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RESEARCH ARTICLE

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A new species of *Metopiellus* (Coleoptera, Staphylinidae, Pselaphinae) from the northern Colombian Amazon

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Abstract

The genus *Metopiellus* (Staphylinidae, Pselaphinae) is confirmed in Colombia with the description of *Metopiellus guanano* **sp. nov.** from the northern Amazon. Major diagnostic characters, a distributional map, and ecological data are given. Finally, a previous taxonomic key to *Metopiellus* is updated to include the new species.

Keywords

Colombian Amazon, *Metopiellus*, Staphylinidae

Introduction

Sakchoowong et al. (2008) stated that Pselaphinae beetles are ubiquitous, diverse, and poorly explored in the tropics. Thirty-nine Pselaphinae tribes show evidence of myrmecophily, and some are composed primarily or exclusively of myrmecophiles, such

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as Arnyliini, Attapseniini, Clavigerini, Colilodionini, Ctenistini, Metopiasini, Tiracerini, and Tmesiphorini (Parker 2016). The Neotropical genus *Metopiellus* Raffray 1908, of the tribe Metopiasini, currently consists of four species: *M. aglenus* Reitter, 1885, *M. hirtus* Reitter, 1885, and *M. painensis* Asenjo et al., 2017, described from Brazil, and *Metopiellus silvaticus* Bruch, 1933, known from Argentina. In this paper, we describe a new species of *Metopiellus* from the Northern Colombian Amazon (from the city of Mitu and the town of Villa Fatima in the Department of Vaupes). These records represent the first species-level documentation of *Metopiellus* in Colombia.

Materials and methods

Samples were examined using a Leica Wild M3C stereo microscope. Z-stepped micrographs were captured using a Leica MC170 HD camera with a Leica 10450528 adapter (0.5x) camera mounted on a Leica M205 A microscope with a 1x objective. Dissections of the apical segments of the abdomen were made under a Motic SMZ-168 microscope (maximum magnification of 80x). The extracted segments were then cleared in a 10% KOH per weight solution for 20 minutes and rinsed in distilled water. Morphological character terminology, including foveation and nomenclature/initials, follows Chandler (2001) and Asenjo et al. (2019). Final plates were edited using Adobe Illustrator CS6 (Adobe Systems Inc., California, USA).

Measurement abbreviations

BL body length (from margin of antennal tubercle of head to posterior margin of tergite VIII).
BW body width (maximum width of elytra).
EL elytral length (maximum).
EW elytral width (maximum).
HL head length (from anterior margin of antennal tubercle of head to posterior margin of head disc).
HW head width (maximum).
NW neck width (minimum).
PL pronotum length (maximum).
PW pronotum width (maximum, without spines).

Repositories

Collections are referred to by the following acronyms:

ICN Instituto de Ciencias Naturales de la Universidad Nacional de Colombia, Bogotá, Colombia.

Results

***Metopiellus guanano* sp. nov.**
http://zoobank.org/579DD444-A6E7-47A5-9394-5648733B4EAB
Figs 1–3

Type material (1 ♂, 1 ♀). ***Holotype*:** Colombia: 1 ♂: Vaupés department, Mitú, kilómetro 16 carretera vía Mitú-Monfort, Cucura. 1°08'41.6"N, 70°08'06.6"W. 10 Aug. 2019, Winkler 48 h. Col. Fernandez Lab. ICN 099808.

***Paratype*:** Colombia: 1♀: Vaupés department, Villa Fatima, Pie de Cerro Tipiaca, 1°01'30.0"N, 69°58'37.2"W. 19 March. 2020, Winkler 40 h. Lote VW01. Col. Fernandez Lab. ICN 099807. Both the holotype and the paratype are deposited in ICN.

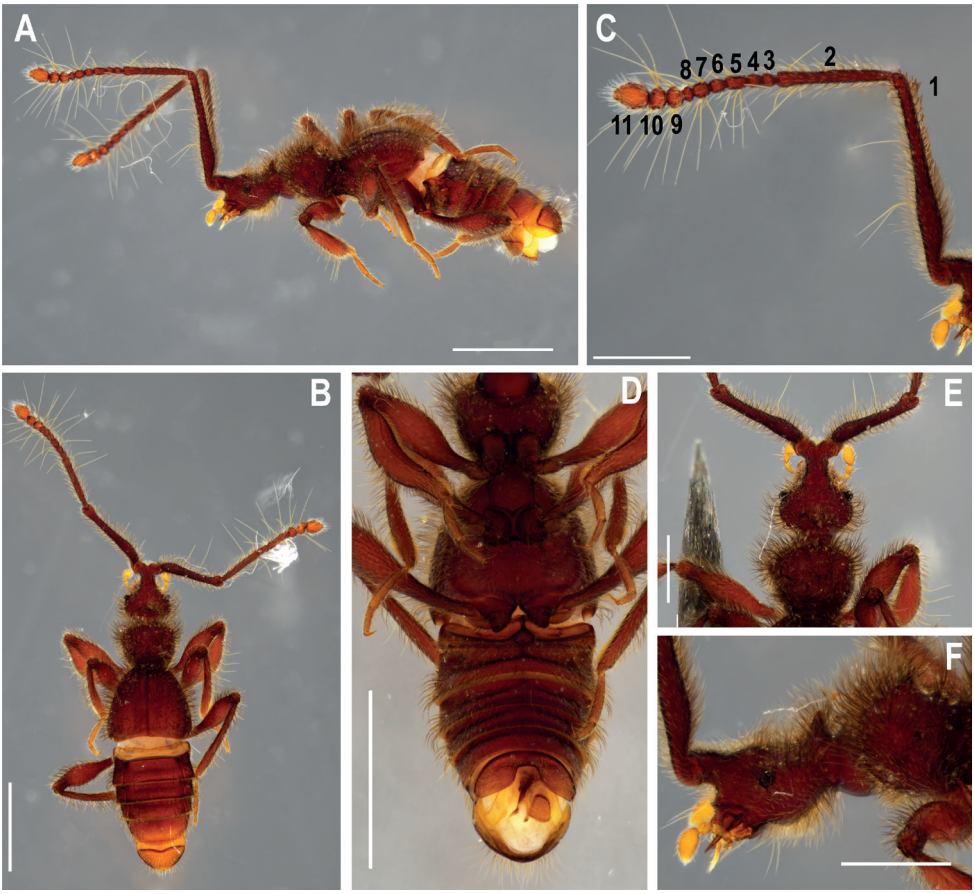

Figure 1. ♂ *Metopiellus guanano* sp. nov., holotype **A** habitus, left lateral view **B** habitus, dorsal view **C** left antenna, lateral view **D** habitus, ventral view **E** head and pronotum, dorsal view **F** head and pronotum, left lateral view. Scale bars: 1 mm (**A, B, D**); 0.5 mm (**C, E, F**).

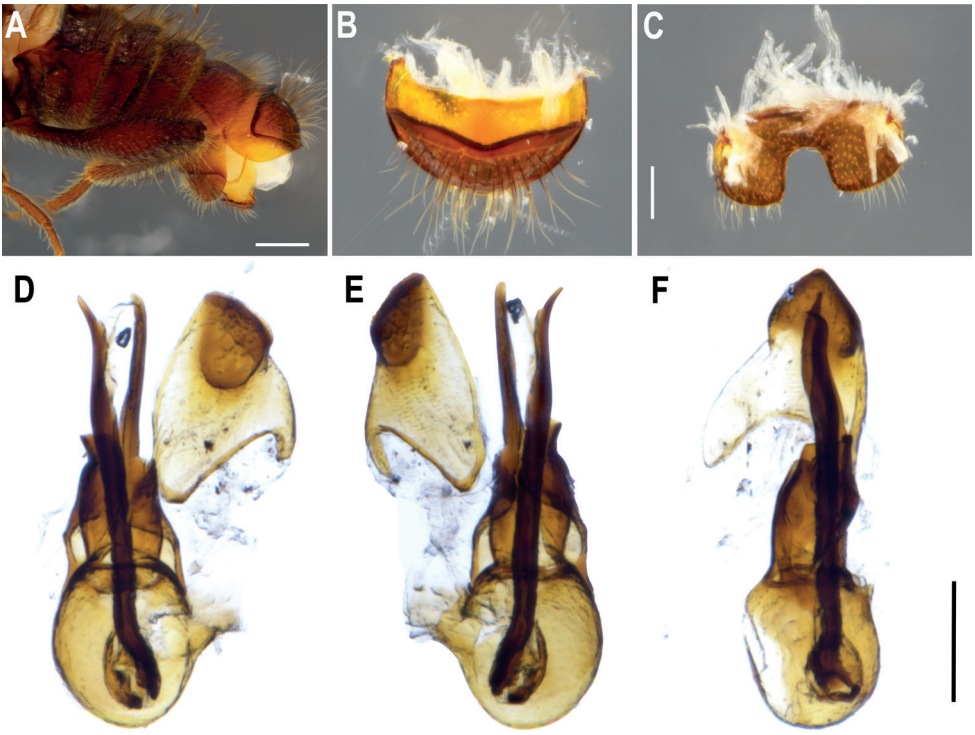

Figure 2. ♂ *Metopiellus guanano* sp. nov., holotype **A** segment VIII (tergum VIII and sternum VIII), lateral view **B** sternum VIII **C** tergum VIII **D**, aedeagus, ventral view **E** aedeagus, dorsal view **F** aedeagus, lateral view. Scale bars: 0.5 mm (**A**); 0.2 mm (**B, C**); 0.2 mm (**D–F**).

Diagnosis. *Metopiellus guanano* sp. nov. is most similar to *M. painensis* Asenjo et. al., 2017. Yet, it can be distinguished by the presence of a significant number of autapomorphic character states, such as: the presence of a prominent, horn-like spine on the vertexal region of the head (Figs 1A,F, 4A,B), the presence of 4 distinct pronotal spines and 2 deep elytral sulci, as well as the shape of the aedeagus (Fig. 2D–F) and thick pilosity covering the entire body (Fig. 1).

Description. Holotype male. Body, mouthparts, antennae, and tarsi reddish light brown (Figs 1–2).

***Measurements*:** BL (2.54 mm), BW (0.7 mm), EL (0.66 mm), EW (0.35 mm), HL (0.4 mm), HW (0.38 mm), NW (0.18 mm), PL (0.35 mm), PW (0.44 mm).

Head (Figs 1E–F, 4A, B): pyriform (HL: 0.4 mm; HW: 0.38), anterior region distinctly narrower, raised at antennal tubercle. Antennal tubercule foveated and coarse. Posterior margin of head abruptly narrowed and with posterior-lateral angles rounded. Neck almost 2/3 width of head, lateral margins slightly obtuse (Fig. 1E). Head with two vertexal foveae [VF] (Fig. 1E) near posterior margin. Medial spine protruding from vertex between the vertexal foveae, similar to spines on pronotum. Vertex longitudinally impressed with sulcus running from anterior margin of antennal tubercle to vertexal fovea, branching out at level of eyes; sulcus narrow. Ventral surface of

head with long, thin gular sulcus, interrupted at posterior third by two large gular foveae [GF]. Head covered in thick curved setae. Compound eyes small and slightly protruding laterally, composed of 12 ommatidia (Fig. 1F). Antennae (Fig. 1C) about 3/4 body length, scape almost half antennal length, last three antennomeres abruptly widened, scape length (all lengths without peduncle) 1.2 mm, width 0.15 mm, pedicel shorter than scape (length 0.59 mm: width 0.07), antennomeres 3–4 and 6–7 about as long as wide, antennomere 5 much longer than wide: 3 (length 0.07 mm: width 0.07 mm), 4 (length 0.06 mm: width 0.07 mm), 5 (length 0.11 mm: width 0.06 mm), 6 (length 0.08 mm: width 0.07 mm), 7 (length 0.08 mm: width 0.07 mm); antennomere 8 wider than long (length 0.04 mm: width 0.08 mm), antennomere 9 subcircular (length 0.08 mm: width 0.10 mm), antennomere 10 almost subquadrate (length 0.09 mm: width 0.11 mm), antennomere 11 longitudinally oval, with pointed apex (length 0.19 mm: width 0.13); all antennomeres with coarse integument and covered by long setae as well as thick, suberect pilosity.

Thorax (Fig. 1B, D–F): pronotum trapezoidal in dorsal view (PL: 0.35; PW: 0.44) widest anteriorly, stair-shaped in profile. Two rounded protuberances on medial region of anterior half, acuminated with two spines. Two smaller spines produced laterally on each side of two rounded protuberances of medial region of anterior half of pronotum. Posterior half well below height of anterior half, demarcated by a deep sulcus connected to two deep, lateral antebasal foveae. Pronotum coarse, covered in thick, curved setae. Pronotum anterior margin slightly convex, basal margin straight. Prosternum with lateral procoxal fovea. Mesoventrite with prepectal fovea and lateral mesosternal fovea. Metaventrite with lateral mesocoxal foveae, a lateral metasternal fovea and a median metasternal fovea. Region of metaventrite in articulation with metacoxae forming a triangular protuberance, inwardly convex. "Waist" between pronotum and elytra strongly produced, with dark, coarsely reticulated integument.

***Elytra*:** subquadrate (EL: 0.66; EW: 0.35), sides gradually broadening apically (Fig. 1A, B). Posterior margins convex, humeri without small longitudinal carina. Elytron uniformly rounded. No conspicuous basal elytral foveae (possibly replaced by sulci). Apicolateral margin of elytra slightly notched.

Legs (Fig. 1A, D): long and robust. Femora thickened in apical half. Tibiae slightly curved and slightly shorter than femora, all tibiae thickened at apex. Protibiae carinate on inner surface and without microsetae on posterior and mesial regions, carinae lined with thick, curved setae. Tarsi 3-segmented, first tarsomeres very short, last 2 tarsomeres longer, tarsomere 2 longer than segment 3; all tarsi with single claw and thick accessory seta. Procoxae conical and prominent, mesocoxae globular-conical, less prominent than procoxae, metacoxae transverse, region that articulates with meta-trochanter conical. Procoxae, mesocoxae and metacoxae contiguous.

Abdomen (Fig. 2A–C): slightly margined, with five visible tergites (morphological tergites IV–VIII), tergite VIII with rounded apex. Tergites and sternites IV–VII fused and bordered by a prominent carina. Sternite III visible as a small transverse plate between metacoxae, with long, transversal sulcus (Fig. 1D). Sternum IX divided longitudinally (Figs 1D, 2B).

***Aedeagus*:** (Fig. 2D–F). Asymmetrical, with median lobe slightly bulbous at base, elongate and narrow, curved at apex. Apical lobe straight in dorsal view (Fig. 2E).

Female with characters of head, pronotum, and elytra as are described for male. Abdominal sternum VIII with posterior margin rounded and without a small prolongation (Fig. 3A, B).

Habitat and ecological notes. The specimens were collected through Winkler sampling in primary forest in the northern Colombian Amazon. The sampled localities correspond to areas with a relative humidity of 84% and an average temperature of 28 °C; at both locations the vegetation was characteristic of a humid tropical forest. The processes that determine the diversity and floristic composition of the forests are not well known (Cano and Stevenson 2008). The sampling in Cucúra was carried out at no more than 20 m from a body of water; the area had been slightly disturbed by the elimination of plants from the understory and the terrain was humid due to recent rains.

Sampling at Villa Fátima was carried out in a submontane primary forest. The collection area was mostly pristine, with predominantly arboreal vegetation with little

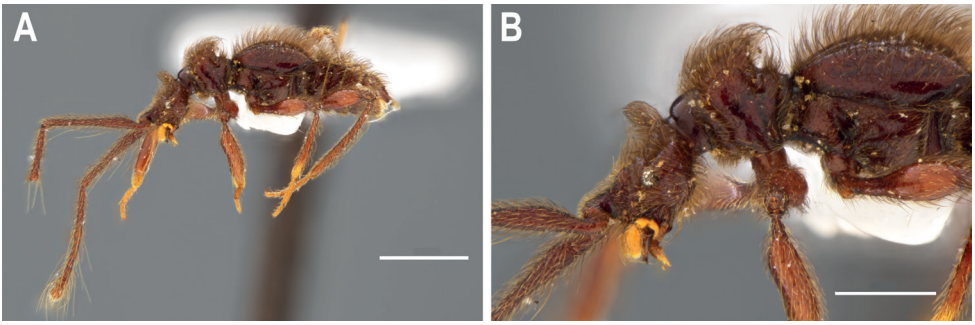

Figure 3. ♀ *Metopiellus guanano* sp. nov., paratype **A** habitus, left lateral view **B** head and pronotum, left lateral view. Scale bars: 1 mm (**A**); 0.5 mm (**B**).

Figure 4. Detail of *Metopiellus guanano* sp. nov., head and pronotal spines, traced in white **A** male head and pronotum, left lateral view **B** female head and pronotum, left lateral view.

understory vegetation. This may be due to the superficial first granite layer of the hill (Tepui) (Gröger 2000). Specimens of the *Apterostigma pilosum* ant complex (Lattke 1997) were abundant in the same samples as the holotype and paratype of the new species. It is important to highlight this morphological similarity to *Apterostigma* species, but further studies are required to indicate any type of relationship between the new beetle and these ants.

Figure 5. Geographic distribution of *Metopiellus guanano* sp. nov. The larger, filled black star denotes the type locality.

Etymology. The new species is named after the indigenous communities located at the type locality. The Guanano people inhabit the Vaupés River region of Colombia, from the Santa Cruz area below Mitú to Ibacaba in the lower Vaupés, near the border with Brazil (Stenzel 2007).

Distribution. *Metopiellus guanano* sp. nov. is known from two localities: the counties of Mitu and Villa Fatima, Department of Vaupes, Colombia (Fig. 5).

Comments. The new species belongs to the genus *Metopiellus* based on the shape of the third antennal segment, which is much shorter than the second (Fig. 1C), the posterior coxae contiguous or nearly so, and the mesial face of the protibia carinate and open at its base and apex (Fig. 1D) (Comellini 1983; Asenjo et al. 2017). However, the new species appears to be unique derived, presenting a horn-like spine on the vertexal margin of the head and spinose protrusions on the pronotum, as well as a medial protrusion on the dorsum of the pronotum (Figs 1F, 3B, 4A, B).

Newton et al. (2005) recorded the genus *Metopiellus* for the first time in Colombia, and Sissa and Navarrete (2016) also documented the genus in a study of the composition and structure of rove beetles in the department of Boyacá. However, neither of these studies identified species and we here provide the first species-level record of *Metopiellus*, and indeed of the tribe Metopiasini, from Colombia.

Key to species of *Metopiellus* (based on Asenjo et al. 2017)

1 Head with a horn-like spine protruding from the vertexal region; mesonotum with 2 acuminate bulbous projections.....***Metopiellus guanano* sp. nov.**

– Head simple, lacking a horn-like spine; mesonotum simple, without spines or projections.....**2**

2 Head similar in width to pronotum; eyes absent..... ..... ***Metopiellus aglenus* (Reitter)**

– Head narrower than pronotum; eyes small or almost absent **3**

3 Pedicel almost half the length of scape; antennomere 5 longer than combined length of antennomeres 3 and 4..... ***Metopiellus painensis* Asenjo et al.**

– Pedicel less than half the length of scape; antennomere 5 shorter than combined length of antennomeres 3 and 4.....**4**

4 Antennomere 8 transverse; eyes small.....***Metopiellus hirtus* (Reitter)**

– Antennomere 8 obconical; eyes almost absent .... ***Metopiellus silvaticus* Bruch**

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their hospitality and help during fieldwork. Finally, we want to give recognition to the indigenous communities of Villa Fatima and Cucúra for their guidance and support, as well as our gratitude for allowing us to work alongside them on their land.

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SHORT COMMUNICATION

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Replacement name for a Panamic bivalve (Mollusca, Bivalvia, Cyrenidae)

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Thomas A. Neubauer of the Systematics & Biodiversity Lab, Justus Liebig University, Giessen, Germany, has called to our attention that *Cyrena acuta* Prime, 1861, published in October, is a junior primary homonym of *Cyrena acuta* Ludwig, 1861, published in January that same year.

Prime's species, currently known as *Polymesoda acuta* (Prime, 1861: 355), ranges from Costa Rica to Ecuador, where it occurs intertidally in mangrove areas (Coan and Valentich-Scott 2012: 464–465). The holotype of this species is deposited in the Museum of Comparative Zoology, USA, Harvard University (MCZ 176951) (Johnson 1959: 441). In Prime's original description the type locality was given only as Central America.

Cyrena acuta Ludwig (1861: 197–199, pl. 72, figs 15, 16) was described from the early Miocene of Münzenberg, Hesse, Germany, where it occurs with other fresh and brackish-water species (Kadolsky 2008). The species is presently considered a junior synonym of *Falsocorbicula faujasii* (Deshayes, 1830: 51) [originally *Cyrena*] (Ott et al. 2009). It also remains in the Cyrenidae.

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The International Code of Zoological Nomenclature's (1999) Article 23.9 [Reversal of Precedence] does not apply in that Prime's name has been used less than ten times in the last 150 years since its publication, athough Ludwig's name has seen little mention because it has been long regarded as a junior synonym.

We hereby rename *Polymesoda acuta* as *Polymesoda neubaueri* Coan & Valentich-Scott, 2022 (Fig. 1). We restrict the type locality to Costa Rica, Guanacaste Province, Lower Río Tempisque; 10.2583°N, 85.2644°W; intertidal zone, because Prime's locality could have been on either the Atlantic or Pacific coast of Central America and there is no additional information accompanying the holotype (International Code of Zoological Nomenclature (1999: Article 76A). Material from this locality in the Santa Barbara Museum of Natural History collection matches the holotype.

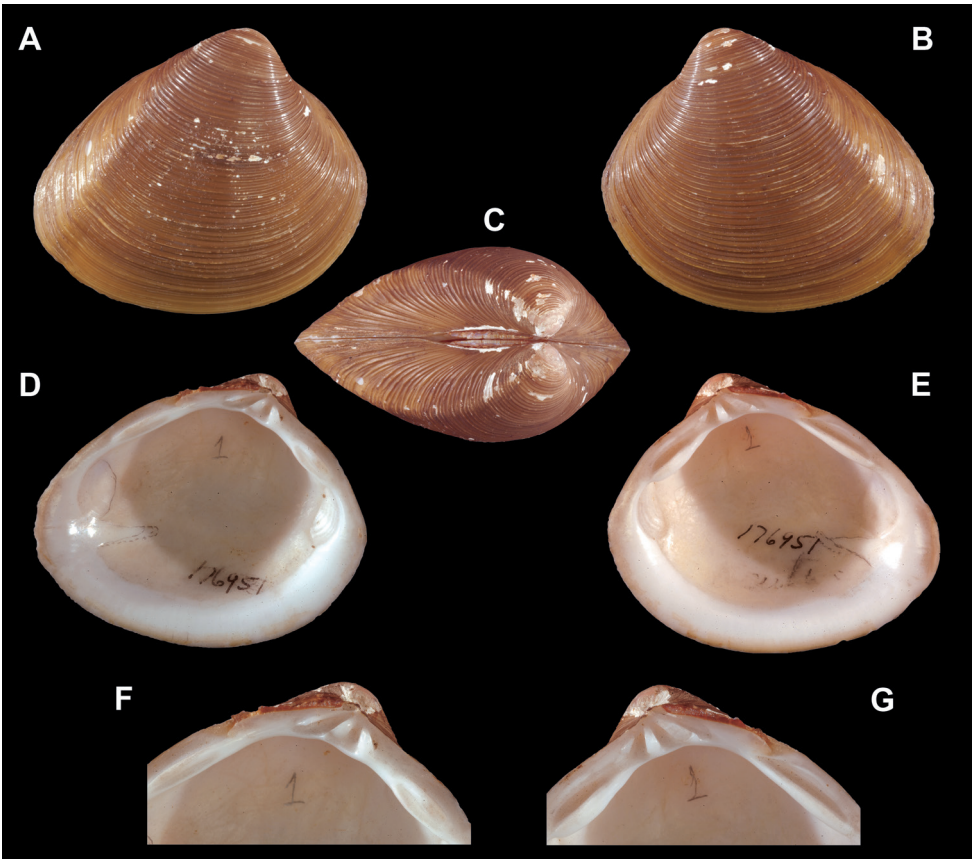

Figure 1. Holotype of *Cyrena acuta* Prime, 1861, renamed herein as *Polymesoda neubaueri* nom. nov. (MCZ 176951), length 41 mm, height 35 mm. **A** exterior of right valve **B** exterior of left valve **C** dorsal view of both valves **D** interior of left valve **E** interior of right valve **F** close up of hinge of left valve **G** close up of hinge of right valve.

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RESEARCH ARTICLE

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Description of a new *Kurixalus* species (Rhacophoridae, Anura) and a northwards range extension of the genus

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Abstract

Knowledge of biodiversity before species become extinct is paramount to conservation, especially when the relevant species are far from their expected distribution and, thus, likely overlooked. Here, we describe a new *Kurixalus* species corresponding to a range extension of *Kurixalus* on the Asian mainland, with the closest population in Taiwan. The species diverged from its closest relative during the Late Pliocene to Pleistocene, ca. 3.06 Mya (HPD 95%: 5.82-0.01), based on calibrations with a relaxed clock species tree of unlinked mtDNA 12S rRNA and nuclear DNA *TYR*. The status of the newly-described species is also supported by a divergence in call properties and morphometrics. We named the species described here as *Kurixalus inexpectatus* sp. nov. due to the nature of the discovery, as well as the adjunct distribution of the species relative to its closest congeners. The species was found in Zhejiang Province and it represents a range extension of 663 km for the *Kurixalus* genus.

Keywords

Bush frog, China, East Asia, species description, taxonomy, Rhacophorid

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Introduction

The taxonomy of the genus *Kurixalus* Ye, Fei & Dubois in Fei (Fei 1999) is still in flux, with the latest species description in 2021 (Zeng et al. 2021). The taxonomy of the family Rhacophoridae follows the same pattern with numerous taxonomic questions still unresolved (Meegaskumbura et al. 2015; Chan et al. 2020; Nguyen et al. 2020). The genus is found throughout south-central and south-eastern Asia, from as far west as northeast India, to as far south as Indonesia, as far east as the Ryuku Islands of Japan and north to the Himalayas and Taiwan (Frost 2020). The genus is often associated with bamboo forests (Chuang et al. 2019; Nguyen et al. 2020) although given the immense range of the genus, it is also present in several other habitat types (Nguyen et al. 2020). Outside of the Himalayas, in mainland China, its northernmost distribution is Chengdu, Sichuan at 30.36°N (Hou et al. 2021) and is also found in northern Taiwan at 25.3°N (Wu et al. 2016; Frost 2020). However, other genera of the family such as *Gracixalus* spp. are found as far north as Mt. Jinggang in Jiangxi Province (Wang et al. 2018) and *Zhangixalus dennysi* is found as far north as Jiangsu (Dijk et al. 2004).

Many anurans in Asia have undergone several taxonomic changes in the last decade and continue to undergo massive re-assignments at the generic level, such as: *Adenopleura*, *Bufo*, *Hyla* sensu lato (s.l.), *Megophrys* s.l., *Polypedates* s.l., *Rana* s.l. and *Theloderma* s.l., just to name a few (Li and Wang 2008; Li et al. 2009; Chen et al. 2017). The genera within Rhacophoridae have undergone similar massive and frequent re-assignments. Within the *Kurixalus* complex, other genera that have been scrutinised include *Aquixalus* Delorme, Dubois, Grosjean and Ohler 2005 (Delorme et al. 2005), *Chiromantis* Peters 1854 (Peters 1854), *Gracixalus* Delorme, Dubois, Grosjean and Ohler 2005 (Delorme et al. 2005), *Liuixalus* Li, Che, Bain, Zhao and Zhang 2008 (Li et al. 2008), *Nasutixalus*, *Nyctixalus*, *Philautus* and *Zhangixalus* Li, Jiang, Ren and Jiang 2019 (Jiang et al. 2019). Species within these genera have bounced around from one genus or another. To compound matters, many authors seem to frequently disagree on the specific arrangement of a species within a single genus, such as *Kurixalus hainanus* Zhao, Wang & Shi, 2005 (Zhao et al. 2005) being considered a junior synonym of *K. odontotarsus* Ye and Fei 1993 (Ye et al. 1993) by Fei et al. (Fei et al. 2010) or a junior synonym of *K. bisacculus* Taylor 1962 (Taylor 1962; Yu et al. 2010). Such doubt in this taxonomic group has made it obvious that more careful inspection of this complex is needed. It is important to note that *Kurixalus* s.l. is now assigned to three independent clades with parapatric distributions (Nguyen et al. 2020): a southern clade on Sundaland assigned to *Zhangixalus appendiculatus* and *K. chaseni*, a continental Asia-restricted clade corresponding to *Aquixalus* (Delorme et al. 2005) and *Kurixalus* s. str. Boettger, 1895 (Boettger 1895) on Taiwan and Ryukus Islands, with some species on southern southeast Asia (Yu et al. 2017b; Lv et al. 2018).

Species in the genus *Kurixalus* are morphologically similar and species identification is difficult (Nguyen et al. 2020). Numerous narrow ranging clades are distributed in South East Asia, with numerous likely undescribed species (Yu et al. 2017a; Lv et al. 2018) and integrated studies that include genetics, call properties, morphology and ecological preferences are required to differentiate the clades (Gonzalez et al. 2014; Yu et al. 2017a; Yu et al.

2018). For instance, only broad sampling was able to highlight the segregated species status between *K. chaseni* from peninsular Malaysia and Borneo and Z. *appendiculatus* from the Philippines (Matsui et al. 2018). The situation is similar in China, where numerous micro-endemics are present and numerous species still need to be described (Yu et al. 2017a).

During herpetological surveys in April and July 2018, we found an unknown frog that could be allocated to family Rhacophoridae, subfamily Rhacophorinae, genus *Kurixalus* Ye et al. (1993), based on serrated dermal fringes of the upper side of the upper arm and tarsus, protruding nostrils, pointed snout and an indistinct tympanum, but could not be assigned to any specific species. Here, we report on a new species of *Kurixalus* from central-eastern China that is highly disjunct (663 km) from the next closest known population of *Kurixalus*. The population represents the northernmost latitude of the genus known to date.

Materials and methods

Sampling

We collected 12 *Kurixalus* samples in April and July 2018 in north-western Zhejiang Province, People's Republic of China (Fig. 1; 31.06°N, 119.85°E). Specimens in April were photographed and had buccal swabs taken for preliminary analysis and subsequently released (Fig. 2), with a follow-up collection expedition taking place in July, pending positive preliminary results for a potential novel species.

Specimens collected in July were humanely euthanised through cooling in line with Shine et al. (2015) and a subsequent application of 20% benzocaine applied to the venter (Torreilles et al. 2009). Specimens were deposited at the Biological Museum at Nanjing Forestry University (institutional code NJFU; Table 1). Genomic materials were collected from buccal swabs for the initial three individuals found in April (photographic vouchers deposited in the repository institution HerpMapper.org (institutional code HM) (HerpMapper 2020): HM 244044, HM 323117 and HM 323118) and thigh muscle tissues for the 11 specimens collected subsequently (Table 1). Genomic DNA was extracted from both swabs and tissues using a Qiagen DNA extraction kit (Blood and Tissue Kit; Qiagen, Germany) according to the manufacturer's protocol.

Molecular analyses

For all 11 individuals from which we extracted tissues, we amplified one mitochondrial and one nuclear gene fragment. For the mtDNA, we sequenced 827 bp from a section of the genes 12S rRNA, the complete tRNA-Valine (Val) and 16S rRNA, using the primer pair F0001 (5'-AGA TAC CCC ACT ATG CCT ACC C-3'), R1169 (5'-GTG GCT GCT TTT AGG CCC ACT-3') (Wilkinson et al. 2002). For the nuclear gene, we sequenced 476 bp of the Tyrosine exon-1 (*TYR*), using the primer pair L2976 (5'-TGC TGG GCR TCT CTC CAR TCC CA-3'), H2977 (5'-AGG TCC TCY TRA GGA AGG AAT G-3') (Bossuyt and Milinkovitch 2000).

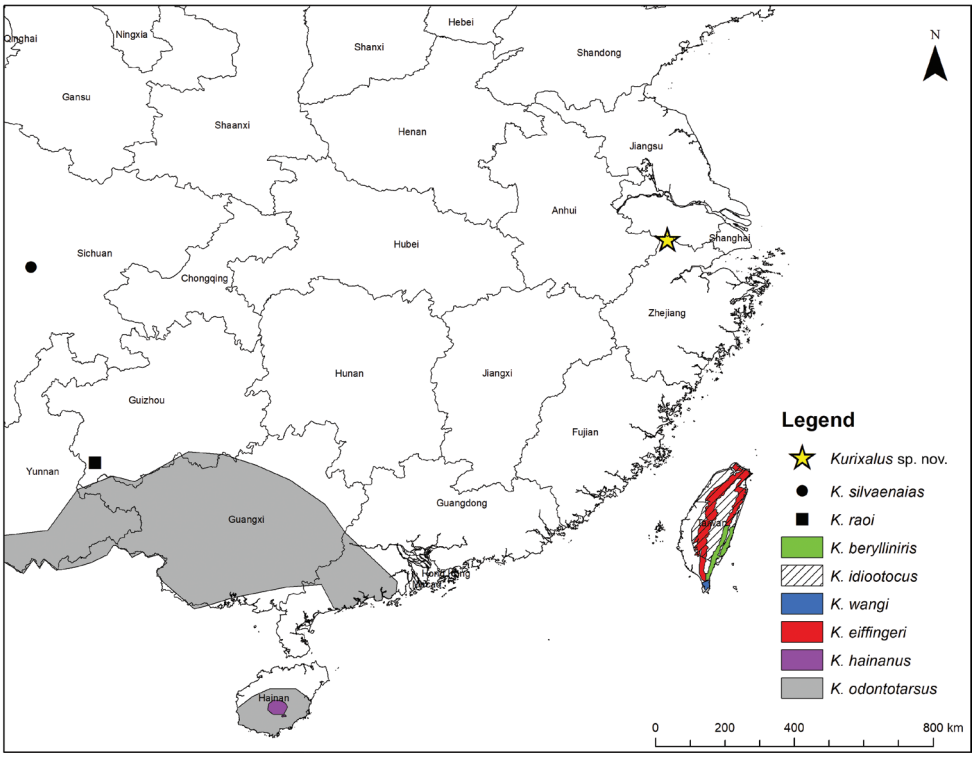

Figure 1. Map of sampling site and *Kurixalus* species. The sample for *Kurixalus* sp. nov. were collected in April and July 2018 in north-western Zhejiang Province, People's Republic of China. Map generated in ArcMap 10.4.

The Polymerase Chain Reactions (PCR) were carried out in 20 µl reaction with 50 to 100 ng of template DNA, with 1.0 µl of each primer (10 mM). The final concentration of each PCR reaction resulted to 1.5 µl of $MgCl_2$ (25 mM), 1.6 µl of dNTP (2.5 mM), 2.0 µl of 10× Buffer and 0.1 µl of TaKaRa Taq DNA polymerase (5 unit/µl). PCR amplifications were performed under the following thermal profiles: initial denaturation at 95 °C for 5 min, followed by 35 cycles with denaturation at 94 °C for 1 min, annealing at 55 °C for the mtDNA genes fragment and 54 °C for *TYR* for 1 min and extension at 72 °C for 1 min. The cycles were followed by a 10 min final extension at 72 °C. The amplified PCR products were sent for purification and sequencing to Cosmo Genetech Co. (Cosmo Genetech, Republic of Korea) on an ABI platform.

Reconstruction of phylogenies and haplotype network

To reconstruct the independent and concantenated genes tree, we relied on two different datasets: (i) 827 bp-long fragments of mtDNA 12S rRNA, tRNA-Val and 16S rRNA (*n taxa* = 98), (ii) 486 bp-long fragments of sequences of protein-coding

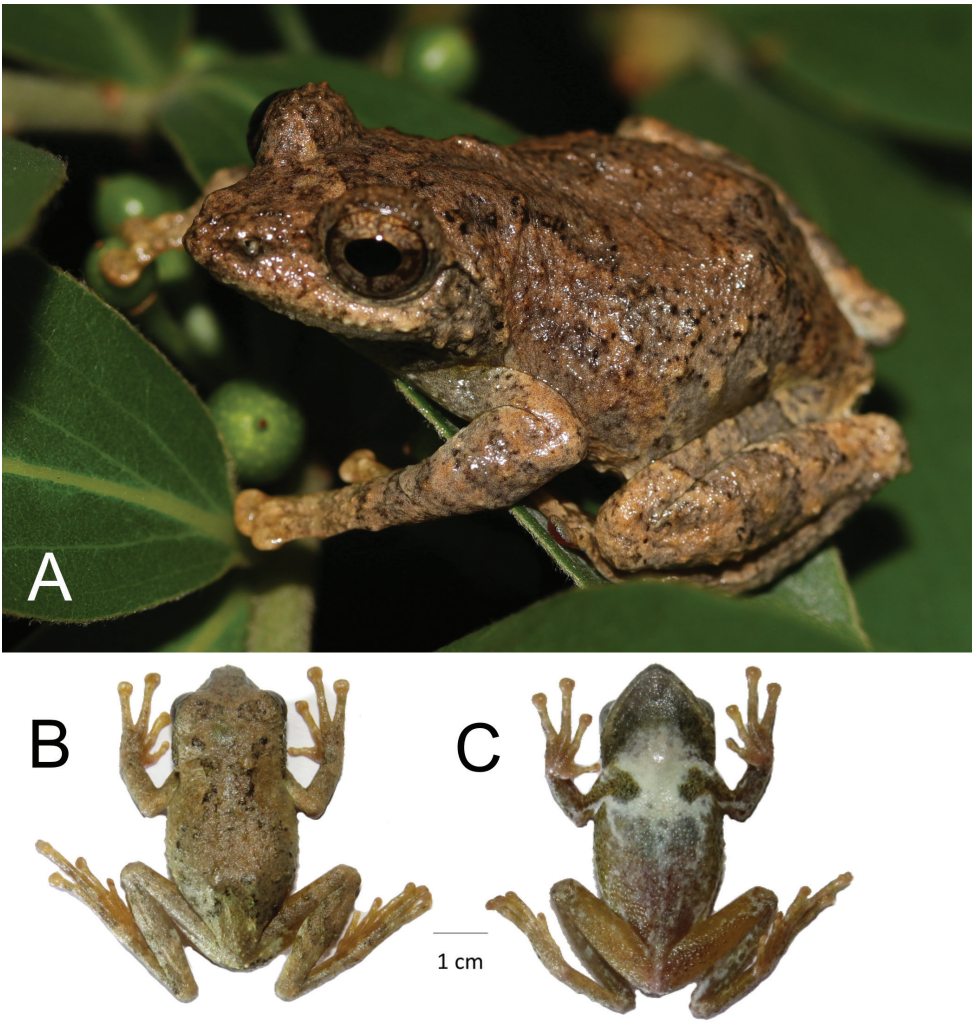

Figure 2. A *Kurixalus* sp. nov. specimen (HM 323117) *in-situ* from 26 April 2018 and **B** dorsal and **C** ventral view of NJFU20180704005.

nuDNA Tyrosinase gene (*TYR*; n taxa = 110); and, (iii) 80 concatenated sequences of partial 12S rRNA (292 bp) and *TYR* (479 bp). We trimmed the sequences in each dataset manually and aligned the three sequences datasets indepedently using Clustal Omega (Sievers et al. 2011) in Geneious Prime (Kearse et al. 2012).

We calculated sequences similarity and estimated the genetic distance (or net evolutionary divergence) on the datasets of mtDNA 12S rRNA-trNA-Val-16S rRNA (*n* sequences = 98) and nuDNA *TYR* (*n* sequence = 110) using MEGA X (Kumar et al. 2018). We estimated the net average of evolutionary divergence between groups of sequences in each dataset; hence, we assigned 19 groups of species for 12S rRNA dataset and 16 groups of species for *TYR* dataset. In MEGA X, we conducted the

Table 1. Samples and sequences used as taxa for the phylogenetic trees in this study.

Species	Sample voucher	GenBank accession number		Localities	Literature
		12S-tRNA val-16S	*TYR*		
Kurixalus inexpectatus sp. nov.	NJFU20180704001	MW115094	MW148393	Huzhou, Zhejiang, China	Present study
Kurixalus inexpectatus sp. nov.	NJFU20180704002	MW115093	MW148394	Huzhou, Zhejiang, China	Present study
Kurixalus inexpectatus sp. nov.	NJFU20180704003	MW115095	MW148395	Huzhou, Zhejiang, China	Present study
Kurixalus inexpectatus sp. nov.	NJFU20180704004	MW115092	MW148396	Huzhou, Zhejiang, China	Present study
Kurixalus inexpectatus sp. nov.	NJFU20180704005	MW115090	MW148397	Huzhou, Zhejiang, China	Present study
Kurixalus inexpectatus sp. nov.	NJFU20180704006	-	MW148398	Huzhou, Zhejiang, China	Present study
Kurixalus inexpectatus sp. nov.	NJFU20180705001	MW115088	MW148400	Huzhou, Zhejiang, China	Present study
Kurixalus inexpectatus sp. nov.	NJFU20180706001	MW115091	MW148401	Huzhou, Zhejiang, China	Present study
Kurixalus inexpectatus sp. nov.	NJFU20180706002	MW115096	MW148402	Huzhou, Zhejiang, China	Present study
Kurixalus inexpectatus sp. nov.	-	-	MW148399	Huzhou, Zhejiang, China	Present study
Kurixalus inexpectatus sp. nov.	NJFU20180706003	MW115089	MW148403	Huzhou, Zhejiang, China	Present study
Kurixalus baliogaster	ROM29862	KX554476	KX554740	Krong Pa, Gia Lai, Vietnam	(Yu et al. 2017b)
Kurixalus baliogaster	ROM29860	KX554475	KX554739	Krong Pa, Gia Lai, Vietnam	(Yu et al. 2017b)
Kurixalus baliogaster	ROM33963	KX554474	KX554738	Krong Pa, Gia Lai, Vietnam	(Yu et al. 2017b)
Kurixalus banaensis	ROM32986	GQ285667	GQ285799	Krong Pa, Gia Lai, Vietnam	(Li et al. 2009)
Kurixalus bisacculus	KUHE 19333	KX554473	KX554737	Phu Luanag, Loei, Thailand	(Yu et al. 2017b)
Kurixalus bisacculus	KUHE 19330	KX554472	KX554736	Phu Luanag, Loei, Thailand	(Yu et al. 2017b)
Kurixalus bisacculus	KUHE 35069	AB933291	KX554734	Pilok, Kanchanaburi, Thailand	(Yu et al. 2017b)
Kurixalus bisacculus	FMNH 261902	KX554471	KX554733	Kampot Dist, Prov, Cambodia	(Yu et al. 2017b)
Kurixalus bisacculus	FMNH 261901	KX554470	KX554732	Kampot Dist, Prov, Cambodia	(Yu et al. 2017b)
Kurixalus bisacculus	FMNH 261900	KX554469	KX554731	Kampot Dist, Prov, Cambodia	(Yu et al. 2017b)
Kurixalus bisacculus	FMNH 257903	KX554458	KX554699	Pakxong Dist, Champasak, Laos	(Yu et al. 2017b)
Kurixalus bisacculus	FMNH 256453	KX554456	KX554697	Nakai Dist, Khammouan, Laos	(Yu et al. 2017b)
Kurixalus bisacculus	FMNH 255656	KX554453	KX554694	Con Cuong Dist, Nghe An, Vietnam	(Yu et al. 2017b)
Kurixalus bisacculus	FMNH 255654	KX554451	KX554692	Con Cuong Dist, Nghe An, Vietnam	(Yu et al. 2017b)
Kurixalus bisacculus	FMNH 255661	KX554450	KX554691	VietnamTuong Duong Dist, Nghe An, Vietnam	(Yu et al. 2017b)
Kurixalus bisacculus	FMNH 255655	KX554452	KX554693	Con Cuong Dist, Nghe An, Vietnam	(Yu et al. 2017b)
Kurixalus bisacculus	FMNH 256452	KX554455	KX554696	Nakai Dist, Khammouan, Laos	(Yu et al. 2017b)
Kurixalus bisacculus	KUHE:19428	AB933290	KX554735	Nakon Sri Tamarat, Thailand	(Yu et al. 2017b)
Kurixalus eiffingeri	UMFS 5969	DQ283122	DQ282931	NanTou, Lu-Gu Chi-Tou, 900–1100 m, Taiwan	(Frost et al. 2006)
Kurixalus eiffingeri		AF458128			(Wilkinson et al. 2002)
Kurixalus idiootocus	UMFS 5702	DQ283054	DQ282905	NanTou, Tung Fu, 750 m, Taiwan	(Frost et al. 2006)
Kurixalus idiootocus		AF458129			(Frost et al. 2006)
Kurixalus idiootocus	SCUM 061107L	EU215547	EU215607	Lianhuachi, Taiwan	(Li et al. 2009)
Kurixalus odontotarsus	YGH 090132	GU227241	KX554683	Caiyanghe, Yunnan, China	(Yu et al. 2017b)
Kurixalus odontotarsus	YGH090130	GU227239	KX554681	Caiyanghe, Yunnan, China	(Yu et al. 2017b)
Kurixalus odontotarsus	Rao 14111401	KX554445	KX554680	Menglun, Yunnan, China	(Yu et al. 2017b)

Species	Sample voucher	GenBank accession number		Localities	Literature
		12S-tRNA val-16S	*TYR*		
Kurixalus odontotarsus	KIZ060821122	EF564456	KX554679	Menglun, Yunnan, China	(Yu et al. 2017b)
Kurixalus odontotarsus	YGH090177	GU227235	KX554677	Mengyang, Yunnan, China	(Yu et al. 2017b)
Kurixalus odontotarsus	YGH090176	GU227234	KX554676	Mengyang, Yunnan, China	(Yu et al. 2017b)
Kurixalus odontotarsus	YGH090175	GU227233	KX554675	Mengyang, Yunnan, China	(Yu et al. 2017b)
Kurixalus odontotarsus	Rao 14111307	KX554443	KX554674	Bada, Yunnan, China	(Yu et al. 2017b)
Kurixalus odontotarsus	Rao 14001643	KX554441	KX554672	Cangyuan, Yunnan, China	(Yu et al. 2017b)
Kurixalus odontotarsus	YGH090179	GU227236	KX554678	Mengyang, Yunnan, China	(Yu et al. 2017b)
Kurixalus odontotarsus	Rao 14102907	KX554442	KX554673	Cangyuan, Yunnan, China	(Yu et al. 2017b)
Kurixalus verrucosus	Rao 14102913	KX554440	KX554671	Yingjiang, Yunnan, China	(Yu et al. 2017b)
Kurixalus verrucosus	Rao 14102912	KX554439	KX554670	Yingjiang, Yunnan, China	(Yu et al. 2017b)
Kurixalus verrucosus	Rao 06308	KX554428	KX554657	Muotuo, Tibet, China	(Yu et al. 2017b)
Kurixalus verrucosus	Rao 06306	KX554427	KX554656	Muotuo, Tibet, China	(Yu et al. 2017b)
Kurixalus verrucosus	Rao 06302	KX554423	KX554654	Muotuo, Tibet, China	(Yu et al. 2017b)
Kurixalus verrucosus	Rao 06301	KX554422	KX554653	Muotuo, Tibet, China	(Yu et al. 2017b)
Kurixalus verrucosus	Rao 06201	KX554419	KX554651	Muotuo, Tibet, China	(Yu et al. 2017b)
Kurixalus verrucosus	Rao 06194	KX554416	KX554650	Muotuo, Tibet, China	(Yu et al. 2017b)
Kurixalus verrucosus	Rao 06193	KX554415	KX554649	Muotuo, Tibet, China	(Yu et al. 2017b)
Kurixalus verrucosus	CAS225128	GU227276	JQ060918	Nagmung, Kachin, Myanmar	(Yu et al. 2017b)
Kurixalus verrucosus	CAS 224381	GU227274	JQ060917	Nagmung, Kachin, Myanmar	(Yu et al. 2017b)
Kurixalus verrucosus	Rao 06202	KX554423	KX554654	Muotuo, Tibet, China	(Yu et al. 2017b)
Kurixalus verrucosus	Rao 06305	KX554426	KX554655	Muotuo, Tibet, China	(Yu et al. 2017b)
Kurixalus verrucosus	Rao 14102902	KX554430	KX554661	Muotuo, Tibet, China	(Yu et al. 2017b)
Kurixalus verrucosus	Rao 14102904	KX554432	KX554663	Nanjingli, Ruili, Yunnan, China	(Yu et al. 2017b)
Kurixalus verrucosus	Rao 14102905	KX554433	KX554433	Nanjingli, Ruili, Yunnan, China	(Yu et al. 2017b)
Kurixalus verrucosus	Rao 14102906	KX554434	KX554665	Nanjingli, Ruili, Yunnan, China	(Yu et al. 2017b)
Kurixalus verrucosus	Rao 14102910	KX554437	KX554668	Yingjiang, Yunnan, China	(Yu et al. 2017b)
Kurixalus verrucosus	Rao 14102909	KX554436	KX554667	Yingjiang, Yunnan, China	(Yu et al. 2017b)
Kurixalus sp.	MVZ Herp 223856	JQ060941	JQ060904	Tam Dao, Vinh Phu, Vietnam	(Yu et al. 2017b)
Kurixalus sp.	MVZ Herp 223863	JQ060943	JQ060921	Tam Dao, Vinh Phu, Vietnam	(Yu et al. 2017b)
Kurixalus sp.	MVZ Herp 223864	JQ060944	JQ060922	Tam Dao, Vinh Phu, Vietnam	(Yu et al. 2017b)
Kurixalus sp.	MVZ Herp 223865	JQ060945	JQ060923	Tam Dao, Vinh Phu, Vietnam	(Yu et al. 2017b)
Kurixalus sp.	MVZ Herp 223867	JQ060946	JQ060924	Tam Dao, Vinh Phu, Vietnam	(Yu et al. 2017b)
Kurixalus sp.	MVZ Herp 223868	JQ060947	JQ060925	Tam Dao, Vinh Phu, Vietnam	(Yu et al. 2017b)
Kurixalus hainanus	HNNU A1180		EU215608	Mt. Diaoluo, Hainan, China	(Li et al. 2008)
Orixalus carinensis	ROM39660	GQ285670	GQ285806	Sa Pa, Lao Cai, Vietnam	(Li et al. 2009)
Romerus ocellatus	HN0806045	GQ285672	GQ285802	Mt. Wuzhi, Hainan, China	(Li et al. 2009)
Romerus romeri	KIZ 061205YP	EU215528	EU215589	Mt. Shiwan, Guangxi, China	(Li et al. 2009)
Zhangixalus appendiculatus	FMNH:267897				(Yu et al. 2017b)
Zhangixalus appendiculatus	FMNH 267896		JQ060926	Bukit Sarang, Sarawak, Malaysia	Yu et al. (2013)
Zhangixalus nigropunctatus	-	EU215533	EU924583	-	(Yu et al. 2017b)

analyses using the Maximum Compo-site Likelihood algorithm (Tamura et al. 2004) and modelled the rate variation amongst sites with a gamma distribution (shape parameter = 1). We considered differences in the composition bias amongst sequences in our evolutionary comparisons (Tamura and Kumar 2002), thus, all ambiguous positions were removed for each sequence pair using pairwise deletion option. These final datasets resulted in the totality of 116 positions, 301 sites for 12S rRNA and 110 positions, 486 sites for *TYR*.

For subsequent phylogenetic analyses, we downloaded supplemental sequences data of 98 homologous sequences of *Kurixalus* and *Zhangixalus* and other Rhacophoridae genera from Genbank (Wilkinson et al. 2002; Frost et al. 2006; Li et al. 2009; Nguyen et al. 2014; Yu et al. 2017a; Yu et al. 2018). GenBank accession numbers for both the new and previously deposited data are given in Table 1. We then created an initial alignment, based on nucleotide sequences with ClustalW2 (Larkin et al. 2007) and refined it manually. The final trimmed sequences resulted in 771 bp of concatenated 12S rRNA and *TYR* (*n* taxa = 79).

We used Partition Finder v. 2.1.1 (Lanfear et al. 2012) to determine the best-fit partitioning of the defined subsets. For the concatenated genes dataset, we defined four subsets by considering a fixed model for non-coding fragment and one subset for every single codon position with respect to the protein coding *TYR* gene fragments. Based on the Bayesian Information Criterion (BIC) values, we selected the following models for the following gene fragments: non-coding 12S rRNA (fixed subset): 1–292 (SYM+G) and protein coding *TYR* (subset 1): 293–771/1 (GTR+G); *TYR* (subset 2): 294–771/2 (K80+I+G) and *TYR* (subset 3): 295–771/3 (GTR+I+G). We used the models selected as a priori in further phylogeny analyses.

We built phylogenetic trees for all three datasets: mtDNA 12S rRNA-tRNA-Val-16S rRNA, protein coding nuDNA *TYR* and concatenated 12S rRNA-*TYR* using Bayesian Inference methodologies with MrBayes v.3.2.6 (Ronquist and Huelsenbeck 2003). For each tree dataset, we performed four separate analyses with 50 million generations of Markov Chain Monte Carlo and discarded the first 20 percent generations as burn-in until a convergence was reached (here we obtained > 0.005 split frequencies).

To test the presence of population differentiation using the *TYR* marker, we ran an analysis of molecular variance (AMOVA; Excoffier et al. 1992) using Arlequin v.3.5.2.2 (Excoffier and Lischer 2010). Here we used the AMOVA to test the three clades recovered from our phylogeny, based on *TYR* (Outgroup, Clade A and Clade B; see phylogenetic tree in Suppl. material 1: Fig. S2). In addition, we phased the diploid sequences of *TYR* gene (486 sites; *n* = 216) and analysed the haplotype using DnaSP v.5.0 (Librado and Rozas 2009). Before analysing the haplotypes, we assigned each haplotype group to its species, resulting 10 *Kurixalus* species and four closely-related species: *K. inexpectatus* sp. nov., *K. banaensis*, *K. baliogaster*, *K. bisacculus*, *Kurixalus* sp., *K. hainanus*, *K. odontotarsus*, *K. verrucosus*, *K. eiffingeri*, *K. idiootocus* and *Zhangixalus appendiculatus*, *R. ocellatus*, *R. romeri* and *Orixalus carinensis*. Out of the 486 sites, we disregarded missing haplotypes and removed all invariable sites. Then, we converted the haplotype analysis in DnaSP v.5.0 to an RDF input

file format and we built the reticulated haplotype network from the phased *TYR* in NETWORK v.10.2.0 (Fluxus Technology Ltd; UK) using the Median-joining method (Bandelt et al. 1995).

Species delimitation and divergence time estimation

Relying solely on a distance-based method is insufficient. The coalescent-based species delimitation was determined as the most efficient method for comparative study of species delimitation in genus of *Kurixalus* (Yu et al. 2017a). To test the assumption that the individuals samples belonged to a new species rather than an exotic or invasive clade of *K. idiootocus*, we employed a topology testing and species delimitation approach using both the coalescent-based methods. First, we designed two competing topology species tree models: model 1 and model 2 with two independent datasets consisting respectively of 79 unlinked sequences of mtDNA 12S rRNA (292 bp) and nuDNA *TYR* (451 bp). Model 1 designated the new *Kurixalus* clade, *K. inexpectatus* sp. nov. as clumped within the clade of most closely related species, *Kurixalus idiootocus*, whereas Model 2 assigned *K. inexpectatus* sp. nov. as a new species, split from *K. idiootocus*. For a comparison between topologies, we ran a nested sampling analyses on species tree Model 1 and Model 2 with NS package implemented in BEAST v.2.6.6 (Bouckaert et al. 2019). We selected MCMC sub-chain length of 10,000 with particle count of 10 and an epsilon of 1.0×10^{-9} as parameters for each nested sampling analysis. Then, we evaluated topology of species trees of Model 1 and Model 2 by comparing the tree marginal L estimate (MLE) value and the Bayes factor obtained from the nested samplings. We calculated the Bayes factor with the following formula: Bayes factor = (MLE value of Model 1) – (MLE value of Model 2). We selected the best species tree model through the Bayes factor value, in which a positive Bayes factor is in favour of that particular model. We visualised the most likely species tree with Densitree (Bouckaert 2010).

Additionally, the recent study on the phylogeography of Taiwanese *Kurixalus* showed that the genus colonised the Island attributes through a land-bridge during the last glacial maxima (Yu et al. 2021). We further inferred the lineage origins and divergence between our focal taxa and *K. idiootocus* distributed in Taiwan Island. To do so, we estimated the time divergence of *Kurixalus* lineage by calibrating the species tree using an uncorrelated lognormal relaxed molecular clock with StarBeast RLC v.2.6.6 (Bouckaert et al. 2019). For both Model 1 and 2 datasets, we enforced three similar calibration points. Due to the absence of fossil records of Rhacophoridae in Asian mainland (Yu et al. 2021), we relied on paleogeological events for our primary calibration source. For secondary calibration, we adapted the range of molecular dating estimations of related literature (Pan et al. 2017; Yu et al. 2021). The three calibration points described as: (i) Emergence of *Zhangixalus nigropunctatus* in Southeast Asian and Chinese mainland ca. 11.39 Mya (High posterior density (HPD) 95%: 8.89–14.16; Pan et al. 2017), (ii) Emergence of stem group of South-eastern Asian clades of *Kurixalus* involves *K. verrucosus* group and its representative members ca. 7.4 Mya (HPD 95%: Yu et al. 2021) and (iii) Emergence of stem group of *K. eiffingeri* and

K. idiootocus in Taiwan ca. 5.50 Mya (HPD 95%: 8.75 -3.25; Yu et al. 2021), simultaneously with the earliest island formation after physically separating from the south -astern mainland of China through the formation of the Taiwan strait (ca. 5.0-2.0 Mya modern shape; Teng 1990). We tested both birth-death and Yule priors on our species tree datasets and finally selected Yule as the best tree prior due to a better pattern of bifurcation for each crown node generated in trees. We ran four independent analyses, with MCMC chains of 20 million generations and 1,000 pre-burn-in steps for each dataset of model. We verified the convergence of the generated trees by evaluating the MCMC outputs with Tracer v.1.7.1 (Rambaut et al. 2018). Here, we ensured the values of effective sample size (ESS) for all parameters to be at least more than 1,000. We summarised a maximum clade credibility (MCC) tree for the calibrated tree time using TreeAnnotator, an application attached to BEAST v.2.6.6.

Finally, we projected the possible dispersal pathways, based on the molecular dating estimates focusing on the clade containing our focal species and Taiwanese *Kurixalus* on paleomaps using QGIS v.2.18.15. The oscillayers used to reconstruct the Plio-Pliocene maps was adapted from datasets provided in Gamisch (2019).

Call data collection and extraction

The acoustic recordings of putative new *Kurixalus* species were recorded between April and July in 2018 at 24 °C with a linear PCM recorder (Tascam DR-40; California, USA) linked to a unidirectional microphone (Unidirectional electret condenser microphone HT-81, HTDZ; Xi'an, China). To determine the relationship with other species, we first compared the number of consecutive calls within a series of calls between the individuals recorded and *K. idiootocus*, *K. eiffingeri*, *K. berylliniris* and *K. wangi*. We then compared the call properties of the new population with that of *K. idiootocus* as it was the most closely-related species and the only species with the same number of calls within a series of consecutive calls (see results). The recordings of *K. idiootocus* were obtained in central Taiwan (23.9240 N, 120.8910 E) in July 2013, using a Tascam DR-70D digital recorder (TEAC Corporation, Tokyo, Japan) and a Sennheiser ME67/K6 directional microphone (Sennheiser Electronic GmbH & Co. KG, Hanover, Germany). All our recordings were recorded at a sampling rate of 44.1 kHz with 16-bit resolution. Temperature was recorded with a Tecpel DIT-517 infrared thermometer (between 22 and 25 °C; TECPEL Corporation, New Taipei, Taiwan). The genus emits a series of continuous notes, pooled in bouts of continuous calls. To compare *K. idiootocus* and the new *Kurixalus* population, we selected one entire series of consecutive calls for each individual and analysed 373 advertisement calls in total, including 238 calls for *K. idiootocus* (9 to 24 calls in a bout from each of 16 males) and 135 calls for the new population (9 to 21 calls in a bout for 9 males).

We used Raven Pro v.1.5 (Cornell Lab of Ornithology 2011) to analyse our recordings. Nine properties, including six temporal and three spectral properties were measured in the two *Kurixalus* species (*K. idiootocus* vs. *K. inexpectatus* sp. nov): number of calls in a bout, bout length (s), call interval (ms), call length (ms), rise time (ms),

fall time (ms), max frequency (kHz), 2nd frequency (kHz), relative amplitude (dB). We measured the number of calls and the length of a series of consecutive calls. Call interval was measured as the duration from the end of a call to the beginning of the next call (Fig. 3A, B).

Call duration refers to the time between the onset and offset of a call. Rise time refers to the time between the onset of call and the local maximum in the waveform. Fall time is the time between the local maximum in the waveform and the offset of a call (Fig. 3B). Dominant frequency is the strongest frequency in the duration of a call and the secondary frequency is the maximum frequency of the second harmonic. Relative amplitude is the difference in amplitude between the dominant frequency and secondary high (dominant – secondary). In both species, the dominant frequency was the primary harmonic, as indicated through preliminary analyses and later confirmed by determining the fundamental frequency for a subset of calls as the reciprocal of the average period of the quasi-periodic fine-temporal waveform. All frequency measurements were based on 1024-point fast Fourier transformation and Hann windows and were made from the average power spectrum computed over the duration of a call.

Call property analyses

We first corrected the calls for temperature variation by adjusting the value of each variable to the average temperature of all recordings using the equation originating from the linear regression of each focal variable in function of temperature. As the contribution of each call property for each individual is not independent of other call properties and consequently correlated, we used a principal component analysis (PCA) to convert those call properties into a set of values of linearly uncorrelated factors. The PCA provided four principal components with Eigenvalues larger than 0.5, explaining 95.7% of the total variance. We used a Discriminant Function Analysis (DFA) to classify the call properties and test for the correctness of group assignment. We then plotted the two significant PCs against each other to illustrate the divergence between the two species. Finally, to determine the differing call variables between the two clades, we used a Multivariate Analysis of Variance (MANOVA) to compare each call property between these two species.

Morphometric measurements

We collected eighteen morphological measurements three times each and averaged the values for further analyses. Morphometric data were taken using digital calipers to the nearest 0.1 mm and included the following characters: snout-vent length (**SVL**), head width (**HDW**), distance between left and right articulations of jaw, head length (**HDL**), from the tip of the snout to the articulation of the jaw, snout length (**SNT**), from tip of snout to the anterior corner of the eye, horizontal eye diameter (**EYE**) from the anterior to the posterior corner of the eye, width of the upper eyelid (**UEW**), the horizontal length of the upper eyelid, internares distance (**IND**), the distance from

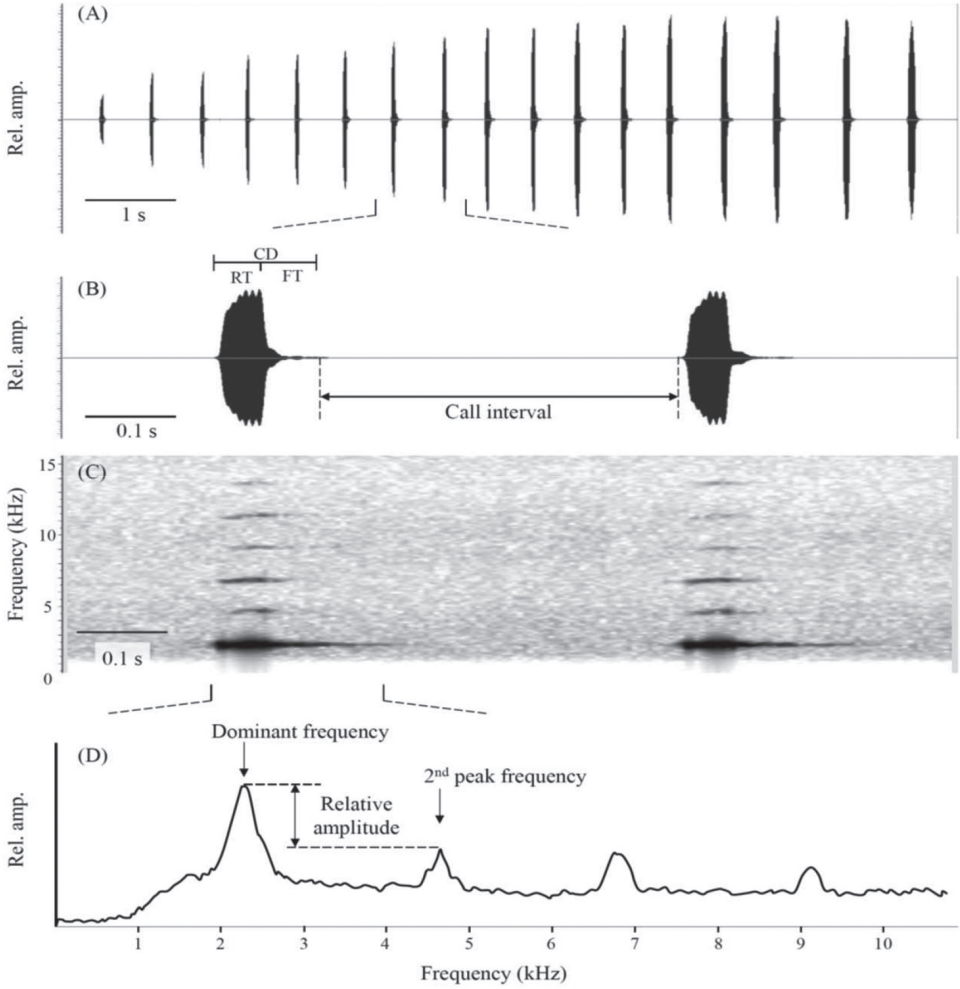

Figure 3. The call property measurements. This figure shows **A** the waveform of entire series of a consecutive call **B** the waveform **C** the spectrogram of two calls and **D** the spectral power distribution of a single call from the new *Kurixalus* population from Zhejiang, China. We extracted the number of calls in a bout, call interval, call duration (CD), rise time (RT), fall time (FT), dominant frequency (here also the max frequency), secondary peak frequency and the relative amplitude of two peaks.

nostril to eye (**DNE**), from the posterior border of nostril to anterior border of the eye, narrowest interorbital distance (**IOD**), greatest horizontal tympanum diameter (**TMP**), tympanum-eye distance (**TEY**) from anterior edge of tympanum to posterior corner of eye, hand length (**HND**) from distal end of radio-ulna to tip of finger III, radio-ulna length (**RAD**), forelimb length (**FLL**), distance from the proximate end of radio-ulna to distal end of finger III, thigh length (**THL**), distance from vent to distal end of femur, tibia length (**TIB**), foot length (**FL**) from proximal end of inner

metatarsal tubercle to tip of toe IV and the length of the foot and tarsus (**TFL**), distance from tibio-tarsal joint to tip of toe IV. All specimens were measured by a single author (YY) to minimise sampling error. The dataset is available Suppl. materials.

To be able to compare with the morphometrics of other clades, we extracted data from the literature for all species available (Suppl. material 1: Table S4): *K. berylliniris*, *K. wangi*, *K. idiootocus*, *K. eiffingeri*, *K. bisacculus*, *K. lenquanensis*, *K. odontotarsus*. *K. yangi*, *K. naso*, *K. viridescens* and *K. ananjevae* (Kuramoto and Wang 1987; Matsui and Orlov 2004; Nguyen et al. 2014; Tao et al. 2014; Wu et al. 2016; Yu et al. 2017b; Yu et al. 2018; Zeng et al. 2021). The data were, however, incomplete for the variables TEY, TFL, THL, HND and RAD and these variables were removed from the analyses. We only kept data points that had a full dataset for the remaining 13 variables and were males to enable further morphological comparison without impact of sexual dimorphism. In total, we harvested data for 68 *Kurixalus* sp. individuals, including our samples. We then removed variation due to size difference between individuals by dividing each of the variables by the SVL of the matching individual. Furthermore and because of the low sample size for most species, we created two categories for the statistical analyses: one including our focal clade and the other one with all other non-focal species. The reasoning behind this segregation being that a clade would have to be extremely divergent to be morphologically different from all other species of the genera. We also created a subsection of the dataset including our focal clade (n = 12) and *K. idiootocus* as it is the most phylogenetically closely-related clade (n = 6).

Morphometric analyses

As the variables were strongly correlated (Pearson's correlation; Table 2), we decided to use a factor reduction statistical analysis to identify the independent dimensions of the morphological characters. The principal component analysis was set such that principal components were to be extracted if their Eigen value was > 1, under a varimax rotation. Variables were selected as loading into a PC if loading > 0.58 (Table 3). Once the PCs were extracted, we tested for significant differences between our focal clade and all other species through one-way ANOVA and then between our focal clade and *K. idiootocus* through a second one-way ANOVA. All analyses were run in SPSS (SPSS, Inc., Chicago, USA). Additionally, after standardising morphological measurements by SVL, we also ran a two-sample t-test comparing the putative new species (n = 12) and *K. idiootocus* (n = 8) on 12 of the morphological characters.

Results

Sequence divergence, phylogenetic relationships and haplotype distribution

Our analyses resulted in minor differences in the evolutionary divergence between the 12S rRNA gene fragments of *K. inexpectatus* sp. nov. and *K. idiootocus* (mean = 0.0004

Table 2. Pearson correlation for all ten selected variables. We run a Pearson Correlation test (n = 68) to highlight the correlation between variables and highlight the need for a variable reduction analysis, such as a PCA. Cells in bold highlight significance.

		HDW	SNT	IND	IOD	UEW	EYE	TD	DNE	FLL	TFL	FL
HDL	r	0.84	0.34	0.23	0.44	0.60	-0.11	-0.13	0.09	0.86	0.45	0.08
	p	**< 0.001**	**0.005**	0.065	**< 0.001**	**< 0.001**	0.365	0.298	0.480	**< 0.001**	**< 0.001**	0.521
HDW	r		0.38	0.22	0.52	0.49	-0.06	-0.15	0.05	0.81	0.54	0.10
	p		**0.001**	0.072	**< 0.001**	**< 0.001**	0.634	0.218	0.698	**< 0.001**	**< 0.001**	0.432
SNT	r			-0.11	0.04	0.38	0.11	-0.25	-0.09	0.34	0.61	-0.05
	p			0.365	0.722	**0.002**	0.394	**0.044**	0.450	**0.005**	**< 0.001**	0.698
IND	r				0.01	0.48	-0.26	0.04	0.15	0.06	0.10	0.16
	p				0.961	**< 0.001**	**0.029**	0.773	0.230	0.644	0.439	0.195
IOD	r					0.07	0.11	-0.04	0.06	0.40	0.15	0.05
	p					0.565	0.383	0.731	0.648	**0.001**	0.209	0.695
UEW	r						-0.04	-0.16	0.09	0.46	0.48	0.06
	p						0.754	0.181	0.455	**< 0.001**	**< 0.001**	0.625
EYE	r							0.39	-0.70	-0.06	0.12	-0.42
	p							0.001	**< 0.001**	0.655	0.330	**< 0.001**
TMP	r								-0.47	-0.14	-0.23	-0.47
	p								**< 0.001**	0.273	0.059	**< 0.001**
DNE	r									-0.06	-0.17	0.73
	p									0.628	0.163	**< 0.001**
FLL	r										0.61	0.09
	p										**< 0.001**	0.471
TFL	r											0.13
	p											0.285

SD ± 0.0004). Similarly, the protein coding nuclear *TYR* between *K. inexpectatus* sp. nov. and *K. idiootocus* showed a comparatively smaller mean of substitution rate (mean = 0.0035 ± 0.0004; value marked with double asterisks (**) in Table 4) than that of other species groups (Table 4).

Overall, the Bayesian Inference (BI) trees inferred from both mtDNA 12S rRNA-tRNA-Val-16S rRNA and nuDNA *TYR* fragments showed strong patterns of genetic structures for the East Asian and Southeast Asian *Kurixalus* phylogeny relationship, recovering four strongly supported clades (Clades A, B, C and D; see the distributions of the clades and the phylogenetic tree in Suppl. material 1: Fig. S1), including two major clades (Clades A and B; Suppl. material 1: Fig. S2) within the *Kurixalus* lineage. Although showing a discordant topology for the clades distributed in Southeast Asia (Suppl. material 1: Figs S1 and S2), the mtDNA and nuDNA trees converged towards a similar phylogenetic position for *K. inexpectatus* sp. nov., highlighting a sister relationship with *K. idiootocus* (Suppl. material 1: Figs S1, S2).

The phylogenetic relationship of concatenated gene fragments of partial 12S rRNA and *TYR* gene fragments recovered the three major clades within the *Kurixalus* genus with a Bayesian Posterior (BP) support of 90% for clade A, 52% for clade B, 71% for clade C (Fig. 4). Monophyletic clade A contained a Vietnamese *Kurixalus* (presumably as *K. carinensis*, but has been considered different from type species of *K. carinensis* in Myanmar) and *K. romeri* and *K. ocellatus* of Chinese mainland (BP = 100%; Fig. 4).

Table 3. Variables and results for the Principal Component Analysis and resulting ANOVA. Principal components were to be extracted if their eigenvalue > 1, under a varimax rotation. Variables were selected as loading into a PC if the value is > 0.58. In bold are variables retained as loading into one if the PCs. Based on the variables loading on to each of the PCs, we assigned PC1 to the general morphology and PC2 to the horizontal head structure. PC1 and PC2 were not significantly different between *Kurixalus inexpectatus* sp. nov. and other *Kurixalus* species under a one-way ANOVA, but they were significantly different between *Kurixalus inexpectatus* sp. nov. and *K. idiootocus*. The sample sizes used in the analysis were such as: *K. inexpectatus* sp. nov. *n* = 12, *K. idiootocus n* = 8; all *n* = 71; details in the Suppl. material 2).

	PC1	PC2
SVL	**0.90**	0.21
HDL	**0.92**	0.14
HDW	**0.96**	0.17
SNT	**0.84**	0.26
IND	**0.74**	-0.08
IOD	**0.85**	0.17
UEW	**0.84**	0.09
EYE	0.33	**0.84**
TMP	0.40	**0.59**
DNE	0.35	**-0.86**
FLL	**0.90**	0.21
TFL	**0.91**	0.25
FL	**0.85**	-0.28
Eigen value	8.25	1.92
Variance (%)	63.49	14.74
ANOVA all clades		
$\chi 2$	0.43	0.69
F	0.47	0.66
df1, df2	1,66	1,66
p	0.494	0.419
ANOVA focal clade-*K. idiootocus*		
$\chi 2$	1.42	1.39
F	13.35	14.56
df1, df2	1,66	1,66
p	**0.002**	**0.017**

Clade B recovered a monophyletic *Kurixalus* originated from Taiwan and south-eastern China. Here, the clades endemic to Taiwan, *K. eiffingeri* (Clade B, BP = 52%; Fig. 4) and *K. idiootocus* (BP = 97%; Fig. 4) were nested to the monophyletic clade of our *Kurixalus* sp. sampled in Zhejiang, south-eastern China (clade B, BP = 63%; Fig. 4). Clade B therefore supported a divergence between focal *Kurixalus* clade and *K. idiootocus*. Clade C comprised of a large nested monophyletic East Asian and Southeast Asian mainland *Kurixalus*, included clades of *K. banaensis* that were ranging in Vietnam and *K. verrucosus* (BP = 100%), *K. baliogaster* (BP = 100%), *Kurixalus* sp. (BP = 74%) *K. bisacculus* (BP = 67%) and *K. odontotarsus* (BP = 96%) originating from Western China, Tibet, Yunnan and the Eastern Indo-Chinese Peninsula: Vietnam, Thailand and Cambodia (Table 1; Fig. 4).

The AMOVA provided support to the genetic differentiation recorded while using the *TYR* marker as it identified 21.80% of variance within clades and 78.20% of

Table 4. Matrix of genetic distances between all pairs of sequences of protein-coding nuclear *TYR* between groups of sequences of 16 species rhacophorids species (*n* = 110). The 16 groups of species consisted of *Kurixalus* and *Rhacophorus* genera. Values in bold in the bottom left of diagonal matrix represent the means of estimate for each species divergence using maximum composite likelihood. Values of the upper right of diagonal matrix represents the standard deviation of each mean of divergence. The mean of distance between our proposed species *K. inexpectatus* sp. nov. and *K. idiootocus* noted with (**), which was higher to mean genetic distance of other pairwise species (values are in bold and marked with *).

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 *K. ocellatus*		0.0071	0.0260	0.0287	0.0292	0.0290	0.0293	0.0292	0.0292	0.0302	0.0340	0.0286	0.0264	0.0206	0.0216	0.0275
2 *K. romeri*	**0.0144**		0.0254	0.0280	0.0290	0.0286	0.0290	0.0291	0.0290	0.0298	0.0338	0.0279	0.0258	0.0201	0.0218	0.0270
3 *K. inexpectatus*	**0.0780**	**0.0752**		0.0047	0.0057	0.0054	0.0056	0.0072	0.0057	0.0063	0.0104	0.0047	0.0025	0.0150	0.0147	0.0221
4 *K. banaensis*	**0.0862**	**0.0832**	**0.0081**		0.0058	0.0056	0.0057	0.0074	0.0059	0.0065	0.0104	0.0058	0.0043	0.0162	0.0147	0.0218
5 *K. baliogaster*	**0.0885**	**0.0876**	**0.0113**	**0.0109**		0.0006	0.0006	0.0035	0.0001	0.0028	0.0100	0.0063	0.0048	0.0171	0.0161	0.0229
6 *K. bisacculus*	**0.0877**	**0.0859**	**0.0108**	**0.0105**	**0.0007**		0.0001	0.0029	0.0004	0.0029	0.0102	0.0060	0.0044	0.0170	0.0157	0.0226
7 *Kurixalus* sp.	**0.0888**	**0.0875**	**0.0113**	**0.0110**	**0.0005**	**-0.0001**		0.0030	0.0001	0.0029	0.0102	0.0062	0.0046	0.0172	0.0160	0.0229
8 *K. hainanus*	**0.0884**	**0.0876**	**0.0161**	**0.0157**	**0.0046**	**0.0037**	**0.0036**		0.0035	0.0048	0.0114	0.0078	0.0064	0.0186	0.0167	0.0235
9 *K. odontotarsus*	**0.0887**	**0.0878**	**0.0114**	**0.0110**	**0.0001***	**0.0004***	**0.0001***	**0.0046**		0.0029	0.0100	0.0063	0.0048	0.0171	0.0161	0.0229
10 *K. verrucosus*	**0.0926**	**0.0906**	**0.0134**	**0.0130**	**0.0035**	**0.0036**	**0.0037**	**0.0081**	**0.0035**		0.0110	0.0068	0.0054	0.0166	0.0162	0.0229
11 *Zhangixalus appendiculatus*	**0.1019**	**0.1009**	**0.0260**	**0.0256**	**0.0240**	**0.0250**	**0.0247**	**0.0290**	**0.0241**	**0.0278**		0.0109	0.0096	0.0224	0.0213	0.0283
12 *K. eiffingeri*	**0.0858**	**0.0829**	**0.0080**	**0.0109**	**0.0125**	**0.0120**	**0.0126**	**0.0173**	**0.0126**	**0.0143**	**0.0272**		0.0037	0.0157	0.0152	0.0227
13 *K. idiootocus*	**0.0797**	**0.0769**	**0.0035#***	**0.0070**	**0.0086**	**0.0081**	**0.0086**	**0.0133**	**0.0086**	**0.0106**	**0.0231**	**0.0054**		0.0144	0.0138	0.0212
14 *K. carinensis*	**0.0605**	**0.0580**	**0.0421**	**0.0456**	**0.0494**	**0.0490**	**0.0497**	**0.0549**	**0.0496**	**0.0479**	**0.0666**	**0.0436**	**0.0401**		0.0111	0.0167
15 *R .nigropunctatus*	**0.0617**	**0.0613**	**0.0400**	**0.0397**	**0.0451**	**0.0438**	**0.0448**	**0.0469**	**0.0452**	**0.0457**	**0.0618**	**0.0412**	**0.0370**	**0.0274**		0.0145
16 *Rhacophorus* sp.	**0.0823**	**0.0794**	**0.0655**	**0.0632**	**0.0691**	**0.0678**	**0.0689**	**0.0711**	**0.0693**	**0.0693**	**0.0850**	**0.0668**	**0.0622**	**0.0460**	**0.0370**	

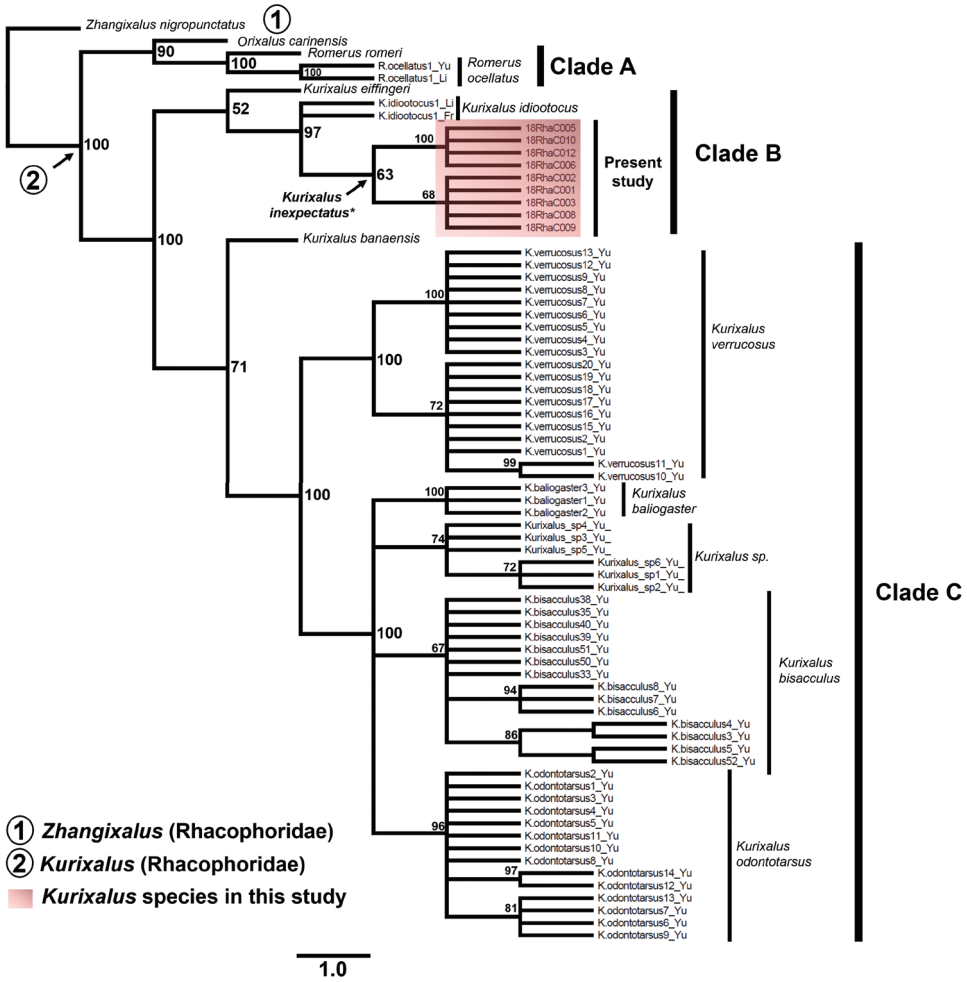

Figure 4. Bayesian Inference (BI) tree inferred from 79 sequences of concatenated 12S rRNA-*TYR* gene fragments. The three clades (Clades **A, B** and **C**) recovered in the phylogenetic tree are labelled accordingly. Clade **B** included the species *K. inexpectatus* sp. nov. described in the present study, indicated by the red box. The value of the node represents the Bayesian posterior probability (BPP) for each clade. The clades are marked with a solid bar and labelled in accordance with their specific name.

variance between clades for the three main clades *Kurixalus* (n = 108; Fig. 4). The results of the AMOVA also provided a significant F_{ST} value (0.782; $p < 0.05$), showing that the three clades were significantly variable. The haplotype generated from the phased *TYR* fragment (n = 216), based in 477 trimmed sites, resulted in 62 haplotypes with a haplotype diversity (Hd) of 0.958 (Fig. 5). The distribution of haplotypes showed that six haplotype groups were representative of *Kurixalus* distributed in Taiwan Island and south-eastern China (Clade A; Fig. 5; see phylogenetic tree in Suppl. material 1: Fig. S2). Out of these six haplotype groups in Clade A, three of them represented *K. inexpectatus*

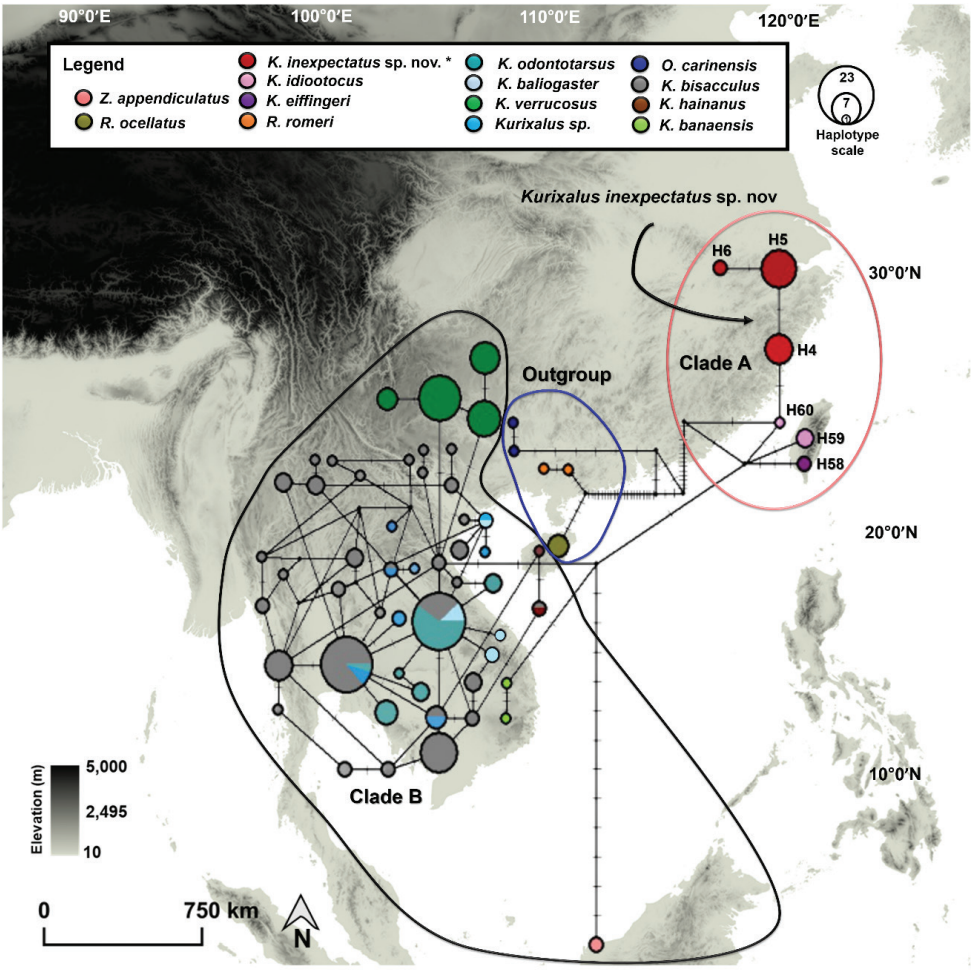

Figure 5. Haplotype network inferred from 216 phased nuDNA *TYR* sequences data (486 sites). The haplotype group for the focal Clade **A** comprised six representative haplotypes of *Kurixalus*. Clade **A** included three *K. inexpectatus* sp. nov. haplotypes. The size of each haplotype marker matches the haplotype scales. The colour coding matches with the name of the taxa in the legend. The colours used for the boundaries of Clade **A** and Clade **B** are coded similarly to the colours of their clades in the phylogenetic tree (Suppl. material 1: Fig. S2).

sp. nov. (H4 – H6; Fig. 5). The origin of the haplotypes of *K. inexpectatus* sp. nov. corresponded to the haplotype groups of Taiwanese *Kurixalus*: *K. idiootocus* (H59- H60; Fig. 5) and *K. eiffingeri* (H 58; Fig. 5), whereas, Clade B contained a large portion of *Kurixalus* haplotype groups originating from south-eastern Asia, including *Z. appendiculatus* from Borneo and six *Kurixalus* species distributed across mainland Southeast Asia: Thailand, Laos, Cambodia and Vietnam (Fig. 5 and Suppl. material 1: Fig. S2). Clade B also included the representative haplotypes of *K. hainanus* distributed in Hainan Island and *K. verrucosus* haplotypes originating from Yunnan, south-western China.

Species delimitation and divergence time estimates

The topology of the coalescent unlinked 12S rRNA and *TYR* tree supported the divergence of the focal *Kurixalus* clade from the most closely-related species, *K. idiootocus*. Nested sampling analyses on both species trees was favoured on the topology proposed by Model 2 (MLE = - 3688.252; Bayes factor: 651.011; Table 5). This topology provided support on the splitting between the lineages of *K. inexpectatus* sp. nov. distributed on south-eastern mainland and *K. idiootocus* distributed on Taiwan Island (Fig. 6), more so than a clumping between *K. inexpectatus* sp. nov. and *K. idiootocus* (see Model 2; Table 5).

Our calibrated species tree of unlinked 12S rRNA and *TYR* gene fragments provided support on the earliest split between Asian lineages of *Kurixalus* and *Zhangixalus* to be dated in Mid-Miocene, ca. 11.17 Mya (Table 6; Fig. 5). This split-off was subsequently followed by the emergence of basal clade of *Kurixalus* in eastern Asia ca. 10.48 Mya [95% Highest Posterior Density (HPD): 8.16 – 12.98; node a; Table 6: Fig. 6]. Stem clade of *Kurixalus* distributed across the eastern Asian mainland, adjacent islands and south-eastern Asian mainland species group, consisting of members, such as *K. banaensis*, *K. verrucosus*. *K. baliogaster*, *K. odontotarsus*, *Kurixalus* sp. *K. hainanus* and *K. bisacculus* may have emerged ca. 9.14 Mya [6.87–11.12; node b; Table 6; Fig. 6]. Later, a stem clade of Taiwanese *Kurixalus* may have emerged, initiated by the isolation of *K. eiffingeri* in Taiwan and Ryukyu Islands ca. 5.66 Mya [3.32–8.07; node c; Table 6; Fig. 6]. Molecular dating estimates the lineage splitting between our proposed species, *Kurixalus inexpectatus* that distributed in south-eastern China and its sister clade, Taiwanese *K. idiootocus* to be in Late Pliocene to Pleistocene, ca. 3.06 Mya (5.82-0.01; node d; Table 6; Fig. 6).

Table 5. Nested sampling analysis results on two competing topology models for combined 12S rRNA and *TYR* using calibrated species trees. The values include summation of estimated Marginal L value with calculated Bayes factor for designated topology Model 1 and model 2. The positive value favoured the designated model. Topology Model 1 clumped *Kurixalus idiootocus* and *K. inexpectatus* sp. nov. as a single species. Topology Model 2 proposed *K. inexpectatus* sp. nov. as a new species and split from *K. idiootocus*. Bold values indicate the mean of nested sampling for each model.

Species tree topology	Nested sampling					Consensus
		Marginal likelihood estimate (MLE)	sqrt (H/N)	Standard deviation	Bayes factor (mean of MLE_1-mean of MLE_2)	
Model 1 (clumping)	1	-4339.441	6.099	6.008	-651.001	Model 1 is not favoured
	2	-4339.114	6.093	5.889		
	3	-4339.123	6.092	5.992		
	4	-4339.334	6.094	5.969		
	Mean	**-4339.253**	**6.095**	**5.965**		
Model 2 (splitting)	1	-3688.305	5.144	5.161	651.001	Model 2 is favoured
	2	-3688.131	5.143	5.458		
	3	-3688.361	5.145	5.279		
	4	-3688.211	5.143	4.925		
	Mean	**-3688.252**	**5.144**	**5.206**		

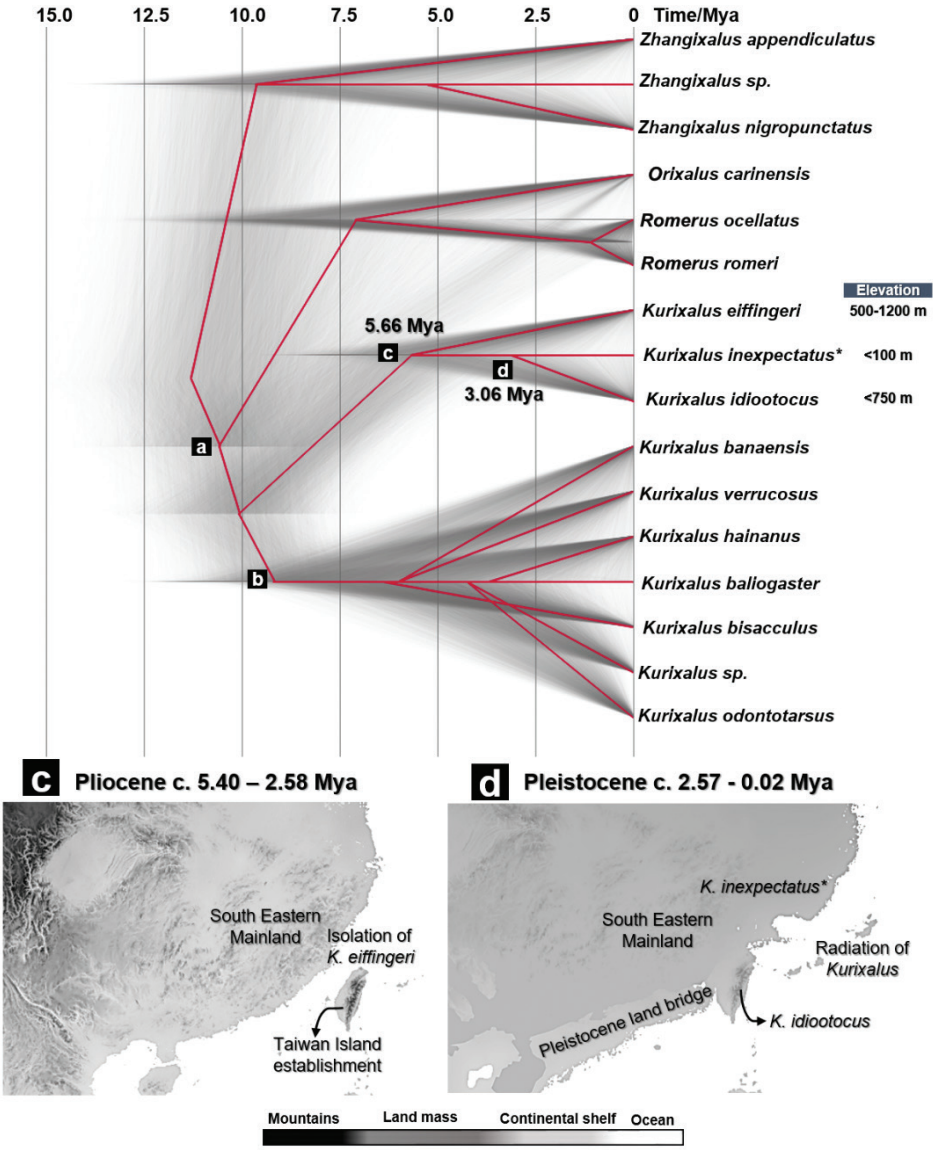

Figure 6. Calibrated species tree of Rhacophoridae represented by *Kurixalus*, *Orixalus*, *Romerus* and *Zhangixalus* distributed over East Asia and Southeast Asia. The species tree reconstructed from unlinked 12S rRNA and *TYR*. The asterisk (*) symbol indicates *Kurixalus inexpectatus* sp. nov. The highlighted lineages divergence noted with (**a–d**) and the time estimates are synchronised with datation in Table 6. Biogeography models **C** and **D** hypothesised early colonisation pathway of *Kurixalus* to Taiwan Island and potential glacial-driven refugia to the south-eastern mainland, projected on the Plio-Pleistocene oscillations models of.

Call properties

As the contribution of each call property for each individual is not independent of other call properties and consequently correlated, we used a principal component anal-

Table 6. Molecular dating of 16 species of Asian rhacophorid frogs estimates the age of lineage separation between *K. inexpectatus* sp. nov. and *K. idiootocus*. The molecular dating estimation was using an uncorrelated lognormal relaxed clock with Yule prior on species tree inferred from unlinked 12S rRNA and *TYR* gene fragments of *Kurixalus* and rhacophorid taxa (*n* taxa = 79) distributed across Southeast Asia and East Asia.

Node	Clade (speciation event)	Node age (Mya)	
		Mean	HPD 95%
a	Emergence of stem clade of *Kurixalus* after split off from *Zhangixalus*	10.48	8.16–12.98
b	Stem clade of south-eastern and eastern Asian mainland group of *Kurixalus* (*K. verrucosus* + *K. baliogaster* + *K. odontotarsus* + *K. hainanus* + *K. bisacculus*)	9.14	6.87–11.50
c	Stem clade of Taiwanese *Kurixalus* group (isolation of *K. eiffingeri*)	5.66	3.32–8.07
d	Split off between Chinese mainland *K. inexpectatus* sp. nov. of south-eastern mainland and *K. idiootocus* of Taiwan Island	3.06	5.82–0.01

ysis (PCA) to convert those call properties into a set of values of linearly uncorrelated factors. We selected the principal components from the results of the PCA to cover as much as possible of the total variance, resulting in four PCs with Eigenvalues larger than 0.5 and explaining 95.7% of the total variance. We used a Discriminant Function Analysis (DFA) to classify the call properties and test for the correctness of group assignment. We then plotted the two significant PCs against each other to illustrate the divergence between the two species. Finally, to determine the differing call variables between the two clades, we used a Multivariate Analysis of Variance (MANOVA) to compare each call property between these two species.

Based on the descriptions of the advertisement call and the number of calls in a series of consecutive calls, we could first segregate the species into two groups matching with the phylogenetic clustering. We grouped the putative new *Kurixalus* species and *K. idiootocus* together, while *K. eiffingeri*, *K. berylliniris* and *K. wangi* were grouped together (Suppl. material 1: Tables S1, S2, S3). From here, when then compared the putative new *Kurixalus* species and *K. idiootocus*.

The DFA on the four resulting PCs highlighted that only two PC1and PC3 were significantly different between the two species (PC1: Wilks' Lambda = 0.93, $F_{(1,19)} = 141.10$, $p < 0.001$; PC3: Wilks' Lambda = 0.17, $F_{(1,19)} = 9.75$, $p = 0.005$) and PC2 (Wilks' Lambda = 0.11, $F_{(1,19)} = 0.61$, $p = 0.442$) and PC4 (Wilks' Lambda = 0.12, $F_{(1,19)} = 0.93$, $p = 0.345$) were not. When plotting PC1 and PC3 against each other, a clear segregation of data was visible (Fig. 7). When comparing variables one by one for *K. idiootocus* and the new clade, the model was significant (MANOVA test; Wilks' value = 0.087, $F_{9,14} = 16.27$, $p < 0.001$) and numerous variables were different from each other. In detail, the new clade had longer call intervals, longer call duration and dominant frequency (Table 7).

Morphometrics

The unique PCA, used to identify the independent dimensions of the morphological characters between the individuals collected in this study and other *Kurixalus* sp. individuals, resulted in two PCs, with eigenvalues of 1.92 and 8.25, explaining a cu-

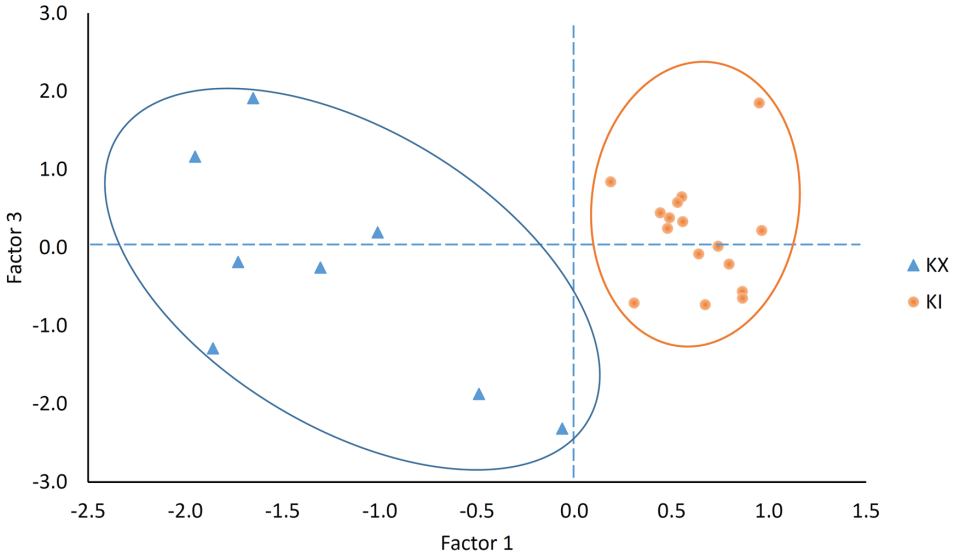

Figure 7. The PCA plot of the *Kurixalus inexpectatus* sp. nov. (KX) and *Kurixalus idiootocus* (KI).

Table 7. The description results of advertisement call properties and the MANOVA test in *Kurixalus inexpectatus* sp. nov. and *Kurixalus idiootocus*. From the MANOVA test, the whole model Wilks' value = 0.087, $F_{9,14}$ = 16.27, p < 0.001. "*" indicate the data are not following the assumption of normal distribution (Shapiro-Wilk test, p < 0.05) and we transformed the data to their natural logarithm before doing statistical tests.

Call property	*K. inexpectatus* sp. nov. (*n* = 8)	*K. idiootocus* (*n* = 16)	$F_{1,22}$	*p*
# of call in a bout	16.9 ± 3.9 (9–21)	14.9 ± 4.2 (9–24)	1.26	0.274
Bout length (s)	7.3 ± 2.9 (3.4–11.2)	3.6 ± 1.1 (1.9–5.5)	21.60	**< 0.001**
Call interval (ms)	376 ± 157 (115–539)*	211 ± 35 (159–278)	9.10	**0.006**
Call length (ms)	76.1 ± 11.1 (58–91)	34.8 ± 4.6 (28–43)	169.71	**< 0.001**
Rise time (ms)	38.1 ± 5.5 (29.5–46.0)	17.5 ± 2.4 (14–22)	167.48	**< 0.001**
Fall time (ms)	37.9 ± 5.5 (29.0–45.0)	17.5 ± 2.4 (14–22)	166.03	**< 0.001**
Max frequency (kHz)	2.30 ± 0.06 (2.20–2.39)	2.54 ± 0.08 (2.35–2.68)	54.20	**< 0.001**
2nd frequency (kHz)	4.59 ± 0.11 (4.41–4.74)	5.05 ± 0.14 (4.71–5.33)	61.20	**< 0.001**
Relative amplitude (dB)	39.7 ± 8.3 (25.3–52)	35.5 ± 2.4 (30–39.7)	3.73	0.067

mulated variation of 78.23% (Table 3). Based on the variables loading on to each of the PCs, we assigned PC1 to the general morphology and PC2 to the horizontal head structure (Table 3).

The results of the one-way ANOVA showed that there was no significant difference between the focal and non-focal groups for either of the PCs (Table 3). However, our focal clade and *K. idiootocus* were significantly different for both of the PCs (Table 3), such as PC1 (general morphology) p = 0.002 and PC2 (horizontal head structure) p = 0.017. When these variables were plotted against each other (Fig. 8), two non-clustering groups were visible, corresponding to variations between the measurements.

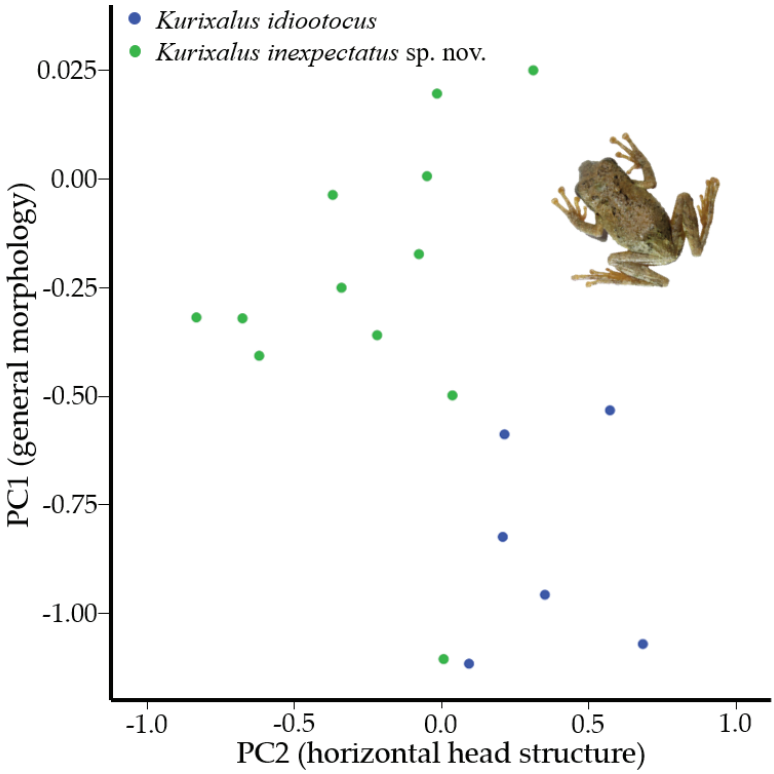

Figure 8. Plot of PC1 and PC2 resulting from the PCA and showing the non-clustering of morphological features between *Kurixalus inexpectatus* sp. nov. and *Kurixalus idiootocus*.

K. inexpectatus is morphologically most similar to *K. idiootocus*, its closest relative and, after standardising measurements by SVL, *K. inexpectatus* differs by having a relatively longer head length (34% vs. 33%), significantly shorter snout (13% vs. 15%; $p < 0.001$), significantly greater internasal distance (11% vs. 10%; $p < 0.001$), significantly smaller eye diameter (13% vs. 16%; $p < 0.001$), nearly significant wider tympanum diameter (7% vs. 6%; $p = 0.06$), significantly greater distance between the eyes and nares (8% vs. 7%, $p = 0.03$), significantly longer forelimb length (50% vs. 48%; $p = 0.03$), shorter tibia length (44% vs. 45%) and longer foot length (42% vs. 40%). Additionally, *K. inexpectatus* is further distinguished from *K. idiootocus* in having a tibio-tarsal articulation that extends beyond the anterior corner of the eye (versus centre of eye). *K. inexpectatus* can be differentiated from *K. bisacculus*, *K. hainanus*, *K. naso*, *K. odontotarsus*, *K. raoi*, *K. silvaenaias*, *K. verrucosus* and *K. yangi* by having an average adult SVL of less than 30 mm (27.5 – 31.8, × = 29.2) (vs. larger) (Yu et al. 2018; Hou et al. 2021; Zeng et al. 2021). *K. inexpectatus* can be further differentiated from *K. absconditus*, *K. baliogaster*, *K. banaensis*, *K. berylliniris*, *K. chaseni*, *K. eiffingeri*, *K. gracilloides*, *K. lenquanensis*, *K. motokawai*, *K. viridescens* and *K. wangi* by the presence of a pair of large, symmetrical dark blotches on the chest (vs. absent; Yu et al.

2018; Hou et al. 2021; Zeng et al. 2021). *Kurixalus inexpectatus* is distinguished from *K. ananjevae* by having limbs with serrated dermal fringes (vs. smooth; Yu et al. 2018; Hou et al. 2021; Zeng et al. 2021).

Species description

***Kurixalus inexpectatus* Messenger, Yang, Borzée, Chuang & Othman, sp. nov.**
http://zoobank.org/02D394DE-BB1C-4C17-BB70-656D68814C8F

Holotype. NJFU20180704001, an adult male (Fig. 9, Table 1), collected by Yi Yang (YY) on a dirt road in Chuanbu Village, north of Changxing 57 m a.s.l. on 4 July 2018 (Fig. 9).

Paratypes. Five adult males, NJFU20180704002 – 20180704006, collected by YY on 4 July 2018 at the type locality. One adult male, NJFU20180705001, collected by YY on 5 July 2018 at the same location. Five adult males, NJFU20180706001-NJFU20180706005 collected by YY on 6 July 2018 at the same location.

Type locality. Chuanbu Village (川步村), Changxing County, Huzhou City, Zhejiang Province, People's Republic of China.

Etymology. The epithet *inexpectatus* is Latin for "the unexpected." This was chosen for several reasons. We selected this name because we had come to survey this region of China for different taxa. KRM came to this locale to survey for *Megophrys*. AB came to this locale to survey for *Dryophytes*. It was not only surprising to find this species while surveying for two other target genera, but upon realising the immense distance to the next closest population of *Kurixalus*, the discovery was even more unexpected. For an English and Chinese common name, we are recommending the name Changxing Treefrog (pronounced "Chang-shing" in English) 长兴原指树蛙 (cháng xīng yuán zhǐ shù wā).

Diagnosis. The specimen matched the genus *Kurixalus*, based on the following characters: tips of digits enlarged to discs, with circum-marginal grooves; small-body size; pointed snout, forming a beak-like appearance; serrated dermal fringes along the outer edge of the forearm and leg; an inverted triangular-shaped dark brown mark between the eyes; dorsal ") (" saddle-shaped marking; and a coarse dorsal and lateral surface with several small, irregular tubercles [7, 18, 29].

Comparisons. *Kurixalus inexpectatus* sp. nov. is characterised and distinct from the majority of its congeners (19) by having a combination of being: (1) a small-sized species with an average adult size below 30 mm (in males); and (2) having two dark symmetrical pectoral blotches.

Genetically, the species is most closely related to *K. idiootocus* and is morphologically distinguished from this species by the combination of features: (1) having a tibiotarsal articulation that extends beyond the anterior corner of the eye (versus the centre of eye); (2) having a significantly shorter snout relative to SVL; (3) a significantly greater internasal distance relative to SVL; (4) a significantly smaller eye diameter relative to SVL; (5) a nearly significantly wider tympanum diameter relative to SVL; (6)

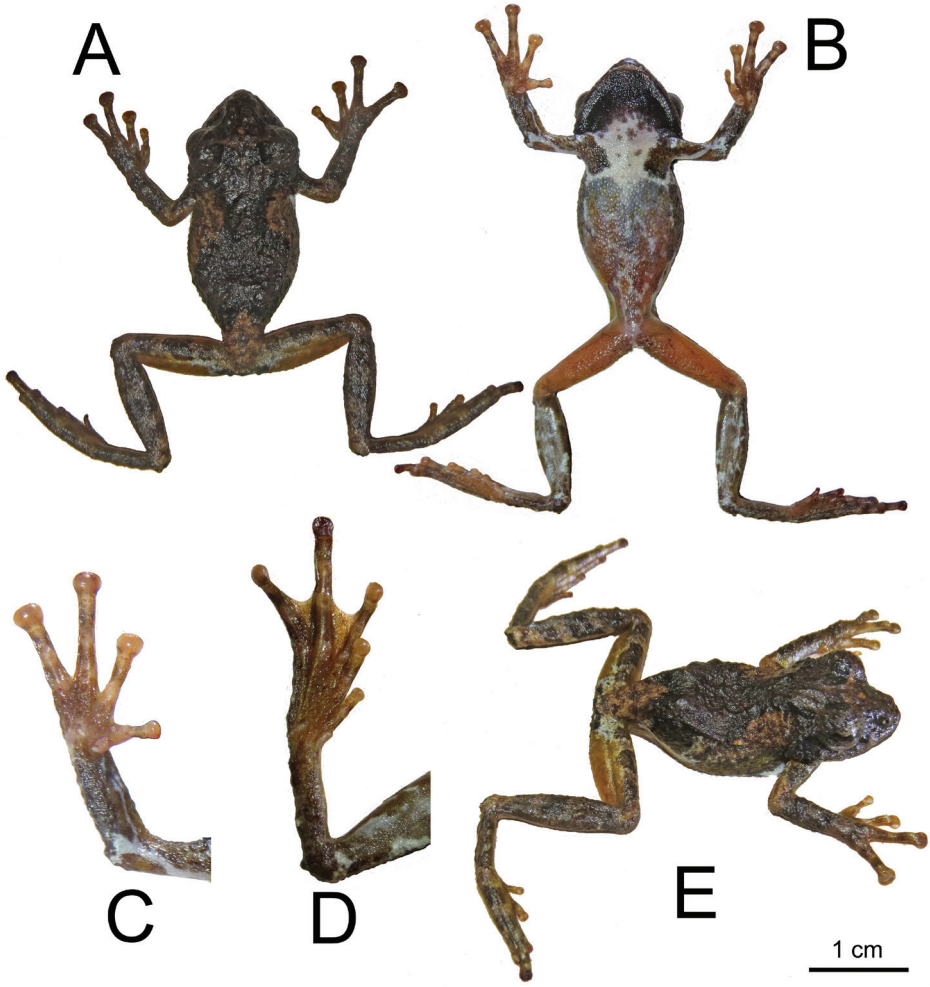

Figure 9. Holotype of *Kurixalus inexpectatus* sp. nov. **A** dorsal view **B** ventral view **C** right hand, ventral view **D** right foot, ventral view **E** dorsolateral view.

having a significantly greater distance between the eyes and the nares; (7) and by having a significantly longer forelimb length.

Description of holotype. Adult male (SVL 29.4 mm); head width about the same as body, its length 37.9% of SVL; head slightly longer than wide in the holotype (11.1 mm vs. 11.0, respectively); snout pointed and slightly turned down, forming a small "beak-like" appearance typical in many rhacophorids; eye large, protuberant, ED 36.3% of HDL, 13.8% of SVL; pupil horizontal; tympanum distinct in form, but not distinct in texture or colour, its diameter 6.8% of SVL; nostrils protuberant; closer to the tip of the snout than the eye; vomerine teeth absent; tongue notched posteriorly; single internal vocal sac.

Relative length of fingers I < II < IV < III. Tips of all four fingers form discs with circum-marginal and transverse ventral grooves; relative width of discs is IV > III > II > I; nuptial pads absent; fingers lacking webbing at base; subarticular tubercles prominent and rounded; series of tubercles forming serrated dermal fringe along outer edge of forearm.

Heels overlapping when legs at right angle to body; relative length of toes is I < II < III < V < IV; toes moderately webbed at base; tips of toes expand to form discs with circum-marginal and transverse ventral grooves; toes discs are smaller than finger discs; relative size of toe discs I < V < IV < III < III; subarticular tubercles present, but not as obvious as hand.

Body is covered in numerous tubercles and dermal ridges. Ridges are present on the dorsum, but absent from the flanks and venter; tympanum also covered in tubercles.

Measurements of holotype (in mm). The average of three measurements for each character is as follows: SVL 29.4, HDL 11.1, HDW 11.0, SNL 3.8, IND 3.3, IOD 3.3, UEW 3.1, ED 4.0, TD 2.0, TEY 0.9, DNE 2.7, FLL 15.7, THL 13.4, TL 14.5, FL 12.9, HND 9.1, RAD 6.7.

Colouration of holotype in life. Light brown dorsum with white patch in the sacral region and extending a bit on to the femurs. Darker brown “) (“ dorsal saddle. Ventrally, white chest with brown colouration in the pectoral and axillary region. Ventral side of forelimbs have streaks of white and brown, almost like a marbled appearance. Ventral side of hind-limbs orange in the thigh and tibia region has the same brown and white marbled appearance present in the forelimbs. Palm of hand primarily light brown; sole of feet slightly darker than hand.

Colouration of holotype in preservation. In preservation, the orange and light brown colours have faded, the darker brown has darkened compared to life. Pattern same as in life. Iris clouded. Chest white, throat black. Ventral side of arms black and white marbled appearance. Ventral side of tibia black and white marbled, similar to ventral aspect of forelimbs.

Variation. As the holotype and paratypes of the new species are all male, sexual dimorphism cannot be ascertained. Aside from SVL, which is to be expected, the next characters which showed the greatest variation were FLL, TL, FL and TFL. Though the holotype has a head length longer than head width, most specimens had a head length shorter than head width. Colour varied between individuals, likely induced by temperature and/or time of day, as we observed this change first-hand. See Table 8 for variation amongst all specimens.

Description of eggs and tadpoles. We did not find any eggs or tadpoles despite being present during the breeding season.

Distribution and ecology. *Kurixalus inexpectatus* sp. nov. has been found calling as early as 26 April. Males would call from shrubs approximately 20 to 160 cm above temporary pools in and along roadside ditches. Temporary pools were 15 cm deep and up to 8 m long. In April, only sparse numbers of individuals were found calling. In

July, full choruses could be heard, yet no individuals were found engaged in amplexus. No females have been found.

The vegetation primarily consisted of shrubs and secondary broad-leaved forest. No specimens were found in the adjacent bamboo forest.

Distribution. Currently, the species is only known from the type locality, on the outskirts of the Wizard of Oz resort in Chuanbu Village, Changxing County, Huzhou City, Zhejiang Province, China. Surveys were made in the surrounding mountains for additional populations without success, including mountain ranges in Anhui and Jiangsu Provinces. The resort is situated at the southeast edge, in a northwest-to-southeast valley lower than 100 m in elevation. A creek comes from the hills, into a reservoir, which then flows about 2 km along the valley through the extent of the resort. The area was intended to be a plantation (unconfirmed, but suspected to be bamboo, based on the number of surrounding bamboo plantations), but in 2013, the land was set aside for the resort (pers. comm.). Now the resort consists of tea plantations, peach orchards, well-manicured grasses, a bamboo forest and miscellaneous shrubbery.

Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature and, hence, the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix "http://zoobank.org/". The LSID for this publication is: urn:lsid:zoobank.org:pub: 3CCB356B-F075-

Table 8. Variation in morphological measurements amongst the holotype* and paratypes. Each character was measured three times, the values in the table represent the average of the three measurements. *Denotes holotype.

Specimen	SVL	HL	HW	SL	IND	IOD	UEW	ED	TD	TEY	DNE	FLL	THL	TL	FL	TFL	HND	RAD
20180704001*	29.4	11.1	11.0	3.8	3.3	3.3	3.1	4.0	2.0	0.9	2.7	15.7	13.4	14.5	12.9	19.9	9.1	6.7
20180704002	31.8	10.3	10.9	3.8	3.4	3.1	3.4	4.3	2.2	0.8	2.6	16.0	14.1	13.9	13.2	19.9	9.5	6.9
20180704003	29.7	10.2	10.1	4.0	3.3	3.0	3.1	3.9	2.1	1.1	2.7	14.7	13.1	12.8	12.5	18.7	8.6	7.0
20180704004	29.5	10.3	11.1	4.0	3.4	2.7	3.1	4.0	1.9	1.0	2.8	15.7	13.9	13.5	13.0	20.1	9.1	7.1
20180704005	29.4	9.6	10.7	3.4	3.1	3.0	2.8	4.0	1.8	1.0	2.5	15.2	13.5	13.6	12.7	19.6	8.6	6.8
20180704006	28.3	10.0	10.1	3.7	3.4	3.1	3.2	3.6	1.7	0.9	2.3	14.1	12.9	12.5	12.1	18.2	8.4	5.9
20180705001	28.6	9.7	10.7	4.2	3.3	3.1	2.9	3.2	1.8	1.2	2.6	14.2	13.3	12.9	10.9	17.6	8.0	6.4
20180706001	29.4	9.3	10.4	3.7	3.3	3.1	3.0	3.7	2.1	1.1	2.5	13.5	12.2	12.0	11.0	17.7	8.0	5.9
20180706002	29.8	10.1	10.9	3.5	3.4	3.4	3.0	4.0	2.0	0.8	2.3	15.1	13.9	13.4	12.9	19.4	8.8	6.3
20180706003	28.5	9.9	10.2	4.0	3.3	3.2	3.1	3.7	1.6	0.9	2.4	13.8	12.2	12.0	11.4	17.6	8.5	5.6
20180706004	29.0	9.9	10.4	3.7	3.4	3.2	2.9	3.8	1.9	0.9	2.5	14.5	12.9	12.9	12.0	18.6	8.7	6.3
20180706005	27.5	9.1	10.1	3.2	3.1	3.0	2.3	3.9	1.8	0.4	1.5	12.3	9.7	10.7	11.4	16.1	8.3	5.7

4EE5-8366-FE96B855F884. The new species name Kurixalus inexpectatus sp. nov. has been registered under LSID: urn:lsid:zoobank.org:act: 02D394DE-BB1C-4C17-BB70-656D68814C8F.

Discussion

The molecular data and phylogeographic patterns presented here are supported by both call properties and morphological data, highlighting a significant segregation between *K. inexpectatus* and other species. The morphological analysis is robust in that *K. inexpectatus* is significantly different from closely-related clades in terms of calls and morphology and it has diverged from the most closely related species ca. 3.06 Mya.

In spite of the high genetic homogeneity between the 12S rRNA gene sequences of *K. inexpectatus* and its homologous species *K. idiootocus*, the haplotype distributions and phylogeny inferred from the nuDNA *TYR* gene fragment showed a distinction between the two clades. The incongruence in pattern of sequence divergences between 12S rRNA gene and *TYR* sequences may result from dissimilarities in the rate of evolution between mitochondrial and nuclear loci in the *Kurixalus* lineage. Accordingly, the phylogenetic tree inferred from nuDNA *TYR* gene and concatenating gene fragments of 12S rRNA and *TYR* also supported the sister species relationship between *Kurixalus inexpectatus* and *K. idiootocus* (subclade B2, BP = 97%) and recovered the monophyly of *K. inexpectatus* (Fig. 4, Suppl. material 1: Figs S1, S2).

Our haplotype network inferred from the nuDNA *TYR* gene sequences demonstrated the absence of identical haplotypes between *K. inexpectatus* and *K. idiootocus* (Clade A; Fig. 5), even if the three haplotype groups of *K. inexpectatus* shared the same ancestral haplotypes as *K. idiootocus* and *K. eiffingeri* (Clade A; Fig. 5). These results unconditionally rejected the possibility of *K. inexpectatus* to be an exotic or invasive population of *K. idiootocus* (Fig. 6). The pre-Pliocene estimates on the emergence of a stem group of Taiwanese *Kurixalus* (ca. 5.86 Mya [8. – 3.32]; Table 6; Fig. 5) matches with the establishment of the Island after the formation of the Taiwan Strait (Mio-Pliocene; ca. 5 Mya) (Teng 1990). The radiations of *Kurixalus* in Taiwan Island, dated to ca. 3.05 Mya, is highly consistent with the time estimates for the colonisation of Taiwan Island by *Kurixalus* (Pliocene, ca. 3.46 Mya)(Yu et al. 2021). Here our estimates regarding the segregation between *K. inexpectatus* and *K. idiootocus* is dated between the Plio-Pleistocene and the Holocene (biogeography models c and d; Fig. 6). This divergence time is supported by the formation of the Quaternary continental shelf which acted as temporary landmass connecting Taiwan Island to the mainland because of sea level fluctuations during glacial oscillations period. A similar phylogeography pattern is found in other anurans distributed in the south-eastern mainland of Asia and Taiwan Island, similarly sharing ancestry and having dispersed over the Pleistocene land-bridge (Yu et al. 2014; Othman et al. 2020). Here, the segregation between the two species is also supported by the difference in the elevational range of *K. idiootocus* (0 – 500 m) and *K. inexpectatus*

(< 100 m; Fig. 6). Additionally, the recently described *K. silvaenaias* from Sichuan, China was discovered at 600 m elevation (Hou et al. 2021), a similar altitudinal preference as *K. idiootocus*.

Our results highlight the importance of advanced genetic analyses to support the conventional distance-based genetic divergence analysis and especially analyses on species delimitation (Table 5). Here, we suggested a splitting of lineage between *K. inexpectatus* and the Taiwanese endemic *K. idiootocus*, a similar taxonomic recommendation as that of Yu et al. (2017a), rejecting the synonymy between *K. hainanus* and *K. bisacculus* (Yu et al. 2010). Nonetheless, we recommend a comparative study including genomic, morphological and acoustic tools for *K. inexpectatus* and all other *Kurixalus* to resolve the taxonomy of the genus.

The lack of clear morphological characteristics is not unexpected for cryptic species and especially in treefrogs. However, identification based on range seems to be a reliable criterion. It is interesting that we did not find any individual in the bamboo forest while the genus is generally associated with this type of vegetation and further surveys may provide a different point of view. We recommend surveys on the contiguous mountain chain to determine the range of the species and the potential connectivity with other geographically related mountain ranges.

Conclusion

Our work revealed a previous undescribed species of *Kurixalus* that was disjunct from the next closest population of the genus by nearly 700 km. The population was found in a highly developed region of northern China, yet surprisingly has gone unnoticed. This discovery reiterates the need to survey regions of the countryside that have been poorly studied. Such efforts should be especially considered in regions of high development, to ensure that potentially critically endangered species, previously unknown to science are not lost.

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Supplementary material 1

Tables S1–S4, Figures S1, S2

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Data type: Docx file.

Explanation note: **Table S1.** The description of advertisement calls of five *Kurixalus* species. **Table S2.** The factor loading of principal analysis on call properties in *Kurixalus inexpectatus* sp. nov. and *Kurixalus idiootocus*. We listed the coefficients of correlation between call properties and their corresponding factors and listed out the Eigen values and accumulate explained variance of each Factor. **Table S3.** The factor scores of each individual for *Kurixalus inexpectatus* sp. nov. and *Kurixalus idiootocus* and the results of Discriminant Function Analysis (Wilks' Lambda = 0.11, F (4.19) = 38.1, $p < 0.001$). This table shows the statistics and coefficient of determination of each factor and the square of Mahalanobis distance from each group centre, the post hoc probability (in brackets) to a group and the assigned group for each observation (individual). **Table S4.** Morphological data for *Kurixalus inexpectatus* sp. nov. used in this analysis. For the analyses, all measurements were adjusted for variations in body size, i.e. each value was divided by the SVL of the individual. The data presented here are not corrected for size. Data extracted from our samples and from the literature ananjevae (Kuramoto and Wang 1987; Matsui and Orlov 2004; Tao et al. 2014; Wu et al. 2016; Yu et al. 2017b; Yu et al. 2018; Zeng et al. 2021). **Figure S1.** Bayesian tree of Asian rhacophorids inferred from 98 sequences of 827 bp of partial mtDNA 12S rRNA-tRNA-Val-16S rRNA. Numbers above the lines or besides the nodes are given as Bayesian posterior probabilities (percentage). The species described in this study, *Kurixalus inexpectatus* sp. nov. are highlighted in the red-coloured box. **Figure S2.** Bayesian inference tree derived from 74 sequences of concatenated partial fragments of TYR. Numbers above the lines or besides the modes are given as Bayesian posterior probabilities (percentage).

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RESEARCH ARTICLE

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Updated species checklist of fishes from Lake Dongting in Hunan Province, South China: Species diversity and conservation

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Abstract

A lack of an updated checklist of freshwater fish species from Lake Dongting is a great hindrance to further biodiversity analysis. A seasonal survey of fishes in the lake was conducted from October 2017 to January 2019. Based on the data obtained during the field survey and coupled with known literature and the latest taxonomic development of relevant taxa, the species checklist of fishes from Lake Dongting was updated. A total of 130 species from 12 orders, 30 families and 76 genera has been documented, containing 126 native species and four alien species. Its fish fauna is dominated by the Xenocyprididae that has the highest number of included species (30), followed by the Gobionidae (25) and Acheilognathidae (11). This checklist comprises 20 species undergoing nomenclatural changes and 11 new records, eight of which are native and three exotic. It excludes 20 species, which have been reported in error in historical works, due to synonyms, erroneous records, taxonomic changes and unconfirmed records. Unsampled in this survey were 34 species that are ecologically specialised: migratory, rheophilic, predatory, shellfish-dependent or pelagic-egg-spawning. While some of these species eluded capture likely due to the paucity of population, others may have been extirpated in Lake Dongting perhaps owing to human perturbations, such as river damming across affluents or the Chang-Jiang mainstem, sand dredging, overfishing or water pollution. The updated checklist lays a sound foundation for biodiversity conservation of fishes in Lake Dongting.

Keywords

Annotated list, biodiversity, ichthyofauna, taxonomy, threatened species

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Introduction

Freshwater ecosystem and freshwater fish may well face one of the greatest threats in the world in the context of global biodiversity crisis (Dudgeon et al. 2006; Strayer and Dudgeon 2010). In comparison with other vertebrates, freshwater fishes are being more severely threatened by human interferences when the usage of water resources is strengthened (Reid et al. 2019; Barbarossa et al. 2021). The biodiversity conservation of freshwater fishes, nevertheless, has received disproportionate attention compared to terrestrial vertebrates (Tedesco et al. 2017). It is imperative and also in urgent need to protect the freshwater ecosystem and its biodiversity (Jackson et al. 2001; Liermann et al. 2012). Species inventories are beyond simply lists of names; they are actually representing an efficient method for obtaining updated information regarding species composition and distributions (Marta et al. 2019). This information provides valuable inputs of biodiversity monitoring which serves many conservational purposes, such as prioritising protection areas and directing conservation actions (Brooks et al. 2004). An update species inventory of a given area is of vital significance for biodiversity conservation.

The freshwater ecosystem of the Chang-Jiang (= Yangtze River; Jiang, Shui and He in Chinese mean river), the third largest river of the world and the largest river of China, supports rich biodiversity of aquatic organisms (Chen et al. 2020). The middle reaches of this river are regarded as one of the hotspots for freshwater fish diversity in Asia (Kottelat and Whitten 1996). Lake Dongting is one of two largest river-linked freshwater lakes in China, lying within the lower Chang-Jiang (= the mid-lower Chang-Jiang) basin which forms a freshwater ecoregion of the world for biodiversity conservation (Abell et al. 2008). This lake is an important portion of fluvio-lacustrine complex ecosystems of the mid-lower Chang-Jiang basin (Wang et al. 2019b), and also one of the priority areas for biodiversity conservation in China (Li et al. 2016a) and the Ramsar-listed floodplain wetlands (Dong et al. 2021), which serves as crucial habitats of migratory birds of East Asia-Australian flyway (Zou et al. 2019). Moreover, Lake Dongting, as a flood buffer zone, provides *Elaphurus davidianus* (Milu or Père David's deer) with seasonal sanctuaries (Yang et al. 2016). It also provides refuge for charismatic mammals like *Neophocaena asiaeorientalis* (Yangtze finless porpoise) (Zhang 2011; Huang et al. 2017) and the feeding grounds of large-sized flagship fishes, such as *Acipenser sinensis* (Chinses sturgeon) and *Psephurus gladius* (Chinese paddlefish) and economically-important potamodromous fishes like four major Chinese carps: *Aristichthys nobilis* (Bighead Carp), *Ctenopharyngodon idella* (Grass Carp), *Hypophthalmichthys molitrix* (Silver Carp) and *Mylopharyngodon piceus* (Black Carp) (Liu et al. 2010; Zhang et al. 2020a). Apparently, the lake plays a vital role in the conservation of aquatic biodiversity of the Chang-Jiang Basin.

The aquatic biodiversity of Lake Dongting is greatly imperilled by anthropogenic activities, like sand dredging, overfishing, alien species invasion, water pollution from industrial, agricultural and domestic sewage discharges and so forth (Dou and Jiang 2000; Fu et al. 2021; Jiang et al. 2022). It is also indirectly impacted by dam building across the Chang-Jiang mainstem and affluents of the lake owing to the continuity of the aquatic ecosystem (Wang et al. 2016; Liu and Wang 2018). Fishes, as top feed-

ers of the aquatic ecosystem and an important source of proteins in human food, are severely threatened by these factors (Zhao et al. 2019; Tregidgo et al. 2021). In the latest Red List assessment of Chinese freshwater fishes (Zhang and Cao 2021a), there are 12 imperilled species from Lake Dongting. *Psephurus gladius* (Martens, 1862) was recently declared to be extinct or functionally extinct (Zhang et al. 2020a). Such species as *Luciobrama macrocephalus* (Lacepède, 1803), *Ochetobius elongatus* (Kner, 1867) and *Tenualosa reevesii* (Richardson, 1846) have been not seen in capture fisheries for decades or are likely extirpated (Wu et al. 2015; Zhang and Cao 2021b). The current status of freshwater fish diversity of Lake Dongting is, therefore, of particular conservation concern.

An updated checklist of fishes from Lake Dongting remains to be provided. The first checklist of freshwater fishes from this lake was given by Tang and Qian (1979), who recorded 114 fish species. A total of 117 species of the lake was later included in Anonymous' (1980) book entitled "Fish of Hunan Province". Subsequent species inventories of fishes from Lake Dongting primarily followed the book and three authoritative monographs of Chinese freshwater fishes authored by Chen (1998), Chu et al. (1999) and Yue (2000). Nevertheless, the species inventory of fishes from this lake needs to be regularly updated for biodiversity conservation, especially with lots of taxonomic revisions of freshwater fishes from the Chang-Jiang basin over the past decades. Therefore, three seasonal field sampling of fishes in Lake Dongting were conducted by us during 2017–2019. Coupled with the data collected in this survey, we aim to synthesise existing knowledge of freshwater fish diversity and systematics to provide an updated checklist of fish of the lake.

History of taxonomic research

The taxonomic history of fishes from Lake Dongting could be traced back to the mid-19th century. Père Heude, a French Jesuit catholic priest, made a collection of fish specimens at the lake from 1869 to 1884 (Luo 2005). Subsequently, Kreyenberg and Pappenheim (1908) reported 22 species from the Chang-Jiang and its tributaries, two of which were new species from Lake Dongting: *Coilia brachygnathus* Kreyenberg & Pappenheim, 1908 and *Culter oxycephaloides* Kreyenberg & Pappenheim, 1908. At the same time, Regan (1908) described three new Chinese species, two of which were *Glyptothorax sinensis* (Regan, 1908) and *Hemisalanx prognathus* Regan, 1908 from the lake. In 1921, Clifford Pope made a collection of fish specimens in Huping College, Yochow (= Yueyang City near East Lake Dongting) (Luo 2005). Nichols (1925a) proposed two new subspecies *Misgurnus anguillicaudatus tungting* Nichols, 1925 and *Misgurnus mohoity leopardus* Nichols, 1925 from Lake Dongting, both being regarded as invalid to date. Nichols (1925b) recorded three species of *Botia* Gray, 1831 from this lake, two of which were new to sciences, namely *B. citrauratea* Nichols, 1925 and *B. purpurea* Nichols, 1925. Both are now placed in *Leptobotia* Bleeker, 1870, but the

latter has been synonymised with *L. taeniops* (Sauvage, 1878). Simultaneously, Nichols (1925c-e) described five new species from Lake Dongting, viz. *Gobio longipinnis* (= *Rhinogobio ventralis* Sauvage & Dabry de Thiersant, 1874), *Gobius cliffordpopei* [= *Rhinogobius cliffordpopei* (Nichols, 1925)], *Hemiculterella engraulis* [= *Pseudolaubuca engraulis* (Nichols, 1925)], *Hemicultur clupeoides* [= *Hemiculter leucisculus* (Basilewsky, 1855)] and *Varicorhnus tungting* [= *Decorus tungting* (Nichols, 1925)]. Later, Nichols and his co-authors (1926, 1927) named a new subspecies *Sarcocheilichthys nigripinnis tungting* Nichols & Pope, 1927 and two new species, i.e. *Acheilognathus gracilis* Nichols, 1926 and *Pseudogobio tungtingensis* [= *Microphysogobio tungtingensis* (Nichols, 1926)] from this lake. Nichols (1928) recorded 71 nominal species from Lake Dongting in his provisional checklist of Chinese freshwater fishes. Subsequent taxonomic contributions to fishes of Lake Dongting were also made by many authors, such as Wu (1930), Tchang (1933) and Kimura (1934), who made a small collection of fish specimens in the lake. Chu (1931) recorded 74 fish species from Hunan Province, the majority of which were from Lake Dongting. Hora (1932) described a new species *Lepturichthys nicholsi* [= *Lepturichthys fimbriatus* (Günther, 1888)]. Nichols (1943), in his book entitled "The fresh-water fishes of China", recognised 79 species or subspecies for fish specimens collected by Clifford Pope in East Dongting Lake in 1921.

More studies were focused on the species inventory of fishes from Lake Dongting following the establishment of P. R. China in 1949. Forty-three species of the lake were involved in Chu's (1955) study on the distribution of fish species in the Yichang section of the Chang-Jiang. Liang and Liu (1959) compiled a species list of 69 fishes from Lake Dongting and its affluent (Xiang-Jiang). Later, Liang and Liu (1966), in their checklist of fishes from Hunan Province, recorded 119 species from Lake Dongting. Anonymous (1976) also reported 84 species from Lake Dongting in the book entitled "Fishes of the Chang-Jiang". Tang and Qian (1979) were the first to provide a checklist of 114 fish species or subspecies from the lake. Anonymous (1980) included 117 species from Lake Dongting in the book entitled "Fishes of Hunan Province". Although Dou and Jiang (2000) compiled a checklist of 104 species from the lake, this work was mainly based on their collections of fish specimens made during 1974–1975.

As from the 1990s, increasing research interests have centred on the fish diversity of Lake Dongting. Survey of fishery resources carried out by Liao et al. (2002), Liao et al. (2006) and Li (2006) into Lake Dongting from 1994 to 2005 found 117, 111 and 117 species, respectively. Ru and Liu (2013) identified 69 species in their surveys conducted into East and South Dongting Lake during 2004–2005. Li (2013) reported a total of 85 fish species, based on his field sampling from March to December in 2012. Eighty species were identified by Jiang et al. (2019) in their research on the spatio-temporal patterns of fish assemblages in Lake Dongting from 2012 to 2014. Sixty-two fish species were recorded by Qin et al. (2019) from the outlet channel of the lake from 2013 to 2015. Eighty-five and 66 fish species were sampled during 2002–2003 and 2012–2014 field surveys to monitor the changes of fish community structure at West Dongting Lake before and after the operation of Three Gorges Dam (Zhu et al. 2014). All these inventories were conducted, particularly in relation to environment impact assessment prepared for hydropower projects or fisheries investigations and the data

collection used for biodiversity analyses. Some surveys were focused on fish resources assessment; small-sized or less commercially valuable species were largely neglected. Others were not based on examination of collected specimens, but compiled through desk review or interview, containing little or no reliable information on fish diversity, although they claimed to have studied biodiversity. More importantly, these inventories required critical scrutiny from an ichthyological perspective as most of them, if not all, were not conducted by trained ichthyologists in the field; information on unrecognised species is impossibly captured through these surveys, therefore giving rise to a grossly underestimated biodiversity value and taxonomic impediment (Sluys 2013).

Material and method

Lake Dongting (28°44'N–29°35'N, 111°53'E–113°05'E) is located in the northern part of Hunan Province, connected to the middle Chang-Jiang mainstem (Wang and Dou 1998). This water-carrying floodplain lake receives not only runoff waters from four main affluents (Xiang-Jiang, Zi-Shui, Yuan-Jiang and Li-Shui), but flood water from the Chang-Jiang mainstem via three inlet channels (Songzi, Hudu and Ouchi Channel) and lake water then flows out into the mainstem of the river again via Chenglingji Channel (Dou and Jiang 2000). Generally, Lake Dongting is divided into three sub-lakes, i.e. East Dongting Lake, South Dongting Lake and West Dongting Lake (Zhao et al. 2005). The lake covers a surface area of 2625 km^2 at a water level of 33.5 m at Chenglingji Station (Dou and Jiang 2000).

Twenty sampling sites were selected in this study, based on habitat heterogeneity (Fig. 1; Suppl. material 1: Table S1). Field sampling was taken from October to November 2017, July to August 2018 and December 2018 to January 2019. Fish surveys were conducted in different types of habitats to ensure maximum representation of species diversity occurring in this area. Multiple sampling methods were thus applied. Three-layer gill nets were used for pelagic fish sampling, while trap nets were applied to catch demersal fishes. Additionally, fish specimens were collected from local fish markets and their sampling localities were restricted to Lake Dongting.

All collected specimens were identified to species level. The initial identification in the field principally followed Chen (1998), Chu et al. (1999) and Yue (2000). The caught specimens were fixed in 95% alcohol solution for molecular analysis, in general and DNA extraction, in particular or initially fixed in 10% formalin and then transferred to 70% ethanol for morphological examination and also for permanent collection. All specimens were deposited in the ichthyolgical collection of the Museum of Aquatic Organisms at the Institute of Hydrobiology, Chinese Academy of Sciences.

In addition to the data collected during our field sampling, known research works were referred. Reference was made to the following main historical records of fishes in the lake: Liang and Liu (1959, 1966), Tang and Qian (1979), Anonymous (1980), Li (2006), Ru (2008), Cao et al. (2012) and Li (2013).

Fish classifications are being transformed greatly as latest molecular phylogenies provide evidence in support for natural groups which were unanticipated by previous

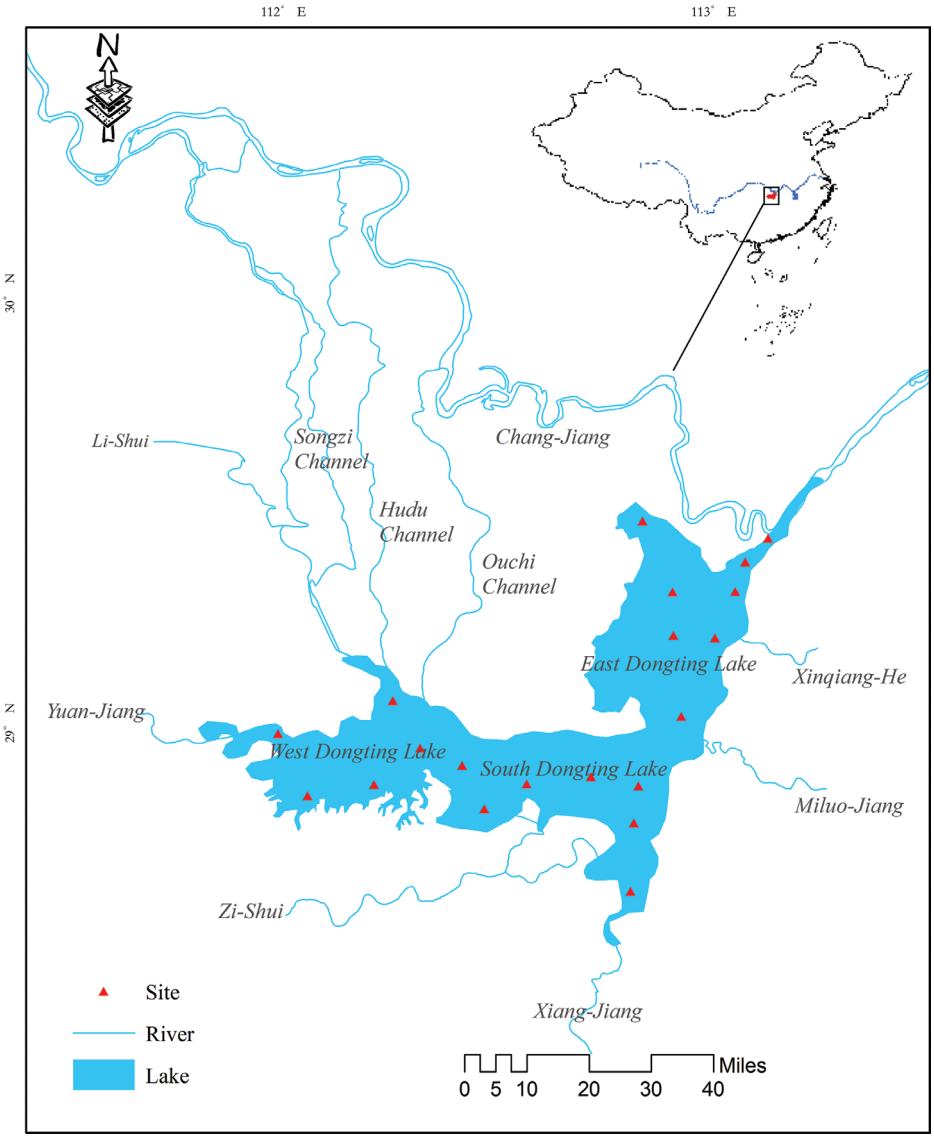

Figure 1. Field sampling sites of Lake Dongting in this study.

studies (Betancur-R et al. 2017). For the order Cypriniformes, the dominant group of freshwater fishes worldwide, significant advances have been made in its familial-level classification; some new familial (Acheilognathidae, Gobionidae and Xenocypridi-dae) and subfamilial names (Acrossocheilinae and Spinibarbinae) have been proposed (Tan and Armbruster 2018). Except for Cypriniformes, the taxonomic revisions of other orders were referred from Van Der Laan et al. (2014). The species checklist of fishes in Lake Dongting was systematically arranged by order, family and subfamily in accordance with the latest developments made in the taxonomic ranks (Van Der Laan et al. 2014).

Table 1. Annotated checklist of the fish fauna from Lake Dongting. The species under each family or subfamily are sorted by alphabetical order. Notes are labelled with taxonomic alteration, synonymisation, misidentification and other meanings.

Valid species name	Previous studies	Note
Acipenseriformes		
Acipenseridae		
001 *Acipenser dabryanus* Duméril, 1869		○P
002 *Acipenser sinensis* Gray, 1835		○D
Polyodontidae		
003 *Psephurus gladius* (Martens, 1862)		○D
Anguilliformes		
Anguillidae		
004 *Anguilla japonica* Temminck & Schlegel, 1846		⊕D
Clupeiformes		
Clupeidae		
005 *Tenualosa reevesii* (Richardson, 1846)		○D
Engraulidae		
006 *Coilia brachygnathus* Kreyenberg & Pappenheim, 1908		⊕P
007 *Coilia nasus* Temminck & Schlegel, 1846		○D
Cypriniformes		
Catostomidae		
008 *Myxocyprinus asiaticus* (Bleeker, 1864)		⊕P
Botiidae		
009 *Leptobotia citrauratea* (Nichols, 1925)		⊕P
	Leptobotia elongata (Bleeker, 1870)	⊙M
010 *Leptobotia rubrilabris* (Dabry de Thiersant, 1872)		○P
011 *Leptobotia taeniops* (Sauvage, 1878)		⊕P
012 *Parabotia banarescui* (Nalbant, 1965)		⊕P
013 *Parabotia fasciata* Dabry de Thiersant, 1872		⊕P
Cobitidae		
014 *Cobitis macrostigma* Dabry de Thiersant, 1872		⊕P
015 *Cobitis sinensis* Sauvage & Dabry de Thiersant, 1874		⊕P
016 *Misgurnus anguillicaudatus* (Cantor, 1842)		⊕P
017 *Paramisgurnus dabryanus* Dabry de Thiersant, 1872		⊕P
Balitoridae		
018 *Lepturichthys fimbriatus* (Günther, 1888)		○P
Cyprinidae		
Labeoninae		
019 *Cirrhinus cirrhosus* Bloch, 1795		+AP
020 *Cirrhinus molitorella* (Valenciennes, 1844)		⊕AP
021 *Decorus tungting* (Nichols, 1925)	*Bangana tungting* (Nichols, 1925)	○TP
022 *Pseudogyrinocheilus prochilus* (Sauvage & Dabry de Thiersant, 1874)		+P
Cyprininae		
023 *Carassius auratus* (Linnaeus, 1758)		⊕P
	Cyprinus carpio Linnaeus, 1758	⊙T
024 *Cyprinus rubrofuscus* Lacepède, 1803		⊕P
	Procypris rabaudi (Tchang, 1930)	⊙M
Acrossocheilinae		
025 *Onychostoma rarum* (Lin, 1933)		○P
026 *Onychostoma simum* (Sauvage & Dabry de Thiersant, 1874)		○P
Spinibarbinae		
027 *Spinibarbus caldwelli* (Nichols, 1925)		○P

Valid species name	Previous studies	Note
	Spinibarbus hollandi Oshima, 1919	⊙T
	Spinibarbus sinensis Bleeker, 1871	⊙M
Xenocyprididae		
028 *Aristichthys nobilis* (Richardson, 1845)		⊕P
029 *Chanodichthys dabryi* (Bleeker, 1871)	*Culter dabryi* Bleeker, 1871	⊕TP
030 *Chanodichthys erythropterus* (Basilewsky, 1855)	*Culter alburnus* Basilewsky, 1855	⊕TP
031 *Chanodichthys mongolicus* (Basilewsky, 1855)	*Culter mongolicus* Basilewsky, 1855	⊕TP
032 *Chanodichthys oxycephalus* (Bleeker, 1871)	*Culter oxycephalus* Bleeker, 1871	○TP
033 *Chanodichthys oxycephaloides* (Kreyenberg & Pappenheim, 1908)	*Culter oxycephaloides* Kreyenberg & Pappenheim, 1908	⊕TP
034 *Culter alburnus* (Basilewsky, 1855)	*Cultrichthys erythropterus* (Basilewsky, 1855)	⊕TP
035 *Ctenopharyngodon idella* (Valenciennes, 1844)		⊕P
036 *Distoechodon tumirostris* Peters, 1881		○P
037 *Elopichthys bambusa* (Richardson, 1845)		⊕P
038 *Hemiculter bleekeri* Warpachowski, 1888		⊕P
039 *Hemiculter leucisculus* (Basilewsky, 1855)		⊕P
040 *Hypophthalmichthys molitrix* (Valenciennes, 1844)		⊕P
041 *Luciobrama macrocephalus* (Lacepède, 1803)		○P
042 *Megalobrama amblycephala* Yih, 1955		⊕P
043 *Megalobrama mantschuricus* (Basilewsky, 1855)	*Megalobrama skolkovii* Dybowski, 1872	⊕SP
044 *Mylopharyngodon piceus* (Richardson, 1846)		⊕P
045 *Ochetobius elongatus* (Kner, 1867)		○P
046 *Opsariichthys bidens* Günther, 1873		⊕P
047 *Parabramis pekinensis* (Basilewsky, 1855)		⊕P
048 *Plagiognathops microlepis* (Bleeker, 1871)	*Xenocypris microlepis* Bleeker, 1871	○TP
049 *Pseudobrama simoni* (Bleeker, 1864)		⊕P
050 *Pseudolaubuca engraulis* (Nichols, 1925)		○P
051 *Pseudolaubuca sinensis* Bleeker, 1864		⊕P
052 *Sinibrama macrops* (Günther, 1868)		⊕P
	Sinibrama wui (Rendahl, 1933)	⊙S
053 *Squaliobarbus curriculus* (Richardson, 1846)		⊕P
054 *Toxabramis swinhonis* Günther, 1873		⊕P
055 *Xenocypris davidi* Bleeker, 1871		⊕P
056 *Xenocypris macrolepis* Bleeker, 1871	*Xenocypris argentea* Günther, 1868	⊕SP
057 *Zacco acanthogenys* (Boulenger, 1901)		⊕P
	Zacco platypus (Temminck & Schlegel, 1846)	⊙T
Acheilognathidae		
058 *Acheilognathus barbatulus* Günther, 1873		+P
059 *Acheilognathus barbatus* Nichols, 1926		○P
060 *Acheilognathus chankaensis* (Dybowski, 1872)		○P
061 *Acheilognathus gracilis* Nichols, 1926		⊕P
	Acheilognathus imberbis Günther, 1868	⊙U
062 *Acheilognathus hypselonotus* (Bleeker, 1871)		○P
063 *Acheilognathus macromandibularis* Doi, Arai & Liu, 1999		+P
064 *Acheilognathus macropterus* (Bleeker, 1871)		⊕P
065 *Acheilognathus polylepis* (Wu, 1964)		⊕P
066 *Acheilognathus tonkinensis* (Vaillant, 1892)		○P
	Acheilognathus taenianalis (Günther, 1873)	⊙S
067 *Rhodeus ocellatus* (Kner, 1866)		⊕P
068 *Rhodeus sinensis* Günther, 1868		⊕P
Gobionidae		
069 *Abbottina rivularis* (Basilewsky, 1855)		⊕P
070 *Coreius heterodon* (Bleeker, 1864)		⊕P

Valid species name	Previous studies	Note
	Coreius guichenoti (Sauvage & Dabry de Thiersant, 1874)	⊙M
071 *Gobiobotia filifer* (Garman, 1912)		⊕P
072 *Gobiobotia meridionalis* Chen & Cao, 1977	*Gobiobotia longibarba meridionalis* Chen & Cao, 1977	⊕TP
073 *Gobiobotia nicholsi* Bănărescu & Nalbant, 1966		○P
074 *Gobiobotia lii* Chen, Wang, Cao & Zhang, 2022		+P
	Gobiobotia pappenheimi Kreyenberg, 1911	⊙M
	Xenophysogobio boulengeri (Tchang, 1929)	⊙M
075 *Hemibarbus labeo* (Pallas, 1776)		⊕P
076 *Hemibarbus maculatus* Bleeker, 1871		⊕P
077 *Microphysogobio tungtingensis* (Nichols, 1926)		⊕P
078 *Paracanthobrama guichenoti* Bleeker, 1864		⊕P
079 *Pseudogobio vaillanti* (Sauvage, 1878)		+P
080 *Pseudorasbora parva* (Temminck & Schlegel, 1846)		⊕P
081 *Rhinogobio cylindricus* Günther, 1888		○P
082 *Rhinogobio typus* Bleeker, 1871		⊕P
083 *Rhinogobio ventralis* Sauvage & Dabry de Thiersant, 1874		○P
	Sarcocheilichthys kiangsiensis Nichols, 1930	⊙T
084 *Sarcocheilichthys nigripinnis* (Günther, 1873)		⊕P
085 *Sarcocheilichthys tungtingensis* Nichols & Pope, 1927		○P
086 *Sarcocheilichthys sinensis* Bleeker, 1871		⊕P
087 *Saurogobio dabryi* Bleeker, 1871		⊕P
088 *Saurogobio dumerili* Bleeker, 1871		○P
089 *Saurogobio gymnocheilus* Lo, Yao & Chen, 1998		⊕P
090 *Saurogobio gracilicaudatus* Yao & Yang, 1977		+P
091 *Saurogobio lissilabris* Bănărescu & Nalbant, 1973		⊕P
092 *Saurogobio xiangjiangensis* Tang, 1980		+P
093 *Squalidus argentatus* (Sauvage & Dabry de Thiersant, 1874)		⊕P
	Squalidus nitens (Günther, 1873)	⊙U
Siluriformes		
Bagridae		
094 *Hemibagrus macropterus* Bleeker, 1870	*Mystus macropterus* (Bleeker, 1870)	⊕P
095 *Tachysurus crassilabris* (Günther, 1864)	*Leiocassis crassilabris* Günther, 1864	⊕TP
096 *Tachysurus dumerili* (Bleeker, 1864)	*Leiocassis longirostris* Günther, 1864	⊕TP
097 *Tachysurus eupogon* (Boulenger, 1892)	*Pelteobagrus eupogon* (Boulenger, 1892)	⊕TP
098 *Tachysurus mica* (Gromov, 1970)	*Leiocassis argentivittatus* (Regan,1905)	+TP
099 *Tachysurus nitidus* (Sauvage & Dabry de Thiersant, 1874)	*Pelteobagrus nitidus* (Sauvage & Dabry de Thiersant, 1874)	⊕TP
100 *Tachysurus sinensis* (Lacepède, 1803)	*Pelteobagrus fulvidraco* (Richardson, 1846)	⊕TP
	Tachysurus tenuis (Günther, 1873)	⊙M
101 *Tachysurus ussuriensis* (Dybowski, 1872)	*Pseudobagrus ussuriensis* (Dybowski, 1872)	○TP
102 *Tachysurus vachellii* (Richardson, 1846)	*Pelteobagrus vachellii* (Richardson, 1846)	⊕TP
103 *Tachysurus zhangfei* Shao, Cheng & Zhang, 2021	*Pseudobagrus albomarginatus* (Rendahl, 1928)	⊕TP
Amblycipitidae		
104 *Liobagrus aequilabris* Wright & Ng, 2008		⊕P
Sisoridae		
105 *Glyptothorax sinensis* (Regan, 1908)		⊕P
Siluridae		
106 *Silurus asotus* Linnaeus, 1758		⊕P
107 *Silurus meridionalis* Chen, 1977		⊕P
Ictaluridae		
108 *Ictalurus punctatus* (Rafinesque, 1818)		+AP

Valid species name	Previous studies	Note
Osmeriformes		
Salangidae		
109 *Hemisalanx prognathus* Regan, 1908		⊕F
	Hemisalanx brachyrostralis (Fang, 1934)	⊙S
110 *Neosalanx brevirostris* (Pellegrin, 1923)		○F
	Neosalanx taihuensis Chen, 1956	⊙S
111 *Neosalanx jordani* Wakiya & Takahashi, 1937		○F
	Neosalanx oligodontis Chen, 1956	⊙S
112 *Protosalanx hyalocranius* (Abbott, 1901)		○F
Gobiiformes		
Odontobutidae		
113 *Micropercops cinctus* (Dabry de Thiersant, 1872)	*Micropercops swinhonis* (Günther, 1873)	⊕SV
114 *Odontobutis sinensis* Wu, Chen & Chong, 2002		⊕V
Gobiidae		
115 *Mugilogobius myxodermus* (Herre, 1935)		○V
	Rhinogobius brunneus (Temminck & Schlegel, 1845)	⊙U
116 *Rhinogobius cliffordpopei* (Nichols, 1925)		○V
117 *Rhinogobius similis* Gill, 1859		⊕V
	Rhinogobius giurinus Gill, 1859	⊙S
Synbranchiformes		
Mastacembelidae		
118 *Sinobdella sinensis* (Bleeker, 1870)		⊕P
Synbranchidae		
119 *Monopterus albus* (Zuiew, 1793)		⊕P
Anabantiformes		
Osphronemidae		
120 *Macropodus opercularis* (Linnaeus, 1758)		⊕P
Channidae		
121 *Channa argus* (Cantor, 1842)		⊕P
122 *Channa asiatica* (Linnaeus, 1758)		⊕P
Beloniformes		
Hemiramphidae		
123 *Hyporhamphus intermedius* (Cantor, 1842)		⊕V
Adrianichthyidae		
124 *Oryzias latipes* (Temminck & Schlegel, 1846)		○F
Tetraodontiformes		
Tetraodontidae		
125 *Takifugu obscurus* (Abe, 1949)		○D
Centrarchiformes		
Centrarchidae		
126 *Micropterus salmoides* (Lacepède, 1802)		+AP
Sinipercidae		
127 *Siniperca chuatsi* (Basilewsky, 1855)		⊕P
128 *Siniperca knerii* Garman, 1912		⊕P
129 *Siniperca roulei* Wu, 1930	*Coreosiniperca roulei* (Wu, 1930)	⊕TP
130 *Siniperca scherzeri* Steindachner, 1892		⊕P

Note: ⊕ Historically recorded species caught in this study; ○ Historically recorded species uncollected in this study; ⊙ Historically recorded species excluded from the updated checklist; + Newly-recorded species; A-Alien or introduced species; S-Junior synonym species; M-Previously misidentified species; T-Taxonomically altered species; U-Unconfirmed species; P-Primary freshwater species; F-Secondary freshwater species; D-Diadromous species; V-Vicarious species.

Results

Analysis of the species checklist

A total of 130 fish species, identified from 12 orders, 30 families and 76 genera, have been documented from Lake Dongting (Table 1). Amongst these species, there are 126 native and four exotic species. Ninety-six species from 10 orders, 24 families and 61 genera collected during 2017–2019 fish survey are included.

For species richness, the order representing the greatest number of species were Cypriniformes (86 species, 66.15% of the total), followed by the Siluriformes (15, 11.54%), Centrarchiformes (5, 3.85%), Gobiiformes (5, 3.85%), Osmeriformes (4, 3.08%), Anabantiformes (3, 2.31%), Clupeiformes (3, 2.31%), Acipenseriformes (3, 2.31%), Synbranchiformes (2, 1.54%), Beloniformes (2, 1.54%), Tetrodontiformes (1, 0.77%) and Anguilliformes (1, 0.77%). The family Xenocyprididae has the highest number (30) of fish species, accounting for 23.08% of the total, followed by the Gobionidae and Acheilognathidae, with 25 and 11 species contributing to 19.23% and 8.46%, respectively. The subsequent families included the Bagridae (10, 7.69%), Cyprinidae (9, 6.92%) and so forth (Table 2).

Lake Dongting harboured 27 migratory fishes, six of which are diadromous and 21 potamodromous, and 103 sedentary fishes, accounting for 20.77% and 79.23% of the total freshwater fishes, respectively. There are 113 (86.92% of the total species) primary freshwater fishes (species spending the whole life in freshwater; Kottelat et al. (2012)), five (3.85%) secondary freshwater fishes (species related to marine families, but living in fresh or sometimes brackish water), six (4.62%) diadromous species (species migrating between fresh and brackish water, but staying in freshwater for part of their life), six (4.62%) vicarious species (species of otherwise largely marine families, but spending their whole life in freshwater, for example, some gobies species) (See Table 1).

The updated checklist of fishes from Lake Dongting includes 49 species endemic to China, 22 endemic to the Chang-Jiang and nine endemic to the mid-lower Chang-Jiang, respectively. This survey yielded 35 Chinese endemics (accounting for 71.43% of the total Chinese endemics from Lake Dongting), 13 endemic species of the Chang-Jiang (59.09% of the total endemic species of the river from the lake) and six endemic species of the mid-lower Chang-Jiang (66.67% of the total endemic species of these reaches from the lake), respectively.

Annotated species checklist

The updated checklist of fishes in Lake Dongting recognises a total of 130 species, based on the data collected in this survey and historical records. Amongst them, 93 native fish species were observed in this fish survey, including eight new records (See Table 1; note '+'). Thirty-four historically recorded species, unsampled in this field surveys, are contained in the updated checklist (Note '○'). Other 20 historically re-

Table 2. The taxonomic composition of fish species in Lake Dongting.

Order	Family	Genus	Species
Acipenseriformes	Acipenseridae	1	2
	Polyodontidae	1	1
Anguilliformes	Anguillidae	1	1
Clupeiformes	Clupeidae	1	1
	Engraulidae	1	2
Cypriniformes	Catostomidae	1	1
	Botiidae	2	5
	Cobitidae	3	4
	Balitoridae	1	1
	Cyprinidae	7	9
	Xenocyprididae	22	30
	Acheilognathidae	2	11
	Gobionidae	12	25
Siluriformes	Bagridae	2	10
	Amblycipitidae	1	1
	Sisoridae	1	1
	Siluridae	1	2
	Ictaluridae	1	1
Osmeriformes	Salangidae	3	4
Gobiiformes	Odontobutidae	2	2
	Gobiidae	1	3
Synbranchiformes	Mastacembelidae	1	1
	Sybranchidae	1	1
Anabantiformes	Osphronemidae	1	1
	Channidae	1	2
Beloniformes	Hemiramphidae	1	1
	Adrianichthyidae	1	1
Tetraodontiformes	Tetraodontidae	1	1
Centrarchiformes	Centrarchidae	1	1
	Sinipercidae	1	4
12	30	76	130

corded species are excluded, including six being synonymised with other species (Note 'S'), seven misidentified or having an erroneous record in the lake (Note 'M'), four experiencing taxonomical changes (Note 'T') and three having unconfirmed records (Note 'U'). A number of nomenclatural changes also occur for valid species included in the updated checklist (20 species). Taxonomic comments were appended to discuss its validity and occurrence where relevant.

Acipenseridae & Polyodontidae

The Acipenseridae has two representatives in the lake, namely *Acipenser sinensis* Gray, 1835 and *A. dabryanus* Duméril, 1869, while the Polyodontidae is presented only by a single species *Psephurus gladius*. All three large-sized sturgeons were not collected in Lake Dongting during this field survey. One juvenile individual (4340 mm SL, 566.0 g) of *A. sinensis* was collected from East Dongting Lake during the 2012–2013 field sur-

vey (unpublished data). The specimen is likely a captive-bred individual released into the wild. This conservation measure has been implemented in the upper Chang-Jiang Basin for nearly twenty years (Du et al. 2013). Nichols (1928) was the first to report on the existence of *A. dabryanus* in Lake Dongting. Liang and Liu (1959, 1966) included the sturgeon in their species inventories of the lake. This species has vanished in Lake Dongting since the Gezhouba Dam was constructed across the Chang-Jiang mainstem (Zhang and Cao 2021a). Likely, *A. dabryanus* became highly depleted as no individuals have been collected in the river as from 1995 (Zhang et al. 2017). The capture record on *P. gladius* showed a similar trend to that of *A. dabryanus* as no records of Chinese paddlefish have been reported since 1995 (Zhang et al. 2020a).

Anguillidae

Anguilla japonica Temminck & Schlegel, 1846, a delicious food fish of economic importance in China and even across the Globe, is the only representative of the family in Lake Dongting. Historically, the lake and its affluents were utilised by this catadromous fish as feeding grounds (Anonymous 1980), but it is hardly seen in fish capture presently (Liu et al. 2013). One small individual (295 mm SL, 33.9 g) was captured at Chenglingji Channel during this field survey. It might be an individual which escaped from reservoirs where cage culture was used to farm this fish, in terms of local fishermen.

Clupeidae & Tetraodontidae

The family Clupeidae and Tetraodontidae are each represented in Lake Dongting by a single species. The two diadromous fishes, *Tenualosa reevesii* and *Takifugu obscurus* (Abe, 1949), are hardly seen in this lake so far. The last capture of *T. reevesii* (one individual) was at Jiangsu provincial section of the Chang-Jiang in 1998 (Liu et al. 2002). *Takifugu obscurus* is occasionally encountered in the lower Chang-Jiang Basin so far (Wang et al. 2016; Chen et al. 2020).

Engraulidae

This family has only two representatives in Lake Dongting: *Coilia brachygnathus* and *C. nasus* Temminck & Schlegel, 1846. So far, *C. brachygnathus* abounds in this lake where it is a delicious food fish of economic importance, but *C. nasus* is a rarely encountered fish. *Coilia nasus* is even regarded to have been extinct due to anthropogenic interferences for nearly two decades (Wang et al. 2016); however, this anadromous fish has recently been found to persist in Lake Dongting (Xuan et al. 2020).

Salangidae

This family has four representatives in Lake Dongting: *Hemisalanx prognathus*, *Neosalanx brevirostris* (Pellegrin, 1923), *N. jordani* Wakiya & Takahashi, 1937 and *Protosalanx hyalocranius* (Abbott, 1901). So far, the taxonomy of Chinese icefishes

still remains controversial (Fu et al. 2005; Zhang et al. 2007). Based on the latest taxonomic advances of this family, three formerly recorded species were removed from the updated checklist. *Neosalanx taihuensis* Chen, 1956 was treated as a synonym of *N. brevirostris* (Zhang et al. 2007). *Hemisalanx brachyrostralis* (Fang, 1934) was synonymised with *H. prognathous* and so was *Neosalanx oligodontis* Chen, 1956 with *N. jordani* (Guo et al. 2011).

Catostomidae

This family has a single representative in China: *Myxocyprinus asiaticus* (Bleeker, 1864). *Myxocyprinus asiaticus* had long been considered as a migratory fish (Anonymous 1976). Nevertheless, Zhang and Zhao's (2001) examination on collection specimens found that all individuals caught from the mid-lower Chang-Jiang Basin were small-sized, but large-sized individuals came from the upper reaches of this river, so concluding that *M. asiaticus* may be not a migratory species. One specimen (382 mm SL and 675.1 g) of this species was captured at the estuary of the Xiang-Jiang into Lake Dongting during our field survey. It is probably a captive-bred individual released into the Xiang-Jiang at Hengyang section yearly, according to local fishermen.

Cyprinidae

The Cyprinidae, as traditionally delimited, contains species with one to three rows of pharyngeal teeth, barbels present or absent and Weberian apparatus (Chen 1998; Nelson et al. 2016). A recent re-classification of the Cypriniformes was provided by Tan and Armbruster (2018), based on Yang et al.'s (2015a) phylogenetic relationships of this order inferred from both mitochondrial and nuclear genes. The Cyprinidae *s. l.* splits into ten families, namely Acheilognathidae, Cyprinidae *s. str.*, Danionidae, Gobionidae, Leptobarbidae, Leuciscidae, Sundadanionidae, Tanichthyidae, Tincidae and Xenocyprididae. The Cyprinidae *s. str.* is further subdivided into eleven subfamilies, i.e. Acrossocheilinae, Barbinae, Cyprininae, Labeoninae, Poropuntiinae, Probarbinae, Schizopygopsinae, Schizothoracinae, Smiliogastrinae, Spinibarbinae and Torinae. Amongst them, four subfamilies have their representatives in this lake: Acrossocheilinae, Cyprininae, Labeoninae and Spinibarbinae.

Acrossocheilinae

This subfamily was newly erected to include species currently designated to *Folifer*, *Onychostoma* and *Acrossocheilus* (Yang et al. 2015a; Tan and Armbruster 2018), but its generic classification still needs in-depth study. According to historical records (Tang and Qian 1979; Li 2006), *Onychostoma* has two representatives in Lake Dongting: *Onychostoma simum* (Sauvage & Dabry de Thiersant, 1874) and *O. rarum* (Lin, 1933). The two rheophilic fishes were not caught in this survey, though.

Cyprininae

Previously, *Cyprinus carpio* Linnaeus, 1758 was extensively utilised as the available specific name for the common carp widespread in China. This species, however, is currently regarded as the endemic species of Europe (Kottelat and Freyhof 2007). The East Asian populations of the common carp represent a distinct species from *C. carpio*. The available specific name for it is *C. haematopterus* Temminck & Schlegel, 1846 (Zhou et al. 2003), a junior synonym of *C. rubrofuscus* Lacepède, 1803 (Kottelat 2006, 2013). Specimens previously reported by Li (2006) as *Procypris rabaudi* (Tchang, 1930) from Lake Dongting are possibly misidentified. This species is a rheophilic fish usually found in headwaters of rivers, but not in the lentic environment (Zhang and Zhao 2016).

Labeoninae

This subfamily has four representatives in Lake Dongting: *Decorus tungting*, *Cirrhinus cirrhosus* Bloch, 1795, *C. molitorella* (Valenciennes, 1844) and *Pseudogyrinocheilus prochilus* (Sauvage & Dabry de Thiersant, 1874). The first species were firstly designated to *Sinilabeo* Rendahl, 1933 (Wu 1977; Tang et al. 2001) and later moved into *Bangana* Hamilton, 1822 (Zhang and Chen 2006). Recently, Zheng et al. (2019) assigned this species, along with *Bangana decora* (Peters, 1881) from the Zhu-Jiang Basin, *B. lemassoni* (Pellegrin & Chevey, 1936) from the Red River Basin and *B. rendahli* (Kimura, 1934) from the upper Chang-Jiang Basin, to their own genus named as *Decorus*. Both *Cirrhinus cirrhosus* and *Pseudogyrinocheilus prochilus* are two new records of this lake. The former was introduced into China as cultured fish from India during the 1990s (Wang and Zhang 2021); it, like *C. molitorella* in southern China, has widely been farmed as food fish for cultured Mandarin fish (Ye et al. 2016). Individuals of two *Cirrhinus* fishes, caught from Lake Dongting in this field survey, probably escaped from farming waters. The latter *P. prochilus* was collected at Chenglingji, the outlet channel from Lake Dongting into the Chang-Jiang mainstem. This means that the species has an extended distribution in this lake. The rheophilic fish is mainly found in the upper Chang-Jiang and Zhu-Jiang presently (Zhang 1994; Zheng et al. 2010). It was even recorded from the Li-Shui, Yuan-Jiang and Xiang-Jiang (Liang and Liu 1966; Anonymous 1980; Cao et al. 2012).

Spinibarbinae

The subfamily is represented in Lake Dongting by a single species: *Spinibarbus caldwelli* (Nichols, 1925). *Spinibarbus caldwelli* was previously recognised as *S. hollandi* Oshima, 1919 (Chu and Chen 1989; Yue 2000), a species widespread in Asian mainland (Tang et al. 2005). Indeed, *S. hollandi* is endemic to Taiwan Island of China (Tang et al. 2005). The available scientific name for Asian mainland specimens of this species is *S. caldwelli* (Tang et al. 2005). *Spinibarbus sinensis* Bleeker, 1871 is a species mainly found in the upper Chang-Jiang Basin (Chu and Chen 1989; Ding 1994; Yue

2000; Zhang and Zhao 2016). It was also reported from Lake Dongting by Tang and Qian (1979) and Li (2006). This identification, nevertheless, needs confirmation when specimens become available.

Xenocyprididae

The family is the dominant group of the ichthyofauna of Lake Dongting, with 30 species identified from 22 genera: *Aristichthys* Oshima, 1919 (one species), *Chanodichthys* Bleeker, 1860 (five), *Ctenopharyngodon* Steindachner, 1866 (one), *Culter* Basilewsky, 1855 (one), *Distoechodon* Peters, 1881 (one), *Elopichthys* Bleeker, 1860 (one), *Hemiculter* Bleeker, 1860 (two), *Hypophthalmichthys* Bleeker, 1860 (one), *Luciobrama* Bleeker, 1870 (one), *Megalobrama* Dybowski, 1872 (two), *Mylopharyngodon* Peters, 1881 (one), *Ochetobius* Günther, 1868 (one), *Opsariichthys* Bleeker, 1863 (one), *Parabramis* Bleeker, 1864 (one), *Plagiognathops* Berg, 1907 (one), *Pseudobrama* Bleeker, 1870 (one), *Pseudolaubuca* Bleeker, 1864 (two), *Sinibrama* Wu, 1939 (one),*Squaliobarbus* Günther, 1868 (one), *Toxabramis* Günther, 1873 (one), *Xenocypris* Günther, 1868 (two) and *Zacco* Jordan & Evermann, 1902 (one). The large majority of these species are widespread in the lowlands of south or east China.

Several previously-recorded species from Lake Dongting have synonymisations or taxonomic changes. *Xenocypris argentea* Günther, 1868 was synonymised with *X. macrolepis* Bleeker, 1871 (Kottelat 2013). *Xenocypris microlepis* Bleeker, 1871 had been referred to *Plagiognathops* Berg, 1907 (Kottelat 2013). *Zacco acanthogenys* (Boulenger, 1901) had long been synonymised with *Zacco platypus* (Temminck & Schlegel, 1846) until Wang (2019) and Zhu et al. (2020) revalidated it. The type locality of *Z. platypus* is in Japan (Liu et al. 2011), but *Z. acanthogenys* occurs in the mid-lower Chang-Jiang Basin. Specimens under the name of *Sinibrama wui* (Rendahl, 1933) from Lake Dongting are referred to as *S. macrops* (Günther, 1868), following Xie et al. (2003) and Zhang et al. (2004). Specimens, previously recognised as *Megalobrama skolkovii* Dybowski, 1872, from the lake are identified as *M. mantschuricus* (Basilewsky, 1855), following Vasil'eva and Makeeva (2003) and Bogutskaya et al. (2008).

The taxonomy of three genera *Chanodichthys* Bleeker, 1860, *Culter* Basilewsky, 1855 and *Cultrichthys* Smith, 1938 is hitherto in a chaotic status in Chinese literature. The type species of *Chanodichthys* is *Leptocephalus mongolicus* Basilewsky, 1855 [type locality: China: Mongolia (presently Inner Mongolia Province) and Manchuria (now northeast China)], that of *Culter* is *C. alburnus* Basilewsky, 1855 [type locality: China: rivers flowing into the Gulf of Tschili (today's Hebei Province)] and that of *Cultrichthys* is *C. brevicauda* Günther, 1868 (type locality: Taiwan, China). Bănărescu (1997) synonymised *Cultrichthys* with *Culter*. This synonymisation was subsequently accepted by some researchers (Bogutskaya and Naseka 2004; Bogutskaya et al. 2008). The type species of *Culter*, though, was misplaced in *Cultrichthys* in Chinese literature (Luo 1994; Luo and Yue 1996; Chen 1998). This misplacement can be traced back to Yi and Zhu (1959), who took it for granted that *Cultrichthys erythropterus* (Basilewsky, 1855), as indicated by the species name, is the available scientific name for the species

with pink pectoral, pelvic and anal fins. This character, along with a long keel extending along the mid-line of the chest and belly, is typical for *Culter alburnus* (Bogutskaya et al. 2008). *Cultrichthys erythropterus* (*sensu* Chen 1998) is, thus, the misidentification of *Culter alburnus* (*sensu* Chen 1998) and vice versa. *Culter*, as here delimited, includes two species: *C. alburnus* and *C. compressocorpus* Yih & Chu, 1959. All other species currently placed to *Culter* by Chinese authors should be referred to as *Chanodichthys*.

Gobionidae

Twenty-five species of gudgeons from Lake Dongting are placed in 12 genera, namely *Abbottina* Jordan & Fowler, 1903 (one species), *Coreius* Jordan & Starks, 1905 (one), *Gobiobotia* Kreyenberg, 1911 (four), *Hemibarbus* Bleeker, 1860 (two), *Microphysogobio* Mori, 1934 (one), *Paracanthobrama* Bleeker, 1864 (one), *Pseudogobio* Bleeker, 1860 (one), *Pseudorasbora* Bleeker, 1860 (one), *Rhinogobio* Bleeker, 1870 (three), *Sarcocheilichthys* Bleeker, 1860 (three), *Saurogobio* Bleeker, 1870 (six) and *Squalidus* Dybowski, 1872 (one). Most of these species are often seen in the mid-lower Chang-Jiang basin or even lowland areas of the southern China.

Sarcocheilichthys is represented in Lake Dongting by three species, namely *S. nigripinnis* (Günther, 1873), *S. tungtingensis* Nichols & Pope, 1927 and *S. sinensis* Bleeker, 1871. Our ongoing taxonomy of this genus demonstrates that *S. kiangsiensis* Nichols, 1930 occurs only in the lake Poyang system and that specimens, formerly identified as this species from Lake Dongting, belong to *S. tungtingtensis* (An 2020). A critical revision of *Sarcocheilichthys* from China is underway; the species diversity of this genus has been highly underestimated so far. *Saurogobio* is presently represented in Lake Dongting by six species, namely *S. dabryi* Bleeker, 1871, *S. dumerili* Bleeker, 1871, *S. gracilicaudatus* Yao & Yang, 1977, *S. gymnocheilus* Lo, Yao & Chen, 1998, *S. lissilabris* Bănărescu & Nalbant, 1973 and *S. xiangjiangensis* Tang, 1980. Both *S. gracilicaudatus* and *S. xiangjiangensis* are new records and so is *P. vaillanti* (Sauvage, 1878). The species status of *S. lissilabris* was suspected by some researchers (Wu 1977; Chen 1998) or even it was synonymised with *S. gymnocheilus* (Dai et al. 2014). Tang et al. (2018) considered it as a valid species on the basis of molecular evidence and their examination on its type. Two historically documented species are removed from the updated species checklist: *Coreius guichenoti* (Sauvage & Dabry de Thiersant, 1874) and *Squalidus nitens* (Günther, 1873). The former is hitherto found only in the upper Chang-Jiang (Zhang et al. 2019; Liu et al. 2020a) and the latter, whose type locality is in Shanghai City (Günther 1873), has not been found in Lake Dongting for decades.

Four species of *Gobiobotia* were formerly reported from Lake Dongting: *G. filifer* (Garman, 1912), *G. meridionalis* Chen & Cao, 1977, *G. nicholsi* Bănărescu & Nalbant, 1966 and *G. pappenheimi* Kreyenberg, 1911. The first species is to date endemic to the Chang-Jiang Basin downstream of Yibin City. The second species had long been treated as a subspecies of *G. longibarba* Fang & Wang, 1931 until Chen (1998) regarded it as a full species. It is extensively known from the middle reaches of the Chang-Jiang and Zhu-Jiang Basins (Chen 1998; Tang et al. 2001; Zhang and Zhao 2016). The two species

were collected from this lake during this field survey. The third species was originally described from Lake Dongting (Bănărescu and Nalbant 1966), but later synonymised with *G. filifer* (Wu 1977). Our ongoing taxonomy of Chinese species of *Gobiobotia* shows that *G. nicholsi* is a valid species of the lake Dongting system, but it is so far known merely by its type specimens. Although Bănărescu and Nalbant (1966) reported on the occurrence of *G. pappenheimi* and *Xenophysogobio boulengeri* (Tchang, 1929) in Lake Dongting, no additional specimens have since been collected. Generally, *G. pappenheimi* (type locality: northern China: Tianjin City) is mainly found in the Hai-He and Huang-He (Wang 1984) and *X. boulengeri* (type locality: southwest China: Sichuan Province) occurred in the upper Chang-Jiang Basin (Zhang et al. 2019). Our photograph examination indicated that specimens, identified by Bănărescu and Nalbant (1966) as *G. pappenheimi* and *X. boulengeri* from Lake Dongting, are conspecific with *G. nicholsi*. Nevertheless, this identification still needs confirmation when topotypical specimens become available. Provisionally, these two species are here regarded to have an erroneous record in the Lake. Recently, a new species from Lake Dongting is here found, based on morphological and molecular evidence (Chen et al. 2022b). Therefore, the eight-barbel gudgeons have four representatives in the lake: *Gobiobotia filifer*, *G. lii*, *G. meridionalis* and *G. nicholsi*.

Acheilognathidae

The bitterlings have eleven representatives in Lake Dongting: *A. macropterus* (Bleeker, 1871), *A. barbatulus* Günther, 1873, *A. macromandibularis* Doi, Arai & Liu, 1999, *A. polylepis* (Wu, 1964), *A. gracilis*, *A. barbatus* Nichols, 1926, *A. chankaensis* (Dybowski, 1872), *A. tonkinensis* (Vaillant, 1892), *A. hypselonotus* (Bleeker, 1871), *Rhodeus ocellatus* (Kner, 1866) and *R. sinensis* Günther, 1868. The first one was formerly misidentified as *A. taenianalis* (Günther, 1873) (Tang and Qian 1979; Li 2006); however, *A. taenianalis* has been shown to be a junior synonym of *A. macropterus* (Kottelat 2013). The second and third bitterlings are new records for this lake (Doi et al. 1999; Li 2013). *Acheilognathus imberbis* Günther, 1868, previously documented from Lake Dongting (Ru 2008), is removed from the species checklist. Its type locality remains unclear, but it is reportedly present in the lower Chang-Jiang Basin so far (Li et al. 2016c; Zhang et al. 2020b). Moreover, this species has not been found in the Lake Dongting system over the past several decades. Although Yu et al. (2005) reported on its distribution in Xiang-Jiang, the identification still needs confirmation.

Botiidae

This family is so far represented in Lake Dongting by five species, three of which are from *Leptobotia* [*L. citrauratea* (Nichols, 1925), *L. rubrilabris* (Dabry de Thiersant, 1872) and *L. taeniops* (Sauvage, 1878)] and two from *Parabotia* [*P. fasciata* Dabry de Thiersant, 1872 and *P. banarescui* (Nalbant, 1965)]. Nichols (1925b) reported on the occurrence of *Botia rubrilabris* in the Lake Dongting system and described *B. purpurea* and *B. citrauratea* as two new species from the lake. The three sympatrically existing

congeneric species were later transferred to *Leptobotia* where *B. citrauratea* and *B. purpurea* were synonymised, respectively with *L. elongata* and *L. taeniops* (Bleeker, 1870) (Chen 1980; Kottelat 2004, 2012). Recently, *L. citrauratea* was resurrected from the synonym of *L. elongata*, based on examination of the type and morphological data (Bohlen and Šlechtová 2017). Guo and Zhang (2021) also affirmed that *L. citrauratea* survives in Lake Dongting (type locality). Only a single small-sized individual of *L. rubrilabris* was collected by Anonymous (1980) in this lake. Our field survey yielded no specimens of this species. Likely, it has been extirpated in Lake Dongting. The taxonomy of *Leptobotia* species from China needs a critical revision.

Bagridae

The taxonomy of the bagrid catfishes from China is notoriously poorly understood. This family is represented in Lake Dongting by two genera: *Hemibagrus* Bleeker, 1862 and *Tachysurus* Lacepède, 1803. Species previously referred to *Mystus* Scopoli, 1777 are misidentification of *Hemibagrus* in Chinese literature (Liu et al. 2013; Yuan et al. 2019; Yang et al. 2020). All species, formerly placed in *Pelteobagrus* Bleeker, 1864 and *Pseudobagrus* Bleeker, 1858, are currently referred to *Tachysurus* (Ng and Freyhof 2007; Kottelat 2013) and so are Chinese species formerly placed in *Leiocassis* Bleeker, 1857 (Cheng and Zhang 2012), which is in fact a genus endemic to Southeast Asia (Ng and Kottelat 2007).

The Bagridae is represented in Lake Dongting by 10 species, namely *Tachysurus crassilabris* (Günther, 1864), *T. dumerili* (Bleeker, 1864), *T. eupogon* (Boulenger, 1892), *T. mica* (Gromov, 1970), *T. nitidus* (Sauvage & Dabry de Thiersant, 1874), *T. sinensis* Lacepède, 1803, *T. ussuriensis* (Dybowski, 1872), *T. vachellii* (Richardson, 1846), *T. zhangfei* Shao, Cheng & Zhang, 2021 and *Hemibagrus macropterus* Bleeker, 1870. *Tachysurus dumerili* is a senior subjective synonym of *T. longirostris* Günther, 1864 (Kottelat 2013). Specimens of *T. mica* were formerly misidentified as the juveniles of other catfishes owing to their small size (Chu et al. 1999), but our ongoing taxonomy of Chinese *Tachysurus* indicates that it is a valid species. *Tachysurus sinensis* is a senior subjective synonym of *T. fulvidraco* (Richardson, 1846) (Ng and Kottelat 2007). Specimens, previously recognised as *T. albomarginatus* (Rendahl, 1928), from Lake Dongting represent an undescribed species, which was named as *T. zhangfei* (Shao et al. 2021). Possibly, Ru's (2012) specimens, under the name of *T. tenuis* (Günther, 1873), from Lake Dongting were misidentified as it is hitherto known merely from the type locality, Chongming Island, Shanghai City (Kottelat 2013; Cheng et al. 2021). This species is tentatively excluded from this updated species checklist.

Ictaluridae & Centrarchidae

The family Ictaluridae and Centrarchidae are each represented in Lake Dongting by a single species. *Ictalurus punctatus* (Rafinesque, 1818) and *Micropterus salmoides* (Lacepède, 1802), introduced as cultured fishes to China, are sporadically found in the lakes from southern China (Li et al. 2016d).

Sinipercidae

This family is so far represented in Lake Dongting by four species of the genus *Siniperca* Gill, 1862: *S. chuatsi* (Basilewsky, 1855), *S. knerii* Garman, 1912, *S. roulei* Wu, 1930 and *S. scherzeri* Steindachner, 1892 (Tang and Qian 1979; Li 2006; Li 2013). This third perch was previously assigned to *Coreosiniperca* Fang & Chong, 1932, but this genus has been shown to be invalid (Liu and Chen 1994).

Gobiidae

Five gobies of *Mugilogobius* Smitt, 1900 and *Rhinogobius* Gill, 1859 were previously recorded from Lake Dongting: *M. myxodermus* (Herre, 1935), *R. brunneus* (Temminck & Schlegel, 1845), *R. cliffordpopei*, *R. giurinus* Gill, 1859 and *R. similis* Gill, 1859 (Tang and Qian 1979; Li 2006). Specimens, under the name of *R. giurinus*, have been shown to be misidentification of *R. similis* (Suzuki et al. 2016; Suzuki et al. 2017). The current identification of *R. brunneus* from this lake remains suspicious. Its type locality is in Japan (Temminck and Schlegel 1845). Chinese specimens of this goby were referred to as different species (Wu and Zhong 2008). Nevertheless, whether the species exists in Chinese freshwaters remains unsolved yet. Temporarily, the goby is removed from this updated checklist. Thus, only three gobies are here recognised from Lake Dongting: *M. myxodermus*, *R. cliffordpopei* and *R. similis*.

Discussion

Species diversity

Lake Dongting, as the second-largest river-connected freshwater lake lying within the floodplain areas of the mid-lower Chang-Jiang Basin, supports diversified freshwater fish species. A total of 130 fish species is here reported from the Lake. This number accounts for ca. 31.48% of the total freshwater fishes of the Chang-Jiang Basin where 413 native species were recently documented (Zhang and Cao 2021a). According to the recently-published book entitled "The fish fauna of Hunan Province", the Dongting Lake system harbours up to 218 freshwater fish species (Wu et al. 2021). The lake alone contributes to 59.63% of the total number of freshwater fishes from the system. In addition to serving as favourable habitats of the Yangtze finless porpoise (Huang et al. 2017) and the crucial stopover and breeding grounds of plentiful migrating birds (Fang et al. 2006; Zou et al. 2019), this lake is also used as sanctuaries or nursery grounds by numerous larvae of drifting-egg-spawning or potamodromous fishes like four major Chinese carps, as spawning grounds by some anadromous fishes like *Coilia nasus* and *Tenualosa reevesii* and as feeding grounds by some catadromous fishes like *Anguilla japonica* and *Takifugu obscurus* (Dou and Jiang 2000). Evidently, Lake Dongting is the key biodiversity area of this lake system or the Chang-Jiang Basin.

The total number of freshwater fish species of Lake Dongting given in this updated checklist is actually comparable to that of Lake Poyang, the first-largest river-connected floodplain lake of the mid-lower Chang-Jiang Basin, where a total of 136 fish species has been recorded so far (Zhang and Li 2007; Yang et al. 2015b; Fang et al. 2016). This number seems to be higher than that of Lake Dongting, but remains doubtful. From the latest species checklist of freshwater fishes from the Gan-Jiang—the largest river flowing into Lake Poyang, 36 historically recorded species were removed (Wang and Zhang 2021). Amongst them, at least ten species were contained in checklists of fish species of Lake Poyang by Zhang and Li (2007) and Fang et al. (2016); these ten species were also components of the ichthyofauna of Lake Poyang system compiled by Huang et al. (2013) and Hu et al. (2019). Both Lakes Dongting and Poyang support rich fish species diversity that is unmatched by any other lake in the mid-lower Chang-Jiang Basin, such as Lake Chao (54 fish species, Guo et al. 2007), Lake Tai (107, Zhu et al. 2007), Lake Hongze (88, Lin et al. 2013) or Lake Hong (84, unpublished data) and far higher than that of lakes located in the Yunnan-Guizhou Plateau (Yuan et al. 2010). This can be plausibly explained by uniqueness of these two large-sized floodplain subtropical lakes: the permanent lateral hydrological connection with the Chang-Jiang mainstem and co-existence of lentic and lotic environments. The assembly mechanism maintaining fish community within Lake Dongting has been addressed in Chen et al. (2022a).

The present study shows that fish species diversity of Lake Dongting remains insufficiently understood. The number of species, collected from the Lake in this survey, is lower compared with the frontrunners (Liang and Liu 1959, 1966; Tang and Qian 1979; Li 2006). Eight newly-recorded native species are added likely due to the multiple sampling methods used and three seasonal samplings during our survey from 2017 to 2019. More sampling efforts lead to the discovery of higher species richness (Hughes et al. 2021; Pompeu et al. 2021). Twenty historically-recorded species are excluded from the checklist, mainly due to the following reasons: (1) Species misidentification. This is the case for seven species which do not exist in the lake presently, namely *Coreius guichenoti*, *Gobiobotia pappenheimi*, *Leptobotia elongata*, *Procypris rabaudi*, *Pseudobagrus tenuis* (= *Tachysurus tenuis*), *Spinibarbus sinensis* and *Xenophysogobio boulengeri*; (2) Taxonomic alteration. Species, formerly identified as *Cyprinus carpio*, *Sarcocheilichthys kiangsiensis*, *Spinibarbus hollandi* and *Zacco platypus* from this lake or China, are now referred to as *Cyprinus rubrofuscus*, *Sarcocheilichthys tungtingtensis*, *Spinibarbus caldwelli* and *Zacco acanthogenys*, respectively; (3) Unconfirmed records. Whether *Acheilognathus imberbis*, *Rhinogobius brunneus* and *Squalidus nitens* occur in Lake Dongting remains controversial; (4) Synonymisation. The following six species are to date regarded as invalid: *Acheilognathus taenianalis*, *Hemisalanx brachyrostralis*, *Neosalanx oligodontis*, *Neosalanx taihuensis*, *Rhinogobius giurinus*, and *Sinibrama wui*. It is apparent that problems with the current identification of some fish species in Lake Dongting still remains.

This checklist includes 20 species which experienced nomenclatural alterations, viz. *Bangana tungting* (= *Decorus tungting*), *Coreosiniperca roulei* (= *Siniperca roulei*), *Culter alburnus* (= *Chanodichthys erythropterus*), *Culter dabryi* (= *Chanodichthys dabryi*), *Culter mongolicus* (= *Chanodichthys mongolicus*), *Culter oxycephaloides*

(= *Chanodichthys oxycephaloides*), *Culter oxycephalus* (= *Chanodichthys oxycephalus*), *Cultrichthys erythropterus* (= *Culter alburnus*), *Gobiobotia longibarba meridionalis* (= *Gobiobotia meridionalis*), *Leiocassis argentivittatus* (= *Tachysurus mica*), *Leiocassis crassilabris* (= *Tachysurus crassilabris*), *Leiocassis longirostris* (= *Tachysurus dumerili*), *Mystus macropterus* (= *Hemibagrus macropterus*) *Pelteobagrus eupogon* (= *Tachysurus eupogon*), *Pelteobagrus fulvidraco* (= *Tachysurus sinensis*), *Pelteobagrus nitidus* (= *Tachysurus nitidus*), *Pelteobagrus vachellii* (= *Tachysurus vachellii*), *Pseudobagrus albomarginatus* (= *Tachysurus zhangfei*), *Pseudobagrus ussuriensis* (= *Tachysurus ussuriensis*), *Xenocypris microlepis* (= *Plagiognathops microlepis*). Two species, *Gobiobotia nicholsi* and *Sarcocheilichthys tungtingensis* are, for the time being, regarded as valid. Their taxonomic status needs to be confirmed when specimens from their type locality (today's East Dongting Lake) become available.

Biodiversity conservation

Amongst 130 freshwater fish species of Lake Dongting, 12 (9.23% of the total) are labelled as threatened species in Zhang and Cao's (2021a) assessment of the Red List of Chinese freshwater fishes, viz. *Acipenser sinensis* (CR), *A. dabryanus* (CR), *Anguilla japonica* (EN), *Decorus tungting* (EN), *Leptobotia rubrilabris* (VU), *Luciobrama macrocephalus* (CR), *Myxocyprinus asiaticus* (CR), *Ochetobius elongatus* (CR), *Onychostoma rarum* (VU), *Psephurus gladius* (CR), *Rhinogobio ventralis* (EN) and *Tenualosa reevesii* (CR) (see Table 3). Three species, *Psephurus gladius*, *Acipenser sinensis* and *A. dabryanus*, are also listed in the Appendices II of the Convention on International Trade in Endangered Species (CITES 2019). *Psephurus gladius* was declared to have been functionally extinct in the Chang-Jiang Basin, due to a permanent lack of reproduction or recruitment since 1993 (Zhang et al. 2020a). No wild individuals on *A. dabryanus* have been monitored since 1995 (Zhang et al. 2017). The critically endangered status of *A. sinensis* was mainly owing to a dramatic decline in population after 2000 (Zhang and Cao 2021a). Field surveys conducted from 2002 to 2009 found a trend of a drastic decrease in its juvenile population year by year (Wang et al. 2011; Wu et al. 2015). No spawning individuals were monitored from 2013 to 2015 into the Chang-Jiang mainstem downstream of the Gezhouba Dam (Wu et al. 2017; Zhang et al. 2020a), therefore indicating that the population of this freshwater megafauna species is extremely impacted by river damming (Zhang et al. 2017). Only one small individual of *A. sinensis* was collected from Lake Dongting during 2012 (unpublished data). This clearly means that the lake can be utilised as nursery or feeding grounds by the juveniles and, hence, plays an important role in the conservation of the sturgeon. Nevertheless, the young sturgeon is also likely the captive-bred juveniles released into the upper Chang-Jiang, given that restocking, one salvaging measure taken to conserve this fish, has been in place for several decades (Du et al. 2013). Two imperilled species, *Anguilla japonica* (EN) and *Myxocyprinus asiaticus* (CR) were collected in this field survey, indicating that both still persist here. More attention should be paid to the remaining threatened fish species unsampled in this survey. Whether they eluded capture or have been extirpated, their populations are in a continuous decline and salvaging actions should be adopted immediately.

Five species are also on the latest List of Key Protected Wild Animals in China, namely *Leptobotia rubrilabris*, *Luciobrama macrocephalus*, *Myxocyprinus asiaticus*, *Rhinogobio ventralis* and *Tenualosa reevesii* (Anonymous 2021). *Tenualosa reevesii* is of importance for capture fisheries, particularly in the mid-lower Chang-Jiang Basin. The population of this anadromous fish had been in remarkable decrease as from 1992 when Wan'an Dam was constructed across the Gan-Jiang, where its spawning grounds were located (Tang et al. 1993; Liu 2002). During the past twenty years, no individuals have been collected (Wang and Zhang 2021). The fish, like *Psephurus gladius*, has probably been extinct in the Chang-Jiang Basin (Zhang and Cao 2021a). *Myxocyprinus asiaticus* is rarely encountered in Lake Dongting due to a sharp decline in population resulting from anthropogenic disturbances (Fang et al. 2006). One small individual of 382 mm SL, which was caught during our field survey, is likely a captive-bred juvenile released into the wild to restock its population. *Luciobrama macrocephalus* used to be widely distributed in southern China, but this food fish of high value has become an occasionally-encountered species. The carnivorous fish has long been regarded as the target species to be eradicated as its juveniles prey on fries of other farmed fishes, thus having negative impacts on lake or reservoir fisheries. Deliberate removal of this apex predator was mainly responsible for its current endangerment status. *Rhinogobio ventralis* was initially described from Lake Dongting (Sauvage and Thiersant 1874), but the gudgeon has vanished since Liang and Liu's (1959, 1966) report on its existence in the lake. *Leptobotia rubrilabris*, originally described from the upper Chang-Jiang Basin, was also recorded from Lake Dongting (Nichols 1925b). The latest report on its survival in the lake was Anonymous (1980), who caught a single specimen of 80 mm SL. Field survey of fishes conducted from 2014 to 2019 into Lake Dongting yielded no specimens of this fish (Guo and Zhang 2021). Likely, it has already been extirpated in this system.

Besides three species (*Luciobrama macrocephalus*, *Myxocyprinus asiaticus* and *Tenualosa reevesii*), there are another 12 species currently included in Hunan provincial key protected wildlife list (The Forest Department of Hunan Province 2015): *Channa asiatica*, *Coilia nasus*, *Decorus tungting*, *Macropodus opercularis*, *Microphysogobio tungtingensis*, *Neosalanx brevirostris*, *Ochetobius elongatus*, *Onychostoma rarum*, *O. simum*, *Saurogobio xiangjiangensis*, *Siniperca roulei* and *Spinibarbus caldwelli* (Table 3). No specific conservation actions, however, have been in place for these species. It is worth pointing out that, except for the *Channa asiatica* and *Macropodus opercularis*, all these species seem to be of local economic importance in the mid-lower Chang-Jiang Basin or Lake Dongting system.

Lake Dongting harbours nine fish species endemic to the mid-lower Chang-Jiang Basin, namely *Acheilognathus hypselonotus*, *A. macromandibularis*, *A. macropterus*, *Coilia brachygnathus*, *Decorus tungting*, *Leptobotia citrauratea*, *Megalobrama amblycephala*, *Microphysogobio tungtingensis* and *Saurogobio gracilicaudatus*. These species have a high risk of being imperilled by anthropogenic perturbation. More efforts should be dedicated to monitor their population size and trend. *Decorus tungting*, a popular food fish of local economic importance in Lake Dongting system before 1980s, is currently restricted only to some sections of the Yuan-Jiang and Zi-Shui, two affluents of Lake Dongting (Bian et al. 2011). Owing to a sharp decrease in population over the past 30 years, this

rheophilic species was assessed as Endangered (EN) in the latest assessment of Chinese freshwater fish Red List (Zhang and Cao 2021a). No doubt, salvaging actions should be taken to conserve this species. All these species, except *D. tungting*, were not included in this Red List. Nevertheless, two fishes were listed as Data Deficient (DD): *Coilia brachygnathus* and *Microphysogobio tungtingensis*. *Leptobotia citrauratea* can also be assessed in this category and was recently revalidated (Guo and Zhang 2021). These three species are possibly under the same threat as *D. tungting* and, thus, deserve special attention.

Table 3. Endemics of the mid-lower Chang-Jiang Basin and protected fish species in Lake Dongting.

Species	CITES	China	Hunan	IUCN	Endemics
Psephurus gladius	√	I		CR	
Acipenser sinensis	√	I		CR	
Acipenser dabryanus	√	I		CR	
Coilia nasus			√	LC	
Coilia brachygnathus				DD	√
Tenualosa reevesii		I	√	CR	
Neosalanx brevirostris			√	DD	
Anguilla japonica				EN	
Myxocyprinus asiaticus		II	√	CR	
Onychostoma simum			√	NT	
Onychostoma rarum			√	VU	
Spinibarbus caldwelli			√	LC	
Luciobrama macrocephalus		II	√	CR	
Ochetobius elongatus			√	CR	
Decorus tungting			√	EN	√
Megalobrama amblycephala				LC	√
Acheilognathus macropterus				LC	√
Acheilognathus hypselonotus				LC	√
Acheilognathus macromandibularis				LC	√
Microphysogobio tungtingensis			√	DD	√
Saurogobio gracilicaudatus				LC	√
Saurogobio xiangjiangensis			√	LC	
Rhinogobio ventralis		II		EN	
Leptobotia citrauratea				DD	√
Leptobotia rubrilabris		II		VU	
Siniperca roulei			√	NT	
Channa asiatica			√	LC	
Macropodus opercularis			√	NT	
Total	3	8	15		9

Thirty-four historically documented fish species were not collected from Lake Dongting during this field survey; their fate is of particular concern. These species fall within five categories. The first one is migrating species, like *Acipenser dabryanus*, *A. sinensis*, *Coilia nasus*, *Psephurus gladius*, *Tenualosa reevesii* and *Takifugu obscurus*. The main reasons for the extirpation of the first four species in the lake are mentioned above. Although the last two species eluded capture during this field survey, both were

reportedly collected in exceptional years (Ren et al. 2015; Wang et al. 2016; Chen et al. 2020). Since 1970s, more and more dams have been built across the affluents of Lake Dongting and also the Chang-Jiang mainstem (Wang et al. 2019a). The blockage of migration ways and the shrinkage of favourable habitats were the key factors leading to a sharp decrease in the population of the two diadromous fishes (Wang et al. 2016). Small population size makes it difficult for them to migrate for such a long distance from the estuary of Chang-Jiang into Lake Dongting, particularly when all fishes, being of economic importance in the river, were under high pressure from fishing during the past 20 years. The second category is such potamodromous or drifting-egg-spawning fishes as *Luciobrama macrocephalus*, *Ochetobius elongatus*, *Pseudolaubuca engraulis*, *Rhinogobio cylindricus*, *R. ventralis* and *Saurogobio dumerili*, which are susceptible to dam construction. River damming makes inundated reaches shift from lotic to lentic habitat, which have adverse impacts on the spawning of these species. The third category is rheophilic species such as *Decorus tungting*, *Leptobotia rubrilabris*, *Lepturichthys fimbriatus*, *Onychostoma rarum*, *O. simum*, *Spinibarbus caldwelli*, *Tachysurus ussuriensis* and *Zacco acanthogenys*. Their extirpation in Lake Dongting is mainly attributed to river damming in its affluents, which not only led to a remarkable decline in the population of these fishes, but also blocked their short migration into the lake. The fourth category is bitterlings, such as *Acheilognathus barbatus*, *A. chankaensis*, *A. hypselonotus* and *A. tonkinensis*, which depend on freshwater mussels for spawning. The absence of these bitterlings in Lake Dongting may be related to the decrease or disappearance of mussels caused by degrading water quality or sand extraction (Meng et al. 2018; Liu et al. 2020b; Wang and Zhang 2021). The fifth category includes some fishes of economic value, such as *Distoechodon tumirostris* Peters, 1881, *Neosalanx brevirostris*, *N. jordani*, *Plagiognathops microlepis* and *Protosalanx hyalocranius*. These fishes eluded capture mainly due to small population size led by overfishing and habitat loss or degradation. Overall, most of the unsampled fish species during this field survey have ecologically specialised preferences, for example, migratory, rheophilic, carnivorous, drifting-egg-producing or mussel-dependent. These fishes are susceptible to human disturbances and, thus, can act as biological indicators of aquatic ecosystem health. Their lack of samples clearly indicates that the freshwater ecosystem of Lake Dongting has been severely threatened by human perturbations including river damming, overfishing, habitat degradation and sanding dredging.

The Chang-Jiang basin is an area with over 400 million residents, highly impacted by anthropogenic interferences. It is also the most rapidly growing area of China's economic development. The loss of aquatic diversity and, thus, its ecological service function in this river is becoming a pressing challenge. It is urgently needed to take practical actions to conserve the freshwater ecosystem of the Chang-Jiang Basin. To this end, the Chinese government made a decision of implementing the conservation measure of 'ten-year fishing ban' in all natural water bodies of the mainstem and major tributaries of the Chang-Jiang since 2020 (Pan and Liu 2021). Whether it is an effective protection action for conserving the fish diversity of Lake Dongting is of much public concern. In this context, adequate information about the current status of fish

diversity, including species composition, distribution, population size and imperilled status, is an urgent requirement in the future to answer the question. This updated species checklist will be very useful for further biodiversity analysis and conservation of freshwater fishes from Chang-Jiang.

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Supplementary material 1

Table S1

Authors: Xiao Chen

Data type: Table (docx. file)

Explanation note: Table S1. Geographical coordinates of 20 sampling sites in Lake Dongting.

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Revision of the *javanicus* species group of the millipede genus *Glyphiulus* Gervais, 1847, with descriptions of five new species from China (Diplopoda, Spirostreptida, Cambalopsidae)

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Abstract

The *javanicus*-group of *Glyphiulus* is re-assessed and its Chinese component species are presently divided between the following two newly-circumscribed species groups, i.e. the *formosus*- and the *sinensis*-group. The two can be differentiated, based on the diagnostic characters of the first pair of legs in the male. In addition, metatergal crests being complete and the carinotaxy formula on the collum being I–III+P+M are only characteristic of the *formosus*-group. A molecular phylogeny of the genus, based on DNA sequencing of four gene fragments of four genes, allows for *Glyphiulus* to be recovered as a monophyletic group, the phylogenetic relationship being ((Clade A, Clade B), Clade C). Molecular evidence is fully congruent with the morphological one. In addition, based on barcoding data, interspecific p-distances between *Glyphiulus* species amount to 11.2–24.9%, vs. 0–8.2% for intraspecific p-distances. Five new species of *Glyphiulus*, all cavernicolous, are described from China: *G. sinuatoprocessus* Zhao & Liu, **sp. nov.**, *G. conuliformis* Zhao & Liu, **sp. nov.** (both from Guangdong Province), *G. xiniudong* Zhao & Liu, **sp. nov.**, *G. scutatus* Zhao & Liu, **sp. nov.** and *G. portaliformis* Zhao & Liu, **sp. nov.** (all three from Guangxi Zhuang Autonomous Region). The known Chinese species of the *formosus*-group appear to mainly be confined to the South China region.

* These authors have contributed equally to this work.

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Keywords
Cave, DNA barcoding, new record, new species, phylogeny, taxonomy

Introduction

Glyphiulus Gervais, 1847 is the largest genus in the millipede family Cambalopsidae, currently comprising 70 species (Likhitrakarn et al. 2017; Liu and Wynne 2019; Jiang et al. 2021, 2022). They range from southern China in the north to Java and Borneo in the south and southeast, being particularly common in caves and usually very narrow in distribution, except for *G. granulatus* (Gervais, 1847) which is pantropical (Likhitrakarn et al. 2021).

The genus *Glyphiulus* has recently been reviewed and divided into two species groups, based on morphological characteristics alone, namely, the *granulatus*-group and the *javanicus*-group (Golovatch et al. 2007a, b; 2011a, b). The main features to distinguish these two species groups lie in the first pair of legs of the male. The *granulatus*-group is represented by a sternum with two widely separated and curved prongs, coupled with 1- or 2-segmented and strongly reduced telopodite rudiments. On the contrary, species in the *javanicus*-group show a sternum with a pair of fused, paramedian prongs, flanked by 2-segmented leg vestiges or nearly normal 4- or 5-segmented telopodites. Besides this, both groups differ in the structure of the gnathochilarium, collum and metatergal crests, as well as anterior and posterior gonopods, but these distinctions are not too stable.

There are presently 103 gene sequences related to Cambalopsidae species that can be found in NCBI. COI and 28S gene fragments used in studies on the genera *Trachyjulus* Peters, 1864, *Glyphiulus* and *Plusioglyphiulus* Silvestri, 1923 have demonstrated the genus *Trachyjulus* to be monophyletic (Likhitrakarn et al. 2020). Jiang et al. (2020, 2021) differentiated some species between *Hypocambala* Silvestri, 1895 and *Glyphiulus*, based on four gene fragments.

China currently supports the largest number of *Glyphiulus* species in the world, with 46 known species which are mainly distributed in South China's karsts (Golovatch and Liu 2020; Jiang et al. 2022). Of these, 25 species are considered to belong to the *granulatus*-group, vs. about 21 in the *javanicus*-group.

After many years of investigation and sampling across southern China, the authors of the present paper have found out that the Chinese species from the *javanicus*-group could further be subdivided into two reliable groups, namely, the *formosus*- and the *sinensis*-group, based both on morphological and molecular evidence. However, the phylogenetic relationship between the *formosus*-, *granulatus*- and *sinensis*-group seems to be unstable. To substantiate the above new information, the present paper puts on record not only five new species, but it also adds new records for two previously-described species of *Glyphiulus* from caves in southern China. Besides this, a key to all 14 species of the *formosus*-group of *Glyphiulus* known from China is given and their distributions are mapped.

Materials and methods

The material underlying the present study was collected by hand from several caves in southern China and preserved in 95% ethanol. The holotypes and most of the paratypes are deposited in the Zoological Collection of the South China Agricultural University (SCAU), Guangzhou, Guangdong Province, China, with a few paratypes shared with the Zoological Research Museum Koenig (ZFMK), Bonn, Germany. A detailed examination of characters and dissections were performed using a Leica S8 APO stereomicroscope. For scanning electron microscopy (SEM), the samples were cleaned by ethanol and then mounted on aluminium columns. Except for the first new species described in this paper, which was sputter-coated with gold in a Cressington 108 automatic sputter coater, the remaining four new species samples were not coated. SEM micrographs were taken using a ZEISS Sigma 300VP scanning electron microscope (based at ZFMK) or Hitachi TM4000 scanning electron microscope (based at Gongbei Port, Zhuhai City, Guangdong Province, China). After the study, dry SEM material was removed from stubs and returned to alcohol. Line drawings were prepared with a ZEISS Axioskop40 microscope with a camera lucida attached. Photographs of specimens were taken with a Keyence VHX-5000 digital microscope and edited using Adobe Photoshop CS6 software. The terminology used in the text is after Golovatch et al. (2007a, b, 2011a, b), Liu and Wynne (2019), Jiang et al. (2017, 2018, 2020) and Likhitrakarn et al. (2017, 2021). The distribution map was created using QGIS 3.20.1 software.

Genomic DNA was extracted from legs and collum tissue of specimen samples with Qiagen DNeasy Blood and Tissue kit following the manufacturer's extraction protocol. Partial sequences of two mitochondrial genes (COI and 16S) and two nuclear genes (18S and 28S) were amplified and sequenced. The PCR amplification was performed using a T100 thermal cycler (BIO-RAD) with a final reaction volume of 25 µl. Raw sequences were edited and assembled using SeqMan Pro software (Lasergene v. 7.1; DNA Star, Inc., Madison, Wis., USA).

Protein-coding gene sequences (COI) were aligned using the codon-aware programme MACSE v. 2.03 (Ranwez et al. 2018), which preserves reading frame and allows incorporation of sequencing errors or sequences with frameshifts. The more variable sequences (16S, 18S, 28S) were aligned using the online version of MAFFT v. 7.0 (Katoh and Standley 2013) using '—auto' strategy and normal alignment mode. Best partitioning scheme and evolutionary models for six pre-defined partitions were selected using PartitionFinder2 (Lanfear et al. 2017), with all algorithm and AICc criteria.

The analysis involved 37 *Glyphiulus*, two *Plusioglyphiulus* and five *Trachyjulus* COI sequences (18 new sequences and 26 from GenBank). Codon positions included were $1^{st}+2^{nd}+3^{rd}$. All positions containing 'N's were removed for each sequence pair. Uncorrected p-distances of COI markers were calculated using MEGA X (Kumar et al. 2018).

The final aligned dataset included 28 sequences, each with 657 bp of COI (one is not available for SCAUG32), 481 bp of 16S rRNA, 627 bp of 18S rRNA and 1182 bp of 28S rRNA. The combined analysis after these exclusions consisted of 2947 positions. Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were executed by PhyloSuite v.1.2.2 (Zhang et al. 2020). ML analysis was conducted using IQ-TREE

with 1000 bootstrap replications. Bayesian Inference (BI) analysis was implemented by MrBayes 3.2.6 using the Markov Chain Monte Carlo technique (MCMC) (Ronquist et al. 2012) under partition model (two parallel runs, 2,000,000 generations), in which the initial 25% of sampled data were discarded as burn-in.

All analysed species, voucher numbers/taxonomy ID, and Genbank accession numbers are listed in Table 1.

Table 1. List of the species used for molecular phylogenetic analyses and their relevant information. *: new sequence; /: absent.

Voucher number	Species	GenBank accession numbers			
		COI	16S	18S	28S
SCAUG33*	*Glyphiulus* sp. 1	ON255879	ON617345	ON263093	ON263226
SCAUWL49*	*Glyphiulus* sp. 1	ON255892	ON617360	ON263096	ON263229
SCAUWL20*	*Glyphiulus* sp. 2	ON256155	ON617353	ON263101	ON263239
SCAUG32*	*Glyphiulus zorzini*	/	ON263092	ON263225	ON263092
SCAUWL23*	*Glyphiulus zorzini*	ON255887	ON263095	ON263228	ON263095
SCAUG39*	*Glyphiulus* sp. 3	ON255880	ON263094	ON263227	ON263094
IBGASJXK051	*Glyphiulus guangnanensis*	MN725096	MN733292	MN733302	MN733282
SCAUWL38*	*Glyphiulus impletus*	ON255889	ON617357	ON263088	ON263235
SCAUWL39*	*Glyphiulus impletus*	ON255890	ON617358	ON263090	ON263236
IBGASJXK002	*Glyphiulus impletus*	MN725095	MN733291	MN733301	MN733281
SCAUG78*	*Glyphiulus xiniudong* Zhao & Liu, sp. nov.	ON255885	ON617351	ON263085	ON263232
SCAUWL37*	*Glyphiulus calceus*	ON255888	ON617356	ON263089	ON263234
IBGASJXK061	*Glyphiulus calceus*	MN725098	MN733294	MN733304	MN733284
SCAUG72*	*Glyphiulus scutatus* Zhao & Liu, sp. nov.	ON255884	ON617350	ON263084	ON263231
SCAUWL30*	*Glyphiulus scutatus* Zhao & Liu, sp. nov.	ON256153	ON617355	ON263087	ON263238
IBGASJXK059	*Glyphiulus foetidus*	MN725097	MN733293	MN733303	MN733283
SCAUWL40*	*Glyphiulus portaliformis* Zhao & Liu, sp. nov.	ON255891	ON617359	ON263091	ON263237
JXK275	*Glyphiulus sinuatoprocessus* Zhao & Liu, sp. nov.	OM746179	/	/	/
SCAUWL02*	*Glyphiulus sinuatoprocessus* Zhao & Liu, sp. nov.	ON255886	ON617352	ON263086	ON263233
SCAUG24*	*Glyphiulus conuliformis* Zhao & Liu, sp. nov.	ON255878	ON617343	ON263083	ON263230
SCAUG15*	*Glyphiulus deharvengi*	ON255877	ON617342	ON263097	ON263221
IBGASJXK310	*Glyphiulus deharvengi*	MN725104	MN733300	MN733310	MN733290
IBGASJXK072	*Glyphiulus quadrohamatus*	MN725099	MN733295	MN733305	MN733285
IBGASJXK196	*Glyphiulus granulatus*	MN725102	MN733298	MN733308	MN733288
SCAUG50*	*Glyphiulus proximus*	ON255881	ON617347	ON263098	ON263222
SCAUG61*	*Glyphiulus proximus*	ON255882	ON617348	ON263099	ON263223
SCAUG62*	*Glyphiulus speobius*	ON255883	ON617349	ON263100	ON263224
CAM022	*Glyphiulus duangdee*	MN893779	/	/	/
CAM030	*Glyphiulus sattaa*	MN893778	/	/	/
JXK282	*Glyphiulus formosus*	MN905180	/	/	/
JXK375	*Glyphiulus fortis*	OM746180	/	/	/
JXK376	*Glyphiulus fortis*	OM746181	/	/	/
JXK377	*Glyphiulus fortis*	OM746182	/	/	/
XK046	*Glyphiulus hainanensis*	OM746174	/	/	/
XK047	*Glyphiulus hainanensis*	OM746175	/	/	/
XK048	*Glyphiulus hainanensis*	OM746176	/	/	/
XK049	*Glyphiulus hainanensis*	OM746177	/	/	/
XK050	*Glyphiulus hainanensis*	OM746178	/	/	/
IBGAS JXK517	*Cambala annulata*	MT683305	MT676457	MT676456	MT676769
IBGAS JXK165	*Hypocambala zizhongi*	MN725101	MN733297	MN733307	MN733287
CAM031	*Plusioglyphiulus erawan*	MN893780	/	/	/
CAM021	*Plusioglyphiulus saksit*	MN893781	/	/	/
CAM059	*Trachyjulus bifidus*	MN893771	/	/	/
CAM061	*Trachyjulus bifidus*	MN893772	/	/	/
CAM027	*Trachyjulus phylloides*	MN893773	/	/	/
CAM079	*Trachyjulus unciger*	MN893774	/	/	/
CAM070	*Trachyjulus magnus*	MN893775	/	/	/

Results

Morphologic analysis

The Chinese *Glyphiulus* species could be divided into three morphologically distinct species groups, mainly based on the structure of male legs 1 (Fig. 1): the *granulatus*-group (Fig. 1A, represented by a coxosternum bearing strongly separated, distally evidently curved prongs, each side with or without a rather small leg vestige), the *formosus*-group (Fig. 1B, showing a pair of small, fused, paramedian, subunciform prongs directed forward, flanked by strongly separated, rudimentary, 2-segmented leg vestiges) and the *sinensis*-group (Fig. 1C, D, telopodites often complete or nearly so, with or without claw; coxosternum with a pair of relatively large and stout, paramedian, basically non-fused outgrowths directed laterad).

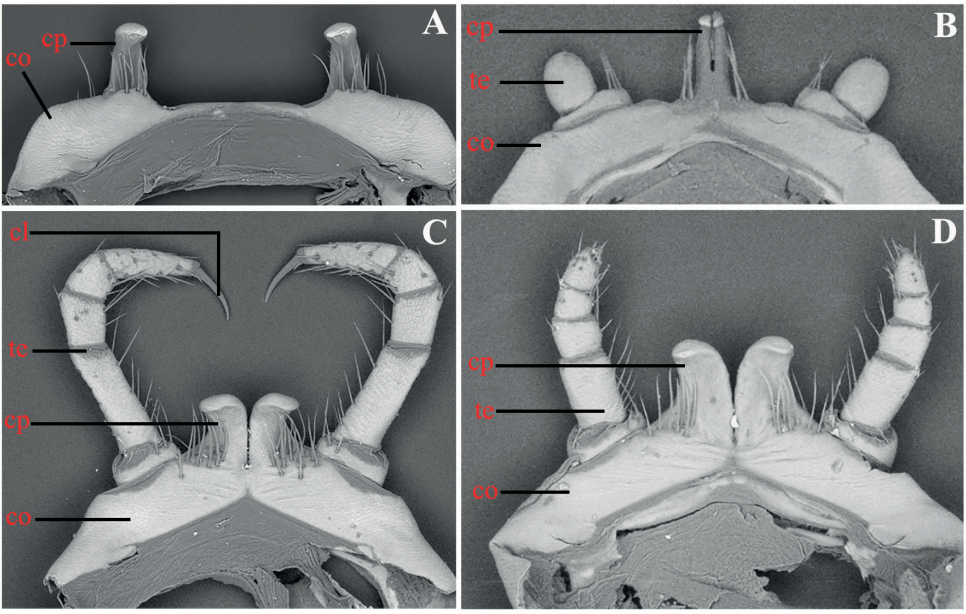

Figure 1. ♂ leg 1 of *Glyphiulus*, frontal view **A** *granulatus*-group **B** *formosus*-group **C, D** *sinensis*-group. Abbreviations: cl: claw, co: coxosternum, cp: coxosternum process, te: telopodite.

Species of the *formosus*-group are also distinguished by the following characteristics (Fig. 2): (1) Rather complete crests on collum, carinotaxy formula I–III+P+M (Fig. 2A), vs. crests incomplete, carinotaxy formula either I–IV+5c+6a+pc+ma (Fig. 2B) or something similar. (2) Metatergal carinotaxy formula 2/2+I/i+3/3 (Fig. 2C) or nearly so, but never 2/2+I/i+4/3. (3) Epiproct basically with a strong central tubercle dorsally (Fig. 2D). (4) Anterior gonopod with a scalloped shield-shaped coxosternum or coxite with an apicomesal process and posterior gonopods always with flagella.

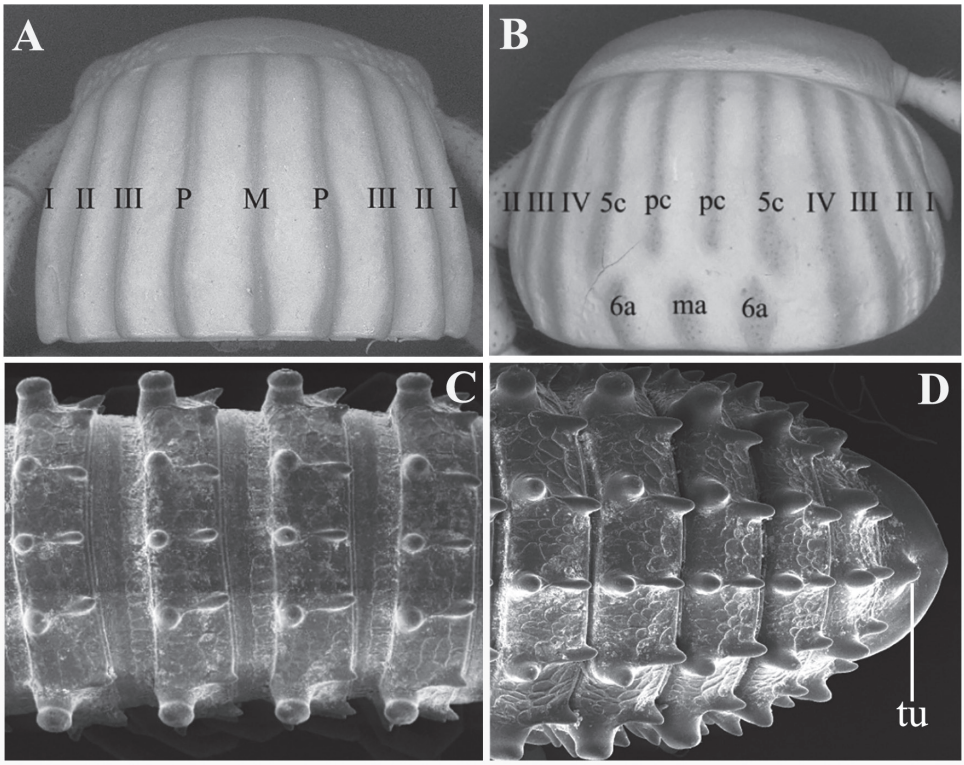

Figure 2. Partial trunk morphology of *Glyphiulus* **A** collum's carinotaxy formula I–III+P+M **B** collum's carinotaxy formula I–IV+5c+6a+pc+ma **C** metaterga carinotaxy formula 2/2+I/i+3/3, dorsal view **D** epiproct with a strong central tubercle dorsally. Abbreviation: tu: tubercle.

Distance analysis

The number of base differences per site between sequences (absolute p-distance) is shown in Suppl. material 1: Table S1. Uncorrected pairwise distances between *Glyphiulus* and the other two genera of cambalopsid species were found to be generally high, varying 17.7–27.1% between *Glyphiulus* and *Trachyjulus* and 17.2–24.7% between *Glyphiulus* and *Plusioglyphiulus*.

Amongst the *Glyphiulus* species concerned, *G. foetidus* showed the highest divergence from the other *Glyphiulus* species, ranging from 14.4–24.9%. The lowest divergence was 11.2% between *Glyphiulus* sp.1 SCAUG33, SCAUWL49 and *G. Glyphiulus* sp. 2 SCAUWL20.

The intraspecific divergence of *Glyphiulus* species was found to range from 0.0–8.2%. Intraspecific distances in our dataset between individuals of *G. impletus* vary 2.4–8.2%, 2.1% in *G. calceus*, 0.0–3.3% in *G. hainanensis* and 6.6% in *G. scutatus* Zhao & Liu, sp. nov.

Phylogenetic analysis

As the phylogenetic tree estimated by both the Maximum Likelihood (ML) and Bayesian Inference (BI) analyses revealed equivalent topologies, we only present the BI tree here (Fig. 3). The monophyly of the genus *Glyphiulus* was strongly supported by 0.98 bpp for BI, but a little weaker at 59% bootstrap values for ML. The analysed species of *Glyphiulus* can be clearly divided into three clades, the phylogenetic relationships amongst them being ((Clade A, Clade B), Clade C). The three clades can be defined as three species groups, namely, the *formosus*-group (Clade A), the *granulatus*-group (Clade B) and the *sinensis*-group (Clade C). The former two clades are sister-groups with 0.58 bpp for BI and a 26% bootstrap support.

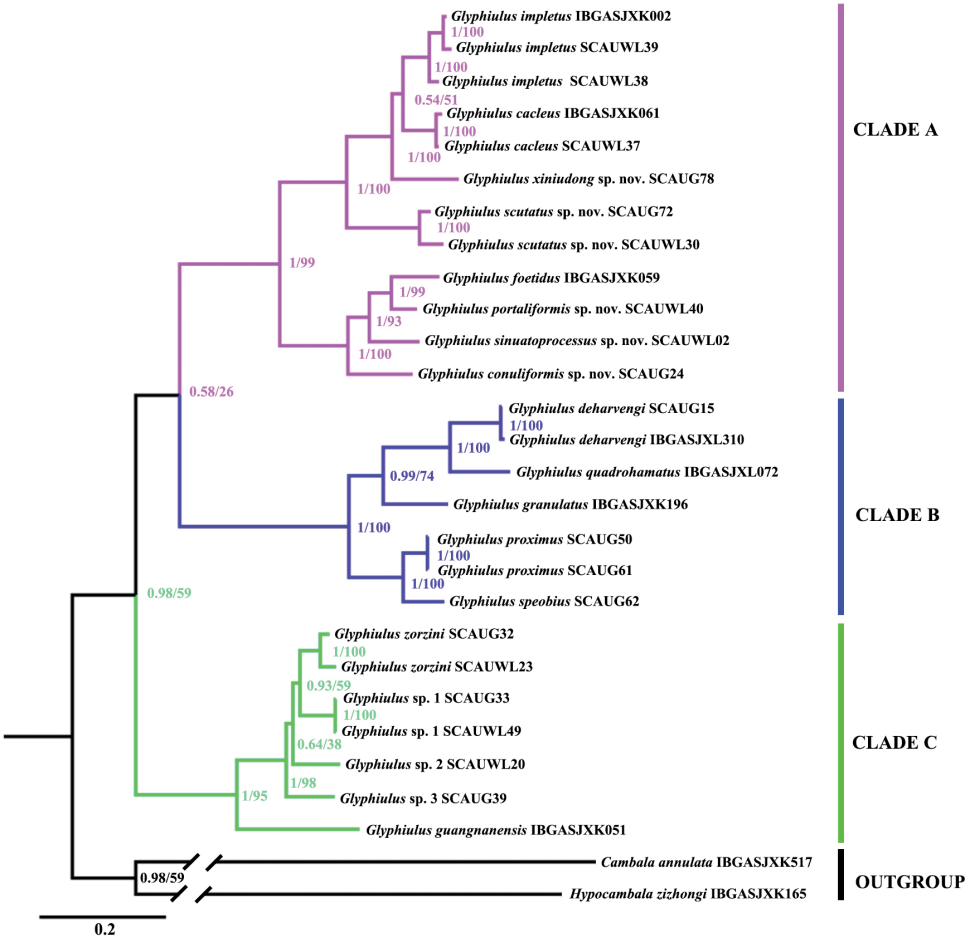

Figure 3. Phylogenetic reconstruction of the genus *Glyphiulus* species, based on four gene fragments. Numbers on nodes indicate Bayesian posterior probability (bpp) from Bayesian Inference analysis (BI) and bootstrap values from Maximum Likelihood (ML).

Within Clade A, almost all internal nodes were strongly supported: 0.54–1 bpp for BI and 51–100% bootstrap values for ML. *Glyphiulus foetidus* and three new species (*G. portaliformis* Zhao & Liu, sp. nov., *G. sinuatoprocessus* Zhao & Liu, sp. nov. and *G. conuliformis* Zhao & Liu, sp. nov.) found their places in the basal part of the tree, followed by *G. scutatus* Zhao & Liu, sp. nov. and a sister clade of *G. xiniudong* Zhao & Liu, sp. nov., *G. calceus* and *G. impletus* (Fig. 3).

In the single gene (COI) tree from the ML analysis, *Trachyjulus* species served as an outgroup and were clearly prioritised (Fig. 4). However, *Plusioglyphiulus* (Clade BC) became the sister clade of the ingroup which, together with the *granulatus*-group (Clade BA) and the *sinensis*-group (Clade BB), formed Clade B. In addition, clade A (the *formosus*-group) was obviously divided into two small clades. Clade AA included *G. impletus*, *G. fortis*, *G. calceus*, *G. xiniudong* Zhao & Liu, sp. nov., *G. hainanensis*, *G. formosus* and *G. scutatus* Zhao & Liu, sp. nov., while Clade AB consisted of *G. conuliformis* Zhao & Liu, sp. nov., *G. sinuatoprocessus* Zhao & Liu, sp. nov., *G. portaliformis* Zhao & Liu, sp. nov. and *G. foetidus*.

Molecular evidence is thereby fully congruent with the morphological one.

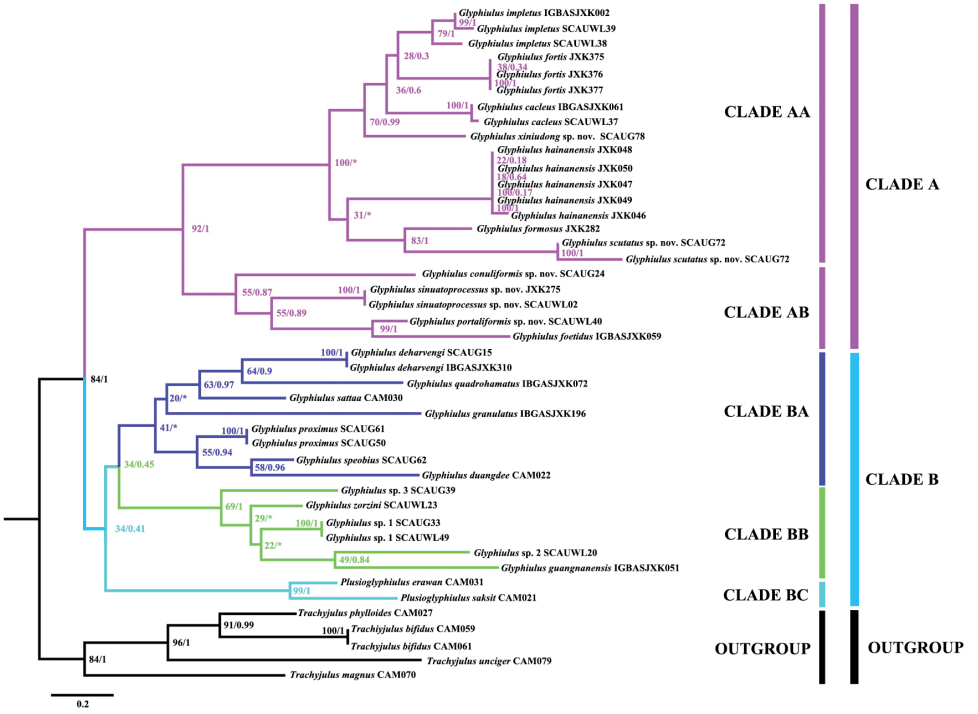

Figure 4. Phylogenetic tree of the mitochondrial COI gene for *Glyphiulus* constructed using Maximum Likelihood analysis. Numbers on branches are estimates of the bootstrap values and bpp of a clade, respectively. *: inconsistent construction of ML and BI.

Taxonomic treatment

Family Cambalopsidae Cook, 1895
Genus *Glyphiulus* Gervais, 1847

***Glyphiulus sinuatoprocessus* Zhao & Liu, sp. nov.**
http://zoobank.org/A8C259A1-4697-4890-8E87-442953B8F104
Figs 5A, 6, 7

Type material. ***Holotype*** ♂ (SCAU GD7), China, Guangdong Province, Qingyuan City, Yangshan County, Taiping Town, Niubi Village, Cave Niubi Yan, 24°10'23.93"N, 112°33'27.50"E, 100 m alt., 2014-XII-27, leg. Tian Mingyi, Liu Weixin, Huang Sunbin & Wang Xinhui. ***Paratypes*:** 1 ♂ (ZFMK), 1 ♂, 1 ♀ (SEM), 2 ♂, 1 ♀ (SCAU GD7), same data as the holotype.

Etymology. To emphasise the apicomesal process of the anterior gonopod coxite being hook-shaped.

Diagnosis. Differs from congeners of the *formosus*-group by the anterior gonopod showing a high and digitiform process, in which the tip is hook-shaped, coupled with a short, distally pectinate flagellum of the posterior gonopod. Based on molecular evidence, *G. sinuatoprocessus* Zhao & Liu, sp. nov. differs from all other *Glyphiulus* species analysed in a > 16.1% uncorrected p-distance of the COI barcoding gene.

Description. Length ca. 33.0–42.0 (♂) or 45.0–50.0 mm (♀), mid-body rings round in cross-section, their width and height similar, 2.2–2.3 mm (♂) or 2.5–2.8 mm (♀). Body with 52–65 (♂) or 70–76 (♀) podous + 1–4 apodous rings + telson. Colouration orange-brownish to brownish, anterior part of body much darker in alcohol, red-brownish (Fig. 5A).

Head surface smooth (Fig. 7A). Labrum with 4 or 5 teeth anteromedially. Ocellaria blackish, with 12–15 (♂) or 17–23 (♀) ommatidia arranged in 1–3 irregular linear rows (Fig. 7A). Antennae relatively long, reaching back to ring 3; in length, antennomeres 5 > 3 > 2 > 4 > 6 > 1 > 7. Antennomeres 5–7 each with a distodorsal field or corolla of bacilliform sensilla (sensory bacilli). Antennomere 7 with four sensory cones (Fig. 7A). Gnathochilarium with a separate promentum, lamellae linguales and promentum densely setose, a few setae on mentum (Fig. 7B). Mandible with a large external tooth and an internal tooth, the latter provided with nine cusps.

***Collum*:** crests complete and evident; carinotaxy formula I–III+P+M (Fig. 7A). Following metaterga strongly crested; carinotaxy formula 2/2+I/i+3/3 (Fig. 6). Ozoporiferous tubercles very large, much higher than wide. Tegument delicately alveolate-areolate, fine longitudinal striations in front of stricture. Metatergal setae absent. Rings 2 and 3 with long pleural flaps (Fig. 6D). Limbus more or less regularly denticulate.

Epiproct simple, very narrow, caudal edge uneven, with a strong central tubercle dorsally (Fig. 6C). Paraprocts rather regularly convex, each with several irregular rows of setae. Hypoproct transversely bean-shaped, with 3+3 small setae near caudal margin.

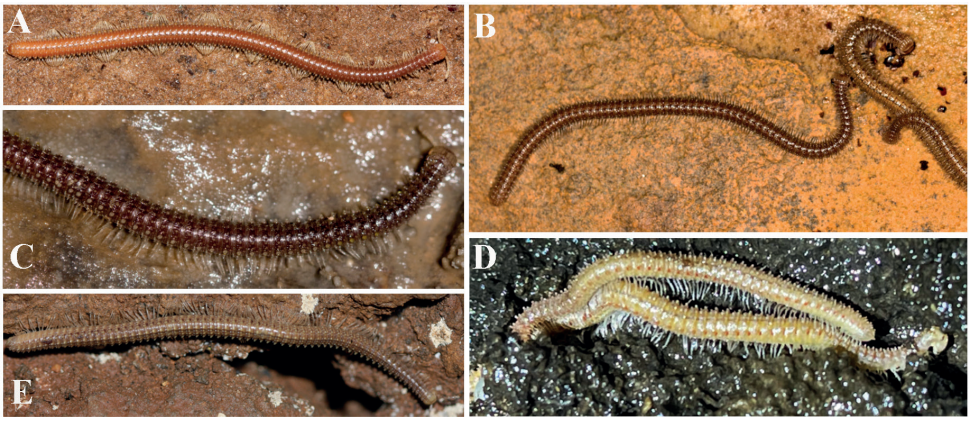

Figure 5. Pictures of live animals **A** *G. sinuatoprocessus* Zhao & Liu, sp. nov. from Cave Niubi Yan **B** *G. scutatus* Zhao & Liu, sp. nov. from Cave Bianfu Dong **C** *G. portaliformis* Zhao & Liu, sp. nov. from Cave Baiyan Dong **D** *G. xiniudong* Zhao & Liu, sp. nov. from Cave Xiniu Dong **E** *G. conuliformis* Zhao & Liu, sp. nov. from Cave Yanzi Dong.

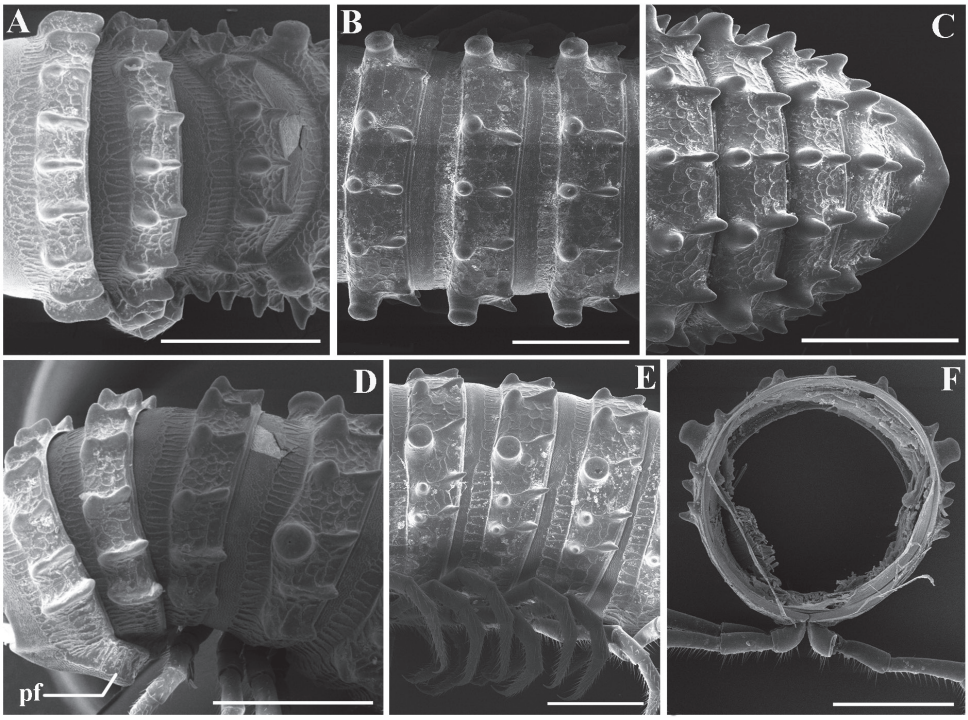

Figure 6. *Glyphiulus sinuatoprocessus* Zhao & Liu, sp. nov., ♂ paratype **A** rings 2–4, dorsal view **B, E** mid-body rings, dorsal and lateral views, respectively **C** posterior body rings, dorsal view **D** rings 2–5, sublateral view **F** cross-section of a mid-body ring, caudal view. Scale bars: 1 mm. Abbreviation: pf: pleural flap.

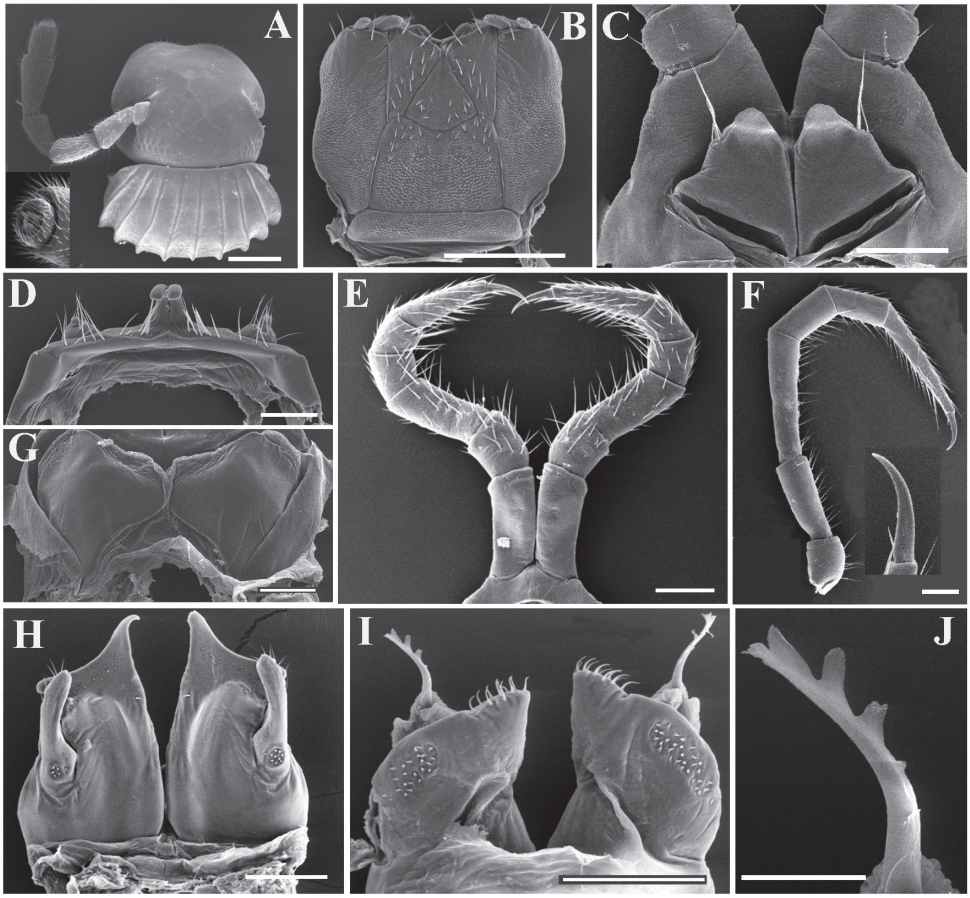

Figure 7. *Glyphiulus sinuatoprocessus* Zhao & Liu, sp. nov., ♂ paratype **A** head, collum and antenna **B** gnathochilarium **C** penes **D** leg 1, frontal view **E** leg 3, frontal view **F** mid-leg **G** ♀ paratype, vulvae **H** anterior gonopods, caudal view **I** posterior gonopod, caudal view **J** flagellum. Scale bars: 0.5 mm (**A, B**), 0.2 mm, (**C–I**), 0.1 mm (**J**).

Legs slender, about 1.2 times as long as mid-body height; claw with a small accessory spine at base, about 1/3 as long as claw (Fig. 7F). ♂ legs 1 very strongly reduced, represented by a sternum showing a pair of small, fused, paramedian, subunciform prongs directed forward, with about 10–11+10–11 long setae at base; flanked by strongly separated, rudimentary, 2-segmented leg vestiges, first segment being much larger (Fig. 7D). ♂ legs 2 slightly hypertrophied, coxae large; penes small, much shorter than coxae, oblong-subtrapeziform, each with two strong setae distolaterally (Fig. 7C). ♂ legs 3 modified through coxae being especially slender and elongate (Fig. 7E). ♂ femora 6 and 7 normal, without modifications.

Anterior gonopods (Fig. 7H) with a broad and plate-shaped coxosternum supporting about 18–20 microsetae near distal margin; apicomesal process of coxite very

high, digitiform, tip narrow and hook-shaped. Telopodite large and coiled, 1-segmented, lateral in position, slightly higher than lateral corner of coxite and with a field of 5–10 microspinules at base and 6–8 strong setae apically.

Posterior gonopods (Fig. 7I) compact. Coxite subquadrate, with a circular field of 12–15 basolateral microspinules in frontal view and an elongated field of 20–25 microspinules in caudal view; apical and mesal parts of coxite with dense, strong and curved setae. Lamelliform lobe membranous, with a short, distally pectinate flagellum (Fig. 7J).

Vulvae very simple, bare, modestly emarginate medially (Fig. 7G).

Remarks. In the absence of direct troglomorphic traits, this species can only be considered as troglophilic at most. In the Guangdong Province and in Hong Kong, only *G. formosus* and *G. granulatus* have been recorded as yet.

Glyphiulus conuliformis Zhao & Liu, sp. nov.

http://zoobank.org/7FCC4E95-5E67-450E-8003-D5F41C71E5B1
Figs 5E, 8, 9

Type material. ***Holotype*** ♂ (SCAU G24), China, Guangdong Province, Yangjiang City, Yangchun, Cave Yanzi Dong, 22°5'N, 111°36'50"E, 400 m alt., 2016-X-29, leg. Tian Mingyi, Chen Mengzhen & Wang Dianmei. ***Paratypes*:** 2 ♂, 26 ♀ (SCAU G24), same data as the holotype.

Etymology. To emphasise the metatergal anterior tubercles being very sharp and coniform.

Diagnosis. Differs from congeners of the *formosus*-group by the conical shape of the anterior tubercles of metaterga and by the first segment of the telopodite being significantly enlarged in ♂ legs 1, combined with the anterior gonopod process being slender, finger-shaped and curved inwards distally. Based on molecular evidence, *G. conuliformis* Zhao & Liu, sp. nov. differs from all other *Glyphiulus* species analysed in a > 17.0% uncorrected p-distance of the COI barcoding gene.

Description. Length ca. 32.5–37.0 (♂) or 47.0–58.0 mm (♀), mid-body rings round in cross-section, their width and height similar, 1.7–2.0 mm (♂) or 2.2–3.0 mm (♀). Body with 48–53 (♂) or 57–67 (♀) podous + 2–4 apodous rings + telson. Colouration dark brownish, head and legs yellowish (Fig. 5E).

Head surface smooth. Labrum with four teeth anteromedially (Fig. 9C). Ocellaria blackish, with 12–22 ommatidia arranged in 2–3 irregular linear rows (Fig. 9A). Antennae slender, slightly clavate, reaching back to middle of rings 2 and 3; in length, antennomeres 5 > 3 > 2 ≈ 4 > 6 > 1 > 7. Antennomeres 5–7 each with a distodorsal field or corolla of bacilliform sensilla (sensory bacilli). Antennomere 7 with four sensory cones (Fig. 9B). Gnathochilarium with a separate promentum, polytrichous on promentum and mentum, lamellae linguales each with 6–7 setae (Fig. 9C). Mandible not dissected.

***Collum*:** crests complete and evident; carinotaxy formula I–III+P+M (Figs 8A, B). Following metaterga strongly crested, anterior tubercles very sharp and coniform;

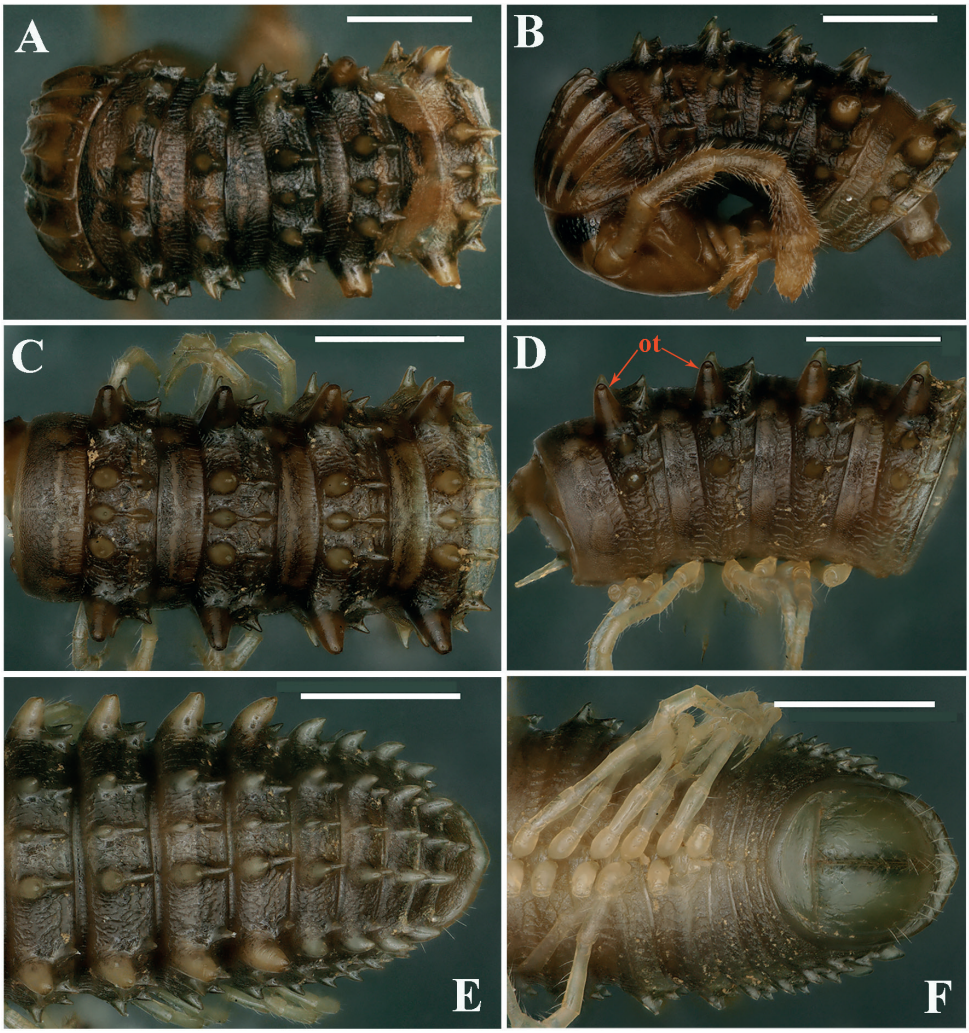

Figure 8. *Glyphiulus conuliformis* Zhao & Liu, sp. nov., ♂ paratype **A, B** anterior body rings, dorsal and lateral views **C, D** mid-body rings, dorsal and lateral views, respectively **E, F** posterior body rings, dorsal and ventral views, respectively. Scale bars: 1 mm. Abbreviation: ot: ozoporiferous tubercle.

carinotaxy formula 2/2+I/i+3/3 (Fig. 8). Ozoporiferous tubercles very large, coniform (Figs 8C, D). Tegument delicately alveolate-areolate, fine longitudinal striations in front of stricture. Rings 2 and 3 with long pleural flaps.

Epiproct simple, caudal edge with a very low central protrusion, dorsally with an obvious sharp tubercle (Fig. 8E). Paraprocts regularly convex, each with several irregular rows of setae (Fig. 8F). Hypoproct transversely bean-shaped, with 3+3 small setae.

Legs slender, about 1.3 times as long as mid-body height; claw with a small accessory spine at base, about 1/6 as long as claw (Fig. 9D). ♂ legs 1 very strongly reduced, represented by a sternum showing a pair of small, unfused, paramedian, subunciform

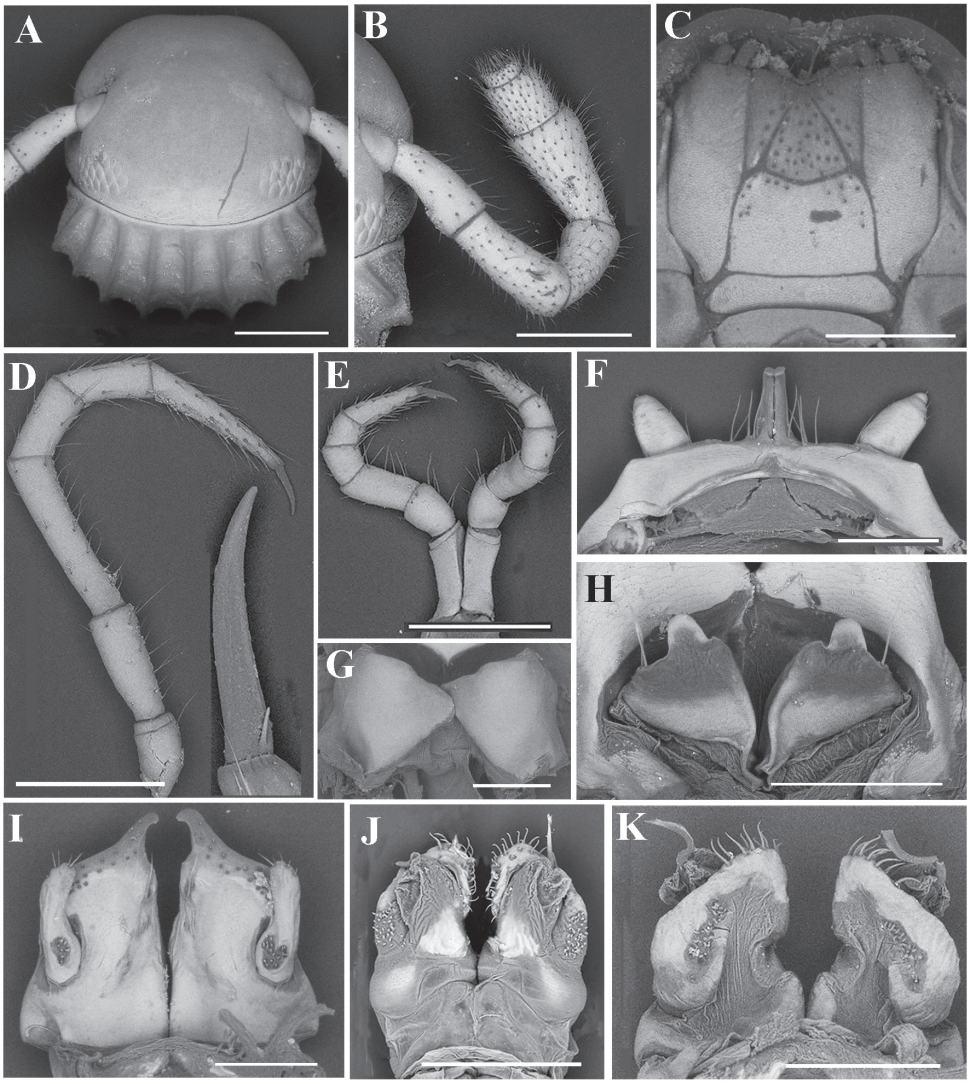

Figure 9. *Glyphiulus conuliformis* Zhao & Liu, sp. nov., ♂ paratype **A** head and collum **B** right antenna, oral view **C** gnathochilarium **D** mid-leg and claw **E** leg 3, caudal view **F** leg 1, frontal view **G** ♀ paratype, vulvae **H** penes **I** anterior gonopods, caudal view **J, K** posterior gonopods, frontal and caudal views, respectively. Scale bars: 0.5 mm (**A–E**), 0.2 mm (**F–K**).

prongs directed forward, with about 4–5+4–5 long setae at base; flanked by strongly separated, rudimentary, 2-segmented, asymmetric leg vestiges, first segment significantly enlarged (Fig. 9F). ♂ legs 2 slightly hypertrophied, coxae large; penes rather small, much shorter than coxae, oblong-subtrapeziform, each with 1 or 2 strong setae distolaterally (Fig. 9H). ♂ legs 3 modified through coxae being especially slender and elongate (Fig. 9E). ♂ femora 6 and 7 normal, without modifications.

Anterior gonopods (Fig. 9I) with a broad and plate-shaped coxosternum supplied with about 14–16 microsetae near distal margin; apicomesal process of coxite high, slender and digitiform, curved inwards distally. Telopodite very large and stout, coiled, 1-segmented, lateral in position, almost parallel to lateral corner of coxite, with a field of 8–16 microspinules at base and five strong setae apically.

Posterior gonopods (Figs 9J, K) compact. Coxite subtrapezoid, with a longitudinal field of 22–24 basolateral microspinules in frontal view and a slanted field of 32–36 median microspinules in caudal view; apical and mesal parts of coxite with dense, strong and curved setae. Lamelliform lobe membranous, with a rather short and broad flagellum.

Vulvae very simple, bare, M-shaped (Fig. 9G).

Remark. In the absence of direct troglomorphic traits, this species can only be considered as troglophilic at most.

Glyphiulus xiniudong **Zhao & Liu, sp. nov.**
http://zoobank.org/6F0C1D58-BCAB-4FF0-AA4E-22D189E7202D
Figs 5D, 10, 11

Type material. ***Holotype*** ♂ (SCAU G78), China, Guangxi Zhuang Autonomous Region, Laibin City, Wuxuan County, Cave Xiniu Dong, 23°33'N, 109°32'55"E, 100 m alt., 2021-I-15, leg. Tian Mingyi, Liu Weixin & Zhao Yi. ***Paratypes*:** 4 ♂, 30 ♀ (SCAU G78), same data as the holotype.

Etymology. To emphasise the provenance of this species from the "Xiniu" Cave, in Chinese meaning "rhinoceros"; noun in apposition.

Diagnosis. Differs from congeners of the *formosus*-group by the metazonae with an obvious, corrugate, carved texture and by the leg claw with a rather large accessory spine, coupled with a long subtriangular coxosternum of the anterior gonopod, the latter process being narrow and digitiform. Based on molecular evidence, *G. xiniudong* Zhao & Liu, sp. nov. differs from all other *Glyphiulus* species analysed in a > 14.7% uncorrected p-distance of the COI barcoding gene.

Description. Length ca. 28.0–31.0 (♂) or 26.0–41.0 mm (♀), mid-body rings round in cross-section, their width and height similar, 1.1–1.3 (♂) or 1.2–1.8 mm (♀). Body with 42–54 (♂) or 45–64 (♀) podous + 0–3 apodous rings + telson. Colouration purple-brownish, legs almost transparent (Fig. 5D).

Head surface smooth. Labrum with four teeth anteromedially. Ocellaria blackish, with 9–13 ommatidia arranged in 1–2 irregular linear rows (Figs 10B, 11A). Antennae short, slightly clavate, reaching back to caudal margin of ring 2; in length, antennomeres 5 > 3 > 4 ≈ 2 > 6 > 1 > 7. Antennomeres 5–7 each with a distodorsal field or corolla of bacilliform sensilla (sensory bacilli). Antennomere 7 with four sensory cones (Fig. 11B). Gnathochilarium with a separate promentum, polytrichous on promentum and mentum, lamellae linguales each with 6–7 setae (Fig. 11B). Mandible not dissected.

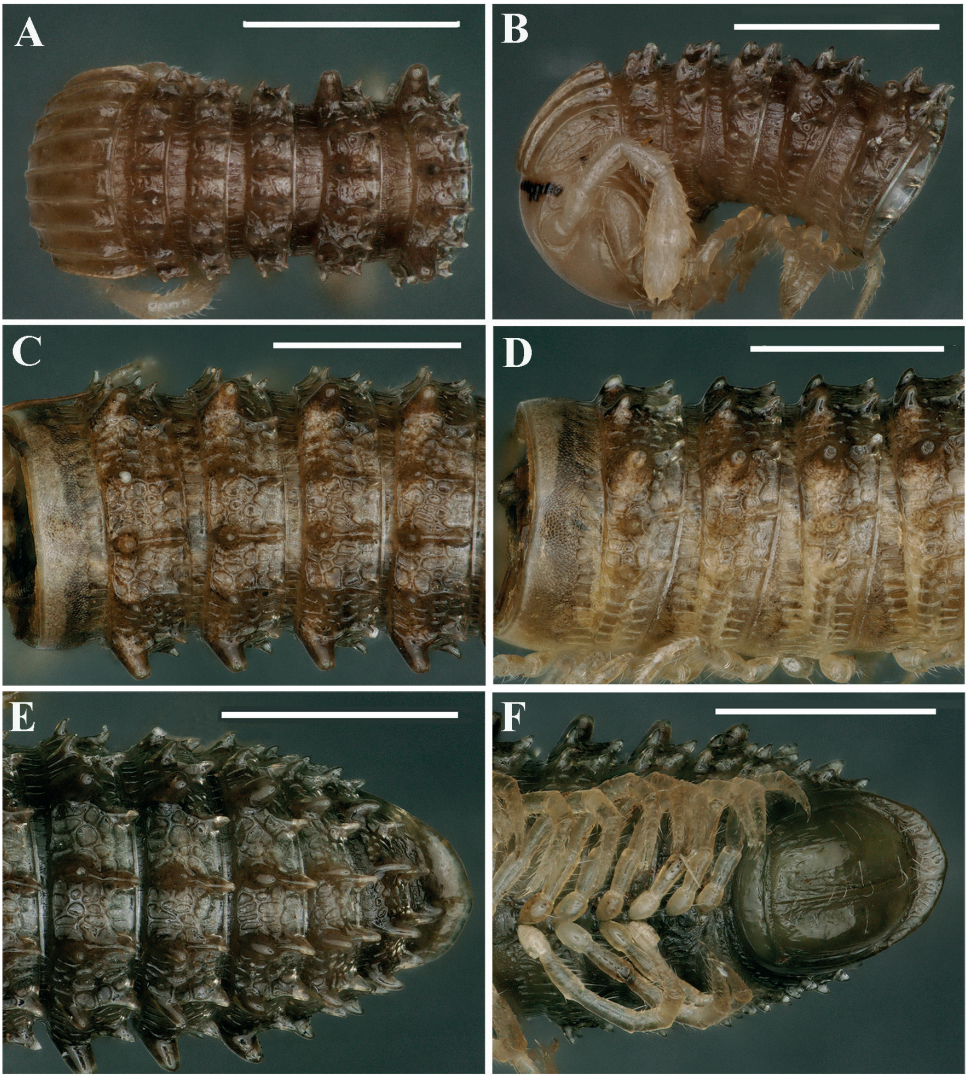

Figure 10. *Glyphiulus xiniudong* Zhao & Liu, sp. nov., ♂ paratype **A, B** anterior body rings, dorsal and lateral views **C, D** mid-body rings, dorsal and lateral views, respectively **E, F** posterior body rings, dorsal and ventral views, respectively. Scale bars: 1 mm.

***Collum*:** crests complete and evident; carinotaxy formula I–III+P+M (Figs 10A, 11A). Following metaterga strongly crested; carinotaxy formula 2/2+I/i+3/3 (Fig. 10). Ozoporiferous tubercles very large, coniform. Prozonae delicately alveolate-areolate, fine longitudinal striations in front of stricture. Metazonae with an obvious, corrugate, carved texture (Fig. 10). Rings 2 and 3 with long pleural flaps.

Epiproct simple, caudal edge uneven, with an obvious central tubercle dorsally (Fig. 10E). Paraprocts regularly convex, each with several irregular rows of setae. Hypoproct transversely bean-shaped, with 4+4 small setae (Fig. 10F).

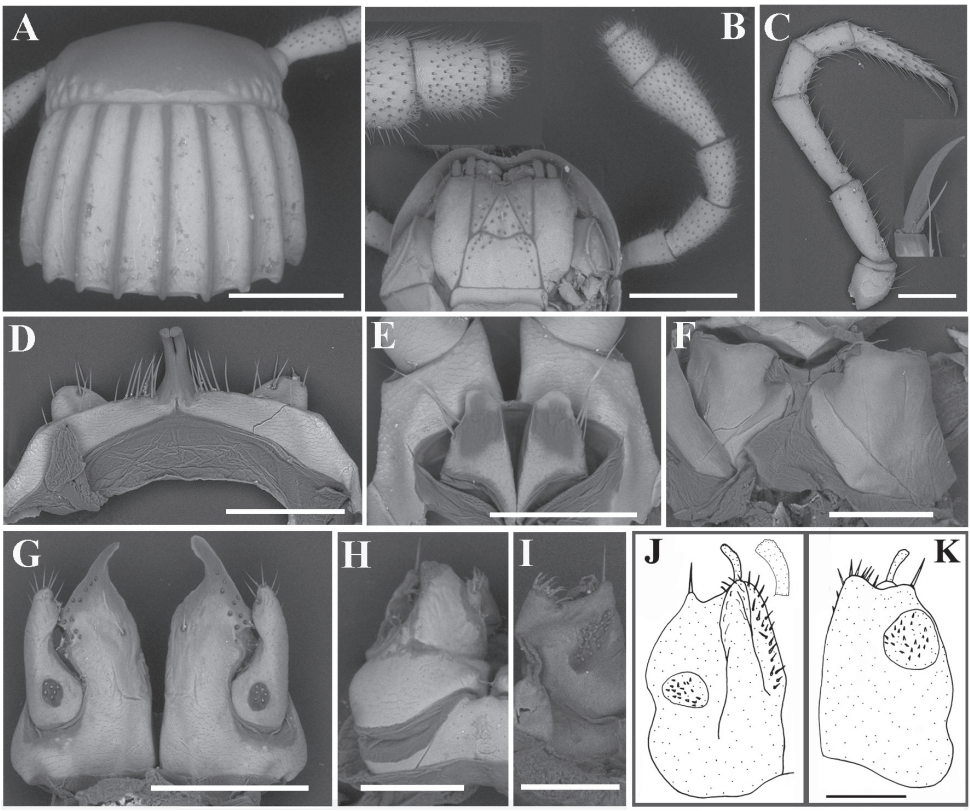

Figure 11. *Glyphiulus xiniudong* Zhao & Liu, sp. nov., ♂ paratype **A** head and collum **B** gnathochilarium and left antenna **C** mid-body leg and claw **D** leg 1, frontal view **E** penes **F** ♀ paratype, vulvae **G** anterior gonopods, caudal view **H, J** posterior gonopod, frontal view **I, K** posterior gonopod, caudal view. Scale bars: 0.5 mm (**A, B**), 0.2 mm (**C–I**), 0.1 mm (**J, K**).

Legs short, about as long as mid-body height; claw with a relatively large accessory spine at base, about half as long as claw (Fig. 11C). ♂ legs 1 very strongly reduced, represented by a sternum showing a pair of small, more slender, fused, paramedian, subunciform prongs directed forward, with about 7–9+7–9 long setae at base; flanked by strongly separated, rudimentary, 1-segmented leg vestiges, with some setae (Fig. 11D). ♂ legs 2 slightly hypertrophied, coxae large; penes rather small, much shorter than coxae, oblong-subtrapeziform, each with three strong setae distolaterally (Fig. 11E). ♂ legs 3 modified through coxae being especially slender and elongate. ♂ femora 6 and 7 normal, neither modifications.

Anterior gonopods (Fig. 11G) with a long subtriangular coxosternum with about 9–11 microsetae near distal margin; apicomesal process of coxite very high, rather narrow and digitiform, modestly curved inwards. Telopodite very large, stout and coiled, 1-segmented, lateral in position, much higher than lateral corner of coxite, with a field of six microspinules at base and 6–7 strong setae apically.

Posterior gonopods (Figs 11H, I) compact. Coxite subquadrate, with a circular field of about 10–15 basolateral microspinules in frontal view; with a field of 19 me-

dian microspinules and an apicolateral very strong and long seta in caudal view; apical and mesal parts of coxite with dense, strong and curved setae. Lamelliform lobe membranous, with a rather small, spine-like flagellum.

Vulvae very simple, bare, modestly emarginate medially (Fig. 11F).

Remark. In the absence of direct troglomorphic traits, this species can only be considered as troglophilic at most.

Glyphiulus scutatus Zhao & Liu, sp. nov.

http://zoobank.org/9D2EE1BE-88CA-43E0-B7EC-41EBA177FD3E
Figs 5B, 12, 13

Type material. ***Holotype*** ♂ (SCAU WL30), China, Guangxi Zhuang Autonomous Region, Hechi City, Du'an Yao Autonomous County, Napang Dong, 24°08'22"N, 107°51'07"E, 650 m alt., 2015-VII-26, leg. Chen Jujian, Wang Xinhui & Tang Mingruo. ***Paratypes*:** 2 ♂, 12 ♀ (SCAU WL30), same data as the holotype. 3 ♂, 4 ♀ (SCAU G72), same County, Cave Bianfu Dong, 24°01'55"N, 108°20'12"E, 550 m alt., 2017-VIII-18, leg. Tian Mingyi, Huang Sunbin, Wang Dianmei & Chen Mengzhen.

Etymology. To emphasise the anterior gonopod showing a scalloped and shield-shaped coxosternum.

Diagnosis. Differs from congeners of the *formosus*-group by both ♂ femora 6 and 7 being slightly inflated and the anterior gonopod without apicomesal process, as well as the posterior gonopod with less than ten microspinules. Based on molecular evidence, *G. scutatus* Zhao & Liu, sp. nov. differs from all other *Glyphiulus* species analysed in a > 15.8% uncorrected p-distance of the COI barcoding gene.

Description. Length of both sexes ca. 25.0–41.0 mm, mid-body rings round in cross-section, their width and height similar, 1.3–1.8 (♂) or 1.8–2.0 mm (♀). Body with 55–68 podous + 1–4 apodous rings + telson. Colouration purple-brownish to dark brownish, legs light brownish to almost transparent (Fig. 5B).

Head surface smooth. Labrum with four teeth anteromedially (Fig. 13A). Ocellaria blackish, with 8–12 ommatidia arranged in two irregular linear rows (Fig. 12B). Antennae short, slightly clavate, reaching back to ring 3; in length, antennomeres 5 > 3 > 4 > 2 > 6 > 1 > 7. Antennomeres 5–7 each with a distodorsal field or corolla of bacilliform sensilla (sensory bacilli). Antennomere 7 with four sensory cones (Fig. 13B). Gnathochilarium with a separate promentum, setae variable in number, polytrichous or smooth on promentum and mentum, lamellae linguales each with 4–6 setae (Fig. 13A). Mandible not dissected.

***Collum*:** crests complete and evident; carinotaxy formula I–III+P+M (Figs 11A, B). Following metaterga strongly crested and extremely sharp; carinotaxy formula 2/2+I/i+3/3 (Fig. 12). Ozoporiferous tubercles very large, subcylindrical. Prozonae delicately alveolate-areolate, fine longitudinal striations in front of stricture. Metazonae with an obvious, corrugate, carved texture (Fig. 12). Rings 2 and 3 with long pleural flaps.

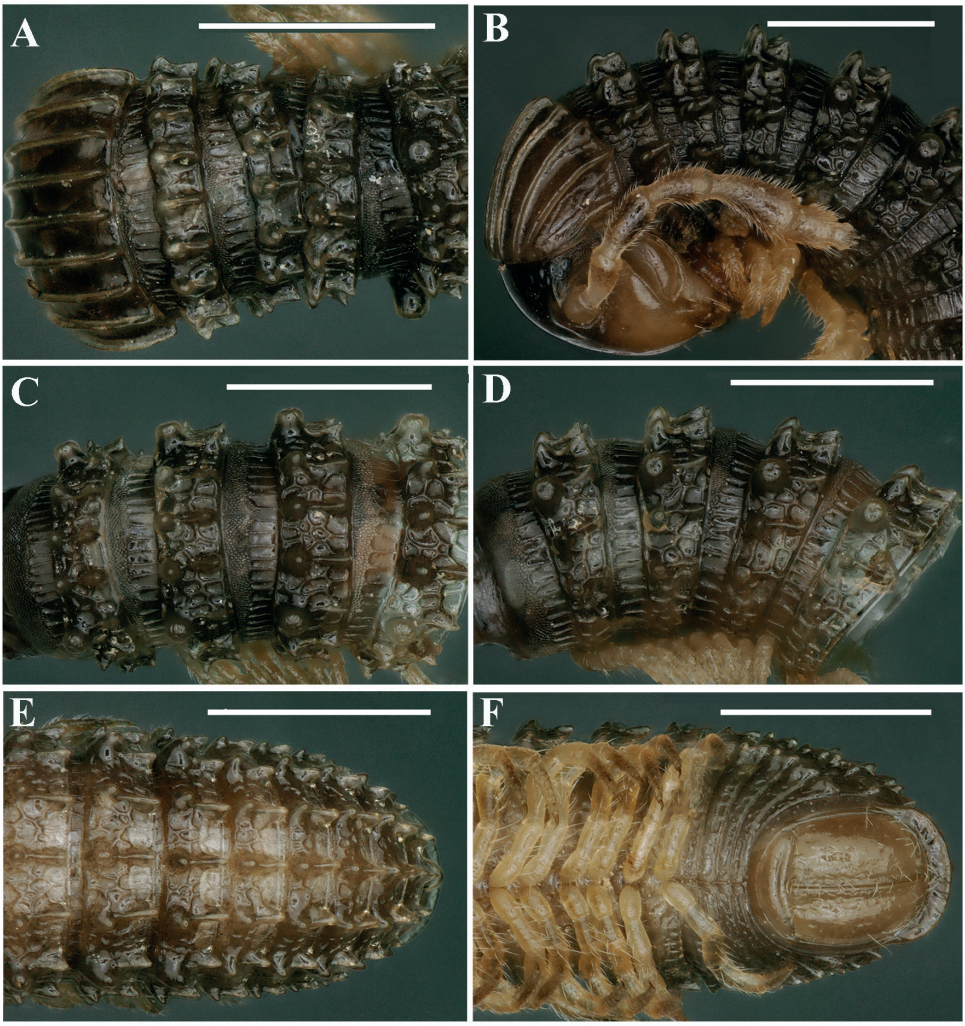

Figure 12. *Glyphiulus scutatus* Zhao & Liu, sp. nov., ♂ paratype from Cave Napang Dong **A, B** anterior body rings, dorsal and lateral views **C, D** mid-body rings, dorsal and lateral views, respectively **E, F** posterior body rings, dorsal and ventral views, respectively. Scale bars: 1 mm.

Epiproct simple, very narrow, caudal edge uneven, with a clear central tubercle dorsally (Fig. 12E). Paraprocts regularly convex, each with several irregular rows of setae. Hypoproct transversely bean-shaped, with 3+3 small setae (Fig. 12F).

Legs slender, about 1.1–1.3 times as long as mid-body height; claw with a small accessory spine at base, about ¼ as long as claw (Fig. 13H). ♂ legs 1 very strongly reduced, represented by a sternum showing a pair of small, fused, paramedian, subunciform prongs directed forward, with 3+3 long setae at base; flanked by strongly separated, rudimentary, 2-segmented leg vestiges, with either a few or no setae (Fig. 13D).

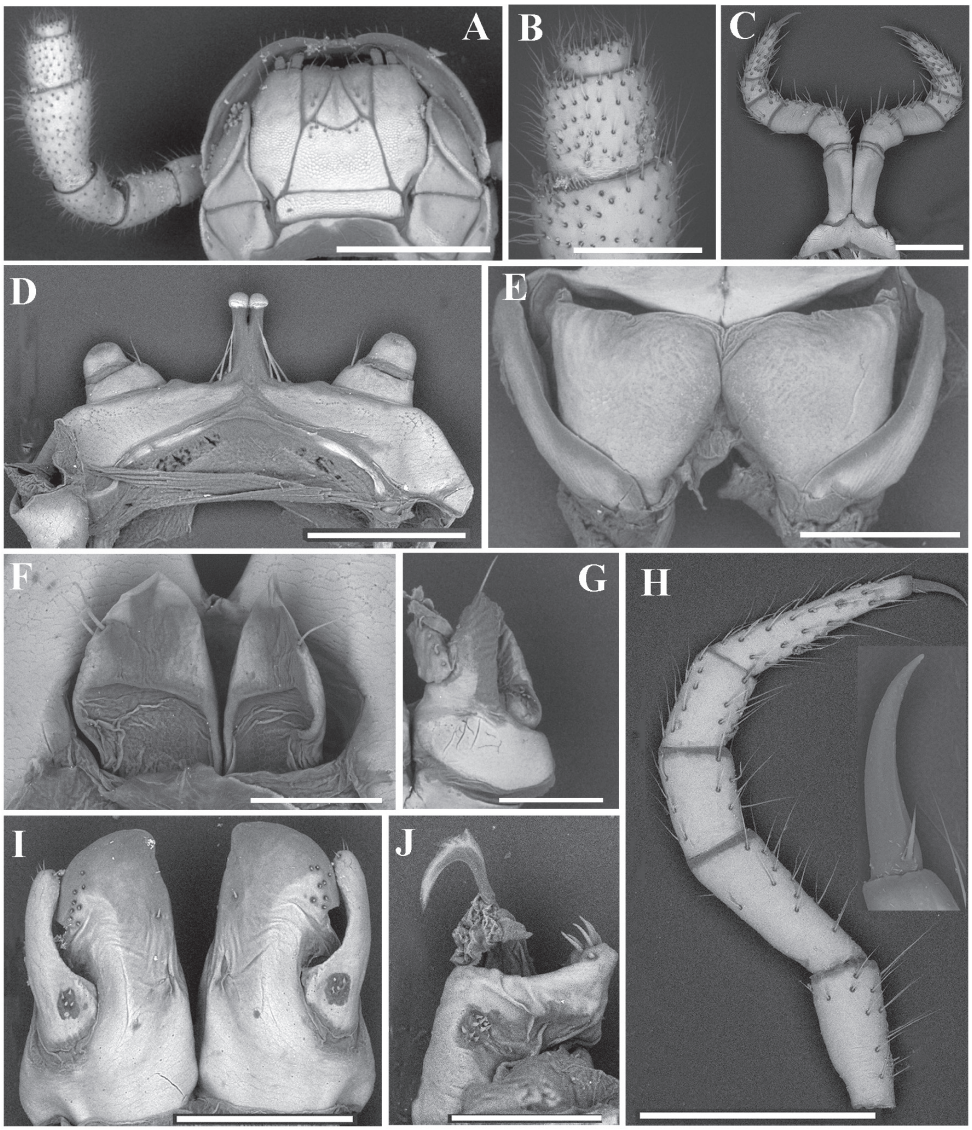

Figure 13. *Glyphiulus scutatus* Zhao & Liu, sp. nov., ♂ paratype from Cave Napang Dong **A** gnathochilarium and right antenna **B** antenna tip **C** leg 3, frontal view **D** leg 1, frontal view **E** ♀ paratype, vulvae **F** penes **G, J** posterior gonopod, frontal and caudal views, respectively **H** mid-body leg and claw **I** anterior gonopods, caudal view. Scale bars: 0.5 mm (**A, H**), 0.2 mm (**B–E**), 0.1 mm (**F, G, I, J**).

♂ legs 2 slightly hypertrophied, coxae large; penes small, much shorter than coxae, oblong-subtrapeziform, each with 1–4 strong setae distolaterally (Fig. 13F). ♂ legs 3 modified through coxae being especially slender and elongate (Fig. 13C). Both ♂ femora 6 and 7 slightly inflated.

Anterior gonopods (Fig. 13I) with a scalloped and shield-shaped coxosternum with about 6–8 microsetae near lateral corner of coxite. Telopodite very large and

coiled, 1-segmented, lateral in position, much higher than lateral corner of coxite, with a field of 5–7 microspinules at base and 4–5 strong setae apically.

Posterior gonopods (Figs 13G, J) compact. Coxite subquadrate, with a field of about 8–10 basolateral microspinules in frontal view and with a field of ten median microspinules in caudal view; apical and mesal parts of coxite with dense, strong and curved setae. Lamelliform lobe membranous, with a short, broad, distally spike-like flagellum.

Vulvae very simple, bare, rather faintly emarginate medially (Fig. 13E).

Remark. In the absence of direct troglomorphic traits, this species can only be considered as troglophilic at most.

Glyphiulus portaliformis **Zhao & Liu, sp. nov.**
http://zoobank.org/5879B2B8-A5DE-46BE-8710-07E3E41BAE46
Figs 5C, 14, 15

Type material. ***Holotype*** ♂ (SCAU WL40), China, Guangxi Zhuang Autonomous Region, Hechi City, Bama County, Cave Baiyan Dong, 24°03'40"N, 107°08'16"E, 400 m alt., 2015-VII-31, leg. Chen Jujian, Wang Xinhui & Tang Mingruo. ***Paratypes*:** 7 ♂, 18 ♀ (SCAU WL40), same data as the holotype.

Etymology. To emphasise the coxosternum of the anterior gonopods being portal-shaped.

Diagnosis. Differs from congeners of the *formosus*-group by the epiproct showing a small caudal protrusion and the anterior gonopods being portal-shaped, combined with a foliate flagellum of the posterior gonopod. Based on molecular evidence, *G. portaliformis* Zhao & Liu, sp. nov. Differs from all other *Glyphiulus* species analysed in a > 14.4% uncorrected p-distance of the COI barcoding gene.

Description. Length of both sexes ca. 41.0–56.0 mm, mid-body rings round in cross-section, their width and height similar, 2.2–3.0 mm. Body with 55–67 podous + 1 apodous ring + telson. Colouration brownish, legs almost transparent (Fig. 5C).

Head surface smooth. Labrum with 4 teeth anteromedially (Fig. 15A). Ocellaria blackish, with 14–17 ommatidia arranged in 2–3 irregular linear rows (Figs 14B, 15B). Antennae short, slightly clavate, reaching back to ring 4; in length, antennomeres 5 > 3 > 2 > 4 > 6 > 1 > 7. Antennomeres 5–7 each with a distodorsal field or corolla of bacilliform sensilla (sensory bacilli). Antennomere 7 with four sensory cones (Fig. 15A). Gnathochilarium with a separate promentum, polytrichous on promentum and mentum, lamellae linguales each with 6–7 setae (Fig. 15A). Mandible not dissected.

***Collum*:** crests complete and evident; carinotaxy formula I–III+P+M (Figs 14A, B, 15B). Following metaterga strongly crested; carinotaxy formula 2/2+I/i+3/3 (Fig. 14). Ozoporiferous tubercles very large, coniform. Prozonae delicately alveolate-areolate, fine longitudinal striations in front of stricture. Metazonae with an obvious, corrugate, carved texture (Fig. 14). Rings 2 and 3 with long pleural flaps.

Epiproct simple, caudal edge with a central conical protrusion and dorsally with a strong central rounded tubercle (Fig. 14E). Paraprocts regularly convex, each with several irregular rows of setae. Hypoproct transversely bean-shaped, no setae visible (Fig. 14F).

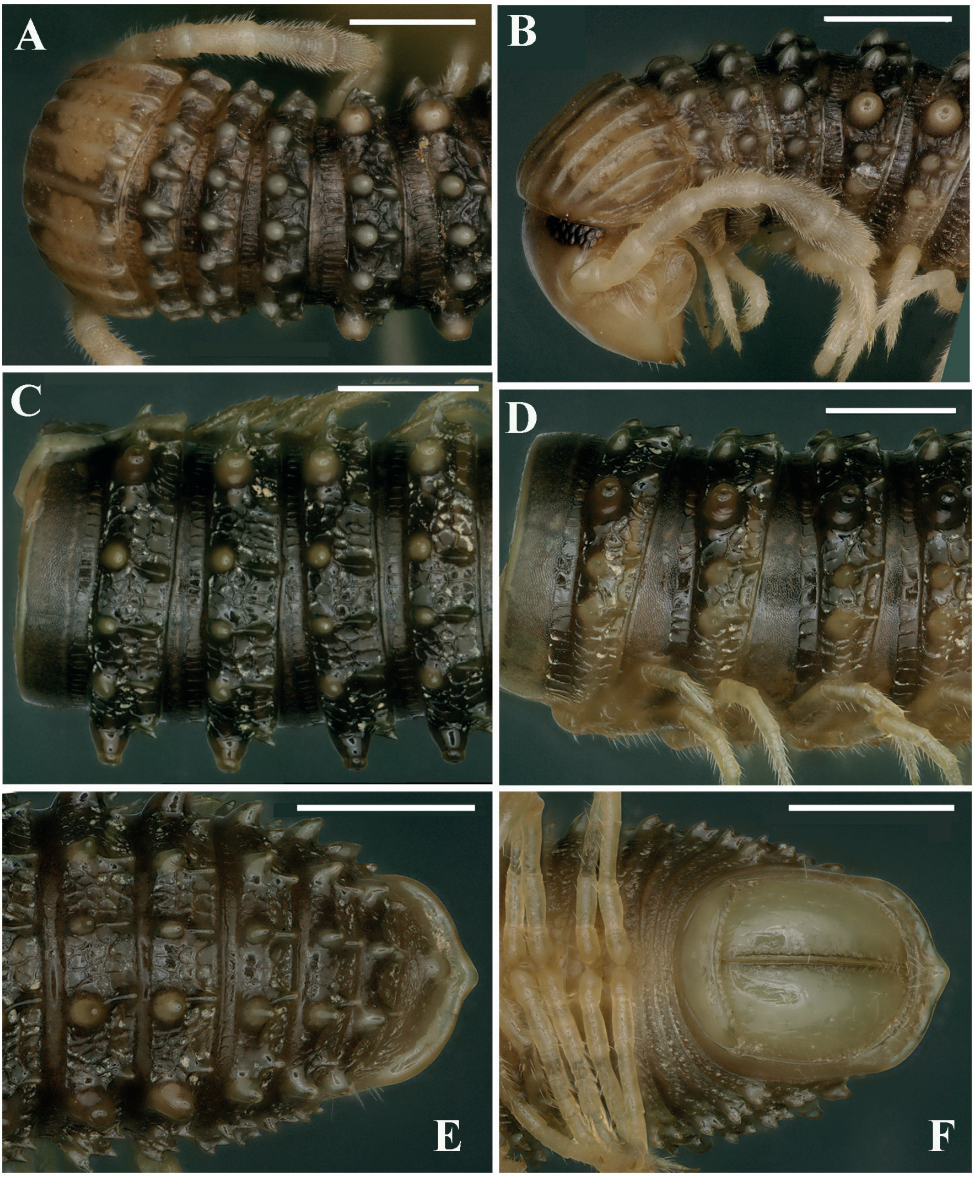

Figure 14. *Glyphiulus portaliformis* Zhao & Liu, sp. nov., ♂ paratype **A, B** anterior body rings, dorsal and lateral views **C, D** mid-body rings, dorsal and lateral views, respectively **E, F** posterior body rings, dorsal and ventral views, respectively. Scale bars: 1 mm.

Legs slender, about 1.1 times as long as mid-body height; claw with a small accessory spine at base, about 1/5 as long as claw (Fig. 15D). ♂ legs 1 very strongly reduced, represented by a sternum showing a pair of small, fused, paramedian, subunciform prongs directed forward, with about ten long setae at base; flanked by strongly separated, rudimentary, 2-segmented leg vestiges, second segment very large, stout, rod-shaped (Fig. 15F). ♂ legs 2 slightly hypertrophied, coxae large; penes small, much

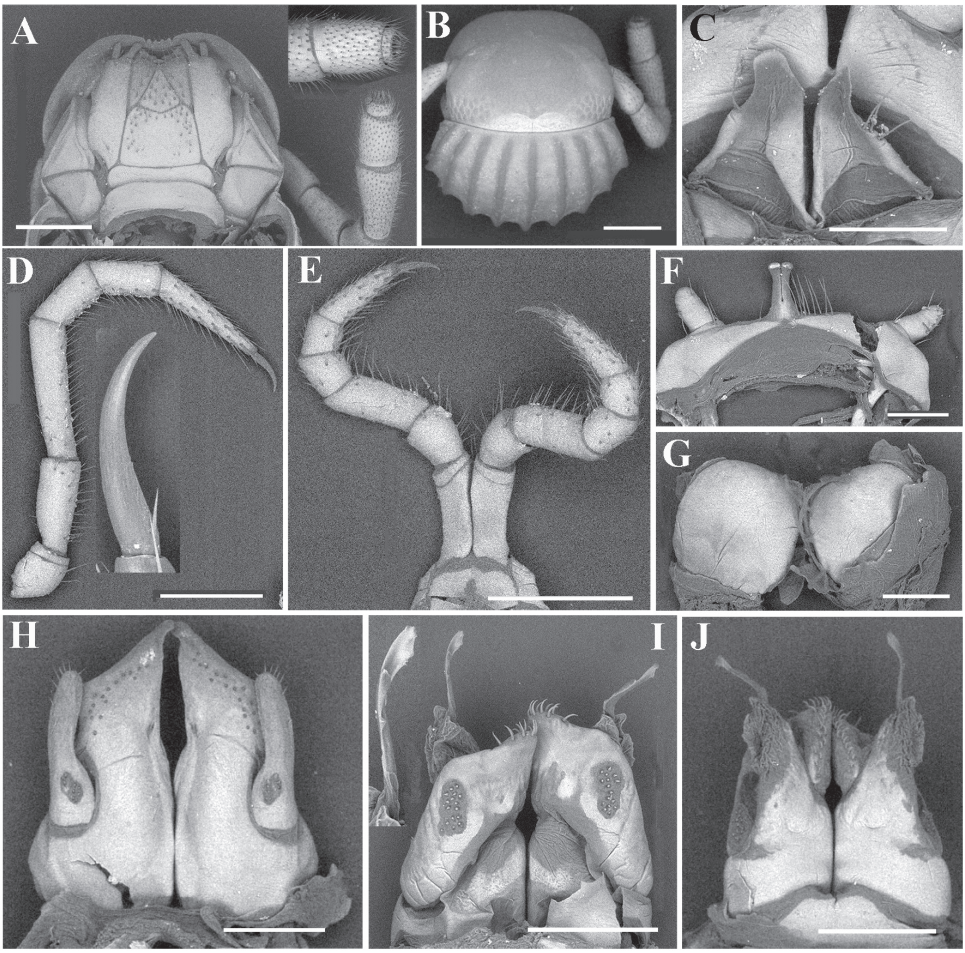

Figure 15. *Glyphiulus portaliformis* Zhao & Liu, sp. nov., ♂ paratype **A** gnathochilarium and left antenna **B** head and collum **C** penes **D** mid-leg and claw **E** leg 3, caudal view **F** leg 1, frontal view **G** ♀ paratype, vulvae **H** anterior gonopods, caudal view **I, J** posterior gonopod, caudal and frontal views, respectively. Scale bars: 0.5 mm (**A, B, D, E**), 0.2 mm (**C, F–J**).

shorter than coxae, oblong-subtrapeziform, each with two or three strong setae distolaterally (Fig. 15C). ♂ legs 3 modified through coxae being especially slender and elongate (Fig. 15E). Both ♂ femora 6 and 7 normal, without modifications.

Anterior gonopods (Fig. 15H) very tall, with a portal-shaped coxosternum with about 16–18 microsetae near distal margin; apicomesal process of coxite subtriangular, tip small, slightly curved inwards. Telopodite very large and clavate, 1-segmented, lateral in position, slightly higher than lateral corner of coxite, with a field of nine microspinules at base and six strong setae apically.

Posterior gonopods (Figs 15I, J) compact. Coxite nearly parallelogram-shaped, with an elongated field of about 14 basolateral microspinules in frontal view and with a field of 16 median microspinules in caudal view; apical and mesal parts of coxite with

dense, strong and curved setae. Lamelliform lobe membranous, with a relatively long, broad, distally foliate flagellum.

Vulvae very simple, bare, faintly emarginate medially (Fig. 15G).

Remark. In the absence of direct troglomorphic traits, this species can only be considered as troglophilic at most.

Glyphiulus calceus Jiang, Guo, Chen & Xie, 2018

Glyphiulus calceus Jiang, Guo, Chen & Xie, 2018: 162.

Material examined. 4 ♂, 7 ♀ (SCAU WL37), China, Guangxi Zhuang Autonomous Region, Hechi City, Fengshan County, Zhaiya Town, Cave Jianbang Dong, 24°43'02.96"N, 107°13'11.21"E, 350 m alt., 2015-VIII-4, leg. Chen Jujian, Huang Sunbin & Tang Mingruo.

Remarks. This species has been described from the Cave Xianren Dong, Bala Town, Tian'e County, Guangxi, China. The new samples were collected from a cave in the neighbouring county, both being located close geographically (Fig. 16). The above material is in good agreement with the original description by Jiang et al. (2018), while intraspecific p-distance is 2.1%, based on DNA-barcoding. Based on molecular evidence, *G. impletus* differs from all other *Glyphiulus* species analysed from between 11.9% (compared to *G. calceus*) and 23.6% (compared to *G. duangdee*).

Glyphiulus impletus Jiang, Guo, Chen & Xie, 2018

Glyphiulus impletus Jiang, Guo, Chen & Xie, 2018: 171.

Material examined. 9 ♂ (SCAU WL38), China, Guangxi Zhuang Autonomous Region, Hechi City, Fengshan County, Jiangzhou Underground Corridor, 24°30'4"N, 106°53'46"E, 850 m alt., 2013-VI-30, leg. Tian Mingyi, Liu Weixin, Lin Wei, Yin Haomin & Huang Sunbin. 4 ♂, 2 ♀ (SCAU WL39), China, Guangxi Zhuang Autonomous Region, Baise City, Linyun County, Cave Fengniu Dong, 24°28'39"N, 106°37'52"E, 700 m alt., 2017-VI-9, leg. Tian Mingyi, Liu Weixin, Wang Xinhui & Tang Mingruo.

Remarks. This species has been found to have a relatively wide distribution in Guangxi, involving the Lingyun, Fengshan, Nandan and Donglan Counties. The new samples add only the records of two cave collections (Fig. 16). The above material is in good agreement with the original description by Jiang et al. (2018), while intraspecific p-distances are 2.4–8.2%, based on DNA-barcoding. Based on molecular evidence, *G. impletus* differs from all other *Glyphiulus* species analysed from between 11.9% (compared to *G. calceus*) and 24.9% (compared to *G. foetidus*).

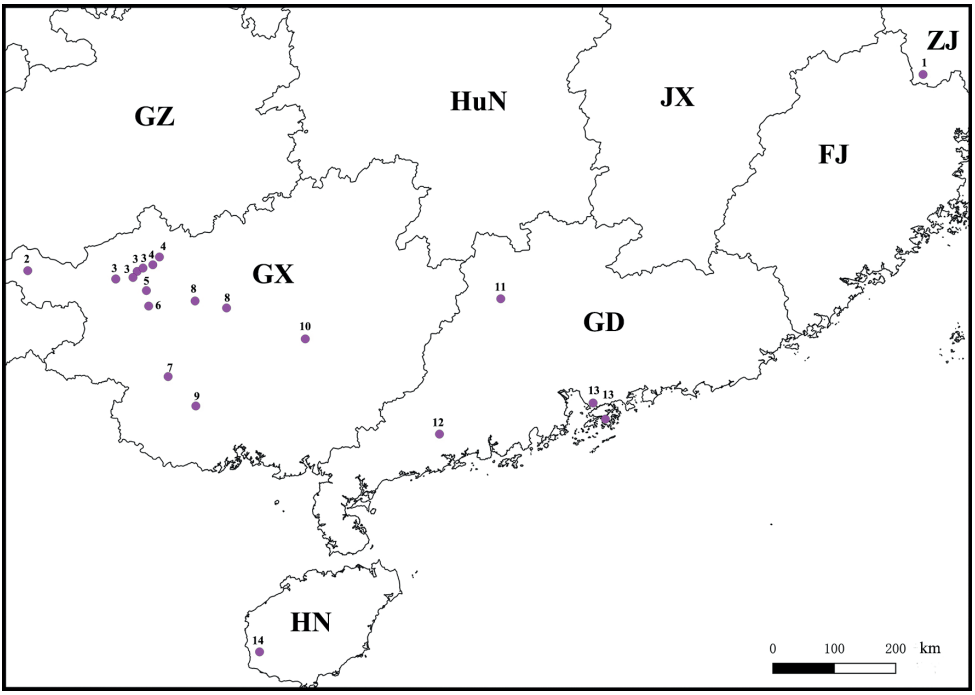

Figure 16. A distribution map of the *Glyphiulus formosus* species group in China. **1** *G. recticullus* **2** *G. foetidus* **3** *G. impletus* **4** *G. calceus* **5** *G. fortis* **6** *G. portaliformis* Zhao & Liu, sp. nov. **7** *G. pulcher* **8** *G. scutatus* Zhao & Liu, sp. nov. **9** *G. echinoides* **10** *G. xiniudong* Zhao & Liu, sp. nov. **11** *G. sinuatoprocessus* Zhao & Liu, sp. nov. **12** *G. conuliformis* Zhao & Liu, sp. nov. **13** *G. formosus* **14** *G. hainanensis*. Abbreviations: FJ: Fujian, HN: Hainan, HuN: Hunan, GD: Guangdong, GX: Guangxi, GZ: Guizhou, ZJ: Zhejiang.

A key to the species of the *Glyphiulus formosus*-group known from China

1 Anterior gonopod with a scalloped and shield-shaped coxosternum (Fig. 13I) **2**

– Anterior gonopod coxosternum not fan-shaped, but with a high apicomesal process of varying shapes (Figs 7H, 9I, 11G, 15H) **6**

2 ♂ leg 1 with a 1-segmented telopodite ..... ***G. pulcher***

– ♂ leg 1 with a 2-segmented telopodite (Fig. 13D) **3**

3 Collum quadrate; telopodite of ♂ leg 1 with a claw ..... ***G. recticullus***

– Collum not quadrate; telopodite of ♂ leg 1 without a claw **4**

4 Neither ♂ femora 6 nor 7 inflated; anterior gonopod coxosternum lower than telopodite ..... ***G. echinoides***

– Both ♂ femora 6 and 7 inflated; anterior gonopod coxosternum higher than telopodite **5**

5 Anterior gonopod coxosternum with 14–15 microsetae along lateral margin; flagellum of posterior gonopod incurved and sawtooth-shaped at inner margin ..... ***G. formosus***

– Anterior gonopod coxosternum with 6–8 microsetae near lateral corner; flagellum of posterior gonopod spike-like (Fig. 13J) ..... ***G. scutatus* Zhao & Liu, sp. nov.**

6 Both ♂ femora 6 and 7 inflated, with a small tubercle distoventrally ..... ***G. hainanensis***

– Both ♂ femora 6 and 7 normal, not modified **7**

7 Metazonae with an obvious, corrugate, carved texture (Figs 10, 12, 14) **8**

– Metazonae relatively smooth, not so markedly carved (Figs 6, 8) **9**

8 Apicomesal process of anterior gonopod coxite narrow and digitiform (Fig. 11G); posterior gonopod coxite with a long apicolateral seta in caudal view (Fig. 11H, I) ..... ***G. xiniudong* Zhao & Liu, sp. nov.**

– Apicomesal process of anterior gonopod coxite subtriangular (Fig. 15H); posterior gonopod coxite devoid of a long apicolateral seta (Fig. 15I) ..... ***G. portaliformis* Zhao & Liu, sp. nov.**

9 Metatergal anterior tubercles coniform, unusually sharp (Fig. 8) ..... ***G. conuliformis* Zhao & Liu, sp. nov.**

– Metatergal anterior tubercles mostly well-rounded **10**

10 Flagellum of posterior gonopod pectinate distally, with several branches at inner margin **11**

– Flagellum of posterior gonopod broad and flat, finely serrate distally at inner margin **12**

11 Apicomesal process of anterior gonopod coxite digitiform, tip narrow and hook-shaped (Fig. 7H) ..... ***G. sinuatoprocessus* Zhao & Liu, sp. nov.**

– Apicomesal process of anterior gonopod coxite subtriangular, tip not hook-shaped ..... ***G. foetidus***

12 Posterior gonopod coxite with a long apicolateral seta ..... ***G. calceus***

– Posterior gonopod coxite without a long apicolateral seta 13

13 Apicomesal process of anterior gonopod coxite very slender, finger-shaped ..... ***G. impletus***

– Apicomesal process of anterior gonopod coxite rather strong, tip rounded ..... ***G. fortis***

Discussion

Morphologically, the Chinese species from the *formosus*-group can presently be considered as well-defined: (1) male leg 1 with a pair of small, fused, paramedian, subunciform prongs directed forward, flanked by strongly separated, rudimentary, 1- or 2-segmented leg vestiges; and (2) collum's carinotaxy formula I–III+P+M. However, although *G. submediator* Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2011, from Vietnam and *G. striganovae* Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2012,

from Borneo, Indonesia, both agree in the above character 1, the carinotaxy formula of the collum in the former species being I–III+4c+5a+pc+ma (Golovatch et al. 2011b), vs. 1c+II+3c+4a+pa+ma in the latter congener (Golovatch et al. 2012). Therefore, we are inclined to treat *G. submediator* as remaining in the original *javanicus*-group. Golovatch et al. (2012) mentioned that *G. striganovae* failed to fit in either the *granulatus*- or the *javanicus*-group. At present, we also have no clear clue for its closer assignment. Nor that *G. striganovae* may not belong to *Glyphiulus* because of its special gonopod structure.

In addition, most species of the Chinese *sinensis*-group show the distal margin of the anterior gonopod coxosternum clearly concave and arcuate centrally and the posterior gonopod sometimes lacks a flagellum. On the contrary, all members of the *formosus*-group either have a scalloped and shield-shaped coxosternum or bear a high apicomesal process of the coxite, while their posterior gonopod always has a flagellum. Based on this, the relationship between the *formosus*-group and the *granulatus*-group may be considered closer from the perspective of the anterior and posterior gonopod structure.

In the single-gene COI phylogenetic tree, the genus *Plusioglyphiulus* may be speculated as being more closely related to *Glyphiulus* than to *Trachyjulus*, because its two species are clustered together with *Glyphiulus* clade BA and clade BB. Golovatch et al. (2011c) also pointed out that some species of *Plusioglyphiulus* appear to be highly peculiar morphologically and there may be transitional groups bridging them with the *javanicus*-group of *Glyphiulus*. Although single-gene COI construction can effectively identify species, a phylogenetic tree, based on the joint construction of multiple genes is deemed to much better resolve the relationship between species (Hebert et al. 2003; Cepeda et al. 2012; Hassan and Hassan 2021).

In our study, *Glyphiulus* proves to be a monophyletic taxon, based on both morphological and molecular phylogenetic evidence. It can presently be divided into three clades, the relationship between them being ((Clade A, Clade B), Clade C), albeit none has gained strong support yet. Therefore, in order to fully verify its reliability, a larger number of samples and a greater amount of information are needed to promote further advance in the study of Cambalopsidae, *Glyphiulus* included.

Most of the *Glyphiulus* species presently known to occur in China appear to largely be confined to the southern parts of the country. Moreover, most of them have been found in caves. Based on the current distribution map (Fig. 16), the *formosus*-group may belong to the South China regionalisation type, mainly covering Guangxi, Guangdong, Hong Kong and Hainan, with solely *G. recticullus* coming from Zhejiang (Zhang and Li 1982). Whether there is indeed an obvious geographic gap/isolation between the *formosus*-group and the other two species groups may become clearer as further species become revealed and/or recorded from intermediate areas.

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Supplementary material 1

Table S1

Authors: Yi Zhao, Wanru Guo, Sergei Golovatch, Weixin Liu

Data type: Uncorrected p-distances

Explanation note: Uncorrected p-distances for the 657 bp barcoding of the COI gene as between *Glyphiulus* and some related Cambalosidae taxa.

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Link: https://doi.org/10.3897/zookeys.1108.85156.suppl1

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RESEARCH ARTICLE

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Molecular phylogeny of Lichen Tiger Moths (Lepidoptera, Erebidae, Arctiinae, Lithosiini): a contribution toward classifying Western Hemisphere genera

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Abstract

This study analyzes molecular sequence data from one mitochondrial (COI) and two nuclear (28S, RPS5) genes to test the monophyly of previously proposed subtribes of the Lithosiini (Erebidae: Arctininae), including subtribal assignment of all North American genera that occur north of Mexico. After transferring *Gardinia* W.F. Kirby from Lithosiina to Cisthenina, there is strong support for a monophyletic Lithosiina, which includes three originally unplaced Nearctic genera: *Agylla* Walker, *Inopsis* Felder, and *Gnamptonychia* Hampson. The result of this study removes *Clemensia* Packard and *Pronola* Hampson from Cisthenina and places them in subtribe Clemensiina. We synonymize Eudesmiina under Cisthenina. After these changes, the phylogeny shows strong support for the monophyly of Cisthenina, which includes a further three unplaced Nearctic genera: *Gardinia* Kirby, *Bruceia* Neumögen, and *Ptychoglene* Felder. The monophyly of Cisthenina (including *Eudesmia* and *Gardinia*) is supported by two apomorphies found in adults: the apodemes of the second abdominal sternite are long and the anterolateral processes are fused with the rest of the sternite.

Keywords

Acsalina, Cisthenina, Clemensiina, Eudesmiina, Lithosiina, molecular sequence data, new subtribal classification, phylogenetic analysis

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Introduction

Lithosiini (Erebidae: Arctiinae), known as Lichen Tiger Moths, consist of approximately 4000 described species, and have the uncommon ability to feed on lichens (Fig. 1). While other lepidopterans are known to facultatively feed on lichens, only a few groups are known to be obligate lichen feeders. Some authors have suggested most of these are feeding primarily on the algal symbiont of the lichen (Wagner et al. 2008). In the New

Figure 1. Representative Lithosiini larvae **A** *Crambidia myrlosea* Dyar **B** *Inopsis modulata* (Edwards).

World, these include members of the Afridini (Nolidae), Elaphriini (Noctuidae) and the Bryophilinae (Noctuidae). Not only do the Lithosiini obligately feed on lichen and algae, they are the only lepidopterans known to sequester phenolics produced by the lichen fungal symbiont (Hesbacher et al. 1995; Wagner et al. 2008; Conner 2009; Scott et al. 2014; Anderson et al. 2017; Scott Chialvo et al. 2018). Lithosiini larvae are secretive, nocturnal, seldom encountered, and poorly known (Wagner 2005; Conner 2009). All Lithosiini larvae that have been examined to date have a mola, a unique flattened, heavily sclerotized area on the inner margin of the mandibles which they use to grind through tough lichen thalli (Fig. 2) (Gardner 1943; Issiki et al. 1965; McCabe 1981; Lafontaine et al. 1982; Rawlins 1984; Garcia-Barros 1985; Habeck 1987; Bendib and Minet 1998; Bendib and Minet 1999; Jacobson and Weller 2002). The ability of the larvae to feed on lichens and sequester associated toxins for their own protection was likely the key innovation that led to the remarkable diversification of this group (Wagner et al. 2008).

Defensive chemicals that the larvae acquire from feeding on lichens are maintained through the pupal stage into the adult (Hesbacher et al. 1995; Anderson et al. 2017; Scott Chialvo et al. 2018). Lithosiini adults are small to medium-sized moths (Fig. 3). Some species have white, gray or brown wing scales and others are brightly and aposematically colored. The audible clicks of some adults warn bats of their distastefulness (Acharya and Fenton 1992). Like their better-studied arctiine relatives, it was suggested that the ability of lithosiines to sequester toxic compounds in the larval stages conveys fitness to the adults (Wagner et al. 2008). Among the arctiines, not only do sequestered toxins provide protection from predators (Eisner and Eisner 1991) and parasites (Singer et al. 2004), they are also critical in pheromone production, courtship success, and can be nuptial gifts that the female passes on to protect her eggs (Conner et al. 1981; Eisner and Meinwald 2003; Jordan at al. 2005). The use of sequestered lichen-derived toxins among members of the Lithosiini remains a wide-open area for research.

Monophyly of the Lithosiini is supported by two larval apomorphies, a mandibular mola (Fig. 2B) and the unique arrangement of labral setae, where M1 is more ventral and

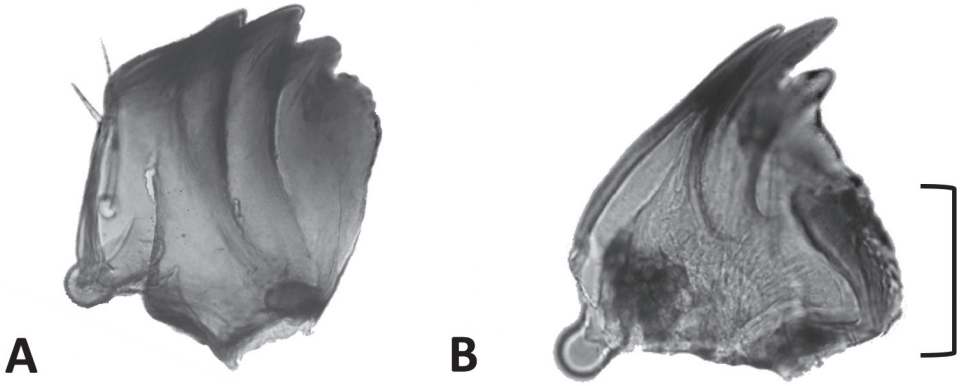

Figure 2. Dissections of the mandible of larvae illustrating two alternate states found among Arctiinae **A** mandible of *Lerina incarnata* Walker with a blade-like inner margin, as found in Arctiinae tribes other than Lithosiini **B** mandible of *Eudesmia arida* (Skinner), bracket indicates the mola, an apomorphy of Lithosiini.

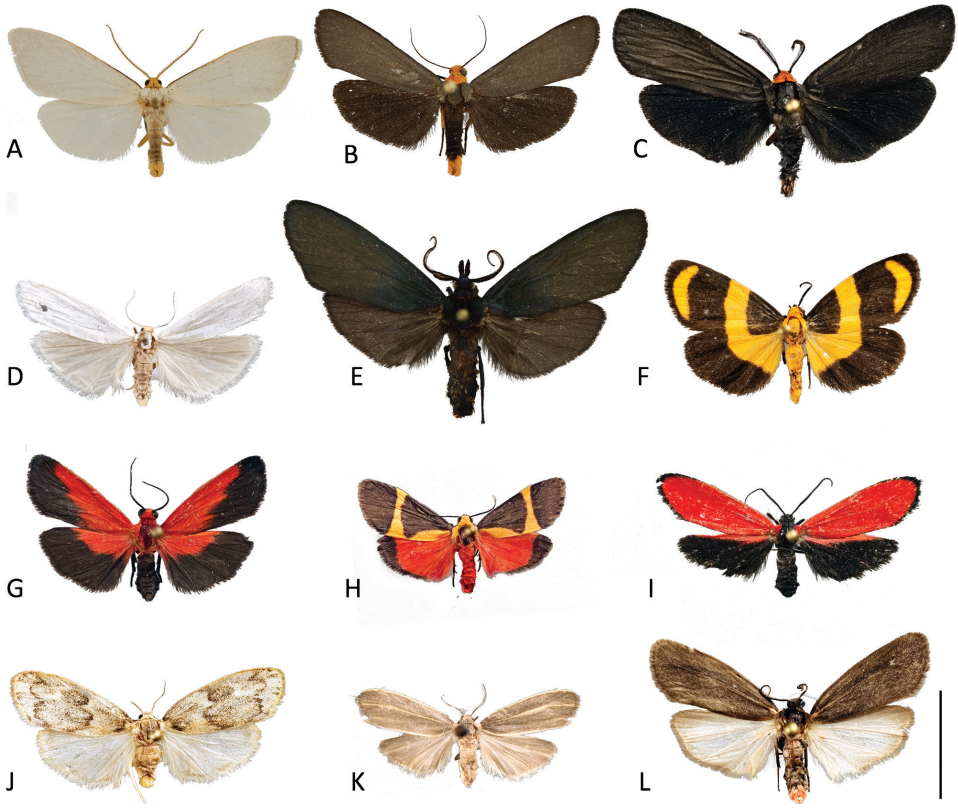

Figure 3. Dorsal views of representative Lithosiini adults from North America **A–D** Lithosiina **E–L** Cisthenina **A** *Agylla septentrionalis* Barnes & McDunnough **B** *Gnamptonychia ventralis* Barnes & Lindsey **C** *Inopsis modulata* (Edwards) **D** *Crambidia cephalica* (Grote & Robinson) **E** *Gardinia anopla* Hering **F** *Eudesmia arida* (Skinner) **G** *Ptychoglene coccinea* (Edwards) **H** *Cisthene tenuifascia* Harvey **I** *Lycomorpha regulus* (Grinnell) **J** *Bruceia pulverina* Neumögen **K** *Haematomis uniformis* Schaus **L** *Hypoprepia inculta* Edwards. Scale bar: 1 cm.

far from M2 (Bendib and Minet 1999). In the plesiomorphic condition (non-Lithosiini), M1 and M2 are either in a horizontal line or M1 is slightly dorsad of M2 (Habeck 1987). Lithosiini monophyly is further supported by several molecular phylogenetic studies of the Arctiinae (Zahiri et al. 2012; Zaspel et al. 2014; Zenker et al. 2016).

One lingering question is the classification of the Neotropical genus *Afrida* Möschler which has a confusing taxonomic history. Several authors considered it to belong to Lithosiini (Hampson 1900; Dyar 1913). While the larvae do feed on lichens, they are morphologically distinct, particularly in the shape of their cocoon and that they weave bits of lichen into the structure (Wagner et al. 2011), something no Lithosiini is known to do. Several authors proposed to move this genus from the Erebidae to the family Nolidae, subfamily Afridinae (Holloway 1998; Kitching and Rawlins 1998). More recently, Lafontaine and Schmidt (2010) placed Afridinae as a subfamily of Nolidae, based on COI sequence data and morphology.

Although Zahiri et al. (2013a) performed a molecular phylogenetic study of the family Nolidae based on eight gene regions, *Afrida* was not included in their taxon sampling, and thus the phylogenetic placement of this genus has not been tested by molecular-based analysis.

Knowledge of the relationships among the 350 genera classified within the Lithosiini is not well-resolved. Seven lineages within the Lithosiini were either redefined or first proposed by the seminal work of Bendib and Minet (1999). Based on their extensive analysis of morphological characters in adults and larvae (where known) they described and assigned 49 Lithosiini genera to six of these lineages (here considered subtribes), including Cisthenina (26 genera), Eudesmiina (four genera), Acsalina (one genus), Nudariina (15 genera), Endrosiina (two genera) and Phryganopterygiina (one genus). While this work established a baseline and laid the groundwork for future studies of Lithosiini, their taxon sampling was far from complete. They did not include all genera in their classification, and they did not treat the Lithosiina or assign any genera to this group. Jacobson and Weller (2002) included some lithosiines in their pioneering cladistical study of arctiid adult and larval characters, while Scott and Branham (2012) conducted the largest morphology-based phylogenetic analysis of the Lithosiini, including 76 species in 49 genera from each of the proposed seven subtribes. While these studies again supported the monophyly of the Lithosiini as a group, morphology alone failed to elucidate subtribal relationships.

In this study we conduct a DNA-based phylogenetic analysis of the Lithosiini that builds upon three previously published studies (Scott et al. 2014; Zenker et al. 2016; Scott Chialvo et al. 2018), with the aim of including representatives of all genera known from North America north of Mexico (Schmidt and Opler 2008) as well as published sequences from other Western Hemisphere taxa. We propose a new subtribal classification based upon our analyses. The resulting phylogenetic framework and classification provide a baseline for future systematic and behavioral studies of this charismatic group and evolutionary studies of their remarkable defensive chemistry.

Materials and methods

Gene selection and taxon sampling

Sequences acquired from previous molecular phylogenetic studies of Erebidae (Zahiri et al. 2011; Zahiri et al. 2012; Zahiri et al. 2013b; Scott et al. 2014; Zenker et al. 2016; Scott Chialvo et al. 2018) were downloaded from GenBank and assembled into single gene matrices. Preliminary phylogenetic analyses of the aligned sequences were conducted to determine which gene markers appeared to be most phylogenetically informative and would provide the most complete taxon sampling for our analyses. Based on the results of these preliminary analyses we chose to proceed with one mitochondrial protein-coding gene, cytochrome oxidase I (COI); one nuclear protein-

coding gene, ribosomal protein S5 (RPS5); and one nuclear structural gene, the large subunit rRNA D2 loop (28S). Sequences from five species classified in the Erebidae subfamily Aganainae, and representative species of the Arctininae tribes Arctiniini, Syntomiini, and Amerillini were downloaded from GenBank and included in the single gene matrices as outgroups. Molecular sequence data for 31 additional species, representing 16 genera from the southwestern United States were added. All voucher specimens have been deposited in the University of Arizona Insect Collection (UAIC).

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from the right mesothoracic leg or the abdomen of single specimens using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) according to manufacturer suggested protocols. Total genomic DNA was stored in buffer at -80 °C.

Gene fragments were PCR amplified for COI using the primers LCO1490 and HCO2198 (Hebert et al. 2003); the nuclear protein-coding gene RPS5 and nuclear large subunit 28S were amplified using primers and PCR protocols as provided in Scott et al. (2014). PCR products were cleaned, quantified, normalized and sequenced in both directions at the University of Arizona's Genomic and Technology Core Facility using a 3730 or 3730XL Applied Biosystems automatic sequencer. Chromatograms were assembled into contigs and initial base calls were made for each gene with Phred (Green and Ewing 2002) and Phrap (Green 1999) as orchestrated by Chromaseq ver. 1.5 in Mesquite ver. 3.6 (Maddison and Maddison 2017, 2018). Final base calls were made in Mesquite and ambiguous bases were designated by standard ambiguity codes. GenBank accession numbers for all sequences used in this study are listed in Table 1.

Sequence alignment and phylogenetic analyses

Single gene matrices were aligned using default settings in MAFFT v7.474 (Katoh and Standley 2013) and were concatenated in Mesquite. Maximum likelihood analyses were conducted on each gene individually and on the concatenated dataset using IQ-TREE ver. 1.6.10 (Nguyen et al. 2015), as orchestrated by Mesquite. The ModelFinder feature within IQ-TREE (Kalyaanamoorthy et al. 2017) was used to find the optimal character evolution models. The MFP model option was used for 28S, and the TESTMERGE option for the protein-coding genes. The TESTMERGE option sought the optimal partition of sites, beginning with the codon positions in different parts. Analyses of the concatenated data matrix were conducted using the TESTMERGE option, beginning with each codon position for each gene as a separate part (thus, the analysis began allowing for up to 7 parts, three for both of the protein-coding genes and one for 28S). One hundred searches were conducted for the maximum likelihood tree for each matrix. One thousand replicates were used for bootstrap analyses.

Table 1. Sampling of Lithosiini and outgroup species and GenBank accession numbers for sequences used in this study.

	UAIC Specimen Number	RpS5	28S rDNA	COI
Family Nolidae				
Afrida exegens Dyar USA: AZ, Cochise Co., Huachuca Mts.	UAIC1148036, UAIC1148037	OM990708	ON006455 ON006456	ON000160 ON000161
Family Erebidae				
Subfamily Aganainae				
Asota heliconia (Linnaeus)		KC571142	KC570976	KC571044
Asota orbona Vollenhoven		KC571143	KC570977	GWORG305-08
Neochera dominia Cramer		KC571144	KC570978	JZAGA909-12
Peridrome orbicularis Walker		JN401903		JN401280
Subfamily Arctiinae				
Tribe Amerilini				
Amerilla brunnea Hampson		KX300895		KX300223
Tribe Arctiini				
Cycnia tenera Hübner		KF533651	KF533380	KF533458
Halysidota tessellaris J. E. Smith		KF533658		KF533469
Leucanopsis setosa Rothschild		KJ723700	KF533400	KJ723706
Phragmatobia amurensis Seitz		KF533679	KF533419	KF533492
Pygoctenucha terminalis Walker Mexico: Sonora, SSW Mesa Tres Rios	UAIC1128849	OM990703	ON006450	
Virbia costata (Stretch) USA: AZ, Pima Co., Santa Catalina Mts.	UAIC1128305	OM990695	ON006437	MF923392
Tribe Syntomiini				
Amata phegea (Linnaeus)		HQ006749	KF533352	HQ006238
Apisa canescens Walker		HQ006663		HQ006146
Automolis ferrigera Druce		KF533641		KF533447
Ingroup				
Tribe Lithosiini				
Subtribe Acsalina				
Acsala anomala Benjamin		KC571145	KC570980	KJ378646
Subtribe Cisthenina				
Abrochocis esperanza Dyar			KC570979	KC571047
Ardonea tenebrosa (Walker)		KX361016		KX360798
Arhabdosia sp.		KX361034		KX360800
Balbura dorsisigna Walker			KC570986	KC571053
Balbura intervenata Schaus		KX361017	KC570987	KX360802
Bruceia hubbardi Dyar USA: AZ, Pima Co., Santa Catalina Mts.	UAIC1128313	OM990689	ON006431	ON000141
Bruceia pulverina Neumögen Mexico: Sonora, Sierra del Tigre	UAIC1128312	OM990704	ON006451	KC571055 ON000157
Bruceia sp. 1 Mexico: Sonora, Sierra del Tigre	UAIC1128309	OM990692	ON006434	ON000146
Bruceia sp. 2 USA: AZ, Pima Co., Santa Catalina Mts.	UAIC1148030	OM990697	ON006439	ON000144
Chrysochlorosia magnifica Schaus			KC570996	KC571057
Cisthene angelus (Dyar) USA: AZ, Pima Co., Tucson Mts.	UAIC1128316		ON006426	ON000136
Cisthene sp. USA: AZ, Pima Co., Santa Catalina Mts.	UAIC1148032	OM990690	ON006432	ON000142
Cisthene martini Knowlton USA: AZ, Cochise Co., Huachuca Mts.	UAIC1128318		ON006427	ON000137
Cisthene kentuckiensis (Dyar) USA: Texas, Travis Co., Austin	UAIC1148031	OM990698	ON006440	ON000143
Cisthene tenuifascia Harvey USA: AZ, Pima Co., Santa Catalina Mts.	UAIC1128319		ON006430	ON000140

	UAIC Specimen Number	RpS5	28S rDNA	COI
Clemensia marmorata (Schaus)		KX300811		KX300245
Cloesia digna Schaus			KC570995	JQ561796
Cloesia sp.		KX361038		KX360809
Dipaenae contenta (Walker)		KX361018		KX360815
Dolichesia falsimonia Schaus			KC571000	KC571062
Eudesmia arida (Skinner) Mexico: Sonora, Municipio de Nacori Chico	UAIC1128306	OM990701	ON006448	ON000156
Eudesmia menea (Drury)				MF922663.1
Euthyone grisescens (Schaus)			KC571010	KC571073
Euthyone purpurea (E. D. Jones)		KX361046		KX360823
Gardinia anopla Hering		KC571159	KC571012	KC571075
Gardinia anopla Hering USA: AZ, Pima Co., Santa Catalina Mts.	UAIC1128297		ON006425	ON000135
Gardinia paradoxa Hering		KX361019		KX360825
Hypermaepha sp.		KX361049		KX360828
Hypoprepia cadaverosa Strecker USA: AZ, Apache Co., Greer	UAIC1148028		ON006446	
Hypoprepia fucosa Hübner		KC571162	KC571017	KC571078
Hypoprepia fucosa tricolor (Fitch)		KC571163	KC571018	KC571079
Hypoprepia inculta Edwards USA: AZ, Cochise Co., Chiricahua Mts.	UAIC1128315	OM990706	ON006453	MH337839
Hypoprepia lampyroides Palting & Ferguson USA: AZ, Greenlee Co., Blue Ridge Primitive Area	UAIC1128324		ON006441	MH337834
Hypoprepia miniate (Kirby)				MF923793
Illice endoxantha Hampson		KX361050		KX360831
Lycomorpha fulgens (H. Edwards) USA: AZ, Apache Co., Hannagan Meadow	UAIC1148033		ON006447	
Lycomorpha grotei (Packard) USA: AZ, Apache Co., Greer	UAIC1148029	OM990702	ON006449	
Lycomorpha regulus (Grinnell) USA: AZ, Greenlee Co., Blue Ridge Primitive Area	UAIC1148034	OM990693	ON006435	ON000147
Lycomorphodes correbioides Schaus			KC571027	KC571088
Lycomorphodes sordida (Butler)			KC571028	KC571089
Lycomorphodes strigosa (Butler)		KX361051		KX360833
Metalobosia varda (Schaus)		KX361052		KX360836
Meterythrosia sangala (H. Druce)			KC571030	KC571030
Nodozana cf. coresa Schaus		KX361055		KX360839
Prepiella sesapina (Butler)		KX361057		KX360844
Pronola magniplaga Schaus		KX300812		KX300312
Ptychoglene coccinea (H. Edwards)			KC571036	HQ918634
Ptychoglene phrada H. Druce		KF533681		KF533497
Rhabdatomis cora coroides Schaus			KC571037	KC571094
Rhabdatomis laudamia (H. Druce) Mexico: Sonora, Sierra La Madera	UAIC1128848		ON006429	ON000139
Rhabdatomis mandana (Dyar)		KX361058		KX360845
Rhabdatomis melinda (Schaus)			KC571039	KC571096
Talara cara Schaus			KC571041	KC571098
Talara lepida Schaus			KC571042	KC571099
Talara nr. mona Dyar			KC571043	KC571100
Talara semiflava Walker		KX361060		KX360847
Subtribe Endrosina				
Eugoa bipunctata Walker		JN401906	KF533390	JN401906

	UAIC Specimen Number	RpS5	28S rDNA	COI
Setina irrorella (Linnaeus)		KX050605		KX050282
Stigmatophora micans (Bremer & Grey)				KF704470
Trischalis sp.				HM906475
Subtribe Lithosiina				
Agkonia ovifera Dognin		KX300816		KX300221
Agylla argentea Walker		KX300817		KX300220
Agylla argentifera Walker			KC570981	KC571048
Agylla septentrionalis Barnes & McDunnough USA: AZ, Cochise Co., Chiricahua Mts.	UAIC1148038	OM990705	4167	ON000158
Apistosia judas Hübner		KX300815		KX300230
Areva trigemmis Hübner		KX300814		KX300233
Atolmis rubricollis (Linnaeus)		KC571147	KC570985	ABOLA126-14
Brunia antica (Walker)		HQ006706	KF533366	HQ006193
Calamidia hirta Walker		KC571148	KC570990	KC571056
Crambidia cephalica (Grote & Robinson) USA: AZ, Navajo Co., Showlow	UAIC1128271	OM990699	ON006442	ON000152
Crambidia impura Barnes & McDunnough USA: AZ, Gila Co., N. of Winkelman	UAIC1128280	OM990688	ON006428	ON000138
Crambidia myrlosea Dyar Mexico, Sonora, Sierra Alacran	UAIC1148035	OM990696	ON006438	ON000150
Crambidia pallida Packard USA: NC, Macon Co, Slick Rock	UAIC1128304	OM990691	ON006433	ON000145
Crambidia xanthocorpa Lewis USA: IN, Tippecanoe Co., Purdue University	UAIC1128323	OM990694	ON006436	ON000148
Cybosia mesomella (Linnaeus)			KC570999	ABOLA124-14
Eilema complanum (Linnaeus) Romania: Torda, Torocko	UAIC1128295		ON006443	ON000153
Gnamptonychia flavicollis (H. Druce)		KC571158	KC571013	KC571076
Gnamptonychia ventralis Barnes & Lindsey Mexico: Sonora, Sierra del Tigre	UAIC1128300	OM990707	ON006454	
Hiera gyge H. Druce		KC571161	KC571015	
Inopsis modulata (H. Edwards)		KC571164	KC571020	KC571082
Lithosia quadra (Linnaeus) Bulgaria: Kalimantsi	UAIC1128303		ON006444	ON000154
Manulea bicolor (Grote) USA: CO, Gilpin Co., Golden Gate Canyon	UAIC1128293	OM990700	ON006445	ON000155
Mintopola braziliensis Schaus				KX300290
Subtribe Nudariina				
Asura cervicalis Walker			KC570983	KC571050
Barsine sp.		JN401878	KF533364	JN401286
Cyana meyricki Rothschild & Jordan		KC571151	KC570998	KC571061
Cyana sp.		JN401876	KF533379	JN401285
Lyclene pyraula (Meyrick)		KC571165	KC571022	KC571084
Lyclene quadrilineata (Pagenstecher)		KC571172	KC571035	KC571093
Lyclene reticulata (C. Felder)		KC571166	KC571023	KC571085
Lyclene sp.		KC571168	KC571024	KC571086
Miltochrista miniata (Forster)		KC571170	KC571031	KC571090
Miltochrista sp.		KC571171	KC571032	KC571091
unplaced				
Heliosia jucunda Walker		KC571160	KC571014	KC571077

Results

A summary diagram of the ML tree for the concatenated dataset is shown in Fig. 4. The full ML tree and all bootstrap values recovered from analyses of the concatenated dataset are shown in Suppl. material 1: Fig. S1. Based on our results we propose several changes to the subtribe classification within Lithosiini as discussed below and summarized in Table 2.

Discussion

Afrida exegens Dyar was initially included in the taxon sampling to test its potential placement within the Lithosiini or as an outgroup in this analysis. Including it caused long branch attraction, so the ssequences were removed from the matrices. GenBank BLAST searches of 28S, RPS5 and COI all confirm that *Afrida*, long considered by some an arctiine based on hindwing venation, does not belong to Erebidae, supporting the conclusions of Kitching and Rawlins (1998), Holloway (1998), Lafontaine and Schmidt (2010) and Zahiri et al. (2013a), who regarded the Afridinae as a subfamily of the family Nolidae.

Monophyly of Lithosiini

Lithosiini monophyly is supported by two morphological apomorphies found in the larvae. Both the unique arrangement of labral setae M1 and M2 and the mandibular mola were present in all Lithosiini larvae reared as part of this study, many of which were previously unknown, including *Agylla septentrionalis* Barnes & McDunnough, *Cisthene kentuckiensis* (Dyar), *Gardinia anopla* Hering, *Crambidia myrlosea* Dyar, *Eudesmia arida* (Skinner), *Hypoprepia lampyroides* Palting & Ferguson, *Inopsis modulata* (Edwards) and *Lycomorpha fulgens* (Edwards).

Subtribe Acsalina

Acsala anomala Benjamin occurs on a long branch by itself, supporting the placement of this species in a monotypic subtribe Acsalina. This enigmatic species was placed among the Lymantriidae, however following description of the larval stages feeding on lichens and the presence of a mandibular mola (Lafontaine et al. 1982) it was considered Lithosiini. Bendib and Minet (1999) list many unique apomorphies of the Acsalina, including flightless females, translucent wing vestiture, compound eyes with interommatidial setae, and a primitive hindwing ground plan not found among other Lithosiini. From a biogeographic perspective it is interesting that the Acsalina does not seem to be recently derived from any other temperate lineage contrary to virtually all other Lepidoptera endemic to the Arctic.

Figure 4. Maximum likelihood tree for the concatenated matrix. Branch lengths are proportional to relative divergence, as estimated by IQ-TREE. Bootstrap values are depicted below branches. Western Hemisphere monophyletic genus-level clades are collapsed and subtribes are colored. Clades that do not include Western Hemisphere species are collapsed and colored gray. See Suppl. material 1: Fig. S1 for the full tree.

Table 2. Proposed classification of Western Hemisphere genera of Lithosiini based on this study with reference to their original placement by Bendib and Minet (1999). Plus symbols (+) indicate that that genus was included in one or more of three molecular-based studies of Lithosiini, including Zenker et al. (2016) (column A), Scott Chialvo et al. (2018) (column B), and this study (column C), and that results support its position in the subtribal classification proposed here. Dashes indicate that that genus was not included in the molecular-based studies. When dashes occur in all three columns, that genus was placed in the proposed subtribal classification by morphology alone.

Proposed subtribal classification	Placement by Bendib and Minet 1999	A	B	C
Acsalina Bendib and Minet				
Acsala Benjamin	Acsalina	-	-	+
Cisthenina Bendib and Minet				
Abrochocis Dyar	unplaced	-	-	+
Ardonea Walker	unplaced	+	-	+
Arhabdosia Dyar	Cisthenina	+	+	+
Ascaptesyle Dyar	Cisthenina	+	-	+
Balbura Walker	unplaced	+	-	+
Barsinella Butler	Cisthenina	-	-	-
Bruceia Neumoegen	unplaced	-	+	+
Callisthenia Hampson	Cisthenina	-	-	-
Chrysochlorosia Hampson	unplaced	-	-	+
Chrysozana Hampson	Cisthenina	-	-	-
Cisthene Walker	Cisthenina	+	+	+
Cloesia Hampson	unplaced	+	-	+
Dipaenae Walker	unplaced	+	-	+
Dolichesia Schaus	Cisthenina	-	-	+
Eudesmia Hübner	Eudesmiina	-	-	+
Euryptidia Hampson	Eudesmiina	-	-	+
Euthyone Watson	Cisthenina	+	-	+
Gardinia Kirby	unplaced	+	-	+
Haematomis Hampson	unplaced	-	+	-
Hypermaepha Hampson	Cisthenina	-	-	-
Hypoprepia Hübner	Cisthenina	+	+	+
llice Walker	unplaced	+	-	+
Josiodes Felder	Eudesmiina	-	-	+
Leucorhodia Hampson	Cisthenina	-	-	-
Lycomorpha Harris	Cisthenina	-	-	+
Lycomorphodes Hampson	Cisthenina	+	-	+
Maepha Walker	Cisthenina	-	-	-
Metallosia Hampson	Cisthenina	-	-	-
Metalobosia Hampson	unplaced	+	-	+
Meterythrosia Hampson	unplaced	-	-	+
Neotalara Hampson	Cisthenina	-	-	-
Neothyone Hampson	Cisthenina	-	-	-
Nodozana H. Druce	unplaced	+	-	+
Odozona Walker	Cisthenina	-	-	-
Paratype Felder	Eudesmiina	-	-	+
Prepiella Schaus	Cisthenina	+	-	+
Ptychoglene Felder	unplaced	+	+	+
Rhabdatomis Dyar	Cisthenina	+	+	+
Seripha Walker	Cisthenina	-	-	-
Talara Walker	Cisthenina	+	+	+
Clemensiina Bendib & Minet				
Clemensia Packard	Cisthenina	+	-	+
Pronola Schaus	Cisthenina	+	-	+

Proposed subtribal classification	Placement by Bendib and Minet 1999	A	B	C
Lithosiina Stephens	not treated			
Agylla Walker	not treated	+	-	+
Apistosia Hübner	not treated	+	-	+
Areva Walker	not treated	+	-	+
Atolmis Hübner	not treated	-	-	+
Crambidia Packard	not treated	+	-	+
Cybosia Hübner	not treated	-	-	+
Eilema Hübner	not treated	-	-	+
Gnamptonychia Hampson	not treated	-	-	+
Hiera Druce	not treated	-	-	+
Inopsis Felder	not treated	-	-	+
Lithosia Fabricius	not treated	-	-	+
Manulea Wallengren	not treated	-	+	+
Mintopola Hampson	not treated	+	-	+

Subtribe Cisthenina (includes *Gardinia* and Eudesmiina)

When Bendib and Minet (1999) erected the tribe Cisthenini they divided it into the Cistheniti (containing *Cisthene* Walker, *Clemensia* Packard, *Hypoprepia* Hübner, *Lycomorpha* Harris, *Lycomorphodes* Hampson, and *Rhabdatomis* Dyar) and Clemensiiti (containing *Clemensia* Packard, *Pronola* Schaus, *Siccia* Walker, *Hyposiccia* Hampson and *Parasiccia* Hampson). They noted Cistheniti have an unusual resting posture with the antennae facing forward, while Clemensiiti exhibit the plesiomorphic folding backwards of the antennae. They also noted that Clemensiiti rested with the wings flattened rather than roof-like over their back as in Cistheniti.

We find strong support to remove *Clemensia* and *Pronola* (as discussed below) and include thirteen Neotropical genera that were unplaced by Bendib and Minet (1999). These include nine genera (*Balbura* Walker, *Cloesia* Hampson, *Dipaenae* Walker, *Dolichesia* Schaus, *Ilice* Walker, *Metalobosia* Hampson, *Nodozona* Druce, *Ptychoglene* Felder, and *Talara* Walker) which were found to be cisthenines in previous studies molecular-based studies (Zenker et al. 2016; Scott Chialvo et al. 2018) as well as four genera (*Abrochocis* Dyar, *Bruceia* Neumögen, *Chrysochlorosia* Hampson, *Meterythrosia* Hampson) which we include in a molecular-based study and classify for the first time (Table 2). We were not able to obtain fresh specimens of *Haematomis* Hampson for inclusion in our phylogeny, however we speculate based on its small size and resting posture that *Haematomis* belongs to Cisthenina. The wing pattern, particularly the distinctive pink basal wing markings, are consistent with other cisthenines that are thought to be Mullerian mimics of lampyrid beetles, especially some *Hypoprepia* species (*H. lampyroides* and *H. inculta*, for example).

Contrary to previous classifications, this study finds support to include two genera, *Eudesmia* and *Gardinia*, within Cisthenina. Bendib and Minet (1999) classified *Eudesmia* in subtribe Eudesmiina along with three other Western Hemisphere genera: *Euryptidia* Hampson, *Josiodes* Felder, and *Paratype* Felder. This is the first molecular-based phylogenetic study to include a member of the subtribe Eudesmiina. Results indicate

that recognizing Eudesmiina as a valid subtribe would render Cisthenina polyphyletic. Therefore, as first revisers we place Eudesmiina in synonymy under Cisthenina, with an expanded concept of the latter. Both names, Cisthenina and Eudesmiina, were published at the same time (Bendib and Minet 1999), so neither name has priority (ICZN Article 24.2). We choose Cisthenina since it is a much more diverse lineage. Given the apomorphies presented by Bendib and Minet for uniting the four genera they placed within the Eudesmiini, we tentatively place them all within Cisthenina (Table 2).

While *Gardinia* was unplaced by Bendib and Minet (1999) it was treated as a member of Lithosiina by several authors (Scott and Branham 2012; Scott et al. 2014). *Gardinia* is a Neotropical genus containing five species, including one species from southeastern Arizona, *Gardinia anopla* Hering (Fig. 1E). *Gardinia anopla* is the largest lichen moth in North America with an average forewing length of 25 mm, making it more than twice as large as other Cisthenina (typically with forewing lengths of 10 mm or less). When captured, adults of this nocturnal species produce audible clicks. Among Cisthenina, adults of *Cisthene martini* are known to produce clicks in response to bat echolocation and are generally avoided by bats (Dowdy and Conner 2016). The clicks of *Gardinia* might be used similarly to warn bats of their distastefulness.

Cisthenina larvae generally have short, sparse setae and they lack verrucae, which was proposed as an apomorphy for the subtribe (Bendib and Minet 1999). However, the larvae of *Gardinia* (Fig. 5A) and of *Eudesmia* (Fig. 5B), reared as part of this study, possess verrucae making species in these genera the only members of Cisthenina known to have them. *Eudesmia arida* (Skinner) larvae possess exceedingly long, soft setae, unlike the short, stiff setae characteristic of other Cisthenina (Fig. 5B).

All members of the *Cisthenina*, as defined here, are endemic to the Western Hemisphere. Among Cisthenina adults, apodemes on the second abdominal sternite are long and the anterolateral processes are fused with the rest of the sternum (Fig. 6). We find that these character states are present in *Eudesmia* and *Gardinia* (Fig. 6) which hold as a strong apomorphies of Cisthenina as redefined in this study.

Subtribe Clemensiina Bendib & Minet

Type-genus: *Clemensia* Packard.

When Bendib and Minet (1999) erected the tribe Cisthenini they divided it into the Cistheniti and Clemensiiti, with the latter housing *Clemensia*, *Pronola*, *Siccia*, *Hyposiccia* and *Parasiccia*. In addition to the differences they noted in resting posture between the subtribes, they noted three apomorphies of the Clemensiiti, including the presence of a pair of metascutal membranous areas, sternite A2 possessing curved movable anterolateral processes and the presence of a corethrogyne in females.

In this study *Clemensia* falls outside Cisthenina and it forms a highly supported clade with the small neotropical genus *Pronola* Schaus (5 species), the adults of which are similarly sized and have a similar peculiar rounded wing shape. Zenker et al. (2016) found *Clemensia* + *Pronola* were the sister group of the Oriental genus *Garudinia* Moore. Additional taxon sampling from around the world will be needed to determine

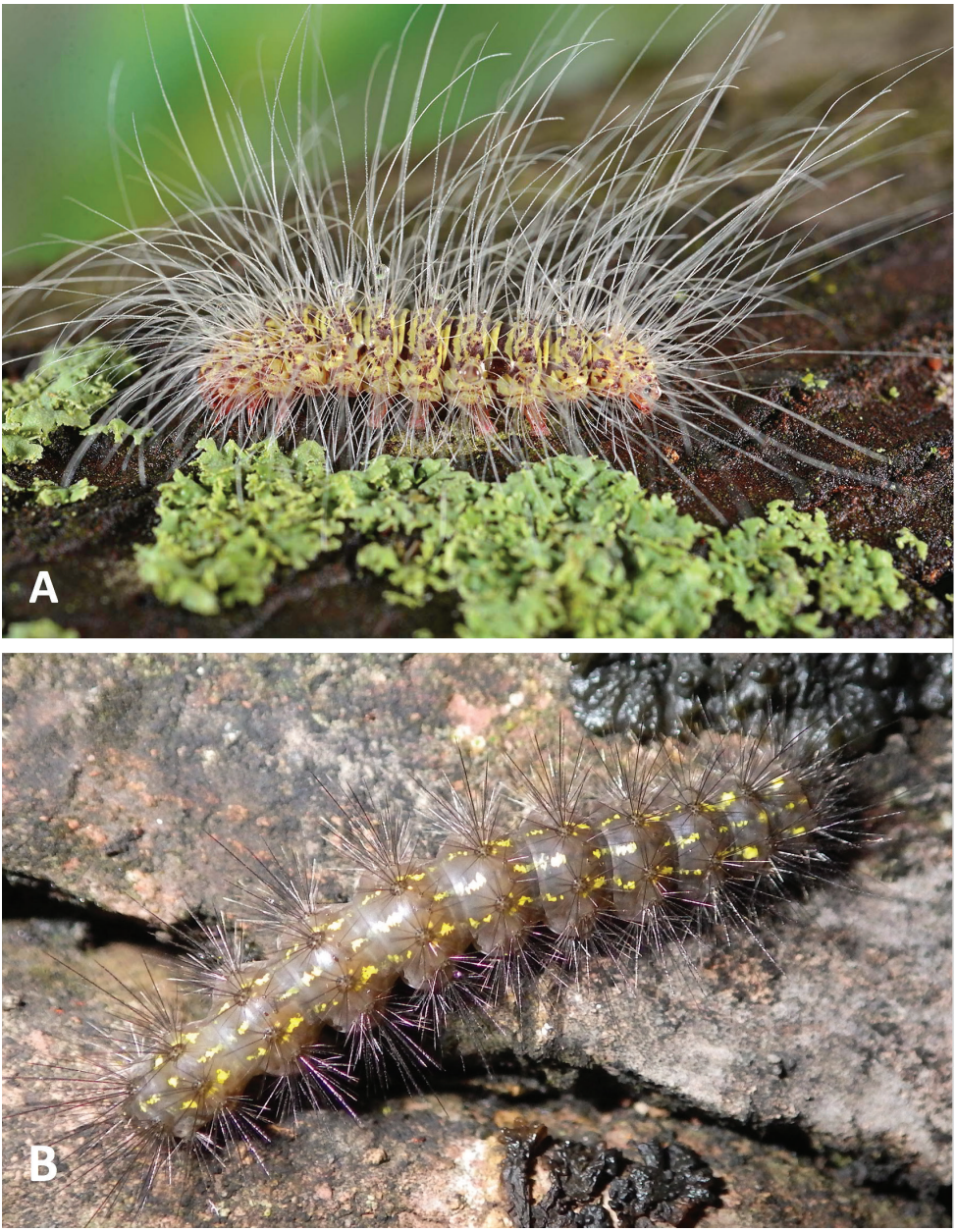

Figure 5. Representative Cisthenina larvae **A** *Eudesmia arida* (Skinner) **B** *Gardinia anopla* Hering.

the extent of this clade, with the genera *Sicia*, *Hyposiccia* and *Parasiccia* from the Western Hemisphere likely to be included within it. Future research on this clade is likely to be fruitful. Not only is a larger molecular and morphological analysis required, the limited information available on the immatures of species in this clade (McCabe 1981) suggests they are strictly algivores, refusing to feed on lichen at all. This observation,

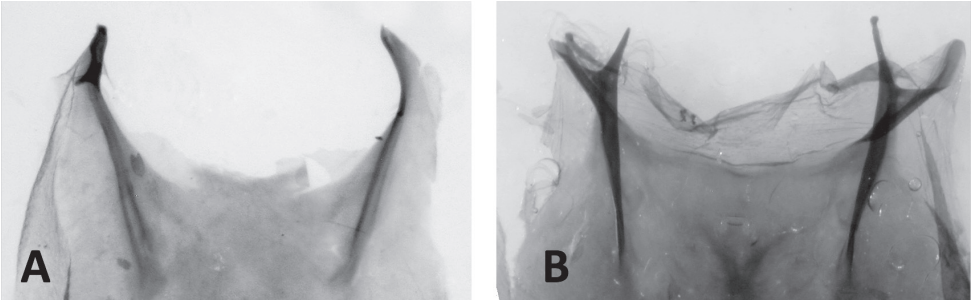

Figure 6. Dissections of the second abdominal sternites of adults illustrating two alternate states found among subtribes of Lithosiini **A** apodemes of *Pygarctia roseicapitis* (Neumoegen & Dyar) are relatively short and the anterolateral processes articulate with the sternal plate as found in most members of Lithosiina (other than *Agylla*) and in Arctiini **B** apodemes of *Gardinia anopla* Hering are relatively long and the anterolateral processes are fused with the rest of the sternum as found in members of the Cisthenina, including *Eudesmia* and *Gardinia.*

combined with their somber coloring, might indicate they do not sequester lichen phenolics for protection as do all other lithosines.

Subtribe Lithosiina

Bendib and Minet (1999) did not treat or assign genera to the Lithosiina. Results of this study indicate the following 13 genera are included in this well-supported clade: *Agylla* Walker, *Apistosia* Hübner, *Areva* Walker, *Atolmis* Hübner, *Crambidia* Packard, *Cybosi*a Hübner, *Eilema* Hübner, *Gnamtonychia* Hampson, *Hiera* Druce, *Inopsis* Felder, *Lithosia* Fabricius, *Manulea* Wallengren, *Mintopola* Hampson.

This is the first study to include specimens of *Gnamtonychia* Hampson and *Inopsis* Felder in a molecular-based phylogenetic analysis. Including them in *Lithosiina* is also supported by the shape of the second abdominal sternite (Fig. 6A) and their resting posture with their wings held somewhat flattened and rolled around their abdomen, two traits typical of Lithosiina. *Gnamtonychia ventralis* Barnes and Lindsey occurs in southeastern Arizona and New Mexico and *Inopsis modulata* Edwards occurs in Mexico and is rarely found in southeastern Arizona. These two species are remarkably similar in external appearance as adults, however side-by-side *I. modulata* is a slightly smaller moth with shorter, more rounded wings than *G. ventralis* (Fig. 3B, C). Both species are evidently part of a Mullerian mimicry complex that includes the arctiine *Pygotenucha terminalis* (Walker) (included here as an outgroup), which is similarly colored and a toxic milkweed-feeder in the larval stages. The larvae of *G. ventralis* are unknown. The larvae of *I. modulata* have distinctive orange to red verrucae and a dark bodies (Fig. 5B), making them conspicuous as they feed on lichens growing on tree branches.

In agreement with Zenker et al. (2016), the molecular phylogeny in this study places *Agylla* within Lithosiina. *Agylla* represents the single largest radiation among Western Hemisphere Lithosiini, with 101 described species found in the Neotropics. Primarily an

Old World group, Zenker et al. (2016) proposed the Lithosiina colonized South America from the Holarctic in one or more events. In fact, results presented here likely confirm that there have been at least three incursions from the Old World to the New World (assuming the group originated in Asia as proposed by Zenker et al. 2016). In our analyses we added *A. septentrionalis* Barnes and McDunnough (Fig. 1A), which is restricted to the mountains of southeastern Arizona. Adults of this species look similar to some European members of the genus *Lithosia* Fabricius such as male *L. quadra* Linnaeus included in our analyses. However, the results of this analysis show that these two genera are not closely related, *Crambidia* is instead closely related to *Manulea*+*Eilema* (Palaearctic/Oriental), whereas *Agylla* is part of a Neotropical clade. We note that adults of *A. septentrionalis* hold their wings "tent-like" over the body rather than flattened and rolled around their abdomens like most Lithosiina. In addition, the adults possess a Cisthenina-like second abdominal sternite (Fig. 6B). Thus, the placement of *Agylla* within Lithosiina, means that these morphological characteristics are more labile than previously thought.

Concluding remarks

With a tribe as large as Lithosiini, it is surprising that a subtribal classification was neglected for so long, yet understandable given their worldwide diversity and confounding variation of morphological characters. Beginning with Bendib and Minet (1999), we started to conceptualize how Lithosiini genera might be related. Some of our placements here, such as *Gardinia* among Cisthenina, show that the appearance of the adults does not belie their phylogenetic relatedness. With the apparent lack of morphological apomorphies identified thus far that support subtribal alliances, molecular techniques provide a useful tool for understanding how their diversity evolved. As additional molecular data are published and made available, their evolutionary relationships will become more apparent and hopefully lead to the discovery of morphological apomorphies in both larvae and adults. Presently the whole life history is known for only a small percentage of species. Thus, we have barely scratched the surface in understanding these remarkable lepidopterans and their unique relationship to their lichen hosts and to each other.

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Supplementary material 1

Figure S1

Authors: John D. Palting, Wendy Moore

Data type: Image.

Explanation note: Maximum likelihood tree for the concatenated matrix. Branch lengths are proportional to relative divergence, as estimated by IQ-TREE, scale bar indicates 0.05 units. Bootstrap values are depicted below branches. Clades are colored by subtribe.

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Taxonomic and nomenclatural notes on Chinese species of *Sarcophaga* Meigen, 1824 (Diptera, Sarcophagidae)

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Abstract

New taxonomic and nomenclatural data are provided for Chinese species of *Sarcophaga* Meigen, 1824. Eight new synonyms are proposed: two at the genus level, *Magnicauda* Wei, 2005 **syn. nov.** = *Sarcophaga* Meigen, 1824 and *Leigongshanophaga* Lehrer & Wei, 2010 **syn. nov.** = *Sarcophaga* Meigen, 1824, two at the subgenus level, *Magnicauda* Wei, 2005 **syn. nov.** = *Pterosarcophaga* Ye, 1981 and *Leigongshanophaga* Lehrer & Wei, 2010 **syn. nov.** = *Cornexcisia* Fan & Kano, 2000, and four at the species level, *Sarcophaga catoptosa* Wei & Yang, 2007 **syn. nov.** = *Sarcophaga suthep* Pape & Bänziger, 2003, *Pierretia daozhenensis* Wei, 2005 **syn. nov.** = *Sarcophaga sichotealini* (Rohdendorf, 1938), *Pierretia autochthona* Wei & Yang, 2007 **syn. nov.** = *Sarcophaga* (*Liosarcophaga*) *kanoi* Park, 1962, and *Parasarcophaga simultaneousa* Wei & Yang, 2007 **syn. nov.** = *Sarcophaga huangshanensis* (Fan, 1964). *Sarcophaga* (*Liosarcophaga*) *aegyptica* Salem, 1935 is considered a senior synonym of *Sarcophaga* (*Liosarcophaga*) *parkeri* (Rohdendorf, 1937). Correct original spellings are established, by First Reviser action, for the genus-group names *Magnicauda* Wei, 2005 and *Pterosarcophaga* Ye, 1981 and for the species-group name *Magnicauda linjiangensis* Wei, 2005. Chinese material of *Sarcophaga* (*Bellieriomima*) *genuforceps*, *S.* (*Robineauella*) *huangshanensis* (holotype and paratype), *S.* (*Liosarcophaga*) *kanoi*, and *S.* (*L.*) *aegyptica* is photographed for the first time.

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Keywords
Leigongshanophaga, *Magnicauda*, new synonyms, nomenclature, original spellings, revision, *Sarcophaga*, taxonomy

Introduction

Sarcophaga Meigen, 1824 (*sensu lato*) is by far the largest genus in the Sarcophagidae, and with upwards of a thousand species it is also one of the largest genera of Diptera (Whitmore et al. 2013; Wang et al. 2019, 2020; Evenhuis and Pape 2021). The genus is widespread, and the adults are very homogeneous in their external morphology and often recognizable at the species level only through a detailed study of the male terminalia (Buenaventura et al. 2017), for which professional skills as well as considerable experience are needed. The uniformity in external appearance stands in strong contrast to the marked structural complexity of the male terminalia, where phallic morphology in particular has diversified through the evolution of variously shaped appendages, the homologies of which are often obscure. The diversity and variability of the male terminalia, combined with the practical need to break up the large *Sarcophaga* (*sensu lato*) into smaller taxa, has brought about a high number of genus-level and species-level synonyms (Pape 1996; Wang et al. 2019, 2020). Ongoing studies of the Chinese fauna of *Sarcophaga* has led to the recognition of several new synonyms, which are presented here together with relevant taxonomic and nomenclatural details.

Material and methods

Specimens examined or otherwise mentioned are deposited at the following institutions:

CDCP Center for Disease Control and Prevention of Anshun city, Guizhou province, China;
MNHN Muséum national d'Histoire naturelle, Paris, France;
MBFU Museum of Beijing Forestry University, Beijing, China;
NHMD Natural History Museum of Denmark;
SECA Shanghai Entomological Museum, Chinese Academy of Sciences, Shanghai, China;
SMNH Swedish Museum of Natural History.

Identifications were aided by the keys in the publication of Fan (1992), combined with extensive comparisons against specimens in the reference collections of MBFU and NHMD, supplemented by a library of images of male terminalia and the original descriptions. We follow Roback (1954), Downes (1965), Pape (1996), Pape and Dahlem (2010), Giroux et al. (2010), Richet et al. (2011), Whitmore et al. (2013), Buenaventura et al. (2017), and Buenaventura and Pape (2018) in a broad definition of the genus *Sarcophaga*. External morphology was examined with an Olympus SZX16 stereomicroscope, and pho-

tographs were taken with a Canon 600D camera mounted on the same microscope. Images were processed in Adobe Photoshop CS 6 (Adobe Systems, Inc., San Jose, CA, USA) and stacked in Helicon Focus 3.2 (Helicon Soft Ltd, Kharkov, Ukraine). Inked illustrations were done by tracing over a photograph or figures from the original descriptions. The International Code of Zoological Nomenclature (ICZN 1999) is referred to as "the Code".

Taxonomy and nomenclature

Genus *Sarcophaga* Meigen, 1824

Sarcophaga Meigen, 1824: 305. Type species: *Musca carnaria* Linnaeus, 1758, by subsequent designation of Partington (1837: 607).

Magnicauda Wei, 2005: 405. Type species: *Magnicauda linjiangensis* Wei, 2005, by original designation. Syn. nov.

Maginicauda: Wei (2005: 409). Incorrect original spelling of *Magnicauda*, by First Reviser action in the present paper.

Leigongshanophaga Lehrer & Wei, 2010: 8. Type species: *Sarcophaga catoptosa* Wei & Yang, 2007 [= *Sarcophaga suthep* Pape & Bänziger, 2003], by original designation. Syn. nov. For other synonyms, see Pape (1996).

Remarks. Verves and Khrokalo (2020: 204) proposed *Leigongshanophaga* Lehrer & Wei, 2010 as a new synonym of the valid genus *Rosellea* Rohdendorf, 1937, but Xue et al. (2011: 320) proposed the same earlier. As argued below, we consider *Sarcophaga catoptosa* Wei & Yang, 2007, which is the type species of *Leigongshanophaga* Lehrer & Wei, 2010, to be a synonym of *Sarcophaga suthep* Pape & Bänziger, 2003, syn. nov., and we follow Wang et al. (2019) in treating this species in *Sarcophaga* subgenus *Cornexcisia* Fan & Kano, 2000.

Subgenus *Bellieriomima* Rohdendorf, 1937

Bellieriomima Rohdendorf, 1937: 164 (as subgenus of *Thyrsocnema* Enderlein, 1928). Type species: *Sarcophaga laciniata* Pandellé, 1896 [= *Sarcophaga subulata* Pandellé, 1896], by original designation.

Sarcophaga (*Bellieriomima*) *genuforceps* Thomas, 1949

Figs 1, 2

Sarcophaga genuforceps Thomas, 1949: 172. China, Sichuan, Chungking, Chinyunshan.
Pierretia catharosa Wei & Yang, 2007: 530. China, Guizhou, Leigongshan.

Material examined. 1♂, China, Zhejiang, Tianmu Mountain, 600–1100 m, 30.vi.1964, Huitai Fang leg. (SECA).

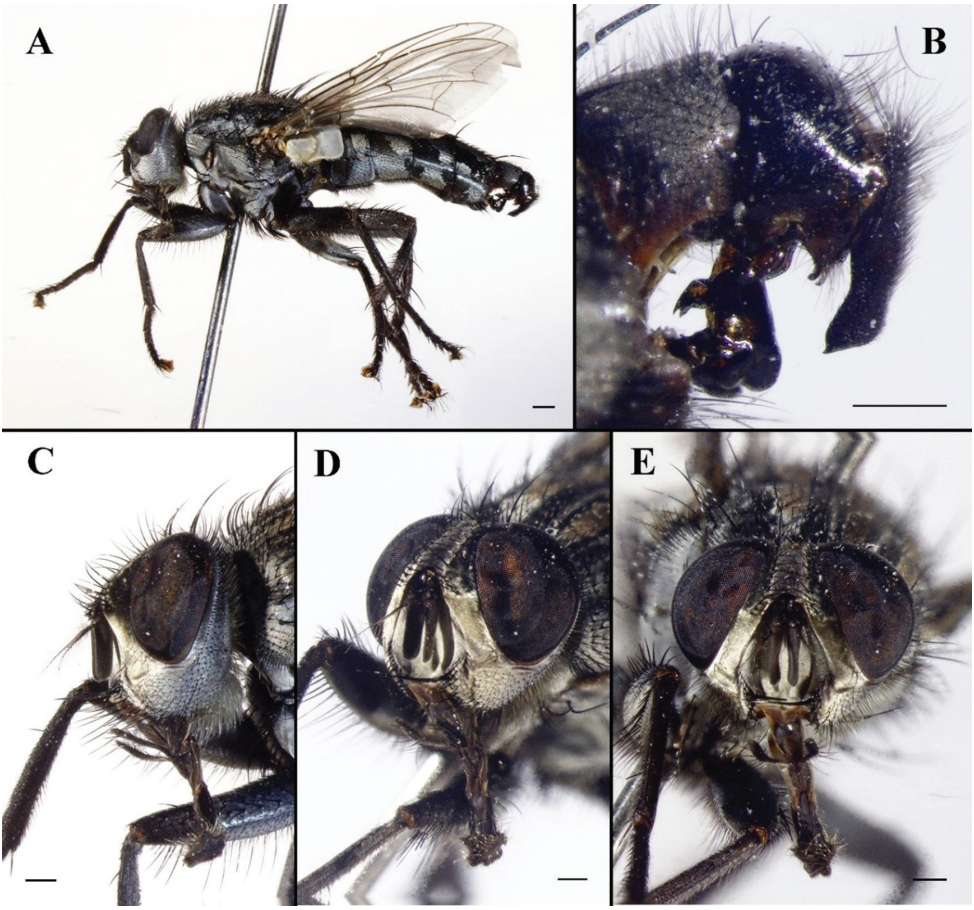

Figure 1. *Sarcophaga* (*Bellieriomima*) *genuforceps* Thomas, 1949; male (China, Zhejiang, Tianmu Mountain; in SECA) **A** habitus, lateral view **B** terminalia, lateral view **C** head, lateral view **D** head, anterolateral view **E** head, anterior view. Scale bars: 1 mm.

Remarks. The holotype of *Pierretia catharosa* is deposited in CDCP and not currently available for loan and study. Verves (2020: 36) listed *P. catharosa* as a junior synonym of *S. genuforceps*, although not as a new synonym. Wei and Yang (2007) gave a detailed description and a somewhat schematical illustration of the phallus (Fig. 2B), which is here considered sufficient justification for the synonymy. Xue and Verves (2009: 53) considered *S. genuforceps* to belong to *Pachystyleta* Fan & Chen, 1992, as a subgenus of *Myorhina* Robineau-Desvoidy, 1830, whereas Lehrer (2010: 18) raised *Pachystyleta* to genus rank. We prefer to follow the classification of Pape (1996), with *Pachystyleta* as a synonym of *Bellieriomima* and the latter as a subgenus of *Sarcophaga* (*sensu lato*).

Figure 2. *Sarcophaga* (*Bellieriomima*) *genuforceps* Thomas, 1949; phallus, lateral view **A** adapted from Lehrer (2012) **B** adapted from Wei and Yang (2007, as *Pierretia catharosa*).

Subgenus *Cornexcisia* Fan & Kano, 2000

Cornexcisia Fan & Kano, 2000: 251. Type species: *Cornexcisia longicornuta* Fan & Kano, 2000, by original designation.

Leigongshanophaga Lehrer & Wei, 2010: 8. Type species: *Sarcophaga catoptosa* Wei & Yang, 2007 [= *Sarcophaga suthep* Pape & Bänziger, 2003], by original designation. Syn. nov.

Sarcophaga (*Cornexcisia*) *suthep* Pape & Bänziger, 2003

Fig. 3

Sarcophaga suthep Pape & Bänziger, 2003: 52. Thailand, Chiang Mai Province, Doi Suthep.

Sarcophaga catoptosa Wei & Yang, 2007: 531. China, Guizhou, Leigongshan. Syn. nov.

Sarcophaga sutbeb: Wei and Yang 2007: 532. Incorrect subsequent spelling of *S. suthep* Pape & Bänziger, 2003.

Material examined. ***Holotype*** of *S. suthep*: ♂, Thailand, Chiang Mai Province, Doi Suthep, above Sangwal School, 1240 m, 28.viii.2000, H. Bänziger (in SMNH;

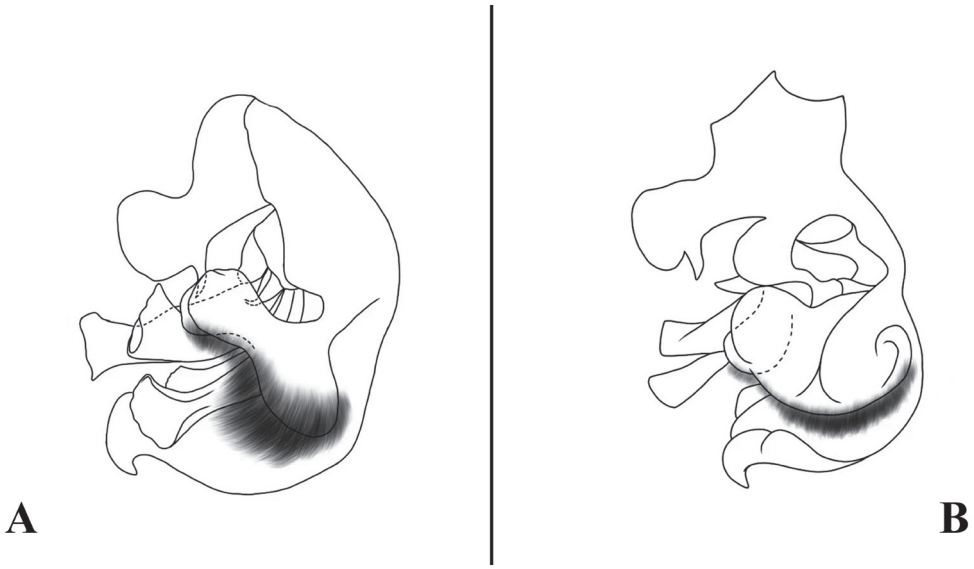

Figure 3. *Sarcophaga* (*Cornexcisia*) *suthep* Pape & Bänziger, 2003; phallus, lateral view **A** adapted from Pape and Bänziger (2003) **B** adapted from Wei and Yang (2007, as *Sarcophaga catoptosa*).

specimen dissected and with terminalia glued to a piece of cardboard pinned below the specimen).

Remarks. The holotype of *Sarcophaga catoptosa* is deposited in CDCP and not currently available for loan and study. Wei and Yang (2007) described the lateral styli as bifurcated at the base and expanded at the apex (Fig. 3). This unique character in *Sarcophaga* is shared by *S. suthep* and other species assigned to the subgenus *Cornexcisia*. We consider the following compelling similarities between the nominal species *S. suthep* and *S. catoptosa*, as assessed from the illustrations of the phallus (Fig. 3), to justify the proposed synonymy: vesica of identical shape; juxta, harpes and lateral styli differing only by small differences in the outline, and this involves membranous parts that are often presenting themselves very differently due to shrinking during drying or other preparation. Wei and Yang (2007) stressed the following difference between *catoptosa* and *suthep*: the protuberance of former cerci is slightly narrower than the latter in dorsal view and the hind margin of former pregonite is wavy bending with a sharper tip, but those differences are minor. They still have the same shape, only varying in degree. Therefore, we consider these to be intraspecific differences.

Subgenus *Liosarcophaga* Enderlein, 1928

Liosarcophaga Enderlein, 1928:18. Type species: *Cynomya madeirensis* Schiner, 1868, by original designation.

***Sarcophaga* (*Liosarcophaga*) *aegyptica* Salem, 1935**
Fig. 4

Sarcophaga dux aegyptica Salem, 1935: 56. Egypt, Alexandria; Egypt, Abbassieh; Egypt, Monsouriah.
Parasarcophaga (*Liosarcophaga*) *parkeri* Rohdendorf, 1937: 217. Ukraine, south shore of Crimea.

Material examined. 1♂, China, Qinghai, Minhe, 22.vii.1976, Shaoyuan Ma leg. (SECA).

Remarks. There has been disagreement among authors as to whether *Parasarcophaga parkeri* is a valid species or a junior synonym of *S. aegyptica*. Rohdendorf (1937) evidently knew Salem's (1935) work on *Sarcophaga* (s.l.) from Egypt, but he did not study any material identified as *S. aegyptica* and therefore quoted Salem's description. Furthermore, the diagnostic differences in the shape of the juxtal arms and

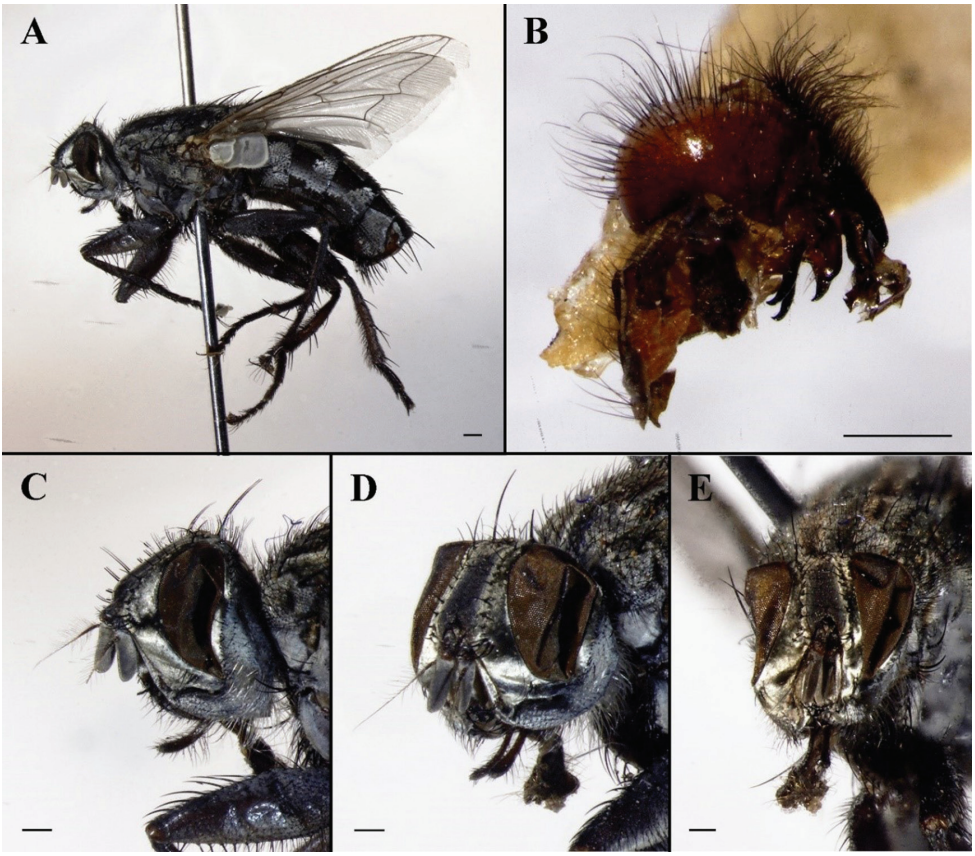

Figure 4. *Sarcophaga* (*Liosarcophaga*) *aegyptica* Salem, 1935; male (China, Qinghai; in SECA) **A** habitus, lateral view **B** terminalia, lateral view **C** head, lateral view **D** head, anterolateral view **E** head, anterior view. Scale bars: 1 mm.

harpes outlined in the key by Rohdendorf (1937: 440) were assessed based on Salem's illustrations. Gregor and Povolný (1960) synonymized the two nominal species, which was accepted by Rohdendorf (1970), and these taxa have since been considered either as separate species, e.g., by Lehrer (1995), Pape (1996), El-Ahmady et al. (2018), and Verves and Khrokalo (2020), or as synonyms, e.g., by Xue and Chao (1998), Nandi (2002), Povolný and Hula (2004), and Richet et al. (2011). The recent conspectus of Egyptian species of *Sarcophaga* (s.l.) by El-Ahmady et al. (2018) separated *aegyptica* and *parkeri* by vesica with two short processes apically and narrow harpes (*aegyptica*) versus vesica with three short processes apically and broad harpes (*parkeri*). The material at our disposal was not sufficient for a thorough assessment of the relevant morphological characters, but we have the impression that both the vesica and the harpes are variable structures, which furthermore present themselves very differently depending on the type of preparation and condition of the specimen. We have therefore chosen a conservative approach and consider the two nominal taxa as synonyms.

***Sarcophaga* (*Liosarcophaga*) *kanoi* Park, 1962**
Fig. 5

Sarcophaga (*Liosarcophaga*) *kanoi* Park, 1962: 6. South Korea, Taegu, Mt Pal-gong.
Pierretia autochthona Wei & Yang, 2007: 529. China, Guizhou, Leigongshan. Syn. nov.
Pierretia autochtona: Verves 2020: 37, incorrect subsequent spelling of *P. autochthona*.

Material examined. 1♂, China, Shanghai (Zi-Ka-Wei), 3.ix.1917, no further data (MNHN). 1♂, China, Hunan, Anxiang, Guandang, 20–21.vii.2012, Ming Zhang leg.; 1♂, China, Hunan, Anxiang, Guandang, 7.vii.2013, Ming Zhang leg.; 3♂♂, China, Hubei, Shishou, Gaoling, 8.vii.2013, Ming Zhang leg.; 1♂, China, Beijing, Beijing Forestry University, 9.vii.2016, Miao Jiang & Yunyun Gao leg. (MBFU).

Remarks. Wei and Yang (2007) considered *P. autochthona* as close to *S.* (*Pseudothyrsocnema*) *caudagalli* Böttcher, 1912, but we are here proposing a synonymy with *S.* (*L.*) *kanoi*. Wei and Yang (2007: fig. 72) illustrated the phallus of the holotype of *P. autochthona* as having a short, arm-like extension arising from the left lateral part of the distiphallus (probably the proximal part of the juxta) and a long, slender, process arising from the right lateral part of the distiphallus (Fig. 6). We consider this apparent asymmetry to be an artefact, and possibly an inaccuracy of the original illustration. This could not be confirmed because the holotype of *P. autochthona*, deposited in CDCP, has not been available for study through ordinary loan.

Subgenus *Phallantha* Rohdendorf, 1938

Phallantha Rohdendorf, 1938: 101. Type species: *Phallantha sichotealini* Rohdendorf, 1938, by original designation.

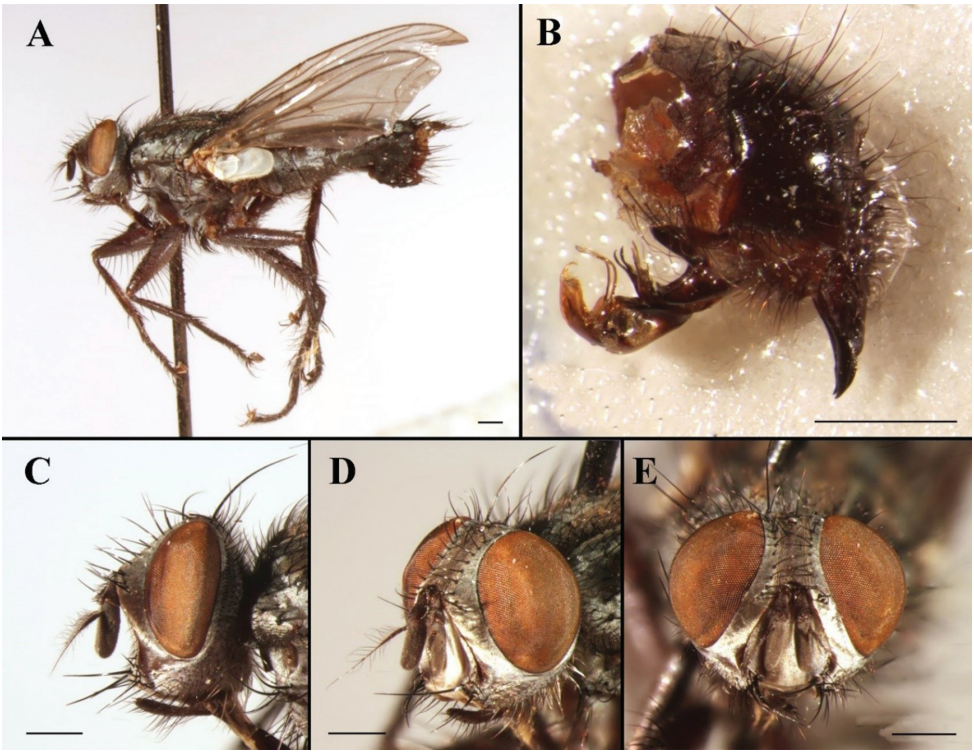

Figure 5. *Sarcophaga* (*Liosarcophaga*) *kanoi* Park, 1962; male (China, Hubei; in MBFU) **A** habitus, lateral view **B** terminalia, lateral view **C** head, lateral view **D** head, anterolateral view **E** head, anterior view. Scale bars: 1 mm.

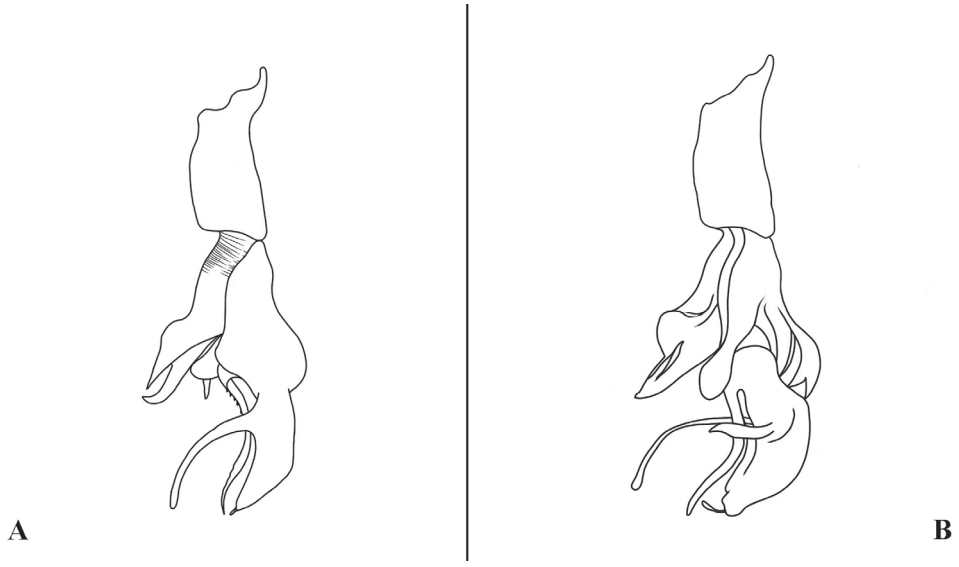

Figure 6. *Sarcophaga* (*Liosarcophaga*) *kanoi* Park, 1962; phallus, lateral view **A** adapted from Lehrer (2012) **B** adapted from Wei & Yang (2007, as *Pierretia autochthona*).

***Sarcophaga* (*Phallantha*) *sichotealini* (Rohdendorf, 1938)**
Fig. 7

Phallantha sichotealini Rohdendorf, 1938: 102. Russia, Primorye, Sikhote-Alin State Reservation.
Pierretia daozhenensis Wei in Wei & Yang, 2005: 424. China, Guizhou, Daozhen, Dashahe. Syn. nov.

Material examined. 1♂, Russia, Primorye, SE Ussurijsk, 8.viii.1983, A. Ozerov leg. (NHMD). 1♂, China, Sichuan, Baoxing, 8.v.1981, unknown leg.; 1♂, China, Sichuan, Ya'an, 29.iv.2002, unknown leg. (SECA).

Remarks. The holotype of *P. daozhenensis* is deposited in CDCP and not currently available for loan and study. This nominal species was not included by Verves (2020), probably in an oversight. Wei (2005) described the vesica as flower-like, the cerci as having pointed apices and being slightly bent in lateral view, and the juxtal

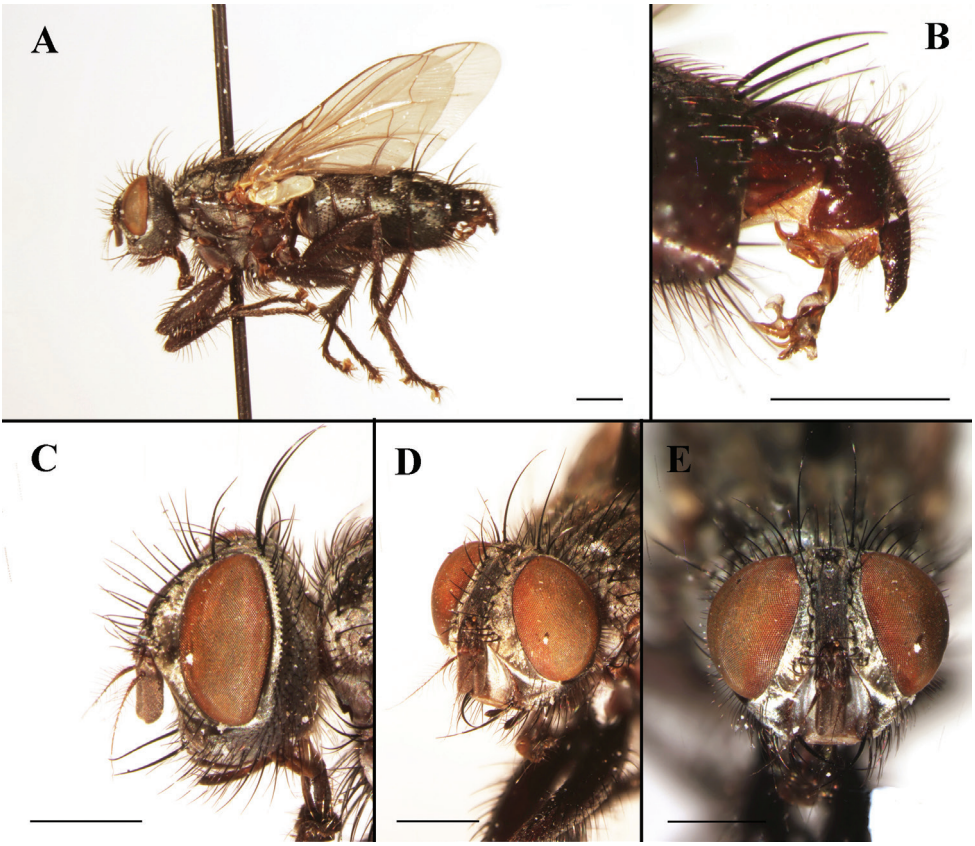

Figure 7. *Sarcophaga* (*Phallantha*) *sichotealini* (Rohdendorf, 1938); male (China, Sichuan; in SECA) **A** habitus, lateral view **B** terminalia, lateral view **C** head, lateral view **D** head, anterolateral view **E** head, anterior view. Scale bars: 1 mm.

Figure 8. *Sarcophaga* (*Phallantha*) *sichotealini* Rohdendorf, 1938; phallus, lateral view **A** illustrated from figure 7B **B** adapted from Wei and Yang (2005, as *Pierretia daozhenensis*).

extension as well developed, flexed at its base and bent forward apically (Fig. 4). All of these features are consistent with *S. sichotealini*, and we consider the illustrations of the phallus provided by Xue and Chao (1998: 677, fig. 1332 m), and Wei and Yang (2005: fig. 3) to be a fully acceptable match (Fig. 8). We notice that Wei and Yang (2005) mentioned that *P. daozhenensis* was assigned to *Pierretia* using the key by Xue and Chao (1998), but the species was not assigned to any of the subgenera applied by Xue and Chao (1998), which includes *Phallantha.* Wei and Yang (2005) made no discussion about the subgeneric affiliation of *P. daozhenensis*, and there is no comparison with *P. sichotealini* in spite of the significant similarities with the illustration provided by Xue and Chao (1998). Vesica and harpes are of the same overall configuration, and as these are composed of flattened, partly membranous structures, even small changes in orientation may result in considerable changes in outline. The juxta has a very characteristic shape, with an almost exact match. *Sarcophaga* (*P.*) *sichotealini* is distributed in China (Guizhou, Hunan, Sichuan, Yunnan), the Russian Far East, South Korea, and temperate Japan (Pape 1996; Xue and Chao 1998; Verves 2020).

Subgenus *Pterosarcophaga* Ye, 1981

Pterosarcophaga Ye, 1981: 229. Type species: *Pterosarcophaga emeishanensis* Ye & Ni, 1981, by original designation.

Pterosacophaga: Ye 1981: 230. Incorrect original spelling of *Pterosarcophaga*, by First Reviser action of Ye (1982: 21).

Magnicauda Wei, 2005: 405. Type species: *Magnicauda linjiangensis* Wei, 2005, by original designation. Syn. nov.

Maginicauda: Wei 2005: 409. Incorrect original spelling of *Magnicauda*, by First Reviser action in the present paper.

Remarks. Monotypic subgenera in *Sarcophaga* (*sensu lato*) are often erected for lack of evidence as to their phylogenetic relationships, and as such they convey little if any information. We prefer a classification based on similarities rather than on differences, and as Wei (2005) considered *Magnicauda* to be closely related to *Pterosarcophaga* due to the male cerci of the type species of both subgenera being expanded, wing-like, in lateral view, we are here treating the two nominal subgenera as synonyms.

Ye in Ye and Ni (1981) provided two different spellings: "*Pterosarcophaga*" (pp. 229, 232, 233) and "*Pterosacophaga*" (p. 230). By using only the spelling "*Pterosarcophaga*", Ye (1982: 21) acted as First Reviser according to Article 24.2.4 of the Code.

Wei (2005) provided two different spellings: "*Magnicauda*" (pp. 404–406, 408) and "*Maginicauda*" (p. 409). Since then, the only mention of this genus-group name we have found is that of Verves (2020: 48); however, as only the spelling "*Maginicauda*" was used, the criteria of Article 24.2.3 for a First Reviser action were not fulfilled. Wei (2005) did not provide an explicit etymology, but the description of a remarkably broad male cercus is here taken to indicate that "*Magnicauda*" was the intended spelling. This is supported by the repeated use of this spelling, whereas the spelling "*Maginicauda*" was used only once. We herewith select "*Magnicauda*" to be the correct original spelling, by First Reviser action.

***Sarcophaga* (*Pterosarcophaga*) *linjiangensis* (Wei, 2005)**

Fig. 9

Magnicauda linjiangensis Wei, 2005: 405. China, Guizhou, Xishui, Linjiang National Nature Reserve.

linjianensis: Wei 2005: 408, incorrect original spelling of *linjiangensis* Wei, 2005, by First Reviser action in the present paper.

Material examined. None.

Remarks. This species can be distinguished from other species of *Sarcophaga* by the flag-like pregonite. Wei (2005) provided two different spellings: "*linjiangensis*" (pp. 404–406, 409) and "*linjianensis*" (p. 408). Since then, the only mention of the species we have found is that of Verves (2020: 48); however, as only the spelling "*linjiangensis*" was used, the criteria for a First Reviser action were not fulfilled (see Art. 24.2.3 of the Code). As the species was evidently named after its type locality, we herewith select "*linjiangensis*" as the correct original spelling by First Reviser action.

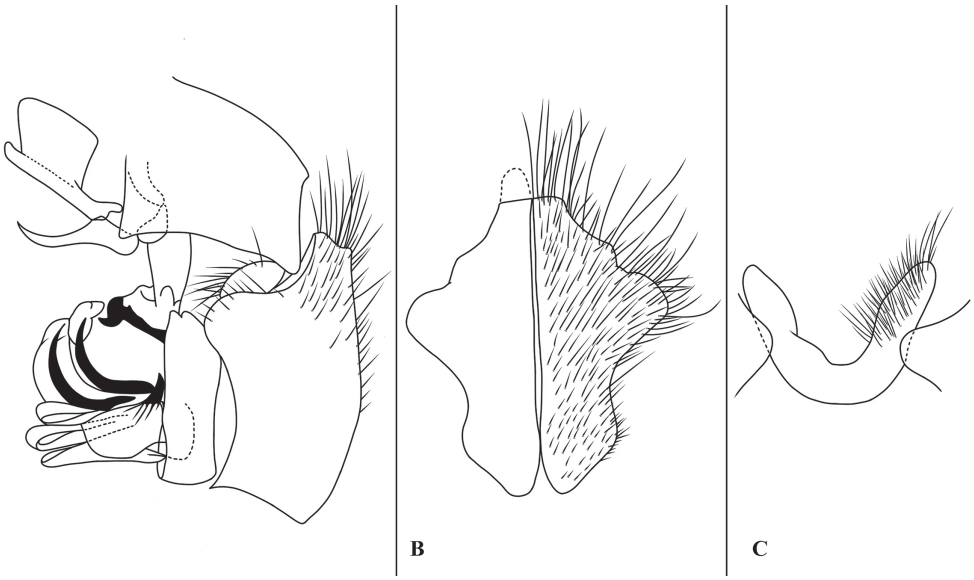

Figure 9. *Sarcophaga* (*Pterosarcophaga*) *linjiangensis* (Wei, 2005); male terminalia **A** terminalia, lateral view **B** cerci, dorsal view **C** sternite 5, ventral view. (Adapted from Wei 2005).

Subgenus *Robineauella* Enderlein, 1928

Robineauella Enderlein, 1928: 23 (as subgenus of *Parasarcophaga* Johnston & Tiegs, 1921). Type species: *Sarcophaga scoparia* Pandellé, 1896 [= *Sarcophaga caerulescens* Zetterstedt, 1838], by original designation.

Sarcophaga (*Robineauella*) *huangshanensis* (Fan, 1964)

Figs 10–12

Parasarcophaga (*Robineauella*) *huangshanensis* Fan, 1964: 312. China, Anhui, Huang-Shan.

Parasarcophaga simultaneousa Wei & Yang, 2007: 528. China, Guizhou, Leigongshan. Syn. nov.

Material examined. ***Holotype*** of *Parasarcophaga* (*Robineauella*) *huangshanensis* Fan, 1964: ♂, China, Anhui, Huangshan, 19.vi.1936, [unknown collector] [terminalia not recovered]. ***Paratypes*:** 2♂♂, China, Zhejiang, Tianmu mountain, 1100 m, 5.vii.1962, Zhizi Chen leg. (SECA).

Remarks. We examined the type series of *S.* (*R.*) *huangshanensis* and found that the male terminalia are a close match with the description and illustrations provided for *P. simultaneousa* (Figs 11, 12). The most important difference would be

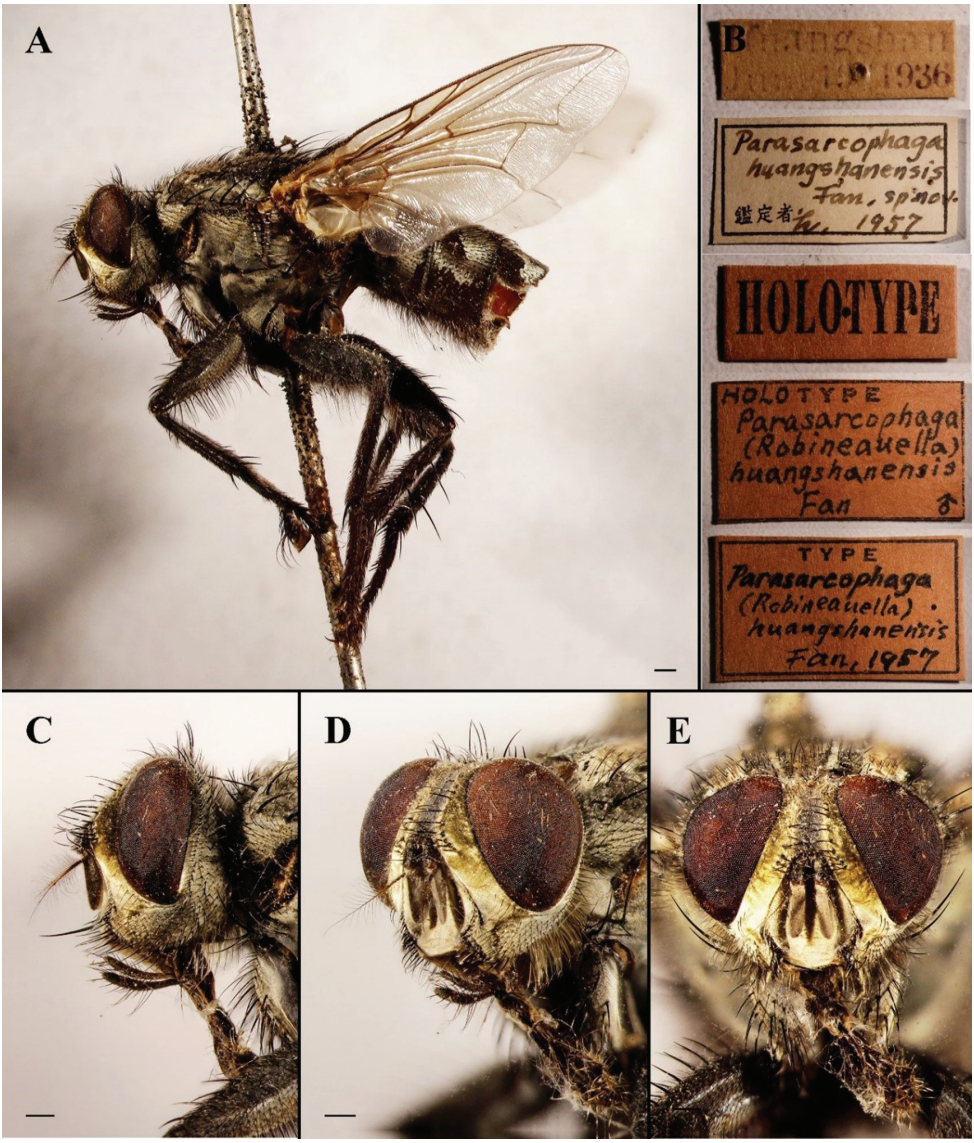

Figure 10. *Sarcophaga* (*Robineauella*) *huangshanensis* (Fan, 1964); holotype (China, Anhui; in SECA) **A** habitus, lateral view **B** labels **C** head, lateral view **D** head, anterolateral view **E** head, anterior view. Scale bars: 1 mm.

the difference in thickness of the proximal part of the juxtal processes, but this is here considered as infraspecific variation. Wei and Yang (2007) noted that this species could be confused with *S.* (*Liosarcophaga*) *kitaharai* Miyazaki, 1958; however, the latter, as a member of *Liosarcophaga* Rohdendorf, 1937, has a distiphallus with a better-developed dorso-median juxtal extension and an almost right-angled apico-dorsal part of juxta (Figs 11b, 12b, c). Lehrer (2012) examined the holotype

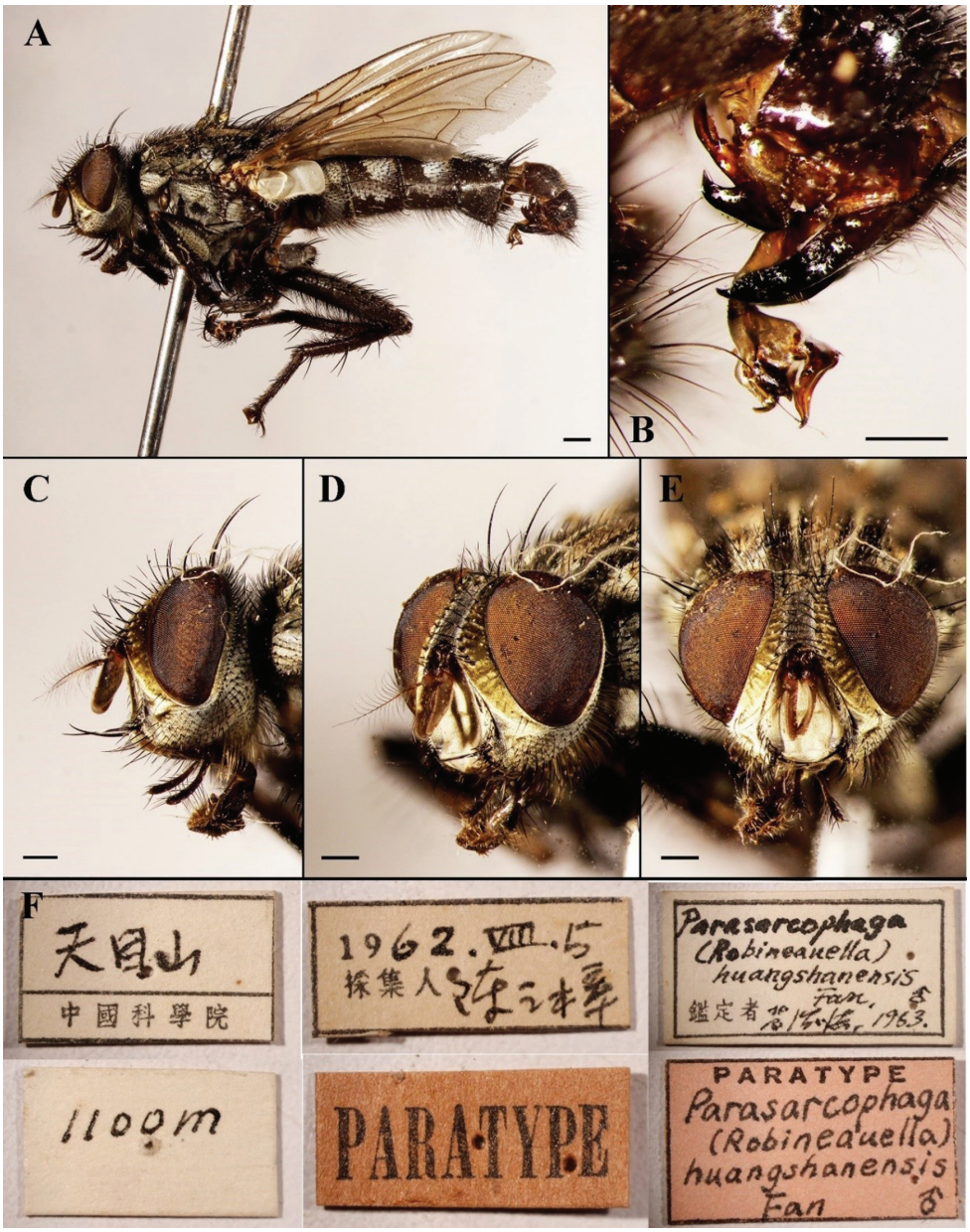

Figure 11. *Sarcophaga* (*Robineauella*) *huangshanensis* (Fan, 1964); paratype (China, Anhui; in SECA) **A** habitus, lateral view **B** terminalia, lateral view **C** head, lateral view **D** head, anterolateral view **E** head, anterior view **F** labels. Scale bars: 1 mm.

of *S. simultaneousa* and mentioned a similarity to *R. daurica* Grunin, 1964 and *R. mendeliana* Lehrer, 2008 (as "*mendelliana*"); however, he did not mention *S.* (*R.*) *huangshanensis*, maybe by an oversight.

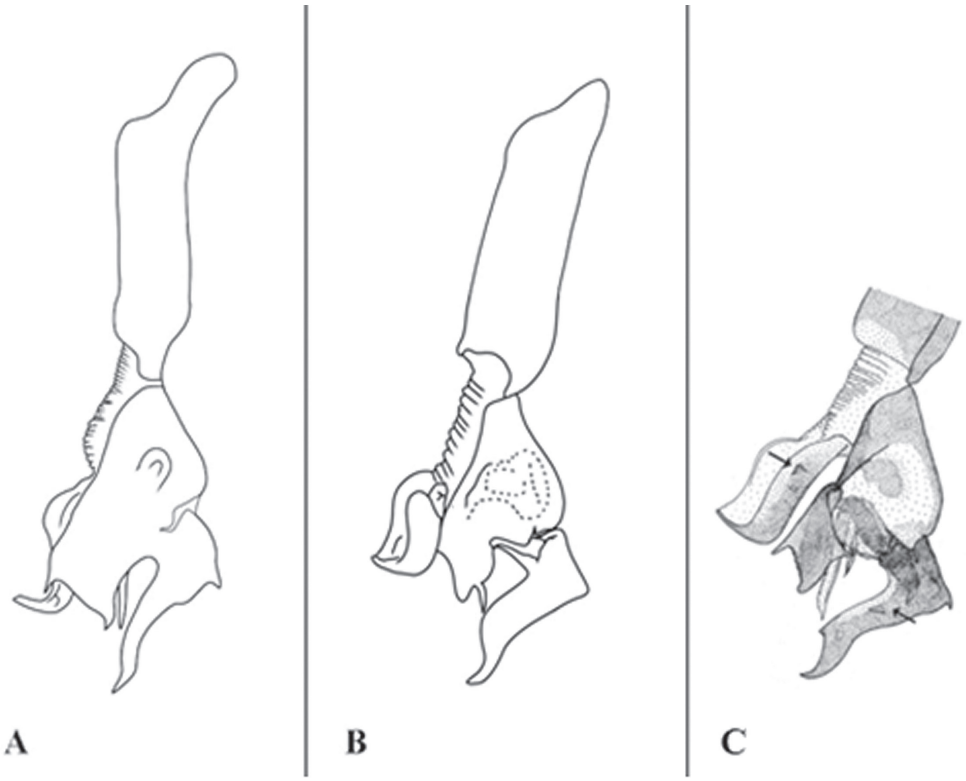

Figure 12. *Sarcophaga* (*Robineauella*) *huangshanensis* (Fan, 1964); phallus, lateral view **A** adapted from Fan (1964) **B** adapted from Wei and Yang (2007, as *Parasarcophaga simultaneousa*) **C** adapted from Lehrer (2012, as *Robineauella simultaneousa*).

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RESEARCH ARTICLE

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Two new cave *Hyleoglomeris* species (Glomerida, Glomeridae) from northern Vietnam

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Abstract

Two new glomerid species from caves in Cao Bang Province, Northern Vietnam, namely, *Hyleoglomeris halang* Kuroda, Nguyen & Eguchi, **sp. nov.** and *Hyleoglomeris alba* Nguyen, Kuroda & Eguchi, **sp. nov.**, are described. The former is characterized by a distinct body color pattern; telopods with a large, quadrate, medially concave, sparsely setose, central syncoxital lobe; and syncoxital horns approximately 1.5–2.0 times as long as the lobe. The latter is distinguished by its completely troglobiotic form without eyes, an unpigmented body, and a roundly triangular syncoxital lobe of telopods. An identification key is also provided for the cave glomerids of Vietnam.

Keywords

Biodiversity, millipede, new species, taxonomy, troglobiont

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Introduction

Currently, 23 glomerid species in six genera (*Annameris* Verhoeff, 1915, *Hyleoglomeris* Verhoeff, 1910, *Hyperglomeris* Silvestri, 1917, *Peplomeris* Silvestri, 1917, *Rhopalomeris* Verhoeff, 1906, and *Tonkinomeris* Nguyen, Sierwald & Marek, 2019) have been recorded and described from Vietnam (Nguyen et al. 2019a, b, 2021). Of these species, five were described from caves, *Hyleoglomeris cavernicola* Golovatch, Geoffroy & VandenSpiegel, 2013 and *Hyleoglomeris speophila* Golovatch, Geoffroy & Mauriès, 2006, both from Cat Ba National Park; *Hyleoglomeris colorata* Golovatch, Geoffroy & VandenSpiegel, 2013 and *Hyleoglomeris spelaea* Golovatch, Geoffroy & VandenSpiegel, 2013 both from Phong Nha - Ke Bang National Park; and *Hyperglomeris depigmentata* Golovatch, Geoffroy & VandenSpiegel, 2013 from Thanh Hoa Province (Golovatch et al. 2006, 2013).

Of the six genera, *Hyleoglomeris* is a rich-species genus, not only in Vietnam (12 species; Nguyen et al. 2019a, 2019b) but also worldwide (~ 100 species) (Golovatch et al. 2006; Wesener 2015; Sierwald and Spelda 2021). The genus is widely distributed from Greece in the west, Japan in the east, and Sulawesi (Indonesia) in the southeast (Golovatch et al. 2006). Therefore, it is not surprising that most Vietnamese cave glomerids belong to this genus.

During our field expeditions in northern Vietnam, glomerid specimens that could not be assigned to the named species were discovered and collected. They were both collected from caves and described in this paper.

Materials and methods

Specimens were collected manually and directly preserved in 85%–90% ethanol and examined under an Olympus SZX16 microscope. Telopods were dissected for morphological examination and photographed. Colored images were taken using a Nikon SMZ800N microscope and NIS-Element BR v. 5.20.00 and stacked using Helicon Focus v. 7.0. Images were assembled into plates using Photoshop CS6. Terminology follows Golovatch et al. (2013) and Nguyen et al. (2019a).

Total DNA was extracted using Qiagen DNeasy Blood and Tissue Kits. A 680-bp fragment of the mitochondrial gene, cytochrome C oxidase subunit I (COI), was amplified and sequenced using a pair of universal primers, LCO1490 and HCO2198 (Folmer et al. 1994). Polymerase chain reaction (PCR) conditions for amplification of the COI gene follow those of Nguyen et al. (2019b). ExoSap IT was used to successfully purify amplified PCR products, which were then sent for sequencing to the GenLab Company (Hanoi, Vietnam) and the Systematic Zoology Laboratory of Tokyo Metropolitan University (Tokyo, Japan). COI sequences were checked and confirmed using BLASTN 2.6.0+ search (Zhang et al. 2000) and deposited in GenBank.

The holotype, paratypes, and DNA vouchers were preserved in 90% ethanol and deposited at the Institute of Ecology and Biological Resources (**IEBR**), Hanoi, Vietnam.

Results

Taxonomy

Order GLOMERIDA
Family GLOMERIDAE Leach, 1815
Subfamily Doderiinae Slivestri, 1904
Genus *Hyleoglomeris* Verhoeff, 1910

***Hyleoglomeris halang* Kuroda, Nguyen & Eguchi, sp. nov.**
https://zoobank.org/91A73C3E-10AC-4273-9757-1996DCFBC138
Figs 1–5

Material examined. *Holotype*. Vietnam: male, Cao Bang Province, Ha Lang District, Duc Quang commune, Quang Hoai village, Nguom Hang cave, 22.7208N, 106.6692E, 10 Oct 2020, coll. AD Nguyen, VD Dang & VT Mai (IEBR-Myr 898H). ***Paratypes*.** Vietnam: 1 male, 1 female; Cao Bang Province, Ha Lang District, Duc Quang commune, Quang Hoai village, Nguom Hang cave, 22.7208N, 106.6692E, 10 Oct 2020, coll. AD Nguyen, VD Dang & VT Mai (IEBR-Myr 898P); 1 male, 1 female; Cao Bang Province, Ha Lang District, Duc Quang commune, Quang Hoai village, Nguom Hang cave, 22.7208N, 106.6692E, 15 March 2022, coll. AD Nguyen & DD Nguyen (IEBR-Myr 926).

Diagnosis. The species differs from its congeners in having a distinct body color pattern of white with oval-black spots on terga 4 and 5 and tadpole-shaped black bands on the thoracic shield and terga 6–9; telopods with a large, square/rectangular, slightly concave medially, sparsely setose, central syncoxital lobe; and syncoxital horns ~ 1.5 × as long as the lobe.

The new species can be keyed out at the 26th node in Golovatch et al. (2006), characterized by a thoracic shield and pygidium that are entirely or mainly light

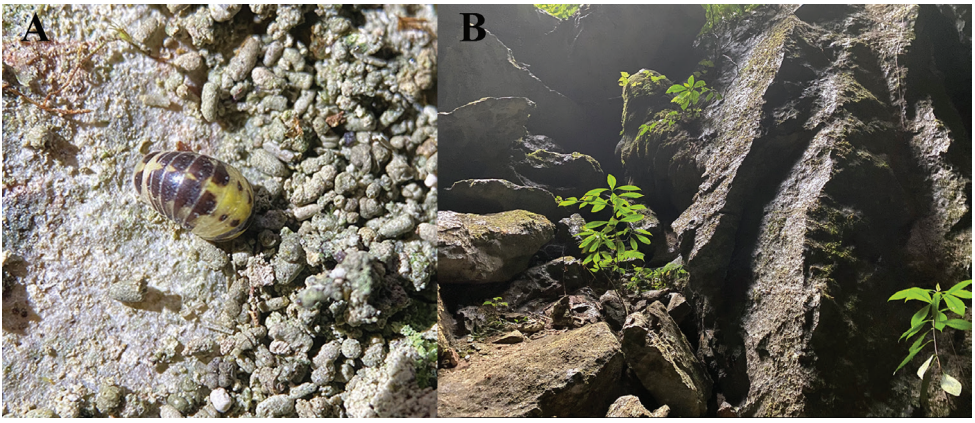

Figure 1. A *Hyleoglomeris halang* Kuroda, Nguyen & Eguchi, sp. nov. **B** Nguom Hang cave, entrance zone. Images not to scale.

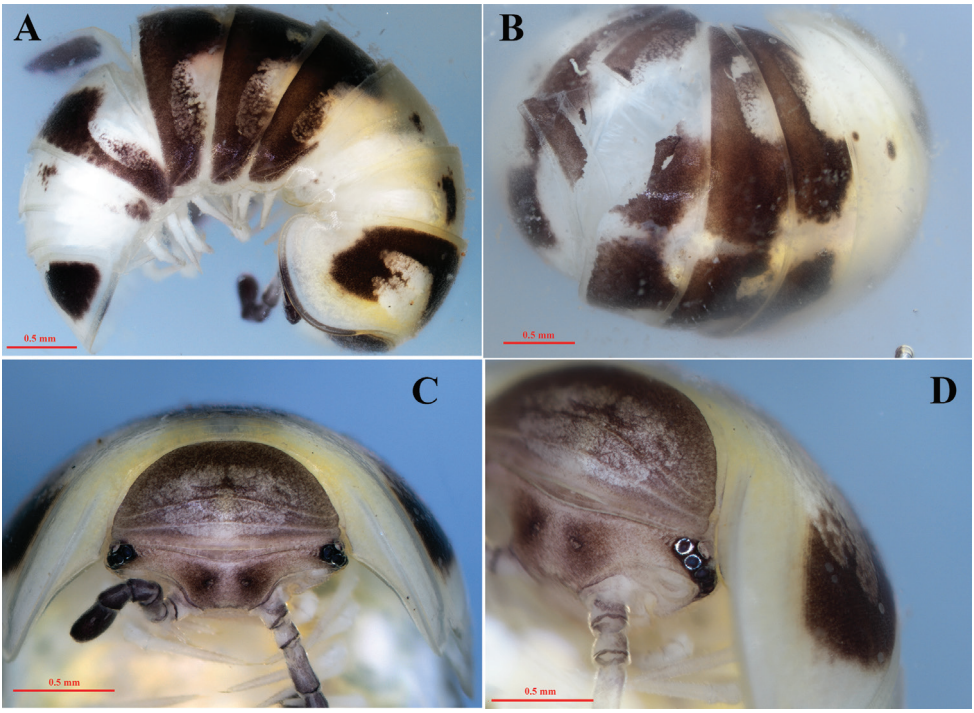

Figure 2. *Hyleoglomeris halang* Kuroda, Nguyen & Eguchi, sp. nov., holotype **A** whole body, lateral view, **B** dorsal view **C** collum, anterior view **D** ocelli, sub-anterior view.

and whitish to brown, as well as spots or markings, when present, that is darker than the background. Thus, this species can be grouped with *Hyleoglomeris triangulifera* Attems, 1938 and *Hyleoglomeris siamensis* (Silvestri, 1917). However, its smaller size distinguishes it from both species (3.5 mm wide vs. 6.5 mm and 5.0 mm wide).

Etymology. The new species is named after the Ha Lang District where the types were found. Noun in apposition.

Description. Body length 6 mm, width of the second segment ca. 3.5 mm. Coloration shown as in Figs 2 and 3. Generally white with a line of symmetrical, marbled, black, oval spots at terga 4 and 5, tadpole-shaped black bands on thoracic shield and terga 6–9. Anal shield white, with two laterally symmetrical triangular black spots.

Head. Ocelli 6+1; lenses convex, black contrasting against to a pale blackish background of the head. Tömösváry's organs transverse and strongly horseshoe-shaped, ~ 2 × as wide and long. Antennae clavate apically; antennomere 6 large, ~ 2.5 × longer than wide; antennal tip with four large, apical sensory cones.

Collum semi-circular, with a very large marbled white spot in the center and two transverse striae; the other parts of the collum pale black. The thoracic shield has a narrow hyposchism, not reaching the caudal margin, with 12 superficial transverse striae, eight of which cross the dorsum.

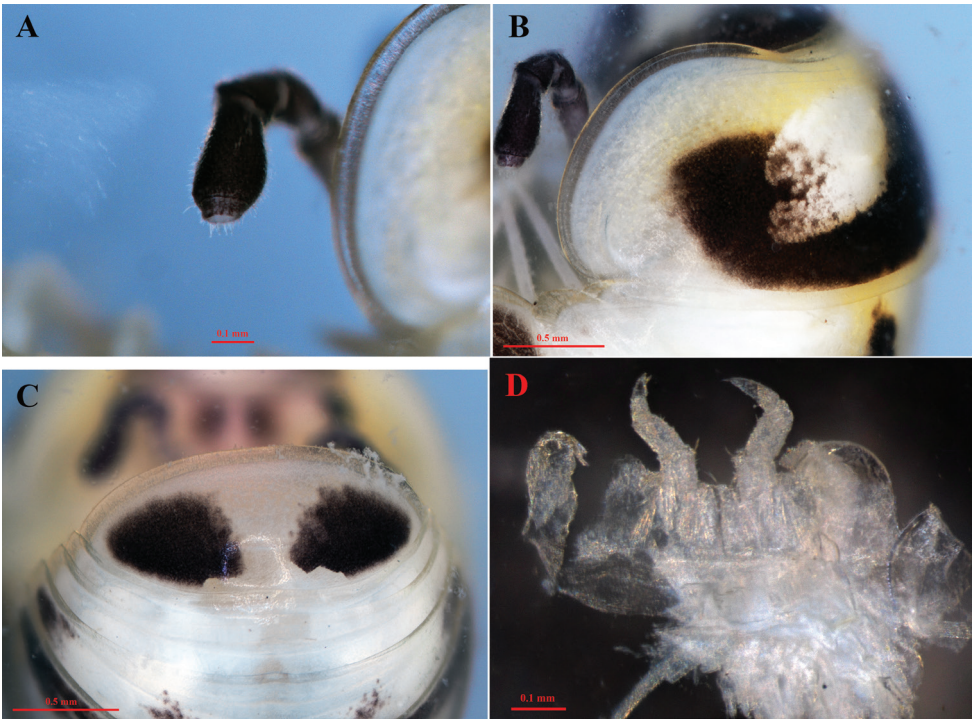

Figure 3. *Hyleoglomeris halang* Kuroda, Nguyen & Eguchi, sp. nov., holotype **A** right antenna **B** hyposchism **C** pygidium, posterior view **D** leg-pair 17.

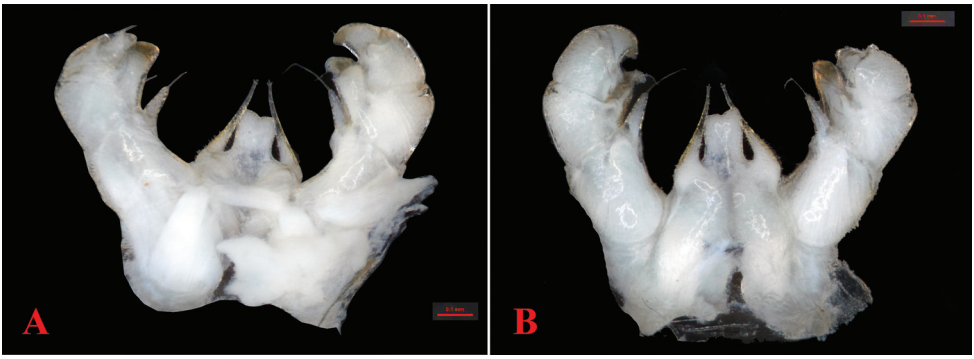

Figure 4. *Hyleoglomeris halang* Kuroda, Nguyen & Eguchi, sp. nov., holotype **A** telopods, anterior view **B** telopods, posterior view.

Leg-pair 17 strongly reduced, 4-segmented with a high, regularly rounded, outer coxal lobe (Figs 3D, 5A). Leg-pair 18 also reduced, but more developed in comparison with leg-pair 17, 4-segmented with a simple V-shaped syncoxital notch.

Telopods (Figs 4, 5B–D) with a large, quadrate, slightly concave medially, sparsely setose, central syncoxital lobe (syl) accompanying two setiferous horns (syh), each di-

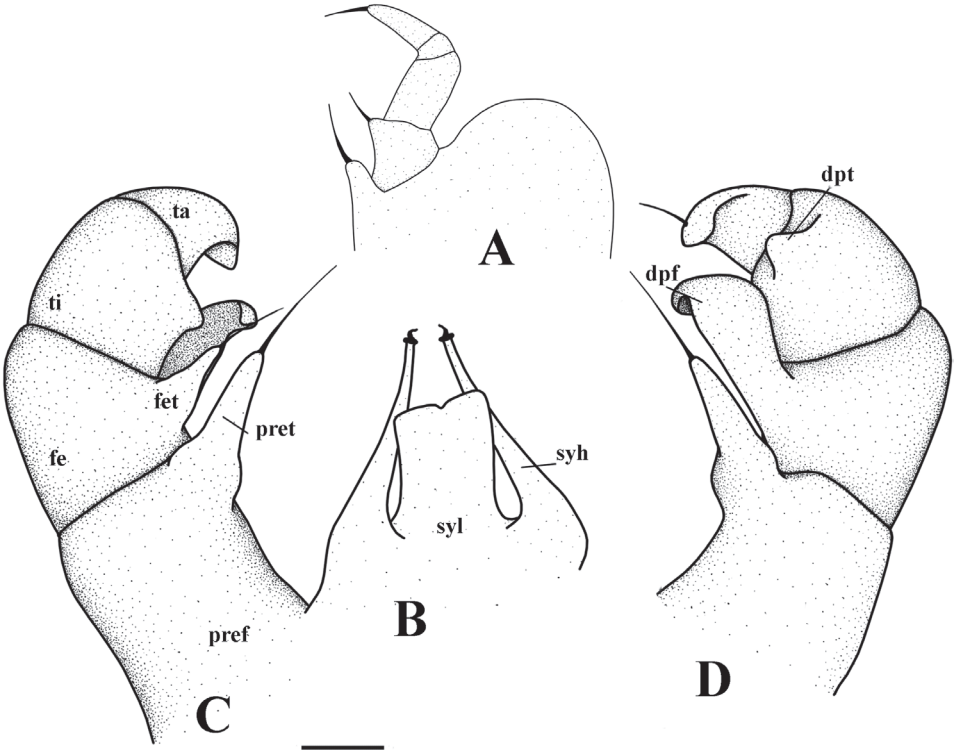

Figure 5. *Hyleoglomeris halang* Kuroda, Nguyen & Eguchi, sp. nov., holotype **A** leg-pair 17 **B** syncoxital lobe and syncoxital horns, posterior view **C** left telopod, posterior view **D** left telopod, anterior view. Scale bar: 0.1 mm. Abbreviations: syl = syncoxital lobe, syh = syncoxital horn, pref = prefemur, fe = femur, pret = prefemoral trichostele, fet = femoral trichostele, dpf = distomesal process of femur, ti = tibia, dpt = distolateral process of tibia, ta = tarsus.

rected subventrad, 1.5 × longer than the syncoxital lobe, tip crowned with an apical setoid. Prefemur (pre) and femur (fe) with long trichosteles (pret and fet), prefemoral one (pret) longer than femoral one (fet). Prefemur without additional processes. Distomesal process of femur (dpf) large, long, straight, lamelliform; distal part tuberculiform and strongly curved down, directed laterodorsad. Tibia (ti) with a shorter triangular distolateral process (dpt). Tarsus (ta) slightly sigmoid anteriomesad, subacuminate apically with a seta distoventrally.

Variability. Syncoxital horns can be 2.0 × longer than the syncoxital lobe, each horn directed ventromesad. Two trichosteles are almost of the same in length.

DNA barcode. The COI barcode data (679 bp fragment of the COI) for the paratype was uploaded to GenBank under the accession numbers ON704753 and ON704754. The new species shares 86.98% and 85.89% of its identity with *Hyleoglomeris lobus* Nguyen, Sierwald & Marek, 2019 (MT749402) and *Hyleoglomeris hoanglien* Nguyen, Eguchi & Hwang, 2019 (MH248038), respectively.

Remarks. This species is not a true cave inhabitant. However, it was discovered in the cave entrance (Fig. 1), and its body is less pigmented with large white areas, suggesting that this species is adapting to a cave-dwelling life.

Hyleoglomeris alba **Nguyen, Kuroda & Eguchi, sp. nov.**
https://zoobank.org/C27C76BA-7AD6-4B59-AFE0-6BD5AC9F831F
Figs 6–11

Material examined. ***Holotype.*** Vietnam: male; Cao Bang Province, Tra Linh District, Quoc Toan commune, Thang Hen lake, Ky Rang cave, 22.7650N, 106.2911E, 2 Nov. 2021, leg. AD Nguyen (IEBR-Myr 919). ***Paratypes.*** Vietnam: 2 females, Cao Bang Province, Tra Linh District, Quoc Toan commune, Thang Hen lake, Ky Rang cave, 22.7650N, 106.2911E, 12 Oct. 2020, leg. AD Nguyen, VT Mai & VD Dang (IEBR-Myr 917); 1 male, Cao Bang Province, Tra Linh District, Quoc Toan commune, Thang Hen lake, Ky Rang cave, 22.7650N, 106.2911E, 17 March 2022, leg. AD Nguyen & DD Nguyen (IEBR-Myr 928).

Diagnosis. The species can be recognized by a completely troglobiotic form with no eyes, an unpigmented body, and a roundly triangular syncoxital lobe.

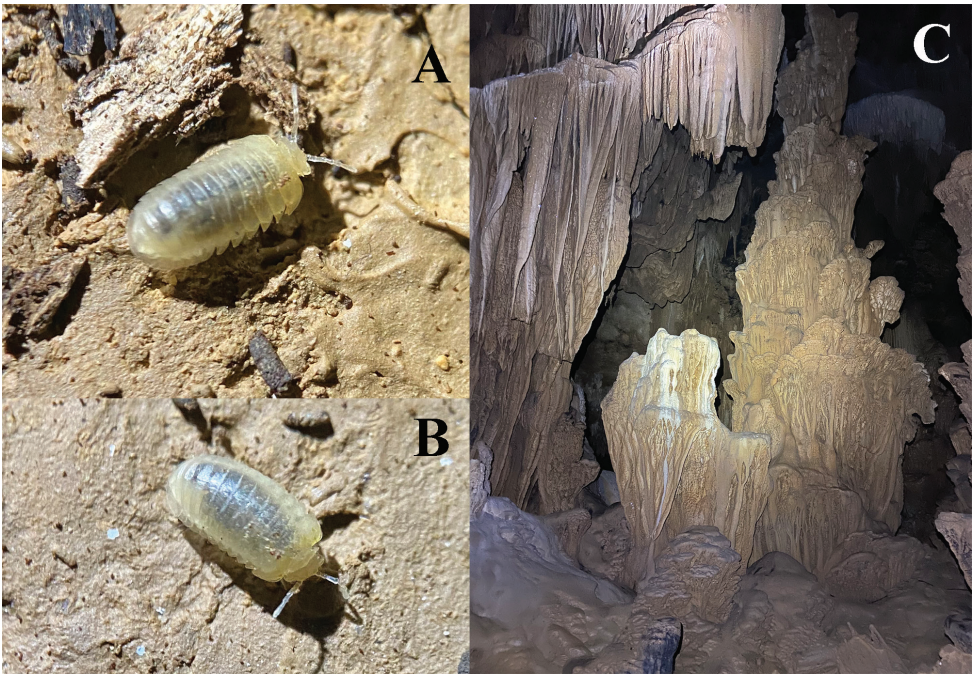

Figure 6. A, B *Hyleoglomeris alba* Nguyen, Kuroda & Eguchi, sp. nov., habitus **C** Ky Rang cave, dark zone. Images not to scale.

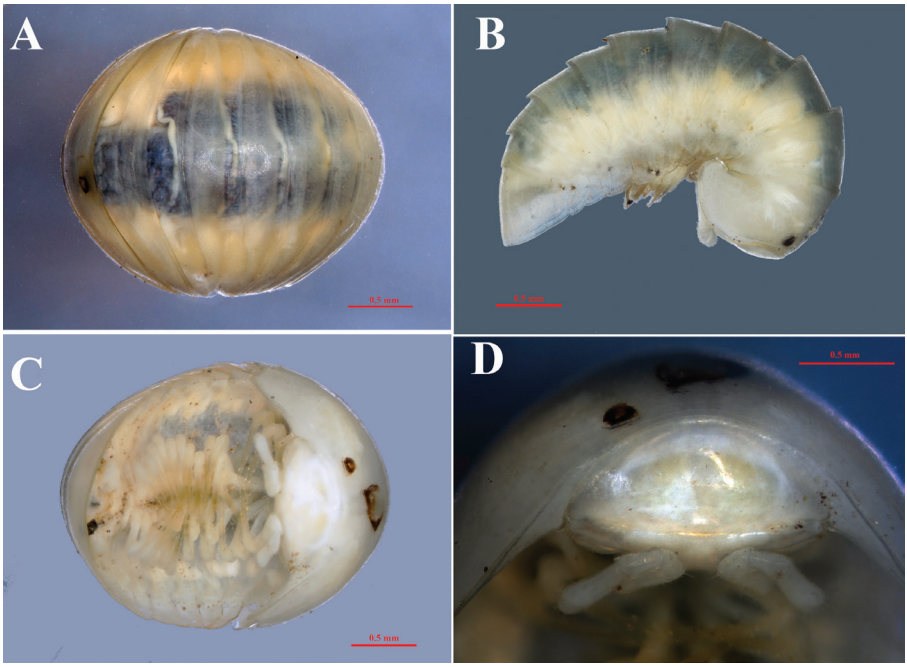

Figure 7. *Hyleoglomeris alba* Nguyen, Kuroda & Eguchi, sp. nov., holotype **A** whole body, dorsal view **B** lateral view **C** ventral view **D** collum, anterior view.

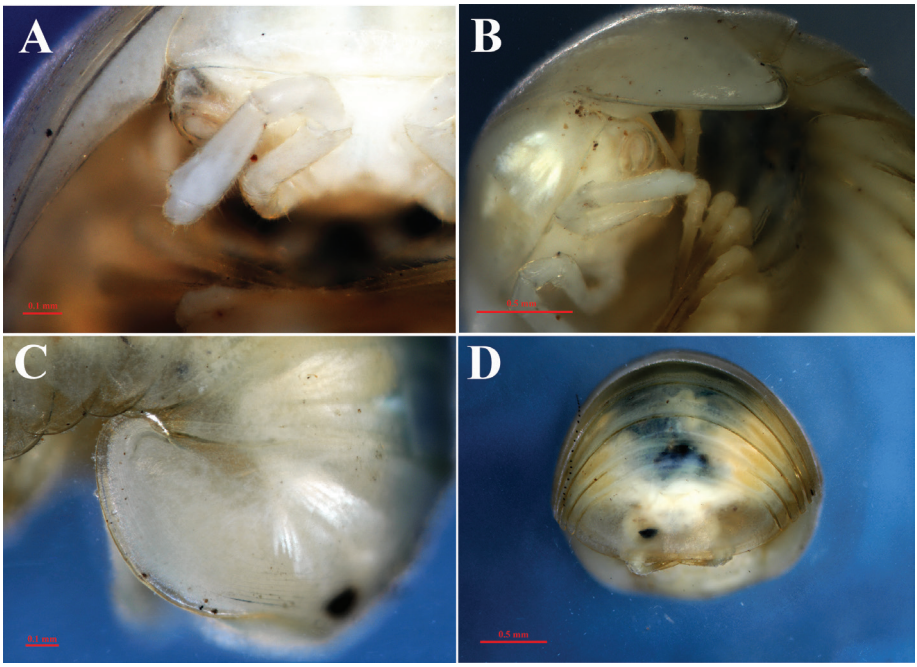

Figure 8. *Hyleoglomeris alba* Nguyen, Kuroda & Eguchi, sp. nov., holotype **A** left antenna **B** right antenna and Tömösváry's organ **C** left hyposchism, dorsal view **D** pygidium, posterior view.

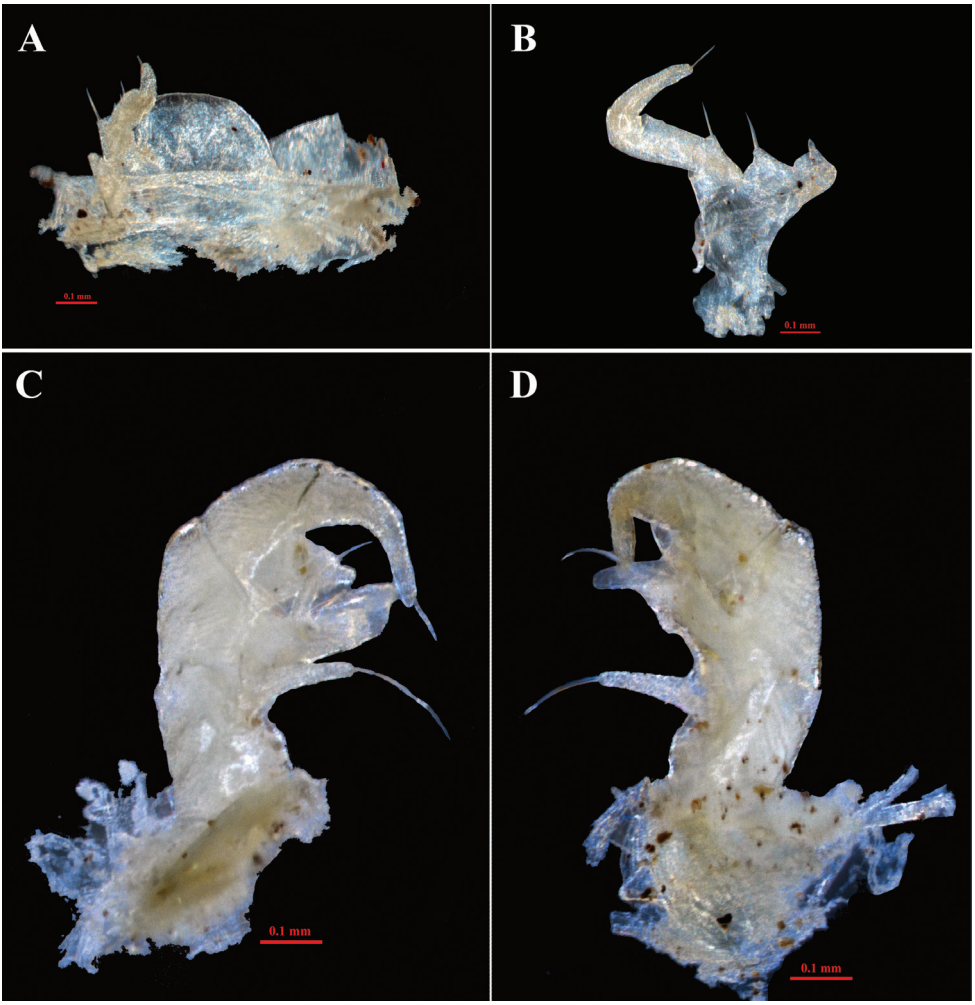

Figure 9. *Hyleoglomeris alba* Nguyen, Kuroda & Eguchi, sp. nov., holotype **A** leg-pair 17 **B** leg 18 **C** left telopod, anterior view **D** subposterior view.

According to Golovatch et al. (2006, 2013), the new species seems to belong to the troglobiont species group containing *H. speophila*, *H. spelaea*, *H. cavernicola*, *H. differens* Golovatch, Geoffroy & Mauriès, 2006, *H. reducta* Golovatch, Geoffroy & Mauriès, 2006, and *H. albicorporis* Zhang & Zhang, 1995. These species are characterized by a totally unpigmented body. However, the new species differs from these species in lacking ocelli and the telopods bearing a roundly triangular syncoxital lobe. In contrast, the other species have convex ocelli, telopods with a roundly subtraperziform syncoxital lobe (*H. speophila*, *H. cavernicola*, *H. reducta*, *H. albicorporis*), or a subquadrate syncoxital lobe (*H. spelaea*), or a roundly triangular syncoxital lobe (*H. differens*).

Etymology. From the Latin *alba*, meaning white. It was used to emphasize the unpigmented body of the new species.

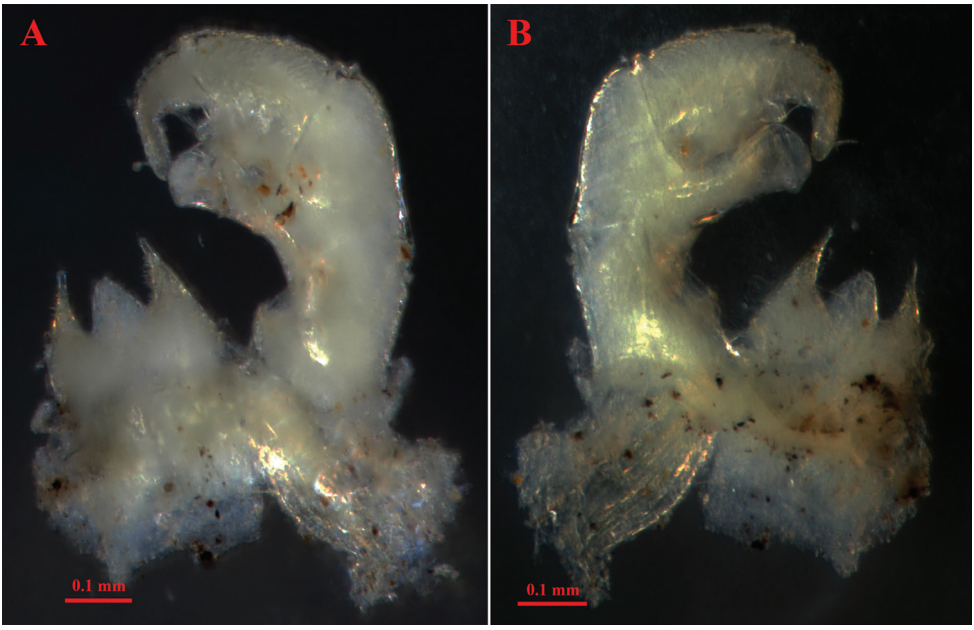

Figure 10. *Hyleoglomeris alba* Nguyen, Kuroda & Eguchi, sp. nov., holotype **A** right telopod and syncoxital lobe, posterior view **B** anterior view.

Description. Body length 4.38 mm, width of the second segment ~ 2.02 mm. Color entirely white, unpigmented (Figs 6–8). Ocelli totally absent. Tömösvary's organ transverse, strongly horseshoe-shaped, ~ 2 × as wide as long (Fig. 8B). Antennae long and slender, antennomere, ~ 3 × longer than wide, antennal tip with four apical sensory cones (Fig. 8A).

Collum semicircular, with a trace of a transverse oval spot in the center and two distinctly transverse striae (Fig. 7D). Second tergum with a narrow hyposchism, not reaching the caudal margin, with seven or eight striae, five or six of which cross the dorsum. Anal shield rounded, very slightly concave medio-caudally (Fig. 8D).

Leg-pair 17 (Figs 9A, 11A) strongly reduced, with four podomeres, with a high, regularly rounded, outer coxal lobe; coxa with an apical setiferous spine; leg-pair 18 (Figs 9B, 11B) also strongly reduced, but more developed in comparison with leg-pair 17, with four podomeres, and a simple V-shaped syncoxital notch.

Telopods (Figs 9C, D, 10, 11C–E) with a roundly triangular, sparsely setose, central syncoxital lobe (syl) accompanying two setiferous syncoxital horns (syh), each directed ventrad, slightly longer than the syncoxital lobe, tip crowned with an apical setoid. Prefemur (pre) and femur (fe) with long trichosteles (pret and fet), prefemoral one (pret) longer than femoral one (fet). Prefemur without additional processes. Distomesal process of femur (dpf) large, long, straight, rectangular; distal part tuberculiform and strongly curved downwards, directed laterodorsad. Tibia (ti) with a shorter triangular distolateral process (dpt). Tarsus (ta) slightly sigmoid anteriomesad, subacuminate apically, with a seta distoventrally.

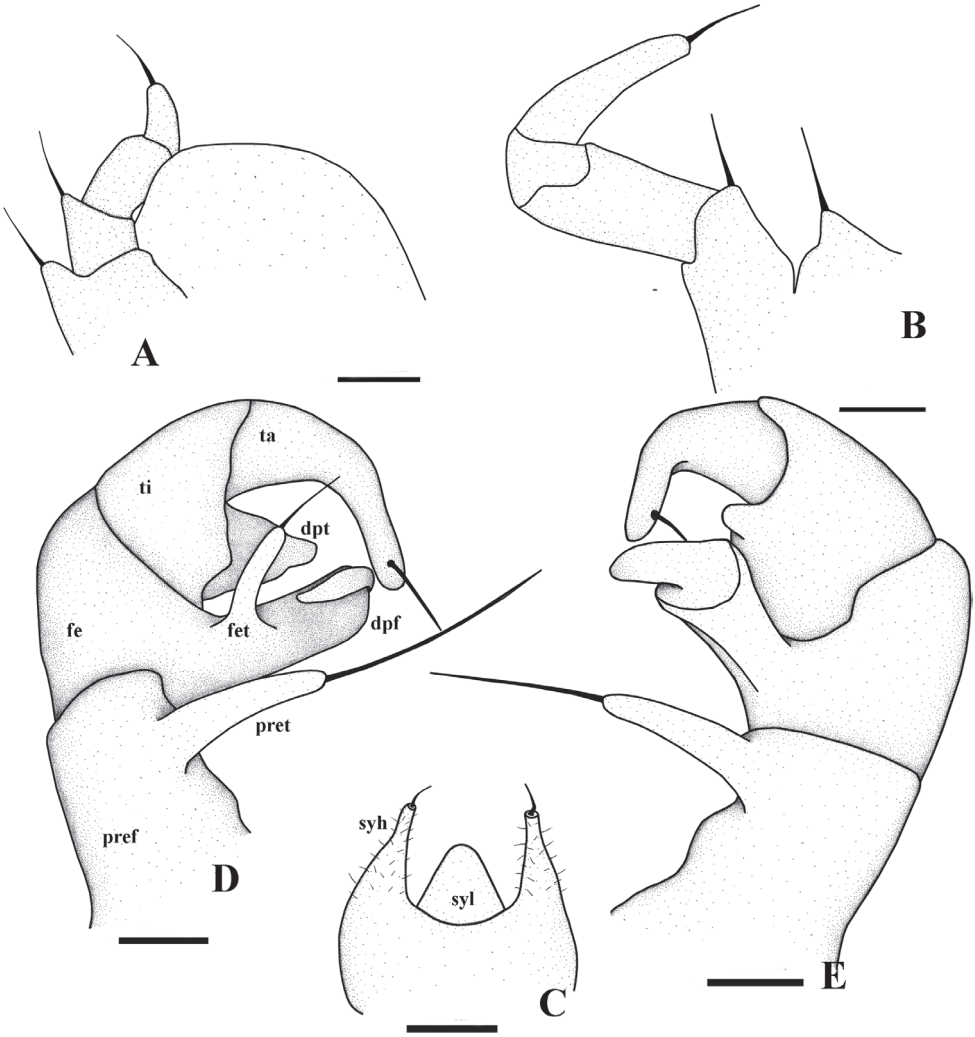

Figure 11. *Hyleoglomeris alba* Nguyen, Kuroda & Eguchi, sp. nov., holotype **A** leg-pair 17 **B** leg-pair 18 **C** syncoxital lobe and syncoxital horns **D** left telopod, anterior view **E** subposterior view. Scale bar: 0.1 mm. Abbreviations: syl = syncoxital lobe, syh = syncoxital horn, pref = prefemur, fe = femur, pret = prefemoral trichostele, fet = femoral trichostele, dpf = distomesal process of femur, ti = tibia, dpt = distolateral process of tibia, ta = tarsus.

DNA barcode. We failed to amplify the COI fragments of this species.

Remarks. The species was collected from the totally dark region in the cave. The completely unpigmented body without ocelli, and with long, slender antennae suggest that this species is a true troglobiont. These characters were also mentioned in Liu et al. (2017) who reviewed the morphological adaptations seen in troglobitic glomerids and other millipedes.

An identification key to cave glomerids in Vietnam

1 Prefemoral and femoral trichosteles of telopods absent or rudimentary. Leg-pair 18 3-segmented ..... ***Hyperglomeris depigmentata***

– Prefemoral and femoral trichosteles of telopods present, well-developed. Leg-pair 18 4-segmented **2**

2 Leg-pair 17 3-segmented. Body pattern peculiar, annulated ..... ***Hyleoglomeris colorata***

– Leg-pair 17 4-segmented. Body pattern with some dark spots or entirely unpigmented **3**

3 Body with dark spots ..... ***Hyleoglomeris halang*** **sp. nov.**

– Body coloration completely white or unpigmented **4**

4 Ocelli completely absent. Syncoxital lobe of telopods roundly triangular ..... ***Hyleoglomeris alba*** **sp. nov.**

– Ocelli present, sometime poorly visible. Syncoxital lobe of telopods differently shaped but not triangular **5**

5 Body size small, up to 6 mm long. Syncoxital lobe subquadrate; horn tip with a minute, elongate lobule with a flagelloid filament .... ***Hyleoglomeris spelaea***

– Body size larger, 8.5–10 mm. Syncoxital lobe slightly concave or roundly subtrapeziform **6**

6 Body length 9–10 mm. Syncoxital lobe slightly concave, broadly subtrapeziform; lateral horns simple, unarmed ..... ***Hyleoglomeris cavernicola***

– Body length 8.5 mm. Syncoxital lobe rounded subtrapeziform; lateral horns each crowned with an apical setoid ..... ***Hyleoglomeris speophila***

General discussion

The northeastern part of the present Indochinese peninsula was covered with a shallow sea from the Late Devonian to the Early Triassic periods (370–220 mya), forming limestone strata. Subsequently, the limestone strata were uplifted by the influence of the Himalayan orogeny after the Late Mesozoic era, which has been eroded by wind and rain for a long time (Clements et al. 2006; Sterling et al. 2006). Thus, Vietnam has vast karst of ~ 60,000 km^2 (~ 15% of the total area of karst in Southeast Asia) and many limestone caves of different sizes, structures, formation history, and degrees of geographical isolation (Sterling et al. 2006), which are rich in troglobites (Deharveng et al. 2001).

The Cao Bang Province is located in a karst region of northern Vietnam and supports hundreds of caves varying in size and environmental parameters (Sterling et al. 2006). Little is known about the cave millipedes in the Cao Bang Province. Golovatch (2019) recently described several new paradoxosomatid species, namely *Tylopus nguyeni* Golovatch, 2019, *Parasundanina faillei* Golovatch, 2019, and *Hylomus srisonchaii* Golovatch, 2019 from the same cave as the new species in the current study.

These species are completely troglobiotic, with unpigmented bodies, long antennae, and legs. Interestingly, these discoveries resulted from the study of several caves and surveys. Thus, more intensive studies are suggested to reveal more new species.

Acknowledgements

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RESEARCH ARTICLE

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Two new species of *Itaphlebia* (Insecta, Mecoptera, Nannochoristidae) from the late Middle Jurassic of China

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Abstract

Two new species of *Itaphlebia* Sukatsheva, 1985, *I. procera* **sp. nov.** and *I. elegana* **sp. nov.**, are described and illustrated from the latest Middle Jurassic Jiulongshan Formation of Daohugou, Inner Mongolia, China. Based on fossil specimens with wings, these new species are established and assigned to *Itaphlebia* by a combination of three forewing characters: Sc with three branches ending at C, the four-branched Rs (R_2 to R_5) originating distad of M (vs. three-branched RS (R_{2+3} undivided) in all other fossil and extant nannochoristids), and M forking with four branches; and a hind wing character of Sc simple and short, terminating at C well before the pterostigma. Furthermore, this is the first report of long and robust setae present on the anal veins of the forewing for *I. elegana* **sp. nov.** in fossil Nannochoristidae.

Keywords

Insect fossil, Jiulongshan Formation, nannochoristid, taxonomy

Introduction

Mecoptera Packard, 1886 is an order of insects, comprising nine extant families with more than 600 species (Soszyńska-Maj and Krzemiński 2013; Lin et al. 2016; Tillyard 1917). The earliest fossil records of mecopterans are known from the Early Permian,

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which occupy an important place in the Insecta. The insect fossil sites of Nannochoristidae Tillyard, 1917 are located in Siberia in Russia, Kazakhstan and Inner Mongolia, Liaoning in China. In the Southern Hemisphere, larval fossils from the Australian Cretaceous and eight extant species of nannochoristids have been documented in Argentina, Chile, New Zealand, Australia, and Tasmania (Jell and Duncan 1986; Byers 1989a; Ren et al. 2009; Cao et al. 2015). Based on conspicuously divergent larval morphology and lifestyle, Nannochoristidae has been suggested as a separate suborder, Nannomecoptera Hinton, 1981, to distinguish it from other families of Mecoptera (Penny 1975; Hinton 1981; Kristensen 1989). In recent phylogenetic studies, the systematic placement of Nannochoristidae has not been accurately settled. As a result of unusual and more primitive morphological characters of larval and immature stages (Beutel and Baum 2008; Beutel et al. 2009), Nannochoristidae together with Boreidae Latreille, 1816 form a clade with fleas, based more on phenetic differences than phylogenetic argumentation (Whiting 2002). Even though such thorough analyses have used extensive molecular data, the precise treatment and phylogenetic position of this separated suborder are still uncertain as well as unpredictable. Meanwhile, biological information on nannochoristids is limited; larvae are campodeiform, prognathous and predaceous, adults are almost aquatic, mostly resting or laying eggs on moist leaf litters at river edges. The adults may be omnivorous or nectarivorous, as documented studies on head morphology distinctly illustrate that they exclusively feed on flowing liquid, mainly nectar (Beutel and Baum 2008; Tillyard 1917; Beutel and Friedrich 2019). With their peculiar method of copulation and lacking detailed observations on feeding habits (Tillyard 1917), the lifestyle and biology of these rather fragile adults are still unclear so far.

Due to limited specimens of well-preserved compression fossils for nannochoristids, it was difficult to study detailed morphological characters to classify, diagnose and describe species or genera of these specimens. Therefore, the classifications of fossil species and genera of Nannochoristidae are in a state change and revision. So far, six genera: *Dahurochorista* Sukatsheva, 1985, *Dahurolarva* Sukatsheva, 1985, *Itaphlebia* Sukatsheva, 1985, *Namdyrus* Sukatsheva, 1993, *Tarantogus* Sukatsheva, 1985, and *Undisca* Sukatsheva, 1990, and one subgenus: *Eunannochorista* Novokshonov, 1997, from the Middle Jurassic and the Lower Cretaceous have been described (Sukatsheva 1985; Sukatsheva 1990; Novokshonov 1997a, b; Liu et al. 2010; Cao et al. 2015). However, the diagnoses of *Dahurolarva* and *Tarantogus* (Sukatsheva 1985) are based only on larvae, as revised by Novokshonov (1997b), and the diagnoses of *Undisca* and *Itaphlebia* (Sukatsheva 1985; Sukatsheva 1990) need revision after new and more well-preserved fossils have become available. In this paper, we describe two new species of *Itaphlebia*: *I. procera* sp. nov. and *I. elegana* sp. nov., and emend the diagnosis of *Itaphlebia*, based on five new and well-preserved fossil specimens.

All specimens were collected from the Jiulongshan Formation at Daohugou Village of Ningcheng County in Inner Mongolia. This locality is one of the richest Middle Jurassic fossil-bearing sites in China. The Jiulongshan Formation has yielded abundant and diverse mecopteran fossils (Cao et al. 2015), highlighting the complex interactions

of mecopterans with the ecosystems (Wang et al. 2012). The deposits are considered as the latest Middle Jurassic (late Callovian) in age (Walker et al. 2013; Ren et al. 2019; Gao et al. 2021), approximately 165–164 Mya.

Materials and methods

The new type specimens are collected from the latest Middle Jurassic, Jiulongshan Formation; Daohugou Village, Shantou Township, Ningcheng City, Inner Mongolia, China and housed in the insect fossil collection of the Key Laboratory of Insect Evolution and Environmental Changes, College of Life Sciences, Capital Normal University, Beijing, China (**CNUB**; Dong Ren, Curator).

These specimens were examined and photographed using a LEICA M165C dissecting microscope with a LEICA DFC 500 digital camera system with cool white transmitted light from double optical fibers, irradiating the specimens from two sides simultaneously, and illustrated with the aid of a drawing tube attachment. Enlarged photos were taken by using a Nikon SMZ 25 microscope with a Nikon DS-Ri 2 digital camera system. The line drawings were edited with Adobe Photoshop CS5. We use the venational nomenclature of Byers (1989b) as a frame of reference.

Systematic palaeontology

Order Mecoptera Packard, 1886
Family Nannochoristidae Tillyard, 1917

Genus *Itaphlebia* Sukatsheva, 1985

Chrysopanorpa Ren, 1995, p. 91.
Netropanorpodes Sun, Ren & Shih, 2007, p. 867.
Stylopanorpodes Sun, Ren & Shih, 2007, p. 865.
Protochoristella Sun, Ren & Shih, 2007, p. 405.

Diagnosis. Small to moderately-sized insect (forewing length 5.1–15.4 mm); body slender; wing nearly oval or rounded apically. Forewing: Sc with two or three branches ending at C; Rs (R_2 to R_5) origination distad of M origination; Rs forking with four (in some cases with five) branches; M forking with four (in some cases with five) branches. Hind wing: Sc simple and short, terminating at C well before pterostigma; Rs forking with four branches; M forking with four branches (in some cases with five).

Type species. *Itaphlebia completa* Sukatsheva, 1985

Species included. Type species, *I. ruderalis* comb. (= *Chrysopanorpa ruderalis* Ren, 1995 = *Stylopanorpodes eurypterus* Sun, Ren & Shih, 2007 = *Protochoristella formosa*

Sun, Ren & Shih, 2007), *I. jeniseica* Novokshonov, 1997 (= *Netropanorpodes sentosus* Sun, Ren & Shih, 2007), *I. multa* Novokshonov, 1997 (= *Protochoristella polyneura* Sun, Ren & Shih, 2007), *I. reducta* Novokshonov, 1997, *I. sharovi* Novokshonov, 1997, *I. generosa* Novokshonov & Sukatsheva, 2003, *I. decorosa* comb. (= *Netropanorpodes decorosus* Sun, Ren & Shih, 2007), *I. exquisita* Liu, Zhao & Ren, 2010, *I. laeta* Liu, Zhao & Ren, 2010, *I. longiovata* Cao, Shih, Bashkuev & Ren, 2015, *I. amoena* Cao, Shih, Bashkuev & Ren, 2015, *I. procera* sp. nov. and *I. elegana* sp. nov.

Itaphlebia procera **Cao, Shih & Ren, sp. nov.**

https://zoobank.org/3771FB50-DDB5-45CA-968A-8DC60CE923CF
Figs 1, 2

Etymology. The specific name is derived from the Latin adjective "procerus", indicating the relatively large body size.

Type material. ***Holotype*****:** sex unknown, No. CNU-MEC-NN2009273 (Fig. 1), preserved in dorsal view, head and thorax well-preserved, with four wings partly preserved, veins of right forewing mostly discernible. ***Paratype*****:** sex unknown, No. CNU-MEC-NN2009168 (Fig. 2), preserved in lateral view, body partly preserved, only one forewing venation clearly discernible.

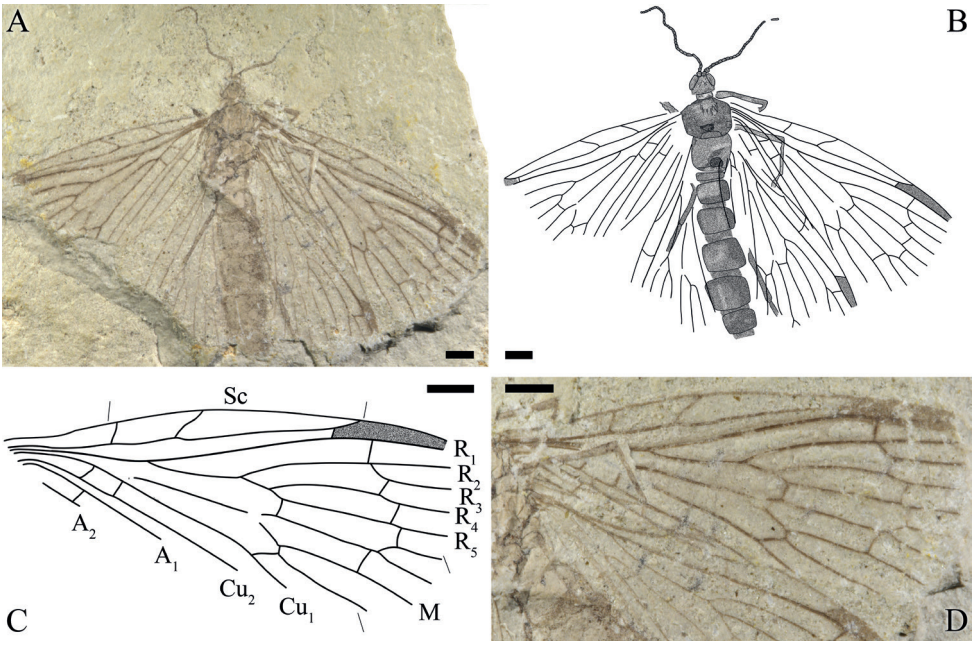

Figure 1. *Itaphlebia procera* sp. nov., holotype, CNU-MEC-NN2009273 **A** photograph of holotype **B** line drawing of holotype **C** line drawing of right forewing **D** photograph of right wing. Scale bars: 1 mm (**A–D**).

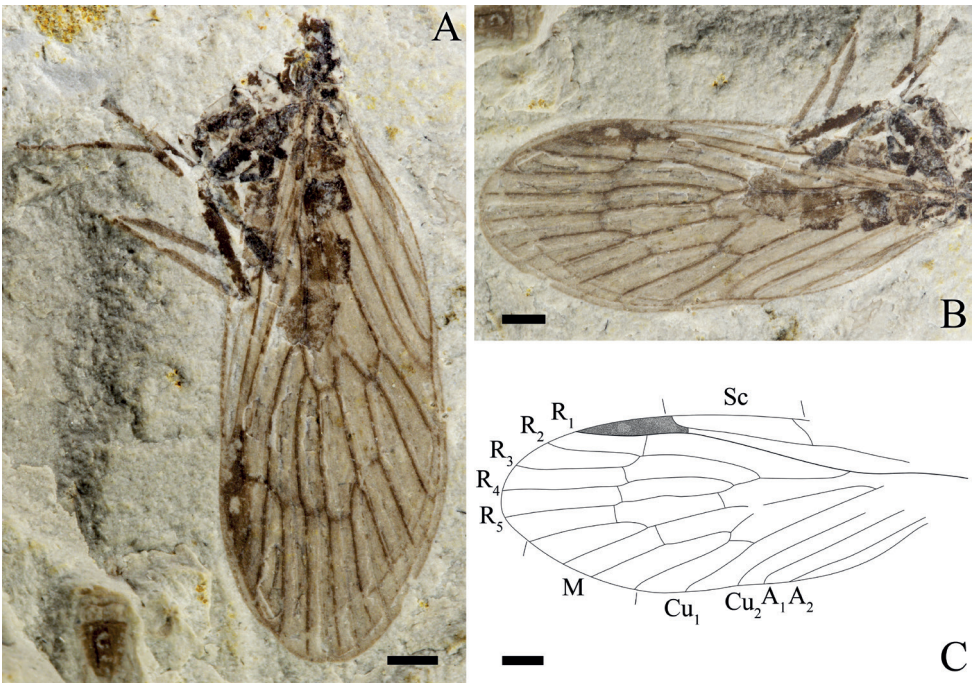

Figure 2. *Itaphlebia procera* sp. nov., paratype, CNU-MEC-NN2009168 **A** photograph of paratype **B** photograph of wings **C** line drawing of forewing. Scale bars: 1 mm (**A–C**).

Locality and horizon. All specimens were collected from the Jiulongshan Formation, latest Middle Jurassic age (Bathonian–Callovian boundary interval) from Daohugou Village, Ningcheng County, Inner Mongolia Autonomous Region in China.

Diagnosis. Body size ca 10 mm. On forewing, Sc forking with three branches; R_{2+3} forking before M_{1+2}; crossvein r_4-r_5 before crossvein r_5-m_1; Cu_1+M forking before the crossvein cu_1-cu_2; and crossvein cu_1-m_4 after M_{3+4} forking.

Description. Mainly based on Holotype, unless indicated as paratype.

***Head*:** Oval with large compound eyes. Antenna partially preserved, filiform with 33 and 27 segments as preserved respectively. Vertex of the head raised, mouthparts long and slender.

***Thorax*:** Long and relatively well-preserved, pronotum (width 0.5 mm, length 0.25 mm), mesonotum (width 1.5 mm, length 1.25 mm), metanotum (width 1.25 mm, length 1.4 mm) and scutellum (heavy shadowed) clearly discernible.

***Leg*:** Leg barely preserved and slender, tibia longer than femur, surfaces of all legs densely covered with short and irregularly arranged setae.

***Wing*:** Forewing: Long and oval, basal part of the wing narrow, gradually broadening from the base toward the apex. Right forewing with the anterior area broad, a distinct pterostigma present; Sc with 3 branches, Sc_1 and Sc_2 ending at C before the middle of the wing length, Sc_3 terminated at C right near pterostigmal area; R forking before Sc_2, Rs forking with 4 branches, one short crossvein sc-r before pterostigma,

crossvein r_1-r_2 under the pterostigma, approximately near the forking of R_{2+3}, crossvein r_3-r_4 after the level of the crossvein r_1-r_2, R_{2+3} 4 times as long as R_{4+5}, crossvein r_5-m_{1+2} emerged after the forking of R_{4+5}, oblique crossvein r_5-m_1 present after the crossvein r_4-r_5; conspicuous thyridium at the forking of M; M with 4 branches, M_{3+4} divided beyond the forking of M_{1+2}; straight crossvein m_{1+2}-m_3 between M_{1+2} and M_3, near the forking of M_{1+2}; crossvein m-cu_1 after the forking of M_{3+4}, somewhat S-shaped; Cu_1 coalesced with M for a long distance and separated from M after the crossvein cu_1-cu_2; A_1 and A_2 are almost parallel; crossvein a_1-a_2 after the level as the base of Cu_1; A_3 absent. Hind wing: Shorter than forewing; the pterostigma slightly darkened. Sc simple, terminated at C in the first one third of wing, one crossvein c-r_1 present at half-length of hind wing; R_1 under pterostigma and connected with C by a short crossvein c-r_1; posterior and anal margins in hind wings not preserved.

***Abdomen*:** With six visible large abdominal segments, segments II–IV and segment VI distinctly smaller than segment V, the distal segments not preserved.

Remarks. The new species shows great differences from other species of *Itaphlebia* in having relatively moderate body size, a broader thoracic notum and the presence and position of the crossvein r_5-m_1 that is in a more distal position than in other species, which is also a new finding for nannochoristid venation. So far, we have found two specimens of the new species, of which characters appear to be stable and therefore sufficient for establishing a new species.

Measurements (in mm). *Holotype*: No. CNU-MEC-NN2009273: head length 0.7 (excluding the antenna), head maximum width 1.0 (excluding the antenna), thorax length ca 3.0, thorax maximum width 1.9, forewing length 9.4, hind wing length 7.5 (all as preserved). ***Paratype*:** No. CNU-MEC-NN2009168: forewing length 11.5, forewing maximum width 4.4 (all as preserved).

Itaphlebia elegana Cao, Shih & Ren, sp. nov.

https://zoobank.org/3D8414C6-F617-4F7B-BC84-13330D7A5A5D
Figs 3–5

Etymology. The specific name is from Latin adjective "elegans", referring to the elegant body posture.

Type material. *Holotype*: female, No. CNU-MEC-NN2008228 p/c (Fig. 3), preserved in lateral view, thorax partly preserved, abdomen well-preserved, with four wings partly preserved, veins of one forewing mostly discernible, veins of one hind wing relatively discernible. ***Paratypes*:** sex unknown, No. CNU-MEC-NN2015026 (Fig. 4), preserved in ventral view, thorax and abdomen partly preserved, only right forewing venation clearly discernible; female, CNU-MEC-NN2016020 p/c (Fig. 5), body nearly complete but not well-preserved, forewing venation partly discernible.

Locality and horizon. All specimens were collected from the Jiulongshan Formation, latest Middle Jurassic age (Bathonian–Callovian boundary interval) from Daohugou Village, Ningcheng County, Inner Mongolia Autonomous Region in China.

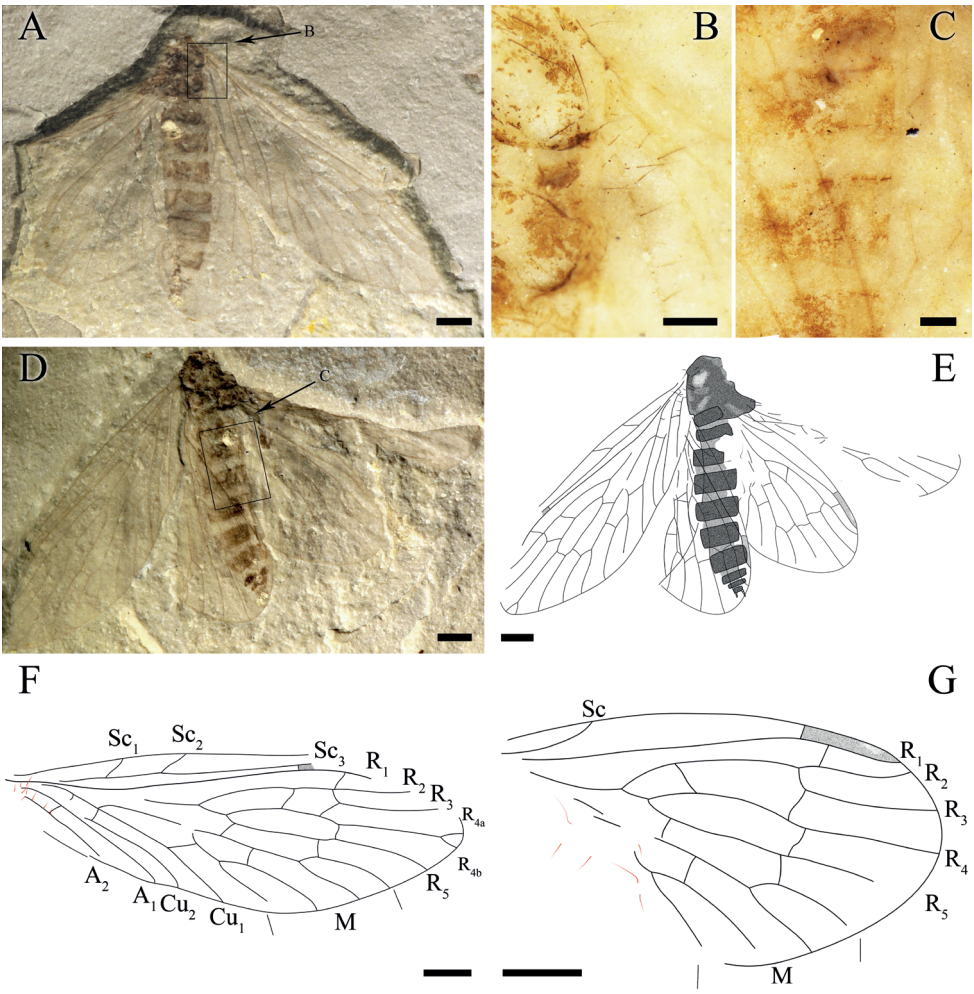

Figure 3. *Itaphlebia elegana* sp. nov., holotype, CNU-MEC-NN2008228 p/c. **A** photograph of part **B** enlarged details of basal forewing of part, anal veins with long and robust setae (under alcohol) **C** enlarged details of basal hind wing of counterpart (under alcohol) **D** photograph of counterpart **E** line drawing of counterpart **F** Line drawing of forewing of part, anal veins with long and robust setae **G** line drawing of hind wing of counterpart. Scale bars: 1 mm (**A–G**).

Diagnosis. On forewing, costal area slightly broad; Sc forking with three branches; Rs (R_2 to R_5) forking with five branches; R_{2+3} forking before the crossvein r_3-r_4; R_{4+5} forking before the crossvein r_5-m_{1+2}; crossvein cu_1-cu_2 near the forking of Cu_1+M, and crossvein cu_1-m_4 far before M_{3+4} forking. Long and robust setae present on anal veins of forewing.

Description. Mainly based on Holotype, unless indicated as paratype.

***Thorax*:** Incompletely preserved, setae discernible.

***Wing*:** Long and oval, basal part of the wing narrow, gradually broadening from the base toward the apex. Forewing with the basal part of C somewhat convex, anterior

area slightly broad, pterostigma incompletely preserved; Sc with three branches, Sc_1 and Sc_2 ending at C before the middle of the wing length, Sc_3 near pterostigmal area; Rs forking with five branches, one short crossvein sc-r before pterostigma, crossvein r_1-r_2 under the pterostigma, approximately near the forking of R_{2+3}, crossvein r_{2+3}-r_4 before crossvein sc-r, oblique crossvein r_3-r_4 between R_3 and R_4, R_{2+3} four times as long as R_{4+5}, R_4 forking with two branches (R_{4a}, R_{4b}), oblique crossvein r_5-m_{1+2} emerged after the forking of R_{4+5}, crossvein r_5-m_1 present before the crossvein r_4-r_5; conspicuous thyridium at the forking of M; M with four branches, M_{3+4} divided beyond the forking of M_{1+2}; straight crossvein m_{1+2}-m_3 forking at the middle length of M_{1+2}; crossvein m-cu_1 before the forking of M_{3+4}; Cu_1 coalesced with M for a relatively short distance and separated from M near the crossvein cu_1-cu_2; long and robust setae present on veins A_1 and A_2, A_1 and A_2 almost parallel; crossvein a_1-a_2 at the same level of Cu_1; A_3 absent. Hind wing shorter and broader than forewing; pterostigma preserved in hind wing. Sc simple, terminated at C in the first one third of wing; R_1 under pterostigma and connecting with C by a short crossvein c-r_1, Rs with four branches, two crossveins between R_{2+3} and R_4. Anal margins in hind wings not preserved, several long and robust setae preserved.

***Abdomen*:** With 11 visible abdominal segments, segments VIII–XI distinctly smaller than segment VII, a pair of cerci at the end of abdomen, each one with three segments, setae discernible.

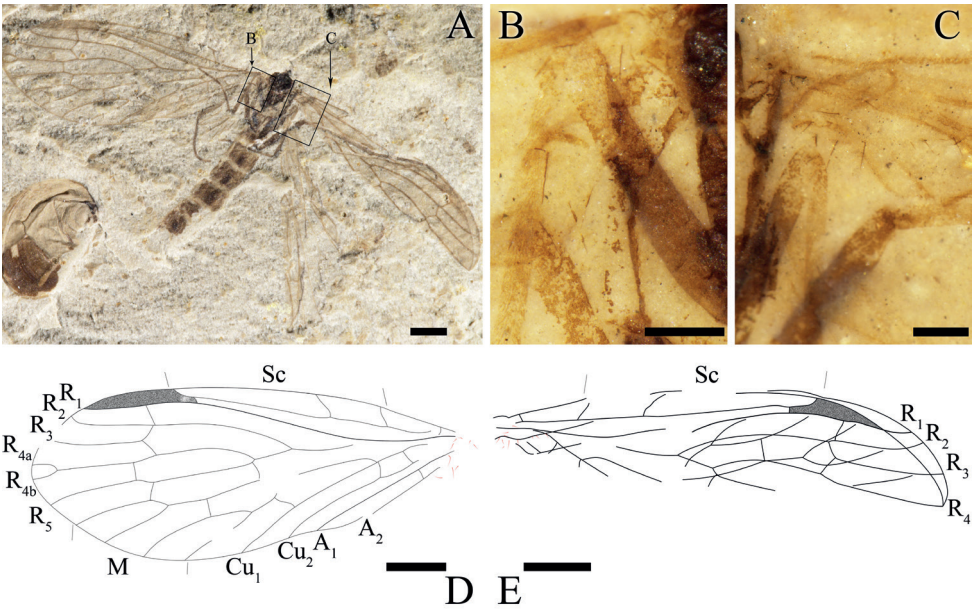

Figure 4. *Itaphlebia elegana* sp. nov., paratype, CNU-MEC-NN2015026 **A** photograph of paratype **B** enlarged details of base of right forewing (under alcohol) **C** enlarged details of base of left forewing (under alcohol) **D** line drawing of right forewing **E** line drawing of left forewing. Scale bars: 1 mm (**A–E**).

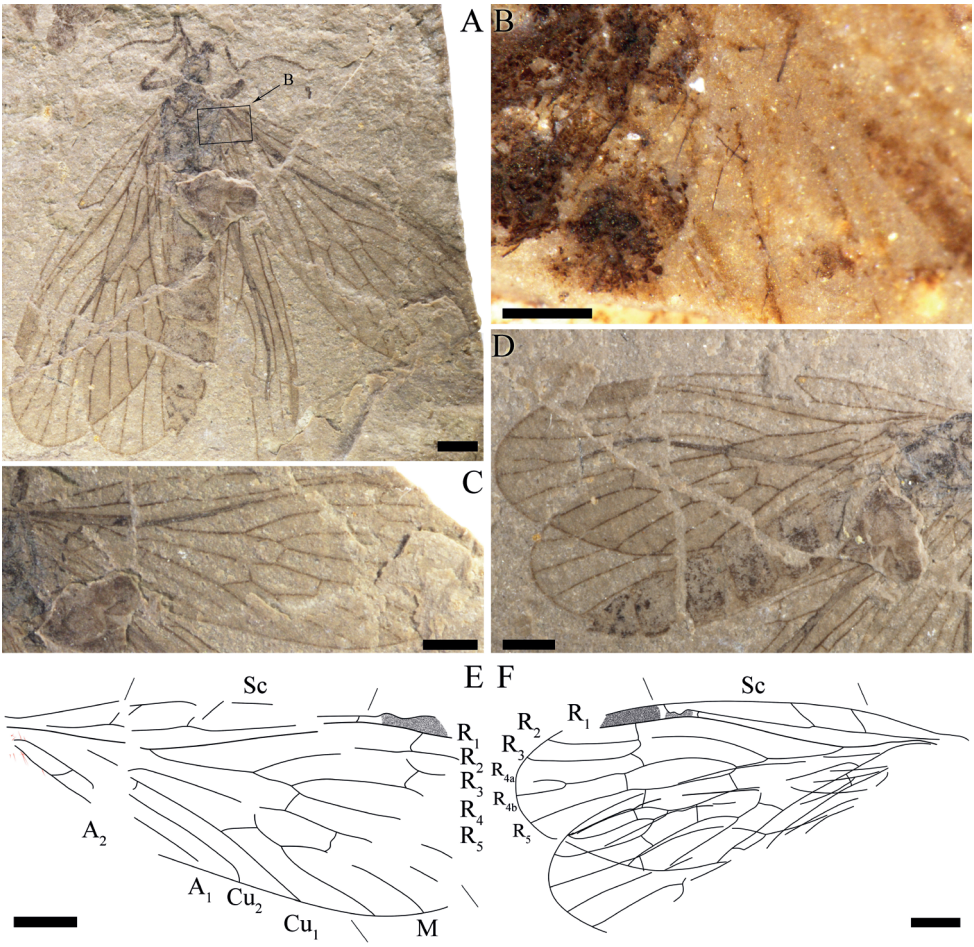

Figure 5. *Itaphlebia elegana* sp. nov., paratype, CNU-MEC-NN2016020 p/c. **A** photograph of part of paratype **B** enlarged details of base of forewing of counterpart of paratype (under alcohol) **C** photograph of right forewing of part **D** photograph of left forewing and part of hind wing of part **E** line drawing of right forewing **F** line drawing of left forewing and part of hind wing. Scale bars: 1 mm (**A–F**).

Remarks. *Itaphlebia elegana* sp. nov. demonstrates an individual aberration of the sixth branch of R vein by the forking of R_4, providing a character for understanding the evolution of wing venation. Although the hind wing has R with five branches, the diagnosis of *Itaphlebia* should be revised to indicate that the forewing Rs forking with four or five branches. CNU-MEC-NN2008228 p/c has two crossveins between R_{1+2} and R_{3+4}, but only one crossvein is visible in CNU-MEC-NN2016020 p/c and CNU-MEC-NN2016026 p/c. Considering these three specimens have many similarities regarding other characters, we regard them as the same species. The difference in crossveins between R_{2+3} and R_4 might have been due to the intraspecific difference or inheritable mutations.

Measurements (in mm). ***Holotype*:** female, No. CNU-MEC-NN2008228 p/c: Abdomen length 6.0, forewing length 9.4, hind wing length 7.5 (all as preserved). ***Paratypes*:** sex unknown, No. CNU-MEC-NN2015026: thorax length 1.8, thorax maximum width 1.1, forewing length 7.5, forewing maximum width 3.0 (all as preserved); female, CNU-MEC-NN2016020 p/c: head length 1.1 (excluding the antenna), head maximum width 0.8 (excluding the antenna), thorax length 2.2, thorax maximum width 1.7, forewing length 8.4, forewing maximum width 3.5 (all as preserved).

Discussion

Taxonomic and morphological characters for fossil nannochoristids from the latest Middle Jurassic Jiulongshan Formation in Northeastern China have been investigated. *Itaphlebia elegana* sp. nov. is unique in having a forewing with R_4 forking with two branches and remarkable long setae on the anal veins. Novokshonov (1997a) thoroughly studied the difference in venation [mainly in Sc branches of forewing and Rs (R_{2+3}) branches of both wings] between Mesozoic nannochoristids and the only extant genus, *Nannochorista* including, *N. dipteroides* Tillyard, 1917 (Tasmania); *N. eboraca* Tillyard, 1917 (New South Wales); *N. holostigma* Tillyard, 1917 (Tasmania); *N. maculipennis* Tillyard, 1917 (Tasmania); *N. neotropica* Navás, 1928 (western Argentina, Neuquén, Chile, Arauco, LLanquihue, Chiloe, Magellanes); *N. edwardsi* Kimmins, 1929 (western Argentina, Chile); *N. andina* Byers, 1989 (western Argentina, Neuquén); *N. philpotti* (= *Choristella philpotti* Tillyard, 1917, = *Microchorista philpotti* Byers, 1974) Kristensen, 1989 (New Zealand). In fossil nannochoristids, Sc has three branches (most species) on the forewing, terminating at C and Rs has four branches (five branches only in *I. elegana* sp. nov.), while all extant species have Sc with two branches, Sc_2 fused with R_1 for a distance (most species) on the forewing; and Rs with three branches. Therefore, the wing venation in Nannochoristidae has become simplified over time. On the other hand, the distance from the forking point of R_{4a+b} to the margin of the forewing is 0.5 mm for the holotype of *I. elegana* sp. nov. (vs. 0.5 mm and 1.1 mm for the paratypes), indicating the occurrence of such a forking of R_{4a} and R_{4b} in the forewing is frequent. Hence, the forking of R_4 should be a character for *I. elegana* sp. nov. and the diagnosis of *Itaphlebia* is also emended.

Itaphlebia elegana sp. nov. has distinct and short setae on the anal veins of the forewing, which was described in only one extant species of Nannochoristidae, *Nannochorista andina* (Byers 1989a). The setae of *I. elegana* sp. nov. are similar in appearance to those of the two fossil species, *Jurassipanorpa sticta* Ding Shih & Ren, 2014 and *Jurassipanorpa impunctata* Ding Shih & Ren, 2014 in Panorpidae Latreille, 1805 (Ding et al. 2014) and the extant *Notiothauma reedi* in Eomeropidae Cockerell, 1909 (Cockerell 1909; Crampton 1930; Mickoleit 1971). The distinct and short setae on forewing anal veins of *I. elegana* sp. nov. are different from microtrichia on the wing membrane or the dense and shorter setae of extant species of Panorpidae as well as the notably long and robust setae for the extinct species of Orthophlebiidae Handlirsch, 1906 (Mickoleit 1971; Ding et al. 2014).

Furthermore, such setae in *Itaphlebia elegana* sp. nov. are apparently shorter and less powerful, compared to the many stout bristles (dinotrichia) on the basal anterior margin of the forewings of fossil eomeropids of *Tsuchingothauma shihi* Ren & Shih 2005 from the latest Middle Jurassic and *Typhothauma yixianensis* Ren & Shih 2005 from the Early Cretaceous, both in Northeastern China (Ren and Shih 2005), and of both fore- and hind wings of the extant *N. reedi* (Carpenter 1909, Crampton 1930; Mickoleit 1971; Carpenter 1972). But such setae in *I. elegana* sp. nov. might be homologous to those of *N. reedi* as they all attach to the basal parts of the wings (Carpenter 1972; Crampton 1930; Mickoleit 1971). The setae of *N. reedi* are located on the middle or anal parts of the forewing, gradually getting smaller and only emerging on the basal region of some veins in the forewing. Such long bristles in the hind wing are usually the forerunners of the frenulum of higher insects on the front edge of the hind wing, which is hardly called a frenulum on the forewing (Crampton 1930). Therefore, the functions of these setae on forewings remain puzzling. Due to the setae occurring only on the anal veins of the forewings for *I. elegana* sp. nov., we surmised that they might have been sensory or used for wing coupling (Byers 1989a; Ding et al. 2014). However, since we have not found any other associated structures preserved on the anterior part of the hind wings of these fossil specimens as well as extant species, the functions of these setae are still unknown.

Compared with other species of *Itaphlebia*, *I. procera* sp. nov. has unique venational characters: crossvein r_5-m_1 distad of the levels of crossveins between R_1 and Rs, namely the forking of M_{1+2} originates basal (for Fig. 2C) or distad (for Fig. 1C) of the forking of R_{2+3}; and crossvein m_{1+2}-m_3 is near the level of the crossvein between R_1 and Rs (vs. basal location in most species). Hence, we propose that the origin of M_{1+2} is variable in *Itaphlebia*, and it should be a diagnosis at the level of species for *Itaphlebia*.

Conclusions

Eight extant species of Nannochoristidae Tillyard, 1917 have been documented in the Southern Hemisphere. The fossil sites of nannochoristids are in Siberia in Russia, Kazakhstan and Inner Mongolia and Liaoning in China. Due to limited specimens of well-preserved compression fossils, it was difficult to study detailed morphological characters to classify, diagnose and describe species or genera of these fossil nannochoristids.

We describe two new species of an extinct genus, *Itaphlebia procera* sp. nov. and *I. elegana* sp. nov., in the family Nannochoristidae, based on five fossil specimens from the latest Middle Jurassic Jiulongshan Formation of Daohugou, Inner Mongolia, in Northeastern China. *Itaphlebia elegana* sp. nov. demonstrates a new and unique forking of R_4 into two branches (R_{4a}, R_{4b}), providing a character for understanding the evolution of wing venation. Furthermore, we report long and robust setae present on the anal veins of the forewing in *I. elegana* sp. nov. It is the first time that these two characters of *Itaphlebia* are documented for the fossil records in Nannochoristidae.

These new species not only broadened the diversity of the *Itaphlebia* in the mid-Mesozoic ecosystems but also provided new taxonomic information to emend the generic diagnosis.

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RESEARCH ARTICLE

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Ground spiders (Araneae, Gnaphosidae) from Jiangxi Province, China

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Abstract

A list of 26 gnaphosid species belonging to 14 genera collected in Jiangxi Province, China, is provided. Three new species of ground spiders from Jiangxi Province of China are diagnosed, described, and illustrated: *Haplodrassus yinae* Liu, **sp. nov.** (♂♀), *Hitobia xiaoxi* Liu, **sp. nov.** (♂), and *Zelotes dingnan* Liu, **sp. nov.** (♂♀). *Haplodrassus yinae* Liu, **sp. nov.** was previously erroneously recorded in Jiangxi Province as *H. montanus* Yin et al., 2012.

Keywords

Asia, biodiversity, distribution, gnaphosid spiders, new species, taxonomy

Introduction

The rich biodiversity of China is likely due to two reasons: cenozoic tectonic evolution in the Tethyan, has greatly changed the landforms and environment of Eurasia and driven the evolution of animals (Zhao et al. 2022), and China's mountainous landscape has provided refuge for organisms, enabling species such as spiders to survive glacial periods (Yao et al. 2021; Lu et al. 2022). Approximately 5700 spider

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species from 69 families have been recorded from China (Biodiversity Committee of Chinese Academy of Sciences 2022). The southwest (Himalayan and Hengduan mountains, and Yunnan-Guizhou Plateau), has been the center of diversification for several spider groups (Li 2020) and has become a biodiversity hotspot for researchers. Many spider species are discovered from there in the last 15 years (Li and Lin 2016; Li 2020). In contrast to these areas, species richness of spiders in non-hotspots, such as Jiangxi Province, has not been given enough attention. Recently, in Jiangxi, which has a mountain terrain, many spider taxa have been discovered, such as Agelenidae (Liu et al. 2020a, 2021a), Dictynidae (Liu et al. 2018), Oonopidae (Liu et al. 2016, 2019), Phrurolithidae (Liu et al. 2020b, 2020c, 2021b, 2022a), Salticidae (Liu et al. 2017b, 2022c), Thomisidae (Liu et al. 2017a, 2022b), and Gnaphosidae (this study). These discoveries lend support to mountainous landscapes being refuges for spiders during glacial periods.

Gnaphosidae Banks, 1892, commonly known as ground spiders, is the sixth largest spider family with a global distribution, comprising 2414 extant species belonging to 144 genera (WSC 2022). Currently, 213 species belonging to 36 genera are known from China (Li and Lin 2016; Li 2020). Although Gnaphosidae is the ninth largest family in China, the number of taxonomic papers dealing with this family in the last decade is few (WSC 2022). Among species of ground spiders recorded from China, more than 60% are recorded from northern China, namely from Qinghai, Xinjiang, Neimenggu, Hebei, Gansu, Beijing, Shanxi, Liaoning, Xizang, and Henan provinces. The family is poorly studied in Jiangxi Province located in southern China, and only four species of Gnaphosidae are recorded from this province: *Allozelotes lushan* Yin & Peng, 1998, *Gnaphosa kompirensis* Bösenberg & Strand, 1906, *Hitobia yasunosukei* Kamura, 1992, and *Zelotes liaoi* Platnick & Song, 1986 (Li and Lin 2016).

While studying ground spiders from Jiangxi Province, we came across several undescribed and poorly known species, as well as many described species. The aims of the present paper are to provide detailed descriptions of three new species and to report findings of 26 species belonging to 14 genera.

Materials and methods

Specimens were examined using a Zeiss Stereo Discovery V12 stereomicroscope with a Zoom Microscope System. Both male palps and female copulatory organs were detached and examined in 80% ethanol, using a Zeiss Axio Scope A1 compound microscope with a KUY NICE CCD. The epigynes were cleared in trypsin enzyme solution to dissolve soft tissues. For SEM photographs, specimens were dried under natural conditions, sprayed with gold with a small ion-sputtering apparatus ETD-2000, or left without coating, and photographed with a ZEISS EVO LS15 scanning electron microscope. Specimens, including detached male palps and epigynes, were stored in

75% ethanol after examination. All the specimens are deposited in Animal Specimen Museum, Life Science of College, Jinggangshan University (**ASM-JGSU**).

Measurements were taken with the AxioVision software (SE64 rel. 4.8.3) and given in millimetres. Terminology of the male and female copulatory organs follows Platnick and Shadab (1982) and Fomichev and Marusik (2021). Leg measurements are given as total length (femur, patella, tibia, metatarsus, tarsus).

The abbreviations used in the text are as follows

Eyes: ALE, anterior lateral eye; **AME**, anterior median eye; **MOA**, median ocular area; **PLE**, posterior lateral eye; **PME**, posterior median eye.
Leg segments: Fe, femur; **Mt**, metatarsus; **Pt**, patella; **Ta**, tarsus; **Ti**, tibia.
Spination: d, dorsal; **p**, prolateral; **r**, retrolateral; **v**, ventral.
Male palp: BP, basal process; **Co**, conductor; **EA**, embolic apophysis; **Em**, embolus; **EP**, embolic projection; **IS**, intercalary sclerite; **LaP**, lamellar process; **MA**, median apophysis; **RTA**, retrolateral tibial apophysis; **SD**, sperm duct; **StP**, subterminal process; **TA**, terminal apophysis; **TP**, terminal process; **UP**, upper process.
Epigyne: AP, anterior pocket; **CD**, copulatory duct; **CO**, copulatory opening; **FD**, fertilization ducts; **Fo**, fovea; **H**, hood; **LG**, lateral gland; **MP**, median pocket; **PP**, posterior pocket; **Se**, septum; **Sp**, spermatheca.

Taxonomic survey

Family Gnaphosidae Banks, 1892

The known gnaphosid spider fauna of Jiangxi Province is complemented by 23 additional species belonging to 13 genera and now numbers 27 species in 14 genera. The full list of gnaphosid spiders recorded in this province is presented in Table 1.

Genus *Haplodrassus* Chamberlin, 1922

Comments. This genus includes 83 species, mainly distributed in the Palaearctic (WSC 2022). A smaller number of species are known from the Nearctic and Oriental realms (WSC 2022). The genus was divided into nine species groups, based on morphological characteristics, by Omelko and Marusik (2012), i.e., the *caspius*, *dalmatensis*, *kulczynskii*, *mediterraneus*, *montanus*, *signifier*, *silvestris*, *tegulatus*, and *umbratilis* groups. To date, only 13 species of *Haplodrassus* are recorded from China. Except for *Haplodrassus guiyangensis* Yan & Yu, 2021, the others were recorded more than 10 years ago.

Table 1. List of Gnaphosidae species recorded in Jiangxi Province. Genera recorded for the first time are marked with an asterisk (*).

Genus	Species	No. of ♂♂	No. of ♀♀	Total
Allozelotes Yin & Peng, 1998	*A. lushan* Yin & Peng, 1998	0	1	1
Aphantaulax Simon, 1878 *	*A. trifasciata* (O. Pickard-Cambridge,1872)	0	1	1
Cladothela Kishida, 1928 *	*C. oculinotata* (Bösenberg & Strand, 1906)	1	0	1
	C. parva Kamura, 1991	1	1	2
Drassyllus Chamberlin, 1922 *	*Drassyllus* sp. 1	0	13	13
	D. sanmenensis Platnick & Song, 1986	14	18	32
	Drassyllus sp. 2	0	7	7
Gnaphosa Latreille, 1804	*G. hastata* Fox, 1937	0	1	1
	G. kompirensis Bösenberg & Strand, 1906	1	1	2
Haplodrassus Chamberlin, 1922 *	*H. yinae* sp. nov.	1	3	4
Hitobia Kamura, 1992	*H. taiwanica* Zhang, Zhu & Tso, 2009	1	0	1
	H. xiaoxi sp. nov.	1	0	1
	H. yasunosukei Kamura 1992	2	0	2
Odontodrassus Jézéquel, 1965 *	*O. hondoensis* (Saito, 1939)	1	0	1
Pseudodrassus Caporiacco, 1935 *	*P. pichoni* Schenkel, 1963	0	1	1
Sanitubius Kamura, 2001 *	*S. anatolicus* (Kamura, 1989)	0	1	1
Scotophaeus Simon, 1893 *	*S. hunan* Zhang, Song & Zhu, 2003	1	1	2
Sernokorba Kamura, 1992 *	*S. fanjing* Song, Zhu & Zhang, 2004	0	2	2
	S. pallidipatellis Bsenberg & Strand, 1906	2	0	2
Synaphosus Platnick & Shadab, 1980 *	*S. daweiensis* Yin, Bao & Peng, 2002	5	2	7
Zelotes Gistel, 1848	*Z. asiaticus* (Bösenberg & Strand, 1906)	12	4	16
	Z. dingnan sp. nov.	4	1	5
	Z. potanini Schenkel, 1963	1	0	1
	Z. sanmen Platnick & Song, 1986	0	6	6
	Z. wuchangensis Schenkel, 1963	2	0	2
	Z. yinae Platnicket & Song, 1986	0	1	1

Haplodrassus yinae **Liu, sp. nov.**

https://zoobank.org/A9BEB099-2D07-4226-B5BF-95D11DD643AC
Figs 1–3

Haplodrassus montanus Yin et al., 2012: 1177, fig. 627a–f (♂♀).

Material examined. ***Holotype*** ♂, China: Jiangxi Province, Ji'an City, Jinggangshan County Level City, Jinggang Mountain National Nature Reserve, Dongshang Town, Jiangshan Village, 26°46'01.56"N, 113°54'53.65"E, 326 m, 4.II.2021, K. Liu et al. leg. ***Paratypes*****:** 2 ♂, 1 ♀, the same data as the holotype.

Etymology. The specific name is a matronym in honour of Prof. Changmin Yin, the first to find and recognise this species, in honour of her great contribution to Chinese arachnology; noun (name) in genitive case.

Diagnosis. The new species belongs to the *montanus* group. The male of the new species is similar to *H. guiyangensis* Yan & Yu, 2021, *H. hatsushibai* Kamura, 2007,

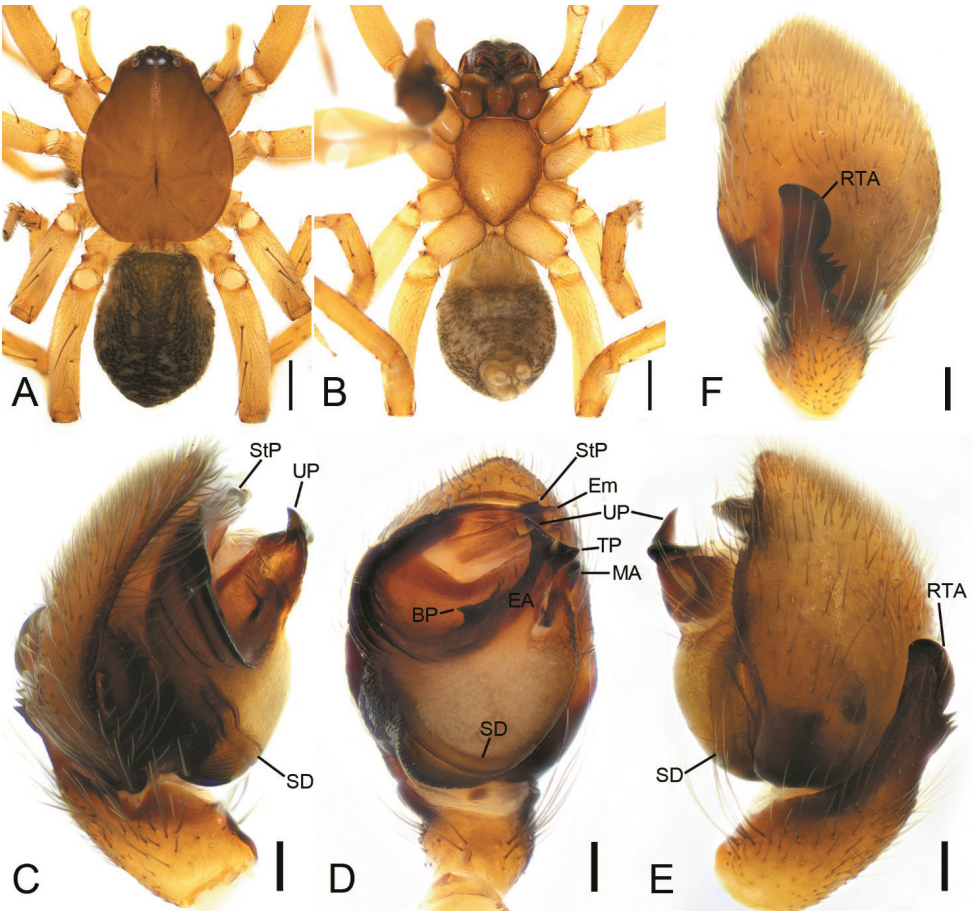

Figure 1. *Haplodrassus yinae* sp. nov., male holotype **A** habitus, dorsal view **B** same, ventral view **C** palp, prolateral view **D** same, ventral view **E** same, retrolateral view **F** same, dorsal view. Abbreviations: BP – basal process, EA – embolic apophysis, Em – embolus, MA – median apophysis, RTA – retrolateral tibial apophysis, SD – sperm duct, StP – subterminal process, TP – terminal process, UP – upper process. Scale bars: 0.5 mm (**A, B**); 0.1 mm (**C–F**).

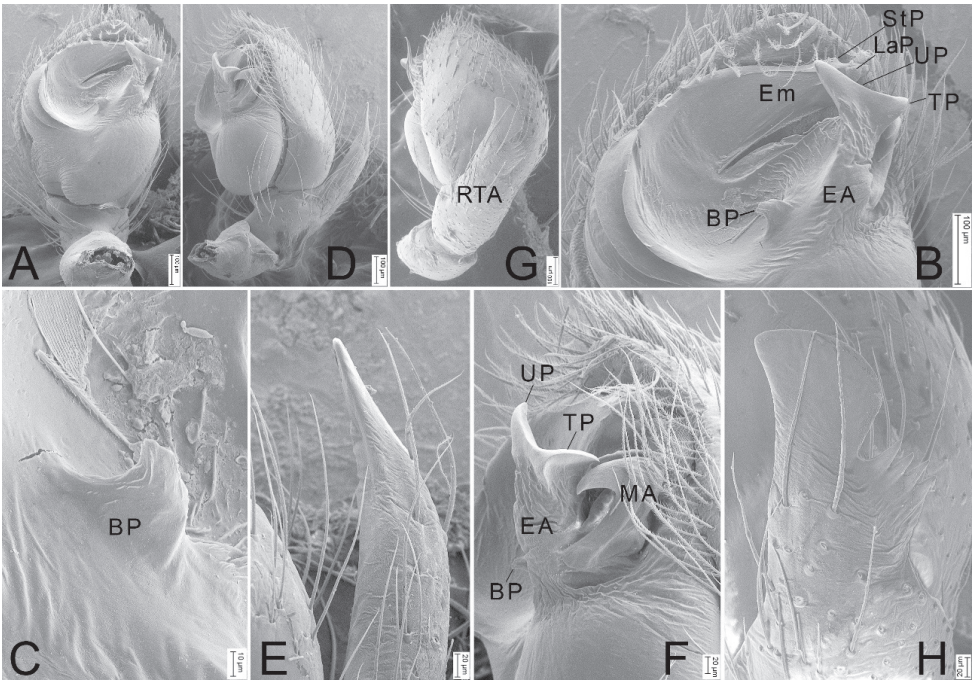

Figure 2. SEM micrographs of *Haplodrassus yinae* sp. nov., male palp (paratype) **A** ventral view **B** same, detail of embolic division **C** same, details of basal process of embolic base **D** retrolateral view **E** same, details of RTA **F** same, detail of embolic division **G** dorsal view, slightly retrolaterally **H** same, details of RTA. Abbreviations: BP – basal process, EA – embolic apophysis, Em – embolus, LaP – lamellar process, MA – median apophysis, RTA – retrolateral tibial apophysis, StP – subterminal process, TP – terminal process, UP – upper process.

H. huarong Yin & Bao, 2012, and *H. montanus* Paik & Sohn, 1984 in having an oval tegulum, a bifurcate embolic apophysis (EA), and a hook-shaped median apophysis (MA), but it can be differentiated from *H. hatsushibai* and *H. montanus* by the absence of the basal tooth on the embolus (cf. Figs 1D, 2A, B vs Omelko and Marusik 2012: figs 7, 10). In addition, *H. yinae* sp. nov. possess 5 or 6 ridges on the embolic base (vs 6–8 ridges in *H. guiyangensis*; 7 or 8 ridges in *H. hatsushibai*; 6 or 7 ridges in *H. huarong*; 3 or 4 ridges in *H. montanus*) (cf. Fig. 1C, D vs Yan et al. 2021: figs 1A, C, 2A, B and Omelko and Marusik 2012: figs 7, 8, 10, 11) and has a small tooth-like basal process (BP) directed at 9 o'clock in ventral view (vs 11 o'clock in *H. guiyangensis*; a large laminar, tooth-like basal process, directed at 11 o'clock position in *H. hatsushibai*, *H. huarong* and *H. montanus*) (cf. Figs 1D, 2A, B vs Yan et al. 2021: figs 1C, 2B and Omelko and Marusik 2012: figs 7, 10 and Yin et al. 2012: fig. 625f, g). The female of the new species resembles those of *H. montanus* in having pair of posterior pockets (PP) located in posterolateral part of the atrium, but it can be distinguished by the septum (Se) narrowing posteriorly (vs septum narrowing anteriorly) (cf. Fig. 3C vs Omelko and Marusik 2012: fig. 20). Also, it can be separated from *H. hatsushibai* by posterior pockets located in posterolateral part of the atrium (vs posterior pockets

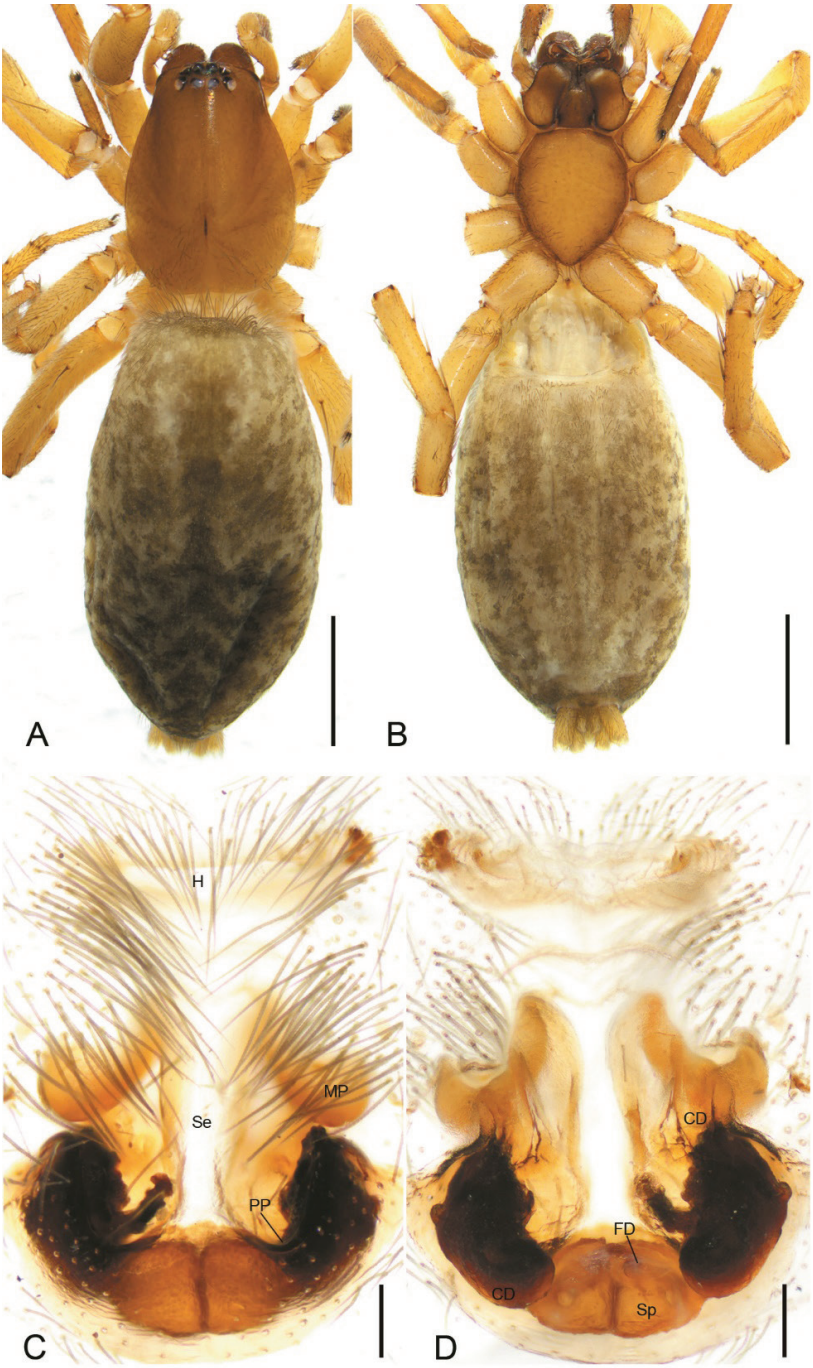

Figure 3. *Haplodrassus yinae* sp. nov., female paratype **A** habitus, dorsal view **B** same, ventral view **C** epigyne, ventral view **D** same, dorsal view. Abbreviations: CD – copulatory duct, FD – fertilization duct, H – anterior hood, MP – median pocket, PP – posterior pocket, Se – septum, Sp – spermatheca. Scale bars: 0.5 mm (**A, B**); 0.1 mm (**C, D**).

located in posteromedial part of the atrium) (cf. Fig. 3C vs Omelko and Marusik 2012: fig. 23) and from *H. huarong* by the copulatory ducts (CD) as wide as spermathecae (Sp) (vs 1/3 of spermathecal width) (cf. Fig. 3D and Yin et al. 2012: fig. 625e).

Description. Male. Habitus as in Fig. 1A, B. Total length 3.65. Carapace: 1.89 long, 1.51 wide. Carapace covered with a few strong setae. Eye sizes and interdistances: AME 0.08, ALE 0.11, PME 0.09, PLE 0.10, AME–AME 0.07, AME–ALE 0.03, PME–PME 0.02, PME–PLE 0.05, AME–PME 0.08, AME–PLE 0.12, ALE–ALE 0.24, PLE–PLE 0.33, ALE–PLE 0.04. MOA 0.21 long, front width 0.20, back width 0.23. Chelicera with 4 promarginal and 2 retromarginal teeth. Abdomen: 1.77 long, 1.21 wide. Leg measurements: I 4.08 (1.1, 0.57, 1.08, 0.69, 0.64); II 3.72 (1.11, 0.62, 0.84, 0.59, 0.56); III 3.06 (0.97, 0.4, 0.56, 0.62, 0.51); IV 4.91 (1.4, 0.58, 1.14, 1.17, 0.62). Leg spination: I Fe: d2, p1; Mt: p1; II Fe: d2; Pa: r1; Ti: v2; III Fe: d2, p1, r1; Ti: d1, p2, r2, v6; Mt: p3, r3, v4; IV: Fe: d2, r1; Pa: r1; Ti: p2, r3, v6; Mt: p2, r2, v6.

Colouration (Fig. 1A, B). Carapace, chelicerae and sternum brown. Maxillae and labium reddish brown. Legs yellow. Palps yellow, cymbium brown. Abdomen dark brown, with two pairs of longitudinal yellowish markings anteromedially and four pairs of chevrons posteromedially. Spinnerets yellow.

Palp as in Figs 1C–F, 2. RTA thumb-shaped, 2× longer than tibia, bearing three strong teeth. Cymbium 2× longer than wide. Tegulum ovate, with a distinct depression in the anterior part. Sperm duct (SD) stretched along the posterior margin of the tegulum. Embolic apophysis (EA) with three well developed processes: the basal one (BP) hook-shaped and small, directed at 9 o'clock; bases of terminal (TP) and upper processes (UP) touching each other together, forming a fishtail-shape. Upper process and terminal process triangular, approximately the same length. Median apophysis (MA) hook-shaped, twice shorter than embolic apophysis. Embolic base with five or six ridges prolaterally. Apex of embolus bears subterminal process (StP) and lamellar process (LaP).

Female. Habitus as in Fig. 3A, B. Total length 5.13. Carapace: 1.8 long, 1.33 wide. Eye sizes and interdistances: AME 0.08, ALE 0.11, PME 0.08, PLE 0.10, AME–AME 0.07, AME–ALE 0.02, PME–PME 0.03, PME–PLE 0.06, AME–PME 0.08, AME–PLE 0.13, ALE–ALE 0.22, PLE–PLE 0.34, ALE–PLE 0.06. MOA 0.24 long, front width 0.19, back width 0.22. Chelicera with 4 promarginal and 2 retromarginal teeth. Abdomen: 3.19 long, 1.86 wide. Leg measurements: I 2.9 (0.88, 0.45, 0.67, 0.44, 0.46); II 3.2 (0.95, 0.49, 0.72, 0.51, 0.53); III 2.49 (0.95, 0.32, 0.41, 0.42, 0.39); IV 3.26 (0.89, 0.43, 0.68, 0.75, 0.51). Leg spination: I Fe: d2, p1; Mt: p1; II Fe: d2; Pa: r1 Ti: v2; III Fe: d2, p1, r1; Ti: d1 p2, r2, v6; Mt: p3, r3, v4; IV: Fe: d2, r1; Pa: r1; Ti: p2, r3, v6; Mt: p2, r2, v6.

Epigyne as in Fig. 3C, D. Epigyne 1.3× longer than width. Anterior hood (H) flat, 7× wider than long. Septum (Se) expands anteriorly. Median pockets (MP) concave backwards. Posterior pockets deep, located at posterolateral part of the atrium. Copulatory openings invisible, arising from median pockets and covered by the margin of median pockets. Copulatory ducts (CD) nearly 3× longer than width. Spermathecae (Sp) stuck together, as long as wide. Fertilisation ducts (FD) directed laterally.

Distribution. China: Jiangxi and Hunan provinces (Fig. 9).

Genus *Hitobia* Kamura, 1992

Comments. The genus includes 21 species, all of which are distributed in south, southeast, or east Asia (China, Thailand, Korea, Japan, Vietnam, India) (WSC 2022). More than two-thirds of all known species have been described and/or reported from China.

Hitobia xiaoxi Liu, sp. nov.

https://zoobank.org/1183FC4C-27BB-41CE-AA87-3B85437FE1D4
Figs 4, 5

Material examined. ***Holotype***: ♂, China: Jiangxi Province, Ji'an City, Jinggangshan County Level City, Jinggang Mountain National Nature Reserve, Huangao Town, Fuxi Village, Xiaoxi Forest Farm, 26°28'22.92"N, 114°11'53.07"E, 413 m, 14.XI.2020, Liu et al. leg.

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

Diagnosis. The male of this new species is similar to *Hitobia shaohai* Yin & Bao, 2012 and *H. taiwanica* Zhang, Zhu & Tso, 2009 in having a short RTA and retrolaterally oriented embolus (Em), but it differs from them in the subtriangular RTA (vs beak-like in *H. shaohai* and *H. taiwanica*) and the embolus with twisted apex (vs twisted apex absent in *H. shaohai* and *H. taiwanica*) (cf. Figs 4E, 5C–F vs Yin et al. 2012: fig. 631d and Zhang et al. 2009: fig. 1E, F).

Description. Male. Habitus as in Fig. 4A, B. Total length 4.97. Carapace: 2.18 long, 1.74 wide. Eye sizes and interdistances: AME 0.09, ALE 0.09, PME 0.08, PLE 0.08, AME–AME 0.06, AME–ALE 0.21, PME–PME 0.1, PME–PLE 0.07, AME–PME 0.12, AME–PLE 0.21, ALE–ALE 0.23, PLE–PLE 0.42, ALE–PLE 0.12. MOA 0.3 long, front width 0.2, back width 0.27. Chelicera with 2 promarginal and 1 retromarginal teeth. Abdomen: 2.64 long, 1.29 wide. Abdomen covered with numerous setae dorsally. Scutum covers more than 2/3 of abdomen. Leg measurements: I 5.09 (1.47, 0.71, 1.21, 1.01, 0.69); II 4.99 (1.47, 0.68, 1.13, 0.98, 0.73); III 5.15 (1.37, 0.74, 1.05, 1.27, 0.72); IV 6.74 (1.78, 0.93, 1.46, 1.91, 0.66). Leg spination: I Fe: d3, p2; Pa: r1; Ti: p2, v6; Mt: v2; II Fe: d3, p2, r1; Ti: p2, v5; Mt: v2; III Fe: d3, p2, r2; Pa: d1, p1, r1; Ti: d1, p3, r2, v5; Mt: d2, p3, r3, v2; IV: Fe: d2, p1, r1; Pa: p1, r1; Ti: p3, r2, v5; Mt: d2, p3, r3, v6.

Colouration (Fig. 4A, B). Carapace, sternum, chelicerae, labium, and maxillae yellow-brown. Legs brown. Palps yellow, cymbium brown. Abdomen dark brown, with one transverse white stripe posteriorly. Spinnerets yellow grey.

Palp as in Figs 4C–F, 5. Femur with three strong spines dorsally. Patella with single spine dorsally. RTA subtriangular, slightly shorter than tibia, apex directed dorsally. Cymbium 2.5× longer than wide. Tegulum simple and smooth, tapers anteriorly. Conductor (Co) membranous. Sperm duct (SD) U-shaped. Embolus longer than conductor (Em), cone-shaped and twisted.

Female. Unknown.

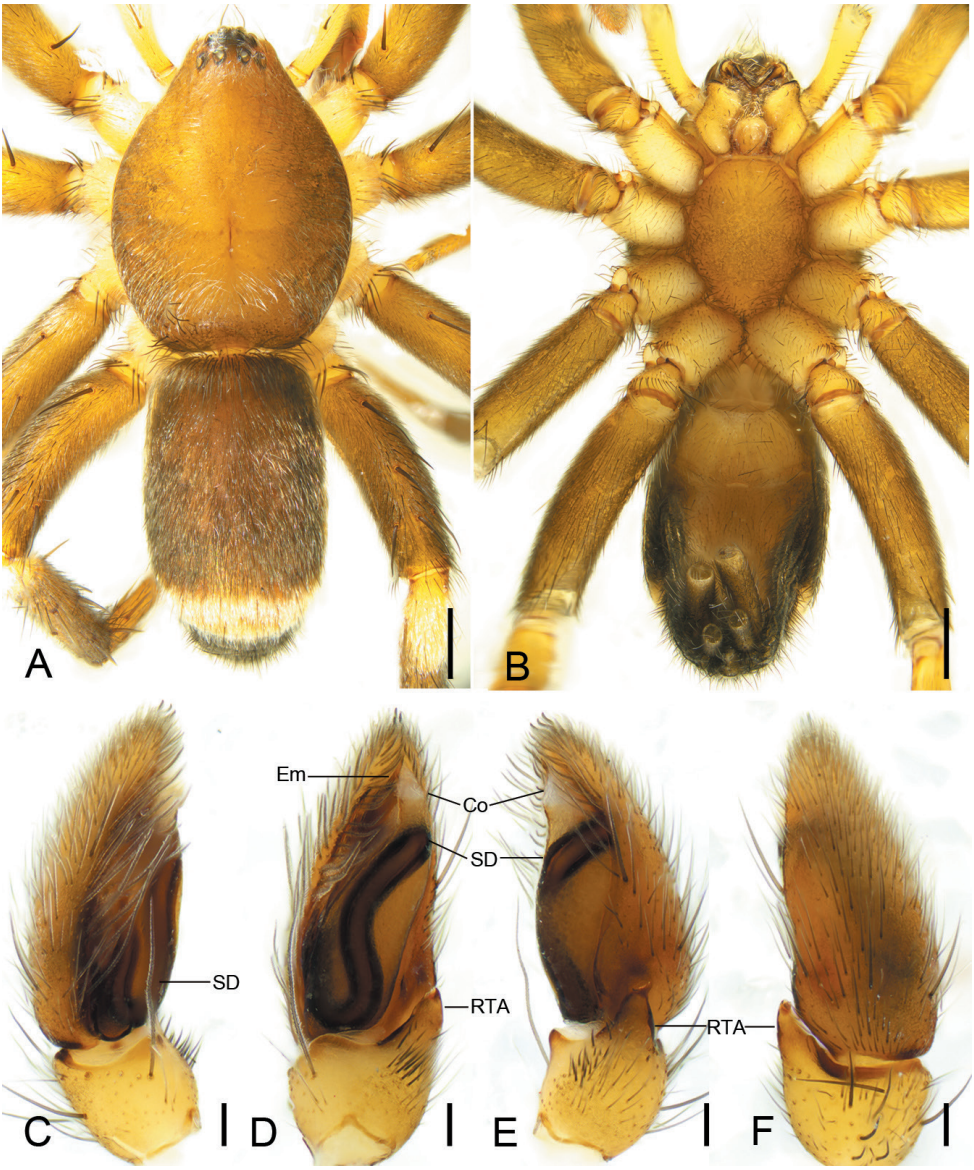

Figure 4. *Hitobia xiaoxi* sp. nov., male holotype **A** habitus, dorsal view **B** same, ventral view **C** palp, prolateral view **D** same, ventral view **E** same, retrolateral view **F** same, dorsal view. Abbreviations: Co – conductor, Em – embolus, RTA – retrolateral tibial apophysis, SD – sperm duct. Scale bars: 0.5 mm (**A, B**); 0.1 mm (**C–F**).

Comments. The new species together with *H. shaohai* and *H. taiwanica* clearly belongs to the same group based on configuration of their male palps. Unfortunately, only one male of *H. xiaoxi* sp. nov. was found and several *Hitobia* species from southern China are known only from females: *H. chayuensis* Song, Zhu & Zhang, 2004, *H. shimen* Yin & Bao, 2012, and *H. yunnan* Song, Zhu & Zhang, 2004. Thus, *H. xiaoxi* sp. nov.

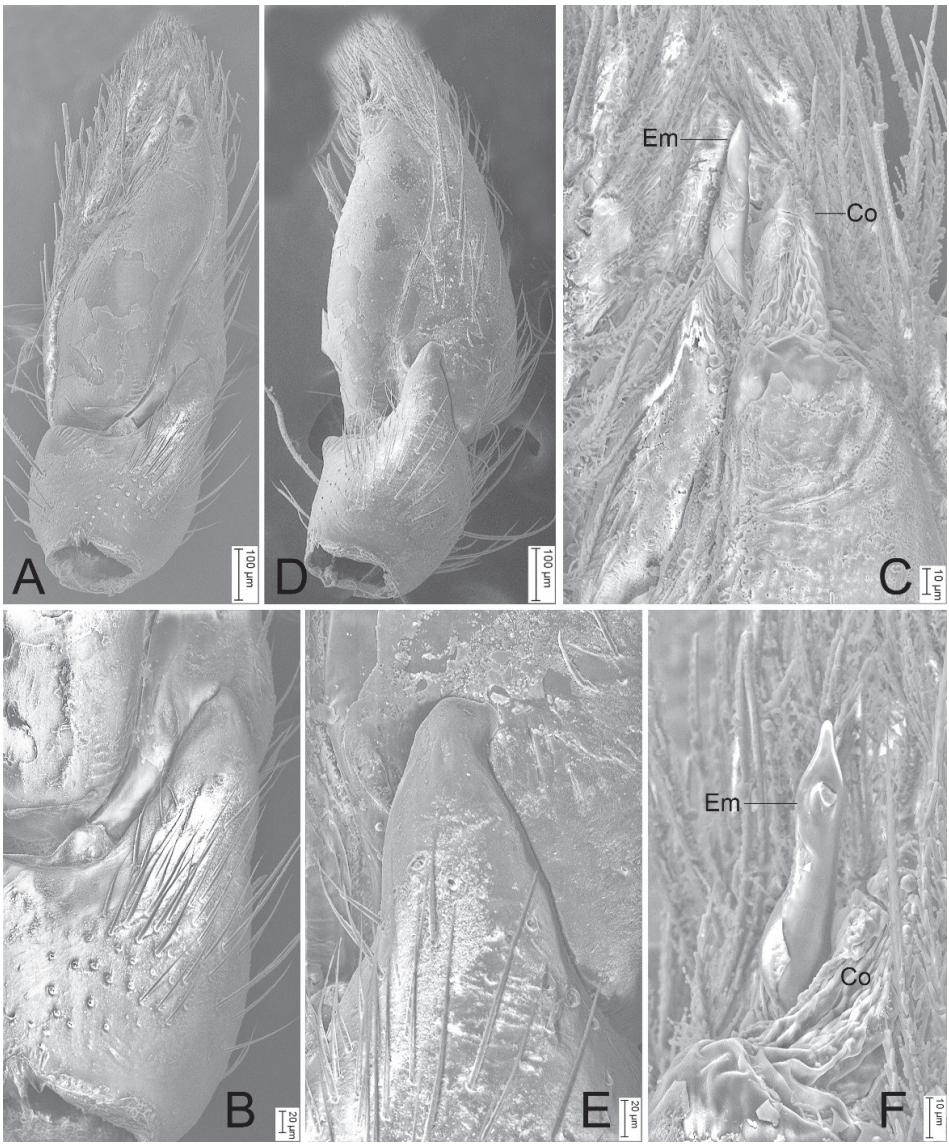

Figure 5. SEM micrographs of *Hitobia xiaoxi* sp. nov., male palp (holotype) **A** ventral view **B** same, details of RTA **C** same, detail of embolic division **D** retrolateral view **E** same, details of RTA **F** same, detail of embolic division. Abbreviations: Co – conductor, Em – embolus.

may be a junior synonym of any of the above-mentioned species. However, it should be noted that the new species differs from all females by the abdominal pattern of a broad arc-shaped white stripe subposteriorly (vs wavy wite stripe in *H. yunnan*, thin transverse white stripe in *H. chayuensis*, and herringbone-pattern in *H. shimen*). For this reason, we consider *H. xiaoxi* sp. nov. as a separate species. This hypothesis will be confirmed or rejected in the future when both sexes of the new species are collected together.

Distribution. Known only from the type locality, Jiangxi Province, China (Fig. 9).

Genus *Zelotes* Gistel, 1848

Comments. With 397 described species and worldwide distribution, *Zelotes* is the most speciose genus of the family (WSC 2022).

Zelotes dingnan Liu, sp. nov.

https://zoobank.org/AA3583D1-87C9-4B52-9983-04AFACAF5E40
Figs 6–8

Material examined. ***Holotype*:** ♂, China: Jiangxi Province, Ganzhou City, Dingnan County, Lingbei Town, Aonao Village, near 42# poles, 25°01'48.95"N, 115°06'11.01"E, 395 m, 5.X.2020, K. Liu et al. leg. ***Paratype*:** 1 ♂: Dayu County, Neiliang Town, Tianhua Mountain, 25°25'38.09"N, 114°01'43.95"E, 1019 m, 3.X.2020, other data same as holotype; 1 ♂: near the county boundary between Xunwu and Anyuan Counties, Guizhumao parking lot, 24°55'35.36"N, 115°27'25.09"E, 716 m, 7.X.2020, other data same as holotype; 1 ♀: Anyuan county, Sanbaishan National Forest Park, hiking trails, 25°00'28.19"N, 115°25'59.45"E, 511 m, other data as same as previous; 1 ♂: Ji'an City, Jinggangshan Level City, Dongshang Town, Jiangshan Village, Qilichuan, 26°46'88.81"N, 113°52'00.83"E, 665 m, 4.II.2021, K. Liu et al. leg.

Etymology. The specific name derived from the county where the type locality is located and is a noun in apposition.

Diagnosis. The male of this new species is similar to *Zelotes liaoi* Platnick & Song, 1986 in having a finger-like RTA, U-shaped sperm duct (SD), a triangular median apophysis (MA) in ventral view and a strong terminal apophysis (TA) with a spine-like apex, but differs by the clearly visible embolic projection (EP) (vs invisible or absent) and by the embolus (Em) with a membranous apex bending toward posterior part of tegulum (vs sclerotized apex bending toward anterior part of tegulum) (Figs 6C–F, 7 vs Song et al. 2004: fig. 158C, D). The female of *Z. dingnan* can be distinguished from that of *Z. liaoi* by the teardrop posterior part of the fovea (Fo) (vs subtriangular) and by the copulatory ducts (CD) divided into three parts (anterior, medial, and posterior) (vs two) (Figs 8C, D vs Song et al. 2004: fig. 158A, B).

Description. Male. Habitus as in Fig. 6A, B. Total length 4.81. Carapace: 2.23 long, 1.75 wide. Eye sizes and interdistances: AME 0.06, ALE 0.09, PME 0.07, PLE 0.08, AME–AME 0.03, AME–ALE 0.03, PME–PME 0.04, PME–PLE 0.04, AME–PME 0.11, AME–PLE 0.12, ALE–ALE 0.17, PLE–PLE 0.23, ALE–PLE 0.02. MOA 0.23 long, front width 0.18, back width 0.19. Chelicera with 3 promarginal and 4 retromarginal teeth. Abdomen: 2.48 long, 1.5 wide. Leg measurements: I 6.04 (1.57, 0.89, 1.37, 1.16, 1.05); II 4.89 (1.39, 0.6, 1.12, 0.94, 0.84); III 5.2 (0.95, 0.68, 1.36, 1.51, 0.7); IV 6.43 (1.77, 0.7, 1.51, 1.57, 0.88). Leg spination: I Fe: d3; II Fe: d2, r1; Ti: v1; Mt: v4; III Fe: d2, p1, r2; Pa: r1; Ti: p2, r2, v5; IV: Fe: d2, r1; Pa: r1; Ti: p2, r1, v6; Mt: d6, p2, r3, v4.

Colouration (Fig. 6A, B). Carapace dark yellow-brown, with radial dark stripes dorsally. Chelicerae, maxillae, labium and sternum dark yellow-brown. Legs brown. Palps yellow-brown. Abdomen dark brown. Spinnerets brown.

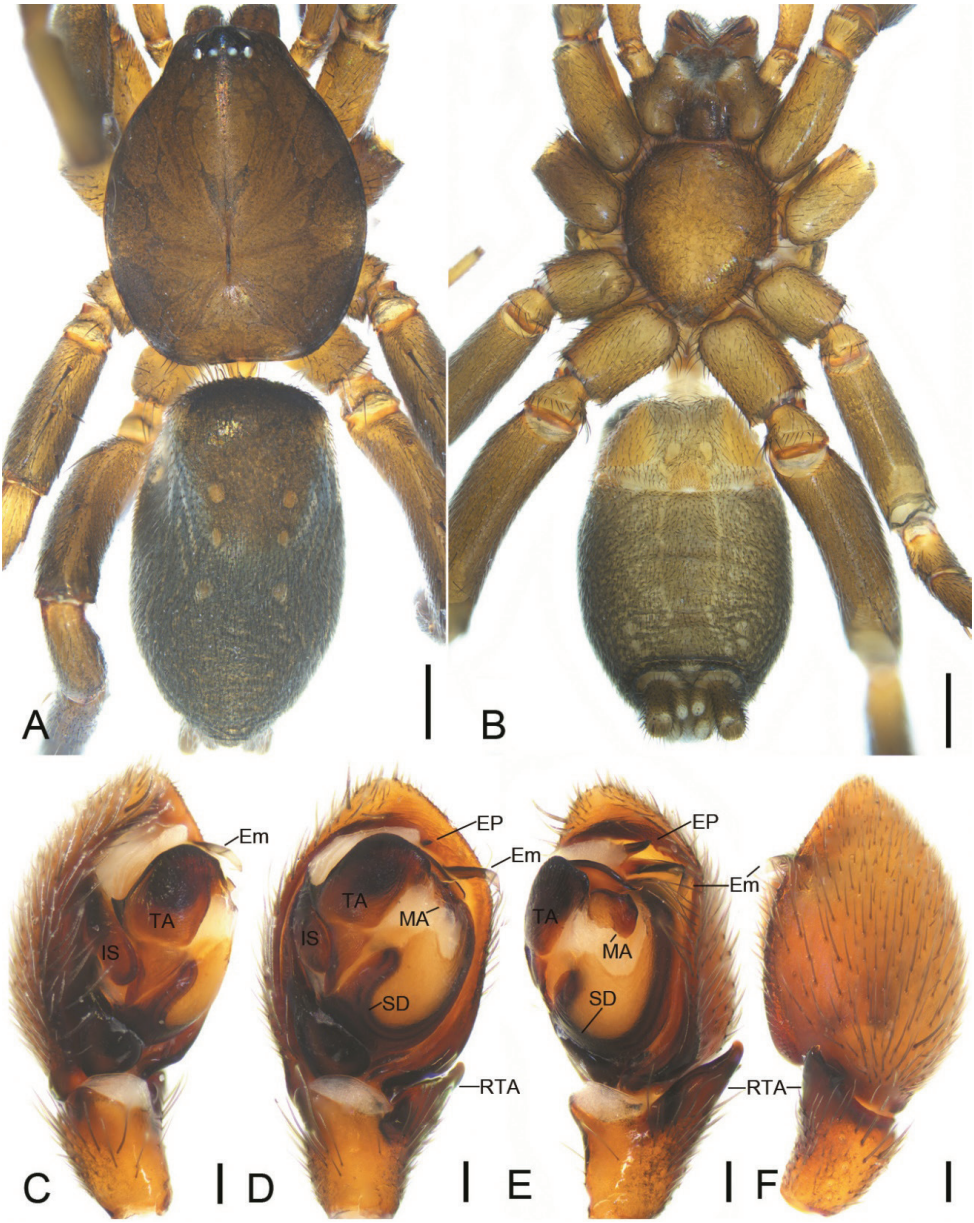

Figure 6. *Zelotes dingnan* sp. nov., male holotype **A** habitus, dorsal view **B** same, ventral view **C** palp, prolateral view **D** same, ventral view **E** same, retrolateral view **F** same, dorsal view. Abbreviations: Em – embolus, EP – embolic projection, IS – intercalary sclerite, MA – median apophysis, RTA – retrolateral tibial apophysis, SD – sperm duct, TA – terminal apophysis. Scale bars: 0.5 mm (**A, B**); 0.1 mm (**C–F**).

Palp as in Figs 6C–F, 7. RTA finger-like, slightly longer than tibia in retrolateral view, apex directed dorsally. Apex of RTA bears two spines. Cymbium 2× longer than wide. Tegulum elliptical. Sperm duct (SD) U-shaped, originating from near median

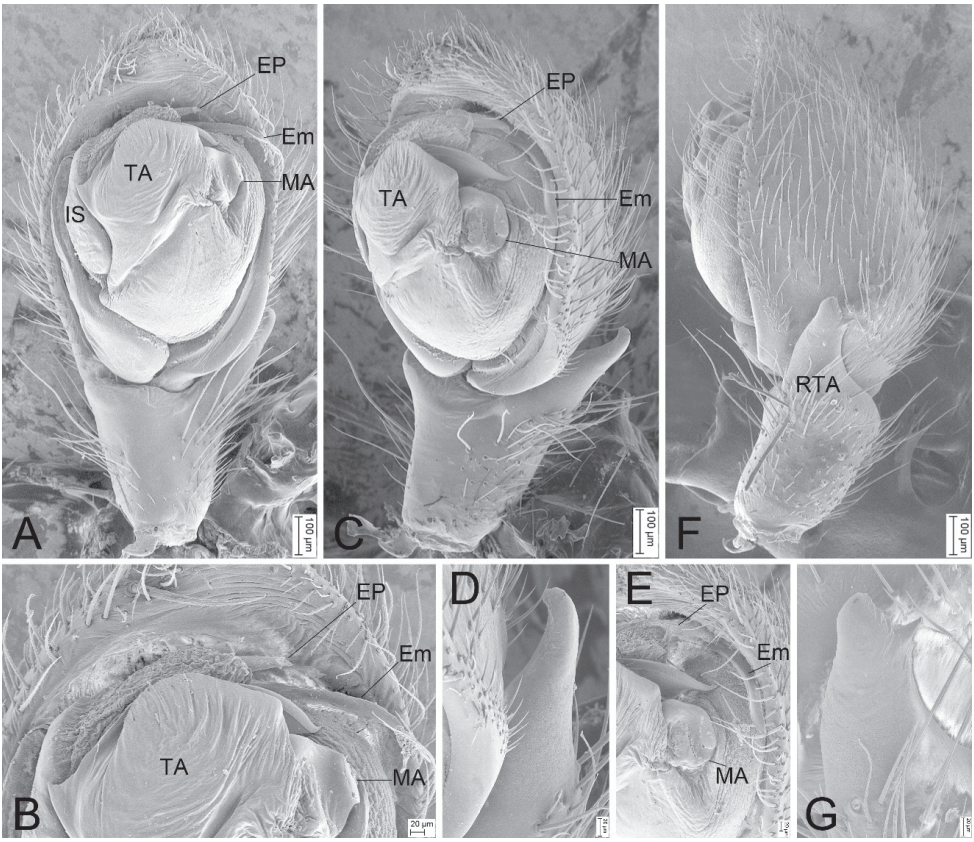

Figure 7. SEM micrographs of *Zelotes dingnan* sp. nov., male palp (paratype) **A** ventral view **B** same, details of embolic division **C** retrolateral view, slightly ventrally **D** same, details of RTA **E** same, details of embolic division **F** dorsal view, slightly retrolaterally **G** same, details of RTA. Abbreviations: Em – embolus, EP – embolic projection, IS – intercalary sclerite, MA – median apophysis, RTA – retrolateral tibial apophysis, TA – terminal apophysis.

apophysis. Median apophysis (MA) with a broad base and retrolaterally curved apex. Terminal apophysis (TA) subquadrangular, with a tooth-like apophysis prolaterally and a spine-like apophysis anteriorly. Intercalary sclerite (IS) longer than terminal apophysis, with membranous part anteriorly. Embolic projection (EP) spine-like, covered by the membranous part of intercalary sclerite. Embolus (Em) with a membranous apex directed posteriorly.

Female. Habitus as in Fig. 8A, B. Total length 5.49. Carapace: 2.39 long, 1.85 wide. Eye sizes and interdistances: AME 0.07, ALE 0.09, PME 0.07, PLE 0.08, AME–AME 0.06, AME–ALE 0.03, PME–PME 0.06, PME–PLE 0.06, AME–PME 0.1, AME–PLE 0.13, ALE–ALE 0.23, PLE–PLE 0.32, ALE–PLE 0.06. MOA 0.21 long, front width 0.19, back width 0.21. Chelicera with 3 promarginal and 4 retromarginal teeth. Abdomen: 3.0 long, 1.65 wide. Leg measurements: I 6.18 (1.63, 0.96, 1.34, 1.2, 1.05); II 3.29 (0.95, 0.47, 0.68, 0.62, 0.57); III 4.7 (1.28, 0.56, 0.99, 1.04, 0.83); IV

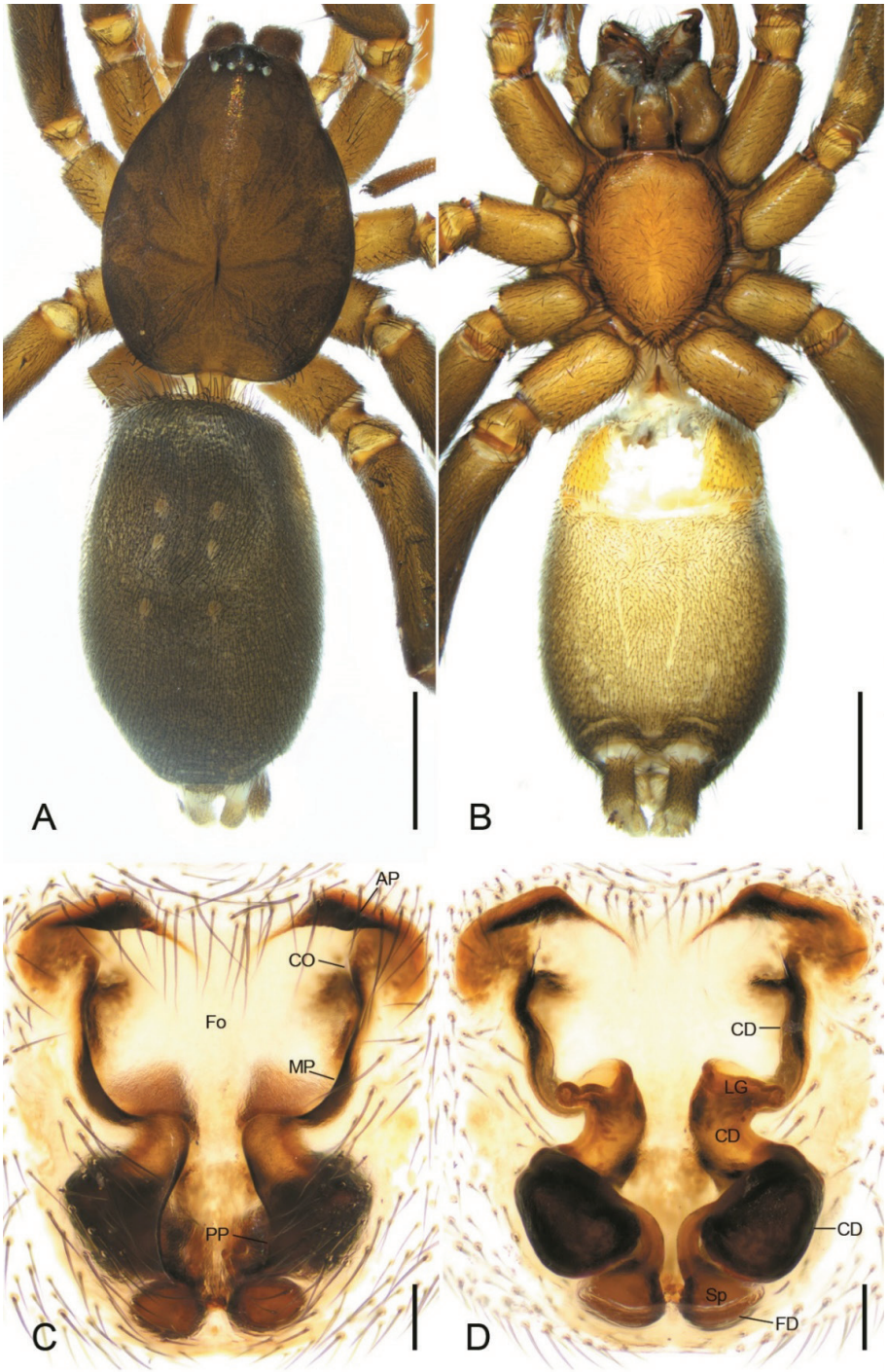

Figure 8. *Zelotes dingnan* sp. nov., female paratype **A** habitus, dorsal view **B** same, ventral view **C** epigyne, ventral view **D** same, dorsal view. Abbreviations: AP – anterior pocket, CD – copulatory duct, CO – copulatory opening, FD – fertilization duct, Fo – fovea, LG – lateral gland, MP – median pocket, PP – posterior pocket, Sp – spermatheca. Scale bars: 0.5 mm (**A, B**); 0.1 mm (**C, D**).

4.91 (1.37, 0.75, 0.94, 1.03, 0.82). Leg spination: I Fe: d3; Pa: r1; Ti: v1; Mt: v4; II Fe: d2; Pa: r1; Ti: v1; Mt: v4; III Fe: d2, p2, r2; Pa: r1; Ti: p3, r2, v6; Mt: d2, p3, r3; IV: Fe: d2, p2, r2; Pa: r1; Ti: p3, r2, v6; Mt: d1, p4, r4, v2.

Colouration as in male, but paler.

Epigyne (Fig. 8C, D). Epigyne 1.3× longer than wide. Anterior pockets (AP) wavy, longer than median pockets (MP). Posterior pockets (PP) poorly visible. Anterior part of fovea (Fo) subrectangular, 1.5× wider than long. Posterior part of the fovea teardrop-like. Copulatory openings (CO) slit-like, located antero-laterally. Copulatory ducts (CD) consist of three parts: longitudinal anterior part, C-shaped median part, which is 2× wider than anterior, and swollen posterior part. Lateral glands (LG) twice shorter than median part of copulatory ducts, directed laterally. Spermathecae (Sp) oval, connected with copulatory ducts by a short bending tube. Fertilisation ducts (FD) slightly curved.

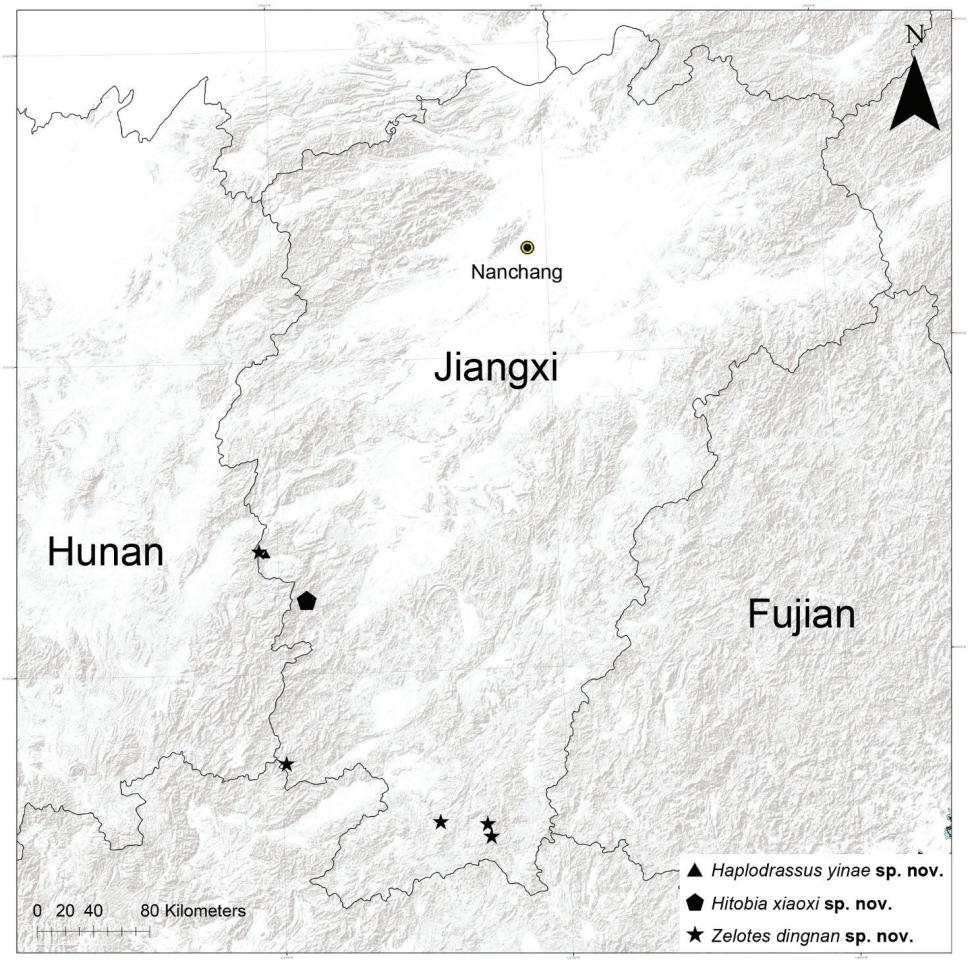

Figure 9. Records of *Haplodrassus yinae* sp. nov., *Hitobia xiaoxi* sp. nov., and *Zelotes dingnan* sp. nov. from Jiangxi Province, China

Comments. The holotype male and the paratype female were collected in different localities. Distance between Guizhumao parking lot and Sanbaishan National Forest Park is about 12 km, which is very close. Moreover, both specimens have similar habitus and coloration. For these reasons, we consider them as conspecific. This hypothesis will be confirmed or rejected in future when both sexes are collected together.

Distribution. Known only from the type locality, Jiangxi Province, China (Fig. 9).

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