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A new Chinese *Ephemera* species (Ephemeroptera: Ephemeridae) from a small muddy canal

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Abstract

Nymphs of the genus *Ephemera* are usually found in sandy/muddy habitats but have not been collected from purely muddy sediments in China. However, a tentative sampling in a small wetland surrounded by agricultural fields revealed a new species *Ephemera aureola* **sp. nov.** The reared adults and collected nymphs demonstrate that this species exhibits mixed characters of the subgenera *Ephemera* and *Sinephemera*. Remarkably, the new species possesses straight stripes on abdominal terga VI–X, while its sterna have oblique streaks. The male genitalia have titillators, and the wings are almost uniformly golden. The nymphs have a shallow median ridge and setal tuft on tergum I, while its frons has a shallowly concave anterior margin. There is only one diagnostic feature separating subgenus *Ephemera*.

Key words: China, ecology, mayfly, muddy habitat, new species

Introduction

Mayfly nymphs are true aquatic insects and they can live in almost all freshwaters or even brackish waters, from headwaters of creek to low reach of big rivers (Bauernfeind & Soldán 2012; Jacobus *et al.* 2019). Normally, the mayfly biomass and its diversity in middle-sized stream is higher than other habitats, but they can also be found in independent ponds, reservoirs, lakes and wetlands (Bauernfeind & Soldán 2012), or even some urban rain ponds (Fischer *et al.* 2000). However, their presence in deep muddy sediments is rarely reported. Johnson *et al.* (2000) reported a baetid species in water of a mitigated wetland of USA. Blouin *et al.* (2004) discovered two ephemerid species which can dig in the bottom of lake bays. Fairchild *et al.* (1999) investigated some man-made marshes with shallow water and reported that mayflies belonging to four genera within three families (Baetidae, Caenidae and Ephemeridae) are capable of inhabiting such environments. Until now, no mayfly species have been reported from shallow muddy habitats in China.

In Ephemeridae, McCafferty (1975) and Blouin *et al.* (2004) found that American nymphs of *Hexagenia* Walsh, 1863 prefer fine sediment substrates (adhesive mud or fine sandy mud with a high content of organic detritus), while those of *Ephemera* prefer sandy substrates with gravel and pebbles. Based on our collections, the Chinese *Eatonigenia* Navás, 1935 prefers muddier sediments of larger rivers than *Ephemera* Linnaeus, 1758, which are usually found in streams or small rivers with sandy/muddy sediments (Sun *et al.* 2016; Lei *et al.* 2024). True lentic habitats with shallow water and dense organic detritus are expected to be sampled.

China has the highest diversity of the genus *Ephemera*, boasting more than 30 species out of the approximately 70 known globally. Some of them bear a series of plesiomorphies, especially the subgenus *Sinephemera* Kluge, 2004. This country is definitely an evolutionary center of the genus *Ephemera* (Hwang *et al.* 2008; Lei & Zhou 2024). In addition, usually we collected Chinese mayflies in montane streams with stony and sandy bottoms, as these habitats have more species and individuals there. China has rich water systems in plains, hills and agricultural areas. Most of

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them are man-made ditches or canals with deep muddy sediments, especially if they do not have concreted banks and/or bottoms. The mayfly fauna of this type of habitats has not been investigated.

In recent years, we have systematically studied the family Ephemeridae (Sun *et al.* 2016; Li *et al.* 2023; Lei *et al.* 2024; Lei & Zhou 2024). At the beginning of 2025, we tried to collect mayflies in a very narrow canals modified from a mountain stream and in a small marsh adjacent to it. Both habitats were located beside agricultural fields or small bamboo groves. Surprisingly, large number of *Ephemera* nymphs were collected from silty substrates in these habitats. After nearly two weeks of rearing, all life stages of this species were obtained. Morphologically, it is a new species of the subgenus *Ephemera*. Ecologically, it represents the first mayfly species in shallow wetland, with nymphs capable of living in very black fine mud. This species not only represents a new evolutionary type of *Ephemera* but also exhibits a combination of morphological characters from the two subgenera *Ephemera* and *Sinephemera*.

Material and methods

Nymphs were collected using hand screen in deep water (about 0.5 m) with muddy bottom. Mature nymphs were reared in a plastic tray with appropriate water, supplied air with a pump used in golden fish culture in the laboratory. All specimens were preserved in 85% alcohol and deposited in the Mayfly Collection at Nanjing Normal University, Jiangsu Province, China.

Photographs of the specimens were taken by a digital camera and subsequently observed and photographed under a stereomicroscope at different magnifications for fine structures, including mouthparts, legs, and gills.

Eggs were obtained by dissecting from female imagos. Samples for scanning electron microscopy were performed following standard protocols: fixed with 4% glutaraldehyde for 5–8 h, rinsed with phosphate-buffered saline (PBS) 2–3 times (10–15 min each time), dehydrated with concentration-graded acetone (30%, 50%, 70%, 80%, 90%, and 100%, 10–15 min each time), and vacuum-coated with a gold film.

The thoracic muscles of two male imagos were dissected to sequence its mitochondrial COI gene. The PCR-amplification adopted Premix Taq (Takara Bio Inc., Beijing, China) with forward primer LCO (5'-TTC AGC CAC TTT ACC GCG-3', see Hrivniak *et al.* 2017) and reverse primer HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-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 final extension at 72˚C for 10 min.

The COI sequence data of this species was uploaded to GenBank to find related ones. As a result, the seven most similar sequences were downloaded for further comparison and analysis. The total eight sequences were aligned with the software Clustal W. The final sequences include 582 bp after deleting the ambiguous positions between them, and their GenBank accession numbers are listed in Table 1.

The genetic distances of those eight sequences were calculated and performed with the Kimura 2-parameter model (Kimura 1980) in the MEGA 11 software (Tamura *et al.* 2021) (Table 2).

Table 1. The COI sequences used in the present study.

Species	GenBank Accession Number	Provider	Specimen Come From
E. aureola sp. nov.	PV605557	This study	Anhui, China
E. japonica	LC815563.1	Takenaka *et al.* 2024	Japan
E. strigata	LC815606.1	Takenaka *et al.* 2024	Japan
E. formosana	MN961289.1	Wakimura *et al.* 2020	Japan
E. serica	OK018134.1	Wang *et al.* 2022	Guangdong, China
E. lota	OQ059010.1	Lei *et al.* 2024	Jiangsu, China
E. longiventris	OR753657.1	Nguyen *et al.* 2023	Vietnam
E. sachalinensis	PP866765.1	Mayorga-Villalobos *et al.* 2024	South Korea

Table 2. K2P genetic distance among the eight COI sequences (%).

	E. aureola sp. nov.	*E. japonica*	*E. strigata*	*E. formosana*	*E. serica*	*E. lota*	*E. longiventris*
E. *aureola* sp. nov. (China)							
E. japonica (Japan)	16.58						
E. strigata (Japan)	17.24	4.28					
E. formosana (Japan)	16.58	19.53	19.12				
E. serica (China)	15.88	17.57	18.24	19.64			
E. lota (China)	16.57	16.79	15.90	16.15	12.72		
E. longiventris (Vietnam)	15.68	18.25	18.92	19.90	2.09	12.11	
E. sachalinensis (South Korea)	15.50	17.68	17.24	14.48	14.99	13.55	15.42

Results

Systematic account

***Ephemera* (*Ephemera*) *aureola* sp. nov.**

Figures 1–8.

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Material examined. CHINA: Holotype: 1 male reared imago, foot of the Fenghuang Hill, Yian District, Tongling City, Anhui Province, 30°87'51.92" N, 118°03'49.04" E, 53.3 m a.s.l., 2025-III-25, leg. Di Wang. **Paratypes:** 8 male imagos, 1 male subimago, 4 female imagos, 1 female subimago (reared), 2 nymphs, same as the holotype; 8 nymphs, same data as holotype, 2025-II-16; 6 male imagos, 1 female imago (reared), same as holotype, 2025-IV-4; 9 female imagos, 1 male subimago, 1 female subimago (reared), 7 nymphs, same data as holotype, 2025-II-27, leg. Di Wang, Yu-Xian Sun, Ning-Ning Wang, Chang-Fa Zhou.

Descriptions

Male imago (in alcohol): Body length 18.0–23.0 mm, paracercus 29.0–31.0 mm, cerci 34.0–38.0 mm, forewing 18.0–19.0 mm, hindwing 7.5–8.0 mm (Fig. 1A). Body generally golden to pale yellowish but with deep brown stripes and lines laterally, from head to abdominal segment X. Head pale yellowish, area between ocelli dark brown; antennae pale gray; compound eyes spherical, with black basal 4/5 and gray apical 1/5. Pronotum yellowish, lateral margins dark; meso- and metathorax with reddish brown to brown pleura (Figs. 2A–C). Foreleg: length 15.5 mm, coxa yellowish with a longitudinal deep brown line on outer surface; trochanter gray, femur brown and tibia to claws dark; length ratio of femur: tibia: tarsus = 1.0: 1.8: 2.0, ratio of five segments of foretarsus from basal to apical = 1.0: 4.2: 3.3: 2.3: 2.0; two claws similar, blunt (Fig. 3E). Mid leg: outer surface of coxa gray to brown, outer surface of trochanter with brown to deep brown spots and lines; other part gray to reddish brown while claws black; length ratio of femur: tibia: tarsus = 1.0: 1.1: 0.5; three basal tarsal segments subequal in length, apical segment ca. 2.0x each of them; two claws dissimilar, one blunt and one acute (Fig. 3F). Hind leg similar to mid leg in length and color pattern except its coxa and trochanter yellowish, paler than mid legs; length ratio of femur: tibia: tarsus = 1.0: 1.1: 0.5 (Fig. 3G).

Forewings pale yellowish with deep longitudinal veins and crossveins, most crossveins washed with deep pigments, especially those between C, Sc and R_1; crossveins between C and Sc in stigmatic area divided into two parts, looking like with an additional veinlet between C and Sc; bullae visible in Sc, Rs_1, Rs_5 and MP_1, crossveins nearby bullae usually more pigmented than others; forewing transparent but C to R_1 area tinted with yellowish, semi-transparent; MP_2 fused with CuA at base, and A with six veinlets (Fig. 3A). Hindwings yellowish to golden; veins dark; MA unforked, MP forked asymmetrically (Fig. 3B).

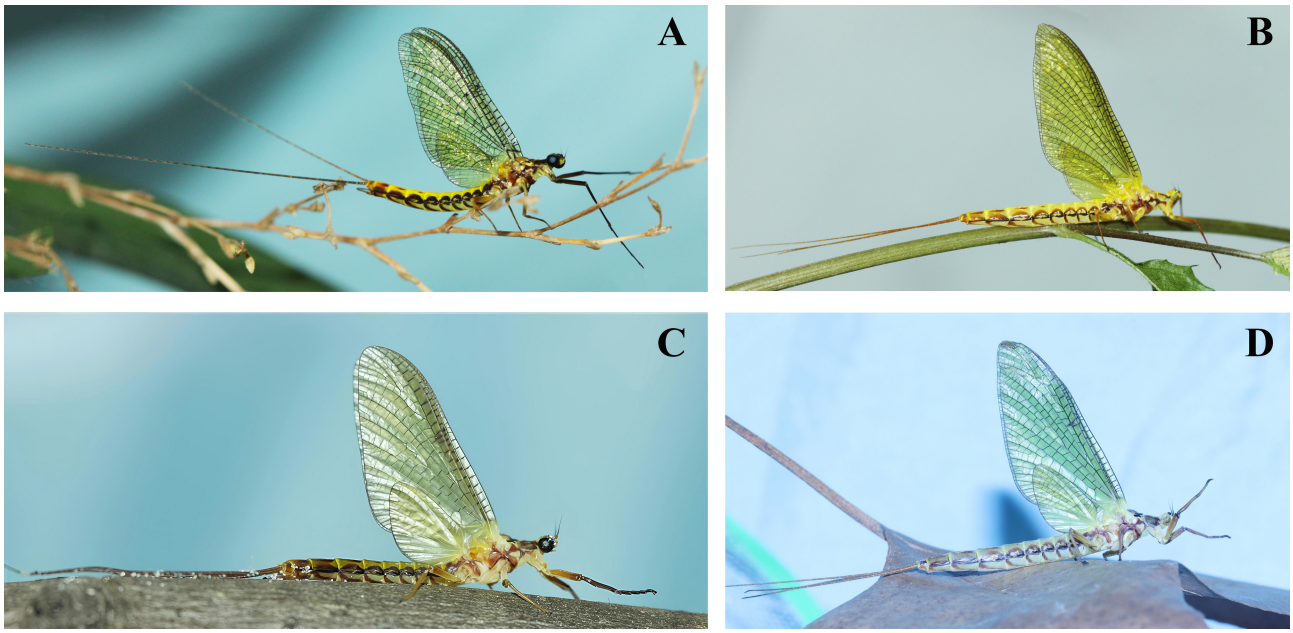

Figure 1. Imaginal habitus of *Ephemera aureola* **sp. nov. A,** Male imago; **B,** Female imago; **C,** Male subimago; **D,** Female subimago.

Abdomen: terga yellowish to golden, lateral margins deep, forming longitudinal stripe; each tergum with a pair of stripes near deep lateral margins, each of them semicircular, those stripes of tergum I extended to sub-median area; tergum II with pair of gray submedian dots, terga VI–X with pair of submedian longitudinal reddish to deep brown stripes. Sterna I–IX each with a pair of oblique stripes, each of them attaching to lateral stripe; those stripes of sternum I paler than others (Figs. 2A–C).

Genitalia: styliger yellowish, with very shallowly concave posterior margin; forceps brown to dark brown, segment I ca. 1/3x of segment II; segment III and IV shorter than segment I respectively; penes shorter than segment I of forceps, each penis with titillator, outer half of penes sclerotized and its apex extended into blunt knob-like structure (Figs. 3K–M). Terminal filament slightly shorter and narrower than cerci; all of them reddish brown to pale brown, articulations usually pale yellowish (Figs. 2A–C).

Female imago (in alcohol): Body length 24.0–29.0 mm, cerci 31.0–34.0 mm, terminal filament 29.0–30.0 mm, forewing 24.0–28.0, hind wing 11.0–14.0 mm (Fig. 1B). Similar to male but slightly paler, wings usually yellower than male and stripes of body slightly narrower (Figs. 3C–D). Posterior margin of sternum VII slightly thickened and expanded (Figs. 2D–F). Forelegs: coxa and trochanter pale yellow; femur to tarsus reddish brown to dark brown, length ratio of femur: tibia: tarsus = 1.0: 1.3: 0.9, length ratio of four tarsal segments = 1.0: 0.7: 0.6: 1.1; two claws dissimilar, one pointed and one blunt (Fig. 3H). Mid legs: coxa and trochanter yellow, somewhat darker than forelegs; two claws, one blunt, one acute but sometimes degenerated to blunt; femur to tarsus light brown, length ratio = 1.0: 1.2: 0.7 (Fig. 3I). Hind legs similar to mid legs in color; length ratio of femur: tibia: tarsus= 1.0: 0.9: 0.5 (Fig. 3J).

Male subimago (in alcohol): Similar to male imago but slightly duller. Cerci 21.0–22.0 mm, terminal filament 22.0–24.0 mm (Fig. 1C). Coxa and trochanter of legs light brown, somewhat lighter than in male imago; femur of foreleg reddish brown to dark brown, significantly shorter than that of imago, with the ratio of femur: tibia: tarsus = 1.0: 0.9: 1.0, length ratio of five tarsal segments = 1.0: 4.0: 3.0: 2.8: 3.0; two claws blunt, but acute apex of one distinct; mid legs light brown, with ratio length of femur: tibia: tarsus = 1.0: 1.3: 0.4; hind legs light brown, slightly longer than mid legs but shorter than forelegs, with ratio length of femur: tibia: tarsus = 1.0: 0.9: 0.5: 0.5; claws of mid and hind leg dissimilar, one blunt, one acute.

Female subimago (in alcohol): Similar to female imago. Cerci 24.0–26.0 mm, terminal filament 22.0–24.0 mm (Fig. 1D). Coxa and trochanter lighter than in female imagos, light brown; femora of forelegs reddish brown, claws similar to those of adults, one sharp and one blunt, length ratio of femur: tibia: tarsus = 1.0: 0.9: 0.6, length ratio of four tarsal segments = 1.0: 0.7: 0.5: 1.1; mid legs light brown, length ratio of femur: tibia: tarsus = 1.0: 1.1: 0.6; hind legs light brown, slightly longer than mid legs but shorter than forelegs, length ratio of femur: tibia: tarsus = 1.0: 0.9: 0.5; basal three segments of mid- and hind tarsi subequal in length, apical segment ca. 2.0x of them; all claws similar, one blunt, one acute.

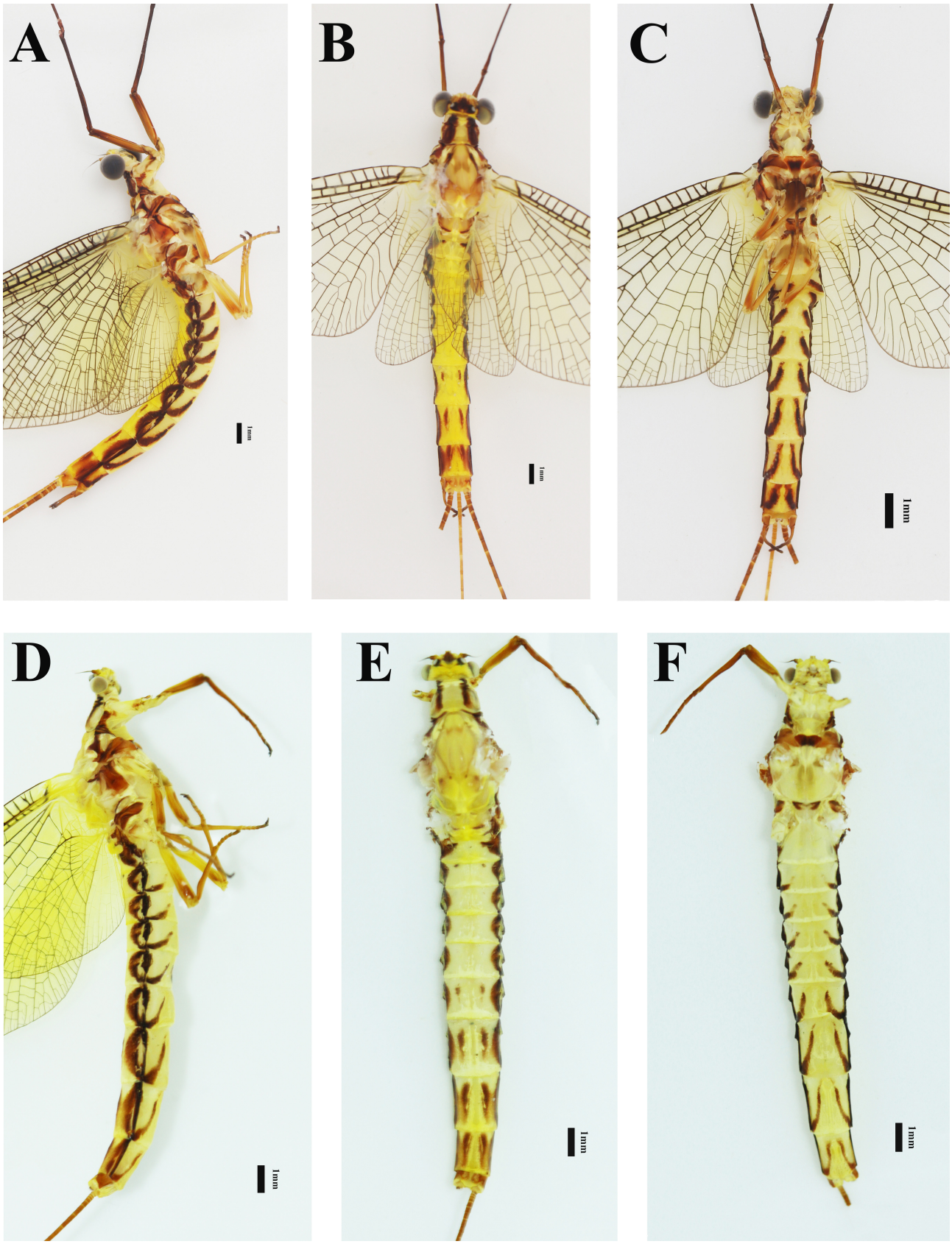

Figure 2. Adults of *Ephemera aureola* **sp. nov. A,** Male imago (lateral view); **B,** Male imago (dorsal view); **C,** Male imago (ventral view); **D,** Female imago (lateral view); **E,** Female imago (dorsal view); **F,** Female imago (ventral view).

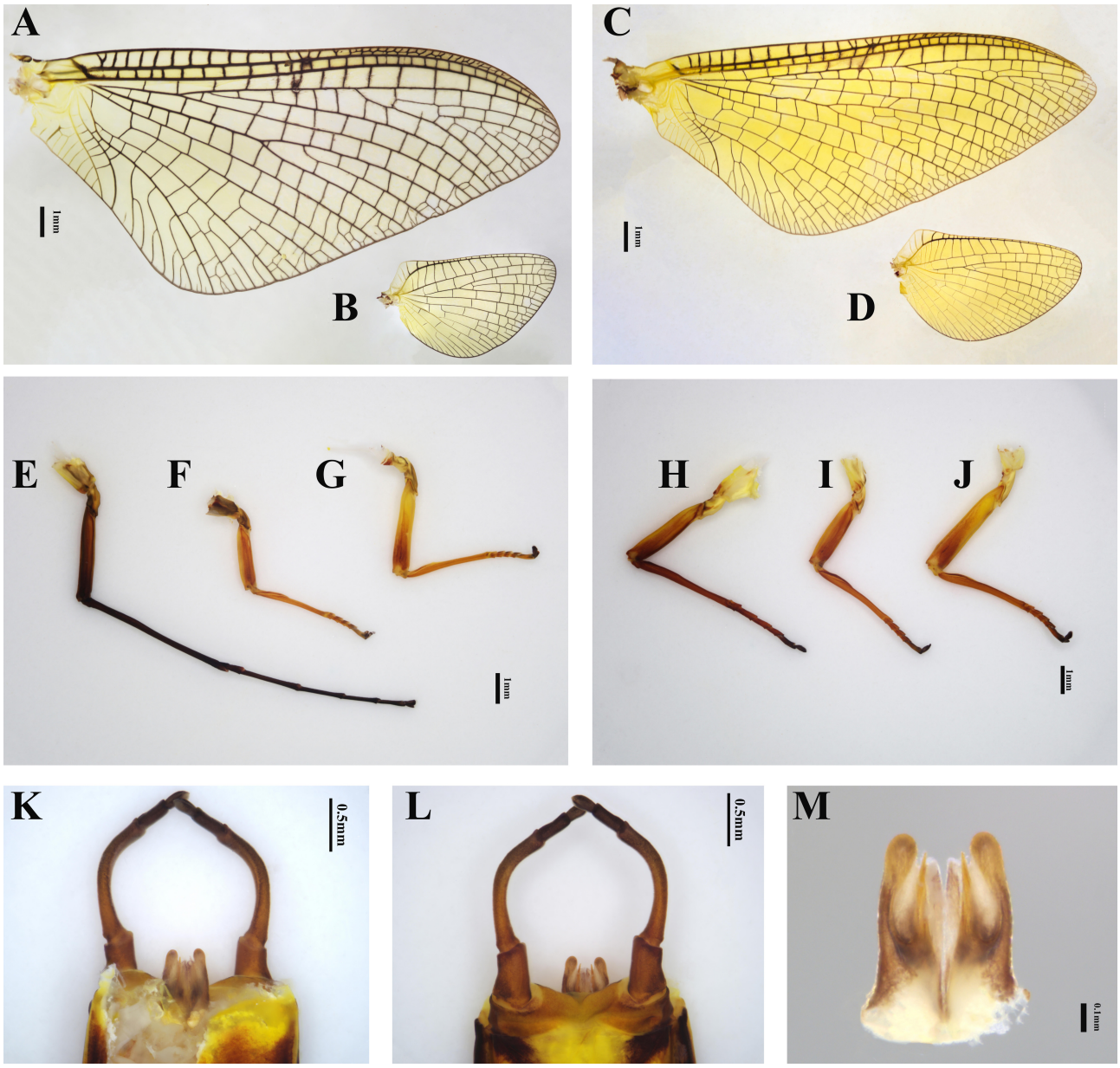

Figure 3. Imaginal characters of *Ephemera aureola* **sp. nov. A,** Forewing of male; **B,** Hindwing of male; **C,** Forewing of female; **D,** Hingwing of female; **E,** Foreleg of male; **F,** Midleg of male; **G,** Hindleg of male; **H,** Foreleg of female; **I,** Midleg of female; **J,** Hindleg of female; **K,** Male genitalia (dorsal view); **L,** Male genitalia (ventral view); **M,** Penes (ventral view).

Nymph (in alcohol): Male body length 22.0–23.00 mm, cerci 12.0 mm, terminal filament 15.0 mm; female body length 27.0–28.0 mm, terminal filament 14.0, cerci 10.0 mm. Body golden with brown to dark brown stripes and dots on thorax and abdomen, those pattern similar to adults, male nymph slightly darker and shorter than female (Figs. 4A–D). Anterior margin of frontal projection concave, its depth ca. 1/3x of whole frons; anterior half of frontal projection slightly wider than posterior half; dorsal surface with sparse golden hair-like setae; dorsal surface and area between three ocelli darker than other place of head; pedicel of antennae with golden hair-like setae on all surface and two spine-like setae on ventral surface; articulations of flagella with hair-like setae, those setae progressively shorter from base to apex. Inner base of antennae with a denticle-like projection (Figs. 6A–B).

Mouthparts: Labrum with hair-like setae on both dorsal and ventral surface, those of dorsal surface longer and denser than ventral ones; anterior margin with distinct median notch (Fig. 5A). Left and right mandibles subequal in length; in dorsal view, mandibular tusks subequal to head length (Figs. 5D–E, 6A). Outer incisor of left mandible divided into four denticles, inner one divided into three; prostheca spine-like, apex divided into two parts, each of them with serrated margins; except mandibular tusks, mandible with very sparse and short hair-like setae, a tuft of setae on mesal margin nearby molar (Fig. 5G). Right mandible similar to left one in setal pattern and length, but its

outer incisor divided into three denticles and inner incisor with two denticle-like apex; prostheca represents by a tuft of spine, much shorter and invisible than left one. Three canines and two dentisetae of maxillae distinct, along with two lines of spine-like setae at apex of galea-lacinia; dorsal, ventral and mesal surfaces of galea-lacinia with a row of hair-like setae; three segments of maxillary palp subequal in length, all of them covered with hair-like setae, those setae progressively longer and denser from base to apex. Surface of cardo with some hair-like setae (Fig. 5H). Apex of hypopharynx concave, with densely covered with hair-like setae; superlinguae with concave lateral margin, with setae on apex (Fig. 5B). Both glossae and paraglossae of labium heart-like in shape but the latter much wider than the former; dorsal surface of them with dense golden hair-like setae, ventral surface with sparse setae (Fig. 5C); length ratio of segment I: II: III of labial palp = 1.0: 0.8: 0.9, segment III expanded apically, with both hair-like and spine-like setae (Fig. 5F).

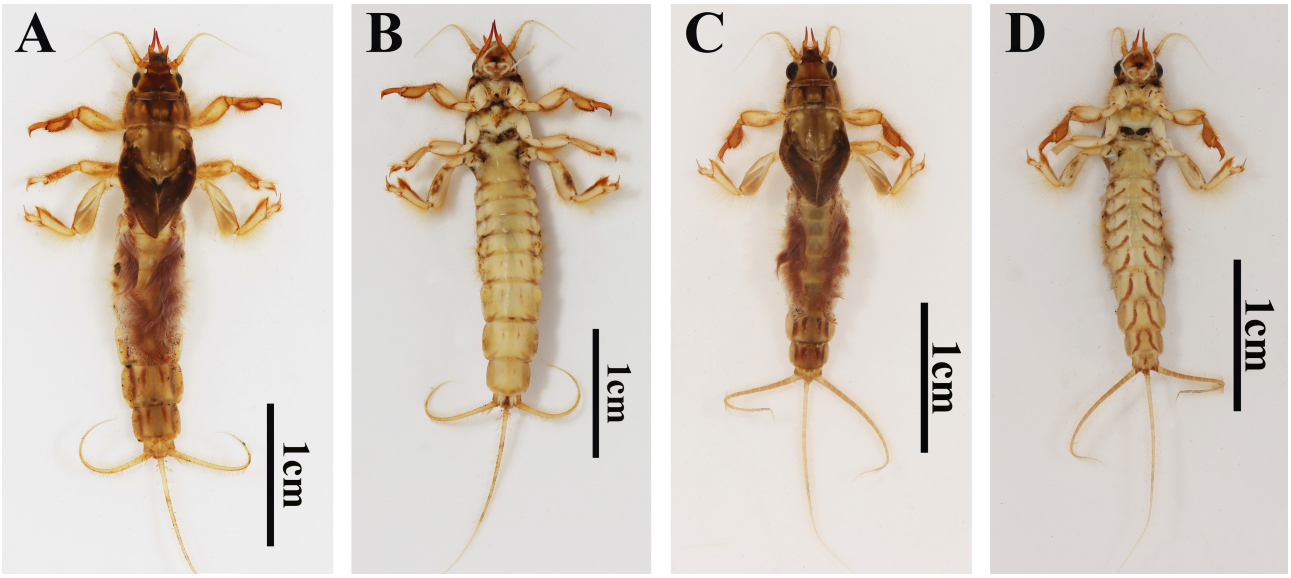

Figure 4. Nymphal habitus of *Ephemera aureola* **sp. nov. A,** Female (dorsal view); **B,** Female (ventral view); **C,** Male (dorsal view); **D,** Male (ventral view).

Forelegs: Coxa with golden hair-like setae on dorsal and ventral margins; trochanter with similar setae on ventral margin, those setae nearby coxa forming setal tuft, much longer than others; femur with two columns of hair-like setae on two margins, additional column of setae on proximal half of midline; tibia with golden hair-like setae on both margins, its free margins with additional reddish brown spine-like setae; tarsus with hair-like setae on dorsal margin and spine-like setae on ventral margin; claw with two lines of denticles; length ratio of femur: tibia: tarsus = 1.0: 1.1: 0.5 (Fig. 6G). Mid leg shorter and slightly thinner than foreleg, especially in tibiae; its setal pattern similar to foreleg but its tarsus with more and denser hair-like setae than on foretarsus; length ratio of femur: tibia: tarsus = 1.0: 1.1: 0.6 (Fig. 6H). Hind leg longer than foreleg and mid leg, with similar setal pattern of mid leg, but its femur without setae on outer surface; with two distinct white stripes intersecting base of femur; claws long and hooked; length ratio of femur: tibia: tarsus = 1.0: 0.7: 0.4 (Fig. 6I). Nota and wingpads of thorax brown to dark brown.

Abdominal color pattern similar to adults. Gills I bifurcated, each one banana shaped, with sparse hair-like setae; gills II–VII similar to each other, pigmented reddish brown (Figs. 6D–F). Tergum I with a shallow median ridge and some with hair-like setae on it (Fig. 6C). Both lateral margins of cerci and terminal filament with hair-like setae (Figs. 4A–D).

Eggs: Generally long and ellipsoid; diameter at midline ca. 110.0–125.0 μm, length ca. 185.0–230.0 μm; surface with dense elliptical projections, each about 1.5–2.5 μm (Fig. 7A).

Etymology: The species name *aureola* comes from the Latin word *aureolus*, meaning "golden" or "gold-colored", here it is indicating the golden wings and body of the new species.

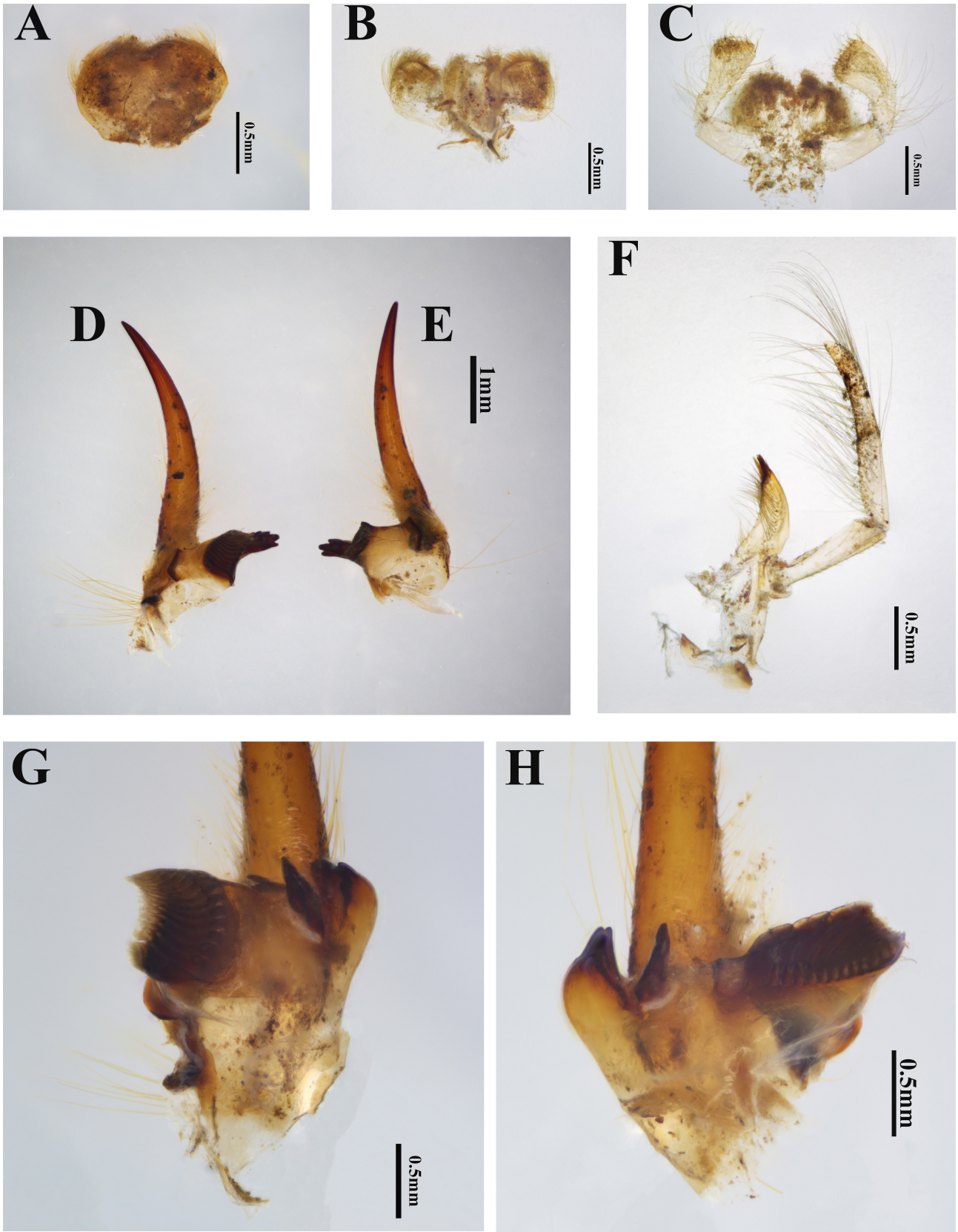

Figure 5. Mouthparts of *Ephemera aureola* **sp. nov.** nymph. **A,** Labrum (dorsal view); **B,** Hypopharynx (ventral view); **C,** Labium (ventral view); **D,** Left mandible (ventral view); **E,** Right mandible (ventral view); **F,** Maxilla (dorsal view); **G,** Left mandible (lateral view); **H,** Right mandible (lateral view).

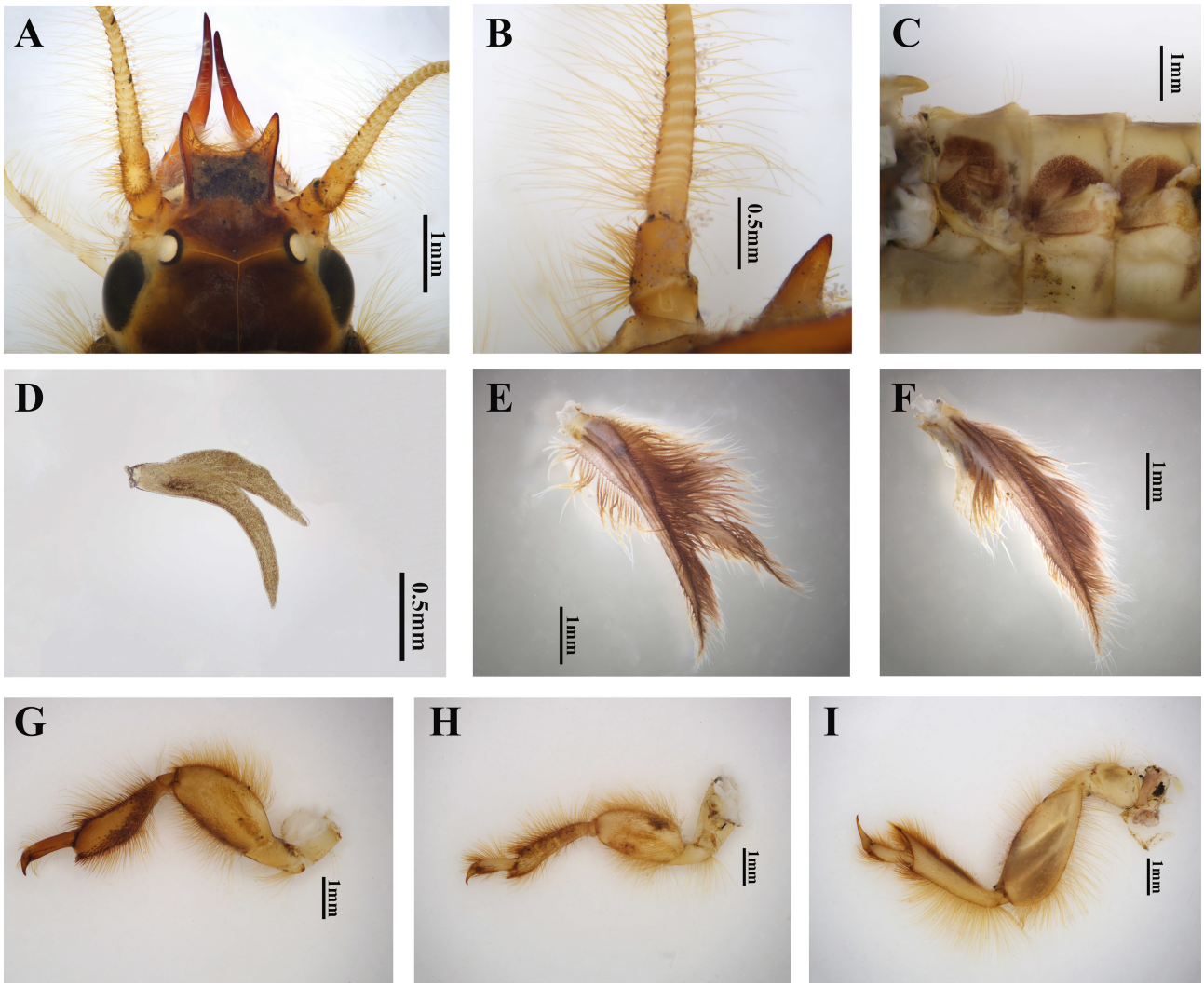

Figure 6. Nymphal structures of *Ephemera aureola* **sp. nov. A,** Male head (dorsal view); **B,** Antennal base (ventral view); **C,** Abdomen (wings and gills removed, showing the stripes and median projections; lateral view); **D,** Gill I (dorsal view); **E,** Gill II (dorsal view); **F,** Gill III (dorsal view); **G,** Foreleg (dorsal view); **H,** Mid leg (dorsal view); **I,** Hind leg (dorsal view).

Molecular similarity

The K2P values of *Ephemera aureola* **sp. nov.** to other seven close congeners are more than 15%, such as 15.50% to Korean *E. sachalinensis* Matsumura, 1911, 15.68% to *E. longiventris* Navás, 1917 from Vietnam, and 15.88% to *E. serica* Eaton, 1871 from Guangdong, China. Those data show that *E. aureola* **sp. nov.** is a new species because the K2P value of those *Ephemera* species is usually higher than 12.11% (Table 2).

Diagnosis

The new species *Ephemera aureola* **sp. nov.** described here is easily recognized by, (i) the adults and nymphs have straight stripes on abdominal terga VI–IX and oblique stripes on sterna. In previously known *Ephemera* species, have either oblique or straight stripes on both terga and sterna. This kind of mixed stripes are first recorded (Figs. 2A–F, 4A–D); (ii) *E. aureola* **sp. nov.** has a pair of dots on tergum II and stripes on terga VI–X, this kind of stripe pattern are not found before (Figs. 2B, 2E, 4A, 4C); (iii) the imaginal wings of this species are almost uniformly yellowish or golden in color, and except bulla of Sc, almost without any dots or markings. This kind of wings are not reported previously (Figs. 3A–D); (iv) genitalia of this new species are dark brown and with distinct titillators, which is almost never seen in the subgenus *Ephemera*, all other genitalia are pale yellowish (Figs. 3K–M).

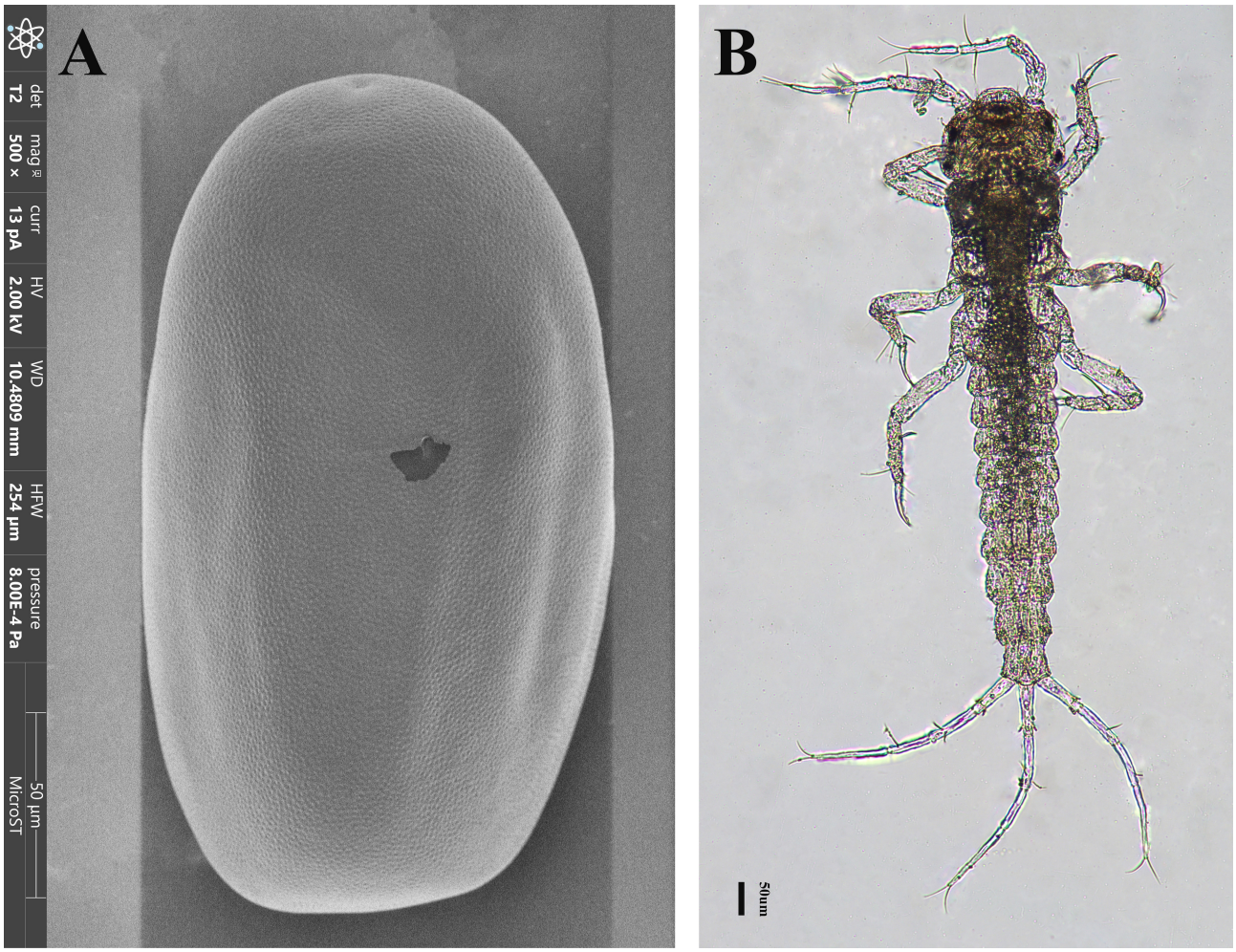

Figure 7. Egg and first instar nymph of *Ephemera aureola* **sp. nov. A,** Whole view of egg; **B,** First instar nymph (dorsal view).

Comparison

In the subgenus *Ephemera*, this new species does not have any closely related congeners. Species within this subgenus usually have straight stripes on terga and sterna, pale yellowish body and genitalia, forewings that usually with some markings in the middle, while the remaining areas are almost totally transparent and colorless.

Interestingly, this new species closely resembled to some Chinese members of the subgenus *Sinephemera* (Lei & Zhou 2024). For example, the males of *Ephemera pictipennis* Ulmer, 1924, have short stripes on terga and oblique stripes on sterna. However, *E. pictipennis* has a white body and darkly pigmented wings, its penes are much longer than those of new species and lack titillators.

Another species, *Ephemera separigata* Bae, 1995 has straight lateral streaks on the terga and oblique stripes on sterna, but it lacks additional stripe on terga and its penes also lack titillator either, its wings are hyaline with median markings which differs from the new species. Besides, *Ephemera rubera* Lei & Zhou, 2024 has two pairs of reddish streaks on the terga and oblique streaks on sterna, most part of its wings are reddish and penes also lacks titillator.

Collections

Nymphs of the new species *E. aureola* **sp. nov.** with well-developed wingpads were first collected on February 16th, 2025, which is typically the coldest period in China. Judging by its size and the local weather conditions, we believe that this species lives through the winter as the nymphs. Between April 4th–6th, well-developed nymphs were still found at the same site. Other mayfly species collected from the same canal are *Cincticostella fusca* Kang & Yang, 1995 (Ephemerellidae) and *Habrophlebiodes zijinensis* You & Gui, 1995 (Leptophlebiidae).

The nymphs of *E. aureola* **sp. nov.** were first found in a very narrow man-made canal, ca. 0.5 m width, with almost still water and a sandy to muddy bottom. Additional nymphs were collected in and around the same locality,

including the heavily muddy sediments of a small marsh. The surrounding plants around the canal and marsh are mainly bamboo bushes. The landscape of the collecting site is a low reach of a reservoir located within agricultural fields. (Fig. 8A–D).

We reared three groups of nymphs in lab to get adults. The first group (18 nymphs) was collected on February 27th, 2025. During the rearing process, seven nymphs died, nine successfully developed into female imagos and two emerged as a male subimago and a female subimago respectively. The first adult appeared on March 21st, and the last one emerged on March 24th. The second group (16 male-like nymphs) was obtained on March 26th, 2025, and the third group (7 male-like nymphs) was collected on April 4th. Of these, fourteen of them became male imagos, five became female imagos, one into a female subimago, one into a male imago and two died. Most adults emerged in early April, with the final emergence occurred on April 13th.

The nymphs show strong tolerance to habitat changes and can survive for nearly a month under laboratory conditions, eventually developing into subimagos.

Figure 8. Habitats of *Ephemera aureola* **sp. nov. A,** Environment of collection site; **B,** Shallow water of collecting site; **C,** Visible burrow of nymphs; **D,** Collectors looking for nymphs in the mud (digged out); **E,** A molted subimago standing on the water.

During our observations, most nymphs did not burrow into the artificial sandy or muddy sediments provided, despite being collected from muddy habitats in the wild. They usually hide and crawl under the leaves in our rearing tray. The rhythm of their gill movement was distinct but they also can holding their gills still for a couple of minutes. When nymphs are disturbed or touched with forceps or tree twigs, they often remain their bodies and gills still for few seconds, resembling death-feigning behaviour observed in other insects (Humphreys & Ruxton 2018). The nymphs are also good swimmers.

The last instar nymphs of this species molted on the water surface in the afternoon, between 13:00 and 17:00, local Beijing time. One individual recorded on video molted at 15:21. The molting process proceeds as follows: the last instar nymph swims to the surface of water, floats there for one or two seconds, then the nymphal cuticle splits at the head and thorax, and the subimaginal body rapidly emerges from the exuviae. Once the entire body and caudal filaments are free, the subimago stands on water surface for several seconds (one individual remained motionless for 10 seconds) before flying away. The whole process lasts about 15 seconds, with the molting movement lasting only 1–2 seconds (Fig. 8E).

The subimagos kept indoor in a net lived for about 18–22 hours before molting into imagos. One recorded female subimago emerged around 16:30 on March 24th and molted into an imago at 13:15 on March 25th. It died later that night. Another female became a subimago at 15:20 and molted into an imago around 14:10 in the next day.

The molting process from subimago to imago proceeds as follows: the subimago remains stationary on the net for a couple of hours, occasionally it moves its abdomen or caudal filaments slightly or sometimes hardly. At last, its wings spread downward along the sides of the body, and the midline of cuticle on head and thorax break. The new head and thorax appears first. They struggles to pull the rest of the body out of the exuviae, using its legs and the downward pull of its own body weight, often bending its head downward to assist in the process. Once the wings are free, it bends up and grasps the net with their legs, and finally pulls out the abdomen and caudal filaments. The whole process lasts about three minutes.

A clear sex bias was observed. In our first collection, there were 16 female nymphs but only 2 males.

On April 14th, 2025, we artificially mated a male and female successfully. The process is involved by gently holding their wings, aligning their abdomens and genitalia closely, and allowing the male penes to intercourse with the female gonopores for five seconds. The male imago died after mating. The female imago was then placed in a petri dish with some water, where it laid thousands of eggs. These eggs were incubated in the lab at about 25°C. On April 22nd, 2025, most of the eggs hatched into nymphs, and the rest egg hatched by April 23rd. The first instar nymphs were milky white in the water, swam vigorously like tadpoles. Their body length ranged from 750–815 μm; terminal filament 280–315 μm, cerci 280–320 μm. The antennae, legs and caudal filaments with long hair-like setae on all segments but body without distinct setae. Claws are slender and abdomen lack gills (Fig. 7B).

Discussion

Most species in the genus *Ephemera* can be attributed to subgenus *Ephemera* or *Sinephemera* easily because in general, the species in the subgenus *Ephemera* have the pale yellowish body, black straight streaks on abdominal terga and sterna, distinct markings on wings and genitalia with titillators. In contrast, those of subgenus *Sinephemera* have more colorful wings and bodies, oblique black or reddish stripes on abdominal terga and sterna, tinted wings without clear markings and genitalia lacks titillator. Certainly, there are exceptions, like species *Ephemera* (*Sinephemera*) *shengmi* Hsu, 1937 and *Ephemera* (*Sinephemera*) *glaucops* Pictet, 1843 have yellowish bodies and black straight abdominal stripes (Lei & Zhou 2024). However, the new species in this study outweighs them greatly on this issue. It has distinct titillators, a crucial character of the subgenus *Ephemera*, but the rest of its characters are similar to most species in *Sinephemera*. (1) Its body is relatively larger than most *Ephemera* species; (2) It has dark streaks on lateral body, but without remarkable markings on wings (Figs. 2A, 2D); (3) the stripes on sterna are oblique and reddish (Figs. 2B, 2E); (4) wings tinted with yellowish (Figs. 3A–D); (5) hindcoxae without distinct dots or markings (Figs. 3G, 3J); (6) forceps darkly pigmented (Figs. 3K–L); (7) terga I–V without distinct submedian stripes, terga VI–X with very short submedian stripes or dots (Figs. 2B, 2E); (8) nymphal terga I with shallow median ridges and hair-like setae. Thus, in taxonomy, the characteristics of this new species bridge the two subgenera together, greatly narrowing the gap between them. It seems except titillator, no any other character can separate them (Fig. 6C).

The borrowing mayflies, especially those of the families Polymitarcyidae and Palingeniidae, have whitish or pale yellowish bodies. But the mayflies of the genus *Ephemera* always have conspicuous markings on their body.

Generally, the abdominal stripes of the genus *Ephemera* can be divided into two types: straight and oblique. The perfect examples are *Ephemera* (*Sinephemera*) *pictiventris* McLanchlan, 1894 and *Ephemera* (*Ephemera*) *pieli* Navás, 1934 (Lei & Zhou 2024; Li *et al.* 2023). The abdominal stripes of the former species are all oblique while those of the latter are totally straight. The stripe pattern of the new species shows a mixed type: those on terga are straight while those of sterna are oblique (Figs. 2A–F).

Where do the stripes of *Ephemera* come from? The stripe pattern observed in the new species provides a possible clue. Similar to typical species in the family Potamanthidae, this species has lateral markings from head to end of the body, submedian stripes on terga I and all stripes of sterna connected to lateral markings (Figs. 2A–F). So in our opinion, the submedian stripes or dots on terga and sterna may be extensions of these lateral markings. In most *Ephemera* species, these connections appear to break off near the base, resulting in isolated paired stripes.

The small canals of shallow water, especially those nearby agricultural fields are often overlooked by aquatic insect collectors because of difficulty of sampling, the rarity of the species and the sparse distribution of individuals. However, the new species reported here shows that certain type of mayfly can live in these environments, and may have unique and primitive characteristics. In future collections, we should not only focus on the natural water bodies but also explore the man-made underwaters.

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