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A new *Neoleptophlebia* Kluge, 1997 species from eastern China (Ephemeroptera: Leptophlebiidae)

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Abstract

The genus *Neoleptophlebia* Kluge, 1997 includes five Asian species. Three of them were reported from northeastern Asia and two were found from Chinese Taiwan Island, leaving a huge geographic gap on the Chinese mainland. Here we find a new one, which is named *N. uncinata* Zhou **sp. nov.**, from Nanjing municipality, eastern China. Via field collecting and indoor rearing, all life stages were obtained, and its nymphs are found living in small creeks (with a width less than 1 m) and shallow waters (with a depth less than 30 cm). Diagnostically, the imago of this new species has larger lateral penial appendages than its congeners, and its nymph has subequal broadened segments II and III of maxillary and labial palpi. Biogeographically, this species bridges two northern and southern groups of the genus.

Key words: mayfly, taxonomy, phylogeny, new species, China

Introduction

The taxon *Neoleptophlebia* was established by Kluge in 1997 as a subgenus and was upgraded to a genus by Tiunova & Kluge in 2016 to include eleven species originally in *Paraleptophlebia* and *Leptophlebia* with Y-shaped gills and lateral penial appendages only. At that time, five Asian species and six Nearctic species were moved into this genus. However, all of them were named before that year, their exact morphology and positions need more researches and pictures to present.

Amongst five Asian species, two of them from Chinese Taiwan Island (*N. erratica* Kang & Yang, 1994 and *N. spina* Kang & Yang, 1994) are known from nymphs only. Additionally, *N. japonica* (Matsumura, 1931), *N. spinosa* (Uéno, 1931) and *N. vladivostokica* (Kluge, 1982) from northeastern Asia have similar nymphal morphology but different penial shapes. Therefore, additional species descriptions would not only increase species diversity but also potentially bridge the morphological gap between known species and provide a clearer definition of the genus.

Geographically, previous Asian species are distributed in two areas. One species was found in Russia (Kluge 1982; Potikha 2015; Tiunova & Kluge 2016), another one in Korea (Bae & Andrikovics 1997), Mongolia (Soldán *et al*. 2009) and northeastern China (Quan *et al*. 2002), two in Japan (Kawai & Tanida 2005), the northeastern part of Palearctic. The other two species are found on the Chinese Taiwan Island (Kang & Yang 1994), a part of the Oriental realm. The most Chinese mainland has had no records so far. New findings of more species from this country will also contribute to the distribution of the genus *Neoleptophlebia* and connection of the two groups.

The genus *Neoleptophlebia*, whose nymph has shallowly and relatively broad bifurcated gills but imago has no costal projection of hindwings, was regarded as plesiomorphic in the clade Leptophlebiinae (Kluge 1997; Tiunova & Kluge 2016). Compared to the related genera *Paraleptophlebia* and *Leptophlebia*, it was also defined by primitive traits (Tiunova & Kluge 2016). In our view, the species of the family Leptophlebiidae usually differ in very fine and unnoticeable characters, especially in nymphs. So more species with real illustrations will provide more details of the species and genus, which will help to find some apomorphies of the genus, and to benefit their generic phylogeny and evolutionary trends too.

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There is just one record of the genus *Neoleptophlebia* from Chinese mainland and two species from Taiwan Island (Kang & Yang 1994; Quan *et al*. 2002). In recent years, thousands of leptophlebiid specimens have been gathered in China. Among them, a species with shallow clefted gills was found in and around Nanjing city, eastern China. After careful comparison to congeners, it is recognized as a new *Neoleptophlebia* one with remarkable appendages on male penis. This finding provides an opportunity to enhance the diversity of Chinese leptophlebiids, to accurately describe the characteristics of the genus, and to deepen our understanding of this genus and family.

Materials and Methods

Nymphs were collected from the creek by hand screen. Imagoes and subimagoes were obtained by rearing in lab (mature nymphs were reared in plastic tray, which is covered by a small net) except one was obtained by sweep net. All materials used in this study were stored in ethanol (about 85%).

Specimens were examined under a stereomicroscope (Nikon SMZ 745T). Habitus of nymphs and winged stages were photographed by a SONY a7R II camera with a LAOWA 25mm 5× macro lens. Details of mouthparts and legs were studied by dissection and observed and photographed with a camera (Nikon 50i) mounted on a microscope. Eggs were dissected from female imagoes. The SEM (scanning electronic microscope) samples were prepared with a standard protocol: fixed in 4% glutaraldehyde for 5–8 hours, rinsed with PBS (physiological saline) 2–3 times (10–15 minutes each), dehydrated in concentration gradient acetone (30%, 50%, 70%, 80%, 90%, 100%, 10 to 15 min each), and coated with gold film in a vacuum.

Compared species: *Neoleptophlebia japonica* (Matsumura, 1931): 4 male imagoes, 1 male subimago, Nagano Prefecture, Japan, 137°59’12.9’’E, 36°13’14.6’’N, 600 m alt., 14.VI.2012, Chang-Fa Zhou.

All specimens used in this study are deposited in the Mayfly collection, College of Life Sciences, Nanjing Normal University (NNU), China.

Neoleptophlebia uncinata Zhou, sp. nov.

(Figs 1–7)

Material examined. H**olotype:** Male imago (reared from nymph), South Gate, creek nearby Parking Lot, Baohua Mountain, Zhenjiang City, Jiangsu Province, China, 8-15.III.2023, colls. Dewen Gong, Xuhongyi Zheng, Xinhe Qiang, Qingyi Yang. **Paratypes**: 7 male and 8 female imagoes (reared from nymphs), 10 nymphs, same place and data as holotype, colls. Dewen Gong, Xuhongyi Zheng, Xinhe Qiang, Qingyi Yang; 1 male imago, Yingtuo Village, Zijin Mountain, Nanjing City, Jiangsu Province, China, 3.IV.2006, colls. Dong Liu, Xin Liu, Xin Wang.

Description. Mature nymph (in alcohol): body length 6.0–8.0 mm, antennae 1.9–2.5 mm, cerci 4.7–5.3 mm, terminal filament 4.6–5.2 mm. Body color amber to dark brown, with some pale dots and stripes on different parts dorsally (Figs 1A–C).

Head generally brown, area between ocelli darker than others; areas between lateral ocelli and compound eyes slightly paler, area between clypeus and median ocellus with an oval pale portion or large dot (Figs 1A, F). Antennae with brown scape and pedicel, but flagella pale (Fig. 1F). Compound eyes dark; basal ocelli dark too and apices of them pale. Vertex smooth, posterior margin of head convex (Figs 1A, F).

Mouthparts: width of labium ca. 0.4x head; width: length 0.5:1.0; dorsal surface and free margins with dense hair-like setae; ventral surface with two sub-median hair-like setal tufts; front margin generally convex but with a shallow median notch (Fig. 2A). Mandibles with smoothly convex outer margins, basal half dorsal surface coarse, with relatively sparse and long hair-like setae (Figs 2D, F); both mandibles with a row of hair-like setae at median line of ventral surface respectively (Fig. 2H); a row hair-like setae on mesal margin of right mandible (Fig. 2F); outer incisor of left mandible divided into four apical denticles, inner one with three; prostheca composed with a distinct spine and a tuft of tine spine (Fig. 2E). Outer incisor of right mandible divided into three apices, inner one with two; prostheca divided into lots of tine spine (Fig. 2G). Almost whole crown of maxillae with dense hair-like setae, a row of 5–6 comb-like setae near inner corner of galea-lacinia; crown wider than base of galea-lacinia; the latter with two rows of feather-like setae on mesal margin; outer margins of stipes and cardo with very sparse and tiny hair-like setae (Figs 2I, J); length ratio of three segments of maxillary palpi from base to apex 1.37: 1.14: 1.00. Free margins of basal segment convex, outer one with a row of spine-like setae, inner margin with a row of hair-like

setae (Fig. 2I); apex of segment II wider than base, apical half inner margin with hair-like setae; segment III near triangular in shape, whole surface with relatively long and dense hair-like setae, especially outer margin (Fig. 2I). Glossae and paraglossae of labium with dense hair-like setae on both surface; width of paraglossae ca. 2.0x glossae; length ratio of segments of labial palpi from base to apex 1.68: 1.00: 1.16; segment I much stronger than others, its inner margin expanded greatly; outer margin with spine-like setae, inner margin with hair-like setae; segment II with relatively long hair-like setae on outer margin only, base of it much narrower than apex; segment III nearly triangular, with spine-like setae on whole surface (Fig. 2C). Superlinguae of hypopharynx with dense hair-like setae on surface but without on slightly concave outer margins; lingua with tiny hair-like setae only (Fig. 2B).

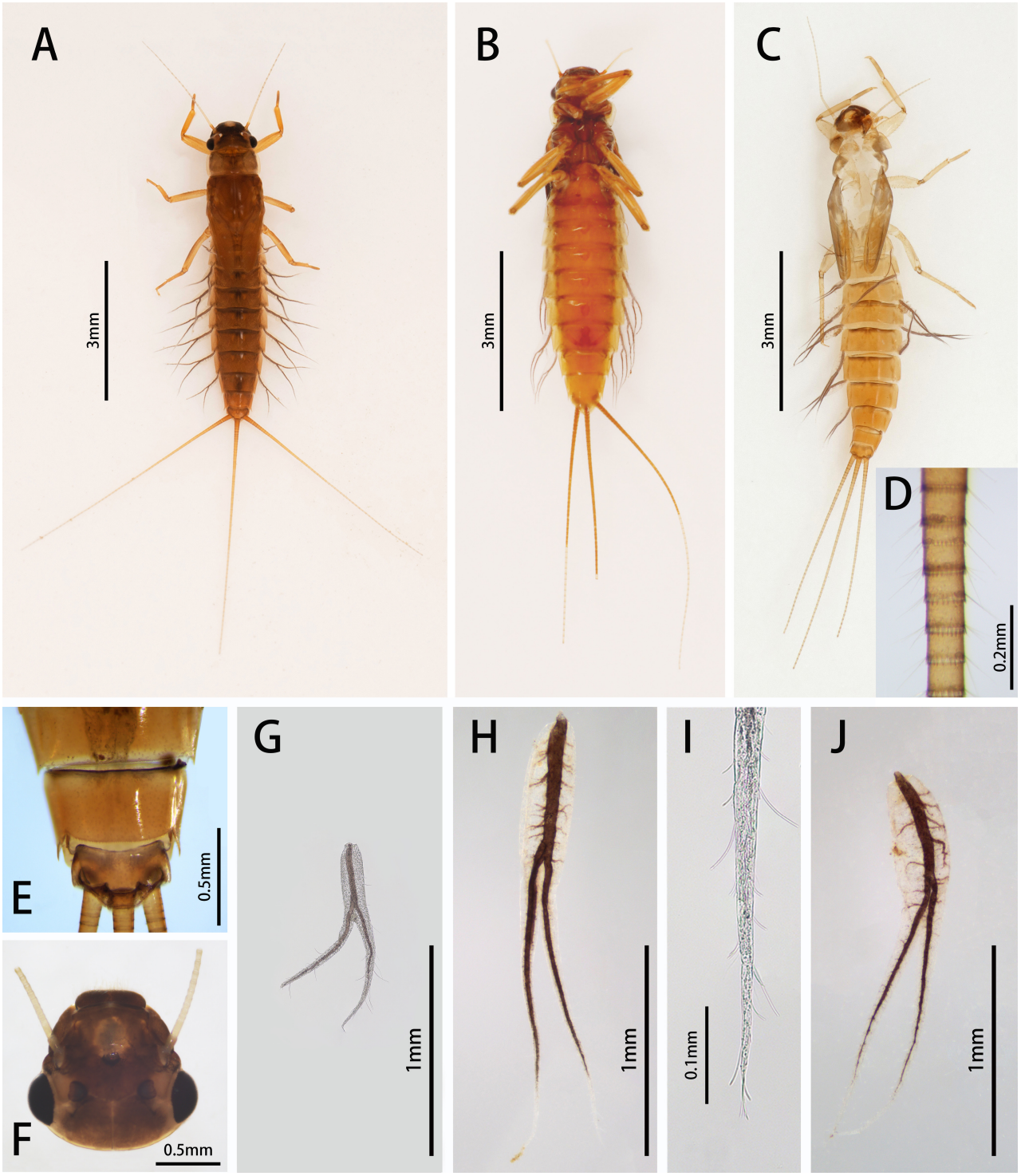

FIGURE 1. Nymphal structures of *Neoleptophlebia uncinata* **sp. nov.**: (**A**) habitus (dorsal view); (**B**) habitus (ventral view); (**C**) nymphal exuviate of holotype (dorsal view); (**D**) terminal filament enlarged; (**E**) terga VIII–X (dorsal view); (**F**) head (dorsal view); (**G**) gill I; (**H**) gill IV; (**I**) gill IV enlarged (showing marginal setae); (**J**) gill VII.

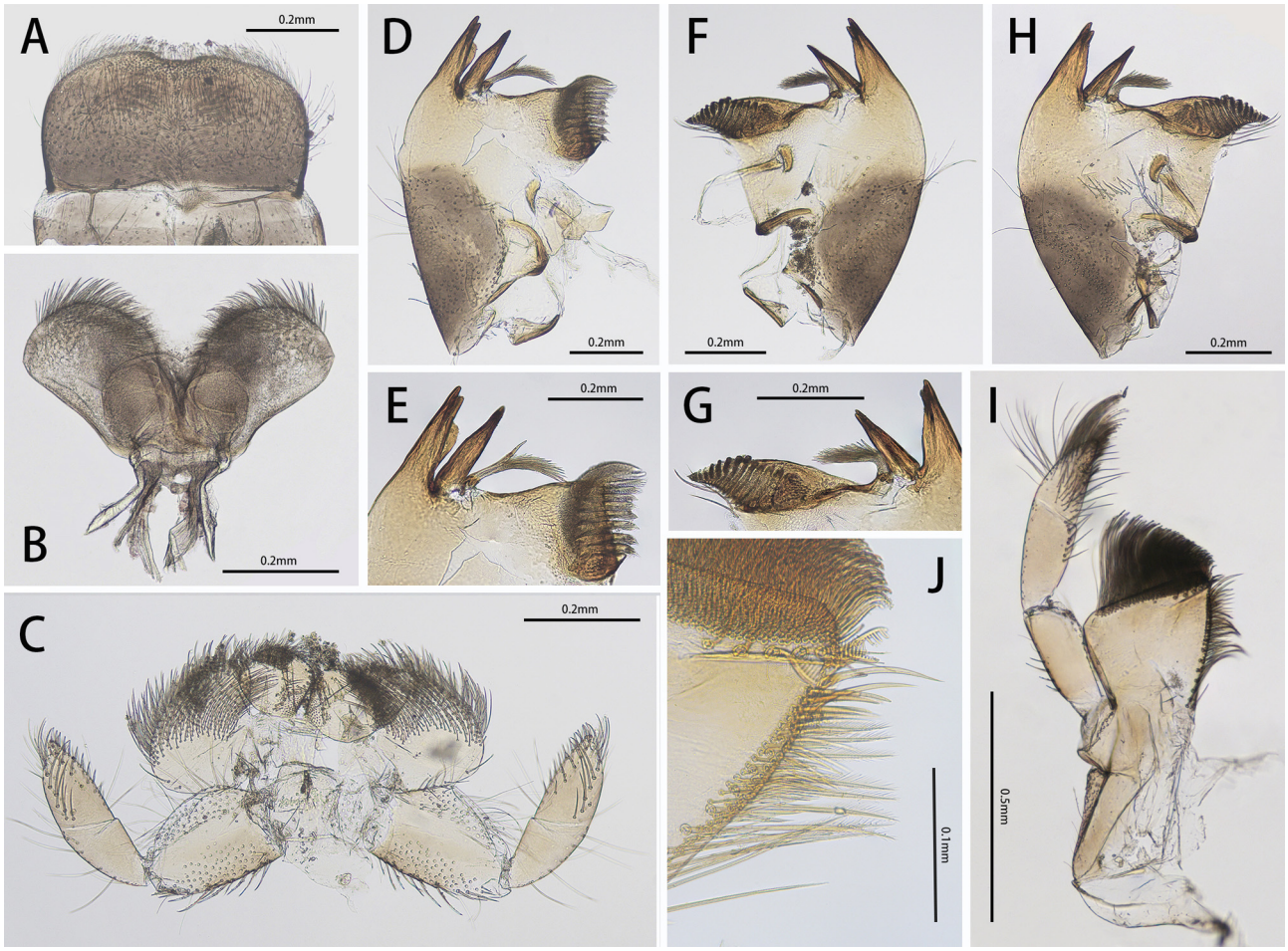

FIGURE 2. Mouthparts of *Neoleptophlebia uncinata* **sp. nov.**: (**A**) labrum (dorsal view); (**B**) hypopharynx (dorsal view); (**C**) labium (dorsal view); (**D**) left mandible (dorsal view); (**E**) left mandible enlarged; (**F**) right mandible (dorsal view); (**G**) right mandible enlarged; (**H**) right mandible (ventral view); (**I**) left maxilla (dorsal view); (**J**) galea-lacinia of maxilla (showing canines and dentisetae).

Pronotum amber to brown, its width slightly wider than head, with pale lateral margins, midline pale too, median area slightly darker than lateral areas; mesonotum slightly darker than pronotum, with two dark and two pale indistinct dots at wingpad base respectively; scutella of mesothorax and metathorax pale (Fig. 1A). Legs deep yellow to amber, joints darker than other parts, width of coxae ca. 3.0x trochanter (Figs 3A–C). Trochanter and femora of foreleg with spatulate-like setae on surface (Fig. 3D), tibiae and tarsus with spine-like setae on inner margins only, length ratio three segments of forelegs 1.75: 1.37: 1.00 (Figs 3A, E). Setal pattern of midlegs similar to forelegs except it with an additional transverse spine-like setae on tibiae apex; length ratio of three segments 2.00: 1.46: 1.00 (Figs 3B, G). Hindlegs similar to midlegs but its femora with sparser setae but tibiae with additional spine-like setae on outer margin; length ratio of three segments 2.01: 1.70: 1.00 (Fig. 3C). Midlegs and hindlegs with patellar-tibial fusion sutures (Figs 3B, C, F). Claws of all legs similar, with a row of denticles (16–23) from base to near apex (Fig. 3H).

Abdomen: terga amber to dark brown, usually with a pale midline and a pair of submedian pale dots respectively; lateral portions near margins pale (Fig. 1A). Posterolateral angles of terga VIII–IX extended into sharp projections (Fig. 1E); posterior margins of each tergum with a row of transverse denticles (Fig. 1E). Sterna uniformly amber to brown (Fig. 1B). Gills I–VII similar, margins with tiny hair-like setae (Fig. 1I). Gills I smaller than others, common base ca. 0.4x gill length; with dark trachea but without distinct tracheal branch (Fig. 1G). Gills II and VII slightly smaller than gills III–VI, all of them with main trachea and tracheal branches (Figs 1H–J). Common base of them ca.0.4x gill length (Fig. 1H). Cerci and terminal filament amber to brown, with rows of hair-like setae and tiny triangular denticles between articulations (Figs 1A,D).

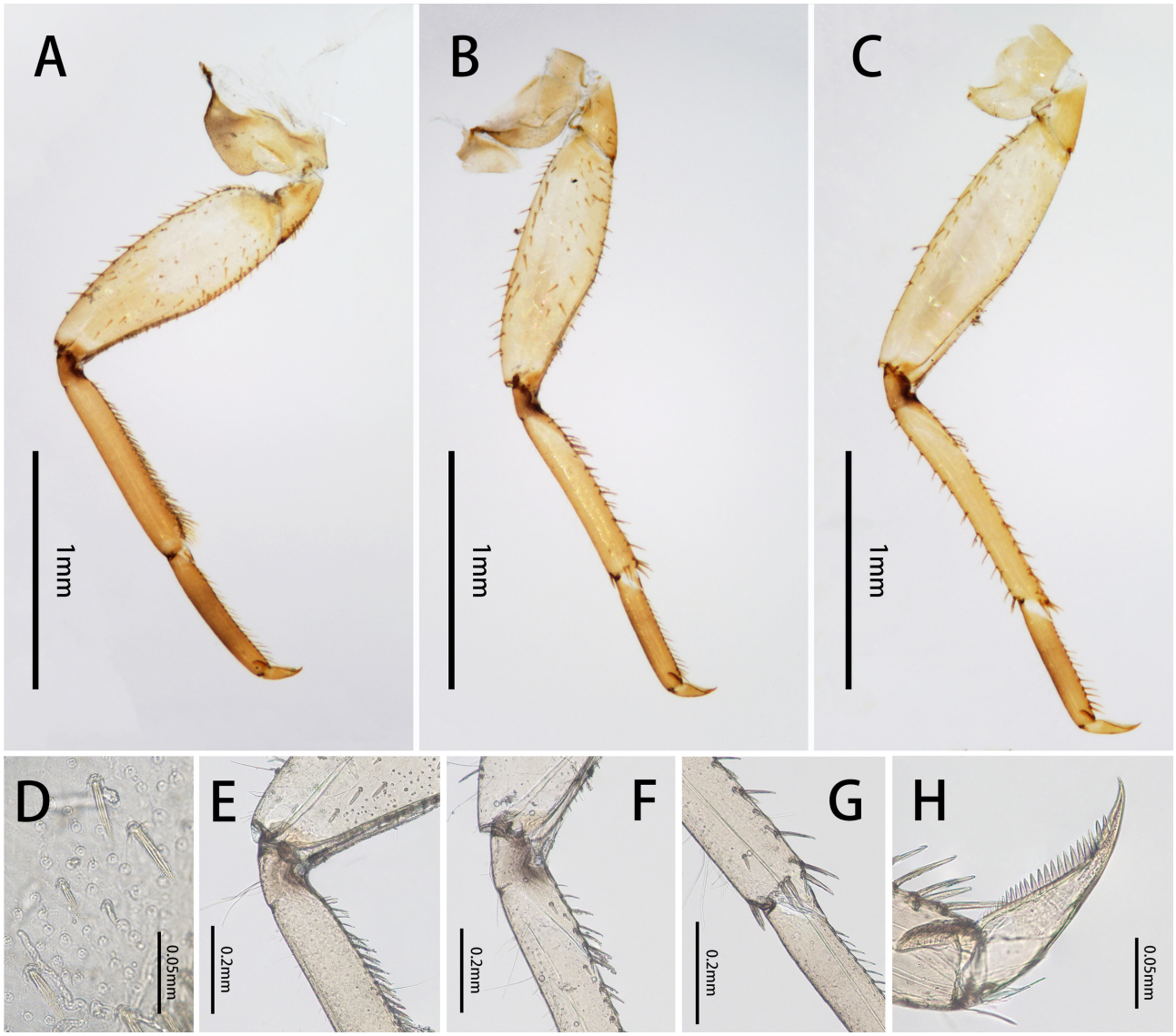

FIGURE 3. Nymphal legs of *Neoleptophlebia uncinata* **sp. nov.**: (**A**) foreleg; (**B**) midleg; (**C**) hindleg; (**D**) forefemur enlarged (showing spatulate-like setae); (**E**) proximal part of foretibia; (**F**) proximal part of midtibia (showing patellar-tibial fusion suture); (**G**) distal part of midtibia; (**H**) midleg claw.

Male imago (in alcohol): body length 9.0–9.5 mm, caudal filaments 7.5–8.0 mm, forewings 8.6–9.0 mm, hindwings 2.3–2.5 mm. Generally body chocolate to dark brown (Figs 4A, 6A–C).

Head chocolate but with pale anterior margin; ocelli with dark base and pale apex; compound eyes clearly divided into dark basal half and reddish brown upper half, the latter semispherical; distance between two eyes less than median ocellus (Fig. 5F).

Thorax chocolate to dark brown; wings transparent except very base with stains (Figs 4A, 6A–C). Veins of forewings amber, crossveins of posterior half more indistinct than other half; bullae of Sc, Rs distinct; MP forked slightly more basally than Rs, MA forked near middle point; all of them forked near symmetrically; 4–5 intercalaries between CuA and CuP; outer margin sclerotized (Fig. 6A). Hindwings near oval in shape, maximum length: width 2.0: 1.0; a slightly concave recession at the middle of anterior margin; MA single, MP forked at basal 1/3 symmetrically. Outer margin sclerotized too (Figs 6B, C). Legs brown to dark brown, joints darker than others; length ratio of femora: tibiae: tarsi of forelegs 1.00: 1.20: 1.55, length ratio of five segments of foretarsi 1.00: 12.67: 11.62: 9.11: 3.40. Ratio of midlegs 1.00: 1.07: 0.45, segment IV of midtarsi slightly longer than segment I-III; Ratio of hindlegs 1.00: 1.07: 0.40; tarsal segments similar to midlegs (Figs 5G–I). Claws of all legs similar, with two claws respectively, one blunt, one acute (Fig. 5J).

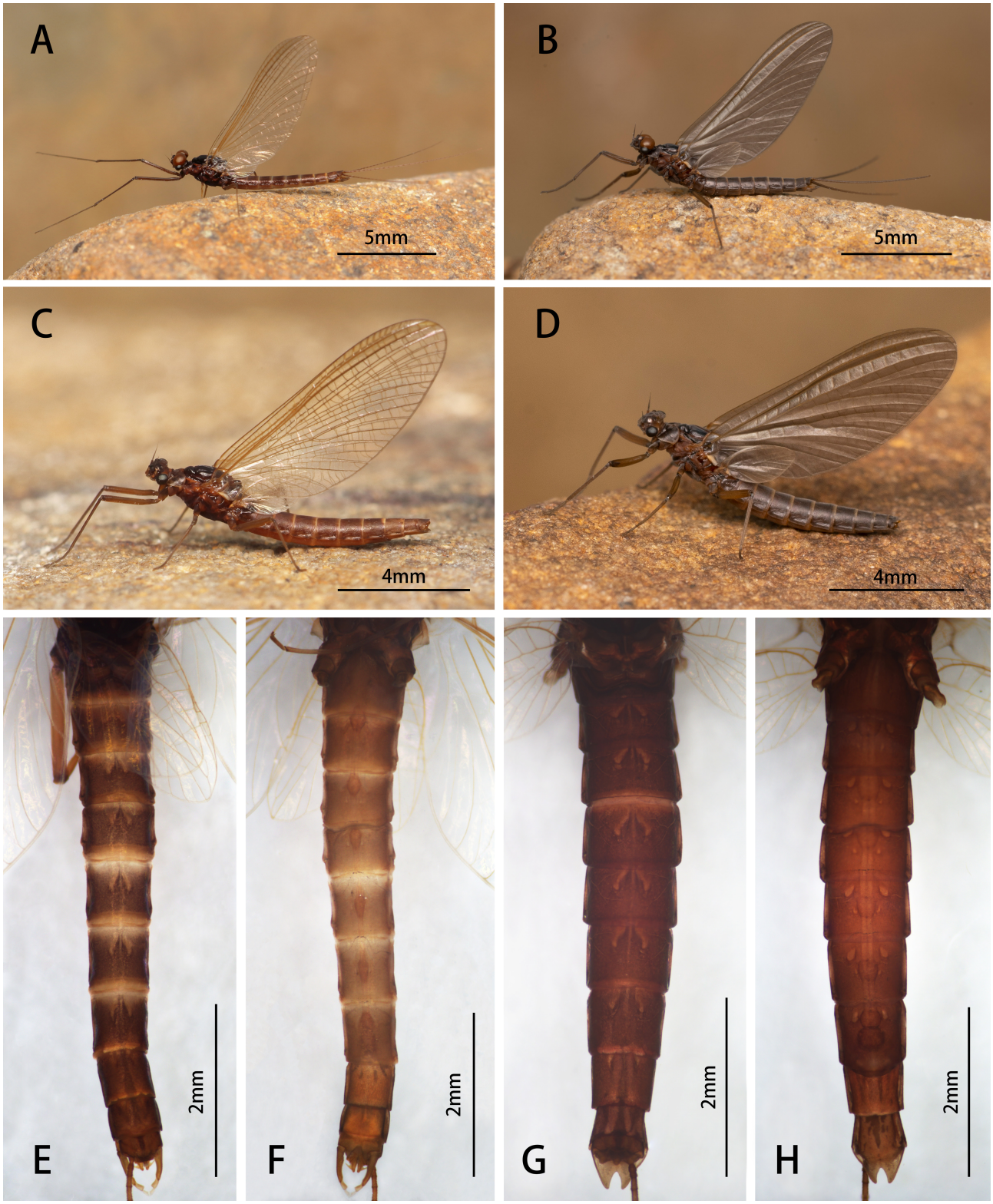

Figure 4. Adults of *Neoleptophlebia uncinata* **sp. nov.**: (**A**) male imago (habitus); (**B**) male subimago (habitus); (**C**) female imago (habitus); (**D**) female subimago (habitus); (**E**) male abdomen (dorsal view); (**F**) male abdomen (ventral view); (**G**) female abdomen (dorsal view); (**H**) female abdomen (ventral view).

Abdomen generally dark brown to dark, area between tergites pale; pale midline very narrow, companied by a pair of oblique pale short stripes on each tergites (Fig. 4E). Sternites reddish brown (Fig. 4F). Genitalia: subgenital plate deeply forked into two triangular lobes, surface with tiny hair-like setae (Fig. 5D); basal half of segment I of forceps wider than apical half, its length ca. 2.3x combined length of segments II–III; segments II slightly longer

and wider than segments III, each length of them ca. 2.0x width (Figs 5A, C). All segments with numbers of tiny projections on inner margins and very tiny hair-like setae on outer margins (Fig. 5C). Gonopores on lateral penes, each with a spear or hook-like projection oriented downward laterally (Figs 5A, E); basal half of penes about 2.0x apical half in width (Fig. 5E). Three caudal filaments brown, subequal in length (Fig. 4A).

Male subimago (alive): body darker than male, near dark brown (Fig. 4B). Tibiae covered with tiny cilia or microtrichia while free segments of tarsi covered with triangular scales or pointed microlepides.

Female imago (in alcohol): body length 9.0–9.2 mm, forewings 8.3–8.5 mm, hindwings 2.1–2.3 mm; color pattern similar to male (Figs 4C, 6D–F). Color pattern similar to males, compound eyes semi-spherical, grey to dark, at posterolateral corners of head; posterior margins of head slightly concave (Fig. 5L). Length ratio of femora: tibiae: tarsi of forelegs 1.57: 1.82: 1.00, ratio of midlegs 2.24: 2.48: 1.00, ratio of hindlegs 2.24: 2.63: 1.00. Length ratio of four segments of foretarsi 1.33: 1.19: 1.00: 1.27, ratio of four segments of midtarsi = 1.33: 1.25: 1.00: 2.23, ratio of four segments of hindtarsi 1.32: 1.14: 1.00: 2.39. Abdomen chocolate to dark, subanal plate forked deeply. Subgenital plate tongue-like, semi-circular; subanal plate narrowed progressively, with deep fork (Figs 4G, H, 5K).

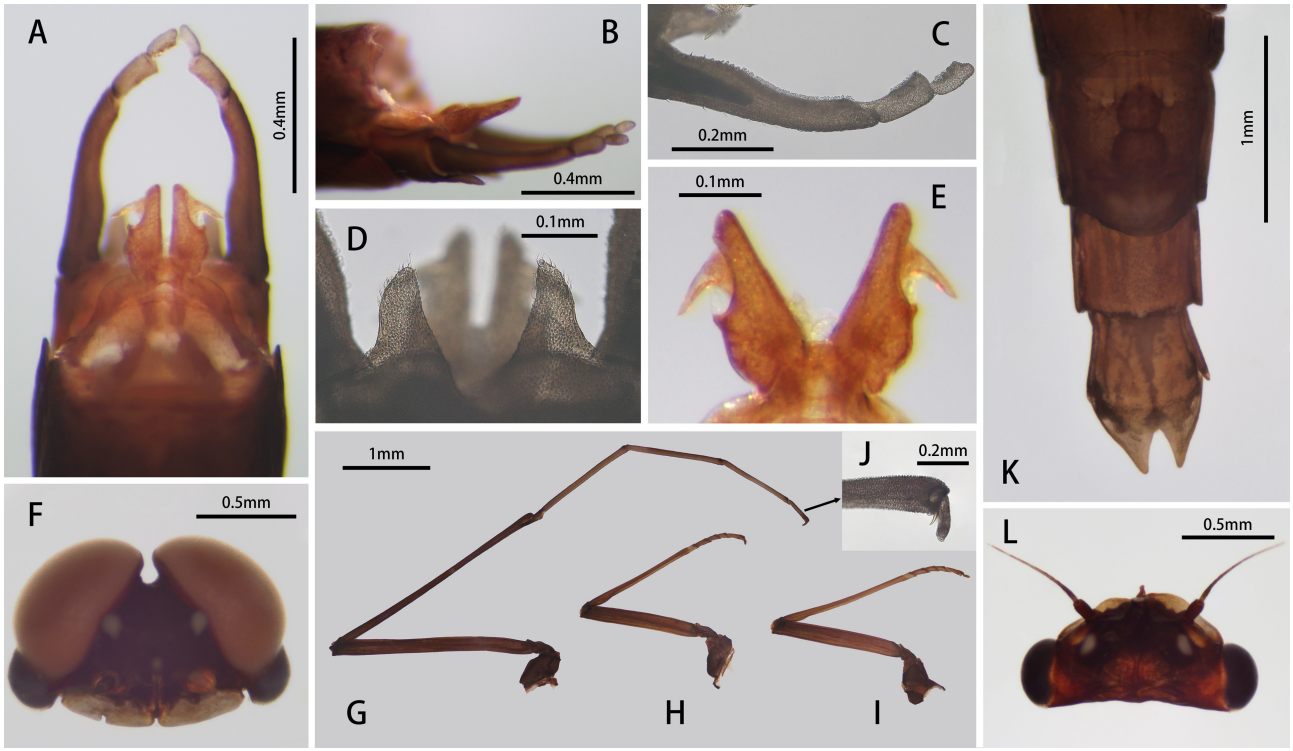

FIGURE 5. Adult structures of *Neoleptophlebia uncinata* **sp. nov.**: (**A**) male genitalia (dorsal view); (**B**) male genitalia (lateral view); (**C**) male forceps (lateral view); (**D**) male subgenital plate (ventral view); (**E**) male penes enlarged (ventral view); (**F**) male head (anterior view); (**G**) male foreleg; (**H**) male midleg; (**I**) male hindleg; (**J**) male foreleg claw; (**K**) female terminal abdomen (ventral view); (**L**) female head (dorsal view).

Female subimago (in alcohol): body length 8.5–8.8 mm, forewings 8.0–8.2 mm, hindwings 2.1–2.3 mm. Darker than female (Fig. 4D). Length ratio of femora: tibiae: tarsi of forelegs 1.44: 1.79: 1.00, ratio of midlegs 2.07: 2.31: 1.00, ratio of hindlegs 2.38: 2.63: 1.00. Length ratio of four segments of foretarsi 1.38: 1.21: 1.00: 1.24, ratio of four segments of midtarsi 1.22: 1.03: 1.00: 1.91, ratio of four segments of hindtarsi 1.24: 1.10: 1.00: 2.30. Abdomen chocolate to dark, subanal plate forked.

Egg: length 150–170 μm, width 70–90 μm, elongated oval; covered with dense small projections (Figs 7A, B).

Etymology: specific name "*uncinate*" comes from the Latin word "*uncinatus*", meaning "hook" or "hook shape", indicating the distinct projections of its penes.

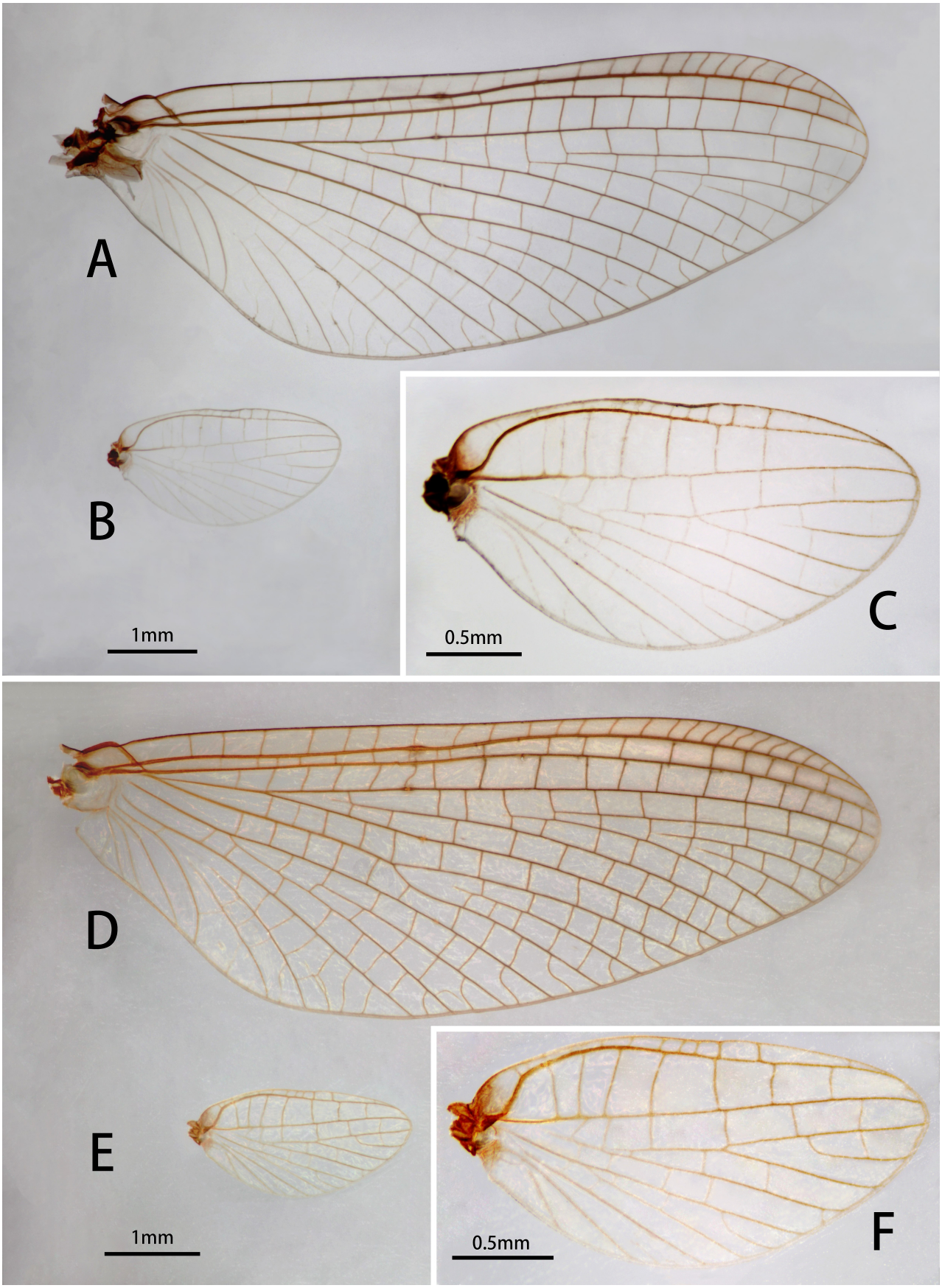

FIGURE 6. Wings of *Neoleptophlebia uncinata* **sp. nov.**: (**A**) forewing of male; (**B**) hindwing of male; (**C**) male hindwing enlarged; (**D**) forewing of female; (**E**) hindwing of female; (**F**) female hindwing enlarged.

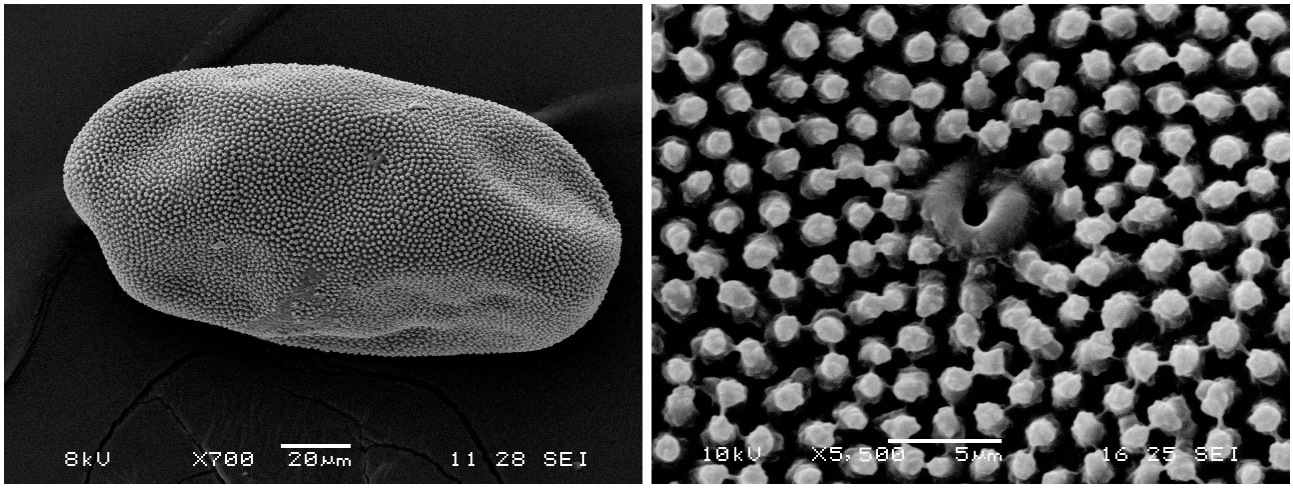

FIGURE 7. Egg of *Neoleptophlebia uncinata* **sp. nov.** (**A**) whole egg; (**B**) egg surface enlarged.

FIGURE 8. Habitat of *Neoleptophlebia uncinata* **sp. nov.** nymph (Baohua Mountain, Jiangsu, eastern China).

Biology

The nymphs were collected from mountain creeks (width less than 1 m, current less than 30 cm, velocity less than 20 cm/s) (Figure 8). They seem appear very early in spring. The earliest adult was recorded on 1st Match. The last instar nymph was observed to become subimagoes around 3:00 pm local time. The subimagoes can live 20 hours in our lab.

Diagnosis

Male imago: 1) body and legs generally brown to dark (Figs 4A, 5G); 2) penes with distinct lateral penial appendages, which is hook-like (Figs 5A, E); 3) the subgenital plate deeply forked (Fig. 5D). Female imago: 1) body and legs reddish brown to dark brown (Fig. 4C); 2) subgenital plate slightly expanded (Fig. 5I); 3) subanal plate deeply forked (Fig. 5I). Mature nymph: 1) maxillary palpi with broadened segments II and III (Fig. 2I); 2) labial palpi with broadened segments I–III, especially the segment I (Fig. 2C); 3) segments II and III of maxillary and labial palpi subequal in length (Figs 2C, I).

Discussion

Although the nymphal stages of leptophlebiid species among same genus are notoriously difficult to differentiate, the new species *Neoleptophlebia uncinata* **sp. nov.** described here has three diagnostic characters. The first one is its subequal segments II–III of maxillary and labial palpi. The second one is sharp posterolateral projections on tergites VIII–IX. The third is its color of gills and abdomen.

In contrast to two nymphal species *N. erratica* and *N. spina*, which have blunt posterolateral projections on tergites VIII–IX (Kang & Yang, 1994), our new species has sharp projections (Fig. 1E). Unlike the Russian *N. vladivostokica* (Kluge, 1982) (with un-branched tracheae), our new species have clear branches of main tracheae in gills (Figs 1G–J).

The nymphs of our new species are very similar to those of Japanese *N. japonica* or *N. spinosa*. Both Gose (1980) and Kawai & Tanida (2005) used tiny characters to key the latter two species, so we assume they are very similar to each other. Two features are adopted here to differentiate our new species *Neoleptophlebia uncinata* **sp. nov.** from two Japanese congeners. (1) The maxillary palpi of our new species are slightly broader than those of *N. japonica* or *N. spinosa* (Fig. 2I). (2) The abdominal midline of *N. japonica* usually have pale dots while tergites of our new species have very narrow pale midline and a pair of submedian stripes (Fig. 1A). Uéno (1959) reported the posterior part of pronotum *N. japonica* is slightly narrowed, but this point was not mentioned by Gose (1980) or Kawai & Tanida (2005).

The male imago of this new species is extraordinary in terms of its penes: they have remarkable lateral appendages with spear or hook-like shape (Figs 5A, E). The similar penes can be found in the males of *N. japonica* (see Kawai & Tanida, 2005), but the latter has lateral appendages near apex of penes in contrast to subapical ones of the *N. uncinata* **sp. nov.**. In addition, the subgenital plate of our new species is more deeply forked (Fig. 5D) than the former species, the apical segment of forceps are slightly longer too (Fig. 5A). Furthermore, their abdominal colors are different either. The *N. japonica* has pale abdominal segments II–VI, other parts are nearly chocolate. Our new species is brown to dark, slightly more reddish than the former species (Figs 4A, E, F). Our new species is larger than the male *N. japonica*. Females of them are different in body size and color pattern too.

Another Japanese species *N. spinosa* has apical appendages on penes instead of subapical ones (see Gose, 1980; Kawai & Tanida, 2005). They can be diagnosed easily. The penes of *N. vladivostokica* are longer and slimmer than our new species. Its appendages are smaller and located on ventral penes (Kluge 1982). No north American species in the genus have similar penes of our new species except *N. assimilis* (Banks, 1914) (see Needham *et al*. 1935). But its color, body size and genitalia are close to those of *N. japonica*.

The eggs of our new species (Figs 7A, B) are similar to those of *N. japonica* and *N. spina* (Ishiwata *et al*. 2013; Kang & Yang, 1994). Those of *N. erratica* have various of ridges on surface (Kang & Yang 1994), which is dissimilar to the former three species.

Kluge (1997) and Tiunova & Kluge (2016) transferred five Asian species (*N. erratica*, *N. japonica*, *N. spina, N. spinosa, N. vladivostokica*) into the genus *Neoleptophlebia* and designated *N. japonica* (=*Paraleptophlebia chocolata* Imanishi, 1937) as the type species. Our new species *Neoleptophlebia uncinata* **sp. nov.** is very close to this type species in both nymphal and imaginal morphology. Our additional description and photos of the new species support the validity of the genus.

Kluge (1997) and Tiunova & Kluge (2016) regarded the shallowly forked single gills, hindwings without costal projection of *Neoleptophlebia* as plesiomorphic, making this genus without apomorphies in the lineage of *Leptophlebia*, *Neoleptophlebia* and *Paraleptophlebia*. In our opinion, the following three characters of the genus *Neoleptophlebia* can be seen as its apomorphies: (1) the broadened segments II–III of maxillary palpi (Fig. 2I).

(2) The modified labial palpi, especially the segment I of it (Fig. 2C). Compared to counterparts of *Leptophlebia* and *Paraleptophlebia*, those palpi are wider, shorter and stronger. (3) In the imaginal stage, the hindwings of *Neoleptophlebia* are slightly longer but narrower (Figs 6B, C, E, F) than those of *Leptophlebia* and *Paraleptophlebia* (see Peters & Edmunds, 1970).

Kluge (2022) reported the subimagoes of Leptophlebiinae have microtrichia on tibiae and first tarsal segment (fused with tibiae) but the left tarsal segments (unfused segments I–IV or original segments II–V) covered with pointed microlepides. Our new species supports this observation.

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