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The morphology of the Chinese Mayfly *Ephoron nanchangi* (Hsu, 1936) (Ephemeroptera: Polymitarcyidae)

JIA-HAO PANG^{1,2,3}, JUN-KAI XU^{1,2,4}, YING-SHU WANG^{1,5}, XU-HONG-YI ZHENG^{1,6} & CHANG-FA ZHOU^{1,7*}

¹College of Life Sciences, Nanjing Normal University, Nanjing 210023, China

²Danyang High School of Jiangsu Province, Danyang 212309, China

³✉ 867235900@qq.com; <https://orcid.org/0009-0009-5983-971X>

⁴✉ 961184938@qq.com; <https://orcid.org/0009-0009-8593-0277>

⁵✉ 2383342494@qq.com; <https://orcid.org/0009-0007-2118-8398>

⁶✉ 1445148819@qq.com; <https://orcid.org/0000-0002-1520-2540>

⁷✉ zhouchangfa@njnu.edu.cn; <https://orcid.org/0000-0001-8785-5228>

*Corresponding author

Abstract

The Chinese polymitarcyid mayfly *Ephoron nanchangi* (Hsu, 1936) was originally named upon females, which were lost. The exact morphology has not been presented until now. The newly collected materials of this species, which include nymphs, female subimagos and male imagos, show it is a valid species with unique morphology, distinguishable from congeners by its short and strong nymphal mandibular tusks, long distance between forks of Rs and MA in imaginal forewings.

Key words: burrowing mayfly, mandibular tusks, taxonomy, biology, China

Introduction

The species *Ephoron nanchangi* was first named by Hsu (1936) as *Polymitarcys nanchangi*. Since its establishment, this species has been listed and mentioned by several Chinese researchers (Gui 1985; You & Gui 1995; Zhou 2013; Zhou *et al.* 2015), but no further information about it was added.

The exact morphology of this species *Ephoron nanchangi* is unclear because: (1) the types of this species are female subimagos, and they were lost during the World War II (Zhou *et al.* 2015). (2) In this genus, the females usually have vestigial structures, like legs and tails. Their morphology provide very limited information, especially those about species delimitation. This is a possible reason that Hsu (1936) did not designate the holotype for this species. (3) The original description and figure of this species are very brief.

The Asian Palearctic *Ephoron* species have been studied and illustrated in excellent level (Tshernova *et al.* 1986; Ishiwata 1996; Sekiné *et al.* 2013), but its Oriental congeners have not been presented systematically. Five species of six ones in this region were known from imaginal stages only and three of them were reported from female adults (Techakijvej *et al.* 2021). More contributions on this issue will improve our understanding on the genus *Ephoron* of Asia.

Geographically, the species *Ephoron nanchangi* was found in central China (Jiangxi Province), an intermediate region between Palearctic and Oriental realms. Its morphology maybe has somewhat transition features of congeners too.

In the year 2022, several places of central China were reported witnessing the mass emergence of mayflies, including a place nearby the type locality of the species *Ephoron nanchangi*. We went all those locations to check out the real situation and to collect specimens. It turns out that they are the species *Ephoron nanchangi* and sweep away the fog around it.

Material and methods

Nymphs were collected from the rivers by hand screen. Imagos and subimagos were obtained by light traps or under the road lights. All materials used in this study were stored in ethanol (about 85%), deposited in the mayfly collection of College of Life Sciences, Nanjing Normal University.

Specimens were examined under a stereomicroscope (Nikon SMZ 745T). Nymphs and winged stages were photographed by a SONY a7R II camera with a LAOWA 25mm 5× macro lens. Eggs were dissected from female subimagos. 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. Terminology mainly follows that of Kluge (2004).

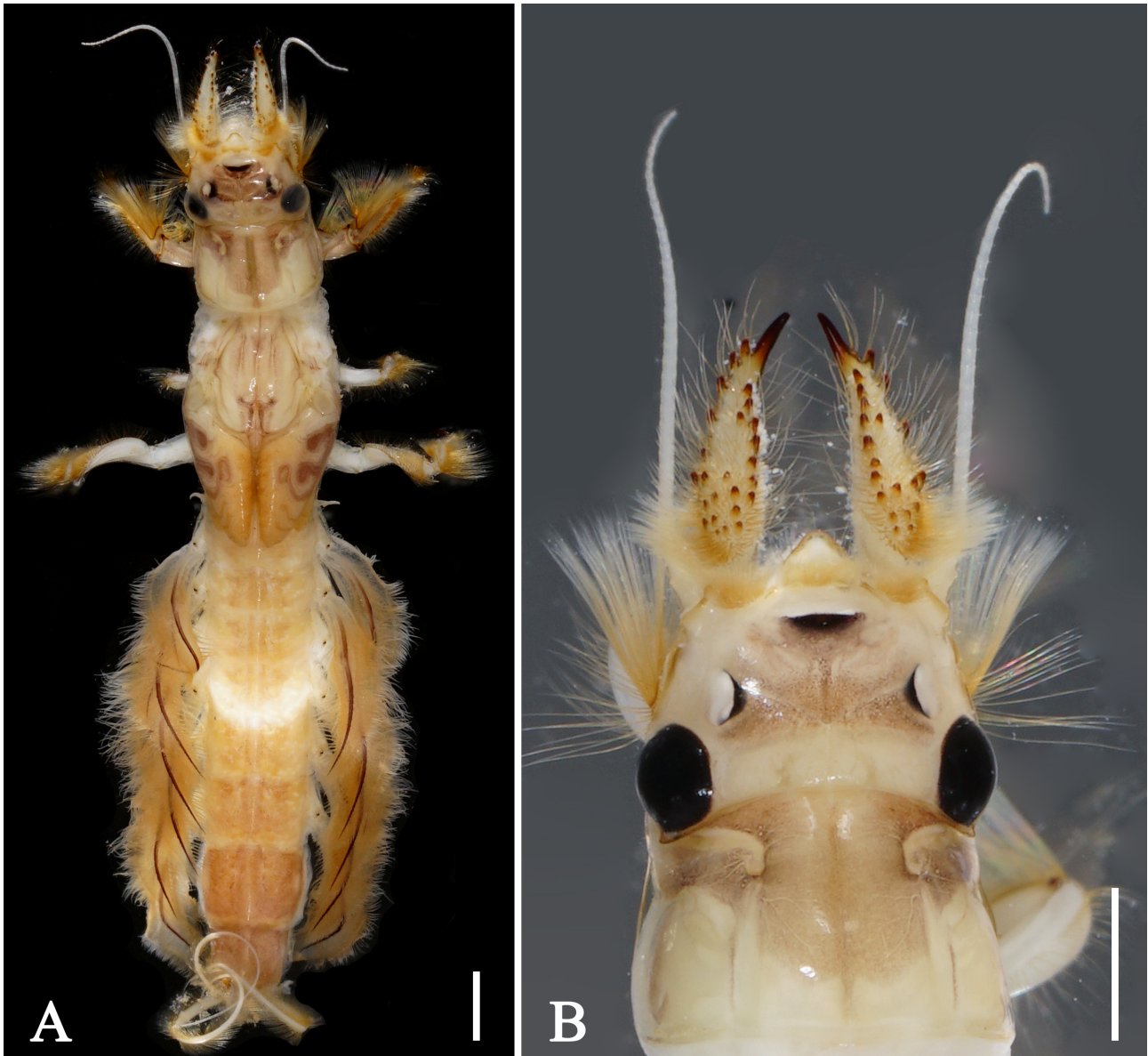


Figure 1. Female nymph of *Ephoron nanchangi*. **A**, habitus (dorsal view); **B**, head (dorsal view). Scale bars: 1.0 mm.

Results

Ephoron nanchangi (Hsu, 1936)

Polymitarcys nanchangi Hsu, 1936: 143 (female). Types: female, Nanchang, Jiangxi Province, China.

Polymitarcys nanchangi - Gui 1985: 92 (list).

Ephoron nanchangi - You & Gui 1995: 90 (female); Ishiwata 1996: 558 (compared); Kluge 2004: 265 (mentioned); Zhou 2013: 201 (list); Zhou *et al.* 2015: 250 (list); Techakijvej *et al.* 2021: 392 (mentioned).

Distribution: China (Hunan Province, Henan Province, Jiangxi Province).

Material examined: 12 nymphs, 20 male imagos, 20 male subimagos, 300 female subimagos, China, Henan Province, Dengzhou City, Jitan Bridge, 32°40'44.3" N, 112°16'3.84" E, 95m a.s.l., 21. VIII. 2022, leg. Changfa Zhou; 3 male imagos, 10 female subimagos, China, Hunan Province, Yiyang City, Yuanjiang River, 28°51'24.7" N, 112°23'24.6" E, 27m a.s.l., 5. IX. 2022, leg. Zhuoxuan Chen.

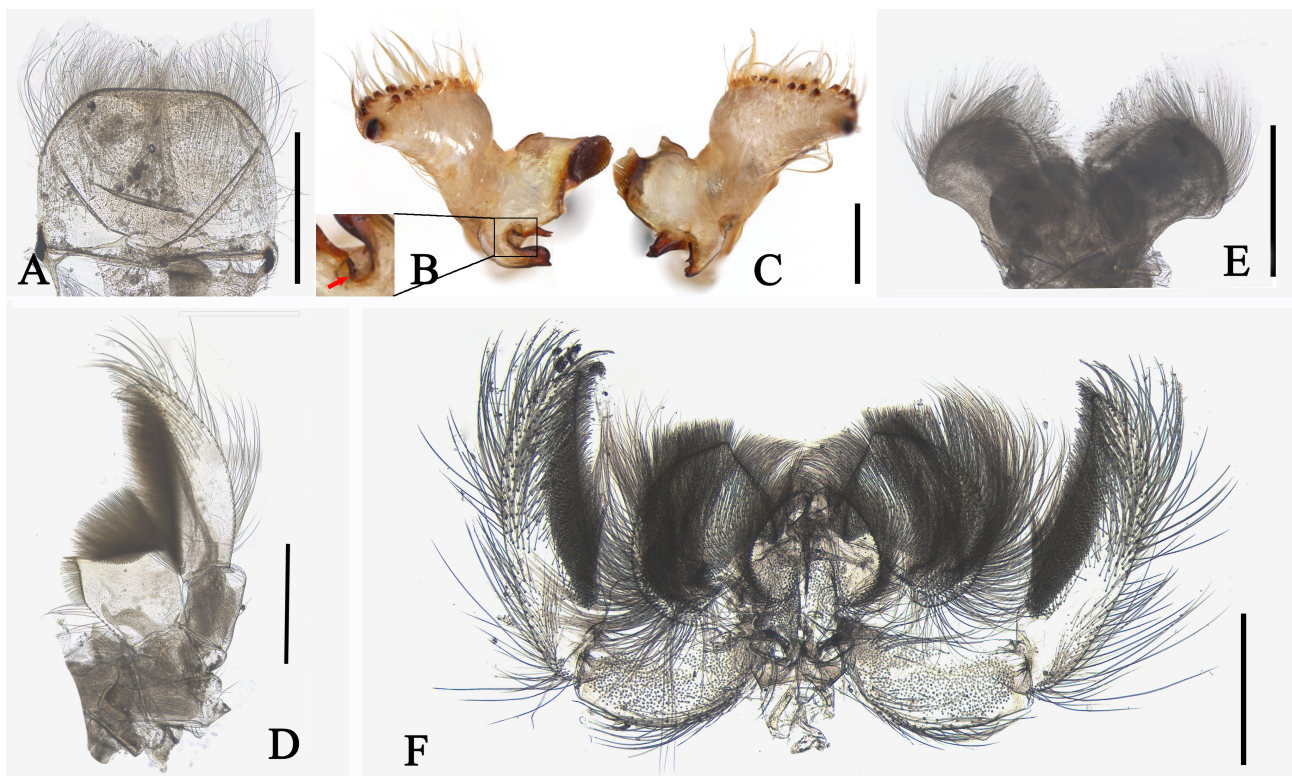


Figure 2. Nymphal mouthparts of *Ephoron nanchangi*. **A**, labrum (dorsal view); **B**, right mandible (front view) and its spine enlarged (arrow indicating the spine); **C**, left mandible (front view); **D**, maxilla; **E**, hypopharynx (dorsal view); **F**, labium (ventral view). Scale bars: 0.5 mm.

Description

Mature nymph (in alcohol, Figs. 1–3, 9): body length 12.0–15.0 mm in males, 20.0–25.0 mm in females (including mandibular tusks, excluding caudal filament and cerci). Body white with brownish markings (Fig. 1).

Head nearly trapezoidal in dorsal view, white to yellowish pale in background with dark brown patch between three ocelli (Fig. 1B). Compound eyes located on posterolateral corners of head, ocelli white with a black basal circle. Anterior margin of head with a triangular median process (frons), densely fringed with hair-like setae (Fig. 1B). Labrum rounded, dorsal surface covered with dense hair-like setae (Fig. 2A). Mandibular tusks pale to golden in color, subequal to head length in dorsal view, apex brownish and slightly bent inwards; dorsal side with dense hair-like setae, over 20 brownish blunt tubercles on lateral margins and dorsal base (Figs. 1A–B). Right mandible with a strongly curved outer incisor, apex rounded with a small protrusion blunt spine, base of the outer incisor with a stout spine, inner incisor short and forked apically (Fig. 2B). Outer incisor of left mandible stout, apex divided into two denticles;

inner incisor bifurcated apically into a rounded anterior lobe and blunt posterior tooth, a slender spine present near the base of the inner incisor (Fig. 2C). Base of each mandible with a row of long setae on outer margin. Maxilla with nearly trapezoidal galea-lacinia, front margin of galea-lacinia densely covered with long bristles, upper half of inner margin with rows of short bristles, lower half sparsely set with long hair-like setae; maxillary palp two-segmented, segment II ca. $3\times$ segment I in length, slightly curved and covered with setae, those on inner margin shorter and extremely dense (Fig. 2D). Outer margins of superlinguae slightly concaved, lingua rounded (Fig. 2E). Labium densely covered with setae, glossa slender drop-shaped, paraglossa rounded, labial palp two-segmented, segment II ca. $1.7\times$ segment I in length, both covered with setae, shape and setae of segment II similar to maxillary palp (Fig. 2F).

Thorax with dark brown pattern dorsally. Legs pale, dorsal and ventral margin of each leg covered with yellowish long setae (Fig. 1). Foreleg strongest, length ratio of femur: tibia: tarsus ca. 1.0: 1.3: 0.4, tibia and tarsus much wider than midleg and hindleg, basal half of ventral margin of femur and outer surface of tibia with lines of tubercles, claw stout (Fig. 3A). Midleg and hindleg similar in structures, length ratio of femur: tibia: tarsus ca. 1.0: 1.2: 0.5, claws much slender than foreleg, midleg shortest among legs (Figs. 3B–C).

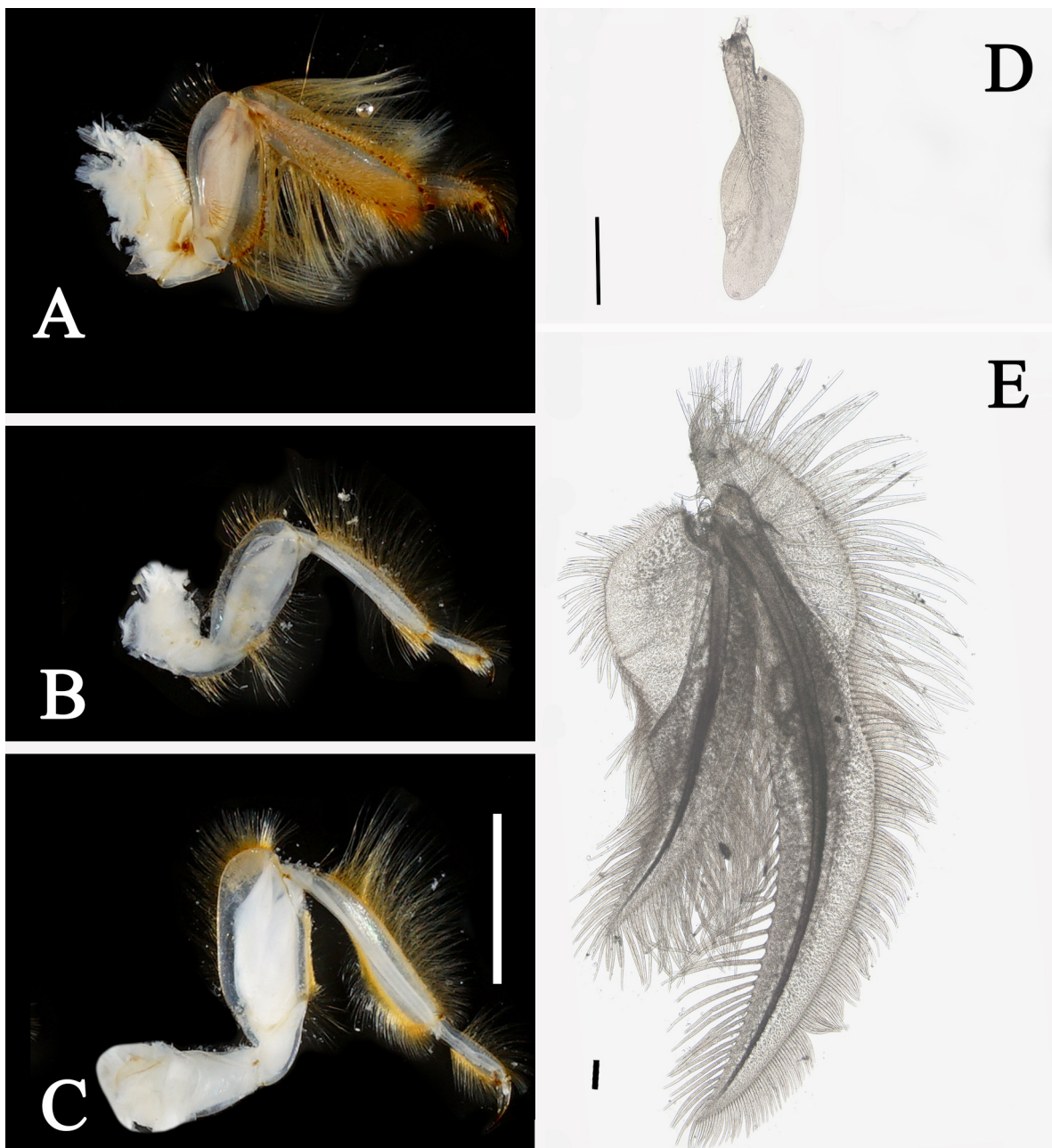


Figure 3. Nymphal structures of *Ephoron nanchangi*. **A**, foreleg; **B**, midleg; **C**, hindleg; **D**, gill I; **E**, gill IV. Scale bars: 1.0 mm in A–C, 0.1 mm in D, E.

Abdomen light brownish, gills on abdominal segments I–VII, pale in color (Fig. 1A); gill I single, leaf-shaped (Fig. 3D); gills II–VII similar in shape, bifurcated at base, both lamellae fringed with hairs, dorsal lamella slender and slightly curved, outer margin with a shallow depression medially, ventral lamella ca. 3/4 dorsal lamella in length, expanded basally (Fig. 3E). Cerci and paracercus subequal in length, ca. 2/3 body length in males, 1/3 body length in females, with dense lateral setae (Fig. 1A).

Male imago (in alcohol, Figs. 4–5): Body length 12.0–16.0 mm (n = 10), predominantly pale in color (Fig. 4A). Head width ca. 2.0 mm (n = 10), pale, washed with dark brown dorsally (Figs. 4A–B). Compound eyes black and globular, located on posterolateral corners of head, narrowed basally; three ocelli rounded and protruded, with white tops and black bases (Fig. 4B). Antennae pale, about 1.5 mm, length ratio of scape: pedicel: flagella ca. 1.0: 1.5: 12.0; scape and pedicel stout cylindrical, pedicel with dark brownish apex; flagella slender filiform.

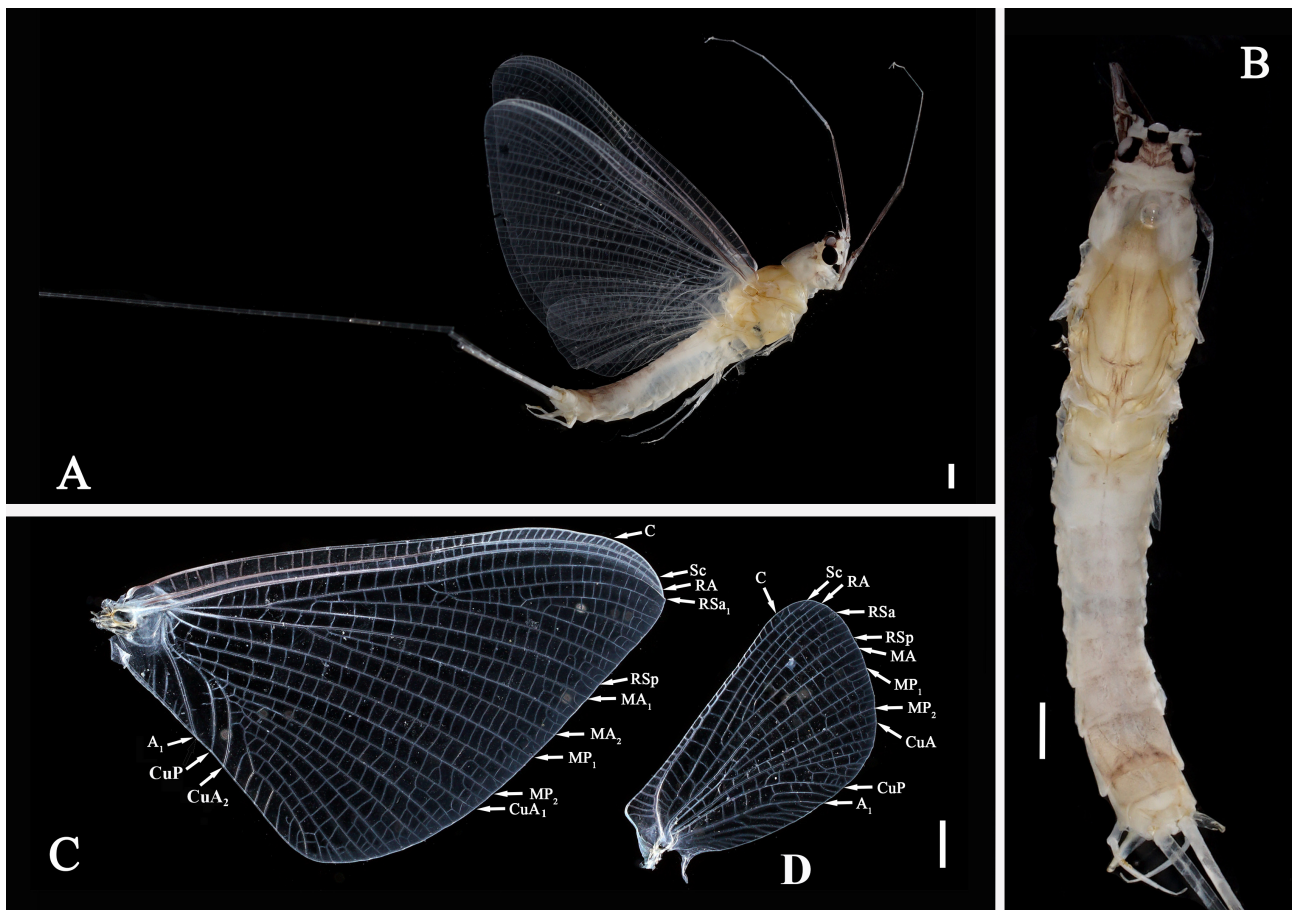


Figure 4. Male imago of *Ephoron nanchangi*. **A**, habitus (lateral view); **B**, body (dorsal view); **C**, forewing; **D**, hindwing. Scale bars: 1.0 mm.

Prothorax pale, slightly washed with grey to brown dorsally, length: width ca. 1.0: 1.0 (Fig. 4B). Mesothorax and metathorax pale yellowish-brown (Figs. 4A–B). Forewings ca. 10.0 mm, transparent, costal margin slightly brownish, apical and cubital angle rounded, cross-veins densely and evenly distributed (Figs. 4A, C). RS forked at basal 1/4, with five intercalaries in RS fork; MA forked at basal 1/3 symmetrically, MP forked at base asymmetrically; base of MP₂ curved posteriorly greatly and fused with CuA for some distance; CuA forked, with 4–6 intercalaries between CuA₁ and CuA₂; CuP and CuA run closely for long distance; A₁ almost straight (Fig. 4C). Hindwings ca. 0.5× forewing in length, transparent, with a blunt costal-basal projection; an intercalary present between RA and RSa, RS connected with MA at base, bifurcated at middle; MA single, MP bifurcated symmetrically ant middle; numerous intercalaries between CuA and CuP; A₁ connected with several veinlets running to the anal margin (Fig. 4D).

Foreleg slender, femur and tibia dark brown, tarsus pale; length ratio of femur, tibia and five tarsomeres = 1.0: 3.0: 0.2: 1.5: 1.0: 1.0: 0.5 (Fig. 5A); claws paired, rounded apically but with a small tubercle (Fig. 5D). Midleg and hindleg poorly developed. Midleg shortest, pale in color, trochanter stretched and projected, length ratio of femur: tibia: tarsus = 1.0: 1.3: 0.4, tarsus flattened and four-segmented, length ratio of four tarsomeres ca. 0.5: 0.8: 0.8: 1.2 (Fig. 5B), with a pair of claws attached on tarsomere IV, one blunt one hooked (Fig. 5E). Coloration, length ratio and shape of hindleg similar to midleg but twice the latter in length (Fig. 5C).

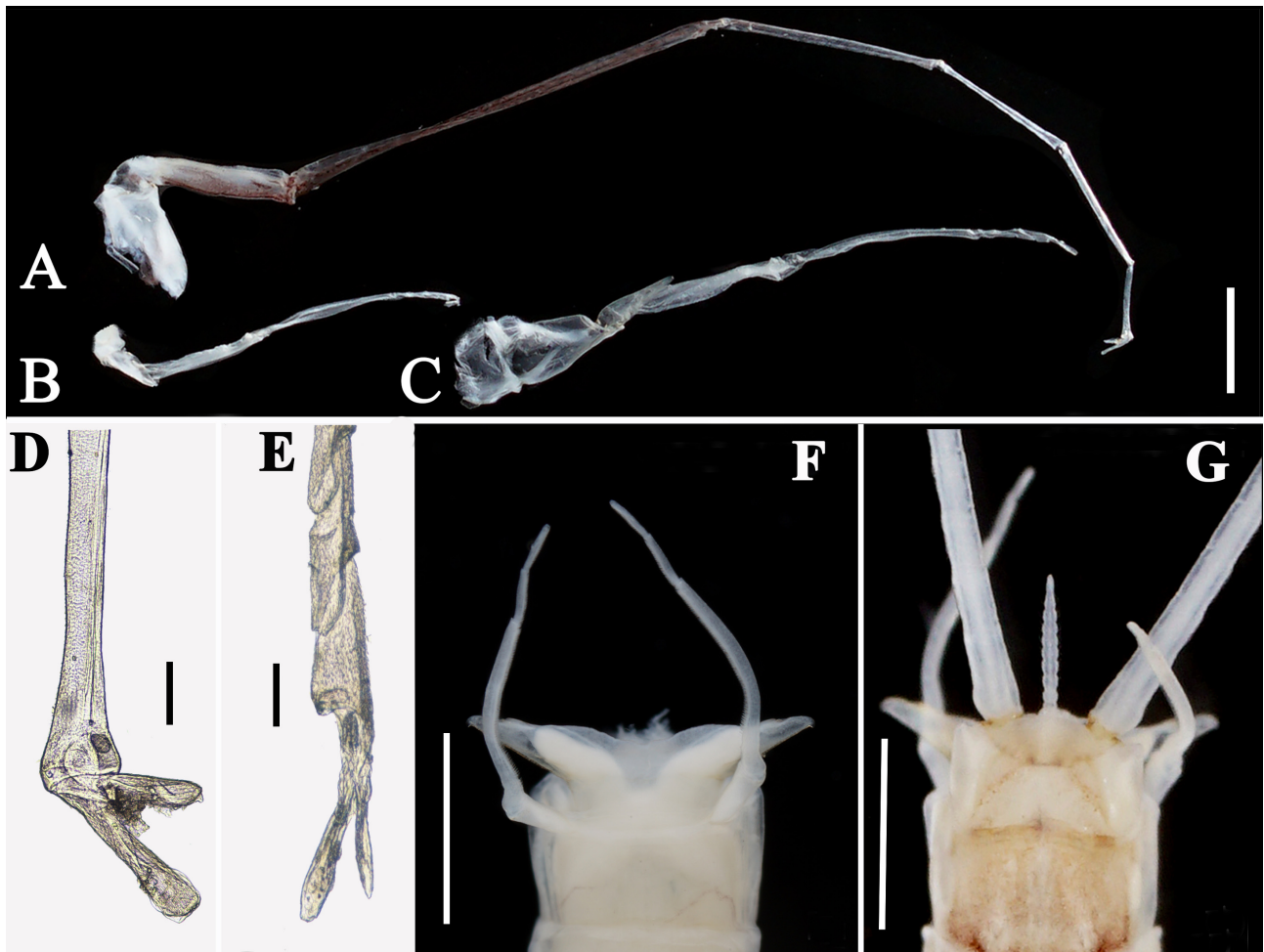


Figure 5. Male imago of *Ephoron nanchangi*. **A**, foreleg; **B**, midleg; **C**, hindleg; **D**, claw of foreleg; **E**, claw of midleg; **F**, genitalia (ventral view); **G**, genitalia and caudal filaments (dorsal view). Scale bars: 1.0 mm in A–C, F, G, 0.1 mm in D–E.

Abdomen pale white, posterior terga washed with grey to brown (Fig. 4B). Forceps ca. 2.0 mm in length, four segmented, length ratio of them = 2.0: 2.0: 0.6: 0.6, gradually narrowed apically, segments II–IV with setae on inner margin (Figs. 5F–G). Penis lobes slender and diverging laterally, posterior margin of each lobe with slightly rounded basal half, and two slight depressions at middle and 3/4 point from base to apex respectively, apex of each lobe hooked (Figs. 5F–G). Cerci poorly developed, less than 1.0 mm, consists of around 12 globular segments, paracercus about 2–3× body length, with sparse short setae (Fig. 5G).

Male subimago (in alcohol, Fig. 6): Body length 12.0–16.0 mm (n = 10), generally similar to male imago except following structures: wings pale translucent. Tarsus of foreleg poorly developed, length ratio of femur: tibia: tarsus = 1.0: 1.1: 0.6 (Fig. 6A). Penes and forceps undeveloped, penes simple and pointing posteriorly (Fig. 6D). Cerci and paracercus densely covered with setae, about twice the body length (Fig. 6D).

Female subimago (in alcohol, Figs. 7–8): Sexually mature in this stage. Body length 19.0–23.0 mm (n = 10), predominantly pale in color (Figs. 7A–B).

Shape and coloration of head and thorax generally similar to male (Fig. 7B). Forewings ca. 18.0 mm, translucent, costal margin slightly brownish, shape and venation similar to male but with only four intercalaries between CuA_1 and CuA_2 (Fig. 8C). Hindwings translucent, venation similar to male (Fig. 8D). Legs poorly developed; foreleg dark brownish, length ratio of femur: tibia: tarsus = 1.0: 1.2: 0.4, tarsus four segmented, length ratio of tarsomeres variable, tarsomere IV expanded apically (Fig. 7C); claws paired, each with a tubercle apically (Fig. 8A); midleg and hindleg similar to male (Figs. 7D), pale in color, femur: tibia: tarsus = 1.0: 1.2: 0.4, tarsus three segmented (Fig. 7D); claws paired, one blunt one hooked (Fig. 8B); hindleg slightly longer than midleg (Fig. 7E).

Abdomen pale ventrally, terga washed with dark brown (Fig. 7B). Cerci and paracercus subequal in length, slightly shorter than body length, pale and densely covered with setae.

Eggs. Length 0.2–0.3 mm, densely and evenly covered with pits, one polar cap with irregular pullets (Fig. 8E). Eggs tightly clustered together into two slender cylinder-shaped masses, they extremely sticky and hard after air-drying (Fig. 7A).

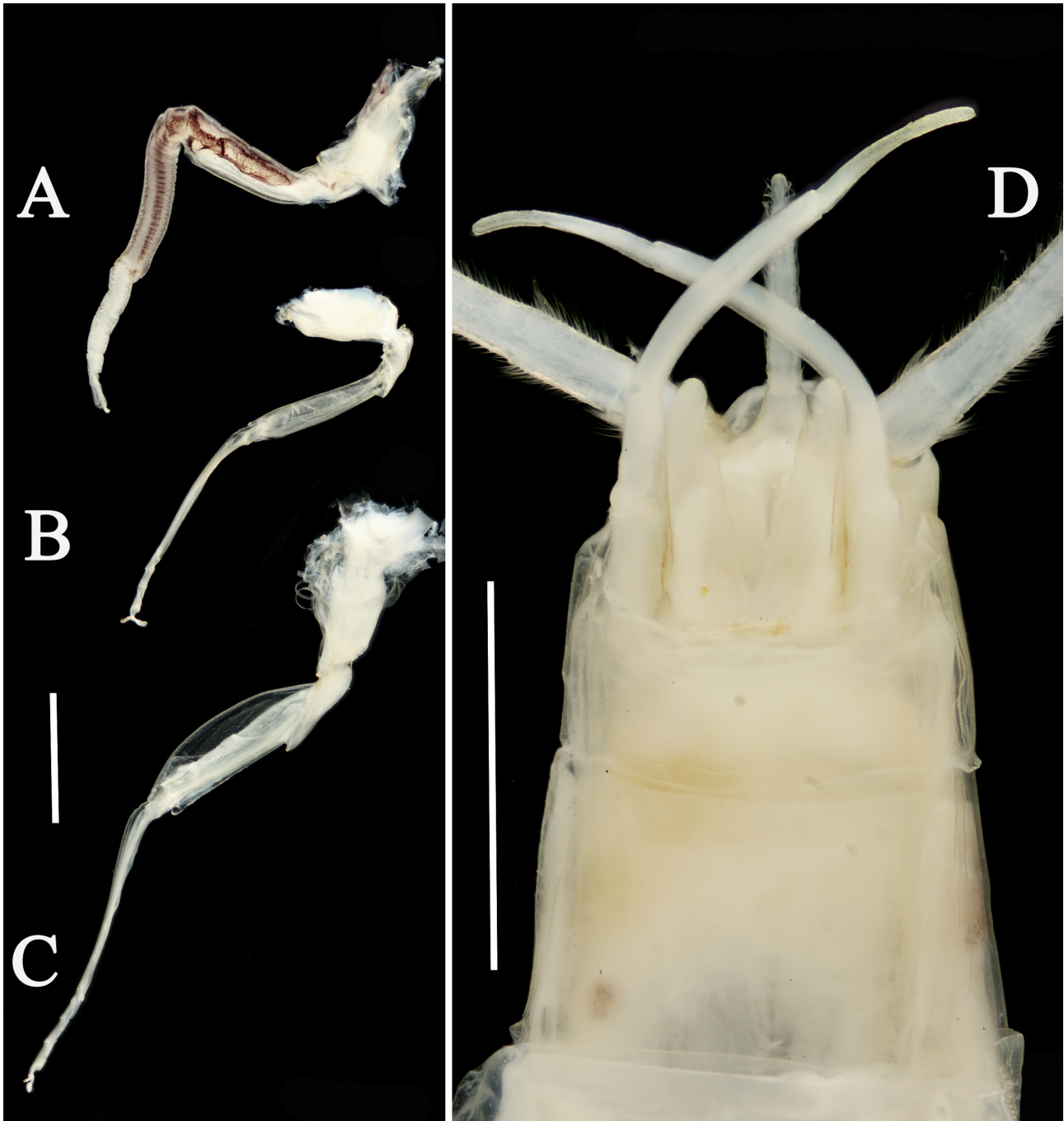


Figure 6. Male subimago of *Ephoron nanchangi*. **A**, foreleg; **B**, midleg; **C**, hindleg; **D**, genitalia and caudal filaments (ventral view). Scale bars: 1.0 mm.

Diagnosis

Compared to other described Asian *Ephoron* species, the nymphs of *E. nanchangi* are unique because their mandibular tusks are shorter, stouter and stronger than any others (Fig. 1). The right mandible has a distinct blunt spine between outer and inner incisors (Fig. 2B). In addition, its nymphal gills have no pigmented tracheal branches (Figs. 1A, 3D–E).

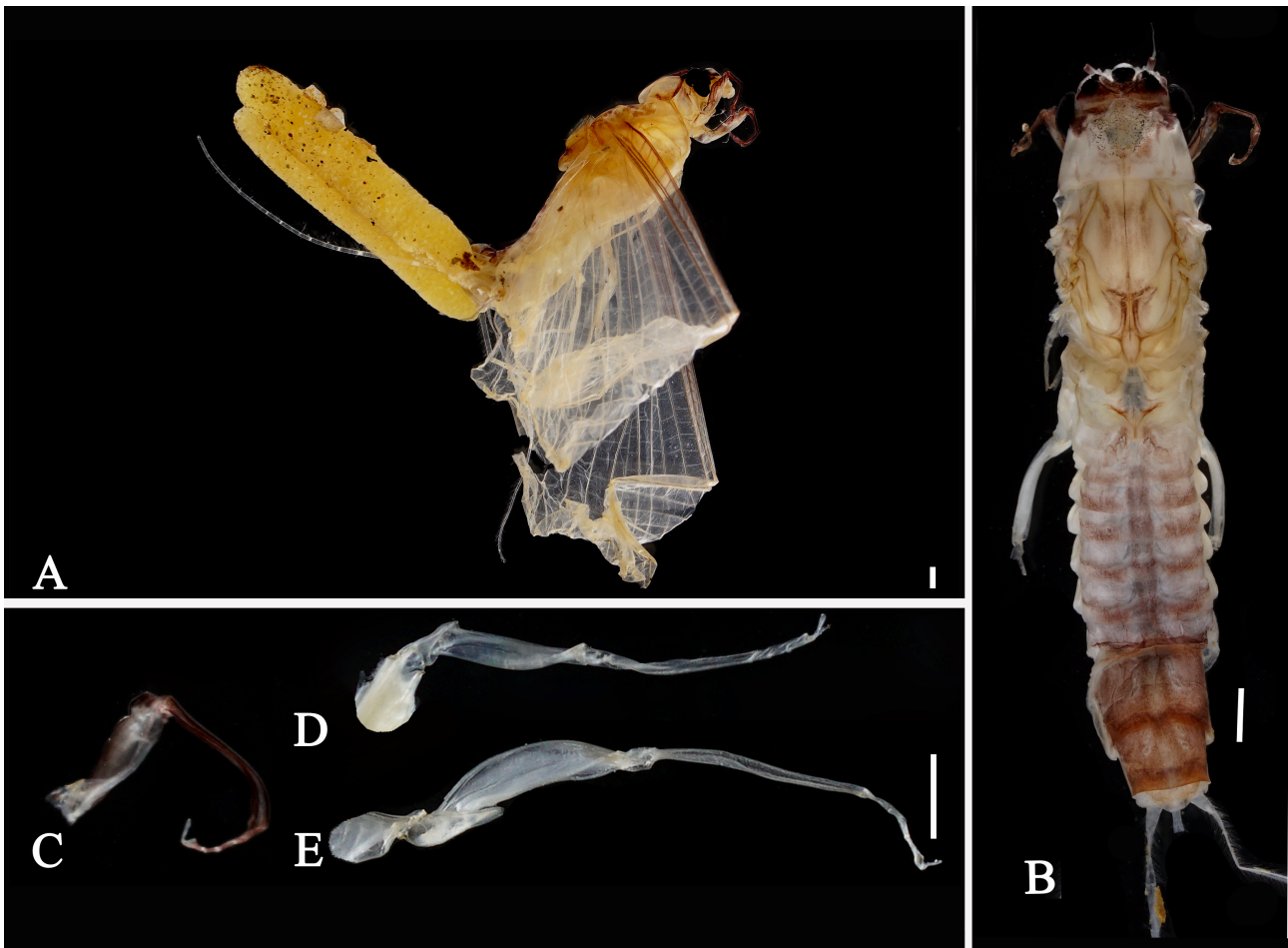


Figure 7. Female subimago of *Ephoron nanchangi*. **A**, lateral view; **B**, dorsal view of body; **C**, foreleg; **D**, midleg; **E**, hindleg. Scale bars: 1.0 mm.

In males, the distance between two forks of Rs and MA of forewings (Fig. 4C), 4–6 intercalaries between CuA section (Fig. 4C), the totally hyaline wings, abdomen without clear color pattern and shape of their genitalia (Figs. 5F–G) are good identification characters of this species. Besides, males of *E. nanchangi* can be recognized through forceps with slender apical segment (Fig. 5F), which are longer than all its Asian congeners (Ishiwata 1996; Techakijvej *et al.* 2021).

The venation of forewings mentioned above of males are also presented in the female forewings (Fig. 8C). Additionally, female subimagos can be recognized through their tarsi: four, three and three tarsomeres in foreleg, midleg and hindleg respectively, tarsomere IV of foreleg expanded apically (Fig. 7C). However, because most female subimagos of Palearctic species were poorly described or illustrated, this character is hard to use in distinguishing.

In eggs, they are drum-shaped with one polar cap and decorated with pit or puncture-like impressions (Fig. 8E).

Nymphs of *E. nanchangi* described here is relatively close to *E. shigae* (Takahashi, 1924) and *E. limnobium* Ishiwata, 1996, all of them have triangular frons and relatively short mandibular tusks in nymphs (Fig. 1) (the morphology of two latter species were shown by Ishiwata 1996). However, mandibular tusks of *E. nanchangi* are stout and without strongly curved apex, right mandible with a large stout spine between outer and inner incisor, dissimilar to all described Asian *Ephoron* nymphs (Ishiwata 1996; Techakijvej *et al.* 2021).

Discussion

Techakijvej *et al.* (2021) reported the Thailand species *Ephoron ookaewae* Techakijvej & Phalaraksh, 2001 has short mandibular tusks and the tusks have few tubercles. The reported Asian Palearctic *Ephoron* species have relatively long tusks and more tubercles on mandibles. Interestingly, the tusks of our species *E. nanchangi* are between them (Fig. 1).

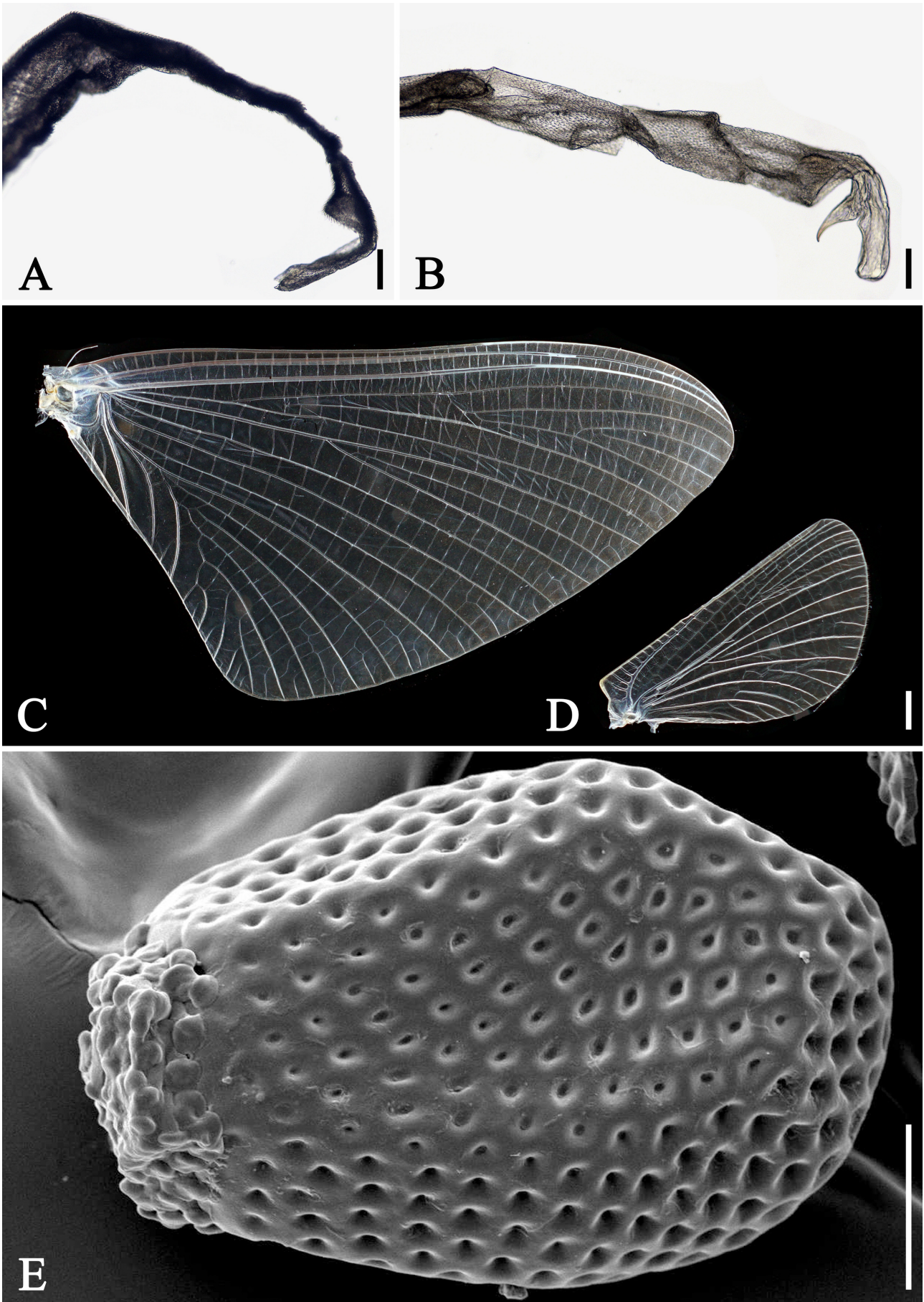


Figure 8. Structures of female subimago of *Ephoron nanchangi*. **A**, tarsus and claw of foreleg; **B**, tarsus and claw of midleg; **C**, forewing; **D**, hindwing; **E**, egg. Scale bars: 0.1 mm in A, B, 1.0 mm in C, D, 0.05 mm in E.



Figure 9. Living habitat of *Ephoron nanchangi* nymph. **A**, one collecting locality of Henan Province, central China; **B**, substrate of nymph living; **C**, mass individuals on road and bridge.

Most Asian *Ephoron* species share similar transparent wings with similar venation, although the number of intercalaries in CuA sections of forewing were used as distinguishable character in some previous studies (Techakijvej *et al.* 2021), it is found unstable in our materials of *E. nanchangi*. The males of this species have six intercalaries in CuA section (Fig. 4C) but females have only four intercalaries and several veinlets (Fig. 8C). So, this feature should be used cautiously.

In recent years, the mass emergences of mayflies were frequently reported in the news. Adults attracted by light sometimes even covered the roads and bridges, made disturbances to normal traffic (Fig. 9C). In this study, we confirmed that those mayfly clouds appeared in Hunan and Henan Province were formed by the species *E. nanchangi*. The nymphs of this species were found living in large rivers (width larger than 50 m) and big lakes. The nymph in this study were collected from sandy-muddy substrate beneath a bridge (Fig. 9).

The Chinese *Ephoron* species are under reviewed. The exact comparison of them will be presented soon morphologically and molecularly.

Acknowledgement

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