

# Two new species of *Deuterophlebia* Edwards, 1922 from Southwestern China (Diptera, Deuterophlebiidae)

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## Abstract

The family Deuterophlebiidae is considered the most plesiomorphic Diptera family, with striking morphology and life history. In this study, we provide detailed descriptions and figures of two new species from Southwestern China: *Deuterophlebia pseudopoda* **sp. nov.** and *Deuterophlebia pachychaeta* **sp. nov.**, along with genetic distances of COI sequences between the seven known Chinese species of *Deuterophlebia* Edwards, 1922. The two new species can be identified by the adults' male terminalia, head structures, male and female antennae and mesothoracic spines or abdominal projections of the pupae. As a result, China is currently the country with the highest *Deuterophlebia* species diversity.

## Key Words

aquatic insects, barcoding, COI, genetic identification, mountain midge

## Introduction

Deuterophlebiidae (or mountain midge) is a monotypic family in the order Diptera. Members of this family show unique morphology and living habits. In contrast to other dipteran groups, the larvae of *Deuterophlebia* Edwards, 1922 have paired pseudopodia and live in torrent streams; adults mate at sunrise, male adults die immediately after mating, while females shed their wings, go back to the water and lay eggs (Courtney 1989; Zheng et al. 2022). This kind of specialized taxon and its living history has attracted more and more attention and studies in recent years.

The biogeographic and phylogenetic status of the family is still enigmatic (Courtney 1994; Zheng et al. 2022). In the latest phylogenetic topology based on mitochondrial sequences, Deuterophlebiidae was considered the earliest branching lineage of Diptera, a sister group of all other dipterans (Zhang et al. 2022). This result differs from previous inferences, which usually consider Blephariceridae and Deuterophlebiidae as sister groups (Courtney 1991). Obviously, many questions are still waiting for answers in the systematic study of this family.

Up to 2022, there were 14 named species in this family, eight species reported from the Palearctic Region (Kitakami 1938; Jedlička and Halgoš 1981; Courtney 1994; Sofi et al. 2020) and six from the Nearctic realm (Pennak 1945, 1951; Shewell 1954; Kennedy 1958, 1960; Courtney 1989, 1990). China was a blank area in the distribution maps (Courtney 1994). In 2022, five species were reported from Southwestern and Eastern China, bringing some new insights into the diversity and biogeography of Deuterophlebiidae (Zheng et al. 2022). Encouraged by that, extensive investigations were carried out, and it was hypothesized that China is possibly the center of the diversity of Deuterophlebiidae. More studies on these Chinese species can provide more information on their evolution and dispersal.

During a trip in Yunnan Province of southwestern China, some *Deuterophlebia* adults and pupae were collected. After careful examination, they were recognized as two new species. We presented detailed images of their adults and pupae, described them in detail and compare them with the known species using COI and morphological characters, including some that have not been mentioned before.

## Materials and methods

Pupae were hand-picked from the surface of stones underwater. Adults were found floating nearby the banks of creeks. Specimens were examined under a stereomicroscope (Nikon SMZ 745T). Habitus of pupae and adults were photographed using a SONY a7R II camera with a LAOWA 25 mm 5× macro lens. Details of heads and legs were studied by dissection and treatment in 10% NaOH (30 °C, 30 min), observed and photographed with a camera (Nikon 50i) coupled on a microscope. Terminology mainly follows that of Courtney (1994). All specimens were preserved in 85% ethanol and deposited in the Diptera collection of College of Life Sciences, Nanjing Normal University and School of Grain Science and Technology, Jiangsu University of Science and Technology.

To associate the pupae and winged stages, total genomic DNA was extracted from the abdomen of specimens using Animal Genomic DNA Kit (TsingKe Biotech Co., Beijing, China). The mitochondrial gene cytochrome *c* oxidase subunit I fragment was PCR-amplified using the Premix Taq (Takara Bio Inc., Beijing, China) with forward primer LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and reverse primer HCO2198 (5'-TAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). PCR conditions included initial denaturation at 94 °C for 5 min, 40 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 40 s, with a final extension at 72 °C for 10 min (Zheng et al. 2022). Sequences were aligned using Muscle, and the K2P genetic distances were estimated in MEGA11 (Tamura et al. 2021). The intraspecific genetic distances were lower than 0.01. The sequence of each species has been uploaded to GenBank (Table 1) with the same specimen information as for the holotype of each species.

**Table 1.** GenBank accession numbers of COI sequences used in this research.

Species	GenBank Accession Number
<i>D. sinensis</i> Zheng et al., 2022	ON637906
<i>D. yunnanensis</i> Zheng et al., 2022	ON637909
<i>D. alatus</i> Zheng et al., 2022	ON637916
<i>D. acutirhina</i> Zheng et al., 2022	ON637914
<i>D. wuyiensis</i> Zheng et al., 2022	ON637910
<i>D. pseudopoda</i> sp. nov.	OR064529 (this study)
<i>D. pachychaeta</i> sp. nov.	OR064528 (this study)

## Results

### *Deuterophlebia pseudopoda* sp. nov.

<https://zoobank.org/E1B5D9F5-9797-4350-815F-197CEBE47826>

**Description. Male adults.** Body length ca. 2.2–2.6 mm (n=5), uniformly brownish black (Fig. 1). Head brownish black, flattened, nearly trapezoidal, width ca. 0.50 mm, folded backward under thorax, hidden in dorsal view (Fig. 1). Head densely covered with microtrichia. Median



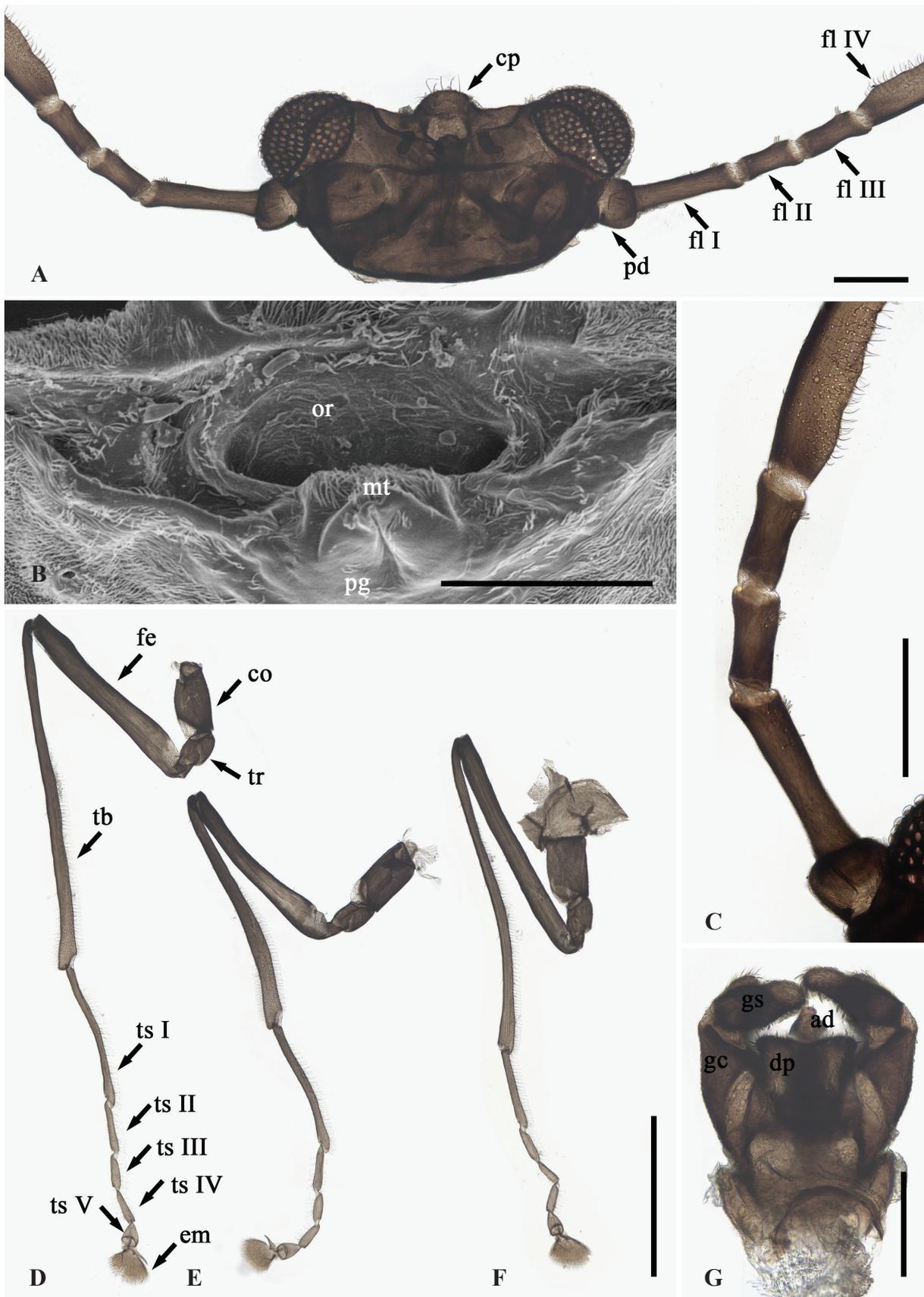
**Figure 1.** Male adult of *D. pseudopoda* sp. nov. Scale bar: 1.0 mm.

clypeal lobe slightly convex, semicircle shaped, with around 20 sharp setae (Fig. 2A). Mouthparts in form of an invaginated tubule, oral region depressed (Fig. 2A, B). Edges of oral region (or mouth opening) ridged, convex medially on ventral ridge, forming a blunt mental tooth (Fig. 2A, B). Postgena and oral region with sparser microtrichia than other regions, a pair of tentorial pits present on each side of oral region (Fig. 2A, B). Compound eyes glabrous, width ca. 0.18 mm, distance between eyes ca. 0.30 mm (Fig. 2A).

Antennae 8.5–10.0 mm (n=5) (Fig. 2A, C). Scape oval shaped, pedicel globular, both covered with microtrichia (Fig. 2A, C). Flagella four segmented, flagellomeres I–III slender cylindrical, each with a subapical tubercle on front margin, bearing 9–12, 6–9 and 4–6 digitiform setae respectively (Fig. 2C). Flagellomere IV flattened and elongated, broader than flagellomeres I–III, narrowed gradually, with curved hair-like setae on the anterior side of basal half, apical half generally glabrous but bearing 4–5 clusters of curved hair-like setae, apex slightly expanded with some curved hair-like setae (Fig. 2C). Antennal ratio: 4.0: 2.0: 5.0: 3.0: 3.0: 238.0 (Figs 1, 2C). Flagellomere IV about 14× combined length of five basal antennal articles or about 4× body length (Fig. 1).

Thorax uniformly brownish black, densely covered with microtrichia (Fig. 1). Pronotum almost hidden, mesonotum strongly expanded (Fig. 1). Wings ca. 4.0 mm, uniformly set with dark micro-tubercles, grayish translucent, cubital area greatly enlarged, costal margin slightly thickened (Fig. 1). Outer margin fringed with soft hair-like setae, denser and longer on cubital margin (Fig. 1). Veins radially arranged, pale and inconspicuous (Fig. 1). Halteres transparent, ca. 0.35 mm (Fig. 1).

Legs brownish black, slender, sharing similar chaetotaxy with four types of setae: (1) microtrichia, densely covered on all segments; (2) sharp macrotrichia, sparsely on dorsal margin of femora and tibiae; (3) long capitate setae, ventrally on tarsomeres I–IV of each leg, distal half of ventral edge of all tibiae, surrounding the top of fore- and midtibiae, and also densely arranged radially on each empodium; (4) digitiform setae, 1–3 pairs for each tarsomere (Fig. 2D–F). In all legs, coxae much broader than trochanters, coxae about twice the length of trochanters (Fig. 2D–F). In foreleg, femur: tibia: tarsus = 9.0: 14.0: 14.0; femur slightly flattened, tibia slender, cylindrical,



**Figure 2.** Male adult of *D. pseudopoda* sp. nov.: **A.** Head (ventral view); **B.** Oral region (ventral view); **C.** Flagellomeres; **D.** Foreleg; **E.** Midleg; **F.** hindleg; **G.** Terminalia (dorsal view). Abbreviations: cp, median clypeal lobe; pd, pedicel; fl, flagellomere; or, oral region; mt, mental tooth; pg, postgena; co, coxa; tr, trochanter; fe, femur; tb, tibia; ts, tarsomere; em, empodium; dp, dorsal plate; gc, gonocoxite; gs, gonostylus; ad, aedeagus. Scale bars: 0.1 mm (**A**, **C**, **G**); 0.05 mm (**B**); 0.5 mm (**D–F**).

and gradually broader apically; tarsomere I: II: III: IV: V = 6.4: 2.3: 1.4: 1.4: 1.0, tarsomeres I–IV cylindrical, tarsomere V conical; empodium shell-shaped, length subequal to tarsomere V; claw slender, tapered, shorter than empodium (Fig. 2D). Midleg shortest among all legs, similar to foreleg, femur: tibia: tarsus = 8.0: 10.5: 12.0, tarsomere I: II: III: IV: V = 6.0: 1.9: 1.1: 1.1: 1.0 (Fig. 2E). In hindleg, femur: tibia: tarsus of hindleg = 10.0: 13.5: 10.0, tarsomere I: II: III: IV: V = 3.5: 1.5: 1.2: 1.2: 1.0 (Fig. 2F).

Abdomen brownish black, densely covered with microtrichia, nine segmented, tapering posteriorly (Fig. 1). First two segments strongly fused with each other, paler and shorter than others (Fig. 1). Segment VIII in form of a

short chitin ring (Fig. 1). Sternite IX almost glabrous, fused with dorsal plate, connected with gonocoxite (Fig. 2G). Gonocoxite with posterior projection which length subequal to gonostylus (Fig. 2G). Gonostylus subequal to the dorsal plate in length, flattened, oval-shaped, flexor surface with numerous curved sharp setae (Fig. 2G). Dorsal plate parallel-sided, posterior margin slightly depressed without cleft with some stout curved setae on margin (Fig. 2G). Aedeagus in form of a smooth tube, length subequal to gonostylus and dorsal plate (Fig. 2G).

**Female adult.** Body length ca. 2.0 mm ( $n = 1$ ). Besides sexual differences, generally similar to the males except following features (Fig. 3A–D).

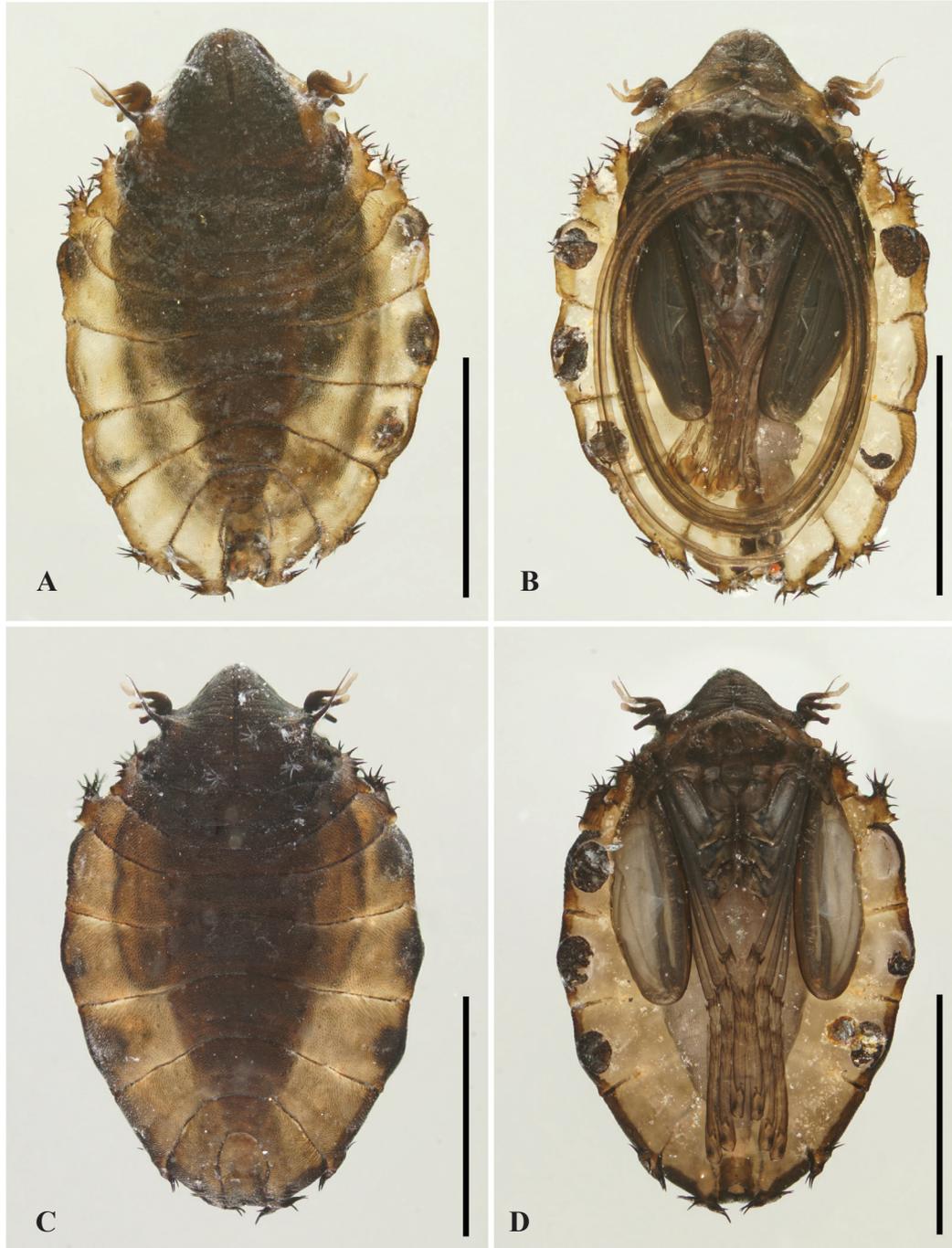


**Figure 3.** Female adult of *D. pseudopoda* sp. nov.: **A.** Head (ventral view); **B.** Foreleg; **C.** Midleg; **D.** Hindleg. Scale bars: 0.1 mm.

Head width ca. 0.36 mm (Fig. 3A). Median clypeal lobe strongly protruded medially with ca. 20 setae (Fig. 3A). Oral region located near anterior margin of head (Fig. 3A). Compound eyes more prominent than males (Fig. 3A). Antenna ca. 0.3 mm. Scape slender oval shaped, pedicel globular, both scape and pedicel covered with microtrichia and bearing several sharp setae (Fig. 3A). Flagellomere I slender cylindrical, flagellomeres II–III slender oval shaped, flagellomere IV dripping shaped, strongly narrowed basally (Fig. 3A). Each of flagellomeres I–III bearing ca. 5 digitiform setae apically, flagellomere IV with 4 sharp setae. Antennal ratio ca. 7.0: 3.0: 10.0: 4.0: 5.0: 4.0 (Fig. 3A).

Legs sharing similar chaetotaxy and exhibiting three types of setae, chaetotaxy similar to males but without capitate setae (Fig. 3B–D). In foreleg and midleg, femur: tibia: tarsus = 1.0: 2.0: 1.3, tarsomere I: II: III: IV: V = 1.0: 0.8: 0.8: 0.8: 2.4. (Fig. 3B, C) In hindleg, femur: tibia: tarsus = 1.0: 1.7: 1.1, tarsomere I: II: III: IV: V = 1.0: 0.9: 0.9: 0.9: 2.7 (Fig. 3D). Claws of all legs similar, paired, stout and curved, with a blunt protrusion in the middle (Fig. 3B–D). Empodium in form of a long and hairy spine, subequal to the length of claw (Fig. 3B–D).

**Male pupae.** Pupae flattened oval shaped, length 2.3 mm (n = 2), width 1.6 mm. Dorsal integument dark brown, divided into 11 segments (Fig. 4A, B).



**Figure 4.** Pupae of *D. pseudopoda* sp. nov.: **A.** Male pupa (dorsal view); **B.** Male pupa (ventral view); **C.** Female pupa (dorsal view); **D.** Female pupa (ventral view). Scale bars: 1.0 mm.



**Figure 5.** Pupa of *D. pseudopoda* sp. nov.: **A.** Thoracic spine (dorsal view); **B.** Gill (ventral view); **C.** Posterior end (ventral view). Abbreviation: pp, posterolateral projections. Scale bars: 0.1 mm.

Prothorax fused with mesothorax, forming a conical segment with a median suture (Fig. 4A, B). Mesothoracic lateral margins each with a sharp spine and a gill (Figs 4A, B, 5A, B). Spines ca. 0.4 mm, slightly curved, dark brown, originated from a round base (Fig. 5A, B). Ventral gills light to dark brown, length subequal to the dorsal spines, hand-shaped and consisting of three filaments: posterior filament shorter, pointing backward; anterior two filaments similar in shape, twisted and light in color apically (Fig. 5A, B). Metathorax completely surrounded by mesothorax and first abdominal segment (Fig. 4A, B). Abdominal segments I–II with a pair of anterolateral projections, each projection pointing forward and bearing ca. 13 spines (Fig. 4A, B). Segments VI–VII with a pair of posterolateral projections, projections foot-shaped and each bearing ca. 8 spines (Fig. 5C). Segment VIII shield-shaped, surrounded by segments VII and IX (Fig. 4A, B).

Adult structures visible on ventral side (Fig. 4A, B). Head present directly below mesothorax; antennal sheaths in form of a large elliptic ring, surrounding body 2.0 times (Fig. 4A, B). Leg sacs extended to posterior end of antennal ring, strongly expanded apically.

Abdominal segments III–V with a pair of black adhesive discs (Fig. 4A, B).

**Female pupae.** Length ca. 2.2 mm ( $n = 2$ ), width ca. 1.5 mm (Fig. 4C, D). Dorsal morphology similar to male except for smaller mesothorax (Fig. 4C, D). Gender can be identified through the absence of antennal ring, apex of female leg sheaths not expanded (Fig. 4C, D).

**Material examined.** *Holotype*: male adult, China: Yunnan Province, Gongshan County, Dulongjiang Township, Dulongjiang River, 27°50'14.16"N, 98°19'54.2"E, 1470 m a.s.l., 4.II.2023, Xuhongyi Zheng leg. *Paratypes*: 6 male adults, 1 female adult, 2 male pupae, 2 female pupae, same locality and data as holotype.

**Diagnosis.** Male adults of *Deuterophlebia pseudopoda* sp. nov. can be identified by their terminalia: gonostylus short, length of gonostylus subequal to the gonocoxite and dorsal plate; posterior margin of dorsal plate slightly depressed but without a median cleft (Fig. 2G). Such a terminalia differs from the 19 named *Deuterophlebia* species (Courtney 1990, 1994; Zheng et al. 2022). The shape of their heads is also distinct among known species: median clypeal lobe slightly convex, inner side of compound

eye without a protruded corner (Fig. 2A) (Courtney 1990, 1994; Zheng et al. 2022).

Female adults of *D. pseudopoda* sp. nov. can be recognized through a combination of the pronounced median clypeal lobe, chaetotaxy of antennae, and shape of flagella (Fig. 3A). Compared to other species, its pronounced median clypeal lobe is similar to *D. oporina* Courtney, 1994 and *D. nipponica* Kitakami, 1938, but can be differentiated from them by its antenna: flagellomeres I–III bearing ca. 5 digitiform setae respectively, flagellomere IV with only 4 sharp setae, antennal ratio = 7.0: 3.0: 10.0: 4.0: 5.0: 4.0 (Courtney 1990, 1994; Zheng et al. 2022).

Pupae can be easily identified by their foot-shaped posterolateral projections of abdominal segments VI–VII (Fig. 5C). This feature is absent in the 15 species with clear pupal stage (Courtney 1990, 1994; Zheng et al. 2022). They can also be separated from other species by their single mesothoracic spines, gills consisting of three filaments, and absence of conspicuous thoracic ridges (Figs 4A–D, 5A, B) (Courtney 1990, 1994; Zheng et al. 2022).

**Etymology.** The specific epithet “*pseudopoda*” means “pseudopodia”, refers to the pseudopodia-like lateral projections of pupal abdominal segments VI–VII.

**Distribution.** China (Yunnan Province).

#### *Deuterophlebia pachychaeta* sp. nov.

<https://zoobank.org/99503019-F78C-49B5-B602-8CE1D53BD96A>

**Description. Male adults.** Body length ca. 3.0 mm ( $n = 2$ ), uniformly brownish black. Head brownish black, flattened and nearly trapezoidal, width ca. 0.50 mm (Fig. 6A). Head densely covered with microtrichia. Median clypeal lobe slightly convex, semicircle shaped with around 20 sharp setae (Fig. 6A). Mouthparts in form of an invaginated tubule, oral region depressed (Fig. 6A, B). Edges of oral region (or mouth opening) ridged, forming a protruded angle on inner side of each eye (Fig. 6A, B). Ventral ridge of oral region convex medially and forming a blunt mental tooth (Fig. 6A, B). Postgena and oral region with microtrichia but more sparse than other regions, a pair of tentorial pits present on each side of oral region (Fig. 6A, B). Compound eyes glabrous, width ca. 0.13 mm, distance between eyes ca. 0.30 mm (Fig. 6A).

Antennae ca. 13.0 mm ( $n=5$ ) (Fig. 6A, C). Scape oval shaped, pedicel globular, both scape and pedicel covered with microtrichia (Fig. 6A, C). Flagella four segmented, flagellomeres I–III slender cylindrical, each with a sub-apical tubercle on front margin and bearing 8–10 digitiform setae (Fig. 6C). Flagellomere IV flattened and elongated, with curved hair-like setae on the anterior side of basal half, apical half generally glabrous but bearing 4–5 clusters of curved hair-like setae, apex slightly expanded with some curved hair-like setae (Fig. 6C). Antennal ratio ca. 4.0: 2.0: 5.0: 3.0: 3.0: 287.0, flagellomere IV about 17× combined length of five basal antennal articles or about 4× body length (Fig. 6C).

Thorax brownish black, densely covered with microtrichia. Mesonotum strongly expanded. Wings ca. 5.5 mm, shape, venation and other features similar to *D. pseudopoda* sp. nov. (Fig. 1).

Legs brownish black, slender, three pairs sharing similar chaetotaxy exhibiting four types of setae: (1) microtrichia, densely covered on all segments; (2) sharp macrotrichia, sparsely on dorsal margin of femora and tibiae; (3) long capitate setae, on ventral side of tarsomere I–IV of each leg, distal half of ventral edge of all tibiae, surrounding the top of fore- and midtibiae, and also densely arranged radially on each empodium; (4) digitiform setae, 1–3 pairs for each tarsomere (Fig. 6D–F). In foreleg, femur: tibia: tarsus = 11.0: 17.0: 16.0; femur slightly flattened, tibia slender cylindrical and gradually broader apically; tarsomere I: II: III: IV: V = 8.5: 2.0: 2.0: 1.5: 0.8, tarsomeres I–IV cylindrical, tarsomere V conical; empodium shell-shaped, length subequal to tarsomere V; claw slender tapered, shorter than empodium (Fig. 2B) (Fig. 6D). Midleg shortest among all legs, features generally similar to foreleg, femur: tibia: tarsus = 10.0: 12.5: 14.0, tarsomere I: II: III: IV: V = 8.5: 2.0: 2.0: 1.5: 0.8 (Fig. 6E). In hindleg, femur: tibia: tarsus of hindleg = 14.0: 17.0: 11.0, tarsomere I: II: III: IV: V = 3.0: 2.0: 2.0: 1.5: 0.8 (Fig. 6F).

Abdomen brownish black, densely covered with microtrichia, nine segmented, tapering posteriorly. Segment VIII in form of a short chitin ring, sternite IX almost glabrous, connected with dorsal plate and gonocoxite (Fig. 6G). Gonocoxite with posterior projection ca. 1/2 length of gonostylus (Fig. 6G). Gonostylus subequal dorsal plate, flattened, slender oval shaped, flexor surface with numerous curved sharp setae (Fig. 6G). Dorsal plate parallel-sided, posterior margin slightly depressed with a median cleft and some stout setae on margin (Fig. 6G). Aedeagus in form of a smooth tube, length subequal to gonostylus, longer than dorsal plate (Fig. 6G).

**Female adult.** Body length ca. 2.3 mm ( $n = 1$ ). Besides sexual differences, generally similar to the males except following features (Fig. 7A–D).

Head width ca. 0.40 mm (Fig. 7A). Median clypeal lobe slightly convex with ca. 20 setae (Fig. 7A). Oral region located near anterior margin of head, postgena and oral region with microtrichia but more sparse than other regions (Fig. 7A). Compound eyes more prominent than males (Fig. 7A). Antenna ca. 0.3 mm. Scape slender oval-shaped, pedicel globular, both scape and pedicel covered with microtrichia with several sharp setae (Fig. 7A). Flagellomere I slender cylindrical, flagellomeres II–III slender oval shaped, flagellomere IV dripping shaped (Fig. 7A). Flagellomeres I–IV bearing 2, 4, 10 and 2 digitiform setae apically respectively, flagellomere III–IV with 1 and 4 sharp setae respectively (Fig. 7A). Antennal ratio = 7.0: 4.0: 10.0: 5.0: 5.0: 4.0 (Fig. 7A).

Legs sharing similar chaetotaxy and exhibiting three types of setae, chaetotaxy similar to males but without capitate setae (Fig. 7B–D). In all legs, coxae about twice longer and broader than trochanters, femora slightly



**Figure 6.** Male adult of *D. pachychaeta* sp. nov.: **A.** Head (ventral view); **B.** Oral region (ventral view); **C.** Flagellomeres; **D.** Foreleg; **E.** Midleg; **F.** Hindleg; **G.** Terminalia (dorsal view). Scale bars: 0.1 mm (**A**, **C**, **G**); 0.05 mm (**B**); 0.5 mm (**D–F**).

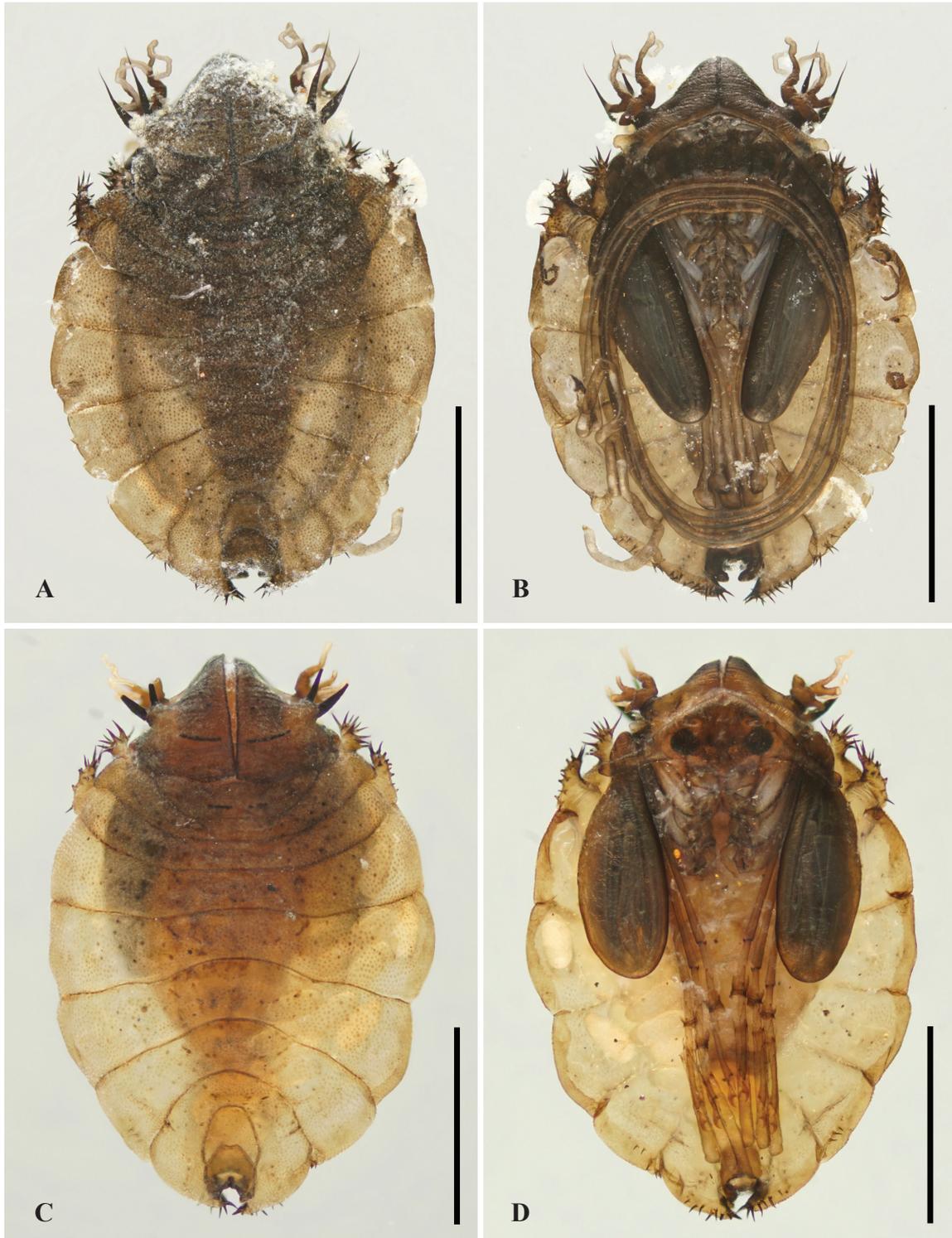


**Figure 7.** Female adult of *D. pachychaeta* sp. nov.: **A.** Head (ventral view); **B.** Foreleg; **C.** Midleg; **D.** Hindleg. Scale bars: 0.1 mm.

flattened, tibiae and tarsomeres slender cylindrical (Fig. 7B–D). In foreleg and midleg, femur: tibia: tarsus = 1.0: 2.0: 1.5, tarsomere I: II: III: IV: V = 1.2: 0.8: 0.8: 0.8: 2.4 (Fig. 7B, C). In hindleg, femur: tibia: tarsus = 1.0: 1.7: 1.1, tarsomere I: II: III: IV: V = 1.0: 0.8: 0.8: 0.8: 2.4 (Fig. 7D). Claws of all legs similar, paired, stout and curved, with a blunt protrusion in the middle (Fig. 7B–D). Empodium in form of a long and hairy spine, subequal to the length of claw (Fig. 7B–D).

**Male pupae.** Pupae flattened oval shaped, length 2.8 mm ( $n = 2$ ), width 2.0 mm (Fig. 8A, B). Dorsal integument dark brown, divided into 11 segments (Fig. 8A, B).

Prothorax fused with mesothorax, forming a conical segment with a median suture (Fig. 8A, B). Mesothoracic lateral margins each with a pair of sharp spines and a gill (Figs 8A, B, 9A, B). Spines ca. 0.4 mm, expanded basally, slightly curved, dark brown, originated from a round base (Fig. 9A). Ventral gills light to dark brown, length subequal



**Figure 8.** Pupae of *D. pachychaeta* sp. nov.: **A.** Male pupa (dorsal view); **B.** Male pupa (ventral view); **C.** Female pupa (dorsal view); **D.** Female pupa (ventral view). Scale bars: 1.0 mm.



**Figure 9.** Male pupa of *D. pachychaeta* sp. nov.: **A.** Thoracic spine (dorsal view); **B.** Gill (ventral view). Scale bar: 0.1 mm.

to the dorsal spines, each including four filaments: posterior filament single and short, pointing backward; anterior three gill filaments on a common base, similar in shape, slender and twisted; second and third filaments fused at base (Fig. 9B). Metathorax completely surrounded by mesothorax and first abdominal segment (Fig. 8A, B).

Abdominal segment I and II similar, each with a pair of anterolateral projections, each projection pointing forward and bearing ca. 18 spines (Fig. 8A, B). Lateral margins of segments VI and VII with several spines on both dorsal and ventral sides. Segment VIII shield-shaped, surrounded by segments VII and IX (Fig. 8A, B).

Adult structures visible on ventral side (Fig. 8B). Head present below mesothorax; antennal sheaths in form of a large elliptic ring, surrounding body 2.0 times (Fig. 8B). Leg sacs extended to posterior end of antennal ring, strongly expanded apically. Abdominal segments III–V each with a pair of black adhesive discs (Fig. 8B).

**Female pupae.** Length ca. 2.8 mm ( $n = 1$ ), width ca. 2.0 mm (Fig. 8C, D). Dorsal morphology similar to male except for smaller mesothorax (Fig. 8C, D). Gender can be identified through the absence of antennal ring, apex of female leg sheaths not expanded (Fig. 8C, D).

**Material examined. Holotype:** male adult, China: Yunnan Province, Nujiang Lisu Autonomous Prefecture, Gongshan County, Dulongjiang Township, Dulongjiang River, 27°50'14.16"N, 98°19'54.2"E, 1470 m a.s.l., 4.II.2023, Xuhongyi Zheng leg. **Paratypes:** 1 male adult, 2 male pupae, same locality and data as holotype; 1 female adult dissected from a pupa, Yunnan Province, Nujiang Lisu Autonomous Prefecture, Fugong County, 26°57'17.32"N, 98°52'3.04"E, 1265 m a.s.l., 3. VII. 2022, leg. Xuhongyi Zheng, Zhenxing Ma, Zhiteng Chen & Pengxu Mu.

**Diagnosis.** Male adults of *Deuterophlebia pachychaeta* sp. nov. can be identified by their head shape and

chaetotaxy of antennae (Fig. 6A, C). Following the keys of Courtney (1994) and Zheng et al. (2022), males of this species, which are similar to *D. mirabilis* Edwards, 1922, *D. blepharis* Courtney, 1994 and *D. sinensis* Zheng et al., 2022, can be separated from other Asian species by their relatively long antennae (longer than 8.0 mm), and post-gena covered with microtrichia (Fig. 6B). Chaetotaxy on flagellomeres can be used to differentiate those four similar species: in the new species, there were ca. 8–10 digitiform setae on each of flagellomeres I–III (Fig. 6A, C) (Courtney 1990, 1994; Zheng et al. 2022).

Female adults of *D. pachychaeta* sp. nov. can be recognized through a combination of the smooth median clypeal lobe, chaetotaxy of antennae, and shape of flagella (Fig. 7A). Its smooth and convex median clypeal lobe is similar to *D. mirabilis* Edwards, 1922, *D. blepharis* Courtney, 1994, *D. sinensis* Zheng et al., 2022, *D. yunnanensis* Zheng et al., 2022 and *D. wuyiensis* Zheng et al., 2022, but can be differentiated from them by its antenna: flagellomeres I–IV bearing ca. 2, 4, 10 and 2 digitiform setae respectively, flagellomere IV with additionally 4 sharp setae; antennal ratio = 7.0: 4.0: 10.0: 5.0: 5.0: 4.0 (Fig. 7A) (Courtney 1990, 1994; Zheng et al. 2022).

Pupae can be identified by their mesothoracic spines: there are two spines on each side, expanded basally (Fig. 9A, B). Compared with the 15 species with a clear pupal stage (Courtney 1990, 1994; Zheng et al. 2022), only *D. acutirhina* Zheng et al., 2022 has similar basally expanded spines, but less expanded than in the new species. They can also be distinguished by the absence of abdominal dots (Zheng et al. 2022).

**Etymology.** The specific epithet “*pachychaeta*” is a combination of the words *pachy* and *chaeta*, which refers to its thickened spines on pupal mesothorax.

**Distribution.** China (Yunnan Province).

## Keys to Asian *Deuterophlebia*

### Key to male adults

Modified from the key by Zheng et al. 2022. Among the 15 Asian species, *D. tyosenensis* and *D. wuyiensis* are not included since male adults of them remain unknown.

- 1 Antennal length 7 mm or less, length of flagellomere IV approximately 3× the body length ..... 2
- Antennal length 8 mm or more, length of flagellomere IV approximately 4× the body length..... 4
- 2 Median clypeal lobe convex ..... *D. oporina*
- Median clypeal lobe indistinct ..... 3
- 3 Flagellomere I with more than 10 digitiform setae..... *D. alata*
- Flagellomere I with fewer than 10 digitiform setae ..... *D. brachyrhina*
- 4 Dorsal plate without a median cleft ..... *D. pseudopoda* sp. nov.
- Dorsal plate with a median cleft ..... 5
- 5 Mid-tibiae dorsally glabrous at the top..... *D. acutirhina*
- Mid-tibiae with capitate setae around top ..... 6
- 6 Postgena with microtrichia ..... 7
- Postgena glabrous..... 10
- 7 Compound eyes with microtrichia between ommatidia ..... *D. blepharis*
- Compound eyes glabrous ..... 8
- 8 8–10 digitiform setae on each of flagellomeres I–III ..... *D. pachychaeta* sp. nov.
- Flagellomere I–III with less than 8 digitiform setae..... 9
- 9 Oral region with microtrichia ..... *D. mirabilis*
- Oral region glabrous..... *D. sinensis*
- 10 Flagellomere I with more than 10 digitiform setae..... *D. sajanica*, *D. bicarinata*
- Flagellomere I with 10 or fewer digitiform setae ..... 11
- 11 Hind-tibiae covered with sharp setae on dorsal margin ..... *D. yunnanensis*
- Hind-tibiae glabrous on dorsal margin ..... *D. nipponica*

### Key to pupae

Modified from the key by Zheng et al. 2022. Contains nine known species with previously described pupal stage and two new species from this work.

- 1 Mesothorax with lateral outgrowths ..... *D. alata*
- Mesothorax without lateral outgrowths ..... 2
- 2 Abdominal tergites VI–VII with posterolateral projections ..... *D. pseudopoda* sp. nov.
- Abdominal tergites VI–VII without posterolateral projections ..... 3
- 3 Mesothorax without spines on anterolateral margin ..... *D. nipponica*
- Mesothorax with spines on anterolateral margin ..... 4
- 4 Mesothorax with one pair of spines on anterolateral margin..... 5
- Mesothorax with two pairs of spines on anterolateral margin ..... 6
- 5 Abdominal tergites with dark bands..... *D. bicarinata*
- Abdominal tergites without dark bands..... *D. sajanica*, *D. yunnanensis*
- 6 Abdominal tergites with dark bands..... *D. wuyiensis*
- Abdominal tergites without dark bands..... 7
- 7 Abdominal tergites with a pair of large dark dots..... *D. acutirhina*
- Abdominal tergites without obvious larger dark dots ..... 8
- 8 Mesothoracic spines expanded basally ..... *D. pachychaeta* sp. nov.
- Mesothoracic spines not expanded basally..... 9
- 9 Gills with elongated posterior filaments ..... *D. sinensis*
- Gills with indistinct posterior filaments ..... *D. tyosenensis*

## Molecular study

We sequenced a fragment of the COI gene of the two new species in this study and analyzed them together

with the other five Chinese species. One female adult, one male adult and one pupa were sequenced for each species, since the intraspecific distances are under 0.01, we submit only one sequence of male adult for

**Table 2.** Values of K2P genetic distance among the DNA barcodes (COI).

Values	<i>D. sinensis</i>	<i>D. yunnanensis</i>	<i>D. alatus</i>	<i>D. acutirhina</i>	<i>D. wuyiensis</i>	<i>D. pseudopoda</i> sp. nov.
<i>D. yunnanensis</i>	0.086					
<i>D. alatus</i>	0.154	0.164				
<i>D. acutirhina</i>	0.154	0.159	0.148			
<i>D. wuyiensis</i>	0.175	0.167	0.156	0.155		
<i>D. pseudopoda</i> sp. nov.	0.129	0.127	0.154	0.158	0.154	
<i>D. pachychaeta</i> sp. nov.	0.118	0.091	0.160	0.163	0.169	0.131

each species with the collecting information same as for the holotype.

Interspecific genetic distances are 0.086–0.175 (Table 2). Two of them are under 0.10, one is between *D. sinensis* and *D. yunnanensis*, the other is between *D. pachychaeta* and *D. yunnanensis*. Morphologically, male adults of the three species share several common characters, including relatively long antennae (longer than 8 mm), similar models of head (flattened and nearly trapezoidal, median clypeal lobe slightly convex) and terminalia (relatively slender, dorsal plate with a median cleft). Pupae and female adults of the three species also show similarity, as mentioned above and in Zheng et al. 2022. As a result, we consider them as closely related species, possibly belonging to the same clade, which may also contain *D. mirabilis*. A reliable phylogenetic tree reconstructed by more specimens and molecular data may provide support for this hypothesis.

## Biological notes

Specimens were collected from Dulong River, in the dry season, water was ca. 20 m wide and 2–3 m deep, unshaded, containing stones of various sizes (Fig. 10). Water temperature was lower than 10 °C. All pupae and adults of these two new species were collected at the same locality. Pupae were collected in torrent areas where the water depth was ca. 0.4 m and we could hardly stand (Fig. 10). Pupae were tightly attached to the stone surfaces with their abdominal discs. Dead adults were found floating near the river bank. These biological aspects are similar to other known *Deuterophlebia* species.

## Discussion

The two new species in this study enrich the Chinese diversity of Deuterophlebiidae, making China the area with the most *Deuterophlebia* species. At present, China holds half of the Asian species, including seven only reported from China and *D. mirabilis*, the type species of the genus that has been collected from Xinjiang Province in the last century (Brodsky 1930; Courtney 1994).

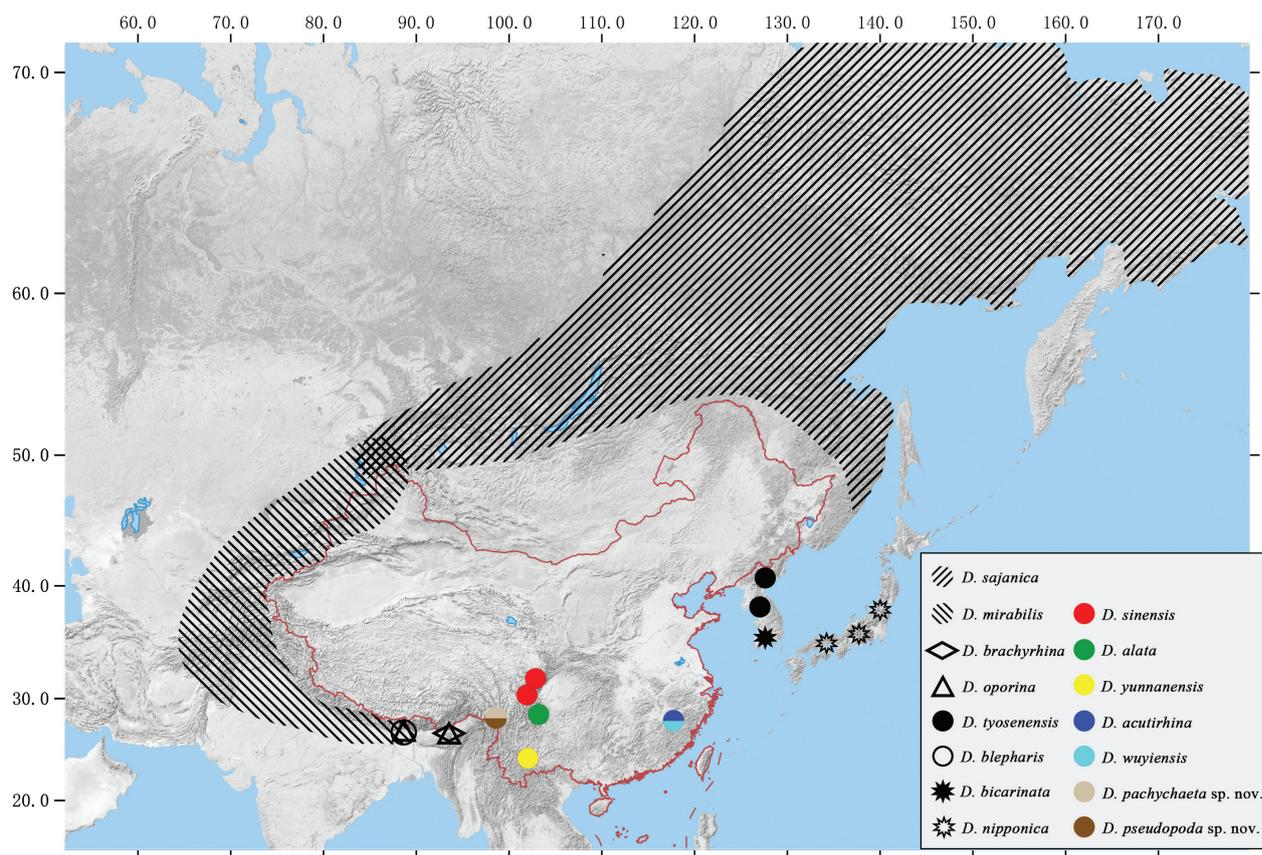
Morphologically, *Deuterophlebia pseudopoda* sp. nov. has more apomorphies than *D. pachychaeta* sp. nov. Both shapes of genitalia (stout, length of gonostylus subequal to the gonocoxite and dorsal plate, dorsal plate without median cleft) and head (nearly trapezoidal, without protruded corners near eyes, median clypeal lobe slightly



**Figure 10.** Habitat stream of *D. pseudopoda* sp. nov. and *D. pachychaeta* sp. nov.

convex) of *D. pseudopoda* sp. nov. are unique among *Deuterophlebia* members (Fig. 2A, G). Its pupae have a prominent autapomorphy: the posterolateral projections on abdominal segments VI–VII (Fig. 5C). Through extensive examination and comparison in recent years, we found that the pupal stage is the easiest for species identification, although most species have a male adult as holotype. All seven Chinese species have their pupal structures described.

In an overview of the distribution map of Deuterophlebiidae (Fig. 11, modified from Courtney 1994 and Zheng et al. 2022), a Himalayan bias can be easily identified. Taking into account the biogeography, the Dulongjiang area (the type locality of the two new species) can be considered as part of the Himalayas (Zhu and Yan 2002). Thus, at least six species can be found in the Himalayas, including *D. mirabilis*, *D. blepharis*, *D. brachyrhina*, *D. oporina* (Courtney 1994; Zheng et al. 2022), and the two new species in this study. In addition, *D. brachyrhina* and *D. oporina*, which are considered sister to the remaining Deuterophlebiidae species, also have their distributions restricted to the Himalayas



**Figure 11.** Distribution of Asian *Deuterophlebia* spp.

(Courtney 1994). This could indicate a Himalayan origin of the genus and family.

However, there are two issues with this hypothesis. One is that the formation of the Himalayas was much later than the origin of early dipteran members. The former event happened about 50 million years ago, but the fossils of early flies can already be found in Cretaceous ambers (about 100 million years ago) (Borkent and Grimaldi 2004; Poinar 2019). The emergence of Deuterophlebiidae, which is possibly the earliest extant fly lineage, can be traced back to the Triassic period (Wiegmann et al. 2011; Zhang et al. 2022). Additionally, the absence of species in Europe remains unexplained. In contrast, the Nearctic realm, which is much farther to the Himalayas than to Europe, hosts six species (Courtney 1994; Zheng et al. 2022).

In conclusion, the possibility of a Himalayan origin is low. At the present time, the only thing we can be sure of is that the Himalayas and its surroundings are one of the speciation centers of *Deuterophlebia* because it harbors the highest diversity and the most plesiomorphic species of the genus. Western North America with six species is another center (Courtney 1990, 1994). A plausible explanation of the Himalayan diversity of *Deuterophlebia* is the unique geography here. Those rapidly raised mountains and distinct climates created abundant streams and diverse habitats, coupled with the geographical barriers of the mountains and habitat changes, possibly accelerated the speciation of Himalayan mountain midges.

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