ZooKeys 1157: 1–42 (2023)
doi: 10.3897/zookeys.1157.98997
https://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

New records for the Western Balkans cranefly fauna (Diptera, Tipuloidea) with the description of a new *Baeoura* Alexander (Diptera, Limoniidae)

Levente-Péter Kolcsár[1], Micha Camiel d'Oliveira[2], Wolfram Graf[3], Clovis Quindroit[4], Kozo Watanabe[1], Marija Ivković[5]

1 *Center for Marine Environmental Studies, Ehime University, Matsuyama, Ehime 790-8577, Japan* **2** *Naturalis Biodiversity Center, Darwinweg 2, 2333, CR Leiden, Netherlands* **3** *Institute of Hydrobiology and Aquatic Ecosystem Management, University of Natural Resources and Life Sciences Vienna, Vienna, Austria* **4** *Groupe d'études des Invertébrés Armoricains, Angers, France* **5** *Division of Zoology, Department of Biology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, 10000, Zagreb, Croatia*

Corresponding authors: Levente-Péter Kolcsár (kolcsar.peter@gmail.com); Marija Ivković (marija.ivkovic@biol.pmf.hr)

Academic editor: Pavel Starkevic | Received 19 December 2022 | Accepted 1 March 2023 | Published 31 March 2023

https://zoobank.org/1685D647-9DDD-45FE-B0AC-70AE8CF295AA

Citation: Kolcsár L-P, d'Oliveira MC, Graf W, Quindroit C, Watanabe K, Ivković M (2023) New records for the Western Balkans cranefly fauna (Diptera, Tipuloidea) with the description of a new *Baeoura* Alexander (Diptera, Limoniidae). ZooKeys 1157: 1–42. https://doi.org/10.3897/zookeys.1157.98997

Abstract

The cranefly (Tipuloidea) fauna of the Western Balkans is still poorly known. In this study, occurrence data of 77 species is reported, of which two species are newly recorded for Albania, eight species for Bosnia and Herzegovina, twelve for Croatia, and seven for Slovenia, respectively. A new species, *Baeoura neretvaensis* Kolcsár & d'Oliveira, **sp. nov.** is described from Bosnia and Herzegovina, Montenegro, and Slovenia. Images of the habitus, wing, and male and female terminalia of the new species are provided. Furthermore, images of male terminalia and wings of thirteen additional species are presented.

Keywords

New records, Pediciidae, taxonomy, terminalia, Tipulidae, wing

Introduction

Craneflies (Tipuloidea) are one of the most species-rich Dipteran group both in Europe and worldwide and represent almost 10% of all known Diptera species worldwide (Evenhuis and Pape 2022; Oosterbroek 2023). At present, 1267 cranefly species belonging to four families (Cylindrotomidae, Limoniidae, Pediciidae, Tipulidae) are reported from Europe (Oosterbroek 2023). In the last decade, our knowledge of the distribution of European craneflies has increased, but nonetheless craneflies are still considered a poorly known insect group. Apart from a few better-studied countries, like the Czech Republic, Finland, or the United Kingdom, the fauna of many countries is still poorly investigated and it is relatively easy to find new or unreported cranefly species (Kolcsár et al. 2021, 2023).

The Western Balkans (Albania, Bosnia and Herzegovina, Croatia, Kosovo, Montenegro, North Macedonia, Slovenia, and Serbia) and within it the Dinaric Mountains are a biodiversity hotspot, especially regarding freshwater and underground taxa (Gaston and David 1994; Bănărescu 2004; Griffiths et al. 2004; Sket et al. 2004; Ivković and Plant 2015). The Dinaric Mountains are the longest uninterrupted karst area in Europe (Mihevc et al. 2010) with one of the most complex hydrological systems. The rivers and streams of Western Balkans are mostly in good or excellent condition and therefore the nickname of this area is "the Blue Heart of Europe" (https://www.balkanrivers.net/en). The Western Balkans are still unexplored and understudied (Kolcsár et al. 2023), especially when it comes to aquatic insect fauna. Therefore, there are many new described species in the last decade, especially regarding Diptera (e.g., Ivković et al. 2012; Kvifte et al. 2013; Pont and Ivković 2013; Andersen et al. 2016; Kvifte and Ivković 2018). For craneflies, the following publications have dealt with the Western Balkans fauna in the last decade: Bilalli et al. (2021), de Jong et al. (2021), Kolcsár et al. (2015a, b, 2017a, b, 2018a, b, 2023), Keresztes et al. (2018a, b), and Starý (2012).

In this paper, we present new records of Limoniidae, Pediciidae, and Tipulidae from the Western Balkans, and we describe a new *Baeoura* Alexander (Limoniidae) species collected along streams and rivers in Bosnia and Herzegovina, Montenegro, and Slovenia. We also present wing and terminalia photos of several species newly recorded from Croatia, Bosnia and Herzegovina, and Montenegro. All the specimens from the Plitvice Lakes were collected as a part of the project "Phenology of aquatic insects" at the National Park Plitvice Lakes, Croatia. Specimens collected from the Neretva River in Bosnia and Herzegovina for this study were collected during the Neretva Science Week 2022 organized by the Scientists for Balkan Rivers Network in July 2022, which forms part of the Blue Heart of Europe campaign.

Materials and methods

The specimens were collected using insect nets, aspirators, light traps, and pyramid emergence traps and are preserved in ethanol. The following format is used for the records collected with the pyramid emergence traps: date referring to the date when traps were emptied after one moth run, trap number is given, e.g., "P1" is pyramid emergence trap number 1 (see Kolcsár et al. 2015a). Description of body coloration based on specimens stored in ethanol. The genital structures were studied using a Zeiss Stemi 508 stereomicroscope and an Olympus CX33 microscope equipped with Canon Kiss M digital camera. Layer photos were combined using Zerene Stacker software. General morphological terminology in this study follows Cumming and Wood (2017) and Ribeiro (2008) in case of male terminalia.

Specimens from the following depositories were examined:

CKLP	Private collection of L.-P. Kolcsár;
PCCQ	Private Collection of C. Quindroit, Angers, France;
PCJS	Private Collection of J. Starý, Olomouc, Czechia;
PCMCO	Private Collection of M.C. d'Oliveira, Haarlem, The Netherlands;
SMOC	Silesian Museum, Opava, Czech Republic;
UZC	Collection of M. Ivković at University of Zagreb.

Results

Taxonomic treatment

Genus *Baeoura* Alexander, 1924

Type species. *Erioptera nigrolatera* Alexander, 1920 by original designation.

Remarks. *Baeoura* is a species rich genus, including 69 recognized species, prior to this article. The majority of the species is known from the Oriental region (43 species), but the genus also occurs in the Afrotropics (11 species), the Palearctic (4 species in Eastern Palaearctic and 10 species in Western Palaearctic) and one species from the Neotropics (Oosterbroek 2023). Recently two new species were described from the Western Palaearctic, *B. staryi* Driauach & Belqat, 2015 from Morocco and *B. rotherayi* Hancock, 2020 from Spain.

Baeoura neretvaensis Kolcsár & d'Oliveira, sp. nov.

https://zoobank.org/F5064F12-97F3-4B8D-AABF-7B02DE749710
Figs 1–5

Type material. ***Holotype*.** Bosnia and Herzegovina • male; Ulog, Neretva River at Ulog Camp site; alt. 650 m; 43.41714°N, 18.31205°E; 28 Jun. 2022; W. Graf leg.; HOLOTYPE *Baeoura neretvaensis* Kolcsár & d'Oliveira, sp. nov. [red label]; SMOC.

***Paratypes*.** Bosnia and Herzegovina • 3 females; Ulog, Neretva at Ulog Camp site; 43.41714°N, 18.31205°E; alt. 650 m; 28 June 2022; leg. W. Graf; SMOC • 2 males, 2 females; Krupac, Krupac Confluence to Neretva River; 43.32942°N, 18.42574°E; alt. 775 m; 29 June 2022; leg. M. Ivković; 1 male in UZC, 1 male and 2 females in SMOC • 1 female; Cerova, Cerova on Neretva; 43.37887°N, 18.35621°E; alt. 695 m; 30 June 2022; leg. M. Ivković; SMOC • 6 males, 13 females; Ulog, Ulog on Neretva River; 43.42414°N, 18.30837°E; alt. 640 m; 29 June 2022; leg. W. Graf; 1 male and 1 female in PCJS; 4 males and 11 females in SMOC, 1 male and 1 female in CKLP. **Montenegro** • 1 female; Berane, on window in town; 42.8436°N, 19.8666°E; alt. 685 m; 07 July 2012; leg. M. Ivković; UZC. **Slovenia** • 2 females; Gorenjska, Juliske alpe, Gozd Martuljek, River Sava; in small woodland on the banks of river Sava; 46.483°N, 13.838°E; alt. 740 m; 20 August 2019; leg. M.C. d'Oliveira; PCMCO.

Diagnosis. General coloration dark brown, with lateral parts of thorax striped. Scutellum posterior margin whitish. Wing without any markings, hyaline. Gonocoxite long and narrow, without prominent dorsal lobe. Gonostylus very long, narrow, and strongly curved, with a long seta at tip and a flat, blade-like lobe at base. Aedeagal sheath large, strongly curved dorsally, laterally flattened, with a forked process at 3/5 of its length from the base. Female terminalia with a pair of finger-like lobes on sternite 8, longer than cercus or hypogynial valve. Genital chamber complex and strongly sclerotized, sternite 9 with a pair of triangular lobes on the posterior edge, and a pair of finger-like anterior invaginations.

Description. Male. Body length 5.5–6.5 mm, wing length 4.5–5.5 mm. General color dark brown, with lighter abdomen (Fig. 1A).

Head. Wider than long. Eyes small and dorsally widely separated, ~ 1/2 as wide as narrowest point between eyes (Fig. 1B); eyes also separated from each other ventrally too, ~ 1/2 of width of eye. Eyes small, separated dorsally and ventrally (Fig. 1B). Dorsal separation ~ 1/2 as wide as narrowest point between eyes (Fig. 1B), ventral separation ~ 1/2 of wide of eye. Vertex dark brown. Rostrum short, pale brown to brown. Palpus 5-segmented, uniformly brown, or slightly paler at apex; palpomeres 2–5 similar in size. Antenna ~ 2–2.5× longer than head, reaching beyond prescutum if bent backward (Fig. 1B, C). Scape brown, cylindrical 2–2.5× longer than wide; pedicel dark brown, shorter than scape, slightly enlarged, drop-shaped, 2× wider than first flagellomere. Flagellum 13-segmented, brown, first flagellomere drop-shaped, subsequent flagellomeres gradually decreasing in wide and increasing in length toward apical segment, last flagellomere cylindrical. Basal flagellomeres with 2–4 longest verticils on dorsal and lateral sides, start from flagellomere 5 with five or six verticils; length of verticils subequal to length of corresponding flagellomeres.

Thorax. Dark brown dorsally (Fig. 1B), lateral parts stripped (Fig. 1C), formed by lighter and darker parts. Cervical sclerite black, roughly angular with a long extension connecting to head. Pronotum flat, anterior part brown posterior part pale brown. Prescutum and anterior part of scutum dark brown with four broad, darker, less distinct, longitudinal stripes (probably more visible on dry specimens). Central two stripes are fused anteriorly on prescutum, stripes cease near transverse suture. Outer stripes start on sides of posterior scutum, ceasing directly at transverse suture. Posterior part of scutum brown, with two longitudinal darker patches, lateral corner of scutal lobe distinctly yellowish brown. Scutellum brown anteriorly, posterior margin conspicuously white. Mediotergite dark brown. Proepisternum yellowish, pleu-

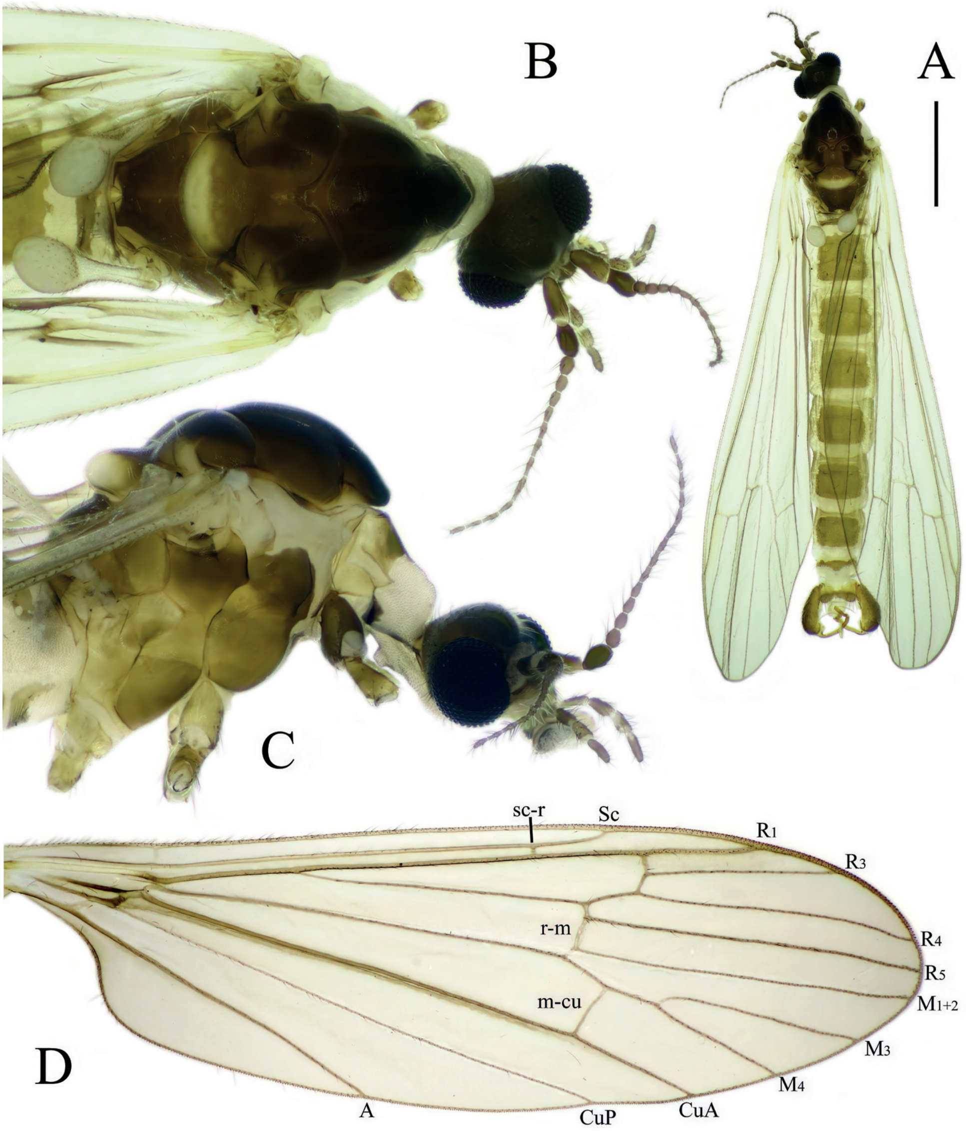


Figure 1. *Baeoura neretvaensis* sp. nov. Paratype: Ulog, Neretva at Ulog Camp site (SMOC) **A** habitus of male **B** head and thorax, dorsal view **C** head and thorax, ventral view **D** wing. Scale bar: 1 mm.

ron, and posterior basalare white to yellowish white. Coxa 1, anepisternum, and anepimeron dark brown. Trochanters 1, 2, katepisternum, and meron brown. Coxae 2, 3, trochanter 3, and metaepisternum light brown. Femora brown, slightly darkening towards apex; tibiae and tarsi brown. Wing yellowish tinged, 3–3.2× as long as wide (Fig. 1D). Stigma inconspicuous, whitish subhyaline with backlight. Vein Sc ending between level of forks of Rs and R2+3+4; crossvein sc-r situated on level of 4/5 of

Sc (measured from crossvein h); R2+3+4 short, ~ 1/5–1/4 length of R4; R2+3 ~ as long as R2, almost perpendicular; M1+2 slightly longer than R5; crossvein r-m, 2–4× longer than basal section of M1+2; cell dm opened, by atrophy of crossvein m-m; M4 ~ 1/4–1/3× longer than M3+4; cross vein m-cu ca. middle of M3+4, relatively long; wing margin between tips of M1+2 and M3 similar in length as between M3 and M4, and between M4 and CuA, and ca. 1/2 in length than distance between CuP and A, CuP and A almost straight. Halter whitish, ~ 0.5 mm (Fig. 1B).

Abdomen. Tergites and sternites uniformly pale brown; terminalia slightly darker (Fig. 1A).

Male terminalia (Fig. 2). Relatively large and prominent. Tergite 8 very narrow, posterior margin fits over anterior part of tergite 9 (Fig. 2C). Tergite 9 narrow, anterior part weakly sclerotized; posterior part with pair of round lobes, bearing long setae (Fig. 2A). Sternite 9 present as narrow band. Gonocoxite long, ~ 3× longer than wide at middle with short ventral lobe (Fig. 2B, C). Gonostylus narrow and very long, directed dorsally (Fig. 2C) and strongly curved (Fig. 2A, B); basally with a flat plate, whose margin round from posterior view (Fig. 2D); tip of gonostylus slightly widened, with a perpendicular, very long, subhyaline gonostylar seta (Fig. 2A). Aedeagal complex long, as long as gonocoxite (Fig. 2 A, B, C). Interbase flattened, blade-like, widely fused with mesal surface of gonocoxite (inseparable from gonocoxite without breaking it), ~ 1/3 length of gonocoxite (Fig. 2A); tip convex or slightly concave in lateral view (Fig. 2E); both interbases medially fused. Aedeagal sheath strongly curved dorsally, narrowest near base, gradually broadening distally, widest at 3/5 of its length, produced into a very long filament-like aedeagus and a dorsal forked extension (Fig. 2E). Ejaculatory apodeme short, rod-shaped, directed ventrally. Parameres short, slightly curved dorsally in later view (Fig. 2E) and directed laterally in ventral view.

Female. Body length 6.0–6.4 mm, wing length 7.4–8.1 mm. Generally resembling male, coloration sometimes somewhat paler.

Female terminalia (Fig. 3). Short and strongly modified. Tergite 9 ~ 2/3 of length and width of tergite 8 (Fig. 3A, C). Posterior margin of tergite 9 slightly concave. Tergite 10 and short, fleshy cerci fused, fused section sub-equal in length to tergite 9. Cerci appearing as two rounded lobes, with numerous setae at apex cerci widely separated with V-shape notch (Fig. 3A). Sternite 8 large, longer than tergites 8 and 9 together (Fig. 3C). Ventral margin of sternite 8 convex, posterolateral corner produced into finger-like process, covered with setae, and slanted upwards at ~ 45°, reaching further than cerci and hypogynial valves (Fig. 3C, D). Hypogynial valves short, subequal in length with tergite 10+cerci (Fig. 3A, C). Parts of genital chamber as genital fork, sternite 9 and genital opening fused and forming a complex strongly sclerotized structure (Fig. 3D). Area of genital opening pale, membranous, surrounded by more sclerotized sternite 9. Sternite 9 with numerous short setae. Posterior part of sternite 9 with a roughly triangular lobe between hypogynial valve and finger-like lobe of sternite 8, subequal in length to hypogynial valve. Anterior part of sternite 9 with a pair of invaginations lateral to genital fork, most probably holding the male gonostylus dur-

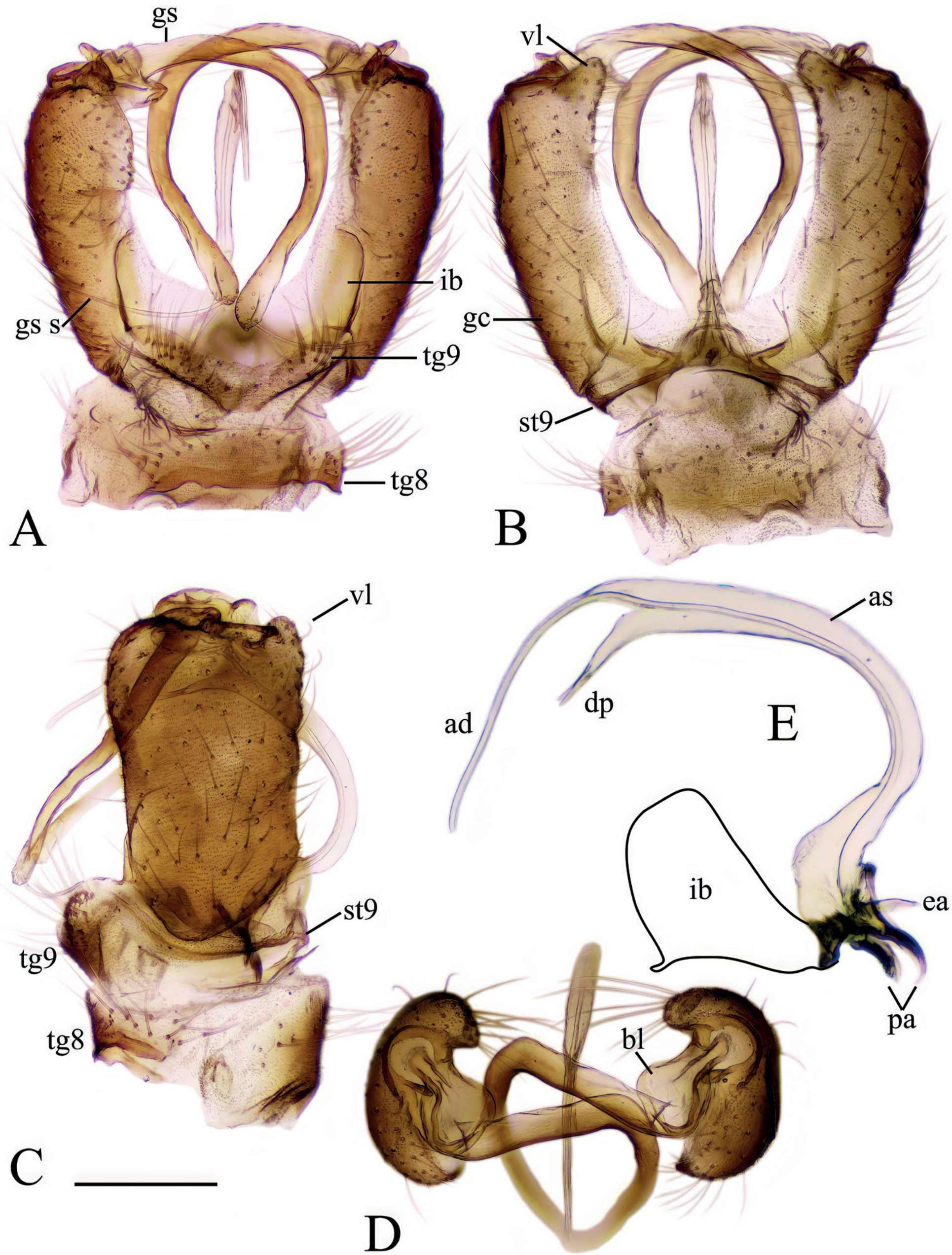


Figure 2. Male terminalia of *Baeoura neretvaensis* sp. nov. Paratype: Ulog, Ulog on Neretva River (SMOC) **A** dorsal view **B** ventral view **C** lateral view **D** caudal view **E** aedeagal complex, lateral view (interbase broken and illustrated with outline). Scale bar: 0.25 mm. Abbreviations: **ea** – ejaculatory apodeme; **ad** – aedeagus; **as** – aedeagal sheath; **bl** – basal lobe of gonostylus; **dp** – dorsal process of aedeagal sheath; **gc** – gonocoxite; **gs** – gonostylus; **gs s** – gonostylar seta; **ib** – interbase; **pa** – paramere; **st** – sternite; **tg** – tergite; **vl** – ventral lobe of gonostylus.

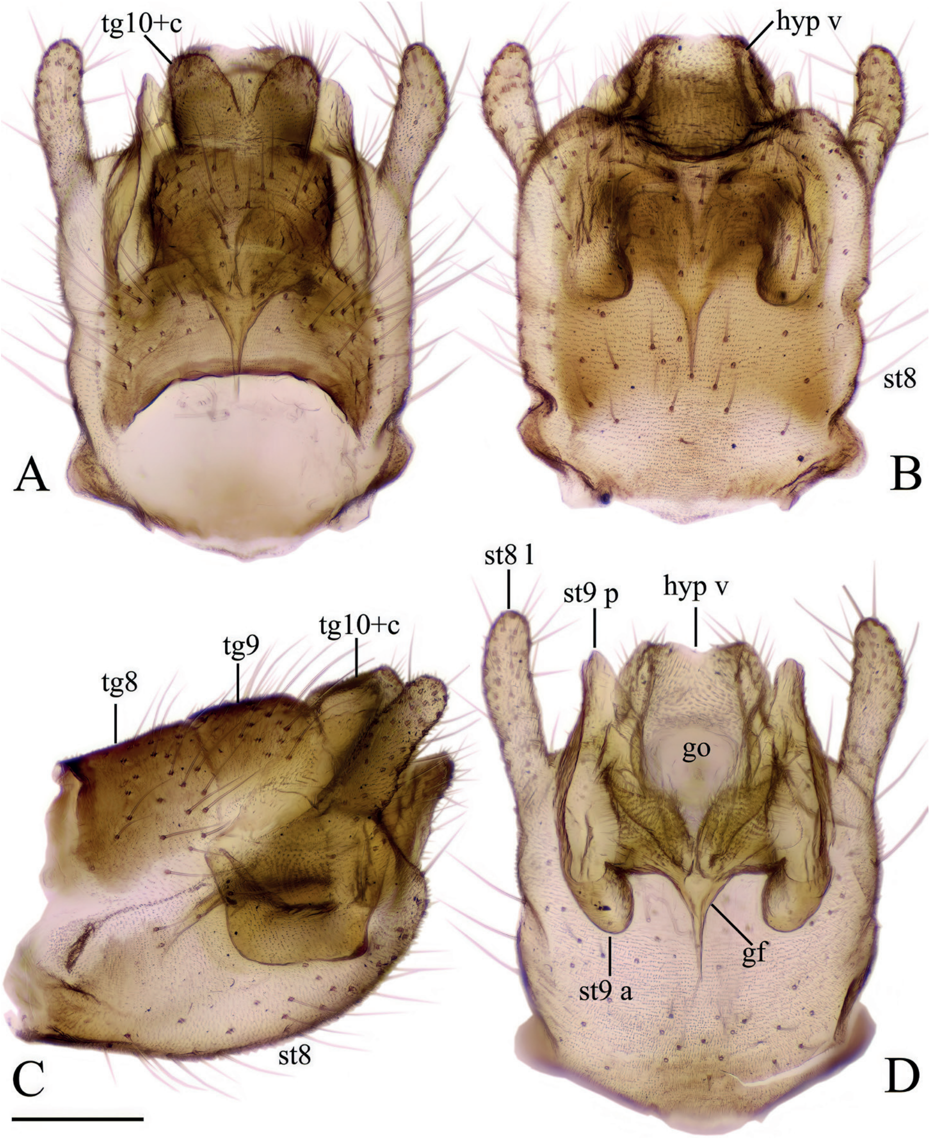


Figure 3. Female terminalia of *Baeoura neretvaensis* sp. nov. Paratype: Ulog, Ulog on Neretva River (SMOC) **A** dorsal view **B** ventral view **C** lateral view **D** sternite 8, hypogynial valvae, sternite 9 and genital fork, inner view. Scale bar: 0.2 mm. Abbr.: **hyp v** – hypogynial valve; **gf** – genital fork; **go** – genital opening; **st** – sternite; **st8 l** – lobe of sternite 8; **st9 a** – sternite 9 anterior invagination; **st9 p** – sternite 9 posterior lobe; **tg** – tergite, **tg10+c** – fused segment of tergite 10 and cerci.

ing the copulation. Genital fork narrowing to a point anteriorly, posteriorly fused with sternite 9 (Fig. 3D). Sternite 10 small, rounded.

Egg. Dark, large, sub-equal in length of tergite 9, tergite 10 and cerci combined; shape oval, cross section roughly triangular.

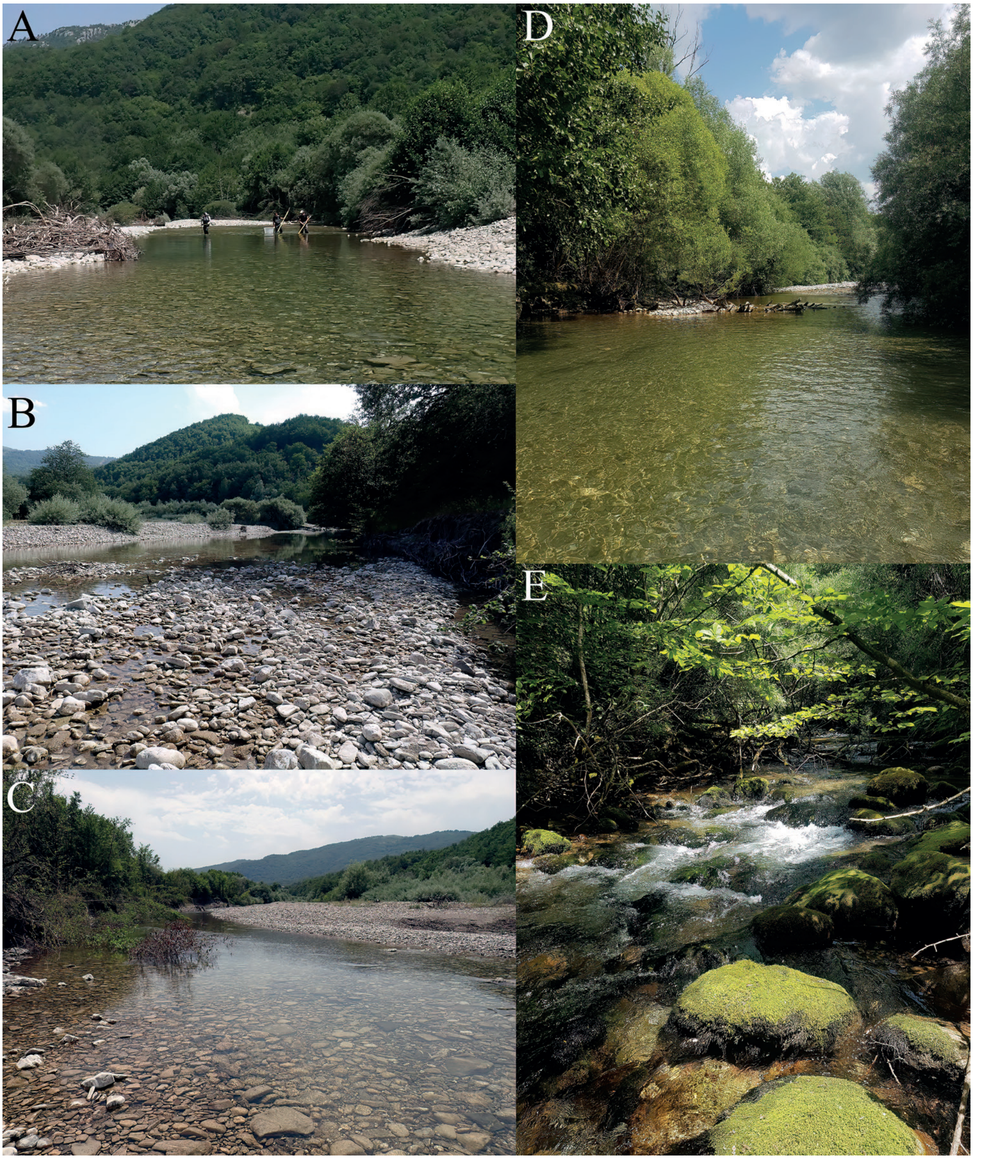


Figure 4. Neretva River and confluence **A–C** ulog on Neretva River; 43.42414°N, 18.30837°E **D** Cerova on Neretva, 43.37887°N, 18.35621°E **E** Krupac, confluence to the Neretva River, 43.32942°N, 18.42574°E. Photographs: Wolfram Graf (**A–C**), Marija Ivković (**D, E**).

Etymology. The name of this small and unique species refers to the Neretva River (Fig. 4), one of the last pristine European rivers, from where it was collected in high numbers.

Distribution. The new species is known from Bosnia and Herzegovina, Montenegro, and Slovenia (Fig. 5).

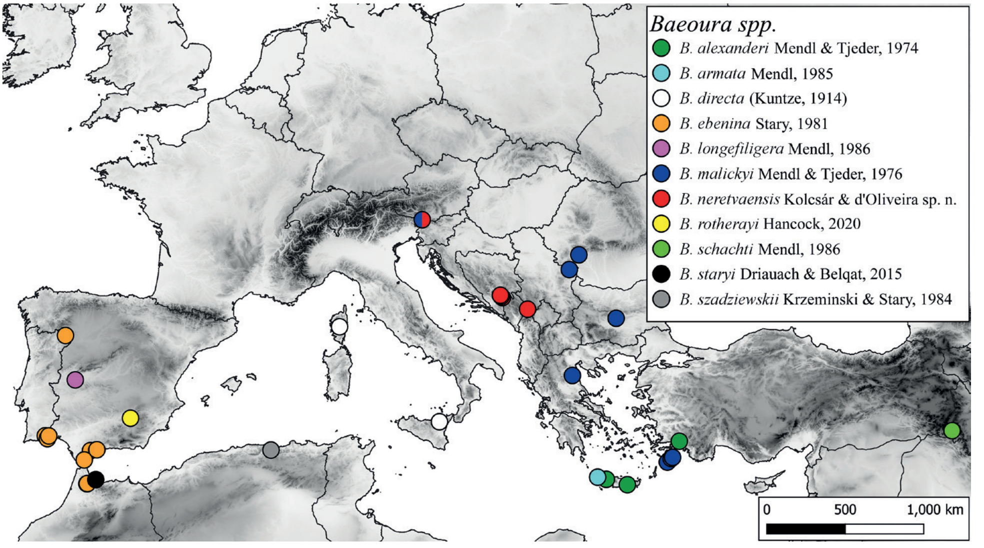


Figure 5. Distribution of *Baeoura* Alexander species in the Western Palearctic.

Remarks. *Baeoura neretvaensis* sp. nov. is unique among the Western Palaearctic species. The closest related species is *Baeoura malickyi* Mendl & Tjeder, 1976, but *B. neretvaensis* can be differentiated from it by the long and slender gonostylus which terminates in a long seta (gonostylus more robust and flattened in *B. malickyi*, with only a short spine-like seta at tip), gonocoxite with a short, apical ventral lobe (long finger-like lobe in *B. malickyi*), a flat lobe at the base of the gonostylus present (no such lobe in *B. malickyi*) (Mendl and Tjeder 1976: figs 1–4). The female of the new species also differs from all described females by the presence of a pair of long finger-like lobes on sternite 8, which is longer than the hypogynial valve (a much shorter lobe is also present in female of *B. malickyi*; however, it is 1/2 as long as the hypogynial valve (Mendl and Tjeder 1976: figs 5–9).

Faunistic records

Limoniidae

1. *Antocha* (*Antocha*) *vitripennis* (Meigen, 1830)

Material examined. Croatia • 1 male; Ličko-Senjska county, Rastovača, Tufa barrier Kozjak-Milanovac, Plitvice Lakes; 44.89416°N, 15.60888°E; alt. 545 m; 28 June 2017; emergence trap, P1; CKLP • 1 male, same locality, 31 May 2016; emergence trap, P5; leg. M. Ivković; UZC. Slovenia • 1 male; Savinjska, Ljubno ob Savinji; 46.332°N, 14.839°E; alt. 490 m; 23 July 2022; leg. M.C. de Haas; PCMCO.

Comments. A common species. Larvae aquatic, associated with clear running water with rocky bottom.

2. *Antocha* (*Orimargula*) *alpigena* (Mik, 1883)
Fig. 6

Material examined. Bosnia and Herzegovina • 6 males, 3 females; Krupac, Krupac 100–600 m from Neretva River upstream; 43.33092°N, 18.42894°E; alt. 805 m; 29 June 2022; leg. M. Ivković; 3 males and 2 females in CKLP, 3 males and 1 female in UZC. Slovenia • 1 male; Gozd Martuljek, Martuljški slapovi; 46.473611°N, 13.829333°E; alt. 850 m; 5 July 2022; leg. C. Quindroit; PCCQ.

Comments. Much rarer species than *Antocha vitripennis*, adults usually found along small, fast-flowing mountain rivers, streams, and waterfalls (Starý 2009). First record from Bosnia and Herzegovina.

3. *Atypophthalmus* (*Microlimonia*) *machidai* (Alexander, 1921)

Material examined. Slovenia • 1 male; Krma Valley; 46.370556°N, 13.88916°E; alt. 900 m; 6 July 2022; leg. C. Quindroit; PCCQ.

Comments. A widely distributed species, known from the Palearctic and Oriental regions. It is relatively rare in Europe, and recently reported from Slovenia (Kolcsár et al. 2021).

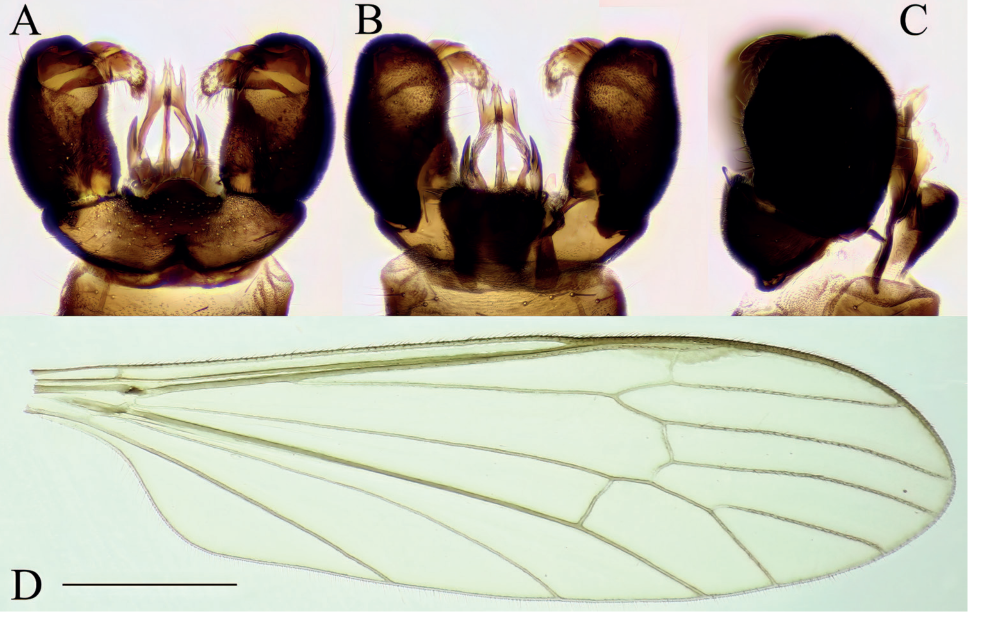


Figure 6. *Antocha* (*Orimargula*) *alpigena*, specimen: Bosnia and Herzegovina, Krupac, Krupac 100–600 m from Neretva River upstream (CKLP) **A–C** male terminalia **A** dorsal view **B** ventral view **C** lateral view **D** wing. Scale bar: 1 mm (**D**).

4. *Austrolimnophila* (*Austrolimnophila*) *ochracea* (Meigen, 1804)

Material examined. **Slovenia** • 1 male; Ljubljana, castle; 46.048°N, 14.50977°E; alt. 340 m; 30 June 2022; leg. C. Quindroit; PCCQ.

Comments. A common and widespread species in the Western Palaearctic.

5. *Dicranomyia* (*Dicranomyia*) *chorea* (Meigen, 1818)

Fig. 7

Material examined. **Croatia** • 1 male; Ličko-Senjska county, Končarev Kraj, Spring of Bijela rijeka, Plitvice Lakes; 44.83472°N, 15.56194°E; alt. 720 m; 31 August 2015; emergence trap, P5 • 1 male, same locality, 31 October 2016; emergence trap, P6 • 1 male, same locality, 31 August 2021; emergence trap, P5; leg. M. Ivković; UZC • 1 male; Ličko-Senjska county, Rastovača, Tufa barrier Kozjak-Milanovac, Plitvice Lakes; 44.89416°N, 15.60888°E; alt. 545 m; 27 July 2017; emergence trap, P3; leg. M. Ivković; CKLP • 1 male, same locality; 28 June 2017; emergence trap, P1 • 2 males, 2 females; same locality; 26 July 2020; emergence trap, P1 • 7 males, 2 females; same locality; 28 June 2020; emergence trap, P1 • 2 males, 1 female; same locality; 31 August 2021; emergence trap, P1; leg. M. Ivković; CKLP.

Comments. A very common and widespread species. Coloration of the specimens very variable from almost fully yellow to dark brown. All specimens examined are yellow.

6. *Dicranomyia* (*Dicranomyia*) *conchifera* (Strobl, 1900)

Fig. 8

Material examined. **Croatia** • 1 male, 1 female; Ličko-Senjska county, Rastovača, Tufa barrier Kozjak-Milanovac, Plitvice Lakes; 44.89416°N, 15.60888°E; alt. 545 m; 30 June 2019; emergence trap, P3 • 4 males, 1 female; same locality; 28 May 2020; emergence trap, P3 • 1 female; same locality; 28 June 2020; emergence trap, P3 • 1 male, 1 female; same locality; 30 June 2021; emergence trap, P1 • 2 males, 4 females; same locality; 30 June 2021; emergence trap, P3; leg. M. Ivković; 2 males in UZC, other specimens in CKLP.

Comments. A rare and poorly known species. In the Balkans it is recorded from Albania, Bulgaria, Greece, North Macedonia, Serbia, and Slovenia. First records from Croatia.

7. *Dicranomyia* (*Dicranomyia*) *didyma* (Meigen, 1804)

Material examined. **Bosnia and Herzegovina** • 1 male, Izvor Ribnik; 44.40222°N, 16.80055°E; alt. 308 m; 24 April 2006; leg. M. Ivković; CKLP. **Croatia** • 1 male, 1 female; Ličko-Senjska county, Končarev Kraj, Spring of Bijela rijeka, Plitvice Lakes; 44.83472°N, 15.56194°E; alt. 720 m; 30 June 2015; emergence trap, P5 • 1 female;

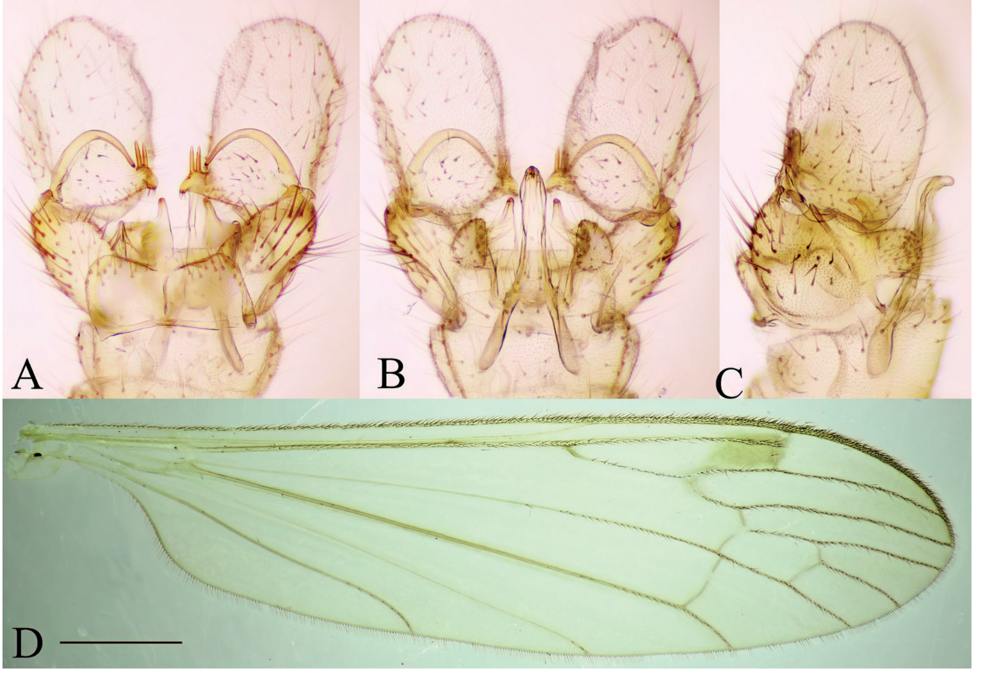


Figure 7. *Dicranomyia* (*Dicranomyia*) *chorea*, specimen: Croatia, spring of Bijela rijeka, Plitvice Lakes (CKLP) **A–C** male terminalia **A** dorsal view **B** ventral view **C** lateral view **D** wing. Scale bar: 1 mm (**D**).

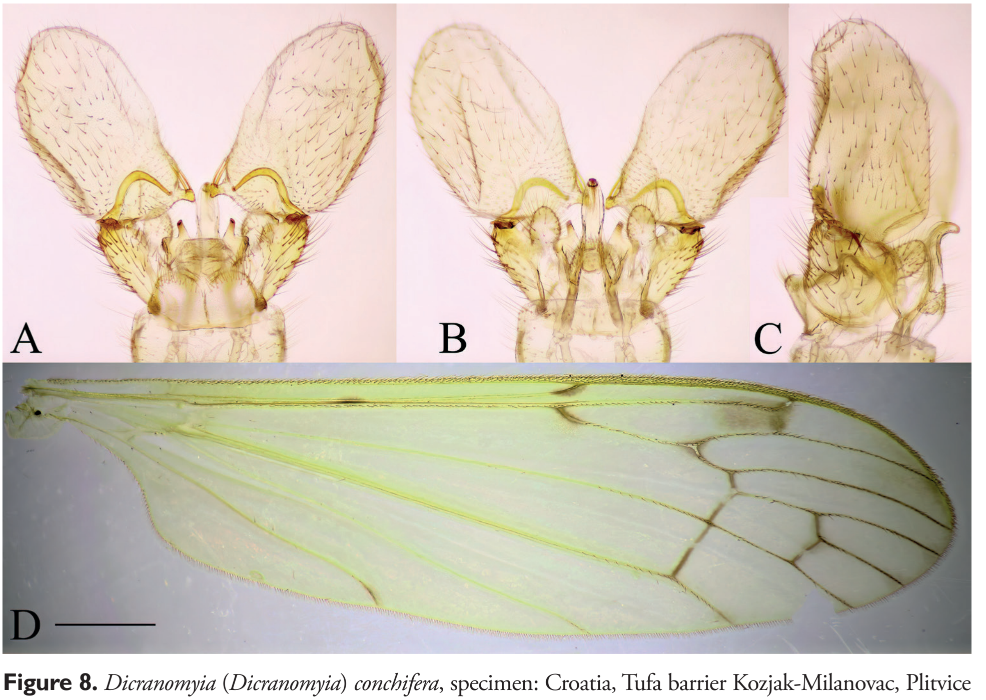


Figure 8. *Dicranomyia* (*Dicranomyia*) *conchifera*, specimen: Croatia, Tufa barrier Kozjak-Milanovac, Plitvice Lakes (CKLP) **A–C** male terminalia **A** dorsal view **B** ventral view **C** lateral view **D** wing. Scale bar: 1 mm (**D**).

same locality; 27 July 2015; emergence trap, P5 • 13 females; same locality; 31 August 2015; emergence trap, P5 • 1 male, 7 females; same locality; 30 September 2015; emergence trap, P5 • 12 males, 6 females; same locality; 31 August 2016; emergence trap, P5 • 5 males, 3 females; same locality; 29 September 2016; emergence trap, P5 • 8 females; same locality; 28 June 2017; emergence trap, P5 • 7 males; same locality; 28 June 2017; emergence trap, P5 • 1 male; same locality; 28 June 2017; emergence trap, P6 • 3 males, 6 females; same locality; 27 July 2017; emergence trap, P5 • 4 males, 1 female; same locality; 31 August 2017; emergence trap, P5 • 1 male; same locality; 29 September 2017; emergence trap, P5 • 1 male, 1 female; same locality; 26 July 2018; emergence trap, P5 • 1 male, 1 female; same locality; 31 August 2018; emergence trap, P5 • 1 female; same locality; 31 August 2018; emergence trap, P6 • 6 males, 1 female; same locality; 31 August 2018; emergence trap, P5 • 2 males, 1 female; same locality; 30 September 2018; emergence trap, P5 • 1 male, 1 female; same locality; 30 June 2019; emergence trap, P5 • 2 males; same locality; 30 June 2019; emergence trap, P5 • 3 males, 5 females; same locality; 27 July 2019; emergence trap, P5 • 1 female; same locality; 30 August 2019; emergence trap, P4 • 2 males, 3 females; same locality; 30 August 2019; emergence trap, P5 • 6 males, 1 female; same locality; 30 September 2019; emergence trap, P5 • 1 male, 1 female; same locality; 29 October 2019; emergence trap, P5 • 2 females; same locality; 26 July 2020; emergence trap, P5 • 1 male; same locality; 29 September 2021; emergence trap, P5; leg. M. Ivković; CKLP • 1 female; Ličko-Senjska county, Plitvica Selo, Stream Plitvica, Plitvice Lakes; 44.90222°N, 15.6075°E; alt. 555 m; 25 July 2008; emergence trap, P6 • 1 female; same locality; 25 July 2008; emergence trap, P3; leg. M. Ivković; CKLP • 1 female; Ličko-Senjska county, Plitvički Ljeskovac, Tufa barrier Labudovac, Plitvice Lakes; 44.87138°N, 15.59972°E; alt. 630 m; 30 October 2014; emergence trap, P3; leg. M. Ivković; CKLP • 2 females; Ličko-Senjska county, Rastovača, Tufa barrier Kozjak-Milanovac, Plitvice Lakes; 44.89416°N, 15.60888°E; alt. 545 m; 30 June 2015; emergence trap, P3 • 5 males, 1 female; same locality; 31 May 2016; emergence trap, P3; leg. M. Ivković; CKLP • 5 males, 1 female; same locality; 31 May 2016; emergence trap, P3 • 1 male; same locality; 30 June 2016; emergence trap, P3 • 2 males, 2 females; same locality; 25 July 2016; emergence trap, P2 • 1 male; same locality; 25 July 2016; emergence trap, P5 • 1 male; same locality; 29 May 2018; emergence trap, P3 • 3 males, 6 females; same locality; 30 August 2019; emergence trap, P4; leg. M. Ivković; 2 males and 2 females in UZC, other specimens in CKLP.

Comments. A widely distributed species, associated with small rivers and streams. The most abundant species in the emergence trap samples from Croatia.

8. *Dicranomyia* (*Dicranomyia*) *imbecilla* Lackschewitz, 1941

Material examined. Albania • 1 male; Dibër, Fushë-Bulqizë; 41.5279°N, 20.2983°E; alt. 780 m; light trap; 26 July 2021; leg. A. de Ketelaere; PCMCO. Slovenia • 1 male; Gorenjska, Gozd Martuljek, Juliske alpe, River Sava; 46.483°N, 13.838°E; alt. 745 m;

light trap; leg. M.C. d'Oliveira; PCMCO • 1 male; Stara fužina, Mostnica river; 46.297889°N, 13.886389°E; alt. 600 m; 3 July 2022; leg. C. Quindroit; PCCQ.

Comments. A poorly known species, only recently reinstated as a valid species and probably a more widely distributed species than thought (Starý and Stubbs 2015). Here we present the first records from Albania and Slovenia.

9. *Dicranomyia* (*Dicranomyia*) *mitis* (Meigen, 1830)

Material examined. Croatia • 1 male, 1 female; Ličko-Senjska county, Končarev Kraj, Spring of Bijela rijeka, Plitvice Lakes; 44.83472°N, 15.56194°E; alt. 720 m; 30 June 2015; emergence trap, P5; leg. M. Ivković; CKLP.

Comments. Common species with a wide distribution across Europe.

10. *Dicranomyia* (*Dicranomyia*) *quadra* (Meigen, 1838)
Fig. 9

Material examined. Croatia • 1 male; Ličko-Senjska county, Končarev Kraj, Spring of Bijela rijeka, Plitvice Lakes; 44.83472°N, 15.56194°E; alt. 720 m; 28 June 2017; emergence trap, P3 • 2 males; same locality; 28 June 2017; emergence trap, P5 • 1 female; same locality; 28 June 2017; emergence trap, P6 • 1 male; same locality; 29 May 2018; emergence trap, P5 • 1 male; same locality; 29 May 2018; emergence trap, P4 • 1 female; same locality; 29 June 2018; emergence trap, P4 • 1 female; same locality; 30 June 2019; emergence trap, P5 • 2 males; same locality; 30 June 2019; emergence trap, P6 • 2 males, 1 female; same locality; 27 July 2019; emergence trap, P6 • 1 female; same locality; 27 July 2019; emergence trap, P4 • 2 males, 2 females; same locality; 28 May 2020; emergence trap, P6 • 1 male; same locality; 30 June 2021; emergence trap, P5 • 1 male; same locality; 30 June 2021; emergence trap, P6; leg. M. Ivković; CKLP • 1 male; Ličko-Senjska county, Plitvički Ljeskovac, Tufa barrier Labudovac, Plitvice Lakes; 44.87138°N, 15.59972°E; alt. 630 m; 29 May 2021; emergence trap, P6; leg. M. Ivković; 2 males and 2 females in UZC, other specimens in CKLP.

Comments. A poorly known species and only recently reinstated as a valid species (Starý and Stubbs 2015). It is known from several countries from the Balkans, and we present the first records from Croatia.

11. *Dicranomyia* (*Melanolimonia*) *morio* (Fabricius, 1787)
Fig. 10

Material examined. Croatia • 1 female; Ličko-Senjska county, Končarev Kraj, Spring of Bijela rijeka, Plitvice Lakes; 44.83472°N, 15.56194°E; alt. 720 m; 30 August 2020; emergence trap, P4 • 5 males, 1 female; same locality; 28 September 2020; emergence trap, P5; leg. M. Ivković; 2 males in UZC, other specimens in CKLP.

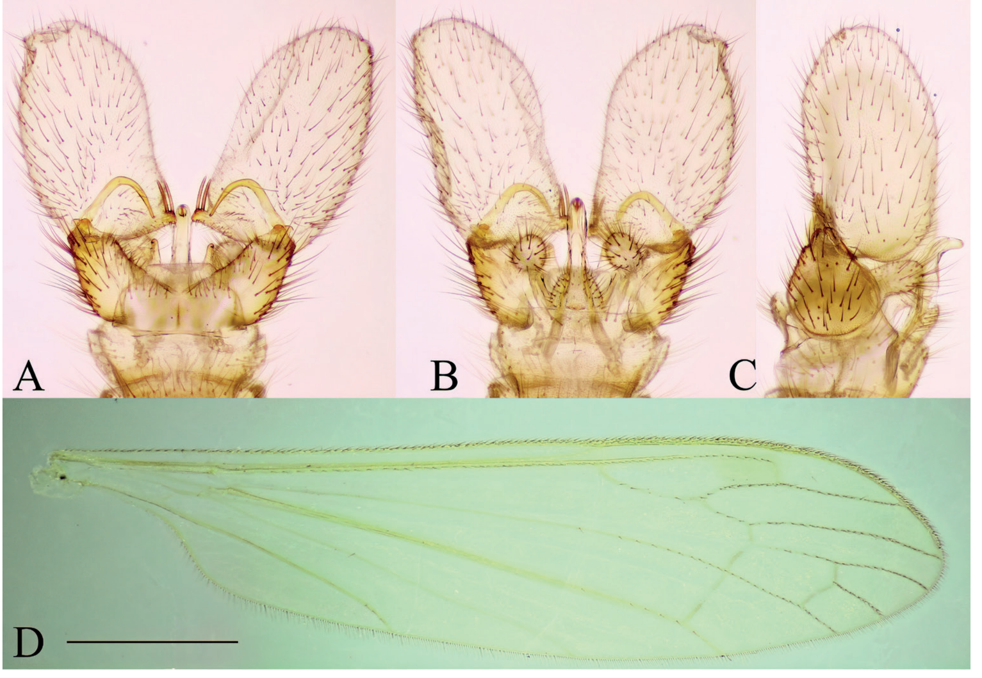


Figure 9. *Dicranomyia* (*Dicranomyia*) *quadra*, specimen: Croatia, spring of Bijela rijeka, Plitvice Lakes (CKLP) **A–C** male terminalia **A** dorsal view **B** ventral view **C** lateral view **D** wing. Scale bar: 1 mm (**D**).

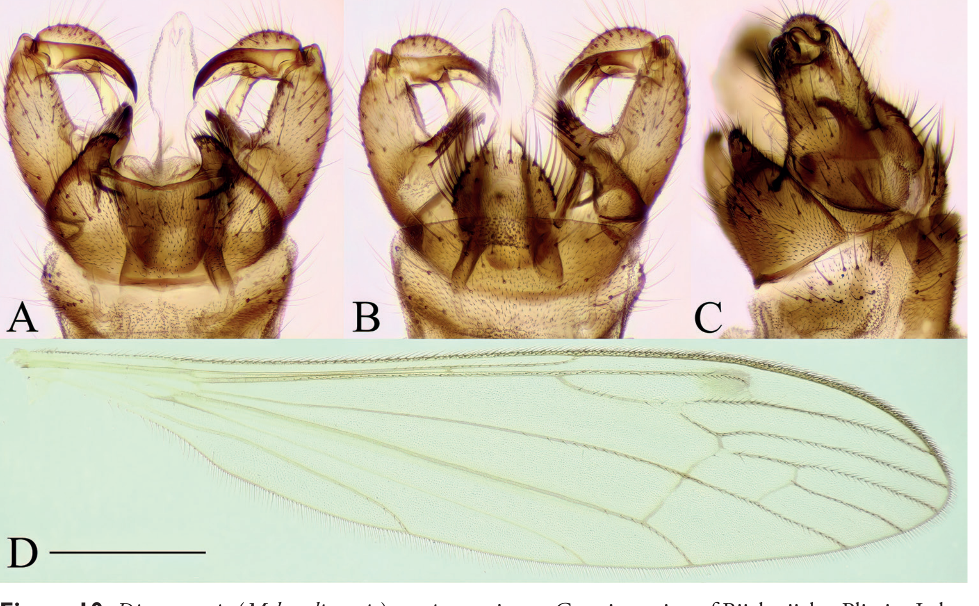


Figure 10. *Dicranomyia* (*Melanolimonia*) *morio*, specimen: Croatia, spring of Bijela rijeka, Plitvice Lakes (CKLP) **A–C** male terminalia **A** dorsal view **B** ventral view **C** lateral view **D** wing. Scale bar: 1 mm (**D**).

Comments. Widely distributed species in the Palearctic, adults usually found around springs and smaller streams, larvae probably live on moss covered wet rocks. First records from Croatia.

12. *Dicranomyia (Melanolimonia) stylifera* Lackschewitz, 1928

Material examined. **Slovenia** • 1 male; Mojstrana, Slap Peričnik; 46.439111°N, 13.894278°E; alt. 900 m; 05 july 2022; leg C. Quindroit; PCCQ.

Comments. A rare and poorly known species, associated with calcareous habitats (Salmela 2008; Stubbs 2021).

13. *Dicranomyia* (*Numantia*) *fusca* (Meigen, 1804)

Material examined. **Slovenia** • 2 males; Savinjska, Ljubno ob Savinji; 46.332°N, 14.839°E; alt. 490 m; 23 July 2022; leg. M.C. de Haas; PCMCO.

Comments. Common species and widely distributed in the Holarctic.

14. *Dicranomyia* (*Sivalimnobia*) *aquosa* Verrall, 1886

Material examined. **Slovenia** • 3 males, 1 female; Gozd Martuljek, Martuljški slapovi; 46.473611°N, 13.829333°E; alt. 850 m; 5 July 2022; leg. C. Quindroit; PCCQ • 1 male; Mojstrana, Slap Peričnik; 46.439111°N, 13.894278°E; alt. 900 m; 5 July 2022; leg C. Quindroit; PCCQ.

Comments. A rare species associated with hygropetric habitats, usually found around waterfalls. Larvae most probably live on the surface of permanently wet rocks (Stubbs 2021).

15. *Dicranophragma* (*Brachylimnophila*) *nemorale* (Meigen, 1818)

Material examined. **Croatia** • 2 males; Ličko-Senjska county, Plitvički Ljeskovac, Tufa barrier Labudovac, Plitvice Lakes; 44.87138°N, 15.59972°E; alt. 630 m; 29 September 2021; emergence trap, P6; leg. M. Ivković; CKLP • 1 male; Ličko-Senjska county, Rastovača, Tufa barrier Kozjak-Milanovac, Plitvice Lakes; 44.89416°N, 15.60888°E; alt. 545 m; 29 September 2017; emergence trap, P4; leg. M. Ivković; 1 male in UZC, other specimens in CKLP. **Slovenia** • 1 male; Stara fužina, Mostnica river; 46.297889°N, 13.886389°E; alt. 600 m; 3 July 2022; leg. C. Quindroit; PCCQ.

Comments. Common.

16. *Dicranoptycha fuscescens* (Schummel, 1829)

Material examined. Slovenia • 1 male; Bovec, junction Soča and Koritnica rivers; 46.330167°N, 13.577028°E; alt. 400 m; 7 July 2022; leg. C. Quindroit; PCCQ.

Comments. A common species with wide distribution range in the Palaearctic.

17. *Elliptera omissa* Schiner, 1863

Material examined. Croatia • 1 male; Primorsko-Goranska county, Gorski Kotar, Spring of River Kupa and just below the spring; 45.4919°N, 14.6925°E; alt. 756 m; 06 August 2021; leg. M. Ivković; CKLP. Slovenia • 1 male; Trenta, Soča source; 46.411972°N, 13.729583°E; alt. 950 m; 7 July 2022; leg. C. Quindroit; PCCQ.

Comments. A rare species, known only from a few European countries, recently reported from Croatia (Kolcsár et al. 2015a). The species is connected to hygropetric habitats, usually found around waterfalls and fast-flowing rocky mountain streams and rivers.

18. *Ellipteroides* (*Ellipteroides*) *lateralis* (Macquart, 1835)

Material examined. Croatia • 1 male; Ličko-Senjska county, Rastovača, Tufa barrier Kozjak-Milanovac, Plitvice Lakes; 44.89416°N, 15.60888°E; alt. 545 m; 29 May 2018; emergence trap, P6; leg. M. Ivković; CKLP.

Comments. Species usually found around streams and rivers with sandy sediments.

19. *Ellipteroides* (*Protogonomyia*) *alboscutellatus* (von Roser, 1840)
Fig. 11

Material examined. Bosnia and Herzegovina • 1 male, 1 female; Krupac, Krupac 100–600 m from Neretva River upstream; 43.33092°N, 18.42894°E; alt. 805 m; 29 June 2022; leg. M. Ivković; CKLP. Slovenia • 1 male, 2 females; Vintgar gorge; 46.393333°N, 14.086056°E; alt. 600 m; 1 July 2022; leg C. Quindroit; PCCQ • 5 males; Stara fužina, Mostnica river; 46.297889°N, 13.886389°E; alt. 600 m; 3 July 2022; leg. C. Quindroit; PCCQ.

Comments. First record from Bosnia and Herzegovina.

20. *Ellipteroides* (*Protogonomyia*) *limbatus* (von Roser, 1840)
Fig. 12

Material examined. Bosnia and Herzegovina • 2 females; Krupac, Krupac 100–600 m from Neretva River upstream; 43.33092°N, 18.42894°E; alt. 805 m; 29 June

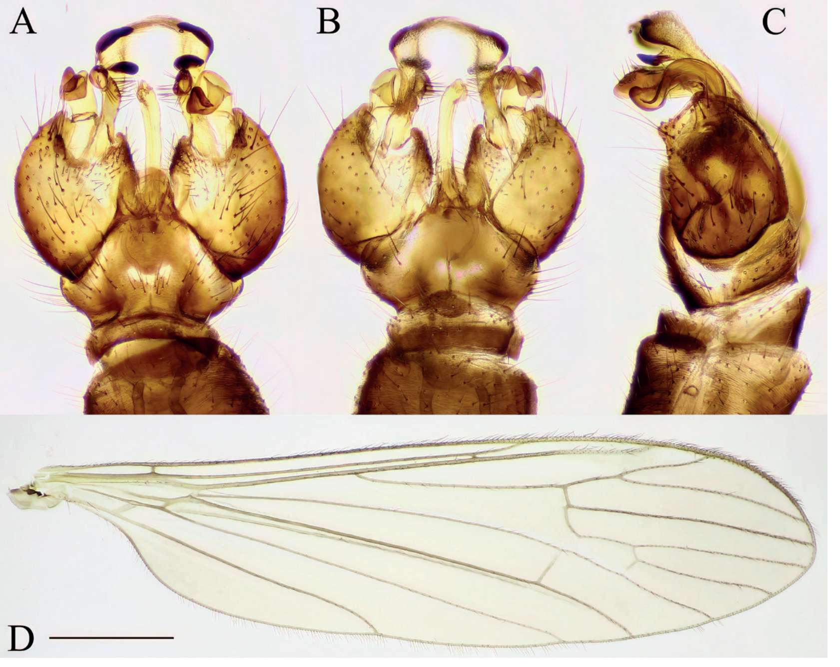


Figure 11. *Ellipteroides* (*Protogonomyia*) *alboscutellatus*, specimen: Bosnia and Herzegovina, Krupac, Krupac 100–600 m from Neretva River upstream (CKLP) **A–C** male terminalia **A** dorsal view **B** ventral view **C** lateral view **D** wing. Scale bar: 1 mm (**D**).

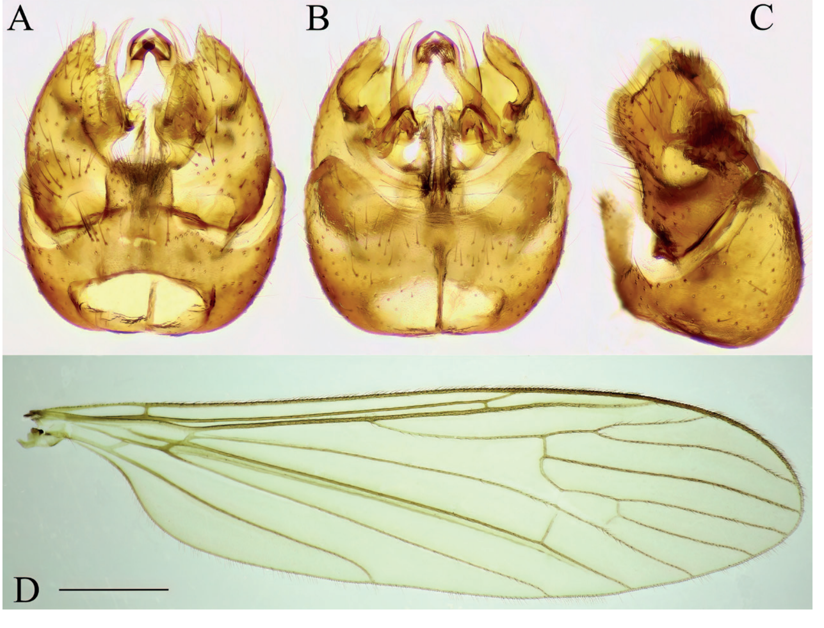


Figure 12. *Ellipteroides* (*Protogonomyia*) *limbatus*, specimen: Montenegro, mouth of stream Desna rijeka to River Mojanska Rijeka (CKLP) **A–C** male terminalia **A** dorsal view **B** ventral view **C** lateral view **D** wing. Scale bar: 1 mm (**D**).

2022; leg. M. Ivković; CKLP. **Montenegro** • 2 males; Mouth of Stream Desna rijeka to River Mojanska rijeka; 42.68888°N, 19.72777°E; alt. 925 m; 08 July 2012; leg. M. Ivković; CKLP • 1 male; River Murinska Rijeka; 42.6525°N, 19.88361°E; alt. 1000 m; 07 July 2012; leg. M. Ivković; 1 male and 1 female in UZC, other specimens in CKLP. **Slovenia** • 1 male, 1 female; Savinjska, Ljubno ob Savinji; 46.332°N, 14.839°E; alt. 490 m; 23 July 2022; leg. M.C. de Haas; PCMCO.

Comments. First record from Bosnia and Herzegovina.

21. *Eloeophila miliaria* (Egger, 1863)

Fig. 13

Material examined. Bosnia and Herzegovina • 1 male; Ulogski Buk, Ulogski Buk on Neretva; 43.40467°N, 18.32423°E; alt. 675 m; 01 July 2022; leg. M. Ivković; CKLP.

Comments. A relatively rare species, and only recently reported from Croatia (Kolcsár et al. 2015a); here we report the species for the first time from Bosnia and Herzegovina.

22. *Erioptera* (*Erioptera*) *lutea lutea* Meigen, 1804

Material examined. Slovenia • 1 male; Savinjska, Ljubno ob Savinji; 46.333°N, 14.8387°E; alt. 745 m; light trap; 20 August 2019; leg. M.C. d'Oliveira; PCMCO.

Comments. A common species with wide distribution range in the Palaearctic.

23. *Gonomyia* (*Prolipophleps*) *abbreviata* Loew, 1873

Material examined. Slovenia • 1 male; Savinjska, Ljubno ob Savinji; 46.332°N, 14.839°E; alt. 490 m; 22 July 2022; light trap; leg. M.C. de Haas; PCMCO.

Comments. A relatively rare and poorly known species with a wide distribution range from Iran to Great Britain; however, only with scattered records. First record from Slovenia.

24. *Helius* (*Helius*) *pallirostris* Edwards, 1921

Material examined. Croatia • 1 female; Ličko-Senjska county, Plitvički Ljeskovac, Tufa barrier Labudovac, Plitvice Lakes; 44.87138°N, 15.59972°E; alt. 630 m; 27 July 2019; emergence trap, P1; leg. M. Ivković; CKLP.

Comments. A relatively common species in Central Europe; however, this is the first record from Croatia and from the Western Balkans.

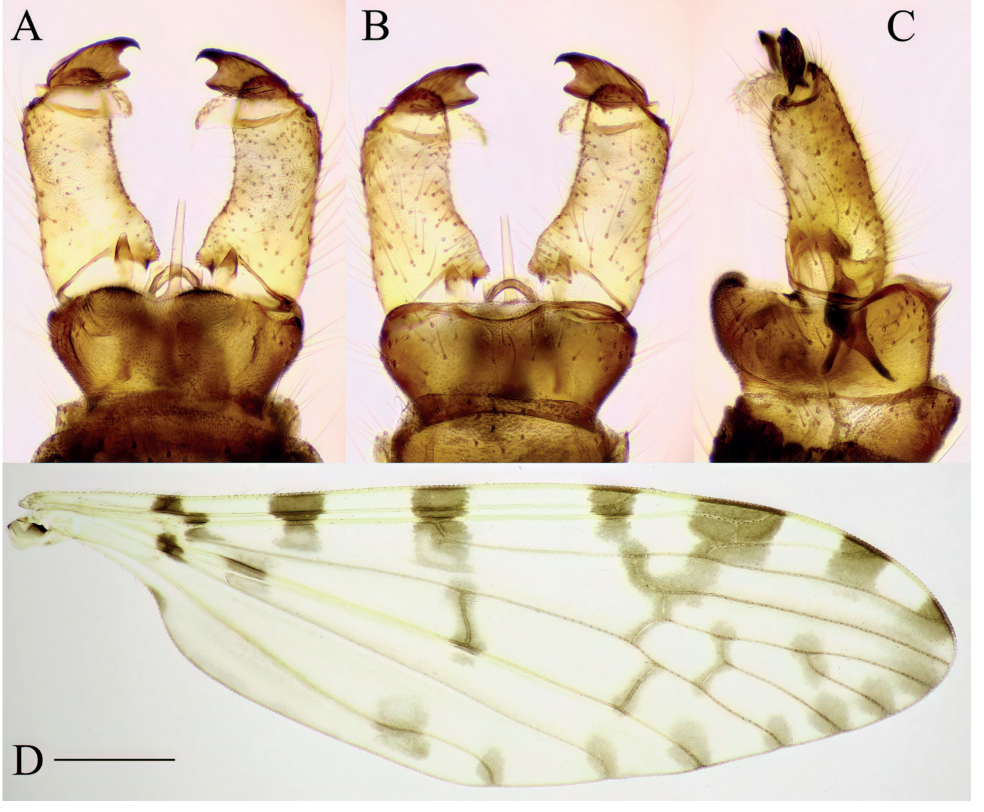


Figure 13. *Eloeophila miliaria*, specimen: Bosnia and Herzegovina, Ulogski Buk, Ulogski Buk on Neretva (CKLP) **A–C** male terminalia **A** dorsal view **B** ventral view **C** lateral view **D** wing. Scale bar: 1 mm (**D**).

25. *Hexatoma* (*Eriocera*) *chirothecata* (Scopoli, 1763)

Material examined. Croatia • 1 male; Ličko-Senjska county, Končarev Kraj, Spring of Bijela rijeka, Plitvice Lakes; 44.83472°N, 15.56194°E; alt. 720 m; 26 July 2020; emergence trap, P6; leg. M. Ivković; CKLP • 1 male; Ličko-Senjska county, Plitvički Ljeskovac, Tufa barrier Labudovac, Plitvice Lakes; 44.87138°N, 15.59972°E; alt. 630 m; 25 July 2014; emergence trap, P2 • 2 males; same locality; 27 July 2015; emergence trap, P2 • 1 female; same locality; 27 July 2015; emergence trap, P7 • 3 females; same locality; 30 June 2021; emergence trap, P6 • 1 male, 1 female; same locality; 30 June 2021; emergence trap, P6 • 2 males; same locality; 31 August 2021; emergence trap, P3; leg. M. Ivković; 1 male and 1 female in UZC, other specimens in CKLP • 1 male; Ličko-Senjska county, Rastovača, Tufa barrier Kozjak-Milanovac, Plitvice Lakes; 44.89416°N, 15.60888°E; alt. 545 m; 30 August 2019; emergence trap, P5; leg. M. Ivković; CKLP. Slovenia • 1 female; Savinjska, Ljubno ob Savinji; 46.332°N, 14.839°E; alt. 490 m; 16 July 2022; light trap; leg. M.C. de Haas; PCMCO.

Comments. A poorly known species. It seems common in the Western Balkans, reported from all countries; however, it is relatively rare in other parts of Europe. The species seems to prefer small calcareous rivers and streams.

26. *Hexatoma* (*Hexatoma*) *bicolor* (Meigen, 1818)

Material examined. Slovenia • 1 male; Savinjska, Luče; 46.357°N, 14.753°E; alt. 515 m; 20 July 2022; leg. M.C. de Haas; PCMCO.

Comments. A poorly known species associated with streams and rivers with sandy or gravelly banks (Starý 2009).

27. *Idiocera* (*Euptilostena*) *jucunda* (Loew, 1873)

Fig. 14

Material examined. Bosnia and Herzegovina • 1 male, 4 females; Sutjeska, Jabučica Stream; 43.29°N, 18.6172°E; alt. 767 m; 04 July 2012; UV Lamp; leg. M. Ivković; CKLP • 1 male; Ulog, Neretva at Ulog Camp site; 43.41714°N, 18.31205°E; alt. 650 m; 28 June 2022; leg. W. Graf; CKLP. Slovenia • 1 male; Gorenjska, Gozd Martuljek, Juliske alpe, River Sava; 46.483°N, 13.838°E; alt. 745 m; 20 August 2019; light trap; leg. M.C. d'Oliveira; PCMCO.

Comments. A rare and poorly known species associated with mountain rivers and streams with sandy and gravelly banks (Starý 2009). We collected the species near similar habitats, with light traps. First records from Bosnia and Herzegovina.

28. *Idiocera* (*Idiocera*) *lackschewitzi* (Starý, 1977)

Fig. 15

Material examined. Bosnia and Herzegovina • 1 female; Sutjeska, Jabučica Stream; 43.29°N, 18.6172°E; alt. 767 m; 04 July 2012; leg. M. Ivković; CKLP • 1 male; Ulog, Neretva at Ulog Camp site; 43.41714°N, 18.31205°E; alt. 650 m; 28 June 2022; leg. W. Graf; CKLP.

Comments. A very rare species, only known from Albania, Greece, Italy (Sicily), and North Macedonia. First records from Bosnia and Herzegovina. Specimens collected along or near rivers with gravelly banks, usually together with *Idiocera jucunda*.

29. *Ilisia maculata* (Meigen, 1804)

Material examined. Slovenia • 1 male; Savinjska, Ljubno ob Savinji; 46.332°N, 14.839°E; alt. 490 m; 22 July 2022; light trap; leg. M.C. de Haas; PCMCO.

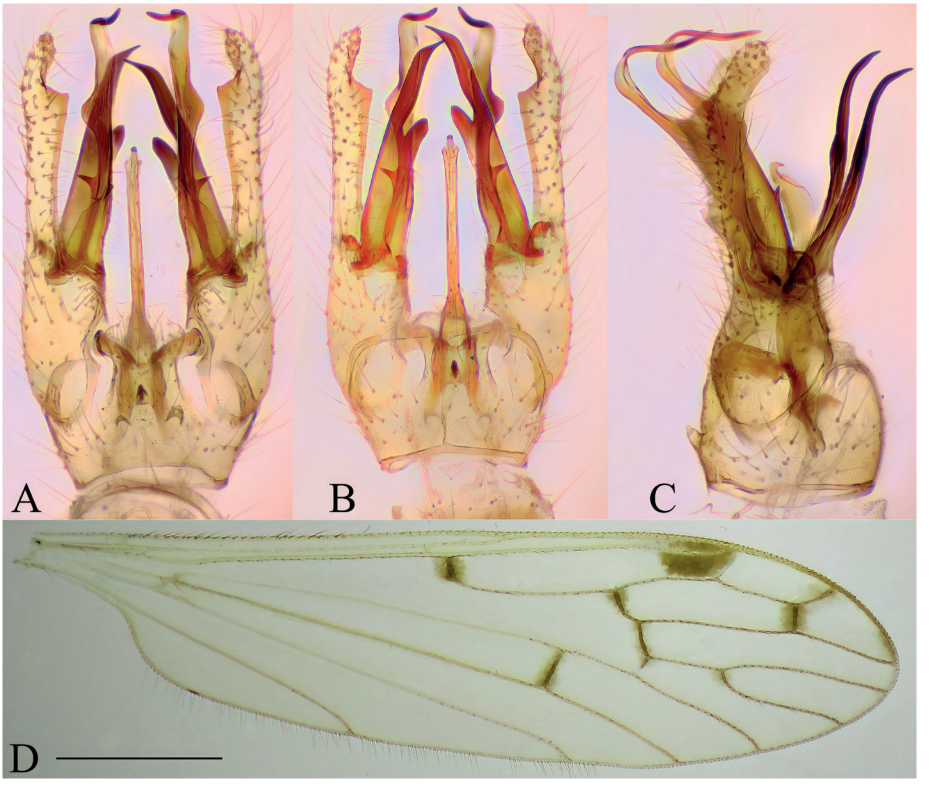


Figure 14. *Idiocera* (*Euptilostena*) *jucunda*, specimen: Bosnia and Herzegovina, Ulog, Neretva at Ulog Camp site (CKLP) **A–C** male terminalia **A** dorsal view **B** ventral view **C** lateral view **D** wing. Scale bar: 1 mm (**D**).

Comments. A common species, associated with rich organic muds (Podeniene 2009), and can be found around various water bodies. First record from Slovenia.

30. *Limonia nubeculosa* Meigen, 1804

Material examined. Croatia • 1 female; Ličko-Senjska county, Končarev Kraj, Spring of Bijela rijeka, Plitvice Lakes; 44.83472°N, 15.56194°E; alt. 720 m; 29 May 2018; emergence trap, P5; leg. M. Ivković; CKLP • 1 female; Zagreb, Stream Jelenovac; 45.82306°N, 15.95235°E; alt. 170 m; 10 April 2020; leg. M. Ivković (not stored)

Comments. A very common and widely distributed species.

31. *Limonia sylvicola* (Schummel, 1829)

Material examined. Slovenia • 1 male; Stara fužina, Mostnica river; 46.297889°N, 13.886389°E; alt. 600 m; 3 July 2022; leg. C. Quindroit; PCCQ.

Comments. A common species.

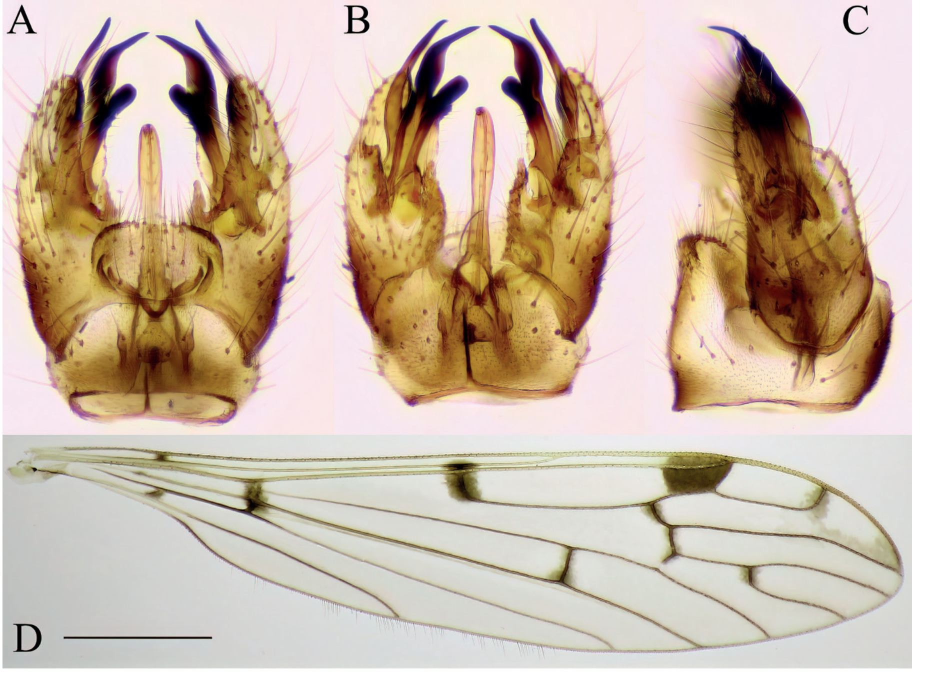


Figure 15. *Idiocera* (*Idiocera*) *lackschewitzi*, specimen: Bosnia and Herzegovina, Ulog, Neretva at Ulog Camp site (CKLP) **A–C** male terminalia **A** dorsal view **B** ventral view **C** lateral view **D** wing. Scale bar: 1 mm (**D**).

32. *Limonia taurica* (Strobl, 1895)

Material examined. Slovenia • 1 male; Stara fužina, Puncrat; 46.303778°N, 13.839917°E; alt. 1400 m; 2 July 2022; leg. C. Quindroit; PCCQ.

Comments. A widely distributed species, known from several European countries and from China.

33. *Limonia phragmitidis* (Schrank, 1781)

Material examined. Croatia • 1 male; Zagreb, Stream Jelenovac; 45.82306°N, 15.95235°E; alt. 170 m; 10 April 2020; leg. M. Ivković; CKLP.

Comments. A very common and widely distributed species.

34. *Lipsothrix nobilis* Loew, 1873

Material examined. Croatia • 1 male; Ličko-Senjska county, Plitvički Ljeskovac, Tufa barrier Labudovac, Plitvice Lakes; 44.87138°N, 15.59972°E; alt. 630 m; 29 May 2015; emergence trap, P5; leg. M. Ivković; CKLP.

Comments. A common Western Palaearctic species, larvae and pupae associated with partly submerged decaying larger wood in streams and rivers (Hancock et al. 2009).

35. *Lipsothrix remota* (Walker, 1848)

Material examined. Croatia • 1 male; Ličko-Senjska county, Končarev Kraj, Spring of Bijela rijeka, Plitvice Lakes; 44.83472°N, 15.56194°E; alt. 720 m; 30 June 2021; emergence trap, P5; leg. M. Ivković; UZC • 1 male; Ličko-Senjska county, Plitvički Ljeskovac, Spring of Crna rijeka, Plitvice Lakes; 44.83714°N, 15.60752°E; alt. 680 m; 30 June 2021; emergence trap, P6 • 1 female; same locality; 29 September 2021; emergence trap, P5; leg. M. Ivković; CKLP • 1 male; Ličko-Senjska county, Plitvički Ljeskovac, Tufa barrier Labudovac, Plitvice Lakes; 44.87138°N, 15.59972°E; alt. 630 m; 30 June 2014; emergence trap, P4 • 1 male; same locality; 30 June 2015; emergence trap, P6; leg. M. Ivković; CKLP.

Comments. A common European species with similar habitat reference as *Lipsothrix nobilis* (Hancock et al. 2009).

36. *Molophilus* (*Molophilus*) *aduncus* Starý, 1978

Material examined. Albania • 1 male; Dibër, Fushë-Bulqizë; 41.5279°N, 20.2983°E; alt. 780 m; 26 July 2021; light trap; leg. A. de Ketelaere; PCMCO.

Comments. A very rare species, reported only from Andorra, Bulgaria, Spain, and Russia: North Caucasus in Europe. First record from Albania.

37. *Molophilus* (*Molophilus*) *appendiculatus* (Staeger, 1840)

Material examined. Croatia • 1 male; Krapinsko-Zagorska county, Mountain Ivanščica, Spring Podbel; 46.20111°N, 16.25611°E; alt. 346 m; 15 July 2014; leg. M. Ivković; CKLP. **Slovenia** • 2 males; Vintgar gorge; 46.39333°N, 14.086056°E; alt. 600 m; 1 July 2022; leg C. Quindroit; PCCQ.

Comments. Common species that occurs in several terrestrial and semi-aquatic habitats.

38. *Molophilus* (*Molophilus*) *bifidus* Goetghebuer, 1920

Material examined. Albania • 3 males; Dibër, Fushë-Bulqizë; 41.5279°N, 20.2983°E; alt. 780 m; 26 July 2021; light trap; leg. A. de Ketelaere; PCMCO.

Comments. A common species, associated with springs and small headwaters.

39. *Molophilus* (*Molophilus*) *corniger* de Meijere, 1920

Material examined. Croatia • 1 male; Ličko-Senjska county, Plitvički Ljeskovac, Tufa barrier Labudovac, Plitvice Lakes; 44.87138°N, 15.59972°E; alt. 630 m; 30 June 2014; emergence trap, P4; leg. M. Ivković; CKLP. Slovenia • 1 male; Savinjska, Ljubno ob Savinji; 46.332°N, 14.839°E; alt. 490 m; 23 July 2022; leg. M.C. de Haas; PCMCO.

Comments. Common European species; however, these are the first records from Croatia and Slovenia.

40. *Molophilus* (*Molophilus*) *crassipygus* de Meijere, 1918

Material examined. Bosnia and Herzegovina • 1 male; Ulog, Neretva at Ulog Camp site; 43.41714°N, 18.31205°E; alt. 650 m; 28 June 2022; leg. W. Graf; CKLP.

Comments. Another relatively common European species, which is recently reported from several countries from the Balkan (Kolcsár et al. 2015b, 2021). Here we report it for the first time from Bosnia and Herzegovina.

41. *Molophilus* (*Molophilus*) *lackschewitzianus* Alexander, 1953

Material examined. Slovenia • 3 males; Vintgar gorge; 46.39333°N, 14.086056°E; alt. 600 m; 1 July 2022; leg C. Quindroit; PCCQ.

Comments. A relatively rare species, associated with calcareous habitats (Stubbs 2021), but rarely it can also be found around non-calcareous streams (Kolcsár and Soltész 2018).

42. *Molophilus* (*Molophilus*) *medius* de Meijere, 1918

Material examined. Slovenia • 3 males, 1 female; Savinjska, Ljubno ob Savinji; 46.332°N, 14.839°E; alt. 490 m; 8 August 2020; light trap; leg. M.C. de Haas; PCMCO • 1 male; Savinjska, Ljubno ob Savinji; 46.332°N, 14.839°E; alt. 490 m; 22 July 2022; light trap; leg. M.C. de Haas; PCMCO.

Comments. A common and widespread species in Europe, here reported for the first time from Slovenia.

43. *Molophilus* (*Molophilus*) *propinquus* (Egger, 1863)

Material examined. Bosnia and Herzegovina • 1 male; Ulog, Ulog on Neretva River; 43.42414°N, 18.30837°E; alt. 640 m; 29 June 2022; leg. W. Graf; CKLP.

Comments. Common and usually abundant species around wet habitats, especially along rivers and streams.

44. *Molophilus* (*Molophilus*) *pullus* Lackschewitz, 1927
Fig. 16

Material examined. Croatia • 1 male, 2 females; Zagreb, Stream Jelenovac; 45.82306°N, 15.95235°E; alt. 170 m; 10 April 2020; leg. M. Ivković; CKLP.

Comments. A rarer *Molophilus* species, known only from few European countries. First record from Croatia. Adults usually found around small muddy springs and slowly flowing streams with rich organic muds.

45. *Neolimonia dumetorum* (Meigen, 1804)

Material examined. Croatia • 2 females; Ličko-Senjska county, near Baške Oštarije, Velebit NP; 44.545°N, 15.152°E; alt. 980 m; 30 July 2021; sweep net; leg. M.C. d'Oliveira; PCMCO. Slovenia • 1 male; Gorenjska, near Spominski Park; 46.336°N, 14.573°E; alt. 730 m; leg. M.C. de Haas; PCMCO.

Comments. A common species.

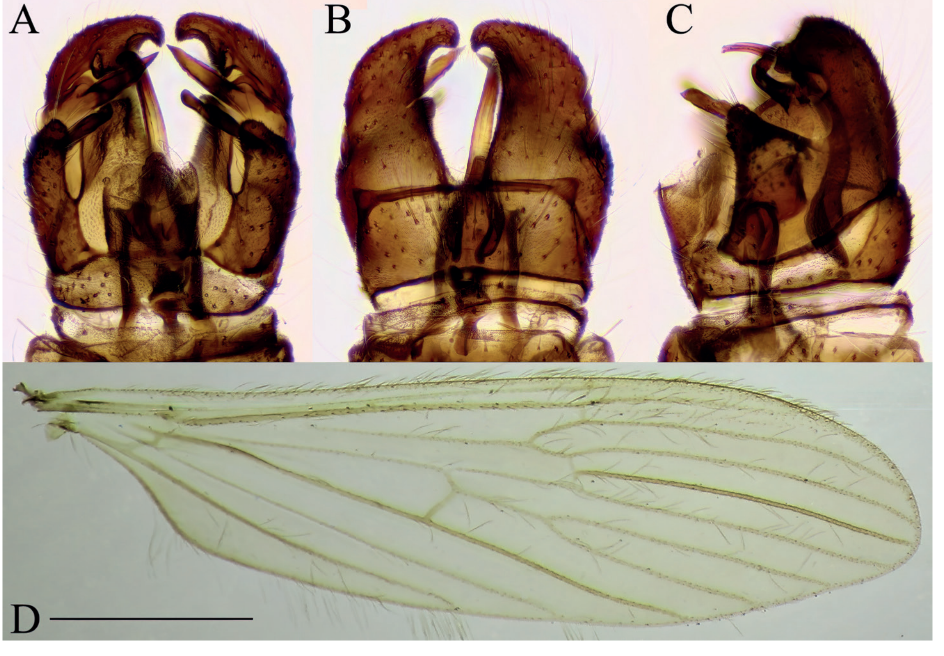


Figure 16. *Molophilus* (*Molophilus*) *pullus*, specimen: Croatia, Zagreb, stream Jelenovac (CKLP) **A–C** male terminalia **A** dorsal view **B** ventral view **C** lateral view **D** wing. Scale bar: 1 mm (**D**).

46. *Orimarga* (*Orimarga*) *juvenilis* (Zetterstedt, 1851)

Material examined. Slovenia • 1 male, 1 female; Gorenjska, Kranjska Gora, Gozd Maturljek, in small woodland near river Sava; in small woodland on the banks of river Sava; 46.483°N, 13.838°E; alt. 740 m; 20 July 2019; light trap; leg. M.C. d'Oliveira; PCMCO.

Comments. A rare species, known from few European countries. *Orimarga* species are associated with hygropetric calcareous habitats. First record from Slovenia.

47. *Orimarga* (*Orimarga*) *virgo* (Zetterstedt, 1851)

Material examined. Slovenia • 1 male; Vintgar gorge; 46.393333°N, 14.086056°E; alt. 600 m; 1 July 2022; leg C.Quindroit; PCCQ.

Comments. Another rare *Orimarga* species.

48. *Ormosia* (*Ormosia*) *albitibia* Edwards, 1921

Material examined. Slovenia • 1 male; Krma Valley; 46.370556°N, 13.88916°E; in low alder tree area, in a rockslide; alt. 900 m; 6 July 2022; leg. C. Quindroit; PCCQ.

Comments. A rare species, usually found around mountain streams in Central Europe. First record from Slovenia.

49. *Ormosia* (*Ormosia*) *lineata* (Meigen, 1804)
Fig. 17

Material examined. Croatia • 1 male, 1 female; Zagreb, Stream Jelenovac; 45.82306°N, 15.95235°E; alt. 170 m; 10 April 2020; leg. M. Ivković; CKLP.

Comments. A common early spring species usually found around muddy, sandy springs and smaller streams. First record from Croatia.

50. *Paradelphomyia fuscula* (Loew, 1873)

Material examined. Croatia • 1 male; Ličko-Senjska county, Plitvički Ljeskovac, Tufa barrier Labudovac, Plitvice Lakes; 44.87138°N, 15.59972°E; alt. 630 m; 30 June 2021; emergence trap, P7; leg. M. Ivković; CKLP.

Comments. A species associated with various aquatic and semi-aquatic habitats. First record from Croatia.

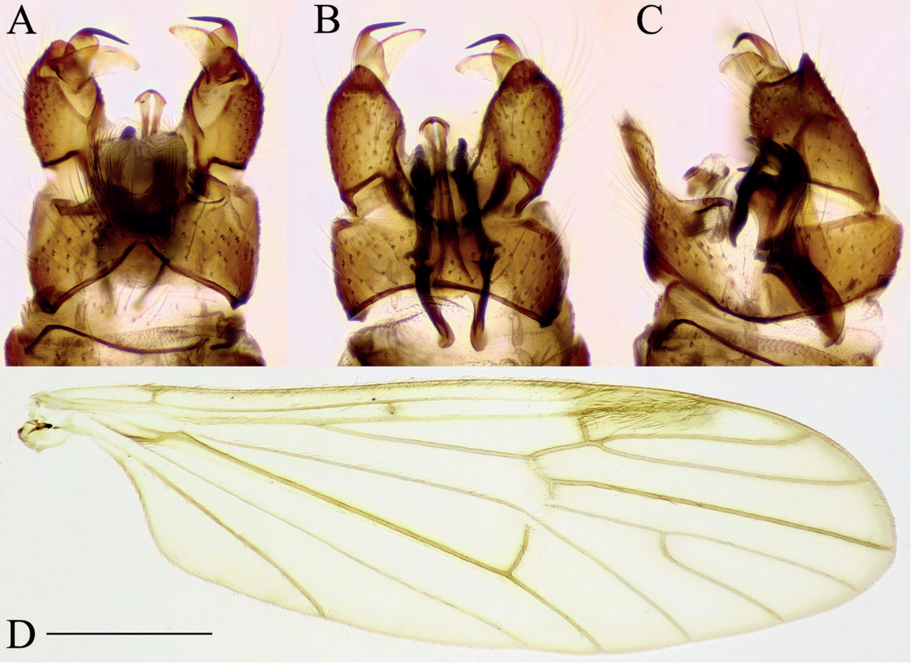


Figure 17. *Ormosia* (*Ormosia*) *lineata*, Specimen: Zagreb, stream Jelenovac (CKLP) **A–C** male terminalia **A** dorsal view **B** ventral view **C** lateral view **D** wing. Scale bar: 1 mm (**D**).

51. *Paradelphomyia senilis* (Haliday, 1833)

Material examined. Croatia • 1 male; Ličko-Senjska county, Plitvički Ljeskovac, Tufa barrier Labudovac, Plitvice Lakes; 44.87138°N, 15.59972°E; alt. 630 m; 30 June 2015; emergence trap, P6; leg. M. Ivković; CKLP.

Comments. A widespread species in Europe.

52. *Prionolabis hospes* (Egger, 1863)

Material examined. Slovenia • 1 male; Vintgar gorge; 46.393333°N, 14.086056°E; alt. 600 m; 1 July 2022; leg C. Quindroit; PCCQ.

Comments. A common species associated with deciduous forests.

53. *Symplecta* (*Psiloconopa*) *stictica stictica* (Meigen, 1818)

Material examined. Albania • 1 female; Shkodër, Lëpushë; 42.5291°N, 19.72654°E; alt. 1290 m; 3 August 2021; leg. A. de Ketelaere; PCMCO.

Comments. A common and widespread species, occurs in different semi-aquatic habitats, also tolerant of salty soils.

54. *Thaumastoptera* (*Thaumastoptera*) *calceata* Mik, 1866

Material examined. **Bosnia and Herzegovina** • 1 male; Ulog, Ulog on Neretva River; 43.42414°N, 18.30837°E; alt. 640 m; 29 June 2022; leg. W. Graf; CKLP. **Croatia** • 1 male; Ličko-Senjska county, Rastovača, Tufa barrier Kozjak-Milanovac, Plitvice Lakes; 44.89416°N, 15.60888°E; alt. 545 m; 30 June 2015; emergence trap, P5 • 1 male; same locality; 30 June 2019; emergence trap, P1; leg. M. Ivković; CKLP.

Comments. A relative rare and tiny species, associated with calcareous rivers and streams usually with sandy banks. First records from Croatia and Bosnia and Herzegovina.

Pediciidae

55. *Dicranota* (*Paradicranota*) *subtilis* Loew, 1871
Fig. 18

Material examined. **Croatia** • 1 male; Ličko-Senjska county, Končarev Kraj, Spring of Bijela rijeka, Plitvice Lakes; 44.83472°N, 15.56194°E; alt. 720 m; 28 April 2017; emergence trap, P5 • 1 male; same locality; 29 October 2020; emergence trap, P5 • 1 male; same locality; 28 October 2021; emergence trap, P1; leg. M. Ivković; CKLP.

Comments. One of the most common *Dicranota* species in Europe, adults frequently collected around different types of small streams and mountain rivers. First records from Croatia.

56. *Pedicia* (*Amalopis*) *occulta* (Meigen, 1830)

Material examined. **Croatia** • 1 female; Ličko-Senjska county, Končarev Kraj, Spring of Bijela rijeka, Plitvice Lakes; 44.83472°N, 15.56194°E; alt. 720 m; 31 March 2014; emergence trap, P5 • 2 males, 2 females; same locality; 30 April 2014; emergence trap, P5 • 2 females; same locality; 30 April 2014; emergence trap, P6 • 1 female; same locality; 29 May 2014; emergence trap, P4 • 1 male; same locality; 30 June 2014; emergence trap, P2 • 1 male; same locality; 30 September 2014; emergence trap, P2 • 1 male; same locality; 29 April 2015; emergence trap, P4 • 1 female; same locality; 29 April 2015; emergence trap, P5 • 1 female; same locality; 30 September 2015; emergence trap, P3 • 1 male; same locality; 31 March 2016; emergence trap, P3 • 1 female; same locality; 31 May 2016; emergence trap, P3 • 1 male; same locality; 31 May 2016; emergence trap, P6 • 1 female; same locality; 31 August 2016; emergence trap, P3 • 1 male, 1 female; same locality; 31 October 2016; emergence trap, P3 • 1 male; same locality; 29 March 2017; emergence trap, P4 • 2 females; same locality; 28 April 2017; emergence trap, P4

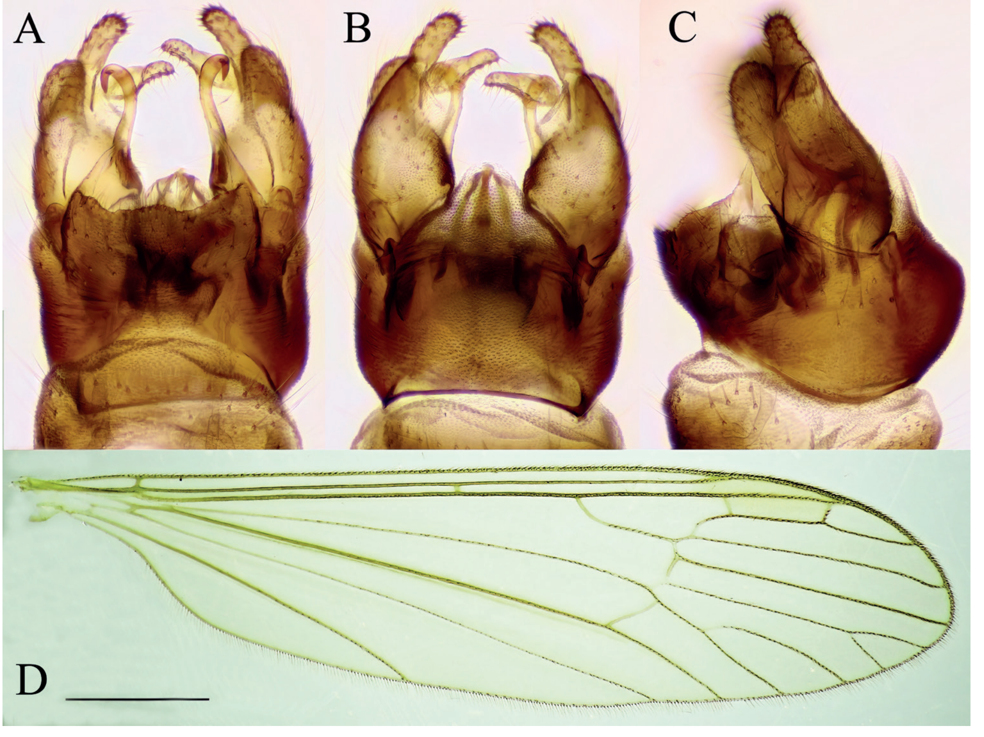


Figure 18. *Dicranota* (*Paradicranota*) *subtilis*, Specimen: Croatia, spring of Bijela rijeka, Plitvice Lakes (CKLP) **A–C** male terminalia **A** dorsal view **B** ventral view **C** lateral view **D** wing. Scale bar: 1 mm (**D**).

• 1 male; same locality; 28 June 2017; emergence trap, P4 • 1 male; same locality; 27 July 2017; emergence trap, P4 • 1 male; same locality; 27 July 2017; emergence trap, P3 • 1 male; same locality; 30 August 2017; emergence trap, P4 • 1 female; same locality; 28 October 2017; emergence trap, P5 • 1 female; same locality; 28 October 2017; emergence trap, P6 • 1 female; same locality; 29 November 2017; emergence trap, P3 • 1 female; same locality; 29 November 2017; emergence trap, P6 • 1 female; same locality; 30 March 2018; emergence trap, P6 • 1 male, 1 female; same locality; 30 April 2018; emergence trap, P6 • 1 male; same locality; 29 June 2018; emergence trap, P5 • 1 female; same locality; 29 June 2018; emergence trap, P6 • 1 male; same locality; 26 July 2018; emergence trap, P6 • 1 female; same locality; 28 May 2019; emergence trap, P5 • 1 female; same locality; 27 July 2019; emergence trap, P4 • 1 male; same locality; 30 April 2020; emergence trap, P5 • 1 female; same locality; 28 May 2020; emergence trap, P6 • 3 males, 3 females; same locality; 29 October 2020; emergence trap, P3 • 1 male; same locality; 28 February 2021; emergence trap, P3 • 1 male; same locality; 30 March 2021; emergence trap, P3 • 1 female; same locality; 29 April 2021; emergence trap, P3 • 1 male; same locality; 29 April 2021; emergence trap, P2 • 1 female; same locality; 29 April 2021; emergence trap, P5 • 1 male; same locality; 29 May 2021; emergence trap, P3 • 1 male, 1 female; same locality; 29 July 2021; emergence trap, P3 • 1 male; same locality; 29 July 2021; emergence trap, P6 • 1 female; same locality; 31 August 2021; emergence trap, P3 • 1 female; same locality; 31 August 2021; emergence trap, P6 • 1 male; same locality; 28 October 2021; emergence trap, P6 • 1 male; same locality; 28 October 2021; emergence trap, P3 • 1 female; same locality; 29 November 2021; emergence trap, P3; leg. M. Ivković; CKLP • 1 male, 1 female; Ličko-Senjska county, Plitvica Selo, Stream Plitvica, Plitvice Lakes; 44.90222°N, 15.6075°E; alt. 555 m; 30 September 2008; emergence trap, P3 • 5 males, 2 females; same locality; 30 September 2008; emergence trap, P2 • 1 female; same locality; 30 October 2008; emergence trap, P2; leg. M. Ivković; UZC.

Comments. A widely distributed species, one of the most common and abundant species occurring together with *Dicranomyia didyma* in samples collected with emergence traps at Plitvice Lakes.

57. *Pedicia* (*Crunobia*) *littoralis* (Meigen, 1804)

Material examined. Slovenia • 1 male; Kobarid, Kozjak waterfall; 46.259139°N, 13.590056°E; alt. 360 m; 9 July 2022; leg C. Quindroit; PCCQ.

Comments. A widely distributed *Crunobia* species, can be found around small, cold-water streams. The males swarm above littoral vegetation.

58. *Pedicia (Crunobia) straminea* (Meigen, 1838)

Material examined. Slovenia • 1 male; Vintgar gorge; 46.393333°N, 14.086056°E; alt. 600 m; 1 July 2022; leg C. Quindroit; PCCQ.

Comments. Another common and widely distributed *Crunobia* species, can be found throughout a wider range of habitats than *P. littoralis*, including springs and small, cold-water rivers.

59. *Pedicia* (*Pedicia*) *rivosa* (Linnaeus, 1758)

Material examined. Croatia • 1 female; Ličko-Senjska county, Končarev Kraj, Spring of Bijela rijeka, Plitvice Lakes; 44.83472°N, 15.56194°E; alt. 720 m; 29 May 2018; emergence trap, P5 • 1 female; same locality; 28 June 2020; emergence trap, P1; leg. M. Ivković; CKLP.

Comments. A relative common and easily recognizable species. Larvae are aquatic and the species can be found around springs and streams, from lowlands to high mountains. Appears to be a very rare species in southern part of Europe and found only in humid mountains in the Western Balkans. Here we present the first records from Croatia.

60. *Tricyphona* (*Tricyphona*) *immaculata* (Meigen, 1804)

Material examined. Croatia • 1 male; Ličko-Senjska county, Končarev Kraj, Spring of Bijela rijeka, Plitvice Lakes; 44.83472°N, 15.56194°E; alt. 720 m; 03 January 2019; emergence trap, P4 • 2 males; same locality; 29 October 2020; emergence trap, P5 • 1 male; same locality; 28 October 2021; emergence trap, P1; leg. M. Ivković; CKLP • 1 male; Ličko-Senjska county, Plitvički Ljeskovac, Tufa barrier Labudovac, Plitvice Lakes; 44.87138°N, 15.59972°E; alt. 630 m; 29 October 2020; emergence trap, P3 • 1 male; same locality; 29 September 2021; emergence trap, P6; leg. M. Ivković; UZC.

Comments. A relative common and wide distributed species, associated with different types of aquatic and semi-aquatic habitats.

Tipulidae

61. *Ctenophora* (*Cnemoncosis*) *ornata* Meigen & Wiedemann, 1818

Material examined. Croatia • 1 female; Zadarska county, Benkovac, Pristeg; 43.96214°N, 15.62876°E; alt. 170 m; 01 April 2021; leg. T. Dražina; CKLP.

Comments. Conspicuous species associated with old decaying standing wood.

62. *Nephrotoma appendiculata appendiculata* (Pierre, 1919)

Material examined. Croatia • 1 male, 1 female; Zagrebačka county, Kuče, Chanel Sava Odra; 45.6725°N, 16.135278°E; alt. 98 m; 14 April 2011; leg. M. Ivković; CKLP.

Comments. A very common species.

63. *Nephrotoma cornicina cornicina* (Linnaeus, 1758)

Material examined. Slovenia • 1 male; Češnjica; 46.296667°N, 13.948917°E; alt. 600 m; 1 July 2022; leg. C. Quindroit; PCCQ.

Comments. A very common species.

64. *Nephrotoma flavescens* (Linnaeus, 1758)

Material examined. Bosnia and Herzegovina • 1 male; Ulog, Ulog on Neretva River; 43.42414°N, 18.30837°E; alt. 640 m; 29 June 2022; leg. W. Graf; CKLP.

Comments. A common species.

65. *Nephrotoma lamellata lamellata* (Riedel, 1910)

Material examined. Slovenia • 1 male; Češnjica; 46.296667°N, 13.948917°E; alt. 600 m; 1 July 2022; leg. C. Quindroit; PCCQ.

Comments. A less common *Nephrotoma* species.

66. *Nephrotoma quadrifaria quadrifaria* (Meigen, 1804)

Material examined. Bosnia and Herzegovina • 1 female; Cerova, Cerova on Neretva; 43.37887°N, 18.35621°E; alt. 695 m; 30 June 2022; leg. M. Ivković; CKLP.

Comments. A common species.

67. *Tipula* (*Acutipula*) *balcanica* Vermoolen, 1983

Material examined. Croatia • 1 male; Ličko-Senjska county, Plitvički Ljeskovac, Tufa barrier Labudovac, Plitvice Lakes; 44.87138°N, 15.59972°E; alt. 630 m; 30 June 2019; emergence trap, P6; leg. M. Ivković; CKLP.

Comments. A relatively common and conspicuous species in the Balkans and some neighboring countries.

68. *Tipula* (*Lunatipula*) *fascipennis* Meigen, 1818

Material examined. Bosnia and Herzegovina • 1 male; Ulog, Ulog on Neretva River; 43.42414°N, 18.30837°E; alt. 640 m; 29 June 2022; leg. W. Graf; CKLP.

Comments. A common species.

69. *Tipula* (*Lunatipula*) *helvola* Loew, 1873

Material examined. Slovenia • 1 male; Ljubljana, Castle; 46.048°N, 14.509778°E; alt. 340 m; 30 June 2022; leg. C. Quindroit; PCCQ.

Comments. A common species.

70. *Tipula* (*Lunatipula*) *truncata* Loew, 1873

Material examined. Bosnia and Herzegovina • 1 male, 2 females; Ulog, Ulog on Neretva River; 43.42414°N, 18.30837°E; alt. 640 m; 29 June 2022; leg. W. Graf; CKLP.

Comments. A common species in central and southeastern Europe, prefers drier habitats.

71. *Tipula* (*Pterelachisus*) *glacialis* (Pokorny, 1887)

Material examined. **Slovenia** • 1 male; Krnica; 46.37333°N, 13.862528°E; alt. 2000 m; 6 July 2022; leg. C. Quindroit; PCCQ • 2 males; Dom Planika; 46.371417°N, 13.846417°E; alt. 2400 m; 6 July 2022; leg. C. Quindroit; PCCQ • 1 male; Rudno Polje, Sreniski preval; 46.360889°N, 13.894472°E; alt. 1900 m; 3 July 2022; leg. C. Quindroit; PCCQ.

Comments. A rare alpine species, known only from the Alps, Dinaric Alps, and Rila-Rhodope massif.

72. *Tipula* (*Savtshenkia*) *benesignata* Mannheims, 1954

Material examined. **Croatia** • 1 male; Ličko-Senjska county, Rastovača, Tufa barrier Kozjak-Milanovac, Plitvice Lakes; 44.89416°N, 15.60888°E; alt. 545 m; 28 September 2020; emergence trap, P1; leg. M. Ivković; CKLP

Comments. A relative common autumnal species.

73. *Tipula* (*Savtshenkia*) *cheethami* Edwards, 1924

Material examined. **Croatia** • 2 males; Ličko-Senjska county, Končarev Kraj, Spring of Bijela rijeka, Plitvice Lakes; 44.83472°N, 15.56194°E; alt. 720 m; 29 May 2018; emergence trap, P5 • 1 male; same locality; 29 May 2018; emergence trap, P5 • 1 male; same locality; 29 May 2021; emergence trap, P6; leg. M. Ivković; CKLP • 1 male; Ličko-Senjska county, Plitvički Ljeskovac, Spring of Crna rijeka, Plitvice Lakes; 44.83714°N, 15.60752°E; alt. 680 m; 29 May 2021; emergence trap, P6; leg. M. Ivković; UZC.

Comments. A rare, late spring species, associated with wet, mossy habitats along rivers and streams. First records from Croatia.

74. *Tipula* (*Savtshenkia*) *rufina rufina* Meigen, 1818

Material examined. **Croatia** • 1 male; Ličko-Senjska county, Končarev Kraj, Spring of Bijela rijeka, Plitvice Lakes; 44.83472°N, 15.56194°E; alt. 720 m; 31 October 2016; emergence trap, P6 • 2 males; same locality; 30 September 2019; emergence trap, P5 • 3 males, 1 female; same locality; 29 September 2021; emergence trap, P6; leg. M. Ivković; CKLP • 1 male; Ličko-Senjska county, Rastovača, Tufa barrier Kozjak-Milanovac, Plitvice Lakes; 44.89416°N, 15.60888°E; alt. 545 m; 28 September 2020; emergence trap, P3 • 1 female; same locality; 29 October 2020; emergence trap, P5; leg. M. Ivković; UZC.

Comments. A common and widespread species, known from different habitats.

75. *Tipula* (*Vestiplex*) *hortorum* Linnaeus, 1758

Material examined. Croatia • 1 female; Ličko-Senjska county, Končarev Kraj, Spring of Bijela rijeka, Plitvice Lakes; 44.83472°N, 15.56194°E; alt. 720 m; 28 June 2017; emergence trap, P5; leg. M. Ivković; CKLP • 1 male; Grad Zagreb County, Zagreb, Stream Jelenovac; 45.82306°N, 15.95235°E; alt. 170 m; 10 April 2020; leg. M. Ivković; CKLP.

Comments. A common species, usually found in wet forest habitats.

76. *Tipula* (*Vestiplex*) *excisa excisa* Schummel, 1833

Material examined. Slovenia • 1 male; Krnica; 46.373333°N, 13.862528°E; alt. 2000 m; 6 July 2022; leg. C. Quindroit; PCCQ.

Comments. A mountain alpine species.

77. *Tipula* (*Yamatotipula*) *lateralis* Meigen, 1804

Material examined. Bosnia and Herzegovina • 1 male; Ulog, Ulog on Neretva River; 43.42414°N, 18.30837°E; alt. 640 m; 29 June 2022; leg. W. Graf; CKLP.

Comments. A very common aquatic/semi-aquatic species.

Discussion

As many other publications have previously demonstrated, the biodiversity of the Balkans remains insufficiently investigated, and the local fauna consists of far more species than what is currently reported (e.g., Ivković et al. 2013; Kvifte et al. 2013; Pont and Ivković 2013; Ivković and Plant 2015; Ivković and Pont 2015; Ivković et al. 2016; Kvifte and Ivković 2018; Keresztes et al. 2018b). Despite historical and more recent publications (Bilalli et al. 2021; de Jong et al. 2021; Kolcsár et al. 2015a, b, 2017a, b, 2018a, b, 2023; Keresztes et al. 2018a, b; Starý 2012) the cranefly fauna of the Western Balkans is still poorly known. In this study we report two species for the first time from Albania, eight from Bosnia and Herzegovina, twelve from Croatia, and seven from Slovenia. Most of the newly reported species are common and widely distributed in Europe, highlighting how understudied the local fauna is. However, some rare and poorly known species such as *Antocha alpigena*, *Ellipteroides limbatus*, *Idiocera jucunda*, *I. lackschewitzi*, and *Orimarga juvenilis* etc. were also collected, primarily around natural streams and rivers. Furthermore, a new species, *Baeoura neretvaensis* sp. nov. is described. *Baeoura* is considered as a rare group in the Western Palaearctic, with a distribution mainly restricted to the Mediterranean region (Fig. 5). Besides the original species descriptions (Kuntze 1914; Mendl and Tjeder 1974, 1976; Starý 1981; Krzemiński and Starý 1984; Mendl 1985, 1986; Driauach and Belqat 2015; Hancock 2020) only few additional faunistic records are known from the Western Palearctic (Erhan-Dincă 1984; Simova-Tosic 1992; Starý and Oosterbroek 1996; Koç 2004; Ujvárosi 2005; Starý 2014; Oosterbroek et al. 2020; Kolcsár et al. 2021).

The biology and ecology of *Baeoura* species are poorly known or even unknown. Larvae and pupae of the South African species *B. claripennis* (Alexander, 1921) were found on the surface of smaller boulders, ~ 30 cm deep in a swiftly flowing stream and the imago was collected from vegetation along the stream (Wood 1952). Adults of *B. malickyi*, *B. schachti* Mendl, 1986, and *B. armata* Mendl, 1985 were collected around small streams with clear water (Mendl and Tjeder 1976; Mendl 1985, 1986; Kolcsár et al. 2021). *Baeoura rotherayi* and *B. szadziewskii* Krzemiński & Starý, 1984 were also collected along streams (Krzemiński and Starý 1984; Hancock 2020). Driauach and Belqat (2015) collected *B. ebenina* Starý, 1981 and *B. staryi* along partly dried out rivers, with minimal water flow in Morocco. Adults of *B. alexanderi* Mendl & Tjeder, 1974 in Crete and *B. longefiligera* Mendl, 1986 in Spain were found in dry valleys of very small streams (Mendl and Tjeder 1974; Mendl 1986). It is assumed that larvae of Western Palaearctic species are also aquatic or semi-aquatic (Mendl and Tjeder 1976; Erhan-Dincă 1984). Adults of *B. neretvaensis* sp. nov. were collected around fast flowing gravelly/rocky streams and rivers (Fig. 4). *Baeoura malickyi* and *B. neretvaensis* sp. nov. were collected from the same habitat at the same time along Sava River in Slovenia (Fig. 5, see also Kolcsár et al. 2021). Based on the above-mentioned observations, the European *Baeoura* species are rheobiont, larvae most probably occurring in fast flowing streams and rivers. Some species seem to be adapted to fluctuating water conditions, tolerating the partly or fully drying up of streams and rivers during the dry periods in the Mediterranean area. The adults migrate farther from the larval habitats along valleys. Based on literature data and our observations, *Baeoura* species are attracted by light sources and surveillance traps could be effective methods to collect these small-sized and poorly known species.

Conclusions

At this time of intensified climate change, that causes streams and rivers to dry up, and the general increasing anthropogenic influences on freshwater habitats, we stipulate that it is of utmost importance to protect the streams and rivers as much as we possibly can. The Western Balkans is a part of Europe with still relatively little human impact and therefore our efforts on exploring and studying this area need to be much more than they are at present. Only by increasing our knowledge of the diversity of this area can we help to protect it more thoroughly. It truly deserves to be protected, so it can remain the Blue Heart of Europe.

Acknowledgements

We are grateful to Herman de Jong and to Pavel Starkevič for their valuable comments on the manuscript. We are grateful to Plitvice Lakes National Park for giving us access and helping us during the last 16 years of research conducted there. We would like to thank Tvrtko Dražina for collecting one of the samples. We are grateful to "Scientists for the Balkan Rivers" initiative that arranged sampling of the Neretva River. We are thankful to Jaroslav Starý (Olomouc, Czech Republic) who confirmed the identification of some specimens. We also thank to Roháček Jindřich (Silesian Museum, Opava, Czech Republic) for his help with deposition of types in the Silesian Museum, Czech Republic. This study was supported by the International Research Fellow of the Japan Society for the Promotion of Science (JSPS), grant numbers: P21094 to L.-P. Kolcsár and project "Phenology of aquatic Insects" (106-F19-00081).

References

Andersen T, Baranov V, Hagenlund LK, Ivković M, Kvifte GM, Pavlek M (2016) Blind Flight? A New Troglobiotic *Orthoclad* (Diptera, Chironomidae) from the Lukina Jama – Trojama Cave in Croatia. PLoS ONE 11(4): e0152884. https://doi.org/10.1371/journal.pone.0152884

Bănărescu PM (2004) Distribution pattern of the aquatic fauna of the Balkan Peninsula. In: Griffiths HI, Kryštufek B, Reed JM (Eds) Balkan Biodiversity Pattern and Process in the European Tspot. Kluwer Academic Publishers, Dordrecht, The Netherlands, 203–219. https://doi.org/10.1007/978-1-4020-2854-0_12

Bilalli A, Ibrahimi H, Musliu M, Grapci-Kotori L, Geci D, Slavevska-Stamenkovic V, Hinic J, Mitic-Kopanja D, Keresztes L (2021) New records of the craneflies (Diptera: Limoniidae, Tipulidae) from the Western Balkans. Journal of Entomological Research Society 23(23): 141–152.

Cumming JF, Wood DM (2017) Adult morphology and terminology. In: Kirk-Spriggs AH, Sinclair BJ (Eds) Manual of Afrotropical Diptera (Vol. 1). Introductory Chapters and Keys to Diptera Families. Suricata (Vol. 4). South African National Biodiversity Institute, Pretoria, 89–133.

de Jong H, Adghir A, Bosch EJ, Kettani K (2021) Taxonomy of *Nephrotoma guestfalica* (Westhoff, 1879) (Diptera, Tipulidae), with the description of a new subspecies from Morocco. Tijdschrift voor Entomologie 163(1): 31–45. https://doi.org/10.1163/22119434-20192086

Driauach O, Belqat B (2015) A new species of the genus *Baeoura* from Morocco, with a key to the West Palaearctic species (Diptera, Tipuloidea, Limoniidae). ZooKeys 532: 99–105. https://doi.org/10.3897/zookeys.532.5994

Erhan-Dincă E (1984) Some new or little known species of the Limoniidae (Diptera-Nematocera) fauna of Romania. Travaux du Museum National d'Histoire Naturelle "Grigore Antipa" / Muzeul National de Istoire Naturala "Grigore Antipa" 26: 163–183.

Evenhuis NL, Pape T (2022) Systema Dipterorum, Version 3.11. http://diptera.org/ [Accessed 15 November 2022]

Gaston KJ, David R (1994) Hotspots across Europe. Biodiversity Letters 2(4): 108–116. https://doi.org/10.2307/2999714

Griffiths HI, Kryštufek B, Reed JM (2004) Balkan Biodiversity. Pattern and Process in the European hotspot. Kluwer Academic Publishers, Dordrecht. [In press] https://doi.org/10.1007/978-1-4020-2854-0

Hancock EG (2020) Some cranefly records from Cazorla National Park, Jaen Province, Spain with description of a new species of *Baeoura* (Diptera, Tipuloidea). Dipterists Digest (2nd series) 27: 91–100.

Hancock EG, Hewitt SM, Godfrey A, Mullin M (2009) Thoracic spiracular gill structure of *Lipsothrix* (Diptera, Limoniidae) in Britain described from scanning electron micrographs. In: Lantsov V (Ed.) Craneflies. History, taxonomy and ecology (Diptera: Tipulidae, Limoniidae, Pediciidae, Trichoceridae, Ptychopteridae, Tanyderidae). Memorial volume dedicated to Dr. Charles Paul Alexander (1889–1981), Dr. Bernhard Mannheims (1909–1971) and Dr. Evgeniy Nikolaevich Savchenko (1909–1994). Zoosymposia 3: 77–87. https://doi.org/10.11646/zoosymposia.3.1.6

Ivković M, Plant A (2015) Aquatic insects in the Dinarides: Identifying hotspots of endemism and species richness shaped by geological and hydrological history using Empididae (Diptera). Insect Conservation and Diversity 8(4): 302–312. https://doi.org/10.1111/icad.12113

Ivković M, Pont AC (2015) New records of Muscidae (Diptera) from Mediterranean countries. ZooKeys 496: 131–144. https://doi.org/10.3897/zookeys.496.9445

Ivković M, Plant A, Horvat B (2012) A new species of *Wiedemannia* (Diptera: Empididae: Clinocerinae) from Balkan Peninsula. Zootaxa 3478(1): 581–585. https://doi.org/10.11646/zootaxa.3478.1.46

Ivković M, Gračan R, Horvat B (2013) Croatian aquatic dance flies (Diptera: Empididae: Clinocerinae and Hemerodromiinae): species diversity, distribution and relationship to surrounding countries. Zootaxa 3686(2): 255–276. https://doi.org/10.11646/zootaxa.3686.2.7

Ivković M, Kúdela M, Kúdelová T (2016) Blackflies (Diptera: Simuliidae) in Croatia: species richness, distribution and relationship to surrounding countries. Zootaxa 4109(1): 16–30. https://doi.org/10.11646/zootaxa.4109.1.2

Keresztes L, Kolcsár L-P, Dénes A-L, Török E (2018a) Revealing unknown larvae of the *maxima* species group of the genus *Acutipula* Alexander, 1924 (*Tipula*, Tipuloidae, Diptera) using an integrative approach. North-Western Journal of Zoology 14: 17–24.

Keresztes L, Menendez JM, Martin L, Török E, Kolcsár L-P (2018b) Description of a new species of *Mediotipula* from Albania, with consideration of the eastern Mediterranean as a diversity hotspot (Diptera, Tipulidae). ZooKeys 792: 99–115. https://doi.org/10.3897/zookeys.792.25683

Koç H (2004) Faunistic and investigation of ecological aspects of Tipulidae and Limoniidae families in Mugla province. Mugla Universitesi Yayinlari 55 (Rektorluk Yayinlari 35): [i–xviii] 1–154. [In Turkish with English abstract]

Kolcsár L-P, Soltész Z (2018) New records of Tipuloidea (Diptera: Insecta) from Hungary. Folia Entomologica Hungarica 79: 163–176. https://doi.org/10.17112/FoliaEntHung.2018.79.163

Kolcsár L-P, Ivković M, Ternjej I (2015a) New records of Limoniidae and Pediciidae (Diptera) from Croatia. ZooKeys 513: 23–37. https://doi.org/10.3897/zookeys.513.10066

Kolcsár L-P, Török E, Keresztes L (2015b) A new species and new records of *Molophilus* Curtis, 1833 (Diptera: Limoniidae) from the western Palaearctic region. Biodiversity Data Journal 3 (e5466): 1–10. https://doi.org/10.3897/BDJ.3.e5466

Kolcsár L-P, Soos A, Török E, Graf W, Rákosy L, Keresztes L (2017a) New faunistic records of the genus *Erioptera* Meigen (Limoniidae, Diptera, Insecta) from Europe. Entomologica Romanica 21: 23–44. https://doi.org/10.24193/entomolrom.21.4

Kolcsár L-P, Oláh T, Veres R, Török E, Keresztes L (2017b) New faunistic records of the genus *Limonia* Meigen (Limoniidae, Diptera, Insecta) from the Balkan Region. Entomologica Romanica 21: 45–59. https://doi.org/10.24193/entomolrom.21.5

Kolcsár L-P, Török E, Keresztes L (2018a) First record of *Cylindrotoma distinctissima* (Meigen, 1818) from Serbia and new data on the occurrence of Cylindrotomidae (Diptera) in Bulgaria and Romania. Fragmenta Faunistica 60(2): 107–112. https://doi.org/10.3161/00159301FF2017.60.2.107

Kolcsár L-P, Török E, Keresztes L (2018b) New faunistic records of Pediciidae (Diptera, Insecta) from Europe. Entomologica Romanica 22: 5–9. https://doi.org/10.24193/entomolrom.22.1

Kolcsár L-P, Oosterbroek P, Gavryushin DI, Olsen KM, Paramonov NM, Pilipenko VE, Starý J, Polevoi A, Lantsov VI, Eiroa E, Andersson M, Salmela J, Quindroit C, d'Oliveira MC, Hancock EG, Mederos J, Boardman P, Viitanen E, Watanabe K (2021) Contribution to the knowledge of Limoniidae (Diptera: Tipuloidea): first records of 244 species from various European countries. Biodiversity Data Journal 9(e67085): 1–247. https://doi.org/10.3897/BDJ.9.e67085

Kolcsár L-P, Oosterbroek P, Olsen KM, Paramonov NM, Gavryushin DI, Pilipenko VE, Polevoi AV, Eiroa E, Andersson M, Dufour C, Syratt M, Kurina O, Lindström M, Starý J, Lantsov VI, Wiedeńska J, Pape T, Friman M, Peeters K, Gritsch W, Salmela J, Viitanen E, Aristophanous M, Janević D, Watanabe K (2023) Contribution to the Knowledge of Cylindrotomidae, Pediciidae and Tipulidae (Diptera: Tipuloidea): First Records of 86 Species from Various European Countries. Diversity 15(3): e336. https://doi.org/10.3390/d15030336

Krzemiński W, Starý J (1984) A new species of *Baeoura* Alexander, 1924 (Diptera, Limoniidae), from Algeria. Polskie Pismo Entomologiczne 54: 359–361.

Kuntze A (1914) Bestimmungstabellen der paläarktischen Eriopterinen (Diptera, Nematocera, Polyneura). Annalen des Naturhistorischen Museums in Wien 28: 361–388.

Kvifte GM, Ivković M (2018) New species and records of the *Pericoma trifasciata* group from Croatia (Diptera: Psychodidae). Zootaxa 4486: 076–082. https://doi.org/10.11646/zootaxa.4486.1.5

Kvifte GM, Ivković M, Klarić A (2013) New records of moth flies (Diptera: Psychodidae) from Croatia, with the description of *Berdeniella keroveci* sp. nov. Zootaxa 3737: 57–67. https://doi.org/10.11646/zootaxa.3737.1.4

Mendl H (1985) Eine neue *Baeoura*-Art aus dem Mittelmeergebiet (Diptera Nematocera: Limoniidae). Articulata 2: 196–198.

Mendl H (1986) Zwei neue *Baeoura*-Arten aus der Westpalaarktis (Diptera Nematocera: Limoniidae). Articulata 2: 293–298.

Mendl H, Tjeder B (1974) A new species of *Baeoura* from the island of Crete (Diptera, Tipulidae). Entomologica Scandinavica 5(3–4): 247–250. https://doi.org/10.1163/187631274X00281

Mendl H, Tjeder B (1976) *Baeoura malickyi* n. sp. from the Greek mainland and the island of Rhodos (Diptera: Tipulidae). Entomologica Scandinavica 7(3): 237–238. https://doi.org/10.1163/187631276X00397

Mihevc A, Prelovšek M, Zupan Hajna N (2010) Introduction to the Dinaric Karst. Karst Research Institute at Research Centre of the Slovenian Academy of Sciences and Arts, Postojna, Slovenia. https://doi.org/10.3986/9789612541989

Oosterbroek P (2023) Catalogue of the Craneflies of the World (Insecta, Diptera, Nematocera, Tipuloidea). https://ccw.naturalis.nl/index.php [Accessed on 14 February 2023]

Oosterbroek P, Starý J, Andrade R, Hancock GE, Ferreira S (2020) The craneflies of continental Portugal (Diptera, Limoniidae, Pediciidae, Tipulidae) including 28 species new for Portugal. Boletin de la Asociacion Espanola de Entomologia 44: 317–358.

Podeniene V (2009) Lithuanian Chioneinae (Limoniidae, Diptera): Larval habitat preferences and problems of identification, with description of last instar larvae of *Molophilus* (*Molophilus*) *crassipygus* de Meijere, 1918, *M.* (*M.*) *griseus* (Meigen, 1804), *M.* (*M.*) *ochraceus* (Meigen, 1818), *M.* (*M.*) *propinquus* (Egger, 1863). Lauterbornia 68: 135–145.

Pont AC, Ivković M (2013) The hunter-flies of Croatia (Diptera: Muscidae, genus *Limnophora* Robineau-Desvoidy). Journal of Natural History 47(15–16): 1069–1082. https://doi.org/10.1080/00222933.2012.750775

Ribeiro GC (2008) Phylogeny of the Limnophilinae (Limoniidae) and early evolution of the Tipulomorpha (Diptera). Invertebrate Systematics 22(6): 627–694. https://doi.org/10.1071/IS08017

Salmela J (2008) Semiaquatic fly (Diptera, Nematocera) fauna of fens, springs, headwater streams and alpine wetlands in the northern boreal ecoregion, Finland. W-album 6: 1–63.

Simova-Tosic D (1992) Contribution to the study of Limoniidae in Yugoslavia. Acta Zoologica Cracoviensia 35: 139–146.

Sket B, Paragamian K, Trontelj P (2004) A census of the obligate subterranean fauna of the Balkan Peninsula. In: Griffiths HI, Krystufek B, Reed JM (Eds) Balkan Biodiversity Pattern and Process in the European Hotspot. Kluwer Academic Publishers, Dordrecht, 309–322. https://doi.org/10.1007/978-1-4020-2854-0_18

Starý J (1981) *Baeoura directa* (Kuntze, 1914), with the description of a new species from Spain (Diptera, Limoniidae). Reichenbachia 19: 97–103.

Starý J (2009) Limoniidae. In: Rohacek J, Sevcik J (Eds) Diptera of Polana Protected Landscape Area / Biosphere Reserve (Central Slovakia). SNC SR, Administration of the PLA / BR Polana, Zvolen, 23–39.

Starý J (2012) The identity of *Geranomyia bezzii* and a description of *G. fuscior* sp. nov. (Diptera: Limoniidae). Acta Entomologica Musei Nationalis Pragae 52: 273–279.

Starý J (2014) Some records of Limoniidae and Pediciidae (Diptera) from Portugal and Spain. Acta Musei Silesiae. Scientiae Naturales 63(1): 83–95. https://doi.org/10.2478/cszma-2014-0010

Starý J, Oosterbroek P (1996) Review of the Limoniidae and Pediciidae of Italy (Diptera, Tipuloidea). Fragmenta Entomologica, Roma 28: 51–95.

Starý J, Stubbs AE (2015) Five species under *Dicranomyia* (*Dicranomyia*) *mitis* (Meigen, 1830) (Diptera, Limoniidae). Zootaxa 3964(3): 321–334. https://doi.org/10.11646/zootaxa.3964.3.2

Stubbs AE (2021) British Craneflies. British Entomological and Natural History Society, Reading, 343 pp.

Ujvárosi L (2005) Additions to the Cylindrotomidae, Limoniidae and Pediciidae species (Diptera) in Romania and Bulgaria. Entomologica Romanica 10: 65–70.

Wood HG (1952) The crane-flies of the South-West Cape (Diptera, Tipuloidea). Annals of the South African Museum 39: 1–327.

ZooKeys 1157: 43–93 (2023)
doi: 10.3897/zookeys.1157.95873
https://zookeys.pensoft.net

REVIEW ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

A decade of amphibian studies (Animalia, Amphibia) at Sekayu lowland forest, Hulu Terengganu, Peninsular Malaysia

Baizul Hafsyam Badli-Sham[1], Muhamad Fatihah Syafiq[1], Mohd Shahrizan Azrul Aziz[2], Natrah Rafiqah Mohd Jalil[3], Muhammad Taufik Awang[1], Muhammad Nouril Ammin Othman[1], Anis Azira Abdul Aziz[1], Khunirah Dzu[1], Nurul Asyikin Abdol Wahab[1], Nor Liyana Jamil[1], Murni Azima Ismail[1], Wan Ahmad Aidil Wan Azman[1], Ooi Xin Wei[1], Nur Ain Nabilah Jamaha[1], Mohamad Aqmal-Naser[1,2], Muhammad Fahmi-Ahmad[1], Noor Shahirah-Ibrahim[4], Syed Ahmad Rizal[1], Daicus M. Belabut[5], Chan Kin Onn[6], Evan Seng Huat Quah[7], Larry Lee Grismer[8], Amirrudin B. Ahmad[1,2]

1 *Biodiversity and Ecology Research Group, Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia* **2** *Institute of Tropical Biodiversity and Sustainable Development, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia* **3** *Academy of Science Malaysia, 902-4, Jalam Tun Ismail, 50480 Kuala Lumpur, Malaysia* **4** *Forestry Biotechnology Division, Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia* **5** *Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia* **6** *Lee Kong Chian Natural History Museum, National University of Singapore, 2 Conservatory Drive, 117377 Singapore, Singapore* **7** *Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia* **8** *Department of Biology, La Sierra University, 4500 Riverwalk Parkway, Riverside, California, 92515-8247 USA*

Corresponding author: Amirrudin B. Ahmad (amirrudin@umt.edu.my)

Academic editor: Thomas Ziegler | Received 1 October 2022 | Accepted 17 November 2022 | Published 31 March 2023

https://zoobank.org/D4FDD1DB-B1EA-46F3-B638-8A3D888F148E

Citation: Badli-Sham BH, Syafiq MF, Aziz MSA, Mohd Jalil NR, Awang MT, Othman MNA, Abdul Aziz AA, Dzu K, Abdol Wahab NA, Jamil NL, Ismail MA, Wan Azman WAA, Xin Wei O, Jamaha NAN, Aqmal-Naser M, Fahmi-Ahmad M, Shahirah-Ibrahim N, Rizal SA, Belabut DM, Kin Onn C, Quah ESH, Grismer LL, Ahmad AB (2023) A decade of amphibian studies (Animalia, Amphibia) at Sekayu lowland forest, Hulu Terengganu, Peninsular Malaysia. ZooKeys 1157: 43–93. https://doi.org/10.3897/zookeys.1157.95873

Abstract

Amphibians of Sekayu lowland forest have been studied more than a decade, with discoveries of new records of species showing no sign of abating between the years 2003 to 2020, indicating the remarkably rich diversity of anurans in this forest. Despite ceaseless anthropogenic activities in this area, this study successfully recorded 52 species of amphibians from 32 genera in the lowland forest of Sekayu. The species composition consisted of a single species from the family Ichthyophiidae and 51 species of anurans of 31 genera and six families. The number of species recorded has steadily increased especially during more recent surveys from 2015 to 2020. This study augments the total number of amphibian species recorded from Hulu Terengganu by ten additional species, increasing the total to 70 species for the district.

Keywords
Biodiversity conservation, herpetofauna, lowland forest, Malaysia, protected areas

Introduction

The earliest herpetological surveys conducted by Dring (1979) at Gunung Lawit, Hulu Terengganu reported 77 species, of which 44 were amphibians. The survey also discovered two new species of geckos, *Cyrtodactylus elok* and *Cnemaspis argus*. Until the 1990s, limited herpetological studies had been conducted, such as the surveys on reptiles in Bukit Labohan in Ma' Daerah by Davison (1993), which enlisted at least ten reptile species, and the surveys on the freshwater turtle trade conducted by Sharma (1999) in several districts in Terengganu. Surveys from 2000 to 2010 consist of amphibian studies by Norhayati et al. (2006) in Pasir Raja Forest Reserve and a follow-up survey on a reptile in Bukit Labohan by Sharma et al. (2007) with photograph records of amphibians. In 2003 and 2008, an Environmental Impact Assessment (EIA) on herpetofauna was done by Tenaga Nasional Berhad Research (TNBR) in Tembat Forest Reserve before the construction of two dams began in this areas (TNBR 2003, 2007). Two new species were made within this period, namely *Cnemaspis perhentianensis* on Pulau Perhentian Besar (Grismer and Chan 2008) and *Cyrtodactylus leegrismeri* on Pulau Tenggol (Chan and Norhayati 2010).

From 2011 to the present, extensive efforts have been made to document the herpetofauna diversity in Terengganu, including lowland to upland areas (Gunung Gagau: Hamidi 2013; Gunung Tebu and Lata Belatan recreational forest: Muin et al. 2014; the base of Gunung Lawit, Gunung Tebu, adjacent lowland forests of Lata Tembakah and Lata Belatan: Sumarli et al. 2015; Tasik Kenyir: Zakaria et al. 2019; Komaruddin et al. 2020), wetlands (Setiu: Tamblyn et al. 2006; Zahidin et al. 2017), urbanised areas (UMT Campus: Badli-Sham et al. 2019) and archipelagic islands (Perhentian archipelago, Pulau Redang, and Pulau Tenggol: Grismer et al. 2011; Pulau Bidong: Zakaria et al. 2015; Fatihah-Syafiq et al. 2020). Still, continuous surveys were carried out in Tembat Forest Reserve to monitor the herpetofauna communities during the construction of hydroelectric dams (Chan 2011; Norhayati et al. 2011; Ummi 2013; Nur Amalina et al. 2017, 2020, 2021). Surprisingly, more new species were discovered during this period, such as *Lipinia sekayuensis* (Grismer et al. 2014, 2016), *Tytthoscincus keciktuek* and *T. monticulous* (Grismer et al. 2018) and *Rentapia flavomaculata* (Chan et al. 2020a), and the discovery of *Philautus davidlabangi* (Quah et al. 2021) in Tasik Kenyir which is a new record for Peninsular Malaysia. Besides, many localities in Terengganu are still under ongoing surveys by a herpetology team from Biodiversity and Ecology Research Group (BERes), Universiti Malaysia Terengganu, particularly in the Hulu Terengganu District.

As part of the Hulu Terengganu Forest Reserve that is adorned with beautiful streams and intact forests, Sekayu lowland forest (SLF) has become the most popular picnic spot in Terengganu amongst locals and tourists alike with nearly 203,000 visitors reported in the year 2010 (Bhuiyan et al. 2011). Accommodated with sufficient facilities, this area has become the most conducive place for recreational and ecotourism activities. SLF is known to house a diverse range of organisms, such as butterflies, aquatic invertebrates (Wahizatul et al. 2011; Wahizatul and Geok 2016) and dragonflies (Wahizatul et al. 2006; Choong et al. 2013), reptiles (Zakaria et al. 2019), fishes (Kottelat et al. 1992; Tan and Ng 2005), and also diverse families of trees (Jarina et al. 2007). Several species of reptiles have been described from this area such as skinks, *Lipinia sekayuensis* (Grismer et al. 2014, 2016), *Tytthoscincus keciktuek* and *T. monticolus* (Grismer et al. 2018), plus a new genus and species of terrestrial crab, *Gempala bilobata* (Ng and Ahmad 2016), and *Johora michaeli* (Ng 2020). Recent fieldwork in SLF has contributed additional locality records for several recently described species in Peninsular Malaysia such as the skink *Sphenomorphus sungaicolus* (Sumarli et al. 2016) and *Rentapia flavomaculata* (Chan et al. 2020a).

Furthermore, the actual diversity of amphibians in SLF remains uncertain as the checklists were not properly reviewed and updated in the latest taxonomy, may contain several erroneous records or misidentifications of specimens, and the existing areas have not been exhaustively surveyed. The objectives of this paper are to: (1) properly compile and update the information from previous and recent fieldwork to produce a comprehensive checklist of amphibians, (2) to examine the previously and recently collected specimens in SLF for accurate species accounts, and (3) to assess the trend of long-term surveys conducted on amphibians throughout the past decade in SLF. In addition, this paper also provides a compiled checklist based on published records on the amphibian fauna in Hulu Terengganu District (Dring 1979; Norhayati et al. 2011; Hamidi 2013; Sumarli et al. 2015; Nur Amalina et al. 2017) to report the current diversity of amphibians in this area.

Materials and methods

Study area

Sekayu lowland forest (**SLF**) is located within the Hulu Terengganu Forest Reserve (Annex) near Kuala Berang to the east, and Taman Negara (= National Park) to the west and south-west (Fig. 1), with a total area of 30 ha. This area consists of Sekayu Recreational Forest (**SRF**) that is covered by tall and old growth lowland dipterocarp trees, cascading waterfalls drained by a pristine stream from the Peres River, and Sekayu Agricultural Park (**SAP**) which is an enormous agricultural area of 85 ha that is also open for recreational activities, and is drained by the Bubu River (Wahizatul et al. 2011). This area receives heavy rainfall from the northeast monsoon that typically occurs between October and March of each year (Khan et al. 2014).

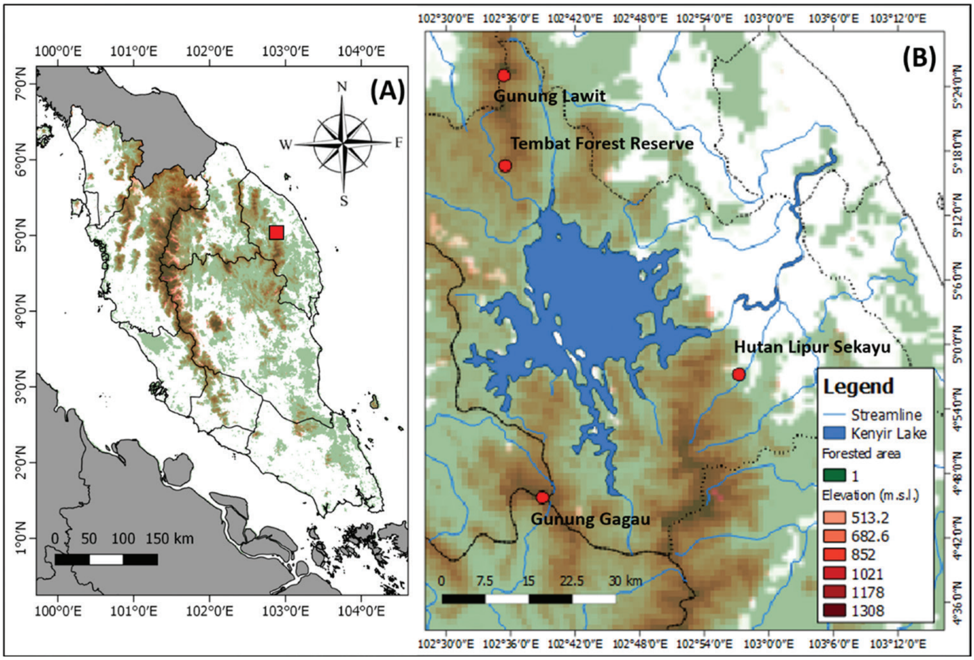


Figure 1. Map **A** shows the location of Sekayu lowland forest (SLF) indicated by red box in Peninsular Malaysia. The detailed location of SLF and several localities with published checklist on amphibians in Hulu Terengganu were displayed on Map **B** and indicated by the red circles for respective localities.

Descriptions of study sites

In SLF, fieldworks were conducted at various locations within SRF and SAP. The Sekayu Recreational Forest area (SRF) (entrance at 4°58'01.1"N, 102°57'35.4"E) contains substantial landscape changes to accommodate facilities for Forestry staff and visitors, such as huts, toilets, changing rooms, chalets, and camping sites. SRF is drained by a stream channel of the Peres River, which remains the main attraction for this area for recreational activities such as picnics and camping (Bhuiyan et al. 2011). The trekking route at SRF is also used as the main hiking route to Gunung Gajah Terom. Minor modifications were observed at certain parts of the Peres River, such as the construction of a dam to create shallow pools and suspension bridges that provided access to the forest within the SRF. However, a large portion of trees families and many others vegetations remain intact and well preserved (Jarina et al. 2007; Rafaai 2007; Nor 2007). In addition, many microhabitats such as rock pools, riparian vegetation, and small streams located beyond the recreational area are untouched. Much of the area in the recreational zone consists of a mixture of natural forest vegetation and garden plants, along with artificial drainage, especially at the Herbal Park. The surveys were conducted at six sampling sites within the SRF, which included the upper stream area and a tributary of the Peres River, recreational zone (vicinity of the car park, chalet, Forestry office, and trekking route), Orchid Garden, Herbal Park, and camping site.

Sekayu Agricultural Park (SAP) (entry point at 4°58'01.8"N, 102°57'28.4"E) is comprised of mostly agricultural lands growing various species of fruits such as *Lansium domesticum*, *Nephelium lappaceum*, *Gracia mangostana*, *Durio zibethinus*, and garden plants. Most of the landscape of this area is significantly altered and surrounded by the forest edge and drained by a stream channel of the Bubu River (Wahizatul et al. 2011). There is an upstream area of the Bubu River (4°58'20.12"N, 102°57'26.86"E) that was predominantly covered by secondary forest as in SRF, along with beautiful cascading waterfalls, an untouched small stream, and an abundance of granitic boulders along the upstream area. Similar to SRF, this area was occasionally visited by locals and has undergone moderate landscape modifications with the construction of recreational facilities such as a suspension bridge, Herbal Park and swimming pool. Half a kilometre from this area, buildings such as rest houses, dormitory and camping sites are provided. However, many of these buildings have been abandoned due to lack of maintenance. The upstream area of the Bubu River was frequently observed to have severe sediment loads, especially in the monsoon season which is assumed to have resulted from logging activities in nearby areas. The surveys were conducted in five sampling sites within the SAP, such as the upper stream area and a tributary of Bubu River, forest trail, recreational zone (area of rest houses, dormitory and camping site), and Herbal Park.

Data collection

Surveys were conducted using standard methods of visual encounter surveys (**VES**) and acoustic surveys, together with drift-fenced pitfall traps in SLF. VES is a time-constrained technique that is frequently employed in herpetological surveys, in which the observers walk along a in standardised route at a standard pace to visually search the entire area for amphibians (Crump and Scott 1994; Doan 2003). Any calling heard during the surveys were identified by the help of experts and/or calls were referenced at AmphibiaWeb (2021) (https://amphibiaweb.org). Five sets of drift-fenced pitfall traps (each set comprised of three pitfall traps and two 2.5-m long aluminium sheets) were set up for each of the small feeder streams of the Peres River (PR1: 4°57'43.15"N, 102°57'12.16"E; PR2: 4°57'42.43"N, 102°57'11.57"E; PR3: 4°57'42.11"N, 102°57'10.24"E; PR4: 4°57'40.03"N, 102°57'9.04"E; and PR5: 4°57'39.73"N, 102°57'7.2"E) at SFR and along the hiking trail at SAP (HT1: 4°58'17.7"N, 102°57'21.25"E; HT2: 4°58'17.34"N, 102°57'21.12"E; HT3: 4°58'16.99"N, 102°57'21.17"E; HT4: 4°58'16.53"N, 102°57'21.08"E; and HT5: 4°58'16.28"N, 102°57'21.24"E). Four days and three nights were spent for each survey occasion in (1) October and November 2015, (2) March to May and July to November 2016, (3) September to December 2017, (4) August to December 2018, and (5) July to December 2020.

Additional information of species from SLF was obtained from the compilation of amphibian checklists conducted by previous undergraduate fieldwork from 2003, 2004, 2006, 2013, 2014 and early 2015. Voucher specimens collected from previous and more recent fieldwork were examined to confirm species identifications based on Berry (1975), Brown and Guttman (2002), Harvey et al. (2002), Wood et al. (2008b), Matsui (2006), McLeod and Norhayati (2007), Nishikawa et al. (2012), Chan et al.

(2014a, b, 2016, 2018, 2020a–c), Rujirawan et al. (2013), Sumarli et al. (2015), Zug (2015), Matsui et al. (2014, 2017, 2018), Sheridan and Stuart (2018), Davis et al. (2018), Garg et al. (2019), Jiang et al. (2019), and Hong et al. (2021). The latest taxonomic nomenclatural follows the Amphibian Species of the World online database (Frost 2022). Measurement of snout-vent length (**SVL**) were taken using digital callipers. All preserved specimens were designated with the code Universiti Malaysia Terengganu Zoological Collections (**UMTZC**) and deposited in the General Laboratory of Biology, Universiti Malaysia Terengganu. The voucher photographs included in species accounts were designated with the code **UMTZCP** (Universiti Malaysia Terengganu Zoological Collections Photograph).

Data analysis

The checklist of amphibians from previous studies (2003–2015) were tabulated and compiled together with the most recent studies (October 2015-December 2020) to obtain the accumulated number of amphibian species that have been recorded in SLF. Species accumulation curve and estimated number of duplicates (species with two samples) and unique species (species represented by one sample) (Colwell and Coddington 1994) of amphibians from SLF was generated from the incidence-based data of species obtained from the year 2003 until 2018 by using EstimateS software 9.0 (Colwell 2013).

Results

Fifty-two species of amphibians were recorded in total, consisting of one caecilian from the family Ichthyophiidae and 51 anurans from 31 genera and six families in SLF (Table 1). Thirty-eight species were recorded from previous fieldwork between the years 2003 until early 2015, and 13 additional species were recorded in recent surveys from late 2015 to 2020 in SLF (Table 1). The additional species obtained in the recent surveys were *Ichthyophis* cf. *asplenius*, *Ansonia lumut*, *Leptophryne borbonica*, *Limnonectes plicatellus*, *L. hascheanus*, *L. utara*, *Occidozyga sumatrana*, *O. martensii*, *Kalophrynus palmatissimus*, *Micryletta dissimulans*, *Pulchrana laterimaculata*, *Polypedates colletti*, and *Theloderma licin*. Species nomenclature was updated based on the latest taxonomy, such as *Kaloula latidisca* (formerly reported as *Kaloula baleata*) (Chan et al. 2014b), *Amolops gerutu* (formerly known as *A. larutensis*) (Chan et al. 2018), *Sylvirana malayana* (formerly known as *H. nigrovittata*) (Sheridan and Stuart 2018), *Kurixalus chaseni* (formerly known as *K. appendiculatus*) (Matsui et al. 2018), *Limnonectes deinodon* (formerly known as *L. laticeps* and *L. khasianus*) (Dehling 2014), *Pulchrana sundabarat* (formerly known as *H. picturata*) (Chan et al. 2020c), *Zhangixalus prominanus* and *Z. tunkui* (formerly known as *Rhacophorus prominanus* and *R. tunkui*) (Jiang et al. 2019) and *Rentapia flavomaculata* (formerly known as *R. hosii*) (Chan et al. 2016; Chan et al. 2020a) were recorded in SLF as well.

Table 1. Checklist of amphibians of Sekayu lowland forest, Hulu Terengganu, Terengganu.

No	Family/Species	Year										
		2003	**2004**	**2006**	**2008**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2020**
	Family Ichthyophiidae											
1	*Ichthyophis* cf. *asplenius*	–	–	–	–	–	–	–	–	+	–	–
	Family Bufonidae											
2	*Ansonia latiffi*	–	–	–	–	+	+	+	+	–	+	–
3	*Ansonia lumut*	–	–	–	–	–	–	–	+	+	+	–
4	*Duttaphrynus bengalensis* (*Duttaphrynus* sp. 1)	–	–	–	+	+	–	–	+	–	+	+
5	*Ingerophrynus parvus*	+	+	+	–	+	+	+	+	+	+	+
6	*Leptophryne borbonica*	–	–	–	–	–	–	–	+	–	–	+
7	*Phrynoidis asper*	+	+	+	+	+	+	+	+	+	+	+
8	*Rentapia flavomaculata*	–	–	–	–	–	–	+	+	–	–	–
	Family Dicroglossidae											
9	*Fejervarya limnocharis*	+	+	+	+	+	+	+	+	+	+	+
10	*Limnonectes blythii*	+	+	+	+	+	–	+	+	+	+	+
11	*Limnonectes hascheanus*	–	–	–	–	–	–	–	+	+	+	+
12	*Limnonectes deinodon*	–	–	–	–	–	–	+	+	+	+	+
13	*Limnonectes malesianus*	+	+	–	–	–	–	+	+	+	+	+
14	*Limnonectes plicatellus*	–	–	–	–	–	–	–	+	+	+	+
15	*Limnonectes utara*	–	–	–	–	–	–	–	–	–	+	–
16	*Occidozyga sumatrana*	–	–	–	–	–	–	–	+	–	+	+
17	*Occidozyga martensii*	–	–	–	–	–	–	–	+	+	+	+
	Family Megophryidae											
18	*Leptobrachium hendricksoni*	+	–	+	+	+	+	+	+	+	+	+
19	*Leptobrachella sola*	–	–	–	–	–	–	+	+	+	+	–
20	*Pelobatrachus nasutus*	–	–	+	–	+	+	–	+	+	+	+
	Family Microhylidae											
21	*Kalophrynus kiewi*	–	–	–	–	–	–	+	+	+	+	+
22	*Kalophrynus palmatissimus*	–	–	–	–	–	–	–	+	–	–	–
23	*Kaloula latidisca*	–	–	–	–	–	–	+	+	–	+	+
24	*Kaloula pulchra*	–	–	–	–	+	+	+	+	+	–	+
25	*Microhyla bedmorei*	–	–	–	–	–	–	+	+	–	–	–
26	*Microhyla butleri*	–	–	–	+	+	+	+	+	+	+	+
27	*Microhyla* cf. *heymonsi*	+	+	+	+	+	+	+	+	+	+	+
28	*Microhyla superciliaris*	–	–	–	–	+	–	–	+	–	+	+
29	*Micryletta dissimulans*	–	–	–	–	–	–	–	+	+	+	+
30	*Phrynella pulchra*	–	–	–	–	–	+	–	–	–	–	–
	Family Ranidae											
31	*Amolops gerutu*	+	–	+	+	+	+	+	+	+	+	+
32	*Chalcorana labialis*	–	–	+	+	+	+	+	+	+	+	+
33	*Humerana miopus*	–	–	–	–	+	–	+	+	+	+	+
34	*Hylarana erythraea*	+	+	+	+	+	+	+	+	+	+	–
35	*Indosylvirana nicobariensis*	+	–	–	–	+	–	+	+	+	+	+
36	*Odorrana hosii*	+	–	+	+	+	+	+	+	+	+	+
37	*Pulchrana glandulosa*	–	–	–	–	+	+	+	+	+	+	+
38	*Pulchrana laterimaculata*	–	–	–	–	–	–	–	–	–	+	+
39	*Pulchrana sundabarat*	+	–	+	–	–	–	+	+	+	+	–
40	*Sylvirana malayana*	+	–	+	–	+	+	+	+	+	–	–
	Family Rhacophoridae											
41	*Kurixalus chaseni*	–	–	–	–	–	+	–	+	+	+	+
42	*Nyctixalus pictus*	–	–	–	–	–	+	+	+	+	+	+

No	Family/Species	Year										
		2003	2004	2006	2008	2013	2014	2015	2016	2017	2018	2020
43	*Polypedates colletti*	–	–	–	–	–	–	–	–	–	–	+
44	*Polypedates discantus*	–	–	–	–	–	–	+	+	+	+	+
45	*Polypedates leucomystax*	+	+	+	+	+	+	+	+	+	+	+
46	*Polypedates macrotis*	–	–	–	–	+	+	+	+	+	+	+
47	*Rhacophorus nigropalmatus*	–	–	–	–	+	–	–	–	–	+	–
48	*Rhacophorus pardalis*	–	–	–	–	+	–	–	–	–	–	–
49	*Theloderma horridum*	–	–	–	–	+	–	–	–	–	–	–
50	*Theloderma licin*	–	–	–	–	–	–	–	+	–	–	–
51	*Zhangixalus prominanus*	–	–	–	–	+	–	–	+	+	+	–
52	*Zhangixalus tunkui*	–	–	–	–	+	–	–	–	–	–	–
	Total number of species	**14**	**8**	**14**	**12**	**28**	**20**	**29**	**43**	**34**	**38**	**34**

Class Amphibia
Order Gymnophiona
Family Ichthyophiidae

Ichthyophis* cf. *asplenius
Fig. 2
Malayan Caecilian

Examined specimens. One specimen was collected from SRF (UMTZC1792, SVL = 198 mm).

Identification. The specimen had elongated and cylindrical body with SVL 198 mm; head as wide as body; snout round; small eyes; body darkish purple with pale yellowish lateral band. Specimen tentatively recognised as *Ichthyophis* cf. *asplenius* as suggested by Nishikawa et al. (2012), until further research to reconfirmed taxonomy of this group in Peninsular Malaysia.

Remarks. The specimen was collected from the downstream areas of the Peres River. The collected individual was spotted amongst piles of wet leaves at the stream edge. This species is a new record for the amphibians in Hulu Terengganu.

Order Anura
Family Bufonidae

***Ansonia latiffi* Wood, Grismer, Norhayati & Juliana, 2008**
Fig. 3A
Latiff's Torrent-dwelling Toad

Examined specimens. Eight specimens were collected from SRF consisted of four males (UMTZC1319, UMTZC1400, UMTZC1461, and UMTZC1575, SVL = 38–60 mm) and four females (UMTZC1318, UMTZC1353, UMTZC1401, and UMTZC1553, SVL = 41–59 mm).

Figure 2. *Ichthyophis* cf. *asplenius*.

Identification. Morphological characters of the specimens from SLF agreed well with the description by Wood et al. (2008b). Size (SVL: 38–60 mm, n = 4 males; 41–59 mm, n = 4 females); snout projecting beyond lower jaw; tympanum distinct; interorbital ridges absent; small warts at jaw; head narrow in females, but wide in males; inner and outer metatarsal tubercles present; first finger reaching tip of second finger; single mandibular asperities in UMTZC1400 and double for other specimens; dorsal tubercles distinct; spotting at gular region, obscured in UMTZC1353 and UMTZC1553; abdomen finely granular; no dorsolateral row of tubercles;

Remarks. All observed and collected *A. latiffi* were found along the banks of the small streams of the Peres River within the areas of SRF. *Ansonia latiffi* is typically found on rocky substrate or the ground of sloping terrain, and sometimes found perched on low vegetation below 1 m from the ground.

Ansonia lumut **Chan, Wood, Anuar, Muin, Quah, Sumarli & Grismer, 2014**
Fig. 3B
Mossy Stream Toad

Examined specimens. Three males were collected from SRF (UMTZC1527 and UMTZC1991, SVL = 15–24 mm) and SAP (UMTZC1615, SVL = 26 mm).

Identification. Morphological characters of the specimens agreed well with the description by Chan et al. (2014a). Size (SVL: 15–26 mm, n = 3 males); distinct tympanum; snout projecting beyond lower jaw; snout wider than long; paratoid gland absent; interorbital ridges absent; large yellow rectal tubercles behind tympanum; limbs with yellow cross bars; venter surface pale grey with fine white spotting; first finger much shorter than second; no dorsolateral row of tubercles; dorsum blackish with greenish-yellow reticulations; and flank with small yellow spots.

Remarks. *Ansonia lumut* was first collected from the Bubu River in SAP by Davis et al. (2016) (LSUHC 11212–13), and a second specimen (UMTZC1615) was collected in the same place during recent surveys. For the Peres River, two specimens were collected from a small stream area (UMTZC1527) and in drift-fenced pitfalls (UMTZC1991). This stream-dwelling species was found on substrates such as granite rock covered with moss, low vegetation at steep edges of the stream, and collected as well in pitfall traps.

Duttaphrynus bengalensis **(*Duttaphrynus* sp. 1) (Daudin, 1802)**

Fig. 3C

Common Asian Toad

Examined specimens. Four males were collected from SRF (UMTZC1147, UMTZC1148, and UMTZC1149, SVL = 47–68 mm) and SAP (UMTZC1065, SVL = 59 mm).

Identification. Morphological characters of the specimens agreed well with the former description by Berry (1975). Size (SVL: 47–68 mm, n = 4 males); stout bodies; head with supraorbital and supratympanic bony ridges; parietal ridges absent; snout obtusely pointed; tympanum distinct; tips of digits blunt; subarticular tubercles distinct; toes more than ½ webbed; paratoid gland ellipsoidal; outer metatarsal tubercles smaller than inner metatarsal tubercles. Based the revision by Jablonski et al. (2022), the specimens resembled the characteristics of *Duttaphrynus bengalensis* comb. nov. and "*hazarensis*" such as the concave interorbital space; interorbital space larger than upper eyelid width and internarial distance; snout longer than horizontal eye diameter; tympanum oval; canthus rostralis with a ridge and sharp (Daudin 1802; Khan 2001).

Remarks. This species was more frequently observed at SAP compared to SRF, probably due to the more disturbed and man-made environment that is favoured by this commensal anuran (Shahriza and Ibrahim 2012; Badli-Sham et al. 2019). The species was commonly observed in drains, irrigation ditches, abandoned ponds, and even along the roadside. Calls of this species could be heard after rains. Recently, Jablonski et al. (2022) carried out the molecular assessment on the *Duttaphrynus melanostictus* complex in the South-East Asia, which revealed the two groups of populations namely, *Duttaphrynus bengalensis* comb. nov. and "*hazarensis*" (*Duttaphrynus* sp. 1) and *Duttaphrynus* sp. 2 sensu Bisht et al. (2021). However, careful inspection of collected specimens shows a resemblance to *Duttaphrynus bengalensis* (*Duttaphrynus* sp. 1) based on five characters by Daudin (1802) and Khan (2001).

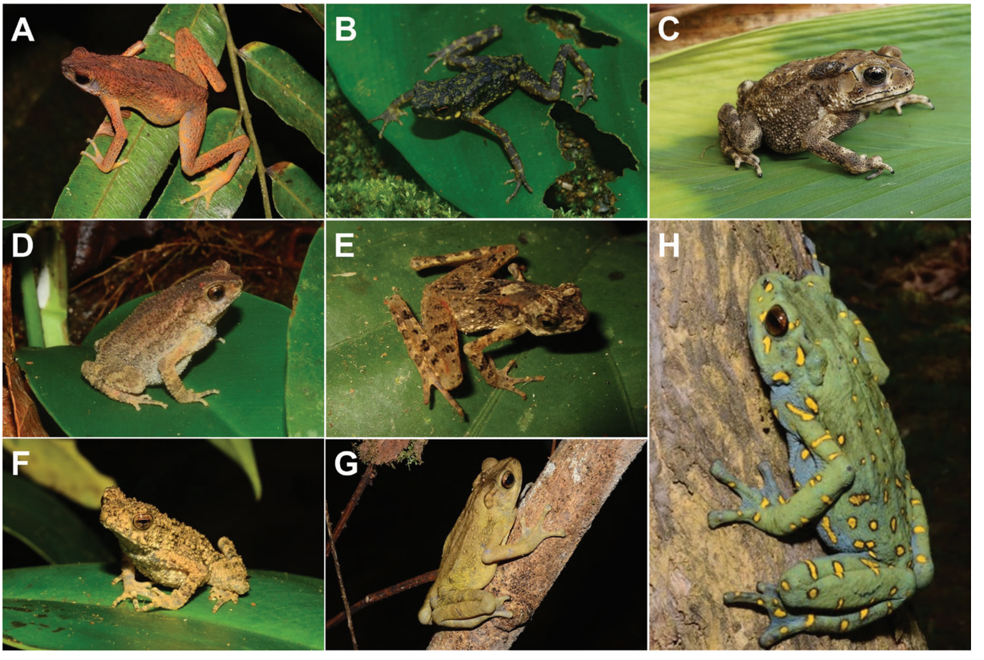


Figure 3. A *Ansonia latiffi* **B** *Ansonia lumut* **C** *Duttaphrynus bengalensis* (*Duttaphrynus* sp. 1) **D** *Ingerophrynus parvus* **E** *Leptophryne borbonica* **F** *Phrynoidis asper* **G** male *Rentapia flavomaculata* **H** female *R. flavomaculata*.

***Ingerophrynus parvus* (Boulenger, 1887)**
Fig. 3D
Malayan Dwarf Toad

Examined specimens. Ten specimens were previously collected from SRF consisted of seven males (UMTZC1021, UMTZC1025, UMTZC1026, UMTZC1033, UMTZC1255, UMTZC1339, and UMTZC1398, SVL = 33–37 mm) and three females (UMTZC1023, UMTZC1158, and UMTZC1625, SVL = 42–45 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975) and Sumarli et al. (2015). Size (SVL: 33–37 mm, *n* = 7 males; 42–45 mm, *n* = 3 females); stout body; head with supraorbital and parietal ridges forming straight lines; supratympanic ridges short; tympanum distinct; snout truncate; finger tips rounded; first finger longer than second; subarticular tubercles distinct; toes ½ webbed; paratoid gland rounded to sub-triangular; dorsum skin with distinct spiny tubercles, venter coarsely granular.

Remarks. Most of the individuals were collected and observed near the stream areas. This species is also common in open recreational areas. *Ingerophrynus parvus* was commonly found on the leaf litter, in rocky crevices and rotten logs, but rarely found on low vegetation.

Leptophryne borbonica (Tschudi, 1838)

Fig. 3E
Javan Tree Toad

Examined specimens. Two male specimens were collected from SRF (UMTZC1500 and UMTZC1733, SVL = 22–24 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975). Size (SVL: 22–24 mm, *n* = 2 males); slender body; head without bony ridges; snout truncate; tympanum distinct; tips of digits swollen into discs; first finger slightly shorter than the second; fingers ½ webbed; subarticular tubercles distinct; skin with small warts; paratoid gland small and almost invisible; dorsum with hourglass shape; pale dorsolateral gland; hind limbs with pinkish red on underside surface.

Remarks. *Leptophryne borbonica* is so far known to occur at the small streams of SRF with the first collected specimen UMTZC1500 from the Peres small stream and UMTZC1733 from the Bubu small stream. This forest-dwelling species was sighted amongst the piles of dead leaves on the forest floor and the banks of the streams.

Phrynoidis asper (Gravenhorst, 1829)

Fig. 3F
Malayan Giant Toad

Examined specimens. Nineteen specimens were collected from SLF consisted of 17 specimens from SRF (Juvenile: UMTZC1136, SVL = 17 mm; Males: UMTZC1020, UMTZC1022, UMTZC1031, UMTZC1083, UMTZC1112, UMTZC1129, UMTZC1135, UMTZC1399, UMTZC1483, UMTZC1508, UMTZC1511, UMTZC1575, UMTZC1576, and UMTZC1577, SVL = 24–111 mm; Females: UMTZC1321 and UMTZC1800, SVL = 93–150 mm) and two specimens from SAP (Males: UMTZC1152 and UMTZC1153, 32–34 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975) and Sumarli et al. (2015). Size (SVL: 17 mm, *n* = 1 juvenile; 24–111 mm, *n* = 16 males; 93–150 mm, *n* = 2 females); stocky body; supraorbital and supratympanic bony ridges distinct; snout obtusely pointed; paratoid gland rounded to sub-triangular; tympanum distinct; first finger slightly longer than second; subarticular tubercles conspicuous and large. Five specimens absent of X-shaped dorsum marking (UMTZC1031, UMTZC1129, UMTZC1321, UMTZC1483, UMTZC1511).

Remarks. *Phrynoidis asper* is common at the stream areas of SLF. This species was typically observed in rocky crevices and the ground near the stream bank. This toad is also amongst the largest species of anuran recorded in this area with a maximum SVL reaching 150 mm.

Rentapia flavomaculata **Chan, Abraham & Badli-Sham, 2020**
Fig. 3G, H
Yellow Spotted Tree Toad

Examined specimens. Two adult females were collected from SRF (UMTZC1404 and UMTZC1495, SVL = 102–104 mm).

Identification. Morphological characters of the specimens agreed well with the description of Chan et al. (2020a). Size (SVL: 102–104 mm, *n* = 2 females); moderately stout bodies; head without bony ridges; tympanum distinct; paratoid gland short and distinct; finger tips long and expended into broad discs; inner metatarsal tubercles larger than outer; body greenish with yellow spots and reticulations; venter finely granular. One male from areas near SRF was observed and photographed (Fig. 3G).

Remarks. The first collected specimen UMTZC1404 (Fig. 2H) was from a small stream of the Bubu River, and UMTZC1495 was from a small stream of the Peres River, and the photographed male (Fig. 3G) was from the latter location. Both preserved specimens were collected during the post-monsoon season at SRF. Both were seen on rotten logs and tree trunks within the vicinity of the small streams.

Family Dicroglossidae

Fejervarya limnocharis **(Gravenhorst, 1829)**
Fig. 4A
Rice Field Frog

Examined specimens. Twenty-four specimens were previously collected from SAP (Males: UMTZC1391, UMTZC1396, UMTZC1397, and UMTZC1598, SVL = 14–40 mm; Female: UMTZC1497, SVL = 50 mm), and SRF (Males: UMTZC1002, UMTZC1019, UMTZC1048, UMTZC1049, UMTZC1088, UMTZC1089, UMTZC1150, UMTZC1215, UMTZC1216, UMTZC1229, UMTZC1230, UMTZC1239, and UMTZC1259, SVL = 23–45 mm; Females: UMTZC1003, UMTZC1058, UMTZC1087, UMTZC1122, UMTZC1151, and UMTZC1324, SVL = 49–58 mm).

Identification. Morphological characters of the specimens agreed well with the description of Berry (1975) and Sumarli et al. (2015). Size (SVL: 23–45 mm, *n* = 17 males; 49–58 mm, *n* = 7 females); vomerine teeth in two oblique series between choanae; head moderate; pointed snout; tympanum distinct; supratympanic fold distinct; first finger longer than second; fingers lacking fringes of skin; finger tips blunt; pointed toe tips; inner and outer metatarsal tubercle with oval-shaped; male specimens with nuptial pads on dorsal portion of first finger; dorsum skin with longitudinal skin folds.

Remarks. This species was ubiquitous in cleared and disturbed areas of SLF and is considered as commensal species of frog in this area. Most of the collected specimens were found on the grassy fields and in puddles. Active calling can be heard after the rains.

Limnonectes blythii (Boulenger, 1920)

Fig. 4B
Blyth's Giant Frog

Examined specimens. Seven specimens were collected from SRF consisted of juveniles (UMTZC1390 and UMTZC1599, SVL = 31–38 mm), males (UMTZC1004 and UMTZC1394, SVL = 84 to 87 mm), and females (UMTZC1393, UMTZC1459, and UMTZC1491, SVL = 48–68 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975) and Sumarli et al. (2015). Size (SVL: 31–38 mm, *n* = 2 juveniles; 84–87 mm, *n* = 2 males; 48–68 mm, *n* = 3 females); stout body; head long and narrow; pointed snout projecting beyond lower jaw; supratympanic fold distinct; supratympanic fold distinct; toes fully webbed; upper eyelids with low and rounded tubercles; dorsum smooth and sometimes scattered with low tubercles; dorsum pattern variable from dark W-shaped marking on back (UMTZC1390, UMTZC1393, UMTZC1459, UMTZC1491, and UMTZC1599) to a broad vertebral stripe from snout to vent (UMTZC1394), or plain dorsum (UMTZC1004).

Remarks. All specimens were collected from the recreational zones of SLF and the small streams of the Peres and Bubu Rivers. This species is usually found on the ground at stream banks.

Limnonectes hascheanus (Stoliczka, 1870)

Fig. 4C
Hill Forest Frog

Examined specimens. Two male specimens were collected from SAP (UMTZC1516, SVL = 29 mm) and SRF (UMTZC1529, SVL = 19 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975) and Hong et al. (2021). Size (SVL: 19–29 mm, *n* = 2 males); vomerine teeth in two oblique oval groups; head moderate; dark crossbar between eyes; rounded snout; tympanum distinct; distinct supratympanic fold from eye to shoulder; inner metatarsal tubercles large; outer metatarsal tubercles absent; digit tips lacking of circum marginal grooves; toes webbed but not reaching discs of second and third toes; dorsum skin smooth with small and low tubercles; dorsolateral fold absent; dorsum colour pale brown with small dark spots and W-shaped marking.

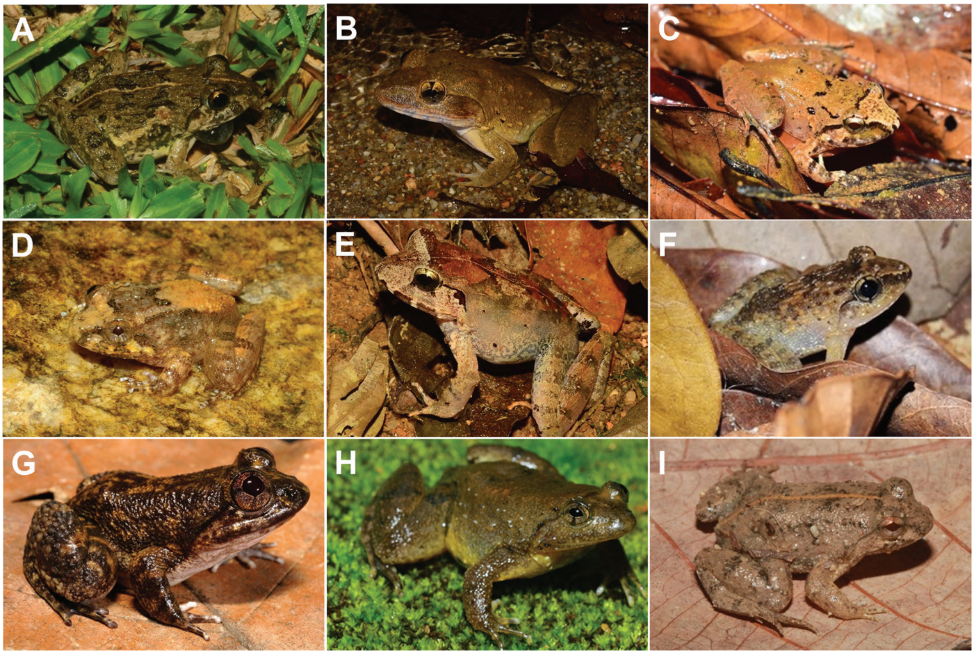


Figure 4. A *Fejervarya limnocharis* **B** *Limnonectes blythii* **C** *L. hascheanus* **D** *L. deinodon* **E** *L. malesianus* **F** *L. plicatellus* **G** *L. utara* **H** *Occidozyga sumatrana* **I** *O. martensii*.

Remarks. UMTZC1516 was collected from hilly terrain at SAP and they are usually seen quietly perched amongst piles of dead leaves. UMTZC1529 was found at the Herbal Park within SRF on similar substrate as the preceding specimen.

Limnonectes deinodon **Dehling, 2014**
Fig. 4D
Flat-headed Corrugated Frog

Examined specimens. Seventeen specimens were collected from SRF consisted of juveniles (UMTZC1386, UMTZC1388, UMTZC1548, and UMTZC1549, SVL = 20–30 mm), males (UMTZC1392, UMTZC1395, UMTZC1458, UMTZC1547, and UMTZC1557, SVL = 43–55 mm) and females (UMTZC1125, UMTZC1370, UMTZC1371, UMTZC1374, UMTZC1387, UMTZC1389, UMTZC1470, and UMTZC1471, SVL = 31 to 53 mm).

Identification. Morphological characters of the specimens agreed well with the description by Dehling (2014). Size (SVL: 20–30 mm, n = 4 juveniles; 43–55 mm, n = 5 males; 31–53 mm, n = 8 females); vomerine teeth in two oblique series behind choanae; head wide and moderately depressed; lower jaw with a pair of prominent odontoids; rounded snout; tympanum hidden; supratympanic fold distinct; digit tips rounded and slightly swollen; first finger shorter than second; fingers with narrow dermal fringes; toes webbing reduced which does not reach toe disc and extend beyond penultimate subarticular tubercle on fourth toe; elongate inner metatarsal tubercle and small outer metatarsal tubercle; dorsum and limbs with longitudinal and corrugated warts; dorsum colour variable from pale brown to bright orange; plain or pale marking on back. This species was previously identified as *L. laticeps* and *L. khasianus* (Ohler and Deuti 2013; Sumarli et al. 2015).

Remarks. The species was ubiquitous at stream areas of SRF and can be found at various microhabitats such as in rocky crevices, dead leaves, rotten logs, or intermittent pools near the streams.

Limnonectes malesianus (Kiew, 1984)

Fig. 4E
Malayan River Frog

Examined specimens. Two male specimens were collected from SRF (UMTZC1123 and UMTZC1628, SVL= 74–81 mm).

Identification. Morphological characters of the specimens agreed well with the description by Grismer (2012). Size (SVL: 74–81 mm, n = 2 males); broad head; rounded snout projecting beyond lower jaw; lower jaws of males with two fang-like projections; upper eyelids with prominent and spiny tubercles; tympanum distinct; supratympanic distinct; dorsum smooth with W-shaped marking, small tubercles, and longitudinal folds. UMTZC1628 had fine and whitish vertebral line from snout to vent and along the upper side of thigh, while UMTZC1123 had no vertebral line on the back.

Remarks. All specimens were collected from a small stream of the Peres River at SRF. Additional individuals of this species can be found on forest floors and stream banks of the small streams and recreational zones in SRF.

Limnonectes plicatellus (Stoliczka, 1873)

Fig. 4F
Rhinoceros Frog

Examined specimens. Two adult specimens were collected from SRF for male (UMTZC1460, SVL = 35 mm) and female (UMTZC1512, SVL = 35 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975) and Sumarli et al. (2015). Size (SVL: 35 mm for male and female); large head; blunted snout; vomerine teeth in two oblique series between choanae; lower jaw with two fang-like projections; head with knob-like bony projection between eyes in males; tympanum distinct; supratympanic fold distinct; finger tips dilated into small disc; first finger slightly longer than second; toes 2/3 to 3/4 webbed; subarticular tubercles well developed; inner metatarsal tubercle elongate; outer metatarsal tubercle absent; dorsum skin with longitudinal folds; dorsum colour bronze to reddish brown.

Remarks. UMTZC1460 was collected from a small stream of Peres River, and UMTZC1512 from the recreational zone within SRF. Both were found on the forest litter.

Limnonectes utara Matsui, Belabut & Ahmad, 2014

Fig. 4G
Warty River Frog

Examined specimen. One male specimen was collected from SLF (UMTZC1964, SVL = 49 mm).

Identification. Morphological character of the specimen agreed well with the description by Matsui et al. (2014). Size (SVL: 49 mm, *n* = 1 male); obtusely pointed snout; head longer than broad; tympanum almost visible; supratympanic fold distinct; finger tips bluntly rounded; first finger slightly longer than second; nuptial pad present on first finger and second finger of males; toes webbed at base; subarticular tubercles oval-shaped; inner metatarsal tubercle large; outer metatarsal tubercle absent; dorsum smooth; less densely arranged circum-cloacal warts; tibia surface densely covered by warts; dark blotches absent on rear thigh.

Remarks. Information of *L. utara* from SLF is limited to a specimen and photograph of a dead specimen, as it was contributed through recent undergraduate sampling. This species was previously reported as *Limnonectes* cf. *kuhlii* in SRF.

Occidozyga sumatrana (Peters, 1877)

Fig. 4H
Yellow Bellied Puddle Frog

Examined specimens. Seven adult male specimens were collected from SRF (UMTZC1507, UMTZC1561, UMTZC1562, UMTZC1610, UMTZC1629, UMTZC1631, and UMTZC1734SVL, SVL = 22–35 mm).

Identification. Morphological characters of the specimens agreed well with the description by Davis et al. (2018) and Hong et al. (2021). Size (SVL: 22–35 mm, *n* = 7 males); depressed head; rounded snout; tympanum present but not visible through skin; weak supratympanic fold; tips of digits blunt and dilated to small disc; elliptical and compressed inner metatarsal tubercle, outer metatarsal tubercle absent; toes webbed and reaching discs of all toes; dorsum patterns variable from having a broad and pale vertebral stripe between eyes and shoulder (UMTZC1629) to indistinct dark marking (UMTZC1507, UMTZC1561, UMTZC1610, UMTZC1631, and UMTZC1734), dense and dark blotching, or whitish marking on snout and interorbital region (UMTZC1562).

Remarks. All specimens were found in temporary stagnant water bodies near the Peres River at SRF, such as puddles or intermittent pools that developed after heavy rains on the forest floor and near stream areas. The species is typically observed with its body partially submerged in water with eyes exposed.

Occidozyga martensii **(Peters, 1867)**
Fig. 4I
Marten's Puddle Frog

Examined specimens. No specimen was collected for this species, but it was recorded by field observation and photographs of two individuals at an artificial pond at Herbal Park in SRF (UMTZCP040519–122).

Identification. All observed individuals had stocky body; flattened heads; tympanum covered by skin; supratympanic fold distinct; first finger longer than second; inner and outer metacarpal tubercles distinct; toes completely webbed; dorsum paler brown with numerous dark blotches and indistinct blackish dorsolateral stripe; venter smooth and yellowish white. Morphological features of these individuals closely resemble *Occidozyga martensii* based on photographic material illustrated in Chan et al. (2010) from Pulau Pangkor, Shahriza and Ibrahim (2014) from Ulu Paip Recreational Forest, and in Hong et al. (2021) from Batu Hampar Recreational Forest.

Remarks. Both individuals were observed perched on a leaf overhanging the pond, and they quickly escaped into the water when approached.

Family Microhylidae

Kalophrynus kiewi **Matsui, Eto, Belabut & Nishikawa, 2017**
Fig. 5A
Kiew's Sticky Frog

Examined specimens. Three specimens were collected from SRF consisted of males (UMTZC1484 and UMTZC1563, SVL 29–36 mm) and female (UMTZC1614, SVL = 43 mm).

Identification. All specimens were previously identified as *Kalophrynus* cf. *pleurostigma* and were re-examined following the description of populations from Peninsular Malaysia by Matsui et al. (2017) as *K. kiewi*. Morphological characters of the specimens agreed well with the description of *K. kiewi* in having size (SVL: 29–36 mm, n = 2 males; 43 mm, n = 1 female); pointed snout which directed downwards; tympanum distinct; first and fourth fingers shorter than second; finger tips rounded and not dilated; fingers with distinct subarticular tubercles (two on third finger and three on other fingers); toes moderately webbed; toes with distinct subarticular tubercles (one on first, second and fifth toes, two on third toe, and three on fourth toe); dorsum skin glandular and spineless; distinct dorsum gland around arm insertion; dorsum pattern with irregular markings extending between eyes and supra-scapular area.

Remarks. All specimens were collected from the trekking trails and artificial pond within the Herbal Park at SRF. No calling was heard, but *K. kiewi* was frequently found on the forest litter and generally towards the monsoon season. This species is a new record for the amphibians in Hulu Terengganu.

***Kalophrynus palmatissimus* Kiew, 1984**
Fig. 5B
Web-footed Sticky Frog

Examined specimens. Two male specimens were collected from SRF (UMTZC1486 and UMTZC1632 = 35–38 mm).

Identification. Morphological characters of the specimens agreed well with the description by Zug (2015). Size (SVL: 35 38 mm, *n* = 2 males); head slightly broader than long; snout moderately broad; tympanum distinct; finger tips rounded and not dilated; fingers with distinct subarticular tubercles; no subarticular tubercles on fifth toe; toes strongly webbed; inner metatarsal tubercle oval-shaped; small and indistinct outer metatarsal tubercle; dorsum pale brown to reddish brown; dorsum patterns with darker hour-glass shaped marking on back.

Remarks. Specimens of this species were collected from the open areas of the camping site, and trekking trail of the Herbal Park at SRF, among leaf litter and rotten logs. Brief calling was heard from a specimen found at the camping site in mid-November. This species is a new record for the amphibians in Hulu Terengganu.

***Kaloula latidisca* Chan, Grismer & Brown, 2014**
Fig. 5C
Wide-Disked Bull Frog

Examined specimens. Five specimens (formerly identified as *K. baleata*) were collected from SRF consisted of juvenile (UMTZC1011, SVL = 20 mm) and adult males (UMTZC1310, UMTZC1316, UMTZC1464, and UMTZC1482, SVL = 35–64 mm).

Identification. Morphological characters of the specimens agreed well with the description of northern populations from Peninsular Malaysia by Chan et al. (2014b). Size (SVL: 20 mm, *n* = 1 juvenile; 35–64 mm, *n* = 4 males); head wider than long; snout obtusely pointed; tympanum hidden; supratympanic fold present; limbs long and robust; digit tips expended into distinct discs lacking of circum-marginal grooves; toes webbed at base; subarticular tubercles distinct; inner metatarsal tubercle large and oval-shaped; outer metatarsal tubercle small and rounded; arm and limb insertion with yellowish patches (pale patches in preserved specimens); dorsum with black speckled marking.

Remarks. *Kaloula latidisca* was frequently spotted on tree trunks around the open and recreational areas, and occasionally at the small streams of the Peres River at SRF. No calling was heard, but *K. latidisca* was typically found in mid-November and possibly until the end of the monsoon season. This species is a new record for the amphibians in Hulu Terengganu.

***Kaloula pulchra* Gray, 1831**
Fig. 5D
Painted Bull Frog

Examined specimens. Four specimens were collected from SRF (Juvenile: UMTZC1414, SVL = 15 mm; Males: UMTZC1082 and UMTZC1415, SVL = 57–62 mm) and SAP (Male: UMTZC1056 = 45 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975) and Davis et al. (2018). Size (SVL: 15 mm, *n* = juvenile; 45–62 mm, *n* = 3 males); stocky body; snout rounded; tympanum distinct; finger tips with expended discs; toes webbed at base; dorsum skin smooth; dorsum colouration medium to dark brown with broad orange, black-edged, or pale stripes which extend from head along each side of body.

Remarks. *Kaloula pulchra* was ubiquitous in open and recreational areas in SLF, with a tendency to be encountered hiding in irrigation ditches, drains, and toilets. The calls of adult males were heard during and after the heavy rains.

***Microhyla berdmorei* (Blyth, 1856)**
Fig. 5E
Berdmore's Narrow-mouthed Frog

Examined specimens. Three male specimens were collected from SRF (UMTZC1457, SVL = 12 mm) and SAP (UMTZC1062 and UMTZC1063, SVL 13–14 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975), Sumarli et al. (2015) and Garg et al. (2019). Size (SVL: 12–14 mm, *n* =3 males); head as long as broad; snout obtusely pointed; tympanum hidden by skin; finger tips swollen into small discs; toes almost completely webbed; dorsum with a pale stripe from eyes to shoulder; dorsum pattern with broad black marking; anal region with black spots.

Remarks. UMTZC1457 was collected from the recreational zone within SRF in leaf litter, UMTZC1062 and UMTZC1063 were found at the roadside within SAP, also in leaf litter. Additional individuals were observed inside the artificial ponds at the Herbal Park within SRF during the mid-monsoon season.

***Microhyla butleri* Boulenger, 1900**
Fig. 5F
Butler's Narrow-mouthed Frog

Examined specimens. Eight male specimens were collected from SAP (UMTZC1064, UMTZC1217, UMTZC1218, UMTZC1219, UMTZC1220, UMTZC1221, UMTZC1222, and UMTZC1223, SVL = 21–25 mm).

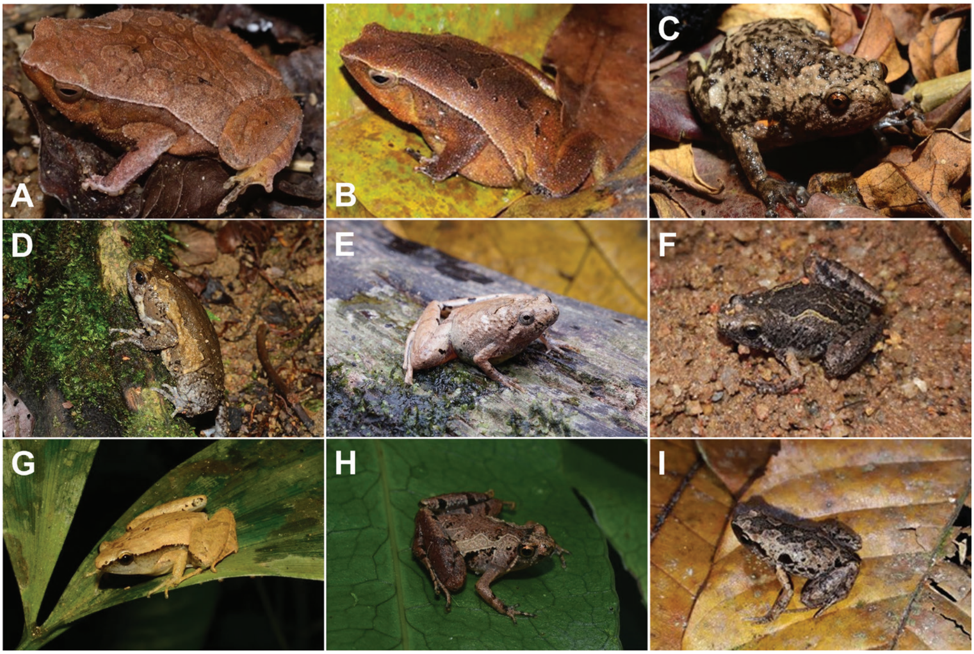


Figure 5. A *Kalophrynus kiewi* **B** *K. palmatissimus* **C** *Kaloula latidisca* **D** *K. pulchra* **E** *Microhyla berdmorei* **F** *M. butleri* **G** *M.* cf. *heymonsi* **H** *M. superciliaris* **I** *Micryletta dissimulans*.

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975) and Garg et al. (2019). Size (SVL: 21–25 mm, *n* = 8 males); rounded snout; tympanum hidden; upper eyelids without dermal tuberculation; tips of digits dilated into small discs bearing circum-marginal grooves; toes webbed at base; subarticular tubercles small; metatarsal tubercles present; head with whitish streak from the eyes to shoulder; dorsum with wavy markings extending from the eyes to posterior region, forming cross bars on hind limbs with pale edges.

Remarks. *Microhyla butleri* was usually found beneath piles of leaf litter and occurs throughout SLF. Despite the fact that all collected specimens were from SAP, many individuals of this species were observed on leaf litter, mostly along roadsides and trekking trails within SRF.

Microhyla cf. *heymonsi* Vogt, 1911

Fig. 5G
Heymon's Narrow-mouthed Frog

Examined specimens. Nine specimens were collected from SRF (Males: UMTZC1028, UMTZC1067, UMTZC1224, and UMTZC1226, SVL = 17–24 mm; Females: UMTZC1008 and UMTZC1066, SVL = 25–27 mm) and SAP (Males: UMTZC1320 and UMTZC1489, SVL = 17–20 mm; Female: UMTZC1343, SVL = 29 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975), Garg et al. (2019) and Sumarli et al. (2015). Size (SVL: 17–24 mm, *n* = 6 males; 25–29 mm, *n* = 3 females); rounded snouts, projecting beyond lower jaw; tympanum barely visible; supratympanic fold distinct; tips of digits dilated to form large disc bearing circum-marginal grooves; toes basally webbed; dorsum with pale coloured vertebral stripe, with black marks on each side, and dark stripe on lateral sides from tip of snout until groin; ventral surface of foot is dark brown.

Remarks. *Microhyla* cf. *heymonsi* was commonly found beneath piles of leaf litter and in rock crevices throughout SLF. This species was also found to occur in similar man-made ponds as with other species of *Microhyla*. Active and loud calling could be heard from this species after rains. The species is considered a commensal species that is tolerant of habitat alteration (Badli-Sham et al. 2019).

Microhyla superciliaris Parker, 1928

Fig. 5H

Batu Cave's Narrowed-mouthed Frog

Examined specimens. One specimen was collected from SRF (UMTZC1761, SVL = 18 mm).

Identification. Morphological characters of the specimen agreed well with the description by Berry (1975), Sumarli et al. (2015), and Manthey et al. (2016). Size (SVL = 18 mm, *n* = 1 male); rounded snout; upper eyelid with dermal tubercles; tympanum hidden; first finger much shorter than second; finger tips lacking discs; toe tips dilated into well-developed discs and bearing circum-marginal grooves; toes fully webbed and reaching to discs of all except fourth toe; subarticular tubercles obscured; distinct metatarsal tubercles; dorsum skin smooth with few tubercles.

Remarks. *Microhyla superciliaris* can be easily mistaken for *M. butleri* that occurs syntopically in the leaf litter at SLF. This species is a new record for the amphibians in Hulu Terengganu.

Micryletta dissimulans Suwannapoom, Nguyen, Pawangkhanant, Gorin, Chomdej, Che & Poyarkov, 2020

Fig. 5I

Camouflaged Paddy Frog

Examined specimens. Five specimens were collected from SRF consisted of males (UMTZC1519, UMTZC1521 and UMTZC1573, SVL = 18–23 mm) and females (UMTZC1520 and UMTZC1523, SVL = 23–26 mm).

Identification. Morphological characters of the specimens closely resembled the newly described *Micryletta dissimulans* from Saba Yoi District, Songkhla Province, Southern Thailand (Suwannapoom et al. 2020). Size (SVL: 18–23 mm, *n* = 3 males; 23–26 mm, *n* = 2 females); head longer than wide; snout round; interorbital distance two times wider than upper eyelid width; upper lips lacking white patches; tympanum small and barely visible; finger tips rounded; toe tips rounded and weakly dilated into small discs; fingers and toes without webbings; dorsum colour pale to reddish brown; dorsal pattern with merging brown blotches with beige edge; body flanks brown with black spots and whitish mottling.

Remarks. *Micryletta dissimulans* was commonly found at open areas with grass or places with piles of dead leaves. This species is typically found in early November of each year until the end of the monsoon season. This species is a new record for the amphibians in Hulu Terengganu.

Phrynella pulchra **Boulenger, 1887**
Fig. 6
Malacca's Narrow-mouthed Frog

Examined specimens. The only female specimen ever collected (UMTZC1302, SVL = 38 mm) which deposited as voucher without much associated information of habitat or site, but with a date of 2014 from SLF.

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975). Size (SVL: 38 mm, *n* = 1 female); small head; snout truncate; tympanum hidden; finger tips depressed and expended into large sub-triangular discs; fingers with distinct subarticular tubercles; toes completely webbed; inner metatarsal tubercles oval-shaped; anal region with large dark spots on that are distinctly separated by white colouration.

Remarks. The available information of this species is limited to a description and photograph of a preserved specimen; however, it is presumed to be found within SRF.

Families Megophryidae

Leptobrachium hendricksoni **Taylor, 1962**
Fig. 7A, B
Spotted Litter Frog

Examined specimens. Fourteen specimens were collected from SRF (Juvenile: UMTZC1406, SVL = 28 mm; Males: UMTZC1051, UMTZC1052, UMTZC1091, UMTZC1159, and UMTZC1256, SVL = 40–54 mm; Females: UMTZC1127, UMTZC1160, UMTZC1161, UMTZC1166, and UMTZC1192, SVL = 60–70 mm) and SAP (Juvenile: UMTZC1468, SVL = 35 mm; Males: UMTZC1455 and UMTZC1603, SVL = 40–48 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975) and Sumarli et al. (2015). Size (SVL: 28–35 mm, *n* = 2 juveniles; 40–54 mm, *n* = 7 males; 60–70 mm, *n* = 5 females); broad head; vomerine teeth absent; tongue notched posteriorly; snout rounded; tympanum distinct; distinct supratympanic fold from eyes to shoulder; tips of digits rounded; first and second finger almost equal in length; two distinct and large metacarpal tubercles; toes ½ webbed; inner metatarsal tubercle oval shaped, outer metatarsal tubercle absent; tibiotarsal joint reaches to shoulder or tympanum;

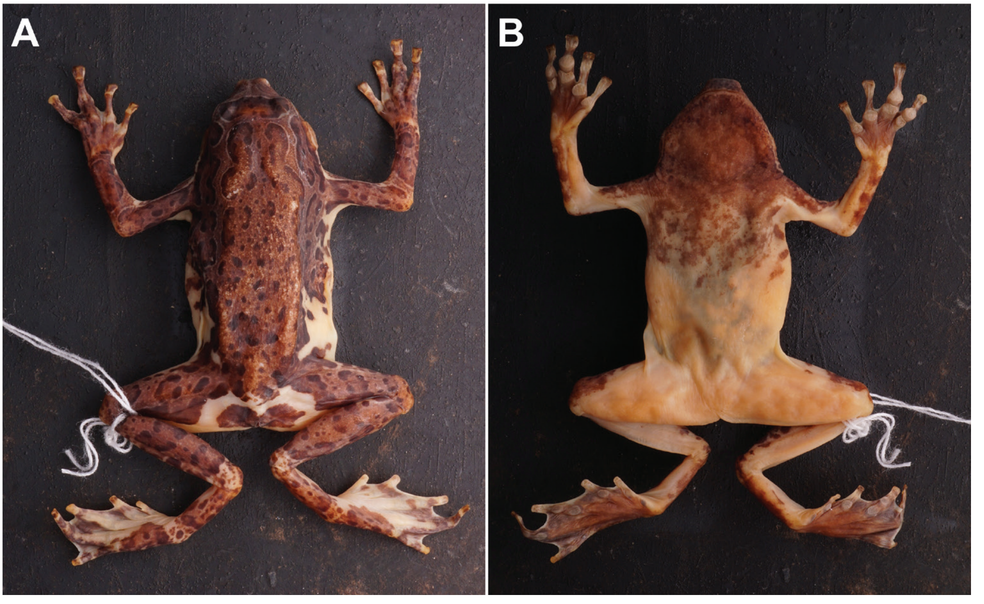


Figure 6. A dorsal view and **B** ventral view of *Phrynella pulchra* preserved specimen.

dorsum and venter smooth; dorsum colour dark brown to greyish; venter whitish with black speckling in adults (Fig. 7B). Juvenile specimens (UMTZC1406 and UMTZC1468) had blackish bodies; stumpy tails; and whitish venters with black dots (Fig. 7A).

Remarks. *Leptobrachium hendricksoni* was common along small streams, cleared areas and man-made ponds in SLF. This species is usually found hiding among grass, on the ground or leaf litter. The tadpoles of *L. hendricksoni* can be found in the small streams, throughout the year.

Leptobrachella sola **(Matsui, 2006)**
Fig. 7C
Spotted Litter Frog

Examined specimens. Eight male specimens were collected from SLF (UMTZC1378, UMTZC1379, UMTZC1408, UMTZC1409, UMTZC1410, UMTZC1411, UMTZC1473, and UMTZC1521, SVL = 20 to 27 mm).

Identification. Morphological characters of the specimens agreed well with the description of *L. sola* by Matsui (2006). Size (SVL: 20–27 mm, *n* = males); head longer than broad; vomerine teeth absent; snout rounded; tympanum distinct; supratympanic fold distinct; finger tips slightly swollen; indistinct subarticular tubercles on fingers; fingers unwebbed with first and fourth almost equal or longer than second; toes basally webbed; tibiotarsal articulation reaching nostril; inner metatarsal tubercle low and small; outer metatarsal tubercle absent; nuptial pads absent; dorsum with indistinct brown markings and blackish blotches on flanks.

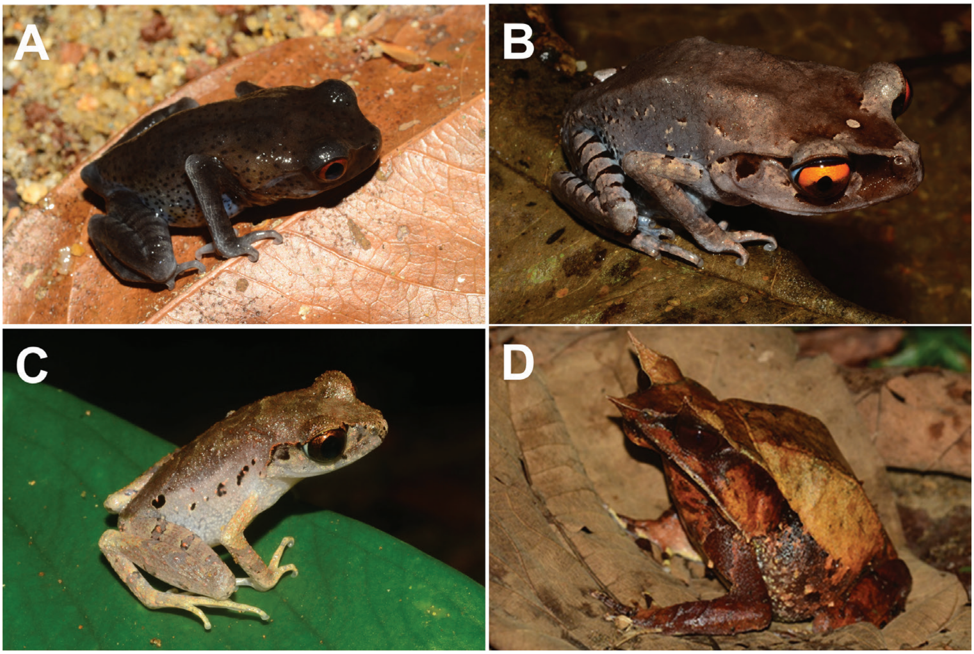


Figure 7. A juvenile *Leptobrachium hendricksoni* **B** adult *L. hendricksoni* **C** *Leptobrachella sola* **D** *Pelobatrachus nasutus*.

Remarks. *Leptobrachella sola* is commonly found near the stream banks, and is usually sighted sitting on low vegetation, bare ground, or piles of dead leaves. This species can be hard to spot during the night as they usually hide amongst the leaf litter and quickly hides beneath the litter when approached.

Pelobatrachus nasutus **(Schlegel, 1858)**
Fig. 7D
Malayan Horned Frog

Examined specimens. Three specimens were collected from SLF (Juveniles: UMTZC1103 and UMTZC1187, SVL = 36–38 mm; Male: UMTZC1494, SVL = 94 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975) and Sumarli et al. (2015). Size (SVL: 36–38 mm, n = 2 juveniles; 94 mm, n = 1 male); large heads; tongue completely or partly notched posteriorly; vomerine teeth present; snout truncate and projecting beyond the lower jaw; upper eyelids and snout form pointed dermal projection; tympanum distinct; distinct supratympanic fold from eyes to shoulders; subarticular tubercles indistinct; metacarpal tubercles distinct; dorsum smooth with few tubercles; two pairs of longitudinal skin fold on back reaching until the vent; venter smooth with small tubercles.

Remarks. *Pelobatrachus nasutus* were found on the forest floor at various locations: UMTZC1494, UMTZC1103 and UMTZC1187 were collected from the small stream of Peres Rivers where it was sighted on the forest floor. Afterwards, two individuals were observed on the forest floor beside the recreational trail at SRF, and three individuals were observed amongst the dead leaves at the banks of the small stream of the Peres River. Loud callings can be heard typically near the monsoon season.

Families Ranidae

Amolops gerutu Chan, Abraham, Grismer & Grismer, 2018

Fig. 8A
Warty Torrent Frog

Examined specimens. Thirteen specimens were collected from the SLF consisted of adult males (UMTZC1030, UMTZC1032, UMTZC1036, UMTZC1041, UMTZC1043, UMTZC1045, UMTZC1046, UMTZC1106, UMTZC1137, UMTZC1378, and UMTZC1505, SVL = 30–38 mm) and females (UMTZC1297 and UMTZC1377, SVL = 48–53 mm).

Identification. Morphological characters of the specimens agreed well with the description by Chan et al. (2018). Size (SVL: 30–38 mm, n = 11 males; 48–53 mm, n = 2 females); dorsum densely covered with irregular sized tubercles; dorsolateral region with slightly enlarged, elongated and ridge-like tubercles; dorsal surfaces of hind limb covered with denser and more pronounce tubercles; indistinct pectoral gland with pale yellowish patches.

Remarks. This species can only be found along the torrential zones of the Peres and Bubu Rivers in SLF. The species can be observed perched on the surfaces of boulder stacks along the streams, and occasionally on adjacent low vegetation. Tadpoles of *A. gerutu* can found clinging to boulders below the waterline.

Chalcorana labialis (Boulenger, 1887)

Fig. 8B, C
White-lipped Frog

Examined specimens. Twelve specimens were collected from SRF consisted of one juvenile (UMTZC1032, SVL = 20 mm), males (UMTZC1029, UMTZC1090, UMTZC1235, UMTZC1236, UMTZC1237, UMTZC1383, and UMTZC1384, SVL = 29–36 mm) and females (UMTZC1132, UMTZC1297, UMTZC1317, and UMTZC1622, SVL = 49–55 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975) and Hong et al. (2021). Size (SVL: 20 mm, n = 1 juvenile; 29–36 mm, n = 7 males; 49–55 mm, n = 4 females); vomerine teeth in oblique groups between choanae; snout pointed; tympanum distinct; digit tips of dilated into discs with circum-marginal grooves; first finger much shorter than second; nuptial pads present on first finger of males; toes webbed and reaching outer edge of first to third toes, inner edge of fifth toe, and fourth toe with one or two phalanges free of webbing; dorsum skin coarsely granular with weak dorsolateral fold; dorsum colour variable from pale green, brownish or pale yellow.

Remarks. This species can be found at many swampy locations and flowing streams within SLF. *Chalcorana labialis* was usually observed perched on the surface of low vegetation along the streams and swampy areas, and was occasionally found at artificial ponds. All collected and observed individuals were found lower than 2 metres from the ground.

***Humerana miopus* (Boulenger, 1918)**
Fig. 8D
Three-striped Frog

Examined specimens. Four specimens were collected from SRF consisted of juvenile (UMTZC1682, SVL = 23 mm), males (UMTZC1007 and UMTZC1379, SVL = 62–65 mm) and female (UMTZC1472, SVL = 70 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975). Size (SVL: 23 mm, *n* = 1 juvenile; 62–65 mm, *n* = 2 males; 70 mm, *n* = 1 female); vomerine teeth in two oblique series between choanae; snout obtusely pointed; tympanum distinct; finger tips expended into small discs bearing circum-marginal grooves; first finger much longer than second; distinct subarticular tubercles on fingers and toes; toes 2/3 to ¾ webbed with two phalanges of fourth toe free from webbing; inner metatarsal tubercles elliptic; outer metatarsal tubercles indistinct or absent; skin smooth with dorsolateral fold; dorsum with two to three diagonal lines on mid-dorsum region.

Remarks. *Humerana miopus* was common at the artificial ponds of the Herbal Park in SRF. The species was frequently observed perched on low vegetation beside the artificial ponds, and quickly leapt into the water when disturbed.

***Hylarana erythraea* (Schlegel, 1837)**
Fig. 8E
Green Paddy Frog

Examined specimens. Eight specimens were collected from SAP consisted of juvenile (UMTZC1264, SVL = 25 mm), males (UMTZC1182, UMTZC1183, UMTZC1184, and UMTZC1186, SVL = 30–40 mm) and females (UMTZC1050, UMTZC1055, and UMTZC1165, SVL = 42–73 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975) and Davis et al. (2018). Size (SVL: 25 mm, *n* = 1 juvenile; 30–40 mm, *n* = 4 males; 42–73 mm, *n* = 3 females); vomerine teeth in two oblique group between choanae; head slightly longer than broad; snout pointed; tympanum distinct; weak supratympanic fold; digit tips of expanded into disc bearing circum-marginal grooves; first finger longer than second; toes webbed reaching base of disc in first to third toes, inner edge of fifth, and fourth toes with two phalanges free; inner metatarsal tubercles oval shaped; outer metatarsal tubercles rounded; dorsum smooth with distinct dorsolateral fold; dorsum colour brown with a pale-coloured dorsolateral stripe and brown flanks; ventral surface white.

Remarks. *Hylarana erythraea* was common in the swampy areas in SAP. The species was also observed by drains, artificial ponds, and sometimes on grassy areas.

***Indosylvirana nicobariensis* (Stoliczka, 1870)**
Fig. 8F
Nicobar Island Frog

Examined specimens. Three specimens were collected from SRF consisted of one male (UMTZC1571, SVL = 50 mm) and two females (UMTZC1563 and UMTZC1676, SVL = 42–43 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975) and Shahriza and Ibrahim (2014). Size (SVL: 50 mm, n =1 male; 42–43 mm, n = 2 females); vomerine teeth in two oblique series between choanae; head longer than wide; snout pointed; tympanum distinct; supratympanic fold absent; first finger longer (UMTZC1571) or equal (UMTZC1563) with second; digit tips expended into small discs bearing circum-marginal grooves; nuptial pad present on first fingers in males; dorsum skin granular; narrowed dorsolateral folds; gravid female with eggs on translucent side of belly.

Remarks. This species was common at the Herbal Park in SRF. The species can be observed perching on rock surfaces at artificial ponds and is abundant at the start of monsoon season. *Indosylvirana nicobariensis* is a pond-breeding frog and can inhabit both natural and altered habitats (Lalremsanga et al. 2016).

***Odorrana hosii* (Boulenger, 1891)**
Fig. 8G
Poisonous Rock Frog

Examined specimens. Twenty adult specimens were collected from SRF (Males: UMTZC1164, UMTZC1170, UMTZC1171, UMTZC1172, UMTZC1173, UMTZC1174, UMTZC1233, UMTZC1299, and UMTZC1304, SVL = 32–66 mm; Females: UMTZC1009, UMTZC1044, and UMTZC1063, SVL = 54–96 mm) and SAP (Males: UMTZC1348, UMTZC1351, UMTZC1385, and UMTZC1504, SVL = 32–50 mm; Females: UMTZC1306, UMTZC1323, UMTZC1380, and UMTZC1481, SVL = 48–98 mm).

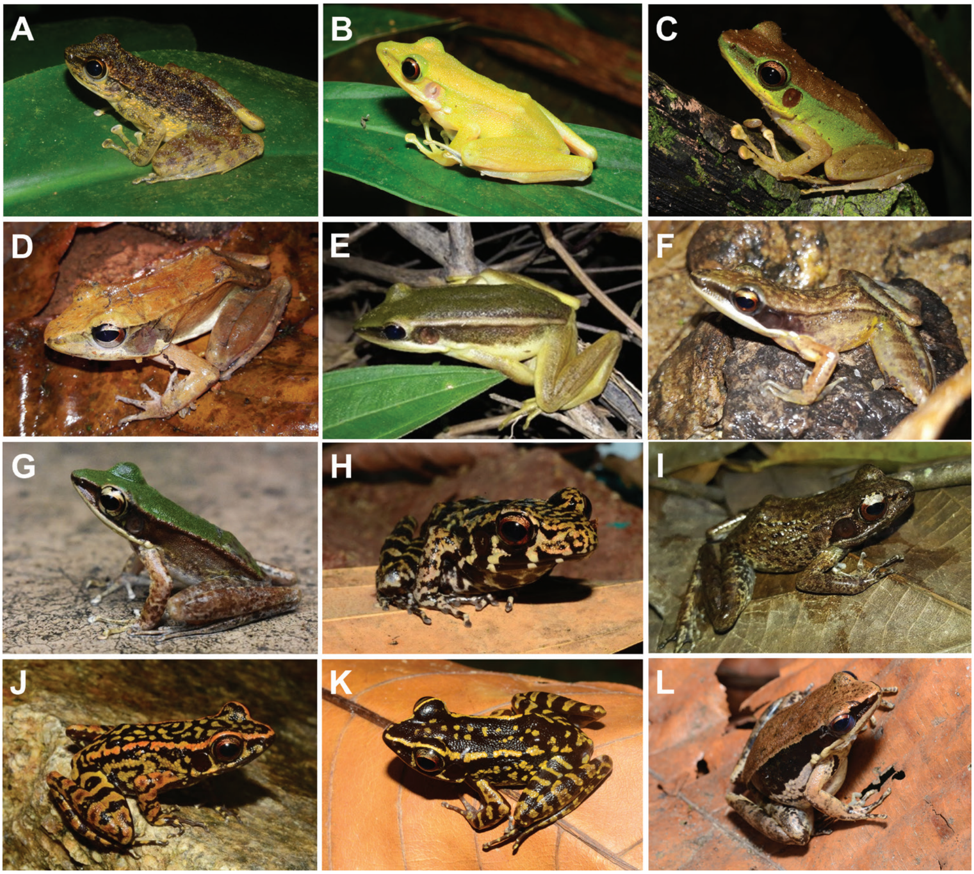


Figure 8. **A** *Amolops gerutu* **B** *Chalcorana labialis* (pale yellow colouration) **C** *C. labialis* (brown colouration) **D** *Humerana miopus* **E** *Hylarana erythraea* **F** *Indosylvirana nicobariensis* **G** *Odorrana hosii* **H** *Pulchrana glandulosa* **I** *P. laterimaculata* **J** *P. sundabarat* (orange dorsolateral stripe) **K** *P. sundabarat* (yellow dorsolateral stripe) and **L** *Sylvirana malayana*.

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975) and Hong et al. (2021). Size (SVL: 32–66 mm, n = 13 males; 48–98 mm, n = 7 females); vomerine teeth in two oblique series behind choanae; head as long as broad with pointed snout; tympanum distinct; supratympanic fold; tips of digits expanded into large discs with circum-marginal grooves; first finger equal or shorter than second, and all marked with narrow fringes of skin; nuptial pads on first fingers of males; broad webbing reaching tips of all toes; dorsum skin smooth with weak dorsolateral fold.

Remarks. *Odorrana hosii* was ubiquitous at the rocky sections of streams with many boulders. All individuals were collected at night but specimens could be observed in the day hiding in the roots of large trees at the stream bank.

***Pulchrana glandulosa* (Boulenger, 1882)**
Fig. 8H
Poisonous Gland Frog

Examined specimens. Four male specimens were collected from SRF (UMTZC1301, UMTZC1350, UMTZC1576, and UMTZC1608, SVL = 43–74 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975) and Sumarli et al. (2015). Size (SVL: 43–74 mm, *n* = 4 males); vomerine teeth in two oblique series between choanae; head large, rounded snout; tympanum distinct; weak supratympanic fold; digit tips dilated into small discs bearing circum-marginal grooves; first finger much longer than second; skin fringes absent on fingers; toes webbing not well-developed; inner metatarsal tubercles oval-shaped; outer metatarsal tubercles small and rounded; dorsum without dorsolateral fold; dorsum surfaces covered with low and rounded glandular warts; dorsum colour greyish to dark brown with indistinct dark blotches; limbs with dark cross bars.

Remarks. *Pulchrana glandulosa* was found in the small streams and recreational areas of SLF. The species was commonly observed hidden amongst piles of dead leaves and rotten logs.

***Pulchrana laterimaculata* (Barbour & Noble, 1916)**
Fig. 8I
Side-spotted Swamp Frog

Examine specimens. One specimen of adult female specimen was collected from SLF (UMTZC1699, SVL = 50 mm).

Identification. Morphological characters of the specimen agreed well with the description by Sumarli et al. (2015) and Leong et al. (2003). Size (SVL: 50 mm, *n* = 1 female); vomerine teeth in two oblique rows bounded by choanae; head moderate; relatively rounded snout; tympanum distinct and entirely black; upper lips with uninterrupted white line; finger and toe tips expended into discs; toes well-developed webbing but not reaching medial subarticular tubercles; dorsum and flanks with raised rounded tubercles, forming discontinuous longitudinal ridges; distinct humeral glands in male; dorsum pale brown.

Remarks. *Pulchrana laterimaculata* can be found in areas similar to *P. glandulosa* in SLF. The species was commonly observed hidden among piles of dead leaves and rotten logs.

***Pulchrana sundabarat* Chan, Abraham, Grismer & Brown, 2020**
Fig. 8J, K
Western Sunda Spotted Stream Frog

Examine specimens. Four specimens were collected from SRF consisted of three males (UMTZC1376, UMTZC1377, and UMTZC1387, SVL = 40 mm) and one female (UMTZC1375, SVL = 57 mm).

Identification. Morphological characters of the specimens agreed well with the description by Chan et al. (2020c). Size (SVL: 40 mm, *n* = 3 males; 57 mm, *n* = 1 female); head longer than wide; snout pointed; tympanum distinct; supratympanic fold absent; digit tips slightly expended into small disc with circum-marginal groove; nuptial pads distinctly separated on first finger in UMTZC1376 and UMTZC1377, and slightly joined in UMTZC1387; toes slightly more than ½ webbed; dorsum smooth and indistinctly glandular; dorsum colour black; dorsum patterns with conspicuous and defined yellowish to bright orange dorsolateral stripe, dorsum and flanks with yellowish blotches; humeral gland raised and blackly pigmented in males; throat and abdomen with white spots.

Remarks. Chan et al. (2020b, c) revised this complex and suggested that the *Pulchrana picturata* from the Malay Peninsula and Sumatra belong to this new species, *P. sundabarat* that is genetically distinct from the true Bornean *P. picturata.* The specimens were all found at the pristine areas in SRF. This species usually hide beneath the roots of large trees on stream banks. The distinct calls of males could be easily heard in those areas.

Sylvirana malayana **Sheridan & Stuart, 2018**
Fig. 8L
Malayan Black-striped Frog

Examined specimens. Seven specimens were collected from SRF (Males: UMTZC1013, UMTZC1014, UMTZC1107, and UMTZC1382, SVL = 43–47 mm; Females: UMTZC1093 and UMTZC1381, SVL = 51–58 mm) and SAP (Males: UMTZC1308, SVL = 45 mm).

Identification. Morphological characters of the specimens agreed well with the description by Sheridan and Stuart (2018). Size (SVL: 43–47 mm, *n* = 5 males; 51–58 mm, *n* = 2 females); head longer than wide; snout obtusely pointed; tympanum distinct; triangular or teardrop shaped fold slightly behind the tympanum; supratympanic fold absent; digit tips of expended into discs with circum-marginal grooves; first finger much longer than second; toes with well-developed webbing; distinct subarticular tubercles; elongated inner metatarsal tubercle; rounded outer metatarsal tubercle; dorsum finely granular; dorsum brown with broad dark band extending from snout to groin.

Remarks. *Sylvirana malayana* can be found in the disturbed areas of SLF. This species was commonly observed hiding between rock crevices within the Herbal Park.

Families Rhacophoridae

Kurixalus chaseni **(Smith, 1924)**
Fig. 9A
Malay Frilled Tree Frog

Examined specimens. Ten adult specimens were collected from SRF (Males: UMTZC1095, UMTZC1345, UMTZC1360, UMTZC1361, UMTZC1463, UMTZC1465, UMTZC1574, and UMTZC1627, SVL = 30–41 mm; Female: UMTZC1359, SVL = 44 mm) and SAP (Male: UMTZC1502, SVL = 22 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975) and Sumarli et al. (2015). Size (SVL: 22–41 mm, *n* = 9 males; 44 mm, *n* = 1 female); vomerine teeth at the anterior edges of choanae; head longer than wide; rounded snout with conical projection on tip; tympanum distinct; digit tips dilated into small discs bearing circum-marginal grooves; fingers webbed at base; toes broadly webbed; fourth fingers, tarsus, heel and vent with crenulated dermal fringes; dorsum smooth and slightly granular; dorsum colour pale brown to mossy green.

Remarks. *Kurixalus chaseni* can be found perched on low vegetation within artificial ponds of the Herbal Park. Active calls of males can be heard from this place during the monsoon season in SLF.

Nyctixalus pictus (Peters, 1871)

Fig. 9B
Cinnamon Tree Frog

Examined specimens. Three adult male specimens were collected from SRF (UMTZC1425 and UMTZC1426, SVL = 29–30 mm) and SAP (UMTZC1601, SVL = 35 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975). Size (SVL: 29–35 mm, *n* = 3 males); no vomerine teeth; head longer than broad; snout obtusely pointed; vertical loreal region; tympanum distinct; digit tips expanded into round or oval-shaped discs; inner metatarsal tubercle oval-shaped, outer metatarsal tubercle absent; dorsum bright orange with whitish spots scattered over the body; limbs with rows of whitish spots forming cross-bars; abdomen with greenish black reticulation.

Remarks. UMTZC1601 was found on low vegetation, less than 1 m from the ground near the suspension bridge at SAP, and UMTZC1426 and UMTZC1425 were found at bushy areas within a small stream off the Peres River in SRF. This species is a new record for the amphibians in Hulu Terengganu.

Polypedates colletti (Boulenger, 1890)

Fig. 9C
Collett's Tree Frog

Examined specimen. One adult male specimen was collected from SAP (UMTZC1871, SVL = 67 mm).

Identification. Morphological characters of the specimen agreed well with the description by Berry (1975) and Rujirawan et al. (2013). Size (SVL: 67 mm, *n* = 1 male);

triangular head; snout acutely pointed; low tubercles around eyes; tympanum distinct; skin of head not co-ossified with skull; skin coarsely granular; small white spots on rear thigh; heel with a distinct conical tubercle; dorsum pattern with an hour-glass marking extending from interorbital region to back, flanks with black vermiculations.

Remarks. This species was found at the waterfall of SAP, perched on low vegetation less than 2 m from the ground.

Polypedates discantus **Rujirawan, Stuart & Aowphol, 2013**
Fig. 9D
Malayan Slender Tree Frog

Examined specimens. Twenty-seven specimens were collected from SLF consisted of males (UMTZC1012, UMTZC1015, UMTZC1016, UMTZC1024, UMTZC1081, UMTZC1094, UMTZC1096, UMTZC1113, UMTZC1115, UMTZC1185, UMTZC1240, UMTZC1273, UMTZC1421, UMTZC1509, UMTZC1510, UMTZC1589, and UMTZC1592, SVL = 40–53 mm) and females (UMTZC1006, UMTZC1027, UMTZC1157, UMTZC1179, UMTZC1241, UMTZC1423, UMTZC1424, UMTZC1496, UMTZC1590, and UMTZC1591, SVL = 60–71 mm).

Identification. Based on description by Rujirawan et al. (2013), 29 specimens were identified as *P. discantus* for having size (SVL: 40–53 mm, *n* = 17 males; 60–71 mm, *n* = 10 females); triangular head; snout obtusely pointed; skin of head not co-ossified with skull; tympanum distinct; supratympanic fold distinct; digit tips with well-developed disc bearing circum-marginal groove; fingers webbed at base; toes fully webbed; inner metatarsal tubercle oval-shaped; outer metatarsal tubercle absent; nuptial pad present on first and second fingers in males; rear thigh with indistinct or absence of white spots; heel with rounded tubercle; dorsum with variable patterns: plain dorsum with scattered dark blotches, 2 to 4 longitudinal stripes, or X-shaped marking on interorbital region.

Remarks. This species was common in both natural and man-made habitats of SLF. Most of the collected and observed individuals were found clinging to shrubs or leaves of low vegetation less than 2 m from the ground. This species is a new record for the amphibians in Hulu Terengganu.

Polypedates leucomystax **(Gravenhorst, 1829)**
Fig. 9E
Four-lined Tree Frog

Examined specimens. Twenty-seven specimens were collected from SLF consisted of males (UMTZC1010, UMTZC1114, UMTZC1128, UMTZC1131, UMTZC1180, UMTZC1181, UMTZC1243, UMTZC1244, UMTZC1246,

UMTZC1328, UMTZC1329, UMTZC1422, UMTZC1485, UMTZC1624, and UMTZC1417, SVL = 40–52 mm) and females (UMTZC1125, UMTZC1130, UMTZC1154, UMTZC1155, UMTZC1156, UMTZC1178, UMTZC1245, UMTZC1263, UMTZC1272, UMTZC1344, UMTZC1347, and UMTZC1444, SVL = 44–67 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975) and Sumarli et al. (2015). Size (SVL: 40–52 mm; *n* = 15 males; 44–67 mm, *n* = 12 females); vomerine teeth in between choanae; head longer than broad; rounded snout; tympanum distinct; supratympanic fold present; digit tips expended into large discs with circum-marginal grooves; fingers without webbing; toes fully webbed; forearm with whitish skin flaps; dorsum skin smooth with colour pale to dark tan; dorsum pattern variable with two to four longitudinal stripes or plain with dark blotches.

Remarks. *Polypedates leucomystax* can be distinguished from *P. discantus* by having the skin of the head fused with the skull, distinct white spots or reticulations on a dark background on the rear thigh, and the absence of a tubercle at the heel. This species known as a commensal species and inhabits all manner of human-made and natural habitats in SLF. This species can be observed clinging onto shrubs or the leaves of low vegetation up to 2.5 m off the ground.

Polypedates macrotis (Boulenger, 1891)

Fig. 9F

Dark-eared Tree Frog

Examined specimens. Nine specimens were collected from SRF consisted of males (UMTZC1327, UMTZC1346, UMTZC1418, UMTZC1419, and UMTZC1497, SVL = 60–68 mm) and females (UMTZC1030, UMTZC1097, UMTZC1416, and UMTZC1454, SVL= 62 to 102 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975) and Sumarli et al. (2015). Size (SVL = 60–68 mm, *n* = 5 males; 62 to 102 mm, *n* = 4 females); vomerine teeth in transverse or slightly oblique series between choanae; head broader than long; rounded snout; tympanum distinct and covered with broad dark stripes; supratympanic fold distinct; digit tips expanded into large discs bearing circum-marginal grooves; fingers free of webbing; nuptial pads present on dorsal portion of first and second fingers; toes fully webbed; dorsum with variable markings from two broad longitudinal stripes (UMTZC1327, UMTZC1419, and UMTZC1497), scattered dark blotches (UMTZC1346, UMTZC1416, and UMTZC1418), plain dorsum (UMTZC1030, UMTZC1097, UMTZC1346, and UMTZC1454), and combination of plain and scattered dark blotches (UMTZC1418).

Remarks. *Polypedates macrotis* is restricted to natural or man-made stagnant water bodies closer to the forested areas. This species can also be observed perched on low vegetation near the ponds in syntopy with other species of *Polypedates*.

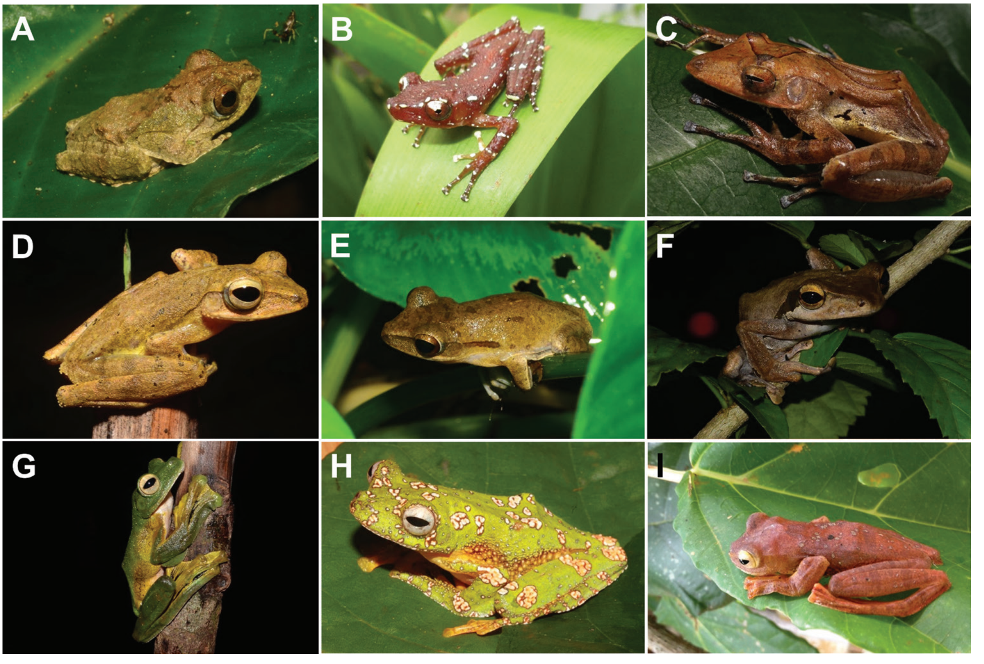


Figure 9. A *Kurixalus chaseni* **B** *Nyctixalus pictus* **C** *Polypedates colletti* **D** *P. discantus* **E** *P. leucomystax* **F** *P. macrotis* **G** adult *Rhacophorus nigropalmatus* **H** subadult *R. nigropalmatus* **I** *R. pardalis*.

***Rhacophorus nigropalmatus* Boulenger, 1895**
Fig. 9G, H
Wallace's Flying Frog

Examined specimens. Two specimens were collected from SRF consisted of subadult male (UMTZC1732, SVL = 60 mm) and adult male (UMTZC1057, SVL = 95 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975). Size (SVL: 60 mm for subadult male; 95 mm for adult male); vomerine teeth in two straight or transversely curved rows between choanae; head longer than broad; rounded snout; tympanum distinct; supratympanic fold absent; digit tips expanded into large and oval-shaped discs and bearing circummarginal grooves; fingers and toes fully webbed; inner metatarsal tubercle oval-shaped; outer metatarsal tubercle absent; dorsum with small clusters of whitish tubercles; broad skin flaps along forearm, rounded on heels, and awning-like flaps on anal region. Head, body, and limbs of subadult male (UMTZC1732) (Fig. 9H) covered with white patches edged in pale brown.

Remarks. UMTZC1057 was collected from a fallen large tree at the Herbal Park within SRF while the second individual UMTZC1732 was found perched on low vegetation at the same location. This species was only encountered during the monsoon season in SLF.

Rhacophorus pardalis Günther, 1858

Fig. 9I
Harlequin Tree Frog

Examined specimens. Two male specimens were collected from SRF (UMTZC1111 and UMTZC1110, SVL = 49–53 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975) and Harvey et al. (2002). Size (SVL: 49–53 mm, *n* = 2 males); vomerine teeth in two or slightly oblique series at inner edges of choanae; head equally longer with width; snout obtusely pointed; tympanum distinct; supratympanic fold reaching angle of jaws; fingers and toes fully webbed; broad skin flaps along forearm, rounded on heels, and absence on anal region; flanks and abdomen of UMTZC1111 displayed black reticulation, but pale orange in UMTZ1110.

Remarks. Both UMTZC1111 and UMTZC1110 were collected from the large trees beside the artificial pond of the camping site in SRF.

Theloderma licin McLeod & Norhayati, 2007

Fig. 10A
Smooth-skinned Warted Tree Frog

Examined specimens. One male specimen was collected from SAP (UMTZC1490, SVL = 28 mm).

Identification. Morphological characters of the specimen agreed well with the description by McLeod and Norhayati (2007). Size (SVL: 28 mm, *n* = 1 male); head equally longer with wide; snout obtusely pointed; tympanum distinct; dorsal and lateral surfaces with fine pearly tipped tubercles; coarsely granular venter; no vomerine teeth; tympanum distinct; supratympanic fold reaching angle of jaws; digit tips expended into large disc bearing circum-marginal grooves; fingers webbed at base; toes fully webbed; inner metatarsal tubercle oval-shaped; outer metatarsal tubercle absent; nuptial pad present on second fingers in males; dorsum colour changed from whitish to pale brown when stressed; inguinal area with dark brown blotches.

Remarks. *Theloderma licin* was found resting on the ground within piles of dead leaves. This species is a new record for the amphibians in Hulu Terengganu.

Theloderma horridum (Boulenger, 1903)

Fig. 10B
Malayan Warted Treefrog

Examined specimens. No specimen was collected, but photographs of this species from from previous fieldwork in 2014 in SRF (UMTZCP070614-392, Fig. 11B).

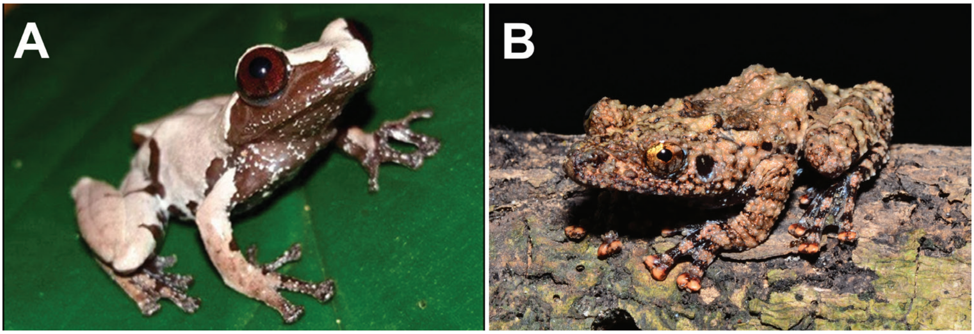


Figure 10. A *Theloderma licin* and **B** *T. horridum*.

Identification. Examined photographs show similar characteristics as described by Berry (1975) and Sumarli et al. (2015) for having stocky body; head wide; distinct tympanum; digit tips expended into distinct disc bearing circum-marginal groove; fingers ½ webbed; toes fully webbed; dorsum rough with warts bearing granular asperities; large lumbar spot; and venter with black-white reticulations.

Remarks. This species was found among the dense vegetation on steep terrain in the forested areas in SRF. *Theloderma horridum* was spotted clinging to the side of a tree trunk facing upwards.

***Zhangixalus prominanus* (Smith, 1924)**
Fig. 11A
Green Tree Frog

Examined specimens. Two male specimens were collected from SRF (UMTZC1469 and UMTZC1299, SVL = 56–57 mm).

Identification. Morphological characters of the specimens agreed well with the description by Berry (1975). Size (SVL: 56–57 mm, n = 2 males); vomerine teeth in two transverse series between choanae; head broader than long; snout obtusely pointed; tympanum distinct; supratympanic fold hidden; tips of digits expanded into large and oval discs with circum-marginal grooves; fingers and toes fully webbed; broad skin flaps along forearm, rounded on heels, and awning-like skin flaps on anal region.

Remarks. Two specimens of *Zhangixalus prominanus* were collected from the flooded rock pools within the recreational zones of SRF. Another observed individual was also seen perched on low vegetation or rock surfaces in the same area, only found during the monsoon season. This species is a new record for the amphibians in Hulu Terengganu.

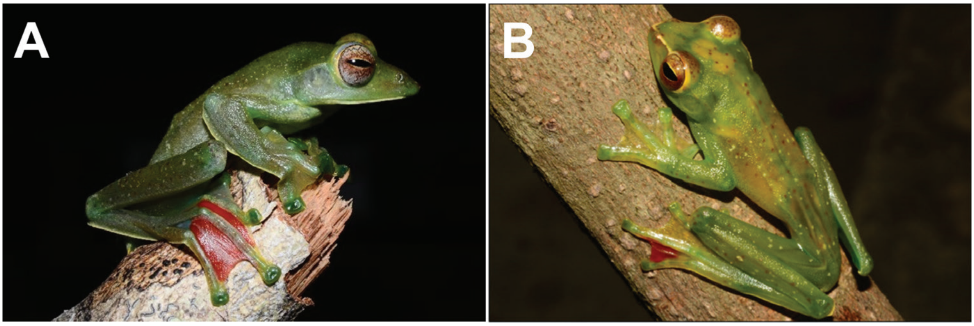


Figure 11. A *Zhangixalus prominanus* and **B** *Z. tunkui*.

***Zhangixalus tunkui* Kiew, 1987**
Fig. 11B
Johore Flying Frog

Examine specimens. UMTZCP110414-419.

Identification. One individual of *Zhangixalus tunkui* was previously recorded in SRF. However, it was only photographed (UMTZCP110414-419, Fig. 11B) and released back to the wild for it was mistaken to be an individual of *Z. prominanus*, which was only later identified as *Z. tunkui* by Evan S.H. Quah. Morphology of photographed individual matched description by Kiew (1987) and Leong (2004) based on toe webbing with reddish colouration between fourth and fifth toes (vs. third to fifth toes in *Z. prominanus*). Photographed individual had body with pale and translucent green colouration; dorsum scattered with whitish spots; smaller SVL, less than 50 mm (M. Taufik Awang, pers. comm.); skin translucent; absence of skin flaps on along forearm; whitish line along the snout, canthus rostralis and around eyes.

Remarks. The individual of this species was found at the flooded rock pools in SRF. This species is a new record for the amphibians in Hulu Terengganu.

From 14 species recorded in the first survey in 2003, the number of species had steadily increased to 18 species in 2008. The surveys continued in 2013 and recorded more species, with a total of 38 species in 2015. This trend of species discoveries kept increasing during the surveys up to the end of 2020. The results shown by the species accumulation curve generated from the list of species recorded between 2003 and 2018 show a constantly increasing trend (Fig. 12). The species accumulation curve has almost reached the asymptote, which indicates that our long-term surveys may have reached the true diversity of amphibians in SLF. The number of unique and duplicate species also kept decreasing over the years. However, even after our exhaustive fieldwork from 2015 to 2020, a few unique species such as *Phrynella pulchra*, *Rhacophorus pardalis*, *Theloderma horridum*, and *Zhangixalus tunkui* have remained unrecorded after their first discovery in 2013-2014. As for the records of amphibians in Hulu Terengganu District, this study contributes an additional 10 new records for this area, which now totals 70 species so far known (Table 2). The new records are *Ichthyophis* cf. *asplenius*, *K. palmatissimus*, *Kaloula latidisca*, *Micryletta dissimulans*, *Microhyla superciliaris*, *Nyctixalus pictus*, *Polypedates discantus*, *Zhangixalus prominanus, Z. tunkui* and *Theloderma licin*.

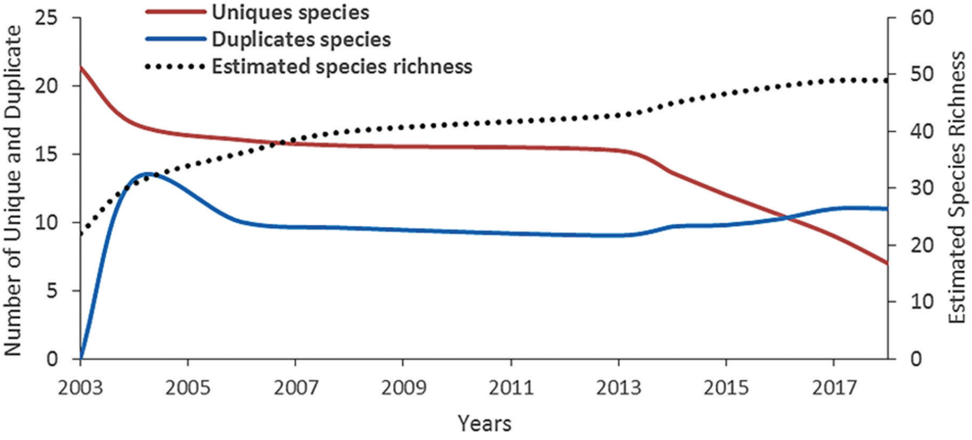


Figure 12. The cumulative species discovery curve of the species (dotted line), species accumulation curve of replicated samples of the amphibians from previous and recent surveys (black line). Red line shows the estimated number of unique, and duplicate species (blue line) of amphibians from long term surveys in Sekayu lowland forest, Hulu Terengganu.

Table 2. Compilation of updated and revised checklist of amphibian fauna from published materials and this study in Hulu Terengganu, Terengganu. Note: DD = Data Deficient, NE = Not Evaluated, LC = Least Concerned, NT = Near Threatened, EN = Endangered, NP = Not Protected, and P = Protected. Symbol * = represents the additional record of species in Hulu Terengganu, 1 = Sekayu (This study), 2 = Gunung Lawit (Dring 1979; Sumarli et al. 2015), 3 = Tembat (Norhayati et al. 2011; Nur Amalina et al. 2017), and 4 = Gunung Gagau (Hamidi 2013).

No	Species	IUCN Status	WCA 2010	1	2	3	4
	Family Ichthyophiidae						
1	*Ichthyophis* cf. *asplenius**	DD	NP	+	–	–	–
2	*Ichthyophis glutinosus*	VU	NP	–	–	+	–
3	*Ichthyophis* sp.	DD	NP	–	+	+	–
	Family Bufonidae						
4	*Ansonia latiffi*	NT	NP	+	+	–	+
5	*Ansonia lumut*	NE	NP	+	+	–	–
6	*Duttaphrynus bengalensis* (*Duttaphrynus* sp.1)	LC	NP	+	–	+	–
7	*Ingerophrynus parvus*	LC	NP	+	+	+	+
8	*Ingerophrynus quadriporcatus*	LC	NP	–	+	+	–
9	*Leptophryne borbonica*	NE	NP	+	+	+	+
10	*Phrynoidis asper*	LC	NP	+	+	+	+
11	*Rentapia flavomaculata*	NE	NP	+	+	+	–
	Family Dicroglossidae						
12	*Fejervarya cancrivora*	LC	NP	–	–	+	–
13	*Fejervarya limnocharis*	LC	NP	+	+	+	+
14	*Ingerana tenasserimensis*	LC	NP	–	–	+	–
15	*Limnonectes blythii*	NT	P	+	+	+	+
16	*Limnonectes hascheanus*	LC	NP	+	–	–	+
17	*Limnonectes utara*	NE	NP	+	+	+	+
18	*Limnonectes deinodon*	NE	NP	+	+	+	–
19	*Limnonectes malesianus*	NT	P	+	–	+	–
20	*Limnonectes tweediei*	LC	P	–	+	–	–

No	Species	IUCN Status	WCA 2010	1	2	3	4
21	*Limnonectes paramacrodon*	NT	P	–	+	–	+
22	*Limnonectes plicatellus*	LC	NP	+	+	+	–
23	*Occidozyga sumatrana*	LC	NP	+	+	+	–
24	*Occidozyga martensii*	LC	NP	+	+	–	–
	Family Megophryidae						
25	*Leptobrachium hendricksoni*	LC	NP	+	+	+	+
26	*Leptobrachella heteropus*	LC	NP	–	–	+	+
27	*Leptobrachella sola*	EN	NP	+	+	–	–
28	*Pelobatrachus nasutus*	LC	P	+	+	+	+
29	*Xenophrys aceras*	LC	P	–	+	–	–
	Family Microhylidae						
30	*Chaperina fusca*	LC	NP	–	–	+	–
31	*Kalophrynus kiewi*	NE	NP	+	+	+	–
32	*Kalophrynus palmatissimus**	EN	P	+	–	–	–
33	*Kaloula latidisca**	DD	NP	+	–	–	–
34	*Kaloula pulchra*	LC	NP	+	–	+	–
35	*Microhyla bedmorei*	LC	NP	+	+	+	–
36	*Microhyla butleri*	LC	NP	+	+	+	–
37	*Microhyla heymonsi*	LC	NP	+	+	+	–
38	*Microhyla superciliaris**	LC	NP	+	–	–	–
39	*Microhyla mantheyi*	LC	NP	–	+	–	–
40	*Micryletta dissimulans**	LC	NP	+	–	–	–
41	*Metaphrynella pollicaris*	LC	NP	–	+	–	–
42	*Phrynella pulchra*	LC	NP	+	–	+	–
	Family Ranidae						
43	*Abavorana luctuosa*	LC	NP	–	+	–	–
44	*Amolops gerutu*	NE	NP	+	+	+	+
45	*Chalcorana labialis*	NE	P	+	+	+	+
46	*Humerana miopus*	LC	NP	+	–	+	–
47	*Hylarana erythraea*	LC	P	+	–	+	+
48	*Indosylvirana nicobariensis*	NE	NP	+	–	+	–
49	*Odorrana hosii*	LC	P	+	+	+	+
50	*Pulchrana baramica*	LC	NP	–	–	+	–
51	*Pulchrana glandulosa*	LC	NP	+	–	+	–
52	*Pulchrana laterimaculata*	LC	NP	+	+	+	–
53	*Pulchrana sundabarat*	LC	P	+	+	+	+
54	*Sylvirana malayana*	NE	NP	+	–	+	+
	Family Rhacophoridae						
55	*Kurixalus chaseni*	NE	NP	+	+	–	–
56	*Nyctixalus pictus**	NT	P	+	–	–	–
57	*Philautus vermiculatus*	LC	NP	–	+	–	–
58	*Polypedates colletti*	LC	P	+	+	+	–
59	*Polypedates discantus**	NE	NP	+	–	–	–
60	*Polypedates leucomystax*	LC	NP	+	+	+	–
61	*Polypedates macrotis*	LC	NP	+	+	+	–
62	*Rhacophorus rhodopus*	LC	NP	–	+	–	–
63	*Rhacophorus nigropalmatus*	LC	P	+	+	–	–
64	*Rhacophorus pardalis*	LC	P	+	+	–	–
65	*Rhacophorus norhayatiae*	NE	NP	–	+	–	–
66	*Theloderma leprosum*	NE	NP	–	+	–	–
67	*Theloderma horridum*	LC	NP	+	+	–	–
68	*Theloderma licin**	LC	NP	+	–	–	–
69	*Zhangixalus prominanus**	LC	P	+	–	–	–
70	*Zhangixalus tunkui**	NE	NP	+	–	–	–
	Total numbers of species			**52**	**44**	**41**	**18**

Discussion

This extensive survey on amphibian fauna in SLF highlights the immense biodiversity that can be found on a local scale through long-term inventory study, hence demonstrating the potential of Hulu Terengganu forests as one of the nation's important biodiversity spots. The approach also affirms the importance of long-term and comprehensive surveys, regardless of substantial time, cost and effort are needed, especially in tropical countries. The long-term surveys in SLF have successfully captured the variation in abundance and composition of amphibians between habitats and the temporal patterns that are apparently impossible in short-term or rapid surveys. In addition, continuous, standardised sampling efforts and multi-habitat surveys could provide the most useful baseline information on the status and trends of amphibian communities in tropical lowland forest for biodiversity monitoring.

Amphibian community particularly in tropical forests are closely related to seasonal factors (Duellman and Trueb 1986; Praderio and Robinson 1990; Pearman et al. 1995), with many of the specialist or cryptic amphibians influenced by changing monsoon patterns (Gibbons and Bennett 1974; Bury and Corn 1987; Crosswhite et al. 1999). This can be indicated by the discovery of several new records during our recent surveys. For instance, seasonal breeding anurans such as *Micryletta dissimulans*, *Kaloula latidisca*, and *Nyctixalus pictus*, (Chan et al. 2014b; Das et al. 2019), and rarer arboreal species like *Rhacophorus nigropalmatus* and *Theloderma licin* were only found between October and early January. We can only assume they are influenced by the northeast monsoon (Muhammad et al. 2007; Mandeep 2008; Varikoden et al. 2011). Heavy rainfall would inundate and raise the temporary water bodies over the open forested areas and serve as a cue for breeding season for most of the tropical species (Rastogi et al. 2011).

Expanding the survey areas that have been overlooked before, such as hilly areas of forests and small streams, has been rewarding as additional species were added to the list. Hilly forested areas and small streams in fact serve as the better ground for foraging and breeding areas for many species of anurans (Pineda and Halffter 2004). As reported here, new records of anurans like *Leptophryne borbonica* and *Limnonectes plicatellus* were for the first time discovered under thick forest litter near the stream bank of the Peres River small stream, while *L. hascheanus* and *Theloderma licin* were encountered for the first time at a long-abandoned hiking trail. We also started to realise that secretive and rarely found species like *Pelobatrachus nasutus*, *Rentapia flavomaculata* and *Nyctixalus pictus* were actually abundant in pristine habitats such as the small stream of Peres River. After the continuous monthly surveys at this habitat, more specimens were obtained.

However, certain species such as *Phrynella pulchra*, *Theloderma horridum*, and *Zhangixalus tunkui* remained undetected since their initial discoveries in 2013–2014, even though continuous surveys have been made since 2015. This might indicate that these secretive species cannot be detected by a simple method such as visual surveys alone and may require alternative techniques that effective on arboreal amphibians. Several studies noted that *T. licin* and *P. pulchra* occupied smaller tree holes closer to the ground, generally in the less disturbed areas (McLeod and Norhayati

2007; Chan and Ahmad 2009). Thus, installing tree hole traps in non-recreational areas within SLF might be effective to sample these tree hole dwellers (Berry 1975; Duellman 1978; Yanoviak and Fincke 2005). Although *Zhangixalus tunkui* shares the same habitat as its congener, which is rock pools along the upper stream areas of SRF, the species remains undetected, possibly because it is only present during the days with the heaviest rainfall in the monsoon season. Unfortunately, conducting fieldwork during such times is impossible due to limited visibility and the risk of fallen trees and wild animals.

The compilation of amphibian records from established inventories in Hulu Terengganu have demonstrated a great diversity of amphibians so far, with a total of 70 species compared to another studied area in Terengganu like Gunung Tebu and adjacent forests in Besut, which have a total of 50 amphibian species (Muin et al. 2014; Sumarli et al. 2015). The records of amphibians in Hulu Terengganu have surpassed the species richness reported from other localities with well-established inventories such as Endau-Rompin National Park (Kiew 1987; Lim 1989; Daicus and Hashim 2004; Norhayati and Shamada 2004; Wood et al. 2008a; Shahriza et al. 2012) and Krau Wildlife Park (Grandison 1972; Jasmi et al. 1999; Norsham et al. 2001; Chan et al. 2008; Zakaria et al. 2014). However, more areas in Hulu Terengganu remain unexplored, especially in Belukar Bukit and a large portion of Lake Kenyir catchment which surely holds vast unreported species diversity.

To date, there are 17 protected species of amphibians under the Wildlife Conservation Act 2010 (Act 716). Five species of 'Near Threatened', two species of 'Endangered' and one species of 'Vulnerable' amphibians found in Hulu Terengganu are listed on the IUCN Red List (IUCN, 2021). This area deserves attention for better protection of the species, especially for the endemic and threatened species in this area. More alarming, frequent changes in taxonomy and the description of many new species has put these species into a vulnerable state as most of them are not evaluated under the IUCN Red List. The problem with the Wildlife Conservation Act 2010 appears to be not comprehensive enough, and is outdated as there are only 17 species listed as protected, while many of the unique and newly described species such as *Rhacophorus norhayatiae*, *Ansonia lumut*, *Limnonectes utara*, and many others are not listed. This legislation requires immediate revision to offer better protection of these species from illegal trade and wildlife trafficking. Furthermore, many areas within Hulu Terengganu, even in SLF, are vulnerable to further deforestation if conservation action is not taken seriously. The benefits of this long-term data collection could be utilised to spread public awareness on the importance of biodiversity conservation and be extended for ecotourism benefits of this forest reserve.

Conclusions

Fifty-two amphibian species from 32 genera were recorded from SLF, making up a total of 70 species recorded in Hulu Terengganu District. Data such as this are a clear indication that extensive study and monitoring is the cogent approach in attempting to reveal the true diversity of a forest reserve. Working repeatedly and systematically in this locality during different months of the years from 2003 to 2020 has resulted in revealing the ecological complexity and high species richness within this area. This finding also denotes that varying sampling efforts influence the knowledge on species diversity of the studied area. This study emphasised that continuous efforts of documenting species diversity is crucial to ensuring the reliability and validity of species diversity harboured by any area or habitat. The available information on amphibian diversity in SLF and Hulu Terengganu can hopefully be used to assist conservation programmes and long-term monitoring of biodiversity. In another way, remarkable species diversity of recreational forests and other areas should be preserved as it can be used to nurture conservation awareness and promote scientific citizenship amongst the local folks to work together to protect the biodiversity at this recreational forest.

Acknowledgements

We wish to thank, Department of Forestry Terengganu for granting permission to conduct research at Sekayu lowland forest and to Faculty of Science and Marine Environment, Universiti Malaysia Terengganu for providing facility and equipment to conduct the field survey and laboratory works. We would like to thank Mr Mazrul Aswady, Izzat Husna, Izwan Anas and Najwa Sawawi for assistances during the fieldwork. We are also deeply grateful to the Department of Wildlife and National Parks Malaysia (DWNP) (research permit: T-00563-16-17), Department of Forestry Peninsular Malaysia (JPSM), Forestry Department of Terengganu, Sekayu Recreational Forest staffs, Agriculture Department of Terengganu, and Sekayu Agricultural Park's authorities for permission to conduct the study. Anuar McAfee (Universiti Sultan Zainal Abidin, UniSZA, Terengganu) reviewed the English language.

References

AmphibiaWeb (2021) AmphibianWeb: Information on amphibian biology and conservation. University of California, California, USA. https://amphibiaweb.org [accessed 30 May 2021]

Badli-Sham BH, Shahirah-Ibrahim N, Goh SX, Syamila-Noh H, Abdul Shukor NSA, Shafie FA, Md Daud A, Abdul Razak FA, Rosli R, Abdul Aziz AA, Mohammad FNF, Kamaruzzaman MF, Mohamad S, Dzu K, Shariffudin A, Najwa-Sawawi S, Ahmad A (2019) Herpetofauna of Universiti Malaysia Terengganu Campus: Sustaining biodiversity in campus green area. Journal of Sustainability Science and Management 14(1): 11–28.

Berry PY (1975) The amphibian fauna of Peninsular Malaysia. Tropical Press, Kuala Lumpur, 130 pp.

Bhuiyan MAH, Siwar C, Ismail SM, Islam R (2011) Ecotourism development in recreational forest areas. American Journal of Applied Sciences 8(11): 1116–1121. https://doi.org/10.3844/ajassp.2011.1116.1121

Bisht K, Garg S, Sarmah A, Sengupta S, Biju SD (2021) Lost, forgotten, and overlooked: systematic reassessment of two lesser-known toad species (Anura, Bufonidae) from Peninsular

India and another wide-ranging northern species. Zoosystematics and Evolution 97(2): 451–470. https://doi.org/10.3897/zse.97.61770

Brown RM, Guttman SI (2002) Phylogenetic systematics of the *Rana signata* complex of Philippine and Bornean stream frogs: Reconsideration of Huxley's modification of Wallace's Line at the Oriental-Australian faunal zone interface. Biological Journal of the Linnean Society 76(3): 393–461. https://doi.org/10.1111/j.1095-8312.2002.tb01704.x

Bury RB, Corn PS (1987) Evaluation of pitfall trapping in northwestern forests: Trap arrays with drift fences. The Journal of Wildlife Management 51(1): 112–119. https://doi.org/10.2307/3801640

Chan KO (2011) Comparison of herpetofauna assemblages in logged and unlogged hill dipterocarp forests in Hulu Terengganu. Master Thesis, Universiti Kebangsaan Malaysia, Selangor, Malaysia.

Chan KO, Ahmad N (2009) Distribution and natural history notes on some poorly known frogs and snakes from Peninsular Malaysia. Herpetological Review 40(3): 294–301.

Chan KO, Norhayati A (2010) A new insular species of *Cyrtodactylus* (Squamata: Gekkonidae) from northeastern Peninsular Malaysia, Malaysia. Zootaxa 2389(1): 47–56. https://doi.org/10.11646/zootaxa.2389.1.2

Chan KO, Norhayati A, Juliana S (2008) Kekayaan spesies herpetofauna di Kuala Gandah menggunakan kaedah perangkap lubang berpagar. In: Sivananthan E, Siti Hawa Y, Abdul Kadir AH, Hasdi H, Lim BL, Rosli H, Noorlidah A, Shukor MN, Rahmah I (Eds) Biodiversity Resources and Conservation Status in Peninsular Malaysia. Department of Wildlife and National Parks, Malaysia, 71–84.

Chan KO, van Rooijen J, Grismer LL, Belabut D, Akil MAMM, Jamaludin H, Ahmad N (2010) First report on the herpetofauna of Pulau Pangkor, Perak, Malaysia. Russian Journal of Herpetology 17(2): 139–146.

Chan KO, Wood Jr PL, Anuar SMS, Muin MA, Quah ES, Sumarli AX, Grismer LL (2014a) A new species of upland stream toad of the genus *Ansonia* Stoliczka, 1870 (Anura: Bufonidae) from northeastern Peninsular Malaysia. Zootaxa 3764(4): 427–440. https://doi.org/10.11646/zootaxa.3764.4.3

Chan KO, Grismer LL, Brown RM (2014b) Reappraisal of the Javanese Bullfrog complex, *Kaloula baleata* (Müller, 1836) (Amphibia: Anura: Microhylidae), reveals a new species from Peninsular Malaysia. Zootaxa 3900(4): 569–580. https://doi.org/10.11646/zootaxa.3900.4.7

Chan KO, Grismer LL, Zachariah A, Brown RM, Abraham RK (2016) Polyphyly of Asian tree toads, genus *Pedostibes* Günther, 1876 (Anura: Bufonidae), and the description of a new genus from Southeast Asia. PLoS ONE 11(1): e0145903. https://doi.org/10.1371/journal.pone.0145903

Chan KO, Abraham RK, Grismer JL, Grismer L (2018) Elevational size variation and two new species of torrent frogs from Peninsular Malaysia (Anura: Ranidae: *Amolops* Cope). Zootaxa 4434(2): 250–264. https://doi.org/10.11646/zootaxa.4434.2.2

Chan KO, Abraham RK, Badli-Sham BH (2020a) A revision of the Asian tree toad complex *Rentapia hosii* (Anura: Bufonidae) with the description of a new species from Peninsular Malaysia. The Raffles Bulletin of Zoology 68: 595–607. https://doi.org/10.26107/RBZ-2020-0075

Chan KO, Hutter CR, Wood Jr PL, Grismer LL, Das I, Brown RM (2020b) Gene flow creates a mirage of cryptic species in a Southeast Asian spotted stream frog complex. Molecular Ecology 29(20): 3970–3987. https://doi.org/10.1111/mec.15603

Chan KO, Abraham RK, Grismer LL, Brown RM (2020c) A systematic review of the *Pulchrana picturata* complex, with the description of a new species from Peninsular Malaysia, Sumatra, and southern Thailand. The Raffles Bulletin of Zoology 68: 880–890. https://doi.org/10.26107/RBZ-2020-0096

Choong CY, Ng YF, Dow RA (2013) Odonata (Insecta) from three forests of central Terengganu, Malaysia. Malayan Nature Journal 64(2): 95–104.

Colwell RK (2013) EstimateS: Statistical estimation of species richness and shared Species from samples. http://viceroy.eeb.uconn.edu/estimates/

Colwell RK, Coddington JA (1994) Estimating terrestrial biodiversity through extrapolation. Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences 345(1311): 101–118. https://doi.org/10.1098/rstb.1994.0091

Crosswhite DL, Fox SF, Thill RE (1999) Comparison of methods for monitoring reptiles and amphibians in upland forests of the Ouachita Mountains. Proceedings of the Oklahoma Academy of Science 79: 45–50.

Crump ML, Scott Jr NJ (1994) Visual encounter surveys. In: Heyer WR, Donnelly MA, McDiarmid RW, Hayak LC, Foster M (Eds) Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Smithsonian Institution Press, Washington, 84–92.

Daicus B, Hashim R (2004) Herpetofauna of the western region of Endau-Rompin, Johore, Peninsular Malaysia. Malaysian Journal of Science 23(1): 65–72.

Das A, Garg S, Hamidy A, Smith EN, Biju SD (2019) A new species of *Micryletta* frog (Microhylidae) from Northeast India. PeerJ 7: e7012. https://doi.org/10.7717/peerj.7012

Daudin FM (1802) Histoire naturelle des rainettes, des grenouilles et des crapauds. Avec planches. Levrault.

Davis HR, Grimser LL, Cobos AJ, Murdoch ML, Sumarli AX, Anuar S, Quah ES (2018) Checklist of the herpetofauna of Hutan Lipur Gunung Senyum, Pahang, Peninsular Malaysia. Russian Journal of Herpetology 25(3): 207–220. https://doi.org/10.30906/1026-2296-2018-25-3-207-220

Davis HR, Grismer LL, Klabacka RL, Muin MA, Quah ES, Anuar S, Sites JW (2016) The phylogenetic relationships of a new stream toad of the genus *Ansonia* Stoliczka, 1870 (Anura: Bufonidae) from a montane region in Peninsular Malaysia. Zootaxa 4103(2): 137–153. https://doi.org/10.11646/zootaxa.4103.2.4

Davison GWH (1993) Ma'Daerah and Cagar Hutan, Terengganu; if, how and where to develop? Unpublished report, WWF-Malaysia, Petaling Jaya.

Dehling JM (2014) Eine neue Fangzahnfroschart der Gattung *Limnonectes* (Anura: Dicroglossidae) vom Gunung Lawit, Malaiische Halbinsel. Sauria 36: 17–30.

Doan TM (2003) Which methods are most effective for surveying rain forest herpetofauna? Journal of Herpetology 37(1): 72–81. https://doi.org/10.1670/0022-1511(2003)037[0072:WMAMEF]2.0.CO;2

Dring JCM (1979) Amphibians and reptiles from northern Trengganau, Malaysia, with descriptions of two new geckos: *Cnemaspis* and *Cyrtodactylus*. Bulletin of the British Museum of Natural History 34: 181–241.

Duellman WE (1978) The biology of an equatorial herpetofauna in Amazonian Ecuador. University of Kansas, Lawrence, 352 pp.

Duellman WE, Trueb L (1986) Biology of Amphibians. McGraw-Hill, New York, 670 pp. https://doi.org/10.2307/1445022

Fatihah-Syafiq M, Badli-Sham BH, Fahmi-Ahmad M, Aqmal-Naser M, Rizal SA, Azmi MSA, Grismer LL, Ahmad AB (2020) Checklist of herpetofauna in the severely degraded ecosystem of Bidong Island, Peninsular Malaysia, South China Sea. ZooKeys 985: 143–162. https://doi.org/10.3897/zookeys.985.54737

Frost DR (2022) Amphibian species of the world: An online reference. American Museum of Natural History, New York. http://research.amnh.org/herpetology/amphibia/index.html [accessed 10 November 2022]

Garg S, Suyesh R, Das A, Jiang J, Wijayathilaka N, Amarasinghe AT, Meegaskumbura M (2019) Systematic revision of *Microhyla* (Microhylidae) frogs of South Asia: A molecular, morphological, and acoustic assessment. Vertebrate Zoology 69(1): 1–71.

Gibbons JW, Bennett DH (1974) Determination of anuran terrestrial activity patterns by a drift fence method. Copeia 1974(1): 236–243. https://doi.org/10.2307/1443029

Grandison AGC (1972) The Gunung Benom expedition, 1967: Reptilia and amphibians of Gunung Benom with a description of a new species *Macrocalamus*. Bulletin of the British Museum, Natural History. Zoology 23(4): 45–101. https://doi.org/10.5962/p.314200

Grismer LL (2012) Amphibians and reptiles of the Seribuat Archipelago (Peninsular Malaysia). Frankfurt am Main, Edition Chimaira, 239 pp.

Grismer LL, Chan KO (2008) A new species of *Cnemaspis* Strauch, 1887 (Squamata: Gekkonidae) from Pulau Perhentian Besar, Terengganu, Peninsular Malaysia. Zootaxa 1771(1): 1–15. https://doi.org/10.11646/zootaxa.1771.1.1

Grismer LL, Grismer JL, Wood Jr PL, Ngo VT, Neang T, Chan KO (2011) Herpetology on the fringes of the Sunda Shelf: A discussion of discovery, taxonomy and biogeography. Bonner Zoologische Monographien 57: 57–97.

Grismer LL, Lukman HBI, Muhammad TA, Syed AR, Amirrudin BA (2014) A new species of lowland skink (genus *Lipinia* Gray, 1845) from northeastern Peninsular Malaysia. Zootaxa 3821(4): 457–464. https://doi.org/10.11646/zootaxa.3821.4.4

Grismer LL, Perry Jr L, Syafiq MF, Baizul HBS, Rizal SA, Ahmad AB, Quah ES (2016) On the taxonomy and phylogeny of the skinks *Lipinia sekayuensis* Grismer, Ismail, Awang, Rizal and Ahmad and *Lipinia surda* Boulenger from Peninsular Malaysia. Zootaxa 4147(1): 59–66. https://doi.org/10.11646/zootaxa.4147.1.3

Grismer LL, Wood PLJ, Ahmad AB, Baizul-Hafsyam BS, Afiq-Shuhaimi M, Rizal SA, Quah ES (2018) Two new *Tytthoscincus* Linkem, Diesmos, Brown (Squamata; Scincidae) from Peninsular Malaysia and another case of microsyntopy between ecologically specialized, unrelated, leaf-litter species. Zootaxa 4425(1): 87–107. https://doi.org/10.11646/zootaxa.4425.1.5

Hamidi J (2013) Herpetofauna di Gunung Gagau, Terengganu. In: Latiff A, Ali CA, Mohamad KR (Eds) Gunung Gagau, Terengganu: Transforming Natural Assets into an Ecotourism Product. Academy of Sciences Malaysia and Ketengah, 165–172.

Harvey MB, Pemberton AJ, Smith EN (2002) New and poorly known parachuting frogs (Rhacophoridae: Rhacophorus) from Sumatra and Java. Herpetological Monograph 16(1): 46–92. https://doi.org/10.1655/0733-1347(2002)016[0046:NAPKPF]2.0.CO;2

Hong Z, Shahrul AMS, Grismer LL, Quah ESH (2021) Preliminary report on the herpetofaunal diversity of Batu Hampar Recreational Forest, Kedah, Malaysia. Check List 17(3): 791–814. https://doi.org/10.15560/17.3.791

IUCN (2021) The IUCN Red List of Threatened Species Version 2021-2. International Union for Conservation of Nature and Natural Resources, Gland.

Jablonski D, Masroor R, Hofmann S (2022) On the edge of the Shivaliks: An insight into the origin and taxonomic position of Pakistani toads from the *Duttaphrynus melanostictus* complex (Amphibia, Bufonidae). Zoosystematics and Evolution 98(2): 275–284. https://doi.org/10.3897/zse.98.79213

Jarina MJ, Wahizatul AA, Jamilah MS, Amiruddin A, Muhammad Razali S (2007) The reinforcement of the roles of recreational forest in Malaysia. In: Zakaria MY, Khan NKN, Baharom Y (Eds) Proceedings of the National Conference on the Management and Conservation of Forest Biodiversity in Malaysia: Forest Biodiversity for Better Life. Forestry Department Peninsular Malaysia, 280–288.

Jasmi A, Shaari MA, Norsham Y, Lim BL (1999) Herpetofauna survey at Sungai Chenderoh, Perlok, Krau Wildlife Researve, Pahang. Journal of Wildlife and Parks 17: 83–88.

Jiang D, Jiang K, Ren J, Wu J, Li J (2019) Resurrection of the genus *Leptomantis*, with description of a new genus to the family Rhacophoridae (Amphibia: Anura). Asian Herpetological Research 10: 1–12. https://doi.org/10.16373/j.cnki.ahr.180058

Khan MS (2001) Notes on cranial-ridged toads of Pakistan and description of a new subspecies (Amphibia: Bufonidae). Pakistan Journal of Zoology 33(4): 293–298.

Khan MMA, Shaari NA, Bahar AMA, Baten MA (2014) Impact of the flood occurrence in Kota Bharu, Kelantan using statistical analysis. Journal of Applied Sciences 14(17): 1944–1951. https://doi.org/10.3923/jas.2014.1944.1951

Kiew BH (1987) An annotated checklist of the herpetofauna of Ulu Endau, Johore, Malaysia. Malayan Nature Journal 41(2–3): 413–424.

Komaruddin SA, Mohamad NA, Fatihah-Syafiq M, Sham BHB, Mamat MA, Zakaria N (2020) Dataset of reptiles in fragmented forests at Tasik Kenyir, Hulu Terengganu, Malaysia. Data in Brief 28: 104994. https://doi.org/10.1016/j.dib.2019.104994

Kottelat M, Ng PKL, Lim KKP (1992) Recent collections of freshwater fish from Terengganu, Peninsular Malaysia. Malayan Naturalist 46(2): 7–12.

Lalremsanga HT, Sailo S, Lalfamkima VC, Hooroo RNK (2016) Choice of ovoposition site by Nicobar frog, *Hylarana nicobariensis* (Amphibia: Anura). International Journal of Current Research 8(5): 31505–31512.

Leong TM (2004) Larval descriptions of some poorly known tadpoles from Peninsular Malaysia (Amphibia: Anura). The Raffles Bulletin of Zoology 52(2): 609–620.

Leong TM, Matsui M, Yong HS, Hamid AA (2003) Revalidation of *Rana laterimaculata* Barbour et Noble, 1916 from the synonymy of *Rana baramica* Boettger, 1901. Current Herpetology 22(1): 17–27. https://doi.org/10.5358/hsj.22.17

Lim KPK (1989) The amphibian fauna of Ulu Kinchin, Pahang, Malaysia. Malayan Nature Journal 43: 322–325.

Mandeep JS (2008) Rain height statistics for satellite communication in Malaysia. Journal of Atmospheric and Solar-Terrestrial Physics 70(13): 1617–1620. https://doi.org/10.1016/j.jastp.2008.06.005

Manthey U, Denzer W, Quah ESH (2016) Südostasiatische Anuren im Fokus: *Microhyla palmipes* Boulenger, 1897 und *Microhyla superciliaris* Parker, 1928 der Malaiischen Halbinsel. Sauria 38: 23–30.

Matsui M (2006) Three new species of *Leptolalax* from Thailand (Amphibia, Anura, Megophryidae). Zoological Science 23(9): 821–831. https://doi.org/10.2108/zsj.23.821

Matsui M, Belabut DM, Ahmad N (2014) Two new species of fanged frogs from Peninsular Malaysia (Anura: Dicroglossidae). Zootaxa 3881(1): 75–93. https://doi.org/10.11646/zootaxa.3881.1.6

Matsui M, Eto K, Belabut DM, Nishikawa K (2017) A new *Kalophrynus* (Amphibia, Anura, Microhylidae) from Peninsular Malaysia. Current Herpetology 36(2): 75–86. https://doi.org/10.5358/hsj.36.75

Matsui M, Kawahara Y, Eto K, Hamidy A, Ahmad N, Hossman MYB (2018) Distinct species status of *Kurixalus chaseni* (Rhacophoridae, Anura) as revealed by mitochondrial phylogeny. Alytes 36: 170–177.

McLeod DS, Norhayati A (2007) A new species of *Theloderma* (Anura: Rhacophoridae) from southern Thailand and peninsular Malaysia. Russian Journal of Herpetology 14(1): 65–72.

Muhammad BG, Jumaat HA, Mohd Ekhwan HT, Sahibin AR, Hafizan HJ, Gasim MB, Adam JH, Toriman MEH, Rahim SA, Juahir HH (2007) Coastal flood phenomenon in Terengganu, Malaysia: Special reference to Dungun. Research Journal of Environmental Sciences 1(3): 102–109. https://doi.org/10.3923/rjes.2007.102.109

Muin MAMA, Shahrul Anuar MS, Quah ESH, Nurolhuda N, Sharma D, Yap WWL, Nur Minura A, Nur Juliani S, Mohd Yusof O, Zakaria R, Mansor A, Mohd Sukor H (2014) Herpetofauna diversity of Gunung Tebu, Terengganu. In: Abdul Rahim R, Abu Hassan MN, Nordin A, Nor Hasliza MB, Latiff A (Eds) Siri 22 Kepelbagaian biologi hutan. Hutan Simpan Gunung Tebu, Terengganu: Pengurusan hutan, persekitaran fizikal dan kepelbagaian biologi. Forestry Department Peninsular Malaysia, 312–318.

Ng PK (2020) Revision of the freshwater crabs of the *Johora tahanensis* (Bott, 1966) species group (Crustacea, Brachyura, Potamidae), with a key to the genus. ZooKeys 994: 1–34. https://doi.org/10.3897/zookeys.994.56810

Ng PK, Ahmad AB (2016) A new genus and new species for an unusual semi-terrestrial potamid crab (Decapoda: Brachyura) with a bilobed mandibular palp from Peninsular Malaysia. Journal of Crustacean Biology 36(6): 823–832. https://doi.org/10.1163/1937240X-00002492

Nishikawa K, Matsui M, Yong HS, Ahmad N, Yambun P, Belabut DM, Yoshikawa N (2012) Molecular phylogeny and biogeography of caecilians from Southeast Asia (Amphibia, Gymnophiona, Ichthyophiidae), with special reference to high cryptic species diversity in Sundaland. Molecular Phylogenetics and Evolution 63(3): 714–723. https://doi.org/10.1016/j.ympev.2012.02.017

Nor ANM (2007) Diversity, abundance and distribution on palmae of Bukit Bauk Forest Reserve and Sekayu Recreational Forest. Undergraduate Thesis, Universiti Malaysia Terengganu, Terengganu, Malaysia.

Norhayati A, Shamada SKS (2004) Fauna amfibia di Taman Negeri Endau Rompin. In: Mohamad Ismail S, Mat Isa M, Ahmad WYW, Rahim Ramli M, Latiff A (Eds) Taman Negeri Endau Rompin: Pengurusan, Persekitaran Fizikal dan Biologi. Jabatan Perhutanan Semenanjung Malaysia, 197–206.

Norhayati A, Eszri S, Juliana S, Shukor MN (2006) An annotated checklist of amphibians at Hutan Simpan Pasir Raja, Terengganu. In: Muda A, Jaafar N, Sabran MR, Md Som J, Nizam MS, Latiff A (Eds) Siri kepelbagaian biologi Hutan. Gunung Mandi Angin, Terengganu: Pengurusan, Persekitaran Fizikal, Kepelbagaian Biologi dan Pelancongan Ekologi. Forestry Department Peninsular Malaysia, 434–450.

Norhayati A, Chan KO, Daicus B (2011) Amphibians and reptiles of Sungai Tembat, Terengganu. In: Latiff A, Ali CA, Mohamad KR (Eds) Gunung Gagau, Terengganu: Transforming Natural Assets into an Ecotourism Product. Academy of Sciences Malaysia and Ketengah, 173–178.

Norsham Y, Leong TM, Gary L (2001) Amphibians checklist of Bukit Rengit Lanchang, Pahang. Journal of Wildlife and Parks 19: 123–124.

Nur Amalina MI, Azhari M, Norshaqinah A, Nor Azrin NA, Shukor MN, Aisah MS, Amirrudin A, Grismer LL, Norhayati A (2017) Species composition of amphibians and reptiles in Tembat Forest Reserve, Hulu Terengganu, Terengganu, Peninsular Malaysia. Malaysian Applied Biology 46(3): 119–129.

Nur Amalina MI, Nazron NS, Nazir NAM, Shukor AM, Ilias R, Ahmad A, Ahmad N (2020) Sustainable dam development: A study of dam environmental impact on the herpetofauna in Hulu Terengganu, Peninsular Malaysia. In: Sidek LM, Salih GHA, Boosroh MH (Eds) International Conference on Dam Safety Management and Engineering. Springer, Singapore, 457–467. https://doi.org/10.1007/978-981-15-1971-0_46

Nur Amalina MI, Ahmad N, Nor SM, Syuhada Nazron N, Adyla N, Abidin Z (2021) Herpetofauna diversity in Sg. Deka, Hulu Terengganu, Peninsular Malaysia. Journal of Science and Technology 4(1): 86–93.

Ohler A, Deuti K (2013) *Pyxicephalus khasianus* Anderson, 1871 and *Rana laticeps* Boulenger, 1882 (Dicroglossidae, Anura, Amphibia) are synonyms. Zoosystema 35(3): 415–424. https://doi.org/10.5252/z2013n3a4

Pearman PB, Velasco AM, López A (1995) Tropical amphibian monitoring: A comparison of methods for detecting inter-site variation in species' composition. Herpetologica 51(3): 325–337.

Pineda E, Halffter G (2004) Species diversity and habitat fragmentation: Frogs in a tropical montane landscape in Mexico. Biological Conservation 117(5): 499–508. https://doi.org/10.1016/j.biocon.2003.08.009

Praderio MJ, Robinson MD (1990) Reproduction in the toad *Colostethus trinitatus* (Anura: Dendrobatidae) in a northern Venezuela seasonal environment. Journal of Tropical Ecology 6(03): 333–341. https://doi.org/10.1017/S0266467400004600

Quah ESH, Badli-Sham BH, Rahman MF-S, Ahmad A, Chan KO (2021) A new record and range extension for *Philautus davidlabangi* Matsui, 2009 from Peninsular Malaysia (Amphibia: Rhacophoridae). Herpetology Notes 14: 1181–1186.

Rafaai NH (2007) Gingers (Zingiberaceae) in Bukit Bauk Forest Reserve and Sekayu Recreational Forest. Undergraduate Thesis, Universiti Malaysia Terengganu, Terengganu, Malaysia.

Rastogi RK, Pinelli C, Polese G, D'Aniello B, Chieffi-Baccari G (2011) Hormones and reproductive cycles in Anuran Amphibians. In: Norris D, Lopez KH (Eds) Hormones and Reproduction of Vertebrates. Academic Press, 171–186. https://doi.org/10.1016/B978-0-12-374931-4.10009-4

Rujirawan A, Stuart BL, Aowphol A (2013) A new tree frog in the genus *Polypedates* (Anura: Rhacophoridae) from southern Thailand. Zootaxa 3702(6): 545–565. https://doi.org/10.11646/zootaxa.3702.6.3

Shahriza S, Ibrahim J (2014) A preliminary checklist of amphibians of Ulu Paip Recreational Forest, Kedah, Malaysia. Check List 10(2): 253–259. https://doi.org/10.15560/10.2.253

Shahriza S, Ibrahim, J (2012) The amphibian diversity of Bukit Jana, Taiping, Perak. Tropical Life Sciences Research 23(2): 49–57.

Shahriza S, Ibrahim J, Anuar S, Muin A (2012) Herpetofauna of Peta area of Endau-Rompin National Park, Johor, Malaysia. Pertanika. Journal of Tropical Agricultural Science 35(3): 553–567.

Sharma DSK (1999) Tortoise and freshwater turtle trade and utilisation in Peninsular Malaysia. TRAFFIC Southeast Asia, Petaling Jaya, Malaysia. http://www.traffic.org/species-reports/traffic_species_reptiles20 [accessed 10 July 2021]

Sharma DSK, Chong DKF, Darmaraj MR, Lau MM, Ng BS, Abdul Wahab AZ, Nurul Ain E, Nor Zalipah M (2007) An annoted preliminary checklist of reptiles of Bukit Labohan Forest Reserve and the Ma'Daerah turtle sanctuary, Kemaman, Terengganu. In: Sharma D, Abdul Wahab AZ, Basri N, Awang Ali Bema DN (Eds) Biodiversity Expedition in Bukit Labohanm, Ma'Daerah, Terengganu. WWF-Malaysia, Petaling Jaya, 73–80.

Sheridan JA, Stuart BL (2018) Hidden species diversity in *Sylvirana nigrovittata* (Amphibia: Ranidae) highlights the importance of taxonomic revisions in biodiversity conservation. PLoS ONE 13(3): e0192766. https://doi.org/10.1371/journal.pone.0192766

Sumarli AX, Grismer LL, Anuar S, Muin MA, Quah ESH (2015) First report on the amphibians and reptiles of a remote mountain, Gunung Tebu in northeastern Peninsular Malaysia. Check List 11(4): 1679. https://doi.org/10.15560/11.4.1679

Sumarli A, Grismer LL, Ahmad AB, Rizal S, Ismail LH, Izam NAM, Linkem CW (2016) The first riparian skink (Genus: *Sphenomorphus* Strauch, 1887) from Peninsular Malaysia and its relationship to other Indochinese and Sundaic species. Zootaxa 4173(1): 29–44. https://doi.org/10.11646/zootaxa.4173.1.3

Suwannapoom C, Van Nguyen T, Pawangkhanant P, Gorin VA, Chomdej S, Che J, Poyarkov NA (2020) A new species of *Micryletta* (Amphibia: Microhylidae) from southern Thailand. Zoological Research 41(5): 581–588. https://doi.org/10.24272/j.issn.2095-8137.2020.139

Tamblyn A, Turner C, Raines P (2006) Malaysia Tropical Forest Conservation Project Report of the Setiu Wetlands Phase. Coral Cay Conservation Ltd, London, United Kingdom, 98 pp.

Tan HH, Ng PKL (2005) The fighting fishes (Teleostei: Osphronemidae: Genus *Betta*) of Singapore, Malaysia and Brunei. Raffles Bulletin of Zoology Supplement 13: 43–99.

Tenaga Nasional Berhad Research [TNBR] (2003) Detailed environmental impact assessment. Report, Tenaga Nasional Berhad Research Sdn. Bhd., Bangi, Selangor, Malaysia, 114 pp.

Tenaga Nasional Berhad Research [TNBR] (2007) Detailed environmental impact assessment. Report, Tenaga Nasional Berhad Research Sdn. Bhd., Bangi, Selangor, Malaysia, 139 pp.

Ummi MA (2013) Species composition and richness of vertebrates (herpetofauna, birds, small mammals) at Hulu Terengganu, Peninsular Malaysia. Master Thesis, Universiti Kebangsaan Malaysia, Selangor, Malaysia.

Varikoden H, Preethi B, Samah AA, Babu CA (2011) Seasonal variation of rainfall characteristics in different intensity classes over Peninsular Malaysia. Journal of Hydrology (Amsterdam) 404(1): 99–108. https://doi.org/10.1016/j.jhydrol.2011.04.021

Wahizatul AA, Geok HA (2016) Aquatic insect communities in relation with water quality of selected tributaries of Tasik Kenyir Terengganu. Journal of Sustainability Science and Management 11(2): 1–10.

Wahizatul AA, Julia J, Amirrudin A (2006) Diversity and distribution of dragonflies (Insecta: Odonata) in Sekayu recreational forest, Terengganu. Journal of Sustainability Science and Management 1(2): 97–106.

Wahizatul AA, Long SH, Ahmad A (2011) Composition and distribution of aquatic insect communities in relation to water quality in two freshwater streams of Hulu Terengganu, Terengganu. Journal of Sustainability Science and Management 6(1): 148–155.

Wood Jr PL, Grismer LL, Youmans TM, Nasir NB, Ahmad N, Senwai J (2008a) Additions to the herpetofauna of Endau-Rompin, Johor, West Malaysia. Herpetological Review 39(1): 112–121.

Wood Jr PL, Grismer LL, Norhayati A, Juliana S (2008b) Two new species of torrent-dwelling toads *Ansonia* Stoliczka, 1870 (Anura: Bufonidae) from Peninsular Malaysia. Herpetologica 64(3): 321–340. https://doi.org/10.1655/07-065.1

Yanoviak SP, Fincke OM (2005) Sampling methods for water-filled tree holes and their artificial analogues. In: Leather S (Ed.) Insect sampling in forest ecosystems. Blackwell, Oxford, 168–185. https://doi.org/10.1002/9780470750513.ch8

Zahidin MA, Candyrilla VB, Abdullah MT (2017) Fauna in setiu wetland forest. Kenyir Research Institute, Universiti Malaysia Terengganu, Kuala Nerus, Terengganu, 11 pp.

Zakaria N, Senawi J, Musa FH, Belabut D, Chan CK, Md Nor S, Ahmad N (2014) Species composition of amphibians and reptiles in Krau wildlife reserve, Pahang, peninsular Malaysia. Check List 10(2): 335–343. https://doi.org/10.15560/10.2.335

Zakaria AA, Rahim NAA, Abdullah MT (2015) Reptile diversity as an ecotourism attraction in Pulau Bidong. In: Mariapan M, Lim EAL, Isa SS, Karim MS, Hakeem KR (Eds) Ecotourism potentials in Malaysia. Faculty of Forestry, Universiti Malaysia Putra, Serdang, 42–47.

Zakaria AA, Rahim NAA, Ahmad A, Abdullah MT (2019) Species richness estimation of reptiles in selected sites of Tasik Kenyir, Hulu Terengganu, Malaysia. In: Abdullah MT, Mohammad A, Nor Zalipah M, Safih Lola M (Eds) Greater Kenyir Landscapes. Springer, Cham, 159–170. https://doi.org/10.1007/978-3-319-92264-5_15

Zug GR (2015) Morphology and systematics of *Kalophrynus interlineatus-pleurostigma* populations (Anura: Microhylidae: Kalophryninae) and a taxonomy of the genus *Kalophrynus* Tschudi, Asian sticky frogs. Proceedings of the California Academy of Sciences 62: 135–190.

ZooKeys 1157: 95–108 (2023)
doi: 10.3897/zookeys.1157.97646
https://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Review of the genus *Hauptenia* Szwedo (Hemiptera, Fulgoromorpha, Derbidae), with descriptions of two new species from China

Yong-Jin Sui[1,2,3], Lin Yang[1,2,3], Jian-Kun Long[1,2,3], Zhi-Min Chang[1,2,3], Xiang-Sheng Chen[1,2,3]

1 *Institute of Entomology, Guizhou University, Guiyang, Guizhou, 550025, China* **2** *The Provincial Special Key Laboratory for Development and Utilization of Insect Resources of Guizhou, Guizhou University, Guiyang, Guizhou, 550025, China* **3** *Guizhou Key Laboratory for Plant Pest Management of Mountainous Region, Guizhou University, Guiyang, Guizhou, 550025, China*

Corresponding author: Xiang-Sheng Chen (chenxs3218@163.com)

Academic editor: Mike Wilson | Received 16 November 2022 | Accepted 23 February 2023 | Published 3 April 2023

https://zoobank.org/ACCD12F3-ED40-4CED-847B-21B01CB7D11B

Citation: Sui Y-J, Yang L, Long J-K, Chang Z-M, Chen X-S (2023) Review of the genus *Hauptenia* Szwedo (Hemiptera, Fulgoromorpha, Derbidae), with descriptions of two new species from China. ZooKeys 1157: 95–108. https://doi.org/10.3897/zookeys.1157.97646

Abstract
The derbid planthopper genus *Hauptenia* Szwedo, 2006 is reviewed. Two new species from China, *H. beibengensis* Sui & Chen, **sp. nov.** and *H. daliensis* Sui & Chen, **sp. nov.**, are described and illustrated. A third species, *H. tripartita* Rahman et al., 2012, is recorded from China for the first time. An updated checklist and identification key to all ten known species of the genus *Hauptenia* are provided.

Keywords
Cedusini, distribution, new record, planthoppers, taxonomy

Introduction

The planthopper family Derbidae (Hemiptera, Fulgoromorpha) was established by Spinola in 1839, containing 22 tribes in three subfamilies (Bourgoin 2023), eight tribes, 38 genera, and 156 species of which are known in China (Yang and Wu 1994;

Sui and Chen 2019; Bourgoin 2023). Most of these taxa are distributed in the Oriental bioregion, especially in southern China.

In the Breddiniolinae Fennah, 1950, Cedusini Emeljanov, 1992 and subtribe Cedusina Emeljanov, 2008, the genus *Hauptenia* was established by Szwedo (2006) for five Chinese species (previously in *Malenia* Haupt, 1924) and with *Malenia magnifica* Yang & Wu, 1994 as its type species. Rahman et al. (2012) described two Korean species and Jhan et al. (2016) described one Bangladeshi species, bringing the total of known species to eight: *H. bandarbanensis* Jhan & Rahman, 2016, *H. fellea* (Yang & Wu, 1994), *H. glutinosa* (Yang & Wu, 1994), *H. idonea* (Yang & Wu, 1994), *H. jacula* (Yang & Wu, 1994), *H. magnifica* (Yang & Wu, 1994), *H. palgongsanensis* Rahman, Kwon & Suh, 2012, and *H. tripartita* Rahman, Kwon & Suh, 2012.

Herein, two new species, *Hauptenia beibengensis* Sui & Chen, sp. nov. and *H. daliensis* Sui & Chen, sp. nov., are described and illustrated from China, bringing the total number of this genus to ten. One species, *H. tripartita* Rahman et al., 2012, is recorded from China for the first time, and a key is provided to all ten species of *Hauptenia*.

Materials and methods

The morphological terminology follows Bourgoin (1987), Bourgoin and Huang (1990) and Yang and Wu (1994). Body length was measured from apex of vertex to tip of fore wing by KEYENCE VHX-1000E system. The standard terminology of venation follows Bourgoin et al. (2015). Dried specimens were used for the descriptions and illustrations. External morphology was observed under a stereoscopic microscope and all measurements were done with an ocular micrometer. Color pictures for adult habitus were obtained by the KEYENCE VHX-6000 system. Genital segments were macerated in 10% NaOH and drawn from preparations in glycerin jelly using a Leica MZ 12.5 stereomicroscope. Illustrations were scanned with a Canon CanoScan LiDE 220 and imported into Adobe Photoshop CS5 for labeling and plate composition. The dissected genitalia were preserved in glycerin in small plastic tubes pinned together with the specimens.

The type specimens and examined specimens are deposited in the Institute of Entomology, Guizhou University, Guiyang, Guizhou Province, China (**GUGC**).

Taxonomy

Taxonomy of the genus *Hauptenia* Szwedo, 2006

Figs 1–22

Hauptenia Szwedo, 2006: 331–332; Rahman et al. 2012: 63; Jhan et al. 2016: 2.

Type species. *Malenia magnifica* Yang & Wu, 1994: by original designation.

Diagnostic characters. Combination of the following characters: head (Figs 1, 3, 5, 14) with eyes distinctly narrower than pronotum. Vertex (Figs 1, 3, 5, 14) trapezoidal, at base slightly wider than at apex, slightly projecting in front of eyes. Frons (Figs 6, 15) longer in middle line than widest part ~ 1.48–1.84: 1 and shorter than clypeus ~ 1: 1.1–1.53, without median carina. Antennae (Figs 6, 7, 15, 16) short, subantennal process well developed. Fore wing (Figs 8, 17) longer than widest part ~ 2.6–3.1: 1, RA with one or two terminal(s). Hind wing (Figs 9, 18) with vein ScP+RA very short, CuA with two or three terminals; spinal formula of hind leg 7–6–5. Male terminalia (Figs 10, 19) with gonostyli symmetrical, short and stout, dorsobasal projection distad; pygofer with dorsocaudal angle not produced into finger-shaped process; anal tube not distinctly elongated, dorsal margin shorter than ventral margin in lateral view, usually with apex not reaching level of apex of gonostyli, epiproct turned ventrad or nearly so, slightly notched at apex in lateral view.

Checklist and distributions of species of *Hauptenia* Szwedo, 2006

H. bandarbanensis Jhan & Rahman, 2016; Bangladesh.
H. beibengensis Sui & Chen, sp. nov.; China (Xizang).
H. daliensis Sui & Chen, sp. nov.; China (Yunnan).
H. fellea (Yang & Wu, 1994); China (Guizhou, Sichuan, Taiwan, Yunnan).
H. glutinosa (Yang & Wu, 1994); China (Chongqing, Fujian, Guizhou, Hainan, Hunan, Taiwan, Zhejiang).
H. idonea (Yang & Wu, 1994); China (Guizhou, Taiwan).
H. jacula (Yang & Wu, 1994); China (Guangxi, Guizhou, Hainan, Taiwan).
H. magnifica (Yang & Wu, 1994); China (Guangxi, Guizhou, Hainan, Taiwan, Yunnan).
H. palgongsanensis Rahman, Kwon & Suh, 2012; Korea.
H. tripartita Rahman, Kwon & Suh, 2012; Korea; China (Anhui, Guangxi, Guizhou, Hunan, Liaoning, Shaanxi, Sichuan, Zhejiang); new record for China.

Key to the species of genus *Hauptenia* Szwedo, 2006 (based on Jhan et al. 2016)

1 Gonostyli with dorsocaudal angle produced into finger-shaped process (Fig. 19; Rahman et al. 2012: fig. 20) **2**
– Gonostyli with dorsocaudal angle not produced into finger-shaped process (Fig. 10; Rahman et al. 2012: fig. 31) **6**
2 Gonostyli with apical hook of dorsobasal projection quadrate, apical margin obliquely truncate (Rahman et al. 2012: fig. 20) **3**
– Gonostyli with apical hook of dorsobasal projection triangular, apical margin truncate (Fig. 19; Yang and Wu 1994: fig. 42E) **4**
3 Endosoma of aedeagus (Rahman et al. 2012: figs 17, 18) with four lobes and four processes, length of middle processes ca. half of left and right processes; mesonotum dark brown to black .... ***H. palgongsanensis* Rahman et al., 2012**
– Endosoma of aedeagus (Yang and Wu 1994: fig. 41H, I) with two lobes and four processes, middle processes as long as left and right processes; mesonotum yellow .... ***H. fellea* (Yang & Wu, 1994)**

4 General color dark brown; hind wing (Fig. 18) with vein CuA with two terminals .... ***H. daliensis*** **Sui & Chen, sp. nov.**

– General color yellowish brown; hind wing (Yang and Wu 1994: fig. 42D) with vein CuA with three terminals **5**

5 Endosoma of aedeagus (Yang and Wu 1994: figs 42H, I) with one large lobe and five processes; body relatively small, body length including fore wing male 3.9–4.3 mm, female 4.9 mm .... ***H. glutinosa*** **(Yang & Wu, 1994)**

– Endosoma of aedeagus (Yang and Wu 1994: figs 43G, H) with one large lobe and four processes; body relatively large, body length including fore wing male 5.4–5.7 mm, female 5.9 mm .... ***H. idonea*** **(Yang & Wu, 1994)**

6 Hind wing with CuA with three terminals (Fig. 9); gonostyli (Fig. 10) with each inner lower surface with a small hook apically.. **7**

– Hind wing with CuA with two terminals (Yang and Wu 1994: fig. 40D); gonostyli with each inner lower surface without hook apically .... ***H. jacula*** **(Yang & Wu, 1994)**

7 General color dark brown; gonostyli with apical margin near truncate (Fig. 10) .... ***H. beibengensis*** **Sui & Chen, sp. nov.**

– General color yellow to yellowish brown; gonostyli with apical margin obliquely truncate (Rahman et al. 2012: fig. 31) **8**

8 Endosoma of aedeagus (Rahman et al. 2012: figs 28, 29) with four lobes, longest one wide and tripartite.............................. ***H. tripartita*** **Rahman et al., 2012**

– Endosoma of aedeagus with four lobes, longest one slender and monopartite....... **9**

9 Endosoma of aedeagus (Yang and Wu 1994: figs 39H, I) with five processes, and two lobes out of four produced into processes .... ***H. magnifica*** **(Yang & Wu, 1994)**

– Endosoma of aedeagus (Jhan et al. 2016: figs 1H, I) with six processes, and three lobes out of four produced into processes... .... ***H. bandarbanensis*** **Jhan & Rahman, 2016**

Descriptions

Hauptenia bandarbanensis Jhan & Rahman, 2016

Hauptenia bandarbanensis Jhan & Rahman, in Jhan et al. 2016: 2–3, figs 1, 2.

Material examined. No specimen examined.

Diagnostic characters. (Based on Jhan et al. 2016). General color yellowish brown. Fore wing light brown, hind wing pale brown. Fore wing longer than widest part ~ 3: 1, RA with two terminals, MP with four sectors. Hind wing with CuA with three terminals. Gonostyli with apical margin obliquely truncate, dorsocaudal angle not produced; each inner lower surface with a hook subapically. Endosoma of aedeagus with six processes and three lobes out of four produced into processes.

Distribution. Bangladesh.

Hauptenia beibengensis **Sui & Chen, sp. nov.**

https://zoobank.org/43354BDE-7248-4623-9A54-0D671318F12D

Figs 1, 2, 5–13

Type material. ***Holotype***, ♂, China: Xizang, Motuo, Beibeng (29.25°N, 95.18°E), 15 August 2020, Y-J Sui. Paratypes, 3♂, same data as holotype.

Measurements. Body length (including fore wing): male 4.86–5.01 mm (n = 4); fore wing length: male 4.45–4.57 mm (n = 4).

Description. ***Coloration.*** General color dark brown. Vertex (Figs 1, 5), frons (Fig. 6), gena (Figs 2, 7), antennae, subantennal process, ocelli, pronotum and tegula (Figs 1, 5, 7) yellowish brown. Rostrum brown, with apex fuscous. Eyes (Figs 1, 2, 5–7) slightly dark brown. Ocelli (Figs 2, 7) slightly yellowish white. Mesonotum (Figs 1, 5) dark brown, with median carina slightly lighter. Fore wing (Figs 1, 2) dark brown, veins same color. Hind wing subhyaline, brownish, veins lightly darker. Thorax with ventral areas yellowish brown. Legs brownish yellow. Genital segment dark brown.

Head and thorax. Head (Figs 1, 5) including eyes distinctly narrower than pronotum (1: 1.49), short. Vertex (Figs 1, 5) trapezoidal, length between basal angles wider than length in middle line (2.42: 1), slightly projecting in front of eyes, posterior margin slightly concave, lateral carinae slightly elevated, median carina absent, disk slightly depressed. Frons (Fig. 6) longer in middle line than at the widest parts (1.54: 1), shorter than clypeus (1: 1.51), near apical 2/5 widest, disk depressed in entire length, lateral carinae keeled. Clypeus (Fig. 6) distinctly carinate medially from near basal 1/3. Apical segment of rostrum longer than wide. Antennae (Figs 2, 6, 7) short, second antennomere oval, flagellum originated from apical point. Subantennal processes (Figs 2, 7) distinct, ear-shaped. Transversely oblique carina across the gena between subantennal process and lateral carina of frons distinct. Eyes (Figs 5–7) semicircular. Lateral ocelli distinct, adjacent to eyes and antennae. Median length of pronotum distinctly less than that of vertex, anterior margin between eyes broadly convex, length behind eyes as long as median length. Mesonotum dorsally elevated, in lateral view raised above vertex, with median carinae reaching to the middle, posterior end triangularly depressed. Fore wing (Fig. 8) narrow, ~ 3.1× as long as the widest point, clavus closed, RA with two terminals, MP with four sectors. Hind wing (Fig. 9) shorter than fore wing, with RP reaching to apical margin, CuA with three terminals. Hind tibia without lateral spine. Spinal formula of hind leg 7–6–5.

Male terminalia. Anal tube (Fig. 10) moderately long, in dorsal view, lateral margin narrowed gradually toward the near middle and then parallel toward apex, width at base larger than the narrowest part ~ 1.9: 1, length in middle line (including epiproct) than widest part at base ~ 1.75: 1, dorsolateral margin convex medially near base; epiproct turned ventrad. Pygofer (Fig. 10) in lateral view distinctly shorter dorsally than ventrally, dorsocaudal angle not produced. Gonostyli (Fig. 10) symmetrical, short and stout, apical margin truncate, dorsocaudal angle not produced; each inner lower surface with small triangular process apically; inner side of laterodorsal margin with broad projection distad, in left lateral view, basal hook shorter and stout, apical hook slightly turned outward at end. Phallic complex (Figs 12, 13) asymmetrical. Periandrium slightly curved,

Figures 1–4. Male habitus (dorsal and lateral views) **1, 2** *Hauptenia beibengensis* Sui & Chen, sp. nov. **3, 4** *Hauptenia daliensis* Sui & Chen, sp. nov. Scale bars: 1 mm.

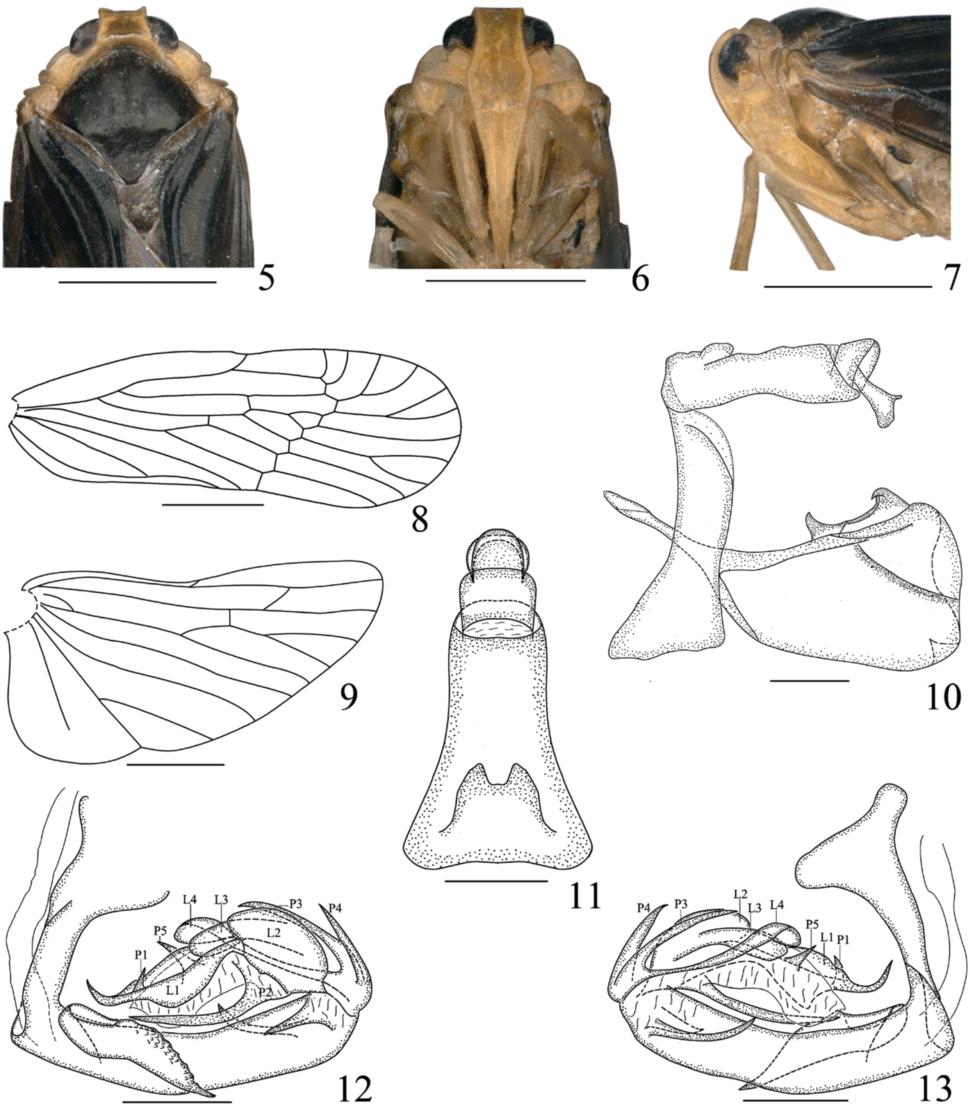
in left view, a big process arising from dorsal margin at base, and a short process arising from end with apex acute; in right view, a long and slender process arising from end near ventral margin. Endosoma more complex, with four lobes, one membrane and five processes of different sizes. Among four lobes, the longest lobe (L1) produced reaching to near base of periandrium, acute at apex; another other three lobes (L2–L4) round at apex, close together. In left lateral view, a small process (P1) arising from the longest lobe near apex, acute at apex; a long process (P2) arising from ventral margin of endosoma near at base, reaching to middle of periandrium; two long and sharped processes (P3, P4) arising from dorsal margin at base, pointed cephalad. In right lateral view, a small triangular process (P5) arising from the membrane one at base near dorsal margin.

Remarks. This species is similar to *H. fellea* (Yang & Wu, 1994), but differs from the latter in the mesonotum (Figs 1, 5) dark brown with median carinae reaching to the middle (mesonotum yellowish brown with median carinae reaching to near end in *H. fellea*); gonostyli (Fig. 10) with dorsocaudal angle not produced (gonostyli with dorsocaudal angle produced into finger-shaped process in *H. fellea*); endosoma (Figs 12, 13) with four lobes, one membrane and five processes of different sizes (endosoma with two lobes and four processes in *H. fellea*).

Etymology. This species is named after the collection site of the holotype, Beibeng Township in Xizang.

Host plants. Unknown.

Distribution. China (Xizang).



Figures 5–13. *Hauptenia beibengensis* Sui & Chen, sp. nov., male **5** head and thorax, dorsal view **6** face **7** head and thorax, left lateral view **8** fore wing **9** hind wing **10** terminalia, left lateral view **11** anal tube, dorsal view **12** phallic complex, left lateral view **13** phallic complex, right lateral view. Scale bars: 1 mm (**5–9**); 0.2 mm (**10–13**).

Hauptenia daliensis **Sui & Chen, sp. nov.**

https://zoobank.org/F6ECE0A6-154E-4F8A-BBF6-A49655AF95CA
Figs 3, 4, 14–22

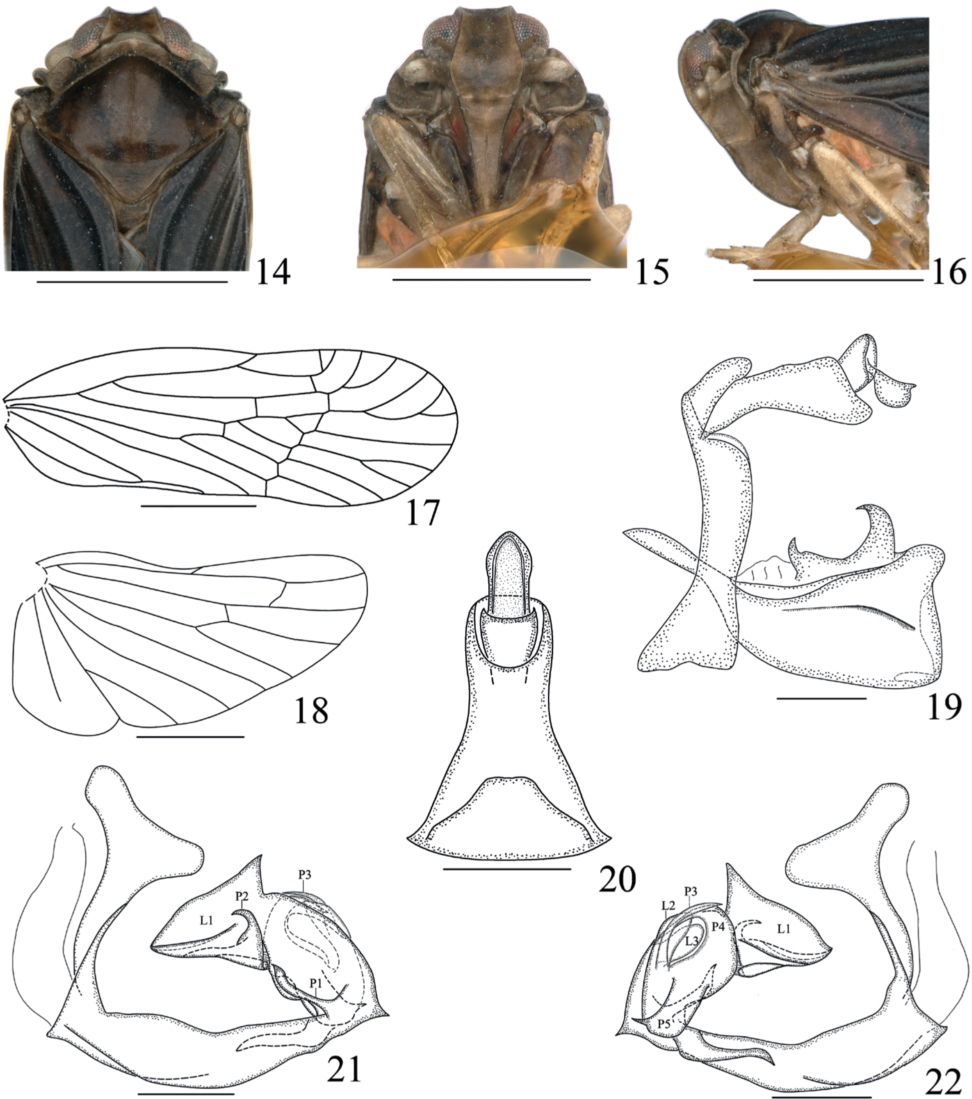
Type material. ***Holotype***, ♂, China: Yunnan, Dali, Mt. Cangshan (25.67°N, 100.13°E), 18 June 2009, B. Li & Z-H. Yang. Paratypes, 3♂, same data as holotype.

Measurements. Body length (including fore wing): male 4.56–4.71 mm (*n* = 4); fore wing length: male 4.02–4.17 mm (*n* = 4).

Description. ***Coloration.*** General color dark brown. Vertex (Figs 3, 14), gena (Figs 4, 16), antennae, subantennal process, pronotum and tegula slightly lighter. Frons (Fig. 15) and clypeus with lateral margin dark. Rostrum brown, with apex fuscous. Eyes (Figs 3, 4, 14–16) slightly dark red. Ocelli (Figs 4, 16) yellowish white. Mesonotum (Figs 3, 14) brown, with median carina slightly lighter. Fore wing (Figs 3, 4) dark brown except cell of ScP lighter, veins concolor. Hind wing subhyaline, brownish, veins lightly darker. Thorax with ventral areas yellow to orange red. Legs brownish yellow. Genital segment dark brown.

Head and thorax. Head (Figs 3, 14) including eyes distinctly narrower than pronotum (1: 1.44), short. Vertex (Figs 3, 14) trapezoidal, length between basal angles wider than length in middle line (3.6: 1), slightly projecting in front of eyes, posterior margin concave, lateral carinae slightly elevated, median carina absent, disk slightly depressed. Frons (Fig. 15) longer in middle line than at the widest parts (1.45: 1), shorter than clypeus (1: 1.48), near apical 2/5 widest, disk depressed in entire length, lateral carinae keeled. Clypeus distinctly carinate medially from near base 1/3. Apical segment of rostrum longer than wide. Antennae (Figs 4, 15, 16) short, second antennomere subglobose, flagellum originated from apical point. Subantennal processes distinct, ear-shaped. Transversely oblique carina across the gena between subantennal process and lateral carina of frons distinct. Eyes (Figs 3, 4, 14–17) semicircular. Lateral ocelli (Figs 4, 16) distinct, adjacent to eyes and antennae. Median length of pronotum distinctly less than that of vertex, anterior margin between eyes broadly convex, length behind eyes slightly greater than median length. Mesonotum (Figs 3, 14) dorsally elevated, in lateral view raised above vertex, with median carinae reaching to the apical 1/3, posterior end triangularly depressed. Fore wing (Fig. 17) narrow, ~ 3× as long as at the widest point, clavus closed, RA with one or two terminal(s), MP with four sectors. Hind wing (Figs 18) shorter than fore wing, with RP reaching to apical margin, CuA with two terminals. Hind tibia without lateral spine. Spinal formula of hind leg 7–6–5.

Male terminalia. Anal tube (Fig. 19) moderately long, in dorsal view, lateral margin narrowed gradually toward the near middle and then wider slightly toward apex, width at base larger than the narrowest part ~ 2.5: 1, length in middle line (including epiproct) than widest part at base ~ 1.62: 1, dorsolateral margin convex medially near base; epiproct turned ventrad. Pygofer (Fig. 19) in lateral view distinctly shorter dorsally than ventrally, dorsocaudal angle not produced. Gonostyli (Fig. 19) symmetrical, short and stout, apical margin truncate, dorsocaudal angle produced into finger-shaped process; each inner lower surface with small hook apically; inner side of laterodorsal margin with broad projection distad, in left lateral view, left hook shorter and smaller than apical hook, apical hook slightly turned outward at end. Phallic complex (Figs 21, 22) asymmetrical. Periandrium curved, with a small acute process at end, visible in both left and right lateral views; in right view, a strong process arising from near end, wavy and parallel with periandrium approximately. En-



Figures 14–22. *Hauptenia daliensis* Sui & Chen, sp. nov., male **14** head and thorax, dorsal view **15** face **16** head and thorax, left lateral view **17** fore wing **18** hind wing **19** terminalia, left lateral view **20** anal tube, dorsal view **21** phallic complex, left lateral view **22** phallic complex, right lateral view. Scale bars: 1 mm (**14–18**); 0.2 mm (**19–22**).

dosoma complex, with three lobes and five processes of different sizes. Among three lobes, in left lateral view, the largest lobe (L1) with dorsal margin produced into triangular process near apical 1/3, and with keel from apex to near apical 1/3 near ventral margin; in right lateral view, the largest lobe ventral margin rolling up at third of apex, another two small lobes (L2, L3) arising from ca. middle of endosoma dorsally. In left lateral view, a long and slender process (P1) arising from basal of endosoma, a process (P2) arising from the ventral margin of the largest lobe near apical 2/5, curved and pointed cephalad; and in right lateral view, a slender process (P3) and a process (P4) broad at base, all curved at dorsal margin, pointed oppositely; another process (P5) arising from ca. middle of endosoma ventrally, abruptly narrowed subapically, acute at apex, pointed caudally.

Remarks. This species is similar to *H. fellea* (Yang & Wu) but differs from the latter in the hind wing (Fig. 18) with CuA with two terminals (CuA with three terminals in *H. fellea*); gonostyli (Fig. 19) with apical margin truncate (gonostyli with apical margin obliquely truncate in *H. fellea*); endosoma (Figs 21, 22) with three lobes and five processes (endosoma with two lobes and four processes in *H. fellea*).

Etymology. This species is named after the collection site of the holotype, Dali City in Yunnan.

Host plants. Unknown.

Distribution. China (Yunnan).

Hauptenia fellea (Yang & Wu, 1994)

Malenia fellea Yang & Wu, 1994: 89, fig. 41.
Hauptenia fellea (Yang & Wu, 1994): Szwdeo 2006: 331.

Material examined. **China:** 5♂♂, Guizhou, Zhijin, 21 June 2019, Z-C Zhou; 4♂♂, Sichuan, Dayi, 20 July 2022, Y-J Sui; 1♂, Yunnan, Mengla, 13 November 2018, L-K Zhong.

Diagnostic characters. General color brown. Fore wing black, hind wing grayish. Fore wing longer than widest part ~ 2.7: 1, RA with two terminals, MP with four sectors. Hind wing with CuA with three terminals. Gonostyli with apical margin obliquely truncate, dorsocaudal angle produced into finger-shaped process; each inner lower surface without hook subapically. Endosoma of aedeagus with four processes and two elongated lobes.

Distributions. China (Guizhou, Sichuan, Taiwan, Yunnan).

Hauptenia glutinosa (Yang & Wu, 1994)

Malenia glutinosa Yang & Wu, 1994: 91, fig. 42.
Hauptenia glutinosa (Yang & Wu, 1994): Szwedo 2006: 331.

Material examined. **China:** 7♂♂, Chongqing, Beibei, Mt. Jinyun, 12 July 2021, Y-J Sui; 1♂, Fujian, Jian'ou, 25 August 2019, Z-C Zhou; 2♂♂, Guizhou, Suiyang, 26 June 2019, Y-J Sui; 1♂, Hainan, Lingshui, 17 July 2007, Z-G Zhang; 2♂♂, Hunan, Yongshun, 21 August 2016, L-J Yang and Y-S Ding; 2♂♂, Zhejiang, Lin'an, Mt. Tianmu, 20 July 2009, Y Chen and Z-H Meng.

Diagnostic characters. General color yellow. Fore wing pale brown, hind wing grayish. Fore wing longer than widest part ~ 2.7: 1, RA with two terminals, MP with four sectors. Hind wing with CuA with three terminals. Gonostyli with apical margin truncate, dorsocaudal angle produced into finger-shaped process; each inner lower surface without hook subapically. Endosoma of aedeagus with one large lobe and five processes.

Distributions. China (Chongqing, Fujian, Guizhou, Hainan, Hunan, Taiwan, Zhejiang).

Hauptenia idonea (Yang & Wu, 1994)

Malenia idonea Yang & Wu, 1994: 94, fig. 43.
Hauptenia idonea (Yang & Wu, 1994): Szwedo 2006: 332.

Material examined. **China:** 1♂, Guizhou, Leishan, Mt. Leigong, 10 July 2011, W-B Zheng; 1♂, Taiwan, Gaoxiong, 21 November 2002, X-S Chen.

Diagnostic characters. General color deep yellow. Fore wing light black, hind wing grayish. Fore wing longer than widest part ~ 2.7: 1, RA with two terminals, MP with four sectors. Hind wing with CuA with three terminals. Gonostyli with apical margin truncate, dorsocaudal angle produced into finger-shaped process; each inner lower surface without hook subapically. Endosoma of aedeagus with one large lobe and four processes.

Distributions. China (Guizhou, Taiwan).

Hauptenia jacula (Yang & Wu, 1994)

Malenia jacula Yang & Wu, 1994: 89, fig. 40.
Hauptenia jacula (Yang & Wu, 1994): Szwedo 2006: 331.

Material examined. **China:** 1♂, Guangxi, Longsheng, 14 May 2021, M Deng; 1♂, Guizhou, Jiangkou, 25 May 2021, Y-J Sui; 1♂, Guizhou, Duyun, 9 June 2017, L-J Yang; 1♂, Hainan, Changjiang, 26 April 2021, Y-J Sui.

Diagnostic characters. General color yellow. Fore wing pale brown, hind wing grayish. Fore wing longer than widest part ~ 2.6: 1, RA with one terminal, MP with four sectors. Hind wing with CuA with two terminals. Gonostyli with apical margin truncate, dorsocaudal angle not produced; each inner lower surface without hook subapically. Endosoma of aedeagus with three processes and two lobes.

Distributions. China (Guangxi, Guizhou, Hainan, Taiwan).

Hauptenia magnifica (Yang & Wu, 1994)

Malenia magnifica Yang & Wu, 1994: 86, fig. 39.
Hauptenia magnifica (Yang & Wu, 1994): Szwedo 2006: 331.

Material examined. **China:** 2♂♂, Guangxi, Huanjiang, 27 July 2019, Y-J Sui; 5♂♂, Guizhou, Wangmo, 29 June 2013, J-C Xing; 1♂, Hainan, Ledong, 12 July 2007, Q-Z Song; 1♂, Yunnan, Mengla, 29 August 2017, Y Zhi.

Diagnostic characters. General color yellow. Fore wing pale brown, hind wing dirty white. Fore wing longer than widest part ~ 2.7: 1, RA with two terminals, MP with four sectors. Hind wing with CuA with three terminals. Gonostyli with apical margin obliquely truncate, dorsocaudal angle not produced; each inner lower surface with a hook subapically, directed basad. Endosoma of aedeagus with five processes, and two lobes out of four produced into processes.

Distributions. China (Guangxi, Guizhou, Hainan, Taiwan, Yunnan).

Hauptenia palgongsanensis Rahman, Kwon & Suh, 2012

Hauptenia palgongsanensis Rahman, Kwon & Suh, 2012: 65, figs 12–22.

Material examined. No specimen examined.

Diagnostic characters. (Based on Rahman et al. 2012). General color dark brown. Fore wing dark brown to black, hind wing grayish white. Fore wing longer than widest part ~ 2.8: 1, RA with two terminals, MP with four sectors. Hind wing with CuA with three terminals. Gonostyli with apical margin obliquely truncate, dorsocaudal angle produced into finger-shaped process; each inner lower surface without hook subapically. Endosoma of aedeagus with four processes and four lobes.

Distribution. Korea.

Hauptenia tripartita Rahman, Kwon & Suh, 2012

Hauptenia tripartita Rahman, Kwon & Suh, 2012: 66, figs 23–33.

Material examined. **China:** 1♂, Anhui, Jinzhai, Tianma National Nature Reserve, 27 June 2013, B Li and B Yan; 2♂♂, Guangxi, Xing'an, 23 July 2015, Q Luo; 3♂♂, Guizhou, Liping, 14 July 2016, Y-J Wang; 1♂, Hunan, Wugang, 11 August 2007, X-S Chen; 2♂♂, Liaoning, Kuandian, 31 August 2010, B Li; 2♂♂, Shaanxi, Foping, 4–9 August 2010, P Zhang; 3♂♂, Sichuan, Yingjing, 28 July 2022, F-E Li; 1♂, Zhejiang, Pan'an, 2 July 2013, B Li.

Diagnostic characters. General color yellowish brown. Fore wing yellowish brown, hind wing grayish white. Fore wing longer than widest part ~ 2.7–2.9: 1, RA with two terminals, MP with four sectors. Hind wing with RP CuA with three terminals. Gonostyli with apical margin obliquely truncate, dorsocaudal angle not produced; each inner lower surface with a hook subapically, directed basad. Endosoma of aedeagus with six processes and four lobes, the largest lobe wide and tripartite.

Distributions. China (Anhui, Guangxi, Guizhou, Hunan, Liaoning, Shaanxi, Sichuan, Zhejiang), Korea.

Note. This species is recorded from China for the first time.

Discussion

The genus *Hauptenia* Szwedo, 2006, belongs to the tribe Cedusini (Hemiptera: Derbidae: Breddiniolinae), which is characterized by sensory pits on head and on wings absent, subantennal process well developed, jugal margin of hind wings without stridulatory plate, and tibia of hind leg without lateral spine (Emeljanov 1996). The tribe Cedusini comprises the subtribes Cedusina Emeljanov, 1992 and Eocenchreina Emeljanov, 2008. The obvious difference between them is that species of Cedusina have cixiid venation of the clavus (fore wing with joined claval veins Pcu + A1 reaching commissural margin of fore wing, reaching vein A2), and species of Eocenchreina have achilid venation of the clavus (fore wing with joined claval veins Pcu +A1 reaching claval suture, reaching CuP, near apex of clavus). As the subtribe Eocenchreina was erected for Cedusini with the achilid venation of the clavus, the genus *Hauptenia* was indirectly placed in the subtribe Cedusina by Emeljanov (2008). Morphologically, the whole subtribe Cedusina are very similar externally, but *Hauptenia* may be easily distinguished from other genera of Cedusina by the short and stout gonostyli, the pygofer with its dorsocaudal angle not produced, and the spinal formula of the hind leg 7–6–5 (Szwedo 2006; Rahman et al. 2012). In terms of geographical distribution, *Hauptenia* may be closely related to *Produsa* and *Muiredusa* in the same subtribe Cedusina. However, for the exact relationships within the subtribe Cedusina, more specimens need to be examined and molecular biology techniques to be used in the future studies.

Due to the original literature not recording host plants of these planthoppers, they are not known. In our study, we found that a few specimens of *Hauptenia* (*H. fellea*, *H. magnifica*, and *H. tripartita*) were collected on bamboo. In addition, some specimens of *H. glutinosa* and *H. jacula* were collected by light traps, and we speculate that some species of the genus *Hauptenia* have positive phototropism.

Based on the diverse natural environment in China, we expect that further collecting will increase the number of new records or species, and suggest that specimens already collected and stored in collections should be reanalyzed.

Acknowledgements

The authors are grateful to associate researcher Dawa at the Institute of Plateau Biology in Xizang Province, China for his help with the specimen collection in Xizang. This work was supported by the National Natural Science Foundation of China (grant nos. 32060343, 31472033), the Science and Technology Support Program of Guizhou Province (grant no. 20201Y129) and the Program of Excellent Innovation Talents, Guizhou Province (grant no. 20154021).

References

Bourgoin T (1987) A new interpretation of the homologies of the Hemiptera male genitalia, illustrated by the Tettigometridae (Hemiptera, Fulgoromorpha). Proceedings 6th Auchenorrhyncha Meeting, Turin, Italy, 7–11 September 1987, 113–120.

Bourgoin T (2023) FLOW (Fulgoromorpha Lists on The Web): a world knowledge base dedicated to Fulgoromorpha. Version 8. http://hemiptera-databases.org/flow/ [Updated 21 March 2023]

Bourgoin T, Huang J (1990) Morphologie comparée des genitalia mâles des Trypetimorphini et remarques phylogénétiques (Hemiptera: Fulgoromorpha: Tropiduchidae). Annales de la Société Entomologique de France. Nouvelle Série 26 (4): 555–564.

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

Emeljanov AF (1996) On the system and phylogeny of the family Derbidae (Homoptera, Cicadina). Entomological Review 75(2): 70–100.

Emeljanov AF (2008) Two new genera of the family Derbidae from the New World, with description of recent and extinct Miocene new species (Homoptera, Fulgoroidea). Entomological Review 88(8): 910–915. https://doi.org/10.1134/S0013873808080046

Jhan PK, Rahman MA, Khan MH, Jahan H (2016) A newly recorded genus *Hauptenia* Szwedo (Hemiptera: Fulgoromorph: Derbidae) in Bangladesh, with description of a new species. International Journal of Innovative Research 1(1): 1–4.

Rahman MA, Kwon YJ, Suh SJ (2012) Two newly recorded genera and three new species of the tribe Cedusini (Hemiptera: Fulgoromorpha: Derbidae) from Korea. Zootaxa 3261: 59–68. https://doi.org/10.11646/zootaxa.3261.1.3

Sui YJ, Chen XS (2019) Review of the genus *Vekunta* Distant from China, with descriptions of two new species (Hemiptera, Fulgoromorpha, Derbidae). ZooKeys 825: 55–69. https://doi.org/10.3897/zookeys.825.31542

Szwedo J (2006) First fossil record of Cedusini in the Eocene Baltic amber with notes on the tribe (Hemiptera: Fulgoromorpha: Derbidae). Russian Entomological Journal 15(3): 327–333.

Yang CT, Wu RH (1994) Derbidae of Taiwan (Homoptera: Fulgoroidea). Cheng Chung Shu Chü, T'ai-pei, 230 pp.

ZooKeys 1157: 109–125 (2023)
doi: 10.3897/zookeys.1157.95986
https://zookeys.pensoft.net

SHORT COMMUNICATION

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Taxonomic identity of *Distaplia stylifera* (Tunicata, Ascidiacea), a new arrival to the eastern Pacific displaying invasive behavior in the Gulf of California, Mexico

Betzabé Moreno-Dávila[1], Leonardo Huato-Soberanis[1], Jaime Gómez-Gutiérrez[2], Carolina Galván-Tirado[3], Carlos Sánchez[4], Teresa Alcoverro[5], Eduardo F. Balart[1], Xavier Turon[5]

1 *Programa de Ecología Pesquera, Centro de Investigaciones Biológicas del Noroeste, C.P. 23096, La Paz, BCS, Mexico* **2** *Departamento de Plancton y Ecología Marina, Centro Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional, C.P. 23096, La Paz, BCS, Mexico* **3** *CONACYT, Universidad Autónoma de Baja California Sur, La Paz, BCS, Mexico* **4** *Departamento de Ciencias Marinas y Costeras, Universidad Autónoma de Baja California Sur, La Paz, BCS, Mexico* **5** *Department of Marine Ecology, Centre for Advanced Studies of Blanes (CEAB-CSIC), Blanes, Spain*

Corresponding author: Xavier Turon (xturon@ceab.csic.es)

Academic editor: Tito Lotufo | Received 7 October 2022 | Accepted 6 March 2023 | Published 5 April 2023

https://zoobank.org/51D4F525-0CD4-4853-A1E1-F2CC78ACE0C7

Citation: Moreno-Dávila B, Huato-Soberanis L, Gómez-Gutiérrez J, Galván-Tirado C, Sánchez C, Alcoverro T, Balart EF, Turon X (2023) Taxonomic identity of *Distaplia stylifera* (Tunicata, Ascidiacea), a new arrival to the eastern Pacific displaying invasive behavior in the Gulf of California, Mexico. ZooKeys 1157: 109–125. https://doi.org/10.3897/zookeys.1157.95986

Abstract

A colonial ascidian of the genus *Distaplia* caused a mass mortality of the pen shell *Atrina maura* (Sowerby, 1835) during June 2016 in the southwest of the Gulf of California (Mexico), with a significant socio-economic cost. Tentatively identified in previous works as *Distaplia* cf. *stylifera*, a precise taxonomic determination was still lacking. In the present work, based on a detailed morphological study, it is confirmed that this aggressive species is *Distaplia stylifera* (Kowalevsky, 1874). Originally described from the Red Sea, the species currently has a wide circumtropical distribution (with the exception of the Eastern Pacific to date) and is reported as introduced in parts of its range. The present account thus represents an important range extension of this species. However, when revising the original description and later observations, the reported variability of several characters makes it likely that the binomen is in fact a complex of species, as is common in other ascidians with wide distributions. A complete morphological and genetic study including populations from the entire range of distribution would be necessary to settle the status of *D. stylifera*. Taxonomic uncertainties hinder a correct interpretation of biogeographical patterns and inference on the origin of the studied population. Nevertheless, the known introduction potential of the species, coupled with an explosive growth in an anthropized environment, and the lack of any previous reports in the Eastern Pacific, strongly suggest that the investigated population represents yet another instance of ascidian introduction. From the point of view of management, its invasive behavior is cause for great concern and warrants mitigation measures.

Keywords
Ascidian, Gulf of California, introduced species, mass mortality, taxonomy, tunicate

Introduction

Taxonomic identification of introduced species is a pre-requisite of any meaningful study of their biology and ecology, including correct ecosystem management (Geller et al. 1997). However, the study of biological introductions in the sea is plagued with taxonomic issues, complicated by the decline of taxonomic expertise (Engel et al. 2021) and the failure to cope with global distributions common in introduced species.

Several species of ascidians, known as sea squirts, are highly successful invaders and cause significant damage, modification, or impact to their new habitats, displacing native species or causing harm in aquaculture farms in several regions of the world (Lambert 2007; Locke and Hanson 2011). However, ascidians are a difficult group from the point of view of taxonomy, with few specialists and scarce diagnostic characters that are also difficult to observe (Monniot et al. 1991). Ascidians with wide distributions have often been showcased as instances of cryptic speciation, whereby several genetic lineages have been lumped under the same nominal species such as *Ciona intestinalis* (Linnaeus, 1767) (Gissi et al. 2017), *Diplosoma listerianum* (Milne Edwards, 1841) (Pérez-Portela et al. 2013), *Styela canopus* (Savigny, 1816) (Barros and Rocha 2020) and *Botryllus schlosseri* (Pallas, 1766) (Bock et al. 2012). Additionally, the conspecificity of populations of other widespread introduced ascidians has been supported by molecular evidence like *Styela plicata* (Lesueur, 1823) (Barros et al. 2009; Pineda et al. 2011) and *Didemnum vexillum* Kott, 2002 (Stefaniak et al. 2009; Casso et al. 2019). Often, widely distributed species translocated to several areas of the world receive local names, and thus fail to be recognized as introduced, constituting instances of the so-called pseudo-indigenous species (Carlton 2009). Upon closer morphological and genetic examination, these species can be shown to correspond to taxa described elsewhere (e.g., Ordóñez et al. 2016; Viard et al. 2019). Detailed morphological observation, coupled whenever possible with genetic data and integration of biogeographic information, are necessary for a reliable identification of potentially introduced ascidian species.

Eleven introduced species of ascidians have been detected in Mexico and five of them in the Gulf of California (Bastida-Zavala et al. 2014). *Styela canopus* was recorded at Estuario de Urías, Sinaloa during 2004 (Salgado-Barragán et al. 2004). Four introduced species of ascidians were recorded (*Botrylloides violaceus* Oka, 1927, *Botryllus schlosseri*, *Lissoclinum fragile* (Van Name, 1902) and *Polyclinum constellatum* Savigny, 1816) several years later in various docks of Mazatlán, and an oyster farm located in Topolobampo, Sinaloa, Mexico (Tovar-Hernández et al. 2010, 2013). *Botrylloides violaceus* is the main species that grows on oyster cultures and natural seabeds in Atlantic Canada (Carver et al. 2006).

A rapid population growth of an ascidian, preliminarily identified as *Distaplia* cf. *stylifera*, was detected in Ensenada de La Paz starting in June 2015 and causing one year later (June 2016) a mass mortality event on *Atrina maura* (Sowerby, 1835), a bivalve whose natural populations are harvested and represents an economically relevant income for regional fishermen (NOS 2015; Moreno-Dávila et al. 2021). The abrupt and rapid colonization of the ascidian was facilitated by the abundant substrate provided by the pen shell, which reached mean densities in the area of more than 47 indvs/250 m^2 (Moreno-Dávila et al. 2021). The pen shell fishery was closed since 2013 to allow the population to recover after years of over-exploitation, thus the rapid tunicate colonization was linked to the success in the recovery of the pen shell population, while other environmental variables (sea surface temperature, sea surface Chlorophyll-*a* concentration, and dissolved oxygen concentration) did not have a significant ecological influence (Moreno-Dávila et al. 2021). Results of a biofouling experiment carried out in Bahía de La Paz reported that *Distaplia* cf. *stylifera* was the most abundant macro-organism in metal panels with sliding coatings based on silicone resins after the second month of the experiment (Galicia-Nicolas et al. 2018). *Distaplia* cf. *stylifera* in Ensenada de La Paz is currently the basibiont of 28 epibiont polychaete species (Cardona-Gutiérrez 2021). These studies noted the increased abundance of *Distaplia* cf. *stylifera* from 2015 to 2016. Its proliferation has thus caused relevant negative impacts, as it is the main biofouling organism in the area (Galicia-Nicolas et al. 2018), and positive impacts such as the availability of habitat for different species of polychaetes (Cardona-Gutiérrez 2021) that are likely to continue over time. However, so far there is no taxonomic work on *Distaplia* specimens collected in the Ensenada de La Paz to infer conclusively its taxonomic status and hence whether it can be a native or introduced species (Valéry et al. 2008, 2009; Warren 2021). Several previous ecological and pharmacological studies used the name *Distaplia stylifera* (Kowalevsky, 1874) for specimens collected in Ensenada de La Paz, BCS, Mexico (Galicia-Nicolas et al. 2018; Mendoza 2019; Cardona-Gutiérrez 2021; Cruz-Escalona et al. 2021). However, these studies only assumed, without any proper taxonomic evaluation, that the individuals collected at Ensenada de La Paz belonged to this species reported in other regions of the world.

The goal of the present study is to describe the morphology of the ascidian (allegedly *D. stylifera*) collected in Ensenada de La Paz, BCS, Mexico, and discuss its taxonomic status as compared with previous descriptions of *D. stylifera* in other regions of the world.

Materials and methods

The study area (Bahía de La Paz) is located southeast of Baja California Sur (24°07'05"N, 110°17'08"W and 24°80'85"N, 110°70'18"W) (Fig. 1A, B). The Ensenada de La Paz is separated from the Bahía de La Paz by a 12 km long (0.4–2.8 km wide) sandy bar known as El Mogote (Cruz-Orozco et al. 1989; Obeso-Nieblas et al. 2008) (Fig. 1A–C). The Ensenada has an area of ~ 45 km^2 and a maximum depth of 10 m and is connected to the Bahía de La Paz through a shallow channel (< 10 m depth) approximately 1 km wide and 4 km long (Fig. 1C). The bottom of the cove ranges from sand to mud-silt (Espinoza 1977). The conurbation of La Paz city, with 300,000 people, has seven yacht docks (Fig. 1C), and a commercial harbor Pichilingue located at the entrance of Bahía de La Paz.

Colonies of the three different color morphs (white, orange, and purple) of *Distaplia* cf. *stylifera* were collected with autonomous diving, using a spatula to extract the colonies from the substrate in four sampling sites of Ensenada de La Paz BCS (24°8'01"N, 110°23'26"W; 24°7'29"N, 110°22'25"W; 24°8'41"N, 110°21'52"W; 24°9'31"N, 110°20'16"W) during December 2017, June 2021, and October 2021, to investigate the identity of the species that caused the mass mortality event of pen shells *Atrina maura* (Moreno-Dávila et al. 2021). The specimens were relaxed with menthol crystals for taxonomic examination. After observing live specimens with a stereoscope, some of the collected ascidians were fixed and preserved in 5% formalin for further morphological observation. Twelve colonies were preserved in 96% undenatured ethanol and the zooids were extracted to obtain sequences of the mitochondrial Cytochrome Oxidase I (COI) gene.

Morphological observations were carried out on relaxed and preserved material using a stereomicroscope with mounted digital camera. Staining was performed when necessary, using Masson's Hemalum. These morphological observations were compared with relevant descriptions from the literature (see Discussion).

For molecular analyses, zooids from 12 ascidian colonies with white, orange, and purple coloration were dissected and their digestive tracts removed to minimize contamination. Their genomic DNA was extracted using the Qiagen DNeasy Blood & Tissue kit (Qiagen, Valencia, CA), following the manufacturer's protocol. To amplify a fragment of the mitochondrial Cytochrome Oxidase I (COI) gene, different primer sets were used, either universal (Folmer et al. 1994) or specific for tunicates (Iannelli et al. 2007; Stefaniak et al. 2009; Brunetti et al. 2017; Salonna et al. 2021). We also tried a nested PCR strategy following Salonna et al. (2021): A first amplification was done with the primer pair dinF/Nux1R, and then a 1:100 dilution of the PCR product was used as template for the second PCR with the primer pair cat1F/ux1R. PCR amplification followed in 20 μl total reaction volume with 2.5 mM MgCl2, 0.3 mM dNTPs, 1X buffer, 0.3 μM of each primer, and 0.16 U of Taq DNA polymerase (Invitrogen, Inc., Carlsbad, CA). The PCR program consisted of an initial denaturing step at 95 °C for 5 min, 35 amplification cycles (denaturing at 95 °C for 1 min, annealing at 43 °C for 1 min and extension at 72 °C for 1 min), and a final extension at 72 °C for 5 min. Positive PCR products were purified and sequenced by Macrogen Inc. in forward and reverse strands.

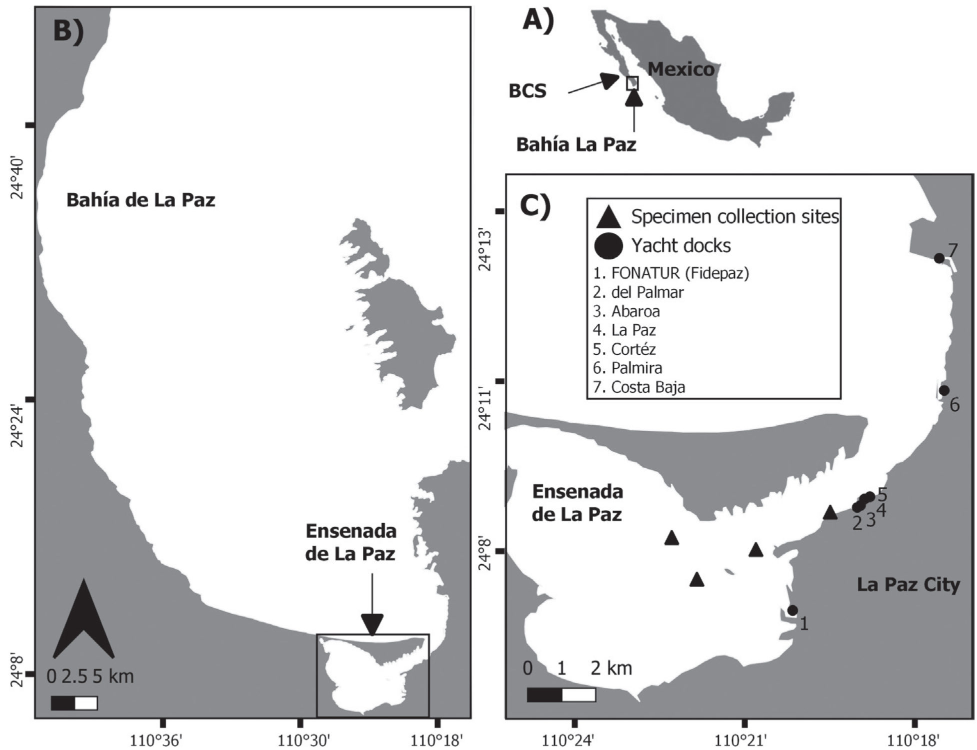


Figure 1. A area of study in Ensenada de La Paz located in the southern part of Bahía La Paz, Baja California Sur, Mexico **B** both bodies of water are located on the southwest coast of the Gulf of California, Mexico **C** sampling sites and potential sources of dispersal of tunicates (circles) [FONATUR (Fidepaz) (24°07'3"N, 110°20'47"W), del Palmar (24°09'09"N, 110°19'39"W), Abaroa (24°09'11"N, 110°19'36"W), La Paz (24°09'17"N, 110°19'31"W), Cortéz (24°09'19"N, 110°19'26"W), Palmira (24°10'58"N, 110°18'09"W) and Costa Baja (24°13'07"N, 110°18'12"W)].

Description

***Distaplia stylifera* (Kowalevsky, 1874)**
Figs 2–4

Didemnium styliferum Kowalevsky, 1874: 443, pl. 30, figs 1–16.
Holozoa bursata Van Name, 1921: 366–368, figs 44–47.
Distaplia bursata – Van Name 1930: 456, fig. 31.
Distaplia mikropnoa – Hartmeyer 1919: 130.
Distaplia stylifera – Hartmeyer 1919: 135; Michaelsen 1930: 502; Van Name 1945: 147, fig. 71.
Polyclinum mikropnous – Sluiter 1909: 94, pl. 5, fig. 1.

Material examined. CEAB.ASC.DIST–001: Ensenada de La Paz, Mexico; two purple colonies, one as epibiont on sea pen *A. maura* and one attached to a PVC pipe, 1–3 m depth, 03/Dec/2017. CEAB.ASC.DIST–002: Ensenada de La Paz, Mexico; two white colonies, one epibiont on *A. maura* and one attached to a buoy, 1–3 m depth, 03/Dec/2017. CEAB.ASC.DIST–003: Ensenada de La Paz, Mexico; three orange colonies epibiont on *A. maura*, on a rope and on a buoy, 1–3 m depth, 03/Dec/2017. CEAB.ASC.DIST–004: Ensenada de La Paz, Mexico; three orange colonies attached to wooden yacht docks, 0.5 m depth, 19/Jun/2021. CEAB.ASC.DIST–005: Ensenada de La Paz, Mexico; three purple colonies attached to wooden yacht docks, 0.5 m depth, 19/Jun/2021. CEAB.ASC.DIST–006: Ensenada de La Paz, Mexico; three white colonies on wooden yacht docks, 0.5 m depth, 19/Jun/2021. CEAB.ASC.DIST–007: Ensenada de La Paz, Mexico; eight orange colonies on wooden yacht docks, 0.5 m depth, 9/Jul/2021. All the colonies examined in the present study have been deposited in the Biological Collection of the Center of Advanced Studies of Blanes (CEAB) with voucher codes CEAB.ASC.DIST–001 to 007.

Morphological characters. Colonies can be mushroom-like with orange color and white mottles marking the common cloacal apertures (Fig. 2A). Purple (Fig. 2B) or white colonies (not shown) can also be found. They are up to 2 cm in height and 2–2.5 cm in head (cormidium) diameter (Fig. 2C, D). The basal part of the colonies is less colored, and only the distal region had pigment after preservation. The tunic is firm and sometimes the stalks are branched so that different heads originate from the same base. Each head had one or several zooid systems, each with a central common cloacal aperture to where double rows of zooids converge. Small systems are made up of a simple circle of zooids. Colonies can also be cushion-shaped, spread over the substrate without a stalk.

The zooids are up to 5 mm in length (excluding the gonadal sacs) (Fig. 2E). They are divided into thorax and abdomen, with two stalked sacs adhered. One sac, smaller than the abdomen and connected to its right posterior side (Fig. 3A), includes the gonads. The second sac contains embryos and larvae in incubation and can be longer than the zooid itself. It is attached by a thin peduncle joining the zooid at the posterior part of the pharynx, on the right-hand side close to the dorsal line (Fig. 2E).

The thorax has a smooth-rimmed oral siphon (or with six slight lobulations), with a large atrial aperture exposing most of the branchial sac. An atrial languet, often consisting of a wide flap-like lid with smooth or lobed margins, is placed at the top of the atrial aperture (Figs 3A, 4A). There are several transverse muscular bands across the atrial languet. Approximately 30 longitudinal muscular bands run over the thorax of each side. The thorax has ~ 14 simple oral tentacles. The pharynx has four stigmata rows clearly divided by parastigmatic vessels (Fig. 4A). All thoraces examined (except for those recently budded) had these parastigmatic vessels. The number of stigmata per half-row are typically between 18 or 19 (reaching 22 in larger zooids) in the first two rows and 15 or 16 (reaching 18) in the two posterior rows. There are three simple dorsal languets between rows, slightly displaced to the left-hand side (Fig. 3B, C).

The abdomen has an elongated and curved stomach. Its wall is marked by fine plications (> 20 per side) that, in section, are visible in both the outer and the inner surface of the wall (Figs 3D, E, 4B). The plications are longitudinal but can be interrupted

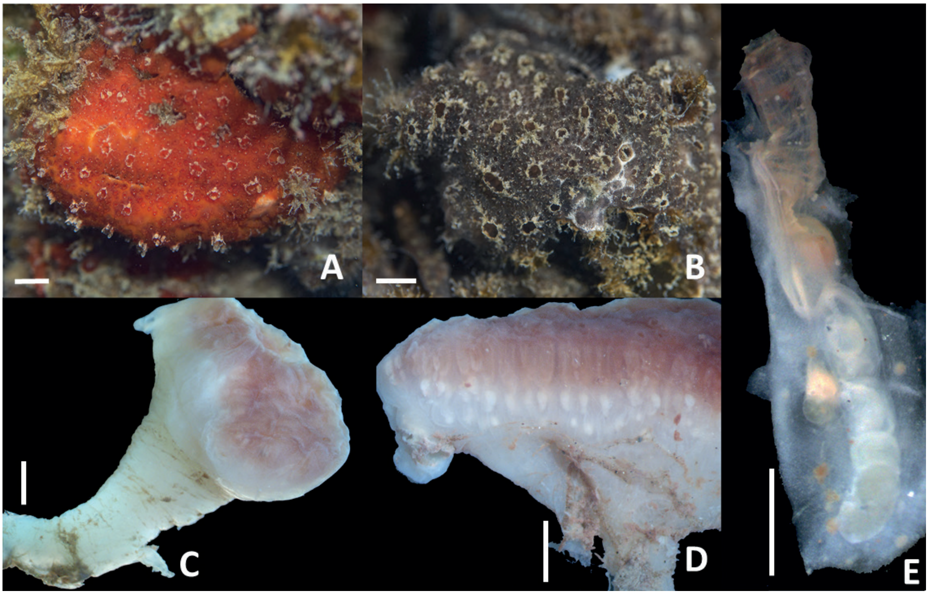


Figure 2. *Distaplia stylifera* **A** live orange colony **B** live purple colony **C, D** typical mushroom-shaped colonies **E** Zooid. Scales bars: 10 mm (**A, B**); 2.5 mm (**C, D**); 1 mm (**E**).

or divided. There is a short post-stomach that connects to an enlarged mid-intestine located at the bottom of the gut loop. The distal intestine runs anteriorly and ends in a bilobed anus at the base of the atrial aperture. The pyloric gland features a vesicle or reservoir between the stomach and the intestine and continues anteriorly forming sinuous tubules over the intestine in front of the stomach (Figs 3D, E, 4A).

The gonads lie in a pedunculated sac, with one well-formed oocyte (sometimes a smaller second one) at the bottom and a cluster of five or six elongated or wedge-shaped testes placed vertically. The common sperm duct arises posteriorly from the cluster of testes, but turns anteriorly at its very beginning, without overlapping the oocytes (Figs 3F, 4B).

All *Distaplia* colonies examined had larvae incubating in long sacs that reach posteriorly deeper than the zooids themselves in the colonies. Usually, several larvae can be seen in the brooding sacs, containing up to two well-formed larvae plus three embryos. Larvae are ~ 1.3 mm length, and when fully developed their body become elongated, reaching up to 1.5 mm in trunk length. Larvae possess three adhesive papillae, two dorsal and one ventral, with a globular ampulla each in the stalks. The oozooid is well-formed, with four rows of stigmata already present and an incipient abdomen folded under the branchial sac (Figs 3H, 4C). The sensory vesicle contains two pigmented spots, a larger one (likely corresponding to the ocellus) and a smaller one (likely the otolith) just under it (Fig. 3G). The pigmented spots are very close and, given that the larvae are not completely transparent, the two pigmented spots can be easily taken as one.

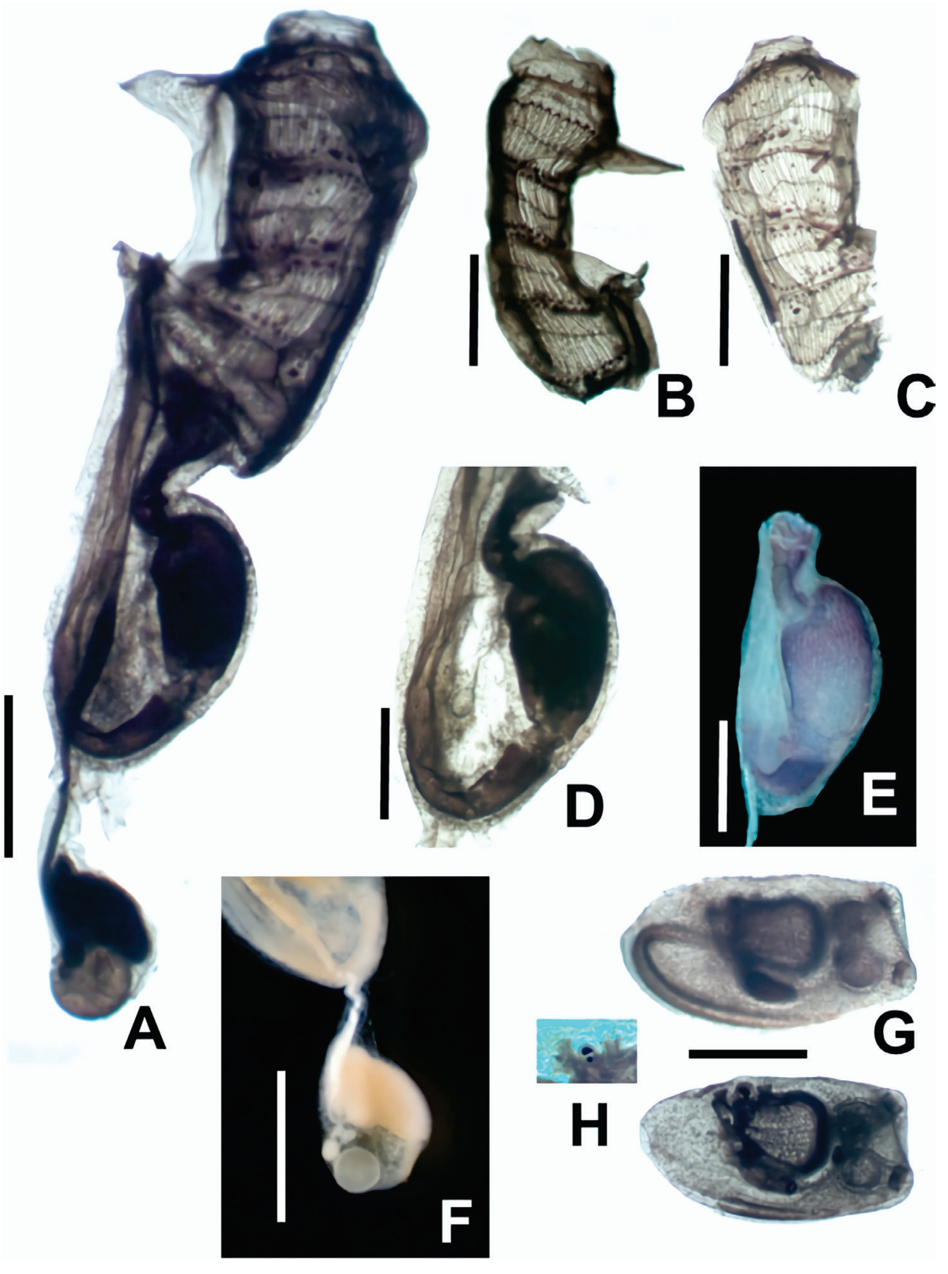


Figure 3. *Distaplia stylifera* **A** zooid (thorax and abdomen) **B** thorax **C** dissected thorax **D, E** stomach **F** gonads **G** larvae **H** enlargement of one larva showing two pigmented spots. Scales bars: 10 mm (**A**); 0.5 mm (**B, C, F, G**); 0.25 mm (**D, E**). All images (except **F**) correspond to stained zooids.

Geographic distribution. *Distaplia stylifera* was described in the Red Sea (Kowalevsky 1874) and has been reported in several areas of the Indo-Pacific. It has been found in Australia (e.g., Brewin 1953; Millar 1963, 1966; Kott 1990, 2002), Philippines (Mil-

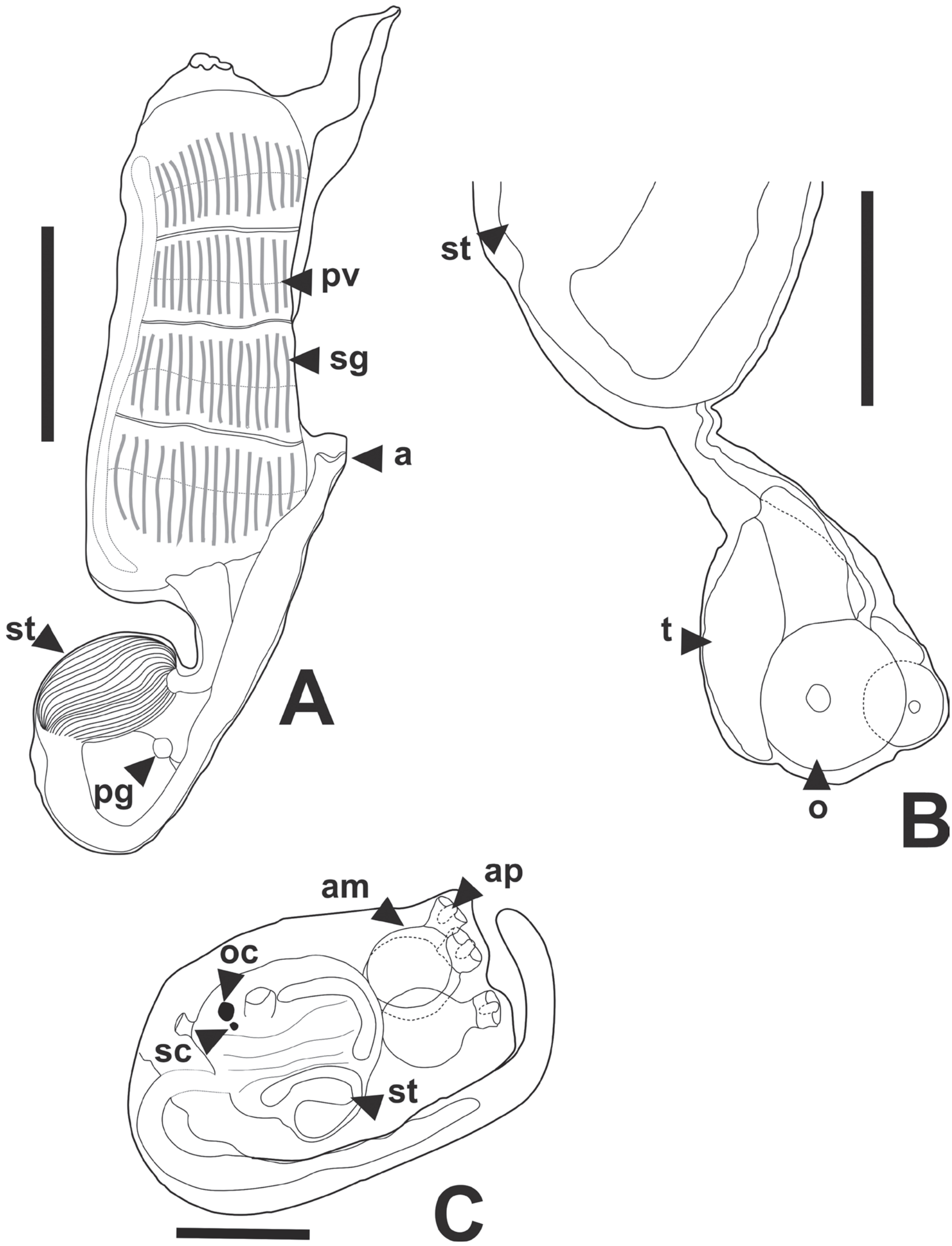


Figure 4. *Distaplia stylifera* **A** zooid (thorax and abdomen) **B** abdomen **C** larva. Abbreviatures: **a.** anus; **am.** ampullae; **ap.** adhesive papillae; **oc.** ocellus; **o.** oocyte; **pv.** parastigmatic vessels; **pg.** pyloric gland vesicle; **sc.** statocyte, **sg.** stigmata; **st.** stomach; **t.** testes. Scales bars: 1 cm (**A**); 0.5 mm (**B, C**).

lar 1975; Monniot and Monniot 2001), South China Sea (Lee et al. 2016), Madagascar (Monniot 2012), and (as *D. mikropnoa*, a debated synonymy, see Discussion) in Palau Islands (Tokioka 1955, 1967). There is a dubious report in the Mediterranean Sea (Pérès

Figure 5. Sites of previous records of *Distaplia stylifera*: 1) Red Sea, Kowalevsky 1874 (type locality). Indo-Pacific, 2) Brewin (1953). 3) Millar 1963 (1966); 4) Millar (1975); 5) Kott 1990 (2002); 6) Monniot and Monniot (2001); 7) Monniot (2012); 8) Shenkar (2012); 9) Lee et al. (2016). Mediterranean, 10) Pérès (1956). Western Atlantic Ocean, 11) Van Name (1921, 1945); 12) Monniot and Monniot (1984, Monniot 1988); 13) Cole and Lambert (2009); 14) Rocha et al. (2010); 15) Villalobos et al. (2017); 16) Rocha et al. (2011); 17) Streit et al. (2021); 18) Dias et al. (2013). Eastern Pacific Ocean, 19) present study. The type locality in the Red Sea and the record of the present study are indicated with stars.

1956). It is also known from the Western Atlantic coast from North Carolina to Jamaica (Van Name 1921, as *D. bursata*, Van Name 1945; Villalobos et al. 2017). It has been reported in several Caribbean locations (Monniot and Monniot 1984; Monniot 1988; Rocha et al. 2010; Streit et al. 2021), and there are recent reports further south in artificial substrates at Sao Paulo region, Brazil (Rocha et al. 2011; Dias et al. 2013). It therefore appears to have a wide circumtropical distribution (Fig. 5, but see discussion for potential taxonomic issues), although it has never been documented in the Eastern Pacific.

Molecular analyses. Despite obtaining positive amplifications with all the primer pairs assayed, no sequence could be obtained that blasted with ascidian mitochondrial COI. Our sequences were closer to algae, mycoparasites or bacteria with an 80–83% similarity. These results may be due to the presence of mutations in the binding sites or contamination. However, we consider contamination unlikely given the care taken during the extraction of the samples.

Discussion

The taxonomy of the genus *Distaplia* is mainly based on characters such as colony shape, arrangement of zooids in systems, presence or not of gonadal sac, stigmata per row, stomach shape and external surface, and muscle arrangement (Van Name 1945; Kott 1990; Monniot and Monniot 2001). According to these morphological characters, the specimens collected at Ensenada de La Paz between 2015 and 2017 reported in Moreno-Dávila et al. (2021) and in the present study match well with the detailed descriptions of *D. stylifera* given by Van Name (1945), Monniot (1988), and Monniot and Monniot (2001). The original description of Kowalevsky (1874) as *Didemnium styliferum* lacks some key characters, as is often the case with old descriptions: the presence of parastigmatic vessels was not mentioned, and the holotype specimen was likely juvenile, with under-developed gonads. In addition, the colonies were lacking larvae.

However, the descriptions of *D. stylifera* reported in the literature are not entirely consistent in several morphological characteristics. As is common in widespread colonial ascidians, it is possible that worldwide reports of *D. stylifera* encompass a group of closely related species. One critical diagnostic morphological characteristic is the presence or absence of parastigmatic vessels. All the colonies collected at Ensenada de La Paz had parastigmatic vessels. Kott (1990) reported that all the specimens collected in Australia, except one colony, lacked parastigmatic vessels. Monniot (1988) reported them in material from the Caribbean but noted the absence of parastigmatic vessels in colonies collected from Madagascar (Monniot 2012). The absence of parastigmatic vessels has been pointed out as the main characteristic distinguishing *D. stylifera* from *D. mikropnoa* (Monniot and Monniot 2001), and Millar (1975) indicated that the variation in this character suggested that these species were synonymous. However, Kott (2002) refers to other differences distinguishing *D. mikropnoa* from *D. stylifera* such as the long double rows of zooids converging to the terminal common cloacal apertures, a long post-pyloric part of the gut loop, and the lack of a gastric reservoir. Another difference is the course of the gastro-intestinal ducts that does not cross from the stomach to the ascending limb of the gut loop but extends down the descending loop. She therefore concluded that both are valid biological species but have been confused in the literature. In particular, the reports of Tokioka (1955, 1967) of *D. mikropnoa* in Palau correspond to *D. stylifera* according to Kott (1990).

Several authors reported *D. stylifera* testis with as many as 15 oval follicles (Tokioka 1955, 1967; under the name *D. mikropnoa* Kott 1990). The specimens from Ensenada de La Paz had only five or six elongated follicles, in accordance with previous reports of *D. stylifera* (Van Name 1945; Monniot 1988; Monniot and Monniot 2001). Further, the sperm duct of *D. stylifera* is sometimes described as running posteriorly and making one or several loops over the oocytes before turning anteriorly (Kott 1990; Monniot 2012), while in other descriptions the sperm duct is straight (Van Name 1945; Monniot and Monniot 2001), as in our specimens. In addition, the gonadal sac can be pedunculated, as in our specimens, or can be almost flush with the abdomen, separated by a wide neck (Kott 1990; Monniot 2012). Millar (1975) reported a single pigmented spot (the ocellus) in the larva, while other authors describe two pigmented spots. The specimens of Ensenada de La Paz have both ocellus and otolith; although it is not easy to discern them as distinct, which can explain previous confusion in the number of pigmented spots.

We consider that these variable morphological characters (presence or absence of parastigmatic vessels, number and shape of testes and sperm duct, gonadal pouch stalked or not stalked) indicate that several species have been mixed under the taxonomic name *D. stylifera*, as suggested also by Dias et al. (2013). This taxonomic uncertainty, as well as the potential synonymy with the morphologically close *D. mikropnoa* species, can be potentially solved by further comparative morphological and genetic analyses of specimens from different regions of the world. Even keeping in mind these shortcomings, the binomen *D. stylifera* has been reported in tropical zones of the Indian Ocean, the western Pacific, and the western Atlantic. Thus, our finding in the eastern Pacific represents an important range expansion of the species.

Unfortunately, we could not obtain sequences of our colonies. There are no sequences of *Distaplia stylifera* available in GenBank and BOLD public databases, either. COI data for the single well-represented species of *Distaplia* in these databases, *D. bermudensis* Van Name, 1902, revealed divergences ~ 14–20% for different morphotypes and distribution areas. This indicates either high intraspecific variability or the existence of multiple species under *D. bermudensis* binomen, as suggested by some authors (Evans et al. 2021; Virgili et al. 2022). The high variability found in the congener *D. bermudensis* suggests that shedding light on the relationships of *D. stylifera* will require extensive sampling of the main areas where the species has been recorded to settle whether it is a single polymorphic biological species with circumtropical biogeographic distribution or a group of related species with distinct (perhaps overlapping) biogeographic distribution patterns.

Interestingly, the species (or some members of the species complex if it proves to be so) has been likely introduced in some parts of its distribution. In western Atlantic it is found from North Carolina to Brazil. Van Name (1921), under the name *D. bursata*, records it in Florida and Jamaica samples collected as early as 1884. However, in North Carolina and in the Caribbean Sea it is mostly found in lagoons and on artificial structures, typical entry points for introduced species (Rocha et al. 2010; Dias et al. 2013; Villalobos et al. 2017; Streit et al. 2021). Its status in the Caribbean has been defined either as cryptogenic (Streit et al. 2021) or as introduced species (Rocha et al. 2010). If the introduced status is eventually confirmed in that area, this species poses a worrisome threat due to its ability to rapidly colonize surfaces and displace other benthic species (Rocha et al. 2010). The species seems to be expanding southwards and was present since the 2000's in the Sao Paulo region of Brazil, where it was found only on artificial substrates (Dias et al. 2013).

Albeit taxonomic uncertainty impedes sound interpretation of biogeographical patterns, given the mass mortality event of pen shells in Ensenada de La Paz caused by *D. stylifera*, it can behave as a typical impactful invasive species. This fact, coupled with a likely previous introduction history in the Western Atlantic and the lack of reports in the Eastern Pacific, strongly suggests that it is a new arrival to the Gulf of California. The species was first noticed by NOS (2015) in June 2015, in spite of periodic monitoring of the area since 2011 for evaluation of the bivalve stocks. It is unclear whether the population detected in this region was introduced from western Pacific populations, or from Atlantic populations via the Panama Channel. Ship traffic seems the most likely introduction vector, and secondary spread by small craft to harbors in the vicinity of the Ensenada de la Paz can account for its arrival there. The origin, the number of introduction events and the existence or not of bottlenecks during the introduction can only be ascertained with detailed genetic studies of worldwide populations. For the time being, constant monitoring and eradication actions seem the only feasible measures to control its growth and to avoid further expansion (Valéry et al. 2008, 2009; Warren 2021).

Acknowledgements

We gratefully acknowledge to CONACyT 421011/263861 PhD scholarship to BBMD. J.G.-G. is a COFAA-IPN fellow, EDI-IPN, and J.G.-G, C.G.T., C.S. and E.F.B. are SNI fellows. Partial funding was provided by project MARGECH (PID2020-118550RB, MCIN/AEI/10.13039/501100011033) from the Spanish Government.

References

Barros RC, Rocha RM (2020) Genetic analyses reveal cryptic diversity in the widely distributed *Styela canopus* (Ascidiacea: Styelidae). Invertebrate Systematics 35(3): 298–311. https://doi.org/10.1071/IS20058

Barros RC, Rocha RM, Pie MR (2009) Human-mediated global dispersion of *Styela plicata* (Tunicata, Ascidiacea). Aquatic Invasions 4(1): 45–57. https://doi.org/10.3391/ai

Bastida-Zavala R, de León-González JÁ, Carballo JL, Moreno-Dávila B (2014) Invertebrados bénticos exóticos: esponjas, poliquetos y ascidias. In: Mendoza R, Koleff P (Orgs) Especies acuáticas invasoras en México. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México, 317–336.

Bock DG, MacIsaac HJ, Cristescu ME (2012) Multilocus genetic analyses differentiate between widespread and spatially restricted cryptic species in a model ascidian. Proceedings of the Royal Society B: Biological Sciences 279(1737): 2377–2385. https://doi.org/10.1098/rspb.2011.2610

Brewin BI (1953) Australian ascidians of the sub-family Holozoinae and a review of the sub-family. Transactions and Proceedings of the Royal Society of New Zealand 81(1): 53–64.

Brunetti R, Manni L, Mastrototaro F, Gissi C, Gasparini F (2017) Fixation, description and DNA barcode of a neotype for *Botryllus schlosseri* (Pallas, 1766) (Tunicata, Ascidiacea). Zootaxa 4353(1): 29–50. https://doi.org/10.11646/zootaxa.4353.1.2

Cardona Gutiérrez MF (2021) Taxocenosis de poliquetos asociados al recurso "hacha" y su evaluación bajo el efecto de *Distaplia stylifera* en la Laguna de La Paz, Baja California Sur, México Master Science thesis, Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas.

Carlton JT (2009) Deep Invasion Ecology and the Assembly of Communities in Historical Time. In: Rilov G, Crooks JA (Eds) Biological Invasions in Marine Ecosystems. Springer-Verlag, Berlin, 13–56. https://doi.org/10.1007/978-3-540-79236-9_2

Carver CE, Mallet AL, Vercaemer B (2006) Biological synopsis of the colonial tunicates, *Botryllus schlosseri* and *Botrylloides violaceus*. Canadian Manuscript Reports of Fisheries of Aquatic Science 2747: 1–42.

Casso M, Turon X, Pascual M (2019) Single zooids, multiple loci: Independent colonisations revealed by population genomics of a global invader. Biological Invasions 21(12): 3575–3592. https://doi.org/10.1007/s10530-019-02069-8

Cole L, Lambert G (2009) Tunicata (Urochordata) of the Gulf of Mexico. In: Felder DL, Camp DK (Eds) Gulf of Mexico–Origins, Waters, and Biota. Biodiversity. Texas A&M Press, College Station, Texas, 1209–1216.

Cruz-Escalona VH, Muñoz-Ochoa M, Ramírez-Luna S, Águila-Ramírez RN (2021) Ascidias invasoras ¿un problema ambiental o una ventana de oportunidades? Repositorio Nacional CONACyT 25(75): 37–43.

Cruz-Orozco R, Rojo P, Godínez L, Nava E (1989) Topografía, hidrología y sedimentos de los márgenes de la Laguna de La Paz, B.C.S. Revista de Investigación Científica de la Universidad Autónoma de Baja California Sur 1: 3–15.

Dias GM, Rocha RM, Lotufo TMC, Kremer IP (2013) Fifty years of ascidian biodiversity research in São Sebastião, Brazil. Journal of the Marine Biological Association of the United Kingdom 93(1): 273–282. https://doi.org/10.1017/S002531541200063X

Engel MS, Ceríaco LMP, Daniel GM, Dellapé PM, Löbl I, Marinov M, Reis RE, Young MT, Dubois A, Agarwal I, Lehmann AP, Alvarado M, Alvarez N, Andreone F, Araujo-Vieira K, Ascher JS, Baêta D, Baldo D, Bandeira SA, Barden P, Barrasso DA, Bendifallah L, Bockmann FA, Böhme W, Borkent A, Brandão CRF, Busack SD, Bybee SM, Channing A, Chatzimanolis S, Christenhusz MJM, Crisci JV, D'elía G, Da Costa LM, Davis SR, De Lucena CAS, Deuve T, Fernandes Elizalde S, Faivovich J, Farooq H, Ferguson AW, Gippoliti S, Gonçalves FMP, Gonzalez VH, Greenbaum E, Hinojosa-Díaz IA, Ineich I, Jiang J, Kahono S, Kury AB, Lucinda PHF, Lynch JD, Malécot V, Marques MP, Marris JWM, Mckellar RC, Mendes LF, Nihei SS, Nishikawa K, Ohler A, Orrico VGD, Ota H, Paiva J, Parrinha D, Pauwels OSG, Pereyra MO, Pestana LB, Pinheiro PDP, Prendini L, Prokop J, Rasmussen C, Rödel MO, Rodrigues MT, Rodríguez SM, Salatnaya H, Sampaio Í, Sánchez-García A, Shebl MA, Santos BS, Solórzano-Kraemer MM, Sousa ACA, Stoev P, Teta P, Trape JF, Van-Dúnem Dos Santos C, Vasudevan K, Vink CJ, Vogel G, Wagner P, Wappler T, Ware JL, Wedmann S, Zacharie CK (2021) The taxonomic impediment: A shortage of taxonomists, not the lack of technical approaches. The Linnean Society of London. Zoological Journal of the Linnean Society 193(2): 381–387. https://doi.org/10.1093/zoolinnean/zlab072

Espinoza A (1977) Los principales parámetros físico-químicos de las aguas de la Ensenada de La Paz B.C.S. Informe de labores. Centro de Investigaciones Biológicas del Noroeste. México, 5–27. https://doi.org/10.13140/RG.2.1.2377.7362

Evans JS, Erwin PM, Sihaloho HF, López-Legentil S (2021) Cryptic genetic lineages of a colonial ascidian host distinct microbiomes. Zoologica Scripta 50(4): 423–438. https://doi.org/10.1111/zsc.12482

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5): 294–299.

Galicia-Nicolas E del C, Águila-Ramírez RN, Rico-Virgen EG, Medina-López MA (2018) Colonización y sucesión de organismos marinos implicados en el proceso de biofouling en paneles sumergidos en La Paz, Baja California Sur, México. In: Estudios recientes en el Océano Pacífico Mexicano. Universidad de Colima, 25–40.

Geller JB, Walton ED, Grosholz ED, Ruiz GM (1997) Cryptic invasions of the crab *Carcinus* detected by molecular phylogeography. Molecular Ecology 6(10): 901–906. https://doi.org/10.1046/j.1365-294X.1997.00256.x

Gissi C, Hastings KEM, Gasparini F, Stach T, Pennati R, Manni L (2017) An unprecedented taxonomic revision of a model organism: The paradigmatic case of *Ciona robusta* and *Ciona intestinalis*. Zoologica Scripta 46(5): 521–522. https://doi.org/10.1111/zsc.12233

Hartmeyer R (1919) Ascidien. Resul. dr. E. Mjöberg Swed. sei. Exp. Australia 1910 – 13. K. svenska Vetensk. Arkiv för Zoologi 60(4): 1–150. https://doi.org/10.5962/bhl.part.20148

Iannelli F, Griggio F, Pesole G, Gissi C (2007) The mitochondrial genome of *Phallusia mammillata* and *Phallusia fumigata* (Tunicata, Ascidiacea): High genome plasticity at intra-genus level. BMC Evolutionary Biology 7(1): e155. https://doi.org/10.1186/1471-2148-7-155

Kott P (1990) The Australian Ascidiacea, part 2, Aplousobranchia (1). Memoirs of the Queensland Museum 29(1): 1–226.

Kott P (2002) Ascidiacea (Tunicata) from Darwin, Northern Territory, Australia. The Beagle. Records of the Museums and Art Galleries of the Northern Territory 18: 19–55. https://doi.org/10.5962/p.286295

Kowalevsky A (1874) Ueber die Knospung der Ascidien. Archiv für mikroskopische Anatomie 10: 441–470. https://doi.org/10.1007/BF02960331

Lambert G (2007) Invasive sea squirts: A growing global problem. Journal of Experimental Marine Biology and Ecology 342(1): 3–4. https://doi.org/10.1016/j.jembe.2006.10.009

Lee S-CS, Chan Y-HJ, Teo L-MS, Lambert G (2016) State of knowledge of ascidian diversity in South China Sea and new records for Singapore. The Raffles Bulletin of Zoology 34: 718–743.

Locke A, Hanson JA (2011) Trends in invasive ascidian research: A view from the 3rd International Invasive Sea Squirt Conference. Aquatic Invasions 6(4): 367–370. https://doi.org/10.3391/ai.2011.6.4.01

Mendoza JM (2019) Didemninas citotóxicas contra la línea leucémica K-562 obtenidas de *Distaplia stylifera* (Kowalevsky, 1874) Tunicata: Ascidiacea. Instituto Politécnico Nacional. Centro Interdisciplinario de Ciencias Marinas, La Paz, BCS, México.

Michaelsen (1930) Ascidiae Krikobranchiae. Die Fauna Südwest Australiens 5(7): 453–659.

Millar RH (1963) Australian ascidian in the British Museum (Natural History). Proceedings of the Zoological Society of London 141(3): 113–221. https://doi.org/10.1111/j.1469-7998.1963.tb01623.x

Millar RH (1966) Port Phillip survey 1957–1963. Memoirs of the Museum of Victoria 27: 357–385. https://doi.org/10.24199/j.mmv.1966.27.17

Millar RH (1975) Ascidians from the Indo-West-Pacific region in the Zoological Museum, Copenhagen (Tunicata, Ascidiacea). Steentrupia Zoological Museum University of Copenhagen 3: 205–336.

Monniot F (1988) Ascidies de Nouvelle-Calédonie. V. Polycitoridae du lagon. Bulletin du Musée d'Histoire Naturelle de Paris 10(2): 1979–235. https://doi.org/10.5962/p.292204

Monniot F (2012) Some ascidians from the southern coast of Madagascar collected during the "AtimoVatae" survey. Zootaxa 3197(1): 1–42. https://doi.org/10.11646/zootaxa.3197.1.1

Monniot C, Monniot F (1984) Ascidies littorales de Guadalupe VII. Espèces nouvelles et complémentaires á I 'inventaire. Bulletin du Musée d'Histoire Naturelle de Paris 6(3): 567–582. https://doi.org/10.5962/p.285903

Monniot F, Monniot C (2001) Ascidians from the tropical western Pacific. Zoosystema 23(2): 201–383.

Monniot C, Monniot F, Laboute P (1991) Coral Reef Ascidians of New Caledonia. ORSTOM editions, Paris, 247 pp.

Moreno-Dávila B, Gómez-Gutiérrez J, Alcoverro T, Ramírez-Luna S, Sánchez C, Balart EF, Huato-Soberanis L (2021) Mass mortality of pen shell *Atrina maura* (Bivalvia: Pinnidae) due to abrupt population increase of tunicate (*Distaplia* sp.) in a subtropical bay, Mexico. Estuarine, Coastal and Shelf Science 260: e107493. https://doi.org/10.1016/j.ecss.2021.107493

NOS (2015) Aprovechamiento sustentable de callo de hacha y almeja catarina en La Ensenada de La Paz, BCS. Resumen Ejecutivo. Noroeste Sustentable A. C: 42.

Obeso-Nieblas M, Shirasago-Germán B, Gaviño-Rodríguez J, Pérez-Lezama E, Obeso-Huerta H, Jiménez-Illescas Á (2008) Variabilidad hidrográfica en Bahía de La Paz, Golfo de California, México (1995–2005). Revista de Biología Marina y Oceanografía 43(3): 559–567. https://doi.org/10.4067/S0718-19572008000300015

Ordóñez V, Pascual M, Fernández-Tejedor M, Turon X (2016) When invasion biology meets taxonomy: *Clavelina oblonga* (Ascidiacea) is an old invader in the Mediterranean Sea. Biological Invasions 18(4): 1203–1215. https://doi.org/10.1007/s10530-016-1062-0

Pérès JM (1956) Etudes sur le seuil Siculo-Tunisien. 2. Ascidies. Résultats scientifiques des campagnes de la Calypso. Annales de l'Institut océanographique de Paris 32(2): 265–304.

Pérez-Portela R, Arranz V, Rius M, Turon X (2013) Cryptic speciation or global spread? The case of a cosmopolitan marine invertebrate with limited dispersal capabilities. Scientific Reports 3(1): e3197. https://doi.org/10.1038/srep03197

Pineda MC, López-Legentil S, Turon X (2011) The Whereabouts of an Ancient Wanderer: Global Phylogeography of the Solitary Ascidian *Styela plicata*. PLoS ONE 6(9): e25495. https://doi.org/10.1371/journal.pone.0025495

Rocha R, Guerra-Castro E, Lira C, Marquez S, Hernández I, Pérez A, Sardi A, Pérez J, Herrera C, Carbonini AK, Caraballo V, Salazar D, Diaz MC, Cruz-Motta JJ (2010) Inventory of ascidians (Tunicata, Ascidiacea) from the National Park La Restinga, Isla Margarita, Venezuela. Biota Neotropica 10(1): 209–218. https://doi.org/10.1590/S1676-06032010000100021

Rocha RM, Dias GM, Lofuto TMC (2011) Checklist of ascidians (Tunicata, Ascidiacea) from São Paulo State, Brazil. Biota Neotropica 11(1): 1–11. https://doi.org/10.1590/S1676-06032002000100009

Salgado-Barragán J, Méndez N, Toledano-Granados A (2004) *Ficopomatus miamiensis* (Polychaeta: Serpulidae) and *Styela canopus* (Ascidiacea: Styelidae), non-native species in Urías estuary, SE Gulf of California, México. Cahiers de Biologie Marine 45: 167–173.

Salonna M, Gasparini F, Huchon D, Montesanto F, Haddas-Sasson M, Ekins M, McNamara M, Mastrototaro F, Gissi C (2021) An elongated COI fragment to discriminate botryllid species and as an improved ascidian DNA barcode. Scientific Reports 11(1): 1–19. https://doi.org/10.1038/s41598-021-83127-x

Shenkar N (2012) Ascidian (Chordata, Ascidiacea) diversity in the Red Sea. Marine Biodiversity 42(4): 459–469. https://doi.org/10.1007/s12526-012-0124-5

Sluiter CP (1909) Die Tunicaten der Siboga-Expedition II. Abteilung. Die Merosomen Ascidien. Siboga-Expeditie 56: 1–109.

Stefaniak L, Lambert G, Gittenberger A, Zhang H, Lin S, Whitlatch RB (2009) Genetic conspecificity of the worldwide populations of *Didemnum vexillum* Kott, 2002. Aquatic Invasions 4(1): 29–44. https://doi.org/10.3391/ai.2009.4.1.3

Streit OT, Lambert G, Erwin PM, López-Legentil S (2021) Diversity and abundance of native and non-native ascidians in Puerto Rican harbors and marinas. Marine Pollution Bulletin 167: e112262. https://doi.org/10.1016/j.marpolbul.2021.112262

Tokioka T (1955) Ascidians from the Palao Islands II. Seto Marine Biological Laboratory 1(3): 115–150. https://doi.org/10.5134/174438

Tokioka T (1967) Pacific Tunicata of the United States National Museum. Bulletin – United States National Museum 251: 1–247. https://doi.org/10.5479/si.03629236.251.1

Tovar-Hernández MA, Suarez-Morales E, Yáñez-Rivera B (2010) The parasitic copepod *Haplostomides hawaiiensis* (cyclopoida) from the invasive ascidian *Polyclinum constellatum* in the Southern Gulf of California. Bulletin of Marine Science 86(3): 637–648.

Tovar-Hernández MA, Villalobos-Guerrero TF, Yáñez-Rivera B, Aguilar-Camacho JM, Ramírez-Santana ID (2013) Guía de Invertebrados Acuáticos Exóticos en Sinaloa, Geomare, A.A. USFWS, INE–SEMARTNAT. Mazatlán, México, 41 pp.

Valéry L, Fritz H, Lefeuvre JC, Simberloff D (2008) In search of a real definition of the biological invasion phenomenon itself. Biological Invasions 10(8): 1345–1351. https://doi.org/10.1007/s10530-007-9209-7

Valéry L, Fritz H, Lefeuvre JC, Simberloff D (2009) Invasive species can also be native…. Trends in Ecology & Evolution 24(11): e585. https://doi.org/10.1016/j.tree.2009.07.003

Van Name WG (1921) Ascidians of the West Indian region and southeastern United States. Bulletin of the American Museum of Natural History 44: 283–494.

Van Name WG (1930) The ascidians of Porto Rico and the Virgin Islands. Scientific Survey. Porto Rico and Virgin Islands. Transactions of the New York Academy of Sciences 10: 401–512.

Van Name WG (1945) The North and South American ascidians. Bulletin of the American Museum of Natural History 84: 1–476.

Viard F, Roby C, Turon X, Bouchemousse S, Bishop J (2019) Cryptic Diversity and Database Errors Challenge Non-indigenous Species Surveys: An Illustration With *Botrylloides* spp. in the English Channel and Mediterranean Sea. Frontiers in Marine Science 6: e615. https://doi.org/10.3389/fmars.2019.00615

Villalobos SM, Lambert G, Shenkar N, López-Legentil S (2017) Distribution and population dynamics of key ascidians in North Carolina harbors and marinas. Aquatic Invasions 12(4): 447–458. https://doi.org/10.3391/ai.2017.12.4.03

Virgili R, Tanduo V, Katsanevakis S, Terlizzi F, Villani G, Fontana A, Crocetta F (2022) The Miseno Lake (Central-Western Mediterranean Sea): An Overlooked Reservoir of Non-Indigenous and Cryptogenic Ascidians in a Marine Reserve. Frontiers in Marine Science 9: e866906. https://doi.org/10.3389/fmars.2022.866906

Warren CR (2021) Beyond 'native v. alien': Critiques of the native/alien paradigm in the Anthropocene, and their implications. Ethics, Policy & Environment 1: 1–31. https://doi.org/10.1080/21550085.2021.1961200

ZooKeys 1157: 127–143 (2023)
doi: 10.3897/zookeys.1157.99642
https://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

First report of the Afrotropical genus *Securiops* Jacobus, McCafferty & Gattolliat (Ephemeroptera, Baetidae) from Southeast Asia, with description of a new species

Thomas Kaltenbach[1,2], Sirikamon Phlai-ngam[3], Chanaporn Suttinun[4], Jean-Luc Gattolliat[1,2]

1 *Muséum Cantonal des Sciences Naturelles, Département de zoologie, Palais de Rumine, Place Riponne 6, CH-1005, Lausanne, Switzerland* **2** *University of Lausanne (UNIL), Department of Ecology and Evolution, CH-1015, Lausanne, Switzerland* **3** *Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand* **4** *Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand*

Corresponding author: Thomas Kaltenbach (thomas.kaltenbach@bluewin.ch)

Academic editor: Ben Price | Received 5 January 2023 | Accepted 7 March 2023 | Published 5 April 2023

https://zoobank.org/872505C2-6DFC-410A-8C77-704A6B3D086B

Citation: Kaltenbach T, Phlai-ngam S, Suttinun C, Gattolliat J-L (2023) First report of the Afrotropical genus *Securiops* Jacobus, McCafferty & Gattolliat (Ephemeroptera, Baetidae) from Southeast Asia, with description of a new species. ZooKeys 1157: 127–143. https://doi.org/10.3897/zookeys.1157.99642

Abstract

Recent collections in Thailand revealed the occurrence of the genus *Securiops* in Asia, formerly known from the Afrotropical Realm only. A new species of *Securiops* is described and illustrated based on larvae and eggs. Eggs of this genus are described for the first time. Morphological differences between the new species and the species from Africa are discussed. The number of species in the genus *Securiops* is augmented to five.

Keywords

Biogeography, COI, eggs, mayflies, taxonomy, Thailand

Introduction

Southeast Asia is one of the regions with the highest diversity worldwide in general, also for mayflies. Much effort has been done in the past years to get a better insight into this yet strongly understudied fauna, including studies of the lesser known, but most diverse mayfly family Baetidae. Emphasis was put on the archipelagos of Indonesia and the Philippines, and continental Thailand. As a result, new genera of Baetidae and many new species were discovered in this region (e.g., Gattolliat 2012; Sutthinun et al. 2018; Suttinun et al. 2020, 2021, 2022; Kaltenbach and Gattolliat 2019; Kaltenbach et al. 2020a, b, 2021; Kluge 2020a, b, 2022; Suttinun 2021; Phlai-ngam et al. 2022; Tungpairojwong et al. 2022; Boonsoong 2022), and more collection efforts and studies are ongoing.

Baetidae are the most diverse family of Ephemeroptera in number of genera (> 118) and number of species (> 1160) worldwide, comprising approximately one third of all mayfly species (Sartori and Brittain 2015; Jacobus et al. 2019; updated by authors). Their distribution is cosmopolitan, with the exception of New Zealand and Antarctica. With continued collections in Southeast Asia and other poorly studied regions with high diversity like New Guinea or the Indian subcontinent, we may expect further new genera and a high number of new species from these regions.

The genus *Securiops* was described by Jacobus, McCafferty and Gattolliat (2006). The type species *S. macafertiorum* (Lugo-Ortiz, 1996) from South Africa was formerly described in the genus *Potamocloeon* Gillies, 1990 (Lugo-Ortiz and McCafferty 1996). Further species are *S. mandrare* Jacobus, McCafferty & Gattolliat, 2006 from Madagascar (formerly described by Gattolliat 2003: 7 as *Potamocloeon* sp. A), *S. megapalpus* Jacobus, McCafferty & Gattolliat, 2006 from Ivory Coast, and *S. mutadens* Jacobus, McCafferty & Gattolliat, 2006 from Gambia, Guinea and Ivory Coast (larva misidentified and described as *Potamocloeon dentatum* by Gillies 1988: 53) (Jacobus et al. 2006). Kluge (2020b) proposed *Securiops* as a subgenus to *Procloeon* Bengtsson, 1915, together with *Oculogaster* Kluge, 2016, *Pseudocentroptiloides* Jacob, 1987 and *Monilistylus* Kluge, 2020. They are all sharing the following autapomorphy: one large, posteriorly directed spine outside laterally on each cercomere in the distal part of the larval cerci; usually, it is spindle-like and thickened and its length exceeds the length of the cercomere (Kluge 2020b). However, we are treating *Securiops* as a separate genus in this study, based on a unique combination of characters, which distinguish it from all other Baetidae genera: (1) labium with strongly reduced glossae, enlarged paraglossae, and very broad, hatchet-like palps; (2) tergalii I–IV with two lamellae; (3) legs elongate, with relatively few short setae on dorsal and ventral margins; (4) claws very elongate, without denticles; and (5) lateral margins of posterior abdominal segments with sharp spines (Jacobus et al. 2006). The imaginal stage remains unknown (Jacobus et al. 2006; Kluge 2020b).

Based on the above-mentioned species, *Securiops* has a widespread distribution across the Afrotropical region. Here, for the first time, we report the presence of this genus additionally in Southeast Asia, based on the discovery of a new species in Thailand, which is described and illustrated in this study. We also provide the first DNA barcode for *Securiops*.

Materials and methods

The larvae were collected in 2017 and 2019, and preserved in 70%-96% ethanol.

The dissection of larvae was done in Cellosolve (2-Ethoxyethanol) with subsequent mounting on slides with Euparal liquid, using an Olympus SZX7 stereomicroscope.

The DNA of some specimens was extracted using non-destructive methods allowing subsequent morphological analysis (see Vuataz et al. 2011 for details). We amplified a 658 bp fragment of the mitochondrial gene cytochrome oxidase subunit 1 (COI) using the primers LCO 1490 and HCO 2198 (Folmer et al. 1994, see Kaltenbach and Gattolliat 2020 for details). Sequencing was done with Sanger's method (Sanger et al. 1977).

GenBank accession numbers are given in the Material examined section.

Drawings were made using an Olympus BX43 microscope.

Photographs of larvae were taken using a Canon EOS 6D camera and processed with Adobe Photoshop Lightroom v. 5 (http://www.adobe.com) and Helicon Focus v. 5.3 (http://www.heliconsoft.com). Photographs of body parts of the larvae were taken with an Olympus BX43 microscope equipped with an Olympus SC50 camera and processed with Olympus (recently Evident) software Cell Sense v. 1.3. All pictures were subsequently enhanced with Adobe Photoshop Elements 13.

The distribution map was generated with SimpleMappr (https://simplemappr.net, Shorthouse 2010). The terminology follows Hubbard (1995) and Kluge (2004).

Abbreviations

KKU-AIC Khon Kaen University, Aquatic Insect Collection (Thailand);
MZL Muséum Cantonal des Sciences Naturelles, Lausanne (Switzerland);
VMCMU Chiang Mai University, Museum of Veterinary Medicine (Thailand).

Results

Securiops primasia **sp. nov.**
https://zoobank.org/CD408527-25B4-4F63-BD52-32AFEECF3514
Figs 1–8

Differential diagnosis. Larva. The following combination of characters differentiate larvae of the new species from other species of *Securiops*: (1) maxillary palp segment II ca. 0.7× as long as segment I (Fig. 3a, d); (2) maxilla ventrolaterally with two groups of simple setae (Fig. 3e); (3) femur approx. twice as long as tibia; tarsus approx. 1.4× as long as tibia; claw approx. 0.7× as long as tarsus (Fig. 5a); (4) hind protoptera absent; (5) tergalii on abdominal segments I–VII, each with two lamellae (Fig. 7b); (6) abdominal segments VIII and IX with large lateral spines, segment VII with minute lateral spines (Fig. 6a); and (7) paraproct with four large, pointed spines (Fig. 7a).

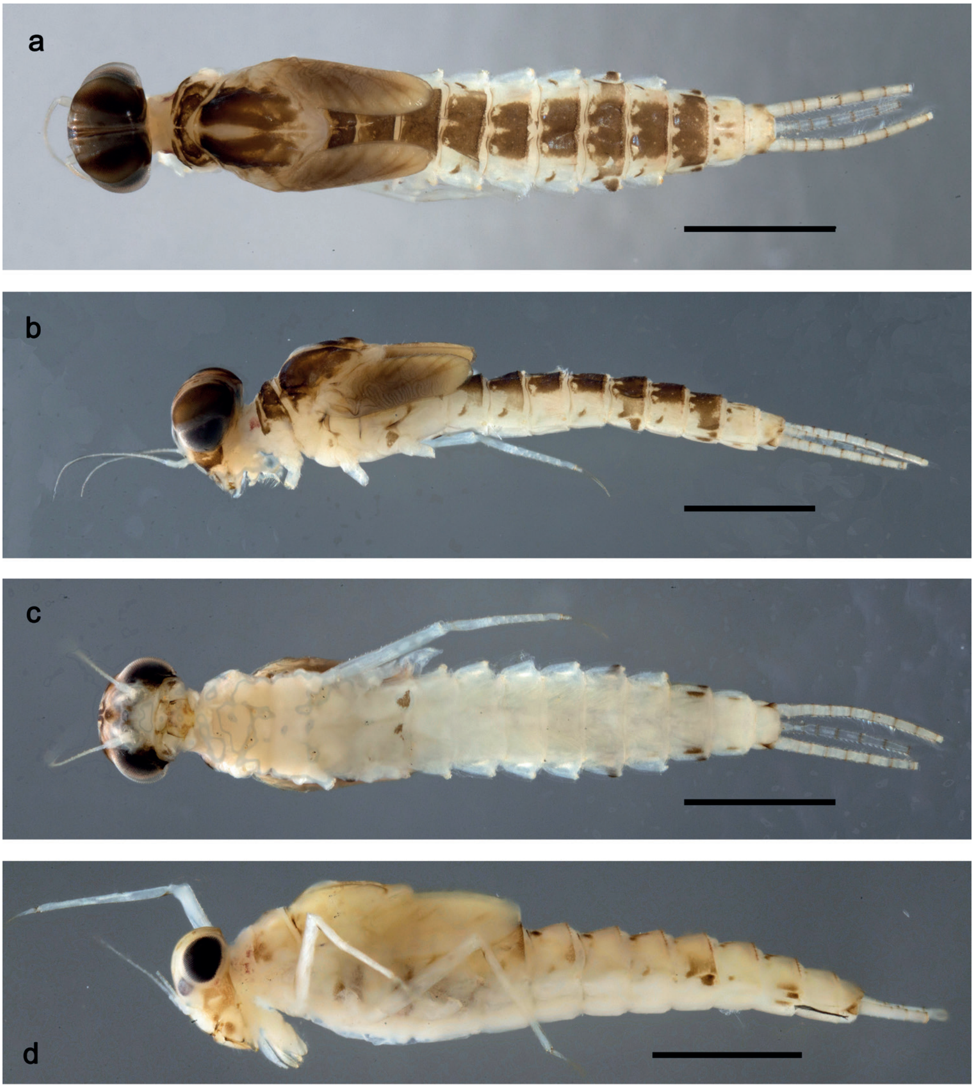


Figure 1. *Securiops primasia* sp. nov., larva habitus **a** male, dorsal view **b** male, lateral view **c** male, ventral view **d** female, lateral view (colour not yet fully developed) **a–c** mouthparts removed. Scale bars: 1 mm.

Description. Larva (Figs 1–7). Body length 3.8–4.7 mm. Cerci approx. ½ body length, slightly longer than paracercus. Antennae somewhat longer than head length.

Colouration (Figs 1a–d). Head, thorax and abdomen dorsally brown, with pattern as in Fig. 1a. Abdomen laterally light brown, with brown spots on most segments (absent or inconspicuous on segments I, IV and X), and larger additional brown spots on segments VI and VIII. Head, thorax and abdomen ventrally light brown, abdomen laterally with brown spots on segments VIII–X (Fig. 1c). Legs light brown, femur with distomedial brown spot, tibia with ventrobasal brown spot, and claw basally darker. Caudalii light brown, with brown annulation at distal margins of segments (Fig. 1a).

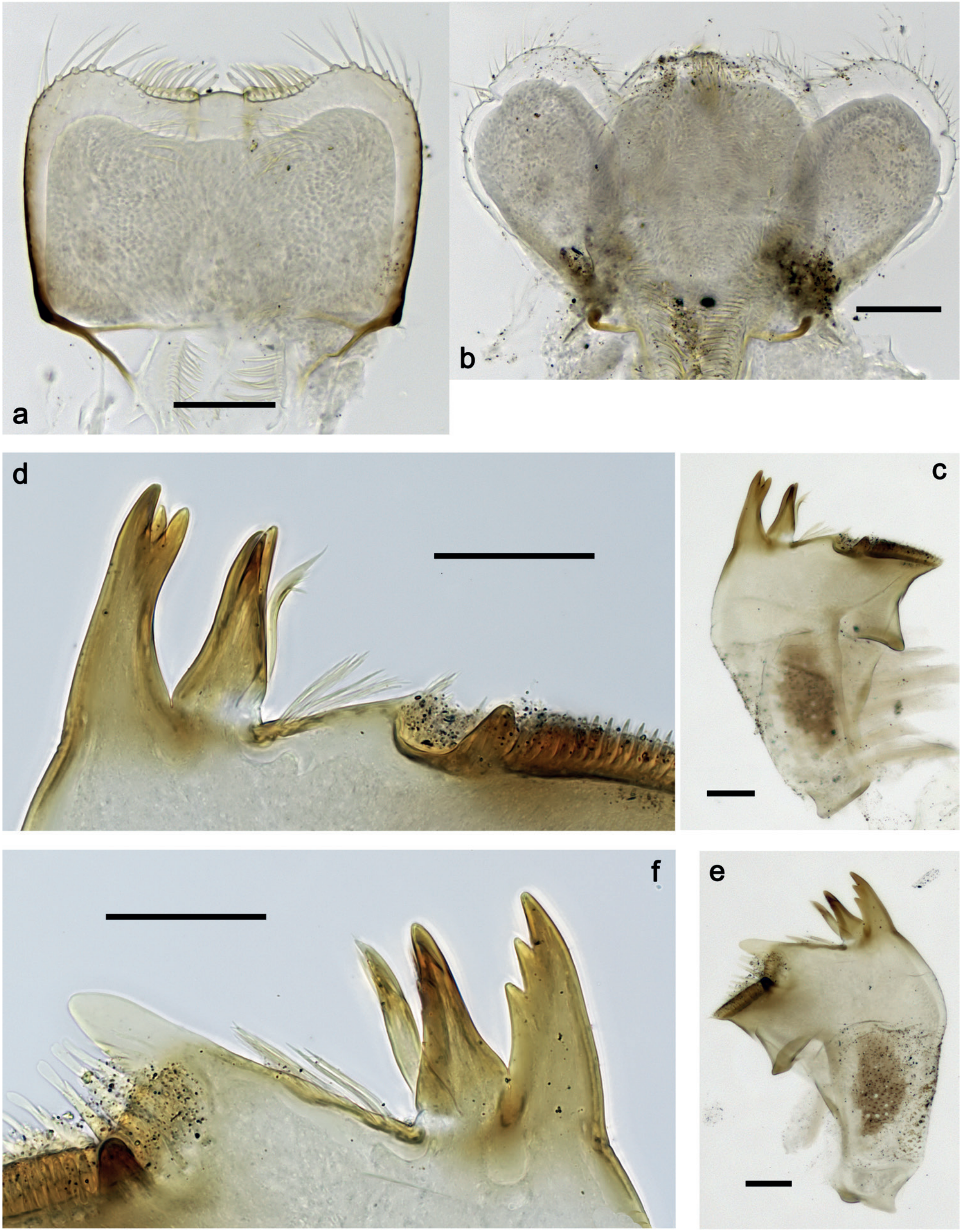


Figure 2. *Securiops primasia* sp. nov., larva morphology **a** labrum **b** hypopharynx and superlinguae **c, d** right mandible **e, f** left mandible. Scale bars: 50 µm.

Labrum (Fig. 2a). Rectangular, length ca. 0.7× maximum width. Distal margin with broad, shallow, medial emargination. Dorsal surface scattered with fine, simple setae; ventral surface with apicolateral patch of long, bifid setae near margin. Anterior margin apicolaterally with row of long, bifid setae, and medially with row of stout, medium, bifid setae.

Right mandible (Fig. 2c, d). Incisor and kinetodontium cleft to base. Incisor with three denticles; kinetodontium with three denticles. Prostheca stick-like, apicolaterally denticulate. With restricted tuft of long setae between prostheca and mola. Tuft of setae at apex of mola present.

Left mandible (Fig. 2e, f). Incisor and kinetodontium cleft to base. Incisor with three denticles; kinetodontium with four denticles. Prostheca stick-like, apicolaterally denticulate. With large tuft of long setae between prostheca and mola. Tuft of setae at apex of mola present.

Hypopharynx and superlinguae (Fig. 2b). Lingua as long as superlinguae, broad; slightly longer than broad; distal margin almost straight, with fine, simple setae, not forming a medial tuft. Superlinguae distally broadly rounded; lateral margins rounded; fine, medium to long, simple setae along distal margin.

Maxilla (Fig. 3a–e). Galea-lacinia ventrally with two simple, apical setae under canines (Fig. 3c). Canines long and very slender (Fig. 3a). Three long, slender, pectinate denti-setae (Fig. 3b). Medially with one bifid, spine-like seta (dorsolateral insertion) and two groups of simple, spine-like setae (ventrolateral insertions) (Fig. 3e). Maxillary palp 2-segmented, more than twice as long as length of galea-lacinia; palp segment II ca. 0.7× length of segment I; setae on maxillary palp long, fine, simple, scattered over surface of segments I and II; apex of last segment pointed (Fig. 3a, d).

Labium (Fig. 4a–e). Glossa much shorter than paraglossa; outer margin with row of simple setae; inner margin distomedially serrate and with fine, simple setae; ventroapically with arc of long, simple setae. Paraglossa slightly curved inward; outer margin with row of long, simple setae; inner margin with medium row of simple setae, and submarginal row of long, simple setae, basally with dense row of long, simple setae. Labial palp 2-segmented. Segment II large, nearly trapezoidal with distal corner prolonged, pointed, curved inward; inner margin with many long, fine setae, in basal half with submarginal row of long, spine-like, setae-like processes; distal corner with dense, long, fine setae.

Hind protoptera absent.

Foreleg (Fig. 5a–k) very slender. Ratio of foreleg segments 2.0:1.0:1.4:0.7. ***Trochanter.*** Ventral margin with row of short, spine-like setae (Fig. 5g). ***Femur.*** Length ca. 6× maximum width. Dorsal margin with row of short, spine-like setae; distally with transverse arc of long, fine setae (difficult to see) (Fig. 5i). Apex rounded. Ventral margin with row of short, spine-like setae; femoral patch absent. ***Tibia.*** Dorsal margin with row of short, spine-like setae; proximally with arc of long, fine setae near margin (difficult to see) (Fig. 5j). Ventral margin with row of short to medium, curved, spine-like setae. Patellatibial suture present in basal 1/2 area. ***Tarsus.*** Dorsal margin bare; proximally with arc of long, fine setae near margin (difficult to see) (Fig. 5k). Ventral margin with row of medium, spine-like setae. ***Claw*** without denticles; subapical setae absent (Fig. 5h).

Abdominal terga (Fig. 6a, b). Lateral margins of terga: VII with some minute spines; VIII with ca. seven small to large spines; IX with five large spines and one small

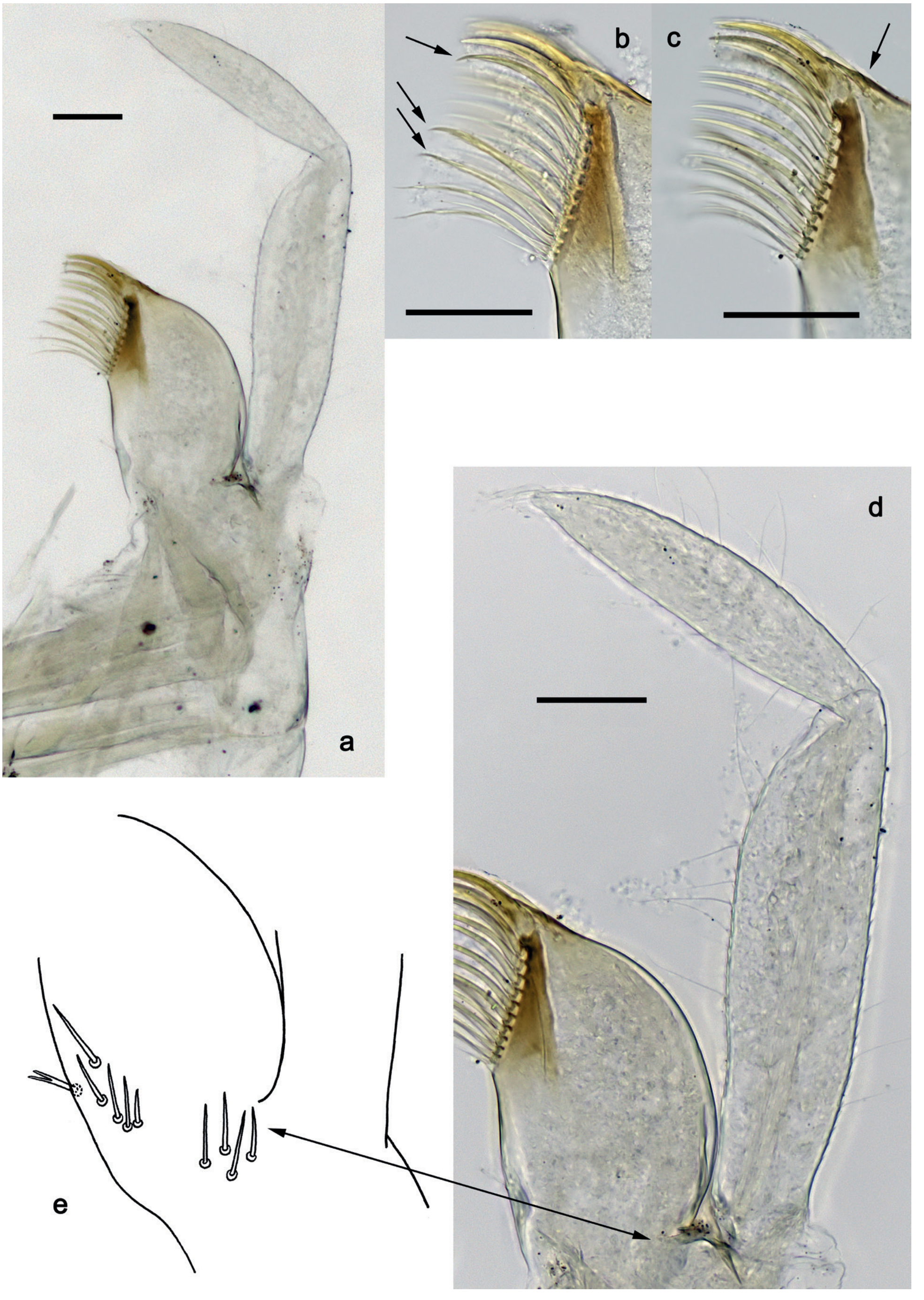


Figure 3. *Securiops primasia* sp. nov., larva morphology **a** maxilla **b** apex of maxilla, dorsal focus (arrows: denti-setae) **c** apex of maxilla, ventral focus (arrow: setae under canines) **d** maxillary palp **e** maxilla, middle part (ventrolateral view). Scale bars: 50 μm.

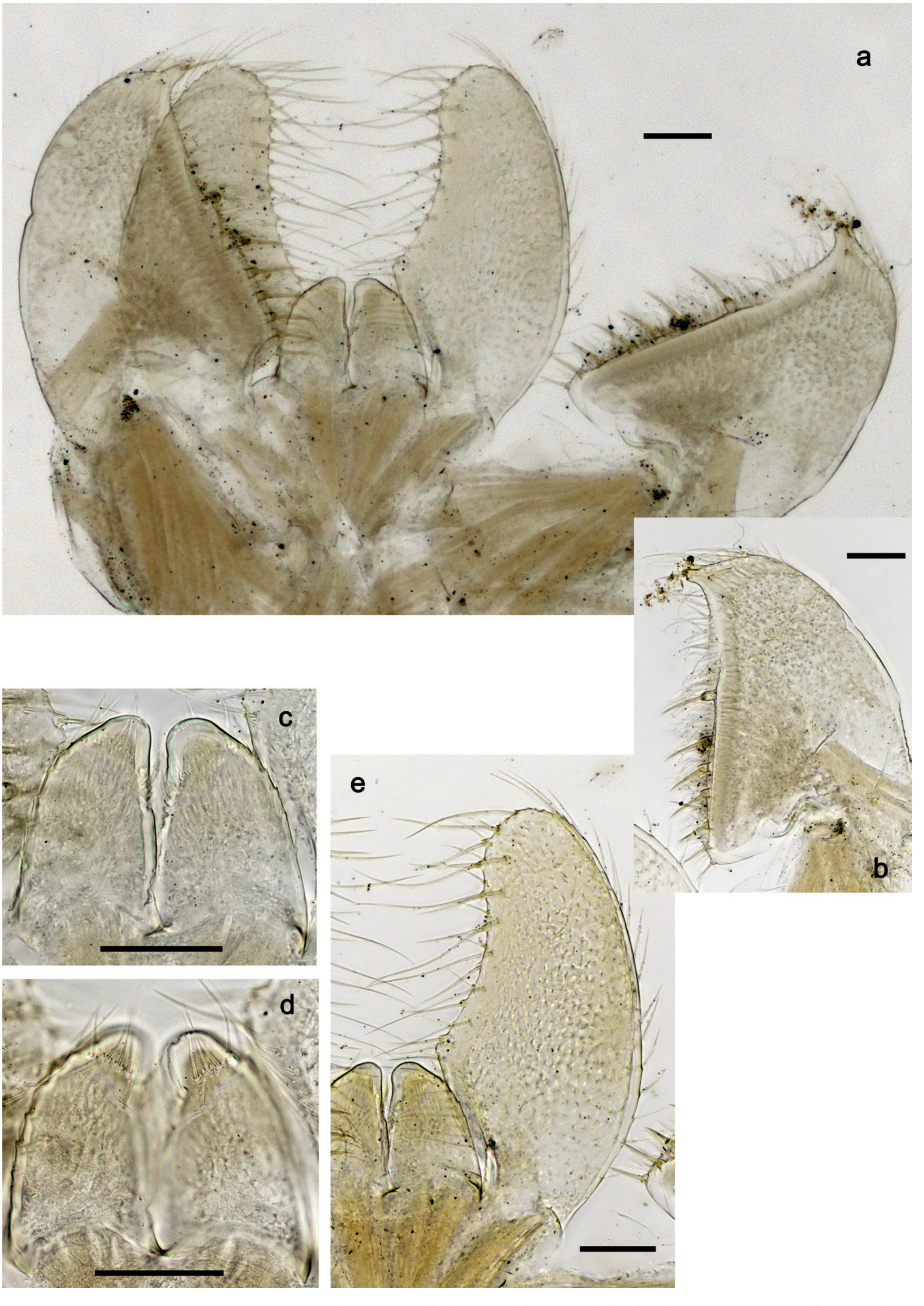


Figure 4. *Securiops primasia* sp. nov., larva morphology **a** labium **b** labial palp segment II **c** glossae (dorsal view) **d** glossae (ventral view) **e** paraglossa. Scale bars: 50 µm.

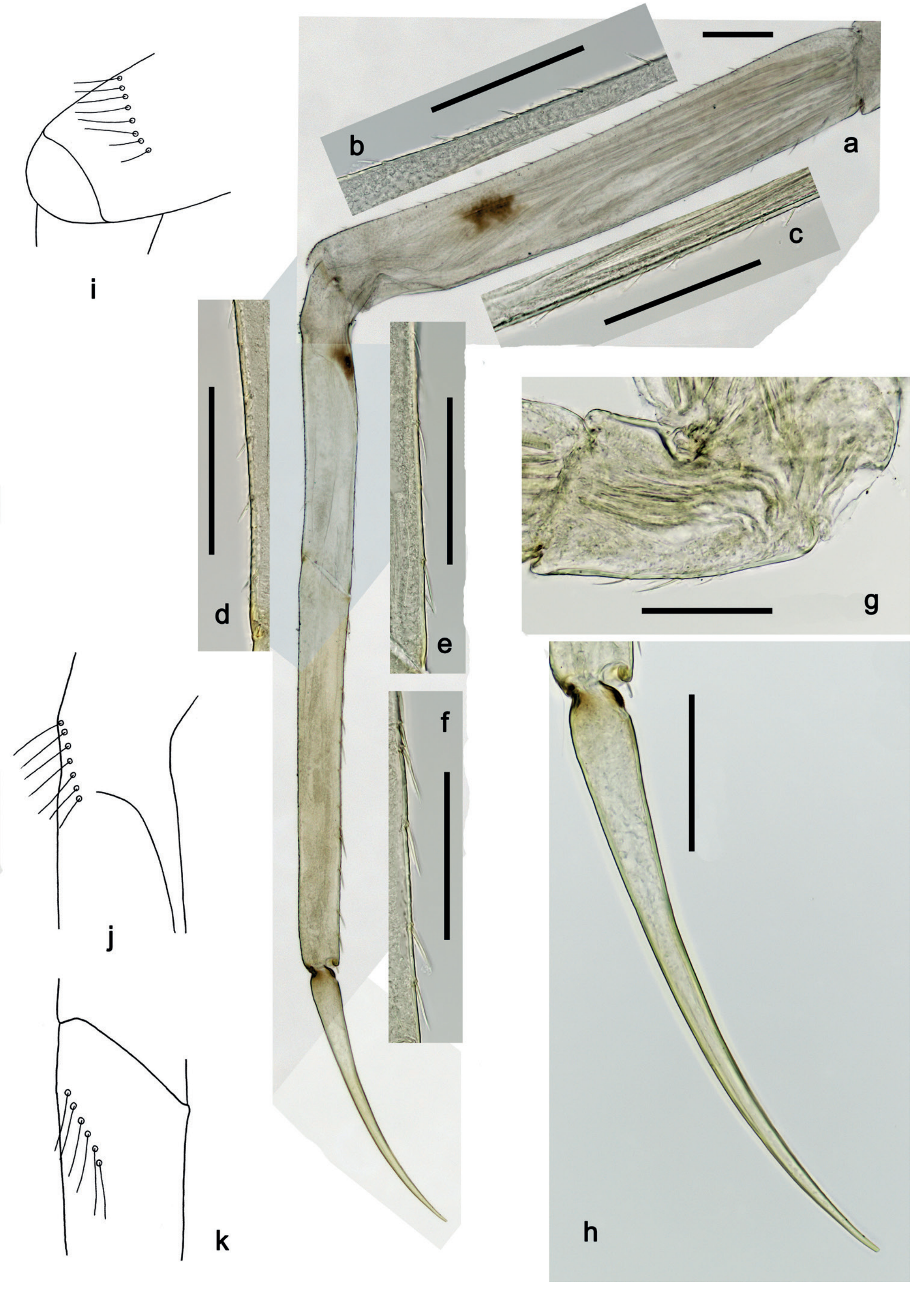


Figure 5. *Securiops primasia* sp. nov., larva morphology **a** foreleg **b** femur dorsal margin **c** femur ventral margin **d** tibia dorsal margin **e** tibia ventral margin **f** tarsus ventral margin **g** trochanter **h** claw **i** femur apex (posterior view) **j** tibia base **k** tarsus base. Scale bars: 100 µm.

spine (spine at posterolateral angle excluded from count); Posterior margins of terga: I smooth, without spines; II with strongly spaced or rudimentary, triangular spines; III–VII with spaced triangular spines, longer than wide.

Abdominal sterna (Fig. 6c). Posterior margin of sterna: I–V smooth, without spines; VI–VIII with triangular spines.

Tergalii (Fig. 7b). Present on segments I–VII; all tergalii with two lamellae, second lamella much smaller. Tracheae restricted to main trunk. Tergalius I as long as length of segment II; tergalius VII as long as length of segments VIII and half IX combined.

Paraproct (Fig. 7a). With four larger, marginal spines, and some additional, minute spines in-between. Cercotractor with minute, marginal spines, hardly to see.

Imago. Unknown.

Eggs (Fig. 7c, d). Barrel-shaped, surface with four longitudinal rows of wide, subrectangular structural elements.

Genetics. We obtained two sequences of 658 bp from specimens of two distinct populations. The K2P distance between them is 0.5%. The closest sequences available on GenBank and Bold system all belong to various species of *Cloeon* which is to be expected as no specimens of *Securiops* were previously sequenced.

Etymology. Combination of the first part of the Latin word "prim-us" (meaning the first), and "asia" (for the continent), to highlight the first discovery of the Afrotropical genus *Securiops* in Asia.

Biological aspects. The specimens were collected at altitudes between 100 m and 300 m.

Distribution (Fig. 8). Thailand.

Material examined. Type-material. *Holotype*. Thailand • larva; Ubon Ratchathani Province, Khong Chiam District, Mekong River; 15°19'29"N, 105°30'07"E; 156 m; 21.v.2017; leg. S. Benjamas; on slide; GBIFCH00592671; KKU-AIC. ***Paratypes*.** Thailand • 3 larvae; Ubon Ratchathani Province, Khong Chiam District, Mekong River; 15°19'29"N, 105°30'07"E; 156 m; 21.v.2019; leg. S. Benjamas; 2 in alcohol; GBIFCH00975670; KKU-AIC; 1 on slide; GBIFCH00592672; MZL • 2 larvae; Ubon Ratchathani Province, Khong Chiam District, Mekong River; 15°19'29"N, 105°30'07"E; 156 m; 18.v.2017; leg. S. Benjamas; 2 on slides; GBIFCH00592670; MZL; GBIFCH00592669; KKU-AIC • 2 larvae; Ubon Ratchathani Province, Khong Chiam District, Mekong River; 15°19'29"N, 105°30'07"E; 156 m; 18.v.2017; leg. S. Benjamas; KKU-AIC.

Other material. Thailand • larva; Kanchanaburi Province, Klong Ta Phoen; 14°06'54"N, 99°23'50"E; 31 m; 09.xi.2018; leg. C. Suttinun; in alcohol; GenBank OQ573687; GBIFCH00763772; KKU-AIC • larva; Tak Province, Huai Pu Ter; 16°37'51"N', 98°37'44"E; 239 m; 27.xii.2017; leg. C. Suttinun; in alcohol; GenBank OQ573688; GBIFCH00763771; MZL • 3 larvae; Ratchaburi Province, Suan Phueng District, Pha Wo Thai; 13°30'56.1"N, 99°20'39.3"E; 118 m; 20.xi.2022; leg. C. Auychida; in alcohol; VMCMU • 2 larvae; Ratchaburi Province, Suan Phueng District, Kang Som Meow; 13°24'37.2"N, 99°16'37"E; 207 m; 20.xi.2022; leg. C. Auychida; in alcohol; VMCMU • 2 larvae; Ratchaburi Province, Suan Phueng District, Ton Nam Pha Chi; 13°20'11.2"N, 99°14'24.8"E; 265 m; 20.xi.2022; leg. C. Auychida; in alcohol; VMCMU.

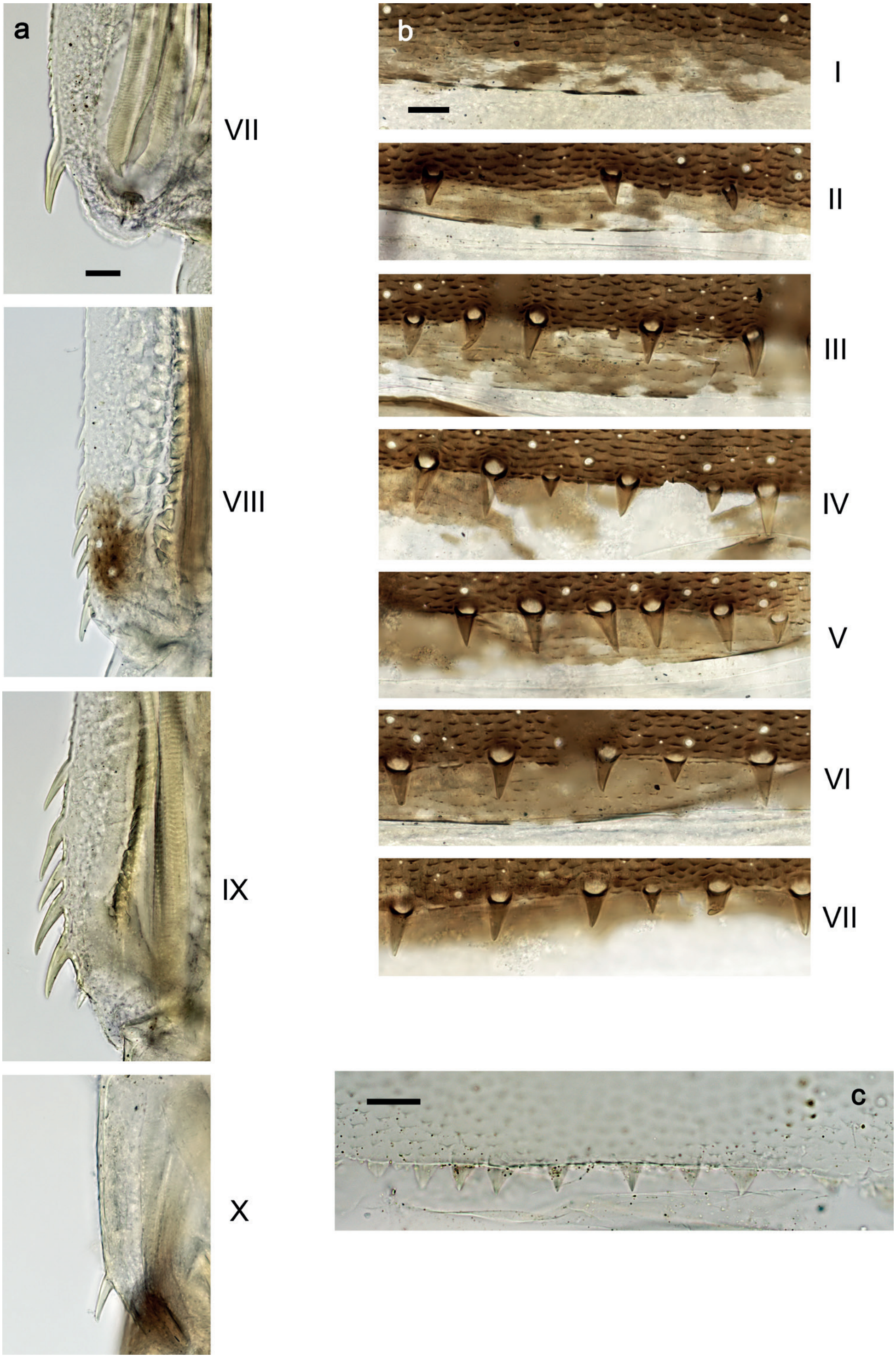


Figure 6. *Securiops primasia* sp. nov., larva morphology **a** abdominal terga VII–X, lateral margins **b** abdominal terga I–VII, posterior margins **c** abdominal sternum VI, posterior margin. Scale bars: 20 µm.

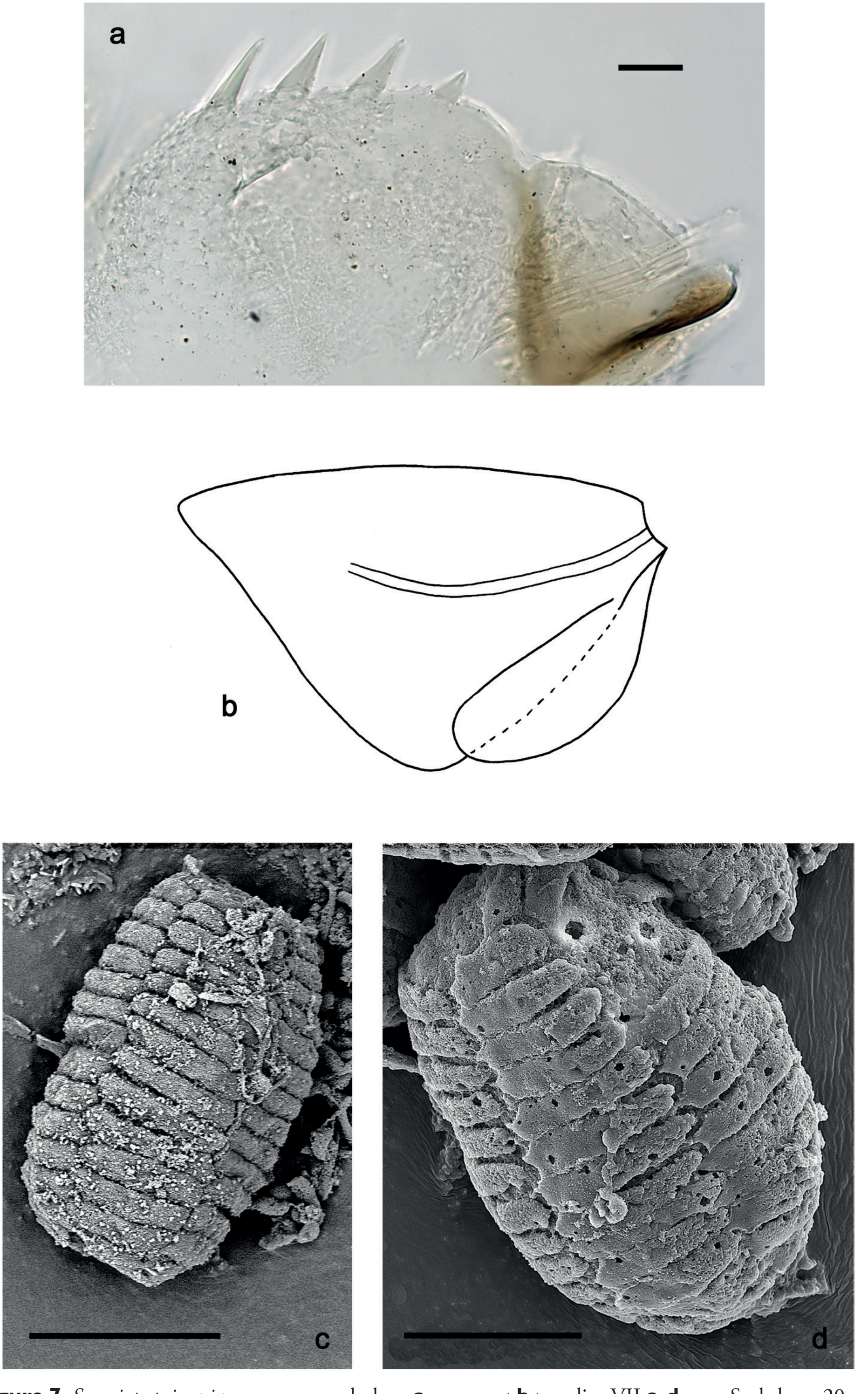


Figure 7. *Securiops primasia* sp. nov., morphology **a** paraproct **b** tergalius VII **c, d** eggs. Scale bars: 20 µm (**a**); 30 µm (**c, d**).

Figure 8. *Securiops primasia* sp. nov., distribution (yellow star: type locality).

Discussion

Assignment to *Securiops*

The new species clearly belongs to the genus *Securiops*, based on the following characters: (1) labrum rectangular, with a broad shallow, medial incision at the distal margin (Fig. 2a); (2) labium with strongly reduced glossae, enlarged paraglossae, and very broad, hatchet-like 2-segmented palps (Fig. 4a); (3) tergalii I–VII with two lamellae

(Fig. 7b); (4) legs very elongate (Fig. 5a); (5) claws very elongate, without denticles (Fig. 5h); and (6) lateral margins of posterior abdominal segments with sharp spines (Fig. 6a) (Jacobus et al. 2006). Additionally, *S. primasia* sp. nov. shares with *S. megapalpus* the arcs of long, fine setae dorsodistally on the femur and dorsoproximally on the tibia and tarsus (Jacobus et al. 2006: figs 15, 16). This combination of characters and especially the shape of the labrum and labium clearly indicate the assignment to *Securiops* and not to other genera or subgenera closely related to *Procloeon* s.l.

Differences to Afrotropical species of *Securiops*

Contrary to the Afrotropical species of *Securiops*, *S. primasia* sp. nov. has complete rows of short setae at the dorsal and ventral margins of the femur and tibia, and not just a few setae. Additional to the marginal setation of the legs, *S. primasia* sp. nov. can be differentiated from the Afrotropical species at least by the following main characters (Lugo-Ortiz and McCafferty 1996; Gattolliat 2003; Jacobus et al. 2006): (1) *S. megapalpus* with maxillary palp segment I much wider (1.9×) and much longer (2.6×) than segment II (*S. primasia* sp. nov. with segment I 1.6× width and 1.5× length of segment II); only tergalii I–IV with two lamellae (I–VII in *S. primasia* sp. nov.); tibia length 1.1× length of claw (1.4× in *S. primasia* sp. nov.); (2) *S. macafertiorum* with hind protoptera present (absent in *S. primasia* sp. nov); abdominal terga V–IX or VI–IX with spines on lateral margins (terga VIII and IX in *S. primasia* sp. nov.); (3) *S. mandrare* with spines on lateral margins of abdominal segments IV–IX (VIII and IX in *S. primasia* sp. nov.); tergalii on abdominal segments I-IV with two lamellae (I–VII in *S. primasia* sp. nov.); and (4) *S. mutadens* with spines on lateral margins of abdominal segments IV–IX (VIII and IX in *S. primasia* sp. nov.); tergalii on abdominal segments I-IV with two lamellae (I–VII in *S. primasia* sp. nov.).

Eggs

The eggs of this genus are described for the first time. They present similarities with the eggs extracted from subimagos of *Baetis alpinus* Pictet, 1843, which should be a convergence (Fig. c, d; Kopelke and Müller-Liebenau 1982: fig. 6).

Distribution

The occurrence of *Securiops* in Southeast Asia in addition to the Afrotropical region, where the four other known species live, is rare for Baetidae as well as for other families of Ephemeroptera. However, apart from the worldwide-distributed genus *Cloeon* Leach, 1815, there are other examples of Baetidae genera, for example, *Labiobaetis* Novikova & Kluge, 1987, *Nigrobaetis* Kazlauskas (in Novikova and Kluge), 1987, *Cheleocloeon* Wuillot & Gillies, 1993, and *Oculogaster* Kluge, 2016 (Barber-James et al. 2013; Kluge 2020a; Kaltenbach and Gattolliat 2021). The latter belongs to *Procloeon* s.l. as *Securiops* (Kluge 2020b). In other families, apart from the worldwide-distributed genus *Caenis* Stephens, 1835 (Caenidae), there are also genera with a distribution in the Afrotropical region as well as in the Oriental region (and mostly in the Palearctic as well), for example, *Ephemera* Linné, 1758 (Ephemeridae), *Afronurus* Lestage, 1924 (Heptagenidae), *Euthraulus* Barnard, 1932 (Leptophlebiidae), *Thraulus* Eaton, 1881 (Leptophlebiidae) and *Povilla* Navás, 1920 (Polymitarcyidae) (Barber-James et al. 2013). However, among the genera mentioned above, only *Oculogaster* and *Cheleocloeon* present a discontinuous distribution including exclusively Afrotropical and Oriental realms. Such a distribution pattern could be explained by a stepwise faunal exchange between Africa and Asia via corridors, which probably were more favourable for the dispersal of mayflies during some periods in the past, or by a fauna present on the Indian subcontinent before its drift to the north (Gattolliat and Nieto 2009).

Securiops in continental Africa, Madagascar and Thailand (present study) is rarely collected during standard protocols and freshwater surveys. It may be due to both its scarcity and its ecological requirements (probably partially psammophilous). We may expect that the occurrence of *Securiops* in Thailand is not an isolated distribution area. More collections in the yet poorly sampled Oriental region may lead to discoveries of a few more new species of *Securiops* in Southeast Asia, and maybe also on the Indian subcontinent.

Acknowledgements

We highly appreciate the efforts of Dr. Benjamas Suksai (Faculty of Science, Khon Kaen University) and Dr. Chonlakarn Auychinda (Faculty of Science, Silpakorn University, Thailand) for the collection of precious specimens (Thailand). The Khon Kaen University Aquatic Insects Collection (KKU-AIC) has to be thanked for permitting us to work with these specimens. Further, we are thankful to Nadir Alvarez (Museum of Zoology Lausanne) for his interest and support of our projects, and to Céline Stoffel and Marion Podolak (Museum of Zoology Lausanne) for her support with lab work and preparation of the COI barcode, and to Antonio Mucciolo (UNIL) for taking the SEM pictures of the eggs.

This research has been reviewed and approved by the Institutional Animal Care and Use Committee of Khon Kaen University, based on the Ethics of Animal Experimentation of the National Research Council of Thailand (Record No. IACUC-KKU-65/63) for collecting mayfly specimens.

The authors are also grateful to the reviewers for their valuable recommendations and comments on the manuscript.

References

Barber-James HM, Sartori M, Gattolliat J-L, Webb J (2013) World checklist of freshwater Ephemeroptera species. http://fada.biodiversity.be/group/show/35

Boonsoong B (2022) Mayfly larvae in Thailand. Pre-One Part., Ltd., 469 pp.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from divers metazoan invertebrates.

Molecular Marine Biology and Biotechnology 3: 294–299. http://www.mbari.org/staff/vrijen/PDFS/Folmer_94MMBB.pdf

Gattolliat J-L (2003) The genera *Demoulinia* Gillies and *Potamocloeon* Gillies (Ephemeroptera: Baetidae) in Madagascar. Zootaxa 184(1): 1–18. https://doi.org/10.11646/zootaxa.184.1.1

Gattolliat J-L (2012) Two new genera of Baetidae (Ephemeroptera) from Borneo (East Kalimantan, Indonesia). International Journal of Limnology 48(2): 187–199. https://doi.org/10.1051/limn/2012012

Gattolliat J-L, Nieto C (2009) The family Baetidae (Insecta: Ephemeroptera): synthesis and future challenges. Aquatic Insects 31(sup1): 41–62. https://doi.org/10.1080/01650420902812214

Gillies MT (1988) Descriptions of the nymphs of some Afrotropical Baetidae (Ephemeroptera). I. *Cloeon* Leach and *Rhithrocloeon* Gillies. Aquatic Insects 10(1): 49–59. https://doi.org/10.1080/01650428809361310

Hubbard MD (1995) Towards a standard methodology for the description of mayflies (Ephemeroptera). In: Corkum LD, Ciborowski JJH (Eds) Current Directions in Research on Ephemeroptera. Canadian Scholar's Press, Toronto, 361–369.

Jacobus LM, McCafferty WP, Gattolliat J-L (2006) Taxonomy of Afrotropical *Securiops*, new genus, and *Cloeodes* Traver (Ephemeroptera: Baetidae). African Entomology 14: 129–140.

Jacobus LM, Macadam CR, Sartori M (2019) Mayflies (Ephemeroptera) and their contributions to ecosystem services. Insects 10(6): 1–26. https://doi.org/10.3390/insects10060170

Kaltenbach T, Gattolliat J-L (2019) The tremendous diversity of *Labiobaetis* Novikova & Kluge in Indonesia (Ephemeroptera, Baetidae). ZooKeys 895: 1–117. https://doi.org/10.3897/zookeys.895.38576

Kaltenbach T, Gattolliat J-L (2020) *Labiobaetis* Novikova & Kluge in Borneo (Ephemeroptera, Baetidae). ZooKeys 914: 43–79. https://doi.org/10.3897/zookeys.914.47067

Kaltenbach T, Gattolliat J-L (2021) *Labiobaetis* Novikova & Kluge in West Africa (Ephemeroptera, Baetidae), with description of a new species. African Invertebrates 62(1): 355–382. https://doi.org/10.3897/afrinvertebr.62.64885

Kaltenbach T, Garces JM, Gattolliat J-L (2020a) The success story of *Labiobaetis* Novikova & Kluge in the Philippines (Ephemeroptera, Baetidae), with description of 18 new species. ZooKeys 1002: 1–114. https://doi.org/10.3897/zookeys.1002.58017

Kaltenbach T, Garces JM, Gattolliat J-L (2020b) A new genus of Baetidae (Insecta, Ephemeroptera) from Southeast Asia. European Journal of Taxonomy 612(612): 1–32. https://doi.org/10.5852/ejt.2020.612

Kaltenbach T, Garces JM, Gattolliat J-L (2021) *Philibaetis* gen. nov., a new genus from the Philippines (Ephemeroptera, Baetidae). Deutsche Entomologische Zeitschrift 68(1): 1–20. https://doi.org/10.3897/dez.68.59462

Kluge NJ (2004) The Phylogenetic System of Ephemeroptera. Academic Publishers, Dordrecht, 442 pp. https://doi.org/10.1007/978-94-007-0872-3

Kluge NJ (2020a) Review of *Oculogaster* Kluge 2016 (Ephemeroptera, Baetidae, *Procloeon* Bengtsson 1915). Zootaxa 4820(3): 401–437. https://doi.org/10.11646/zootaxa.4820.3.1

Kluge NJ (2020b) New subgenus Monilistylus subgen. n. and a new species *Procloeon* (*Monilistylus*) *ornatipennis* sp. n. (Ephemeroptera: Baetidae: *Procloeon*). Zootaxa 4742(3): 573–587. https://doi.org/10.11646/zootaxa.4742.3.11

Kluge NJ (2022) Two new species of *Waynokiops* Hill et al. 2010 (Ephemeroptera: Baetidae) from the Oriental Region. Zootaxa 5182(1): 41–63. https://doi.org/10.11646/zootaxa.5182.1.3

Kopelke J-P, Müller-Liebenau I (1982) Eistrukturen bei Ephemeroptera und deren Bedeutung für die Aufstellung von Artengruppen am Beispiel der europäischen Arten der Gattung *Baetis* Leach, 1815 (Insecta: Baetidae) Teil I: *alpinus*-, *lutheri*-, *pavidus*- und *lapponicus*-Guppe. Gewässer und Abwässer 68/69: 7–25.

Lugo-Ortiz CR, McCafferty WP (1996) The *Bugilliesia* complex of African Baetidae (Ephemeroptera). Transactions of the American Entomological Society 122: 175–197.

Phlai-ngam S, Boonsoong B, Gattolliat J-L, Tungpairojwong N (2022) *Megabranchiella* gen. nov., a new mayfly genus (Ephemeroptera: Baetidae) from Thailand with description of two new species. ZooKeys 1125: 1–31. https://doi.org/10.3897/zookeys.1125.90802

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences of the United States of America 74(12): 5463–5467. https://doi.org/10.1073/pnas.74.12.5463

Sartori M, Brittain JE (2015) Order Ephemeroptera. In: Thorp J, Rogers DC (Eds) Ecology and General Biology: Thorp and Corvich's Freshwater Invertebrates. Academic Press, 873–891. https://doi.org/10.1016/B978-0-12-385026-3.00034-6

Shorthouse DP (2010) SimpleMappr, an online tool to produce publication-quality point maps. https://www.simplemappr.net [Accessed June 2022]

Sutthinun C, Gattolliat J-L, Boonsoong B (2018) A new species of *Platybaetis* Müller-Liebenau, 1980 (Ephemeroptera: Baetidae) from Thailand, with description of the imago of *Platybaetis bishopi* Müller-Liebenau, 1980. Zootaxa 4378(1): 85–97. https://doi.org/10.11646/zootaxa.4378.1.5

Suttinun C (2021) Systematics of family Baetidae (order Ephemeroptera) in southern and western Thailand. Doctoral thesis, Kasetsart University, Bangkok, 170 pp.

Suttinun C, Gattolliat J-L, Boonsong B (2020) *Cymbalcloeon* gen. nov., an incredible new mayfly genus (Ephemeroptera: Baetidae) from Thailand. PLoS ONE 15(10): e0240635. https://doi.org/10.1371/journal.pone.0240635

Suttinun C, Kaltenbach T, Gattolliat J-L, Boonsoong B (2021) A new species and first record of the genus *Procerobaetis* Kaltenbach & Gattolliat, 2020 (Ephemeroptera, Baetidae) from Thailand. ZooKeys 1023: 13–28. https://doi.org/10.3897/zookeys.1023.61081

Suttinun C, Gattolliat J-L, Boonsoong B (2022) First report of the genus *Tenuibaetis* (Ephemeroptera, Baetidae) from Thailand revealing a complex of cryptic species. ZooKeys 1084: 165–182. https://doi.org/10.3897/zookeys.1084.78405

Tungpairojwong N, Phlai-ngam S, Jacobus LM (2022) A new species of *Acentrella* Bengtsson, 1912 (Ephemeroptera: Baetidae) from Thailand. Zootaxa 5125(4): 351–378. https://doi.org/10.11646/zootaxa.5125.4.1

Vuataz L, Sartori M, Wagner A, Monaghan MT (2011) Toward a DNA taxonomy of Alpine *Rhithrogena* (Ephemeroptera: Heptagenidae) using a mixed Yule-Coalescent Analysis of mitochondrial and nuclear DNA. PLoS ONE 6(5): e19728. https://doi.org/10.1371/journal.pone.0019728

ZooKeys 1157: 145–161 (2023)
doi: 10.3897/zookeys.1157.99686
https://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Description of the first species of *Scutigerella* (Symphyla, Scutigerellidae) from China, with mitogenomic and genetic divergence analysis

Ya-Li Jin[1], Nerivania Nunes Godeiro[1], Yun Bu[1]

1 *Shanghai Natural History Museum, Shanghai Science & Technology Museum, Shanghai, 200041, China*

Corresponding author: Yun Bu (buy@sstm.org.cn)

Academic editor: Bruce A. Snyder | Received 6 January 2023 | Accepted 17 March 2023 | Published 5 April 2023

https://zoobank.org/0FF8F1DF-9CE2-4104-ACF2-FAA9929D261A

Citation: Jin Y-L, Godeiro NN, Bu Y (2023) Description of the first species of *Scutigerella* (Symphyla, Scutigerellidae) from China, with mitogenomic and genetic divergence analysis. ZooKeys 1157: 145–161. https://doi.org/10.3897/zookeys.1157.99686

Abstract

Scutigerella sinensis Jin & Bu, **sp. nov.** from China is described and illustrated. It is characterized by a deeply emarginated posterior margin of tergite 2, less differentiated marginal setae on all tergites, absence of seta a3 around the antennal base, and 6–8 setae on the first tergite. The complete mitochondrial genome of the new species is also analyzed and compared with the mitogenome of *Scutigerella causeyae*. In the reconstructed Neighbor-Joining tree based on COI gene sequences, *S. sinensis* **sp. nov.** clusters with *S. causeyae*, however, with big distances. The genetic divergence among *S. sinensis* **sp. nov.** and congeners, species of *Hanseniella* and *Scutigerella*, and both families of Symphyla was analyzed using COI gene sequences.

Keywords

DNA barcode, genetic distance, mitochondrial genome, Myriapoda, symphylans, taxonomy

Introduction

The class Symphyla Ryder, 1880 is a monophyletic group of myriapods with worldwide distribution (Szucsich and Scheller 2011). However, both taxonomic and molecular studies are widely missing. There are only about 250 species reported worldwide (https://www.itis.gov/; Jin and Bu 2020, 2023) and ten species recorded in China until now (Bu and Jin 2018; Jin and Bu 2018, 2019, 2020, 2023; Jin et al. 2019). Only 65 mitochondrial gene sequences and two complete mitogenomes are available in GenBank (accessed in February 2023). The sub-cosmopolitan genus *Scutigerella* Ryder, 1882 is the second largest group of the family Scutigerellidae. It includes 36 valid species registered in the Integrated Taxonomic Information System (ITIS; https://www.itis.gov/) (accessed in February 2023). However, *Scutigerella gratiae* (Ryder, 1881) is missing in the database of ITIS, but it is recorded in the virtual research environment Myriatrix, The Fellegship of the Rings (2020 onwards) (http://myriatrix.myspecies.info), thus 37 valid *Scutigerella* species in total. The occurrence of *Scutigerella* in China (Hunan Province) was only once mentioned in a monograph (Zhang and Wang 1992), but the species remained undetermined.

In recent years, many specimens of Scutigerellidae Bagnall, 1913 were obtained from Shanghai and Beijing and were carefully studied, and most of them belong to the genus *Hanseniella* Bagnall, 1913. Among those specimens, one species of *Scutigerella* was identified as new to science and is described in the present paper. It is also the first species of the genus from China. In order to provide further evidence for the new species and clarify its taxonomic position, its complete mitogenome was sequenced and analyzed. In addition, the phylogenetic relationship and genetic divergence of symphylans were analyzed based on DNA barcode sequences.

Materials and methods

Sample collection and taxonomy

Soil and litter samples from broad-leaf and bamboo forests from Dajinshan Island, Shanghai were collected during several ecological surveys of soil fauna between 2015–2018, and specimens were extracted using Berlese-Tullgren funnels and preserved in 80% ethanol. Materials from Beijing were collected in Yuan-Ming Yuan Imperial Garden by Mr Rui-Qing Wang in 2021. They were mounted on slides using Hoyer's solution and dried in an oven at 50 °C. Morphological observations were performed under a phase contrast microscope (Leica DM 2500). Photographs were taken with a digital camera installed on the microscope (Leica DMC 4500). Line drawings were done using a drawing tube. All specimens are deposited in the collections of the Shanghai Natural History Museum (**SNHM**), Shanghai, China.

Molecular analyses

The specimens used for the experiment were collected by Ya-Li Jin and Yun Bu from Dajinshan Island on 11 November, 2017. Samples were preserved in absolute ethanol at -20 °C for DNA extraction. Prior to DNA extraction, a single individual was mounted on a temporary slide using absolute ethanol to confirm the species identification. One specimen, preserved in alcohol, was sent to Shanghai Yaoen Biotechnology Co., Ltd, China, where all laboratory procedures, including DNA extraction and library construction were made following custom procedures. DNA was extracted from a single individual of the species using the TIANamp MicroDNA extraction kit (Tiangen Co., Ltd, China). Libraries were constructed using KAPA Hyper Prep Kit (Roche). An Illumina NovaSeq platform was used to produce paired-end reads with 150 bp length. Approximately 10 Gb of data from the species was generated and used to assemble the mitogenomes.

Sequence analysis

NovoPlasty v.3.8.3 (Dierckxsens et al. 2016) was used to assemble the mitogenome using the COI sequence from *Scutigerella causeyae* Michelbacher, 1942 retrieved from GenBank as a seed (accession number NC008453). Genes annotation was performed using MitoZ v.2.4-alpha (Meng et al. 2019). The final mitogenome sequence with annotations and the raw sequencing data were submitted to the National Center for Biotechnology Information database (NCBI), accession numbers are listed in Table 1. The mitogenomic data of *Scutigerella causeyae* were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/), and the length, genes arrangement, nucleotides content, and other genomic features were compared with *Scutigerella sinensis* sp. nov.

Table 1. Taxonomical and collection information of the species used in the analysis.

Species and voucher	Family	Genus	Country	GenBank number	Reference
Scutigerella sinensis sp. nov. JYL-DJS2017011	Scutigerellidae	*Scutigerella*	China	OQ165321	Present study
Scutigerella causeyae	Scutigerellidae	*Scutigerella*	Germany	NC008453	Podsiadlowski et al. 2007
Scutigerella sp. WAMT144261	Scutigerellidae	*Scutigerella*	Australia	MW021294	Cullen and Harvey 2020 (unpublished)
Scutigerella sp. WAMT144298	Scutigerellidae	*Scutigerella*	Australia	MW021295	Cullen and Harvey 2020 (unpublished)
Scutigerella sp. WAMT145461	Scutigerellidae	*Scutigerella*	Australia	MW021296	Cullen and Harvey 2020 (unpublished)
Scutigerella sp. WAMT145462	Scutigerellidae	*Scutigerella*	Australia	MW021297	Cullen and Harvey 2020 (unpublished)
Scutigerella sp. WAMT145463	Scutigerellidae	*Scutigerella*	Australia	MW021298	Cullen and Harvey 2020 (unpublished)
Scutigerella sp. PU234	Scutigerellidae	*Scutigerella*	Australia	MT457863	Cullen and Harvey 2020 (unpublished)
Hanseniella sp. BMR00202	Scutigerellidae	*Hanseniella*	Australia	MT902530	Gunawardene et al. 2020 (unpublished)
Hanseniella sp. BMR00229	Scutigerellidae	*Hanseniella*	Australia	MT902546	Gunawardene et al. 2020 (unpublished)
Hanseniella sp. BMR00230	Scutigerellidae	*Hanseniella*	Australia	MT902547	Gunawardene et al. 2020 (unpublished)
Hanseniella sp. BMR00231	Scutigerellidae	*Hanseniella*	Australia	MT902548	Gunawardene et al. 2020 (unpublished)
Hanseniella sp. BMR00232	Scutigerellidae	*Hanseniella*	Australia	MT902549	Gunawardene et al. 2020 (unpublished)
Hanseniella sp. BMR00243	Scutigerellidae	*Hanseniella*	Australia	MT902557	Gunawardene et al. 2020 (unpublished)
Hanseniella sp. BMR00364	Scutigerellidae	*Hanseniella*	Australia	MT902595	Gunawardene et al. 2020 (unpublished)
Hanseniella sp. BMR01208	Scutigerellidae	*Hanseniella*	Australia	MT902776	Gunawardene et al. 2020 (unpublished)
Scutigerellidae sp. FRL-2015	Scutigerellidae	undetermined	Colombia	KP696390	Salazar-Moncada et al. 2015
Scutigerellidae sp. BMR00070	Scutigerellidae	undetermined	Australia	MT902426	Gunawardene et al. 2020 (unpublished)
Scutigerellidae sp. BMR00071	Scutigerellidae	undetermined	Australia	MT902427	Gunawardene et al. 2020 (unpublished)
Scutigerellidae sp. BMR00241	Scutigerellidae	undetermined	Australia	MT902555	Gunawardene et al. 2020 (unpublished)
Scutigerellidae sp. BMR00242	Scutigerellidae	undetermined	Australia	MT902556	Gunawardene et al. 2020 (unpublished)
Scutigerellidae sp. BMR00244	Scutigerellidae	undetermined	Australia	MT902558	Gunawardene et al. 2020 (unpublished)
Scutigerellidae sp. BMR00641	Scutigerellidae	undetermined	Australia	MT902704	Gunawardene et al. 2020 (unpublished)
Scutigerellidae sp. BMR01199	Scutigerellidae	undetermined	Australia	MT902772	Gunawardene et al. 2020 (unpublished)
Scutigerellidae sp. BMR01576	Scutigerellidae	undetermined	Australia	MT621062	Gunawardene et al. 2020 (unpublished)
Scutigerellidae sp. BMR01578	Scutigerellidae	undetermined	Australia	MT621064	Huey and Floeckner 2020 (unpublished)
Scutigerellidae sp. BMR01587	Scutigerellidae	undetermined	Australia	MT621072	Huey and Floeckner 2020 (unpublished)
Symphylella sp. YG-2006	Scolopendrellidae	Symphylella	China	NC011572	Gai et al. 2008

In order to make a comprehensive analysis of genetic divergences among symphylans, DNA barcode sequences (COI gene, 658 base pairs) of 26 sequences of the family Scutigerellidae and one sequence of the family Scolopendrellidae Newport, 1844 (outgroup) were downloaded from GenBank and analyzed. The detailed information and accession numbers of the 28 sequences analyzed in this study are listed in Table 1. To infer the position of the new species described, the Neighbor-Joining tree was constructed based on COI gene sequences by MEGA X (Kumar et al. 2018) with the Jukes-Cantor model (Jukes and Cantor 1969) and 1000 bootstrap replicates. The genetic distance (K2P-distance) was calculated using MEGA X (Kimura 1980; Kumar et al. 2018) and the genetic divergence was analyzed for different taxonomic levels of Symphyla.

Data availability statement

After publication, mitogenome sequence and raw sequencing data will be available in GenBank (NCBI) at https://www.ncbi.nlm.nih.gov/ under the accession numbers OQ165321/PRJNA900014.

Results

Taxonomy

Class Symphyla Ryder, 1880
Family Scutigerellidae Bagnall, 1913

Genus *Scutigerella* Ryder, 1882

Type species. *Scolopendrella immaculata* Newport, 1845. Valid name: *Scutigerella immaculata* (Newport, 1845).

Diagnosis. Trunk with 15 tergites. Four macrosetae (a1–a4) around the antennal base, rarely seta a3 absent. Posterior margins of tergites emarginated. Last tergite with a deep cavity between cerci. First pair of legs with 4 segments, others with 5 segments. Styli present at the base of legs 3–12. Coxal sacs present at the base of legs 3–10.

Distribution. Sub-cosmopolitan (Szucsich and Scheller 2011).

***Scutigerella sinensis* Jin & Bu, sp. nov.**
https://zoobank.org/21204C04-5009-40AE-B37F-2DC2532C62F3
Figs 1–3, Table 2

Diagnosis. *Scutigerella sinensis* sp. nov. is characterized by absence of a3 seta around the antennal base, 6–8 setae on the first tergite, deeply emarginated posterior margin of tergite 2, 28–37 marginal and 41–57 inner setae on tergite 2, less differentiated marginal setae on all tergites, femur of first pair of legs without a conspicuous ventral

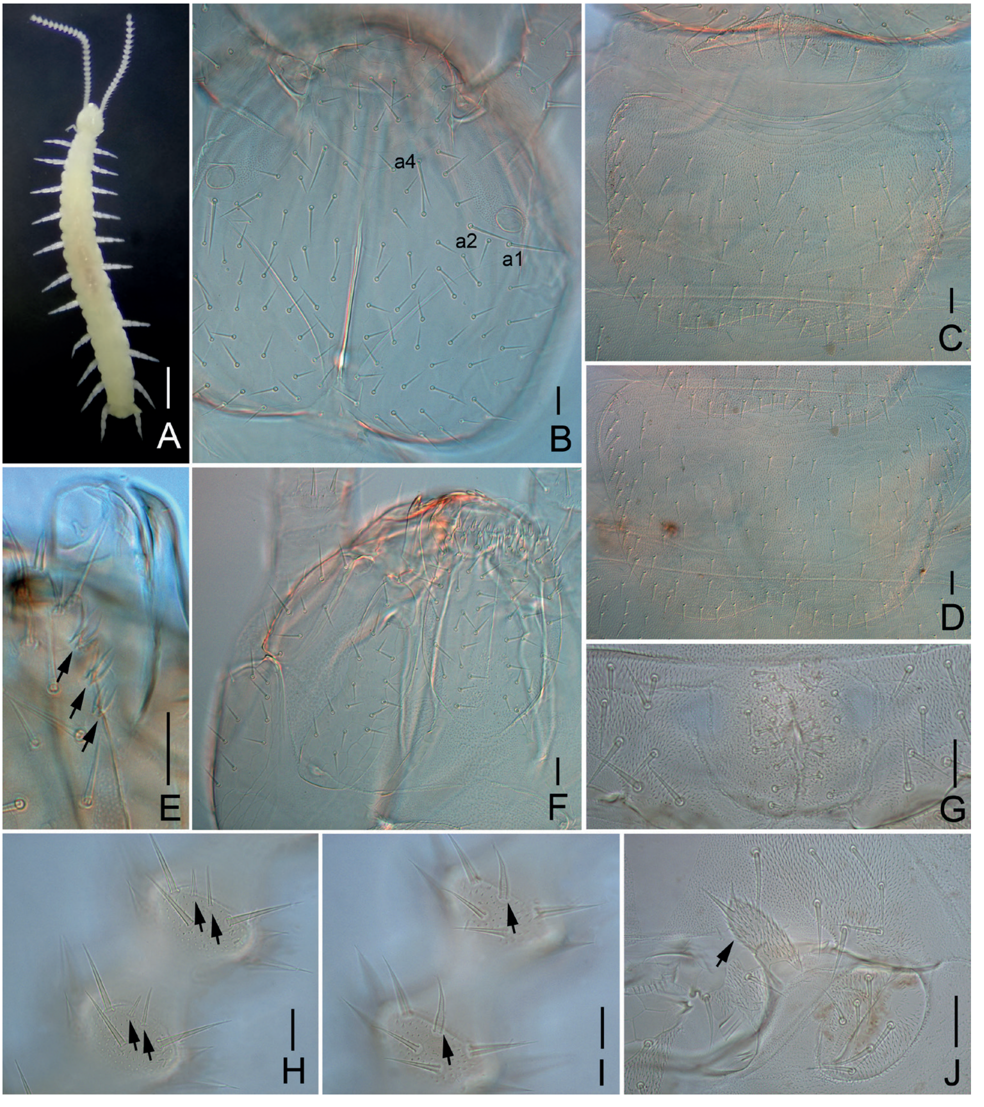


Figure 1. *Scutigerella sinensis* Jin & Bu, sp. nov. **A** habitus, dorsal view in alcohol **B** head, dorsal view (a1, a2 and a4–macrosetae around antennal base) **C** tergites 1–2 **D** tergite 3 **E** first maxilla and the right part of second maxilla (arrows indicate spined organs) **F** head, ventral view **G** male genitalia **H** right 10–11 antennomeres, dorsal view (arrows indicate spiniform sensory organs) **I** right 13–14 antennomeres, ventral view (arrows indicate sensory setae) **J** stylus and coxal sac on base of leg 5 (arrow indicates stylus). Scale bars: 500 μm (**A**); 20 μm (**B–J**).

process, posterior styli without a lateral seta, cavity of fifteenth tergite V-shaped, tarsus of last pair of legs moderately set with setae, cerci 2.7–3.4 times as long as width, cerci densely covered with subequal setae, cerci without expansion in terminal area.

Material examined. ***Holotype***: male (slide no. SH-DJS-SY2015009) (SNHM), China, Shanghai, Dajinshan Island, extracted from soil samples of bamboo forest, alt.

Table 2. Numbers of normal setae and sensory organs on antennae of *Scutigerella sinensis* Jin & Bu sp. nov. (holotype).

Antennomere	Normal setae	Spiniform sensory organs	Spined sensory organs	Conical sensory organs	Ventral sensory setae
1	5				
2	9				1
3	10				1
4	10	1	1		1
5	11	3	1		1
6	14	4	1		1
7	17	4	1	1	1
8	18	4	1		1
9	19	4	1	1	1
10	19	4	1		1
11	20	4	1	1	1
12	20	4	1		1
13	20	5	1		1
14	20	4	1		1
15	19	6	1	1	1
16	18	6	1		1
17	17	5	1		1
18	17	5	1		1
19	17	5	1	1	1
20	18	5	1		1
21	17	6	1	1	1
22	16	5	0		1
23	17	6	1		1
24	17	6	1		1
25	18	6	1	1	1
26	18	6	0		1
27	14	8	1	1	1
28	16	5	1		1
29	22	7	3		

103 m, 30°41'N, 121°26'E, 30-VI-2015, coll. Y. Bu & Y. L. Jin. ***Paratypes*:** 1 female (slide no. SH-DJS-SY2017001), ibidem, 11-XI-2017; 1 female (slide no. SH-DJS-SY2017002), ibidem, extracted from soil samples of broad-leaf forest, 11-XI-2017, coll. Y. Bu & Y. L. Jin; 1 female (slide no. SH-DJS-SY2018003), ibidem, 24-IV-2018, coll. Y. Bu & J. Y. Li; 1 male (slide no. SH-DJS-SY2018001), ibidem, extracted from soil samples of broad-leaf forest, 24-X-2018, coll. Y. Bu & J. Y. Li; 1 female (slide no. BJ-YMY-SY2021001), China, Beijing, Yuan-Ming Yuan Imperial Garden, extracted from soil samples of a deserted field with herbaceous plants, alt. 60 m, 40°1'N, 116°17'E, 15-IV-2021, coll. R. Q. Wang. ***Non-type specimens*:** 1 juvenile with 8 pairs of legs (slide no. SH-DJS-SY2015002), same data as holotype; 1 juvenile with 9 pairs of legs (slide no. SH-DJS-SY2015111), ibidem, 22-IX-2015, coll. Y. Bu & Y. L. Jin; 1 juvenile with 11 pairs of legs (slide no. SH-DJS-SY2018002), ibidem, 24-IV-2018, coll. Y. Bu.

Description. Adult body 3.4 mm long on average (3.0–4.3 mm, $N = 6$), holotype 3.2 mm (Fig. 1A).

Head length 300–350 μm, width 320–420 μm, broadest part just posterior of midlength, dorsally covered with straight setae of varying lengths (Fig. 1B). Antennal

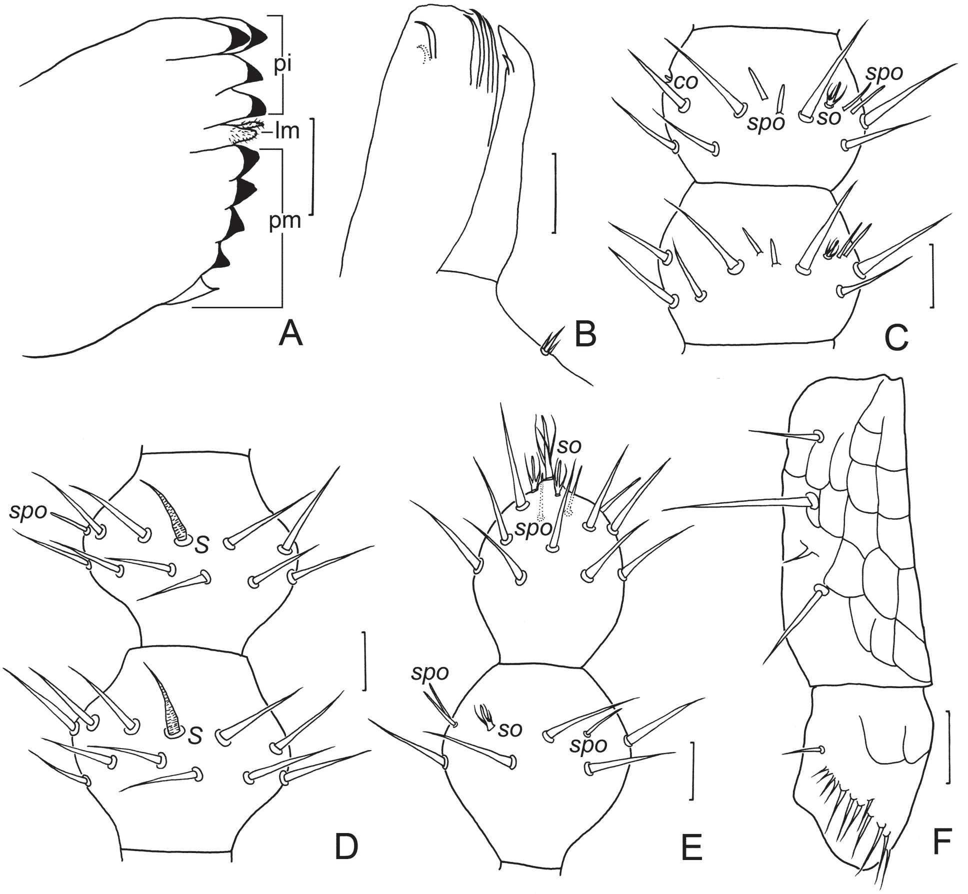


Figure 2. *Scutigerella sinensis* Jin & Bu, sp. nov. **A** mandible, lateral view (*pi*–pars incisivus, *pm*–pars molaris, *lm*–lacinia mobilis) **B** first maxilla **C** right 6–7 antennomeres, dorsal view (*co*–conical sensory organ, *spo*–spiniform sensory organs, *so*–spined sensory organs) **D** right 13–14 antennomeres, ventral view (*S*–sensory setae) **E** terminal two antennomeres, dorsal view **F** femur and tibia of leg 1, ventral view. Scale bars: 20 µm.

base with 3 macrosetae: a1, a2 and a4 (33–50 µm), a3 absent (Fig. 1B). Longest seta (43–53 µm) located between Tömösváry organ and spiracle, same length with greatest width of third antennomere. Reticulation of cuticular thickenings present on frons. Central rod complete (150–187 µm), less distinct at most anterior portion, 0.5 times the length of head, with distinct ovoid swollen end (Fig. 1B). Dorsal cuticle of head glabrous.

Tömösváry organ subspherical, length 20–22 µm, width 15–20 µm, 0.3–0.4 times as wide as greatest diameter of third antennomere (Fig. 1B).

Mouthparts. Mandible similar to *Hanseniella*. Pars incisivus with four distinct thick teeth, pars molaris with four smaller teeth and one proximal spine, lacinia mobilis with 2 pubescent processes observed from lateral view under light microscope (Fig. 2A). First maxilla has two lobes, inner lobe with 4 hook-shaped dorsal teeth and 1 tiny ventral tooth, palp small, with three pointed branches, middle one distinctly longer than lateral ones (Fig. 2B). Second maxilla with many small protuberances anteriorly, each carrying one seta, distal setae thicker and spiniform, posterior part with sparse setae, 3+3 spined organs present on anterolateral margin (Fig. 1E, F). Cuticle of second maxilla covered with dense pubescence.

Antennae with 19–29 antennomeres (holotype with 29), about 0.4 of body length. First antennomere cylindrical, 1.3–2.5 times wider than long (length 30–45 μm, width 50–75μm), with 5–6 setae in one whorl, longest seta 28–30 μm, about half of antennomere width. Second antennomere 1.3–1.9 times wider than long (width 48–65 μm, length 33–40 μm), with 7–9 setae evenly inserted, longest seta inserted outer-dorsally, about 0.5–0.7 times as long as antennomere width. Third antennomere 1.3–1.9 times wider than long (length 25–40 μm, width 45–65μm), with primary whorl of 7–10 setae, longest seta 0.5–0.7 times as long as antennomere width. Setae on proximal antennomeres longer and on distal antennomeres shorter. Proximal antennomeres each with only primary whorl of setae. Secondary whorl setae appear from antennomeres 6–8 to penultimate antennomere (Figs 1H, I, 2C, D). Four kinds of sensory organs observed on antenna: spiniform sensory organs present on antennomeres 3–5 to distal antennomere increasing in number from 2 to 8, short and thick on proximal antennomeres (Fig. 2C, D), long and slender on distal ones (Figs 1H, 2E); one small spined sensory organ consists of several spine and central stub present on dorsal side from fourth antennomere onwards to subdistal antennomere, rarely absent (Fig. 2C, E); single tiny conical sensory organ present on outer side of several antennomeres discontinuously distributed along the antenna (Fig. 2C); one huge spined sensory organ only present on distal antennomere, distinctly bigger than small ones (Fig. 2E). Additionally, one sensory seta decorated with transverse stripes always present on ventral side from second antennomere onwards to penultimate antennomere (Figs 1I, 2D). Distal antennomere longer than wide (length 58–70 μm, width 50–58 μm), with 1 huge spined sensory organ consisting of three or four curved spines stemming from one central stalk on elevated base about 0.3–0.4 times as long as width of antennomere and two neighboring medium ones, 4–7 spiniform organs and 15–22 normal setae on distal half (Fig. 2E). Cuticular reticulation present on first and second antennomere, mainly transverse. All antennomeres pubescent. Chaetotaxy and sensory organs of antennae of holotype are given in Table 2.

Tergites. Tergite 1 rudimentary, with 6–8 subequal setae in one row (Fig. 1C). Tergite 2 complete, 1.6 times wider than long (width 295–245 μm, length 155–185 μm), posterior margin deeply emarginated, with 28–37 subequal marginal setae, longest one (25–27 μm) 1.7–1.8 times as long as shortest one (15–17 μm), 0.4–0.6 and 0.2 times as third antennomere respectively; areas surrounded by marginal setae covered by 41–57 inner setae, similar to marginal setae; anterior half with short pubescence on mesh-work covered cuticular thickenings, posterior half with fine dense pubescence (Fig. 1C). Tergite 3 complete, broader and longer than tergite 2, 1.5–1.6 times wider than long (width 305–382 μm, length 190–240 μm), posterior margin deeply emarginated, with 36–42 subequal marginal setae, longest one (22–29 μm) 1.7–1.8 times as long as shortest one (12–17 μm), with 58–89 subequal inner setae

(Fig. 1D). Tergite 4 broader than tergite 3, with 32–39 subequal marginal setae and 44–66 subequal middle setae. Shape of tergites 5–7, 8–10, and 11–13 similar as tergites 2–4. Pattern of alternating tergite lengths of two short-tergites followed by one long-tergite but disrupted at tergite 13. Last tergite with a V-shaped cavity located medially on posterior border. Anterolateral setae on all tergites not differentiated. Cuticle of all tergites densely pubescent. Posterior border of tergites glabrous (Fig. 1C, D).

Legs. First pair of legs with 4 segments, trochanter absent; femur 1.8–2.0 times longer than wide (length 38–85 μm, width 28–43 μm), with cuticular reticulation (Fig. 2F), with 9–11 setae (Fig. 3A), longest seta (33–43 μm) 0.8–1.1 times as long as greatest width of femur; tibia 1.1–1.4 times as long as wide (40–50 μm, 30–38 μm), with total 4 or 5 setae, long pectinate setation (tibial pecten) present distolaterally (Fig. 2F), dorsal longest seta (23–28 μm) about 0.6–0.9 times as long as greatest diameter of tibia; tarsus about 3.3–4.6 times as long as wide (65–115 μm, 20–25 μm), slowly tapering towards distal end, with 8–14 setae, longest dorsal setae (18–25 μm) 0.8–1.0 times as long as greatest width of tarsus (Fig. 3A). Two slightly curved claws, anterior one somewhat broader and longer than posterior one (Fig. 3A). Leg 12 with 5 segments, 1.0–1.6 times as long as length of head; trochanter 1.4–1.7 times as long as wide (113–150 μm, 75–95 μm), dorsal side with cuticular reticulation, with 11–25 setae in total, longest one (20–21 μm) 0.2–0.3 times of greatest width of podomere (Fig. 3B); femur 1.1–1.3 times as long as wide (55–95 μm, 58–75 μm), with 6–14 setae and dorsal longest seta (23–28 μm) about 0.3–0.5 times as long as width of podomere (Fig. 3B); tibia nearly 1.5–1.9 times longer than wide (80–125 μm, 55–68 μm), with 5 or 6 longitudinal rows of setae, each row with 2–5 setae, longest outer seta (23–30 μm) 0.2–0.3 times as long as greatest width of podomere (Fig. 3B); tarsus 2.9–4.0 times as long as wide (105–150 μm, 38–43 μm) with 5 or 6 longitudinal rows of setae, each row with 2–6 setae, outer rows of setae straight and protruding, other setae slightly curved and short, longest seta (23–25 μm) 0.6–0.7 times as long as greatest width of podomere, 4–6 spiniform setae in a row present on ventral surface (Fig. 3B). Two claws slightly curved, almost same size. All legs covered with dense pubescence except areas with cuticular reticulation.

Coxal sacs present at bases of legs 3–10, fully developed, each with 5 or 6 setae on surface (Fig. 1J). Corresponding area of leg 1, 2, 11, and 12 replaced by 2–3, 2–6, 2–3 and 1 seta respectively.

Styli present at base of legs 3–12, 2.6–4.3 times as long as wide (33–58 μm, 10–20 μm), pubescent, with two distal setae, subapical seta (15–23 μm) 0.3–0.5 times as long as stylus, apical seta (8–10 μm) 0.2–0.3 times as long as stylus, both with pointed apex (Fig. 1J).

Sense calicles located on two ventral protuberances of last tergite, posterior to base of leg 12, with smooth margin around pit. Sensory seta inserted in cup center, extremely long (235–275 μm).

Cerci about 0.7–0.9 of head length, distinctly shorter than leg 12, 2.7–3.4 times as long as its greatest width (240–300 μm, 80–110 μm), moderately covered with subequal setae (Fig. 3C). Dorsally with 30–59 setae, ventrally with 26–57 setae; longest

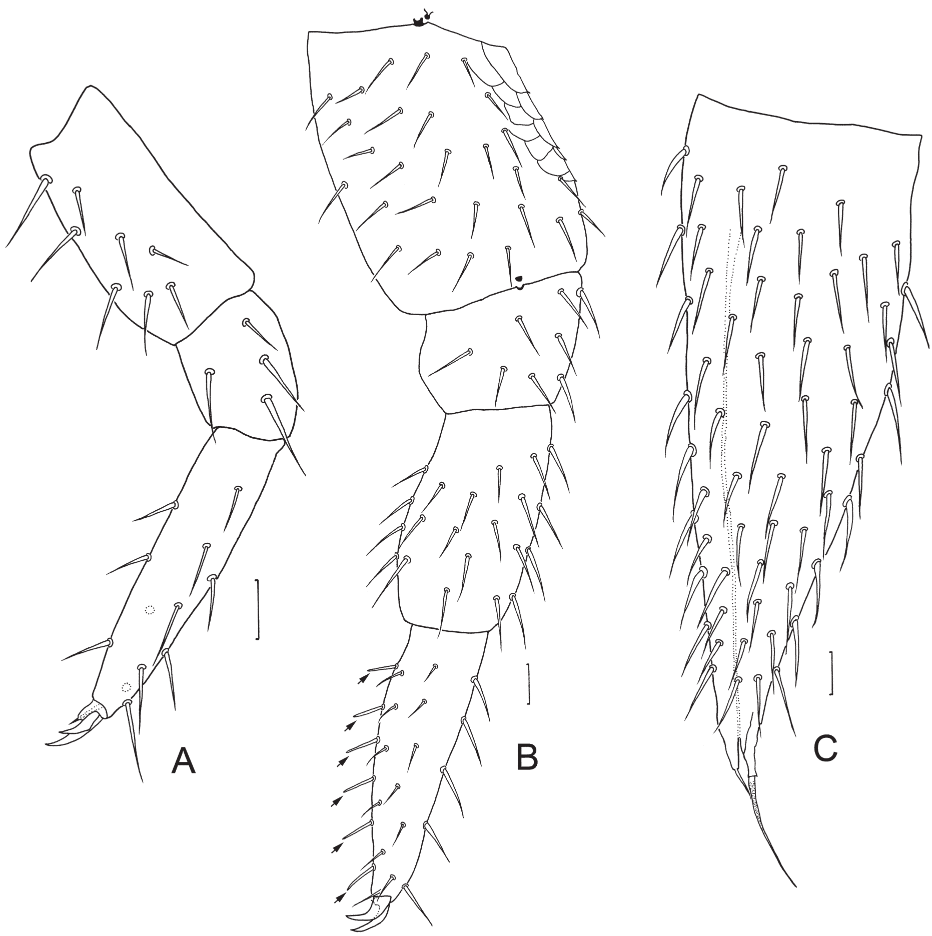


Figure 3. *Scutigerella sinensis* Jin & Bu, sp. nov. **A** leg 1, dorsal view **B** leg 12, ventro-lateral view (arrows indicate spiniform setae) **C** left cercus, dorsal view. Scale bars: 20 µm.

seta (28–33 µm) 1.3–1.5 times as long as shortest seta (20–28 µm), 0.3–0.4 times as long as greatest width of cercus and 0.1 times as length of cercus. Terminal area glabrous, 0.1 times as long as cercus. Two apical setae pointed, longer seta (43–58 µm) 0.2 times as long as cercus, with granules; shorter seta about a third of the length of longer one. Cuticle with dense pubescence.

Male genitalia with 30 setae in total in holotype, without specialized setae (Fig. 1G).

Etymology. This new species is named after the country of origin, from the Latin adjective sinensis, meaning Chinese.

Distribution. China (Shanghai, Beijing).

Ecology. Our current investigation indicates that *Scutigerella sinensis* sp. nov. is a rare species in natural habitats with very low density. We found about ten individuals among several hundred symphylans from plots of different vegetation. This *Scutigerella* is often coexisting with other dominant species of *Hanseniella* and *Symphylella* in the upper soil layer (0–10 cm) or humus.

Remarks. The head chaetotaxy was briefly described in the previous studies of *Scutigerella*, usually with only shapes and numbers mentioned. The macrosetae around the base of the antenna of *Scutigerella* have been noticed and named by former researchers, and all species examined until now have a complete set of four macrosetae (a1–a4), which was deemed as a good diagnostic character in the taxonomy of *Scutigerella* (Hinschberger 1950; Juberthie-Jupeau and Tabacaru 1968; Scheller 1986). *Scutigerella sinensis* sp. nov. has three macrosetae (a1, a2, and a4) around the antennal base, with a3 seta absent, which can be easily distinguished from all other congeners. Our observation indicates this character is stable in both adults and juveniles and can be a unique feature of the new species. The present new species is most similar to the cosmopolitan species *Scutigerella immaculata* (Newport, 1845) in the shapes and chaetotaxy of tergites and legs, but differs in the absence of the a3 seta on the head (present in *S. immaculata*), number of marginal setae on tergite 2 and 3 (less than 50 in *S. sinensis* sp. nov. vs. more than 50 in *S. immaculata*), and the shape of the stylus (tapering in *S. sinensis* sp. nov. vs. cylindrical in *S. immaculata*).

Mitogenomic analysis

The mitochondrial genome of Symphyla until now was only known from two species: *Scutigerella causeyae* and one undetermined species of *Symphylella* (Podsiadlowski et al. 2007; Gai et al. 2008). In the present study, we sequenced the complete mitogenome of *Scutigerella sinensis* sp. nov. The mitogenome of *S. sinensis* sp. nov. is 14 512 bp long and contains the control region (CR) and all 37 genes typically found in Arthropoda (Fig. 4, Table 3). The nucleotide composition varies along its length, being AT-rich for the entire mitogenome with: A – 36.41% (5284); T – 34.46% (5001); C – 19.41% (2817) and G – 9.71% (1410). With an AT content of 81% the AT rich or control region is 297 bp long and is located between trnQ and trnM. Three different start codons were present in the protein coding genes: ATG (6×) and ATA (2×), canonical codons encoding Methionine, and ATT (5×) encoding Isoleucine. Two different stop codons were present: TAA (11×) and TAG (2×). No truncated stop codon was observed (Table 3).

Compared to the mitogenome of the congeneric species *S. causeyae*, the new sequence is 125 bp smaller and differs in the relative position of three tRNA genes (Q, M, I) located next to the control region. Additionally, the tRNA-Valine is located between the rRNAs, like in the inferred arthropod ground pattern (Staton et al. 1997) (Fig. 5). This is the first report of the occurrence of this ground plan in *Scutigerella*, which is an important similarity between the new species and the hypothetical ancestor. On other hand, the translocation between the tRNA genes P and T is shared between *S. sinensis* and *S. causeyae*, but not observed in the ground pattern; it can be a step toward for further studies on character evolution in *Scutigerella*.

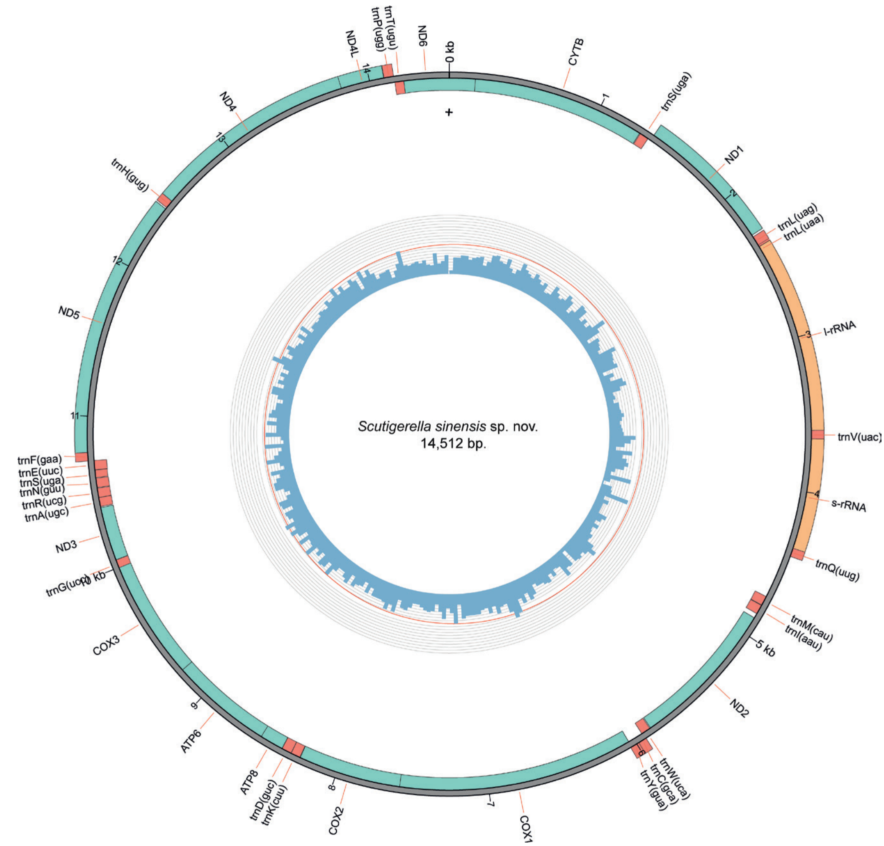


Figure 4. Circular representation of the mitogenome of *Scutigerella sinensis* sp. nov. The innermost circle shows the GC content (the red line marks 50%) and the outermost circle shows the gene order, rRNAs, tRNAs, and PCGs. Plus (+) indicates the side of the major J-strand.

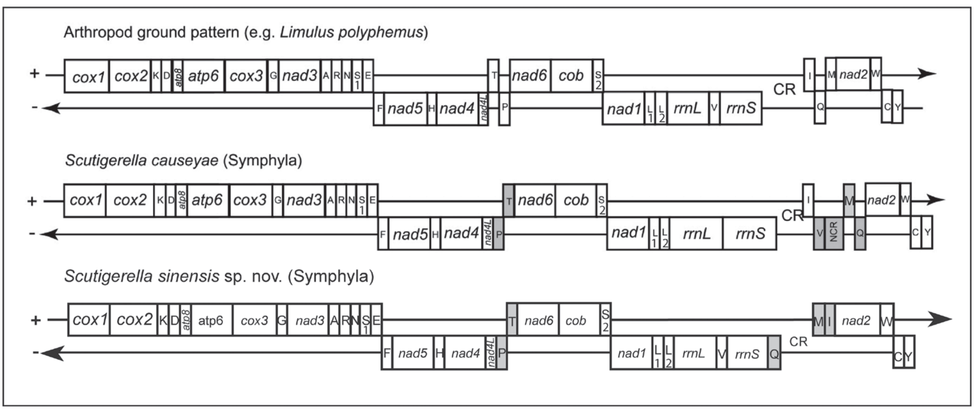


Figure 5. Mitochondrial gene arrangements of *Scutigerella sinensis* sp. nov. compared to *S. causeyae* and the arthropod ground pattern (adapted from Podsiadlowski et al. 2007). Genes shaded grey have different relative positions compared to the ground pattern. Upper line with (+)–strand genes, lower line with (–) –strand genes. CR: putative control region.

Table 3. Organization of *Scutigerella sinensis* sp. nov. mitochondrial genome.

Start	End	Length (bp)	Direction	Start/Stop codons	Gene name	Gene product
181	1303	1122	+	ATG/TAA	CYTB	Cytochrome C Oxidase 1
1307	1368	62	+		trnS2 (uga)	tRNA-Ser
1386	2286	901	-	ATA/TAA	ND1	NADH Dehydrogenase 1
2298	2360	63	-		trnL1 (uag)	tRNA-Leu
2360	2425	66	-		trnL2 (uaa)	tRNA-Leu
2371	3656	1286	-		l-rRNA	16S ribosomal RNA
3604	3665	62	-		trnV (uac)	tRNA-Val
3659	4408	750	-		s-rRNA	12S ribosomal RNA
4369	4425	57	-		trnQ (uug)	tRNA-Gln
4425	4722	297			CR	Control Region
4723	4786	64	+		trnM (cau)	tRNA-Met
4788	4851	64	+		trnI (aau)	tRNA-Ile
4881	5871	991	+	ATT/TAA	ND2	NADH Dehydrogenase 2
5876	5939	64	+		trnW (uca)	tRNA-Trp
5931	5986	56	-		trnC (gca)	tRNA-Cys
5985	6046	62	-		trnY (gua)	tRNA-Tyr
6038	7582	1545	+	ATA/TAA	COX1	Cytochrome C Oxidase I
7581	8256	676	+	ATG/TAA	COX2	Cytochrome C Oxidase II
8258	8321	64	+		trnK (cuu)	tRNA-Lys
8321	8387	67	+		trnD (guc)	tRNA-Asp
8387	8549	163	+	ATT/TAA	ATP8	ATP synthase F0 subunit 8
8542	9214	673	+	ATG/TAA	ATP6	ATP synthase F0 subunit 6
9213	10014	802	+	ATG/TAA	COX3	Cytochrome C Oxidase III
9997	10052	56	+		trnG (ucc)	tRNA-Gly
10052	10406	355	+	ATT/TAA	ND3	NADH Dehydrogenase 3
10412	10472	61	+		trnA (ugc)	tRNA-Ala
10472	10534	63	+		trnR (ucg)	tRNA-Arg
10537	10599	63	+		trnN (guu)	tRNA-Asn
10599	10653	55	+		trnS1 (gcu)	tRNA-Ser
10653	10715	63	+		trnE (uuc)	tRNA-Glu
10713	10769	57	-		trnF (gaa)	tRNA-Phe
10768	12436	1669	-	ATT/TAG	ND5	NADH Dehydrogenase 5
12445	12499	55	-		trnH (gug)	tRNA-His
12498	13821	1324	-	ATG/TAG	ND4	NADH Dehydrogenase 4
13814	14093	280	-	ATG/TAA	ND4L	NADH Dehydrogenase 4L
14095	14157	63	-		trnP (ugg)	tRNA-Pro
14158	14218	61	+		trnT (ugu)	tRNA-Thr
14217	181	477	+	ATT/TAA	ND6	NADH Dehydrogenase 6

COI-tree reconstruction

In the Neighbor-Joining tree constructed based on DNA barcoding sequences, *S. sinensis* sp. nov. clustered with *S. causeyae* (Fig. 6). However, these two species very much differ in their morphological characters: the posterior margin of tergite 2 (deeply emarginated in *S. sinensis* sp. nov. vs. truncate and barely emarginated in *S. causeyae*), chaetotaxy on the head (a3 seta absent in *S. sinensis* sp. nov. vs. a3 seta present in *S. causeyae*) and shape of the stylus (both apical and subapical setae pointed in *S. sinensis* sp. nov. vs. apical seta truncate and subapical seta pointed in *S. causeyae*). The sister group of this cluster is a mixed assemblage of species determined as *Hanseniella*, *Scutigerella*

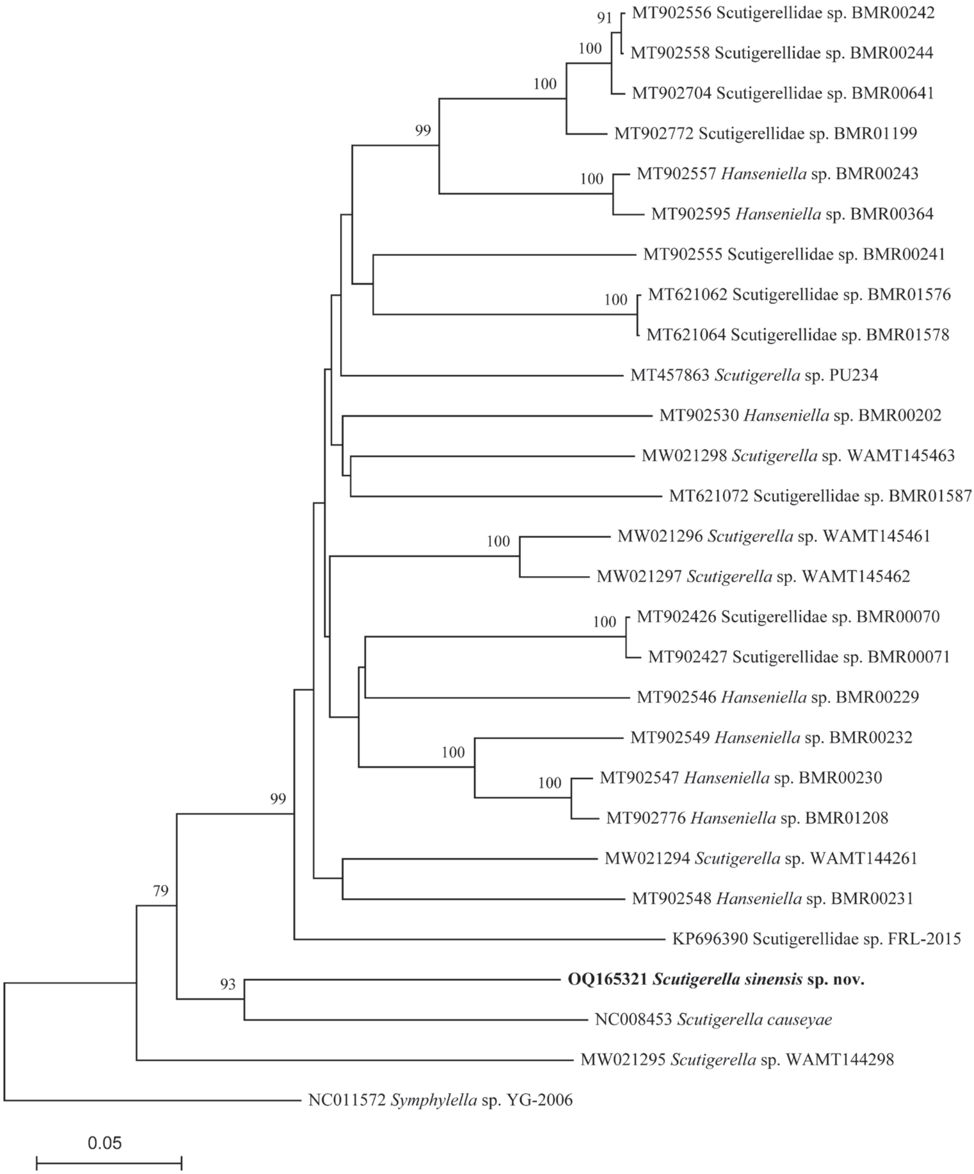


Figure 6. Neighbor-Joining tree (Jukes-Cantor model, Bootstrap 1000 replicates) of symphylans inferred from COI gene sequences. Numbers at the nodes show the bootstrap values > 50%.

and Scutigerellidae. Since most sequences downloaded from GenBank are from the individuals only primarily determined to family or genus levels and the validation of identification cannot be confirmed, we refrain from questioning the monophyletic status of *Hanseniella* and *Scutigerella*.

Table 4. Genetic distances of Symphyla analyzed by mitochondrial COI gene (K2P model).

Level	Mean	Minimum	Maximum
Scutigerella sinensis sp. nov. vs congeners	0.2747	0.2280	0.2946
Interspecific distances within the genus *Scutigerella*	0.2638	0.1883	0.3248
Interspecific distances within the genus *Hanseniella*	0.2084	0.1738	0.2343
Conspecific distances of the genus *Hanseniella*	0.0550	0.0160	0.0961
Intergeneric distances between *Scutigerella* and *Hanseniella*	0.2376	0.1700	0.3364
Interfamiliar distance between Scutigerellidae and Scolopendrellidae	0.3170	0.2947	0.3636

Genetic divergence

The pairwise genetic distance of 28 sequences of symphylan species based on the K2P model is given in the Suppl. material 1. The genetic distance between *S. sinensis* sp. nov. and other congeners is 0.2747 on average (0.2280–0.2946), which gives further support for our morphological identification, however the coverage of species does not allow us to draw too many conclusions. The genetic distances of the COI gene among different taxonomic levels of Symphyla are given in Table 4 (but determinations of species downloaded from GenBank are questionable).

Acknowledgements

We cordially thank Dr Bi-Cheng Li for his careful organization of the expedition to Dajinshan Island, and Dr Jing-Yang Li and Mrs Si-Qi Yang for their generous help in the collection. Special thanks are given to Mr Rui-Qing Wang for the collection of specimens from Yuan-Ming Yuan Imperial Garden in Beijing. We appreciate Prof. José G. Palacios-Vargas (Mexico) for the linguistic corrections of the manuscript and valuable advice. We sincerely thank Dr Nikolaus Szucsich (Austria) and Dr Carlos Alberto Martínez Muñoz (Finland) for their valuable comments in review of the manuscript. This research was supported by the National Natural Science Foundation of China (no: 32170471) and the Research Foundation of Shanghai Science and Technology Museum.

References

Bagnall RS (1913) On the classification of the order Symphyla. Zoological Journal of the Linnean Society 32(216): 195–199. https://doi.org/10.1111/j.1096-3642.1913.tb01775.x

Bu Y, Jin YL (2018) Progress on the systematic study of Symphyla. Chinese Bulletin of Life Sciences 30(5): 500–509. [In Chinese with English summary] https://doi.org/10.13376/j.cbls/2018060

Dierckxsens N, Mardulyn P, Smits G (2016) NOVOPlasty: De novo assembly of organelle genomes from whole genome data. Nucleic Acids Research 45(4): e18. https://doi.org/10.1093/nar/gkw955

Gai Y, Song D, Sun H, Yang Q, Zhou K (2008) The complete mitochondrial genome of *Symphylella* sp. (Myriapoda: Symphyla): Extensive gene order rearrangement and evidence in favor of Progoneata. Molecular Phylogenetics and Evolution 49(2): 574–585. https://doi.org/10.1016/j.ympev.2008.08.010

Hinschberger P (1950) Contribution à l'étude des Symphyles du Mexique. Bulletin du Muséum national d'Histoire naturelle 2e Série 22(2): 256–263. https://www.biodiversitylibrary.org/part/330360

Jin YL, Bu Y (2018) First record of *Scolopendrellopsis* from China with the description of a new species (Myriapoda, Symphyla). ZooKeys 789: 103–113. https://doi.org/10.3897/zookeys.789.27356

Jin YL, Bu Y (2019) Occurrence of *Millotellina* (Myriapoda, Symphyla) in China with the description of two new species. Zootaxa 4691(3): 215–224. https://doi.org/10.11646/zootaxa.4691.3.2

Jin YL, Bu Y (2020) Two new species of the genus *Symphylella* (Symphyla, Scolopendrellidae) from East China. ZooKeys 1003: 1–18. https://doi.org/10.3897/zookeys.1003.60210

Jin YL, Bu Y (2023) Two new species of the genus *Symphylella* (Symphyla, Scolopendrellidae) from China and the significance of the frons chaetotaxy. ZooKeys 1138: 143–160. https://doi.org/10.3897/zookeys.1138.96424

Jin YL, Bu Y, Jiang Y (2019) Two new species of the genus *Symphylella* (Symphyla, Scolopendrellidae) from Tibet, China. ZooKeys 845: 99–117. https://doi.org/10.3897/zookeys.845.33566

Juberthie-Jupeau L, Tabacaru I (1968) Symphyles de Roumanie. Bulletin du Muséum national d'Histoire naturelle 2e Série, 40(3): 500–517. https://www.biodiversitylibrary.org/part/254980

Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: Munro HN (Ed.) Mammalian Protein Metabolism. Academic Press, New York, 21–132. https://doi.org/10.1016/B978-1-4832-3211-9.50009-7

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16(2): 111–120. https://doi.org/10.1007/BF01731581

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35(6): 1547–1549. https://doi.org/10.1093/molbev/msy096

Meng G, Li Y, Yang C, Liu S (2019) MitoZ: A toolkit for animal mitochondrial genome assembly, annotation and visualization. Nucleic Acids Research 47(11): e63. https://doi.org/10.1093/nar/gkz173

Michelbacher AE (1942) A synopsis of the genus *Scutigerella* (Symphyla: Scutigerellidae). Annals of the Entomological Society of America 35(3): 267–288. https://doi.org/10.1093/aesa/35.3.267

Myriatrix, The Fellegship of the Rings (2020 onwards) Myriatrix, The Fellegship of the Rings. http://myriatrix.myspecies.info [Accessed February 2023]

Newport G (1844) Monograph of the class Myriopoda, order Chilopoda, with observations on the general arrangement of the Articulata. Part I. Transactions of the Linnean Society of London 19(3): 265–302. [pl. 303] https://doi.org/10.1111/j.1096-3642.1842.tb00368.x

Newport G (1845) Monograph of the class Myriopoda, order Chilopoda, with observations on the general arrangement of the Articulata. Part II. Transactions of the Linnean Society of London 19(4): 349–439. [pl. 440] https://doi.org/10.1111/j.1096-3642.1842.tb00370.x

Podsiadlowski L, Kohlhagen H, Koch M (2007) The complete mitochondrial genome of *Scutigerella causeyae* (Myriapoda: Symphyla) and the phylogenetic position of the Symphyla. Molecular Phylogenetics and Evolution 45(1): 251–260. https://doi.org/10.1016/j.ympev.2007.07.017

Ryder JA (1880) *Scolopendrella* as the type of a new order of Articulates (Symphyla). American Naturalist 14: 375–376.

Ryder JA (1882) Genera of the Scolopendrellidae. Proceedings of the United States National Museum 5(15): e234. https://doi.org/10.5479/si.00963801.5-280.234

Salazar-Moncada DA, Calle-Osorno J, Ruiz-Lopez F (2015) Morphological and molecular study of Symphyla from Colombia. ZooKeys 484: 121–130. https://doi.org/10.3897/zookeys.484.8363

Scheller U (1986) Symphyla from the United States and Mexico. Texas Memorial Museum, Speleology. Monograph 1: 87–125.

Staton JL, Daehler LL, Brown WM (1997) Mitochondrial gene arrangement of the horseshoe crab *Limulus polyphemus* L.: Conservation of major features among arthropod classes. Molecular Biology and Evolution 14(8): 867–874. https://doi.org/10.1093/oxfordjournals.molbev.a025828

Szucsich N, Scheller U (2011) Chapter 20. Symphyla. In: Minelli A (Ed.) Treatise of Zoology–Anatomy, Taxonomy, Biology: The Myriapoda (Vol. 1). Brill, Leiden, 445–466. https://doi.org/10.1163/9789004188266_021

Zhang CZ, Wang DQ (1992) Symphyla. In: Yin WY, et al. (Eds) Subtropical Soil Animals of China. Science Press, Beijing, 383–387. [In Chinese]

Supplementary material 1

K2P distances between different speices of Symphyla analyzed by the mitochondrial COI gene

Authors: Ya-Li Jin, Nerivania Nunes Godeiro, Yun Bu

Data type: table (excel file)

Link: https://doi.org/10.3897/zookeys.1157.99686.suppl1

ZooKeys 1157: 163–176 (2023)
doi: 10.3897/zookeys.1157.99674
https://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Three new species of the *Clubiona corticalis* group (Araneae, Clubionidae) from China

Panlong Wu[1,2], Yang Chen[2,3], Feng Zhang[2]

1 *Collaborative Innovation Center for Grassland Ecological Security, Ministry of Education of China, School of Ecology and Environment, Inner Mongolia University, Hohhot 010021, China* **2** *The Key Laboratory of Zoological Systematics and Application, Institute of Life Science and Green Development, College of Life Sciences, Hebei University, Baoding, Hebei 071002, China* **3** *Shanxi Yuncheng Vocational and Technical College of Agricultural, Yuncheng, Shanxi 044000, China*

Corresponding author: Feng Zhang (dudu06042001@163.com)

Academic editor: Yuri Marusik | Received 6 January 2023 | Accepted 24 March 2023 | Published 5 April 2023

https://zoobank.org/3BD5CC1E-BB5B-4AA9-88F4-26F63F96CBC0

Citation: Wu P, Chen Y, Zhang F (2023) Three new species of the *Clubiona corticalis* group (Araneae, Clubionidae) from China. ZooKeys 1157: 163–176. https://doi.org/10.3897/zookeys.1157.99674

Abstract

Three new species of the *Clubiona corticalis* group in China are described: *Clubiona bidactylina* **sp. nov.**, *C. camela* **sp. nov.**, and *C. subhuiming* **sp. nov.**

Keywords

Clubionids, sac spiders, species diversity, taxonomy

Introduction

Clubiona Latreille, 1804, the largest genus in the family Clubionidae, currently includes 519 species distributed on most continents of the world (WSC 2023). Due to its high species diversity and worldwide distribution, several arachnologists have proposed to subdivide this genus into subgenera and species groups (e.g., Simon 1932; Lohmander 1944; Edwards 1958; Wiehle 1965; Dondale and Redner 1982; Mikhailov 1995, 2012; Deeleman-Reinhold 2001; Wunderlich 2011).

The *Clubiona corticalis* group, first recognised by Simon (1932), has recently been one of the most frequently reported species groups of the family (Marusik and

Omelko 2018; Zhang et al. 2021; Zhong et al. 2022). In China, at least 51 species of the *Clubiona corticalis* group have been recorded to date, and these are mainly distributed in south China (Table 1). While examining Chinese clubionids, we found three new species belonging to the *Clubiona corticalis* group, and we describe them in this paper.

Materials and methods

All specimens studied are stored in 75% ethanol and stored in the Museum of Hebei University (**MHBU**), Baoding, China. We identified these specimens using a Tech XTL-II stereomicroscope, and drew, photographed, and measured them using a Leica M205A stereomicroscope equipped with a drawing tube and a DFC450 CCD camera. Carapace length was measured from the anterior margin to the posterior margin of the carapace medially. Eye sizes were measured as the maximum diameter of the lens in dorsal or frontal view. The leg measurements are shown as total length (femur, patella, tibia, metatarsus, tarsus). The epigyne was cleared in a warm 10% potassium hydroxide (KOH) solution and transferred to 75% ethanol for drawing, photographing, and measuring. All measurements are in millimetres.

The following abbreviations are used: **ALE**, anterior lateral eye; **AME**, anterior median eye; **B**, bursa; **C**, conductor; **CD**, copulatory duct; **CO**, copulatory opening; **E**, embolus; **FD**, fertilisation duct; **LPA**, lateral patellar apophysis; **MOA**, median ocular area; **PLE**, posterior lateral eye; **PME**, posterior median eye; **RFA**, retrolateral femoral apophysis; **RPA**, retrolateral patellar apophysis; **RTA**, retrolateral tibial apophysis; **S**, spermatheca; **SD**, sperm duct; **TA**, tegular apophysis; **VFA**, ventral femoral apophysis; **VPA**, ventral patellar apophysis; **VTA**, ventral tibial apophysis.

Taxonomy

***Clubiona bidactylina* sp. nov.**
https://zoobank.org/3D8E3EC9-2306-4EA9-929F-2B4DCF38743D
Figs 1, 2

Type material. ***Holotype*:** China • ♂; Tibet, Zayu County (28°39.59'N, 97°27.96'E, 2323 m elev.), 25 June 2018, Yannan Mu leg. ***Paratypes*:** • 2♀, same data as the holotype.

Etymology. The specific name is the combination of the *bi-* (two) and *dactylina* (finger-shaped), referring to two finger-shaped apophyses on the femur; adjective.

Diagnosis. The new species resembles *C. pianmaensis* Wang, Wu & Zhang, 2015 (Wang et al. 2015: figs 1–14), but it differs by the presence of femoral ventral apophysis (vs. absent), 2 retrolateral tibial apophyses (vs. 1), the wider embolic base (embolic base width/genital bulb width ratio 0.4 vs. 0.2), conductor extended to the middle part of embolus (vs. tip of embolus), the smaller copulatory openings (copulatory opening width/interdistance ratio 0.2 vs. 4.7), and the boot-shaped bursae (vs. almost spherical).

Table 1. Species belonging to the *Clubiona corticalis* group recorded in China.

	Species name	Known sex	Distribution
1	*C. pyrifera* Schenkel, 1936	♂♀	Gansu
2	*C. kurosawai* Ono, 1986	♂♀	Taiwan
3	*C. parallela* Hu & Li, 1987	♂♀	Tibet
4	*C. yaginumai* Hayashi, 1989	♂♀	Taiwan
5	*C. lyriformis* Song & Zhu, 1991	♀	Hubei
6	*C. moralis* Song & Zhu, 1991	♂♀	Hubei
7	*C. taiwanica* Ono, 1994	♂♀	Yunnan, Taiwan
8	*C. didentata* Zhang & Yin, 1998	♂♀	Yunnan
9	*C. qiyunensis* Xu, Yang & Song, 2003	♂♀	Fujian, Anhui
10	*C. altissimoides* Liu et al., 2007	♂♀	Yunnan
11	*C. applanata* Liu et al., 2007	♂♀	Yunnan
12	*C. cylindrata* Liu et al., 2007	♂♀	Yunnan
13	*C. lamina* Zhang, Zhu & Song, 2007	♂	Yunnan
14	*C. tengchong* Zhang, Zhu & Song, 2007	♂	Yunnan
15	*C. cordata* Zhang & Zhu, 2009	♂♀	Sichuan, Tibet
16	*C. kai* Jäger & Dankittipakul, 2010	♂♀	Yunnan
17	*C. submoralis* Wu, Zheng & Zhang, 2015	♂♀	Yunnan
18	*C. pollicaris* Wu, Zheng & Zhang, 2015	♂♀	Yunnan
19	*C. pianmaensis* Wang, Wu & Zhang, 2015	♂♀	Yunnan
20	*C. cochleata* Wang, Wu & Zhang, 2015	♂♀	Sichuan
21	*C. biforamina* Liu, Peng & Yan, 2016	♂♀	Yunnan
22	*C. dactylina* Liu, Peng & Yan, 2016	♂♀	Yunnan
23	*C. falciforma* Liu, Peng & Yan, 2016	♂♀	Yunnan
24	*C. gongshan* He, Liu & Zhang, 2016	♂♀	Yunnan
25	*C. lucida* He, Liu & Zhang, 2016	♂♀	Hunan
26	*C. multidentata* Liu, Peng & Yan, 2016	♂♀	Yunnan
27	*C. tangi* Liu, Peng & Yan, 2016	♂♀	Yunnan
28	*C. bifurcata* Zhang, Yu & Zhong, 2018	♂♀	Guizhou
29	*C. dichotoma* Wang, Chen & Zhang, 2018	♂♀	Guizhou
30	*C. globosa* Wang, Chen & Zhang, 2018	♂♀	Guizhou
31	*C. lamellaris* Zhang, Yu & Zhong, 2018	♂♀	Guizhou
32	*C. subapplanata* Wang, Chen & Zhang, 2018	♂♀	Guizhou
33	*C. fanjingshan* Wang, Chen & Zhang, 2018	♂	Guizhou
34	*C. huiming* Wang, Chen & Zhang, 2018	♂	Guizhou
35	*C. subcylindrata* Wang, Chen & Zhang, 2018	♂	Guizhou
36	*C. cochlearis* Yu & Li, 2019	♂♀	Yunnan
37	*C. tiane* Yu & Li, 2019	♂♀	Yunnan
38	*C. subrama* Yu & Li, 2019	♂♀	Yunnan
39	*C. subyaginumai* Yu & Li, 2019	♂♀	Yunnan
40	*C. caohai* Zhang & Yu, 2020	♂♀	Guizhou
41	*C. dakong* Zhang & Yu, 2020	♀	Tibet
42	*C. yanzhii* Zhang & Yu, 2020	♀	Hunan
43	*C. dengpao* Yu & Li, 2021	♀	Yunnan
44	*C. subdidentata* Yu & Li, 2021	♀	Yunnan
45	*C. tixing* Yu & Li, 2021	♀	Yunnan
46	*C. xiaoci* Yu & Li, 2021	♂♀	Yunnan
47	*C. xiaokong* Yu & Li, 2021	♀	Yunnan
48	*C. yejiei* Yu & Li, 2021	♀	Yunnan
49	*C. zhaoi* Yu & Li, 2021	♀	Yunnan
50	*C. zhigangi* Yu & Li, 2021	♂♀	Yunnan
51	*C. xianning* Zhong & Yu, 2022	♂♀	Hubei
52	*C. bidactylina* sp. nov.	♂♀	Tibet
53	*C. camela* sp. nov.	♂♀	Guangxi
54	C. *subhuiming* sp. nov.	♂♀	Hunan

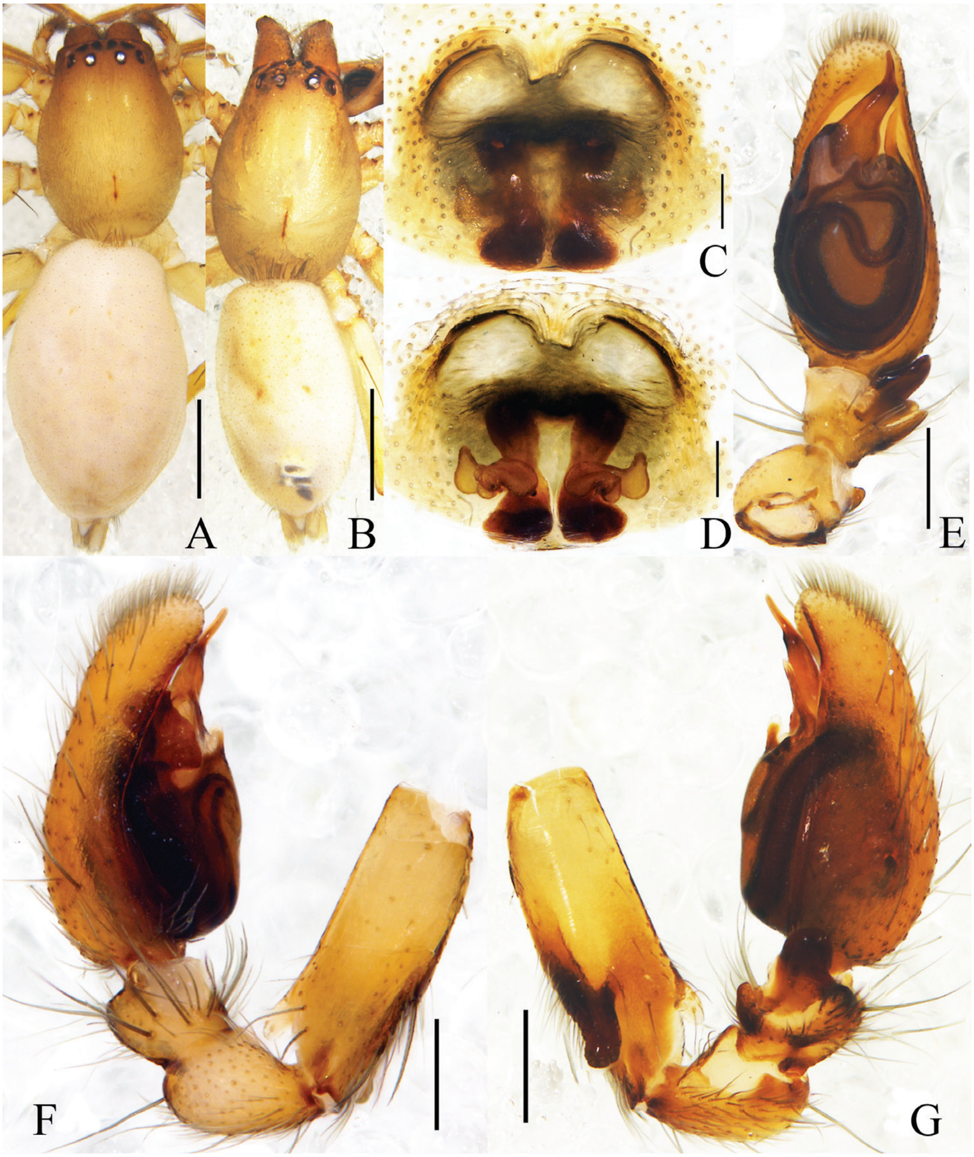


Figure 1. *Clubiona bidactylina* sp. nov. **A** female habitus **B** male habitus **C** epigyne, ventral view **D** vulva, dorsal view. Left male palp: **E** ventral view **F** prolateral view **G** retrolateral view. Scale bars: 1 mm (**A, B**); 0.1 mm (**C, D**); 0.2 mm (**E–G**).

Description. Male (Fig. 1B). Total length 4.11: carapace 2.04 long, 1.44 wide; abdomen 2.16 long, 1.31 wide. Carapace yellowish brown. Eye sizes and interdistances: AME 0.08, ALE 0.11, PME 0.10, PLE 0.12; AME–AME 0.09, AME–ALE 0.04, ALE–ALE 0.34, PME–PME 0.22, PME–PLE 0.12, PLE–PLE 0.66, ALE–PLE 0.11.

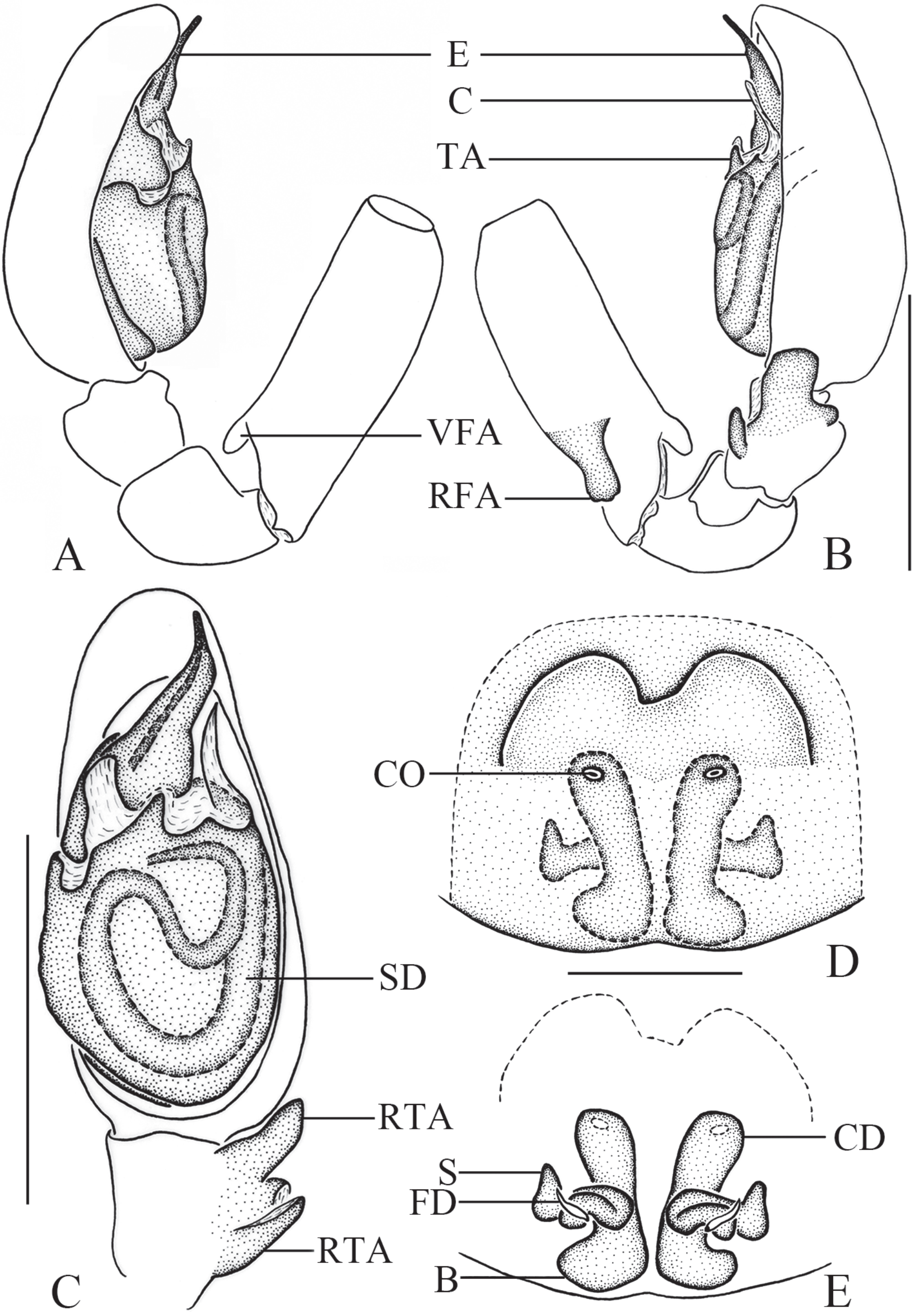


Figure 2. *Clubiona bidactylina* sp. nov. Left male palp: **A** prolateral view **B** retrolateral view **C** ventral view **D** epigyne, ventral view **E** vulva, dorsal view. Scale bars: 0.5 mm (**A–C**); 0.1 mm (**D, E**).

MOA 0.23 long, front width 0.27, back width 0.42. Clypeus height 0.05. Chelicerae yellow brown, promargin with 4 teeth, retromargin with 3 teeth. Endites paler than chelicerae. Labium yellow-brown, 0.31 long, 0.24 wide. Sternum 1.07 long, 0.62 wide. Abdomen oval, light yellow, with conspicuous anterior tufts of setae. Legs, yellow, except tarsi and metatarsi yellowish brown, both tibia I and II with two pairs of ventral spines, both metatarsi I and II with 1 pair of ventral spines. Leg measurements: I 4.25 (1.25, 0.59, 1.09, 0.84, 0.48), II 5.01 (1.46, 0.67, 1.35, 1.00, 0.53), III 3.87 (1.15, 0.58, 0.79, 0.97, 0.38), IV 5.58 (1.62, 0.63, 1.29, 1.55, 0.49). Leg formula: 4-2-1-3.

Palp (Figs 1E–G, 2A–C). Femur length/width ratio 2.9, modified, with 2 apophyses, retrolateral apophysis digitiform, located medially, ventral apophysis transparent, located distally. Patella length/width ratio 1.5, unmodified. Tibia as long as wide, with 2 retrolateral apophyses, wedge-shaped apophysis located distally, with a small, triangular process at base in retrolateral view, digitiform apophysis located proximally. Cymbium length/femur length ratio 1.2. Cymbium length/width ratio 2.3, oblong. Tegulum uninflated, sperm a-shaped in ventral view. Conductor lance-shaped in ventral view, extended to the middle part of embolus. Embolus with wide base, embolic base width/genital bulb width ratio 0.4, the upper third sharply pointed and extended towards tip of cymbium.

Female (Fig. 1A). Total length 4.71–4.79; body length 4.79: carapace 2.04 long, 1.48 wide; abdomen 2.79 long, 1.83 wide. Eye sizes and interdistances: AME 0.08, ALE 0.11, PME 0.09, PLE 0.11; AME–AME 0.11, AME–ALE 0.04, ALE–ALE 0.36, PME–PME 0.23, PME–PLE 0.15, PLE–PLE 0.71, ALE–PLE 0.10. MOA 0.23 long, front width 0.27, back width 0.43. Clypeus height 0.05. Labium 0.30 long, 0.26 wide. Sternum 1.11 long, 0.74 wide. Leg measurements: I 3.52 (1.05, 0.56, 0.82, 0.66, 0.43), II 3.92 (1.18, 0.63, 0.92, 0.76, 0.43), III 3.35 (1.02, 0.44, 0.72, 0.81, 0.36), IV 5.19 (1.49, 0.72, 1.16, 1.40, 0.42). Leg formula: 4-2-1-3. Other characters as in male.

Epigyne (Figs 1C, D, 2D, E). Epigynal plate length/width ratio 0.9, the anterior part membranous, with 2 semicircular hoods. Copulatory openings small and circular, located medially, width/interdistance ratio 0.2. Copulatory ducts almost straight, descended obliquely, then connected with boot-shaped bursae. Bursae situated posteriorly, length/width ratio 1.6. Spermathecae tubular, almost located at the middle position between copulatory openings and bursae. Fertilisation ducts short and lance-shaped, located on dorsal-lateral surface of spermathecae.

Distribution. Known only from the type locality.

Clubiona camela **sp. nov.**

https://zoobank.org/1BF9FAAE-71AD-41DA-B59C-8D38521FFB89
Figs 3–5

Type material. ***Holotype*:** China • ♂; Yunnan Province, Pingbian County, Daweishan National Forest Park (22°59'16"N, 103°57'01"E, 2124 m elev.), 28 October 2016, Guiqiang Huang leg. ***Paratypes*:** • 3♀2♂, same data as holotype.

Etymology. The species name is derived from the Latin *camela* (camel), referring to hump-shaped retrolateral tibia apophyses in retrolateral view; noun.

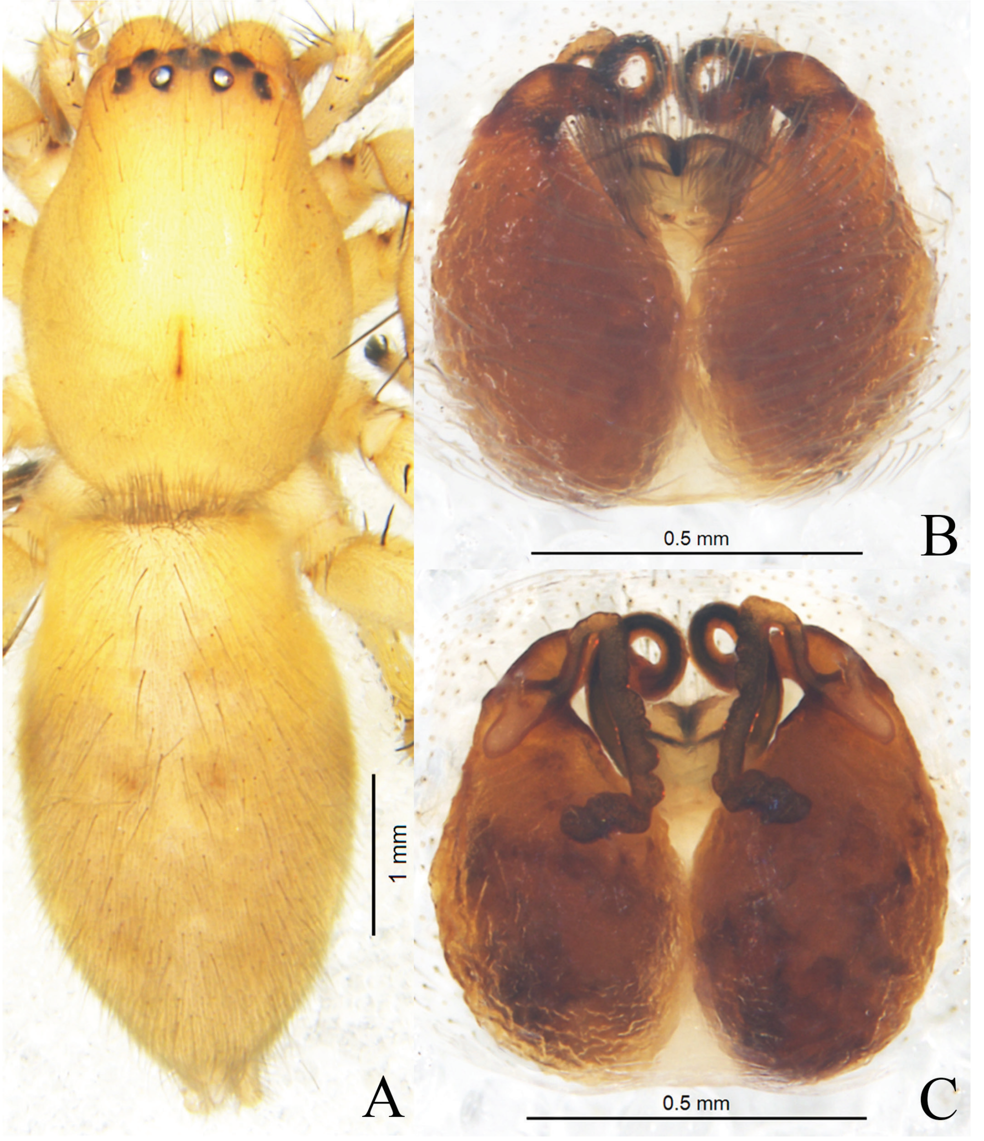


Figure 3. *Clubiona camela* sp. nov. **A** female habitus **B** epigyne, ventral view **C** vulva, dorsal view. Scale bars: 1 mm (**A**); 0.5 mm (**B, C**).

Diagnosis. The new species resembles *C. biforamina* Liu, Peng & Yan, 2016 (Liu et al. 2016: figs 1–12), but it differs by the larger embolus length/conductor length ratio (1.6 vs. 0.6), triangle distal retrolateral tibia apophyses (vs. trapezoidal), the copulatory openings located almost centrally (vs. anteriorly), the coiled copulatory ducts (vs. U-shaped), the pear-shaped bursae (vs. oblong).

Description. Male (Fig. 4A). Holotype total length 6.21. Carapace 2.80 long, 1.99 wide; abdomen 3.51 long, 1.70 wide. Carapace yellowish brown. In dorsal

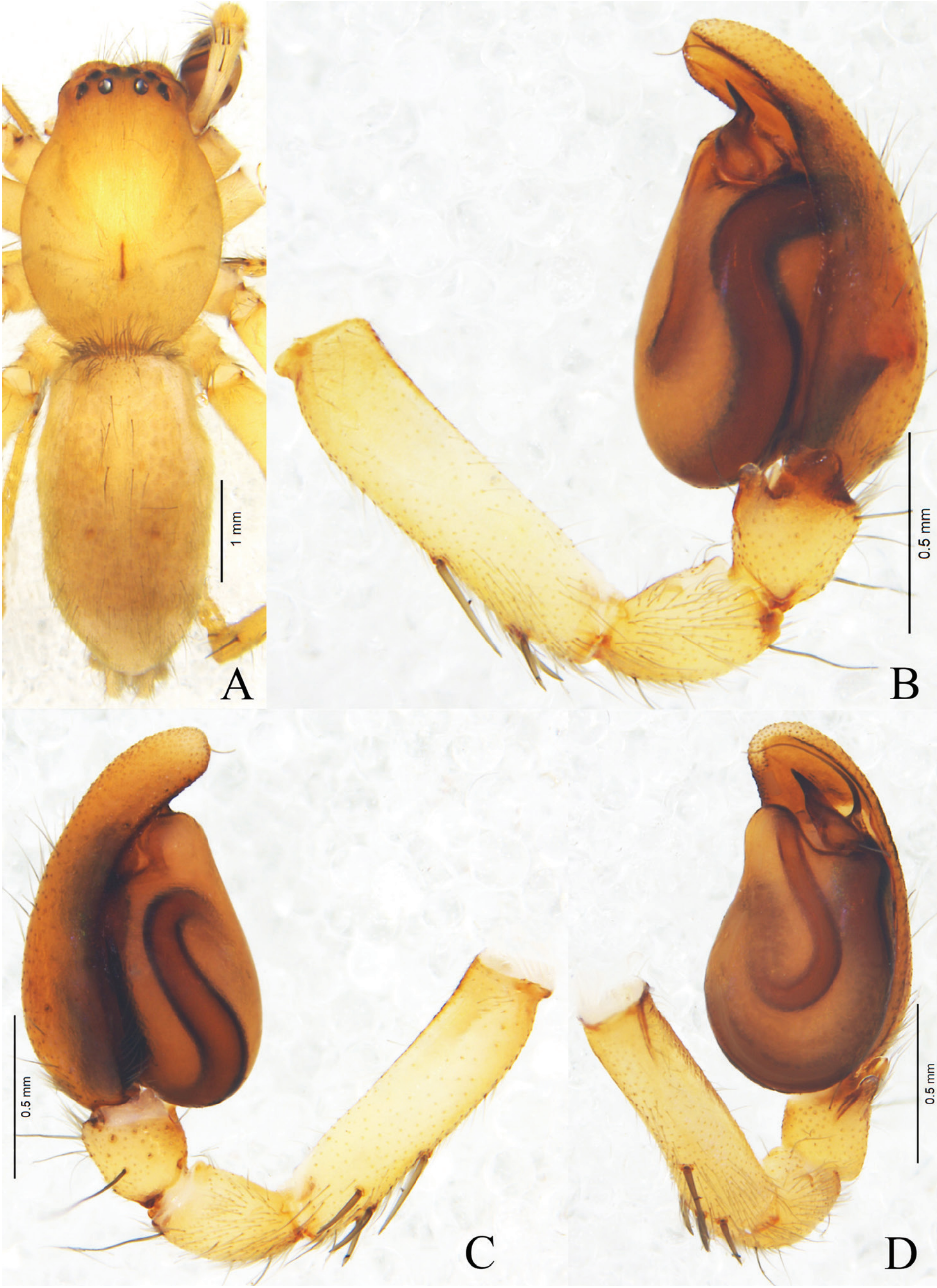


Figure 4. *Clubiona camela* sp. nov. **A** male habitus. Left male palp: **B** retrolateral view **C** prolateral view **D** ventral view. Scale bars: 1 mm (**A**); 0.5 mm (**B–D**).

view, anterior eye row recurved, posterior eye row almost straight. Eye sizes and interdistances: AME 0.12, ALE 0.15, PME 0.12, PLE 0.14; AME–AME 0.11, AME–ALE 0.06, PME–PME 0.25, PME–PLE 0.17, ALE–PLE 0.08. MOA 0.38

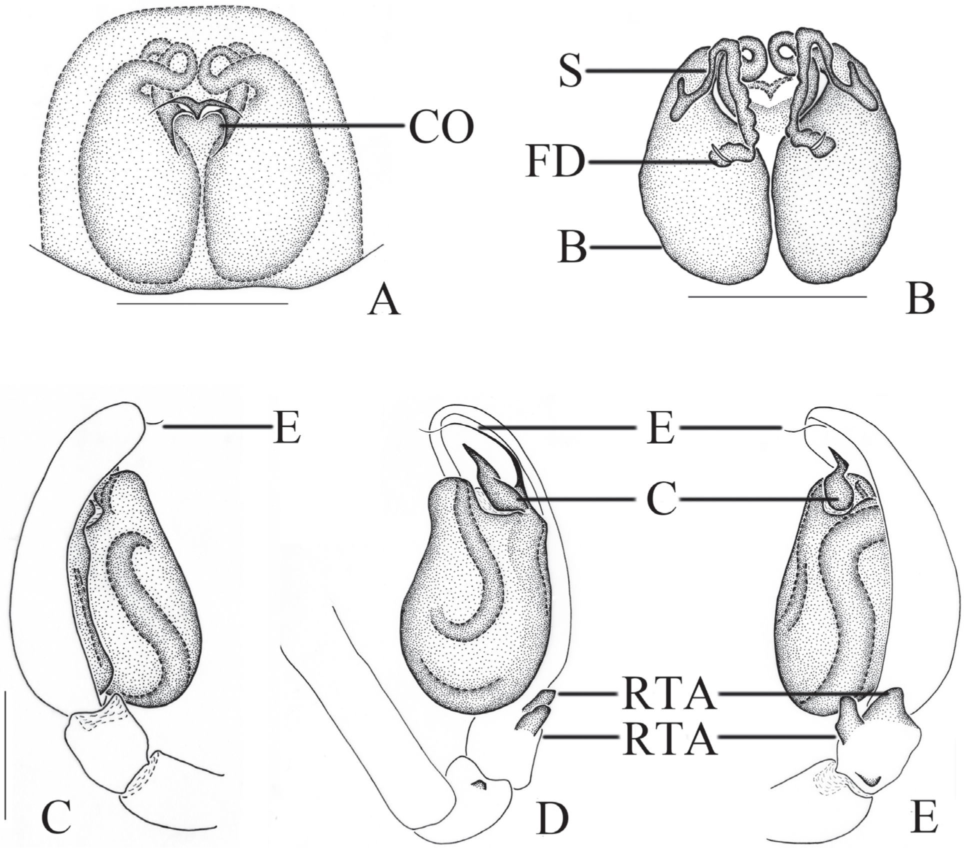


Figure 5. *Clubiona camela* sp. nov. **A** epigyne, ventral view **B** vulva, dorsal view. Left male palp: **C** prolateral view **D** ventral view **E** retrolateral view. Scale bars: 0.5 mm (**A–E**).

long, front width 0.34, back width 0.50. Clypeus height 0.09. Chelicerae yellowish, promargin with 5 teeth, retromargin with 6 teeth. Labium 0.52 long, 0.36 wide. Sternum 1.46 long, 1.03 wide. Endites yellow, longer than wide. Abdomen oval, brownish yellow, with conspicuous anterior tufts of setae; dorsum of abdomen with fine, yellow hairs; cardiac pattern brown. Spinnerets and legs yellow brown. Leg measurements: I 8.58 (2.40, 1.05, 2.43, 1.81, 0.89), II 12.31 (3.48, 1.20, 3.56, 2.83, 1.24), III 8.10 (2.67, 0.89, 1.80, 2.05, 0.69), IV 10.14 (2.92, 0.93, 2.42, 2.98, 0.89). Leg formula: 2-4-1-3.

Palp (Figs 4B–D, 5C–E). Patella length/width ratio 1.7, with a small ventral apophysis. Patella length/tibia length 1.4. Tibia length/width ratio 1.1, with 2 thumb-shaped RTA in ventral view, hump-like in retrolateral view. Cymbium length/width ratio 3.1. Conductor length/width ratio 2.4, with oval base and beak-shaped distal part in retrolateral view, arose from retrolateral tegulum, then extended prolaterally. Embolus slender, rising from retrolatero-distal tegulum, gradually tapering, then extending to prolateral part of cymbium. Sperm duct obvious, S-shaped in prolateral view.

Female (Fig. 3A). Total length 6.12–6.51. One paratype measured: total length 6.51, carapace 2.77 long, 2.06 wide; abdomen 3.78 long, 2.03 wide. Eyes sizes and interdistances: AME 0.11, ALE 0.15, PME 0.12, PLE 0.14; AME–AME 0.12, AME–ALE 0.08, PME–PME 0.28, PME–PLE 0.19, ALE–PLE 0.11. MOA 0.40 long, front width 0.35, back width 0.52. Clypeus height 0.07. Labium 0.48 long, 0.34 wide. Sternum 1.52 long, 1.07 wide. Leg measurements: I 6.57 (1.90, 0.98, 1.73, 1.25, 0.71), II 7.33 (2.10, 1.00, 1.98, 1.50, 0.75), III 6.18 (1.84, 0.83, 1.35, 1.56, 0.60), IV 8.41 (2.32, 0.99, 1.95, 2.35, 0.80). Leg formula: 4-2-1-3. Coloration slightly lighter than in male. Other characters as in male.

Epigyne (Figs 3B, C, 5A, B). Epigynal plate as long as wide, obviously sclerotised. Copulatory openings close to each other, located almost centrally. Copulatory ducts curved, connected with bursae at anterior part of epigyne. Bursae pear-shaped, close to each other, 1.6× longer than wide. Spermathecae long, tubular, located on dorsal-inner surface of bursae. Fertilisation ducts thin and short.

Distribution. Presently known only from Yunnan, China.

Clubiona subhuiming **sp. nov.**

https://zoobank.org/529B6482-C138-4A26-BA5F-61CDB136F2D2

Figs 6, 7

Type material. ***Holotype*****:** China • ♂; Hunan Province, Sangzhi County, Bamaoxi Town, Xiaozhuangping Village (29°47'30''N, 110°05'15''E, 1582 m elev.), 3 November 2018, Yang Chen leg. ***Paratypes*****:** • 1♀, same data as holotype.

Etymology. The specific name is the combination of the prefix *sub-* (near) and *huiming*, referring to its similarity to *C. huiming* Wang, Zhang & Zhang, 2018.

Diagnosis. The male of the new species is similar to *C. huiming* (Wang et al. 2018: figs 8, 9), but differs from the latter by the presence of 2 patellar apophyses (vs. 0), the smaller conductor width/genital bulb width ratio (0.4 vs. 0.7). The female of the new species is similar to *C. subapplanata* Wang, Zhang & Zhang, 2018 (Wang et al. 2018: figs 14, 15), but differs from the latter by having 2 separate copulatory openings (vs. conjoined) and coiled copulatory ducts (vs. s-shaped).

Description. Male (Fig. 6B). Holotype total length 3.31. Carapace 1.59 long, 1.18 wide; abdomen 1.83 long, 1.04 wide. Carapace pale yellow. Median furrow longitudinal, slightly elevated above on the carapace. In dorsal view, both anterior eye row and posterior eye row recurved. Eye sizes and interdistances: AME 0.08, ALE 0.10, PME 0.09, PLE 0.10; AME–AME 0.06, AME–ALE 0.03, PME–PME 0.14, PME–PLE 0.10, ALE–PLE 0.05. MOA 0.22 long, front width 0.20, back width 0.32. Clypeus height 0.05. Chelicerae yellowish, promargin with 5 teeth, retromargin with 4 teeth. Endites yellow, longer than wide. Labium yellow-brown, 0.20 long, 0.20 wide. Sternum 0.93 long, 0.63 wide. Abdomen oval, pale yellow, with conspicuous anterior tufts of setae; abdomen dorsum with fine, yellow hairs; venter yellow. Spinnerets and legs yellowish brown. Leg measurements: I 3.93 (1.19, 0.49, 1.06, 0.77, 0.42), II 4.26 (1.29, 0.50, 1.20, 0.81, 0.46), III 3.66 (1.07, 0.34, 0.96, 0.93, 0.36), IV 5.14 (1.47, 0.54, 1.20, 1.48, 0.45). Leg formula: 4-2-1-3.

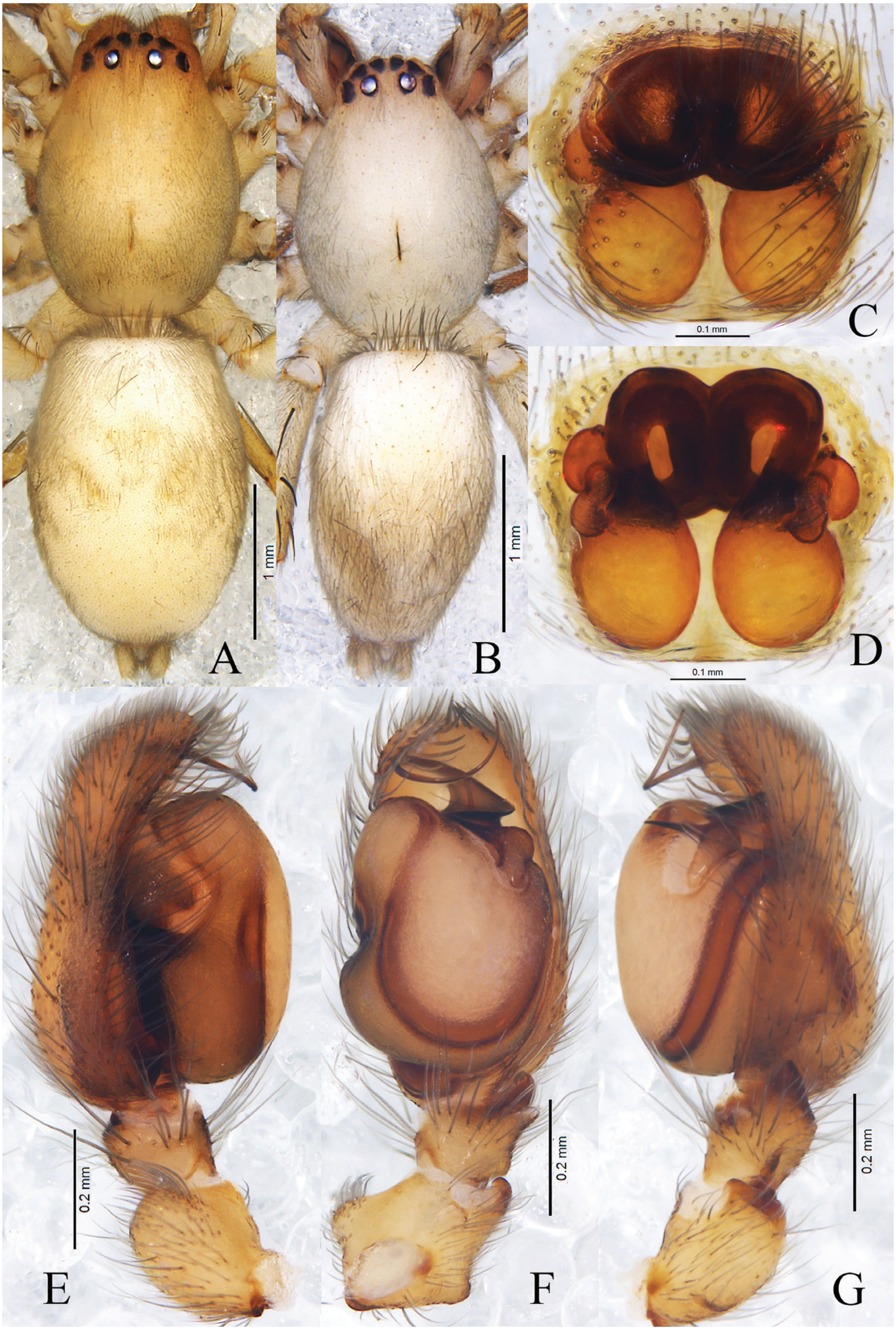


Figure 6. *Clubiona subhuiming* sp. nov. **A** female habitus **B** male habitus **C** epigyne, ventral view **D** vulva, dorsal view. Left male palp: **E** prolateral view **F** ventral view **G** retrolateral view. Scale bars: 1 mm (**A, B**); 0.1 mm (**C, D**); 0.2 mm (**E–G**).

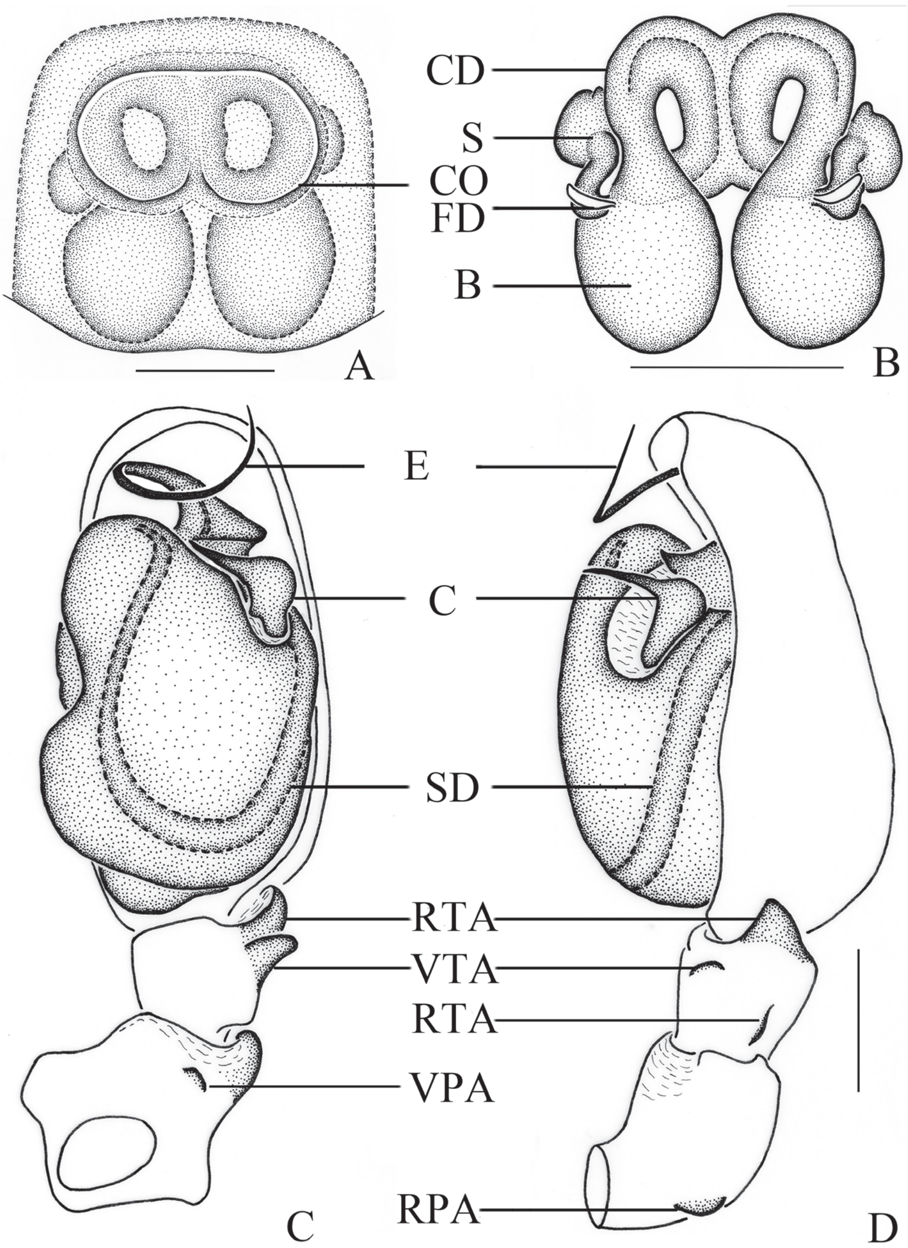


Figure 7. *Clubiona subhuiming* sp. nov. **A** epigyne, ventral view **B** vulva, dorsal view. Left male palp: **C** ventral view **D** retrolateral view. Scale bars: 0.25 mm (**A–D**).

Palp (Figs 6E–G, 7C, D). Patella length/width ratio 1.1, with 2 small, wide apophyses; RPA almost as long as VPA. Patella length/tibia length ratio 2.1. Tibia length/width ratio 1.4, with three apophyses, distal RTA almost conical in retrolateral view; basic RTA short, wide, and located retrolaterally; VTA thumb-shaped in ventral view, almost as long as distal RTA. Cymbium length/tibia length ratio 3.0. Cymbium length/width ratio 1.8. Genital bulb inflated, with a depression at about the 9 o'clock position. Embolus long and coiled, originated at about the 12 o'clock position, then circled clockwise, and terminated at about the 1 o'clock position. Conductor V-shaped in retrolateral view, wide in mid-part, tapering toward its apex. Sperm duct obvious, U-shaped in ventral view.

Female (Fig. 6A). Total length 4.04. Carapace 1.85 long, 1.33 wide; abdomen 2.16 long, 1.41 wide. Eyes sizes and interdistances: AME 0.06, ALE 0.11, PME 0.09, PLE 0.10; AME–AME 0.10, AME–ALE 0.06, PME–PME 0.20, PME–PLE 0.13, ALE–PLE 0.10. MOA 0.24 long, front width 0.24, back width 0.39. Clypeus height 0.05. Labium 0.29 long, 0.27 wide. Sternum 1.07 long, 0.72 wide. Leg measurements: I 3.71 (1.11, 0.54, 0.97, 0.66, 0.43), II 4.01 (1.18, 0.59, 1.06, 0.71, 0.47), III 3.33 (0.87, 0.47, 0.79, 0.86, 0.34), IV 5.20 (1.47, 0.61, 1.19, 1.36, 0.57). Leg formula: 4-2-1-3. Coloration darker than in male. Other characters as in male.

Epigyne (Figs 6C, D, 7A, B). Epigynal plate as long as wide, with strong sclerotised anterior part. Copulatory openings separated from each other, and meso-laterally located. Copulatory duct coiled, almost as long as bursa perimeter. Spermathecae duct-shaped, located on ventral-lateral surface of bursae. Bursae almost spherical, situated posteriorly. Fertilisation ducts lance-shaped, membranous, located on dorsal-anterior surface of bursae.

Distribution. Presently known only from Hunan, China.

Discussion

To date, nearly one-third of *Clubiona* species in China belong to *Clubiona corticalis* species group, and more than half of them have been reported for the first time in the last five years. It is likely that this species group is not monotypic, although we follow previous studies (Mikhailov 1995; Deeleman-Reinhold 2001; Zhang et al. 2021) and temporarily place these three new species in the *Clubiona corticalis* species group. More studies, therefore, are needed to further investigate the species diversity and morphological characteristics of this group of species.

Acknowledgements

We are grateful to Yuri M. Marusik (Institute for Biological Problems of the North RAS, Magadan, Russia) and Kirill G. Mikhailov (Zoological Museum MGU, Moscow, Russia) for providing constructive comments on the manuscript. We thank Guiqiang Huang (Liupanshui Normal University, Guizhou, China) and Yannan Mu (Hebei University, Baoding, China) for collecting specimens. We are grateful to the National Natural Science Foundation of China (32170468, 42101102), the Natural Science Foundation of Inner Mongolia Autonomous Region (2021BS03031), and Yinshanbeilu Grassland Eco-hydrology National Observation and Research Station, China Institute of Water Resources and Hydropower Research, Beijing 100038, China, Grant NO.YSS2022016 for the financial support.

References

Deeleman-Reinhold CL (2001) Forest Spiders of South East Asia: with a Revision of the Sac and Ground Spiders (Araneae: Clubionidae, Corinnidae, Liocranidae, Gnaphosidae, Prodidomidae and Trochanteriidae). Brill, Leiden, 591 pp. https://doi.org/10.1163/9789004475588

Dondale CD, Redner JH (1982) The insects and arachnids of Canada, Part 9. The sac spiders of Canada and Alaska, Araneae: Clubionidae and Anyphaenidae. Research Branch Agriculture Canada Publication 1724: 1–194.

Edwards RJ (1958) The spider subfamily Clubioninae of the United States, Canada and Alaska (Araneae: Clubionidae). Bulletin of the Museum of Comparative Zoology. Harvard 118(6): 365–436.

Liu P, Peng XJ, Yan HM (2016) Five new species of the *Clubiona corticalis* species group (Araneae, Clubionidae) from China. Zootaxa 4184(3): 561–575. https://doi.org/10.11646/zootaxa.4184.3.10

Lohmander H (1944) Vorläufige Spinnennotizen. Arkiv för Zoologi 35(A, 16): 1–21.

Marusik YM, Omelko MM (2018) A survey of the *Porrhoclubiona* Lohmander, 1944 from Central Asia (Araneae, Clubiondae). ZooKeys 802: 19–38. https://doi.org/10.3897/zookeys.802.30236

Mikhailov KG (1995) Erection of infrageneric groupings within the spider genus *Clubiona* Latreille, 1804 (Aranei Clubionidae): A typological approach. Arthropoda Selecta 4(2): 33–48.

Mikhailov KG (2012) Reassessment of the spider genus *Clubiona* (Aranei, Clubionidae). Vestnik Zoologii 46(2): 177–180. https://doi.org/10.2478/v10058-012-0015-z

Simon E (1932) Les Arachnides de France. Tome VI. Synopsis générale et catalogue des espèces françaises de l'ordre des Araneae; 4e partie. Paris, 6: 773–978.

Wang M, Wu PL, Zhang F (2015) Descriptions of two new species of the *Clubiona corticalis*-group (Araneae: Clubionidae) from China. Acta Arachnologica 64(2): 83–89. https://doi.org/10.2476/asjaa.64.83

Wang LY, Chen HM, Wu PL, Zhang F, Zhang ZS (2018) Spider diversity in Fanjing Mountain Nature Reserve, Guizhou, China, II: Clubionidae (Araneae). Zoological Systematics 43(4): 317–333. https://www.zootax.com.cn/EN/Y2018/V43/I3/317

Wiehle H (1965) Die *Clubiona*-Arten Deutschlands, ihre natürliche Gruppierung und die Einheitlichkeit im Bau ihrer Vulva (Arach., Araneae). Senckenbergiana Biologica 46: 471–505.

WSC (2023) World Spider Catalog. Natural History Museum Bern. Version 19.0. http://wsc.nmbe.ch [Accessed 2 January 2023]

Wunderlich J (2011) On extant and fossil (Eocene) Holarctic sac spiders (Araneae: Clubionidae), with descriptions of new taxa. Beiträge zur Araneologie 6: 121–157.

Zhang JS, Yu H, Li SQ (2021) Taxonomic studies on the sac spider genus *Clubiona* (Araneae, Clubionidae) from Xishuangbanna Rainforest, China. ZooKeys 1034: 1–163. https://doi.org/10.3897/zookeys.1034.59413

Zhong Y, Gong XS, Yu H (2022) A new species of the *Clubiona corticalis*-group (Araneae, Clubionidae) from Jiugong Mountains, Hubei Province, central China. Biodiversity Data Journal 10: e94735. https://doi.org/10.3897/BDJ.10.e94735

ZooKeys 1157: 177–191 (2023)
doi: 10.3897/zookeys.1157.97921
https://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

The mitochondrial genome of the bioluminescent fish *Malacosteus niger* Ayres, 1848 (Stomiidae, Actinopterygii) is large and complex, and contains an inverted-repeat structure

Romain Gastineau[1], Christian Otis[2], Brian Boyle[2], Claude Lemieux[3], Monique Turmel[3], Jérôme St-Cyr[2], Marcel Koken[4]

1 *Institute of Marine and Environmental Sciences, University of Szczecin, Szczecin, Poland* **2** *Plateforme d'Analyse génomique, Institut de Biologie Intégrative et des Systèmes, Université Laval, Québec City, Québec, Canada* **3** *Département de biochimie, de microbiologie et de bio-Informatique, Institut de Biologie Intégrative et des Systèmes, Université Laval, Québec, Québec City, Canada* **4** *LABOCEA R&D – Centre national de la recherche scientifique'CNRS, 120 Avenue Alexis de Rochon, 29280, Plouzané, France*

Corresponding authors: Romain Gastineau (romain.gastineau@usz.edu.pl), Marcel Koken (marcel.koken@labocea.fr)

Academic editor: N. Bogutskaya | Received 23 November 2022 | Accepted 17 January 2023 | Published 5 April 2023

https://zoobank.org/0AE93C95-BD55-4310-9E1C-E3C2E6652D0F

Citation: Gastineau R, Otis C, Boyle B, Lemieux C, Turmel M, St-Cyr J, Koken M (2023) The mitochondrial genome of the bioluminescent fish *Malacosteus niger* Ayres, 1848 (Stomiidae, Actinopterygii) is large and complex, and contains an inverted-repeat structure. ZooKeys 1157: 177–191. https://doi.org/10.3897/zookeys.1157.97921

Abstract

We determined the complete mitogenome sequence of the bioluminescent fish *Malacosteus niger* using long-read sequencing technologies. The 21,263 bp mitogenome features a complex structure with two copies of a 1198-bp inverted-repeat and a region of 2616-bp containing alternating copies of 16 and 26 bp repeat elements. Whole mitogenome phylogenies inferred from both nucleotide and amino-acid datasets place *M. niger* among Melanostomiinae. The need for additional complete mitogenome sequences from the subfamily Malacosteinae is discussed.

Keywords

Bioluminescence, Deep Sea Dragonfish, inverted-repeat, long-read sequencing, Malacosteinae, mitogenome, Stomiidae

Introduction

Sunlight is filtered by seawater and only blue/green light with wavelengths between 460 and 490 nm penetrates into the deep sea. Many deep-sea animals with well-adapted eyes can see this very weak light down to about 1500 m (Warrant 2004; de Busserolles et al. 2020). Most of them detect only these blue-green colors, and often produce themselves similar colors by bioluminescence for three main functions: attack, defense/camouflage, and communication.

The peculiar mesopelagic black loose-jaw dragonfish, *Malacosteus niger* Ayres 1848 (Figs 1, 2), represents one of the very few exceptions to this “blue-light rule” (Ayres 1848, 1849; Crossman 1960). This animal lives between 600 and 1000 m and possesses two light-emitting eye glands (Figs 1, 2) (Kenaley 2007, 2008). The suborbital deep-red-emitting photophore is thought to function in intra-specific communication and as a “private torchlight” to detect prey items that are unable to see red light (Mensinger and Case 1988). A bioluminescence reaction in this red eye gland produces blue light that is never emitted, because its energy is transferred to red light-emitting fluorescent proteins via a Bioluminescence Resonance Energy Transfer (BRET) reaction (Campbell and Herring 1987; Herring and Cope 2005). The postorbital eye gland emits blue-green light and is thought to serve intra- and inter-specific exchanges.

Like almost all deep-sea fish, *M. niger* encode only blue opsin photoreceptor genes in order to detect the down welling as well as the bioluminescent light omnipresent in the environment. However, *M. niger* adapted its eyes by linking antenna-pigments (a red-absorbing bacterio-chlorophyll c) to its blue opsin proteins (Douglas et al. 1998a), in order to detect its own red-light emission. Chlorophyll use by vertebrates is very rare and *M. niger* shares this capacity thus far only with a salamander (Isayama et al. 2006). How the fish obtains or produces this chlorophyll is also still an enigma, but it is hypothesized to be retrieved through the food-chain by consuming small copepods which have eaten bacterio-chlorophyll c – containing bacteria (Douglas et al. 1998b, 1999, 2000; Schwab and Marshall 2004). Finally, *M. niger* is the only known vertebrate lacking a hyoid membrane (the membrane that closes the space between the lower jaw), a feature which comes in addition to the unique occipito-vertebral gap observed in several Stomiidae (Schnell et al. 2010; Kenaley 2012; Schnell and Johnson 2017 a, b). For a long time, this 15–20 cm long fish was thought to be a top-predator that mainly eats “big fish”. However, stomach content analysis showed that the animal prefers smaller prey, including the 3–5 mm calanoid copepod *Chirundina streetsi* Giesbrecht, 1895 (Sutton 2005; Drazen and Sutton 2017). This raises the question about how this animal retains its meal in a seemingly open mouth.

We initiated this study by analyzing the mitogenome of *M. niger* with short read sequencing technologies. However, all our efforts to assemble the complete *M. niger* mitogenome proved unsuccessful. The mitogenome always came out as two distinct contigs of similar coverage and all attempts to join them were fruitless whatever parameters were used for assembly. It quickly appeared that repeated sequences were at the

Figure 1. *Malacosteus niger* model made by 10TONS (www.10tons.dk). These most peculiar and unique fishes are frequently damaged by fishing gear and this model allows for the first time to visualize a faithful representation. Courtesy of Esben Horn.

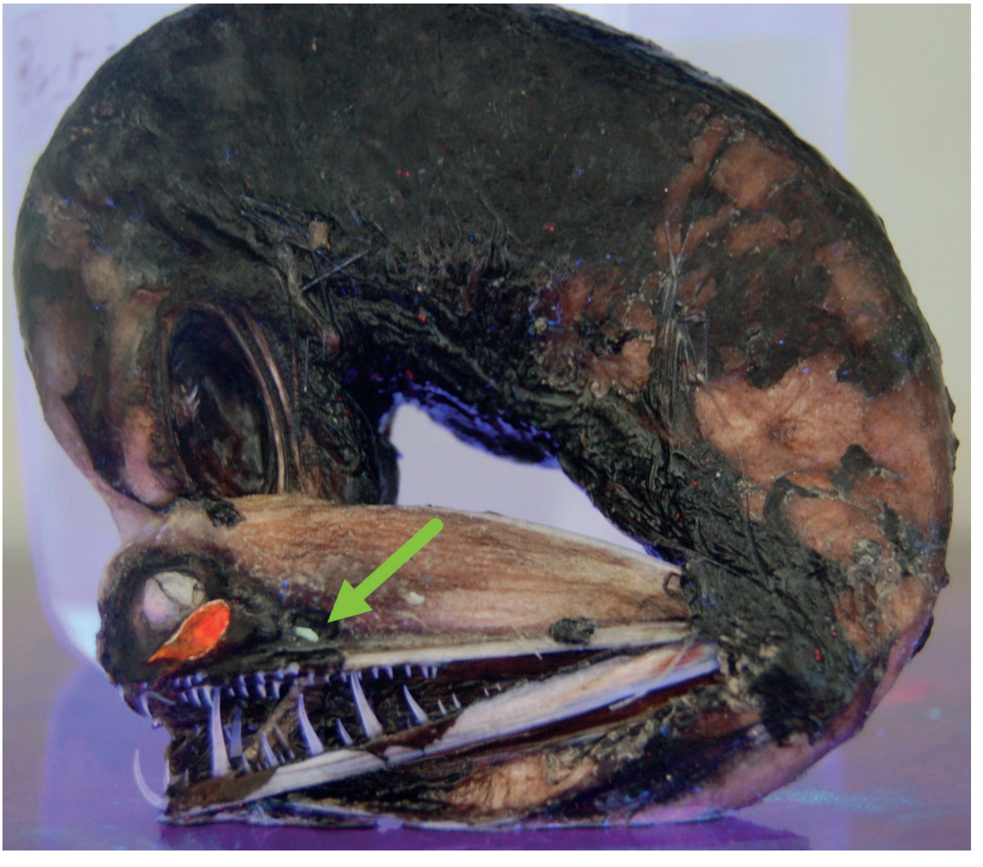


Figure 2. A specimen of *Malacosteus niger* illuminated from below by UV light (360 nm) clearly showing the fluorescence of the suborbital red and the green postorbital eye gland (arrow). The specimen has been damaged during capture.

base of this problem and that long-read sequencing technologies were clearly needed to resolve the complex mitochondrial genome. The results presented here show yet another peculiarity of *M. niger* with the discovery of a large and complex mitochondrial genome harboring inverted-repeat-like structures.

Materials and methods

Biological material and DNA extraction

The *Malacosteus niger* specimen used in this study was caught during the Bear Seamount cruise DE200611 (station 012, 18/6/2006). Pieces of the caudal fin and muscle were sent to the "Plateforme d'Analyses Génomiques" of the "Institut de Biologie Intégrative et des Systèmes" of the Université Laval (Québec, Canada) for DNA library preparation and sequencing. For DNA extraction, 300 mg of muscle were crushed in liquid nitrogen and digested at 65 °C for 30 min in 1.0 ml lysis buffer containing 50 mM Tris-HCl pH 8.0, 200 mM NaCl, 20 mM EDTA, 2.0% SDS and 20 mg/ml proteinase K. An equal volume of CTAB buffer containing 50 mM Tris-HCl pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2.0% CTAB, 1.0% PVP 40,000 was added to the lysate and incubation was pursued for an additional 30 min at 65 °C. This mixture was extracted with phenol: chloroform: isoamylalcohol (25:24:1), and following centrifugation, 5 µl of RNase A (100 mg/ml) was added to the aqueous phase and incubated at room temperature for 20 min. This mixture was then extracted twice with an equal volume of chloroform: isoamylalcohol (24:1) and DNA was precipitated with two volumes of EtOH, dried and dissolved in 100 µl of TE buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA).

Short reads sequencing

The library preparation protocol for short reads sequencing was as follows. Genomic DNA (500 ng in 55 ul TE buffer) was mechanically fragmented for 40 s using a Covaris M220 (Covaris, Woburn MA, USA) with default settings. Fragmented DNA was transferred to PCR tubes and library synthesis was performed using a NEB Next Ultra II kit (New England Biolabs) according to the manufacturer's instructions. To barcode the samples, TruSeq HT adapters (Illumina, SanDiego, CA, USA) were used. The library was sequenced on the Illumina MiSeq platform (300-bp paired-end reads). Of the 15 335 342 raw paired-end reads obtained, 11 121 576 remained after elimination of low-quality reads.

Long reads sequencing

For long-read sequencing, DNA was quantified using a Qubit fluorometer (ThermoFisher) and quality checked on a Femto Pulse System with a genomic DNA 165-kb kit (Agilent, Santa Clara, CA, USA). A DNA aliquot of 8 µg was fragmented with a Covaris g-tube (Covaris Woburn, MA, USA) and small fragments were removed using

Short Read Eliminator XS (Circulomics/PacBio, Menlo Park, CA, USA). The library for Oxford Nanopore MinIon sequencing was prepared using 1.69 µg of DNA and the LSK-109 ligation sequencing kit (Oxford Nanopore, Littlemore, UK), following repair and end-polishing of the sheared DNA using the NEBNext Companion Module for Oxford Nanopore Technologies ligation kit (New England Biolabs, Ipswich, MA, USA). Finally, 0.595 µg of library were loaded on a R9.4.1 MinION flow cell and sequencing was performed on a GridIon benchtop platform (Oxford Nanopore).

Assembly and annotation

All bioinformatics analyses were performed on the THOT superdome flex server at "Université Laval". MiSeq reads were first processed with AfterQC (Chen et al. 2017) to remove adapters and low-quality reads. They were assembled using SPAdes v.3.15.5 (Bankevich et al. 2012), with a k-mer parameter of 125. Data mining in the pool of contigs was done with blastn command line (Camacho et al. 2009). Boundaries of the contigs were extended using the addSolexaReads.pl script of Consed (Gordon and Green 2013). Basic statistics of the Nanopore reads were obtained with NanoStat (De Coster et al. 2018). Nanopore reads were first filtered using Filtlong v.0.2.1 (https://github.com/rrwick/Filtlong), with the two contigs obtained from short reads as a reference. The filtered reads were assembled using Canu v.2.2 (Koren et al. 2017) with a genome size parameter of 0.05M. The contig obtained from Canu was polished with Pilon v.1.24 (Walker et al. 2014) using the MiSeq paired-end reads. Polishing with Pilon was stopped after four iterations, the last one leading to a single base correction.

Annotation was performed with the help of MITOS (Bernt et al. 2013) and manually curated. tRNA genes were identified using Arwen v.1.2 (Laslett and Canbäck 2008). The exact boundaries of the inverted-repeat-like structure were found using the LAST aligner (Kiełbasa et al. 2011). The map of the organellar genome was obtained with OGDRAW (Lohse et al. 2013). The sequences corresponding to the inverted-repeat like structure and the simple repeat portion were displayed with WebLogo v.3 (Crooks et al. 2004).

Maximum likelihood phylogeny

Protein-coding genes and the corresponding amino-acid sequences were extracted from the mitochondrial genomes of 15 taxa of Stomiiformes, including *M. niger*. *Xiphias gladius* Linnaeus, 1758 was used as an outgroup. Nucleotide and inferred amino-acid sequences of all conserved mitochondrial genes (*ATP6*, *ATP8*, *cox1*, *cox2*, *cox3*, *cytB*, *ND1*, *ND2*, *ND3*, *ND4*, *ND4L*, *ND5*, *ND6*) were first concatenated for each species/dataset and then aligned using MAFFT 7 (Katoh and Standley 2013) with the "-auto" option. Poorly aligned regions were filtered out with trimAl (Capella-Gutiérrez et al. 2009) using the "-automated1" option, and the trimmed version of each data set was used to determine the best model of nucleotide and amino-acid evolution with ModelTest-NG (Darriba et al. 2020). Maximum likelihood phylogenies were obtained using IQ-TREE v.2.2.0 (Minh et al. 2020), with 10 000 bootstrap replications in both cases.

Data resources

The mitochondrial genome has been submitted to GenBank with accession number OP326280. The raw fasta file, the annotated gbk file and a fastq file containing the longest Oxford Nanopore read supporting the assembly can be found on Zenodo following this link: https://doi.org/10.5281/zenodo.7330521.

Results

Malacosteus niger mitogenome assembly using short sequencing reads

The size and sequence coverage of the mitogenome contigs obtained after SPAdes assembly of short sequencing reads are indicated in Table 1. Two contigs containing protein-coding and ribosomal RNA genes were retrieved by blastn analyzes using mitogenome data from other Stomiiformes as queries, while the two other contigs were found by extending the end sequences of the former contigs using Consed. The 1198 bp contig displayed twice the coverage of other contigs and could be placed in an inverted-repeat position at both ends of the 12 311 bp contig. One copy of the inverted repeat also proved to be linked to the 4467 bp contig. The 750 bp contig shared short sequences with the inverted repeat but this overlap remained ambiguous.

Assembly of the complete *M. niger* mitogenome using long sequencing reads

Oxford Nanopore sequencing was undertaken to confirm and resolve the contig overlaps that were identified using the short read approach. Table 2 shows the statistics of the Nanopore reads obtained before and after Filtlong filtering on the reference 12 311 and 4467 bp contigs. Canu assembly of the filtered reads returned a single 24 086 bp contig with overlapping end sequences. A 21 263 bp contig representing the complete mitogenome remained after trimming this overlap. It is worth noting that a single Nanopore read of 20 972 bp covered nearly the complete genome (Table 2).

Table 1. Sizes, coverage and gene contents of the *M. niger* mitogenome contigs obtained after assembly of short sequencing reads.

Size of the contig (bp)	**12 311**	**4467**	**1198**	**750**
Coverage	162.46X	156.70X	270.75X	121.78X
Gene content	*ND2*, *cox1*, *cox2*, *ATP8*, *ATP6*, *cox3*, *ND3*, *ND4L*, *ND4*, *ND5*, *ND6*, cob, 18 tRNA genes	*ND1*, *rrnS*, *rrnL*, 3 tRNA genes	None	None

Table 2. Basic statistics of the Nanopore reads before and after Filtlong filtering.

	Nanopore reads (before filtering)	Nanopore reads (after filtering)
Mean read length (bp)	3854	4093
Mean read quality	13.2	14.4
Median read length (bp)	1754	3304.0
Median read quality	13.2	14.4
Number of reads	212 934	153
Read length N50 (bp)	8515	6327
Total bases (bp)	820 590 847	626 269
Top 5 longest reads (bp)	78 432, 77 491, 63021, 58 837, 58 554	20 972, 17 597, 14 526, 14 300, 12 821

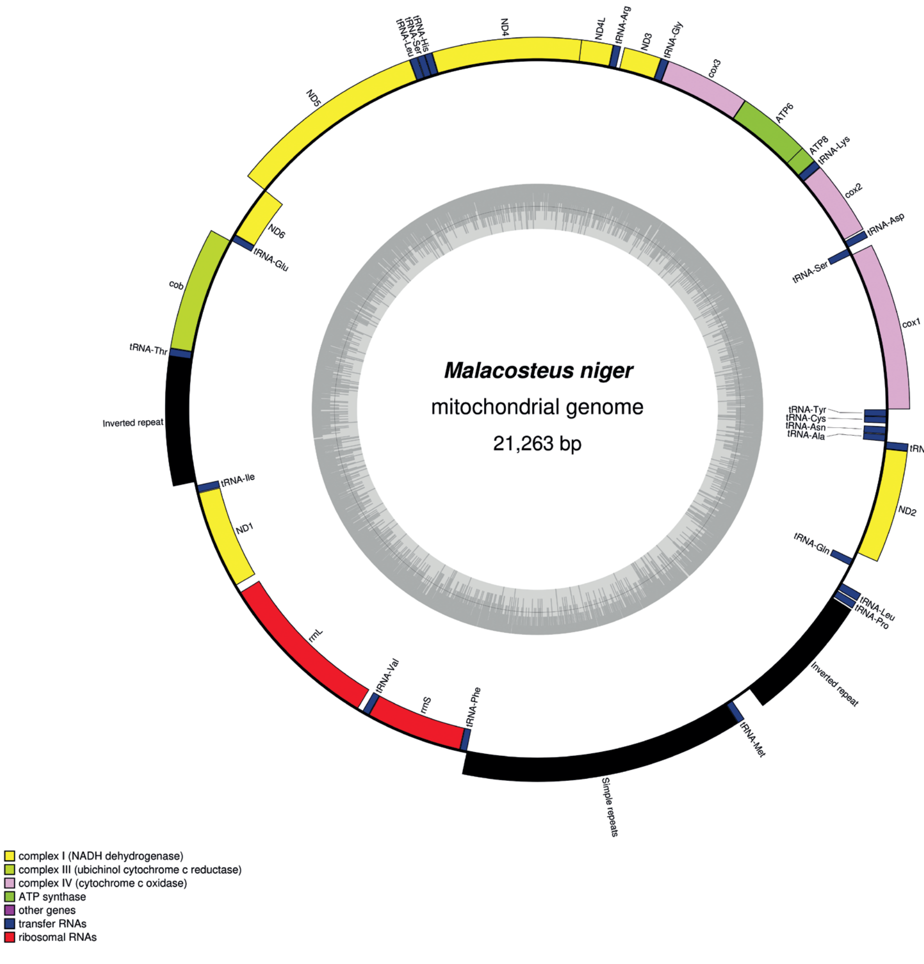


Figure 3. Map of the mitochondrial genome of *Malacosteus niger*. The type of genes are indicated in the legend. The additional black boxes correspond to the two inverted-repeat structures and the long single-repeats fragment.

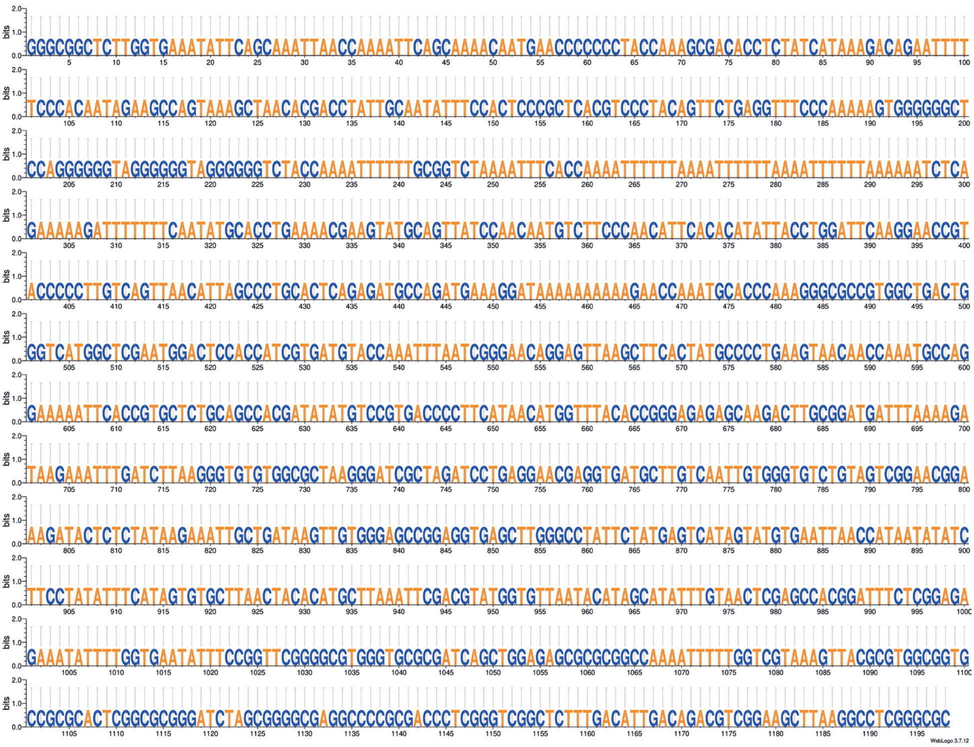


Figure 4. Sequence of the 1198 bp inverted-repeat structure found in the mitochondrial genome of *Malacosteus niger*.

Table 3. Sequences of the repeat elements identified in the region of the *M. niger* mitogenome located between the *tRNA-Met* and *tRNA-Phe* genes.

Sequence of the repeated motif	Number of occurrences
(5’-CATATATCAATATCGACATATGTCAATATTGACATATATCA-3’)	16
(5’-GTCAATACAAACGCATGTGTTTTTAT-3’)	26

Structure and gene content of the *M. niger* mitogenome

The 21 263 bp mitogenome of *M. niger* (GenBank: OP326280) contains 46% G+C and encodes 13 proteins, 22 tRNAs and 2 rRNAs (Fig. 3). It features a 1198 bp inverted-repeat structure with 44% G+C (Fig. 4); one copy of this repeat is located between the genes encoding tRNA-Thr and tRNA-Ile, while the second copy is located between the genes encoding *tRNA-Met* and *tRNA-Pro*. Another region rich in repeated elements is found between the genes encoding *tRNA-Phe* and *tRNA-Met*. This region of 2616 bp contains 36% G+C and features repeat elements of 41 and 26 bp (Table 3): The 41-bp and 26-bp elements are repeated 16 and 26 times, respectively, at positions that alternate between the two elements. There is an overlap between *ATP6* and *ATP8* and also between *ND4L* and *ND4*. Finally, it should be noted that the *cox2*, *ND4* and *cob* genes feature stop codons that are carried by flanking sequences of tRNA genes. Genes are coded on both strands. Our long-read supported assembly proves that *ND1*, *rrnL* and *rrnS* are on the opposite strand in contrast to most other genes.

Phylogenic position of *M. niger* among the Stomiiformes

Phylogenetic analyses of the 11 411 nucleotides and 3795 amino-acid datasets were conducted using the GTR+I+G and mtMAM+I+G+F evolutionary models, respectively. Separation between the Stomiidae and Gonostomatidae was weakly supported in trees inferred from both datasets, but several nodes within the Stomiidae clade proved to be more robust, especially in the amino acid inferred phylogeny. Both the nucleotide and amino-acid trees revealed that the Malacosteinae *M. niger* is sister to the Melanostomiinae *Tactostoma macropus* Bolin, 1939 (LC377784), forming a clade with the Melanostomiinae *Photonectes margarita* (Goode & Bean, 1896) (AP018417) and *Trigonolampa miriceps* Regan & Trewavas, 1930 (AP012961). This group of taxa is sister to a clade containing the Stomiinae *Stomias atriventer* Garman, 1899 (MG321595) and the Chauliodontinae *Chauliodus sloani* Bloch & Schneider, 1801 (AP002915) in the amino-acid phylogeny (Fig. 5), and to the Astronesthinae *Astronesthes lucifer* Gilbert, 1905 (AP012959) in the nucleotide phylogeny (Fig. 6).

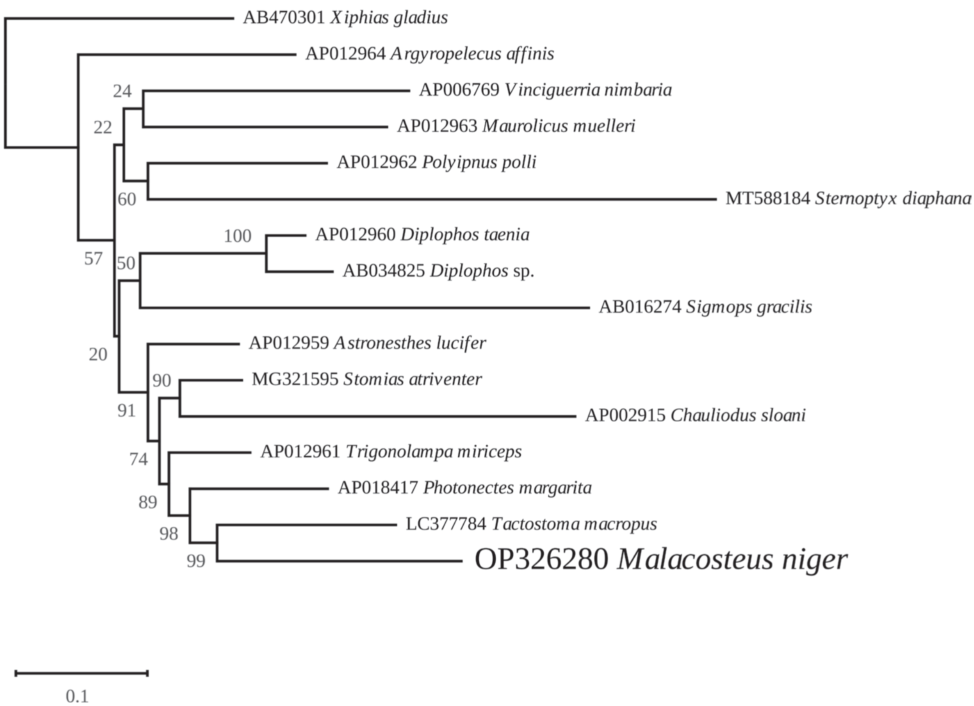


Figure 5. Maximum likelihood phylogenetic tree obtained from concatenated amino-acid sequences of the mitochondrial proteins of *Malacosteus niger* and other Stomiiformes, with a phylogenetically very distant carangiform *Xiphias gladius* as an outgroup.

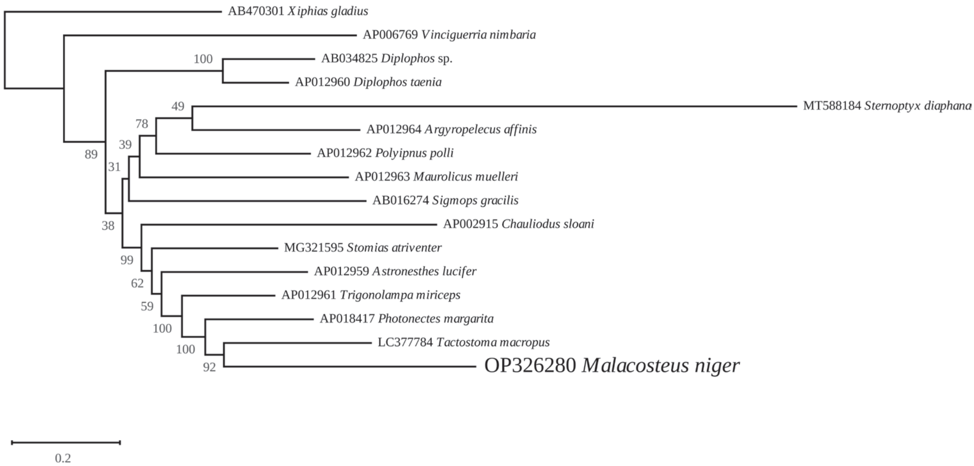


Figure 6. Maximum likelihood phylogenetic tree obtained from concatenated nucleotide sequences of the mitochondrial proteins of *Malacosteus niger* and other Stomiiformes, with a phylogenetically very distant carangiform *Xiphias gladius* as an outgroup.

Discussion

With 21 263 bp, the mitogenome of *M. niger* is among the largest described so far among the Actinopterygii. Recent studies have unveiled very large mitochondrial genomes among metazoan invertebrates, with a current record of 93 065 bp being held by the parasitic cnidarian *Polypodium hydriforme* Ussov, 1885 (MN794187) (Novosolov et al. 2022), closely followed by the 67 195 bp of the zebra mussel *Dreissena polymorpha* (Pallas, 1771) (CM035931) (McCartney et al. 2022). Among Chordata, the largest known mitogenomes belong to the amphibian *Breviceps adspersus* Peters, 1882 (AB777216) and the Actinopterygii *Drepane punctata* (Linnaeus, 1758) (KM273123), which reach 28 757 and 23 152 bp, respectively.

Inverted-repeat structures are a common feature among plastomes (Turmel and Lemieux 2018), with a few exceptions such as the microalgae Pelagophyceae (Ong et al. 2010) and some species of the Dictyochophyceae (Han et al. 2019). However, the presence of such structures in mitogenomes is unusual. To our knowledge, mitogenome inverted repeats have been reported so far only among Stramenopiles and Basidiomycetes (Nieuwenhuis et al. 2019), and a few Chlorophyceae (Robbens et al. 2007; Worden et al. 2009; Pombert et al. 2013; Satjarak et al. 2017; Turmel et al. 2020). Inverted repeats have been identified in some metazoan mitogenomes but their sizes rarely exceed 30 bp (Čechová et al. 2018).

Unveiling unusual features in mitogenomes often faces technical limitations, such as those described in the current study that resulted from the use of short sequencing reads. Discovery of metazoan mitogenomes with anomalous characteristics will certainly become more common with increased use of long-read sequencing. In recent studies, long-read sequencing has been decisive in resolving the complex control regions of mitogenomes from Gastropoda (De Vivo et al. 2022) and trematodes (Kinkar et al. 2021), and to assess the presence of two mitochondrial chromosomes in the isopod *Isocladus armatus* H. Milne Edwards, 1840 (Pearman et al. 2022) and the Tuatara lizard, *Sphenodon punctatus* Gray, 1842 (Macey et al. 2021).

Additional mitogenome sequences from the Malacosteinae are clearly needed to resolve the phylogenetic position of these bioluminescent fishes. *Malacosteus niger* is the only representative of the Malacosteinae that has been sampled so far among the three genera described in this subfamily. It will be particularly important to analyze the mitogenomes from the two remaining genera (*Aristostomias* Zugmayer, 1913 and *Photostomias* Collett, 1889) that harbor a total of 14 valid species. These studies are expected to shed light not only on the phylogenetic positions of these bioluminescent fishes but also on the putative presence, origin and evolution of the inverted-repeat structure among the mitogenomes of Malacosteinae.

Acknowledgements

Dr Tracy Sutton (Oceanic Ecology lab, Nova South-Eastern University, FL, USA) is thanked for determination and provision of several *M. niger* specimens. We thank Stéphane Larose of the bioinformatics platform from Laval Université for his constant and patient help with IT. We would like to thank Esben Horn (https://www.10tons.dk/) for his marvellous model of *Malacosteus niger* and for letting us use a photo of it in this publication.

This work was supported by the 2017–2022 research funds granted for implementation of a co-financed international research project from the Polish Ministry of Science and Higher Education. CL and MT are supported by grant RGPIN-2017-04506 from the Natural Sciences and Engineering Research Council of Canada. BB, JSC and CO are supported by the "Programme d'appui aux plateformes technologiques stratégiques" from the Ministère de l'Économie, de l'Innovation et de l'Énergie Québec.

References

Ayres WO (1848) On a very curious fish. Proceedings of the Boston Society of Natural History 3: 69–70.

Ayres WO (1849) Description of a new genus of fishes, *Malacosteus*. Boston Journal of Natural History 6: 53–64.

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA (2012) SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology 19(5): 455–477. https://doi.org/10.1089/cmb.2012.0021

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

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL (2009) BLAST+: Architecture and applications. BMC Bioinformatics 10(1): e421. https://doi.org/10.1186/1471-2105-10-421

Campbell AK, Herring PJ (1987) A novel red fluorescent protein from the deep sea luminous fish *Malacosteus niger*. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 86B(2): 411–417. https://doi.org/10.1016/0305-0491(87)90314-2

Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T (2009) trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25(15): 1972–1973. https://doi.org/10.1093/bioinformatics/btp348

Čechová J, Lýsek J, Bartas M, Brázda V (2018) Complex analyses of inverted repeats in mitochondrial genomes revealed their importance and variability. Bioinformatics 34(7): 1081–1085. https://doi.org/10.1093/bioinformatics/btx729

Chen S, Huang T, Zhou Y, Han Y, Xu M, Gu J (2017) AfterQC: Automatic filtering, trimming, error removing and quality control for fastq data. BMC Bioinformatics 18: e80. https://doi.org/10.1186/s12859-017-1469-3

Crooks GE, Hon G, Chandonia JM, Brenner SE (2004) WebLogo: A sequence logo generator. Genome Research 14(6): 1188–1190. https://doi.org/10.1101/gr.849004

Crossman EJ (1960) Notes on the original description of *Malacosteus niger* Ayres and subsequent references to it. Copeia 1960(3): 261–262. https://doi.org/10.2307/1439682

Darriba D, Posada D, Kozlov AM, Stamatakis A, Morel B, Flouri T (2020) ModelTest-NG: A New and Scalable Tool for the Selection of DNA and Protein Evolutionary Models. Molecular Biology and Evolution 37(1): 291–294. https://doi.org/10.1093/molbev/msz189

de Busserolles F, Fogg L, Cortesi F, Marshall J (2020) The exceptional diversity of visual adaptations in deep-sea teleost fishes. Seminars in Cell & Developmental Biology 106: 20–30. https://doi.org/10.1016/j.semcdb.2020.05.027

De Coster W, D'Hert S, Schultz DT, Cruts M, Van Broeckhoven C (2018) NanoPack: Visualizing and processing long-read sequencing data. Bioinformatics 34(15): 2666–2669. https://doi.org/10.1093/bioinformatics/bty149

De Vivo M, Lee HH, Huang YS, Dreyer N, Fong CL, de Mattos F, Jain D, Wen YV, Mwihaki JK, Wang TY, Machida RJ, Wang J, Chan B, Tsai IJ (2022) Utilisation of Oxford Nanopore sequencing to generate six complete gastropod mitochondrial genomes as part of a biodiversity curriculum. Scientific Reports 12(1): e9973. https://doi.org/10.1038/s41598-022-14121-0

Douglas RH, Partridge JC, Marshall NJ (1998a) The eyes of deep-sea fish. I: Lens pigmentation, tapeta and visual pigments. Progress in Retinal and Eye Research 17(4): 597–636. https://doi.org/10.1016/S1350-9462(98)00002-0

Douglas RH, Partridge JC, Dulai K, Hunt D, Mullineaux CW, Tauber AY, Hynninen PH (1998b) Dragon fish see using chlorophyll. Nature 393(6684): 423–424. https://doi.org/10.1038/30871

Douglas RH, Partridge JC, Dulai KS, Hunt DM, Mullineaux CW, Hynninen PH (1999) Enhanced retinal longwave sensitivity using a chlorophyll-derived photosensitiser in

Malacosteus niger, a deep-sea dragon fish with far red bioluminescence. Vision Research 39(17): 2817–2832. https://doi.org/10.1016/S0042-6989(98)00332-0

Douglas RH, Mullineaux CW, Partridge JC (2000) Long-wave sensitivity in deep-sea stomiid dragonfish with far-red bioluminescence: Evidence for a dietary origin of the chlorophyll-derived retinal photosensitizer of *Malacosteus niger*. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 355(1401): 1269–1272. https://doi.org/10.1098/rstb.2000.0681

Drazen JC, Sutton T (2017) Dining in the deep: the feeding ecology of deep-sea fishes. Annual review of marine science 9(1): 337–366. https://doi.org/10.1146/annurev-marine-010816-060543

Gordon D, Green P (2013) Consed: A graphical editor for next-generation sequencing. Bioinformatics 29(22): 2936–2937. https://doi.org/10.1093/bioinformatics/btt515

Han KY, Maciszewski K, Graf L, Yang JH, Andersen RA, Karnkowska A, Yoon HS (2019) Dictyochophyceae plastid genomes reveal unusual variability in their organization. Journal of Phycology 55(5): 1166–1180. https://doi.org/10.1111/jpy.12904

Herring PJ, Cope C (2005) Red bioluminescence in fishes: On the suborbital photophores of *Malacosteus*, *Pachystomias* and *Aristostomias*. Marine Biology 148(2): 383–394. https://doi.org/10.1007/s00227-005-0085-3

Isayama T, Alexeev D, Makino CL, Washington I, Nakanishi K, Turro NJ (2006) An accessory chromophore in red vision. Nature 443(7112): e649. https://doi.org/10.1038/443649a

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

Kenaley CP (2007) Revision of the Stoplight Loosejaw genus *Malacosteus* (Teleostei: Stomiidae: Malacosteinae), with description of a new species from the Temperate Southern Hemisphere and Indian Ocean. Copeia 2007(4): 886–900. https://doi.org/10.1643/0045-8511(2007)7[886:ROTSLG]2.0.CO;2

Kenaley CP (2008) Diel vertical migration of the loosejaw dragonfishes (Stomiiformes: Stomiidae: Malacosteinae): a new analysis for rare pelagic taxa. Journal of Fish Biology 73(4): 888–901. https://doi.org/10.1111/j.1095-8649.2008.01983.x

Kenaley CP (2012) Exploring feeding behaviour in deep-sea dragonfishes (Teleostei: Stomiidae): jaw biomechanics and functional significance of a loosejaw. Biological Journal of the Linnean Society, Linnean Society of London 106(1): 224–240. https://doi.org/10.1111/j.1095-8312.2012.01854.x

Kiełbasa SM, Wan R, Sato K, Horton P, Frith MC (2011) Adaptive seeds tame genomic sequence comparison. Genome Research 21(3): 487–493. https://doi.org/10.1101/gr.113985.110

Kinkar L, Gasser RB, Webster BL, Rollinson D, Littlewood D, Chang B, Stroehlein AJ, Korhonen PK, Young ND (2021) Nanopore sequencing resolves elusive long tandem-repeat regions in mitochondrial genomes. International Journal of Molecular Sciences 22(4): e1811. https://doi.org/10.3390/ijms22041811

Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM (2017) Canu: Scalable and accurate long-read assembly via adaptive *k*-mer weighting and repeat separation. Genome Research 27(5): 722–736. https://doi.org/10.1101/gr.215087.116

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

Lohse M, Drechsel O, Kahlau S, Bock R (2013) OrganellarGenomeDRAW – A suite of tools for generating physical maps of plastid and mitochondrial genomes and visualizing expression data sets. Nucleic Acids Research 41(W1): W575–W581. https://doi.org/10.1093/nar/gkt289

Macey JR, Pabinger S, Barbieri CG, Buring ES, Gonzalez VL, Mulcahy DG, DeMeo DP, Urban L, Hime PM, Prost S, Elliott AN, Gemmell NJ (2021) Evidence of two deeply divergent co-existing mitochondrial genomes in the Tuatara reveals an extremely complex genomic organization. Communications Biology 4(1): e116. https://doi.org/10.1038/s42003-020-01639-0

McCartney MA, Auch B, Kono T, Mallez S, Zhang Y, Obille A, Becker A, Abrahante JE, Garbe J, Badalamenti JP, Herman A, Mangelson H, Liachko I, Sullivan S, Sone ED, Koren S, Silverstein K, Beckman KB, Gohl DM (2022) The genome of the zebra mussel, *Dreissena polymorpha*: a resource for comparative genomics, invasion genetics, and biocontrol. G3 Genes|Genomes|Genetics 12(2): jkab423. https://doi.org/10.1093/g3journal/jkab423

Mensinger AF, Case JF (1988) Dual role of the orbital photophores in the stomiatoid fish, *Malacosteus niger*. American Zoologist 28(4): 90A. [Meeting Abstract]

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R (2020) IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. Molecular Biology and Evolution 37(5): 1530–1534. https://doi.org/10.1093/molbev/msaa015

Nieuwenhuis M, van de Peppel L, Bakker FT, Zwaan BJ, Aanen DK (2019) Enrichment of G4DNA and a large inverted repeat coincide in the mitochondrial genomes of *Termitomyces*. Genome Biology and Evolution 11(7): 1857–1869. https://doi.org/10.1093/gbe/evz122

Novosolov M, Yahalomi D, Chang ES, Fiala I, Cartwright P, Huchon D (2022) The phylogenetic position of the enigmatic, *Polypodium hydriforme* (Cnidaria, Polypodiozoa): insights from mitochondrial genomes. GBE Genome Biology and Evolution: evac112. https://doi.org/10.1093/gbe/evac112

Ong HC, Wilhelm SW, Gobler CJ, Bullerjahn G, Jacobs MA, McKay J, Sims EH, Gillett WG, Zhou Y, Haugen E, Rocap G, Cattolico RA (2010) Analyses of the complete chloroplast genome sequence of two members of the pelagophyceae: *Aureococcus anophagefferens* CCMP1984 and *Aureoumbra lagunensis* CCMP1507. Journal of Phycology 46(3): 602–615. https://doi.org/10.1111/j.1529-8817.2010.00841.x

Pearman WS, Wells SJ, Dale J, Silander OK, Freed NE (2022) Long-read sequencing reveals atypical mitochondrial genome structure in a New Zealand marine isopod. Royal Society Open Science 9(1): e211550. https://doi.org/10.1098/rsos.211550

Pombert JF, Otis C, Turmel M, Lemieux C (2013) The mitochondrial genome of the prasinophyte *Prasinoderma coloniale* reveals two trans-spliced group I introns in the large subunit rRNA gene. PLoS ONE 8(12): e84325. https://doi.org/10.1371/journal.pone.0084325

Robbens S, Derelle E, Ferraz C, Wuyts J, Moreau H, Van de Peer Y (2007) The complete chloroplast and mitochondrial DNA sequence of *Ostreococcus tauri*: Organelle genomes of the smallest eukaryote are examples of compaction. Molecular Biology and Evolution 24(4): 956–968. https://doi.org/10.1093/molbev/msm012

Satjarak A, Burns JA, Kim E, Graham LE (2017) Complete mitochondrial genomes of prasinophyte algae *Pyramimonas parkeae* and *Cymbomonas tetramitiformis*. Journal of Phycology 53(3): 601–615. https://doi.org/10.1111/jpy.12521

Schnell N, Johnson GD (2017a) The first functional fish head joint discovered in deep sea Dragonfishes. Fisheries 42(5): 296–296. https://doi.org/10.1080/03632415.2017.1305195

Schnell N, Johnson GD (2017b) Evolution of a functional head joint in deep-sea Fishes (Stomiidae). PLoS ONE 12(2): e0170224. https://doi.org/10.1371/journal.pone.0170224

Schnell NK, Britz R, Johnson GD (2010) New insights into the complex structure and ontogeny of the occipito-vertebral gap in barbeled dragonfishes (Stomiidae, Teleostei). Journal of Morphology 271: 1006–1022. https://doi.org/10.1002/jmor.10858

Schwab IR, Marshall J (2004) Blue light special in a red light district. The British Journal of Ophthalmology 88(3): e318. https://doi.org/10.1136/bjo.2003.041293

Sutton T (2005) Trophic ecology of the deep-sea fish *Malacosteus niger* (Pisces: Stomiidae): An enigmatic feeding ecology to facilitate a unique visual system? Deep-sea Research. Part I, Oceanographic Research Papers 52(11): 2065–2076. https://doi.org/10.1016/j.dsr.2005.06.011

Turmel M, Lemieux C (2018) Evolution of the Plastid Genome in Green Algae. In: Chaw S-M, Jansen RJ (Eds) Advances in Botanical Research. Plastid Genome Evolution, Academic Press, 157–193. https://doi.org/10.1016/bs.abr.2017.11.010

Turmel M, Otis C, de Cambiaire JC, Lemieux C (2020) Complete mitogenomes of the chlorophyte green algae *Scherffelia dubia* and *Tetraselmis* sp. CCMP 881 (Chlorodendrophyceae). Mitochondrial DNA, Part B, Resources 5(1): 138–139. https://doi.org/10.1080/23802359.2019.1698349

Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM (2014) Pilon: An integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS ONE 9(11): e112963. https://doi.org/10.1371/journal.pone.0112963

Warrant E (2004) Vision in the dimmest habitats on earth. Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology 190(10): 765–789. https://doi.org/10.1007/s00359-004-0546-z

Worden AZ, Lee JH, Mock T, Rouzé P, Simmons MP, Aerts AL, Allen AE, Cuvelier ML, Derelle E, Everett MV, Foulon E, Grimwood J, Gundlach H, Henrissat B, Napoli C, McDonald SM, Parker MS, Rombauts S, Salamov A, Von Dassow P, Badger JH, Coutinho PM, Demir E, Dubchak I, Gentemann C, Eikrem W, Gready JE, John U, Lanier W, Lindquist EA, Lucas S, Mayer KF, Moreau H, Not F, Otillar R, Panaud O, Pangilinan J, Paulsen I, Piegu B, Poliakov A, Robbens S, Schmutz J, Toulza E, Wyss T, Zelensky A, Zhou K, Armbrust EV, Bhattacharya D, Goodenough UW, Van de Peer Y, Grigoriev IV (2009) Green evolution and dynamic adaptations revealed by genomes of the marine picoeukaryotes *Micromonas*. Science 324(5924): 268–272. https://doi.org/10.1126/science.1167222

ZooKeys 1157: 193–206 (2023)
doi: 10.3897/zookeys.1157.90792
https://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Description of two new species of *Dicranomyia* (*Erostrata*) crane fly (Diptera, Limoniidae) from Korea, with remarks on DNA barcoding and updated taxonomic key

Jisoo Kim[1], Yeon Jae Bae[1]

1 *Department of Environmental Science and Ecological Engineering, Graduate School, Korea University, Seoul 02841, Republic of Korea*

Corresponding author: Yeon Jae Bae (yjbae@korea.ac.kr)

Academic editor: Pavel Starkevic | Received 29 July 2022 | Accepted 27 March 2023 | Published 11 April 2023

https://zoobank.org/632A33A8-0232-4CF3-A7FA-D9B295EC027B

Citation: Kim J, Bae YJ (2023) Description of two new species of *Dicranomyia* (*Erostrata*) crane fly (Diptera, Limoniidae) from Korea, with remarks on DNA barcoding and updated taxonomic key. ZooKeys 1157: 193–206. https://doi.org/10.3897/zookeys.1157.90792

Abstract

Two new crane fly species, *Dicranomyia* (*Erostrata*) *jejuensis* **sp. nov.** and *D.* (*E.*) *koreana* **sp. nov.**, from Korea are described on the basis of morphology and mitochondrial *COI* sequences. DNA barcode sequences for other four *D.* (*Erostrata*) species from Korea are also provided for the first time. The identification key for all known *D.* (*Erostrata*) species is presented.

Keywords

Dicranomyia (*Erostrata*) *jejuensis*, *Dicranomyia* (*Erostrata*) *koreana*, DNA barcode, Limoniinae, taxonomy

Introduction

Genus *Dicranomyia* Stephens, 1829, is the largest genus of the Limoniidae and, as such, contains 1,136 species and 24 subgenera, including the subgenus *D.* (*Erostrata*) Savchenko, 1976. Twelve species of this subgenus have been reported from the Palearctic, Nearctic, and Oriental regions (Oosterbroek 2023). Adult crane flies are typically found in moist deciduous forests, shrubs along small streams, and abandoned farmlands near streams (Alexander 1931a, b; Podenas et al. 2019, 2020). Meanwhile, larval *D*. (*E*.) *globithorax* has been reported in fungus on decaying logs (Rogers 1930). Data on DNA barcoding for this subgenus is unknown so far.

Alexander (1934) first reported *D*. (*E*.) *tabashii* (as *Limonia* (*Limonia*) *tabashii*) from Suigen (= Suwon) in Korea, and Podenas et al. (2019, 2020) added three and one additional species, respectively, to the recognized fauna of Korea. In Japan, Kato et al. (2018) revised six *Dicranomyia* species of the subgenus *Erostrata* including three new species and suggested that *Limonia congesta* Alexander, 1976 and *Limonia striopleura* Edwards, 1919 be classified as members of subgenus *Erostrata* based on non-genitalic characters.

Here, two new *D*. (*Erostrata*) crane fly species are described from Korea, providing identification for all Korean members of the subgenus. A DNA barcode (*COI*) dataset for six *Dicranomyia* species of the subgenus *Erostrata* from Korea is also presented for the first time.

Materials and methods

Crane fly sampling and examination

Crane fly adults were collected using insect nets or Malaise traps and preserved in 80% ethanol (Table 1). Wings and legs of selected adults were slide mounted using Euparal. Meanwhile, the genitalia and ovipositors of male and female specimens, respectively, were cleared overnight using 10% KOH and then preserved in micro-vials with glycerol. Specimens were examined using a microscope Olympus SZ51 with a digital camera Canon EOS 6D (Tokyo, Japan) and Olympus BX53 with camera Nikon Z7 (Tokyo, Japan).

The terminologies used to describe the morphology generally follow Cumming and Wood (2017), and de Jong (2017) for wing venation. The species distribution is given according to Oosterbroek (2023).

Specimen depositories are as follows: **KUEM** – Korea University Entomological Museum, Seoul, Republic of Korea; **NIBR** – National Institute of Biological Resources, Incheon, Republic of Korea.

DNA extraction and sequence generation

Total genomic DNA was extracted from the leg muscle of using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. *COI* sequences were amplified and sequenced following Suh et al. (2019), except for the use of primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3'; Folmer et al. 1994) and C1-N-2191 (5'-CCC GGT AAA ATT AAA ATA TAA ACT TC-3'; Simon et al. 1994), which targeted a 676-bp region of *COI*. All sequences were submitted to GenBank (accession numbers: OM102975–OM102983; OP081140; OP093621).

Table 1. Collection data of the Korean *Dicranomyia* (*Erostrata*) species used in the barcode analyses of this study.

Species*	GenBank accession number	Specimen code	Locality	Date	Collector(s)	Coordinates
D. (*E.*) *globithorax*	OM102980	CF21-0149	Gangwon-do, Wonju-si	3 Sep. 2021	J. Kim, D. Lee	37°29'50.88"N, 130°53'23.78"E
D. (*E.*) *jejuensis* sp. nov.	OM102981	CF21-0150H	Jeju-do, Seoguipo-si	14 Jun.–4 Aug. 2021	Y. J. Bae	33°19'49.07"N, 126°37'28.08"E
	OM102982	CF21-0150P	Jeju-do, Seoguipo-si	4 Aug.–8 Sep. 2021	Y. J. Bae	33°19'49.07"N, 126°37'28.08"E
	OM102983	CF21-0151	Jeju-do, Seoguipo-si	4 Aug.–8 Sep. 2021	Y. J. Bae	33°19'49.07"N, 126°37'28.08"E
D. (*E.*) *koreana* sp. nov.	OM102979	CF21-0148	Jeju-do, Seoguipo-si	13 Jun.–4 Aug. 2021	Y. J. Bae	33°20'57.10"N, 126°29'43.29"E
	OP081140	CF21-0152	Gyeongsangnam-do, Sancheong-gun	28 Jul. 2021	J. Kim, C. Lim, D. Lee, W. Lee	35°18'37.83"N, 127°45'05.47"E
D. (*E.*) *submelas*	OM102978	CF21-0133	Jeju-do, Seoguipo-si	4 Aug.–8 Sep. 2021	Y. J. Bae	33°19'49.07"N, 126°37'28.08"E
D. (*E.*) *tabashii*	OM102975	CF21-0115	Gyeongsangnam-do, Sancheong-gun	28 Jul. 2021	J. Kim, C. Lim, D. Lee, W. Lee	35°18'37.83"N, 127°45'05.47"E
	OM102976	CF21-0115f	Gyeongsangnam-do, Sancheong-gun	28 Jul. 2021	J. Kim, C. Lim, D. Lee, W. Lee	35°18'37.83"N, 127°45'05.47"E
D. (*E.*) *yazuensis*	OM102977	CF21-0132	Gangwon-do, Pyeongchang-gun	28 Jul.–15 Sep. 2020	Y. J. Bae	37°47'05.67"N, 128°34'16.97"E
D. (*D.*) *kandybinae*	OP093621	CF21-0099	Gangwon-do, Wonju-si	23 Jul.–3 Sep. 2021	Y. J. Bae	37°17'26.50"N, 128°04'54.77"E

*Specimens were morphologically identified by J. Kim.

DNA barcode sequence analysis

DNA barcode analysis was performed using 11 *COI* sequences (Table 1), which were generated from six Korean *D.* (*Erostrata*) species (10 sequences), and the outgroup species *D.* (*Dicranomyia*) *kandybinae* Savchenko, 1987 (1 sequence). Phylogenetic analyses were conducted using the neighbor-joining (NJ) method and Kimura-2-parameter model (Kimura 1980), with 1,000 bootstrap replicates, in MEGA X (Kumar et al. 2018). Sequence divergence was estimated via pairwise comparison of the uncorrected genetic distances (*p*-distances) in MEGA X, using the complete deletion option.

Checklist of the world *Dicranomyia* (*Erostrata*) crane flies (Oosterbroek, 2023)

Dicranomyia (*Erostrata*) *canis* (Alexander, 1931b)
Dicranomyia (*Erostrata*) *cnephosa* (Alexander, 1959)
Dicranomyia (*Erostrata*) *congesta* (Alexander, 1967)
Dicranomyia (*Erostrata*) *cynotis* (Alexander, 1931a)
Dicranomyia (*Erostrata*) *globithorax* Osten Sacken, 1869
Dicranomyia (*Erostrata*) *globulithorax* Alexander, 1924
Dicranomyia (*Erostrata*) *jejuensis* sp. nov.
Dicranomyia (*Erostrata*) *koreana* sp. nov.
Dicranomyia (*Erostrata*) *melas* (Alexander, 1934)

Dicranomyia (*Erostrata*) *reniformis* Kato, Tachi & Gelhaus, 2018
Dicranomyia (*Erostrata*) *striopleura* (Edwards, 1919)
Dicranomyia (*Erostrata*) *submelas* Kato, Tachi & Gelhaus, 2018
Dicranomyia (*Erostrata*) *tabashii* (Alexander, 1934)
Dicranomyia (*Erostrata*) *yazuensis* Kato, Tachi & Gelhaus, 2018

Key to the species of *Dicranomyia* (*Erostrata*), updated from Kato et al. (2018) and Podenas et al. (2020)

1 Tarsi with white bands .... ***Dicranomyia* (*Erostrata*) *congesta* (India)**
– Tarsi without bands **2**
2 Pleuron with broad, blackish brown lateral stripe .... ***Dicranomyia* (*Erostrata*) *striopleura* (Indonesia, Malaysia)**
– Pleuron without lateral stripe **3**
3 Scutellum obscure yellow **4**
– Scutellum yellowish brown to blackish brown **6**
4 Rostrum black. Wing strongly blackened .... ***Dicranomyia* (*Erostrata*) *cnephosa* (Nepal)**
– Rostrum pale. Wing tinged with pale brown **5**
5 Palpus 2-segmented. Male seventh sternite with strongly darkened internal sac .... ***Dicranomyia* (*Erostrata*) *tabashii* (Japan, Korea, Russia)**
– Palpus 3-segmented. Male seventh sternite with slightly darkened internal sac with rounded entrance ... ***Dicranomyia* (*Erostrata*) *koreana* sp.nov. (Korea)**
6 Gonostylus with black spines on mesal face **7**
– Gonostylus without black spines **12**
7 Gonostylus narrowed to a point, triangular **8**
– Gonostylus not as above **9**
8 Mesal face of gonostylus densely covered with black setae .... ***Dicranomyia* (*Erostrata*) *cynotis* (Philippines)**
– Mesal face of gonostylus with black setae restricted to distal 1/2 .... ***Dicranomyia* (*Erostrata*) *canis* (Philippines)**
9 Paramere distally with rounded tip **10**
– Paramere distally with pointed tip **11**
10 Gonostylus elongate with truncated apex .... ***Dicranomyia* (*Erostrata*) *globithorax* (Canada, Japan, Korea, USA)**
– Gonostylus elongate with angled apex, length of angled apex ca 1/5 of gonostylus .... ***Dicranomyia* (*Erostrata*) *globulithorax* (Japan, Korea, Russia)**
11 Gonocoxite with apically rounded ventromesal lobe. Gonostylus shallowly concaved at inner apical edge, the area 1/5 as long as gonostylus .... ***Dicranomyia* (*Erostrata*) *melas* (Taiwan)**
– Gonocoxite with apically truncated ventromesal lobe. Gonostylus deeply emarginated at inner apical edge, the area 1/3 as long as gonostylus .... ***Dicranomyia* (*Erostrata*) *submelas* (Japan, Korea)**

12 Gonostylus stout and reniform, tip of ventral surface covered with dense, fine setae .... ***Dicranomyia* (*Erostrata*) *reniformis* (Japan)**
– Gonostylus not reniform, tip of ventral surface without dense, fine setae.... **13**
13 Palpus 2-segmented. Gonostylus tapered in distal 1/3, without apical spine.. .... ***Dicranomyia* (*Erostrata*) *yazuensis* (Japan, Korea)**
– Palpus 3-segmented. Gonostylus tapered in distal 1/2, with black apical spine .... ***Dicranomyia* (*Erostrata*) *jejuensis* sp. nov. (Korea)**

Taxonomic accounts

Family Limoniidae Speiser, 1909
Subfamily Limoniinae Speiser, 1909
Genus *Dicranomyia* Stephens, 1829

Subgenus *Erostrata* Savchenko, 1976

Dicranomyia (*Erostrata*) Savchenko in Savchenko and Krivolutskaya 1976: 131–132; Kato et al. 2018: 182; Podenas et al. 2019: 72–73.

Type species. *Dicranomyia globithorax* Osten Sacken, 1869 (original designation).

Diagnosis. Rostrum is very short or reduced. Number of palpomeres ranges from one to three. Wings have no patterns, even in stigmal region. Third and fourth tarsomere are slightly swollen. Internal sac or notch is located on the male seventh sternite. Gonocoxite has ventromesal lobe. Gonostylus is one paired, with one or two setae arising from small tubercle on outer surface.

***Dicranomyia* (*Erostrata*) *jejuensis* sp. nov.**
https://zoobank.org/9659D5F8-C578-4FAA-B16A-6AF8A9AE39E5
Figs 1, 2

Type material. ***Holotype*:** Korea • ♂; Jeju-do, Seogwipo-si, Namwon-eup, Sillye-ri, Iseungi-oreum Volcanic Cone; 33°20.24'N, 126°37.25'E; alt. 450 m; 4 Aug.–8 Sep. 2021; Y. J. Bae leg.; Malaise trap; GenBank: OM102981; CF21-0150H; NIBR.

***Paratypes*:** Korea • 1 ♀; same data as holotype, 14 Jul.–4 Aug. 2021; GenBank: OM102983; CF21-0151; KUEM • 1 ♂; same data as holotype; GenBank: OM102982; CF21-0150P; KUEM.

Diagnosis. Palpus is 3-segmented. Male seventh sternite has shallow V-shaped notch. Outer face of gonostylus has single seta arising from tubercle. Distal lobe of paramere has a hooked tip with a subapical process.

Description. Male (holotype). Body length 4.3 mm, wing length 4.6 mm, antenna length 0.9 mm. General body coloration pale yellow to yellowish brown (Fig. 1A).

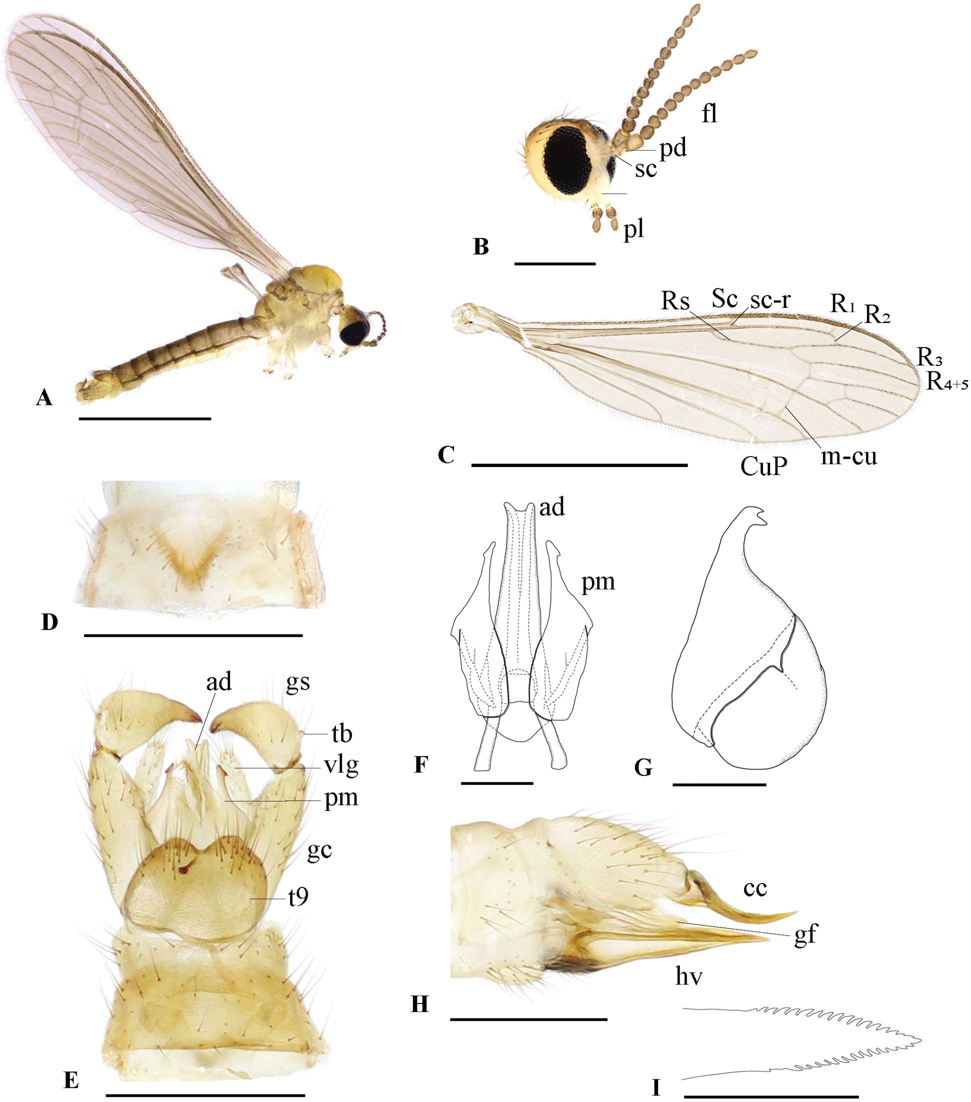


Figure 1. *Dicranomyia* (*Erostrata*) *jejuensis* sp. nov. **A** habitus, male (paratype) **B** head, female (paratype) **C** wing, male (paratype) **D** male seventh sternite, ventral view **E** male terminalia, dorsal view **F** aedeagal complex, dorsal view **G** paramere, lateral view **H** female terminalia, lateral view **I** tip of hypovalva, lateral view. Abbreviations: ad – aedeagus; cc – cercus; fl – flagellum; gc – gonocoxite; gf – genital fork; gs – gonostylus; hv – hypovalva; pd – pedicel; pl – palpus; pm – paramere; sc – scape; tb – tubercle; t9 – ninth tergite; vlg – ventromesal lobe of gonocoxite. Scale bars: 2 mm (**A, C**); 0.5 mm (**B, D, E, H**); 0.1 mm (**F, G, I**).

Head (Fig. 1B). Dark brown dorsally, pale ventrally. Antennae 14-segmented; scape pale yellow; pedicel yellowish brown; flagellum brown. Rostrum pale, rudimentary. Palpus 3-segmented; basal 2/3 of first palpomere pale; remainder of palpus brown.

Figure 2. Habitat of *Dicranomyia* (*Erostrata*) *jejuensis* sp. nov.

Thorax. Prescutum and presutural scutum yellow. Postsutural scutum, scutellum and mediotergite yellowish brown. Pleuron uniformly dull yellow, without lateral stripes. Wing (Fig. 1C) tinged with pale brown; veins brown; Sc ending before middle of Rs; sc-r at tip of Sc; Rs arched at base; R_1 and R_2 nearly transverse, at the same level; R_3 and R_{4+5} parallel to each other; discal medial cell closed; m-cu before fork of M; CuP ending beyond tip of Sc. Halter pale brown. Legs with coxae and trochanters pale; base of femora pale, remainder of femora brown; tibiae and tarsi brown. Femur II 3.3 mm; femur III 3.8 mm; tibia II 3.5 mm; tibia III 4.0 mm; tarsus II 3.2 mm; tarsus III 2.8 mm. Claw without additional tooth.

Abdomen. Tergites yellowish brown, sternites 1–4 yellow, remaining yellowish brown. Seventh sternite with shallow V-shaped notch with darkened margin (Fig. 1D).

Male terminalia (Fig. 1E–G). Yellow. Ninth tergite with posterior margin rounded (Fig. 1E), medially with distinct emargination, distal part covered with setae. Gonocoxite elongated, approximately 3× as long as width at base, with elongated, setose ventromesal lobe. Gonostylus yellowish brown at base, turning dark distally; distal 1/2 of gonostylus gradually tapered toward apex, with short black spine at tip; dorsal margin near the base with small tubercle bearing pale, stout seta. Paramere (Fig. 1F, G) with basal 1/2 pale and apical 1/2 yellowish, distal lobe darkened apically with hooked tip and subapical projection. Aedeagus (Fig. 1F) as long as gonocoxite, bifid, curved outwards at tip.

Female. Body length 4.5 mm, wing length 4.8 mm, antenna length 0.9 mm (N = 1). General body coloration brighter than male. Femur I 2.8 mm; II 3.2 mm; III 3.4 mm; tibia I 3.2 mm; I: 3.1 mm; III 3.4 mm; tarsus I 3.0 mm; II 2.6 mm; III 2.4 mm.

Female terminalia (Fig. 1H–I). Yellow. Cercus curved upwardly (Fig. 1H), wider at base, narrowing towards acute tip. Genital fork long, ca 1.5× as long as width, extending to base of cercus. Hypovalva wedge-shaped, reaching to ca 2/3 of cercus, with distinct black spot at base. Dorsal and ventral margin of hypovalva serrated near tip (Fig. 1I).

Etymology. Specific name "*jejuensis*" refers to the type locality, Jejudo Island, Korea.

Distribution. The species is currently only known from Jejudo Island, Korea.

Habitats. Adults of this species are found in deciduous forests with moss-covered rocks along intermittent, rocky mountain streams (Fig. 2) and co-occur with *D.* (*E.*) *submelas*.

Period of activity. Adults were collected from June through early September.

Remarks. *Dicranomyia* (*E.*) *jejuensis* sp. nov. is morphologically similar to *D.* (*E.*) *yazuensis* based on the male genital structures, but it can be distinguished by the following characters: pleuron entirely dull yellow (vs dark dorsally); palpus 3-segmented (vs 2-segmented); distal 1/2 of gonostylus tapered to tip (vs distal 2/3 strongly narrowed toward tip); posterior margin of male seventh sternite with shallow V-shaped notch (vs long triangular notch); distal part of paramere with hooked tip (vs straight tip).

Dicranomyia (*Erostrata*) *koreana* sp. nov.

https://zoobank.org/163BF75E-826C-4E64-AAE4-CFAA23F2E22D

Figs 3, 4

Type material. ***Holotype*:** Korea • ♂; Jeju-do, Seogwipo-si, Hawon-dong, Mt. Hallasan; 33°20.95'N, 126°29.72'E; alt. 1220 m; 13 Jun.–4 Aug. 2021; Y. J. Bae leg.; Malaise trap; GenBank: OM102979; CF21-0148; NIBR.

***Paratypes*:** Korea • 1 ♂; Gyeonggi-do, Gapyeong-si, Buk-myeon, Jeokmok-ri, Garim-gyo (Br.); 37°58.60'N, 127°26.55'E; alt. 300 m; 25 Jul.–1 Aug. 2015; Y. J. Bae leg.; Malaise trap; published as *D.* (*E.*) *tabashii* by Podenas et al. (2019); KUEM • 2 ♂♂, 1 ♀; same data as for preceding; 2–8 Aug. 2015; published as *D.* (*E.*) *tabashii* by Podenas et al. (2019); KUEM • 1 ♂; same data as for preceding; 23–29 Jul. 2016; published as *D.* (*E.*) *tabashii* by Podenas et al. (2019); KUEM • 1 ♂; Gangwon-do, Inje-gun, Girin-myeon, Bangdong-ri, Mt. Bangtaesan; 37°54.50'N, 128°24.41'E; alt. 690 m; 30 Jul.–16 Sep. 2019; Y. J. Bae leg.; Malaise trap; KUEM • 1 ♂; Gyeonsang-nam-do, Sancheong-gun, Sicheon-myeon, Jungsan-ri, Jungsan-ri Campsite, Mount Jirisan; 35°18.63'N, 127°45.09'E; alt. 700 m; 28 Jul. 2021; J. Kim, C. Lim, D. Lee, W. Lee leg.; sweeping; GenBank: OP081140; CF21-0152; KUEM.

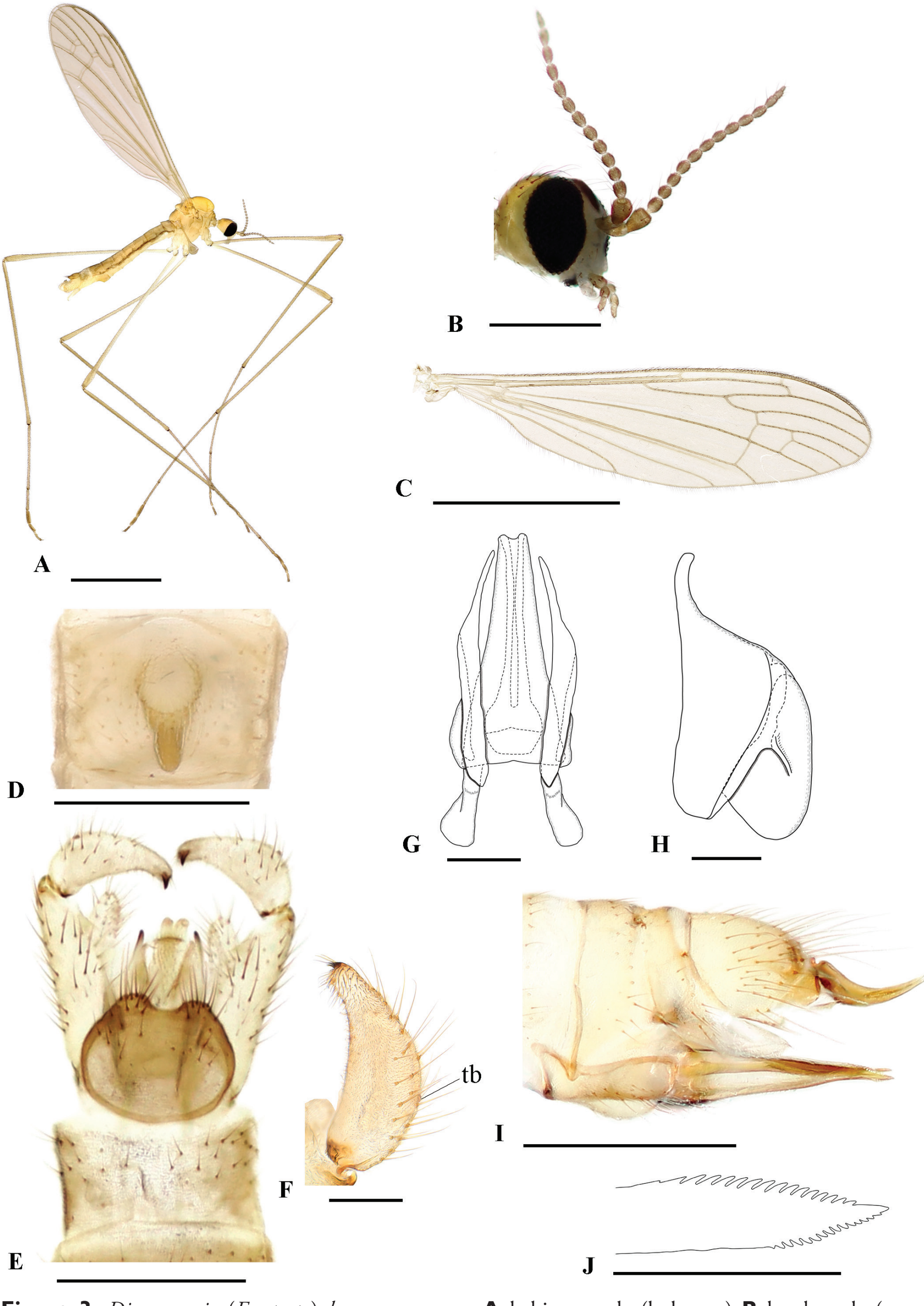


Figure 3. *Dicranomyia* (*Erostrata*) *koreana* sp. nov. **A** habitus, male (holotype) **B** head, male (paratype) **C** wing, male (paratype) **D** male seventh sternite, ventral view **E** male terminalia, dorsal view **F** gonostylus, ventral view **G** aedeagal complex, dorsal view **H** paramere, lateral view **I** female terminalia, lateral view **J** tip of hypovalva, lateral view. Abbreviation: tb – tubercle. Scale bars: 2 mm (**A, C**); 0.5 mm (**B, D, E, I**); 0.1 mm (**F, G, H, J**).

Diagnosis. Palpus is 3-segmented. Center of male seventh sternite has a deep conical internal sac that has a wide, round entrance. Outer face of gonostylus has two setae arising from a small tubercle. Paramere is elongated and narrow, distally with a darkened tip.

Description. Male (holotype). Body length 3.5 mm, wing length 4.5 mm, antenna length 0.7 mm. General body coloration yellow (Fig. 3A).

Head (Fig. 3B). Dark brown dorsally, yellow ventrally. Vertex with a distinct black spot between compound eyes. Antennae 14-segmented; scape pale brown; pedicel yellowish brown; flagellum brown. Rostrum pale, reduced. Palpus 3-segmented, yellowish brown; basal 1/2 of first palpomere pale.

Thorax. Prescutum, scutum and scutellum yellow. Mediotergite yellowish brown. Pleuron entirely pale yellow, without lateral stripes. Wing (Fig. 3C) tinged with pale brown; veins brown; tip of Sc reaching ca 1/3 of Rs; sc-r at tip of Sc; R_1 indistinct; R_2 ending distinctly beyond tip of R_1; discal cell closed; m-cu slightly beyond fork of M. Halter pale brown. Legs with coxae and trochanters pale yellow; femora and tibiae brownish yellow; tarsal segments light brown. Femur I 2.4 mm; femur II 2.8 mm; femur III 3.1 mm; tibia I 2.8 mm; tibia II 2.4 mm; tibia III 2.8 mm; tarsus I 2.9 mm; tarsus II 2.5 mm; tarsus III 2.4 mm. Claw without additional tooth.

Abdomen. Tergites yellow except pale eighth tergite; sternites paler. Seventh sternite (Fig. 3D) with central deep, conical, slightly darkened sac, and rounded entrance.

Male terminalia (Fig. 3E–H). Yellow. Ninth tergite rounded (Fig. 3E), wider basally, narrower apically; posterior margin with two short, setose lateral lobes separated by shallow U-shaped incision. Gonocoxite elongated, ventromesal lobe margin rounded and covered with setae, reaching beyond tip of aedeagus. Gonostylus (Fig. 3E, F) widened at base and narrowed at apex, with short, black dorsal spine at tip; dorsal margin near the base with small protuberance bearing two pale setae. Paramere (Fig. 3G, H) with basal part bilobed, distal lobe elongated and narrow, slightly darkened at tip. Aedeagus (Fig. 3G) apically bent downwards, bifid at tip.

Female. Body length 3.8 mm, wing length 4.7 mm, antenna length 0.7 mm (N = 1). General body coloration lighter than male.

Female terminalia (Fig. 3I–J). Yellow. Cercus curved dorsally (Fig. 3I), gradually tapered to pointed tip. Genital fork broad, as long as width, not extending to base of cercus. Hypovalva elongated, blade-shaped reaching slightly before tip of cercus, with distinct dark spot at basal area. Distal end bearing dorsal and ventral serration (Fig. 3J).

Etymology. Specific name “*koreana*” refers to the country of its discovery, Korea.

Distribution. The species is widely distributed in Korea, including Jejudo Island.

Habitats. This species is found along intermittent mountain streams in moist mixed forests with grassy vegetation (Fig. 4A) and in wet deciduous forest along the rocky margins of small mountain streams (Fig. 4B). Adults share their habitats with *D.* (*E.*) *globulithorax* on Mount Bangtaesan and with *D.* (*E.*) *tabashii* on Mount Jirisan.

Period of activity. Adults are mainly active from July through August.

Figure 4. Habitats of *Dicranomyia* (*Erostrata*) *koreana* sp. nov. **A** Jejudo Island **B** Mt. Jirisan.

Remarks. In terms of the shape of the male terminalia, *D.* (*E.*) *koreana* sp. nov. is similar to *D.* (*E.*) *tabashii*, but it can be distinguished by the following characters: palpus 3-segmented (vs 2-segmented); male seventh sternite with weakly darkened, conical internal sac with round entrance (vs strongly darkened, U-shaped internal sac); paramere with darkened tip (vs without). This species is also similar to another species, *D.* (*E.*) *jejuensis* sp. nov. based on the male genital structures, but it can be distinguished by the following characters: male seventh sternite with a deep, conical internal sac (vs shallow, V-shaped notch); gonostylus with two setae from tubercle (vs a single seta); paramere without hook at tip (vs with hook).

The male genitalia of *D.* (*E.*) *koreana* sp. nov. from Mount Bangtaesan differs from other materials of the species based on the shape of the seventh sternite internal sack (shallow conical without rounded mouth) and paramere distal lobe (pointed tip). However, additional specimens are needed to determine whether this difference is due to intra- or interspecific variation.

DNA barcode analysis

The 676-bp *COI* sequences contained 190 variable sites, of which 156 were parsimony-informative. The interspecific divergences (*p*-distances) within subgenus *D.* (*Erostrata*) ranged from 11.54% to 16.42%, with a mean distance of 13.17% across the entire dataset (Table 2), whereas the intraspecific genetic distances ranged from 0% to 0.59%: from 0% to 0.15% in *D.* (*E.*) *jejuensis* sp. nov., 0.59% in *D.* (*E.*) *koreana* sp. nov., and 0.15% in *D.* (*E.*) *tabashii*. The maximum intraspecific genetic distance (0.59%) was much smaller than the minimum interspecific one (11.54%). The NJ tree (Fig. 5) indicated that the monophyly of each of the new species was highly supported, as was that of subgenus *D.* (*Erostrata*) (Fig. 5).

Table 2. Estimates of genetic divergence (%) between sequences. The number of base differences per site from between sequences are shown. Standard errors (%) are shown above the diagonal and were obtained by a bootstrap procedure (1,000 replicates). All positions containing gaps and missing data were eliminated (complete delete option).

	Species	Accession number	1	2	3	4	5	6	7	8	9	10	11
1	*D.* (*E.*) *globithorax*	OM102980	–	1.36	1.36	1.36	1.36	1.37	1.28	1.42	1.41	1.37	1.34
2	*D.* (*E.*) *jejuensis* sp. nov.	OM102981	13.61	–	0	0.15	1.37	1.35	1.36	1.34	1.33	1.33	1.45
3		OM102982	13.61	0	–	0.15	1.37	1.35	1.36	1.34	1.33	1.33	1.45
4		OM102983	13.76	0.15	0.15	–	1.36	1.34	1.36	1.34	1.33	1.34	1.45
5	*D.* (*E.*) *koreana* sp. nov.	OM102979	15.24	14.94	14.94	14.79	–	0.30	1.38	1.21	1.20	1.31	1.45
6		OP081140	15.38	14.64	14.64	14.50	0.59	–	1.40	1.22	1.21	1.31	1.45
7	*D.* (*E.*) *submelas*	OM102978	11.54	14.64	14.64	14.79	15.09	15.68	–	1.43	1.43	1.37	1.36
8	*D.* (*E.*) *tabashii*	OM102975	16.12	14.94	14.94	14.79	11.69	11.83	16.42	–	0.15	1.29	1.44
9		OM102976	15.98	14.79	14.79	14.64	11.54	11.69	16.27	0.15	–	1.30	1.44
10	*D.* (*E.*) *yazuensis*	OM102977	15.24	12.43	12.43	12.57	13.46	13.46	13.76	12.87	13.02	–	1.30
11	*D.* (*D.*) *kandybinae*	OP093621	14.50	16.27	16.27	16.42	16.12	16.12	14.50	15.68	15.53	12.57	–

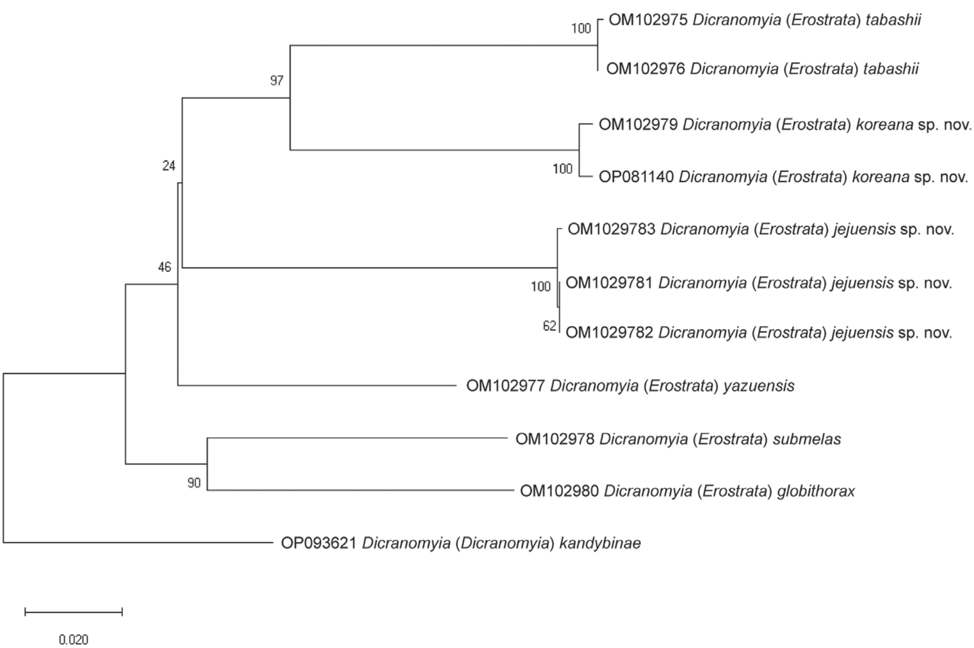


Figure 5. Neighbor-joining (NJ) Kimura-2-parameter tree based on the analysis of the *COI* of six Korean *Dicranomyia* (*Erostrata*) species and *D.* (*Dicranomyia*) *kandybinae* as outgroup. Numbers at the nodes indicate NJ bootstrap support values.

Discussion

This is the first study to use DNA barcoding for the delimitation of the *D.* (*Erostrata*) species. The present study identified two new species using both morphological and molecular data. According to the NJ tree (Fig. 5), the subgenus includes two major clades, which can be distinguished based on the presence or absence of numerous black, strong spines on the mesal face of gonostylus. Indeed, *D.* (*E.*) *jejuensis* sp. nov., *D.* (*E.*) *koreana* sp. nov., *D.* (*E.*) *tabashii*, and *D.* (*E.*) *yazuensis* can be distinguished from other members of their subgenus based on the shape and mesal face (without lots of black, strong spines) of their gonostyli. Two hypotheses may be considered: i) this clade can be classified into morphological species groups, or ii) it can be elevated to a new subgenus. Additional materials are needed to more accurately reconstruct phylogenetic relationships within genus *Dicranomyia*.

Based on our morphological examinations of the materials, we also found that some specimens identified as *D.* (*E.*) *tabashii* by Podenas et al. (2019) are actually specimens of *D.* (*E.*) *koreana* sp. nov. Based on our observation, unknown cryptic species of crane flies could also be detected and identified using molecular data.

Acknowledgements

We are deeply grateful to Dr Sigitas Podenas (Nature Research Centre and Vilnius University, Vilnius, Lithuania), who provided photographs of the *D.* (*E.*) *tabashii* type specimens, and to Dr Pjotr Oosterbroek (University of Amsterdam, Amsterdam, the Netherlands), who provided valuable information through his website, Catalogue of the Crane flies of the World (https://ccw.naturalis.nl/index.php). We are very grateful to members of the Laboratory of Biodiversity and Ecology, and the Korean Entomology Institute (Korea University, Seoul, Korea) for their assistance during field trips. We are also grateful to Dr Pavel Starkevich and Dr Daichi Kato for offering valuable comments and improving the manuscript. This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR202102204 and NIBR202203201).

References

Alexander CP (1924) New or little-known crane flies from northern Japan (Tipulidae, Diptera). Philippine Journal of Science 24: 531–611.

Alexander CP (1931a) New or little-known Tipulidae from the Philippines (Diptera). IX. Philippine Journal of Science 45: 415–448.

Alexander CP (1931b) New or little-known Tipulidae from the Philippines (Diptera). X. Philippine Journal of Science 46: 9–38.

Alexander CP (1934) New or little-known Tipulidae from eastern Asia (Diptera). XXI. Philippine Journal of Science 55: 19–60. https://doi.org/10.1093/aesa/27.1.55

Alexander CP (1959) Undescribed species of crane-flies from the Himalaya mountains (Tipulidae, Diptera). IV. Journal of the New York Entomological Society 67: 223–235.

Alexander CP (1967) New or little-known Tipulidae from eastern Asia (Diptera). LX. Philippine Journal of Science 95: 227–266.

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

de Jong H (2017) Limoniidae and Tipulidae (Crane Flies). In: Kirk-Spriggs AH, Sinclair BJ (Eds) Manual of Afrotropical Diptera. Vol. 2. Nematocerous Diptera and Lower Brachycera. Suricata 5. South African National Biodiversity Institute, Pretoria, 427–477.

Edwards FW (1919) Results of an expedition to Korinchi Peak, Sumatra. Part III. Invertebrates. II. Diptera. Collected in Korinchi, West Sumatra, by Messrs. H. C. Robinson and C. Boden Kloss. Journal of the Federated Malay States Museums 8: 7–59.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5): 294–299.

Kato D, Tachi T, Gelhaus J (2018) Revision of the subgenus *Dicranomyia* (*Erostrata*) Savchenko, 1976 (Diptera, Limoniidae) of Japan. Zootaxa 4441(1): 181–194. https://doi.org/10.11646/zootaxa.4441.1.11

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16(2): 111–120. https://doi.org/10.1007/BF01731581

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35(6): 1547–1549. https://doi.org/10.1093/molbev/msy096

Oosterbroek P (2023) Catalogue of the Craneflies of the World (CCW). https://ccw.naturalis.nl/ [Accessed on 24.03.2023]

Osten Sacken CR (1869) Monographs of the Diptera of North America. Part IV. Smithsonian Miscellaneous Collections 8(219): 1–345.

Podenas S, Seo HY, Kim T, Hur JM, Kim AY, Klein TA, Kim HC, Kang TH, Aukštikalnienė R (2019) *Dicranomyia* crane flies (Diptera: Limoniidae) of Korea. Zootaxa 4595(1): 1–110. https://doi.org/10.11646/zootaxa.4595.1.1

Podenas S, Park S, Byun H, Kim A, Klein TA, Kim H, Aukštikalnienė R (2020) New data on Limoniinae and Limnophilinae crane flies (Diptera: Limoniidae) of Korea. Journal of Species Research 9(4): 492–531. https://doi.org/10.12651/jsr.2020.9.4.492

Rogers JS (1930) The summer crane-fly fauna of the Cumberland Plateau in Tennessee. Occasional Papers of the Museum of Zoology 215: 1–50.

Savchenko EN, Krivolutskaya GO (1976) Limoniid flies (Diptera, Limoniidae) of the South Kuril and South Sakhalin. Akad. Nauk. Ukr. SSR, Kiev, 160 pp. [in Russian]

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87(6): 651–701. https://doi.org/10.1093/aesa/87.6.651

Suh KI, Hwang JM, Bae YJ, Kang JH (2019) Comprehensive DNA barcodes for species identification and discovery of cryptic diversity in mayfly larvae from South Korea: Implications for freshwater ecosystem biomonitoring. Entomological Research 49(1): 46–54. https://doi.org/10.1111/1748-5967.12334