three groups: the *birmanicus*-group, the *bristowei*-group, and the *trang*-group (Schwendinger 1990; Schwendinger et al. 2022; Sivayyapram et al. 2024).

Following an examination of specimens collected from Myanmar, we identify and describe four new *Liphistius* species, each restricted to a limited range, all belong to the *birmanicus*-group.

Material and methods

All specimens were collected alive in Myanmar (Figs 1, 2) and transported the subadult individuals to the laboratory, where they were reared until reaching maturity. Right legs were removed from adults, preserved in absolute ethanol, and stored at -80 °C for genome DNA extraction. The remaining parts of each specimen were preserved in 80% ethanol as vouchers for morphological examination. These vouchers are currently deposited at the School of Life Sciences, Hubei University, Wuhan, Hubei Province, China (**HUBU**). In the future, the specimens will be deposited at two locations: School of Life Sciences, Hubei University, Wuhan, Hubei Province, China, and the Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences, Yezin, Nay Pyi Taw, Myanmar (CAS-SEABRI).



Figure 1. Map showing the collecting localities of the *Liphistius* species in Myanmar, China (*L. nabang*), and Thailand (*L. lahu*, *L. metopiae*). Blue circles refer to four new species described in this study, black circles indicate known and one putative species.



Figure 2. Microhabitat and general somatic morphology of four new *Liphistius* species. **A** microhabitat **B** burrow with trapdoor open **C**, **E**–**G** male dorsal view **C** *L*. *kanpetlet* sp. nov. **D**, **E** *L*. *rostratus* sp. nov. **F** *L*. *kalaw* sp. nov. **G** *L*. *nawngau* sp. nov. Scale bars: 0.5 mm (**D**); 5 mm (**C**, **E**–**G**).

For morphological examination, we used an Olympus SZ51 stereomicroscope to dissect the specimens. Soft tissues of vulvae were removed using 10 mg/ml pancreatin, allowing for a minimum 3-hour digestion period at room temperature. Male palps and female genitalia were photographed using an Olympus BX53 compound microscope equipped with a digital camera CCD. Compound-focused images were generated using Helicon Focus v. 6.7.1. Specimen coloration was described in alcohol. All measurements were carried out under a Leica M205C stereomicroscope using Leica Application Suite v. 4 software and are given in millimeters. Measurements of legs and palp are presented in the following order: leg total length (femur, patella, tibia, metatarsus [absent on palp], tarsus).

Genital anatomical terminology used in this paper follows Aung et al. 2019 and Schwendinger et al. 2019, 2022. Abbreviations used in the text are: ALE = anterior lateral eyes; AME = anterior median eyes; PLE = posterior lateral eyes; PME = posterior median eyes; BL = body length (excluding chelicerae); CL = carapace length; OL = opisthosoma length; CW = carapace width; OW = opisthosoma width.

Taxonomy

Family Liphistiidae Thorell, 1869

Genus Liphistius Schiödte, 1849

Type species. Liphistius desultor Schiödte, 1849 from Malaysia.

Diagnosis. *Liphistius* can be distinguished from the other seven genera by having the male palps bearing tibial apophysis (TiA) (Figs 3A, 5C, 7C, 9A), clavate trichobothria on the dorsal side of cymbia and leg tarsi, and vulvae possessing median receptacular cluster (RC) and sclerotized poreplate (PPI) (Figs 4, 6, 8, 10).

Distribution. China (Yunnan Province), Indonesia (Sumatra), Laos, Myanmar, Peninsular Malaysia, and Thailand.

Comments on Liphistius in Myanmar. Eleven named Liphistius species in Myanmar were grouped into three species-groups: the *birmanicus*-group, comprising nine valid species; the *bristowei*-group, consisting of one known species; and the *trang*-group, also containing one known species (Schwendinger 1990; Schwendinger et al. 2022; Sivayyapram et al. 2024). All named Liphistius species of the *birmanicus*-group were described based on both sexes, except for *L. hpruso* Aung, Xu, Lwin, Sang, Yu, Liu, Liu & Li, 2019, which is only known from females. In this study, four new *Liphistius* species belonging to the *birmanicus*-group are described, identified according to the morphology of their copulatory organs in both sexes.

Composition of the birmanicus-group: *L. birmanicus, L. cupreus* Schwendinger & Huber, 2022, *L. ferox* Schwendinger & Huber, 2022, *L. hpru-so, L. lahu* Schwendinger, 1998, *L. lordae* Platnick & Sedgwick, 1984, *L. me-topiae* Schwendinger, 2022, *L. nabang* Yu, Zhang & Zhang, 2021, *L. pinlaung* Aung, Xu, Lwin, Sang, Yu, Liu, Liu & Li, 2019, *L. platnicki* Schwendinger & Huber, 2022, *L. pyinoolwin* Xu, Yu, Aung, Yu, Liu, Lwin, Sang & Li, 2021, *L. tung* Schwendinger, 2022.

Liphistius kalaw Zhan & Xu, sp. nov.

https://zoobank.org/DE92B21F-6A12-44AA-97C8-18EE985DB623 Figs 3, 4

Type material. *Holotype*: MYANMAR ♂, Shan State, 16 km W of Kalaw Township; 20.70°N, 96.52°E, alt. 944 m; 27.07.2019; D. Li et al. leg.; XUX-2019-061A. *Para-types*: 8♀♀, same data as for holotype; 27.07.2019 and 15.07.2018; XUX-2019-062/063/064/065/066/067A/068; XUX-2018-124.

Diagnosis. The male of *L. kalaw* sp. nov. resembles those of *L. birmanicus* and *L. pinlaung* in having a distinct contrategular process (cp) (Fig. 3B, E), but it can be distinguished from *L. birmanicus* by the relatively wider base of the contrategular process (cp) (Fig. 3E vs fig. 13E in Schwendinger et al. 2022), and from *L. pinlaung* by the cumulus (Cu) which has shorter spines (Fig. 3A–C vs fig. 4C in Aung et al. 2019). The female of *L. kalaw* sp. nov. can be distinguished from that of *L. hpruso* by the relatively wider posterior stalk (PS) (Fig. 4C vs fig. 3B–E in Aung et al. 2019).

Description. Male holotype. Carapace yellowish brown, with few short, scattered bristles; opisthosoma yellowish brown, with 12 brown tergites, close to each other, 2–6th larger than others, 5th largest; chelicerae robust, promargin with 13 denticles of variable size; labium and sternum yellowish brown, with few short setae on anterior margin and many long setae on elongated posterior margin; legs yellowish brown, with strong setae and spines. Measurements: BL 13.65, CL 5.94, CW 6.07, OL 8.00, OW 5.36; eye sizes and interdistances: AME 0.08, ALE 0.54, PME 0.27, PLE 0.43, AME–AME 0.11, AME–ALE 0.15, PME–PME 0.08, PME–PLE 0.10, ALE–PLE 0.17, ALE–ALE 0.12, PLE–PLE 0.34, AME–PME 0.10; labium 1.06 long and 0.98 wide; sternum 3.08 long and 0.96 wide; legs: I 18.36 (4.81, 2.57, 3.87, 4.60, 2.51), II 20.24 (5.64, 2.56, 4.23, 5.07, 2.74), III 20.46 (5.64, 2.37, 4.42, 6.00, 3.03), IV 27.56 (6.97, 2.84, 5.57, 8.17, 4.01).

Palp: tibial apophysis (TiA) with 4 tapering setae of similar length (Fig. 3C); paracymbium (PC) with many setae situated on tip (Fig. 3A–C); cumulus (Cu) slightly elevated, with 8 tapering spines (Fig. 3A–C); subtegulum with weakly developed apophysis (Fig. 3B); contrategulum (CT) with arched projection distally, and large triangular process in prolateral view (Fig. 3A, B, D); tegulum (T) fan-shaped with serrated edge and longitudinal ridges (Fig. 3C, F); paraembolic plate (PeP) narrow, sclerotized (Fig. 3A, F); embolus (E) with 4 distinct longitudinal ridges reaching tip in retrolateral view, and with several denticles along longitudinal ridges (Fig. 3B–F).

Female paratype (XUX-2019-062). Carapace yellowish brown, with few short, scattered bristles; opisthosoma brown, with 12 brown tergites, close to each other, with gray patches, 2–6th larger than others, 5th largest; chelicerae robust, reddish brown; promargin of cheliceral groove with 12 denticles of variable size; labium yellowish brown, sternum with several setae; legs with strong setae and spines. Measurements: BL 11.56, CL 5.82, CW 5.22, OL 6.23, OW 4.62; eye sizes and interdistances: AME 0.07, ALE 0.56, PME 0.29, PLE 0.42, AME–AME 0.09, AME–ALE 0.15, PME–PME 0.06, PME–PLE 0.09, ALE–PLE 0.11, ALE–ALE 0.07, PLE–PLE 0.35, AME–PME 0.08; labium 3.00 long and 0.61 wide; sternum 1.19 long and 1.13 wide; palp 10.25 (3.62, 1.74, 2.36, 2.53), legs: I 12.78 (4.29, 2.03, 2.63, 2.37, 1.46), II 12.89 (4.17, 2.03, 2.56, 2.59, 1.54), III 13.42 (4.13, 1.84, 2.75, 2.78, 1.92), IV 19.33 (5.57, 2.32, 3.81, 4.91, 2.72).

Vulva: poreplate (PPI) slightly wider than long, with pair of large anterior lobes and pair of small anterolateral lobes; central dorsal opening (CDO)



Figure 3. Male palp of *Liphistius kalaw* sp. nov. **A**, **E** prolateral view **B** ventral view **C**, **F** retrolateral view **D** dorsal view, red arrows show arched projection and triangular process of CT **G** distal view. Abbreviations: CT = contrategulum; cp = contrategular process; Cu = cumulus; E = embolus; PC = paracymbium; PeP = paraembolic plate; ST = subtegulum; T = tegulum; TiA = tibial apophysis. Scale bars: 0.5 mm.





circular, racemose receptacular cluster (RC) slightly long; posterior stalk (PS) axe-shaped; lateral margins of genital atrium (GA) membranous (Fig. 4).

Variation. Females vary in body size. The range measurements of females (N = 8): BL 8.55–16.11, CL 4.48–7.20, CW 3.90–6.43, OL 4.18–8.56, OW 3.00–7.36. The number of denticles on the promargin of cheliceral groove varies from 11 to 14 (N = 8).

Etymology. The species epithet "kalaw" refers to the type locality, Kalaw Township; it is treated as a noun in apposition.

Distribution. Mandalay Region (Kalaw Township), Myanmar.

Liphistius kanpetlet Zhan & Xu, sp. nov.

https://zoobank.org/8982C984-06DB-4636-9C22-92E2C69A62CB Figs 5, 6

Type material. *Holotype*: MYANMAR ♂, Chin State, Kanpetlet Township, Kanpetlet Matupi Rd; 21.19°N, 94.05°E, alt. 1469 m; 17.07.2019; D. Li et al. leg.; XUX-2019-026. *Paratypes*: 5♀♀, same locality as for holotype; 17–18.07.2019; XUX-2019-020/021/024/031/033.



Figure 5. Male palp of *Liphistius kanpetlet* sp. nov. **A**, **E** prolateral view **B** ventral view; **C**, **F** retrolateral view, red arrow shows distal process of PC **D** dorsal view, red arrows show arched projection and triangular process of CT **G** distal view. Abbreviations: CT = contrategulum; cp = contrategular process; Cu = cumulus; E = embolus; PC = paracymbium; PeP = paraembolic plate; ST = subtegulum; T = tegulum; TiA = tibial apophysis. Scale bars: 0.5 mm.

Diagnosis. The male of *L. kanpetlet* sp. nov. can be distinguished from those of *L. birmanicus* by having the paracymbium (PC) bearing a distal process (Fig. 5C vs lacking a distal process, fig. 13H in Schwendinger et al. 2022), and from *L. kalaw* sp. nov. by the contrategulum (CT) which has a blunt process (Fig. 5D vs slightly sharp process, Fig. 3E). The female of *L. kanpetlet* sp. nov. differs from that of *L. hpruso* by having two relatively larger anterior lobes (Fig. 6 vs fig. 3B–E in Aung et al. 2019).

Description. Male holotype. Carapace yellowish brown with few short, scattered bristles; opisthosoma brown, with 12 brown tergites, close to each other, 2–6th larger than others, 4th largest; chelicerae robust, promargin of cheliceral groove with 13 denticles of variable size; labium yellow, sternum yellow, with few short setae on anterior tip and many long setae on elongated posterior tip; legs reddish brown, with strong setae and spines; 8 spinnerets. Measurements: BL 12.41, CL 6.01, CW 6.06, OL 6.30, OW 4.19; eye sizes and interdistances: AME 0.06, ALE 0.56, PME 0.32, PLE 0.42, AME–AME 0.06, AME–ALE 0.16, PME–PME 0.10, PME–PLE 0.11, ALE–PLE 0.14, ALE–ALE 0.14, PLE–PLE 0.34, AME–PME 0.08; labium 0.96 long and 0.50 wide; sternum 2.63 long and 1.00 wide; legs: I 17.51 (4.82, 2.52, 3.79, 4.14, 2.24), II 18.36 (5.20, 2.19, 3.95, 4.72, 2.3), III 20.63 (5.46, 2.20, 4.41, 5.86, 2.70), IV 26.62 (7.18, 2.64, 5.40, 7.71, 3.69).

Palp: tibial apophysis (TiA) with 5 setae of different lengths (Fig. 5A–C); paracymbium (PC) with some setae situated on tip, and with pointed lateral process (Fig. 5C); and several tapering spines on slightly elevated cumulus (Cu) (Fig. 5B); subtegular apophysis weakly developed (Fig. 5B, F); contrategulum (CT) with arched projection distally, and wide triangular process in prolateral view (Fig. 5D); fan-shaped tegulum (T) with 6 longitudinal ridges (Fig. 5C); paraembolic plate (PeP) widely rounded, short, with curved margin in ventral view (Fig. 5A, F); embolus (E) sclerotized basally, with several denticles along longitudinal ridges reaching tip (Fig. 5C–G).

Female paratype (XUX-2019-024). Carapace brown with few short, scattered bristles; opisthosoma gray, with 12 brown tergites, close to each other, with gray patches, 2–6th larger than others, 5th largest; chelicerae robust, reddish brown; promargin of cheliceral groove with 10 denticles of variable size; labium yellow, sternum yellow with several setae; legs with strong setae and spines, without distinct annulations. Measurements: BL 11.71, CL 5.26, CW 5.15, OL 6.36, OW 4.70; eye sizes and interdistances: AME 0.09, ALE 0.50, PME 0.34, PLE 0.40, AME–AME 0.06, AME–ALE 0.19, PME–PME 0.07, PME–PLE 0.10, ALE–PLE 0.09, ALE–ALE 0.14, PLE–PLE 0.35, AME–PME 0.06; Labium 1.38 long and 0.59 wide; sternum 2.31 long and 1.19 wide; palp 8.05 (2.76, 1.36, 2.15, 1.78), leg I 10.24 (3.21, 1.60, 2.30, 2.01, 1.12), II 10.82 (3.43, 1.68, 2.27, 2.14, 1.30), III 11.14 (3.49, 1.51, 2.16, 2.51, 1.47), IV 15.54 (4.45, 1.94, 3.13, 3.71, 2.31).

Vulva: approximately rectangular poreplate (PPI) wider than long with smoothly curved posterior margin; with pair of large anterior lobes and pair of small anterolateral lobes, 2 anterior lobes separated from each other, but close to anterolateral lobes; central dorsal opening (CDO) small, situated in center of poreplate (PPI); racemose receptacular cluster (RC) long and narrow; posterior stalk (PS) axe-shaped; genital atrium (GA) with slightly sclerotized lateral margins (Fig. 6).

Variation. Females vary in body size. The range of measurements of females (N = 5): BL 9.70-14.85, CL 4.86-6.55, CW 4.20-8.63, OL 3.82-8.63, OW 3.82-7.09. The number of denticles on the promargin of cheliceral groove varies from 10-13 (N = 5).



Figure 6. Vulva of *Liphistius kanpetlet* sp. nov. **A–C** dorsal view **D–F** ventral view. Abbreviations: CDO = central dorsal opening; GA = genital atrium; PS = posterior stalk; PPI = poreplate; RC = receptacular cluster. Scale bars: 0.5 mm.

Etymology. The species epithet "kanpetlet" refers to the type locality, Kanpetlet Township; it is treated as a noun in apposition.

Distribution. Chin State (Kanpetlet Township), Myanmar.

Liphistius nawngau Zhan & Xu, sp. nov.

https://zoobank.org/1ADFCE8F-66EE-43F9-B61A-927F6B88189F Figs 7, 8

Type material. *Holotype*: MYANMAR 3, Shan State, Kyaukme Dist., Nawnghkio Township, Nawng Au Vill.; 22.26°N, 96.83°E, alt. 1096 m; 26.07.2019; D. Li et al. leg.; XUX-2019-054A. *Paratypes*: 9332, same data as for holotype; XUX-2019-054/054A/055/055A/055B/055D/056A/057/058/059/060; 53322, same township as for holotype; 22.30°N, 96.73°E, alt. 845 m; 26.07.2019 and 14.07.2018; XUX-2019-049/051/052/053, XUX-2018-116/119/123.

Diagnosis. The male of *L. nawngau* sp. nov. resembles those of *L. lordae* and *L. pyinoolwin* in having an adpressed proximal tegular margin (Fig. 7C, F), but it can be distinguished from them by the tegulum (T), which bears a distinct transversal ridge in retrolateral view (Fig. 7C, F vs lacking transversal ridge, figs 5, 6 in Schwendinger 1990; fig. 4F in Xu et al. 2021), and by the embolus (E), which has smooth longitudinal ridges reaching tip (Fig. 7E vs having several denticles along longitudinal ridges, fig. 4F in Xu et al. 2021). The female of *L. nawngau* sp. nov. can be distinguished from that of *L. hpruso* by having the posterior stalk triangular (PS) (Fig. 8 vs nearly oval, fig. 3B–E in Aung et al. 2019).

Description. Male holotype. Carapace brown with few short, scattered bristles; opisthosoma brown, with 12 brown tergites, close to each other, 2-6th


Figure 7. Male palp of *Liphistius nawngau* sp. nov. **A**, **E** prolateral view **B** ventral view **C**, **F** retrolateral view **D** dorsal view, red arrows show arched projection and triangular process of CT **G** distal view. Abbreviations: CT = contrategulum; cp = contrategular process; Cu = cumulus; E = embolus; PC = paracymbium; PeP = paraembolic plate; ST = subtegulum; T = tegulum; TiA = tibial apophysis. Scale bars: 0.5 mm.



Figure 8. Vulva of *Liphistius nawngau* sp. nov. **A, C** dorsal view **B, D** ventral view. Abbreviations: CDO = central dorsal opening; GA = genital atrium; PS = posterior stalk; PPI = poreplate; RC = receptacular cluster. Scale bars: 0.5 mm.

larger than others, 5th largest; chelicerae robust, promargin of cheliceral groove with 11 denticles of variable size; labium and sternum yellowish brown, sternum with few short setae on anterior tip and many long setae on elongated posterior tip; legs yellowish brown, with strong setae and spines. Measurements: BL 15.47, CL 6.63, CW 6.57, OL 7.73, OW 5.56; eye sizes and interdistances: AME 0.08, ALE 0.72, PME 0.35, PLE 0.49, AME-AME 0.08, AME-ALE 0.20, PME-PME 0.08, PME-PLE 0.07, ALE-PLE 0.81, ALE-ALE 0.11, PLE-PLE 0.16, AME-PME 0.12; labium 0.81 long and 0.58 wide; sternum 2.59 long and 1.01 wide; legs: I 19.58 (5.67, 2.78, 4.03, 4.68, 2.42), II 20.42 (5.66, 2.78, 4.13, 4.96, 2.89), III 22.62 (5.96, 2.63, 4.54, 6.29, 3.20), IV 28.26 (7.16, 2.75, 5.82, 8.55, 3.98).

Palp: tibial apophysis (TiA) with four setae of similar length (Fig. 7A–C); paracymbium (PC) with several setae situated on tip (Fig. 7A–C); cumulus (Cu) slightly elevated, with 8 tapering spines (Fig. 7A–C); subtegulum (ST) with weakly developed apophysis (Fig. 7B, F); contrategulum (CT) with an arched projection distally, and triangular process with wide base (Fig. 7D); tegulum (T) with distinct transversal ridge in retrolateral view (Fig. 7C, F); paraembolic plate (PeP) narrow, sclerotized (Fig. 7A, F); embolus (E) with several longitudinal ridges reaching tip (Fig. 7E, F).

Female paratype (XUX-2019-053). Carapace yellowish brown with few short, scattered bristles; opisthosoma brown, with 12 brown tergites, close to each other, with gray patches, 2–6th larger than others, 5th largest; chelicerae robust, reddish brown; promargin of cheliceral groove with 12 denticles of variable size; labium and sternum yellowish brown; legs with strong setae and spines. Measurements: BL 14.24, CL 6.10, CW 5.30, OL 7.12, OW 4.96; eye sizes and interdistances: AME 0.08, ALE 0.57, PME 0.31, PLE 0.45, AME–AME 0.10, AME–ALE 0.17, PME–PME 0.09, PME–PLE 0.08, ALE–PLE 0.09, ALE–ALE 0.10, PLE–PLE 0.46, AME–PME 0.06; labium 1.23 long and 0.77 wide; sternum 2.68 long and 1.13 wide; palp 10.42 (3.63, 1.84, 2.77, 2.18), leg I 13.33 (4.16, 2.15, 2.90, 2.58, 1.54), II 16.85 (4.35, 2.26, 2.84, 2.84, 1.72), III 15.04 (4.37, 2.35, 2.90, 3.31, 2.11), IV 20.39 (5.92, 1.92, 4.48, 5.33, 2.74).

Vulva: poreplate (PPI) with pair of large anterior lobes and pair of relatively small anterolateral lobes; central dorsal opening (CDO) located at center of poreplate (PPI); receptacular cluster (RC) racemose, protrudes upper edge of poreplate (PPI); posterior margin of triangular posterior stalk (PS) almost straight; lateral margins of genital atrium (GA) slightly sclerotized (Fig. 8).

Variation. Males (N = 14): BL 12.64–16.98, CL 6.27–7.56, CW 6.02–7.56, OL 6.95–8.50, OW 4.60–6.40; females (N = 5): BL 10.55–14.24, CL 5.26–6.37, CW 4.47–5.63, OL 5.24–7.12, OW 4.33–4.96. The number of denticles on the promargin of male cheliceral groove varies from 10 to 13 (N = 12); in females, the number of denticles on the promargin of cheliceral groove varies from 11 to 12 (N = 5). The number of setae on tibial apophysis varies from 4 to 6.

Etymology. The species epithet "nawngau" refers to the type locality, Nawng Au Village; it is treated as a noun in apposition.

Distribution. Shan State, (Kyaukme District), Myanmar.

Liphistius rostratus Zhan & Xu, sp. nov.

https://zoobank.org/35E36D61-84CB-4C01-830F-BCFE53FBAA6E Figs 9, 10

Type material. *Holotype*: MYANMAR ♂, Mandalay Region, War Phyu Taung Vill.; 22.88°N, 96.12°E, alt. 553 m; 25.07.2019; D. Li et al. leg.; XUX-2019-038. *Paratypes*: 2♂♂ 3♀♀, same data as for holotype; XUX-2019-034–037/041.

Diagnosis. The male of *L. rostratus* sp. nov. resembles those of *L. cupreus*, *L. nabang*, and *L. platnicki* in having distinctly elevated cumulus (Cu) (Fig. 9C), but it can be distinguished from them by the relatively larger paraembolic plate (PeP) (Fig. 9A, E vs figs 21A, 23K in Schwendinger et al. 2022; fig. 3A in Yu et al. 2021), and an indistinct contrategular process (cp) (Fig. 9B vs relatively large contrategular process, figs 21E, 23E in Schwendinger et al. 2022; fig. 3B in Yu et al. 2021). The female of *L. rostratus* sp. nov. resembles to that of *L. cupreus* in having vesicle clusters along the anterior margin of the poreplate (PPI), but it can be distinguished by the relatively longer posterior stalk (PS), with a length/width ratio 0.4–0.6 (Fig. 10 vs length/width ratio about 0.2–0.4, fig. 22 in Schwendinger et al. 2022).

Description. Male holotype. Carapace yellowish brown, with few short, scattered bristles; opisthosoma brown, with 12 brown tergites, close to each other, 2–6th larger than others, 4th largest; chelicerae robust, promargin of cheliceral groove with 12 denticles of variable size; labium and sternum yellowish brown,



Figure 9. Male palp of *Liphistius rostratus* sp. nov. **A**, **E** prolateral view **B** ventral view **C**, **F** retrolateral view **D** dorsal view **G** distal view, red arrow shows beak-like contrategular projection. Abbreviations: CT = contrategulum; cp = contrategular process; Cu = cumulus; E = embolus; PC = paracymbium; PeP = paraembolic plate; ST = subtegulum; T = tegulum; TiA = tibial apophysis. Scale bars: 0.5 mm.



Figure 10. Vulva of *Liphistius rostratus* sp. nov. **A**, **C** dorsal view **B**, **D** ventral view. Abbreviations: CDO = central dorsal opening; GA = genital atrium; PS = posterior stalk; PPI = poreplate; RC = receptacular cluster. Scale bars: 0.5 mm.

sternum with few short setae on anterior tip and many long setae on elongated posterior tip; legs yellowish brown, with strong setae and spines. Measurements: BL 9.34, CL 4.57, CW 4.26, OL 5.02, OW 3.59; eye sizes and interdistances: AME reduced, ALE 0.40, PME reduced, PLE 0.24, ALE–PLE 0.07, ALE–ALE 0.05, PLE–PLE 0.30; labium 0.98 long and 0.48 wide; sternum 2.09 long and 0.83 wide; legs: I 12.63 (3.80, 1.32, 2.60, 3.07, 1.84), II 13.19 (3.80, 1.32, 2.60, 3.07, 1.84), III 14.35 (3.79, 1.58, 3.33, 3.44, 2.21), IV 19.76 (4.76, 1.66, 4.13, 6.01, 3.20).

Palp: tibial apophysis (TiA) with 4 setae of similar length (Fig. 9A–C); paracymbium (PC) with several setae situated on tip (Fig. 9B, C); cumulus (Cu) distinct elevated with 5 tapering spines (Fig. 9C); subtegulum (ST) without apophysis (Fig. 9F); contrategulum (CT) with beak-like projection distally in distal view and small process in ventral view (Fig. 9B, G); tegulum (T) semicircular with flat surface and serrated edges, and proximal tegular margin with an elevated transverse ridge (Fig. 9B, C, F); paraembolic plate (PeP) sclerotized, narrow (Fig. 9A, E); embolus (E) with 3 distinct longitudinal ridges reaching tip retrolaterally, and several denticles along these longitudinal ridges (Fig. 9B, F).

Female paratype (XUX-2019-034). Carapace brown with few short, scattered bristles; opisthosoma brown, with 12 brown tergites, close to each other, 2–6th larger than others, 5th largest; chelicerae robust, reddish brown; promargin of cheliceral

groove with 12 denticles of variable size; labium yellowish brown, sternum yellowish brown with several setae; legs with strong setae and spines. Measurements: BL 10.54, CL 4.5, CW 3.93, OL 6.47, OW 4.74; eye sizes and interdistances: AME 0.05, ALE 0.37, PME 0.19, PLE 0.29, AME-AME 0.07, AME-ALE 0.12, PME-PME 0.06, PME-PLE 0.06, ALE-PLE 0.09, ALE-ALE 0.10, PLE-PLE 0.32, AME-PME 0.08; labium 0.95 long and 0.38 wide; sternum 2.41 long and 0.99 wide; palp 7.10 (2.44, 0.98, 1.86, 1.82), leg I 8.89 (2.94, 1.34, 2.00, 1.62, 0.99), II 9.69 (3.40, 1.09, 2.02, 1.98, 1.20), III 9.48 (2.29, 1.05, 2.59, 2.38, 1.17), IV 13.71 (3.21, 1.44, 3.11, 3.80, 2.21).

Vulva: about rectangular poreplate (PPI) with pair of anterior lobes; central dorsal opening (CDO) located below center of poreplate (PPI); racemose receptacular cluster (RC) small; posterior stalk (PS) as wide as poreplate (PPI), lateral margins of genital atrium (GA) slightly sclerotized (Fig. 10).

Variation. Males (N = 3): BL 9.34–10.31, CL 4.57–5.24, CW 4.26–5.83, OL 4.73–5.10, OW 3.40–3.40; females (N = 4): BL 10.54–12.35, CL 4.50–6.31, CW 3.93–5.47, OL 6.47–6.87, OW 4.64–4.79. The number of denticles on the cheliceral promargin varies from 12 to13 (N = 6). In addition, the male lacking an AME and PME, which may be degenerate during molting, is chosen as the holotype, because all paratype male palps are deformed.

Etymology. The species name is derived from the Latin word *"rostratus"*, referring to the beak-like contrategulum of the male palp in distal view.

Distribution. Mandalay Region (War Hpyu Taung), Myanmar.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

Two new species of the family Aoridae (Crustacea, Malacostraca, Amphipoda) from Korean waters

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Abstract

Two new species of the family Aoridae, one from the genus *Aoroides* Walker, 1898, and other from the genus *Grandidierella* Coutière, 1904, are reported from Korean waters. *Aoroides gracilicrus* **sp. nov.** is similar to *A. longimerus* in having numerous plumose setae on the basis and carpus of gnathopod 1. However, the new species can be distinguished from *A. longimerus* by the numerous plumose setae on the bases of pereopods 3 and 4 and the slender basis of pereopod 7. *Grandidierella naroensis* **sp. nov.** is morphologically most similar to *G. pseudosakaensis*. However, the new species can be distinguished by the presence of small distal and proximal processes and a large middle process on the carpus of gnathopod 1, and the subovate propodus of gnathopod 1. Both new species are illustrated and compared to related species. A key to species in the family Aoridae from Korean waters is also provided.

Key words: Amphipod, Aoridae, Aoroides, Grandidierella, Korea, new species

Introduction

The family Aoridae Stebbing, 1899 includes amphipod species that are abundant in coastal and sublittoral waters. Aorid amphipods usually feed on generalized organic detritus and algal debris, but some aorids can be opportunistic predators. *Microdeutopus gryllotalpa* Costa, 1853 will seize and consume small crustaceans passing the opening of its tube, and an undescribed species of *Grandidierella* from northwestern Australia has been observed to feed on insect larvae (Myers and Lowry 2003).

The family Aoridae was first established by Stebbing (1899) with *Aora typica* Krøyer, 1845 as its type species. Aorid amphipods are characterized by an enlarged male gnathopod 1 and are easily distinguished from congeners by pereopod 7, which is disproportionately longer than pereopod 6. According to the ratio of the length of pereopod 7, groups with pereopod 7 relatively longer than pereopods 5 and 6 have been classified as the family Aoridae, whereas groups with pereopods 5–7 of similar length have been classified as the family Unciolidae (Myers and Lowry 2003). In addition, due to strong sexual dimorphism in the family, the female gnathopod 1 is subequal in size to gnathopod 2, without an especially enlarged or elongated article.



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Copyright: [©] June Kim et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). The Aoridae includes 244 species in 26 genera (Horton et al. 2024). The genus Aoroides Walker, 1898 consists of 20 species and was established by Walker (1898), with A. columbiae Walker, 1898 as its type species. Aoroides is characterized by the absence of an accessory flagellum and an elongated merus of the male gnathopod 1 (Barnard 1962). The genus Grandidierella Coutière, 1904 comprises 52 species. This genus was established by Coutière (1904), with G. mahafalensis Coutière, 1904 as its type species. Grandidierella is characterized by the enlarged male gnathopod 1 carpus, which typically has 1–3 processes.

In this paper, we add a new species each of the genera Aoroides and Grandidierella to the Korean aorid amphipod fauna. A total of 32 species belonging to seven genera have been reported to date in Japanese waters adjacent to Korea, including two species of Aora Krøyer, 1845, 11 species of Aoroides, one species of Bemlos Shoemaker, 1925, 13 species of Grandidierella, three species of Paragrandidierella Ariyama, 2002, one species of Pseudobemlos Ariyama, 2004, and one species of Tethylembos Myers, 1988 (Ariyama and Kawabe 2022; Ariyama and Kohtsuka 2022). In Chinese waters, 23 species belonging to seven genera have been reported, including one species of Aora Krøyer, 1845, two species of Aoroides, five species of Bemlos, five species of Globosolembos Myers, 1985, seven species of Grandidierella, two species of Lembos Bate, 1856, and one species of Xenocheira Haswell, 1879 (Hou and Li 2002; Ren 2006). However, only three species of Aoroides and two species of Grandidierella have been recorded in Korean waters: Aoroides columbiae Walker, 1898, A. ellipticus Ariyama, 2004, A. semicurvatus Ariyama, 2004, Grandidierella fasciata Ariyama, 1996, and G. japonica Stephensen, 1938 (Kim and Kim 1987; Kim 2011; Jung and Yoon 2013; Jung, Kim and Yoon 2016; Kim and Heo 2016). Therefore, additional species of the family Aoridae are to be expected in Korean waters. This study also provides a key to species in the family Aoridae from Korean waters.

Materials and methods

Specimens were collected with a light trap and hand net from subtidal waters of the East and South Sea, Korea (Fig. 1). The specimens were fixed in 95% ethanol and dissected in glycerol on Cobb's aluminum hole slides. The materials were examined under stereoscopic (Olympus SZX 10) and compound microscopes (Olympus BX 51), and drawings and measurements were made with the aid of a drawing tube. Body length was measured from the end of the rostrum to the end of the urosome, along the dorsal parabolic line of the body. The examined specimens are deposited at the Honam National Institute of Biological Resources (**HNIBR**), Mokpo, Korea, and the Department of Biological Science, Dankook University (**DKU**), Cheonan, Korea.

Taxonomy

Order Amphipoda Latreille, 1816 Family Aoridae Stebbing, 1899

Genus Aoroides Walker, 1898

Type species. Aoroides columbiae Walker, 1898.



Figure 1. Distribution of the Aoridae species (black star: *Aoroides gracilicrus* sp. nov., black circle: *Grandidierella naroensis* sp. nov.; 1, Sogagido Is.; 2, Dolsando Is.; 3, Beol-po port; 4, Bijin-ri; 5, Daejin port; 6, Dokdo Island; 7, Narodo Island).

Aoroides gracilicrus sp. nov.

https://zoobank.org/DACEFB72-5A24-4CD4-A7CF-3E9CFEAD88A0 Figs 2A, 3–5 Korean name: Ga-neun-da-ri-teol-keun-ap-son-yeop-sae-u, new

Type material. *Holotype*, SOUTH KOREA • 3.3 mm • 1 ♂; Bijin-ri, Han-san-myeon, Tongyeong-si, Gyeongsangnam-do; 34°42'59"N, 128°27'37"E; 1 August 2019; collected from hand net; Y.H. Kim leg.; HNIBRIV2426.

Additional material. SOUTH KOREA • 3.1 mm • 1 ♂; Dolsando Island, Yeosu-si, Goheung-gun, Jeollanam-do; 34°37'19"N, 127°47'55"E; 20 March 2004; collected from hand net; Y.H. Kim leg., DKUAMP202402 • 1 ♂; Beol-po port, Sanyang-eup, Tongyeong-si, Gyeongsangnam-do; 34°49'56"N, 128°21'21"E, collected from light trap; 24 August 2005; Y.H. Kim • 1 ♂; Sogagido Island, Nagwol-myeon, Yeonggwang-gun, Jeollanam-do; 35°15'08"N, 126°06'04"E; collected from hand net; 27 June 2007; Y.H. Kim leg. • 1 ♂; Daejin port, Hyeonnae-myeon, Goseong-gun, Gangwon-do; 38°29'56"N, 128°25'34"E; collected from light trap; 11 June 2009; Y.H. Kim • 1 ♂; Dokdo Island, Ulleung-eup, Ulleung-gun, Gyeongsangbuk-do; 37°26'01"N, 131°56'52"E; collected from hand net; 22 February 2019; Y.H. Kim leg.



Figure 2. Aoroides gracilicrus sp. nov. A male, 3.3 mm B Grandidierella naroensis sp. nov., male, 4.9 mm. Scale bars: 1.0 mm (A, B).

Diagnosis. Eyes well developed, oval. Antenna 1 slender, except peduncular article 1, elongated, moderately setose; flagellum longer than peduncle. Antenna 2 densely setose, stout, gland cone bluntly pointed; flagellum short, 3-articulate, with two robust setae on each article distally. Mandible, incisor with five dentate, tricuspidate lacinia mobilis, molar triturative. Maxilla 1, outer plate with 10 dentate setae apically. Gnathopod 1 merochelate, massive, greatly larger than gnathopod 2, covered by plumose setae; coxa with a long robust and a plumose seta on anteroventral corner. Pereopod 7 slender, elongated, longer than pereopods 5 and 6. Uropod 1 with a large inter-ramal process. Uropod 3, outer ramus biarticulate. Telson short, fleshy, truncate distally.

Description. Holotype, adult male, HNIBRIV2426.

Body (Figs 2A, 3A) 3.3 mm long, dorsally smooth, laterally compressed; eyes well developed, oval.

Antenna 1 (Fig. 3B) slender, elongated, moderately setose, length ratio of peduncular articles 1-3 = 1.00: 0.95: 0.43; flagellum 10-articulate, terminal article minute, length 0.87 × peduncle; accessory flagellum absent.

Antenna 2 (Fig. 3C) densely setose; gland cone bluntly pointed, short; peduncular articles 1-3 short, peduncular articles 4, 5 subrectangular; length ratio of peduncular articles 3-5 = 1.00: 1.22: 0.41; flagellum (Fig. 3D) 3-articulate, with two robust setae distally on each article.

Upper lip (Fig. 3E) subovate, apical margin round and pubescent.

Lower lip (Fig. 3F), inner plate elongate-ovate, densely pubescent medially and distally; outer plate distally expanded, mediodistal margin round and pubescent; mandibular process developed.

Left mandible (Fig. 3G), incisor with 5 blunt teeth and lacinia mobilis with 3–4 teeth; accessory setal row composed of two multicuspidate setae; molar triturative with pappose seta; palp slender, 3-articulate; article 1 short, unarmed, length 0.28 × article 3; article 2 unarmed, length 0.84 × article 3; article 3 with two long setae distally.

Maxilla 1 (Fig. 3H), inner plate small with long pinnate seta apically; outer plate, apical margin with 10 dentate setal teeth; palp biarticulate, article 1 short,



Figure 3. *Aoroides gracilicrus* sp. nov., holotype, male, 3.3 mm **A** habitus, lateral view **B** antenna 1 **C** antenna 2 **D** flagellum of antenna 2 **E** upper lip **F** lower lip **G** mandible **H** maxilla 1 **I** maxilliped **J** maxilla 2. Scale bars: 1.0 mm (**A**); 0.3 mm (**B**–**D**); 0.1 mm (**E**–**J**).



Figure 4. Aoroides gracilicrus sp. nov., holotype, male, 3.3 mm A gnathopod 1 B gnathopod 2. Scale bars: 0.6 mm (A); 0.3 mm (B).

unarmed, article 2 swollen distally, apex round and weakly serrate, with seven robust setae, subapical portion with one pinnate seta.

Maxilla 2 (Fig. 3I), inner plate slender, slightly narrowing distally, apex and medial margin setose, with oblique row of plumose setae on surface; outer plate large, broad, with row of mediodistal setae, apex round, slightly notched subapically, pubescent laterally.

Maxilliped (Fig. 3J), inner plate subrectangular, medial margin with seven plumose setae submarginally, apex with four plumose and two stout robust setae; outer plate subrectangular, apex expending beyond end of palp article 2, medial margin straight with seven blunt robust setae gradually increasing in size distally and two elongate setae apically; palp 4-articulate, article 1 short, articles 2 and 3 subrectangular, medial and laterodistal margins lined with long setae, article 4 falcate, with a nail.

Gnathopod 1 (Fig. 4A) merochelate, densely setose, moderate, greatly larger than gnathopod 2; coxa wider than long, width 2.51× length, with one robust and one plumose setae on anteroventral corner; basis relatively long, slender, moderately broad, anterior and lateral margins with numerous plumose setae, posterior margin with 17 setae; ischium short, length 0.28× basis, with five plumose setae anterolaterally; merus elongated, located below carpus, length 0.86× basis, apex acute, ventral surface with numerous plumose setae; carpus moderate, subrectangular, length 1.05× merus, anterior and posterior margins with numerous plumose setae; propodus slightly curved anteriorly, length 0.57× carpus, posterior margin with numerous plumose setae; dactylus elongated, falcate, length 1.14× propodus.

Gnathopod 2 (Fig. 4B) subchelate; coxa subquadrate, width 1.11× length; basis subrectangular, width 0.32 × length, slightly curved posteriorly, anterior margin with plumose and simple setae, posterior margin with simple setae; merus 1.25× ischium, half of posterodistal margin setose; carpus subrectangular,



Figure 5. Aoroides gracilicrus sp. nov., holotype, male, 3.3 mm **A** pereopod 3 **B** pereopod 4 **C** pereopod 5 **D** pereopod 6 **E** pereopod 7 **F** uropod 1 **G** uropod 2 **H** uropod 3 I telson. Scale bars: 0.3 mm (**A**–**H**); 0.2 mm (**I**).

widening distally, ventrally setose; propodus subrectangular, length 0.83× carpus, anterior margin with five clusters of setae and posterior margin setose, palm steeply angled, defined by one robust seta; dactylus falcate, overreaching palm, length 0.66× propodus. **Pereopod 3** (Fig. 5A) setose; basis subrectangular, width 0.27× length, anterior margin with 13 plumose and 10 simple setae, posterior margin with six simple setae; merus subquadrate, length 1.82× ischium, with seven plumose and 12 simple setae anteriorly, 19 simple setae posteriorly; carpus subrectangular, length 1.29× merus, with one robust seta posteroproximally; propodus rectangular, slender, length 1.04× carpus, posterior margin setose; dactylus falcate, length 0.49× propodus.

Pereopod 4 (Fig. 5B) similar to pereopod 3, but merus with plumose setae along with simple setae on posterior margin.

Pereopod 5 (Fig. 5C), coxa bilobate, wider than long; basis subrectangular, slightly narrowing distally, length 2.52× width, both margins with unequal simple setae; merus widening distally, length 1.59× ischium, posterior margin with five plumose and four simple setae; carpus rectangular, length 0.77× merus, with two proximal and three distal robust setae; propodus slender, slightly widening distally, length 1.52× carpus, anterior margin with 1-2-3-4 robust setae formula; dactylus falcate, length 0.48× propodus.

Pereopod 6 (Fig. 5D) similar to pereopod 5, but less setose, unarmed.

Pereopod 7 (Fig. 5E) elongated, much longer than either pereopods 5 or 6; basis characteristic in form, subrectangular, slender, width 0.14× length; anterior margin with four robust and one simple setae; ischium short, length 0.23× basis, posterodistal corner with two setae; merus rectangular, length 2.29× ischium; carpus rectangular, length 0.86× merus; propodus elongate, length 1.64× carpus; dactylus falcate, length 0.43× propodus.

Uropod 1 (Fig. 5F), peduncle subrectangular, length 0.73× inner ramus, with three dorsolateral and two dorsomedial robust setae, and a large inter-ramal process; outer ramus length× 1.09 inner ramus, with two robust setae on both margins, four robust setae on apex; inner ramus with three dorsal and four apical robust setae.

Uropod 2 (Fig. 5G), peduncle subrectangular, subequal in length to inner ramus, with one dorsomedial robust seta; outer ramus length 1.26× inner ramus, with three dorsal and four apical robust setae; inner ramus with three dorsal and four apical robust setae.

Uropod 3 (Fig. 5H) short, length 0.52× uropod 2; peduncle subequal to outer ramus, with two dorsolateral and two dorsomedial robust setae; outer ramus 0.73× inner ramus, biarticulate, proximal article with six long setae subapically, terminal article minute, with two long setae on apex; inner ramus with two long setae on apex.

Telson (Fig. 5I) short and fleshy, longer than broad, concave distally, each lobe with two lateral robust setae and 5–6 unequal setae near the apex.

Female. Unknown.

Remarks. The new species *Aoroides gracilicrus* sp. nov. resembles *A. longimerus* Ren & Zheng, 1996 from Dayawan, China, *A. myojinensis* Ariyama, 2004 from Myojin-zaki, Japan, and *A. secunda* Gurjanova, 1951 from Primorskii Krai, Russia, in having densely setose anterior margins of the basis and carpus of gnathopod 1. However, *A. gracilicrus* sp. nov. is distinguished from its congeners by the characters and character states shown in Table 1 and the following features (different characters and character states of *A. longimerus*, *A. myojinensis*, and *A. secunda* in brackets): 1) gnathopod 1, coxa with an anterior robust and a plumose setae (vs four plumose setae and a robust seta in *A. longimerus*, unarmed in *A. myojinensis*, and two robust setae in *A. secunda*); 2) gnathopod 1,

Table 1. Morphological characters of *Aoroides gracilicrus* sp. nov. and related species with numerous plumose setae on anterior margin of basis and carpus of gnathopod 1.

Species Characters	A. longimerus	A. myojinensis	A. secunda	A. gracilicrus sp. nov.
Body length	3.6 mm	2.8 mm	3.9 mm	3.3 mm
Coxa 1	1 robust seta & 4 plumose setae	Unarmed	2 long robust setae	1 robust seta & 1 plumose seta
Gnathopod 1, carpus	Moderate	Ovate	Elongate ovate	Subrectangular
Pereopod 3, anterior margin of basis	Sparse simple setae	Sparse simple setae	Dense Plumose setae	Dense Plumose setae
Pereopod 3, posterior margin of carpus	2 robust setae	5 robust setae	3 robust setae	1 robust seta
Pereopod 4, anterior margin of basis	Sparse simple setae	Sparse simple setae	Dense Plumose setae	Dense Plumose setae
Pereopod 7, basis	Elongate ovate	Elongate ovate	Elongate ovate	Slender, Subrectangular
Uropod 3, both rami	Lateral robust setae	Lateral robust setae	Unarmed	Unarmed
Distribution	Osaka, Japan	Myojin-zaki, Japan	Primorskii Krai, Russia	Bijin-ri, Korea
Reference	Ariyama 2004	Ariyama 2004	Gurjanova 1951	Present study

carpus slender and subrectangular (vs moderate in *A. longimerus*, and ovate in *A. myojinensis* and *A. secunda*); 3) pereopods 3 and 4, anterior margins setose with plumose setae (vs sparse with simple setae in *A. longimerus* and *A. myojinensis*); 4) pereopod 3, carpus with a robust seta posteriorly (vs two robust setae in *A. longimerus*, five robust setae in *A. myojinensis*, and three robust setae in *A. secunda*); 5) pereopod 7, basis slender and subrectangular (vs elongate ovate in *A. longimerus*, *A. myojinensis*, and *A. secunda*); and 6) uropod 3, both rami unarmed (vs both rami with robust setae in *A. longimerus* and *A. myojinensis*).

Etymology. The species name is derived from the Latin *gracilis* (= slender) and *crus* (= leg) in reference to the relatively slender basis of pereopod 7.

Distribution. Korea (East Sea, South Sea).

Genus Grandidierella Krøyer, 1845

Type species. Grandidierella mahafalensis Coutière, 1904.

Grandidierella naroensis sp. nov.

https://zoobank.org/436FEB52-FEF7-447A-9E3B-F2EE9C532141 Figs 2B, 6–8 Korean name: Na-ro- do-keun-ap-son-yeop-sae-u, new

Type material. *Holotype*, SOUTH KOREA • 4.9 mm • 1 ♂; Yeom-po beach, Narodo Island, Goheung-gun, Jeollanam-do; 34°25'57"N, 127°29'31"E; collected from hand net; 01 April 2022; Y.H. Kim leg.; HNIBRIV2427.

Diagnosis. Antenna 1 slender, elongated. Antenna 2 relatively stout, flagellum short. Gnathopod 1 carpochelate, enlarged, ischium without posterodistal process, merus with weak process posterodistally, carpus with three processes on posterodistal corner, distal and proximal processes short, middle process elongate. Gnathopod 2, carpus longer than propodus, palm steeply angled. Pereopod 6, basis anterior margin with two plumose and three setae. Uropod 1, peduncle lacking inter-ramal process ventrodistally. Uropod 3 uniramus, ramus biarticulate.



Figure 6. *Grandidierella naroensis* sp. nov., holotype, male, 4.9 mm **A** habitus **B** antenna 1 **C** antenna 2 **D** upper lip **E** lower lip **F** right mandible **G** right mandible teeth **H** left mandible teeth **I** maxilla 1 **J** maxilliped **K** maxilla 2. Scale bars: 1.0 mm (**A**); 0.4 mm (**B**, **C**, **F**); 0.2 mm (**D**, **E**, **I**–**K**); 0.05 mm (**G**, **H**).

Description. Adult male, HNIBRIV2427.

Body (Figs 2B, 6A) 4.9 mm long, subcylindrical, dorsally smooth; eye small, rounded, composed of ommatidia.

Antenna 1 (Fig. 6B) slender, elongated; length ratio of peduncular articles 1–3 = 1.00: 1.03: 0.44; flagellum 16-articulate; accessory flagellum short, 2-articulate.

Antenna 2 (Fig. 6C) shorter than antenna 1; peduncular articles 4 and 5 subrectangular, setose; length ratio of peduncular articles 3-5 = 1.00: 2.85: 3.14; flagellum short, 5-articulate, each article with 1-2 robust setae ventrally.



Figure 7. *Grandidierella naroensis* sp. nov., holotype, male, 4.9 mm **A** gnathopod 1 **B** gnathopod 2 **C** pereopod 3. Scale bars: 0.4 mm (**A**–**C**).

Upper lip (Fig. 6D) subrounded, apical margin truncate and pubescent.

Lower lip (Fig. 6E), inner plate elongate-ovate, pubescent medially and distally; outer plate expanded, distal margin rounded and pubescent; mandibular process well developed.

Right mandible (Figs. 6F, G), incisor with five blunt teeth and lacinia mobilis with six teeth; accessory setal row composed of seven setae; molar triturative; palp stout, 3-articulate; article 1 relatively short, unarmed, length 0.58 × article 3; article 2 with five setae medially and one seta laterally, length 0.77× article 3; article 3 with 13 unipectinate and two simple setae distally and five simple setae laterally.

Left mandible (Fig. 6H) similar to right mandible, but lacinia mobilis with four teeth and accessory setal row composed of eight setae.

Maxilla 1 (Fig. 6I), inner plate vestigial; outer plate, apical margin with eight dentate setal teeth (two simple, one bifid, two tricuspidate, and three multicuspidate); palp biarticulate, article 1 short, unarmed, article 2 swollen distally, apex round, with five robust and three simple setae.

Maxilla 2 (Fig. 6J), inner plate apex and medial margins setose; outer plate subequal to inner plate, with row of distal setae.

Maxilliped (Fig. 6K), inner plate subrectangular, mediodistal corner slightly produced, medial margin with five setae, apex with six simple and three stout robust setae; outer plate elongate-ovate, medial margin straight with four blunt robust and four simple setae, apical margin with four robust setae gradually increasing in size; palp 4-articulate, article 1 subtriangular, article 2 subrectangular, medial margin with long setae, article 3 slender, lateral margin with two setae, subapical margin with 10 setae, article 4 falcate, with a nail.



Figure 8. *Grandidierella naroensis* sp. nov., holotype, male, 4.9 mm A pereopod 4 B pereopod 5 C pereopod 6 D pereopod 7 E uropod 1 F uropod 2 G uropod 3 H telson. Scale bars: 0.2 mm (A–D); 0.1 mm (E–H).

Gnathopod 1 (Fig. 7A) carpochelate, enlarged; coxa small, subquadrate, width 1.36× length; basis subrectangular, length 1.67× width, expended posteriorly, widening distally; ischium short, length 0.12× basis, without posterodistal process; merus located below carpus, with weak process posterodistally; carpus characteristic in form, massive, length 1.54× width, length 2.01× merus, with three processes on posterodistal corner, distal and proximal processes short, middle process elongate, with seven setae between middle and proximal processes; propodus subovate, width 0.67× length, convex posteriorly, length 0.65× carpus; dactylus falcate, posterior margin with two accessory teeth.

Gnathopod 2 (Fig. 7B), coxa subquadrate; basis slender, subrectangular, widening distally; merus located below carpus, length 1.42× ischium; carpus moderate, elongate-ovate, posterior margin setose; propodus subquadrate, length 0.74× carpus, posterior margin with three robust setae, palm steeply angled, defined by two robust setae; dactylus falcate, fitting palm.

Pereopod 3 (Fig. 7C), coxa subovate; basis elongate, slightly widened in the middle, both margins with four anterior and three posterior short setae; merus widening distally, length 0.71× basis; carpus slender, short, length 0.59× merus; propodus slender, narrowing distally, subequal to carpus; dactylus falcate.

Pereopod 4 (Fig. 8A) similar to pereopod 3, but coxa with one robust seta on ventral margin.

Pereopod 5 (Fig. 8B), coxa wider than long, bilobate; basis subovate, length 1.72× width, anterior margin with a row of six short setae, posterior margin with three robust setae; merus subrectangular, widening distally, length 1.88× ischium; carpus slender, length 0.80× merus, with two robust setae; propodus slender, length 1.33× carpus, anterior margin with a row of five robust setae; dactylus short, falcate.

Pereopod 6 (Fig. 8C), coxa similar but slightly smaller than coxa 5; basis elongate-ovate, length 1.91× width, anterior margin with two plumose and three setae, posterior margin with row of unequal plumose setae; merus subrectangular, length 0.84× basis, posterior margin with one robust and five simple setae, posterodistal corner with one long and two robust setae; carpus with three robust setae on posterior margin, posterodistal corner with one robust and three setae; propodus slender, length 1.79× carpus, with a row of five robust setae anteriorly; dactylus falcate.

Pereopod 7 (Fig. 8D) similar to pereopod 6, but slightly longer, coxa small, weakly bilobate.

Uropod 1 (Fig. 8E), peduncle subrectangular, with two dorsolateral robust setae, ventrodistal end lacking inter-ramal process, length 1.27× inner ramus; outer ramus with two dorsal and three terminal robust setae; inner ramus sub-equal to outer ramus, with three dorsal and four terminal robust setae.

Uropod 2 (Fig. 8F), peduncle short, subrectangular, subequal to inner ramus in length, with two dorsolateral and one dorsomedial robust setae; outer ramus with three dorsal and four terminal robust setae; inner ramus subequal to outer ramus, with two dorsal and three terminal robust setae.

Uropod 3 (Fig. 8G) uniramus, peduncle short, length 0.83× ramus, unarmed; ramus biarticulate, proximal article with five setae, terminal article minute, with one long seta on apex.

Telson (Fig. 8H) entire, short, broader than long, truncate distally, each lobe with two subapical setae and 1–2 unequal robust setae on the apex.

Remarks. Grandidierella naroensis sp. nov. is morphologically similar to G. pseudosakaensis Ariyama, 2020, G. osakaensis Ariyama, 1996, and G. fasciata Ariyama, 1996 in the following characters and character states: 1) gnathopod 1, merus with a small posterodistal notch; 2) gnathopod 1, carpus with three processes posterodistally. However, G. naroensis sp. nov. is distinguished from its congeners by the characters and character states listed in Table 2 and the following features (different characteristics of G. pseudosakaensis, G. osakaensis, and G. fasciata in brackets): 1) maxilla 1, outer plate with eight teeth (vs with nine teeth in G. pseudosakaensis and with 10 teeth in G. fasciata); 2) gnathopod 1, ischium without a posterodistal process (vs with a process in G. osakaensis); 3) gnathopod 1, carpus with small distal and proximal processes and a large middle process (vs with large middle and proximal processes and a small distal process in G. pseudosakaensis, G. osakaensis, and G. fasciata); 4) gnathopod 1, propodus subovate (vs subrectangular in G. pseudosakaensis, G. osakaensis, and G. fasciata); 5) gnathopod 2, posterior margin of propodus with 3 robust setae (vs with 5 robust setae in G. pseudosakaensis and G. osakaensis and with 4 robust setae in G. fasciata); 6) pereopod 7, basis anterior margin without plumose setae (vs with plumose setae in G. pseudosakaensis, G. osakaensis, and G. fasciata), and 7) uropod 1, peduncle without an inter-ramal process (vs with an inter-ramal process in G. fasciata).

 Table 2. Morphological characters of Grandidierella naroensis sp. nov. and related species with three processes on posterodistal margin of carpus of gnathopod 1.

Species Characters	G. pseudosakaensis	G. osakaensis	G. fasciata	G. naroensis sp. nov.
Body length	3.9 mm	6.9 mm	8.4 mm	4.9 mm
Maxilla 1, outer plate	9 teeth	8 teeth	10 teeth	8 teeth
Gnathopod 1, ischium Absent posterodistal process		Present	Absent	Absent
Gnathopod 1, merus posterodistal process	Prominently produced	Prominently produced	Prominently produced	Weakly produced
Gnathopod 1, carpus	Large middle and proximal processes and small distal process	Large middle and proximal processes and small distal process	Large middle and proximal processes and small distal process	Small distal and proximal processes and large middle tooth
Gnathopod 1, propodus	Subrectangular	Subrectangular	Subrectangular	Subovate
Gnathopod 2, propodus posterior margin	5 robust setae	5 robust setae	4 robust setae	3 robust setae
Pereopod 7, basis anterior margin	With plumose setae	With plumose setae	With plumose setae	Without Plumose setae
Uropod 1, peduncle inter-ramal process	Absent	Absent	Present	Absent
Distribution	lriomote island, japan	Osaka, japan	Osaka, japan	Narodo island, korea
Reference	Ariyama 2020	Ariyama 1996	Ariyama 1996	Present study

Etymology. The species name is derived from the type locality, Narodo Island, located on the south coast of Korea.

Distribution. Korea (Narodo Island).

Key to species of the family Aoridae from Korean waters

1	Antenna 1, accessory flagellum present and elongate
	Aora pseudotypica Hirayama, 1984
-	Antenna 1, accessory flagellum absent or short2
2	Gnathopod 1 carpochelate; uropod 3 uniramus 3
-	Gnathopod 1 merochelate; uropod 3 biramus6
3	Antenna 1, accessory flagellum absent; maxilliped, inner plate without dis-
	tal robust seta Paragrandidierella minima Ariyama, 2002
-	Antenna 1, accessory flagellum present; maxilliped, inner plate with distal
	robust seta4
4	Gnathopod 1, carpus with one posterodistal and two medial processes
	Grandidierella japonica Stephensen, 1938
-	Gnathopod 1, carpus with three posteromarginal processes 5
5	Gnathopod 1, propodus subrectangular; uropod 1, peduncle with inter-ra-
	mal process Grandidierella fasciata Ariyama, 1996
-	Gnathopod 1, propodus subovate; uropod 1, peduncle without inter-ramal
	processGrandidierella naroensis sp. nov.
6	Gnathopod 1 weakly setose; gnathopod 2, propodus posterior margin
	curvedAoroides semicurvatus Ariyama, 2004
_	Gnathopod 1 densely setose; gnathopod 2, propodus posterior margin
	straight7
7	Gnathopod 1, basis and carpus with simple setae; pereopod 3, basis with-
	out plumose setaeAoroides punctatus Ariyama, 2004
_	Gnathopod 1, basis and carpus with plumose setae; pereopod 3, basis
	with plumose setae

- 8 Gnathopod 1, coxa with one robust seta; pereopod 7, basis elliptical...... Aoroides ellipticus Ariyama, 2004

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

Altimella gen. nov., a new genus of Cicurinidae (Arachnida, Araneae) from Xizang, China, with description of two new species

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Abstract

Altimella Wang & Zhang, **gen. nov.**, a new genus belonging to Cicurinidae, is established, and two new species are described, Altimella nedong Wang & Zhang, **sp. nov.** (\mathscr{F} , type species) and A. ngamring Wang & Zhang, **sp. nov.** (\mathscr{F}), from Xizang, China. Detailed descriptions of somatic features and genital characteristics, photos of the habitus, photos and drawings of the copulatory organs, and a distribution map are provided.

Key words: description, distribution, morphology, taxonomy, Tibet



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Introduction

The spider family Cicurinidae F.O. Pickard-Cambridge, 1893 was restored and three genera (*Cicurina* Menge, 1871, *Brommella* Tullgren, 1948, and *Chorizomma* Simon, 1872) were confirmed by Gorneau et al. (2023). Currently, the type genus, *Cicurina*, comprises 144 species, most of which are distributed in North America and Asia, but with a few recorded from Europe. The genus *Brommella* includes 22 species, of which 17 have been reported from China, one from South Korea and Japan, three from the USA, and two from Europe. The third genus, *Chorizomma*, found in Spain and France, is monotypic and was once considered a subgenus of *Cicurina* (WSC 2024).

Gorneau et al. (2023) proposed synapomorphies for Cicurinidae: the absence of a cribellum, replaced by a reduced colulus with several setae; three tarsal claws, and legs without scopulae or claw tufts; the male palp with a variable retroventral tibial apophysis (RvTA); and the RTA enlarged (in some cases as long as the cymbium length), usually with an RTA-conductor. However, some Chinese *Brommella* species like *B. baiseensis* Li, 2017 and *B. casseabri* Li, 2017 possess obvious cribella (Li and Wang 2017: figs 6D, 8D).

We describe a new genus to accommodate two new species of Cicurinidae from Xizang, China, which also possess a cribellum like *Brommella* species, but they differ from *Brommella* by having a longer retrolateral tibial apophysis.

Materials and methods

All specimens are preserved in 75% ethanol and were examined, illustrated, photographed, and measured using a Leica M205A stereomicroscope equipped with a drawing tube, a Leica DFC450 Camera, and LAS v. 4.6 software. Male palps and epigynes were examined and illustrated after they were dissected. Epigynes were cleared by immersing them in pancreatin for about 1 h (Álva-rez-Padilla and Hormiga 2007). Eye sizes were measured as the maximum dorsal diameter. Leg measurements are shown as: total length (femur, patella and tibia, metatarsus, tarsus). All measurements are in millimeters. Specimens examined here are deposited in the Collection of Spiders, School of Life Sciences, Southwest University, Chongqing, China (**SWUC**).

Abbreviations used in the text: **ALE** = anterior lateral eye; **AME** = anterior median eye; **PLE** = posterior lateral eye; **PME** = posterior median eye; **RTA** = retrolateral tibial apophysis.

Taxonomy

Family Cicurinidae F.O. Pickard-Cambridge, 1893

Altimella Wang & Zhang, gen. nov.

https://zoobank.org/22CB1EAD-C4FC-4860-8C5F-F98F21ABC763 高朗蛛属

Type species. Altimella nedong Wang & Zhang, sp. nov.

Diagnosis. Altimella Wang & Zhang, gen. nov. species resemble those of *Brommella* (Li and Wang 2017: 126, figs 1–61) in having an undivided cribellum, a large, simple retrolateral tibial apophysis with a single fold (Figs 2C, 3E, 4C, 5E), a tibial apophysis (spur) arising from the base of the tibia retro-ventrally, a long and filiform embolus, and long and complexly winding copulatory ducts, but they differ from *Brommella* by the long retrolateral tibial apophysis, as long as the cymbium (Figs 2B, C, 3D, E, 4B, C, 5D, E; vs shorter than the cymbium), the simple distal part of the conductor (Figs 2B, 3D, 4B, 5D; vs complex, spiral-shaped, or with three branches), and the obvious spermathecae (Figs 2E, 3G, 4E, 5G; vs inconspicuous).

Etymology. The generic name is composed of the prefix "*alti-*" (high) and the suffix "*-mella*" (from *Brommella*), referring to the high-altitude type locality and similarity to *Brommella*. The gender is feminine.

Description. Small size (male (n = 2): 2.30–3.55, female (n = 11): 2.70–5.19). Carapace yellowish brown to brown. Eight eyes (Fig. 1A, D, E). Cervical groove and radial furrows distinct. Chelicerae yellowish brown, with 3 promarginal and 3–4 retromarginal teeth. Labium and endites yellowish brown. Sternum yellowish brown and scutellate with sparse black setae. Legs yellowish brown. Calamistrum absent in male and weak (10 setae) in female (Fig. 1B, C). Opisthosoma oval, dorsum and venter yellowish brown, with small (about 0.2 mm) and undivided cribellum (Fig. 1F, G). Leg formula: 1423 or 4123.

Male palp (Figs 2A–C, 3C–E, 4A–C, 5C–E): tibia with two apophyses, ventral (TA) and retrolateral (RTA): ventral lobe originates from base of tibia, longer

than tibia, slightly bent near tip; retrolateral apophysis as long cymbium, with fold along entire apophysis. Bulb longer than wide. Anterior part of tegulum rounded, posterior part hidden by conductor (C), sperm duct (Sd) narrow, running along margin of tegulum; conductor with two arms, posterior claw-like (CP) and anterior spine-like (CA); embolus with large base (Eb), long and filiform, originating at 6–7 o'clock position, its anterior part resting in a long groove of anterior part of conductor.

Epigyne (Figs 2D, E, 3F, G, 4D, E, 5F, G): epigynal plate wider than long or as long as wide. Copulatory openings wide, located anteriorly or posteriorly. Copulatory ducts long and complex, winding. Spermathecae convoluted or kidney-shaped. Fertilization ducts hook-like.

Composition. Comprises two new species, *A. nedong* Wang & Zhang, sp. nov. and *A. ngamring* Wang & Zhang, sp. nov.

Biology. Found in high altitude areas of the Qinghai-Tibet Plateau (Xizang). They construct sheet webs under stones.

Distribution. China (Xizang) (Fig. 6).

Remarks. This is the fourth genus of Cicurinidae and has a cribellum similar to species of *Brommella*. The epigynes of all four cicurinid genera are very similar, while male palps have more differences. Additionally, *Cicurina* and *Chorizomma* do not have cribella, while *Brommella* and *Altimella* gen. nov. do.

Altimella nedong Wang & Zhang, sp. nov.

https://zoobank.org/205BC42B-3D4A-43A3-9EEF-AA43AB0384F7 Figs 1-3, 6 乃东高朗蛛

Type material. *Holotype* \bigcirc (SWUC-T-CI-08-01), China, Xizang, Shannan City, Nedong District, Zedang Town, 29°13'56"N, 91°46'36"E, elev. 3616 m, 25.08.2002, M.S. Zhu leg. (SWUC). *Paratypes:* 6 \bigcirc (SWUC-T-CI-08-02 to 07) with same data as for holotype (SWUC) • 2 \bigcirc (SWUC-T-CI-08-08 to 09), Zedang Town, 21.08.2002, F.X. Liu leg.

Etymology. The specific name is derived from the type locality; a noun in apposition.

Diagnosis. The new species differs from those of *A. ngamring* Wang & Zhang, sp. nov. (Figs 4, 5) by the ventral tibial apophysis as long as tibia (Figs 2A–C, 3C, D; vs short and with claw-like tip); posterior part of conductor (Cc) bifurcated (Figs 2B, 3D; vs pocket-like); copulatory openings located in anterior half of epigyne (Figs 2D, 3F, vs near epigastral fold); spermathecae convoluted (Figs 2E, 3G, vs kidney-shaped).

Description. Male holotype (Fig. 3A) total length 3.55. Carapace 1.69 long, 1.33 wide; opisthosoma 1.92 long, 1.10 wide. Eye sizes and interdistances: AME 0.06, ALE 0.14, PME 0.10, PLE, 0.11; AME-AME 0.05, AME-ALE 0.03, PME-PME 0.12, PME-PLE 0.08, ALE-PLE 0.03. MOA 0.24 long, anterior width 0.19, posterior width 0.31. Clypeus height 0.19. Chelicerae with three promarginal and four retromarginal teeth. Leg measurements: I 7.32 (2.06, 2.64, 1.62, 1.00); II 5.69 (1.68, 1.97, 1.27, 0.77); III 4.80 (1.38, 1.53, 1.18, 0.71); IV 6.14 (1.74, 2.06, 1.54, 0.80). Leg I longer than leg IV.



Figure 1. Altimella nedong Wang & Zhang, sp. nov. B, D, F holotype male A, C, E, G paratype female A male eyes and chelicerae, frontal view B, C metatarsus of Leg I, lateral view D, E eyes, front view F, G cribellum ventral view.

Palp (Figs 2A–C, 3C, D). Patella as long as tibia. Tibia: ventral tibial apophysis (TA) originates from base of tibia, longer than tibia, distal half partially grooved and bent posteriorly; retrolateral apophysis (RTA) as long as cymbium, almost six times longer than maximal width. Posterior arm of conductor (CP) claw-like, anterior arm (CA) membranous and groove-like. Embolus long filiform, with a broad base, originating at 6 o'clock position, anterior part resting in long groove of conductor.

Female (paratype, Fig. 3B, SWUC-T-CI-08-02) total length 4.79. Carapace 1.74 long, 1.32 wide; opisthosoma 3.32 long, 2.41 wide. Eye sizes and interdistances: AME 0.08, ALE 0.13, PME 0.10, PLE, 0.12; AME-AME 0.05, AME-ALE 0.03, PME-PME 0.08, PME-PLE 0.03, ALE-PLE 0.04. MOA 0.29 long, anterior width 0.20, posterior width 0.34. Clypeus height 0.15. Chelicerae with three



Figure 2. Altimella nedong Wang & Zhang, sp. nov. A-C holotype male D, E paratype female A left male palp, ventral view B same, retrolateral view C same, dorsal view D epigyne, ventral view E epigyne, dorsal view. Abbreviations: CA = anterior arm of conductor; CD = copulatory duct; CO = copulatory opening; CP = posterior arm of conductor; E = embolus; Eb = embolic base; FD = fertilization duct; RTA = retrolateral tibial apophysis; Sd = sperm duct; Sp = spermathecal; TA = tibial apophyses.

promarginal and four retromarginal teeth. Leg measurements: I 5.86 (1.75, 2.05, 1.26, 0.80); II 5.00 (1.47, 1.72, 1.09, 0.72); III 4.39 (1.27, 1.39, 1.06, 0.67); IV 5.74 (1.59, 1.97, 1.45, 0.73).

Epigyne (Figs 2D–E, 3F–G). Epigynal plate 1.5 times wider than long. Copulatory openings located in anterior hood (Ah). Copulatory ducts long and complexly winding. Spermathecae convoluted.

Variation. Females (n = 8): total length 3.60–5.19.

Distribution. Known only from the type locality, Xizang, China (Fig. 6).

Altimella ngamring Wang & Zhang, sp. nov.

https://zoobank.org/E4E657ED-49D2-44C1-B5FB-293D4437F0DC Figs 4-6 昂仁高朗蛛

Type material. *Holotype* ♂ (SWUC-T-CI-09-01), China, Xizang, Shigatse City, Ngamring County, Kaga Town, Nanma Village, 29°12'41"N, 87°19'42"E, elev. 4265 m, 21.07.2020, L.Y. Wang et al. leg. *Paratypes:* 3♀ (SWUC-T-CI-09-02 to 04) with same data as for holotype (SWUC).



Figure 3. Altimella nedong Wang & Zhang, sp. nov. A, C–E holotype male B, F, G paratype female A male habitus, dorsal view B female habitus, dorsal view C left male palp, ventral view D same, retrolateral view E same, dorsal view F epigyne, ventral view G same, dorsal view.

Etymology. The specific name is derived from the type locality; a noun in apposition.

Diagnosis. The new species differs from *A. nedong* Wang & Zhang, sp. nov. (Figs 2, 3) by the short ventral tibial apophysis, shorter than tibia width (Figs 4B, C, 5D, E; vs longer than tibia, tip with groove); the posterior part of conductor pocket-like (Figs 4B, 5D; vs claw-like); copulatory openings located posteriorly in a joint fovea and not hidden by hood (Figs 4D, 5F; vs located anteriorly and hidden by hood); spermathecae kidney-shaped (Figs 4F, 5G; vs convoluted).



Figure 4. Altimella ngamring Wang & Zhang, sp. nov. A-C holotype male D, E paratype female A left male palp, ventral view B same, retrolateral view C same, dorsal view D epigyne, ventral view E epigyne, dorsal view. Abbreviations: CA = anterior arm of conductor; CD = copulatory duct; CO = copulatory opening; CP = posterior arm of conductor; E = embolus; Eb = embolic base; FD = fertilization duct; RTA = retrolateral tibial apophysis; Sd = sperm duct; Sp = spermathecal; TA = tibial apophyses.

Description. Male holotype (Fig. 5A) total length 2.30. Carapace 1.09 long, 0.88 wide; opisthosoma 1.26 long, 0.88 wide. Eye sizes and interdistances: AME 0.05, ALE 0.08, PME 0.06, PLE, 0.08; AME-AME 0.03, AME-ALE 0.01, PME-PME 0.06, PME-PLE 0.05, ALE-PLE 0.02. MOA 0.16 long, anterior width 0.12, posterior width 0.18. Clypeus height 0.11. Chelicerae with three promarginal and three retromarginal teeth. Leg measurements: I 3.21 (0.90, 1.07, 0.70, 0.54); II 2.87 (0.84, 0.98, 0.60, 0.45); III 2.54 (0.73, 0.81, 0.56, 0.44); IV 3.24 (0.93, 1.01, 0.87, 0.43).

Palp (Figs 4A–C, 5C, D). Tibia longer than patella. Ventral tibial apophysis (TA) shorter than the tibia, with hook-like tip; retrolateral apophysis (RTA) large and as long as cymbium with single fold; posterior arm of conductor (CP) pock-et-like, lamelliform with sharp tip, anterior arm of conductor (CA) unobvious; embolus long, filiform, with a broad base, originating at 6 o'clock position, anterior part resting in the long groove of conductor.

Female (paratype, Fig. 5B, SWUC-T-CI-09-02) total length 2.96. Carapace 1.08 long, 0.85 wide; opisthosoma 1.87 long, 1.32 wide. Eye sizes and interdistances: AME 0.05, ALE 0.09, PME 0.06, PLE, 0.08; AME-AME 0.04, AME-ALE 0.02, PME-PME 0.05, PME-PLE 0.06, ALE-PLE 0.03. MOA 0.16 long, anterior width 0.15, posterior width 0.19. Clypeus height 0.08. Chelicerae with three promarginal and three retromarginal teeth. Leg measurements: I 2.88 (0.79, 1.05, 0.61, 0.43); II 2.59 (0.72, 0.87, 0.54, 0.46); III 2.33 (0.68, 0.65, 0.55, 0.45); IV 3.19 (0.93, 1.07, 0.75, 0.44).



Figure 5. Altimella ngamring Wang & Zhang, sp. nov. A, C–E holotype male B, F, G paratype female A male habitus, dorsal view B female habitus, dorsal view C left male palp, ventral view D same, retrolateral view E same, dorsal view F epigyne, ventral view G same, dorsal view.

Epigyne (Figs 4D, E, 5F, G). Epigynal plate longer than wide. Copulatory openings located posteriorly in a wide fovea. Copulatory ducts long, C-shaped in dorsal view, together forming an X-shape. Spermathecae kidney-shaped. Fertilization ducts hook-like.

Variation. Females (n = 3): total length 2.70–2.96.

Distribution. Known only from the type locality, Xizang, China (Fig. 6).



Figure 6. Distribution of Altimella in China.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

A protocol to evaluate the taxonomic health of Neotropical species of *Nasutitermes* (Termitidae, Nasutitermitinae)

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Abstract

Herein a protocol is proposed to summarize the taxonomic situation for species, using the Neotropical *Nasutitermes* Dudley (Nasutitermitinae) as a test. The objective of this protocol is to allow comparisons between the available taxonomic information for species, and to provide objective criteria for assessing the information gaps for each taxon in order to prioritize topics for future investigation. Key aspects of taxonomic practice (condition of type specimens, helpfulness of descriptions and figures, compilation of distribution data, molecular data) were noted, the data were tabulated, and the taxa ranked. In addition, specific notes for each species have been included that may help to improve the solutions to the problems raised here.

Key words: Collections, revisionary work, rich taxa, species description, taxonomic protocol, taxonomic ranking

Introduction

Taxonomy as a science is misunderstood by most people, and worse yet, this includes many professional biologists, including those responsible for policy decisions and financial allocations. It is a descriptive science, like many others that are more easily recognized to many, such as astronomy with its stellar descriptions and particle physics with the description, albeit "high-tech", of fundamental particles. Grimaldi and Engel (2007) made a vigorous defense of the comparative scientific approach for all descriptive disciplines, arguing against the "pejorative" view regarding the term descriptive in science.

There are many papers in defense of taxonomy and discussing the concept of a "Taxonomic Impediment" (e.g., de Carvalho et al. 2007; Engel et al. 2021), although some interpretations are equivocal, like the idea that the impediment can be solved merely with faster descriptions. Taxonomy is a scientific discipline based on hypotheses that may be challenged and tested using different tools, some are low-tech (anatomical and morphological studies) or "high-tech" (molecular data, nano-CT scans, etc.), and this distinction alone does not mean "more or less scientific". All are equally valuable scientific approaches and each is good science to be considered on equal footing.



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Copyright: [©] Mauricio M. Rocha et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). Taxonomy and nomenclature are not the same thing, they are different disciplines. Taxonomy describes species or higher taxa, searching for evolutionary lineages, that is, each species description is an evolutionary hypothesis. Nomenclature and its rules are used to achieve universal, consistent, and unambiguous communication among the scientific community. In this meaning, shared by all taxonomists, taxonomy is a science, whereas nomenclature is a technical discipline, an assemblage of rules to attribute names to organisms and their ranks.

Current taxonomy is not a "typological science", as the types are merely a system by which the data of taxonomy are vouchered. Many biologists confuse these taxonomic vouchers as typological concepts simply because the word "type" is employed, but this is not the case at all. Nomenclatural types are used to link a name to one biological entity, and it does not "represent" this species. In this sense, it is fundamental to examine nomenclatural types, and when one is lost or damaged, this could be an actual problem to the attribution of that name. Names matter and confusion or misapplication of a name to a biological entity can have repercussions in everything from conservation and medicine to international treaties and national economies. A deeper discussion of scientific names and their importance and connection to good taxonomy was made by Engel (2022), from whom we copy here: "A lack of understanding that taxonomy and nomenclature are the foundation of all other biological disciplines has created a climate in which these sciences are dismissed and underfunded (Britz et al. 2020; Engel et al. 2021)". An interesting and seminal book on practical taxonomic procedures by Winston (1999) is still exceptionally useful and should be consulted by all taxonomists and biologists.

A concise history of termite systematics was given by the treatise of Krishna et al. (2013). This 7-volume work covered all aspects of termite taxonomy up to that date, and the fifth volume was focused on the subfamilies Cubitermitinae and Nasutitermitinae, in with the species of the rich termite genus *Nasutitermes* Dudley occupying 164 of its 488 pages. *Nasutitermes* is the richest genus among termites and it is the type genus of the exceedingly rich subfamily Nasutitermitinae. Constantino (2002a) reviewed its fraught nomenclatural history, and Krishna et al. (2013) can be consulted for a detailed explanation.

Nasutitermes is one of the most frequently encountered termite genera in most Neotropical biome. Occupying a great distributional range across a remarkable variety of habitats, building different kinds of nests, and utilizing several food items, although most are xylophagous, some species are recognized as pests and others have the potential to become pestiferous. The group has evolved sophisticated defense strategies, with a large number of soldiers, equipped with effective chemical weapons. Despite the considerable biological and economic importance of species in the genus, the identification of species remains a great challenge, particularly as the genus comprises a vast number of described species, for which existing descriptions are mostly inadequate. As many species of Nasutitermes construct conspicuous nests, they were originally described by early entomologists and these antique scientific accounts have rarely been updated. Moreover, the existing data and descriptions are not uniform, sometimes they are exceedingly short and failed to include characters today considered of critical value, and in several cases the nomenclatural types were pinned instead of being preserved in alcohol, as is current best practice.
Holmgren (1906, 1910) described and redescribed many species of *Nasutitermes*. Although his descriptions are not uniform, he does mention head and nasus shapes and gives characters of the pilosity, which in the case of *Nasutitermes* may be helpful, as long as they are supplemented with other characters. His illustrations are simple and also not uniform as some of them show the pilosity, while others do not. In general, the head illustrations are effectively useless by modern standards. This author did describe the color, but this is now seldom helpful given that it is highly variable in most species. Because the measurements of head width and length are always present, if an expert considers all the characters together, and makes detailed comparison with other species, they can sometimes reach the right identification. Still, it is possible to suspect that there are many synonyms within *Nasutitermes*, and this situation is pointed out herein.

It is a consensus that the taxonomy of species of *Nasutitermes* is highly confusing and consequently the identification of its species is rendered difficult. The use of cuticular hydrocarbons was of some use to differentiate quite similar species, such as *N. corniger* and *N. ephratae* (Howard et al. 1988). Mitochondrial makers have demonstrated the synonymy of *N. corniger*, *N. costalis*, and *N. polygynus* (Scheffrahn et al. 2005a, 2005b). Pursuing the same goal, that is, to establish species delimitation among species of *Nasutitermes* from French Guiana, Roy et al. (2014) applied exploratory methods of species delimitation that use DNA sequences as their primary information source to establish group membership and estimate putative species boundaries. In the supplementary material of Roy et al. (2014) the authors explain how the species were named.

Gonzalez et al. (2013), with a very similar objective, utilized many of the criteria mentioned here (referred to as "taxonomic products") to evaluate the taxonomic status of bumble bees in literature spanning from 1912 to 2011. They utilized Zoological Records and Web of Science, employing the search terms 'bumble bees'. They categorized levels of operations (I to VIII), which represent combinations of taxonomic products [= diagnoses, descriptions, illustrations, keys, historical review, phylogeny, biogeography, molecular analyses] resulting from taxonomic work. Consequently, the levels of operation increase with the number of products combined. The paper offers more than just an example and should be referenced for a comprehensive understanding.

It is important to highlight that these criteria (their taxonomic products) are similar to our criteria, as they represent an expected standard in taxonomic work, arranged in a sequence, as mentioned there and proposed here. However, the protocol of Gonzalez et al. (2013) tries to evaluate the status of the literature/databases associated to a group of taxa, while our objective is to evaluate how robust the taxonomy associated to each taxon name is, in other words, the number and quality of elements/procedures of the taxonomic practice used to evaluate the species hypothesis represented by each name.

Kitchener et al. (2022) also explored a similar concept, aiming to establish a framework for assessing taxonomic certainty in mammals. They proposed a "traffic-light system" that integrates morphological, genetic, and biogeographical evidence, reflecting a conservation-oriented protocol.

Throughout the history of taxonomy, it took many decades for the concept of evolution to be more harshly incorporated by taxonomists (this is the reason why revisionary works are constantly relevant). Some groups were worked on by "excessively prolific" taxonomists, for example Maurice Pic, who by 1956 had described more than 18,500 beetle species, and was criticized by his contemporaries for his "chaotic methods". Even today, with the use of modern techniques, some authors insist to propose uninformative species names. Some of these cases are discussed in Wheeler (2023), several associated with inappropriate use of molecular data.

The profusion of these "unsubstantiated names" creates difficulties to access information about biodiversity. Despite taxonomic vandalism cases, information about species, retrieved by their names, remains heterogeneous. For termites, Eggleton (1999) and Constantino (2018) conclude, by cumulative species description curves estimations, that the atypical high rates of description from China between 1980–2000 resulted in distorted estimates for the number of species expected for Oriental region. Similar problems occur in almost all groups of Hexapoda.

In face of the actual biodiversity crisis (Dirzo and Raven 2003; Pereira et al. 2010; Barnosky et al. 2011), much is discussed about species description rates (Bini et al. 2006; Dubois 2010; Mora et al. 2011). Although the lack of taxonomists is at the root of the taxonomic impediment, the problem is commonly misinterpreted as "slow species rate descriptions", when the problem is also one of accessibility to names and associated information.

Considering these questions, we expect that this protocol can contribute to assess whether the names are a good reflection of the diversity of a group. Genera with many doubtful names "pollute" the information, artificially altering the perception of diversity, and are a problem. This is particularly relevant now that species databases are automatically generated by algorithms.

We also hope it will be useful for establishing priorities (among species and among their information) for updating knowledge. Some genera are very abundant in collections. Given the dynamics of how the research is practiced today, they will hardly be able to be completely revised. A very superficial estimate is that there are more than 12,000 samples of *Nasutitermes* in the major collections that cover the Neotropics, so it is necessary to establish priorities to work with its taxonomy. As a comparison, Sands (1965), in his review of the Ethiopian Nasutitermitinae, examined 2,400 samples.

In the case of *Nasutitermes*, one of the most frequently encountered and abundant genera in termite surveys, several names may prove problematic. However, assessing whether a genus has all its species names linked to robust taxonomic entities is also an interesting result. This information can be used to select taxa that are more appropriate targets for other studies, such as behavior or ecological approaches.

Materials and methods

To evaluate the taxonomic health status of species, we developed a practical protocol that takes into account seven criteria applied to each species. Each criterion received a numeric value (elaborated in detail below). We used the following sources to evaluate each criterion: personal observations and information from institutional curators or collection managers; information compiled from the literature, referenced in Krishna et al. (2013); the Constantino Online termite database (Constantino 2023); and Google Scholar. In addition, we included any pertinent personal observations on each species (refer to Results, below).

The evaluation of the condition of type specimens was made via personal information [EMC visited the collections of the American Museum of Natural History (**AMNH**) and Naturhistoriska Riksmuseet (**NHRM**)], personal information from curators or collection managers [Museum of Comparative Zoology (**MCZ**), Naturhistorisch Museum Maastricht (**NHMM**), and Zoologisches Museum für Hamburg (**ZMH**)], and online institutional databases.

Explanation of the criteria

1 Type specimens

- 0 Lost or doubtful.
- 1 Badly conserved (e.g., pinned individuals).
- 2 Well preserved.

Many of the original descriptions of Isoptera were made before the broad implementation of the International Code of Zoological Nomenclature, so many species names are linked to syntype series. This practice may prove to be problematic when the type series includes more than one species.

Some of the first termite collectors used the old practice of preserving all insects on pins. Thus, some syntype series include pinned imagoes, workers, and soldiers, as exemplified in part by type material of Rambur in the Muséum national d'Histoire naturelle (**MNHN**, Paris) (EMC pers. obs.). In this kind of preservation, even if the specimen does not get broken, some relevant characters are impossible to examine due to the shrinkage of the soft integument.

2 Description based on

- 0 Imagoes and/or workers only
- 1 Soldiers only, soldiers and workers, or soldiers and imagoes
- 2 Soldiers, workers, and imagoes

Descriptions based on imagoes and/or workers are less useful for identification purposes, as the imagoes are uncommonly collected and congeneric workers recurrently have few distinctive characters. Moreover, for most species the imagoes are unknown, so descriptions associated with soldiers are much more useful. If the description includes worker characters (external sclerites and gut anatomy) this is a step that contributes to a more precise identification.

3 Description

- 0 Generic, i.e., do not allow species identification.
- 1 Clear, based on informative characters.

Species descriptions are not timeless: with the increasing number of described species, and more characters evaluated, old diagnoses tend to be relatively less informative and applicable. This does not mean that the author of the description was "unqualified", simply that the description has become outmoded by the growth of biological information. All descriptions need to eventually be re-evaluated in the light of current data, methods, and concepts – hence the critical value of revisionary monographs.

The evaluation is not restricted to the first description; redescriptions and revisions may solve many species-identity problems and raise the rank. Some descriptions are wordy, with excessive generic characteristics that do not allow for meaningful identification (e.g., "nasus conical").

Criteria used to evaluate a description as informative

- · clear diagnostic characters mentioned in descriptions.
- inclusion of close congeneric species comparison.
- · delimitation of the species through discrete characters.

A good species delimitation can be improved by quantitative characters (e.g., measurements), but this necessitates the availability of a large series of specimens and the evaluation of regional variations.

4 Description, illustrations

0 Illustrations/photographs missing or of poor quality.1 With informative illustrations/photographs.

Illustrations and photographs that show clear informative characters are considered good quality. Even if present, some old illustrations are restricted to the soldier head silhouettes, which are not always useful.

5 Information about biology

- 0 Without information.
- 1 Some available data.

Data about nests can significantly increase identification rates since some species build quite particular nests or forage in specific substrates. In some cases, this information can solve a species identification, for example in *N. ephratae* and *N. corniger*, as reported by Thorne (1980). The soldiers have a similar morphology, while the nests exhibit typical features.

6 Data about species distribution

- 0 Known only from the type locality or sparse occurrences in the literature.
- 1 Partially compiled records.
- 2 The species occurrences are compiled and mapped.

Although some species of *Nasutitermes* are widespread (e.g., *N. corniger* occurs throughout South, Central, and insular America), several species have distributions correlated with environmental factors, and good knowledge about their distribution may be helpful for species discrimination and identification.

Records of species not based on vouchers in zoological collections have not been considered since such vouchers cannot be revised to identify potential misidentifications The same applies to citations of occurrences only by country, without georeferenced localities (checklists).

Partial records are distributions mapped based on records restricted a priori to a geographic vicinity (e.g., only to one country or only one biome). Papers with regional compilations have a more restricted utility to help in species identification but are better than the absence of information.

Well-mapped species distributions are a good indication that the species might have been properly revised.

7 Species delimited by DNA data

0 No data.

1 Species included in phylogenies or population-based studies.

DNA data here means that the species has been the subject of studies evaluating its identity and checking for species monophyly, rather than merely being a terminal included in an analysis or having sequences published or included in a DNA database. Examples are Scheffrahn et al. (2005a, 2005b) and Santos et al. (2017, 2022), which evaluated distinct populations.

Taxonomic health ranks

The scores of each criterion are assessed for each species (Table 1); species are then ranked according to the sum of their individual scores. The taxonomic health of each species is classified into one of the following situations:

- 0: Irresolvable;
- 1-4: The species identity is confusing, identifications with this name are doubtful and a revision and redescription of the type specimen(s) is necessary;
- 5–7: The identification is possible by a specialist through comparison with reference material (type specimens or specimens identified by direct comparison with types);
- 8-10: The identification is possible by a specialist with literature references.

Results

Of the 66 species evaluated, none scored 0, 30 scored 1-4, 26 scored 5-7, 10 scored 8-10, and none scored 10 (Table 1).

Nasutitermes acajutlae (Holmgren, 1910)

The identity of this species relative to *N. nigriceps* was debated for a long time. Thorne et al. (1994) summarized the taxonomic discussion and, based on a few morphological characters of the soldiers and biogeographical data (including an erroneous assumption) from the examined material, considered the two species valid. In a subsequent paper, Thorne et al. (1996) clarified the misunderstanding about the distribution of *N. acajutlae* and the resulting confusion regarding the localities of the samples examined in their previous paper.

Table 1. Evaluation of criteria scores for each Neotropical species of *Nasutitermes* [1-Type specimens (0. Lost or doubtful, 1. Badly conserved, 2. Well-preserved); 2- Castes used for description (0. Imagoes and/or workers only, 1. Soldiers only, soldiers and workers, or soldiers and imagoes, 2. Soldiers, workers, and imagoes); 3- Description (0. Generic, not allowing species identification, 1. Clear, based on informative characters); 4- Description, illustrations (0. Illustrations/photos missing or of poor quality, 1. With informative illustrations/photos); 5- Information about biology (0. Without information, 1. Some available data); 6- Data about species distribution (0. Known only from the type locality or sparse records in the literature, 1. Partially compiled records, 2. The species registries are compiled and mapped); 7- Species delimited by DNA data (0. No data, 1. Species included in phylogenies or population studies)].

Species	1	2	3	4	5	6	7	Score
Nasutitermes lividus	1	0	0	0	0	0	0	1
Nasutitermes feytaudi	2	0	0	0	0	0	0	2
Nasutitermes meinerti	1	1	0	0	0	0	0	2
Nasutitermes montanae	2	0	0	0	0	0	0	2
Nasutitermes aduncus	2	1	0	0	0	0	0	3
Nasutitermes brevioculatus	0	2	0	0	1	0	0	3
Nasutitermes colimae	2	0	0	1	0	0	0	3
Nasutitermes crassus	2	1	0	0	0	0	0	3
Nasutitermes glabritergus	2	1	0	0	0	0	0	3
Nasutitermes jaraguae	2	1	0	0	0	0	0	3
Nasutitermes maximus	2	1	0	0	0	0	0	3
Nasutitermes myersi	2	1	0	0	0	0	0	3
Nasutitermes nordenskioldi	2	1	0	0	0	0	0	3
Nasutitermes peruanus	2	1	0	0	0	0	0	3
Nasutitermes pictus	2	0	0	1	0	0	0	3
Nasutitermes pilosus	2	1	0	0	0	0	0	3
Nasutitermes sanctaeanae	2	1	0	0	0	0	0	3
Nasutitermes tipuanicus	2	1	0	0	0	0	0	3
Nasutitermes arenarius	1	2	0	0	1	0	0	4
Nasutitermes bivalens	2	1	0	0	1	0	0	4
Nasutitermes bolivianus	2	2	0	0	0	0	0	4
Nasutitermes dendrophilus	2	2	0	0	0	0	0	4
Nasutitermes ecuadorianus	2	2	0	0	0	0	0	4
Nasutitermes itapocuensis	2	2	0	0	0	0	0	4
Nasutitermes longiarticulatus	2	1	1	0	0	0	0	4
Nasutitermes longirostratus	2	2	0	0	0	0	0	4
Nasutitermes major	2	2	0	0	0	0	0	4
Nasutitermes minor	2	2	0	0	0	0	0	4
Nasutitermes mojosensis	2	2	0	0	0	0	0	4
Nasutitermes tredecimarticulatus	2	2	0	0	0	0	0	4
Nasutitermes bolivari	2	2	1	0	0	0	0	5
Nasutitermes chaquimayensis	2	2	0	0	1	0	0	5
Nasutitermes comstockae	2	1	1	1	0	0	0	5

Species	1	2	3	4	5	6	7	Score
Nasutitermes ehrhardti	2	2	0	0	0	1	0	5
Nasutitermes globiceps	2	2	0	0	1	0	0	5
Nasutitermes hubbardi	2	1	0	0	1	1	0	5
Nasutitermes minimus	2	2	0	0	1	0	0	5
Nasutitermes pluriarticulatus	2	2	0	0	1	0	0	5
Nasutitermes proximus	2	2	0	0	1	0	0	5
Nasutitermes rotundatus	2	1	0	0	1	1	0	5
Nasutitermes tatarendae	2	1	0	1	1	0	0	5
Nasutitermes araujoi	2	1	1	1	1	0	0	6
Nasutitermes callimorphus	2	1	1	1	0	0	1	6
Nasutitermes llinquipatensis	2	2	1	1	0	0	0	6
Nasutitermes macrocephalus	0	2	1	1	1	1	0	6
Nasutitermes maniseri	2	1	1	1	1	0	0	6
Nasutitermes obscurus	2	1	0	1	1	0	1	6
Nasutitermes rippertii	0	2	1	1	1	1	0	6
Nasutitermes stricticeps	2	1	1	1	1	0	0	6
Nasutitermes unduliceps	2	1	1	1	0	0	1	6
Nasutitermes acangussu	2	1	1	1	1	0	1	7
Nasutitermes banksi	2	2	1	1	1	0	0	7
Nasutitermes gaigei	2	1	1	1	1	1	0	7
Nasutitermes nigriceps	0	2	1	1	1	1	1	7
Nasutitermes octopilis	2	1	1	1	1	0	1	7
Nasutitermes wheeleri	2	1	1	1	1	0	1	7
Nasutitermes acajutlae	2	1	1	1	1	1	1	8
Nasutitermes dasyopsis	2	2	1	1	1	0	1	8
Nasutitermes guayanae	2	2	1	1	1	0	1	8
Nasutitermes kemneri	2	2	1	1	1	0	1	8
Nasutitermes similis	2	2	1	1	1	0	1	8
Nasutitermes surinamensis	2	2	1	1	1	0	1	8
Nasutitermes aquilinus	2	2	1	1	1	2	0	9
Nasutitermes corniger	1	2	1	1	1	2	1	9
Nasutitermes coxipoensis	2	2	1	1	1	1	1	9
Nasutitermes ephratae	2	2	1	1	1	1	1	9

They concluded that Holmgren's imagoes from Acajutla are probably *N. nigriceps*, closely related to *N. acajutlae*, and that at this point, the imagoes of both species are indistinguishable. They also determined that the syntype series of *N. acajutlae* includes two species. To correct the earlier mistake, they designated the soldier from Holmgren's St. Thomas syntype series as the lectotype of *Nasutitermes acajutlae*.

Scheffrahn et al. (2005a, 2005b) and Roy et al. (2014) performed an identity assessment of the species based on DNA. Scheffrahn et al. (1994, 2003) made a good compilation of records for this species in the Antilles, but the records for the continent were not compiled. Perhaps this case deserves another deeper examination of all the material of both species *N. nigriceps* and *N. acajutlae*.

Nasutitermes acangussu Bandeira & Fontes, 1979

There is only the original illustration and description (including soldiers and workers). As for other Amazonian species, the known distribution is limited. There is no map with full records from collections. Nonetheless, the species was not recorded outside the Amazon, and as it is easily identified, it is probably restricted to this biome. Cuezzo et al. (2017) provided images of the worker enteric valve. Roy et al. (2014) performed an identity assessment based on DNA.

Nasutitermes aduncus Snyder, 1926

The description is poor and the only available illustration (a soldier) is a simple one. The workers are mentioned in the description, but without details.

Nasutitermes aquilinus (Holmgren, 1910)

The species was redescribed by Fontes and Terra (1981) and many later papers provide information on its nest, biology, and distribution. The compilation of records in Fontes and Terra (1981) and from Torales et al. (2005) gives an overview of the species' distribution.

Nasutitermes araujoi Roonwal & Rathore, 1976

The original description is quite complete, although the validity of the species is debatable [Constantino (2002b) points out it may be a synonym of N. *corniger*]. There are no photos of the species, although the original illustrations are detailed.

Nasutitermes arenarius (Hagen, 1858)

Hagen (1858) described the imago, soldier, worker, and nymphs from Santarém, Pará State. In fact, Hagen reported Bates' description. Holmgren (1910) copied Hagen's description of the imago ipsis litteris, and added his own description of the soldier and worker, with a figure (Holmgren 1910: fig. 43). Holmgren (1910) reported this species from two localities: Santarém, Pará, Brazil (Bates, ex-Hagen) and Coxipó, Mato Grosso, Brazil (from Silvestri 1903). Holmgren affirmed that it is questionable whether the specimens described by Silvestri as *Eutermes arenarius* were indeed of the same species described by Bates. This challenge could only be solved after comparison of their respective material, which he did not do.

Snyder mentioned Holmgren's (1910) work under this species in Snyder (1949) catalog, but considered only imagoes from Santarém as *N. arenarius*; Holmgren's worker and soldier were considered *N. kemneri*, a new name applied by them.

There are no figures of the nest, but in the original description Hagen (1858), mentioned that they are formed of polycalic constructions in the soil, covering some clumps of vegetation.

Nasutitermes banksi Emerson, 1925

Constantino (1991) gave a good redescription of the species. There is no nest description, just some notes on the microhabitat where the species is found; it probably does not construct any defined nest and lives in diffuse galleries. Cuezzo et al. (2017) provided images of the worker enteric valve. Although there is no compilation of species records, several papers mention its occurrence.

Nasutitermes bivalens (Holmgren, 1910)

The soldier caste dimorphism was originally the main diagnostic character used to delimit this species, but this character can easily induce an error relative to intracolonial size variation in samples, especially true for small samples. The original illustrations by Holmgren are simple and uninformative. Milano and Fontes (2002) provided photos of nests attributed to this species and included a map, but the species identification is dubious and the compilation of occurrences is not helpful.

Nasutitermes bolivari (Snyder, 1959)

The soldier was illustrated by Mathews (1977), and because the species was originally described in the genus *Velocitermes*, without illustration, the original diagnosis does not include comparisons with other species of *Nasutitermes*.

Nasutitermes bolivianus (Holmgren, 1910)

The original description is short and made in comparison with *N. chaquimayensis* and *N. peruanus*. Holmgren (1910) indicated that *N. bolivianus*, *N. chaquimayensis*, *N. peruanus*, *N. tambopatensis*, and *N. mojosensis*, all described by him, may be races of the same species. Accordingly, these may all be synonyms but this necessitates a revision of the species concepts and characters for each of these names.

Nasutitermes brevioculatus (Holmgren, 1910)

Krishna et al. (2013) mentioned that the syntype series of this species may be a mixture of species. The species description is generic and unhelpful. Torales et al. (2005) mapped some records for Argentina, but the identifications are dubious.

Nasutitermes callimorphus Mathews, 1977

Mathews' description and illustrations are reasonable and include data about the micro-habitats where the species is found, in an abandoned nests and another group of workers and soldiers from a covered runway in a Gallery Forest. Roy et al. (2014) performed an identity assessment based on DNA.

Nasutitermes chaquimayensis (Holmgren, 1906)

Holmgren (1906) provided a discussion about the distribution of this species and its disjunction relative to other species of *Nasutitermes*, but the morphological comparison was made relative to *"Eutermes ripperti"*(= *Eutermes Ripperti* f. Ehrhardti", in Holmgren 1910: 276), a taxon whose identity is confused. Holmgren (1910) redescribed the imagoes, soldiers, and workers, with illustrations of all castes. See notes under *N. bolivianus*.

Nasutitermes colimae Light, 1933

This species was described based only on imagoes (just two individuals), and the delimitation was made solely in comparison with other species described in the same work (*N. pictus*), also described only from imagoes. The species has never been recorded since the original description.

Nasutitermes comstockae Emerson,1925

The original description is reasonably detailed, but the species has been mentioned only in catalogs since then. Cuezzo et al. (2017) provided images of the worker enteric valve. The soldier of this species is similar to *N. surinamensis* (EMC pers. obs.).

Nasutitermes corniger (Motschulsky, 1855)

There are abundant data for this species, including DNA population studies. One of the major problems with the taxonomy of this species is that its distribution overlaps with practically all other species of the genus in the New World. Moreover, it can be misidentified with some similar species (e.g., *N. ephratae* and *N. coxipoensis*), and many other species names are probably synonyms. A reliable identification requires a sample with a good number of individuals and, preferably, data on the nest. There are some suggestions from taxonomists (personal information) that *N. globiceps*, *N. tatarendae*, and *N. araujoi* may be synonyms of *N. corniger*, but this need to be investigated. The imagoes described by Banks (1918) are not from the type series, but he made the description with Motschlsky specimens in hands.

Nasutitermes coxipoensis (Holmgren, 1910)

The redescription of this species by Mathews (1977) provided more detailed information about nesting and biology. Holmgren (1910) described this species based on samples considered by Silvestri as a *"Eutermes arenarius* subsp. *proximus* var. β ", Silvestri described the queen and king, but Holmgren did not mention the imagoes in his *N. coxipoensis* description. Nonetheless, the identification of this species remains difficult, and it can easily be misidentified with other close species. For an accurate identification a good number of specimens and preferably nest data are needed. Species identity was assessed based on DNA by Roy et al. (2014). Torales et al. (2005) mapped records for Argentina, but for the remainder South America such occurrences are spread across the literature and have yet to be compiled.

Nasutitermes crassus Snyder, 1926

The species has a poor description and the species has been mentioned only in catalogs since then.

Nasutitermes dasyopsis Thorne, 1989

The original description (Thorne and Levings 1989) is precise and based on all castes. It is also well illustrated, with a photograph of the nest. In addition, it was mentioned in papers with molecular data, but the species has been sparsely cited in the literature. The COII sequence in GenBank DQ442177 generated by Inward et al. (2007) is 99.27% identical to *N. nigriceps*, which suggested it could be junior synonym of *N. nigriceps*. (R. Scheffrahn information), so is desirable to verify the possibility of synonymy.

Nasutitermes dendrophilus (Desneux, 1906)

Desneux described the three castes without illustrations and the description is currently insufficient for proper identification. Desneux mentioned that this is a "subspecies of *T. Ripperti* Rambur", and the imagoes were similar, while the soldiers were different. Holmgren (1910) re-described imagoes, soldiers, and workers, including some simple figures. Although both descriptions give some measurements, which can help, some of them are today uninformative. Krishna et al. (2013) cited material at the AMNH and the United States National Muse-um (USNM) as syntypes without any further information.

Nasutitermes ecuadorianus (Holmgren, 1910)

Holmgren described it originally as a "forma" (in the taxonomic sense) of *N. peruanus*. The species has been mentioned only in catalogs or species lists since the original description. Following Holmgren's (1910) concepts mentioned in his introduction, we may consider that all those designated by him as "forma" are probably synonyms of the mentioned species (in this case, *N. peruanus*). See notes under *N. bolivianus*.

Nasutitermes ehrhardti (Holmgren, 1910)

The syntypes series are hosted in five different institutions, and become from distinct collection sites (Krishna et al. 2013), so it would be necessary revise these specimens. Although Holmgren's species delimitation is not clear, it could be identified by the description of the soldier's pilosity as it differs from other species from the same region (states of Santa Catarina and Rio Grande do Sul, Brazil), such as *N. jaraguae*. This species is mentioned in catalogs and a few surveys. Torales et al. (2005) mapped records for Argentina, but for the remainder of South America occurrences are spread across the literature.

Nasutitermes ephratae (Holmgren, 1910)

Krishna et al. (2013) took three pages to summarize the works that referenced this species, which reflect the large number of studies involving it using different biological approaches. Recently, a phylogeographic study of this species in the Neotropics was published (Santos et al. 2022). This species was described by Holmgren (1910) based on imagoes; the first soldier description was made by Banks (1918), but it was not based on type material. In addition, the specimens used in the work are not from the type locality and the figures are poor. Most authors follow the species delimitation made by Emerson (1925). Occurrences are listed in several works but have never been compiled.

Nasutitermes feytaudi (Holmgren, 1910)

Holmgren noted that, "it is very similar to *N. itapocuensis*, although considerably smaller, almost consonant with it" (see notes under *N. itapocuensis*). After the original description, based only on imagoes, this species was cited only in catalogs. Probably a synonym of *N. jaraguae* (EMC pers. obs.).

Nasutitermes gaigei Emerson, 1925

There is some information on this species in the literature, but it has not been compiled. Based on personal observations (EMC pers. obs.), this species should be removed from Nasutitermes and placed into a new genus. However, this reclassification requires further investigation and formal study. The imago and soldiers described are from the same type series (Emerson 1925).

Nasutitermes glabritergus Snyder & Emerson, 1949

The species does not have a formal description, only a mention in Snyder's (1949) catalog of one character that made it distinct from *N. rotundatus* (absence of setae on the tergites). Nickle and Collins (1992) give a partial diagnosis for the species, although it is not clear if they consulted any type specimens.

Nasutitermes globiceps (Holmgren, 1910)

The original description is not precise and the illustrations are simple. Holmgren mentioned that the species is similar to *N. meinerti* from Venezuela, but the soldiers are larger and the nasus shorter and broader (characters that have a large intraspecific variation). Costa-Leonardo (2000) made a description of the nest and reported on the biology, but it is not clear how the species was identified. This species is possibly synonymous with *N. corniger* (see notes for *N. corniger*). Krishna et al. (2013), affirm that part of the specimens described by Silvestri (1901, 1903) as *Eutermes rippertii* are in true *N. globiceps* (Holmgren descriptions not include the imagoes, but Silvestri's do), so we count the imago caste as described, anyway, is very desirable clarify this situation in a proper investigation.

Nasutitermes guayanae (Holmgren, 1910)

Since the original description more data on the species have been collected by several authors (see Krishna et al. 2013). This includes the (indirect) characterization of the species by associated termitophiles (Emerson 1935). The imagoes are described after the original description (Emerson 1925, 1935; Adamson 1940), based on non-type specimens.

Nasutitermes hubbardi Banks, 1919

The only description for this species is the original (Banks 1919). The nest was briefly mentioned (epigeal in the soil), and available figures are poorly detailed. Cuezzo et al. (2017) provided images of the worker enteric valve. The morphology of this species indicate that it is not related with the Neotropical "Nasutitermes-group" indicated by Bourguignon et al. 2017, and should be transferred to another genus, or placed in a new one (Dr. Carolina Cuezzo, IER (Conicet-UNT), Argentina, pers. comm.), a dedicated investigation is required to solve this question in the future.

Nasutitermes itapocuensis (Holmgren, 1910)

Krishna et al. (2013) reported some series of syntypes whose localities are all from the State of Santa Catarina, Brazil, except for one from São Paulo State, Brazil, housed in different institutions. The species description is reasonable, although the figures are poor. E.M.C. has examined the types from the Holmgren collection at the NHRM, and it seems to be synonymous with *N. jaraguae* (the proper taxonomic and nomenclatural changes will be done elsewhere).

Nasutitermes jaraguae (Holmgren, 1910)

This species was described based on soldiers and workers, and the characters are similar to those of *N*. *itapocuensis*, differing by soldier size. It is under study at our laboratory and molecular data suggest that this is part of a species complex (Barbosa 2018).

Nasutitermes kemneri Snyder & Emerson, 1949

Snyder and Emerson (in Snyder 1949) gave this name to specimens from Coxipó, Mato Grosso, Brazil, that had been previously misidentified by Silvestri (1901) as *"Eutermes arenarius"*. However, they did not redescribe it; they only mentioned Silvestri's text, which while validating the name left it confused. Mathews (1977) made a good redescription of this species and added some data about its biology.

Nasutitermes lividus (Burmeister, 1839)

The description is based on imagoes only, the soldier caste is unknown. The types at the MCZ are pinned and are basically uninformative.

Nasutitermes Ilinquipatensis (Holmgren, 1906)

Holmgren (1906, 1910) presented only simple illustrations; the species has been mentioned nearly only in catalogs since it was described.

Nasutitermes longiarticulatus (Holmgren, 1910)

The description of the soldier (Silvestri 1903) is brief and the only illustration of the species is a poorly detailed plate in the same work. The species has been mentioned only in catalogs since it was described.

Nasutitermes longirostratus (Holmgren, 1906)

The original description was based on the soldier and worker with simple illustrations (Holmgren 1906: figs W¹, X¹; 1910: 208). The imago was described by the same author, but later (Holmgren 1910). The author included it in the subgenus *Subulitermes* with the description and illustration of the imago and soldier (Holmgren 1910: 300, fig. 69). In fact, this species appear does not belong to *Nasutitermes*, which can be suspected even by examining the simple figures from Holmgren. A deeper investigation is necessary to affirm whether it is a species of *Subulitermes*.

Nasutitermes macrocephalus (Silvestri, 1903)

Described originally as a subspecies of *Eutermes rippertii*. The syntype series appears to be a mixture of species (Krishna et al. 2013). Most works with information about biology follow Holmgren's (1910) species delimitation, which includes a simple figure but with distinctive characters. Cuezzo et al. (2017) provided images of the worker enteric valve. Torales et al. (2005) mapped some records for Argentina, but for other countries occurrences are scant.

Nasutitermes major (Holmgren, 1906)

The original description and illustrations are poor, and the species has been mentioned only in catalogs since then. The imago was described by the same author, but later (Holmgren 1910).

Nasutitermes maniseri (John, 1920)

Krishna et al. (2013) mentioned a personal note of Emerson that this species is a probable synonym of *N. globiceps*. Nevertheless, the species description mentions microscopic setae on the head, absent in *N. globiceps*, which disagrees with Emerson's observation (EMC pers. obs.). The nest was described as brown and arboreal, 3 m from the ground. The species has been mentioned only in catalogs since the original description.

Nasutitermes maximus (Holmgren, 1910)

Holmgren (1910) considered that *Eutermes major* (currently *N. major*) was similar to this species, but larger. The locality given by Holmgren is the same for both: Chaquimayo, Peru. The description of both species suggests they are synonymous. *Nasutitermes maximus* has only been mentioned in catalogs since the original description.

Nasutitermes meinerti (Wasmann, 1894)

The original description was a footnote that mentions only the worker, but Holmgren (1910) included a description and illustration of the soldier based on Wasmann's samples, although the figures and description are poor in details. The type specimens are pinned workers. See the notes for *N. globiceps*, above.

Nasutitermes minimus (Holmgren, 1906)

Holmgren (1910) considered that the species as similar to *N. chaquimayensis* but even smaller, lighter, and with the same pilosity. There is a description of color, which is not useful, as it is the same for many species of *Nasutitermes*. The locality given by Holmgren is the same of *N. chaquimayensis*, Chaquimayo, Peru, besides San Firmino, Bolivia. He indicated that this species may be a, "race of *N. chaquimayo*", although the nest is quite different. Again, it seems both may be synonymous.

Nasutitermes minor (Holmgren, 1906)

Holmgren's (1910) description is short, but it could be useful in comparison with other species as he describes the pilosity, which in this case is quite peculiar. He mentions a thin head covered by microscopic setae and a few long bristles at the base of the nasus and vertex, and he also provided an account of the shape of the head and nasus and measurements, with a simple illustration. Snyder (1926) described the imago, but the description is not that helpful.

Nasutitermes mojosensis (Holmgren, 1910)

Krishna et al. (2013) mentioned a personal note of Emerson that this species is a probable synonym of *N. chaquimayensis*. Holmgren's description is not help-ful to recognize it. The species has been mentioned only in catalogs since the original description. See notes under *N. bolivianus*.

Nasutitermes montanae (Holmgren, 1910)

Described by the imago and mentioned only in catalogs since its original description. The locality is Montana, Suriname.

Nasutitermes myersi Snyder, 1933

Snyder mentioned it as, "close to *N. macrocephalus*". The species has been mentioned only in catalogs since the original description.

Nasutitermes nigriceps (Haldeman, 1854)

A large number of works referred to this species. However, the identifications follow the diagnosis of Light (1933), which included the imago description and was provided well after the original description, the types are almost certainly lost (Krishna et al. 2013). Like *N. corniger*, it is a widespread species (Dr. R. Rudolf H. Scheffrahn, University of Florida, U.S., pers. comm.). Distribution records are scattered through the literature.

Nasutitermes nordenskioldi (Holmgren, 1910)

Holmgren's description does not mention clear distinctive characters for this species. Nevertheless, the shape, size, and peculiar pilosity, allied to localities mentioned by Holmgren, may help an expert to solve the species' identity. The species has been mentioned only in catalogs since the original description.

Nasutitermes obscurus (Holmgren, 1906)

This species was renamed by Snyder and Emerson (in Snyder 1949) as *Nasuti-termes lighti*, an action that was dropped by Krishna et al. (2013), who reverted it to the previous name. Krishna et al. (2013) mentioned that the syntypes at the AMNH agree well with the description in Holmgren (1910). Nevertheless, they also mention a note by Emerson considering that the original description (Holmgren 1906) may be based on two different species. Regardless, the existing species description is too generic. The nest is described as an arboreal carton. Roy et al. (2014) characterized the species by DNA.

Nasutitermes octopilis Banks, 1918

The species is well characterized, it can easily be identified by Emerson's (1925) description (that included the imago), and it has been characterized by DNA data (Roy et al. 2014). Cuezzo et al. (2017) provided images of the worker enteric valve. There is no description of the nest and field notes suggest that this species lives in diffuse galleries in the soil and dead wood (Constantino 1991).

Nasutitermes peruanus (Holmgren, 1910)

Holmgren described the soldier of this species as, "very similar to *N. chaquim-ayensis*, but larger", which makes this diagnosis even more problematic since the identity of *N. chaquimayensis* is unclear. The soldier illustration is simple and the species has been mentioned only in catalogs since the original description. See notes under *N. bolivianus*.

Nasutitermes pictus Light, 1933

Nickle and Collins (1990) provided figures of this species but the species was described based only on imagoes (see notes under *N. colimae*). It has never again been recorded since the original description.

Nasutitermes pilosus Snyder, 1926

The original description characterized this species relative to *N. cayennae* (considered a junior synonym of *N. corniger*), with few characters and without illustrations. The species has been mentioned only in catalogs since the original description.

Nasutitermes pluriarticulatus (Silvestri, 1901)

Holmgren's (1910) illustrations are simple but they clearly differ from Silvestri's (1903) plates. Silvestri (1903) mentions the nesting and feeding habits of this species as the, "same as *N. proximus*", another taxon of dubious identity.

Nasutitermes proximus (Silvestri, 1901)

Holmgren (1910) provided a simple illustration of the soldier. Silvestri (1903) gave a poor illustration of the imago and mentioned the nest and feeding habits of this species as the "same as of *Eutermes arenarius*" (samples that which were later renamed as *N. kemneri*).

Nasutitermes rippertii (Rambur, 1842)

The species was originally described based on the imago. Later, Banks (1919) synonymized *Eutermes cubanus* and *Eutermes bahamensis* [both described by Holmgren (1910)] with *N. rippertii*, and the species diagnosis (including the soldier description) came from this material determined by Banks. Since then, some works have increased the available data for the species. Scheffrahn et al. (2006) made a compilation of records in the Antilles. The holotype is probably lost. The Institut Royal des Sciences Naturelles de Belgique (RIB) online catalog (RBINS Virtual Collections 2023) does not list this specimen in their collection. If the type exists, it is certainly pinned, as is the case for other material from Rambur.

Nasutitermes rotundatus (Holmgren, 1906)

The species illustration is simple (Holmgren 1910) and its delimitation was made in comparison with *Eutermes robustus* (currently *Sandsitermes robustus*). Bandeira and Vasconcellos (2002) related that the species lives and feeds in wood, and the MZUSP field notes reinforce that this species does not build a conspicuous nest. Moreira et al. (2008) provided a detailed study of the worker gut. Torales et al. (2005) mapped records in Argentina but occurrences for other countries remain fragmented.

Nasutitermes sanctaeanae (Holmgren, 1910)

Holmgren described this species based on Silvestri's (1903) specimens determined as a variety "alpha" of *Eutermes arenarius proximus*. There are no illustrations and the species has been mentioned only in catalogs since the original description. Torales et al. (2005) listed this species for Argentina, but the record refers only to Silvestri's specimens.

Nasutitermes similis Emerson, 1935

Emerson's characterization of this species included the associated termitophiles, which were distinct from those of *N. guayanae* (a morphologically close species). Cuezzo et al. (2017) provided images of the worker enteric valve. Roy et al. (2014) characterized the species by DNA.

Nasutitermes stricticeps Mathews (1977)

Mathews made a detailed description of this species but it hasn't been registered again since the original description. Cuezzo et al. (2017) provided images of the worker enteric valve.

Nasutitermes surinamensis (Holmgren, 1910)

The imago was described latter, by Emerson (1925), based on non-type specimens. Mathews (1977) published photographs and described the species' nest. Roy et al. (2014) characterized the species by DNA. Cuezzo et al. (2017) provided images of the worker enteric valve.

Nasutitermes tatarendae (Holmgren, 1910)

Holmgren (1910) mentioned the soldier as, "being very similar to *N. major*, but darker". Mathews (1977) redescribed this species and some additional biological data were subsequently published. However, several authors indicate that this species is a probable synonym of *N. corniger*. See notes under *N. bolivianus*.

Nasutitermes tipuanicus (Holmgren, 1910)

The original description characterizes this species as similar to *N. pluriarticulatus*, a species of dubious identity (see above). The curator of the ZMH confirmed that the types are preserved in alcohol. There are no illustrations of the species and it has only been mentioned in catalogs since the original description.

Nasutitermes tredecimarticulatus (Holmgren, 1910)

Holmgren described the soldier as, "nearly identical to *Eutermes major*" (currently *N. major*; see the comments about this species above), without more detailed information. There is only an illustration of the imago and the sole record of the species apart from the original description was made by Bandeira and Macambira (1988), with notes on its feeding habits and occurrence in different vegetative habitats.

Nasutitermes unduliceps Mathews, 1977

Mathews made a complete description of the soldier, with a simple illustration of its head. Roy et al. (2014) characterized the species by DNA.

Nasutitermes wheeleri Emerson, 1925

Emerson's illustrations show some distinctive characters of this species. Mathews (1977) published photographs and described the nest. Cuezzo et al. (2017) provided images of the worker enteric valve. Roy et al. (2014) characterized the species by DNA.

Discussion

About the criteria and their scores

We intentionally tried to keep the criteria evaluations as restrictive as possible. It is clear that some criteria are more easily recognized as discrete, while others are more continuous. Type specimens and the castes used for description can be comfortably accommodated within the listed options, but evaluating species descriptions and illustrations is challenging due to potential subjectivity biases.

Opting for fewer categories was a tentative approach to maintain clarity. We recognize that intermediate situations exist for descriptions and illustrations. In practice, they follow a progressive scale of "obsolescence", with older descriptions failing to consider characters discussed in more recent descriptions and thus becoming progressively outdated. Our practical solution was to maintain a few states for these cases.

The same applies to information about biology. The quality and type of information about each species are certainly very heterogeneous. However, trying to qualify all types of information would significantly increase the workload for assessments with little return. This is why we have included a list of notes to complement the information.

The ranks are intended to assess the viability of species identity for taxonomic studies and establish priorities for resolution, rather than to evaluate the robustness of species identifications. We aim for an operational response by describing what we consider informative. In this way, if the criteria contribute to the robustness of the species hypothesis, the score is achieved.

Nasutitermes results for species

It is necessary to reiterate that species of *Nasutitermes* are challenging to identify, even when good descriptions do exist. In several cases a reliable identification is impossible without direct comparison with type specimens or material previously compared against types. Ultimately, this may be impossible for many species under currently available data. Comparison with determined material in any collection should be undertaken with caution, because not all samples in a collection, even a well-curated one, are correctly identified. It is necessary to know who has determined them and at what time (as species concepts can change over time). Any examiner should be critical as to when the material was determined and if more data for the species has since been accumulated. The best is to compare with types, or if lacking, to search for material compared with types by an expert researcher (typically referred to as a "metatype" by Emerson, although it should be noted that such specimens hold no nomenclatural standing under the ICZN). Sometimes a curator may identify specimens even tentatively, considering that it is better than to leave the sample in a mass of "unidentified material". It is a common practice and a non-specialist should be cautious doing this.

Nearly half of the species evaluated scored between 1 and 4 (i.e., 30 species were considered "confusing"), and the majority of these cases (24) consisted of species that scored only in the "Type specimens" and/or "Castes used for description" criteria; none of the species were considered irresolvable.

Low-ranked species have been recurrently cited only in catalogs after the original description, which suggests that some of these names are synonyms, but this cannot be used as a rule. It became evident that species checklists based on literature records extend the longevity of these "taxonomically confusing species" in databases, which implies some noise for biodiversity evaluations and certainly is problematic for researchers who only mine data rather than verify it from the original sources.

The majority of these low-ranked species have type specimens and it is therefore imperative to clarify their identity as this will allow for advances in understanding these species.

Among the species with a total score comprised between 5 and 9, the most relevant criteria, after the "Type specimens" and "Castes used for description", are the accuracy of descriptions and illustrations, and the existence of information about the species' biology. This is expected since the compilation of records is possible only after the accumulation of good numbers of correctly determined samples in collections, and the identification is dependent on well characterized species. This can be observed more clearly in the species with a total score comprised between 5 and 7, the presence of Information about biology is the more frequent criteria that pulls up the scores.

No species scored 10. Among the highest scores (8 and 9), the absence of information on the distribution of the species is the most frequent criterion that reduces the scores. This makes sense, as most of the *Nasutitermes* species in the collections have not been compiled and plotted on a map. Perhaps in the near future this gap will be reduced, as many databases are currently in progress.

Some peculiar cases are interesting to mention, specifically *N. corniger*, *N. macrocephalus*, *N. nigriceps*, and *N. rippertii*. These species scored low in the type criterion (types lost or badly conserved) and high in the remaining criteria. In these cases, the species identity is anchored only on redescriptions published long after the original description.

DNA studies are prevalent in species of higher taxonomic marks, as expected. As DNA sequencing becomes more accessible and cost-effective, there has been a widespread misconception regarding the utility of these data for taxonomy, particularly the notion that DNA can facilitate "rapid taxonomy". Some of this misconception is based on the widespread perception that taxonomy was not "integrative" before DNA data become available. However, even since Darwin's time when he demonstrated strong relationships between distribution, life habits, and morphology in species of the Galapagos Islands, suggesting distinct evolutionary lineages, taxonomy has been integrative. Nevertheless, it took considerable time for evolutionary concepts to be widely incorporated into taxonomic practices. Molecular data is the latest tool to become available for investigation and has been extensively utilized.

The primary significance of DNA data in taxonomy lies in providing a new set of characters to test species hypotheses and more inclusive groupings, specifically to test the monophyly of genera and higher taxonomic ranks. It is expected that prior to the use of DNA data for species hypothesis testing, all preceding criteria have been investigated and evolutionary lineages have been proposed. Wheeler (2004) extensively discusses these concepts, while Engel (2022) provides a concise and clear summary of these discussions.

The sequential chain of taxonomic steps, which enhances the robustness of a species hypothesis (including nomenclatural stability, species characterization, species data, and inherited characters evidencing natural lineages), becomes routine for experienced taxonomists. Gonzalez et al. (2013) elaborate on all these aspects related to the quality of taxonomic research and propose a similar cumulative sequence of operations to evaluate the status of taxonomic literature, specifically focusing on bumblebees as mentioned in the introduction.

Unfortunately, today there is an effort to reverse the taxonomic process, largely fueled by a false controversy of "morphology vs DNA". Again, as Engel (2022) states: "A consensus barcode for any given cluster is assumed to "diagnose" the given species. In this way, hundreds of species could be wholesale sequenced for COI, clustered by BOLD, and then simply summarized by a consensus barcode sequence and given a name. Quick and easy. And yet, is it meaningful? One of the fundamental and critical roles of a taxonomist is to test species hypotheses. These rely on data, and ideally as much data as can be called upon to formulate a concept for any given species and what features, be they anatomical, behavioral, chemical, molecular, etc., serve to circumscribe that biological unit from others in nature."

The way the criteria scores were presented (Table 1) supports a virtuous cycle of taxonomic practices. The criteria are not independent; they tend to form a 'ladder' in the matrix when ordered by ascending score (see in the Suppl. material 1, the numerical values of each criterion colored according to their rank).

Mathematically, various scoring combinations would be possible, but the scores, in general, do not behave independently. For example, there is no plausible scenario where two species score a total of "2", with one species meeting only the criterion "Type specimens well-preserved (2)" and another "Data about species distribution, the species registries are compiled and mapped (2)". The same applies to higher sums.

Use of this protocol for other taxa

In this work, we used the Neotropical species of *Nasutitermes* as a test case. But it can be adapted to nearly any other taxon, with simple alterations to the evaluation criteria. It would be important and informative to see the results with other taxa to evaluate its broader practicality and effectiveness. The criterion "castes used for description" makes sense primarily for social insects. However, for insects with ametabolous and hemimetabolous development, species are sometimes described based only on immature stages. It is reasonably clear that taxa with incomplete knowledge of developmental stages would have lower taxonomic health than those with more comprehensive information across various instars. The relevance of this criterion varies depending on the taxonomic practices of the group being studied.

The same applies to species described based on only one sex or form. Polymorphism within populations, such as sexual dimorphism or melanistic forms, has been a source of taxonomic confusion, leading to cases where all the species of one genus were synonymized once these gaps were understood.

In some cases, knowing the specific environment is more relevant than having highly precise geographical coordinates. For certain groups, the soil depth or the arboreal stratum in which the specimen was collected is crucial information that can help differentiate species with sympatric distributions.

The existence of voucher specimens of hosts is particularly relevant for inquiline/parasitic organisms. It is very common for the host organism to be mentioned only in the description of their inquilines/parasites. When the identity of hosts needs to be checked (e.g., when it is discovered that what was thought to be a single species of a parasite is actually two species), the information about host specimens becomes inaccessible.

Another criterion to consider is whether an evolutionary species concept was employed or if the only description available was made before the evolutionary theory consistently be accepted. This is not the case for the *Nasutitermes* species we have been working on. The first comprehensive review of Neotropical *Nasutitermes* was conducted by Holmgren in 1910. Despite the limited tools available at the time, Holmgren worked from an evolutionary perspective. Although he tended to be a "splitter" in his work and was occasionally contradictory in his critiques of Silvestri's concepts, Holmgren reviewed almost all the material in collections up until 1900. Hagen published his treatise in 1858, one year before "On the Origin of Species", so evolutionary concepts were not mentioned. Nevertheless, Holmgren later revised the same taxa that Hagen had worked on. Subsequent authors, most notably Emerson, Snyder, and Light, clearly worked under an evolutionary scenario.

We believe that it would be interesting to evaluate older species descriptions, particularly those that remain outdated, to see how many species concepts have been updated from a Linnean/Aristotelian framework to an evolutionary perspective.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Supplementary information

Authors: Mauricio M. Rocha, Karina S. S. Lima, Eliana M. Cancello Data type: xls

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Research Article

Two new species of freshwater goby (Teleostei, Gobiidae) from the Upper Youshui River, Chongqing, China

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Abstract

Two previously unknown species of Rhinogobius have been discovered in the streams of the Upper Youshui River, within the Yuan River Basin, Xiushan County, Chongqing, China. These new species are named as Rhinogobius sudoccidentalis and Rhinogobius lithopolychroma. Phylogenetic analysis based on mitochondrial genomes revealed that R. sudoccidentalis is genetically closest to R. reticulatus, while R. lithopolychroma shares the greatest genetic similarity with R. leavelli. Morphological distinctions allow for the clear differentiation of these species. Rhinogobius sudoccidentalis sp. nov. is characterized by having VI-VII rays in the first dorsal fin and I, 8-9 rays in the second dorsal fin. The longitudinal scale series typically consists of 22-24 scales, while the transverse scale series comprises 7-8 scales. Notably, the predorsal scale series is absent and the total vertebrae count is 12+17=29. Rhinogobius lithopolychroma sp. nov. can be distinguished from other species by the presence of 13-15 rays on the pectoral fin. Its longitudinal scale series ranges from 30 to 33 scales, with no scales in the predorsal area. The total vertebral count is 30, with 12 precaudal and 18 caudal vertebrae. The head and body of this species are light gray with irregular orange markings on the cheeks and opercle. Through morphological and molecular analyses, it has been confirmed that R. lithopolychroma and R. sudoccidentalis represent novel species within the Rhinogobius genus.

Key words: China, fish taxonomy, Gobiidae, Gobionellinae, mitochondrial genome, Yuanjiang River Basin

Introduction

The genus Rhinogobius, belonging to the subfamily Gobionellinae within the family Gobiidae, is widely distributed across East and Southeast Asia. First described by Gill in 1859, with Rhinogobius similis Gill, 1859 as the type species, this genus is known for its high species richness. Over 92 valid species have been described, with an increasing number of new species being discovered. In recent years, several new species of *Rhinogobius* have been found in China, including R. houheensis Kunyuan et al., 2020, R. coccinella Endruweit, 2018, R. maculagenys



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Wu et al., 2018, *R. maxillivirgatus* Xia et al., 2018, *R. nanophyllum* Endruweit, 2018, *R. wuyanlingensis* Huang et al., 2016, *R. niger* Huang et al., 2016, *R. immaculatus* Li et al., 2018, *R. lintongyanensis* Chen et al., 2022 and *R. lianchengensis* Wang & Chen, 2022. To date, a total of 47 species of *Rhinogobius* have been recorded in China (Chen et al. 2022a). The significant diversity of *Rhinogobius* species in China suggests that the overall species diversity within this genus may be underestimated. Notably, the recent discoveries of *Rhinogobius* species have been concentrated in East China, with fewer new species found in other regions.

The Upper Yuanjiang River Basin benefits from a favorable climate and encompasses numerous stream habitats within its mountainous areas. The biodiversity in Xuan'en and Fanjingshan, traversed by the Upper Yuanjiang River Basin, is exceptionally high and potentially serves as a glacial refuge (Fei et al. 2017). Consequently, it is inferred that the biodiversity in other regions of the Upper Yuanjiang River Basin, particularly within its stream habitats, may have been underestimated.

During surveys conducted between June 2023 and January 2024 in the streams of the Upper Youshui River within the Yuanjiang River Basin in Chongqing, two species of *Rhinogobius* were discovered. Historically, only *R. similis* and *Rhinogobius cliffordpopei* (Nichols, 1925) were documented in the Yuanjiang River Basin in Chongqing, with these species primarily adapted to lake and reservoir environments (Wu et al. 2008; Suzuki et al. 2016). In contrast, the newly discovered species exclusively inhabit streams and are characterized by large eggs, unlike *R. similis* and *R. cliffordpopei*, which produce small eggs (Li 2011). The Upper Youshui River features a diverse stream ecosystem where species distribution is influenced by factors such as substrate composition, temperature, and current velocity. This study delves into the habitat of *Rhinogobius* in the Upper Youshui River to explore the habitat segregation of *Rhinogobius*, building upon previous research concerning the ecological preferences of *Rhinogobius* species (Sone et al. 2001; Ito et al. 2006).

Materials and methods

Samples

A total of 44 specimens were collected from Chongqing Municipality and Guizhou Province (Fig. 1) using a hand net. All specimens were preserved in 75% ethanol and are stored at Southwest University in Rongchang District, Chongqing, China.

Morphometrics and meristic methods

Morphological measurements were primarily based on a previous study (Wu et al. 2008). Data were collected from the left side of each fish using vernier calipers, measuring 27 traits to the nearest 0.1 mm. Measurements included the first dorsal fin, second dorsal fin, pectoral fin, anal fin, longitudinal scales, transverse scales, and predorsal scales. Abbreviations for the cephalic sensory pore system followed Chen and Kottelat (2005). The pattern of interdigitation of the dorsal-fin pterygiophores and neural spines (P-V) was observed from radiographs. The P-V method and vertebral counting were expressed using a specific formula to describe the goby's interdigitation pattern of dorsal-fin pterygiophores and neural spines (Akihito et al. 1984). For example, in the formula



Figure 1. Map of the distribution of *Rhinogobius sudoccidentalis* sp. nov. and *Rhinogobius lithopolychroma* sp. nov. in Upper Youshui River, with locations in southwest China shown in the lower right corner. Maps were prepared using ArcMap 10.8.

(P-V) 3/II II II 10/9: (P-V) stands for dorsal-fin pterygiophores and neural spines; "3" indicates that three neural spines are anterior to the first pterygiophore; "II II I I" indicates there are 2 pterygiophores between the neural spine of the 3rd and 4th vertebrae; 2 between the neural spine of the 4th and 5th vertebrae; 1 between the neural spine of the 5th and 6th vertebrae; and 1 between the neural spine of the 5th and 6th vertebrae; "0" indicates no pterygiophore between the neural spines of the 7th and 8th vertebra; "9" indicates that the first pterygiophore of the 1st ray of the 2nd dorsal fin is inserted above the 9th vertebral body. Color in life was described based on samples and photographs taken in fish tanks.

DNA sequencing and phylogenetic analysis

Four specimens were used for DNA barcoding. Total DNA was extracted from the caudal fin following Maeda et al. (2021a) and Wanghe et al. (2020). Briefly, single-stranded circular DNA molecules were amplified into a DNB (DNA Nanoball) containing more than 300 copies via rolling circle replication. These DNBs were then applied to mesh pores on the chip using high-density DNA nano-chip technology. Sequencing was performed by cPAS. Identification of complete mitochondrial genomes from assembled contigs was achieved through two criteria: 1) comparison with the complete mitochondrial genome of *Stiphodon alcedo* Maeda, Mukai, & Tachihara, 2011 (accession: AB613000.1) (BLASTN e-value \leq 1e-100), and 2) confirmation that 100 bp of both the head and tail DNA sequences of a contig were identical, indicating that the sequence was circular.

Complete mitochondrial genomes were aligned using MAFFT v.7.244 (Katoh and Standley 2013). The obtained mitochondrial gene was compared with *Rhinogobius wuyanlingensis* Yang, Wu & Chen, 2008 (accession: NC_062781.1), confirming identical sequences at the head and tail DNA regions, indicative of circularity. Aligned mitochondrial genomes underwent phylogenetic analysis using maximum likelihood (ML) methods with RAxML v. 8.2.3 (Stamatakis 2014), incorporating mitochondrial gene data from the GenBank library (Table 1).

	Accession number	Length of sequence (bp)	Remarks
Rhinogobius estrellae	LC648292	16682	Maeda et al. (2021b)
Rhinogobius estrellae	LC648294	16504	Maeda et al. (2021b)
Rhinogobius estrellae	LC648295	16505	Maeda et al. (2021b)
Rhinogobius estrellae	LC648296	16504	Maeda et al. (2021b)
Rhinogobius tandikan	LC648297	16691	Maeda et al. (2021b)
Rhinogobius tandikan	LC648298	16690	Maeda et al. (2021b)
Rhinogobius tandikan	LC648299	16918	Maeda et al. (2021b)
Rhinogobius tandikan	LC648300	16690	Maeda et al. (2021b)
Rhinogobius similis	LC648303	16499	Maeda et al. (2021b)
Rhinogobius similis	LC648304	16499	Maeda et al. (2021b)
Rhinogobius formosanus	MT363639	16500	Yang et al. (2020)
Rhinogobius formosanus	MN549279	16502	Genbank
Rhinogobius szechuanensis	OM617727	16492	Liu WZ et al. (2023)
Rhinogobius leavelli	MH729000	16499	Zhang and Shen (2019)
Rhinogobius davidi	OM617724	16627	Song et al. (2023)
Rhinogobius rubromaculatus	KU674802	16503	Genbank
Rhinogobius flumineus	LC648305	16504	Maeda et al. (2021b)
Rhinogobius flumineus	LC648306	16503	Maeda et al. (2021b)
Rhinogobius yaima	LC648307	16500	Maeda et al. (2021b)
Rhinogobius yaima	LC648308	16500	Maeda et al. (2021b)
Rhinogobius yonezawai	LC648309	16500	Maeda et al. (2021b)
Rhinogobius yonezawai	LC648310	16500	Maeda et al. (2021b)
Rhinogobius nagoyae	LC648315	16498	Maeda et al. (2021b)
Rhinogobius sp. MO	LC648314	16499	Maeda et al. (2021b)
Rhinogobius brunneus	LC648311	16500	Maeda et al. (2021b)
Rhinogobius brunneus	LC648312	16500	Maeda et al. (2021b)
Rhinogobius wuyiensis	OM678441	16502	Chen XJ et al. (2022b)
Rhinogobius lentiginis	OM617725	16633	Chen XJ et al. (2022b)
Rhinogobius niger	OM791349	16496	Genbank
Rhinogobius maculagenys	OK545540	16500	Hu J et al. (2023)
Rhinogobius shennongensis	OM961050	16500	Genbank
Rhinogobius cliffordpopei	KX898434	16511	Genbank
Rhinogobius cliffordpopei	KP694000	16529	Genbank
Rhinogobius cliffordpopei	KT357638	16525	Genbank
Rhinogobius duospilus	MH127918	16496	Tan et al. (2020)
Rhinogobius filamentosus	OM678440	16510	Chen XJ et al. (2022b)
Rhinogobius wuyanlingensis	OM617722	16491	Song et al. (2022)
Rhinogobius wuyanlingensis	OM961051	16491	Genbank
Rhinogobius sp. Xiushan	SRR28284919	16486	Collected in Xiushan, Chongqing
Rhinogobius lithopolychroma	SRR28284920	16493	Collected in Xiushan, Chongqing
Rhinogobius sudoccidentalis	SRR28284918	16480	Collected in Xiushan, Chongqing
Rhinogobius reticulatus	SRR28284917	16497	Collected in Fuzhou, Fujian Province
Tridentiger kuroiwae	LC653489	16501	Maeda et al. (2021b)
Tridentiger kuroiwae	LC653490	16501	Maeda et al. (2021b)

Table 1. List of accession numbers and sequence length of mitochondrial genome sequences in this study.

Outgroup specimens were analyzed using *Tridentiger kuroiwae* Jordan & Tanaka, 1927 (accessions: LC653489.1 and LC65349.1). The aligned mitochondrial genomes from this study have been deposited in the GenBank library under accession numbers SRR28284917-SRR28284920.

Results

Morphological analyses

Rhinogobius sudoccidentalis sp. nov.

https://zoobank.org/975F33AC-F810-4F32-8D57-26A583D924BB Table 2, Figs 2–7

Type materials. *Holotype*. CHINA • 1 ♂; Chongqing City, Xiushan County; 28°23'23"N, 108°53'16"E; 1 July. 2023; Lingzhen Li & Chaoyang Li leg.; RS20230001.

Paratypes. CHINA - Chongqing City • 7 ♂♂, 3 ♀♀; Xiushan County; 28°23'23"N, 108°53'16"E; 1 July. 2023; Lingzhen Li & Chaoyang Li leg.; RS20230101 to 20230110. • 4 ♂♂ ; Xiushan County; 28°26'17"N, 108°59'12"E; 1 July. 2023; Lingzhen Li & Chaoyang Li leg.; RS20230111 to 20230114. • 1 ♂ , 1 ♀ ; Xiushan County; 28°24'51"N, 109°7'13"E ; 3 July. 2023; Lingzhen Li & Chaoyang Li leg.; RS20230115, 20230116. • 1 ♂ , 2 ♀♀ ; Xiushan County; 28°22'30"N, 108°53'18"E; 4 July. 2023; Lingzhen Li & Chaoyang Li leg.; RS20230118, 20230120. - Guizhou Province • 1 ♂ ; Tongren City; 28°8'50"N, 108°59'13"E; 3 July. 2023; Lingzhen Li & Chaoyang Li leg.; RS20230117.

Diagnosis. *Rhinogobius sudoccidentalis* can be distinguished from other species in the genus by the following characteristics: it possesses VI–VII rays in the first dorsal fin and I, 8–9 rays in the second dorsal fin. The longitudinal scale series typically consists of 22–24 scales (most commonly 23), while the transverse scale series typically comprises 7–8 scales (most commonly 8). The predorsal scale series is absent. The total number of vertebrae counts is 12+17=29. Additionally, it features a black line stripe beneath the eye that extends to the mandible. Morphometrics Reference Table 2.

Description. *Fins*: The fins display distinct features: the first dorsal fin typically bears VI rays (18) or VII rays (2), while the second dorsal fin exhibits either I, 8 rays (2) or I, 9 rays (18). The 3rd or 4th spine of the first dorsal fin is the longest and lacks filamentous. In males, the depressed first dorsal fin extends to the base of the 1st or 2nd branched ray of the second dorsal fin; in females, it reaches only the base of the second dorsal fin anteriorly. The anal fin has I, 6 rays (1) or I, 7 rays (19), originating at a vertical line between the 2nd and 3rd branched soft ray of the second dorsal fin. The pectoral fin typically has 14 rays (2) or 15 rays (18) and is broad. In males, the rear tip of the pectoral fin aligns parallel to the anus, a feature absent in females.

Scales: The body is covered with ctenoid scales, with enlarged mid-trunk scales. The anterior predorsal area lacks scales, while the posterior occipital region is adorned with cycloid scales. The belly is covered with small cycloid scales. The longitudinal scale series ranges from 22 to 24 (mode: 23), and the transverse scale series ranges from 7 to 8 (mode: 8). No scales are present in the predorsal area.

Head canals: Pores σ are located between the anterior and posterior nares. The anterior interorbital sections of oculoscapular canal are separated, fea-

Table 2.	Morphometrics	of the ty	pes of R	. sudoccidentalis	expressed	as a	percentage
of stand	lard length.						

Variable	Holotype	Paratypes					
Sex	males	males (<i>N</i> = 14)	Females (N = 6)				
Morphometry							
Standard length (mm)	33.1	33.1-40.6(36.5)	30.2-36.5(32.1)				
Head length (mm)	8.9	8.9-11.5(10.3)	7.3-9.9(8.1)				
Percent standard length (%)			'				
Head length	26.9	26.5-30.3(28.4)	23.7-27.1(25.2)				
Predorsal length	37.8	31.7-43.1(37.4)	34.5-39.0(36.9)				
Snout to second dorsal fin origin	53.8	53.6-59.2(56.2)	57.1-59.4(58.4)				
Snout to anal fin origin	59.5	55.4-64.9(58.9)	59.3-64.7(62.7)				
Snout to anus	54.1	51.2-56.9(53.5)	50.5-59.0(56.0)				
Pre pelvic length	28.7	28.7-35.7(31.3)	28.8-33.7(30.6)				
Caudal peduncle length	26.9	21.8-29.2(25.7)	17.3-27.5(23.2)				
Caudal peduncle depth	8.8	8.2-10.5(9.2)	8.0-11.9(9.5)				
First dorsal-fin base	8.5	8.5-17.3(12.8)	8.6-13.5(11.2)				
Second dorsal-fin base	16.3	13.1-19.9(16.9)	14.6-19.6(16.1)				
anal fin base	14.2	8.5-14.3(11.7)	8.7-11.7(10.0)				
Caudal fin length	20.8	18.4-26.1(22.1)	13.5-23.8(18.5)				
Pectoral fin length	20.2	19.6-24.1(21.8)	16.6-21.0(18.7)				
Pelvic fin length	14.5	13.5-19.2(15.7)	12.5-18.1(15.8)				
Body depth of pelvic fin origin	9.1	9.1-14.2(11.5)	9.8-12.9(11.7)				
Body depth of anal fin origin	9.4	8.3-13.0(10.4)	9.3-11.5(10.6)				
Pelvic fin origin to anus	26.9	22.0-27.2(25.2)	25.6-30.8(27.4)				
Head depth	9.7	9.7-12.2(11.0)	9.6-12.9(11.0)				
Percent head length (%)			'				
Snout length	31.5	22.8-37.4(30.6)	19.2-31.6(25.5)				
Eye diameter	14.6	11.3-19.3(14.2)	10.4-16.5(12.0)				
Cheek depth	56.2	20.7-32.2(25.5)	21.2-29.1(24.1)				
Postorbital length	55.1	43.1-60.4(51.7)	49.5-58.9(54.4)				
Lower jaw length	31.5	27.9-48.7(38.7)	24.2-37.7(31.0)				
Interorbital width	22.5	11.9-24.0(20.8)	12.1-19.5(16.3)				
Head width in maximum	51.7	45.5-61.7(52.7)	50.5-65.8(58.0)				

turing paired pore λ . A single pore κ is situated in the posterior region, with ω present near posterior edge of eyes. There is an absence of ω 1. The lateral section of anterior oculoscapular canal exhibits pores α and terminal pore ρ . The posterior oculoscapular canal ends with two terminal pores θ and τ . Preopercular canals are presented, featuring pores ϵ , γ , and δ .

Sensory papillae: Row a extends anteriorly to just before the middle of the eye. Row b is oblique and reaches forward to the posterior margin of the eyes. Rows c and d are longer, extending behind the orbit, with Row cp positioned between Rows c and d. Row f is paired. Opercular papillae include Rows ot, oi, and os, with oi nearly reaching ot.

Vertebrae: The total vertebrae count is 12 + 17 = 29 (N = 5), with a (P–V) pattern of 3/II II I I 0/9 (N = 5).



Figure 2. Dorsal (A), lateral (B), and ventral (C) views of preserved holotype of *Rhinogobius sudoccidentalis* sp. nov. (RS20230001 male) and dorsal (D), lateral (E), and ventral (F) views of preserved paratype of *Rhinogobius sudoccidenta- lis* sp. nov (RS20230101 female).



Figure 3. The skeletal system of *R. sudoccidentalis* sp. nov. Radiograph graphs of the whole body for paratype RS20230102, male.

Coloration of preserved specimens: In males, the head and body of *R. sudoccidentalis* exhibit a yellowish-brown color. There are paired brown stripes on the snout converging at the tip, while the cheeks and opercle are adorned with small black spots. A black stripe extends from under the eye to the mandible. The ventral side displays dens coverage of small black spots. The membrane of the first dorsal fin is gray, the second dorsal fin has a transparent membrane with dense black mottling, and the anal fin exhibits a black membrane. The pectoral fin is transparent. In females, the head and body are yellowish, with a single black diagonal line below each eye. Irregular black patches are present on the ventral side, and both the dorsal and anal fins are transparent.

Color in life: In males, the head and body of the *R. sudoccidentalis* are creamy white. There are paired reddish-brown stripes on the snout meeting at the tip, and the cheeks and opercle feature small black spots. A black stripe extends from under the eye to the mandible. The ventral side is densely covered with small orange spots. The membrane of the first dorsal fin is red with a blue mot-



Figure 4. Dorsal (**A**), lateral (**B**), and ventral (**C**) views of the head of the preserved holotype of *R. sudoccidentalis* sp. nov. Red circles indicate sensory canal pores; red dots represent sensory papillae. Abbreviations: AN, anterior nare pore; PN, posterior nare pore


Figure 5. Photographs of *R. sudoccidentalis* sp. nov. captured underwater in a tank **A** male **B** female. Photographed by Mr Zhi.

tling pattern between the 1st and 2nd spinous rays. The second dorsal fin has a transparent membrane with dense black mottling and a white outer edge. The anal fin exhibits a white margin with reddish dots on the ventral part of the reddish membrane. The pectoral fin is transparent, with a milky white basal portion. In females, the head and body are yellowish, with paired brown stripes on the snout meeting at the tip. There are single black diagonal lines below the eyes, and irregular black patches on the ventral side. Both the dorsal and anal fin are transparent, and the pectoral fin is transparent with a milky white basal portio.



Figure 6. Stream environment in Xiushan, Chongqing where *R. sudoccidentalis* sp. nov. was collected.



Figure 7. Eggs of *R. sudoccidentalis* sp. nov. at the type locality.

Distribution and habitat. *Rhinogobius sudoccidentalis* was initially discovered in a small stream in Xiushan, Chongqing, where it predominantly inhabits areas characterized by large cobblestone substrates and slow-flowing water at depths ranging from approximately 30 to 50 cm. Additionally, small populations of this species were also observed in Tongren, Guizhou Province. In the Xiushan area, *R. sudoccidentalis* is the dominant fish species, utilizing the

cobblestone bottom as an egg deposition site, with eggs characterized as large (size 1.6–2.1 mm). During periods of high water levels in the creek, individuals aggregate near the shore to seek refuge from the rapids.

Etymology. This species, discovered in Chongqing and Guizhou Province in the southwestern region of China, has been named *R. sudoccidentalis*. The Latin roots "sud" meaning "south" and "occidentalis" meaning "western" combine to signify "southwestern". The suggested Chinese name for this species is 西南吻虾虎鱼.

Rhinogobius lithopolychroma sp. nov.

https://zoobank.org/C1F210C4-1623-4B50-BB2A-F9DBAD7F197A Table 3, Figs 8–13

Type materials. *Holotype*. CHINA • 1 ♂; Chongqing City, Xiushan County; 28°21'21"N, 108°52'16"E; 2 July. 2023; Lingzhen Li & Chaoyang Li leg.; RL20230001.

Paratypes. CHINA • Chongqing City • 6 33, 4 9; Xiushan County; 28°21'21"N, 108°52'16"E; 2 July. 2023; Lingzhen Li & Chaoyang Li leg.; RL20230101 to 20230110. • 11 33, 1 9; Xiushan County; 28°19'56"N, 108°52'17"E; 4 July. 2023; Lingzhen Li & Chaoyang Li leg.; RL20230111 to 20230122.

Diagnosis. *Rhinogobius lithopolychroma* can be distinguished from other species in the *Rhinogobius* by the following characteristics: It typically possesses 13–15 rays on the pectoral fin. The longitudinal scale series count ranges from 30 to 33, with the predorsal area lacking scales. The total vertebrae count is 30, comprising 12 precaudal and 18 caudal vertebrae. The head and body of this species are light gray, adorned with irregular orange markings on the cheeks and opercle. Morphometrics Reference Table 3.

Description. *Fins*: The fin configuration includes 6 rays on the first dorsal fin (VI), with a 22 total rays. The second dorsal fin consists of one spine and either 9 or 10 branched rays, totaling 15 rays. The fourth or fifth spine of the first dorsal fin is the longest and non-filamentous. In males, when the first dorsal fin is depressed, the rear tip extends to the base of the second branched ray of the second dorsal fin, while in females it reaches only to the base of the second dorsal fin anteriorly. The anal fin has 1 spine and either 7 or 8 branched rays, totaling 13 rays. The origin of the anal fin is inserted at a vertical line between the first and second branched soft ray of the second dorsal fin. The pectoral fins range from 13 to 15 rays, with 13 rays most common (present in 8 specimens), 14 rays in 13 specimens, and 15 in 1 specimen. The pectoral fins are broad in shape.

Scales: The body covered with ctenoid scales, with enlarged mid-trunk scales. The anterior predorsal area lacks scales, while the posterior part of the occipital region is covered by cycloid scales. The belly is adorned with small cycloid scales. The longitudinal scale series count ranges from 30 to 33, with a mode of 31. The transverse scale series count ranges from 7 to 9, with a mode of 8.

Head canals: pores σ are located parallel to the anterior nares. The anterior interorbital sections of the oculoscapular canal are separated, featuring paired pore λ . There is a single pore κ in the posterior region, with ω present near posterior edge of eyes and a lack of ω 1. The lateral section of anterior oculoscapular canal includes pores α and a terminal pore ρ . The posterior oculoscapular canal possesses two terminal pores θ and τ . Preopercular canals are presented, with pores ϵ , γ , and δ .

Table 3	. Morphometrics	of the typ	es of R	. lithopolychroma	expressed	as a	percentage
of stand	lard length.						

Variable	Holotype	Paratypes		
Sex	males	males (<i>N</i> = 17)	Females (N = 5)	
Morphometry				
Standard length (mm)	28.2	28.2-38.8(31.1)	27.5-36.4(33.6)	
Head length (mm)	9.5	7.9-11.6(9.6)	7.9-10.7(9.7)	
Percent standard length (%)			1	
Head length	33.7	25.8-33.7(28.9)	25.8-30.3(28.8)	
Predorsal length	36.5	28.2-43.4(37.2)	32.4-41.6(37.1)	
Snout to second dorsal fin origin	58.2	42.0-58.5(54.6)	54.2-62.0(58.9)	
Snout to anal fin origin	66.7	56.7-66.7(61.8)	63.5-66.9(65.1)	
Snout to anus	56.4	51.5-57.2(55.2)	55.6-61.2(58.5)	
Pre pelvic length	31.9	26.2-34.8(30.5)	28.3-34.9(31.6)	
Caudal peduncle length	18.8	18.8-23.1(21.1)	18.4-24.1(21.4)	
Caudal peduncle depth	10.6	9.0-12.2(10.6)	9.5-11.4(10.6)	
First dorsal-fin base	13.5	10.3-15.2(13.0)	9.9-14.2(11.9)	
Second dorsal-fin base	22.3	16.1-22.8(19.1)	14.5-21.2(16.6)	
anal fin base	15.2	10.8-15.8(13.8)	10.4-15.7(12.2)	
Caudal fin length	28.0	15.4-28.0(22.7)	17.8-23.4(20.3)	
Pectoral fin length	25.5	19.3-26.7(22.4)	20.5-21.2(21.0)	
Pelvic fin length	11.7	9.6-13.8(11.3)	9.9-12.7(11.5)	
Body depth of pelvic fin origin	11.3	9.2-16.3(13.2)	12.0-15.6(14.0)	
Body depth of anal fin origin	9.6	9.2-14.6(12.0)	11.3-15.4(12.9)	
Pelvic fin origin to anus	25.5	19.3-25.9(22.9)	20.3-26.9(23.5)	
Head depth	12.1	10.1-13.8(12.4)	11.3-14.2(13.4)	
Percent head length (%)				
Snout length	21.1	19.4-30.1(24.6)	15.2-28.6(20.3)	
Eye diameter	15.8	11.4-19.5(14.9)	13.1-21.5(17.1)	
Cheek depth	23.2	15.2-28.4(24.1)	17.7-25.5(22.2)	
Postorbital length	42.1	41.7-54.0(45.7)	45.7-58.2(49.3)	
Lower jaw length	26.3	18.8-37.0(29.6)	15.3-25.5(22.7)	
Interorbital width	33.7	25.9-39.3(32.4)	26.6-31.6(28.7)	
Head width in maximum	49.5	43.3-65.5(54.9)	48.6-64.9(56.5)	

Sensory papillae: The sensory papillae arrangement is as follows: Row a extends to before the middle of the eye. Row b is oblique and reaches forward to the orbit. Rows c and d extend to the posterior margin of the eyes, and Row cp is absent. Row f is paired. In the opercular region, there are rows ot, oi, and os. Rows oi and ot are not connected.

Vertebrae: The total vertebrae count is 12 + 18 = 30 (*N* = 5) and (P–V) 3/II II II 0/9 (*N* = 5).

Coloration of preserved specimens: In males, the head and body are gray with irregular markings on the cheeks and operculum. The ventral side is densely covered with tiny black spots and has six large, sometimes inconspicuous, horizontal black lines. The first dorsal fin is yellowish, While the second dorsal fin is yellow-



Figure 8. Dorsal (**A**), lateral (**B**), and ventral (**C**) views of preserved holotype of *R. lithopolychroma* sp. nov. (RL20230001 male) and dorsal (**D**), lateral (**E**), and ventral (**F**) views of preserved paratype of *R. lithopolychroma* sp. nov. (RL20230101 female).



Figure 9. The skeletal system of *R. lithopolychroma* sp. nov. Radiograph graphs of the whole body for paratype RL20230201, females.

ish-brown. The anal fin is yellowish. Females exhibit a gray head and body, with the first dorsal fin being yellowish and displaying blue spots between the 1st and 2nd spiny rays. The second dorsal fin is yellowish-brown, and the anal fin is yellowish.

Colour in life: Males display a light gray head and body with irregular orange markings on the cheeks and operculum, along with three smaller orange lines along the eyes. The ventral side is densely covered with tiny orange spots and has six large, sometimes inconspicuous, horizontal black lines. The first dorsal fin shows orange outlines on spines IV – VII with a white outer edge and blue spots between the 1st and 2nd spiny rays. The second dorsal fin is orange with irregular blue markings internally and on the outer edge, as well as blue spots on the 1st and 2nd spiny rays and a wide white margin. The anal fin is orange at the base, transitioning to black with a wide white margin. Females also exhibit a light gray head and body with irregular orange markings on the cheeks and operculum, and three smaller orange lines along the eyes. The ventral side is densely covered with tiny orange spots and features six large horizontal black



Figure 10. Dorsal (**A**), lateral (**B**), and ventral (**C**) views of the head of the preserved holotype of *R*. *lithopolychroma* sp. nov. Red circles indicate sensory canal pores; red dots represent sensory papillae. Abbreviations: AN, anterior nare pore; PN, posterior nare pore.



Figure 11. Photographs of *R. lithopolychroma* captured underwater in a tank **A** male and **B** female. Photographed by Mr Zhi.

lines. The first dorsal fin displays orange outlines on spines IV–VII with a yellow outer edge and blue spots between the 1^{st} and 2^{nd} spiny rays. The second dorsal fin is orange, and the anal fin is orange at the base, transitioning to black with a wide white margin.



Figure 12. Stream environment in Xiushan, Chongqing where *R. lithopolychroma* sp. nov. was collected.



Figure 13. Eggs of R. lithopolychroma sp. nov. at the type locality.

Distribution and habitat. *Rhinogobius lithopolychroma* is restricted to fast-flowing, shallow streams with a cobble substrate in Xiushan, Chongqing. The surveyed streams ranged from 10 to 30 cm in depth. This goby species is characterized by its large eggs (1.5–2.1 mm in size), which it deposits on the bottom surface of the cobblestones.

Etymology. *Rhinogobius lithopolychroma* was discovered in a small stream with a colorful cobble substrate. Accordingly, we named this species after its

habitat. In Ancient Greek, "litho" means "stone," and "polychroma" means rich in color. We combined these two words to christen this species. We suggest the Chinese name of this species as "彩石吻虾虎鱼".

Discussion

Rhinogobius sudoccidentalis and *R. lithopolychroma* are found in close geographical proximity and share some environmental commonalities, yet their morphology differs considerably. *Rhinogobius sudoccidentalis* typically features a longitudinal scale series of 30–33, while *R. lithopolychroma* exhibits 22–24 scales. In body coloration, *R. sudoccidentalis* appears creamy white with black spots on the cheeks and operculum, and a densely spotted ventral side. Conversely, *R. lithopolychroma* is light gray with irregular orange markings on the cheeks and operculum, and a ventral side densely covered with tiny orange spots, often accompanied by six large, occasionally inconspicuous, horizontal lines of black.

Morphologically, *R. sudoccidentalis* bears the closest resemblance to *Rhinogobius reticulatus* Li, Zhong & Wu, 2007 (Fig. 14A, B). They can be distinguished from other *Rhinogobius* species by their similar creamy white body coloration, reddish-brown stripes on the snout, and densely spotted ventral sides. To differentiate *R. sudoccidentalis* from *R. reticulatus*, one should observe traits such as the absence of predorsal scales in *R. sudoccidentalis* compared 3–6 in *R. reticulatus*, and the presence of a lower jaw stripe absent in *R. reticulatus*. The closest morphological match to *R. lithopolychroma* is *R. cliffordpopei*. *Rhinogobius lithopolychroma* and *R. cliffordpopei* share several distinguishing characteristics, including VI rays in the first dorsal fin, I,7–8 rays in the anal fin, and a predorsal scale series count of 0. They also exhibit similar body coloration. However, *R. lithopolychroma* differs from *R. cliffordpopei* in having 13–15 pectoral fin rays compared to 17–21 in *R. cliffordpopei*, and a total vertebrae count of 30 versus 26 in *R. cliffordpopei* (Li 2011).

As depicted in the phylogenetic tree, R. lithopolychroma is closest to Rhinogobius leavelli (Herre, 1935) and Rhinogobius davidi (Sauvage & Dabry de Thiersant, 1874), whereas R. sudoccidentalis is closest to Rhinogobius filamentosus (Wu, 1939), R. wuyanlingensis, R. reticulatus and Rhinogobius duospilus (Herre, 1935) (Fig. 15). Rhinogobius lithopolychroma shares morphological similarities with R. leavelli and R. davidi, but distinguishes itself with a higher vertebrae count and a naked predorsal area (Table 4), setting it apart from these species. Notably, R. sudoccidentalis also exhibits a high vertebrae count compared to closely related Rhinogobius species, and similarly features a naked predorsal area and a lower count of longitudinal scale (Table 5). Akihito et al. (2000) suggest that vertebrae counts may correlate with Rhinogobius ecotypes, with species inhabiting continental streams and rivers often displaying higher vertebrae counts (Chen and Miller 2008; Wanghe et al. 2020). The present study supports this view, noting that R. leavelli, R. davidi, R. filamentosus, R. wuyanlingensis, R. reticulatus and R. duospilus are primarily found in coastal provinces of southern China (Wu et al. 2008), while both new species are located in inland China. These two new species represent further evidence of vertebral and environmental adaptations within the genus Rhinogobius.



Figure 14. Pictures of *R. reticulatus* and *R. sudoccidentalis* sp. nov. with the latter having black lines under the eyes **A** *R. reticulatus* **B** *R. sudoccidentalis*.

According to studies by Yamasaki et al. (2015) and Li (2011) on *Rhinogobius* species, there is a correlation between egg size and species habitat preferences. Yamasaki defined small eggs as 0.6-0.9 mm and larger eggs as 1.4-2.1 mm. Li's research in 2011, conducted in the Qiantang River, demonstrated that species like *R. duospilus* and *R. davidi* inhabited streams and produced large eggs, whereas *R. similis*, typically was found in pond reservoirs and produced small eggs. Yamasaki et al. (2015) further highlighted that species with small eggs generally have an amphidromous lifestyle (Takahashi and Yanagisawa 1999; Keith et al. 2015), while those with large eggs tend to exclusively inhabit streams.





Table 4. Morphological comparison of *Rhinogobius lithopolychroma* with the genetically closest species.

Variable	R. lithopolychroma	R. leavelli	R. davidi
1 st dorsal fin	VI	VI	VI
2 nd dorsal fin I 9-10		I 8-9	I 9-10
Anal fin	I 7-8	18-9	l 6-8
Pectoral fin	13-15	14-15	14-15
Longitudinal scale	30-33	28-34	30-32
Transverse scale	7-9	9-11	11-12
Predorsal scale	0	6-12	0-4
Total vertebrae	30	26	28
References	This study	Wu et al. 2008; Li 2011	Wu et al. 2008; Li 201

Variable	R. sudoccidentalis	R. filamentosus	R. wuyanlingensis	R. reticulatus	R. duospilus
1 st dorsal fin	VI-VII	V-VI	V-VI	VI	VI
2 nd dorsal fin	I 8-9	I 8-9	I 8-9	I 8-9	I 8-9
Anal fin	l 6-7	18	18	l 7-8	l 6-7
Pectoral fin	14-15	15-17	17-18	15-17	15-16
Longitudinal scale	22-24	30-33	30-32	27-29	30-32
Transverse scale	7-8	8-10	9-10	8-9	8-10
Predorsal scale	0	5-11	7-9	3-6	6-10
Total vertebrae	29	27	27	26-27	27
References	This study	Wu et al. 2008	Huang et al. 2016	Li et al. 2007	Wu et al. 2008; Li 201

Table 5. Morphological	comparison of	Rhinogobius su	doccidentalis with	the genetically	v closest s	pecies
					/	

In the Upper Youshui River catchment, previously documented *Rhinogobius* species include *R. similis* and *R. cliffordpopei*, known to favor lakes, reservoirs, and stagnant water environments. Conversely, the new species discovered in this study exclusively inhabit streams. These newly identified species are all classified as large-egg types, indicating their better adaptation to stream habitats compared to the small-egg types like *R. similis* and *R. cliffordpopei* (Li 2011). Furthermore, the four newly uncovered species exhibit distinct preferences within stream habitats. For instance, *R. lithopolychroma* thrives in environments characterized by strong currents and low temperatures, specifically alpine streams with chilly waters, where it represents the predominant *Rhinogobius* species. On the other hand, *R. sudoccidentalis* demonstrates a broader distribution and adaptability, being found in streams with warmer water temperatures, including urban streams. This diversity in habitat preferences suggests ecological niche differentiation, likely playing a pivotal role in the formation of *Rhinogobius* species.

Presently, the survival of the two recently discovered *Rhinogobius* species faces certain threats. For instance, manganese ore collection in the headwaters of streams where *R. sudoccidentalis* resides may have significant implications for the species survival. Additionally, *R. lithopolychroma* is restricted to a narrow habitat and is only found in alpine streams, underscoring the importance of prioritizing its protection and conducting further detailed studies on its biology and ecology.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Lingzhen Li: methodology, formal analysis, validation, writing-original draft, writing-review, editing, investigation. Chaoyang Li: methodology, investigation, formal analysis, formal analysis. Weihan Shao: data curation, project administration, resources, supervision, writing-review and editing. Suxing Fu: data curation, project administration, resources, supervision, writing-review and editing. Chaowei Zhou: data curation, project administration, resources, supervision, writing-review and editing.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

A new species of the genus *Serangium* Blackburn (Coleoptera, Coccinellidae) from China, with description of the immature stages

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Abstract

A new species *Serangium xinpingensis* Huang & Wang, **sp. nov.** is described from Yunnan Province, China, as a newly discovered predator on *Bemisia tabaci* Gennadius (Hemiptera, Sternorrhyncha, Aleyrodidae). The new species is a valuable addition to the 14 species of this genus in China known before. A diagnosis, detailed description, including the structure of its immature stages, illustrations, and the distribution of the new species are provided.

Key words: Coccinelloidea, larva, Microweiseinae, pupa, taxonomy



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Introduction

Coccinellidae Latreille, 1807 is the largest family within the superfamily Coccinelloidea, comprises 6896 species recorded worldwide (Wang and Chen 2022). This family has been divided into three subfamilies: Microweiseinae, Monocoryninae and Coccinellinae (Che et al. 2021).

The genus *Serangium* was originally established by Blackburn (1889), with the description of the type species *Serangium mysticum* Blackburn, 1889 from Australia. Chapin (1940) suggested that *Serangium* and similar genera formed a closely related group. Blackwelder (1945) erected for them a separate tribe Serangiini in his checklist, and this was validated by Pope (1962). Currently, the tribe Serangiini is assigned to the subfamily Microweiseinae by Ślipiński (2007). The subfamily Microweiseinae includes four tribes: Carinodulini, Madeirodulini, Serangiini and Microweiseini (Szawaryn et al. 2020).

Serangium is the largest genus of the tribe Serangiini, comprising 48 extant species, and mostly occurring in the Oriental and Australian regions (Miyatake 1994; Raimundo and van Harten 2000; Ślipiński and Burckhardt 2006; Wang et al. 2011, 2014). Prior to this study, 14 species of *Serangium* had been known to occur in China (Wang et al. 2011, 2014). In this paper, we described a new species, *Serangium xinpingensis* sp. nov., from southwestern China. Descriptions of the adult, as well as the fourth instar larva and pupa, are provided.

Materials and methods

The adult samples examined were collected from Yunnan Province, China. The larval specimens used for this work were obtained by rearing from eggs laid by females of *S. xinpingensis*. We observed this ladybird preying on *Bemisia tabaci* Gennadius infesting *Tithonia diversifolia* (Hemsl.) A. Gray (Fig. 1). The adults were collected, as well as the plants, and they were raised under laboratory conditions (25 ± 1 °C, 70% ± 10 % R.H. and 14:10 h L:D). All examined specimens are deposited in the Department of Entomology of South China Agricultural University, Guangzhou, China (**SCAU**).

The adult morphological terminology used in this paper follows Ślipiński and Tomaszewska (2010), and the larval terminology follows Kamiya (1965) and Gordon and Vandenberg (1991). External structures were observed with a dissecting stereoscope (Zeiss SteREO Discovery V20). The following measurements were made with a micrometer:

- TL total length, from apical margin of clypeus to apex of elytra;
- TW total width, across both elytra at widest part;
- TH total height, through the highest point of elytra to metaventrite;
- **HW** head width, including eyes;
- **PL** pronotal length, from the middle of the anterior margin to the base of the pronotum;
- PW pronotal width at widest part;
- **EL** elytral length, along the suture, from the apex to the base including the scutellum;

The examined specimens were stored in 75% ethanol. The specific structures of larvae including mouthparts, head, and tibiotarsus, as well as abdomen of adult, were detached and cleaned with 10% NaOH at 56 C for 1 h. Subsequently, these structures were dissected and rinsed with distilled water. Following processing, the genitalia of both males and females were transferred to neutral balsam, and the specific structures of larvae were transferred to glycerol. Photographs of these structures were taken with an Axiocam 506 color digital camera attached to a Zeiss Image M2 microscope using ZEN 2.3 software. The habitus photographs of larvae and adults were taken with a Canon EOS 5DSR digital camera and processed by using Helicon Focus 7. All photos were processed by using Adobe Photoshop 2023 and Adobe Illustrator 2020.

Taxonomy

Serangium Blackburn, 1889

- Serangium Blackburn, 1889: 187, 209. Type species, monotypy: Serangium mysticum Blackburn, 1889.
- Serangium: Sicard 1909: 150, 151; Chapin 1940: 268; Miyatake 1961: 50; Sasaji 1971: 52; Pang and Mao 1979: 27; Miyatake 1994: 238; Ślipiński and Burckhardt 2006: 39; Ślipiński 2007: 53; Wang et al. 2011: 33.



Figure 1. Habitat and ecological photographs of *Serangium xinpingensis* sp. nov. **a** habitat **b** host plant of *Bemisia tabaci* (Gennadius, 1889) **c** egg **d** second instar larva **e** fourth instar larva **f** adult.

Semichnoodes Weise, 1892: 15. Type species, monotypy: Semichnoodes kunowi Weise, 1892. Synonymized by Weise 1908: 13.

Catana Chapin, 1940: 266. Type species, original designation, *Catana clauseni* Chapin, 1940. Synonymized by Ślipiński and Burckhardt 2006: 39

Diagnosis. Body minute, length ranging 1.0–2.5 mm, hemispherical, with head resting closely against prosternal anterior margin at rest; dorsum glossy, bearing sparse, long, thin setae. Mandible small, triangular, with single apical tooth and reduced mola (Fig. 2g); maxillary palps geniculate, palpomeres 2 and 3 closely fitting along stipes, and terminal maxillary palpomere conical or barrel-shaped, always longer than wide (Fig. 2e). Antenna composed of nine antennomeres, antennomere 1 club-shaped and antennomere 3 moderately to strongly elongate (Fig. 2h).



Figure 2. Serangium xinpingensis sp. nov. $\mathbf{a} - \mathbf{c}$ habitus photos: \mathbf{a} dorsal view \mathbf{b} lateral view \mathbf{c} front view \mathbf{d} prothorax, ventral view \mathbf{e} maxilla, ventral view \mathbf{f} labium, ventral view \mathbf{g} mandible, dorsal view \mathbf{h} antenna, dorsal view \mathbf{i} posterior leg, ventral view. Scale bars: 0.2 mm ($\mathbf{a}-\mathbf{d}$, \mathbf{i}); 0.1 mm (\mathbf{e} , \mathbf{h}); 0.05 mm (\mathbf{f} , \mathbf{g}).

Pronotum short, strongly transverse (Fig. 2d). Scutellum large, triangular. Prosternum strongly protruding medially, forming a broad lobe partly covering mouthparts; prosternal process subtruncate apically, broad. Femora, particularly profemora, broad and flat, closely fitting into depressions on ventral surface and shielding tibiae and tarsi. Elytra smooth, strongly convex, without visible punctures; epipleura extending completely to apex with well-defined cavities accommodating tips of meso- and metafemora (Fig. 2a-c).

Abdomen with ventrites 1 and 5 notably longer than ventrites 2–4 together (Fig. 3a). Postcoxal line on abdominal ventrite 1 complete, extending to metanepisternum, without associated pits or pores (Fig. 3a).

Serangium xinpingensis Huang & Wang, sp. nov. https://zoobank.org/E37802CD-7FFD-40E7-96DD-1084584F0F7D

Diagnosis. This species can be identified by its brown body, dark-brown pronotum with sparse setae and two subparallel longitudinal rows of punctures along the lateral elytral margin, and body integument covered with long, thin setae (Fig. 2a); the penis is strongly arched (Fig. 3b).

This species is very similar to *Serangium clauseni* (Chapin, 1940) and *Serangium parcesetosum* Sicard, 1929, but it differs from *S. clauseni* in its more arched penis, squarer bases of parameres, and abdominal ventrite 1 with more densely incised punctures (Wang et al. 2011). It is distinguished from *S. parcesetosum* by its more sharply angular antennomere 2 and curvature of its penis (Booth and Polaszek 1996).

Description of adult. TL: 2.04–2.35 mm, TW: 1.78–2.06 mm, TH: 1.28–1.34 mm, TL/TW: 1.14–1.15; PL/PW: 2.31–3.35; EL/EW: 1.84–1.87.

Body hemispherical; dorsum strongly convex (TH: 1.28–1.34 mm), glabrous (Figs 2a–c). Head brown or reddish-brown. Pronotum brown or reddish-brown, with black margins. Scutellum light brown. Elytra brown, slightly paler than head and pronotum, with one dark-brown band-like marking present along lateral margin; surface with sparse, shallow punctures, demonstrating one row along suture and two subparallel longitudinal rows along lateral margin (Fig. 2a, b).

Head transverse and ventrally flattened, 0.41× elytral width (HW/EW = 2.45). Frons with long sparse setae. Eyes large and coarsely faceted, greatest interocular distance 0.36× of head width. Antenna with nine antennomeres, terminal antennomere large, and spatulately elongate (Fig. 2h).

Pronotum short and strongly transverse, 0.69× of elytral width (PW/EW = 1.96), sparsely covered with long setae. Prosternum with dense setae along anterior margin; prosternal process apically rounded.

Male genitalia. Penis strongly curved, arched, gradually narrowing, with blunt tip (Fig. 3b); penis capsule with one small inner process and concave on outer margin (Fig. 3b). Penis guide relatively slender and elongated, wide at base in ventral view, narrowing near distal 1/3 and having tongue-like shape; thin in lateral view, gradually tapering (Fig. 3d). Parameres asymmetrical; one extending from base to approximately 1/3 of penis guide, tapering apically, bearing few long setae; another extending from base to approximately 2/3 of penis guide, wider at base and gradually narrowing apically, with blunt tip, covered with small protuberances and setae (Fig. 3d).

Female genitalia. Genital plate (coxites) subtriangular (Fig. 3f), rounded at apex, bearing sparse setae; styli rather long (Fig. 3f), with few setae. Spermatheca consisting of two parts, including one globular, with faint constriction and two small pinch-like projections, and another elongate tubular shaped, slightly tapering distally (Fig. 3e).

Description of fourth instar larva. Length 2.98 mm; width 1.34 mm. Body fusiform, bright yellow overall, dorsal surface with two longitudinal rows of pigmented spots laterally, long setae concentrated on body sides and with sparse, short setae on dorsum.

Head subovoid. Mouthparts light brown; frontal arms U-shaped, distinct. Three bulging stemmata presented on each side at antennal insertions, arranged in triangle (Fig. 5e). Antenna with two antennomeres, antennomere 2 Chu-Yang Huang et al.: A new species of the genus Serangium from China



Figure 3. Genitalia of adults of *Serangium xinpingensis* sp. nov. **a** abdomen, ventral view **b**-**d** male genitalia: **b** penis, lateral view **c** tegmen, ventral view **d** tegmen, lateral view **e**-**f** female ovipositor: **e** spermatheca **f** coxites, ventral view. Scale bars: 0.2 mm.



Figure 4. Habitus of adults of *Serangium xinpingensis* sp. nov., lateral view, showing intraspecific variations of the bandlike marking along the outer margin of elytron. Scale bar: 0.2 mm.

twice as long as antennomere 1, with one long apical sensorium (ca 26.2 μ m) and inconspicuous papillae (Fig. 5e). Mandibles heavily sclerotized, subtriangular, without basal tooth, but with one long seta at condyle (Fig. 5g). Maxillae subtriangular (Fig. 5i). Maxillary palp 3-segmented, palpomere 3 nearly subequal in length with palpomere 2, elongate, with sensilla at apex (Fig. 5j), mala transverse and with anterior margin rounded (Fig. 5i). Labrum nearly trapezoidal,



Figures 5. The fourth instar larva and pupa of *Serangium xinpingensis* sp. nov. **a** fourth instar larva, dorsal view **b** fourth instar larva, lateral view **c** pupa, dorsal view \mathbf{d} -**j** larval structures: **d** head capsule, dorsal view **e** antenna, dorsal view **f** labrum. dorsal view **g** mandible, dorsal view **h** tibiotarsus and claw. dorsal view **i** maxillary mala, ventral view **j** mouthparts, ventral view. Scale bars: 1 mm (**a**-**c**); 0.05 mm (**d**-**f**, **h**-**j**); 0.025 mm (**g**).

with sparse setae on anterior margin. Labium with sparse tomentum and four long setae evenly spaced around apex (Fig. 5f). Labial palps moderately separated, 2-segmented, palpomere 2 distinctly longer than palpomere 1 (Fig. 5j).

Thorax with parallel anterior and posterior margins of each segment, strongly convex on lateral margins; laterally with pigmented spots, bearing long setae. Prothorax narrower than meso- and metathorax, meso- and metathorax almost equal in length and width. Tibiotarsus light brown, elongate, and translucent, with sparse setae; tarsal claws sickle-shaped; basal teeth subtriangular, with one long seta at base (Fig. 5h).

Abdomen 9-segmented, each segment with lateral margin strongly convex, pigmented spots, bearing setae (Fig. 5a, b).

Description of pupa. Length 2.73 mm; width 2.11 mm. Body oval, light yellow, bearing flexible setae.

Type materials. *Holotype*: 1♂, China, Yunnan: Musha Township, Yuxi, 23.8507°N, 101.7782°E, ca 475 m elev., 22.v.2023, Huang CY leg (SCAU). *Paratypes* (15): 9♂♂6♀♀, same data as holotype (SCAU).

Distribution. China (Yunnan).

Etymology. The species epithet "*xinpingensis*" refers to the Xinping County where the type series was collected.

Remarks. This is the first time that individual variation in appearance was found in *Serangium*. Based on our examination of 16 specimens, the male genitalia are highly uniform. The species displays distinct intraspecific variations in the coloration of adults. The head and pronotum coloration frequently subuniform, while the elytra along suture and outer margins may vary from brown to blackish-brown among individuals (Fig. 4).

Larvae of the subfamily Microweiseinae have been given little attention, and only two species have been recorded so far, namely *Scymnomorphus japonicus* (Kamiya, 1960) and *Serangium japonicus* Chapin, 1940, belonging to the tribes Microweiseini and Serangiini, respectively. The larva of *Scymnomorphus japonicus* was described by Kamiya (1965) and Sasaji (1968), although it was originally described under the name *Sukunahikona japonicus* Kamiya, 1960. The known larvae of the two tribes Serangiini and Microweiseini are very similar, but they differ greatly in the antennomere 2, which is much shorter in the tribe Microweiseini than Serangiini (Kamiya 1965). Within the tribe Serangiini, the larva of the new species can be distinguished from *S. japonicus* by its distinct U-shaped frontal arms. In *S. japonicus*, the frontal arms are invisible (Jiaming Zhuang pers. obs.)

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: CYH, XMW. Investigation: QJD, PL, YLS, WF, CYH, LLZ. Writing - original draft: CYH, LLZ. Writing - review and editing: XMW.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Research Article

Two new species and a new record of the ant genus *Meranoplus* Smith, 1853 (Hymenoptera, Formicidae, Myrmicinae) from Thailand

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Abstract

Meranoplus Smith, 1853 is distributed in the Old World tropics, from Africa, Asia, New Guinea to Australia. There are four species *Meranoplus bicolor* (Guérin-Méneville, 1844), *M. castaneus* Smith, 1857, *M. laeviventris* Emery, 1889, and *M. mucronatus* Smith, 1857 previously recorded from Thailand. In the present paper, two new species of the genus, *M. siamensis* Yodprasit & Jaitrong, **sp. nov.** and *M. tanomtongi* Yodprasit & Jaitrong, **sp. nov.**, are described based on the worker caste. Additionally, *M. malaysianus* Schödl, 1998 is recorded for the first time for Thailand. A key to the Oriental and Indo-Australian species, based on the worker caste, is provided. The new species and the new record were found to nest in soil.

Key words: Biodiversity, distribution, Oriental and Indo-Australian regions, taxonomy

Introduction

Meranoplus Smith, 1853 is distributed in the Old World tropics from Africa, Asia, and New Guinea to Australia (Bolton 2024). Members of this genus nest in the soil, in rotten wood, or under stones (Bolton 1981; Jaitrong et al. 2020) and are known to be active both day and night (Gross et al. 1991). The genus has been revised and reviewed across its entire distribution over the past few decades (Australasia and New Guinea: Donisthorpe 1947; Andersen 2006; Taylor 2006; Schödl 2007; Africa: Bolton 1981; Madagascar: Boudinot and Fisher 2013; and Asia: Chapman and Capco 1951; Wu and Wang 1995; Schödl 1998, 1999; Terayama 2009; Guénard and Dunn 2012; Bharti and Akbar 2014; Bharti et al. 2016; Jaitrong et al. 2016; Dias et al. 2020). Currently, 91 valid species of the genus are known (Bolton 2024). Among them, 60 species have been recorded from the Australasian region, 13 from the Oriental region, eight from the Afrotropical region, and four from the Malagasy region (Boudinot and Fisher 2013; AntWiki 2024; Bolton 2024). Until now, only four species, Meranoplus bicolor (Guérin-Méneville, 1844), M. castaneus Smith, 1857, M. laeviventris Emery, 1889, and M. mucronatus Smith, 1857, were known from Thailand (Jaitrong and Nabhitabhata 2005; Jaitrong et al. 2020; Khachonpisitsak et al. 2020).



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We examined *Meranoplus* specimens from Thailand and recognized seven species. *Meranoplus malaysianus* Schödl, 1998 is newly recorded from the country, and *M. siamensis* Yodprasit & Jaitrong, sp. nov. and *M. tanomtongi* Yodprasit & Jaitrong, sp. nov. are new to science and described here based on the worker caste. A key to the Oriental and Indo-Australian species of *Meranoplus*, based on the worker caste, is presented.

Materials and methods

This study was mainly based on the specimens deposited in the Natural History Museum of the National Science Museum, Thailand. Almost 500 specimens of the genus *Meranoplus* were examined. Specimens of the new species and the new record were compared with the images available on Antweb (2024) of holo-types and paratypes of small species distributed in Asia: *M. borneensis* Schödl, 1998; *M. loebli* Schödl, 1998; and *M. malaysianus* Schödl, 1998. A paratype of *M. malaysianus* deposited in the Seiki Yamane Collection, Kagoshima, Japan was also examined.

Most morphological observations were made with a Zeiss Discovery V12 stereoscope. Multi-focused montage images were produced using NIS-Elements-D from a series of source images taken with a Nikon Digital Sight-Ri1 camera attached to a Nikon AZ100M stereoscope. Specimens were measured for the following parts using a micrometer on a Zeiss Discovery V12 stereoscope. All measurements are given in millimeters and recorded to the second decimal place.

The abbreviations for the measurements and indices used are as follows: (edited from Bolton 1981; Hölldobler and Wilson 1990; Schödl 1998):

- **HL** Head length, straight-line length of head in full-face view, measured from the mid-point of the anterior clypeal margin to the midpoint of the posterior margin. In species where one or both of these margins are concave, the measurement is taken from the mid-point of a transverse line that spans the apices of the projecting portions.
- **HW** Head width, maximum width of head in full-face view, excluding the compound eyes.
- **ML** Mesosomal length, the diagonal length of the mesosoma in profile from the point at which the pronotum meets the cervical shield to the posterior basal angle of the metapleuron.
- **PML** Length of promesonotal shield, measured from anterior mid-point of pronotum behind collar that is the mid-point of a virtual line, where the anterior pronotal margins meet, to mid-point of behind margin of mesonotum above propodeum.
- **PW** Pronotal width, measured right behind base of anterolateral pronotal projection (angle) in dorsal view.
- **SL** Scape length, straight-line length of the antennal scape, excluding the basal constriction or neck.
- **TL** Total length, total outstretched length of the individual, from the mandibular apex to the gastral apex.
- CI Cephalic index, HW/HL×100.
- **PMI** Pronotum index, PW/PML×100.
- **SI** Scape index, SL/HW×100.

Abbreviations of the ant collections are as follows

AMK	Ant Museum, Faculty of Forestry, Kasetsart University, Thailand
BMNH	The Natural History Museum, London, United Kingdom
MCZC	Museum of Comparative Zoology, Cambridge, USA
NHMB	Naturhistorisches Museum, Basel, Switzerland
NHMW	Naturhistorisches Museum Wien, Vienna, Austria
SKYC	Seiki Yamane Collection, Kagoshima, Japan
THNHM	Natural History Museum of the National Science Museum, Thailand

Scanning electron microscope images were made at the Microscopic Center, Faculty of Science, Burapha University with a Leo 1450 VP scanning electron microscope with gold-coated specimens.

For general terminology in the worker caste of ants, see Bolton (1994) and Hölldobler and Wilson (1990). The terminology of the ant genus *Meranoplus* follows Schödl (1998).

Taxonomy

Meranoplus Smith, 1853

- *Meranoplus* Smith, 1853: 224. Type species: *Cryptocerus bicolor*, by subsequent designation of Bingham (1903: 166).
- *Tricytarus* Donisthorpe, 1947: 187. Type species: *Tricytarus parviumgulatus*, by original designation (junior synonym of *Meranoplus* by Boudinot 2014: 96).

Diagnosis of worker. Bolton (1981) and Sharaf et al. (2014) defined characteristics of this genus as follows: 1) worker is distinctly monomorphic; 2) antennae 9-segmented, with three apical segments forming a club; 3) frontal scrobes distinct, deep, and long; 4) palp formula 5,3; 5) masticatory margin of mandibles with 4–5 teeth; 6) compound eyes present, usually strongly convex, located below antennal scrobes; 7) pronotal spines present, dentiform; 8) mesonotal spines present; 9) mesosomal dorsum fused to form a shield; 10) propodeal spines present; 11) petiolar spines present or absent; 12) with dense, long, erect hairs on body surface.

Meranoplus malaysianus Schödl, 1998

Figs 1A–F, 7F–I

Meranoplus malaysianus Schödl, 1998: 385, figs 4, 18, 32 (workers and queen). *Meranoplus malaysianus*: Pfeiffer et al. 2011: 47; Satria and Herwina 2020: 83.

Types. *Holotype* • (BMNH, CASENT0902029, images examined), West Malaysia (Malaya), Kuala Lumpur, 8 October 1973, B. Bolton. *Paratypes*: • 10 workers and 1 queen, same locality data as holotype (BMNH, NHMW) • 3 workers, Berlese funnel, Malaysia (MALAYA), K. Lumpur, 8 October 1973, B. Bolton, *Meranoplus* sp. det. B. Bolton, 1974 (NHMB, NHMW) • 5 workers, MALAYSIA Neg. Sembilan Pasoh For. Res. November 1994, litt (= litter?) sample M. Brendell, K. Jackson,



Figure 1. *Meranoplus malaysianus* **A**, **C**, **E** holotype (CASENT0902029) **B**, **D**, **F** non-type worker (THNHM-I-00028943) **A**, **B** head in full-face view **C**, **D** promesonotal shield in dorsal view **E**, **F** body in profile.

S. Lewis (BMNH, NHMW) • 4 workers, 2 queens, 1 male (head missing), Damm., Depok 7.1. [1 ex. 30.111.] 1923, MCZC Museum of Comparative Zoology (MCZC, NHMW) • 3 workers, F192-387, Kebun Raya, Bogor, W-Java, Indonesia, 11–31 Janaury 1992, F. Ito (NHMW).

Non-type material examined. Southern: • 1 worker (THNHM-I-00028943, THNHM), Songkhla Province, Hat Yai District, Thung Tam Sao Subdistrict, evergreen rain forest, 27 July 2002, N. Noon-anant leg. • 1 worker (THNHM-I-00027334, THNHM), Narathiwat Province, Wang District, Lo Chood Subdistrict, 5.8455°N, 101.8756°E, 25 September 2001, R. Poonjampa leg., hand collecting • 1 worker (AMK), same locality and date, S. Hasin leg., general collection. **Measurements and indices.** Workers (*n* = 2): HL 0.68–0.69, HW 0.68–0.70, ML 0.65, PML 0.45–0.49, PW 0.65, SL 0.44, TL 2.79–2.84, CI 99–104, PMI 133–142, SI 63–64.

Diagnosis of worker. Small species (HW 0.68–0.70 mm, TL 2.79–2.84 mm). Promesonotal shield shorter than broad and distinctly margined, broadly transparent at sides, overhanging lateral face of mesosoma; anterior corners of pronotum almost right angles; lateral margins of pronotum parallel, slightly sinuate. Petiole in profile tapered, the crest obliquely and narrowly truncate. Postpetiole nodiform, almost as long as high and roundly convex dorsal outline. Dorsa of head and promesonotal shield densely reticulate-rugulose; lateral portion of pronotum reticulate-rugulose; mesopleuron, metapleuron, and lateral faces of propodeum sparsely reticulate-rugulose, with smooth interspaces. Anterior face of petiole smooth and shiny, dorsum and lateral faces rugulose, posterior face smooth. First gastral tergite smooth, with an occasional faint shagreening around piliferous punctures.

The Thai specimens agreed well with the holotype (CASENT0902029) in structure, sculpturing, and pilosity. However, body color of the specimens collected from Thailand are reddish brown, while the holotype is paler, yellow (Fig. 1). Reticulations on dorsum of head rather denser and smaller than that on the holotype (see Fig. 1A, B for comparison).

Distribution. Thailand (Songkhla and Narathiwat, new record, Fig. 4), Malaysia (West Malaysia, Sabah, Sarawak), Singapore, Indonesia (Java).

Habitat. Two specimens from Narathiwat Province were collected from the ground in a disturbed area near a lowland evergreen forest, near the Thai–Malay border. A specimen (THNHM-I-00028943, THNHM) from Songkhla Province was collected in a primary evergreen forest.

Meranoplus siamensis Yodprasit & Jaitrong, sp. nov.

https://zoobank.org/752DDF38-72E5-4325-9B3D-4C70BEEFA1DF Figs 2A-D, 5H-I, 6A

Types. *Holotype*: • worker (THNHM-I-00027303, THNHM), eastern Thailand, Chonburi Province, Sri Racha District, Kasetsart Sriracha Campus, dry evergreen forest, 13.2837°N, 100.9238°E, 18 October 2003, W. Jaitrong leg., TH03-WJT-313.

Paratypes: • 6 workers (THNHM-I-00027304 to THNHM-I-0002730, THNHM-I-00027310, THNHM-I-00028942), same data as holotype • 4 workers (THNHM-I-00027309, THNHM-I-00027311 to THNHM-I-00027313) • 3 workers (THNHM-I-00027314), same locality as holotype, but 19.IV.2003, A. Suwanasri leg., AS190403-01. The paratypes are deposited in THNHM.

Non-type material examined. Central: • 1 worker (THNHM-I-00028919, THNHM), central Thailand, Uthai Thani Province, Ban Rai District, Kaen Ma Kurd Village, dry dipterocarp forest, 15.1225°N, 99.2755°E, 1 June 2002, W. Jaitrong leg., TH02-WJT-039; 21 workers (THNHM-I-00028920 to THNHM-I-00028940, THNHM), same data locality. **Western:** • 3 workers (THNHM-I-00028941, THNHM), Kanchanaburi Province, Sai Yok District, Ban Chong Keab, dry dipterocarp forest, 25 May 2019, W. Jaitrong leg. **Northeastern:** • 7 workers (THNHM-I-00027315, THNHM), Nakhon Ratchasima Province, Wang Nam



Figure 2. *Meranoplus siamensis* sp. nov. (holotype, THNHM-I-00027303) **A** head in full-face view **B** body in profile view **C** promesonotal shield in dorsal view **D** body in dorsal view.

Kheao District, Sakaerat Environmental Research Station (ERS), dry dipterocarp forest, 14.5031°N, 101.9368°E, 5 June 2022, W. Jaitrong leg., TH22-WJT-264 (THNHM); **Eastern:** • 4 workers (THNHM-I-00027316, THNHM), Chachoengsao Province, Tha Takiab District, 6 April 2003, W. Jaitrong leg., WJT260403-01.

Measurements and indices. Holotype worker: HL 0.63, HW 0.65, ML 0.62, PML 0.48, PW 0.63, SL 0. 0.45, TL 2.58, CI 102.38, PMI 130.21, SI 69.77. Paratype workers (*n* = 5): HL 0.60–0.65, HW 0.63–0.66, ML 0.60–0.65, PML 0.46–0.51, PW 0.62–0.65, SL 0.43–0.48, TL 2.56–2.82, CI 100–106.45, PMI 127–136.26, SI 67.72–71.97.

Description of worker. *Head* in full-face view subquadrate, slightly shorter than broad, with sides broadly convex, posterior margin distinctly convex, posterolateral corner bluntly angulate. Antennal scapes short, reaching level of posterior margin of compound eyes, apical half incrassate; antennal segment II slender, longer than each of segments III–VI, and almost as long as III+IV+V; segment VI broader than each of segments II–V. Clypeus roughly subrectangular, shorter than broad, its anterior margin feebly concave medially,

while posterior clypeal margin almost straight. Mandibles subtriangular, masticatory margin with four teeth. Compound eyes large and convex when seen in full-face view, located laterally and well behind mid-length of head, with 8 or 9 ommatidia along longest axis, each facet hexagonal (Fig. 6A). Frontal lobes broad, its anterior corners right angled and lateral margin almost straight (Fig. 5H). Frontal carinae long reaching posterolateral corners of head.

Mesosoma in dorsal view, promesonotal shield distinctly shorter than broad, its lateral margin convex, serrate, margined and slightly overhanging mesosoma; lateral and posterior portions of promesonotal shield with translucent fins; posterior margin of promesonotal shield sinuate and distinctly concave; anterolateral corners of promesonotal shield bluntly angulate and posterolateral corners of promesonotal shield roundly angulate; promesonotal shield with two pairs of fenestrae laterally; metanotal groove absent. Declivity of propodeum almost invisible from above, mostly overhung by posterior margin of promesonotal shield (propodeal spines are visible in profile). Mesosoma in profile subquadrate, dorsal outline weakly convex, lateral face of mesosoma relatively flat; lateral portion of pronotum subtriangular; metapleuron not clearly demarcated from mesopleuron and lateral face of propodeum. Propodeal spines long and acute, longer than wide at its base, located at middle of propodeal declivity length.

Petiole in profile subtriangular. Subpetiolar process low, its ventral outline weakly convex, with small anterior denticle. **Postpetiole** in profile subquadrate, shorter than high; in dorsal view, distinctly shorter than broad, anterior margin weakly convex, posterior margin distinctly convex; dorsum of postpetiole somewhat flat, marginated with distinct ridge, posterior face convex. **Gaster** about as large as head and mesosoma combined; first gastral tergite largest, in dorsal view, its anterior margin distinctly concave.

Sculpture. Mandibles striate, shiny. Antennal scapes superficially striate. Head dorsally sparsely reticulate-rugulose laterally, while median portion weakly sculptured; half posterior portion of antennal scrobes shagreened mixed with few transverse ridges. Promesonotal shield more weakly sculptured than dorsum of head, with median portion smooth, shiny, and lacking any rugae; in profile, upper one-third portion of pronotum shagreened, while lower two-third portion with sparse irregular ridges; upper one-third portion of mesopleuron shagreened, lower two-thirds longitudinal weakly striate; metapleuron, and lateral faces of propodeum somewhat smooth and shiny. Propodeum declivity shagreened. Petiole smooth and shiny, postpetiole somewhat smooth but posterior face of postpetiole scabrous. First gastral tergite superficially shagreened with smooth and shiny interspaces.

Pilosity and coloration. Dorsa of head and mesosoma with dense erect hairs mixed with sparse longer hairs; antennae with dense suberect hairs; in profile, lower two-thirds of pronotum with sparse suberect hairs; lower one-third of mesopleuron and metapleuron with sparse suberect hairs; area around propodeal spiracle with sparse suberect hairs; femora and tibiae with numerous long outstanding hairs as well; petiole with weakly sparse erect hairs on its anterior face and dorsum; postpetiole with dense long erect hairs, except anterior face without hairs; gaster with dense long erect hairs. Body mainly reddish brown; mandibles, antennae, legs, and tip of gaster yellowish brown.

Distribution. Thailand (Uthai Thani, Chonburi, Nakhon Ratchasima and Kanchanaburi Provinces, Fig. 4).

Etymology. The specific name is after Thailand where the type locality is located; Thailand was called "Siam" in the past.

Habitat. This species can be found in dry evergreen and dry dipterocarp forests. The specimens collected from northeastern Thailand (colony code TH22-WJT-264) nested in the soil. Workers moved slowly on the ground.

Differential diagnosis. *Meranoplus siamensis* sp. nov. is a small species that is most similar to *Meranoplus tanomtongi* sp. nov. in general appearance, having a pair of fenestrae along each lateral margin of the promesonotal shield, and having a subrectangular postpetiole when seen in profile. However, M. siamensis can be distinguished from *M. tanomtongi* by: 1) anterior corners of frontal lobes right angled and lateral margin almost straight (round and lateral margin weakly convex in *M. tanomtongi*, see Figs 5E, H for comparison); 2) compound eyes with 8 or 9 ommatidia along longest axis, each facet hexagon (each facet round or elliptical in *M. tanomtongi*, see Figs 5G, 6A for comparison); 3) dorsum of head weakly sculptured (dorsum of head entirely and distinctly reticulate in *M. tanomtongi*, see Fig. 5F, I for comparison); 4) dorsum of postpetiole somewhat flat, marginated with distinct ridge (shallowly concave, marginated with distinct ridge in *M. tanomtongi*); 5) entire head with dense short hairs mixed with sparse longer hairs (hairs along head margin clearly longer than hairs on middle of head in *M. tanomtongi*, see Fig. 5F, I for comparison).

The type series of *M. siamensis* sp. nov. is very similar to the non-type specimens from Central Thailand (TH02-WJT-039). However, the two colonies have some variations: 1) compound eyes with 9 ommatidia along longest axis in the type series (8 ommatidia in colony no. TH02-WJT-039); 2) promesonotal shield shorter than broad in the type series (almost as long as broad in colony no. TH02-WJT-039); 3) posterior half of head with sparse and weak reticulation in the type series (dense distinct reticulations in colony no. TH02-WJT-039); 4) propodeal declivity somewhat shagreened in the type series (smooth and shiny in colony no. TH02-WJT-039); 5) first gastral tergite superficially shagreened with smooth and shiny interspaces in the type series (distinctly shagreened in colony no. TH02-WJT-039). These characters are not clear enough to distinguish the two populations.

Meranoplus tanomtongi Yodprasit & Jaitrong, sp. nov.

https://zoobank.org/7D7848E3-8BA7-49E5-AC8A-3A09BCE7A96B Figs 3A-D, 5A, B, E-G

Types.*Holotype*: • worker (THNHM-I-00028903, THNHM), northeastern Thailand, Kalasin Province, Kuchinarai District, Nong Hang Subdistrict, dry dipterocarp forest, 16.5559°N, 104.1089°E, 10 December 2007, W. Jaitrong leg., TH07-WJT-1010, honey baiting trap. *Paratypes*: • 10 workers (THNHM-I-00028904 to THNHM-I-00028913), same data as holotype. The paratypes are deposited in THNHM.

Non-type material examined. LAOS. Central: • 4 workers (THNHM-I-00028914, THNHM), Vientiance Province, Pak Ngum District, Ban Pha Dang, dry evergreen forest, 18.2716°N, 102.9639°E, 12 June 2010, W. Jaitrong leg., WJT10-LAO111 • 1 worker (THNHM-I-00028915, THNHM), same locality and date, Sk. Yamane leg., LA10-SKY-096, sandy soil. **THAILAND. Northeastern:** •



Figure 3. *Meranoplus tanomtongi* sp. nov. (holotype, THNHM-I-00028903) **A** head in full-face view **B** body in profile view **C** promesonotal shield in dorsal view **D** body in dorsal view.

1 worker (THNHM-I-00028916, THNHM), Mukdahan Province, Kham Cha-e District, Kheang Chang Niam Village, mixed deciduous forest, 16.5698°N, 104.2703°E, 8 June 2007, unknown collector • 1 worker (THNHM-I-00028917, THNHM), same locality, 4 August 2007, P. Kosonpanyapiwat leg. • 4 workers (THNHM-I-00028918, THNHM), same locality and collector, 2 September 2007.

Measurements and indices. Holotype worker: HL 0.63, HW 0.65, ML 0.65, PML 0.51, PW 0.65, SL 0.45, TL 2.72, CI 103, PMI 129, SI 70. Paratype workers (*n* = 5): HL 0.62–0.65, HW 0.61–0.65, ML 0.62–0.68, PML 0.48–0.52, PW 0.61–0.70, SL 0.45–0.46, TL 2.62– 2.85, CI 98–104, PMI 122–140, SI 68–74.

Description of worker. *Head* in full-face view subquadrate, almost as long as broad, with sides weakly convex, posterior margin weakly convex, posterolateral corners bluntly angulate. Antennal scapes short, only reaching level of posterior margin of compound eyes, apical half incrassate; antennal segment II slender, longer than each of segments III–VI, and almost as long as III+IV+V; segment VI broader than each of segments II–V. Clypeus roughly subrectangular, shorter than broad, its anterior margin feebly concave, while posterior clypeal margin almost straight. Mandibles subtriangular, masticatory margin with four teeth. Compound

eyes large, strongly convex in full-face view, located laterally behind mid-length of head, with eight ommatidia along longest axis, each facet round or elliptical (Fig. 5G). Frontal lobes broad, its anterior corners round, its lateral margin weakly convex (Fig. 5E). Frontal carinae long, reaching posterolateral corners of head.

Mesosoma in dorsal view promesonotal shield distinctly shorter than broad, laterally convex, sinuate, margined and slightly overhanging mesosoma; lateral and posterior portions of promesonotal shield with translucent fins; posterior margin of promesonotal shield sinuate and distinctly concave; anterior corners of pronotum and posterior corners of mesonotum bluntly angulate; promesonotal shield with two pairs of fenestrae laterally; metanotal groove absent. Declivity of propodeum almost invisible from above, overhung by posterior margin of promesonotal shield (propodeal spines are visible in profile). Mesosoma in profile subquadrate, weakly convex dorsal outline, lateral face of mesosoma flat; lateral face of pronotum subtriangular; metapleuron not clearly demarcated from mesopleuron and lateral face of propodeum. Propodeal spines long and acute, located at middle of propodeal length, in profile.

Petiole in profile subtriangular, both anterior and posterior faces weakly convex; when viewed from behind, dorsal margin transverse and smoothly convex. Subpetiolar process low, its ventral outline weakly convex, with small anterior denticle. **Postpetiole** in profile subquadrate, shorter than high; in dorsal view, distinctly shorter than broad, anterior margin almost straight, while posterior margin distinctly convex; dorsum of postpetiole shallowly concave marginated with sinuate ridge, posterior face convex. **Gaster** larger than head and mesosoma combined; first gastral tergite largest, in dorsal view, its anterior margin distinctly concave.

Sculpture. Mandibles striate but shiny. Antennal scapes superficially striate. Dorsum of head in full-face view entirely reticulate; posterior half of antennal scrobes shagreened mixed with a few transverse ridges. Dorsum of promesonotal shield distinctly reticulate but median region with weaker reticulation than elsewhere; in profile, upper half portion of lateral faces of pronotum shagreened, while lower half portion with sparse irregular ridges; upper one-third portion of mesopleuron shagreened, lower two-third portion weakly longitudinally striate; metapleuron and lateral face of propodeum smooth and shiny. Propodeum declivity superficially shagreened. Petiole smooth and shiny. First gastral tergite superficially shagreened with smooth and shiny interspaces.

Pilosity and coloration. Dorsum of head with dense erect hairs (usually a closed cell with a hair), hairs along head margin clearly longer than hairs on middle of head; antennae with dense, suberect hairs; promesonotal shield with dense, erect hairs; legs with dense suberect hairs; in profile, lower two-thirds of pronotum with sparse suberect hairs; lower one-third of mesopleuron and metapleuron with sparse suberect hairs; area around propodeal spiracle with sparse, suberect hairs; femora and tibiae with numerous long, outstanding hairs as well; petiole with sparse, erect hairs on its dorsum; postpetiole with dense, long, erect hairs, except anterior face without hairs; femora and tibiae with numerous long, outstanding hairs. Dorsum of body (head, mesosoma, and gaster) and waist yellowish brown; mandibles, antennae, legs, and tip of gaster yellow.

Distribution. Laos (Vientiane Province), Thailand (Kalasin and Mukdahan Provinces, Fig. 4).


Figure 4. Distribution of Meranoplus Thai species in Thailand.

Etymology. The specific name is dedicated to Professor Alongklod Tanomtong of Khon Kaen University, who is an excellent specialist in biological sciences in Thailand, who helped and inspired many young biologists.

Habitat. This species can be found in lowland primary forest (300–600 m a.s.l.). The type series was collected from a dry dipterocarp forest. Lao specimens (colony code WJT10-LAO111) were collected from a dry evergreen forest. Specimens from Mukdahan Province, northeastern Thailand were collected in a mixed deciduous forest.

Differential diagnosis. *Meranoplus tanomtongi* sp. nov. is a small species that is most similar to *M. siamensis* sp. nov., see differential diagnosis under *M. siamensis*. This species is also similar in general appearance to *M. malaysianus* and *M. borneensis* from Sundaland, in having two pairs of fenestrae along each lateral margin of the promesonotal shield and having a concave anterior margin of first gastral tergite. However, *M. tanomtongi* can be distinguished from *M. malaysianus* and *M. borneensis* by 1) anterior corners of frontal lobes round and lateral margin weakly convex (right angled and lateral margin almost straight in *M. malaysianus* and *M. borneensis*); 2) petiole in profile subguadrate, almost flat dorsally (round, usually

convex dorsal outline in *M. malaysianus* and *M. borneensis*); 3) entire lateral margin of the promesonotal shield is serrate and convex (parallel sides in *M. malaysianus* and *M. borneensis*); 4) in profile, the tip of petiole acute (truncate in *M. malaysianus* and *M. borneensis*); 5) head in full-face view entirely reticulate (densely reticulate-rugulose in *M. malaysianus* and *M. borneensis*); 6) the petiole and postpetiole are smooth and shiny (sculptured in *M. malaysianus* and *M. borneensis*).

Key to the Oriental and Indo-Australian species based on worker caste (modified from Schödl 1998)

- 1 Postpetiole in profile subquadrate (Fig. 5A); in dorsal view, postpetiole marginated with distinct ridge (Fig. 5B)......**2**
- Postpetiole in profile nodiform and convex dorsal outline (Figs 5C, 6B, C, 7I); in dorsal view, postpetiole not marginated with distinct ridge (Fig. 5D)......3
- In full-face view, anterior corners of frontal lobes right angled and lateral margin almost straight (Fig. 5H); median of head with weak reticulation (Fig. 5I); compound eyes with 8 or 9 ommatidia along longest axis, each facet hexagonal (Fig. 6A)......*M. siamensis* Yodprasit & Jaitrong, sp. nov.
- Petiolar crest never bidentate; postpetiole without an acute, posteriorly directed short spine (Figs 6C, 7I, 8E)

- 5 Propodeum never overhung by the posterior mesonotal margin (Fig. 6G); compound eyes strongly reduced, consisting of 1–2 ommatidia (compound eyes completely absent in some specimens) (Fig. 6H)

- long spines at the corners of the promesonotal shield (Fig. 7E, F). Smaller species (HL < 1.18 mm, TL < 5 mm)**8**

8	Promesonotal shield rectangular, lacking any armament (Fig. 7F). (TL <
	3.0 mm)
-	Promesonotal shield always with conspicuous, specific outstanding projections (Fig. 7G) 10
9	In profile, petiole distinctly obliquely truncate (Fig. 7H); first gastral tergite distinctly shagreened; pilosity consisting of short pubescence and longer outstanding hairs
-	In profile, petiolar crest only narrowly truncate (Fig. 7I); first gastral tergite either entirely smooth or occasionally with shagreening; pilosity on dorsal surfaces consisting of a pelt of equal sized, short hairs
10	Promesonotum with only one pair of posteriorly directed mesonotal
10	spines, without additional posterolateral and/or posterior paramedian mesonotal projections (Fig. 8A)
-	Promesonotum of different shape, always with additional posterolateral
11	and/or posterior paramedian mesonotal projections (Fig. 8B)13 Small species (HL 0.65-0.80 mm); promesonotal shield with a pair
	of posteriorly directed shorter, blunt or acute projections in posterior mesonotal corners (Fig. 8C); dorsal surfaces and appendages without
_	Larger species (HL 0.79–0.96 mm): promesonotal shield with a single
	pair of posteriorly directed longer spines in posterior mesonotal corners
	(Fig. 8D); dorsal surfaces and appendages with long hairs 12
12	Dorsal surfaces of head and promesonotal shield rugose to rugulose-
	reticulate (Fig. 8A); petiole in profile ± an equilateral triangle (Fig. 5C)
_	Dorsal surfaces of head and promesonotal shield shiny, with rugae and
	<i>M hirmanus</i> Schödl 1999
13	Outline of lateral margins of promesonotum convex in dorsal view, each
-	margin with two large translucent fenestrae; promesonotal shield con-
	spicuously shorter than broad, foliaceous (PMI 178–191) (Fig. 8B)
-	Outline of lateral margins of promesonotum in dorsal view not convex,
	with lateral constrictions; margins never provided with four translucent
	fenestrae of that size; promesonotal shield usually longer than broad (PMI
	134–155), rectangular or narrowed towards hind margin, never foliaceous
11	(FIG. 8F)
14	a weak constriction at the level of lateral fenestrae (Fig. 8G)
_	Mesosoma in dorsal view not rectangular, lateral margins never paral-
	lel-sided, conspicuously narrowed towards posterior margin, with distinct
	lateral constrictions at the level of lateral fenestrae (Fig. 8H)17
15	Posterior margin of mesonotum sinuate, with blunt rounded projections,
	lacking distinct spines (Fig. 8G); anterior margin of clypeal mid-portion
	produced into a serrate apron (Fig. 8I) <i>M. biliran</i> Schödl, 1998
-	Posterior margin of mesonotum with distinct acute paramedian spines
	(Fig. 9A); anterior margin of clypeal mid-portion produced into an entire,
	וומווטיי מאוטוו (רוט. אס)וס

- First gastral tergite entirely shagreened, anteriorly sometimes with a faint, minute reticulum; body dark brown (Fig. 9E)....*M. montanus* Schödl, 1998

- In dorsal view, promesonotal shield with spine-like projections only posteriorly (Fig. 9F); anterior margin of clypeal mid-portion produced into a narrow, medially excavated apron (Fig. 9H)......*M. nepalensis* Schödl, 1998



Figure 5. Characters used in key **A**, **B**, **E** *Meranoplus tanomtongi* (holotype, THNHM-100028903) **F**, **G** *M. tanomtongi* (non-type worker, THNHM-1-00028904) **C**, **D** *M. bicolor* (non-type, THNHM-1-00027270) **H** *M. siamensis* (holotype, THNHM-1-00027303) **I** *M. siamensis* (non-type worker, THNHM-1-00028942) **A**, **C** petiole and postpetiole in profile **B**, **D** petiole and postpetiole in dorsal view **E**, **H** anterior corners of frontal lobes **F**, **I** head in full-face view **G** eye.



Figure 6. Characters used in key **A** *Meranoplus siamensis* (non-type worker, THNHM-100028942) **B**, **F** *M*. *castaneus* (non-type, THNHM-1-00027323) **C** *M*. *laeviventris* (non-type, THNHM-1-00027329) **D**, **E** *M*. *bellii* (lectotype, CASENT0908933) **G**, **H** *M*. *dlusskyi* (in Zryanin 2015) **I** *M*. *levis* (holotype, CASENT0902025) **A** eye **B**, **C** petiole and postpetiole in profile **D**, **F**, **G**, **I** mesosoma in profile **E** gaster in dorsal view **H** head in profile.

Discussion

Until now, 19 species (including the two new species) of the genus *Meranoplus* have been known from the Oriental and Indo-Australian regions. Among them, seven species are found in Thailand (*Meranoplus bicolor, M. castaneus, M. laeviventris, M. malaysianus, M. mucronatus, M. tanomtongi* sp. nov., and *M. siamensis* sp. nov.). The presence of a pair of spines or teeth upon the petiolar dorsum, shape of propodeal spines, shapes of petiole and postpetiole were used by Bolton (1981) to distinguish the three species groups (*M. magrettii, M. nanus,* and *M. spininodis* groups) of the genus *Meranoplus* in the Ethiopian zoogeographical region. For Thai species, *M. castaneus* share a pair of spines or teeth on the petiolar dorsum with the members of *M. spininodis* group, but the other characters, such as shapes of promesonotal shield and postpetiole are different from the species group. For the moment, we do not place *M. castaneus* in the *M. spininodis* group. The other Thai species do not



Figure 7. Characters used in key **A**, **B** *Meranoplus levis* (holotype, CASENT0902025) **C**, **D** *M*. *mucronatus* (non-type, THNHM-I-00027335) **E**, **H** *M*. *borneensis* (paratype, CASENT0902030) **F**, **I** *M*. *malaysianus* (non-type, THNHM-I-00028943) **G** *M*. *periyarensis* (in Bharti and Akbar 2014) **A** eye **B C** head in profile **D**, **E**, **F**, **G** promesonotal shield in dorsal view **H**, **I** petiole and postpetiole in profile.

fit in with any species groups from Ethiopian region. Schödl (2007) separated the *M. diversus* species group from other groups in Australia by the clypeal morphology. So far, no Thai species belongs to the *M. diversus* group. Boudinot and Fisher (2013) divided the Madagascan *Meranoplus* species into two species groups (*M. mayri* and *M. nanus* groups) using sculpturing of the head and promesonotal shield, and the length of propodeal spines. The *M. nanus* species group has the subtriangular petiole and the round postpetiole, thus *M. bicolor* from Southeast Asia should belong to *M. nanus* species group. Schödl (1998) revised the oriental species of *Meranoplus* based on external morphology of the worker caste. He did not mention the species groups. We followed his morphological characters to distinguish the Thai species of the genus.

The body size, the length of posterior corners of mesonotum and propodeal spines, the shape of promesonotal shield, and the sculpturing on the first



Figure 8. Characters used in key **A** *Meranoplus bicolor* (non-type, THNHM-I-00027254) **B** *M. loebli* (paratype, CASENT0902032) **C** *M. rothneyi* (lectotype, CASENT0915542) **D**, **E** *M. birmanus* (holotype, CASENT0919716) **F** *M. laeviven-tris* (non-type, THNHM-I-00027329) **G**, **I** *M. biliran* paratype, CASENT0902033) **H** *M. boltoni* (holotype, CASENT0902031). **A–D**, **F–H** promesonotal shield in dorsal view **E** petiole and postpetiole in profile I clypeus in full-face view.

> gastral tergite were used to distinguish the species of *Meranoplus* in previous papers (Bolton 1981; Schödl 1998, 2007; Boudinot and Fisher 2013). We also use these characters to separate the Thai species. The shape of frontal lobes is an important characteristic that can be used to distinguish the two new species (see couplet 2 in the key; see also Fig. 5E, H for comparison). This character was not used in the previous works. The two new species are small, and they have the subrectangular postpetiole when seen in profile and its dorsum is almost flat or shallowly concave. These characters are unique within *Meranoplus*, and thus the new species are placed in a distinct group (*Meranoplus siamensis* species group).

> Members of the ant genus *Meranoplus* can be found throughout Thailand from lowland to highland (Fig. 4). *Meranoplus castaneus*, *M. malaysianus*, and *M. mucronatus* were mainly found in Sundaland (Borneo, Indonesia, and



Figure 9. Characters used in key **A**, **B**, **E** *Meranoplus montanus* (in Bharti and Akbar 2014) **C**, **G** *M*. *boltoni* (holotype, CASENT0902031) **D** *M*. *periyarensis* (in Bharti and Akbar 2014) **F**, **H** *M*. *nepalensis* (paratype, CASENT0902026) **A**, **F** promesonotal shield in dorsal view **B**, **G**, **H** clypeus in full-face view **C** petiole and postpetiole in profile **D**, **E** body in profile.

Malaysia) (Schödl 1998). Recently, only *M. castaneus* and *M. mucronatus* were recorded in Thailand. *Meranoplus malaysianus* is recorded for the first time in the country. In Thailand, these three species are restricted to the south. The northernmost limit distribution range of *M. malaysianus* is in Songkhla Province (ca 530 km south of the Isthmus of Kra).

All species of the ant genus *Meranoplus* in Thailand nest in soil and are usually found walking on the ground except *M. castaneus*, which nests in dead branches in the canopy (ca 35 m above the ground in evergreen and swamp forests). Itino and Yamane (1995) collected *M. castaneus* in the canopy (25–35 m above ground) in mixed dipterocarp forest in Malaysia. Jantarit et al. (2009) and Watanasit et al. (2007) also found *M. castaneus* high on trees in evergreen forests. *Meranoplus castaneus* can be identified as an arboreal ant.

The two new species were found to nest in soil and walk on the ground. *Meranoplus siamensis* sp. nov. was found in the dry evergreen forest in eastern

Thailand and in the dry dipterocarp forest in the western, northeastern, and central parts of the country. *Meranoplus tanomtongi* sp. nov. was collected from dry dipterocarp and mixed deciduous forests in northeastern Thailand. This species was also found in a dry evergreen forest in Laos (colony No. WJT10-LAO111 and WJT10-LAO111), but the body size of Lao population is slightly larger than the type series.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

This research was approved by the Institutional Animal Care and Use Committee, Mahidol University, certificate number FTM-ACUC 001/2024E.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

Morphological and molecular data warrant the description of a new species of the genus *Scutiger* (Anura, Megophryidae) from the Central Himalaya

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Abstract

Recent phylogenetic studies in Himalayan lazy toads of the genus *Scutiger* Theobald, 1868 revealed the presence of genetically deeply divergent lineages. The taxonomy of *S. nepalensis* sensu lato was re-assessed based on museum material considering molecular and morphological data. The results strongly support the recognition of a new species, *S. kanjiroba* **sp. nov.** distributed along the Nepalese Kanjiroba massif. It is further shown that *S. sikimmensis* has an apparently much more restricted distribution range than previously thought. The frequent misidentification of *Scutiger* across large areas of the Himalaya-Tibet area highlights the significance of careful taxonomic evaluation of collection material and the need for the direct morphological comparison of closely related species when describing new species.

Key words: Genetics, Himalaya, lazy toad, montane forests, morphology, taxonomy

Introduction

The Himalaya, recognised as one of the world's biodiversity hotspots (Myers et al. 2000; Mittermeier et al. 2011), hosts a unique assemblage of flora and fauna, characterised by exceptional species richness and high levels of endemism. However, many species in this region remain taxonomically and biogeographically understudied, leading to knowledge gaps that hinder our understanding of fundamental aspects of the evolutionary history of Himalayan biota and the development of effective conservation strategies. Compounding these challenges is the escalating threat to Himalayan biodiversity posed by human activities such as deforestation, habitat fragmentation, poaching, and climate change (Pandit 2013; Paudel et al. 2018; Kattel 2022). Consequently, species and data collected from the Himalayan region hold immense conservation value and are crucial for unravelling broad-scale biodiversity patterns and their underlying mechanisms (e.g., Agarwal et al. 2014; Schmidt et al. 2016, 2023).



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Lazy toads of the genus Scutiger Theobald, 1868, family Megophryidae, are endemic to the Himalaya-Tibet orogenic system and adapted to high montane and alpine areas. They occur from northern Pakistan, through Nepal, Bhutan, northern India, Myanmar, in the valleys of southern and eastern Tibet, and eastwards to southwestern China. According to Frost (2024), the genus comprises 29 recognised species, most of which distributed in the Hengduan Shan (Fig. 1). Only eight species are known from the Himalaya Mountain range: S. occidentalis Dubois, 1978 from its western part; S. nepalensis Dubois, 1974, S. sikimmensis (Blyth, 1855), and S. ghunsa Khatiwada, Shu, Subedi, Wang, Ohler, Cannatella, Xie, & Jiang, 2019 from the central Himalayan region; S. bhutanensis Delorme & Dubois, 2001, S. nyingchiensis Fei, 1977, S. spinosus Jiang, Wang, Li, & Che, 2016, and S. wuguanfui Jiang, Rao, Yuan, Wang, Li, Hou, Che, & Che, 2012 from the eastern part. Most of them show a disjunct distribution pattern which is hypothesised to result from the displacement of the species' habitats from ancestral Tibet during the surface uplift of the Himalaya-Tibet orogen (HTO). The uplift-associated aridification of a warm temperate Miocene-Tibet, coupled with high extirpation rates of ancestral populations, and species range shifts along the drainage systems and epigenetic transverse valleys of the rising Himalaya explain the evolution of the present-day Himalayan Scutiger fauna most parsimoniously (Hofmann et al. 2024).



Figure 1. Overview of the Himalaya-Tibet orogenic area and occurrences of *Scutiger* species. Holotype localities are indicated by symbols with a central black dot. Some species lack the type locality, others could only be approximated due to imprecise information in the original description.

Interestingly, the assumed wide distribution range of the long-known species S. nepalensis differs considerably from the limited occurrences of other Himalayan Scutiger species. The type locality of this species is given as Khaptar [= Khaptad] area, in the Doti District of Chainpur in western Nepal. The species is assumed to occur in mountains between 3000 and 5000 m across a wide area of western Nepal (Jetz et al. 2012: https://mol.org/species/map/Scutiger_nepalensis). This large distribution contrasts with the above-mentioned biogeographic scenario that would lead us to expect a significant genetic structure and divergences with clear geographical pattern among the Himalayan Scutiger taxa. Recent advances in the phylogeography of Scutiger have revealed deeply divergent lineages with disjunct ranges within Himalayan taxa (Hofmann et al. 2017), necessitating a morphological and genetic re-evaluation of existing museum material considering the new phylogenetic findings. The presence of these genetically distinct lineages advocates the need for a taxonomic intervention, and we describe one of them as a new species herein, based on morphological and molecular evidence.

Materials and methods

Sampling

All voucher specimens and tissue samples for newly generated molecular data were obtained from museum holdings [Muséum national d'Histoire naturelle, Paris (**MNHN**); Natural history museum, Erfurt (**NHME**)] and had been collected in Nepal between 1973 and 1999. In total, the paratype series of *Scutiger nepalensis* (n = 5), and four adult specimens, one subadult, and one tadpole individual of *S. nepalensis* sensu lato (s. l.) were investigated morphologically. For all these *S. nepalensis* s. l. we obtained molecular data. Additional DNA sequencing data of *S. nepalensis* s. l. were generated from tissue samples, available at the NHME, collected along with the voucher specimens by capture and release of individuals. The list of samples and their associated metadata are presented in Suppl. material 1: tables S1–S3.

Laboratory work and molecular analysis

Total genomic DNA was isolated from tissues preserved in ethanol using the Qiagen DNeasy kit (Qiagen Inc.) following the manufacturer's protocol. We amplified partial sequences of the following three mitochondrial (mt) and three nuclear (nu) loci via the polymerase chain reaction (PCR): 16S rRNA (550 bp), cytochrome oxidase subunit 1 (COI, 668 bp), and cytochrome b (Cytb, 985 bp), as well as beta-fibrinogen intron 7 (Bfib7, 508 bp), cyclin B2 gene intron 3 (Ccnb2, 777 bp), and recombination activating protein 1 gene (Rag1, 957 bp); for primers and PCR conditions see Hofmann et al. (2017). Heterozygotes in electropherograms of the nuclear loci were identified based on secondary peak calling. All protein coding gene fragments were translated into amino acids; no frameshift mutations or premature stop codons were observed. Nuclear alleles were not phased because most populations were represented by only a few or single individuals which did not allow a robust statistical inference of haplo-types. Therefore, polymorphic sites were encoded with the appropriate IUPAC

ambiguity code. All newly generated sequences were deposited in GenBank (accession numbers: Suppl. material 1: tables S2, S3). Additional *Scutiger* and appropriate outgroup sequences corresponding to the six molecular target loci were retrieved from GenBank, combined with our new sequences, and subsequently aligned for each marker using MEGA11 software (Tamura et al. 2021).

DNA sequences from the three mitochondrial loci of each species were concatenated and used for Bayesian inference (BI) analysis, while the alignments of the three nuclear gene fragments were used separately for network analysis. Because several *Scutiger* species are represented in GenBank only by COI DNA sequence information, we additionally constructed a BI tree based on that single barcoding gene.

Phylogenetic trees were inferred with MrBayes using *Oreolalax chuanbeiensis* Tian, 1983 and *O. omeimontis* (Liu & Hu, 1960) (concatenated mtDNA dataset), or *Oreolalax xiangchengensis* Fei & Huang, 1983 (COI dataset) as outgroup. Mr-Bayes was run for up to 5 million generations, sampling every 500th generation. We used four parallel Markov chain Monte Carlo simulations with four chains and discarded the first 25% of the samples of each run as burn-in. Chain convergence was monitored with Tracer v. 1.7.1 (Rambaut et al. 2018). Phylogenetic networks were generated based on the nuDNA sequence data and uncorrected p-distances using the Neighbor-Net algorithm (Bryant and Moulton 2004) implemented in SplitsTree v. 4.19.2 (Huson and Bryant 2006). We also calculated genetic distances [uncorrected p-distance and based on maximum composite likelihood (MCL)] between species, and the mean genetic distance between samples within the new species using MEGA11.

Morphological analysis

The following measurements were taken with dial callipers and recorded to the nearest 0.1 mm: Snout vent length (SVL), distance from tip of snout to posterior edge of vent; head length (HL), distance from angle of jaws to snout-tip; head width (HW), measured at posterior angle of jaws; snout length (SL), from tip of snout to anterior corner of eye; distance of naris to tip of snout (NSD); horizontal diameter of eye (ED); eyelid-naris distance (END), from naris to anterior edge of the eye; internarial distance (IND); forearm length (FAL), from flexed elbow to base of inner metacarpal tubercle; hand length (HAL), from base of inner metacarpal tubercle to tip of 3rd finger; femur (thigh) length (FEL), from vent to outer edge of flexed knee; tibia (shank) length (TIBL), distance from outer edge of flexed knee to tip of heel; length of tarsus (TaL), distance from heel to proximal edge of inner metatarsal tubercle; foot length (FOL), distance from proximal edge of inner metatarsal tubercle to tip of 4th toe. We further recorded the relative length of fingers and toes, presence/absence of subarticular tubercles, presence/absence of vocal sac, presence/absence of vomerine (v)/maxillary (m) dentation, presence/absence of tympanum, webbing of toes (developed, weak, rudimentary, absent), finger(s) with nuptial spines, presence/absence of pectoral (p)/axillary (ax) glands, gland size relation (p>>/>/= ax), presence/absence of spines on p/ax, presence/absence of spines on inner forearms, presence/absence of spines at belly, presence/absence of tubercles/warts on dorsal (and lateral) surfaces of body and/or limbs, presence/ absence of black spines on dorsal tubercles. Sex was determined by the presence of nuptial spines on fingers and chest in males in breeding condition.

A Principal Component Analysis (PCA) was performed in R 4.3.3 (RCoreTeam 2024) using the packages FactoMineR (Le et al. 2008) and ggplot2 v. 3.5.0 (Wickham 2016). The PCA was based on metric data (values were normalised by SVL prior to PCA, except ratios) and included the unknown taxon (*Scutiger nepalensis* s. l.) together with the phylogeographical closest species, namely *S. ghunsa* and *S. nepalensis*. For *S. sikimmensis*, comparable morphometric data for individual features were not available to us (see also Discussion). We further assessed SVL and the normalised metric characters for equal variances (F \geq 0.05) across species via Levene's test and analysed them by an analysis of variance (ANOVA) and TukeyHSD post hoc test. Because of the small sample size per group, we combined males and females for analysis.

The labial tooth row formula (**LTRF**) of the tadpole was recorded according to Dubois (1995), and developmental stage was determined following Gosner (1960).

Comparative specimens

Scutiger nepalensis Dubois, 1974 [1974.1096–1974.1098 and 1989.3361– 1989.3362, paratypes; one specimen of *S. nepalensis* (A1724/09); six specimens of *S. nepalensis* s. I. (A0570/99, A0574/99, A0576/99, A1248/05, A1249/05, one tadpole A1250/05) stored at the NHME]. Morphological data of other *Scutiger* species were extracted from original species descriptions and/ or recompiled treatises, particularly (Fei et al. 2009, 2012).

All adult specimens were assigned to the genus *Scutiger* based on the following combination of features: toad-like morphology, tympanum, and tympanic ring entirely absent, pupil vertically elliptic, femoral glands indistinct, tongue rounded or slightly indented behind, fingers free, toes free or with some webbing, dorsum warty, pectoral (and axillary) glands and fingers with nuptial spines (in breeding males) (Myers and Leviton 1962; Fei et al. 2009; Fei and Ye 2016). Additional specimens had their species identity confirmed through molecular data.

Results

mtDNA analysis and phylogenetic networks of nuclear genes

Phylogenetic trees of the mtDNA genes (Fig. 2, concatenated, 2203 bp: 16S, COI and Cytb; Suppl. material 1: fig. S1, 631bp: COI) and networks of the nuclear genes Bfib7, Ccnb2, and Rag1 (Fig. 3) recover specimens from the Kanjiroba Himal as distinct, deeply divergent sister clade of *S. nepalensis*, which is maximal supported in the tree from the concatenated data. The placement of the Kanjiroba/*S. nepalensis* clade in the tree might be discordant depending on the amount and type of molecular data (as is the case for the majority of known *Scutiger* taxa). However, its distinctness is robust and supported by COI uncorrected genetic distances to congeners which range between 7.4% and 15.5% (MCL: 5.9% and 13.5%). Noteworthy, the overall range of COI genetic distance between *Scutiger* species is from 2.4% to 16.7% (MCL: 1.9% to 14.4%; Suppl. material 1: tables S4, S5). Mean uncorrected p-distance within the Kanjiroba clade was $1.6\% \pm 0.3$ (MCL: $1.6\% \pm 0.4$).





Figure 2. Bayesian inference tree based on the concatenated mtDNA sequence data of 16S, COI and Cytb. Node values are Bayesian posterior probabilities \geq 0.7. Holotype (*) and paratype specimens are indicated bold. Only samples with data for more than one locus were included.



Figure 3. Neighbor-Net networks (Bryant and Moulton 2004) of the nuclear **A** Bfib7 **B** Ccnb2, and **C** Rag1 sequence information retrieved in *Scutiger*.

Due to low quality and concentration of the DNA and resulting difficulties in amplification, the following specimens were only barcoded through 16S rRNA: for A0570/99 and A1254/05 we recovered > 99% identity and coverage to/of sequences of *S. kanjiroba* sp. nov., e.g., KY310787– KY310792 (Suppl. material 1: table S2), while A1724/09 (PP766266) matched unambiguously *S. nepalensis*.

Morphological comparison

The morphological comparisons between the *Scutiger* specimens from the Kanjiroba range and their congeners are comprehensively outlined in Suppl. material 1: table S5. Considering the geographic distribution of *Scutiger* species (Fig. 1) and our phylogenetic findings, the differentiation between the Kanjiroba lineage and *S. nepalensis*, *S. ghunsa*, and *S. sikimmensis*, holds significant relevance; all details of the morphological comparison among these taxa (except *S. sikimmensis*) are presented in Suppl. material 1: table S6. In the PCA analysis, specimens from the Kanjiroba range formed a distinct cluster, with the first component (PC1) explaining 48% of the data variance and the second (PC2) explaining 16% (Fig. 4). Subsequent ANOVA and TukeyHSD post hoc tests uncovered several statistically significant differences among the Kanjiroba lineage, *S. ghunsa*, and *S. nepalensis* (Suppl. material 1: fig. S2, table S7).

The *Scutiger* specimens from the Kanjiroba Himal can be readily distinguished from the geographically neighbouring *S. nepalensis* by a narrower and smaller head shape [HW/SVL: 0.34 (0.33-0.35) vs 0.40 (0.37-0.42); HL/SVL: 0.28 (0.27-0.29) vs 0.32 (0.30-0.34), and in a shorter foot length [FOL/SVL: 0.42 (0.41-0.43) vs 0.45 (0.40-0.47)] (Suppl. material 1: fig. S3, tables S6, S7). Of note, the head in *S. nepalensis* seems to be larger than in any other *Scutiger* species (Suppl. material 1: fig. S4). From other congeners the Kanjiroba lineage can be separated by a suite of morphological features as follows:

- S. adungensis the Kanjiroba lineage is distinguished by absence of vocal sac in adult males (vs presence); smaller body size (in males; SVL 55.4 mm vs 71.0–73.0 mm); the absence of maxillary teeth (vs presence); presence of numerous small, densely arranged spines on the surface of fingers I, II, and inner surface of finger III of males in breeding condition (vs large spines on inner two fingers); two pairs of spine patches on chest of breeding male (vs one pair).
- S. bangdaensis the Kanjiroba lineage is distinguished by rudimentary webbing between toes (vs developed), and larger body size (SVL: males 55.4 mm vs 45.5–50.0 mm; females 54.2–66.7 mm vs 48.0–49.5 mm).
- S. bhutanensis the Kanjiroba lineage is distinguished by presence of numerous small, densely arranged spines on surface of fingers I, II, and inner surface of finger III of males in breeding condition (vs large nuptial spines on first two fingers of males).
- S. biluoensis the Kanjiroba lineage is distinguished by absence of vomerine and maxillary teeth (vs presence); presence of nuptial spines on dorsal surface of fingers I, II, and inner surface of finger III of males in breeding condition (vs on inner two fingers); smaller male body size (SVL 55.4 mm vs 73.0 mm).
- S. boulengeri the Kanjiroba lineage is distinguished by rudimentary webbing between toes (vs developed); absence of spine patches on belly of males in breeding condition (vs presence).

- S. brevipes the Kanjiroba lineage is distinguished by rudimentary webbing between toes (vs developed); presence of nuptial spines on dorsal surface of fingers I, II, and inner surface of finger III of males in breeding condition (vs on inner two fingers).
- S. chintingensis the Kanjiroba lineage is distinguished by larger body size (SVL: males 55.4 mm vs 42.0–42.4 mm; females 54.2–66.7 mm vs 48.0–52.8 mm); absence of maxillary teeth (vs presence); rudimentary webbing between toes (vs developed, large or reduced); absence of spines on inner surface of upper arm and forearm of males in breeding condition (vs presence).
- S. feiliangi the Kanjiroba lineage is distinguished by larger body size (SVL: males 55.4 mm vs 45.7–50.2 mm; females 54.2–66.7 mm vs 48.9–51.5 mm); absence of spines on inner surface of forearm of males in breeding condition (vs presence); the absence of maxillary teeth (vs presence); absence of pectoral/axillary glands with spines in females in breeding condition (vs presence); absence of spines on belly of females in breeding condition (vs presence).
- S. ghunsa the Kanjiroba lineage is distinguished by larger body size (male: 55.4 mm vs 42.1-47.8 mm; female: 54.2-66.7 mm vs 50.2-53.9 mm); longer head [HL/SVL: 0.28 (0.27-0.29) vs 0.25 (0.24-0.26)]; shorter hand length [HAL/SVL: 0.25 (0.24-0.26) vs 0.46 (0.43-0.54)]; longer snout (SL/SVL male: 0.15 (0.14-0.15) vs 0.11 (0.10-0.12)]; relative length of toes 4>3>5>2>1 (vs 4>5>3>2>1); absence of tympanum (vs hidden tympanum); rudimentary webbing between toes (vs absence of webbing).
- S. glandulatus the Kanjiroba lineage is distinguished by smaller body size (SVL, male: 55.4 mm vs 67.0-81.0; female: 54.2-66.7 mm vs 77.0-81.0); relative length of toes 4>3>5>2>1 (vs 4>5>3>2>1); absence of subarticular tubercles (vs presence); rudimentary webbing between toes (vs developed); presence of nuptial spines on dorsal surface of fingers I, II, and inner surface of finger III of males in breeding condition (vs on inner two fingers); presence of spines on male axillary glands (vs absence).
- S. gongshanensis the Kanjiroba lineage is distinguished by absence of vocal sac in adult males (vs presence); absence of maxillary teeth (vs presence); presence of numerous small, densely arranged spines on the surface of fingers I, II, and inner surface of finger III of males in breeding condition (vs large spines on inner two fingers); rudimentary webbing between toes (vs absence of webbing); presence of a pair of pectoral and axillary patches in breeding male (vs one pair); presence of spine on tubercles on dorsum (vs absence);
- S. *jiulongensis* the Kanjiroba lineage is distinguished by smaller male body size (SVL, 55.4 mm vs 67.4–81.5 mm; absence of subarticular tubercles (vs presence); presence of nuptial spines on dorsal surface of fingers I, II, and inner surface of finger III of males in breeding condition (vs on inner two fingers).
- *S. liupanensis* the Kanjiroba lineage is distinguished by absence of maxillary teeth (vs presence); rudimentary webbing between toes (vs developed).
- S. luozhaensis the Kanjiroba lineage is distinguished by absence of more than one and up to six separated spines on top of each dorsal tubercle of males in breeding condition (vs presence of up to six spines on tubercles); absence of yellow tubercles scattered around cloaca of males in breeding condition (vs presence).
- S. maculatus the Kanjiroba lineage is distinguished by absence of subarticular tubercles (vs presence); rudimentary webbing between toes (vs developed).

- S. mammatus the Kanjiroba lineage is distinguished by absence of subarticular tubercles (vs presence); rudimentary webbing between toes (vs developed, large or reduced); presence of nuptial spines on dorsal surface of fingers I, II, and inner surface of finger III of males in breeding condition (vs on inner two fingers); presence of spines on male axillary glands (vs absence).
- S. meiliensis the Kanjiroba lineage is distinguished by absence of maxillary teeth (vs presence); presence of nuptial spines on dorsal surface of fingers I, II, and inner surface of finger III of males in breeding condition (vs on inner two fingers).
- *S. muliensis* the Kanjiroba lineage is distinguished by presence of nuptial spines on dorsal surface of fingers I, II, and inner surface of finger III of males in breeding condition (vs on inner two fingers); presence of a pair of pectoral and axillary patches in breeding male (vs one pair).
- S. ningshanensis the Kanjiroba lineage di is distinguished by absence of maxillary teeth (vs presence); absence of spine patches on belly of males in breeding condition (vs presence).
- S. nyingchiensis the Kanjiroba lineage is distinguished by absence of maxillary teeth (vs presence); rudimentary webbing between toes (vs developed).
- S. occidentalis the Kanjiroba lineage is distinguished by a smaller head (HL: 14.5–19.1 mm vs 23.5 mm; HW: 18.2–23.2 mm vs 24.5 mm); absence of spines on male forearm in breeding condition (vs presence); absence of dorsal tubercles with 1–6 coloured tips (corresponding to spines in breeding condition) on top (vs presence, Suppl. material 1: fig. S5); rudimentary webbing between toes (vs developed).
- *S. pingwuensis* the Kanjiroba lineage is distinguished by absence of spines on male forearm in breeding condition (vs presence); absence of spine patches on belly of males in breeding condition (vs presence).
- S. sikimmensis the Kanjiroba lineage is distinguished by relative length of toes 4>3>5>2>1 (vs 4>5>3>2>1); presence of spines on dorsal tubercles of males (vs absence); see also Suppl. material 1: fig. S6 for syntypes of S. sikimmensis and Discussion.
- S. spinosus the Kanjiroba lineage is distinguished by absence of spines on male upper arm in breeding condition (vs presence); absence of spines on dorsal tubercles of females (vs presence); wider internarial distance (5.6–6.8 mm vs 4.0–4.8 mm).
- S. tengchongensis the Kanjiroba lineage is distinguished by larger male body size (SVL: 55.4 mm vs 36.0-40.1 mm); absence of small patches of black spines near armpit of males in breeding condition (vs presence).
- S. tuberculatus the Kanjiroba lineage is distinguished by smaller male body size (SVL: 55.4 mm vs 68.0–76.0 mm); presence of nuptial spines on dorsal surface of fingers I, II, and inner surface of finger III of males in breeding condition (vs on inner two fingers).
- S. wanglangensis the Kanjiroba lineage is distinguished by absence of maxillary teeth (vs presence); absence of spine patches on belly of males in breeding condition (vs presence).
- S. wuguanfui the Kanjiroba lineage is distinguished by absence of vocal sac in adult males (vs presence); absence of small black spines on upper chest (vs presence).



Figure 4. PCA of 15 metric variables in 17 adult specimens **A** with 95% confidence ellipses **B** with loadings. Abbreviations: SVL snout–vent length, HL head length; HW head width; SL snout length; ED horizontal diameter of eye; END eyelid-naris distance; IND internarial distance; FAL forearm length; HAL hand length; FEL femur (thigh) length; TIBL tibia (shank) length; TaL length of tarsus; FOL foot length.

Taxonomy

Based on the synthesis of molecular phylogenetic findings and observed morphological distinctions, the *Scutiger* populations inhabiting the Kanjiroba Himal region in western Nepal are deemed to constitute a novel species. Consequently, we provide a detailed description of this species.

Scutiger kanjiroba sp. nov.

https://zoobank.org/5C6D6A08-E956-43C6-B2E8-3F505AC8F38A Fig. 5; Suppl. material 1: figs S3, S7, tables S5, S6 Suggested common name: Kanjiroba lazy toad

Scutiger nepalensis: Hofmann et al. (2017), partim.

Type material. Holotype. NEPAL • 1 ♂; NHME A0576/99; adult; collected at Juphal in the direction to Tripurakot, Dolpa district, NEPAL (29°01'N, 82°47'E, 2600 m a.s.l.) on 31/05/1997 by Marco Fischer; deposited in the NHME. Paratypes. NE-PAL • 1 2; NHME A0570/99; adult; and 1 juvenile; NHME A1247/05; collected at Talphi, Jumla district, NEPAL (29°18'N, 82°20'E, 3400 m a.s.l.) on 16/06/1997 by Marco Fischer; deposited in the NHME. 1 2; NHME A0574/99; adult; collected at Kaigaon, Jumla district, NEPAL (29°06'N, 82°35'E, 3600 m a.s.l.) on 03/06/1997 by Marco Fischer; deposited in the NHME. 1 ♀; NHME A1248/05, adult, collected at Dhauli Lake, Jumla district, NEPAL (29°21'N, 82°23'E, 4400 m a.s.l.) on 05/07/1999 by Marco Fischer, deposited in the NHME. 1 subadult; NHME A1249/05; collected at Dhauli Lake, Jumla district, NEPAL (29°21'N, 82°23'E, 4400 m a.s.l.) on 05/07/1999 by Marco Fischer, deposited in the NHME. 1 tadpole; NHME A1250/05; collected at Dhauli Lake, Jumla district, NEPAL (29°21'N, 82°23'E, 4400 m a.s.l.) on 05/07/1999 by Marco Fischer, deposited in the NHME. 2 juveniles; NHME A1251/05 and A1252/05; collected at Dhauli Lake, Jumla district, NEPAL (29°21'N, 82°23'E, 4400 m a.s.l.) on 05/07/1999 by Marco Fischer, deposited in the NHME. 1 juvenile; NHME A1253/05; collected at Bumra [Bumramadichaur], Jumla district, NEPAL (29°23'N, 82°08'E) on 22/06/1999 by Ulrich Scheidt, deposited in the NHME. 1 juvenile; NHME A1254/05; collected at Maharigaon, Jumla district, NEPAL (29°20'N, 82°23'E) on 08/07/1999 by Andreas Weigel, deposited in the NHME. 1 metamorp; NHME A2014/13; collected near Khari La, Jumla district, NEPAL (29°21'N, 82°09'E, 3285 m a.s.l.) in 1999 by Marco Fischer, deposited in the NHME.

Diagnosis. Scutiger kanjiroba sp. nov. is assigned to the genus Scutiger based in the above-mentioned morphological features and by its molecular phylogenetic position relative to other Scutiger species. It can be separated from all other species of the genus Scutiger by the combination of the following characters (for character states in other species see section Morphological comparison, above): (1) moderate body size, SVL adult male 55.4 mm (n = 1), adult female 54.2–66.7 mm (n = 3); (2) vomerine and maxillary dentation absent; (3) vocal sac absent; (4) subarticular tubercles absent; (5) presence of numerous small, densely arranged spines on the surface of fingers I, II and inner surface of finger III of males in breeding condition; (6) relative length of fingers 3>4>2>1; (7) spines on (inner) (fore)arms absent; (8) toes rudimentary





webbed; (9) relative length of toes 4>3>5>2>1; (10) a pair of pectoral glands and a pair of axillary glands; (11) spines on pectoral and axillary glands present in males; (12) belly without spines; (13) tubercles/warts on dorsal and lateral surfaces of body present; (14) dorsal tubercles with black spines of males in breeding condition.

Description of holotype. (Fig. 5): Measurements are provided in Suppl. material 1: table S6; all morphological measurements in mm. Adult male SVL 55.4; head moderate, wider than length (HW/HL 1.3); snout short (SL 7.9) and rounded, slightly protruding beyond jaw; canthus rostralis obvious; nostril dorsolateral, closer to tip of snout than eyes (SND 3.4, END 4.0); loreal region slightly oblique and concave; internarial distance larger than distance from anterior margin of eye to nostril (IND/END 1.4); eye moderately in size (EHD/HL 0.4); pupil vertical; interorbital space flat; tympanum and tympanic ring absent; supratympanic ridge moderately thick, from posterior part of upper eyelids to shoulder; tongue oval; choanae oval, widely separated, visible when viewed from below; vomerine and maxillary teeth absent; vocal sac absent.

Forelimbs strong; forearm of median length (FAL/SVL 0.25) and of similar length as the hand (HAL/SVL 0.24), without spines; fingers slender, without webbing and lateral dermal fringes; all fingertips rounded, not dilated; relative finger lengths: 3>4>2>1; subarticular tubercles absent; inner metacarpal tubercle flat and distinct, lager than the outer, indistinct metacarpal tubercle; nuptial spines on dorsal and lateral surface of first and second fingers, and on inner side of third finger.

Hindlimbs robust, moderate in length (TIBL/SVL = 0.38), thighs slightly shorter than shanks FEL/TIBL = 1.1), heels do not touch when folded at right angles to the body (see also Fig. 5A); foot longer than shank (FOL/TIBL = 1.1); tips of toes round; toes not webbed and without lateral fringes; toes relative lengths 4>3>5>2>1; subarticular tubercles absent; moderately large inner metatarsal tubercle, outer metatarsal tubercle indistinct.

Dorsal and lateral skin with distinct tubercles in preservative, each tubercle with creamy coloured keratinised tip; tubercles with small, keratinised tips present below and on supratympanic fold and on shank; forehead and surfaces of lower arm relatively smooth; throat and belly surface smooth; a pair of pectoral glands and a pair of axillary glands present on chest, pectoral glands only slightly larger than axillary glands, all covered by tiny spines.

Sexual dimorphism. In males, a pair of pectoral glands and a pair of axillary glands present on breast (absent in females), all of them covered by spines in breeding season.

Colour in preservative. The background colouration of the dorsum fades to grey, tubercles with creamy white point, dorsal surface of finger I, II and inner surface of finger III pale brown; ventral belly pale greyish; ventral limbs, ventral chest, and ventral head greyish pale brown.

Tadpole. One tadpole (NHME A1250/05) of *Scutiger kanjiroba* sp. nov. was collected at stage 36 (Gosner, 1960) (Suppl. material 1: fig. S7); LTRF: 1:2+2/2+2:1.

Distribution and ecological notes. *Scutiger kanjiroba* is a forest-dwelling species that is currently known from high-montane areas of the Kanjiroba Himal at altitudes between ~ 3300 and 4400 m a.s.l. in west Nepal (Fig. 1).

Etymology. The specific epithet *kanjiroba* is a geographical proper name referring to the Kanjiroba Himal in Nepal. The southern area of the Kanjiroba Himal is so far the only known occurrence region of this new species.

Discussion

Lazy toads of the genus *Scutiger* are a characteristic faunal element of the Himalaya-Tibet orogen. These toads possess limited dispersal capabilities, are adapted to high altitudes, and are typically found throughout the Himalaya Mountain range, exhibiting allopatric distribution with a high degree of local endemism. Previous research has revealed extensive geographically structured relationships among Himalayan *Scutiger* species, indicating significant physical barriers not only between species but also among populations (Hofmann et al. 2017). As taxonomic efforts intensify, we anticipate more frequent

re-evaluation of *Scutiger* populations and taxonomic descriptions across the Himalaya. Ideally, this process will yield precise taxonomic conclusions and (hopefully) contribute to resolving the taxonomic confusion within this group.

The newly identified species *Scutiger kanjiroba* sp. nov. is phylogenetically distinct from the geographically neighbouring *S. nepalensis* Dubois, 1974, forming its sister lineage, as confirmed by both morphology and nuclear molecular analysis. It can be easily distinguished from *S. nepalensis* in the field by a much smaller and narrower head and an overall smaller body size. The two species are geographically separated by the Karnali River gorge, which probably acts as physical barrier to dispersal for terrestrial, non-flying organism groups.

The other species long known from the Central Himalaya, S. sikimmensis (Blyth, 1855), lacks a specified type locality in Sikkim. A neotype (BMNH 1887.11.2.15) had been assigned by Dubois (Dubois 1987 "1986") from Yak La, Sikkim, but was set aside by the discovery of two syntypes ZSIC 9854-55 which originated from the Sikkim area (see Suppl. material 1: fig. S6). The (invalid) neotype is separated by a straight-line distance of <20 km from vouchers recently sampled in Yadong, Tibet, China. The latter had been sequenced and identified as S. sikimmensis. The S. kanjiroba sp. nov. populations from west Nepal do not share a common distribution area with S. sikimmensis and do not form a common phylogenetic clade with this species. Additionally, S. ghunsa Khatiwada, Shu, Subedi, Wang, Ohler, Cannatella, Xie & Jiang, 2019 is geographically situated between S. kanjiroba sp. nov. and S. sikimmensis. This implies that even if the exact location of S. sikimmensis is not precisely known, it can be assumed that S. kanjiroba sp. nov. represents a different species, which can be clearly distinguished morphologically and at the molecular level from S. sikimmensis. It further implicates that the geographic range of S. sikimmensis is vastly overestimated, e.g., by the International Union for Conservation of Nature (IUCN) Red List, scientific web applications like Map of Life (Jetz et al. 2012), and in the pertinent literature (e.g., Schleich and Kästle 2002). We assume that the distributional area of S. sikimmensis is mainly restricted to the Chumbi valley, with temperate climate, at the intersection of India (Sikkim), Bhutan, and China (Tibet) in the eastern part of the Himalaya (Suppl. material 1: fig. S8). The Chumbi valley corresponds largely to the administrative unit Yadong County in the Tibet Autonomous Region of China and is separated by the Dongkya (Chola) Mountain range from the southeastern frontier of Sikkim; the Dongkya range is cut by several passes, e.g., Cho La, Yak La, Nathu La, and Jelep La.

The discovery of *S. kanjiroba* sp. nov. in the Kanjiroba massif, west Nepal, adds to the recent description of *S. ghunsa* in east Nepal (Khatiwada et al. 2019), underscoring the high degree of amphibian diversity in Himalayan cloud forests and the conservation importance of these habitats.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: SH. Data curation: SH, DJ. Formal analysis: SH. Funding acquisition: SH. Investigation: SH. Methodology: SH, JS. Project administration: SH. Resources: DJ, JS. Visualization: SH. Writing – original draft: SH. Writing – review and editing: DJ, JS.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Supplementary information

Authors: Sylvia Hofmann, Daniel Jablonski, Joachim Schmidt Data type: pdf

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Research Article

Four new earthworm species of the genera *Amynthas* and *Metaphire* (Oligochaeta, Megascolecidae) from Hunan and Anhui provinces, China

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Abstract

This paper describes four new species earthworms from Hunan and Anhui provinces, China, *Amynthas xiangtanensis* Qiu & Jin, **sp. nov.**, *Amynthas taoyuanensis* Qiu & Jin, **sp. nov.**, *Amynthas xuanchengensis* Jin & Li, **sp. nov.** and *Metaphire donganensis* Jin & Jiang, **sp. nov.** *Amynthas xiangtanensis* **sp. nov.**, and *A. taoyuanensis* **sp. nov.** belong to the *Amynthas corticis* group. Both have four pairs of intersegmental spermathecal pores in 5/6–8/9; male pores in segment XVIII, separated by 1/3 of body circumference, each on top of a slightly raised porophore, surrounded by several tiny genital papillae. *Amynthas taoyuanensis* **sp. nov.** belongs to the *Amynthas morrisi* group, it has two pairs of spermathecal pores in 5/6 and 6/7; male pores in XVIII, separated by 1/3 of body circumference, each on top of a slightly raised, circular porophore. *Metaphire donganensis* **sp. nov.** belongs to the *Metaphire houlleti* group. It has three pairs of spermathecal pores in 6/7–8/9; male pores in XVIII, separated by 1/3 of body circumference, each on top of a slightly raised, circular porophore. *Metaphire donganensis* **sp. nov.** belongs to the *Metaphire houlleti* group. It has three pairs of spermathecal pores in 6/7–8/9; male pores in XVIII, separated by 1/3 of body circumference, each on top of a slightly raised, circular porophore. *Metaphire donganensis* **sp. nov.** belongs to the *Metaphire houlleti* group. It has three pairs of spermathecal pores in 6/7–8/9; male pores in XVIII, separated by 1/3 of body circumference, each on the bottom center of the longitudinal copulatory chamber.

Key words: Barcode, COI gene, Megascolecidae, new species, Oligochaeta, taxonomy

Introduction

Earthworms belonging to the family Megascolecidae are the most important and widely distributed in China. Hunan Province (24°38'-30°08'N, 108°47'-114°15'E) is located in the south of China and has a subtropical monsoon climate. Anhui Province (29°41'-34°38'N, 114°54'-119°37'E) is located in the east of China, a transitional region between the warm temperate zone and the subtropical zone. Both provinces are among the most abundant biological diversity areas in China, yet only a few earthworm species have been reported there. Previously, only five (*Amynthas moniliatus moniliatus* (Chen, 1946), *A. triastriatus triastriatus* (Chen, 1946), *Metaphire biforatum* Tan & Zhong, 1987, *M. bifoliolare* Tan & Zhong, 1987 and *M. hunanensis* Tan & Zhong, 1986) and eight (*A. carnosus carnosus* (Goto & Hatai, 1899), *A. corticis* (Kinberg, 1867), *A. hupeiensis* (Michaelsen, 1895), *A. loti* (Chen & Hsu, 1975), *A. pectieniferus*



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Copyright: © Qing Jin et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). (Michaelsen, 1931), *A. robustus* (Perrier, 1872), *M. guillelmi* (Michaelsen, 1895) and *M. tschiliensis tschiliensis* (Michaelsen, 1928)) Megascolecidae species have been recorded from Hunan and Anhui, respectively (Chen 1946, 1959; Chen and Hsu 1977; Zeng et al. 1982; Tan and Zhong 1986, 1987).

In 2015, 2016, and 2019, we investigated earthworm diversity in the two provinces and more than 31 and 24 Megascolecidae species were recorded from Hunan and Anhui, respectively. In this paper, we describe three new species of the genus Amynthas and one new species of the genus Metaphire found in those surveys (Fig. 1). Among them, A. xiangtanensis sp. nov. and A. taoyuanensis sp. nov. belong to the A. corticis group with intersegmental spermathecal pores in 5/6-8/9, which is widely distributed in China (such as Hainan, Yunnan, and Guangxi provinces) (Sun et al. 2012, 2018, 2021; Dong et al. 2019; Yuan et al. 2019). Amynthas xuanchengensis sp. nov. belongs to the A. morrisi group with intersegmental spermathecal pores in 5/6 and 6/7, which is widely distributed in China including Hainan, Yunnan, and Guangdong provinces (Zhao et al. 2013; Jiang et al. 2015; Sun et al. 2015, 2021). Metaphire donganensis sp. nov. belongs to the M. houlleti group, with intersegmental spermathecal pores in 6/7-8/9, which is also widely distributed in China including Jiangxi, Gansu, and Fujian provinces (Feng 1984; Sun et al. 2018). DNA barcodes of the four new species are provided (PP497092-PP497100) in this paper.

Materials and methods

The earthworms were collected in 2015, 2016, and 2019. Specimens were anaesthetized in a 10% ethanol solution and preserved in a 95% ethanol solution. Holotypes and paratypes are deposited in the Shanghai Natural History Museum.



Figure 1. Distribution of four species in Hunan Province and Anhui provinces, China.

DNA was extracted from several specimens of A. xiangtanensis sp. nov., A. taoyuanensis sp. nov., A. xuanchengensis sp. nov., M. donganensis sp. nov. by using the E.Z.N.A. Mollusc DNA Kit (Omega Bio-tek, Norcross, GA, USA). The gene cytochrome c oxidase subunit I (COI) was amplified by polymerase chain reaction (PCR). The PCR amplification mixture (50 µL total) consisted of 1 µL of DNA template, 2 µL of each primer, 35.4 µL double-distilled H₂O, and 9.6 µL Trans Taq[™] Polymerase High Fidelity containing 0.6 µL TransTaqTM HiFi DNA polymerase, 4 µL 2.5 mM dNTPs and 5 µL 10 × TransTaq[™] HiFi Buffer I. Primers used in the research were COI,5'-GGTCAACAAATCATAAGATATTGG-3' and 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et al. 1994). The PCR was carried out as follows: 5 min at 94 °C followed by 32 cycles 94 °C for 30 s, 50 °C for 30 s and 72 °C for 60 s, with an extension of 10 min at 72 °C. Sequencing was performed in the Beijing Genomics Institute (Shanghai, China). COI sequences of the new species were submitted to the NCBI GenBank databases under the accession numbers provided in Table 1, and sequences of other similar known species were retrieved from GenBank (Table 1). All the DNA sequences were aligned using ClustalX 2.0 (Thompson et al. 1997). The genetic pairwise distances between these species were calculated using the Kimura two-parameter model (Kimura 1980) in MEGAX with 1000 bootstrap replicates (Kumar et al. 2018).

 Table 1. Specimens with molecular data used in this study. Some species have no molecular data in GenBank. Abbreviations, HT holotype, PT paratype.

Species	Species number	GenBank acc. no.
Amynthas xiangtanensis sp. nov. (HT)	P1CJHUSH190510083 N1-05A	PP497097
Amynthas xiangtanensis sp. nov. (PT)	P1CJHUSH190519808 N5-02	PP497098
Amynthas xiangtanensis sp. nov. (PT)	P1CJHUSH190526781 N5-01	PP497099
Amynthas taoyuanensis sp. nov. (HT)	P1CJHUSH190517069 N8-01A	PP497095
Amynthas taoyuanensis sp. nov. (PT)	P1CJHUSH190526781 R8-04	PP497096
Amynthas xuanchengensis sp. nov. (PT)	P1CJHUSH190521800 Q6-03	PP497100
Metaphire donganensis sp. nov. (PT)	P1CJHUSH190511779 N9-03	PP497092
Metaphire donganensis sp. nov. (PT)	P1CJHUSH190512778 N11-01	PP497094
Metaphire donganensis sp. nov. (PT)	P1CJHUSH190512096 Q3-04	PP497093
Amynthas corticis (Kingberg, 1867)	HN201035-02	KF205966
Amynthas maximus Qiu & Dong, 2019	GX201304-01	MG450707
Amynthas tortuosus Qiu & Dong, 2019	GX201306-06	MG450708
Amynthas stricosus Qiu & Sun, 2012	HN201104-04	JX315345
Amynthas homosetus (Chen, 1938)	-	No data in GenBank
Amynthas genitalis Qiu & Sun, 2012	-	No data in GenBank
Amynthas recavus Yuan & Jiang, 2019	YN201109-09	KF205473
Amynthas endophilus Zhao & Qiu, 2013	HN201011-03	KF240560
Amynthas fucatus Zhao & Qiu, 2013	HN201114-01	KF151185
Amynthas infuscuatus Jiang & Sun, 2015	-	No data in GenBank
Amynthas zonarius Sun & Qiu, 2015	HN201114-06	JQ982486
Amynthas baikmudongensis Hong, 2017	-	No data in GenBank
Metaphire vulgaris agricola (Chen,1930)	-	No data in GenBank
Metaphire tschiliensis lanzhouensis (Feng, 1984)	-	No data in GenBank
Metaphire viridis Feng & Ma, 1987	-	No data in GenBank
Metaphire ptychosiphona Qiu & Zhong, 1993	-	No data in GenBank
Metaphire sanmingensis Sun & Jiang, 2018	FJ201008-02	KY774380

Taxonomy

Family Megascolecidae Rosa, 1891 Genus *Amynthas* Kinberg, 1867

Amynthas xiangtanensis Qiu & Jin, sp. nov.

https://zoobank.org/63FF5853-828D-40A1-A933-8A108DEF9196 Fig. 2

Material examined. Holotype. • 1 clitellate (P1CJHUSH190510083 N1-05A), CHI-NA, Hunan Province, Xiangtan City (27.98312°N, 112.81616°E), 47 m elevation, brownish yellow soil under shrub in farmland, 10 May 2019, JB Jiang, JL Li and BY Yin. Paratypes. 10 clitellates in total • 2 clitellates (P1CJHUSH190510083 N1-05B), CHINA, Hunan Province, Xiangtan City (27.98312°N, 112.81616°E), 47 m elevation, brownish yellow soil under shrub in farmland, 10 May 2019, JB Jiang, JL Li and BY Yin • 2 clitellates (P1CJHUSH190519808 N5-02), CHINA, Hunan Province, Yongzhou City (25.83566°N, 112.27331°E), 163 m elevation, brown soil under weeds in field, 19 May 2019, JB Jiang, JL Li and Y Wang • 2 clitellates (P1CJHUSH190526781 N5-01), CHINA, Hunan Province, Liling City (27.54671°N, 113.54837°E), 74 m elevation, brown soil under weeds in farmland, 26 May 2019, JB Jiang, JL Li and Y Wang • 2 clitellates (P1CJHUSH190514791 N13-01), CHINA, Hunan Province, Yueyang City (29.07035°N, 113.23038°E), 57 m elevation, yellow soil under vegetable field in farmland, 14 May 2019, Y Dong, YF Qin and YZ Wu • 2 clitellates (AH201612-02), CHINA, Anhui Province, Huangshan City (30.58531°N, 117.87033°E), 506 m elevation, brown soil under vegetable field in farmland, 8 May 2016, JB Jiang, J Sun, Y Dong and Y Zheng.

Diagnosis. Size medium to large. Spermathecal pores in 5/6–8/9, separated by 1/3 of body circumference. Male pores in XVIII, separated by 1/3 of body circumference, each on the top of a slightly raised, circular porophore. Spermathecae four pairs in VI–IX, ampulla heart-shaped, duct thick and ~ 1/4 of ampulla. Diverticulum is ~ 2/3 of main pouch (duct and ampulla together), terminal 1/2 dilated into rod-shaped seminal chamber. Intestinal caeca are simple. Prostate glands are well developed.

External characters. Yellowish brown dorsal pigmentation, pale yellowish brown ventral pigmentation. Dimensions 110–184 mm by 5.0–7.0 mm at clitellum, segments 111-133. Annulus present on VIII-XIII. The dorsal midline is clearly visible and purplish brown. First dorsal pore of all examined individuals in 9/10. Prostomium 1/2 epilobous. Clitellum annular, taupe, in XIV- XVI, swollen, setae invisible externally, but dorsal pores visible on clitellum. Setae numbering 16-28 at III, 26-32 at V, 40-46 at VIII, 46-54 at XX, 54-58 at XXV; 14-16 between male pores; 8-12 (V), 10-12 (VI), 12-14 (VII) and 14-17 (VIII) between spermathecal pores; setal formula, aa = 1.0-1.2ab, zz = 1.4-2.0zy. Male pores one pair in XVIII, separated by 1/3 of body circumference, each on the top of a slightly raised, circular porophore. A pair of oval medium-sized flat-topped papillae on XVIII, after the setae ring near male pores, the interval is $\sim 1/4$ of body circumference (Fig. 2A). Sometimes another pair of similar papillae present after male pores, the interval is ~ 1/3 of body circumference (specimen P1CJHUSH190519808 N5-02) (Fig. 2E); another pair similar papillae present on the ventrum of XVIII, but near to the ventral line (specimen P1CJHUSH190514791 N13-01) (Fig. 2F). Female



Figure 2. A. xiangtanensis sp. nov. A ventral view showing spermathecal pores, and male pores B prostate glands C intestinal caeca D spermathecae E male pores of paratypes (P1CJHUSH190519808 N5-02) F male pores of paratypes (P1CJHUSH190514791 N13-01). Abbreviations: sp, spermathecal pores; mp, male pores.

pore single in XIV, oval, milky white. Spermathecal pores four pairs in 5/6-8/9, ventral, small eye-like, milky white porophore in center, separated by 1/3 of body circumference. A pair of oval medium-sized flat-topped papillae before the setae ring on the ventrum of IX, the interval of two papillae separated by ~ 1/9 of body circumference (Fig. 2A). Sometimes another pair of similar papillae present on the same position of VIII (specimen P1CJHUSH190526781 N5-01).

Internal characters. Septa 5/6–7/8 thick and muscular, 10/11–12/13 slightly thickened, 8/9 and 9/10 absent. Gizzard long bucket-shaped, in IX–X. Intestine enlarged distinctly from XV. Intestinal caeca paired in XXVII, extending anteriorly to XXIII, simple, smooth on both sides or sometimes weakly constricted on ventral margin (Fig. 2C). Four esophageal hearts in X–XIII, the latter three are more developed than the first pair. Male sexual system holandric, testis sacs two pairs in X and XI, well developed, left and right lobes separated on the ventral side. Seminal vesicles two pairs in XI and XII, well developed, left and right lobes separated on the ventral side. Prostate glands well developed, inserting in XVIII and extending to XIV and XXI, coarsely lobate, prostatic duct U-shaped, slightly thicker at the distal part, a large lumpy accessory gland beside the prostatic duct (Fig. 2B). Spermathecae four pairs in VI–IX, ampulla heart-shaped, ~ 2.5–4.5 mm long in holotype; ampulla duct is thick and ~ 1/4 of ampulla. Diverticulum is ~ 2/3 of main pouch (duct and ampulla together), slender, terminal 1/2 dilated into rod-shaped seminal chamber. A pair of large lumpy accessory glands present on the ventrum of IX, corresponding to the position of papillae (Fig. 2D). Sometimes another pair of large lumpy accessory glands present on the ventrum of VIII, corresponding to the position of papillae. **Etymology.** The species is named after its type locality.

Remarks. Amynthas xiangtanensis sp. nov., with four pairs of spermathecal pores in 5/6–8/9, keys to the Amynthas corticis group (Sims and Easton 1972) which consists of 111 species (Nguyen et al. 2020a, 2020b; Sun et al. 2021; Li et al. 2024); it is similar to A. taoyuanensis sp. nov. in setae number, spermathecal pores, male pores, and simple intestinal caeca (Table 2). However, A. xiangtanensis sp. nov. (110-184*5.0-7.0, yellowish brown dorsum and pale yellowish brown ventrum) is easily distinguished from A. taoyuanensis sp. nov. (length 41-120*3.5-4.5, colorless) by its larger body size and pigmentation. In addition, the first dorsal pore of A. xiangtanensis sp. nov. is in 9/10, but 10/11 in A. taoyuanensis sp. nov.; clitellum in XIV-XVI of A. xiangtanensis sp. nov., while XIV-2/3XVI in A. taoyuanensis sp. nov.; paired papillae before setae in VIII or IX in A. xiangtanensis sp. nov., but paired papillae after setae in VI, VII or VIII in A. taoyuanensis sp. nov.; two or more papillae near male pore of A. xiangtanensis sp. nov., whereas two pairs or more in XVI, XVII, XVIII or XIX of A. taoyuanensis sp. nov.; prostate gland well developed in A. xiangtanensis sp. nov., nevertheless prostate glands degenerated in A. taoyuanensis sp. nov.; spermathecae of A. xiangtanensis sp. nov. (~ 2.5-4.5 mm long, ampulla heart-shaped, duct ~ 1/4 of ampulla) are different from A. taoyuanensis sp. nov. (~ 0.6-1.8 mm long, ampulla oval-shaped, duct is thick and $\sim 1/2$ of ampulla) by size and shape; diverticulum terminal 1/2dilated into rod-shaped seminal chamber in A. xiangtanensis sp. nov., but terminal 3/4 dilated into bag-shaped seminal chamber in A. taoyuanensis sp. nov.

The new species is also fairly close to *Amynthas corticis* (Kinberg, 1867) by having medium to large size, spermathecal pores location and simple intestinal caeca (Table 2). However, the new species differs from *A. corticis* in pigmentation (yellowish brown dorsum and pale yellowish brown ventrum), first dorsal pore in 9/10, paired papillae within spermathecal pore region before setae in VIII or IX, male pores middle and round by 1/3C, two or more papillae near male pore, prostate glands in XIV–XXI with a large lumpy accessory gland, ampulla heart-shaped, diverticulum terminal 1/2 dilated into rod-shaped seminal chamber, paired large lumpy accessory glands on VIII or IX. Whereas *A. corticis* has a greenish brown dorsum and an unpigmented ventrum, first dorsal pore in 11/12, paired papilla before or after setae in some or all near spermathecal pores, male pores small and circular to transverse elliptical disc by 1/4C–1/3C, one or more papillae near male pore, prostate glands in XVII–XX without accessory glands, ampulla ovoid, diverticulum straight stalked, terminally dilated into a blunt ovoid seminal chamber, accessory glands stalked, coelomic, bound down to the parietes or retained within body wall.
pores, mp, male pore	S.									
Character	A. xiangtanensis	A. taoyuanensis	A. corticis	A. maximus	A. tortuosus	A. stricosus	A. homosetus	A. genitalis	A. recavus	
Body size (mm)	110-184*5.0-7.0	41-120*3.5-4.5	45-170*3.0-6.0	145-170*5.8-6.2	55-86*2.5-2.8	72-97*2-2.8	116*5.2	83-97*2.3-2.5	58-64*2.1-2.3	
Pigment dorsum	Yellowish brown	None	Greenish brown	Pale purple-brown to brown	Purple-brown to pale purple-brown	None	Dark chocolate to grey	None	Pink to pale brown	
Pigment ventrum	Pale yellowish brown	None	None	None to yellowish	Pale purple-brown to None	None	Grey	None	None	
First dorsal pore	9/10	10/11	11/12	13/14	13/14	11/12 or 12/13	12/13	12/13	12/13	
Clitellum	XIV-XVI	XIV-2/3XVI	XIV-XVI	2/5XIV-XVI	XIV-XVI	XIV-XVI	XIV-XVI	XIV-XVI	XIV-XVI	
Setae numbering	16–28/III, 40–46/VIII, 46–54/XX	32-42/III, 44-50/VIII, 50-58/XX	36-40/VII, 40-46/XXV	33–38/III, 29–33/VIII, 18–22/XX	24–26/III, 34–36/VIII, 32–36/XX	30–54/III, 62–72/VIII, 40–70/XX	44/VIII	30–36/III, 32–36/VIII, 38–46/XX	21-22/III, 34-36/VIII, 34-36/XX	
Setae number between sp	14–17 (VIII)	19-22 (VIII)	12 (VIII)	18–22 (VIII)	12–13 (VIII)	23-29 (VIII)	1	8-11 (VIII)	12 (VIII)	
Setae number between mp	14-16 (XVIII)	13-14 (XVIII)	10–14 (XVIII)	9–13 (XVIII)	(III/X) 6–8	10-12 (XVIII)	(III/X)6	11–12 (XVIII)	9-10 (XVIII)	
Ventral distance of sp	1/3C	1/3C	1/3C	1/3C	1/4C	2/5C	1/4C	1/3C	2/5C	
Papillae within sp region	Paired before setae in VIII or IX	Paired after setae in VI, VII or VIII	Paired before or after setae near sp	Two pairs after setae in VII and VIII	Four pairs after setae in VI-IX	None	Invisible	None	None	
Papillae within mp region	Two or more near male pore	Two paired or more in XVI, XVII, XVIII, or XIX	One or more papillae near male pore	Paired before setae medial of male pore	Paired medial of male pore	Single or paired in XVII, XIX, and XX	Invisible	4 paired in XVII, XVIII and XIX	Paired in XVII, 3 papillae in XIX	
Prostate glands	Well developed with accessory gland	Degenerated with accessory glands	Developed	Underdeveloped	Developed	Developed,	Developed	Developed	Developed	
Diverticulum	2/3 of main pouch, terminal 1/2 dilated into rod-shaped seminal chamber	2/3 of main pouch, terminal 2/3 dilated into bag-shaped seminal chamber	Shorter than main pouch and terminal dilated into round or elongate oval seminal chamber	Shorter, lightly twist in middle, terminal 2/5, dilated into rod-shaped seminal chamber	Shorter, terminal 4/5 dilated into S-shaped twisted seminal chamber	As long as main pouch, slender, terminal 2/5 dilated into a band shaped chamber	Shorter, terminal dilated into rod- shaped seminal chamber	Longer than main pouch, terminal 0.29 dilated into rod-shaped seminal chamber	~ 3/5 of main pouch, terminal 1/4 dilated into ovoid-shaped seminal chamber	
Accessory glands	Paired on VIII or IX	Two paired on VII or VIII	Bound down to parietes or retained within body wall	One or two on VI, VII, VII, VII, and IX	One near the each spermatheca	None	Invisible	None	None	

Another similar species with four pairs of spermathecal pores in 5/6-8/9 is Amynthas maximus Qiu & Dong, 2019. The two species share some similarities, such as body size, pigmentation, spermathecal pores, simple intestinal caeca (Table 2). However, A. xiangtanensis sp. nov. has first dorsal pore in 9/10, clitellum XIV-XVI, spermathecal pores, paired papillae before setae in VIII or IX, male pores without ridges, separated by 1/3 of body circumference, two or more papillae near male pore, prostate glands in XIV-XXI with a large lumpy accessory gland, spermathecae larger, ampulla heart-shaped, duct ~ 1/4 of ampulla, diverticulum terminal 1/2 dilated into rod-shaped seminal chamber, paired large lumpy accessory glands on VIII or IX. While A. maximus has first dorsal pore in 13/14, clitellum in 2/5XIV-XVI, fewer setae at VIII and XX, two pairs papillae after setae in VII and VIII, male pores surrounded by 3-4 circular ridges, separated by 2/5 of body circumference, paired papillae before setae medial of male pore, prostate glands in XVII-XIX with accessory glands invisible, spermathecae smaller, ampulla elongate-oval, duct 3/5 of ampulla, diverticulum terminal 2/5 dilated into a swollen, club-shaped seminal chamber, one or two stalked accessory glands on VI, VII, VIII and IX.

Regarding the pigmentation, clitellum, the position of spermathecal and male pores, simple intestinal caeca, and characteristics of the spermathecae, the new species is similar to *Amynthas tortuosus* Qiu & Dong, 2019. However, the two species are distinguished by body size, the position of the first dorsal pore, setae number, the number and position of papillae within the spermathecal pore and male pore region, the characteristics and ventral distance of male pores, the position of prostate glands and the existence or nonexistence of accessory glands, and the characteristics of the diverticulum and accessory glands (Table 2).

In addition, the new species is somewhat similar to *Amynthas stricosus* Qiu & Sun, 2012 in the clitellum, the position of spermathecal and male pores, the ventral distance of male pores, and the simple intestinal caeca. Nevertheless, the two species are different in body size, pigmentation, the position of the first dorsal pore, setae number, the ventral distance of spermathecal pores and the existence or nonexistence of papillae, the characteristics of male pores, the number and position of papillae within the male pore region, the position of prostate glands and the existence or nonexistence of accessory glands, the characteristics of spermathecae, diverticulum and existence or nonexistence of accessory glands (Table 2).

Amynthas taoyuanensis Qiu & Jin, sp. nov.

https://zoobank.org/99EAAAC1-F761-4082-A96E-645F864F631E Fig. 3

Material examined. *Holotype*. • 1 clitellate (P1CJHUSH190517069 N8-01A), CHI-NA, Hunan Province, Yongzhou City (25.30170°N, 111.63432°E), 201 m elevation, brown soil under weeds in dry farmland, 17 May 2019, JB Jiang, JL Li and BY Yin. *Paratypes*. 7 clitellates in total • 3 clitellates (P1CJHUSH190517069 N8-01B), CHINA, Hunan Province, Yongzhou City (25.30170°N, 111.63432°E), 201 m elevation, brown soil under weeds in dry farmland, 17 May 2019, JB Jiang, JL Li and BY Yin • 1 clitellates (P1CJHUSH190526781 R8-04), CHINA, Hunan Province, Liling City (27.63491°N, 113.54768°E), 55 m elevation, yellow soil under weeds in woodland, 26 May 2019, JB Jiang, JL Li and Y Wang • 2 clitellates (HU201601-03), CHINA, Hunan Province, Changde City (28.70779°N, 111.38225°E), 66 m elevation, brown soil under moss and fern in farmland, 2 May 2016, JB Jiang, J Sun, Y Dong and Y Zheng • 1 clitellates (HU201602-04), CHINA, Hunan Province, Changde City (28.65267°N, 111.34672°E), 69 m elevation, reddish brown soil under moss and fern in farmland, 2 May 2016, JB Jiang, J Sun, Y Dong and Y Zheng.

Diagnosis. Size small to medium. Spermathecal pores in 5/6-8/9, separated by 1/3 of body circumference. Male pores in XVIII, separated by 1/3 of body circumference, each on the top of a slightly raised, circular porophore. Spermathecae four pairs in VI–IX, ampulla heart- or rod-shaped, duct is thick and ~ 1/3 of ampulla. Diverticulum is ~ 3/4 of main pouch (duct and ampulla



Figure 3. *A. taoyuanensis* sp. nov. **A** ventral view showing spermathecal pores, and male pores **B** intestinal caeca **C** spermathecae **D** male pores of paratypes (HU201601-03) **E** male pores of paratypes (HU201602-04). Abbreviations: sp, spermathecal pores; mp, male pores.

together), terminal 3/4 dilated into bag-shaped chamber intestinal caeca are simple. Prostate glands are degenerated.

External characters. No pigmentation on dorsal and ventral. Dimensions 41-120 mm by 3.5-4.5 mm at clitellum, segments 49-112. Annulus present on X-XIII. The dorsal midline is clearly visible and green-brown. First dorsal pore of all examined individuals in 10/11. Prostomium 1/2 epilobous. Clitellum annular, grey, in XIV-2/3XVI, smooth, setae numbering 8 at XIV (P1C-JHUSH190517069 N8-01 and HU201602-04), 11 at XV (HU201602-04), 13 at XVI (P1CJHUSH190517069 N8-01) or 14 at XVI (P1CJHUSH190526781 R8-04), dorsal pores visible on clitellum. Setae numbering 32-42 at III, 40-48 at V, 44-50 at VIII, 50-58 at XX, 56-62 at XXV; 13-14 between male pores; 18-19 (V), 18-21 (VI), 19-21 (VII) and 19-22 (VIII) between spermathecal pores; setal formula, aa = 1.0-1.4ab, zz = 1.0-1.4zy. Male pores one pair in XVIII, separated by 1/3 body circumference, each on the top of a slightly raised, circular porophore. Two pairs of circular medium-sized flat-topped papillae present after the setae ring in XVI and XVII, the interval is ~ 1/3 of body circumference. Two pairs of circular medium-sized flat-topped papillae present before the setae ring on XVII and XVIII, the interval is ~ 1/9 of body circumference (Fig. 3A). Sometimes a similar papilla presents in the center of XVII (specimen HU201601-03) (Fig. 3D). Sometimes another similar papilla presents in the center of XIX (specimen HU201602-04) (Fig. 3E). Female pore single in XIV, oval, milky white. Spermathecal pores four pairs in 5/6-8/9, ventral, small eye-like, milky white porophore in center, separated by 1/3 body circumference. A pair of oval medium-sized flat-topped papillae after the setae ring between the spermathecal pores in VII, the interval is ~ 1/4 of body circumference. Sometimes another pair of similar papillae present in the same position of VI (specimen HU201601-03 and HU201602-04). Sometimes another pair of similar papillae present in the same position of VIII (specimen P1CJHUSH190526781 R8-04).

Internal characters. Septa 5/6–7/8 thick and muscular, 10/11–12/13 slightly thickened, 8/9 and 9/10 absent. Gizzard spherical, in IX-X. Intestine enlarged distinctly from XV. Intestinal caeca paired in XXVII, extending anteriorly to XXIV, simple, smooth on both sides (Fig. 3B). Four esophageal hearts in XI-XIII, well developed. Male sexual system holandric, testis sacs two pairs, in X and XI, well developed, left and right lobes separated on the ventral side. Seminal vesicles two pairs, extending in XI and XII, well developed, left and right lobes separated on the ventral side. Prostate glands degenerated, prostatic duct U-shaped inserting in XVIII, several small lumpy accessory glands in center of ventral XVI, XVII, and XVIII; the position is consistent with the position of the ventral mastoid on the body surface. Spermathecae four pairs in VI-IX, ampulla oval-shaped, ~ 0.6-1.8 mm long in holotype; ampulla duct is thick and ~ 1/2 of ampulla. Diverticulum is ~ 2/3 of main pouch (duct and ampulla together), terminal 3/4 dilated into bag-shaped chamber. Two pairs of large lumpy accessory glands present on the ventrum of VII and VIII (Fig. 3C). Sometimes pair of large lumpy accessory glands present on the ventrum of VII (specimen HU201601-03 and HU201602-04).

Etymology. The species is named after its type locality.

Remarks. Amynthas taoyuanensis sp. nov., with four pairs of spermathecal pores in 5/6–8/9, also belongs to the Amynthas corticis group. Amynthas taoyuanensis sp. nov. is close to Amynthas xiangtanensis sp. nov. in setae number, the position and characteristics of spermathecal pores and male pores, and the

simple intestinal caeca. However, the differences between the two new species are body size, pigmentation, the position of the first dorsal pore, the position of clitellum, the position of papillae within spermathecal pores and male pore region, the existence or nonexistence of prostate glands, and the characteristics of the spermathecae and diverticulum (Table 2).

The new species is similar to *Amynthas homosetus* (Chen, 1938) in terms of body size, position and characteristics of spermathecal pores and male pores, and simple intestinal caeca. Whereas, the new species differs from *A. homosetus* in being unpigmented, the first dorsal pore in 10/11, clitellum in XIV–2/3XVI, 13 or 14 setae between male pores, the ventral distance of spermathecal pores is 1/3C, papillae paired after setae in VI, VII, or VIII, two paired or more papillae in XVI, XVII, XVIII, or XIX, prostate glands degenerated, spermathecae ~ 0.6–1.8 mm long, ampulla oval-shaped, duct is thick and ~ 1/2 of ampulla, diverticulum terminal 3/4 dilated into bag-shaped seminal chamber, two paired large lumpy accessory glands on VII or VIII. *Amynthas homosetus* is dark chocolate-colored anteriorly and grey on other parts of dorsum, grey on the ventrum, the first dorsal pore in 12/13, clitellum without setae in XIV–XVI, nine setae between male pores, the ventral distance of spermathecal pores is 1/4C, papillae invisible within spermathecal pores and male pore regions, prostate glands developed in XVI–XXI, spermathecae heart-shaped, diverticulum seminal chamber ovoid (Table 2).

Amynthas taoyuanensis sp. nov. is also close to Amynthas stricosus Qiu & Sun, 2012 by body size, pigmentation, setae number, the characteristics of spermathecal pores and male pores, the ventral distance of male pores, simple intestinal caeca. However, the new species is distinguished from A. stricosus by the first dorsal pore, the position and existence or nonexistence setae of clitellum, and the ventral distance of spermathecal pores; A. taoyuanensis sp. nov. has paired papillae after setae in VI, VII, or VIII, but A. stricosus has no papillae; in addition, A. taoyuanensis sp. nov. has two paired papillae or more in XVI, XVII, XVIII, or XIX, but A. stricosus has a single or paired after setae in XVII, XIX, and XX; prostate glands of A. taoyuanensis sp. nov. are degenerated with several lumpy accessory glands in XVI, XVII, and XVIII, while developed in XVI-XX with accessory glands invisible in A. stricosus; furthermore, spermathecae of A. taoyuanensis sp. nov. are ~ 0.6–1.8 mm long, ampulla oval-shaped, duct is thick and ~ 1/2 of ampulla, whereas ~ 1.6 mm long, ampulla heart-shaped, gradually slender duct as long as ampulla in A. stricosus; diverticulum of A. taoyuanensis sp. nov. is ~ 3/4 of main pouch, terminal 3/4 dilated into bag-shaped seminal chamber, but as long as main pouch, slender, terminal 2/5 dilated into band-shaped seminal chamber in A. stricosus; two paired large lumpy accessory glands on VII or VIII in A. taoyuanensis sp. nov., but no accessory glands in A. stricosus (Table 2).

According to body size, pigmentation, setae ventrally in clitellum, the position and characteristics of spermathecal pores and male pores, simple intestinal caeca, and the characteristics of spermathecae, *A. taoyuanensis* sp. nov. is somewhat similar to *Amynthas genitalis* Qiu & Sun, 2012. However, the new species is characterized by the first dorsal in 10/11, clitellum in XIV–2/3 XVI, 19–22 setae between spermathecal pores (VIII), papillae paired within spermathecal pore region, two paired or more papillae in XVI, XVII, XVIII, or XIX, prostate glands degenerated with several lumpy accessory glands in XVI, XVII, and XVIII, diverticulum ~ 3/4 of main pouch, terminal 3/4 dilated into bag-shaped seminal chamber, two paired large lumpy accessory glands on VII or VIII; whereas *A. genitalis* has 8–11 setae between spermathecal pores (VIII), no papillae within spermathecal pore region, paired papillae before setae annulet in XVIII and XIX, after setae annulet in XVII and XVII, prostate glands developed with accessory glands invisible in XVII–XX, diverticulum longer than main pouch, slender, terminal 0.29 dilated into rod-shaped seminal chamber, no accessory glands (Table 2).

Another similar species with four pairs of spermathecal pores in 5/6–8/9 is *Amynthas recavus* Yuan & Jiang, 2019. The two species share some similarities, such as the position of male pores and simple intestinal caeca. However, the two species are distinguished by body size, pigmentation, the first dorsal pore position, setae number, the ventral distance of spermathecal pores, existence or nonexistence of papillae within spermathecal pore region, the characteristics of male pores and papillae within male pore region, prostate glands, the characteristics of spermathecae and diverticulum, and the existence or nonexistence of accessory glands (Table 2).

Amynthas xuanchengensis Jin & Li, sp. nov.

https://zoobank.org/0CB4FE26-B76E-43AE-95F6-0A2B42139212 Fig. 4

Material examined. *Holotype.* • 1 clitellate (AH201517-06), CHINA, Anhui Province, Xuancheng City (30.89694°N, 118.48889°E), 860 m elevation, black sandy soil under shrubbery in front of the house, 11 October 2015, Y Dong, Z Yuan, MS Chen and YL Wang. *Paratype.* • 1 clitellate (P1CJHUSH190521800 Q6-03), CHINA, Hunan Province, Chenzhou City (26.16809°N, 113.05648°E), 79 m elevation, sandy soil under weeds beside roadways, 21 May 2019, JB Jiang, JL Li and Y Wang.

Diagnosis. Size small. Spermathecal pores in 5/6 and 6/7, separated by 1/3 of body circumference. Male pores in XVIII, separated by 1/4 of body circumference, each on the top of a slightly raised, circular porophore. Spermathecae two pairs in VI and VII, ampulla heart-shaped, duct is thick and ~ 1/2 of ampulla. Diverticulum as long as main pouch (duct and ampulla together), terminal 1/2 dilated into ovoid-shaped seminal chamber. Intestinal caeca are simple. Prostate glands are developed.

External characters. No dorsal or ventral pigmentation. Dimensions 26–32 mm by 1.5-2.0 mm at clitellum, segments 60–65. The dorsal midline is not clearly visible. First dorsal pore of all examined individuals in 12/13. Prostomium 1/2 epilobous. Clitellum annular, taupe, in XIV– XVI, smooth, setae invisible externally. Setae numbering 32–40 at III, 36–44 at V, 40–48 at VIII, 50–54 at XX, 56–58 at XXV; 4 between male pores; setal formula, aa = 1.0-1.4ab, zz = 1.4-2.0zy. Male pores one pair in XVIII, separated by 1/4 body circumference, each on the top of a slightly raised, circular porophore (Fig. 4A). Female pore single in XIV, oval, milky white. Spermathecal pores two pairs in 5/6 and 6/7, ventral, not clearly, milky white porophore in center, separated by 1/3 body circumference.

Internal characters. Septa 5/6–7/8 thick and muscular, 10/11–12/13 slightly thickened, 8/9 and 9/10 absent. Gizzard spherical in IX–X. Intestine enlarged distinctly from XV. Intestinal caeca paired in XXVII, extending anteriorly to XXVI, simple, smooth on both sides (Fig. 4C). Four esophageal hearts in X–XIII, not well developed. Male sexual system holandric, testis sacs two pairs, in X and XI, well developed, left and right lobes separated on the ventral side. Seminal





vesicles two pairs, extending in XI and XII, well developed, left and right lobes separated on the ventral side. Prostate glands well developed, inserting in XVIII and extending to XVI and XIX, coarsely lobate, prostatic duct U-shaped, slightly thicker at the distal part (Fig. 4B). No accessory glands observed. Spermathecae two pairs in VI–VII, ampulla heart-shaped, ~ 0.7 mm long in holotype; ampulla duct is thick and ~ 1/2 of ampulla. Diverticulum as long as main pouch (duct and ampulla together), terminal 1/2 dilated into ovoid-shaped seminal chamber. No accessory glands observed (Fig. 4D).

Etymology. The species is named after its type locality.

Remarks. Amynthas xuanchengensis sp. nov., with two pairs of spermathecal pores in 5/6 and 6/7, can be assigned to Amynthas morrisi group (Sims and Easton 1972) with 55 species included in this group (Sun et al. 2009, 2015, 2021; Shen et al. 2014; Jiang et al. 2015; Hong 2017), such as Amynthas nanulus (Chen & Yang, 1975) (Chen et al. 1975), Amynthas parvus (Chen & Hsu, 1977), Amynthas piagolensis Hong & James, 2001, Amynthas diaoluomontis Qiu & Sun, 2009, Amynthas endophilus Zhao & Qiu, 2013. Amynthas xuanchengensis sp. nov. is very similar to Amynthas endophilus Zhao & Qiu, 2013 by lacking pigmentation, first dorsal pore in 12/13, clitellum in XIV-XVI, the position of spermathecal pores and male pores, no papillae within spermathecal pore and male pore regions, simple intestinal caeca. However, the new species differs from A. endophilus by smaller body size and fewer setae. And beyond that, the ventral distance of spermathecal pores and male pores is 1/3C and 1/4C in A. xuanchengensis sp. nov., but 2/5C and 1/3C in A. endophilus; spermathecal pores of A. xuanchengensis sp. nov. are inconspicuous, but obvious in A. endophilus; male pores of A. xuanchengensis sp. nov. not surrounded by folds, but A. endophilus surrounded by four folds; prostate glands in XVI-XIX in A. xuanchengensis sp. nov., but XVII-XXI in A. endophilus; spermathecae ~ 0.7 mm long, ampulla heart-shaped, duct is thick and ~ 1/2 of ampulla of A. xuanchengensis sp. nov., while spermathecae longer, ampulla elongated ovoid, duct a little shorter than ampulla in A. endophilus; diverticulum as long as main pouch in A. xuanchengensis sp. nov., nevertheless shorter than main pouch in A. endophilus (Table 3).

Another similar species with two pairs of spermathecal pores in 5/6 and 6/7 is Amynthas fucatus Zhao & Qiu, 2013. The two species share some similarities, such as clitellum in XIV-XVI, the position of spermathecal pores and male pores, invisible papillae within spermathecal pore region, and simple intestinal caeca. By contrast, the new species and A. fucatus can be separated on the basis of smaller body size and fewer setae at VIII. In addition, A. xuanchengensis sp. nov. is unpigmented, but dark red brown before clitellum and pale after clitellum in dorsum, pale red brown before clitellum and pale after clitellum in ventrum in A. fucatus; the first dorsal pore of A. xuanchengensis sp. nov. in 12/13, but 11/12 in A. fucatus; the ventral distance of spermathecal pores and male pores are 1/3C and 1/4C in A. xuanchengensis sp. nov., but 2/5C and 2/5C in A. fucatus; spermathecal pores of A. xuanchengensis sp. nov. are inconspicuous, but obvious in A. fucatus; male pores of A. xuanchengensis sp. nov. not surrounded by folds, but A. fucatus surrounded by three to five folds; invisible papillae within male pore region of A. xuanchengensis sp. nov., whereas two papillae on the inner side of male pore, another paired in XVIII in A. fucatus; prostate glands in XVI-XIX of A. xuanchengensis sp. nov., while XV-XXI with accessory glands invisible in A. fucatus; spermathecae ~ 0.7 mm long, ampulla

Table 3. A comparison of characters of A. xuanch	<i>engensis</i> sp. nov	I. and similar species	of the Amynthas morri	si group.
Abbreviations: sp, spermathecal pores, mp, male p	pores.			

Character	A. xuanchengensis	A. endophilus	A. fucatus	A. infuscuatus	A. zonarius	A. baikmudongensis
Body size (mm)	26-32*1.5-2.0	96*3	137*4.0	60-78*1.4-1.6	52-103*1.6-3.1	75*4.3
Pigment dorsum	None	None	Dark red brown to pale	Purple to pale brown	None	None
Pigment ventrum	None	None	Pale red brown to pale	None	None	-
Setae numbering	32-40/III, 40-48/VIII, 50-54/XX	56/III, 72/V, 84/VIII	44/III, 60/VIII, 40/XX	46-48/III, 49-52/VIII, 42-46/XX	38-56/III, 46-52/VIII, 34-54/XX	24/VII, 53/XX
Setae number between mp	4 (XVIII)	2 (XVIII)	8 (XVIII)	8-10 (XVIII)	6-12 (XVIII)	7 (XVIII)
Spermathecal pores	Inconspicuous, 1/3C	Obvious, 2/5C	Obvious, 2/5C	Obvious, 1/3C	Inconspicuous, 2/5C	Inconspicuous
Male pores	1/4C	Surrounded by folds, 1/3C	Surrounded by folds, 2/5C	Surrounded by folds, 1/3C	Surrounded by folds, 1/3C	Large circular raised pads in XVIII
Papillae within mp region	None	None	Two in the inner side of male pore., another paired in XVIII	Two in the inner side of male pore	One or two in the inner side of male pore, sometimes another two on XVIII	None
Prostate glands	Developed	Developed	Well developed	Developed	Developed	Underdeveloped
Diverticulum	As long as main pouch, terminal 1/2 dilated into ovoid-shaped seminal chamber	4/5 of main pouch, terminal 3/5 dilated into zonal seminal chamber	1/3 of main pouch, terminal 2/5 dilated into virgulate seminal chamber	Slightly longer than main pouch, terminal 1/3 dilated into swollen seminal chamber	Long as main pouch, terminal 1/2 dilated into band-shaped seminal chamber	1/2 of main pouch, terminal 1/2 dilated into club-shaped seminal chamber

heart-shaped in *A. xuanchengensis* sp. nov., nevertheless spermathecae longer, ampulla elongated ovoid in *A. fucatus*; diverticulum is as long as main pouch, terminal 1/2 dilated into ovoid-shaped seminal chamber in *A. xuanchengensis* sp. nov., but diverticulum ~ 1/3 of main pouch, straight, terminal 3/4 dilated into virgulate seminal chamber in *A. fucatus* (Table 3).

Amynthas xuanchengensis sp. nov. appears to be closely related to A. infuscuatus Jiang & Sun, 2015 in the combined characters of ventrum pigmentation, first dorsal pore in 12/13, clitellum in XIV-XVI, the position and characteristics of spermathecal pores, invisible papillae within spermathecal pore region, the position of male pores, simple intestinal caeca, and ampulla heart-shaped. Conversely, they still have some differences, such as body size, dorsum pigmentation, and setae number. Amynthas xuanchengensis sp. nov. smaller, without pigment on dorsum, but purple before VIII; pale brown after VIII in A. infuscuatus; four setae between male pores of A. xuanchengensis sp. nov., but 8-10 setae in A. infuscuatus; the ventral distance of male pores is 1/4C in A. xuanchengensis sp. nov., but 1/3C in A. infuscuatus; spermathecal pores of A. xuanchengensis sp. nov. are inconspicuous, but obvious in A. infuscuatus; male pores of A. xuanchengensis sp. nov. surrounded by no folds, but A. infuscuatus surrounded by three or four folds; invisible papillae within male pore region of A. xuanchengensis sp. nov., whereas two papillae on the inner side of male pore in A. infuscuatus; prostate glands in XVI-XIX of A. xuanchengensis sp. nov., while XVI-1/2XX with accessory glands invisible in A. infuscuatus; spermathecae ~ 0.7 mm long, duct is thick and ~ 1/2 of ampulla in A. xuanchengensis sp. nov., but spermathecae longer, duct slender, twice as long as ampulla in A. infuscuatus; diverticulum is as long as main pouch, terminal 1/2 dilated into ovoid-shaped seminal chamber in A. xuanchengensis sp. nov., but diverticulum longer than main pouch, slender, terminal 1/3 dilated into swollen seminal chamber in A. infuscuatus (Table 3).

Amynthas xuanchengensis sp. nov. and Amynthas zonarius Sun & Qiu, 2015 share some common characters in pigmentation, first dorsal pore in 12/13, clitellum in XIV-XVI, the characteristics of spermathecal pores, invisible papillae within spermathecal pore region, the position of male pores, simple intestinal caeca. In contrast, A. xuanchengensis sp. nov. differs from A. zonarius in smaller body size and fewer setae. Additionally, the ventral distance of spermathecal pore and male pores are 1/3C and 1/4C in A. xuanchengensis sp. nov., but 2/5C and 1/3C in A. zonarius; male pores of A. xuanchengensis sp. nov. surrounded by no folds, but A. zonarius surrounded by five folds; invisible papillae within male pore region of A. xuanchengensis sp. nov. whereas one or two papillae on the inner side of male pore, sometimes another two on XVIII in A. zonarius; prostate glands in XVI-XIX in A. xuanchengensis sp. nov., while XVI-1/2XX with accessory glands invisible in A. zonarius; spermathecae ~ 0.7 mm long, ampulla heart-shaped, duct is thick and ~ 1/2 of ampulla in A. xuanchengensis sp. nov., whereas spermathecae longer, ampulla ovoid, duct as long as ampulla in A. zonarius; ovoid-shaped seminal chamber in A. xuanchengensis sp. nov., but band-shaped seminal chamber in A. zonarius (Table 3).

In terms of pigmentation, clitellum, setae number, the positions of spermathecal pores and male pores, inconspicuous spermathecal pores, invisible papillae within spermathecal pore and male pore regions, simple intestinal caeca. *Amynthas xuanchengensis* sp. nov. is somewhat similar to *Amynthas baikmudongensis* Hong, 2017. On the contrary, the new species is easily distinguished from *A. baikmudongensis* by body size, prostate glands, the characteristics of male pores, spermathecae, and diverticulum (Table 3).

Genus Metaphire Sims & Easton, 1972

Metaphire donganensis Jin & Jiang, sp. nov.

https://zoobank.org/25F88443-9F42-49B6-AC05-114236A5D710 Fig. 5

Material examined. Holotype. • 1 clitellate (HU201613-01A), CHINA, Hunan Province, Dongan City (26.35499°N, 111.19531°E), 172 m elevation, brown soil under vegetable field in farmland, 4 May 2016, JB Jiang, J Sun, Y Dong, and Y Zheng. Paratypes. 13 clitellates in total • 1 clitellate (HU201613-01B), CHINA, Hunan Province, Dongan City (26.35499°N, 111.19531°E), 172 m elevation, brown soil under vegetable field in farmland, 4 May 2016, JB Jiang, J Sun, Y Dong and Y Zheng • 2 clitellates (P1CJHUSH190511779 N9-03), CHINA, Hunan Province, Liuyang City (28.32795°N, 113.52008°E), 112 m elevation, red soil under vegetable in vegetable garden, 11 May 2019, Y Dong, YF Qin and YZ Wu • 7 clitellates (P1CJHUSH190512778 N11-01), CHINA, Hunan Province, Yueyang City (28.91995°N, 113.70132°E), 179 m elevation, brown soil under rape in rape field, 12 May 2019, Y Dong, YF Qin and YZ Wu • 1 clitellate (P1CJHUSH190512096 Q3-04), CHINA, Hunan Province, Linxiang City (29.33550°N, 113.40176°E), 51 m elevation, brown sandy soil under shrub in grove, 12 May 2019, Y Dong, YF Qin and YZ Wu · 2 clitellates (P1CJHUSH190518092 Q6-01), CHINA, Hunan Province, Yuanjiang City (29.04369°N, 112.29798°E), 48 m elevation, brown soil under litter next to the house, 18 May 2019, Y Dong, YF Qin and YK Li.



Figure 5. *M. donganensis* sp. nov. **A** ventral view showing spermathecal pores, and male pores **B** prostate glands **C** intestinal caeca **D** male pores of paratype (P1CJHUSH190511779 N9-03) **E** spermathecae. Abbreviations: sp, spermathecal pores; mp, male pores.

Diagnosis. Size medium to large. Spermathecal pores in 6/7–8/9, separated by 1/3 of body circumference. Male pores in XVIII, separated by 1/3 of body circumference, each on the bottom center of the longitudinal copulatory chamber. Sometimes the copulatory chamber eversion is ridged. Spermathecae three pairs in VII–IX, ampulla heart- or rod-shaped, duct thick and as long as ampulla. Diverticulum as long as main pouch (duct and ampulla together), slender and straight at proximal part, terminal 1/2 dilated into twisted in zigzag fashion. Intestinal caeca are simple. Prostate glands are well developed.

External characters. Pale brown dorsal and ventral pigmentation. Dimensions 72–159 mm by 4.6–7.0 mm at clitellum, segments 78–111. Annulus present on IX–XVIII. The dorsal midline is clearly visible and purplish brown. First dorsal pore of all examined individuals in 12/13. Prostomium 1/2 epilobous. Clitellum annular, pale taupe, in XIV– XVI, smooth, setae invisible externally. Setae numbering 20–34 at III, 20–46 at V, 28–58 at VIII, 52–66 at XX, 58–70 at XXV; 8–13 between male pores; 14–17 (VI), 16–20 (VII) and 17–21 (VIII) between spermathecal pores; setal formula, aa = 1.0–1.6ab, zz = 1.2–1.8zy. Male pores one pair in XVIII, separated by 1/3 body circumference, each on the bottom center of the longitudinal copulatory chamber, multiple radioactive folds on the outer edge (Fig. 5A). Sometimes the copulatory chamber eversion is ridged (specimen P1CJHUSH190511779 N9-03, P1CJHUSH190512096 Q3-04 and P1CJHUSH190518092 Q6-01) (Fig. 5D). Female pore single in XIV, oval, milky white. Spermathecal pores three pairs in 6/7–8/9, ventral, large eye-like, milky white porophore in center, separated by 1/3 body circumference.

Internal characters. Septa 5/6-7/8 thick and muscular, 10/11-12/13 slightly thickened, 8/9 and 9/10 absent. Gizzard spherical, in IX-X. Intestine enlarged distinctly from XV. Intestinal caeca paired in XXVII, extending anteriorly to XXIII, simple, smooth dorsal margin, weakly constricted on ventral margin (Fig. 5C). Four esophageal hearts in X-XIII, the latter three are more developed than the first pair. Male sexual system holandric, testis sacs two pairs, in X and XI, well developed, left and right lobes connected on the ventral side. Seminal vesicles two pairs, extending in XI and XII, well developed, left and right lobes separated on the ventral side. Prostate glands well developed, inserting in XVIII and extending to XVI and XXII, strip lobate, prostatic duct U-shaped, slightly thicker at the distal part (Fig. 5B). No accessory glands observed. Spermathecae three pairs in VII-IX, ampulla heart- or rod-shaped, ~ 3.2–8.0 mm long in holotype; ampulla duct is swollen and as long as ampulla. Diverticulum as long as main pouch (duct and ampulla together), slender and straight at proximal part, terminal 1/2 dilated into twisted in zigzag fashion. No accessory glands observed (Fig. 5E).

Etymology. The species is named after its type locality.

Remarks. Metaphire donganensis sp. nov. with three pairs spermathecal pores in 6/7-8/9, keys to the Metaphire houlleti group, which includes 44 species (Sims and Easton 1972; Feng 1984; Feng and Ma 1987; Qiu and Zhong 1993; Sun et al. 2018). Metaphire vulgaris agricola (Chen, 1930) is slightly akin to the new species in the respects of body size, first dorsal pore in 12/13, clitellum in XIV-XVI, setae number, the position and characteristics of spermathecal pores, simple intestinal caeca, and the characteristics of diverticulum. Instead, the difference between the two species is in the pigmentation, pale brown in M. donganensis sp. nov., earthy yellow in M. vulgaris agricola (Feng, 1981). Further, the ventral distance of spermathecal pore and male pores are 1/3C and 1/3 C in M. donganensis sp. nov., but 1/4C and 1/4C in M. vulgaris agricola; no papillae within spermathecal pore region of *M. donganensis* sp. nov., but paired papillae in VII of *M. vulgaris agricola*; no papillae within male pore region of M. donganensis sp. nov., while paired papillae on the inner side of male pore in M. vulgaris agricola; prostate glands well developed in XVI-XXII of M. donganensis sp. nov., nevertheless in XVII-XX with accessory glands invisible in M. vulgaris agricola; spermathecae ~

3.2–8.0 mm long, ampulla heart- or rod-shaped, duct swollen, as long as ampulla of *M. donganensis* sp. nov., while spermathecae ~ 4.5 mm long, ampulla pear-shaped, ampulla duct 1/2 of ampulla in *M. vulgaris agricola*; no accessory glands in *M. donganensis* sp. nov., while paired accessory glands in the VIII of *M. vulgaris agricola* (Table 4).

We compare the new species to Metaphire tschiliensis lanzhouensis (Feng, 1984), which has three pairs of spermathecal pores in 6/7-8/9. They share several common characters in the first dorsal pore position, clitellum in XIV-XVI, setae number, the position of spermathecal pores, the position and characteristics of male pores, no papillae within spermathecal pore region, simple intestinal caeca, and the characteristics of the diverticulum. Quite the contrary, the new species is smaller than M. tschiliensis lanzhouensis. Beyond that, coloration is pale brown in M. donganensis sp. nov., earthy yellow in M. tschiliensis lanzhouensis; spermathecal pores are obvious in M. donganensis sp. nov., but inconspicuous in M. tschiliensis lanzhouensis; no papillae within male pore region of M. donganensis sp. nov., but one in pouch of M. tschiliensis lanzhouensis; prostate glands well developed in XVI-XXII of M. donganensis sp. nov., while underdeveloped in XVII-XIX with a large lumpy accessory gland in M. tschiliensis lanzhouensis; spermathecae ~ 3.2-8.0 mm long, ampulla heartor rod-shaped of M. donganensis sp. nov., but spermathecae ~ 4 mm long, ampulla spherical-shaped in *M. tschiliensis lanzhouensis* (Table 4).

Considering the three pairs of spermathecal pores in 6/7–8/9, we compared the new species with *Metaphire viridis* Feng & Ma, 1987. Both have first dorsal pores in 12/13, clitellum in XIV–XVI, the same position and characteristics of spermathecal pores, simple intestinal caeca, and the same characteristics of diverticulum. However, the new species differs from *M. viridis* in many respects. *Metaphire donganensis* sp. nov. is smaller than *M. viridis* with pale brown coloration; setae number of *M. donganensis* sp. nov. is less than *M. viridis*; the ventral distance of spermathecal

Character	M. donganensis	M. vulgaris agricola	M. tschiliensis lanzhouensis	M. viridis	M. ptychosiphona	M. sanmingensis
Body size (mm)	72-159*4.6-7.0	118*6.0	245-310*6-7	192-230*9.5-10	196-295*6.0-9.0	55-113* 4-5.5
Pigment dorsum	Pale brown	Earthy yellow	Earthy yellow	Dark green	Grey-brown	Pale brown to brown
Pigment ventrum	Pale brown	Earthy yellow	Earthy yellow	Pale green	Grey-brown	None
First dorsal pore	12/13	12/13	12/13	12/13	11/12	11/12, 12/13 or 13/14
Setae numbering	20-34/III, 28-58/VIII, 52-66/XX	29/III, 54/VIII, 62/ XXV	32-40/III, 46-55/VIII	47–50/III, 64–67/ VIII, 92–95/XXV	57–61III, 64–79/ VIII, 87–117/XX	16-24/III, 33-40/VIII, 44-48/XX
Setae number between sp	17-21 (VIII)	-	16-24 (VIII)	-	28-34 (VIII)	12 (VIII)
Setae number between mp	8-13 (XVIII)	-	8-14 (XVIII)	-	15-27 (XVIII)	8-9 (XVIII)
Ventral distance of sp	1/3C	1/4C	1/3C	1/2C	2/5C	1/3C
Papillae within sp region	None	Paired in VII	None	Paired in VII, VIII, and IX	None	Two in VII and VIII, or extra two paired in VIII
Ventral distance of mp	1/3C	1/4C	1/3C	1/2C	1/3C	1/3C
Papillae within mp region	None	Paired in the inner side of male pore	One in pouches	Four in pouches	None	Three in the inner side of male pores, extra three in XVIII
Prostate glands	Well developed	Developed	Underdeveloped with accessory gland	Developed with accessory gland	Well developed	Developed
Diverticulum	As long as main pouch, terminal 1/2 dilated into zigzag fashion	Shorter, terminal 2/3 dilated into twisted zigzag fashion	As long as main pouch, terminal 2/3 dilated into zigzag fashion	Longer, terminal 1/2 dilated into zigzag fashion	Shorter, terminal 0.6 dilated into zigzag fashion	Shorter, terminal dilated into rod-shaped seminal chamber
Accessory glands	None	Paired in VIII	None	Paired in VII, VIII, and IX	None	Invisible

Table 4. A comparison of characters of *M. donganensis* sp. nov. and similar species of the *Metaphire houlleti* group. Abbreviations: sp, spermathecal pores, mp, male pores.

pore and male pores are 1/3C and 1/3 C in *M. donganensis* sp. nov., but 1/2C and 1/2C in *M. viridis*; no papillae within spermathecal pore region of *M. donganensis* sp. nov., while paired in VII, VIII, and IX of *M. viridis*; no papillae within male pore region of *M. donganensis* sp. nov., but four in pouches in *M. viridis*; prostate glands in XVI–XXII of *M. donganensis* sp. nov., while XV–XX or XVII–XIX with a lumpy accessory gland in *M. viridis*; no accessory glands in *M. donganensis* sp. nov., whereas paired in the VII, VIII and IX of *M. viridis*; spermathecae ~ 3.2–8.0 mm long, ampulla heart- or rod-shaped of *M. donganensis* sp. nov., nevertheless spermathecae ~ 4 mm long, ampulla spherical- or oval-shaped in *M. viridis*; no accessory glands of *M. donganensis* sp. nov., while paired in VII, VIII, and IX of *M. viridis*; no accessory glands in *M. viridis*; no accessory glands of *M. donganensis* sp. nov., nevertheless spermathecae ~ 4 mm long, ampulla spherical- or oval-shaped in *M. viridis*; no accessory glands of *M. donganensis* sp. nov., while paired in VII, VIII, and IX of *M. viridis*; no accessory glands of *M. donganensis* sp. nov., while paired in VII, VIII, and IX of *M. viridis*; no accessory glands of *M. donganensis* sp. nov., while paired in VII, VIII, and IX of *M. viridis* (Table 4).

We also compare the new species with *Metaphire ptychosiphona* Qiu & Zhong, 1993 on the basis of the position and characteristics of male pores, no papillae within spermathecal pore and male pore regions, simple intestinal caeca, and the same characteristics of the diverticulum. Nevertheless, the new species is smaller than *M. ptychosiphona*. In addition, pale brown in *M. donganensis* sp. nov., but grey brown in *M. ptychosiphona*; first dorsal pore in 12/13 of *M. donganensis* sp. nov., while in 11/12 of *M. ptychosiphona*; setae number of *M. donganensis* sp. nov. less than *M. ptychosiphona*; the ventral distance of spermathecal pore is 1/3C in *M. donganensis* sp. nov., but 2/5C in *M. ptychosiphona*; spermathecal pores obvious in *M. donganensis* sp. nov., but inconspicuous in *M. ptychosiphona*; and spermathecae ~ 3.2–8.0 mm long, ampulla heart- or rod-shaped, duct swollen, as long as ampulla in *M. donganensis* sp. nov., nevertheless spermathecae shorter, ampulla rod-shaped, duct shorter than ampulla in *M. ptychosiphona* (Table 4).

The new species is also close to *Metaphire sanmingensis* Sun & Jiang, 2018 in body size, clitellum in XIV–XVI, the position of spermathecal pores and male pores, simple intestinal caeca, prostate glands, and the characteristics of the ampulla and diverticulum. However, the new species is easily distinguished from *M. sanmingensis* in pigmentation, first dorsal pore position, setae number, papillae within spermathecal pore region, and the characteristics of male pores and papillae within male pore region (Table 4).

Molecular results

The COI mitochondrial DNA gene, considered as a barcode for earthworm identification (Huang et al. 2007; Novo et al. 2010), is an effective complement to morphological analyses. Comparisons of COI gene sequences among *A. xiangtanensis* sp. nov., *A. taoyuanensis* sp. nov., *A. xuanchengensis* sp. nov. *M. donganensis* sp. nov., *A. corticis*, *A. maximus*, *A. tortuosus*, *A. stricosus*, *A. recavus*, *A. endophilus*, *A. fucatus*, *A. zonarius* and *M. sanmingensis* yielded high pairwise distances (Table 5). According to Chang and James (2011), values above 10–15%, most probably indicate different species. It is clear that the new species and other species in Table 5 have large genetic divergences. In general, pairwise distances between the four new species and the other group species are greater than 17.17%. Together with the different morphological characteristics of each, we can conclude that *A. xiangtanensis* sp. nov., *A. taoyuanensis* sp. nov., *A. xuanchengensis* sp. nov., and *M. donganensis* sp. nov. are different from previously described species and each other.

Table 5. Perc	entage	of pai	rwise d	listance	es of C(JI gene	s betwe	een 13	specie	ú								
	S1 HT	S1 PT	S1 PT	S2 HT	S2 PT	S3 PT	S4 PT	S4 PT	S4 PT	A. corticis	A. maximus	A. tortuosus	A. stricosus	A. recavus	A. endophilus	A. fucatus	A. zonarius	M. sanmingensis
S1 HT																		
S1 PT	0.00%																	
S1 PT	0.16%	0.16%																
S2 HT	21.78%	21.78%	21.55%															
S2 PT	21.78%	21.78%	21.55%	0.00%														
S3 PT	21.32%	21.32%	21.10%	17.25%	17.25%													
S4 PT	22.53%	22.53%	22.29%	18.81%	18.81%	20.64%												
S4 PT	22.29%	22.29%	22.06%	18.60%	18.60%	20.42%	0.16%											
S4 PT	22.27%	22.27%	22.04%	18.82%	18.82%	19.95%	5.62%	5.44%										
A. corticis	23.16%	23.16%	22.92%	20.83%	20.79%	23.25%	20.68%	20.45%	19.55%									
A. maximus	21.05%	21.05%	20.79%	19.13%	19.09%	18.25%	18.16%	17.91%	18.38%	15.54%								
A. tortuosus	19.78%	19.78%	19.55%	18.75%	18.71%	18.78%	17.05%	17.26%	17.25%	20.18%	19.18%							
A. stricosus	18.06%	18.06%	17.85%	16.53%	16.50%	16.62%	17.16%	16.95%	16.31%	16.91%	15.31%	14.18%						
A. recavus	18.54%	18.54%	18.32%	15.90%	15.87%	19.85%	15.08%	14.88%	15.93%	19.58%	19.57%	16.12%	14.69%					
A. endophilus	17.17%	17.17%	16.96%	17.58%	17.54%	17.61%	18.99%	18.77%	18.37%	18.29%	16.59%	17.68%	15.04%	17.32%				
A. fucatus	22.48%	22.48%	22.07%	21.67%	21.67%	23.34%	19.67%	19.29%	21.24%	17.82%	20.52%	18.22%	20.22%	18.67%	18.22%			
A. zonarius	18.04%	18.04%	17.82%	16.00%	15.97%	16.79%	19.05%	19.27%	21.04%	18.43%	16.02%	15.61%	14.38%	15.72%	13.66%	18.73%		
M. sanmingensis	20.81%	20.81%	20.57%	19.96%	19.92%	19.24%	19.30%	19.06%	20.00%	15.30%	19.69%	17.71%	15.00%	17.96%	18.58%	21.54%	15.88%	
Notes: S1 represe	nt A. xian	gtanensis	sp. nov.,	S2 repres	ent A. tao	/uanensis	sp. nov., §	s3 represe	ent A. xuar	nchengensis	sp. nov., S4 re	epresent M. don	janensis sp. nov	; Abbreviations	: HT holotype, P	T paratype.		

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: QJ, JBJ and JPQ. Software: QJ. Formal analysis: QJ. Investigation: JBJ and JLL. Data Curation: QJ. Writing - Original draft: QJ. Writing - Review and Editing: QJ, JBJ and JPQ. Supervision: JBJ and JPQ. Project administration: JBJ and JPQ.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

One new species and two new records of Pyrrhocoridae (Hemiptera, Heteroptera) from China

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Abstract

A new species, *Dindymus albonotum* Zhao & Cao, **sp. nov.**, and two newly recorded species, *Euscopus robustus* Stehlík, 2005 and *Brancucciana* (*Rubriascopus*) orientalis Stehlík & Jindra, 2008, belonging to the family Pyrrhocoridae Amyot & Serville, 1843 (Hemiptera, Heteroptera, Pyrrhocoroidea) from China are described and illustrated.

Key words: Oriental region, red bugs, South China, taxonomy, tropical zone



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Introduction

The currently known pyrrhocorid fauna (Hemiptera, Heteroptera, Pyrrhocoroidea) of China includes 15 genera and 40 species (Zhao et al. 2022). Distant (1903) was the first to comprehensively study the pyrrhocorids of the Oriental region and reported 52 species, including 20 from China. Between 1929 and 2022, there has been a gradual accumulation of taxonomic research on the Pyrrhocoridae of China, including works by Hussey (1929), Blöte (1931), Hsiao (1964), Liu (1981a), Stehlík and Kerzhner (1999), Kerzhner (2001), Stehlík and Jindra (2006a, 2006b), Rédei et al. (2009), and Zhao et al. (2022). Despite this, the number of pyrrhocorid species documented in China is less than 10% of the worldwide total of 525 species (49 genera) (Zhao et al. 2022). Considering the diversity of habitats across China, including the species-rich Oriental portion, it is likely that the Chinese pyrrhocorid fauna has not yet been fully documented.

In this paper we describe a pyrrhocorid species new to science from the Oriental part of China: *Dindymus albonotum* sp. nov. We also report the first distribution records for *Brancucciana* (*Rubriascopus*) *orientalis* Stehlík & Jindra, 2008 and *Euscopus robustus* Stehlík, 2005 in China.

Materials and methods

All studied materials are deposited in the Entomological Museum of China Agricultural University (CAU), Beijing, China. External structures were examined using a binocular dissecting microscope. Species descriptions were based on naturally dried specimens. Male genitalia were put into a 1.50 ml centrifuge tube and soaked in pure lactic acid heated to 80 °C in constant temperature metal bath for ~15 min to remove soft tissue, then rinsed in boiling distilled water for ~1 min and dissected under a microscope. Dissected parts of the genital structures were placed in a plastic microvial with lactic acid under each corresponding specimen. Habitus photographs of all the species were taken with a Canon D60 SLR camera (Canon Inc., Tokyo, Japan). Male external genitalia were photographed with the aid of a Nikon SMZ25 stereomicroscope (Nikon Corporation, Tokyo, Japan). Measurements were obtained using a calibrated micrometer. All measurements are presented in millimeters (mm). The classification system mainly follows that of Rédei et al. (2009), Aukema et al. (2013), Stehlík and Kerzhner (1999), and Zhao et al. (2022). The morphological terminology of male genitalia follows Schaefer (1977) and Ahmad and Zaidi (1986); the terminology of the female genitalia is according to the paper published by Zhou and Rédei (2020). The generic and specific names in the text are arranged alphabetically.

Taxonomy

Family Pyrrhocoridae Amyot & Serville, 1843

Genus Brancucciana Ahmad & Zaidi, 1986

Brancucciana Ahmad & Zaidi, 1986: 423; Stehlík 2007: 109; Aukema et al. 2013: 402. Type species by original designation: *Brancucciana bhutanensis* Ahmad & Zaidi, 1986.

Ascopus Hsiao, 1964: 402, 405 (junior homonym of Ascopus Marshall, 1951, Coleoptera). Type species by original designation: Ascopus rufus Hsiao, 1964: 403, 405. Ascopocoris Stehlík & Kerzhner, 1999: 123; Stehlík and Jindra 2006a: 61. Un-

necessary new name for Ascopus Hsiao, 1964.

Generic character. Body generally sanguineous, broadly oval. Head as long as broad and nearly triangular, apical part anteriorly produced; eyes smaller and bulging, posterior margin of eyes adjacent to anterior margin of pronotum; antennae shorter and subequal in length to half of body; first antennal segment subequal to head length, apical half dilated; labium reaching to hind coxae, second segment longest, fourth shortest, first subequal in length to third. Length of pronotum distinctly shorter than its width, lateral pronotal margins reflexed; scutellum equilateral triangular; metathoracic scent gland ostiole large; membrane of hemelytron reaching just beyond the apex of abdomen, costal margin of corium reflexed. Sterna of thorax centrally with longitudinal ridge. Femora thickened, ventral side of subapcial part of fore femora with several dentate processes; third segment of tarsus of hind leg longer than first and second segments together. Pygophore somewhat rounded, ventroposterior margin medially with a knob; paramere F-shaped; aedeagus with both thecal and conjunctival appendages.

Distribution. Oriental region.

Remark. Hsiao (1964) established the genus Ascopus Hsiao, 1964 with Ascopus rufa Hsiao, 1964 as type species. Liu (1987) found a second species, Ascopus sinuaticollis Liu, 1987. However, the genus name, Ascopus was already preoccupied by Ascopus Marshall, 1951 in the order Coleoptera. Stehlík and Kerzhner (1999) proposed a new name for the genus, Ascopocoris Stehlík & Kerzhner, 1999, and placed Brancucciana bhutanensis Ahmad & Zaidi, 1986, Euscopes gestroi Distant, 1903, and Antilochus pygmaeus Distant, 1903 in it. Additionally, Stehlík and Jindra (2006a) established a new subgenus, Rubriascopus, with Antilochus pygmaeus Distant, 1903 as type species and found a sixth species, Ascopocoris constanti Stehlík & Jindra, 2006. Stehlík and Jindra (2006a) thought that Liu's (1987) description of Ascopus sinuaticollis Liu, 1987 was insufficient and suggested it might be Ascopocoris gestroi (Distant, 1903), but confirmation of this awaits further study. Stehlík (2007) found that Brancucciana is indeed the oldest valid name for this genus-level taxon and placed the above six species in it as new combinations. The seventh species, Brancucciana (Rubriascopus) orientalis Stehlík & Jindra, 2008 is distributed in Philippines and Indonesia (Stehlík and Jindra 2008).

In China, two species of the genus *Brancucciana* have been previously reported. Hsiao (1964) reported *B.* (*Brancucciana*) *rufa*, and Liu (1987) reported *B.* (*Brancucciana*) *sinuaticollis*, both from Yunnan Province. We collected one female and two males at the Nonggang National Nature Reserve, in the Guangxi Zhuang Autonomous Region, southwestern China, and one female from Hainan Island, South China. These specimens are morphologically consistent with *Brancucciana* (*Rubriascopus*) *orientalis* and represent the first report of the species in China. In addition, we reviewed the generic characters according to Hsiao (1964) and Ahmad and Zaidi (1986).

Key to the Chinese species of Genus Brancucciana Ahmad & Zaidi, 1986

- Body generally reddish brown to black; hemelytron reddish brown to black, anterior margin of corium red to reddish brown; pronotum reddish brown to black, lateral margin red to reddish brown; body almost parallel-sided; body with dense, deep punctures; legs bicolor and mostly yellowish brown......2
- 2 Middle part of lateral margin of pronotum nearly straight; labium and legs nearly uniformly reddish brown **B. (Brancucciana) rufa (Hsiao, 1964)**

Brancucciana (Rubriascopus) orientalis Stehlík & Jindra, 2008 Figs 1, 2

Chinese common name: 东方华红蝽

Brancucciana (Rubriascopus) orientalis Stehlík & Jindra, 2008: 632: Aukema et al. 2013: 402.



Figure 1. *Brancucciana* (*Rubriascopus*) *orientalis* Stehlík & Jindra, 2008, habitus **a**, **b** female **c**, **d** male **a**, **c** dorsal view **b**, **d** ventral view.

Diagnosis. The subgenus *Rubriascopus* has two species, *Brancucciana* (*Rubriascopus*) *pygmaeus* Distant, 1903 and *Brancucciana* (*Rubriascopus*) *orientalis* Stehlík & Jindra, 2008. In *B.* (*R.*) *pygmaeus*, the legs and antennomeres are greyish yellow according to Stehlík and Jindra (2006a: fig. 5) [vs legs and antennomeres black in *B.* (*R.*) *orientalis*].

The specimens recently collected from Guangxi and Hainan are morphologically mostly consistent with the original description of *B*. (*R*.) orientalis



Figure 2. Brancucciana (Rubriascopus) orientalis Stehlík & Jindra, 2008, male external genitalia **a**, **b** pygophore **c**, **d** cuplike sclerite of pygophore **e** paramere **f** phallobase **g**–**i** phallosoma **c**, **h** dorsal view **a**, **d**, **i** lateral view **b**, **g** ventral view.

according to the description by Stehlík and Jindra (2008). However, based on these Chinese specimens, the body is mostly red, with black markings (vs pale brown with a reddish tinge in the specimen of this species from Indonesia and the Philippines (Stehlík and Jindra 2008). The structure of their male genitalia is consistent with *B*. (*R*.) orientalis, therefore we consider the differences in colouration as intraspecific variation.

The male genitalia are redescribed in detail here (Fig. 2). The pygophore is oval, the median pygophore process is armed with a posteriorly produced protrusion, its two lateral margins are parallel and the posterior margin of apical part is nearly straight and medially a little concave (Fig. 2a, b); the ventral rim infolds and falls into genital chamber, and forms a special structural interior process, a cup-like sclerite of pygophore, its apical part acute, as shown in Fig. 2c, d; the paramere is clavate, gradually attenuated, its apical part has a pair of minute denticles (Fig. 2e); the phallosoma is elliptic; the phallobase is shown as in Fig. 2f; the endosoma is shown in Fig. 2g–i, the vesical seminal duct (gonopore) extends to the apical part of the vesica.

Measurements [male (n = 1) / female (n = 1), in mm]. Body length 9.88 / 11.66; maximal width of abdomen 4.13 / 4.70. Head length 1.30 / 1.70; Head width 1.78 / 1.94; length of synthlipsis 0.97 / 1.30; length of antennal segments I–IV = 1.27 / 1.46, 1.36 / 1.62, 0.91 / 0.97, 1.82 / 1.78; length of labial segments I–IV = 1.13 / 1.16, 1.30 / 1.30, 1.13 / 1.13, 1.13 / 0.97; length of pronotum 3.29 / 3.56; width of pronotum 3.40 / 3.73; length of anterior pronotal lobe 0.81 / 0.81; length of posterior pronotal lobe 1.13 / 1.30; length of scutellum 1.13 / 1.62; length of hemelytron 7.45 / 8.42.

Material examined. 2 males, 1 female, China, Guangxi, Longzhou, Nonggang, 2021-VI-14, by light, Ping Zhao & Yingqi Liu leg., deposited in CAU; 1 female, China, Hainan, Dongfang city, Guangyin road, 2023-VI-28, Jianyun Wang leg., deposited in CAU.

Distribution. China [Guangxi (Longzhou), Hainan]; Indonesia, Philippines (Stehlík and Jindra 2008). New record from China

Biology. *B.* (*R.*) *orientalis* demonstrates positive phototaxis when exposed to artificial light sources during nocturnal periods.

Genus Dindymus Stål, 1861

Dindymus Stål, 1861: 196; Liu 1981a: 228; Rédei et al. 2009: 23. Type species by subsequent designation (Distant 1903: 110): *Dysdercus thoracicus* Stål, 1855 (= *Pyrrhocoris bicolor* Herrich-Schäffer, 1840).

Generic character. Body medium-sized, smooth. Head triangular, vertex bulged, posterior lobe of head quickly constricted into the neck, head not wider than anterior pronotal lobe, apical part of head slightly declined downward; first antennal segment longest, fourth separately longer than second and third segment, second slightly longer than third; eyes sessile, protruded laterally; apical part of labium usually extending beyond base of abdomen, first labial segment slightly thickened. Pronotum trapezoidal, lateral pronotal margins strongly reflexed. Inter-segmental sutures of third to fifth abdominal segments moderately curved laterally.

Distribution. Oriental and Australian region.

Remark. There are six species of the genus *Dindymus* reported from China prior to this study. *Dindymus brevis* Blöte, 1931 was reported in Taiwan Province (Rédei et al. 2009), *D. medogensis* Liu, 1981 was reported from Xizang Autonomous Region (Liu 1981b), *D. (Dindymus) chinensis* Stehlík & Jindra, 2006 was described based on materials from Hubei Province (Stehlík and Jindra 2006b), *D. lanius* Stål, 1863, *D. rubiginosus* (Fabricius, 1787), and *D. sanguineus*

(Fabricius, 1794) in China are widespread species in continental areas of Oriental region. Herein, we found seventh species from Yunnan Province of China, reported below.

Key to the Chinese species of Genus Dindymus Stål, 1861

- 1 Legs black, apical half of femora and basal 1/4 of tibiae red**2**
- 2 Posterior pronotal lobe red......D. brevis Blöte, 1931
- Posterior pronotal lobe yellowish-white.....D. albonotum Zhao & Cao, sp. nov.

- 4 Labium almost extending to middle part of third abdominal sternum, first segment thicker, significantly exceeding beyond anterior margin of pronotum; lateral margin of pronotum and costal margin of corium strongly expanded laterally, almost lamellate......**D. medogensis Liu, 1981**
- Labium not extending to middle part of third abdominal sternum, first segment not extending beyond anterior margin of pronotum; lateral margin of pronotum and costal margin of corium only slightly expanded laterally...5

- 6 Posterior margins of all thoracic pleura milk-white to yellowish white, outer side of coxae with distinct white spot D. rubiginosus (Fabricius, 1787)
- Posterior margins of metapleura and posterior acetabula milk-white, outer side of coxae paler......D. sanguineus (Fabricius, 1794)

Dindymus albonotum Zhao & Cao, sp. nov.

https://zoobank.org/489A818E-C49C-404B-8C65-C2B5C7CFD463 Fig. 3 Chinese common name: 黄胸光红蝽

Diagnosis. The new species, from Yunnan, China, is similar in body shape and coloration to *Dindymus brevis* Blöte, 1931, which is distributed in Taiwan, China. However, for *D. albonotum* sp. nov, the posterior lobe of pronotum is milk-white to yellowish white; the fore-wing membrane is golden yellow, semitransparent, and its posterior part has scattered black markings; the abdominal sterna are black, except for the milk-white posterior margin of fifth segment, and sixth and seventh segments (Fig. 3a, b). In *D. brevis*, the pronotum is completely red, the membrane of





fore wing is golden yellow, with a large black round spot; the sternum of abdomen is white except the basal part is black, and the apical part is red (Rédei et al. 2009).

The new species also resembles *Dindymus* (*Dindymus*) *flavinotum* Stehlík, 2013 in the following characters: the posterior pronotal lobe is milk-white, and the membrane of fore wing is golden yellow and with fewer black markings; the legs are black, with apices of the femora and bases of the tibiae red; the second to fifth abdominal sternites are black; the sixth and seventh are white. In *D. flavinotum*, the legs are completely black, and the abdominal sternites are red (except black basal parts) (Stehlík 2013).

Type species. *Holotype*, female, China, Yunnan, Puer, 2022-VI, Zhang Guirong leg., deposited in CAU.

Description. Coloration. Body red with black and milk-white markings. Antennae black, basal part of first segment red; labium blackish brown, first segment red; posterior pronotal lobe milk white; pleura and sterna of thorax black, posterior margin of pleura and posterior margin of acetabula milk white; leg black, apical part of femora and basal part of tibiae red; abdomen black, posterior margin of fifth sternum of abdomen, sixth and seventh abdominal sternites milk white.

Structure. Body oval. Pronotum and fore wing widened transversely. Head length subequal to width, eyes laterally protruded; anterior and posterior lobe of pronotum gibbose; anterior margin of pronotum not wider than head, and subequal to 1/2 of distance between lateral pronotal angles. Posterior margin of anterior pronotal lobe convex anteriorly; posterior pronotal lobe sparsely punctured. Costal margin of corium laterally dilated and smooth, corium (except costal margin) and clavus densely punctured.

Measurements [female (*n* = 1), in mm]. Body length 14.78; maximal width of abdomen 7.13. Head length 2.78; head width 2.61; length of synthlipsis 1.39;

length of antennal segments I-IV = 4.35, 2.61, 1.91, 3.48; length of labial segments I-IV = 2.52, 2.52, 2.09, 1.22; length of pronotum 2.86; width of pronotum 4.96; length of anterior pronotal lobe 1.22; length of posterior pronotal lobe 1.74; length of scutellum 2.73 length of hemelytron 12.17.

Etymology. The specific name alludes to the yellow posterior lobe of pronotum of the new species. The Latin noun *albonotum* means "yellowish-white thorax".

Distribution. China [Yunnan (Puer)].

Biology. The specimen was collected from the forest near Wanmu tea garden, in Puer, Yunnan, China.

Genus Euscopus Stål, 1870

Euscopus Stål, 1870: 102: Distant 1903: 105; Liu 1981a: 231. Type species by monotypy: *Euscopus rufipes* Stål, 1870.

Generic character. Body oblique and covered with dense, short setae. Head length approximately equal to width, its apical part downward declining, slightly bulging at top, head width approximately equal to width of anterior pronotal lobe; labium extending to or over coxae of the middle leg, first segment not longer than head; antennae slightly thicker and shorter, first segment longest, second to fourth segments approximately equal in length; pronotum wider than long, lateral margin slightly upward upturned, transversal constriction obviously continuous. Costal margin of corium of the fore wing nearly straight; fore femora slightly thickened, subapical part of the ventral surface with two or three small spines; intersegmental sulcus of the fourth and fifth segments of abdominal sterna obviously bent forward, not straight to its lateral margin.

Remark. A total of 16 species are currently recognized worldwide (Zhao et al. 2022), with four species reported in China. A new record species is reported from China in this paper.

Distribution. Oriental region.

Key to the Chinese species in genus Euscopus Stål, 1870

Corium of fore wing black, anterior margin red
- Corium of fore wing red, middle part with a large black spot, apical angle
black4
Posterior margin of pronotum red3
Posterior margin of pronotum not red
A smooth white point near the centre of the apical edge of the corium
E. distinguendus Blöte, 1933
- Apical part of the corium without with white spot E. fuscus Hsiao, 1964
Apical angle of corium with a small black spot on inner side; membrane of
fore wing blackish brown, its outer and inner margin light brown; abdomen
ventrally completely blackE. rufipes Stål, 1870
- Apical angle of corium with larger black markings on inner side; mem-
brane of fore wing greyish brown; abdomen ventrally black with median
longitudinal part red E. chinensis Blöte, 1932

Euscopus robustus Stehlík, 2005

Figs 4, 5 Chinese common name: 黑锐红蝽

Euscopus robustus Stehlík, 2005: 157.

Diagnosis. The species is similar to *Euscopus fuscus* Hsiao, 1964 in body shape and coloration. However, the species reported here lacks the yellow to red posterior margin on the pronotum, and the body is nearly parallel-sided (in *E. fuscus*, the posterior margin of pronotum is yellowish brown, and the body is oblong-elliptical).

Redescription. *Coloration.* Body dorsally black, ventrally reddish brown. Head, thorax, scutellum, and legs black; lateral margin of pronotum, costal margin of corium yellow to red; antennae black, basal half of fourth white (Fig. 4).



Figure 4. Euscopus robustus Stehlík, 2005, habitus a, b male c, d female a, c dorsal view b, d ventral view.



Figure 5. *Euscopus robustus* Stehlík, 2005, male external genitalia **a**–**c** pygophore **d** paramere **e**–**g** phallus **a**, **e** dorsal view **b**, **f** lateral view **g** ventral view **c** caudal view.

Structure. Body clothed with procumbent short setae. Posterior pronotal lobe, clavus, corium, rim of callus of anterior pronotal lobe, scutellum punctate. Body oblong, nearly parallel-sided (Fig. 4). Head subangular, compound eyes laterally produced, apical part of head forward and downward sloping; frons bulged; clypeus thickened and longer than paraclypei; first antennal segment longest, second sub-equal to fourth in length, third shortest; first to third labial segments nearly equal in length, fourth shortest, first and fourth thickened. Pronotum slightly gibbous, lateral pronotal margin moderately wide and reflexed; anterior pronotal lobe subequal to in length posterior lobe. Profemora ventrally in apical half with several minute denticles, middle part with a large spine. Third and fourth abdominal sternites with four round black spots, and two sides of each abdominal sternum with two black spots.

Male genitalia. Pygophore gibbous, posterior margin concaved medially, posterior rim infolding and distinctly sloping into genital chamber, and forming an internal cuplike sclerite of pygophore, its apical margin straight (Fig. 5a–c); parameres short and thick, middle part with many oblique setae, apical part with a hook-shaped process (Fig. 5d); phallosoma shown in Fig. 5e–g, a pair of dorsal conjunctival appendages long horn-shaped.

Measurements [male (n = 1) / female (n = 1), in mm]. Body length 9.56 / 11.04; maximal width of abdomen 3.74 / 4.00. Head length 1.22 / 1.48; Head width 1.74 / 1.74; length of synthlipsis 0.87 / 0.96; length of antennal segments I–IV= 2.00 / 2.26, 1.22 / 1.30, 0.87 / 0.87, 1.30/1.30; length of labial segments I–IV = 0.78 / 0.78, 0.78 / 0.87, 0.70 / 0.87, 0.43 / 0.43; length of pronotum 1.74 / 2.26; width of pronotum 3.22 / 3.65; length of anterior pronotal lobe 0.70 / 0.70; length of posterior pronotal lobe 1.04 / 1.39; length of scutellum 1.30 / 1.22; length of hemelytron 6.96 / 8.00.

Material examined. 1 male, 1 female, China, Guangxi, Nonggang National Nature Reserve, Ningming, Huashan, Ping Zhao, Zhuo Chen & Yingqi Liu leg., 2022-VII-11, collected by using a light trap, deposited in CAU.

Distribution. China [Guangxi(Nonggang)]; Laos. New record from China **Biology.** *Euscopus robustus* exhibits positive phototaxis when exposed to artificial light sources during nocturnal periods.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

Two new species of *Macropelopia* (Diptera, Chironomidae) from Oriental China, delineated with morphology and COI sequences

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Abstract

Two new species, *Macropelopia* (*Macropelopia*) excavata Xu & Fu, **sp. nov.** and *Macropelopia* (*Macropelopia*) quadrimacula Xu & Fu, **sp. nov.**, are described as male adults. A key to identify the males of *Macropelopia* from China is provided. Furthermore, in order to ascertain the genetic distance between these species and their morphological characteristics, mitochondrial cytochrome c oxidase subunit I gene sequences were uploaded to the National Center for Biotechnology Information. These COI sequences were then utilized to infer the relationships between the species, employing the neighborjoining method.

Key words: DNA barcoding, Macropelopia, morphology, non-biting midge, taxonomy

Introduction

Thienemann and Kieffer (1916) established the genus *Macropelopia*, with *Isoplastus bimaculatus* Kieffer as the type species. *Macropelopia* is classified in the tribe *Macropelopiini* and is further divided into two subgenera: *Bethbilbeckia* Fittkau et Murray and *Macropelopia* s. str. (Cranston and Epler 2013). Previous studies described a total of 18 species within the subgenus *Macropelopia* s. str. and three species in the subgenus *Bethbilbeckia*. The main difference between the two subgenera in the adult stage is that *Macropelopia* s. str. has a tibial comb, while *Bethbilbeckia* does not. Among these, 11 species of the subgenus *Macropelopia* are found in the Palearctic region (Kieffer 1912, 1916; Tokunaga 1937; Fittkau 1962; Lencioni and Marziali 2005), one in the New North region (Roback 1971), two in the Oriental region (Tang and Niitsuma 2020; Hazra and Chaudhuri 2001), three in the Neotropical region (Andersen 2018; Silva and Pinho 2020; Dantas et al. 2023), and one in the African region (Freeman 1955).

According to Wang (2000), only one species of *Macropelopia*, *M. nebulosa* (Meigen), has been described based on adult, and four other species have been recorded based on larvae. Wang et al. (2011) recorded three additional species of *Macropelopia*, namely *M. galbina* Wang, Cheng & Wang, and *M. gran-divolsella* Wang, Cheng & Wang which were both found in Hubei Province, and



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Copyright: [©] Xiangliang Fang et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). *M. rotunda* Wang, Cheng & Wang, which was discovered in Fujian Province. However, recent examinations conducted by Tang and Niitsuma (2020) have resulted in significant taxonomic revisions. The species *M. grandivolsella* has been synonymized with *Macropelopia paranebulosa* Fittkau, while *Macropelopia rotunda* is now referred to as *M. kibunensis* (Tokunaga), with these two species considered synonymous. Additionally, *M. galbina* was transferred to the genus *Brundiniella*. Furthermore, Tang and Niitsuma (2020) have described a previously unknown species, *Macropelopia* (*Macropelopia*) pergrandis, originating from Yunnan Province.

In this study, we report the discovery of two new species within the subgenus *Macropelopia* s. str., sourced from the remarkable Dabie Mountain National Nature Reserve situated in Hubei Province. The discovery holds significant importance as it contributes to the diversity of the genus. In addition, we have also assembled a key for distinguishing and identifying the known adult males of the subgenus in China. Moreover, we have conducted an analysis utilizing the mitochondrial cytochrome *c* oxidase subunit I (COI) gene to infer genetic distance and determine the differences between the species within the genus *Macropelopia*,further enhancing our understanding of their morphological characteristics.

Material and methods

Specimens were collected using the light trap induction method and preserved in 85% alcohol. Subsequently, they were sent to the laboratory for preliminary species identification and assigned individual numbers under a microscope. Images of slide mounts were obtained using a Nexcope NE930 compound microscope equipped with Capture 2.1 software. Genomic DNA was extracted from the thorax and legs of the specimens using the Qiagen DNA Blood & Tissue Kit. PCR amplification of the COI gene was performed following the primers and temperature regimes described by Folmer et al. (1994). After DNA extraction, the transparent exoskeleton was rinsed with 96% ethanol and mounted in Euparal on microscope slides, along with the corresponding antennae, head, wings, and legs, following the protocol outlined by Sæther (1969). Morphological nomenclature adheres to Sæther (1980), and measurements include the minimum, maximum, and average values for at least three specimens. All specimens are currently housed at the College of Biology and Agricultural Resources, Huanggang Normal University, China. Evolutionary analyses were conducted using MEGA 11.

The main abbreviations and corresponding English terms used in this study (with the value of '*N*' representing the number of measured specimens mentioned in the text) are as follows: TL, total length; WL, total wing length; Pfe, length of the forefoot; VR, Venarum ratio = length of Cu / length of M; Cu₁ cubitus 1; AR, Antennal ratio = length of ultimate flagellomere / combined lengths of flagellomeres one to penultimate; Fe, femur; ta₁-ta₅, tarsomeres ta₁-ta₅; LR, ta(basal segment) / ti; BV, Fe + ti +ta₁ / ta₂ + ta₃ + ta₄ + ta₅; SV, Fe + ti / ta₁; R, Radius; R₁, Radius 1; R₄₊₅, Radius four and five; BR, largest bristle/width of ta₁ about 1/3 from distal end; HR, length of gonocoxite / length of gonostylus; HV, total length (TL) / length of gonostylus × 10.
Taxonomy

Genus Macropelopia Thienemann

- Macropelopia Thienemann in Thienemann & Kieffer, 1916: 497. Fittkau 1962: 102; Roback 1971: 87, 1978: 159; Fittkau and Murray 1986: 50; Murray and Fittkau 1989: 61; Epler 2001: 4.53; Niitsuma et al. 2004: 44; Cranston and Epler 2013: 53; Silva and Pinho 2020: 575; Tang and Niitsuma 2020.
- *Bethbilbeckia* Fittkau et Murray, 1988: 253; Murray and Fittkau 1989: 46; Epler 2001: 4.29.

Type species. *Isoplastus bimaculatus* Kieffer [*=Tanypus nebulosus* Meigen] by original designation.

Macropelopia excavata Xu & Fu, sp. nov.

https://zoobank.org/2AA64D33-4F08-444C-AC77-FA080DBFBCAF Fig. 1A-K

Type material. *Holotype* male (HNU: Cdbs60), CHINA: Hubei Province, Huanggang City, Yingshan County, Dabie Mountain, Longtan Gorge, light trap, 31.0867°N, 115.8138°E, 486.71 m a. s. l., 7. IX. 2022, Zigang Xu.

Etymology. The new species is named "*excavata*" derived from the Latin term "*excavatus*", meaning concave, which aptly describes the inward concave shape of tergite IX's posterior edge.

Diagnostic characters. The distinguishing characteristics of this new species are the presence of two prominent longitudinal thick spots positioned in the middle of tergites II to IV, and the wing with brown markings on the distal end of Cu_1 and basal part of cell an. Additionally, the tergites from V to IX display a distinctive brown hue, adding to their identification. The posterior edge of tergite IX is concave in shape, and the anal point is absent. The gonostylus is prominently curved at a right angle.

Description. Adult male (*N* = 1)

Total length 4.69 mm, thorax length 1.45 mm, wing length 2.66 mm, TL/WL 1.76, WL/Pfe 2.19.

Coloration (Fig. 1D). The head and thorax are uniformly dark brown. The femur of the legs is also dark brown, while the remaining parts of the legs are yellow. The wings exhibit two significant gray spots positioned near the Cu_1 and An veins. There is a longitudinal color spot present in the middle of tergites II to IV, the tergites V to IX and hypopygium are all brown in coloration.

Head (Fig. 1A). Antenna (Fig. 1B) with 13 flagellomeres, with an antennal ratio (AR) measuring 2.24. The flagellomeres taper towards their ends and ultimate flagellomere with a single apical seta. Temporal setae 24, comprising 8 inner verticals, 12 outer verticals, and 16 postorbitals. Tentorial length 234 μ m, width 43 μ m. Stipes length 192 μ m, width 82 μ m. Clypeus with 16 setae. Length of palpomeres (in μ m): 69; 100; 161; 210; 342. Length ratio of palpomeres 5/3: 2.13.



Figure 1. *Macropelopia* (*Macropelopia*) excavata Xu & Fu, sp. nov., male imago A head B antenna C wing D whole body of male adult E spotted shape of the tergites F thorax G fore tibial apex H hypopygium, dorsal view I photo of hypopygium, dorsal view J hypopygium, ventral view K gonostylus.

Thorax (Fig. 1F). Antepronotals with 9 setae, the acrostichals and dorsocentrals arranged in multiple columns, acrostichals with 41 setae, dorsocentrals with 62 setae. Postnotals with 6 setae, humerals with 8 setae, prealars with 32 setae. Scutal tubercle present and obvious, scutellars with 32 setae.

Wing (Fig. 1C). The wing membrane area hairy, distinctive gray spots present near Cu₁ and An veins, the arculus hook-like shape, and the anal lobe mainly developed. VR: 0.91, brachiolum with 3 setae, veins with many setae, squama with 34 setae. Costal extension 128 μ m.

Legs. The fore tibia possesses a single spur measuring 85 μ m in length and features 15 side teeth. The width of the fore tibia at its apex is 84 μ m, and the fore tibial comb consists of 5 setae (Fig. 1G). Two spurs of mid tibia are observed, measuring 87 μ m and 51 μ m long, bearing 16 and 11 lateral teeth, respectively, and has a width at the apex of 73 μ m. The hind tibia exhibits two spurs, measuring 75 μ m and 45 μ m, with 18 and 12 lateral teeth, respectively. The hind tibial comb composed of 10 setae, with the longest seta 73 μ m and the shortest 45 μ m. The width of the hind tibia at its apex is recorded as 80 μ m. The lengths and proportions of each leg are shown in Table 1.

Table 1. Lengths (in μ m) and proportions of leg segments of male *M. excavata* Xu & Fu, sp. nov. (*N* = 1).

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄
P ₁	1215	1502	1168	600	428	293
P ₂	1295	1461	886	428	317	222
P ₃	1193	1654	1144	592	414	267
	ta ₅	LR	BV	SV	BR	
P ₁	208	0.78	2.54	2.32	3.96	
P ₂	164	0.61	3.23	3.11	4.45	
P ₃	104	0.00	0.74	0.40	4.50	

Hypopygium (Fig. 1G, H). The anal point absent. The phallapodeme, although short, measures at a clearly discernible length of 69 μ m. The gonocoxite showcases a cylindrical shape and spans 206 μ m in length. The gonostylus (Fig. 1K) is 125 μ m long and exhibits a curved inward shape at approximately two-thirds of its length. It displays protrusions on both the inside and outside, gradually narrowing towards the tip. The megaseta is 16 μ m long. The inferior volsella unconspicuous, along the inside of gonocoxite, and contains concentrated long inner microhairs. HR: 1.64, HV: 3.74.

Remarks. This new species is similar to *M. kibunensis* (Tokunaga) because their anal point is absent and the inferior volella is undeveloped, but can be distinguished by AR 2.24, wing with brown markings on distal end of Cu₁ and basal part of cell an, otherwise unmarked, and a concave rear edge line of tergite IX, while AR 1.7–1.9, wing with brown markings on distal end of Cu₁, M₃₊₄ and M₁₊₂. The pairwise distance based on the COI sequence of *M. kibunensis* and *M. excavata* sp. nov. is 0.105–0.107, further distinguishing them from each other.

Distribution. Hubei Province, Oriental China.

Macropelopia quadrimacula Xu & Fu, sp. nov.

https://zoobank.org/13C57663-77BF-4852-988D-F05C38B51B33 Fig. 2A-K

Type material. *Holotype*, male (HNU: Cdbs7602), CHINA: Hubei Province, Huanggang City, Yingshan County, Dabie Mountain, Longtan Gorge, 31.0867°N, 115.8138°E, 486.71 m a. s. l., 8. VI. 2022, light trap, leg. Zigang Xu. *Paratypes:* 3 males (HNU: Cdbs7601, Cdbs7603, Cdbs7604), same as *holotype*; 3 males (HNU: Cdbs8901, Cdbs8902, Cdbs8903), Hubei Province, Huanggang City, Yingshan County, Dabie Mountain, Wujiashan National Forest Park, 31.1047°N, 115.7913°E, 931.91 m a. s. l., 9. VI. 2022, light trap, leg. Zigang Xu.

Etymology. The name of this new species is derived from the Latin words "*quartri*" and "*macula*", meaning "*four*" and "*spot*", "*stain*" or "*mark*", respectively. The name specifically pertains to the presence of four distinctive black spots found on the tergites of this species.

Diagnostic characters. This species has two short longitudinal striped spots on each side of tergites II to V, as well as two elliptical spots in the center. Wing with brown markings on apical of Cu1, M3+4 and basal part of cell an. Additionally, tergite IX with a triangular anal point beyond the margin of tergite IX. Lastly, the gonostylus is curved inward at two-thirds of its length, and the apex is markedly tapered.

Description. Adult males (*N* = 7)

Total length: 4.49–5.55, 5.02 mm, Wing length 2.72–3.27, 2.99 mm, TL/WL 1.65–1.76, 1.71, WL/Pfe 2.08–2.59, 2.34.

Coloration (Fig. 2D). The head and thorax of this species are uniformly brown. The femurs of all legs are also brown, while the other sections display a yellow coloration. The wings are adorned with various color spots. Notably, there is a longitudinal color band on both sides of tergites II to V, accompanied by two elliptical spots at the center. Tergites VI to VII exhibit a dark brown hue, while tergites VII to IX and the hypopygium are brown in color.

Head (Fig. 2A). Antenna with 13 flagellomeres (Fig. 2B), AR: 2.01–2.05, 2.03, The end of flagellomere narrow, with approximately one-third of the end easily foldable, and the ultimate flagellomere possessing a single apical seta. Temporal setae 20–22, 21. The inner verticals and outer verticals are arranged in two or three columns, with 4–8, 6 inner verticals, 10–16, 13 outer verticals, and 12–18, 15 postorbitals. Tentorial length 250–258, 254 µm, width 36–43, 40 µm. Stipes length 271–303, 287 µm, width 62–95, 78 µm. Clypeus with 16–18, 17 setae. Length of palpomeres (in µm): 67–78, 72; 91–105, 98; 173–190, 182; 241–279, 260; 334–378, 356. Length ratio of palpomeres 5/3: 1.93–1.99, 1.96.

Thorax (Fig. 2F). Thorax length 1.36–1.73, 1.55 mm, antepronotals with 12–12, 12 setae. The acrostichals and dorsocentrals are arranged in multiple columns, acrostichals with 40–60, 50 setae, dorsocentrals with 60–80, 70 setae. Anepisternals with 2–3, 3 setae, postnotals with 4–6, 5 setae, humerals with 8–14, 11 setae, prealars with 22–26, 24 setae. Scutal tubercle was present and obvious, scutellars with 32–40, 36 setae.

Wing (Fig. 2C). The wing membrane area is hairy, Cu₁ and An with obvious gray spots around the veins, arculus hook-shaped, and anal lobes particularly developed. VR: 0.91-0.96, 0.94, brachiolum with 3 setae, R₁ with 30-40, 35 setae, R with 40-60, 50 setae, R₄₊₅ with 50-60, 55 setae, squama with 54-60, 57 setae. Costal extension 157-160, 158 µm.



Figure 2. *Macropelopia (Macropelopia) quadrimacula* Xu & Fu, sp. nov., male imago A head B antenna C wing D whole body of male adult E spotted shape of the tergites F thorax G fore tibial apex H hypopygium, dorsal view I photo of hypopygium, ventral view J hypopygium, ventral view K gonostylus.

Legs. The fore tibia possesses a single spur measuring 85-112, $98 \mu m$ in length and features 19-20, 20 side teeth. The width of the fore tibia at its apex is 79-86, $82 \mu m$, and fore tibial comb consists of 5-6 setae (Fig. 2G). Two spurs on mid tibia are observed, measuring 87-95, $91 \mu m$ and 50-56, $53 \mu m$ long, bearing 18-20, 19 and 11-16, 14 lateral teeth, respectively, and has a width at the apex of 75-81, $78 \mu m$. The hind tibia exhibits two spurs, measuring 88-96, $92 \mu m$ and 49-53, $51 \mu m$, with 19-21, 20 and 15-17, 16 lateral teeth, respectively. The hind tibial comb is composed of 13-15, 14 setae, with the longest comb seta 73-81, $77 \mu m$ and the shortest 40-46, $43 \mu m$. The width of the hind tibia at its apex is recorded as 76-86, $81 \mu m$. The lengths and proportions of each leg are shown in Table 2.

Hypopygium (Fig. 2H–K). The anal point is small, forming an obtuse triangle. Phallapodeme short and obvious, 72–81, 77 μ m long. Gonocoxite cylindrical shape, 253–265, 259 μ m long. Gonostylus (Fig. 2K) 126–146, 136 μ m long, curved inward at two-thirds of its length and possessing protrusions both on the inner and outer sides, the inner protrusion was located at a quarter of the apex of the gonostylus, while the outer protrusion was located halfway along the apex. Tergite IX with 16–20, 18 setae, megaseta 16–17 μ m long. Inferior volsella small and protuberant. HR: 1.91–2.09, 2.00. HV: 3.56–3.94, 3.75.

Remarks. This new species can be identified by the presence of two short longitudinal color bands on the sides of tergites II to V, along with two elliptical spots in the middle. These distinctive characteristics set it apart from other species within the genus. However, the abdominal spots of this species may sometimes be indistinct and appear blurry. In tergites III to V, these spots may be partially obscured by brown spots, but tergite II consistently displays four clearly visible elliptical spots. The overall shape of this new species is similar to that of *M. kibunensis*, and it shares the same gonostylus morphology. However, *M. kibunensis* lacks an inferior volsella and anal point, while this new species possesses a protrusion on the inferior volsella. The shape of the inferior volsella is comparable to that of *M. excavata* sp. nov., but this new species can still be differentiated by the presence of color spots on the tergites, the presence of the anal point, and a higher HR value (1.91–2.09) compared to *M. excavata* sp. nov. Based on COI sequences, the pairwise distances between *M. quadrimacula* and *M. kibunensis*, and between *M. quadrimacula* and *M. excavata*, are 0.119–0.125 and 0.131, respectively, further setting it apart from them.

Distribution. Hubei Province, Oriental China.

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄
P ₁	1260-1321, 1291	1606–1803, 1705	1195–1346, 1271	628–712, 670	455–517, 486	300-342, 321
P ₂	1342–1526, 1434	1546-1732, 1639	944–1086, 1015	474–533, 504	343–394, 368	229–257, 243
P ₃	1285–1338, 1311	1795–1935, 1865	1167–1355, 1261	637–723, 680	455–498, 477	285–319, 302
	ta₅	LR	BV	SV	BR	
P ₁	192–215, 203	0.73–0.75, 0.74	2.47-2.61, 2.54	2.28–2.46, 2.37	2.57-3.84, 3.21	
P ₂	165–184, 175	0.61-0.63, 0.62	3.16-3.21, 3.19	3.01-3.11, 3.06	2.56-4.37, 3.47	
P ₃	176-204, 190	0.63-0.71, 0.67	2.65–2.83, 2.74	2.42-2.67, 2.55	2.84-3.47, 3.16	

Table 2. Lengths (in μ m) and proportions of leg segments of male *M. quadrimacula* Xu & Fu, sp. nov. (*N* = 6).

Key to adult males of genus Macropelopia from China

1	Wings with distinct color spots2
_	Wings without color spots; only dark markings on cross-vein r-m
2	Tergites with brown spots
_	Tergites without brown spots6
3	Foretibial comb with 9–15 small bristles, inferior volsella significantly larger
-	Foretibial comb with 5–7 small bristles, inferior volsella absent or unde- veloped4
4	Tergites IX is uncovered gonocoxite, base of the anal point is wide, pre- senting a triangular anal point; posterior setae on tergite IX are mainly distributed in the central part M . (M .) quadrimacula Xu & Fu. sp. nov.
-	Tergites IX covered parts of gonocoxite, anal point absent; posterior setae on tergite IX are mainly distributed at the posterior margin
5	Coxa, trochanter, part femur and apex of tibia dark brown, and apex of femur pale; antennal ratio 1.7–1.9; whole wing with dense setae on membrane
-	Coxa, trochanter, femur and apex of tibia dark brown; antennal ratio 2.24; upper part of the wings with dense setae on membrane, but they are sig- nificantly reduced below the CU vein and M2+3 vein
6	Tergites pale brown, foretibial comb with $11-15$ small bristles, and anal point absent M (M) pergraphics (Tang & Niitsuma)
-	Tergites all brown, foretibial comb with 6 bristles and anal point present <i>M. (M.) decedens (Walker)</i>

Discussion

Based on the statistical data presented in this study, it has been revealed that there are currently seven species of the genus *Macropelopia* known to be distributed in China. However, it is important to note that the species *Macropelopia* (*M.*) *notata* and *M.* (*M.*) *decedens*, as reported by Wang et al. (2020), lack sufficient morphological characteristics for definitive identification. While we have included these species in the key provided in this study to reflect the current research records, further investigations are necessary to verify their distribution with more certainty.

We successfully obtained eight COI sequences for two new species and downloaded an additional eight sequences for seven species from the National Center for Biotechnology Information (NCBI). Their taxonomic names and Gen-Bank accession numbers can be found in Fig. 3. By utilizing the neighbor-joining method (Tamura et al. 2021) for constructing a phylogenetic tree, our analysis revealed that *M. excavata* sp. nov. and *M. kibunensis* are closely related, as they appeared on the same branch of the tree. This finding is consistent with their shared morphological characteristics, such as the similar coloration of the tergites, anal point absent, and thorax features. *Macropelopia quadrimacula* sp. nov. is shown to be the sister group to (*M. excavata* sp. nov. + *M. kibunensis*); the primary distinguishing features between *M. quadrimacula* sp. nov. and *M. excavata* sp. nov. lie in their hypopygium and the presence or absence of



Figure 3. Neighbor-joining tree based on cytochrome *c* oxidase subunit I (COI) of sixteen *Macropelopia* specimens. Numbers on branches refer to the evolutionary distances computed using the Kimura 2–parameter method (Kimura 1980) and represent number of base substitutions per site. Taxa names include scientific names and GenBank accession numbers of corresponding COI gene fragments.

a dorsal stripe on their tergites. These results exemplify a strong congruence between the molecular and morphological data. Interestingly, our findings contrast with the description of *M. kibunensis*, which includes yellow femora and the wing with dense setae. In contrast, the two new species possess brown femora and a dorsal stripe on the tergites. Thus, within the genus *Macropelopia*, key criteria for morphological classification encompass the characteristics of the hypopygium and dorsal stripe patterns on the tergites, followed by the markings and macrotrichia of the wing, and the color and features of the legs.

Chironomids offer the advantage of having three distinct stages (larvae, pupae, and adults); the morphology of the larvae and pupae of *Macropelopia* also plays an important role in species delineation (Fittkau 1962, Roback 1978, Fittkau and Murray 1986, Tang and Niitsuma 2020). This study primarily focuses on morphological differences in the adult stage; collecting specimens from multiple life stages simultaneously remains a challenge. However, previous studies have demonstrated that COI is suitable for summarizing sequence diversity and detecting taxonomically challenging species within *Macropelopia* (Silva and Pinho 2020). Therefore, we anticipate further studies on species delineation using the COI gene segment to enhance the reliability of new species establishment. The analysis of partial DNA barcode sequences supports *Macropelopia excavata* sp. nov. and *Macropelopia quadrimacula* sp. nov. as valid species.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: YF. Data curation: ZX. Formal analysis: XF. Funding acquisition: YF. Investigation: YY, XF. Methodology: ZX. Supervision: YF. Visualization: ZX. Writing - original draft: XF. Writing - review and editing: YF.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

The description of the first rock-dwelling species of butterfly lizard *Leiolepis* Cuvier, 1829 (Squamata, Agamidae) from the Khorat Plateau in northeastern Thailand

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Abstract

A new species of rock-dwelling *Leiolepis* is described from the Khorat Plateau in northeastern Thailand. *Leiolepis glaurung* **sp. nov.** can be differentiated from all other sexual species of *Leiolepis* by a combination of having a black gular region with a wide medial yellow stripe, a yellow ventrum with black mottling, bright red to orange subcaudal coloration, having reduced to no expandable flanks, and having only one black transverse bar on the flanks. This is the first rocky habitat-adapted *Leiolepis*. *Leiolepis glaurung* **sp. nov.** demonstrates numerous ecological adaptations to survive in these rocky habitats. *Leiolepis* are known for their expandable flanks with bright display colors, however *Leiolepis glaurung* **sp. nov.** has reduced or no ability to expand its flanks. We hypothesize this is an adaptation to reduce their body diameter to better fit into smaller rocky burrows unlike the larger and deeper burrows constructed in looser soils by other *Leiolepis* species. This discovery increases the number of *Leiolepis* species in Thailand to six, and worldwide to 11.

Key words: Conservation, Indochina, Leiolepis, phylogenetics, rock-dwelling

Introduction

Butterfly lizards of the genus *Leiolepis* Cuvier, 1829 contain six sexual species, *L. belliana* (Hardwicke & Gray, 1827), *L. guttata* Cuvier, 1829, *L. ocellata* Peters, 1971, *L. peguensis* Peters, 1971, *L. reevesii* (Gray, 1831), and *L. rubritaeniata* Mertens, 1961, and four all-female parthenogenetic species, *L. boehmei* Darevsky & Kupriyanova, 1993, *L. guentherpetersi* Darevsky & Kupriyanova, 1993, *L. ngovantrii* Grismer & Grismer, 2010, and *L. triploida* Peters, 1971. These ten species represent a

unique lineage of acrodont lizards that collectively range from southern China to Vietnam, Laos, Cambodia, Myanmar, Thailand, and south throughout the Malay Peninsula to Banda Island, Indonesia (Fig. 1). All species of Leiolepis are moderately sized (maximum snout-vent length 180 mm), diurnal omnivores that prefer coastal and inland savannah habitats with loose soil used to construct deep interconnected, subterranean burrow systems used for refugia and eggs deposition (Taylor 1963; Peters 1971; Cox et al. 1998; Aranyavalai et al. 2004; Grismer and Grismer 2010; Grismer 2011; Hartmann et al. 2012, 2013; Grismer et al. 2014). The six sexual species are sexually dimorphic and except for L. guttata, males have elongated ribs used to expand their flanks to display bright orange coloration (Hartmann et al. 2012). These expandable flanks and their colorations are used for courting females, warding off rival males, and anti-predator displays (Aranyavalai et al. 2004; Hartmann et al. 2012; Grismer et al. 2014). Conversely, the four parthenogenetic species are all similar in morphology and color pattern, resembling the coloration of their maternal ancestor, L. guttata (Schmitz et al. 2001; Grismer and Grismer 2010). Currently, four of the six sexual species, L. belliana, L. ocellata, L. pequensis, and L. rubritaeniata have populations within, or distributions that span much of Thailand (Fig. 1). All these species are morphologically diagnosable from each other and are restricted to various regions of Thailand with L. belliana being found in the south, central, and southeast, L. rubritaeniata in the northeast, and *L. ocellata* and *L. peguensis* in the extreme northwest (Fig. 1). These species appear to be ecologically and geographically isolated due to their different temperature and moisture thresholds due to the historical formation of rivers through Thailand over past 20,000 years (Promnun et al. 2021a).

The Khorat Plateau in northeastern Thailand (Fig. 1) is the remnant of the Cimmerian microcontinent and has been a center of endemism since the early Cretaceous (Fernandez et al. 2009; Ridd et al. 2011; Meynell 2017; Yang and Grote 2018; Shen and Siritongkham 2020). Extinct endemic species range from ray-finned fishes, sauropod dinosaurs, crocodiles, turtles, and fossil hominids which are sister to modern orangutans (Chaimanee et al. 2003, 2006 2015; Wongko et al. 2019; Manitkoon et al. 2022; Chaimanee and Jaeger 2023). Given the Khorat Plateau's modern climate with extreme wet and dry seasons and its unique geology, this pattern of endemism is still seen in contemporary groups such as freshwater bivalves, melon flies, blunt-headed burrowing frogs, puddle frogs, land snails, mud snakes, keelback snakes, and wolf snakes (Tumpeesuwan et al. 2007; Stuart and Chuaynkern 2007; Tumpeesuwan and Tumpeesuwan 2014; Boontop et al. 2017; Muanta et al. 2019; Nonsrirach and Lauprasert 2019; Vogel and David 2019; Jirapatrasilp et al. 2021; Köhler et al. 2021; Bernstein et al. 2022). Unfortunately, despite the endemism of this area, there are currently few conservation measures in place to protect its unique historical and contemporary biodiversity. On two recent expeditions to the Khorat Plateau during the dry season we discovered a new sexually reproducing population of Leiolepis that is morphology distinct from all other sexual species. Additionally, this new population of Leiolepis appears to be ecologically restricted to the elevated rocky habitats of the Khorat Plateau and seems to have adapted to these rocky environments by having the ability to dig interconnected, subterranean burrows into and beneath large, isolated rock piles. This type of natural history is unknown for any population of Leiolepis, which as stated above, are all adapted to sandy or loose soils in coastal and inland grassland environments.



Figure 1. The distribution of all *Leiolepis* species and the localities of specimens used in this study. Question marks indicate an area where new populations could possibly occur. The different shades of the green circles for *Leiolepis peguensis* correspond to their phylogenetic positions in Fig. 2.

In this study we use a combination of morphological and molecular datasets to test the hypothesis that this new population of *Leiolepis* from the Khorat Plateau represents a distinct species from all other sexual species of *Leiolepis*. Additionally, we will use the results and examples from the literature to demonstrate the Khorat Plateau's conservation importance and its unique biogeographic connections with other regions of Southeast Asia.

Materials and methods

All *Leiolepis* specimens were collected, photographed, and had liver tissue samples preserved in 95% ethanol for subsequent DNA sequencing. All specimens were fixed in 10% formalin and subsequently transferred into 70% ethanol. Color notes were taken from living specimens and digital images of living specimens. Scale counts and measurements were taken with Mitutoyo digital calipers to the nearest 0.1 mm under a Nikon SMZ 1500 dissecting microscope on the right side of the body where appropriate. Characters were obtained from Darevsky and Kupriyanova (1993) and Grismer and Grismer (2010) with new characters found here for sexual species (Suppl. material 1). We examined 104 specimens from five of the six sexual species for 54 characters (Suppl. material 1).

Measurements taken were: Snout-vent length (SVL), taken from the tip of the snout to the vent; head length (HL), measured from the posterior end of the retroarticular process of the mandible to the tip of snout; head width (HW), measured at the widest part of the head anterior to the tympanum; head height (HH), measured from the ventral surface of the mandible below the center of the eye to the dorsal surface of the head posterior to the eye; rostrum height (RH), measured from the ventral surface of the mandible to the dorsal surface of the rostrum above the nares; height of ear (HE), measured from the ventral margin of the tympanic opening to its dorsal margin; distance between nares (DN), measured with the tips of the calipers inserted into the narial openings; forearm length (FA), measured from the wrist to the outer edge of the flexed elbow; brachium length (BL), measured from the elbow to the limb insertion on the body; axilla-groin length (AG), measured from the posterior margin of the forelimb insertion to the anterior margin of the hind limb insertion on the body; hind limb length (FL), measured from the knee to the limb insertion on the body; tibia length (TIB), measured from the ankle to the flexed knee; length of the first enlarged, subdigital lamella on the third toe (ESL), measured from its base to its apex; third toe length (TE) measured from the base of the third toe to the base of the claw; chest width (CW), taken across the chest between brachium insertions on the body; pelvis width (PW), taken across the pelvic region between the hind limb insertions.

Meristic characters counted were the number of supralabials (SL), counted from the first enlarged scale at the angle of the jaw to the first enlarge scale contacting the postrostral scale; the number of infralabials (IL), counted from the first enlarged scale at the angle of the jaw to the first enlarge scale contacting the postmental scale; the number of scales across the frontal bone between the midpoint of the supraorbital regions that are not a supraorbital scales (FB); the number of postrostral scales contacting the rostral scale (PR); the number of supraorbital scales across dorsal surface of supraorbital region (SO); the number of enlarged, keeled suboculars below the ventral margin of the orbit (SBO); the number of enlarged scales along the ventral angle of the mandible (ESM); the number of transverse gular scales between the fourth enlarged mandibular scales along the angle of the jaw (GBESM); the ventral margin of the rostral scale forming a straight line with ventral margin of the first supralabial or not (VMR); the number of enlarged, keeled scale rows across the forearm midway between the elbow and wrist (KSFA); the number of dorsal scales between the inner margins of the left dorsolateral stripe at the widest part of the dorsolateral stripe (DSS); the number of non-keeled scale across the ventral side of the tibia midway between the ankle

(STIB); the number of enlarged, plate-like scales along the dorsal surface of the first toe (L1T); the number of enlarged, subdigital lamellae on third toe (ESL3T); the number of enlarged, subdigital lamellae on the fourth toe (SL4T); the number of enlarged, plate like scales along the dorsal surface of the fourth toe (L4T); the number of ventral scale across the belly contacting the apex of the umbilical scar (VSB); number of femoral pores per side (FMP); the number of transverse non-pore-bearing scales between pore-bearing-femoral scales (NPBS); the number of scales that make up width of the dorsal tail coloration posterior to the pelvis (NST).

Color pattern and categorical characters recorded were the presence (1) or absence (0) of a black banding or dark mottling in the pectoral region (BY); ventral pattern (VM), banded or mottling (1), spotted (2), or no coloration (3); presence (1) or absence (0) of pale colored spots on flanks (SF); presence (1) or absence (0) of a Y-shape marking on the nape of the neck (YN); the number of pale colored ocelli or spots between the dorsolateral stripes at widest part of the body (NSS); gular coloration (GC), white (1), blue and black (2), yellow and black (3), red (4), black and white (5); ventral color (VC), yellow (1), white (2), black and white (3), black and blue (4); the number of pale colored dorsal stripes (DS); flank color (FC), orange and black (1), orange (2), yellow and orange (3), black and white (4), grey (5); subcaudal coloration (TC), white (1) or red (2); presence (1) or absence (0) of a pale colored ventrolateral stripe contacting axillary region (VNSA); the number of black transverse bars on the flanks (TB); presence (1) or absence (0) of a pale colored ventrolateral caudal stripe (CS); presence (1) or absence (0) of thin, transverse, dorsal, caudal bars (CTB); presence (1) or absence (0) of a white eyespot at the center of the ocelli (WCES); color of plantar surfaces (PC), white (1), red and black (2), white and black (3); mottling coloration of the ventral surface of the legs (BMFT), black (1), red (2), white (0); and presence (1) or absence (0) of constricted tail base (CTAB).

Species concept and phylogenetic analyses

In this study we adopt the general lineage concept (de Queiroz 2007) and hypothesize that monophyletic clades in a phylogeny are evolutionary independent due to a lack of genetic admixture between populations of closely related lineages (Barraclough et al. 2003; de Queiroz 2007). Therefore, we identify any newly collected populations from the Khorat Plateau that form a well-supported monophyletic group distinct from all others in the phylogeny as a lineage that could potentially be a distinct species.

For all phylogenetic analyses we used sequence data from 135 samples representing all 10 species including seven samples from the newly discovered population from the Khorat Plateau and four previously published studies (Grismer and Grismer 2010; Grismer et al 2014; Promnun et al. 2021a, 2021b). Genomic DNA was extracted from liver tissues with the Qiagen DNeasy tissue kit (Valencia, CA, USA). We used double-stranded PCR to amplify roughly 800 aligned bases of the mitochondrial ND2 gene region using the primers METF6 (AAGCAGTTGG-GCCCATACC) and CO1R1 (AGRGTGCCAATGTCTTTGTGRTT) which are listed in Macey et al. (1997) and Grismer and Grismer (2010). Amplification of 25 µl PCR reactions were executed on an Eppendorf Mastercycler gradient thermocycler. Amplification of genomic DNA began with an initial denaturation for 2 min at 95 °C followed by 95 °C for 35 s, annealing at 50 °C for 35 s, and extension at 72 °C for 150 s with four seconds added to the extension per cycle for 32 cycles. PCR products were visualized using electrophoresis through a 1.2% agarose gel, marker 100 bp, 1X TAE and stained with Red- Safe Nucleic Acid Staining Solution and photographed under UV light of Geldoc system (Quantum CX5, Villber, France). Successful amplifications were purified using innuPREP Gel Extraction Kit (Analytik Jena, Germany). Cleaned PCR products were sent to Genewiz from Azenta Life Sciences for sequencing in both directions. The accuracy of sequences was ensured by incorporating negative controls and sequencing complementary strands. New sequences were combined with the mitochondrial datasets of Grismer and Grismer (2010), Grismer et al. (2014), Promnun et al. (2021a, 2021b), aligned into a single dataset using MUSCLE (Edgar 2004) in Geneious 6.1 (Kearse et al. 2012), and checked for nucleotide ambiguities. All new sequences were deposited in GenBank under accession numbers PP987908 to PP988006.

The resulting mtDNA dataset was used to estimate relationships among all 135 samples in RAxML 8.1.1 (Stamatakis 2014), unpartitioned, with the GTR-GAMMA model for sequence evolution, and assessed branch support using the automatic rapid bootstrap function on the CIPRES Science Gateway (Stamatakis 2014; Miller et al. 2010). We complimented the maximum likelihood analysis with a Bayesian analysis in BEAST 2.63 (Bouckaert et al. 2019). We used bModelTest to select the model of evolution, a Relaxed Log Normal Clock for the clock model, a Yule Model for the tree model, and each analysis was run for 350 million generations logging every 15,000. The BEAST log files were visualized in Tracer v. 1.7.0 (Rambaut et al. 2018) to ensure effective sample sizes (ESS) were well above 200 for all parameters. Maximum clade credibility trees using mean heights at the nodes were generated using TreeAnnotator v. 1.8.0 (Rambaut and Drummond 2007) with a burn-in of 10%. Nodes with Bayesian posterior probabilities (BPP) of 0.95 and above were considered strongly supported (Huelsenbeck et al. 2001; Wilcox et al. 2002). We considered nodes with values with a posterior probability of 0.90–0.94 as well-supported.

Statistical analyses

All statistical analyses were conducted using R Core team 2021. We employed a multiple factor analysis (MFA) using the R package FactorMineR (Husson et al. 2017) and visualized using the Factoextra package (Kassambara and Mundt 2017). The MFA was conducted on a concatenated data set comprised of 16 normalized morphometric (SVL, HL, HW, HH, RH, HE, DN, FA, BL, AG, FL, TIB, ESL, TE, CW, and PW), 18 meristic (SL, IL, FB, PR, SO, SBO, ESM, GBESM, KSFA, DSS, STIB, L1T, ESL3T, L4T, VSB, FMP, NPBS, and NST), and 17 categorical characters (TB, BY, VM, SF, YN, NSS, GC, VC, DS, FC, TC, CS, CTB, WCES, PC, CTAB, and BMFT). To remove potential effects of allometry in the morphometric characters, size was normalized using the following equation: X adj = $log(X) - \beta [log(SVL)]$ $-\log(SVL mean)$], where X adj = adjusted value; X = measured value; β = unstandardized regression coefficient for each population; and SVL mean = overall average SVL of all populations in the R package GroupStruct (Thorpe 1975, 1983; Turan 1999; Lleonart et al. 2000; Chan and Grismer 2022). The morphometrics of each species are normalized separately and then concatenated so as not to conflate intra- with interspecific variation (Reist 1986). All data were scaled to their standard deviation to insure they were analyzed based on correlation and

not covariance. A non-parametric permutation multivariate analysis of variance (PERMANOVA) in the package vegan 2.5-3 (Oksanen et al. 2020) was used to determine if the centroid locations and group clusters of each species/population from the MFA were statistically different from one another (Skalski et al. 2018) based on the load scores of dimensions 1-5. Using loading scores as opposed to raw data, allows for the incorporation of the categorical characters which cannot be run in a PERMANOVA untransformed. The analysis calculates a Euclidean (dis)similarity matrix using 20,000 permutations. Lastly, Analyses of variance (ANOVA) were conducted on meristic and normalized morphometric characters to search for the presence of statistically significant mean differences (p < 0.05) among species across the data set. Characters bearing statistical differences were subjected to a TukeyHSD test to ascertain which species pairs differed significantly from each other for those characters.

Results

All phylogenetic analyses returned matching topologies and near identical levels of nodal support (Fig. 2). The topology matches the relationships in the most recent nuDNA and mtDNA phylogenies of *Leiolepis* from Grismer et al. (2014) and Promnun et al. (2021b), respectively. All samples of rock-dwelling *Leiolepis* from the Khorat Plateau were recovered as a monophyletic group with strong support (bootstrap of 100) and lineage independence from all other species (Fig. 2). The Khorat Plateau clade is at the end of a long branch and sister to all populations of the sister species *L. rubritaeniata* and *L. reevesii* from eastern Indochina, with strong support (bootstrap of 100) in the RaxML and moderate support (posterior probability of 0.85) from the BEAST analyses (Fig. 2).

The MFA plot shows that the Khorat Plateau samples cluster outside all other species, including the closely related species *L. rubritaeniata* and *L. reevesii* (Figs 2, 3). Dimension 1 accounted for 28.8% of the variation and dimension 2 accounted for 19.6% of the variation in the dataset (Fig. 3). Across the first five dimensions (D1–D5) the morphometric variables accounted for 40% (D1), 7% (D2), 7% (D3), 12% (D4), and 12% (D5) of the variability of those dimensions (Fig. 4). Likewise, for the first five dimensions the categorical data accounted for 40% (D1), 30% (D2), 50% (D3), 52% (D4), and 25% (D5) of the variation (Fig. 4). Lastly, the contributions of the meristic variables for the first five dimensions accounted for 17% (D1), 62% (D2), 43% (D3), 37% (D4), and 60% (D5) of the variation (Fig. 4).

The results from the PERMANOVA further demonstrate the morphological differences between Khorat Plateau *Leiolepis* and the other clades of sexual species (Table 1). Although our low sample size of *L. reevesii* (*n* = 4) restricted us from including it in our analyses, we expect *L. reevesii* to be statistically distinct from the Khorat Plateau samples as its morphology is similar to that of *L. rubritaeniata*, which was once a subspecies of *L. reevesii* (Böhme 2003). In addition, the ANOVAs and subsequent TukeyHDS tests demonstrated that the Khorat Plateau has statistically different combinations of mean values of AG, BL, CW, DN, FA, HE, HH, HL, HW, PW, TIB, TE, RH, DS, VSB, TB, NST, NPBS, KSFA, FMP, FB, STIB, L1T, and L4T from all other sexual species (Suppl. material 2). Given the morphological differences (Figs 3, 4; Table 1) and their independent phylogenetic position (Fig. 2), we hypothesize the Khorat Plateau population represents a new distinct sexual species of *Leiolepis*.



Figure 2. The mitochondrial gene tree for all 135 samples of *Leiolepis* from the Maximum Likelihood and Bayesian analyses.

Taxonomy

Leiolepis glaurung sp. nov.

https://zoobank.org/22E2BD02-9672-434B-B4DF-43AD636677B7 Figs 5, 6, 9, 11; Table1; Suppl. materials 1, 2 Suggested English name: Khorat Plateau Butterfly Lizard

Type material. *Holotype*. Adult male (THNHM 30909; Fig. 5A) collected from just northeast of Wat Phu Noi in Kaeng Kheng Subdistrict, Kut Khaopun District, Ubon

Pratyaporn Wanchai et al.: Description of the first rock-dwelling Leiolepis from Khorat Plateau



Ratchathani Province, Thailand (15°48'10.7"N, 105°09'24.9"E) on 20 March 2023, at 10:00 am by Pratyaporn Wanchai, Anchalee Aowphol, Attapol Rujirawan, Akrachai Aksornneam, Jesse L. Grismer, L. Lee Grismer, Evan S. H. Quah, and Matthew L. Murdoch. *Paratypes.* Adult female (THNHM 30908; Fig. 5B) and two adult males (THNHM 30910–30911; Fig. 5C, D) bear the same locality and collectors as the holotype. Three adult males (THNHM 30902–30903, THNHM 30907) and three adult females (THNHM 30904–30906; Fig. 6) were collected by Pratyaporn Wanchai between 4–10 July 2020 from the same locality as the holotype.

Diagnosis. Leiolepis glaurung sp. nov. can be diagnosed from all sexual species of Leiolepis by having a black gular region with a wide medial yellow stripe, a yellow ventrum with black mottling, bright red to orange ventral tail coloration, having reduced to no expandable flanks, and having a maximum of one black transverse bar on the flank (Suppl. material 1). Leiolepis glaurung sp. nov. can be further diagnosed from all other sexual species by having the combination of a AG of 61.0-88.0 mm; BL of 11.0-16.8 mm; CW of 22.3-33.1 mm; DN of 4.3-6.7 mm; FA of 14.4-19.0 mm; HE of 5.6-7.1 mm; HH of 14.0-21.3 mm; HL of 28.8-41.9 mm; HW of 19.2-29.7 mm; PW of 10.5-17.0 mm; TIB of 24.34-29.0 mm; TE of 14.0-18.0 mm, RH of 9.7-14.3 mm; three dorsal stripes; 28-29 ventral scales; 21-24 dorsal caudal scales; 19-26 non-pore bearing scales between the pore-bearing femoral scales across the pelvis; maximum number of seven keeled scale rows across the forearm; 17-20 femoral pores per side; 6-8 scales across the frontal bone; maximum of nine subtibial scales; seven scales long the dorsal surface of the first toe; three enlarged subdigital lamellae on the third toe; and 32–34 scales along the dorsal surface of the fourth toe (Figs 7, 8).

Description of holotype. Head large, (HL 41.9 mm; HL/SVL 0.25) obtusely rounded in lateral profile, triangular in dorsal profile (HW 29.7 mm; HW/HL 0.71);



Figure 4. A The percent contribution of meristic and normalized morphometric characters of the first two dimensions of the MFA B The percent contribution of categorical, normalized morphometric, and meristic characters of the first five dimensions of the MFA.

interorbital, frontal region, and rostrum, convex (HH 21.3 mm; RH 14.3 mm; RH/ HH 0.67), sloped anteriorly, covered with small, undifferentiated keeled scales; occipital and supraorbital regions covered with 19 keeled, granular scale rows half the size as dorsal head scales; canthus rostralis short, rounded; dorsal head scales strongly keeled, eight keeled scales across the frontal bone between supraorbital regions; rostral large, triangular (wider than long), bordered posteriorly





by six smaller scales; external nares large, set wide apart (DN 6.7 mm; DN/HW 0.23) rounded, directed laterally, set in single, oval, nasal scale surrounded by several small scales; elongate, keeled, large fused, suborbitals (on five right side; five on the left) extend from anterior margin of eye to posterior margin of eye; superciliary scales elongate, keeled, imbricate, continuous with canthal scales; eyelid scales granular; tympanum naked, deeply set, surrounded by granular scales; temporal scales keeled, small, slightly raised; nine rectangular supralabials whose contact with one another produces an distinct labial margin, bordered ventrally by small granular scales; mental longer than wide, pointed posteriorly, larger than adjacent infralabials; two large postmentals in contact medially, being first of a series of 14 enlarged scales along the angle of jaw (left side: right side was damaged); 10 rectangular infralabials; gular scales small, rounded, granular; two distinct anterior and posterior gular folds present; dewlap absent; antehumeral fold continuous with posterior gular fold (Figs 5A, 6A).

Body elongate (AG 88.0 mm; AG/SVL 0.52) somewhat dorsoventrally compressed; expandable flanks reduced to absent; body scales small, granular, slightly keeled; 43 scales between dorsolateral stripes; 20 scales between vertebral stripe and dorsolateral stripes at widest point of trunk; scales of flanks abruptly transition into much larger, flat scales of belly and pectoral region; 28 scales across middle of belly contacting apex of the umbilical scar; precloacals smooth



Figure 6. Dorsal and ventral views of paratypes THNHM 30906, THNHM 30907, THNHM 30904, THNHM 30902, THNHM 30905, and THNHM 30903 *Leiolepis glaurung* sp. nov. Specimen tags bear field numbers.

and much smaller than ventral scales; forelimbs short, robust (FA 19.0 mm; BL 16.8 mm); dorsal surface of forelimbs and posterior surface of brachia covered with large, keeled, imbricate scales; six rows of enlarged, keeled scales across forearm; ventral surface of forelimbs covered with granular scales; plantar scales



Figure 7. Comparisons of the normalized morphometric characters showing the ranges, frequencies, mean (white dots), and 50% quartiles represented by black bars.

small, granular; subdigital lamellae of fingers are composed of a single wide transversely elongated scales; claws long; hind limbs relatively long (FM 33.6 mm; TIB 27.2 mm); dorsal scales on hind limbs small, weakly keeled; scales on anterior surface of thighs large, flat, weakly keeled, imbricate; those on forelegs slightly enlarged, keeled; postfemoral scales small, granular; eight longitudinal rows of large, smooth, flat, imbricate subtibial scales; 36 total femoral pores; each pore set in larger scale; 25 non-pore-bearing scales between pore-bearing-femoral scales across the pelvis; plantar scales small, raised; subdigital lamellae of toes bicarinate, 32 enlarged, plate-like scales along dorsal surface of the fourth toe; three enlarged, triangular scales on posterior surface at base of third toe (ESL



Figure 8. Comparisons of statistically different meristic characters among well-supported clades of *Leiolepis*. White circles are means and the black horizontal bars are medians.

2.6 mm; ESL/TE 0.16); seven enlarged, plate-like scales along length of first toe; tail dorsoventrally compressed, noticeably wider at base, constricted at its contact point with body, covered dorsally with small, keeled scales grading ventrally into larger, flat, weakly keeled, subcaudals; caudal scales in transverse rows encircle the tail; and the last 110 mm of the tail is regenerated (Fig. 5A).

Coloration in life. Dorsal ground color of head, body, limbs, and tail is grey to almost black (when animal is cold base color is black); anterior portion of the head pale grey with no pattern; three white lines radiate from the posterior region of the orbit with one extending posteriorly to the parietal region, one to the tympanic region, one to the corner of the mouth; four yellow stripes extending posteriorly from the parietal region of the head, the two central stripes connect on the

nape of the neck forming a yellow Y-shape and extend posteriorly as a vertebral stripe that terminating at the anterior margin of the pelvis, the two lateral stripes extend dorsolaterally from the parietal region running the entire length of the body terminating at the anterior margin of pelvis; dorsal pattern is composed of three yellow dorsal stripes (mentioned above) separated by distinct darker regions with yellow spots; the base color of the flanks are yellowish orange with one black transverse bar in the axillary region, followed by pale yellow transverse bands composed of small yellow spots; the hind limbs have small white ocelli with diffuse edges; the dorsal caudal coloration is composed of the same small ocelli as on the hind limbs and the coloration turns into dark transverse caudal bars approximately 30% down the length of the tail; the gular region is black with a wide medial yellow strip; pectoral region is bright red to orange and extends laterally to the dorsolateral coloration of the tail (Figs 5A, 6, 9, 11)

Etymology. The specific epithet *glaurung* is in reference to the large, terrestrial, golden-colored, non-winged dragon, Glaurung in Middle-earth – a character created by J.R.R. Tolkien in *The Silmarillion* (1977). Glaurung the Golden is the father of all dragons and tunneled into the sides of mountains forming burrows. The reduced expandable lateral flanks, yellow ventral and dorsal colors, with the construction of burrows beneath rocky outcrops is similar to the descriptions of Glaurung mentioned above, from 'The Silmarillion' and 'The Children of Húrin' (Tolkien 1977, Tolkien and Tolkien 2007). Additionally, in Thailand the word "Yae" is used to refer to *Leiolepis belliana*, *L. ocellata*, and *L. rubritaeniata*. However, on the Khorat Plateau the populations of *Leiolepis glaurung* sp. nov. are called "Yarb".

Distribution. *Leiolepis glaurung* sp. nov. is currently known from three locations on the Khorat Plateau (Fig. 1). We have only collected specimens from the type locality in Ubon Ratchathani Province (Fig. 1) from the Phu Phan Formation (Jin-geng and Meesook 2013), however a population from Chaiyaphum Province (15°54′7.92″N, 102°9′50.4″E) in the western region of the Khorat Plateau was observed and photographed, but no specimens were collected (AK, AR, and PP pers. obs.), and from southern extent of the plateau on the border of Cambodia (PW pers. obs.) in Ubon Ratchathani Province (Fig. 1). Lizards from both populations are very similar in appearance to those from the type locality.

Variation. Differences in scale counts and measurements are presented in Suppl. material 1. The coloration of the males THNHM 30911 and THNHM 30910 are similar to the holotype but their subcaudal coloration is a much more vibrant coral-red and they have complete original tails (Fig. 5C, D). The coloration of the yellow stripes and spots that composed the dorsal pattern, and the flank coloration are not as pronounced in the female specimens THNHM 30904–30906 and 30908 as they are in the males (Figs 6, 9; Suppl. material 1). Additionally, the dorsal caudal pattern is only faintly visible in the female specimens (Figs 6, 9).

Natural history. Similar to other species of *Leiolepis*, *Leiolepis glaurung* sp. nov. constructs subterranean tunnels. However, given that they live exclusively in rocky habitats, individuals make compressed and shallow burrows in patches of loose soil underneath rocks or rockpiles (Figs 10–12). Males tend to forage during the hottest part of the day and the feces we found appeared to be mostly composed of vegetation and arthropods. *Leiolepis glaurung* sp. nov. is a food source for local people in the area (as many *Leiolepis* species are across Indochina; Grismer and

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Figure 9. A Female paratype THNHM 30908 B male paratype THNHM 30911 of Leiolepis glaurung sp. nov.

Grismer 2010) and talking with local collectors, they report that individuals are highly philopatric. The collectors say that if the burrow is open that means they are out foraging and if the burrow is plugged with soil they are inside. Additionally, local people say that *Leiolepis glaurung* sp. nov. is mostly active during the



Figure 10. The rocky habitat of the Khorat Plateau from the type locality.

dry season (November–March) and estivates during the rainy season (April–November) but will come out of their burrows after rains to eat arthropods. Lastly, all individuals observed and collected (specimens THNHM 30908–30911) were found during the day at 36 °C and 77% humidity. Other species of reptiles observed sharing the same habitat at the type locality included *Calotes versicolor* (Daudin, 1802), *Dixonius siamensis* (Boulenger, 1899), *Gekko petricolus* Taylor, 1962, *G. gecko* (Linnaeus, 1758), *Scincella melanosticta* (Boulenger, 1887), *S. rupicola* (Smith, 1916), *Eutropis macularia* (Blyth, 1853), *Chrysopelea ornata* (Shaw, 1802), *Lycodon laoensis* Günther, 1864 and *Calloselasma rhodostoma* (Kuhl, 1824).

Discussion

Our results indicate that the samples of *Leiolepis* from the Khorat Plateau reported here are phylogenetically, ecologically, and morphologically distinct from all other sexual species of *Leiolepis* and as such, represent a new species. We hypothesize that *Leiolepis glaurung* sp. nov. has undergone multiple mor-



Figure 11. A Male paratype THNHM 30911 displaying the dorsal and lateral display colors and its rocky habitat in the background **B** adult male *Leiolepis rubritaeniata* from southeastern Cambodia **C** male paratype THNHM 30911 of *Leiolepis glaurung* sp. nov.

phological adaptions to balance living in a rocky environment with pressures of sexual selection. Others have demonstrated that the color combination and expandable flanks of male *Leiolepis* are important for courtship and antagonistic confrontations and are likely to be under some degree of sexual selection (Hartmann et al. 2012; Grismer et al. 2014). Grismer et al. (2014) further hypothesized that the amount and combination of colors on the expandable flanks are what drove the hybridization events that created the parthenogenetic species of *Leiolepis*. *Leiolepis glaurung* sp. nov. is distinct from all other sexual species in that individuals retain the bright display colors on the flanks. We



Figure 12. A Two burrow entrances going beneath a large rock **B** multiple small burrow entrances in the soil underneath and around a rock pile **C** a single entrance hole to a burrow beneath a large rock **D** the rock from panel C lifted to show the shallow subterranean burrow of an individual *Leiolepis glaurung* sp. nov.

hypothesize this is an adaptation to reduce their body diameter to allow access into the smaller burrows beneath rocks in shallow condensed soils (Fig. 12), unlike the larger and deeper burrows constructed in looser soils in savannahs and coastal areas by other Leiolepis species in Indochina. As a result of the reduced expandable lateral display area, we hypothesize that Leiolepis glaurung sp. nov. has evolved contrasting brightly colored subcaudal, ventral, and gular regions (Fig. 5) to act as secondary sexual displays. Given the dark base color of the dorsum, these colors and associated displays would possibly stand out to females against the dark color of the substrate (Fig. 10). Field observations indicate that Leiolepis glaurung sp. nov. lives adjacent to L. rubritaeniata which occupies the intervening sandy savannah habitats in the area and appear to not interact with each other. We hypothesize that the unique ecology and display coloration between the two species (Fig. 11) has kept these two species genetically isolated (Fig. 2). Additionally, these ecological and morphological differences discussed above between them has led to local collectors calling them different names - "Yarb" for Leiolepis glaurung sp. nov. and "Yae" for L. rubritaeniata, L. belliana, and L. ocellata.

Our field work indicates that *Leiolepis glaurung* sp. nov. is endemic to the Khorat Plateau (Fig. 1) further highlighting the growing pattern of endemism in this unique region of Thailand. The Khorat Plateau is broadly composed of

Table 1. Results from the PERMANOVA analysis comparing pairs of sexual species of *Leiolepis* bearing statistical mean morphospatial differences at the *p*.adjusted threshold based on the load scores of Dim1–Dim5 of the MFA. * = significant difference.

Clade comparisons	p.value	p.adjusted	sig
L. glaurung sp. nov. vs L. rubritaeniata	0.00004000	0.00083998	*
L. glaurung sp. nov. vs L. belliana	0.00002000	0.00041999	*
L. glaurung sp. nov. vs L. peguensis South	0.00022000	0.00461991	*
L. glaurung sp. nov. vs L. peguensis North	0.00409992	0.08609828	
L. glaurung sp. nov. vs L. guttata	0.00057999	0.01217976	*
L. rubritaeniata vs L. belliana	0.00002000	0.00041999	*
L. rubritaeniata vs L. peguensis South	0.00012000	0.00251995	*
L. rubritaeniata vs L. peguensis North	0.00309994	0.06509870	
L. rubritaeniata vs L. guttata	0.00035999	0.00755985	*
L. belliana vs L. peguensis South	0.00010000	0.00209996	*
L. belliana vs L. peguensis North	0.00179996	0.03779924	*
L. belliana vs L. guttata	0.00025999	0.00545989	*
L. peguensis South vs L. peguensis North	0.01179976	0.24779504	
L. peguensis South vs L. guttata	0.00209996	0.04409912	*
L. peguensis North vs L. guttata	0.01785714	0.37500000	

moist deciduous forest and the environment drastically changes from the dry season to the rainy season. During the rainy season, regions of the plateau flood as the Mun and Chi rivers swell and drain the plateau into the southern Mekong River, creating additional unique microhabitats only available during the wet season. Most of the contemporary endemic species or genetic lineages on the Khorat Plateau are from groups such as freshwater bivalves, land snails, frogs, and water snakes that are most active during the wet season (Tumpeesuwan et al. 2007; Muanta et al. 2019; Jirapatrasilp et al. 2021; Köhler et al. 2021; Bernstein et al. 2022). However, *Leiolepis glaurung* sp. nov. is most active during the dry season and may be a good bioindicator that there are other undescribed species active only during this time of the year. This could mean that there are groups of species adapted to either the wet or dry season on the Khorat Plateau.

Lastly, irrespective of annual activity times, the endemic species and lineages on the Khorat Plateau are generally sister to species or populations from either Sundaland or Indochina (Fernandez et al. 2009; Boontop et al. 2017; Muanta et al. 2019; Jirapatrasilp et al. 2021; Bernstein et al. 2022; Chaimanee and Jaeger 2023). *Leiolepis glaurung* sp. nov. mirrors this pattern being recovered as sister to the remaining populations of *Leiolepis* east of the Chao Phraya River (Figs 1, 2). These conflicting biogeographic patterns indicate that during its formation, the Khorat Plateau may have been colonized multiped times from different regions of Sundaland and Indochina. These biogeographic connections may be the key to understanding why the species composition on the Khorat Plateau has historically been and currently is a mixture of common, wide-ranging, and locally endemic species.

The Khorat Plateau needs further biodiversity surveys during the wet and dry seasons to (1) obtain an understanding of which species are active at what times of the year; and (2) to collect additional specimens and genetic samples for taxonomic and broader biogeographic studies. These types of data would be vital to forming effective conservation measures to provide some level of protection for the unique species and ecosystems of the Khorat Plateau.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

The research protocol was approved by Institutional Animal Care and Use Committee, Kasetsart University (ACKU66-SCI-019).

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Author contributions

Conceptualization: P. Wanchai, J.L. Grismer, A. Aowphol. Formal analysis: P. Wanchai, J.L. Grismer, L.L. Grismer, A. Rujirawan. Investigation: P. Wanchai, A. Rujirawan, M.L. Murdoch, A.Aksornneam, P. Promnun, A. Kaatz, J.J. Gregory, E. Nguyen, W. Van Iderstein, E.S.H. Quah, L.L.Grismer, J.L. Grismer, A. Aowphol. Writing – Original draft: P. Wanchai, J.L. Grismer, A. Aowphol. Writing – Review and Editing: P. Wanchai, A. Rujirawan, M.L. Murdoch, A.Aksornneam, P. Promnun, A. Kaatz, J.J. Gregory, E. Nguyen, W. Van Iderstein, E.S.H. Quah, L.L.Grismer, P. Promnun, A. Kaatz, J.J. Gregory, E. Nguyen, W. Van Iderstein, E.S.H. Quah, L.Grismer, J.L. Grismer, A. Aowphol. Visualization: J.L. Grismer, A. Rujirawan. Supervision: J.L. Grismer, A. Aowphol. Project administration: P. Wanchai, J.L. Grismer, A. Aowphol.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Raw meristic, categorical, and color pattern data from all specimens of *Leiolepis* used in this study

- Authors: Pratyaporn Wanchai, Attapol Rujirawan, Matthew L. Murdoch, Akrachai Aksornneam, Pattarapon Promnun, Amanda Kaatz, Jeren J. Gregory, Eddie Nguyen, William Van Iderstein, Evan S. H. Quah, L. Lee Grismer, Jesse L. Grismer, Anchalee Aowphol Data type: xlsx
- Explanation note: m = male; f = female; = data not taken; NA = specimen was damaged, and character could not be taken.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/zookeys.1210.127557.suppl1

Supplementary material 2

Significant adjusted *p*-values from the results of the ANOVA and TukeyHDS analyses comparing all pairs of sexual species of *Leiolepis* for all morphological characters

- Authors: Pratyaporn Wanchai, Attapol Rujirawan, Matthew L. Murdoch, Akrachai Aksornneam, Pattarapon Promnun, Amanda Kaatz, Jeren J. Gregory, Eddie Nguyen, William Van Iderstein, Evan S. H. Quah, L. Lee Grismer, Jesse L. Grismer, Anchalee Aowphol Data type: xlsx
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/zookeys.1210.127557.suppl2


Research Article

Description of *Nilssondytes diversus* gen. et sp. nov. from Venezuela (Coleoptera, Adephaga, Dytiscidae, Cybistrinae)

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Abstract

A new genus, *Nilssondytes* **gen. nov.**, is described for a unique new species, *Nilsson-dytes diversus* **sp. nov.**, from Venezuela. This paper corrects an inadvertent mistake in a paper by the authors (Miller et al. 2024) in which the collection of deposition of the holotype of the species was not indicated making the new species unavailable which, therefore, made the new genus unavailable. A review of the relevant parts of the International Code of Zoological Nomenclature is included. Diagnostic features of the new genus and species are discussed and illustrated.

Resumen

Un nuevo género, *Nilssondytes* **gen. nov.** se describe para la única nueva especie *Nilssondytes diversus* **sp. nov.** de Venezuela. Este artículo corrige un error involuntario en un artículo de los autores (Miller et al. 2024) en el que no se indicaba la colección de depósito del holotipo de la especie, lo que hacía que la nueva especie no estuviera disponible y, por lo tanto, el nuevo género no estuviera disponible. Se incluye una revisión de las partes relevantes del Código Internacional de Nomenclatura Zoológica. Se discuten e ilustran las características diagnósticas del nuevo género y especie.

Key words: Diving beetle, phylogeny, South America, taxonomy, water beetle

Introduction

In a recent paper revising the classification of the diving beetle (Dytiscidae) subfamily Cybistrinae, a new genus and new species were described, "*Nilsson-dytes diversus*" (Miller et al. 2024). However, a statement indicating the name and location of the type depository of the new species was inadvertently not included (Miller et al. 2024). According to Article 16.4.2 of the International Code of Zoological Nomenclature (hereafter, "The Code" (ICZN 1999)):

"[Art. 16.4] Every new specific and subspecific name published after 1999, except a new replacement name (a *nomen novum*), for which the name-bearing type of the nominal taxon it denotes is fixed automatically [Art. 72.7], must be accompanied in the original publication.



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Copyright: [©] Kelly B. Miller et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). [Art. 16.4.2] where the holotype or syntypes are extant specimens, by a statement of intent that they will be (or are) deposited in a collection and a statement indicating the name and location of that collection (see Recommendation 16C)." (ICZN 1999).

Because of lack of a statement indicating, "... name and location of that collection," it seems objectively clear that the species name was not made available (Miller et al. 2024).

Despite the species name not being made available by Miller et al. (2024), it is possible the genus name was made available. However, according to Article 67.2. of The Code, "Species eligible for type fixation (originally included nominal species)" for nominal genera, the type species must be an available name cited in the original publication (ICZN 1999). The complete, relevant section of the Code reads as follows:

"67.2.1. In the meaning of the Code the "originally included nominal species" comprise only those included in the newly established nominal genus or subgenus, having been cited in the original publication by an available name (including citation by an incorrect spelling [Art. 67.6]) of a species or subspecies (see Articles 45.6 and 68.2), or having been cited there as the deliberate application of a previous misidentification (see Articles 11.10, 67.13 and 69.2.4)" [emphasis ours]. (ICZN 1999).

Since the only species name included in the genus, and the one explicitly designated as the type species, was not available, the genus name was also not available (Miller et al. 2024).

This paper seeks to correct the errors introduced by that paper and formally introduce a new genus and new species of Cybistrinae from northern South America. The following largely replicates Miller et al. (2024) in order to correct the lapse in leaving out the type depository of the new species and the ramifications of that oversight. Additional discussions about relationships of this species and genus to others including a phylogenetic analysis, diagnostics, and keys to Neoptropical Cybistrinae are provided by Miller et al. (2024).

Materials and methods

Methods for specimen preparation and examination follow Miller et al. (2024).

Specimens of known species of South American Cybistrinae genera were examined from several collections (Miller et al. 2024). Collections including examined specimens of the new species described here (holotype and paratypes) are the following:

- **MIZA** Museo del Instituto de Zoología Agrícola Francisco Fernández Yépez, Universidad Central de Venezuela, Maracay, Venezuela (L. Joly).
- **MSBA** Museum of Southwestern Biology, Division of Arthropods, University of New Mexico, Albuquerque, NM, USA (K.B. Miller).
- **SEMC** Snow Entomological Collection, University of Kansas, Lawrence, KS, USA (A.E.Z. Short).

Measurements follow Miller et al. (2024) and are based on the range of available specimens and/or published values and were taken either using a standard steel ruler (longer measurements) or an ocular scale on a Zeiss Discovery V8 dissecting microscope at 50× magnification (shorter measurements). All examined specimens of the new species were measured. Measurements include: (1) total length (TL); (2) greatest width across elytra (GW); (3) greatest pronotal width (PW); (4) greatest width of the head (HW); (5) distance between the eyes (EW); (6) narrowest width of metaventral wing (MV, Fig. 6); and (7) width across lateral portion of metacoxal (MC, Fig. 6). The ratios TL/GW, HW/EW, and MC/ MV were also calculated to provide an indication of overall shape, eye size, and relative sizes of morphological features.

Male and female genitalia were dissected using methods similar to Miller (2001), Miller and Bergsten (2014, 2016), Miller et al. (2007, 2009, 2024). Line drawings were created by sketching the structure using pencil and a drawing tube attached to a Zeiss Discovery V8[™] microscope then scanning and digitizing the sketch, "inking", and editing using Adobe Illustrator[™].

Taxonomy

Nilssondytes gen. nov.

https://zoobank.org/2477607A-7AA0-4DE8-A865-56563D3D29D9 Figs 1-11

Remark. The following description is reproduced with some modifications from Miller et al. (2024).

Type species. *Nilssondytes diversus* sp. nov., by current designation.

Diagnosis. From other Cybistrinae this genus differs in having: (1) the metatibial spurs apically simple, (2) metacoxal lines clearly present, (3) the pronotum and elytron with broad, distinct lateral yellow bands along margins (Fig. 1), (4) males and females each with two metatarsal claws, the posterior much reduced in both sexes (Figs 2, 3), (5) the prosternum and prosternal process relatively shallowly but distinctly sulcate (Fig. 4), (6) the medial margins of the male sternite IX straight, not emarginate (Fig. 10), (7) no cluster or line of setae at the apicodorsal angle of the posterior surface of the mesotarsomeres (Fig. 5), and (8) the ventral surface of the metatrochanter with an oblique, transverse groove. The single species in this genus (described below) is somewhat similar in size, shape, and coloration to *Metaxydytes laevigatus* (Olivier) and may be present among series of that species in collections. *Nilssondytes* differ from *M. laevigatus* in several features (see above) including the presence of yellow lateral elytral margins (Fig. 1) which are absent in *M. laevigatus*. Larvae are unknown.

Etymology. This genus is named *Nilssondytes* from the Greek *dytes* meaning "diver," and *Nilsson*, after the great diving-beetle worker and excellent friend, Anders N. Nilsson, in honor of his inestimable contribution to the science of diving-beetle biology.

Phylogenetic relationships. The single species of *Nilssondytes* gen. nov. is part of the clade that includes species with an oblique metatrochanteric groove, but it has an unresolved position with respect to other genera (Miller et al. 2024). The presence of a reduced posterior metatarsal claw in both males and females (Figs 2, 3) with straight medial margins of the male abdominal

sternite IX (Fig. 10) is a unique combination of features within Cybistrinae. Unique among this larger clade is also the sulcate prosternum and prosternal process (Fig. 4) which is somewhat similar to the Australian genera *Spencerhy-drus* Sharp, 1882 and *Sternhydrus* Brinck, 1945.

Nilssondytes diversus sp. nov.

https://zoobank.org/EE6E7F8D-5B43-4131-BF61-9692E3F9047E Figs 1-11

Remark. The following description is reproduced with some modifications from Miller et al. (2024).

Type locality. Venezuela, Amazonas State, roadside pond ca. 7 km S Samariapo 5°10.900'N, 67°46.078'W, 95 m elev.

Diagnosis. This is the only species in the genus and is characterized by its diagnostic combination (see above). Typically, species-level features include the shape of the male median lobe which is unique. In ventral aspect the apex is abruptly constricted with the apex narrowly truncate with laterally pointed processes (Fig. 6). In lateral aspect, the median lobe is moderately evenly curved on the dorsal margin, apically abruptly narrowed with the apex elongate and slender, apex narrowly rounded (Fig. 7).

Description. *Measurements.* TL = 16.7-19.4 mm, GW = 9.6-10.7 mm, PW = 7.0-8.1 mm, HW = 4.2-4.7 mm, EW = 2.7-2.9 mm, TL/GW = 1.7-1.8, HW/ EW = 1.6-1.7, WC/WV = 3.1-3.2. Body shape suboval, slightly expanded posteriorly, widest at ~ 3/5 of length (Fig. 1); lateral margins evenly, continuously curved between pronotum and elytron. Depressed and somewhat flattened in lateral aspect.

Coloration (Fig. 1). Head dark green, anterior clypeal margin yellow, more so laterally, testaceous near eyes. Pronotum dark green with broad lateral yellow margin, posteriorly interrupted and green in three of the four examined specimens, in other specimen yellow extending to posterior angle. Elytron dark green with broad lateral yellow band, separated narrowly from lateral margin, slightly expanded near apex. Ventral surfaces largely black, testaceous on head, basal leg segments and elytral epipleuron.

Sculpture and structure. Head broad, frontoclypeal lines elongate, straight, strongly oblique; anterior clypeal margin broadly, shallowly and evenly concave; dorsal surface evenly covered with fine microsculpture and micropunctures. Pronotum with lateral margins evenly and broadly curved; surface similar to surface of head in microsculpture and micropunctation. Elytral lateral margin evenly and slightly curved for most of length, apically broadly curved; surface of elytron similar to surface of head in microsculpture and micropunctation. Prosternal process apically rounded, ventral surface distinctly sulcate (Fig. 4), apex robust, acutely pointed. Metaventral wing broad, slightly less than 1/3 width of lateral portion of metacoxa (WC/WV = 3.1-3.2); surface smooth, without sculpturing; metacoxal lines short, extending less than half distance across metacoxa. Abdominal ventrites smooth, unsculptured.

Male genitalia. Male median lobe in ventral aspect broad throughout most of length, apically abruptly narrowed, apex laterally produced, submedially with



Figures 1–10. *Nilssondytes diversus* sp. nov. 1 dorsal habitus 2, 3 metatarsal claws, posterior aspect: 2 male 3 female 4 prosternal process, ventral aspect 5 right mesothoracic leg, posterior aspect 6–8 male genitalia: 6 median lobe, ventral aspect 7 median lobe, right lateral aspect 8 right lateral lobe, right lateral aspect 9 female genitalia (gonocoxae, laterotergites, right gonocoxosternite and internal genitalia), ventral aspect 10 male sternite IX, ventral aspect. Scale bars: 5.0 mm (1); 1.0 mm (2–5, 9, 10)

broad, elongate lobes on each side, ventral sclerite short, apically sharp, acuminate, extending to 3/5 length of median lobe, apex sharply pointed (Fig. 6). In lateral aspect shallowly curved, apically abruptly narrowed, apex narrowed, slightly curved, apically narrowly rounded, broad medially (Fig. 7). Lateral lobe broad in



Figure 11. Distribution of Nilssondytes diversus sp. nov. in northern South America.

basal half, apically distinctly narrowed, apex narrowly rounded, with series of elongate setae along more than apical half of dorsal margin of lateral lobe (Fig. 8).

Female genitalia. With a single genital opening, vagina elongate, slender, with enlarged, bulbous region at base of common oviduct; spermatheca short, curved, at apex of enlarged region, with soft tissue region on each side of enlarged region (Fig. 9); gonocoxae together broad, apically broadly pointed (Fig. 9); gonocoxosternite broad, with elongate anterolateral lobe, with medial margin sublinear, without conspicuous setae (Fig. 9).

Sexual dimorphism. Males have a characteristic broad protarsal palette with ventral adhesive setae. Males also have mesotarsomeres with clumps of posteroventral setae. Females lack pro- and mesotarsal expansions or adhesive setae. Both males and females have two metatarsal claws with the posterior shorter than the anterior (Figs 2, 3), but females have the posterior somewhat more curved than in males (Fig. 3). Females have distinctive microsculpture on the surface of the elytron anteriorly in the form of a field of short striae which is absent in males.

Variation. Five specimens were examined. One specimen has the lateral pronotal yellow band extending to the posterior margin of the pronotum, the others have a narrow dark green separation from the posterior margin.

Distribution. This species is known from few localities in Venezuela along the northwestern margins of the Guiana Shield craton (Fig. 11).

Natural history. The only natural history information available from labels is "roadside pond," "river margin," and "rock outcropping."

Etymology. The species is named from the Latin *diversus*, meaning "different," in recognition of the different lengths of the metatarsal claws in both males and females (Figs 3, 4).

Material examined. *Holotype*, male deposited in MIZA (see above) labeled, "VENEZUELA: Amazonas State 5°10.900'N, 67°46.078'W, 95 m ca. 7 km S. Samariapo 15.i.2009; leg. Short, Miller, García, Camacho, Joly VZ09-0115-02X: roadside pond/ SM0846115 KUNHM-ENT [barcode label]/ Holotype: *Nilssondytes diversus* Miller, Michat & Ferreira-Jr., 2024 [red label with double black line border]." *Paratypes*, 1 male (SEMC) labeled, "Suapure VENEZ. Caura River 4.20.1900 [handwritten] E.A. Klages.", 1 female (MIZA) labeled "VENEZUELA: Bolivar State 7°41'23.6"N, 64°1'56.0"W, 134 m ca. 14 km E Rio Aro; 5.viii.2008 leg. A. Short \$ M. García AS-08-073; rock outcropping/ SM0829328 KUNMH-ENT [barcode label]," 1 female (SEMC) labeled "VENEZUELA: Guárico State 8°6.226'N, 66°26.228'W, 52m UCV San Nicolasito Field Station: Rio Aguaro; 10.i.2009 leg. Short, Miller, Joly, García, Camacho; VZ09-0110-01A/ SEMC0852602 KUNHM-ENT," 1 male (MSBA) labeled "VENEZUELA: Bolivar State 6.58694°N, 67.02912°W Rio Caripito 12.i.2009; leg. Short Miller VZ09-0112-02A: river margin/ SM0844405 KUNHM-ENT [barcode label]." All paratypes with, "...Paratype *Nilssondytes diversus* Miller, Michat and Ferreira-Jr., 2024 [blue label with black line border].

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

Expanding the knowledge of the bat fauna of the Brazilian Caatinga: new geographical records of molossid bats (Chiroptera, Molossidae) for the Chapada Diamantina region, with taxonomic notes

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Abstract

The Caatinga, an exclusive biome in Brazil, is the largest tropical dry forest area in the Americas. It is characterized by a semi-arid climate and various soils that harbor a great diversity of flora and fauna. Novel records of aerial insectivorous bat species in the family Molossidae in the Chapada Diamantina, northeastern Brazil are presented. The study is based on field sampling of 115 molossid bat specimens from six genera and 12 taxonomically confirmed species, along with four taxa requiring further evaluation for definitive species identification. All specimens were obtained using mist nets around a small freshwater lagoon surrounded by semideciduous dry forest. The verified genera were Cynomops, Eumops, Molossops, Molossus, Neoplatymops, and Nyctinomops. Our findings enhance the understanding of bat diversity in the Brazilian Caatinga, with the first records of Europs delticus, E. bonariensis, and Molossus currentium. The most abundant species were Molossus rufus, Eumops glaucinus, Cynomops planirostris, Nyctinomops laticaudatus, and Molossus molossus. Previously unreported morphological and morphometric variations for these Caatinga taxa were examined. Additionally, information on sexual dimorphism in craniodental characteristics of Molossops temminckii and variations in the presence of the sagittal crest in Neoplatymops mattogrossensis are provided. Based on the voucher specimens from this study, the recognized number of species of Molossidae known from the Caatinga has increased to 21. Our results offer new insights into the taxonomy and biogeography of Neotropical molossids, highlighting their importance as members of bat communities in dry forest ecosystems from northeastern South America.

Key words: Distributional records, dry forests, insectivorous bats, morphological diversity, Neotropical Molossidae, northeastern Brazil

Introduction

The Caatinga is an exclusive biome in Brazil and is considered the largest area of tropical dry forests in the Americas (Gutiérrez and Marinho-Filho 2017). This biome is characterized by a semi-arid climate with a wide variety of soils that harbor a great diversity of flora and fauna, including many endemic taxa (Garcia et al. 2014; Gutiérrez and Marinho-Filho 2017).

After Colombia, with 217 bat species (Rosero-Taramuel et al. 2023), Brazil has the second-highest richness of mammals belonging to the order Chiroptera (186) in the New World (Barros and Bernard 2023), with nearly 15% of the world's bats (Bernard et al. 2011; Garbino et al. 2024). In the Caatinga, a range of 69 to 95 bat species have been reported (Garcia et al. 2014; Carvalho-Neto et al. 2017; Carmignotto and Astúa 2017; Silva et al. 2018), representing approximately 53% of the bat diversity of the country (Delgado-Jaramillo et al. 2020). Compared to other biomes comprised of the dry diagonal of non-forest formations in South America, the Caatinga stands out for its significant bat diversity, especially insectivores (Gregorin et al. 2008; Silva and Bernard 2017).

New World free-tailed bats (Molossidae) are a diverse family of aerial insectivorous species predominantly occurring in tropical and subtropical regions, with limited species diversity in temperate zones (Simmons 2005). They are considered the most highly adapted aerial foragers, specifically hawking high-flying insects (Norberg and Rayner 1987). Morphologically, they have relatively long and narrow wings with a high aspect ratio and wing loading. Their flight patterns include high-speed flight in open spaces with relatively low maneuverability (Norberg and Rayner 1987; Jung et al. 2014).

Previously, the diversity of molossids in the Caatinga was estimated to range between 14 and 17 species, based on recent checklists (Carvalho-Neto et al. 2017; Silva et al. 2018). However, many of those reported taxa require a careful, and detailed review of the captured individuals due to recent nomenclatural changes and new species descriptions (Gregorin et al. 2016; Loureiro et al. 2018a; Arenas-Viveros et al. 2021). Additionally, it is necessary to verify the taxonomic identification of many cryptic taxa (Bernardi et al. 2009; Loureiro et al. 2018b), given the lack of comprehensively verified voucher specimens represents a challenge in recognizing intra-specific variations.

As aerial insectivorous, most molossids are known to forage over forest canopies and open spaces, flying from medium to high strata where they feed on relatively large prey (Jung et al. 2014). Given their foraging preferences and feeding strategies, they are difficult to capture with conventional techniques, usually set at ground level (mist nets and harp traps). However, mist nets and harp traps can more effectively capture molossids at the canopy level. As a result, many of the Neotropical aerial insectivorous bats, including most of the molossids are poorly represented in field studies or as vouchers in zoological collections, and biological information is scarce (Ochoa-G et al. 2000; Moras et al. 2018; Miller et al. 2023).

As part of an evaluation of the bat community occurring within the Caatinga, an important number of molossids were captured during nine nights of mist net sampling at a single location associated with a freshwater lagoon. The results of this study confirm the taxonomic richness of this family in the region and provide additional data on an important number of species considered "cryptic" (Bernardi et al. 2009; Gregorin 2009) and highly variable in their morphological and physiological characteristics (Loureiro et al. 2018b); some of these taxa have not been reported in previous publications related to the Caatinga biome (Silva et al. 2018). We underscore the importance of accurately documenting voucher specimens in scientific collections to corroborate taxa identification for future works (e.g., Poma-Urey et al. 2023).

Materials and methods

The survey was conducted within the Caatinga biome in Lençóis, Chapada Diamantina region, Bahia State, northeastern Brazil (12.54822°S, 41.37928°W, 351 m. a.s.l., Fig. 1). The dominant vegetation of the study area corresponds to a primary semideciduous forest. All geographic coordinates are in decimal degrees with the base datum WGS84.

Fieldwork was conducted from 6 to 14 December 2023 using seven mist nets of varying sizes ($6 \times 3 \text{ m}$, $9 \times 3 \text{ m}$, and $12 \times 3 \text{ m}$) set around a freshwater lagoon formed by the drying of an intermittent river within the Chapada Diamantina National Park (Fig. 2).

All bats were captured following the guidelines of Sikes (2016), and those retained as vouchers were humanly euthanized (injecting a barbiturate anesthetic via intraperitoneal), then fixed in formaldehyde (10%), and preserved in ethanol (70%). All handling protocols were approved by the Animal Use Eth-



Figure 1. Map of Chapada Diamantina National Park, northeastern Brazil, showing the geographic location of the sampling site in Lençóis.



Figure 2. Freshwater lagoon used as capture site of the molossid bats reported in this study for Lençóis (Chapada Diamantina National Park). Associated vegetation corresponds to a primary semideciduous forest.

ics Committee of the Universidade Estadual de Santa Cruz (CEUA-UESC Nos. 004/2020 and 024/2021). All capture and collecting were under permit number 17131-4, provided by the Sistema de Autorização e Informação em Biodiversidade – SISBIO of the Instituto Chico Mendes de Conservação da Biodiversidade – ICMBio, Brazil; this permit was granted to one of the researchers (MRAV). The following authorization allowed us to conduct fieldwork within the study area: ICMBio – SISBIO 79060-1 for PARNA Chapada Diamantina. Vouchers were deposited in the Coleção de Mamíferos Alexandre Rodrigues Ferreira (**CMARF**) at the Universidade Estadual de Santa Cruz (**UESC**), Bahia, Brazil. All skulls were extracted and measured to compare cranial morphology and verify identifications.

Species-level identifications were made by the authors, who have extensive experience with the taxonomy of Neotropical bats and the management of natural history collections. We did not include a review of voucher specimens deposited in local collections for this study. This was primarily because most collections related to specimens from the Caatinga have incomplete representation of species in the family Molossidae. However, we did a comprehensive review of the literature to verify the accuracy of our identifications. Secondly, as these included cryptic species, many identifications of local collection-based specimens could be in error as most Latin American institutions do not have specialists for this group. Therefore, all the identifications reported here are based on our broad taxonomic knowledge and supported by published sources.

Preliminary identifications were made based on keys for Neotropical bats (Eger 2008; Díaz et al. 2016, 2021), as well as those specifically for species of Molossidae occurring in Brazil (Gregorin and Taddei 2002; Loureiro et al. 2018b). To verify the preliminary identifications, qualitative and quantitative characteristics were compared to comprehensive resources related to the taxa of molossid bats. These included taxonomic revisions, descriptions of new species, and phylogenetic reviews of the genera Eumops (Marinkelle 1970; Eger 1977; Freeman 1981; Bernardi et al. 2009; Gregorin 2009; Gregorin and Cirranello 2015; Gregorin et al. 2016; Ruelas and Soria 2021), Molossus (Freeman 1981; Dolan 1989; Gregorin and Cirranello 2015; Loureiro et al. 2018a, 2018b), Cynomops (Gregorin and Cirranello 2015; Moras et al. 2018), Molossops (Gamboa Alurralde and Díaz 2019; Ramírez-Chaves et al. 2023), Nyctinomops (Bianconi et al. 2009; Gregorin and Cirranello 2015; Rocha et al. 2015; Oliveira et al. 2019; Portugal-Zegarra et al. 2020; Barquez et al. 2023), and Neoplatymops (Sazima and Taddei 1976; Willig 1985; Willig and Jones 1985; Gregorin and Cirranello 2015).

The following morphological parameters previously reported for molossids (Bernardi et al. 2009; Gregorin et al. 2016; Loureiro et al. 2018b; Moras et al. 2018; Ruelas and Soria 2021), were considered: total length of body (TLB); tail length (TL); length of hind limb (LHL); ear length (EL); weight (W); forearm length (FA); greater length of skull with incisors (GSLI); greater length of skull excluding incisors (GSL); condylobasal length (CBL); condylo-incisor length (CIL); palatal length (PL); zygomatic breadth (ZB); mastoidal breadth (MB); braincase width (BCW); interorbital width (IOW); length of maxillary toothrow (C-M3); width across upper canines (C-C); width across upper molars (M3-M3); greatest length of mandible (LM); and length of mandibular toothrow (c-m3). All measurements are in millimeters and mass in grams.

Due to the pronounced sexual dimorphism reported for members of the family Molossidae (Eger 2008; Gregorin et al. 2011; Moras et al. 2018; Loureiro et al. 2018b), principally body and cranial dimensions, morphometric analysis was conducted separately for males and females. The results are presented for each species based on a structure that includes sex, CMARF catalog number, external and cranial measurements by sex, morphological variations, and a brief description of the diagnostic traits used to verify identification. All bats included in the diagnosis were adults unless otherwise noted.

Results

Of the 335 bats captured during this survey, 115 were molossids, of which 102 were identified to the species level, and 13 were equivocal and only identified to genus. Six genera were recorded (*Cynomops* Thomas, 1920; *Eumops* Miller, 1906; *Molossops* Peters, 1866; *Molossus* É. Geoffroy St.-Hilaire, 1805; *Neoplatymops* Peterson, 1965, and *Nyctinomops* Miller, 1902) and 12 confirmed species.

Sampled specimens included notable representative series of *Molossus rufus* (26 spcms), *Eumops glaucinus* (21 spcms), *Molossus molossus* (15 spcms), *Cynomops planirostris* (10 spcms), *Nyctinomops laticaudatus* (eight spcms), *Neoplatymops mattogrossensis* (seven spcms), and *Molossus aztecus* (five spcms). The remaining captures corresponded to five specimens of *Molossus currentium*, two medium-sized specimens with morphological and metric characteristics documented for the *Eumops bonariensis* complex, two *Molossops temminckii* specimens, and one *Nyctinomops macrotis* specimen. Several captured individuals did not match previously known species and only were identified as morphospecies. They include one specimen consistent with the genus *Molossops* (referred to here as *Molossops* sp.) and three morphotypes whose characteristics correspond to the genus *Molossus* (*Molossus* sp. 1, 2, and 3).

Species accounts

Cynomops planirostris (W. Peters, 1866)

Summary of captures. Six females (CMARF 2111–2116) and four males (CMARF 2117–2120).

External measurements and weights. The average and range of external measurements and weights for females: TLB: 90.00 (80.00–97.00), TL: 31.00 (27.00–36.00), LHL: 5.97 (5.40–7.00), EL: 13.17 (13.00–14.00), W: 9.50 (9.00–10.00). Males: TLB: 90.00 (89.00–92.00), TL: 30.00 (28.00–32.00), LHL: 5.88 (5.34–6.01), EL: 13.80 (13.08–14.05), W: 9.75 (9.00–10.00).

Morphological description. Dorsal fur varies from chocolate brown to yellowish brown, contrasting with the paler ventral coloration. Skulls with a relatively low and short rostrum (Fig. 3). Lacrimal ridge conspicuous with the anterior face sloped smoothly to the forehead. Incisive foramina positioned closer to the accessory foramen and the three foramina (incisive and accessory) forming an equilateral triangle (seen with magnification from above). Basisphenoid pits are shallow. Values of some skull measurements for the females and males are shown in Table 1.

Identification. Externally, the forearm length (< 40 mm) and the ventral coloration paler than the dorsum in all specimens, in addition to cranial measurements and the arrangement of incisive and accessory foramina in the shape of an equilateral triangle, distinguish this species from its most similar congeners in the area (*Cynomops greenhalli* Goodwin, 1958, and *C. milleri* (Osgood, 1914), Moras et al. 2018; López Berrizbeitia and Díaz 2021).

Eumops bonariensis (W. Peters, 1874)

Summary of captures. One female (CMARF 2121).

Table 1. External and cranial measurements for eight species of bats of the family Molossidae with confirmed identities and one unidentified morphospecies. Data based on specimens collected in the Caatinga biome (Lençóis, Chapada Diamantina region, northeastern Brazil), according to this study (catalog numbers are indicated) and previous information in the literature. Abbreviations are described in the Materials and methods.

ТАХА	FA	GSLI	GSL	CBL	CIL	PL	ZB	MB	BCW	IOW	C-M3	C-C	M3-M3	LM	c-m3
Cynomops planirostris															
Female (CMARF 2111)	31.19	15.49	15.00	14.34	14.95	6.86	9.98	9.41	7.86	4.30	5.82	3.93	7.20	10.99	5.66
Female (CMARF 2112)	31.93	16.35	16.04	15.23	15.87	6.98	10.64	10.33	7.79	3.96	6.04	4.33	7.55	11.57	6.97
Female (CMARF 2113)	31.46	15.92	15.30	15.69	15.29	6.05	10.28	9.93	7.73	4.03	5.69	4.15	7.39	10.91	6.60
Female (CMARF 2114)	31.61	15.33	14.89	14.34	14.81	6.38	10.31	9.93	7.88	4.08	5.62	4.11	7.40	10.59	6.63
Female (CMARF 2115)	32.09	15.89	15.71	15.06	15.66	6.21	10.43	9.90	7.38	4.06	5.80	4.44	7.46	11.39	6.48
Female (CMARF 2116)	32.21	15.94	15.60	14.73	15.30	6.42	10.11	10.05	7.78	4.07	5.86	4.21	7.51	11.13	6.85
Male (CMARF 2117)	32.94	17.07	16.98	15.92	16.57	7.63	11.13	10.86	8.05	4.07	6.19	4.57	7.83	12.02	6.50
Male (CMARF 2118)	32.63	16.85	15.95	14.51	15.24	6.72	10.58	10.26	7.76	3.92	5.74	4.81	7.20	11.78	6.05
Male (CMARF 2119)	33.68	16.85	16.10	15.83	16.66	7.51	11.03	10.74	7.87	4.10	6.22	4.80	7.70	11.69	6.32
Male (CMARF 2120)	32.78	17.20	15.00	15.83	16.64	7.29	11.19	10.84	8.10	4.40	6.36	4.68	7.94	12.04	7.32
Eumops bonariensis															
Female (CMARF 2121)	44.63	18.15	17.55	17.54	17.18	7.17	10.63	9.91	8.89	3.99	6.76	4.36	7.59	12.15	7.81
Female (Bernardi et al. 2009)	47.60	19.20	-	17.80	18.40	-	11.10	10.50	9.10	4.20	7.30	4.60	8.10	13.00	7.70
Female (Bernardi et al. 2009)	45.90	19.40	-	17.60	18.00	-	11.40	10.60	9.20	4.00	7.20	4.60	8.10	12.80	7.70
Female (Ruelas and Soria 2021)	49.30	18.86	-	18.09	18.72	7.80	11.69	10.45	9.01	4.29	7.50	0.96*	1.90*	14.48	8.78
Eumops delticus															
Female (CMARF 2122)	47.53	18.34	18.10	17.27	17.46	7.23	10.58	10.12	8.83	3.80	7.15	4.53	7.64	12.36	7.60
Female (Carter and Dolan 1978, Holotype: BMNH 23.8.9.7)	-	-	18.80	17.90	-	-	10.90	10.30	9.10	4.20	7.20	4.70	7.90	12.70	7.90
Female (Ruelas and Soria 2021)	47.68	18.38	-	-	18.11	7.13	11.08	10.17	8.78	4.42	6.93	-	-	13.18	7.79
Eumops glaucinus			1		1	1			1						
Female (CMARF 2123)	60.98	24.08	23.60	22.21	23.22	9.08	14.41	13.05	11.62	5.02	9.26	5.65	10.26	17.29	10.47
Female (CMARF 2124)	56.54	23.70	22.80	21.41	22.74	10.35	13.90	12.89	10.69	4.82	8.93	5.80	9.92	16.46	10.26
Female (CMARF 2125)	60.20	23.82	23.50	22.26	23.52	10.09	14.52	12.96	11.12	4.94	8.91	5.83	10.05	17.50	10.66
Female (CMARF 2126)	59.22	24.45	23.99	22.38	23.41	10.66	14.30	12.92	11.30	5.02	9.27	5.92	10.02	17.32	10.63
Female (CMARF 2127)	60.80	24.43	23.78	22.53	23.61	10.29	14.39	13.11	11.28	5.04	9.30	5.81	9.97	17.53	10.49
Female (CMARF 2128)	60.45	23.90	23.50	22.07	23.32	10.16	14.13	12.60	11.02	5.17	9.22	5.65	9.97	17.35	10.52
Female (CMARF 2129)	62.61	23.93	23.66	22.09	23.16	10.45	14.87	13.11	11.57	4.93	8.81	5.91	10.33	17.14	10.23
Female (CMARF 2130)	63.23	24.12	23.78	22.17	23.27	10.03	14.27	12.95	11.37	4.96	9.01	5.65	10.05	17.15	10.72
Male (CMARF 2131)	59.41	24.43	24.17	22.49	23.99	10.45	14.86	13.74	11.25	5.17	9.55	6.38	9.55	18.15	10.97
Male (CMARF 2132)	59.13	24.45	24.25	22.79	24.03	10.26	14.45	13.14	11.29	4.88	9.15	6.12	9.78	17.35	10.65
Male (CMARF 2133)	58.50	24.78	23.99	22.57	23.94	10.66	14.37	12.86	11.19	4.98	8.82	5.72	9.53	17.21	10.43
Male (CMARF 2134)	60.02	24.63	24.10	22.60	24.01	10.21	14.82	13.56	11.13	5.31	9.37	5.82	10.33	17.72	10.70
Male (CMARF 2135)	60.20	24.66	23.95	22.85	23.80	9.94	15.34	13.51	11.59	5.13	9.21	6.14	10.01	17.28	10.50
Male (CMARF 2136)	59.74	24.30	23.93	22.65	23.78	10.15	15.20	13.30	11.24	4.90	9.54	5.95	10.15	17.53	10.24
Male (CMARF 2137)	58.55	24.38	23.80	24.42	23.74	10.00	14.54	13.25	10.86	4.87	8.86	6.04	9.79	17.43	10.20
Male (CMARF 2138)	59.65	24.68	24.10	22.61	24.00	10.35	14.48	13.34	11.37	5.26	9.29	6.01	10.40	17.46	10.75
Male (CMARF 2139)	64.33	24.50	24.20	23.23	24.17	10.38	14.39	13.21	11.31	4.96	9.12	6.47	10.30	17.50	10.90
Male (CMARF 2140)	64.16	23.81	23.66	22.30	23.26	10.08	14.16	12.87	10.71	4.98	8.84	5.78	10.17	17.11	10.11
Male (CMARF 2141)	59.75	24.69	24.50	22.76	24.25	10.99	14.34	13.11	10.92	4.96	9.57	5.59	10.12	17.81	10.92
Male (CMARF 2142)	52.38	24.63	23.86	22.67	23.76	10.57	14.62	13.28	11.45	5.43	9.39	5.85	9.92	17.15	10.67
Male (CMARF 2143)	59.28	24.55	24.00	22.59	23.96	10.70	14.95	13.35	11.25	5.18	9.18	5.87	10.11	17.51	10.35
Molossops temminckii	1	1	1	1	1	1	1	1	1		1	1	1	1	
Female (CMARF 2144)	31.53	14.27	14.00	13.38	13.83	6.05	9.21	8.73	6.86	3.73	5.35	3.75	6.64	10.03	5.84
Male (CMARF 2145)	32.33	14.85	13.50	13.94	14.52	6.66	9.44	8.72	7.25	3.78	5.51	4.32	6.71	10.86	6.19

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		GSLI	GSL	CBL	CIL	PL	ZB	MB	BCW	IOW	C-M3	C-C	M3-M3	LM	c-m3
Molossops sp.															
Female (CMARF 2146)	31.73	14.29	14.00	13.44	13.76	6.28	9.51	8.58	7.22	3.78	5.07	4.17	6.69	10.15	5.91
Neoplatymops mattogrossensis															
Female (CMARF 2210)	29.39	14.64	14.40	13.62	14.30	6.70	-	9.27	7.06	3.69	5.40	3.70	6.59	9.92	5.80
Female (CMARF 2211)	29.39	14.34	14.21	13.60	14.19	6.70	-	8.51	6.78	3.42	5.49	3.47	6.52	9.52	5.97
Female (CMARF 2212)	30.01	14.85	14.40	13.71	14.30	6.61	9.56	8.98	7.14	3.55	5.56	3.52	6.87	9.84	5.84
Female (CMARF 2213)	29.71	14.62	14.18	13.42	14.12	6.82	9.29	8.52	6.88	3.51	5.14	3.70	6.68	10.08	5.72
Female (CMARF 2214)	24.31	14.62	14.28	13.52	14.08	6.49	9.91	8.65	7.47	3.59	5.21	3.74	6.69	9.48	5.91
Female (CMARF 2215)	23.69	14.47	14.20	13.51	14.13	6.31	9.21	8.63	7.26	3.54	5.21	3.77	6.76	9.92	5.75
Female (CMARF 2216)	28.69	14.01	13.99	13.32	13.96	6.46	9.47	8.96	7.06	3.68	5.18	3.73	6.54	9.78	5.67
Nyctinomops laticaudatus		-						-							
Female (CMARF 2217)	44.26	17.93	17.00	15.95	16.78	7.35	10.07	9.57	8.51	3.50	6.55	3.89	7.68	11.64	7.11
Female (CMARF 2218)	45.90	18.10	17.00	15.78	16.68	6.54	10.25	9.47	8.69	3.50	6.17	3.93	7.48	12.17	7.56
Female (CMARF 2219)	44.02	17.70	17.05	15.87	16.58	6.95	10.21	9.67	8.46	3.75	6.54	3.91	7.33	11.83	7.10
Female (CMARF 2220)	44.97	17.43	17.10	15.44	16.19	6.14	9.66	9.65	8.20	3.65	6.09	3.68	7.07	11.91	7.16
Female (CMARF 2221)	44.47	18.16	17.00	16.00	16.95	7.02	10.39	9.58	8.72	3.64	6.67	3.93	7.63	12.15	7.43
Female (CMARF 2222)	44.67	17.61	17.00	15.56	16.34	6.95	10.19	9.86	8.48	3.57	6.44	3.75	7.42	11.68	6.72
Male (CMARF 2223)	44.47	17.78	16.98	16.03	16.57	7.26	9.74	9.37	8.38	3.37	6.27	3.66	7.16	12.01	7.23
Male (CMARF 2224)	45.19	18.62	17.96	16.41	17.23	7.70	10.43	10.03	8.65	3.54	6.67	4.00	7.39	12.17	7.30
Nyctinomops macrotis															
Female (CMARF 2225)	59.83	23.36	22.68	21.38	22.29	9.46	12.54	11.80	10.34	4.35	8.27	4.91	8.63	16.27	9.31



Figure 3. Dorsal, ventral, and lateral views of the skull of a male (CMARF 2118 **A**, **C** lower) and a female (CMARF 2115 **B**, **C** upper) of *Cynomops planirostris* collected in the Caatinga (northeastern Brazil), showing sexual dimorphism in some dimensions **D** external body features of a female of *Cynomops planirostris* from the same locality (CMARF 2115). Scale bar: 5 mm.

External measurements and weight. TLB: 108.00, TL: 43.00, LHL: 7.00, EL: 18.00, W: 10.00.

Morphological description. Dorsal fur is chocolate brown, with the basal portion of hairs paler than the tips. Ventral and dorsal coloration show slight contrast, with the hairs around the neck and shoulders darker than the rest (Fig. 4). Upper lips are slightly wrinkled. The upper border of the narial region is

surrounded by small and obtuse warts. Small hairs cover internarial ribs. Ears broad, rounded, and joined in a common point. The upper border of ears with pointed and fleshy warts. The inner keel of the ears reaching the antitragus, not extending beyond the posterior part of this structure. Antitragus long and semicircular. The tragus is slightly squared, with the upper extreme narrower, giving the appearance of an obelisk shape.

The skull is broader across the rostrum, with an evident depression near the mastoid bone; the braincase is deeper (globular shape), and the lateral region is curved (Fig. 5). Interparietal bones are not elongated and not visible in the lateral view of the skull. The sagittal crest is less developed than the lambdoidal crest. Upper incisors with divergent tips projected forward, forming an angle with the canines of 45°. Upper canines and first premolars in contact. The second upper premolar is protocone-wide and robust (Fig. 5). The third upper molar has a well-developed commissure. The posterior part extends beyond the maxillary bone (Fig. 6). The palate does not extend beyond the level of the third upper molars (Fig. 6). Mesopterygoid fossa with basisphenoid pits deep, narrow anteriorly and broader posteriorly, with an oval shape (Fig. 5). Rib between basisphenoid pit wide. Incisive foramen diminutive. The mandible is slender, with an articular process wider and more developed than the condylar and coronoid processes. Lower incisors bilobed. Values of some cranial measurements of the collected female and the data for the same variables provided by other studies are shown in Table 1.

Identification. Externally, the forearm length (> 40 mm) and the shape and depth of the basisphenoid pits (deep, narrowing anteriorly, and broader posteriorly) separate this species from *Eumops hansae* Sanborn, 1932 (Eger 2008). Compared to the closest morphologically similar species, *Eumops patagonicus* 0. Thomas, 1924, and *Eumops delticus* 0. Thomas, 1923, *E. bonariensis* can be differentiated from *E. patagonicus* by being larger in some external and cranial measurements, including the lengths of the forearm and skull. Additionally, the extension of the internal keel of the ears, not reaching the anterior antitragus area in *E. patagonicus*, is a discreet characteristic that can help differentiate *E. patagonicus* from *E. bonariensis* (Díaz et al. 2021). However, Bernardi et al. (2009) identified an individual as *E. patagonicus* with an internal keel reaching the middle region of the antitragus. With respect to *E. delticus*, cranial morphological characters are provided in the Discussion to aid in distinguishing both species.

Eumops delticus O. Thomas, 1923

Summary of captures. One female (CMARF 2122).

External measurements and weight. TLB: 107.00, TL: 42.00, LHL: 6.00, EL: 19.00, W: 11.00.

Morphological description. Dorsal fur is cinnamon brown, with the basal portion of the hairs paler than the tips. The color of ventral and dorsal fur shows slight contrast, with the hairs around the neck and shoulders darker than the rest (Fig. 4). The upper lips are slightly wrinkled. The upper border of the nares is surrounded by small and obtuse warts. Small hairs cover internarial ribs. Ears broad, rounded, and joined at a common point. The upper border of ears



Figure 4. Females of *Eumops bonariensis* (CMARF 2121; **A**) and *Eumops delticus* (CMARF 2122; **B**) showing the body coloration, the antitragus shape, and the extension of the internal ear keel that reaches the posterior part of the antitragus (indicated by the white bar).

with pointed and fleshy warts. The inner keel of the ears reaching the antitragus, not extending beyond the posterior part of this structure. Antitragus long and semicircular. The tragus is slightly subquadrate, with the upper extreme narrower, giving an obelisk appearance. Franger J. García et al.: New Molossidae records for the Chapada Diamantina



Figure 5. Dorsal, ventral, and lateral views of the skulls of two species of *Eumops* collected in the Caatinga, northeastern Brazil. **A, C** (upper)- *Eumops bonariensis* (CMARF 2121) **B, C** (lower)- *Eumops delticus* (CMARF 2122). White arrows indicate the morphological differences between both taxa, highlighted in the species' account. Scale bar: 5 mm.

The skull is broader across the rostrum with an evident depression near the mastoid bone, the braincase is deeper (globular shape), and the lateral region is curved (Fig. 5). Interparietal bones are elongated, clearly visible in the lateral view of the skull. Sagittal and lambdoidal crests developed. Upper incisors with divergent tips projected forward, forming an angle of 45° with the canines. Upper canines and first premolars in contact. The second upper premolars have protocones that are thin and not robust (Fig. 5). The third upper molars have a well-developed commissure. The posterior part of the third upper molar does not extend beyond the maxillary bone (Fig. 6). Palate extends beyond the level of the third upper molar (Fig. 6). Mesopterygoid fossa deep and narrower anteriorly, with basisphenoid pits deep, wider posteriorly and oval in shape (Fig. 5). Rib between basisphenoid pit thin. Incisive foramen large. The mandible is slender, with an articular processes. Lower incisors are bilobed. The values of



Figure 6. Enlarged views (not to scale) of some cranial and dental characteristics of *Eumops bonariensis* (**A**, **C**) and *Eumops delticus* (**B**, **D**). The discontinuous red lines and white arrows indicate the differences in palatal and maxillary lengths between both species, respectively.

some cranial measurements of the female collected and comparable data from other studies, are shown in Table 1.

Identification. Externally, the forearm length (> 40 mm) and the shape and depth of the basisphenoid pits (deep and wider posteriorly) separate this species from *Eumops hansae* (Eger 2008). When comparing differences to the closest congeners morphologically, *Eumops patagonicus* and *Eumops bonariensis*, *E. delticus* can be differentiated from *E. patagonicus* by being larger in some external and cranial measurements, including the lengths of forearm and skull, respectively. Additionally, the extension of the internal keel of the ears, not reaching the anterior antitragus area in *E. patagonicus* from *E. delticus* (Díaz et al. 2021). However, Bernardi et al. (2009) identified an individual as *E. patagonicus* with an internal keel reaching the middle region of the antitragus. Concerning differentiation of *E. bonariensis*, cranial morphological characters are provided in the Discussion to aid in distinguishing both species.

Eumops glaucinus (J.A. Wagner, 1843)

Summary of captures. Eight females (CMARF 2123–2130) and 13 males (CMARF 2131–2143).

External measurements and weights. Females: TLB: 142.25 (135.00–150.00), TL: 51.32 (41.46–58.00), LHL: 9.86 (7.91–11.00), EL: 22.33 (21.00–25.00), W: 33.12 (27.00–36.00). Males: TLB: 144.00 (137.00–148.00), TL: 51.84 (45.00–58.00), LHL: 10.53 (9.00–12.00), EL: 23.93 (22.18–26.00), W: 31.46 (28.00–36.00).

Morphological description. The fur color is dark brown to cinnamon, grayish dorsally, and pale brown ventrally, showing slight contrast between both sides (Fig. 7). The ears are wider than long and joined at the forehead. Antitragus is well developed, with a broad base. The tragus is small, wide, and square in shape. Snout elongated, with smooth upper lips. Gular-thoracic gland present in males.

Skull elongated, with variations in the posterior portion of the braincase; in some specimens, the posterior region is more elevated, while in others, it is flatter (Fig. 7). Similarly, the sagittal crest in some specimens is notably well-developed. In contrast, it is less perceptible to others. The lambdoidal crest is developed and evident. The basisphenoid pits are deep. Palate arched. The upper incisors are fused at the bases and divergent at the tips. Some skull measurements are presented in Table 1.

Identification. Externally, the forearm < 65 mm, and the short ears (averaging < 34 mm in both sexes) differentiate this species from the largest members



Figure 7. Dorsal, ventral, and lateral views of the skull of a female (CMARF 2127 **A**, **C** upper) and a male (CMARF 2131 **B**, **C** lower) of *Eumops glaucinus* collected in the Caatinga (northeastern Brazil), showing sexual dimorphism in some dimensions **D** external body features of a female of *E. glaucinus* from the same locality (CMARF 2127). Scale bar: 5 mm.

of *Eumops* (*E. chimaera*, Gregorin, Moras, Acosta, Vasconcellos, Poma, dos Santos & Paca, 2016, *E. dabbenei* O. Thomas, 1914, *E. perotis* (Schinz, 1821), and *Eumops trumbulli* O. Thomas, 1901), while the small, wide, square-shaped tragus, besides the pale brown pelage coloration, separates it from *Eumops auripendulus* (G. Schaw, 1800), which presents a pointed tragus and a blackish pelage (Eger 2008).

Molossops temminckii (Burmeister, 1854)

Summary of captures. One female (CMARF 2144) and one male (CMARF 2145). External measurements and weights. Female: TLB: 85.00, TL: 33.00, LHL: 5.00, EL: 12.00, W: 6.00. Male: TLB: 80.00, TL: 28.00, LHL: 5.00, EL: 13.00, W: 6.00.

Morphological description. Dorsal pelage is chestnut-brown at the tips and yellow at the bases, while ventrally, the coloration is slightly paler (Fig. 8). Ears triangular, with a small and triangular tragus. Antitragus broad and slightly inclined posteriorly. Snout elongated, flat, wide, and blunt, featuring a slightly prominent tip and an obtuse projection between the nasal orifices. Lips smooth and bordered by a fine fringe of hook-shaped hairs, accompanied by a tuft of bristles below the nostrils. Small warts or papillae are notably present on the upper edge of the nostrils.

Skull with dorsoventral flattening (Fig. 8), characterized by an elevation extending from the nasal tip to the posterior part of the braincase. Postorbital constriction is prominently defined, accompanied by deep lacrimal canals. The sagittal crest is distinctive, reaching its peak at the junction with a well-developed lambdoidal crest. Tympanic bullae small. Basisphenoid pits are shallow. Third upper molar is well-developed in the female, surpassing the maxillary bone (Fig. 9). Third upper molar is less developed in the male, not extending beyond the maxillary bone (Fig. 9). Lower incisors are bilobed. Some skull measurements are shown in Table 1.

Identification. The relatively small size (forearm < 33 mm) and condylobasal length < 15 mm allowed for the assignment of the denomination *M. temminckii* to the specimens referred to here, distinguishing them from the only known congener in Brazil (M. neglectus S.L. Williams & Genoways, 1980), whose reported distribution corresponds to the southeastern region (Gregorin et al. 2020). Molossops griseiventer Sanborn, 1941 a taxon recently validated as a full species (Ramírez-Chaves et al. 2023), has an Andean distribution in Colombia. It can be differentiated from *M. temminckii* by the less pronounced shape of the nasal septum. However, it is important to consider that Ramírez-Chaves et al. (2023) did not provide information on sex differences in their comparisons. The variations between M. temminckii and M. griseiventer, suggested by Ramírez-Chaves et al. (2023), could be related to sexual dimorphism, as reported in our study. These authors compared both species without considering the marked sexual dimorphism in Molossidae (Eger 2008); future studies could clarify the possible separation between M. griseiventer and M. temminckii.

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Figure 8. Dorsal, ventral, and lateral views of the skull of a female (CMAR 2144 **A**, **C** upper) and a male (CMARF 2145 **B**, **C** lower) of *Molossops temminckii* collected in the Caatinga, northeastern Brazil **D** external body features of a female of *Molossops temminckii* from the same locality. Scale bar: 5 mm.

Molossops sp.

Summary of captures. One female (CMARF 2146).

External measurements and weight. TLB: 71.00, TL: 21.14, LHL: 4.87, EL: 11.03, W: 6.60.

Morphological description. The dorsal pelage is chocolate brown at the tips and yellow at the base, while the coloration is slightly paler ventrally. Ears triangular, with small and triangular tragus. Antitragus broad and slightly inclined posteriorly. Snout elongated, flat, wide, and blunt, featuring a slightly prominent tip and an obtuse projection between the nasal orifices. Lips smooth and bordered by a fine fringe of hook-shaped hairs, accompanied by a tuft of bristles below the nostrils.

The skull exhibits dorsoventral flattening, with a slight elevation from the tip of the nasals to the back of the braincase. Postorbital constriction is prominently defined, accompanied by deep lacrimal canals. The sagittal crest is present but is low at the junction with the lambdoidal crest. Lambdoidal crest weakly developed. Tympanic bullae are small, and the basisphenoid pits are shallow. The third upper molar has two well-developed posterior commissures, surpassing the maxillary bone. Lower incisors are trilobed. Some skull measurements are shown in Table 1.

Identification. The presence of three-lobed lower incisors and two well-developed posterior commissures in the third upper molar, surpassing the maxillary, represent unique characteristics that differentiate this specimen from the



Figure 9. Magnified view (not to scale) of upper molar teeth in specimens of *Molossops temminckii*, showing the sexual dimorphism in the development of the third molar indicated with white arrows (**A** female **B** male).

two specimens previously assigned to *Molossops temminckii* or its other congener, *Molossops griseiventer*.

Molossus aztecus Saussure, 1860

Summary of captures. Three females (CMARF 2147–2149) and two males (CMARF 2150 and 2151).

External measurements and weights. Females: TLB: 105.00 (103.00–107.00), TL: 40.66 (38.00–44.00), LHL: 6.33 (6.00–7.00), EL: 11.00 (10.00–12.00), W: 11.33 (10.00–12.00). Males: TLB: 110.00 (110.00–110.00), TL: 40.44 (38.89–42.00), LHL: 6.00 (6.00–6.00), EL: 12.30 (12.00–12.60), W: 12.50 (12.00–13.00).

Morphological description. Dorsal pelage with a uniform chocolate brown color and shorter at the shoulders (2–3 mm). Ventral fur is slightly bicolored, with the basal portion of hairs chocolate brown and the tips varying between dark brownish and black shade.

Skull with an inflated rostrum and a rounded braincase. Infraorbital foramen opens laterally in frontal view. Upper incisors spatulated. Occipital complex with a rectangular shape (Fig. 10). Canines projected anteriorly. The nasal process in males is well-developed. Basioccipital pits moderately depth. Lambdoidal and sagittal crests well-developed. Mastoid process oriented ventrally in posterior view. The mandible has a developed angular process and a pair of bilobed incisors. Some skull measurements are shown in Table 2.

Identification. The unicolored dorsal pelage, the infraorbital foramen opens laterally in frontal view, the upper incisors spatulated, and the rectangular-shaped occipital complex differentiate the individuals of this species from their morphologically closest related congeners: *Molossus coibensis* J.A. Allen, 1904, *M. currentium* O. Thomas, 1901, and *M. molossus* (Pallas, 1766) (Gregorin et al. 2011; Loureiro et al. 2018a, 2018b).

Molossus currentium O. Thomas, 1901

Summary of captures. Five females (CMARF 2152–2156).

External measurements and weights. TLB: 103.20 (102.00–105.00), TL: 42.80 (42.00–45.00), LHL: 6.60 (5.00–7.00), EL: 12.00 (11.00–12.00), W: 10.20 (10.00–11.00).

Morphological description. Dorsal fur coloration shows the following variations: unicolored chocolate brown in two specimens, slightly bicolored in two (hairs with a short pale brown basal band and a broader chocolate brown portion), and notably bicolored in one (with a pale brown base and chocolate brown tips); in all these cases, the dorsal coloration slightly contrasts with the venter. Length of dorsal hairs at the shoulders with the following variations: 4 mm in two specimens, 2 mm in one, and 3 mm in two.

Skull with elongated rostrum and a rounded braincase. Upper incisors spatulate. Infraorbital foramen varies in position: laterally positioned and slightly expanded in two specimens (making it visible in frontal view). In contrast, it is laterally positioned in the other three but not expanded. Occipital complex with a triangular shape (Fig. 10). Canines anteriorly projected in lateral view. The nasal process developed in three specimens and undeveloped in the rest. Basioccipital pits are present but shallow in two specimens and absent in the remaining three. The mastoid process is long and oriented ventrally. Presence of two bilobed lower incisors. Some skull measurements are shown in Table 2.

Identification. The upper incisors are spatulate, the infraorbital foramen is laterally positioned, not expanded, or slightly expanded, and the occipital complex is triangular. These features differentiate the individuals of this species from their morphologically closest related congeners: *Molossus aztecus* Saussure, 1860, and *M. molossus* (Gregorin et al. 2011; Loureiro et al. 2018a, 2018b).

Molossus molossus (Pallas, 1766)

Summary of captures. 13 females (CMARF 2157–2169) and one male (CMARF 2171).

Table 2. External and cranial measurements for four species of bats of the family Molossidae with confirmed identity and three unidentified morphos. Data based on specimens collected in the Caatinga bioma (Lençóis, Chapada Diamantina region, northeastern Brazil), according to this study (catalog number are indicated) and previous information in literature. Abbreviations are described in the section of materials and methods.

TAXA	FA	GSLI	GSL	CBL	CIL	PL	ZB	MB	BCW	IOW	C-M3	C-C	M3-M3	LM	c-m3
Molossus aztecus															
Female (CMARF 2147)	39.75	16.61	15.90	14.56	15.66	5.44	10.90	10.11	8.72	3.77	6.12	4.26	7.75	11.66	7.14
Female (CMARF 2148)	38.79	16.81	15.50	14.93	15.04	5.85	10.40	9.84	8.55	3.38	5.99	4.51	7.94	11.28	6.83
Female (CMARF 2149)	40.76	16.28	15.50	14.99	15.20	5.86	10.57	9.84	8.42	3.42	5.73	4.19	7.63	11.29	6.62
Females (average of Loureiro et al. 2018b)	39.00	16.68	16.41	-	13.37*	5.29	10.65	-	9.02	3.78	6.04	4.32	7.62	-	-
Male (CMARF 2150)	39.46	16.74	16.00	14.90	15.07	6.01	10.28	9.89	8.44	3.35	5.69	4.42	7.90	11.64	6.18
Male (CMARF 2151)	40.74	17.04	16.00	15.86	15.47	6.26	10.68	10.52	8.61	3.69	6.17	4.34	8.02	11.73	7.32
Male (CMUFV 1668, Gregorin et al. 2011)	38.50	-	-	-	16.04	-	-	-	9.50	4.0	6.30	-	7.80	-	6.70
Male (CMUFV 1664, Gregorin et al. 2011)	37.80	-	-	-	16.00	-	-	-	9.30	4.0	6.40	-	8.10	-	6.70
Male (CMUFLA 399, Gregorin et al. 2011)	38.80	-	-	-	16.50	-	-	-	9.60	3.90	6.30	-	8.00	-	6.90
Male (CMUFLA 400, Gregorin et al. 2011)	39.30	-	-	-	16.20	-	-	-	9.40	3.90	6.40	-	8.10	-	6.70
Male (CMUFLA 416, Gregorin et al. 2011)	39.00	-	-	-	16.40	-	-	-	9.60	3.90	6.50	-	8.30	-	6.70
Males (average of Loureiro et al. 2018b)	39.50	17.62	17.28	-	14.01*	5.53	10.91	-	9.10	3.85	6.21	4.66	7.88	-	-
Molossus currentium															
Female (CMARF 2152)	38.82	15.04	14.98	14.93	14.84	5.82	10.02	10.08	8.38	3.55	5.66	4.21	7.35	11.30	6.64
Female (CMARF 2153)	38.98	17.04	15.58	15.07	14.74	5.20	10.18	10.15	8.72	3.34	5.84	4.32	7.62	11.19	6.46
Female (CMARF 2154)	39.59	16.14	15.60	14.97	14.67	5.65	10.67	10.08	8.34	3.73	5.71	4.35	7.80	11.25	6.52
Female (CMARF 2155)	40.39	16.59	15.78	14.94	14.54	5.87	10.29	10.04	8.62	3.57	5.77	4.16	7.74	11.21	6.76
Female (CMARF 2156)	37.81	16.67	15.75	14.58	14.92	5.67	10.36	10.06	8.70	3.67	5.98	4.23	7.02	11.13	6.15
Females (average of Loureiro et al. 2018b)	41.90	17.97	17.57	-	14.34*	5.54	11.62	-	9.32	4.13	6.61	4.82	8.25	-	-
Molossus molossus															
Female (CMARF 2157)	38.00	15.70	15.00	14.20	14.44	5.95	10.23	10.10	8.44	3.71	5.56	4.07	7.53	11.07	6.55
Female (CMARF 2158)	40.49	16.51	15.46	15.05	15.07	6.03	9.80	10.27	8.43	3.69	5.84	4.52	7.49	11.72	6.65
Female (CMARF 2159)	39.82	16.42	15.56	14.97	15.07	6.06	10.32	10.03	8.55	3.60	6.04	4.47	7.80	11.41	6.87
Female (CMARF 2160)	38.43	16.83	15.98	15.20	15.55	5.81	10.22	9.94	8.64	3.57	5.89	4.36	7.69	11.33	6.69
Female (CMARF 2161)	40.93	16.98	15.99	15.43	15.47	5.78	10.47	10.51	8.58	3.58	6.12	4.40	7.93	11.84	6.82
Female (CMARF 2162)	38.97	16.39	15.98	14.98	15.06	5.53	10.71	10.43	8.45	3.76	6.00	4.30	7.83	11.44	6.73
Female (CMARF 2163)	39.45	16.54	15.78	15.04	15.10	5.50	10.71	10.54	8.66	3.64	5.81	4.32	7.92	11.26	6.76
Female (CMARF 2164)	38.23	16.11	15.78	15.03	15.20	5.87	10.17	10.06	8.35	3.67	6.31	4.03	7.62	11.40	6.75
Female (CMARF 2165)	40.30	16.30	15.95	14.99	15.05	5.63	10.24	10.04	8.37	3.60	5.94	4.10	7.60	11.31	6.79
Female (CMARF 2166)	38.23	16.29	15.78	14.57	14.63	5.27	10.16	10.13	8.37	3.54	5.88	4.15	7.82	11.21	6.97
Female (CMARF 2167)	39.19	16.06	15.65	14.60	14.86	5.91	10.25	10.08	8.78	3.52	5.99	4.12	7.64	11.41	6.67
Female (CMARF 2168)	39.51	16.27	15.78	14.85	14.87	5.42	10.30	10.06	8.38	3.62	5.88	4.18	7.96	11.21	6.83
Female (CMARF 2169)	39.02	16.24	15.50	13.99	14.55	5.41	10.11	9.90	8.79	3.65	5.91	4.34	7.53	11.26	6.77
Female (CMARF 2170)	40.57	16.08	15.98	14.44	14.86	5.18	10.33	9.91	8.57	3.54	5.87	4.26	7.45	11.75	6.89
Females (average of Loureiro et al. 2018b)	39.45	16.95	16.49	-	13.49*	5.39	10.26	-	8.84	3.70	6.13	4.36	7.65	-	-
Male (CMARF 2171)	39.28	16.72	16.00	15.19	15.21	5.98	10.65	10.41	8.88	3.86	6.10	4.27	8.02	11.22	6.73
Males (average of Loureiro et al. 2018b)	40.21	17.65	17.22	-	14.04*	5.54	11.02	-	9.05	3.80	6.30	4.58	7.92	-	-
Molossus rufus															
Female (CMARF 2172)	50.75	21.49	21.00	19.15	19.72	7.02	13.26	13.32	10.61	4.38	8.00	5.72	10.03	15.56	8.89
Female (CMARF 2173)	50.16	20.32	19.10	17.92	18.92	7.48	13.34	12.88	10.65	4.55	7.73	5.73	9.95	15.20	8.81
Female (CMARF 2174)	50.76	21.27	19.00	18.24	18.86	6.62	13.06	12.34	10.42	4.29	7.78	5.49	9.84	14.69	8.46
Female (CMARF 2175)	50.41	20.67	19.10	18.79	19.48	6.66	13.62	13.55	10.57	4.39	7.69	5.69	10.14	14.93	8.67

TAXA	FA	GSLI	GSL	CBL	CIL	PL	ZB	MB	BCW	IOW	C-M3	C-C	M3-M3	LM	c-m3
Female (CMARF 2176)	50.94	20.86	19.00	18.34	18.75	6.73	13.40	13.01	10.59	4.41	7.69	5.69	10.12	14.84	9.09
Female (CMARF 2177)	49.25	20.37	19.10	17.84	18.57	7.31	13.37	13.25	10.76	4.06	7.67	5.55	9.96	14.87	8.87
Female (CMARF 2178)	50.73	21.54	19.57	18.61	19.38	6.89	13.35	12.62	10.46	4.37	7.64	5.51	9.81	14.51	8.59
Female (CMARF 2179)	50.03	21.95	21.10	19.15	19.93	7.19	13.32	13.11	10.07	4.98	7.93	6.23	9.95	15.29	8.93
Female (CMARF 2180)	50.67	20.68	19.56	18.23	19.22	6.79	13.22	12.62	10.43	4.39	7.48	5.56	10.10	14.81	8.72
Female (CMARF 2181)	49.94	20.58	19.98	18.13	19.25	6.80	13.25	12.70	10.44	4.35	7.50	5.55	10.15	14.71	8.12
Females (average of Loureiro et al. 2018b)	50.00	21.30	20.69	-	17.07*	6.78	12.90	-	10.62	4.38	7.74	5.58	9.67	-	-
Male (CMARF 2182)	51.03	22.57	21.99	19.82	20.51	7.03	14.28	13.61	10.99	4.88	8.03	6.08	10.21	15.86	9.19
Male (CMARF 2183)	52.26	21.71	21.00	19.99	20.69	7.42	13.85	13.99	10.69	4.36	7.79	6.03	10.11	16.03	8.26
Male (CMARF 2184)	52.42	22.20	21.98	19.98	20.58	7.08	14.45	14.30	10.70	4.53	8.12	6.04	10.69	16.18	9.26
Male (CMARF 2185)	50.59	23.26	21.98	19.88	20.66	6.92	14.37	13.79	10.86	4.49	8.29	6.32	10.48	16.17	9.47
Male (CMARF 2186)	50.98	21.68	21.00	18.89	19.73	7.67	13.43	13.72	10.51	4.58	7.79	5.94	10.28	15.43	9.02
Male (CMARF 2187)	51.12	22.87	21.56	20.02	20.60	6.84	13.86	14.00	10.68	4.41	7.93	6.30	10.40	15.89	9.16
Male (CMARF 2188)	52.55	22.84	21.50	20.17	20.85	7.98	14.38	14.22	11.16	4.71	8.02	6.19	10.62	16.08	9.68
Male (CMARF 2189)	51.14	22.41	21.78	19.96	20.67	7.42	14.72	14.00	11.09	4.80	8.28	6.33	10.56	16.09	9.34
Male (CMARF 2190)	53.21	22.75	21.54	19.40	20.56	8.00	14.25	13.87	10.68	4.62	7.93	6.07	10.20	16.27	9.23
Male (CMARF 2191)	53.14	23.04	21.00	19.51	20.28	7.24	14.63	14.22	11.08	4.74	8.10	6.61	10.34	16.57	9.10
Male (CMARF 2192)	53.14	22.04	21.15	19.56	19.86	7.15	13.62	13.44	11.15	4.59	7.88	5.66	10.38	15.69	9.17
Male (CMARF 2193)	50.38	22.38	21.00	19.47	20.19	7.34	14.29	14.18	10.79	4.53	7.75	6.06	10.38	15.65	9.05
Male (CMARF 2194)	52.37	22.36	21.45	19.46	20.40	7.70	14.06	13.75	10.75	4.41	7.89	5.94	10.30	15.41	8.98
Male (CMARF 2195)	53.42	22.82	21.20	19.46	20.20	7.19	14.02	13.74	10.70	4.64	7.97	6.24	10.51	15.77	9.29
Male (CMARF 2196)	45.83	21.50	21.00	19.00	19.46	6.73	14.26	13.65	10.66	4.29	7.71	6.17	10.40	15.60	9.06
Male (CMARF 2197)	45.83	21.55	21.00	19.00	19.00	6.50	14.25	13.80	10.50	4.69	7.90	6.07	10.51	15.70	9.20
Males (average of Loureiro et al. 2018b)	49.55	22.9	22.19	-	17.85*	7.09	14.05	-	10.91	4.54	8.04	6.07	9.95	-	-
Molossus sp. 1															
Female (CMARF 2198)	39.19	16.48	15.96	14.79	15.02	5.77	10.51	10.26	8.78	3.70	5.65	4.09	7.66	11.17	6.58
Male (CMARF 2199)	40.53	17.56	16.00	15.50	15.55	5.93	10.76	10.41	8.45	3.66	5.94	4.36	8.11	11.65	6.49
Male (CMARF 2200)	41.02	17.31	16.00	15.12	15.20	6.01	10.57	10.70	8.79	3.67	6.15	4.34	7.89	11.47	6.81
Male (CMARF 2201)	41.70	17.15	16.16	14.99	15.51	5.63	10.54	10.21	8.46	3.53	6.05	4.49	8.00	11.75	7.00
Male (CMARF 2202)	38.96	16.00	15.78	15.14	15.37	5.85	10.15	9.69	8.35	3.67	5.92	4.09	7.62	11.41	6.92
Male (CMARF 2203)	40.19	17.15	16.55	15.88	16.20	6.10	11.06	10.61	8.81	3.81	5.99	4.60	7.98	11.91	6.98
Male (CMARF 2204)	40.48	17.69	16.76	16.06	16.08	6.03	11.03	10.61	8.80	4.00	6.19	4.49	8.10	12.15	7.34
Male (CMARF 2205)	38.47	17.12	16.19	15.48	15.32	5.41	10.37	10.00	9.48	3.81	5.95	4.39	7.56	12.11	6.76
Molossus sp. 2															
Male (CMARF 2206)	40.16	16.61	16.00	15.28	15.50	6.18	10.70	10.75	8.62	3.80	6.00	4.45	7.71	11.54	6.66
Male (CMARF 2207)	39.58	17.56	16.17	15.17	15.27	5.34	10.83	10.59	8.59	3.72	6.06	4.34	8.07	11.39	7.67
Male (CMARF 2208)	39.05	16.00	15.96	14.49	14.69	5.00	10.29	9.78	8.53	3.45	5.87	4.05	7.53	10.77	6.53
Molossus sp. 3															
Female (CMARF 2209)	40.26	16.15	16.00	15.21	15.90	5.09	10.08	10.25	8.58	3.51	5.89	4.58	7.63	11.43	6.65
*This variable was incorrectly measured	Loureir	o et al. ()	2018b) r	neasure	d from th	e base c	of the tvn	npanic b	ullae an	d not fro	om the b	ase of t	he condyle	٠. •	

External measurements and weights. Females: TLB: 102.38 (100.00–115.00), TL: 40.68 (36.00–45.00), LHL: 6.68 (5.95–7.88), EL: 11.92 (10.00–16.00), W: 11.30 (10.00–13.00). Male: TLB: 105.00, TL: 30.00, LHL: 6.68, EL: 12.70, W: 12.00.

Morphological description. Rostrum narrow with a developed keel (Fig. 11). Dorsal hairs are notably bicolored in some females, with broad white bases and dark brown or chocolate hues tips (Fig. 11). Dorsal pelage is slightly bicolored in the male, with a pale brown base and a broader dark brown or chocolate hues



distal band. Ventral coloration is paler brown, subtly contrasting the dorsal side in all specimens. Length of dorsal hairs at the shoulders: 2–3 mm in females and 3 mm in the male.

Figure 10. A *Molossus aztecus* (CMARF 2147) **B** *Molossus molossus* (CMARF 2158) **C** *Moloussus currentium* (CMARF 2154), collected in the Caatinga (northeastern Brazil), showing differences in the shape of the upper incisors (left), and the occipital complex shape in the posterior region of the skulls (right).

Upper incisors with parallel tips, projecting anteriorly from the rostrum. Infraorbital foramen anteriorly positioned. Nasal process present. The occipital complex is triangular (Fig. 10). Basioccipital pits are absent in some females and in the male, while they are present but barely visible in other females. Sagittal and lambdoidal crests are present but with different degrees of development, showing the maximum degree in the male. The mastoid process developed and oriented ventrally. Presence of a pair of bilobed lower incisors. Some skull measurements are shown in Table 2.

Identification. The rostrum is narrow with a developed keel, the dorsal hairs notably bicolored to slightly bicolored, the infraorbital foramen anteriorly



Figure 11. A, B Molossus molossus (CMARF 2159) and C, D Molossus rufus (CMARF 2182) A, C dorsal and ventral views of the skull in two species of *Molossus* collected in the Caatinga, northeastern Brazil B, D individuals of *M. molossus* and *M. rufus* photographed in the field (not to scale). Scale bar: 5 mm.

positioned, and the occipital complex triangular in shape, differentiate the individuals of this species from their morphologically closest related congeners: *Molossus aztecus* and *M. currentium* (Gregorin et al. 2011; Loureiro et al. 2018a, 2018b).

Molossus rufus E. Geoffroy St.-Hilaire, 1805

Summary of captures. Nine females (CMARF 2172–2181) and 16 males (CMARF 2182–2197).

External measurements and weights. Females: TLB: 130.09 (120.00–140.00), TL: 47.13 (38.45–50.00), LHL: 9.16 (6.28–11.00), EL: 14.96 (13.00–16.73), W: 28.60 (25.00–35.00). Males: TLB: 137.75 (130.00–145.00), TL: 48.48 (39.43–58.00), LHL: 8.74 (6.59–9.96), EL: 15.68 (14.69–18.02), W: 30.75 (25.00–38.00).

Morphological description. Dorsal and ventral pelage with dark coloration, with predominantly dusky hue unicolored hairs (Fig. 11). Dorsal hairs longer than 4 mm. Skull with an inflated rostrum and an elongated braincase (Fig. 11). Mastoid process extending laterally in posterior view. The occipital region has a square shape. Lambdoidal crests are highly developed and inclined. Sagittal crests are present and well-developed, with a more pronounced development in males. Infraorbital foramen opens laterally in frontal view. Basioccipital pits moderately depth. Rostrum with triangular shape in frontal view. Upper incisors are short and spatulated, with convergent tips. Presence of a pair of bilobed lower incisors. Some skull measurements are shown in Table 2.

Identification. The relatively larger forearm length and skulls, along with the unicolored dorsal and ventral pelage, serve as characteristics to distinguish individuals of *M. rufus* from its smaller congeners (Loureiro et al. 2018b). The short and spatulated upper incisors, with convergent tips, differentiate this species from *Molossus pretiosus* Miller, 1902 (Loureiro et al. 2018b). The length of hairs longer than 4 mm, average forearm lengths of 51.98 mm in males and 50.36 mm in females, along with the greater skull lengths for both sexes of *M. rufus*, distinguish this species from *M. fluminensis* (Loureiro et al. 2020). Furthermore, a pair of lower incisors and other externally visible characters distinguish *M. rufus* from members of the genus *Promops* P. Gervais, 1856 (Eger 2008), which can be confused due to their morphological similarities.

Molossus sp. 1

Summary of captures. One female (CMARF 2198) and seven males (CMARF 2199–2205).

External measurements and weights. Female: TLB: 103.00, TL: 40.00, LHL: 9.00, EL: 12.00, W: 10.00. Males: TLB: 104.00 (98.00–111.00), TL: 39.85 (35.00–46.00), LHL: 6.55 (5.66–7.52), EL: 12.07 (10.00–15.40), W: 13.00 (12.00–14.00).

Morphological description. Dorsal fur is unicolored (dark brown), with hairs at the shoulders measuring 2 mm in length. Ventral coloration subtly contrasts with the dorsum, showing pale brown bases. Rostrum broad and convex, with a developed keel. Upper incisors elongated (similar to *M. molossus*) and anterior-ly projected. The infraorbital foramen is exposed anteriorly in the female, while

in some males, this structure is anteriorly exposed, and in others, it is laterally positioned. Nasal process present. Occipital complex with a triangular shape. Mastoid processes developed (large) and ventrally oriented. Presence of a pair of bilobed lower incisors. Some skull measurements are shown in Table 2.

Identification. The morphology of these specimens exhibits unique characteristics in the facial morphology (rostrum broad and convex, with a developed keel), distinguishing them from *M. aztecus*, *M. currentium*, and *M. molossus*. Furthermore, the mastoid processes are developed (large) and oriented ventrally, differentiating them from *Molossus* sp. 2, while the uniform coloration of the dorsal pelage (unicolored) and the broad, convex rostrum with a developed keel, distinguish them from *Molossus* sp. 3.

Molossus sp. 2

Summary of captures. Three males (CMARF 2206-2208).

External measurements and weights. TLB: 102.66 (100.00–106.00), TL: 35.00 (32.00–37.00), LHL: 6.14 (5.23–7.00), EL: 12.28 (11.91–12.95), W: 13.00 (12.00–14.00).

Morphological description. Rostrum broad and convex, with a developed keel. Dorsal fur is weakly bicolored (hairs with pale brown bases and a broad band of dark chocolate brown in the rest). Hairs at the shoulders 2 mm in length. Ventral and dorsal coloration with slight contrast shows pale brown bases and chocolate brown tips.

Infraorbital foramen laterally positioned in two specimens and anteriorly positioned in one. Upper incisors elongated (similar to *M. molossus*) and projected anteriorly. Nasal process undeveloped. Basioccipital pits are absent in one, while in two specimens they are present but scarcely visible. Occipital complex with triangular shape. The mastoid process is scarcely developed (short) and ventrally oriented. Presence of a pair of bilobed lower incisors. Some skull measurements are shown in Table 2.

Identification. Like *Molossus* sp. 1 (see above), the morphology of these specimens exhibits unique characteristics in facial morphology (broad and convex rostrum with a developed keel), distinguishing them from *M. aztecus*, *M. currentium*, and *M. molossus*. However, the scarcely developed (short) and ventrally oriented mastoid processes set them apart from *Molossus* sp. 1. Additionally, the uniform, weakly bicolored dorsal pelage, combined with the broad and convex rostrum with a developed keel, differentiates them from *Molossus* sp. 3.

Molossus sp. 3

Summary of captures. One female (CMARF 2209).

External measurements and weight. TLB: 101.00, TL: 41.00, LHL: 6.00, EL: 11.00, W: 10.50.

Morphological description. Rostrum narrow, with a slightly undeveloped keel. Dorsal fur is bicolored, with a white basal band and dark chocolate brown color in the rest. Ventral fur contrasts with the dorsum, with hairs showing

broad gray bases and pale brown tips. Hairs at the shoulders is 3 mm in length. Upper incisors show only the bases (completely worn). Frontal foramen laterally positioned. The nasal process developed. Occipital complex with triangular shape. Mastoid process elongated and ventrally oriented. Basioccipital pits are present but shallow. Presence of a pair of bilobed lower incisors. Some skull measurements are shown in Table 2.

Identification. This specimen shows unique characteristics in its facial morphology (narrow rostrum, with poorly developed keel) and in the coloration of the ventral pelage, which contrasts with the dorsum. This morphological and chromatic pattern distinguishes it from other small species of *Molossus* (e.g., *M. aztecus, M. currentium, M. molossus, Molossus* sp. 1, and *Molossus* sp. 2).

Neoplatymops mattogrossensis (Vieira, 1942)

Summary of captures. Seven females (CMARF 2210-2216).

External measurements and weights. TLB: 78.85 (73.00–82.00), TL: 29.16 (25.14–32.00), LHL: 5.95 (5.00–7.00), EL: 12.65 (10.00–13.95), W: 5.37 (5.00–6.60).

Morphological description. Small granulations on the dorsal surface of the forearm (wart-like granular structures that represent a distinctive generic characteristic). Dorsal fur is pale brown, contrasting with the ventral coloration composed of hairs with yellowish brown tips and whitish bases. The head is dark brown, with the ears distinctly separated on the forehead (Fig. 12). There is a presence of long and conspicuous vibrissae at the bases of the fingers.

Skull flattened (Fig. 12). The sagittal crest is barely visible with magnification only in two specimens; in the remaining five, this structure is absent (Fig. 12). Lambdoidal crests are present and visible. Upper incisors project anteriorly, separated from each other and the canines. First upper and lower premolars are smaller than the other two homologous teeth. The slender mandible shows a high and triangular coronoid process with a rounded apex and a low condyle. Lower incisors are deeply bifid in six specimens and barely bifid in only one case. Some skull measurements are shown in Table 1.

Identification. The short forearm, the presence of granulations on the dorsal surface of the forearm, and the skull, which is flattened and relatively small, are diagnostic characteristics for individuals of this species (Willig and Jones 1985).

Nyctinomops laticaudatus (E. Geoffroy St.-Hilaire, 1805)

Summary of captures. Six females (CMARF 2217–2222) and two males (CMARF 2223, 2224).

External measurements and weights. Females: TLB: 107.15 (100.00-110.87), TL: 46.86 (41.00-50.17), LHL: 8.30 (7.00-10.00), EL: 14.42 (15.56-19.00), W: 11.66 (9.00-15.00). Males: TLB: 106.00 (105.00-107.00), TL: 49.00 (48.00-50.00), LHL: 7.00 (7.00-7.00), EL: 17.50 (17.00-18.00), W: 10.00 (9.00-11.00).

Morphological description. The dorsal pelage is dark brown chocolate, with a paler belly (Fig. 13). The ears are relatively long and rounded, with the upper edges converging the forehead. The tragus is small and square, and the



Figure 12. Neoplatymops mattogrossensis (CMARF 2212 D) collected in the Caatinga (northeastern Brazil) A, B dorsal and ventral views of the skull in a female, in addition to lateral views C in three females (from the same locality) with different levels of development of the sagittal crest (indicated by the white arrow): absent (above); present but less developed and of lesser extent (medium); and present, more developed and covering a greater extent on the posterior portion of the skull (below). Scale bar: 5 mm.

antitragus is well-developed and wider at the base. The muzzle is pointed and upturned. Upper lips are deeply furrowed, with vertical folds that overhang the lower lips. Nostrils raised with rows of rectangular papillae on the edge, forming a crest on both sides.

Skull robust (Fig. 13), with sagittal and lambdoidal crests present but not well-developed (only discernible under magnification). Basisphenoid pits deep. Anterior border of palate emarginated. Premaxillaries scarcely separated at the anterior portion. The upper incisors are barely parallel. Lacrimal processes are well-developed. The first lower premolar is in contact with the canine, and the larger second premolar. Third lower molar is diminutive, with a complete commissure. Some skull measurements are shown in Table 1.

Identification. The forearm length < 47 mm, the greatest length of the skull < 19 mm, and the shallow basisphenoid pits distinguish individuals of this species from its congeners, *N. macrotis* (Gray, 1839) and *N. aurispinosus* (T. R. Peale, 1848) (Eger 2008).

Nyctinomops macrotis (Gray, 1839)

Summary of captures. One female (CMARF 2225).

External measurements and weight. TLB: 137.00, TL: 60.00, LHL: 7.00, EL: 27.00, W: 22.00.

Morphological description. Dorsal and ventral pelage reddish brown. Ears large, fused at the midline of the forehead and nearly reaching the nostrils. Nostrils directed laterally. The upper lip is deeply furrowed by wrinkles (Fig. 14).



Figure 13. Female of *Nyctinomops laticaudatus* (CMARF 2217; **D**) collected in the Caatinga, northeastern Brazil **A** dorsal **B** ventral, and **C** lateral views of the skull. Scale bar: 5 mm.

The skull is large, with a narrow rostrum (Fig. 14). Sagittal and lambdoidal crests are present and prominent. Upper incisors parallel. First upper premolar diminutive. Lacrimal processes are well-developed. Basisphenoid pits are large and deep. Lower incisors bifid. Some skull measurements are shown in Table 1. **Identification.** The forearm length is > 55 mm, the total length of the skull is > 22 mm, and the basisphenoid pits are large and relatively deep, distinguishing the only captured individual of this species from *N. laticaudatus* and *N. aurispi*

Discussion

nosus (Eger 2008).

Our results document the confirmed presence of at least 12 species from the family Molossidae in the Caatinga biome: *Cynomops planirostris, Eumops bonariensis, Eumops delticus, Eumops glaucinus, Molossops temminckii, Molossus aztecus,*



Figure 14. Female of *Nyctinomops macrotis* (CMARF 2225; **D**) collected in the Caatinga, northeastern Brazil **A** dorsal **B** ventral, and **C** lateral views of the skull. Scale bar: 5 mm.

Molossus currentium, Molossus molossus, Molossus rufus, Neoplatymops mattogrossensis, Nyctinomops laticaudatus, and Nyctinomops macrotis. Among them, nine were known for this region based on voucher specimens (Loureiro et al. 2018b; Silva et al. 2018), two (Eumops bonariensis and Molossus currentium) had been reported informally without conclusive evidence (Silva et al. 2018), and one (Eumops delticus) represents the first record for the Caatinga. Four additional specimens identified as morphospecies belonging to the genera Molossops (one) and Molossus (three) were only identified at the genus level, with species-level identification requiring further detailed morphological and molecular analysis.

The morphological patterns and measurements of those specimens representative of Cynomops planirostris, Eumops glaucinus, Molossus rufus, Nyctinomops laticaudatus, N. macrotis, Neoplatymops mattogrossensis, Molossops temminckii, Molossus aztecus, and Molossus molossus, align with previous data reported within their global distributions (Willig and Jones 1985; Milner et al. 1990; Best et al. 1997; Avila-Flores et al. 2002; Gregorin et al. 2011; Souza et al. 2016; Loureiro et al. 2018b; Gamboa Alurralde and Díaz 2019; López Berrizbeitia and Díaz 2021) and the Caatinga biome (Gurgel-Filho et al. 2015; Rocha et al. 2015; Souza et al. 2016). However, the length of dorsal hairs in specimens of Molossus aztecus collected in this study is relatively short. It does not exceed 3 mm, contrary to the morphological diagnosis presented for the species in other areas of the Caatinga (Ceará, Piauí, and Pernambuco) and non-Caatinga regions, where individuals of this species are characterized by long fur reaching 6 mm in length (Gregorin et al. 2011; Loureiro et al. 2018b). In the case of N. mattogrossensis, the ear lengths in females collected during our study do not correspond to the values reported by Gurgel-Filho et al. (2015) in Ceará. These authors indicated an average ear length of 19 mm for three individuals, in contrast to our average of 12.65 mm for seven specimens; this last value corresponds to the range reported by Willig and Jones (1985) for females of this species.

The presence of *C. planirostris* in the Caatinga, based on voucher material, has been confirmed in Ceará (Fabián 2008; Novaes and Laurindo 2014; Gurgel-Filho et al. 2015; Feijó and da Rocha 2017), Minas Gerais (Oliveira et al. 2003; Oliveira 2004; Nogueira et al. 2015), Paraíba (Feijó and Langguth 2011; Leal et al. 2013; Beltrão et al. 2015), Pernambuco (Mares et al. 1981; Oliveira et al. 2003; Oliveira 2004; Feijó and Langguth 2011), and Piauí (Novaes et al. 2013); the specimens reported here would be the first records of this molossid for the Bahian Caatinga.

In the case of *Eumops glaucinus*, information on the geographic distribution in Brazil was recently updated based on a new record in the Maranhão state within the Cerrado biome (Souza Cardoso et al. 2022). These authors do not document the presence of this species in the Caatinga; however, there are previous published records for this biome, based on museum specimens (Astúa and Guerra 2008; Feijó and Langguth 2011) and observations at roosting sites (Vilar et al. 2016). The specimens reported here confirm the presence of *Eumops glaucinus* in the Bahian Caatinga and represent the second record for the state of Bahia (Sá-Neto and Marinho-Filho 2013).

Both *Eumops bonariensis* and *Molossus currentium* were previously reported in the Caatinga by Silva et al. (2018); however, these authors did not include morphological characters or other evidence to corroborate the identifications, nor did they provide details on the voucher specimens. Additionally, two previous studies conducted acoustic sampling that complemented the capture data (Silva and Bernard 2017; Leal et al. 2022), recording *Molossus cf. currentium*. This record suggests that the acoustic identification is believed to be similar to that of *Molossus currentium*, although it could not be confirmed. Due to their high flight capacity and the difficulty in capturing them for identification, it is estimated that nearly 75% of New World molossid species remain acoustically unverified (Miller, pers. comm.), including *M. currentium* among them. This is further complicated by early published accounts of hand-released bats and recordings at roosts, especially for molossids, as it is now understood that they usually do not emit diagnostic search phase calls useful for free flight identification, but "clutter" calls typically recorded when entering or departing roosts.
For example, O'Farrell and Miller (1999) described the calls of *M. alvarezi* (then recognized as *M. sinaloae*) characterized as having pulses in triplets vs. the paired calls of *M. molossus* and *M. nigricans*. Subsequently, such triplets emitted by species of *Molossus* are now known to be typical "clutter calls" emitted as bats enter or leave roosts and not the typically paired diagnostic search phase pulses emitted when free-flying. This also has a bearing on other published molossid call parameters that included triple pulses vs. diagnostic paired-pulse for species of *Molossus*, as reported by Jung et al. (2014).

The uncertainty of acoustic identification may result from various factors, including a small sample size, the lack of verified vocal signatures, an understanding of vocal repertoires of a given species, or the call variation seen under varied recording conditions, e.g., hand releases, roost, or enclosure recordings (Jung et al. 2014; Miller et al. 2023). Given that many species of Molossidae are cryptic or highly variable in their morphology this may result in uncertainty of identification even with bats in hand or, in some cases, may be based on older taxonomy along with a lack of comprehensive confirmed vocal signatures matching known species (Jung et al. 2014; Eger 2008; Loureiro et al. 2018b; Miller et al. 2023). Therefore, we suggest that the previous records of *E. bonariensis* and *M. currentium* are not sufficiently supported to be accepted.

The average measurements reported by Loureiro et al. (2018b) for both sexes of *M. currentium* in Brazil, Colombia, and Panama are greater than those of the specimens from the Caatinga. This species was previously reported in Brazil by a single individual collected at Corumbá, Matto Grosso do Sul (Loureiro et al. 2018b). A second record was subsequently published from the Carlos Botelho State Park (São Paulo State) by Cláudio et al. (2020). Our record is the first occurrence in the Caatinga based on voucher specimens, expanding its distribution 1,825 km SE from Corumbá to Lençóis and 1,487 km SE from Carlos Botelho State Park to Lençóis.

Despite the similarities between E. delticus and E. bonariensis measurements, morphological variations, and coloration (Eger 1977; Carter and Dolan 1978; Hunt et al. 2003; Gregorin et al. 2016), the differences described here for specimens assigned to these two species agree with the characters described by other authors (Marinkelle 1970; Bernardi et al. 2009) and further confirm these are distinct taxa. The specimens can be differentiated on the base of the following characters: posterior region of the skull more curved in E. bonariensis, with the interparietal bones not project posteriorly; incisive foramen diminutive in E. bonariensis and large in E. delticus; protocone of the second upper premolars wide and robust in E. bonariensis, in contrast to a thin and non-robust protocone in E. delticus (visible in lateral view of the skull); palate not extending beyond the level of the upper third molar in E. bonariensis, with the posterior part of this tooth extending beyond the maxillary bone; in E. delticus the palate surpasses the level of the upper third molar (Marinkelle 1970) and the posterior part of this tooth does not extend beyond the maxillary bone. In addition to these cranial features, the space between the basisphenoid pits is wide in E. bonariensis and narrow in E. delticus, although according to Bernardi et al. (2009) this character exhibits variability in the first species; however, the female of E. bonariensis reported here notably show the basisphenoid pits separated by a wider space with respect to E. delticus, such as is documented by this author.

The geographic distribution of *Eumops bonariensis* in Brazil has been confirmed for the northeastern region in Bahia (but not in the Caatinga; Tavares et al. 2010; Ferreira et al. 2024), Paraná (Miretzki 2003), Rio Grande do Sul (Eger 1977; Pacheco and Freitas 2003; Bernardi et al. 2009; Medina et al. 2014; Gregorin et al. 2016), and São Paulo (Eger 1977; Medina et al. 2014; Ruelas and Soria 2021). Its global distribution also includes Argentina and Uruguay (Eger 2008). The female from the Caatinga extends the geographic range of this species 318 km NE from the nearest documented localities (Santana do Sobradinho, Carinhanha, 14°18'S, 43°45'W, Tavares et al. 2010).

Regarding *Eumops delticus*, the holotype was collected in Caldeirão, Marajó Island, Pará, Brazil (Eger 1977; Carter and Dolan 1978). Several authors previously considered this taxon as a subspecies or synonym of *E. bonariensis* (Sanborn 1932; Eger 1977) due to the morphological similarity of both taxa; however, it is recognized as a distinct species (Simmons and Cirranello 2024). Complementary specimens from Brazil, all of them examined and annotated by Eger (1977, 2008), Medina et al. (2014), and Gregorin et al. (2016), correspond to the following locations: Bahia (but not in the Caatinga) - São Marcelo and Ilha Madre de Deus; Pará-Boim, Tapajós River, and Marajó Island, Caldeirão; Amazonas-Faro, north bank of the Amazon River, Umaitá and Itacoatiara; and Minas Gerais-Uberlândia. The species is also known in southern Colombia and northern Peru. The female collected at Caatinga extends its geographic range 302 km NW from the nearest documented locality (Ilha Madre de Deus, 12°44'22.9"S, 38°36'47.0"W, Eger 2008).

Among the vouchers collected was a group of specimens with unequivocal characteristics that could only be identified to the genus *Molossus*. Defined species limits within this genus are unclear, with tenuous descriptions of many species and subspecies making the taxonomy of *Molossus* confusing and unstable (Loureiro et al. 2018b). The combination of characters of these specimens suggests a probable relation to *Molossus molossus*. However, these specimens represent three clearly differentiated morphospecies requiring further evaluation to resolve species-level identification. Loureiro et al. (2018b) noted the possible presence of an undescribed species of *Molossus* in the Caatinga without further clarification of how it was differentiated from known species. In addition to those specimens noted above, a female with external and cranial features corresponding to the genus *Molossops* but not matching any known species suggests the possibility of an undescribed species differing from *M. temminckii* based on external and cranial features.

Previous information on the distribution of *Molossus aztecus* in Brazil indicated it occurred at three states within the Caatinga biome: Ceará, Piauí, and Pernambuco (Loureiro et al. 2018b); our records are the first for the Bahian Caatinga. A widespread species, *Molossus rufus* is known from seven of the 10 states within the Caatinga (Alagoas, Bahia, Ceará, Paraíba, Pernambuco, Minas Gerais, Piauí), based on historical records (Mares et al. 1981; Oliveira et al. 2003; Oliveira 2004; Astúa and Guerra 2008; Feijó and Langguth 2011) and recent information (Gurgel-Filho et al. 2015; Souza et al. 2016; Silva and Bernard 2017; Leal et al. 2022). Previous reports of this species in Bahia correspond to Carinhanha (Cerrado biome) and Atlantic Forest (Souza et al. 2016). Our records are the first verified occurrence of this species for the Bahian Caatinga.

Among the small bats of the genus *Molossus*, *M. molossus* was the most captured species in this study. Its distribution in the Caatinga is documented

over an extensive area, including all federal units (Loureiro et al. 2018b). However, due to the considerable phenotypic variability of this species (Loureiro et al. 2018a, 2018b), identifications are often erroneously assigned to other species of similar size and morphology. Other species captured during this study have restricted distributions within Brazil or are poorly represented as vouchers in collections, e.g., *C. planirostris, E. glaucinus, N. laticaudatus, N. macrotis, M. aztecus*, and *M. rufus*. Our records for these species provide additional points contributing to a better understanding of these species distributions.

The sexual dimorphism found in the posterior lobe of the third upper molar morphology of *Molossops temminckii* is especially interesting. This structure extends beyond the maxillary bone in the female, while in the male, it is less developed and does not extend beyond the maxillary bone; this has not been previously documented (Gamboa Alurralde and Díaz 2019). Prior to our records, the distribution of this species in the Caatinga included these states: Ceará (Mares et al. 1981; Oliveira et al. 2003; Oliveira 2004; Eger 2008; Gurgel-Filho et al. 2015; Feijó and da Rocha 2017), Minas Gerais (Pinto 2010), Paraíba (Leal et al. 2013; Beltrão et al. 2015), Pernambuco (Mares et al. 1981; Oliveira et al. 2003; Oliveira 2004), Piauí (Gregorin et al. 2008; Louzada et al. 2014), and Rio Grande do Norte (Silva and Bernard 2017; Vargas-Mena et al. 2018).

The absence of a sagittal crest in *Neoplatymops mattogrossensis* was noted by Willig and Jones (1985), although Gurgel-Filho et al. (2015) indicated it was present in some specimens; however, they noted that this structure is difficult to see without magnification. Two individuals captured during this study had obvious sagittal crests, suggesting this is a variable trait within the species. This species has a broad geographical distribution in the Caatinga, with previous records reported from Alagoas (Leal et al. 2022), Ceará (Oliveira et al. 2003; Oliveira 2004; Eger 2008; Novaes et al. 2013; Gurgel-Filho et al. 2015; Feijó and da Rocha 2017), Paraíba (Leal et al. 2013), Pernambuco (Mares et al. 1981; Oliveira et al. 2003; Oliveira 2004; Astúa and Guerra 2008; Novaes et al. 2013; Carvalho-Neto et al. 2017), Piauí (Novaes et al. 2013), Rio Grande do Norte (Silva and Bernard 2017; Vargas-Mena et al. 2018), and Bahia (Oliveira et al. 2003; Oliveira 2004; Astúa and Guerra 2008; Novaes et al. 2013).

Previously, *Nyctinomops laticaudatus* was only known in the Caatinga from the Ceará (Feijó and Langguth 2011), Minas Gerais (Oliveira et al. 2003; Oliveira 2004), Paraíba (Feijó and Langguth 2011), Pernambuco (Mares et al. 1981; Oliveira et al. 2003; Oliveira 2004; Eger 2008; Astúa and Guerra 2008; Feijó and Langguth 2011; Carvalho-Neto et al. 2017), Piauí (Oliveira et al. 2003; Oliveira 2004), and one record of Bahia (Eger 2008). Our captures are the second record of the species in the Bahian Caatinga. *N. macrotis* has been reported from the Rio Grande do Norte (Vargas-Mena et al. 2018) and three locations in Bahia (Czaplewski and Cartelle 1998; Salles et al. 2014; Rocha et al. 2015). The single specimen we collected represents the fourth record of the species in the Bahian Caatinga, updating its distribution.

This study has increased the known number of species of Molossidae for the Caatinga biome to 21, with identifications verified by vouchers, including the confirmation of *E. bonariensis*, *E. delticus*, and *M. currentium* (in this study), *N. macrotis* (Czaplewski and Cartelle 1998; Salles et al. 2014; Rocha et al. 2015; Vargas-Mena et al. 2018), and *M. aztecus* (Loureiro et al. 2018b) not considered in the most recent species list (Silva et al. 2018) and *Cynomops greenhalli* recently reported from Paraíba and Pernambuco (Souza 2022).

The number could increase if a further analysis of the specimens listed here, classified only at the generic level, reveals new or additional taxa. The presence in our inventory of a relatively high number of species whose flight strategies make their detection difficult with conventional methods represents an important contribution, facilitated by the use of small lagoons as sampling sites, which many bats utilize for water consumption and, in some cases, for the capture of insects. Other studies have highlighted the importance of this methodological strategy for detecting Neotropical aerial insectivorous bats, which are usually considered cryptic (Ochoa-G 2000; Silva and Bernard 2017; Hintze et al. 2020).

As a complement to this survey, we included acoustic sampling, which also revealed a relatively high diversity of sonotypes, many of which were species of both Molossidae and Vespertilionidae. The taxonomic relationships of captures during this study to the acoustic sampling are being addressed in another publication. However, given the difficulty in capturing these elusive species, it is possible that some species that were acoustically recorded may have eluded capture during this study.

The data provided in the first stage of this research expand knowledge about the taxonomy and distribution of some members of the family Molossidae, including their presence in seasonal environments where dry forests conform the dominant vegetation. Future research is required to improve knowledge of this and other poorly studied Neotropical bat families, in order to provide information and insights for the design of conservation strategies (Cassano et al. 2017), in addition to a better representation of some taxonomic groups in scientific collections (Moras et al. 2018).

Conclusions

This study provides a robust framework for future research on the ecology and conservation of bats in the Caatinga biome, thus contributing to the understanding and preserving biodiversity in this unique natural region. We further documented the diversity and importance of this biome as a habitat for species of molossids, with implications for biodiversity conservation. Additionally, our morphological assessments offer valuable insights into intraspecific variation and sexual dimorphism for several species, enhancing the understanding of their biology and evolution.

Our results underscore the need for continued research on such species' morphological variations, ecological preferences, and conservation priorities. Finally, this study highlights the significance of small lakes or water bodies as sampling sites for documenting a high diversity of otherwise undersampled aerial insectivores.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

All bats were captured following the guidelines of Sikes (2016) and those retained as vouchers were humanly euthanized, fixed in formaldehyde (10%), and preserved in ethanol (70%). All handling protocols were approved by the Animal Use Ethics Committee of the Universidade Estadual de Santa Cruz (CEUA-UESC Nros. 004/2020 and 024/2021).

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Author contributions

FJG designed the project, conducted all field sampling, identified the biological material, and wrote the first draft of the manuscript. JOG improved the project, participated in identifying biological material, and enhanced the first draft of the manuscript. JLPU contributed to identifying biological material and improved the first draft of the manuscript. BWM assisted in identifying biological material and enhanced the first draft of the manuscript. FCF participated in identifying biological material and improved the first draft of the first draft of the manuscript. MRVA enhanced the initial project design, participated in identifying biological material, and improved the final version of the manuscript

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Data availability

All of the data that support the findings of this study are available in the main text.

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