Dtsch. Entomol. Z. 70 (1) 2023, 1–11 | DOI 10.3897/dez.70.96986

PENSOFT

für Natur MUSEUM FÜR NATURKUNDE BERLIN

First nymph-imago association in *Polyploica* confirming the distribution of Euthyplociidae (Ephemeroptera) in China

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https://zoobank.org/2F0692D8-1056-4A39-AA08-5BE57E928360

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Academic editor: Susanne Randolf ♦ **Received** 1 November 2022 ♦ **Accepted** 12 January 2023 ♦ **Published** 26 January 2023

Abstract

In this study, the imagoes and subimagoes of *Polyplocia orientalis* Nguyen & Bae, 2003 (Ephemeroptera, Euthyplociidae) were reared from nymphs in the lab and described for the first time. Nymphal structures are also photographed and described. The imagoes of this species can be identified by having transparent wings (except costal and subcostal areas), sterna without regular markings and penis divergent in apical half and basal half fused. The K2P distance between newly-sequenced COI gene of Chinese materials and a stored Vietnamese one is 0.10. This report not only confirms the presence of the genus and family in China, but also provides the first nymph-imago association in the genus.

Key Words

aquatic insects, association, genetic identification, mayfly, new record

Introduction

The tropical and subtropical mayfly family Euthyplociidae, which has setose elongated mandibular tusks in nymphs and a distinctive wing venation in imagoes, plays a key role in the taxon Pinnatitergaliae *sensu* Kluge, 2000 or burrowing mayfly group. It links the Potamanthidae, Ephemeridae, Palingeniidae and Polymitarcyidae together, with their flat fossorial nymphs bearing tusks (like Potamanthidae), dense cross-veins of A_1 (like Ephemeridae), multiple intercalaries between CuA and CuP (like Polymitarcyidae) and deeply forked Rs and MA in most species (similar to Palingeniidae) (Kluge 2004). This group of mayflies was once placed as a subfamily in Polymitarcyidae (Lestage 1921; McCafferty 1991) and is currently recognised as the family Euthyplociidae (Edmunds and Traver 1954; Edmunds et al. 1976; Kluge 2004; McCafferty 2004). Gillies (1980) proposed the family to be closely related to the common ancestor of Ephemeroidea and Polymitarcyidae.

The family Euthyplociidae has eight genera and approximately 25 species in two subfamilies (Kluge 2004; Gonçalves and Peters 2016; Gonçalves et al. 2017; Gonçalves et al. 2020). The Afrotropical subfamily Exeuthyplociinae has two species in two genera. The Pantropical subfamily Euthyplociinae now includes four genera in the Neotropical Region: *Euthyplocia* Eaton, 1871 (two species), *Campylocia* Needham & Murphy, 1924 (six species), *Mesoplocia* Demoulin, 1952 (two species) and *Dasyplocia* Gonçalves et al., 2020 (one species), an African genus *Proboscidoplocia* Demoulin, 1966 with eight species and an Oriental genus *Polyplocia* Lestage, 1921 with four species (Ulmer 1939, 1942; Demoulin 1952, 1953, 1966; Elouard et al. 1999; Gonçalves et al. 2017; Gonçalves et al. 2020). In China, however, only an unnamed species was reported by Zhou et al. (2015), based on an immature nymph and there are no nominal species described.

Amongst those genera and species, most euthyplociid species have not been associated between imagoes and nymphs by rearing or molecular evidence. For instance, the genus *Dasyplocia* from Ecuador was established upon nymphs and a partially dissected subimaginal wing, but two species of six present in the genus *Campylocia*

have no report on their nymphs (Gonçalves et al. 2017). Similarly, amongst the four *Polyplocia* species, nymphs of *P. vitalisi* Lestage, 1921 and imagoes of *P. orientalis* Nguyen & Bae, 2003 are still unknown; *P. nebulosa* Gonçalves & Peters, 2016 and *P. campylociella* Ulmer, 1939 have descriptions of males and possible nymphs (Lestage 1921, 1924; Ulmer 1939; Demoulin 1952, 1966; Nguyen and Bae 2003; Gonçalves and Peters 2016). No *Polyplocia* species are described from reared or exactly associated imagoes and nymphs. In addition, recent research further proved that the study of this genus is far from complete (Gonçalves and Peters 2016).

In 2013, a very young *Polyplocia* nymph was collected in Yunnan Province, south-western China. It triggered a series of field investigations of this family in China. Consequently, in February 2022, we collected more than 30 nymphs of Euthyplociidae from a stream in a tropical rainforest in the same province of China. Later, a subimago was reared from the nymphs in the lab and more subimagoes and imagoes were collected at the same site. After careful examination and comparison, we recognise those specimens as *Polyplocia orientalis*, a species known from nymphs only. In this paper, detailed descriptions of imagoes and nymphs are given. They provide not only the first concrete nymph-imago association in the genus *Polyplocia*, but also the first nominal species record of the family in China and also provide some valuable biological information.

Materials and methods

Material examined

CHINA – 30 nymphs, Yunnan Province, Xishuangbanna Prefecture, Mengla County, Menglun Town, Xishuangbanna Botanical Garden, 21°56'8.63"N, 101°16'35.14"E, 200 m a.s.l., 9.II.2022, Xu-Hong-Yi Zheng, Long-Yi Chen, Tian-Yu Zhang leg.; 1 female subimago, collected from same locality and data, reared from nymph in laboratory, 20.III.2022; 3 male imagoes, same locality, 26.IV.2022, Jiao-Long Ai leg.; 1 female subimago, 1 female imago and 1 male imago, same locality, 12.VI.2022, Jiao-Long Ai leg.; 1 nymph, Yunnan Province, Xishuangbanna Prefecture, Mengla County, Nanben, 22°14'35.51"N, 100°35'29.04"E, 800 m a.s.l., 13.III.2013, Jie Zhang leg.

All specimens used in this study are stored in ethanol (about 85%) and deposited in the mayfly collection, College of Life Sciences, Nanjing Normal University. Nymphs were collected from a creek and imagoes were collected from trees near the creek by net. Specimens were examined under a stereomicroscope and photographed with a digital camera (Sony a7r2) with a Sony FE 90 mm macro lens. Eggs were dissected from the female imago and prepared with a standard protocol: fixed in 4% glutaraldehyde for 5–8 h, rinsed with PBS (physiological saline) 2–3 times (10–15 min each), dehydrated in concentration gradient acetone (30%, 50%, 70%, 80%, 90%, 100%, 10–15 min each), coated with gold film in a vacuum and photographed by a Scanning Electron Microscope (Apreo 2S, Thermo Fisher Scientific Company, Waltham, MA, USA). Specimens were identified by morphology, based on previous studies and descriptions (Demoulin 1952; Nguyen and Bae 2003; Kluge 2004; Gonçalves and Peters 2016). Terminology follows that of Kluge (2004) and Gonçalves and Peters (2016). The distribution map was produced using QGIS and the Natural Earth quick start kit from Natural Earth (https://www.naturalearthdata.com/).

Total genomic DNA of a nymph specimen was extracted from nymphal legs using Animal Genomic DNA Kit (TsingKe Biotech Co., Beijing, China). The mitochondrial genes of cytochrome c oxidase subunit I were PCR-amplified using the Premix Taq (Takara Bio Inc., Beijing, China) with forward primer F (5'–TTC AGC CAC TTT ACC GCG–3', see Hrivniak, Sroka, Godunko and Žurovcovà (2017)) and reverse primer HCO2198 (5'–TAA ACT TCA GGG TGA CCA AAA AAT CA–3', Folmer, Black, Hoeh, Lutz and Vrijenhoek (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 final extension at 72 °C for 10 min. The sequence was submitted to GenBank (accession number OP962407) and COI sequences of *P. orientalis* from Vietnam were obtained from GenBank (accession number OP347109.1). They were aligned using MUSCLE (Edgar 2004) and the K2P genetic distance were estimated in MEGA7 (Kumar, Stecher and Tamura 2016).

Results

***Polyplocia orientalis* Nguyen & Bae, 2003**

Polyplocia orientalis Nguyen & Bae, 2003: 280.
Polyplocia orientalis: Gonçalves and Peters 2016: 554.

Redescription of mature nymphs. See also Nguyen and Bae (2003). Body length 25.0–35.0 mm in males (n = 5), 35.0–40.0 mm in females (n = 5) (tusks included, caudal filaments excluded) (Fig. 1), mandibular tusks about 1/5–1/6 body length (Figs 1, 2A), body parts generally yellowish-brown to reddish-dark (Figs 1, 2A–K).

Head brownish-yellow, flattened, nearly trapezoidal, length subequal to width, slightly narrower than pronotum. Lateral ocelli pale, but with dark base, median ocellus indistinct; compound eyes round, at posterolateral corner of head, distance between compound eyes ca. 4× each eye width. Antennae around 10.0 mm (n = 5), longer than mandibular tusks, almost glabrous; scape and pedicel subequal in length, their length slightly larger than width; anterior margin of clypeus convex (Fig. 2A).

Mouthparts light yellow (Fig. 3A–H), except the reddish-brown mandibular tusks. Labrum slightly concave on anterior margin, with setae along anterior and lateral margins, those on anterior margin stouter and

Figure 1. Nymphal habitus of *Polyplocia orientalis* from China. Scale bar: 5.0 mm.

denser, also with a tuft of longer setae on dorsal surface (Fig. 3C). Anterolateral corner of mandibles elongated into tusks; tusks flattened, pointed at apex, 4.0–6.5 mm, ca. 3× the head width (n = 5), densely set with setae (Fig. 3F), divided into longer hair-like setae on both inner and outer margins which are longer near the base and stout setae on apical 1/3 of outer margin; 3–12 denticles set on outer margin of apical tusks (n = 5) (Fig. 3D); incisor and kinetodontium each with 4 and 3 blunt denticles, respectively on left mandible, incisor with 4 blunt denticles and kinetodontium with 2 sharp denticles on right mandible (Fig. 3A, B). Maxillae with hair-like setae on both medial and lateral margins, three canines sharp, a row of bristles present on top of crown near the canines (Fig. 3H). Maxillary palpi three-segmented, length ratio of segments I: II: III = 1.0: 1.0: 2.0, basal segment with setae on outer margin, apical two segments surrounded by long hair-like setae (Fig. 3H). Hypopharynx rounded, front margin concave, hair-like setae set sparsely along margins; superlinguae extended anterolaterally, with setae on margins, denser on the leading margin (Fig. 3E). Labium: glossae and paraglossae with hair-like setae on dorsal surface, ventral surface and margins, longer and denser on margins; paraglossae expanded laterally, glossae pointed ventrally apically; labial palpi three-segmented, 2.5: 1.0: 1.6 in length, long hair-like setae present on margins of basal and apical segment and outer margin of second segment; apical segment expanded, with a row of stout setae on anteromedial margin (Fig. 3G).

Pronotum reddish-brown with dark markings, subquadrate, lateral margins expanded slightly (Figs 1, 2A); anterolateral corners projected into acute points; lateral flange also extended into acute points (Figs 1, 2A). Mesonotum and metanotum reddish-brown, width subequal to pronotum (Fig. 1). Prolegs situated closely at prosternum, making the latter triangular (Fig. 1).

Legs yellowish-brown; foreleg longest while mid-leg shortest (Fig. 1). Length ratio of femora: tibia: tarsus = 1.0: 1.1: 0.6 in foreleg (Figs 1, 2D), 1.0: 1.0: 0.3 in mid-leg (Figs 1, 2C), 1.0: 0.7: 0.3 in hind-leg (Figs 1, 2B). Coxae and trochanters of all legs with hair-like setae on margins, mid-coxae with expanded supracoxal projections (Fig. 1). Femora of legs expanded, fore-femora and mid-femora densely covered with both short spine-like setae and long hair-like setae on margins and dorsal surface, hind-femora with hair-like setae on outer margin, but very sparse setae on inner margin and dorsal surface (Fig. 2B–D); hind-femora with subapical setae patch on ventral surface (Fig. 2G). Fore- and mid-tibiae with an apical, triangular, pyramidal spine or spur overlapping tarsi to about 1/3 of tarsal length (larger on fore-tarsi), margins with bristle-like setae (Figs 1, 2I); fore- and mid-tibiae with hair-like setae on both margins, some short spine-like setae at apex; hind-tibiae with sparse hair-like setae (Fig. 2B); mid-tibiae with clear patello-tibial suture. Fore-tarsi with a sharp apical projection covering claw, with hair-like setae on margins and dorsal surface, additional spine-like setae on margin and projection (Fig. 2H); mid-tarsi with hair-like setae and a row of short spine-like setae dorsally (Fig. 2C); hind-tarsi with sparse hair-like setae. Claws stout and hooked (Fig. 2H).

Abdomen dark brown with light markings, including a pale mid-line, two pairs of pale medial dots and two pairs of lateral pale dots on each tergum, median pairs of dots progressively shortening from anterior to posterior segments (Fig. 2J); sterna light to pale brown (Fig. 2K). A pair of gills on abdominal segments I–VII (Fig. 1); gills I light grey, smaller than others (less than 2 mm), divided into two lobes, dorsal lobe larger and expanded distally, about 1.5× length of ventral one (Fig. 2E); gills II–VII similar, light to dark grey, bifurcated at base, dorsal lamella slightly shorter than ventral lamella, both lamellae slender and fringed with hairs (Fig. 2F). Terminal filament sub-equal to body length, cerci ca. 3/5 of body length (Fig. 1), all with short spine-like setae on articulations and hair-like setae on both margins, progressively shorter from base to apex.

Description of male imagoes. Body length 16.0–18.0 mm (n = 3) (Fig. 4A). Head dark brown, compound eyes black, width between eyes subequal to eye width (Fig. 4B). Three ocelli white, but with dark basal band, rounded, medial one smallest. Antennae sub-equal to head

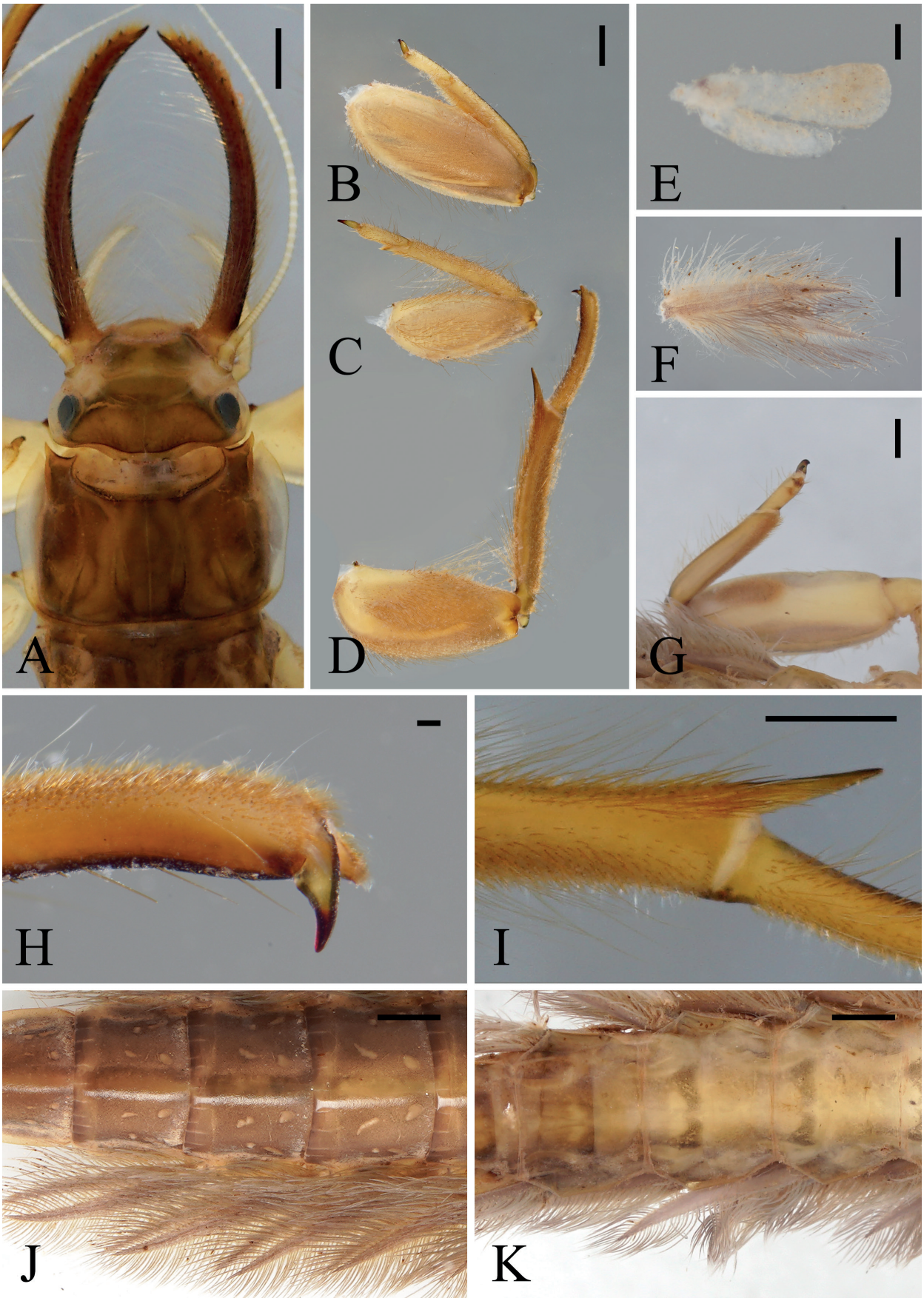

Figure 2. Nymph of *Polyplocia orientalis* from China **A** Head and prothorax; **B** Hind-leg; **C** mid-leg; **D** Foreleg; **E** Gill I; **F** Gill IV; **G** Hind-leg (ventral view); **H** Fore-tarsus and claw; **I** Apex of fore-tibia; **J** Abdomen (dorsal view); **K** Abdomen (ventral view). Scale bars: 1.0 mm (**A–D, F, G, I–K**); 0.1 mm (**E, H**).

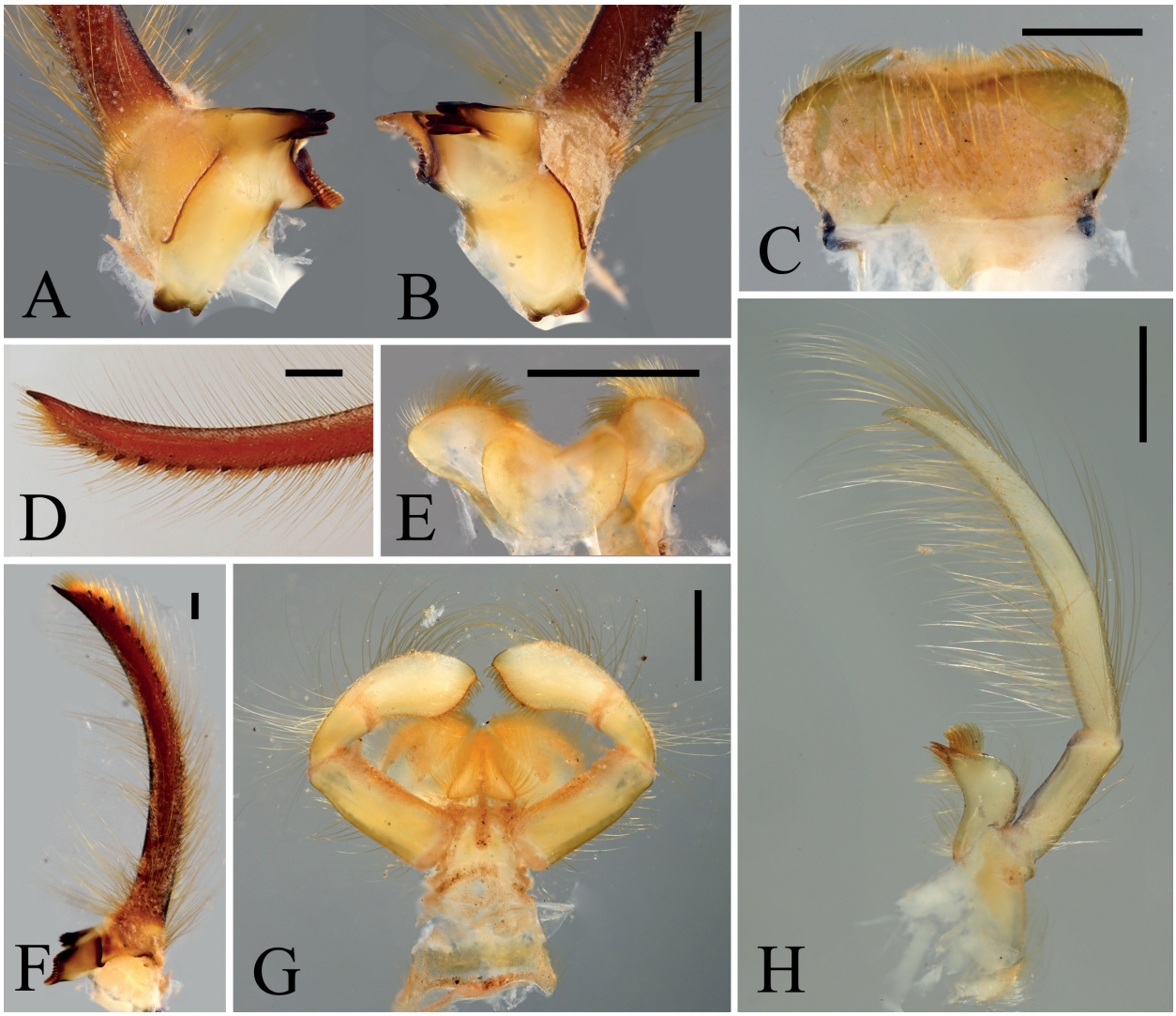

Figure 3. Nymphal mouthparts of *Polyplocia orientalis* from China **A** Right mandible (ventral view); **B** Left mandible (ventral view); **C** Labrum (dorsal view); **D** Apex of mandibular tusk; **E** Hypopharynx (ventral view); **F** Right mandible (dorsal view); **G** Labium (ventral view); **H** Left maxilla (ventral view). Scale bars: 0.5 mm.

width, scape and pedicel stout, dark brown, flagella filamentous, light brown (Fig. 4B). Pronotum yellowish-black with light markings, nearly trapezoidal in dorsal view, length sub-equal to width, lateral and posterior margins ridged, posterior one slightly wider than head (Fig. 4B). Mesonotum and metanotum yellowish-brown (Fig. 4B).

Forewings 19.0–22.0 mm, hindwings 7.0–8.0 mm (n = 3) (Fig. 5A–C). Forewings translucent, base, costal and subcostal areas tinged brown (Fig. 5A); longitudinal veins light to dark brown; forking point of MA at about 1/3 distance from base to margin, same level as fork of Rs; MP forked near base. Two longitudinal intercalaries (ICu) share a common stem attaching to CuP, some sigmoid veins originating from posterior ICu going to hind-margin; CuP and A_1 also with veinlets connecting them to hind-margin of wing (Fig. 5A). Hindwings hyaline, but with pigmented base; costal process round; Rs forked at about 1/3 distance from base to margin, MP forked at about 1/4 distance from base to margin, MA simple; anal region with some veinlets directed towards margin (Fig. 5B, C).

Legs grey to light brown with dark markings, femora of all legs with pale base, tarsi paler than tibiae (Fig. 6A–C). Forelegs sub-equal to body length, hind-legs longer than mid-legs (Fig. 6A–C). Femora: tibiae: tarsi = 1.0: 1.5: 1.3 in forelegs (Fig. 6A), 1.0: 1.1: 0.3 in mid-legs (Fig. 6B), 1.3: 1.0: 0.3 in hind-legs (Fig. 6C). Ratios of tarsomeres I: II: III: IV: V in forelegs = 1.0: 15.0: 12.5: 10.0: 7.0 (Fig. 4A); mid- and hind-tarsi four-segmented, length ratio of tarsomeres = 1.0: 1.0: 1.0: 3.0 (Fig. 6B, C). Claws paired, fore-claws lamellar-shaped, a very small hook present apically (Fig. 6D). Mid- and hind-claws similar, sharply hooked (Fig. 6E).

Abdomen grey to brown dorsally, with light markings as in larvae, two pairs of pale dots on each segment, lateral margins pale (Fig. 4B). Styliger plate yellowish-brown, posterior margin straight (Fig. 7A). Forceps yellowish-brown with a dark marking near apex, about 3.5 mm (n = 3), one segmented, slender and cylindrical, but flattened apically, about 4× length of penis (Fig. 7A, B). Penis yellowish with dark shading, flattened; basal 2/3

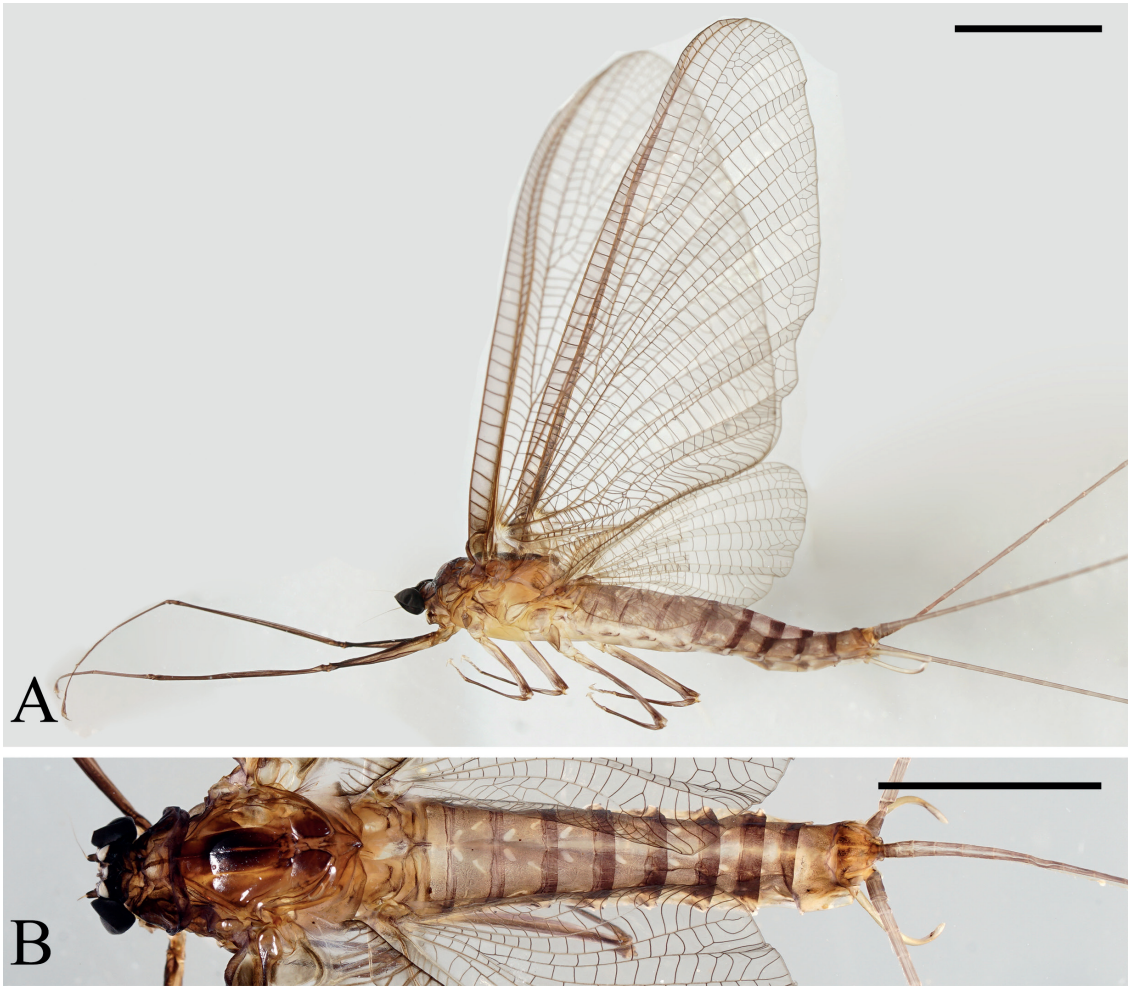

Figure 4. Male imago of *Polyplocia orientalis* from China: **A** Lateral view; **B** Dorsal view. Scale bars: 5.0 mm.

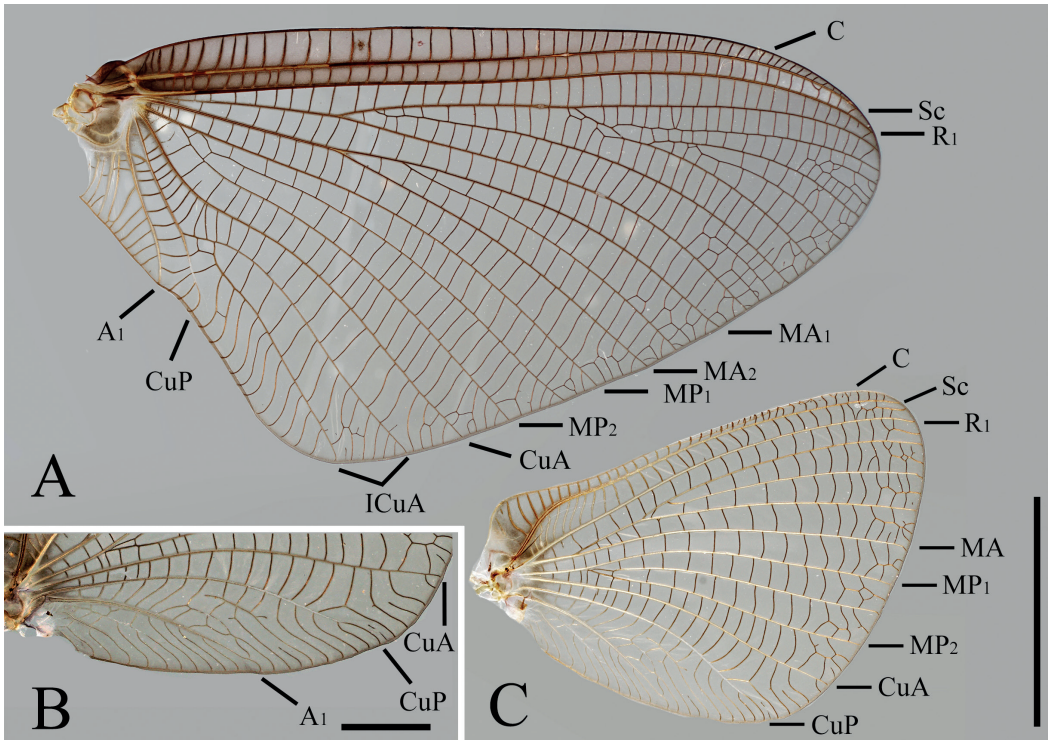

Figure 5. Wings of male *Polyplocia orientalis* from China: **A** Forewing; **B** Posterior area of hind-wing; **C** Hind-wing. Scale bars: 5.0 mm (**A, C**); 1.0 mm (**B**).

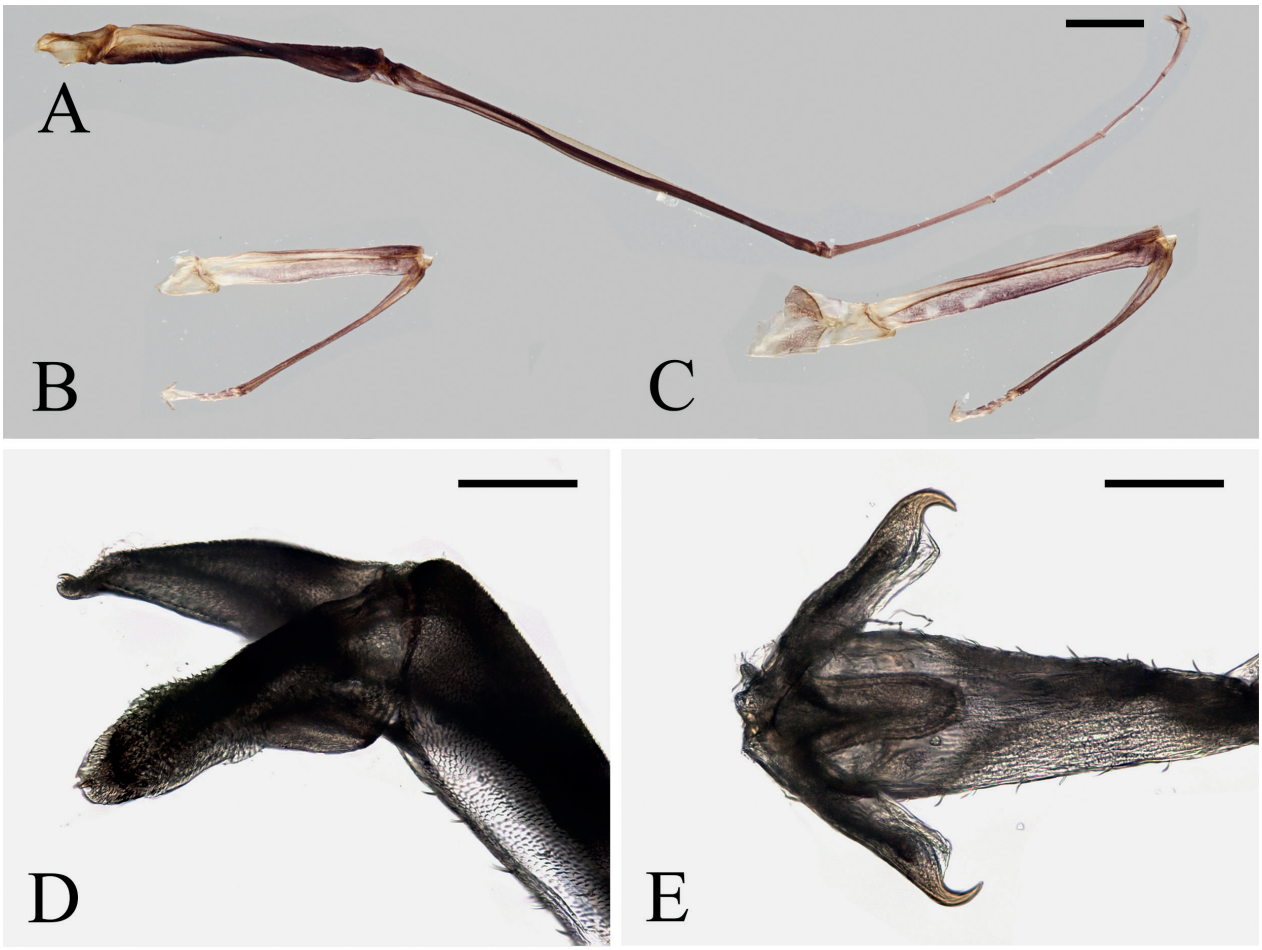

Figure 6. Legs of male *Polyplocia orientalis* from China: **A** Foreleg; **B** Mid-leg; **C** Hind-leg; **D** Claw of foreleg; **E** claw of mid-leg. Scale bars: 1.0 mm (**A–C**); 0.1 mm (**D, E**).

fused together, apical 1/3 divided with a V-shape cleft; each lobe has a deep apical depression and extended apico-laterally into beak-like structure, margins sclerotised (Fig. 7C, D). Caudal filaments grey brown, about 3× body length, some short setae on surface (Fig. 4A, B).

Description of female subimagoes. Body length 28 mm, similar to female imago, except dorsal surface and lateral margin of pronotum smooth, length/width of pronotum larger in female subimago than in imago, scutellum more protruded (Fig. 8A).

Description of female imagoes. Colour pattern, veins and other characters similar to male. Body length 26.0 mm (Fig. 8B). Head dark, compound eyes black, width between eyes about 2× width of each eye (Fig. 8B). Ocelli white, rounded, medial ocellus smallest. Pronotum black with light markings, corrugated in dorsal view. Mesonotum yellowish-brown, scutellum slightly protruded. No obvious sexual dimorphism on wings.

Legs brownish-grey, Femora: tibiae: tarsi = 1.0: 1.2: 0.8 in forelegs (Fig. 8C), 1.0: 1.0: 0.3 in mid-legs (Fig. 8D), 1.3: 1.0: 0.3 in hind-legs (Fig. 8E). Tarsomere I: II: III: IV: V in forelegs = 1.0: 4.0: 2.0: 1.5: 2.0 (Fig. 8C); length ratio of mid- and hind-tarsomeres = 0.7: 1.0: 1.0: 1.0: 2.8 (Fig. 8D–E). Claws paired, all claws similar, sharply hooked.

Abdomen dark brown dorsally, with light markings as in male imagoes, with four pairs of light dots and a light mid-line on each segment (Fig. 8B). Posterior margin of sternum VII and subanal plate smooth, not obviously protruding or cleft (Fig. 8B).

Description of eggs. Dissected from female imago, barrel-shaped, smooth without apparent polar cap or ridges (Fig. 9).

Distribution. China (Yunnan Province), Vietnam (Dak Lak Province, Lam Dong Province, Thua Thien Hue Province).

Diagnosis. The mature nymphs of *Polyplocia orientalis* are characterised by a large body (25.0–40.0 mm) and long mandibular tusks (4.0–6.5 mm) (Fig. 1), acute anterolateral apex of pronotum (Fig. 2A), uniformly pale abdominal sterna (Fig. 2K) and apically expanded dorsal lobe of gill I (Fig. 2E). Their imagoes can be easily recognised by transparent wings (Fig. 4A), smooth eggs in females (Fig. 9) and T-shaped male penis with apical depression on both lobes (Fig. 7A–D).

Gonçalves and Peters (2016) examined specimens of almost all known *Polyplocia* species and provided details of their comparison. We use their information as the basis of this comparative discussion.

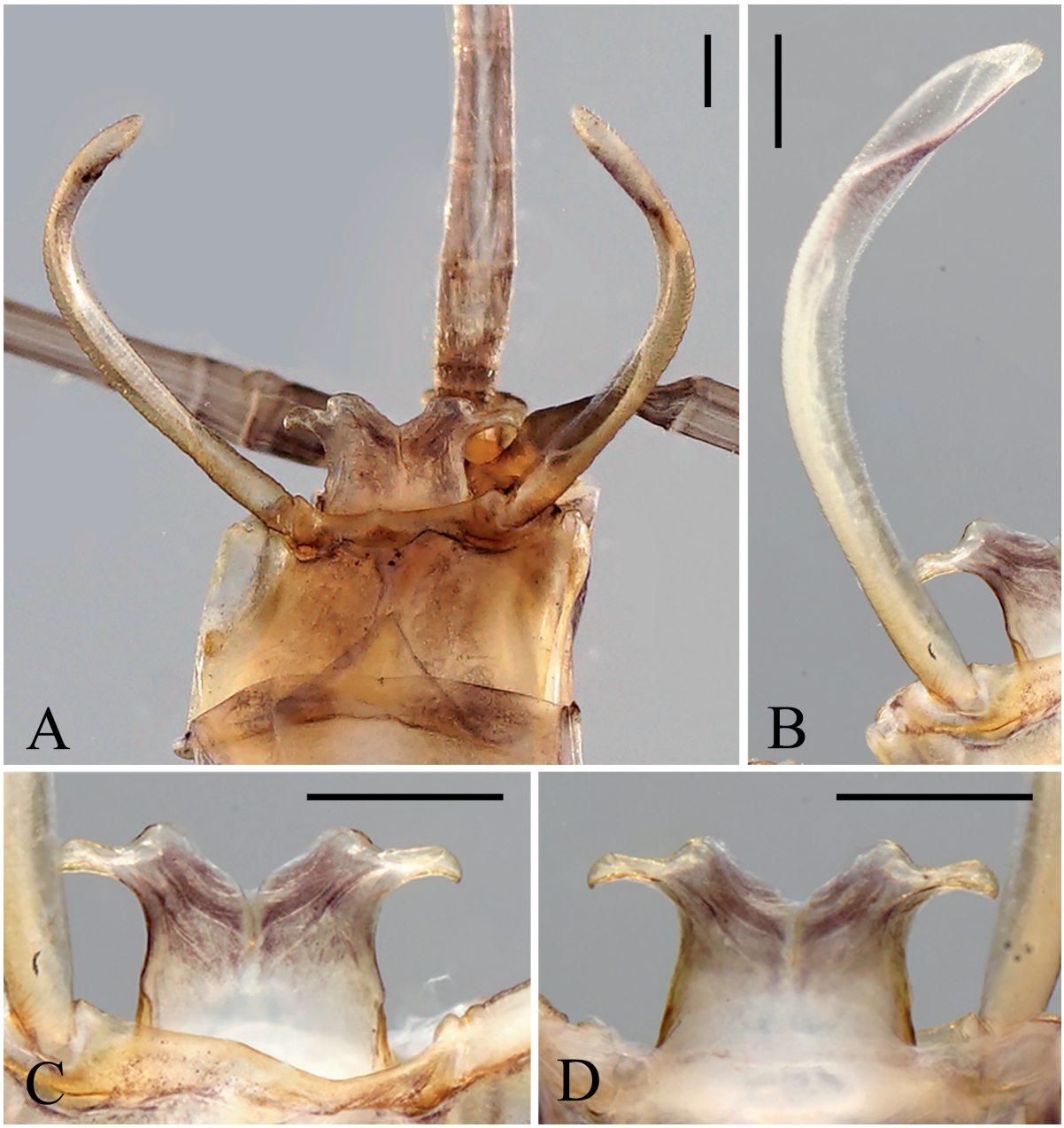

Figure 7. Genitalia of *Polyplocia orientalis* from China **A** Ventral view; **B** Detail of forceps (ventral view); **C** Penis (ventral view); **D** Penis (dorsal view). Scale bars: 0.5 mm.

The males of *P. orientalis* resemble *P. vitalisi* in most characters. Both have transparent wings (except costal and subcostal sections of forewings), two intercalaries between CuA and CuP, cross-veins without surrounding clouds (Fig. 5). There are two differences between them: 1) body size of the *P. orientalis* is 16.0–18.0 mm, while that of *P. vitalisi* is around 12 mm; 2) the penis lobes of *P. orientalis* are extended laterally and pointed with deeply concave lateral margins (Fig. 7), but the penis lobes of the *P. vitalisi* are almost straight.

Males of *P. campylociella* have penis lobes similar to *P. vitalisi*. However, the cross-veins of forewings are pigmented and the membrane has a brown tinge. It can be differentiated from *P. orientalis* easily.

The males of *P. nebulosa* have pigmented cross-veins, both the abdominal terga and sterna have distinct brown markings. Further, its penis lobes are almost entirely fused with a very shallow emargination between the two lobes. In contrast, the males of *P. orientalis*, described in the present study, have clear wings (Fig. 5), somewhat uniform abdominal color (except terga with two pairs of pale dots) (Fig. 4B) and slimmer penial lobes (Fig. 7).

Nymphs of *P. orientalis* and the possible nymph of *P. nebulosa* are very similar to each other. Only three points are found to separate them. First, in mature nymphs, the lateral margins of the pronotal flanges of *P. nebulosa* are almost straight and parallel to the

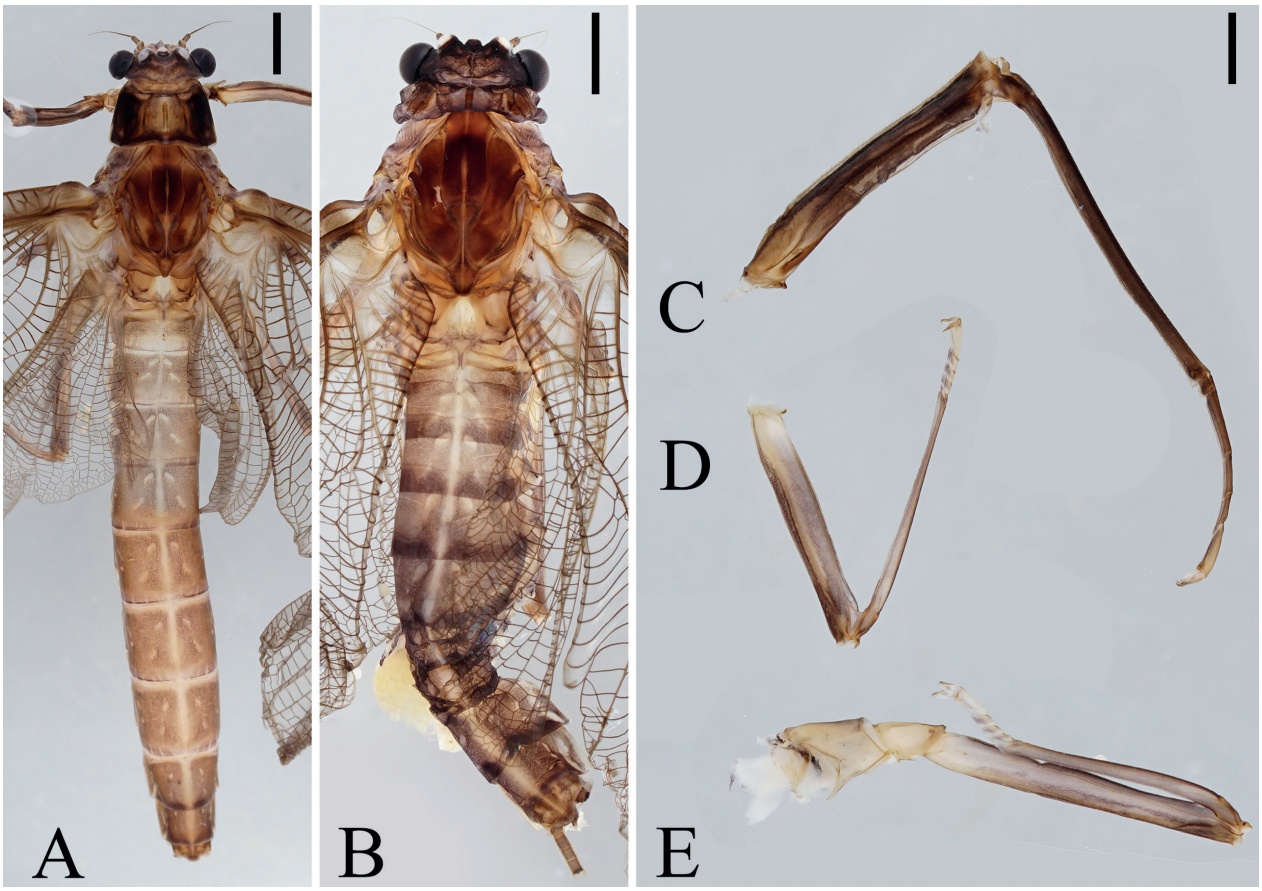

Figure 8. Females of *Polyplocia orientalis* from China **A** Female subimago, dorsal view; **B** Female imago, dorsal view; **C** Foreleg of female imago; **D** Mid-leg of female imago; **E** Hind-leg of female imago. Scale bars: 2.0 mm (**A–B**); 1.0 mm (**C–E**).

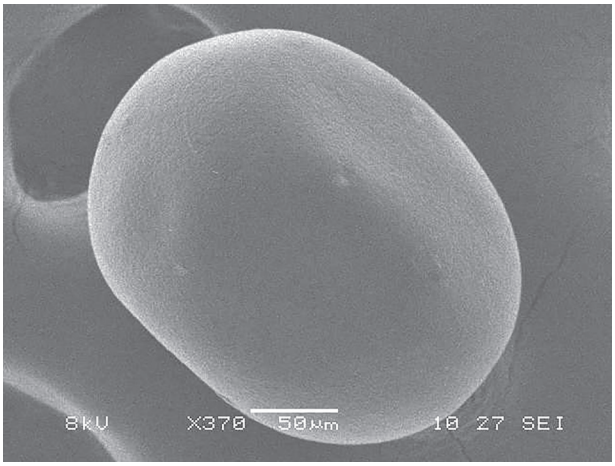

Figure 9. Egg of *Polyplocia orientalis* from China. Scale bar: 0.05 mm.

margin of the pronotum, the anterolateral corner tapers abruptly forming a relatively blunt apex; while those of *P. orientalis* are slightly sinuous and the anterolateral corner tapers gradually forming a more acute apex (Fig. 2A). Second, the dorsal lobe of the first pair of gills of *P. nebulosa* have nearly parallel lateral margins but those of *P. orientalis* have an apically expanded larger lobe (Fig. 2E). Third, the abdominal sterna of *P. nebulosa* have brown markings, while those of *P. orientalis* lack such markings (Fig. 2K). In addition, their eggs are different: eggs of *P. nebulosa* have mesh with raised ridges, while eggs are smooth and glabrous in *P. orientalis*.

The nymphs of *P. campylociella* were only tentatively associated and have not been confirmed, not being discussed here.

Genetic identity. Morphologically, our Chinese nymphal specimens match perfectly with the original description of *P. orientalis* given by Nguyen and Bae (2003) and some figures provided by colleagues. To ensure our identification, we also sequenced the COI gene of our specimen. The K2P genetic distance is 0.10 between our sequence (GenBank accession number OP962407) and a sequence stored in the GenBank of *P. orientalis* (GenBank accession number OP347109.1).

Biological notes. Nymphs were found in small forested streams narrower than 0.6 m, some sections of the stream had almost no flow, width less than 0.2 m and depth 1–15 cm. Nymphs of different instars were found at the same time (length from 0.5–40 mm), hidden in mud under stones (Fig. 10). Imagoes were collected at night sporadically, the rest on nearby vegetation. Judging from all the available information, it is assumed to be a univoltine and highly asynchronous population.

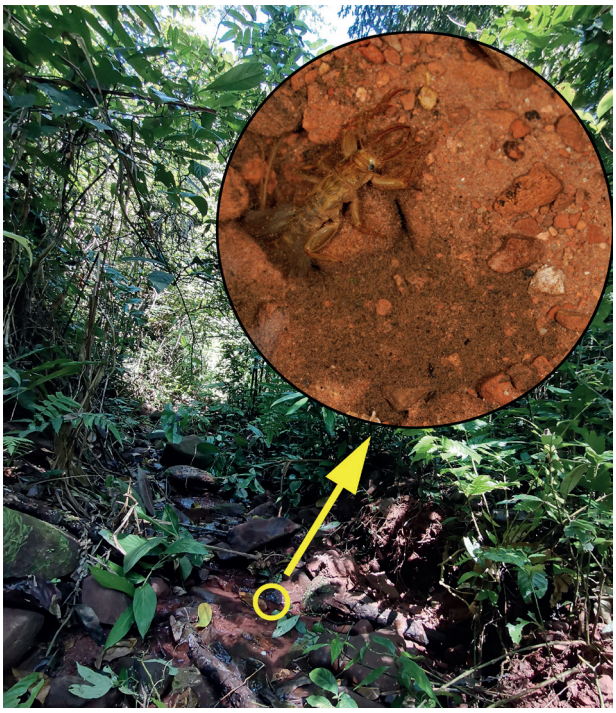

Figure 10. Habitat of *Polyplocia orientalis* nymphs from China.

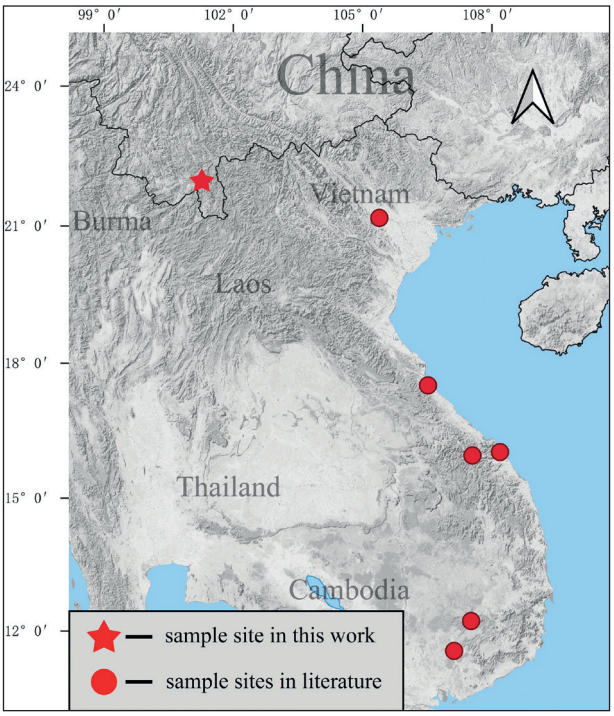

Figure 11. Distribution map of *Polyplocia orientalis*.

Key to male imagoes of *Polyplocia*

1 Wings with dark clouds around cross-veins and margins 2
– Membrane of wings transparent and colourless 3
2 Styliger plate rounded and projected, penis V-shaped ..... *P. campylociella* Ulmer, 1939
– Styliger plate not projected, short and straight, penis T-shaped ..... *P. nebulosa* Gonçalves & Peters, 2016
3 Both penis lobes have an apical depression ..... *P. orientalis* Nguyen & Bae, 2003
– Penis lobes with smooth apical margin ..... *P. vitalisi* Lestage, 1921

Discussion

Morphologically, our Chinese nymphal specimens match perfectly with the original description given by Nguyen and Bae (2003) and some figures provided by colleagues. The K2P genetic distance of COI gene is 0.10 between our sequence and a sequence stored in the GenBank of *P. orientalis*. This data is lower than the average mayfly interspecific distance, which is higher than 0.15 in most cases (Zheng and Zhou 2021).

The Oriental genus *Polyplocia* was reported previously from Vietnam, Malaysia, Indonesia, Laos and Thailand (Lestage 1921, 1924; Ulmer 1939, 1942; Demoulin 1952, 1953, 1966; Nguyen and Bae 2003; Gonçalves and Peters 2016) and the species *P. orientalis* has only been found in Vietnam. We provide the first nominal record of this family in China and expand the distribution of this species slightly northwards (Fig. 11).

Gonçalves and Peters (2016) suggested the morphology of mouthparts (particularly the labium), pronotum and forelegs may contain potential characters to identify *Polyplocia* nymphs. However, except for fine structures mentioned above, the nymphs of *P. orientalis* and possible *P. nebulosa* are extremely similar to each other. This may result from the strong natural selection of the tropical climate and a similar niche. We believe such a problem might be solved through more detailed photographs of nymphs and nymph-imago associations of more species.

Conclusion

In the present study, the reared imagoes from nymphs of the euthyplociid mayfly *Polyplocia orientalis* from China show both mature and immature stages of this species with good identification characters, which are photographically presented herein. The imagoes of *P. orientalis* have unstained wings and cross-veins (except costal and subcostal sections), male penis divergent laterally with a pair of apical depressions. The nymphs have the largest size amongst *Polyplocia* species, with slightly sinuous flanges on pronotum which are with acute anterolateral apex and uniformly pale abdominal sterna.

The present finding confirms the distribution of the genus *Polyplocia* and the family Euthyplociidae in China, extends its distribution range slightly northwards and provides the first solid nymph-imago association in the genus. It also shows an unusual shallow water habitat for nymphs. As a result, it improves our knowledge of taxonomy, biology, biogeography and evolution of the family Euthyplociidae.

Acknowledgements

We would like to express our sincere gratitude to Jérôme Constant (the Institut Royal des Sciences Naturelles de Belgique, Belgium), Inês Gonçalves (Universidade Federal do Rio de Janeiro, Brazil), Janice Peters (Entomology, Florida A&M University, USA) and Luke Jacobus (Biology, Purdue University, USA) for taking and sharing pictures of species in the genus and Zhi-Teng Chen for helping us to check our language. We also thank Mr. Jun-Feng Guo, Long-Yi Chen, Tian-Yu Zhang and Jiao-Long Ai for assistance in specimen collection and rearing.

This work is founded by the National Natural Science Foundation of China (31750002, 32070475) and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

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