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https://doi.org/10.11646/zootaxa.5271.2.8 http://zoobank.org/urn:lsid:zoobank.org:pub:742EC353-E486-4C8E-9734-032F361843A8

The Exact Morphology of the Species *Ephemera pieli* Navás, 1934 from Eastern China (Ephemeroptera: Ephemeridae)

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Abstract

The original description and outline figure of the mayfly *Ephemera pieli* Navás, 1934, were based on male subimagoes, and its morphology and taxonomic status have not been confirmed since then. However, recently collected materials from eastern China, including all stages of this mayfly, reveal several unique characteristics that indicate *E. pieli* is a valid species in the subgenus *Ephemera* (*Ephemera*). These characteristics include forewings with numerous dark dots, hindwings or hind wingpads with one or two distinct median dots, penes covered mostly by the subgenital plate, and the apex of the penis bluntly extended. A neotype is designated.

Key words: *Ephemera*, mayfly, neotype, morphology, life history

Introduction

The species *Ephemera pieli* was first named and described by Navás (1934) in Latin without mentioning the sex of his material. The types were deposited in the Musée Heude (Heude Museum), Shanghai, China. Later, Hsu (1937) re-checked those specimens and re-described them in English, stating clearly those types were two male subimagoes. Both descriptions of the species note that it has hindwings with two distinct median spots and abdominal terga I-II without longitudinal stripes. These two characteristics are crucial for identifying the species. However, from then on, although it has been mentioned by a series of researchers (Gui, 1985; You et Gui, 1995; Zhang *et al.*, 1995; Kluge, 2004; Hwang *et al.*, 2008), neither new information nor more descriptions were added to this species.

In 2017, the senior author of this paper visited the former Musée Heude, finding two dry useless damaged specimens with the label "*Ephemera pieli*" but no type marking on it. We believe those types have been lost. So new materials must be collected to clarify this issue.

During the past ten years, we collected multiple times in more than 15 locations in and around Jiangsu Province, where the types of the species *Ephemera pieli* came from, getting 500 individuals in the genus *Ephemera*. Among them, some nymphs and adults, which have brown to dark spots on hindwing pads or hindwings, remind us the species *Ephemera pieli*. After careful examination, we confirmed this identification. The new material provide us a good opportunity not only to ensure the status of this species but also to describe its exact morphology.

Materials and methods

Photos were taken by a digital camera (Mshot MSX 11, single lens Reflex, Mingmei photoelectric, Guangzhou, China) coupled to a stereomicroscope (Mshot MZ81, Mingmei photoelectric, Guangzhou, China). Photos were edited and imporved with Adobe Photoshop CC.

All adults were attracted by light (small road lights set nearby water), nymphs were collected by hand net. They were stored directly in ethanol (more than 80%).

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

(Figs.1–8)

Ephemera pieli Navás, 1934: 5, fig. 44 (adult). Types: adults, Loubou, Kiangsu (Jiangsu), China. *Ephemera pieli*—Navás, 1935: 99; Wu, 1935: 248; Ulmer, 1936: 212; Hsu, 1937: 295, fig. 24 (male subimago); Gui, 1985: 95; You et Gui, 1995: 111, fig. 118 (male subimago); Zhang *et al.*, 1995: 75; Kluge, 2004: 237; Hwang *et al.*, 2008: 163.

Distribution: China (Jiangsu, Zhejiang, Fujian).

Material examined: 1 male imago (neotype designed), County Road 211, Baishinan, Hanjiang Region, Putian City, Fujian Province, China, 25°31′40.29″N, 119°11′8.29″E, 22m a.s.l., 1–10-V-2022, leg. Zhiming Lei; 1 male imago, 19 female imagoes, 5 nymphs, same data as neotype: 1 male imago, Jiulong Wetland Park, Lishui City, Zhejiang Province, China, 22-VIII-2022, leg. Peng Li, Jianhua Dai, Hong Li; 86 Nymphs and 5 female imagoes, Baishinan, Hanjiang Region, Putian City, Fujian Province, China, 25°31′40.29″N, 119°11′8.29″E, 22 m a.s.l., 20-VIII-2022, leg. Zhiming Lei; 3 female imagoes, County Road 201, Nanping City, Fujian Province, China, 27°50′14.38″N, 117°58′11.46″E, 283.4 m a.s.l., 20-VII-2022, Xinhe Qiang; 31 male subimagoes, 8 female subimagoes, Baiyun National Park, Lishui City, Zhejiang Province, China, 119°54′56.3″E, 28°29′ 30.10″N, 260 m a.s.l., 29-VI-2021, leg. Changhai Sun, Xinyu Ge, Lang Peng; 19 female subimagoes, 1 male subimagoes, Tianmu Lake Wetland Park, Changzhou City, Jiangsu Province, China, 31°14′10.52″N, 119°25′55.24″E, 24-V-2022, leg. Peng Li.

Mature nymph (in alcohol): Body length:19.0–20.0 mm, caudal filaments 4.0–5.0 mm (Figs. 1A–B); body yellowish to amber dorsally (Fig. 1C), hair-like setae on legs yellow to golden (Figs. 1A–B); gills reddish brown. Ventral body pale to cream (Fig.1D). Vertex of head smoothly convex, with seven dark brown stripes or dots (Fig. 2A): anterior one at base of frons midline, first pair triangular, second pair short stripe-like, nearby lateral ocelli; third pair dot-like, close to compound eyes; base of ocelli brown too; compound eyes located posterolaterally while antennae anterolaterally (Fig. 2A); ventral sufaces of antennal base, scape and pedicel with a dark dot respectively (Fig. 2B); base of antennae with a projection dorsally (Fig. 2C), scape ca. 1/2× pedicel in length (Figs. 2A–C), whole dorsal surface of pedicel with golden hair-like setae, ventral surface with two golden spines (Fig. 2B); flagellum with a transverse row of hair-like setae too; antennae progressively narrowed from base to apex. Frons distinct, length ca. 0.88× width, anterior margin concave, with relatively long hair-like setae; dorsal surface with shorter hair-like setae, lateral margins with very sparse setae (Fig. 2A).

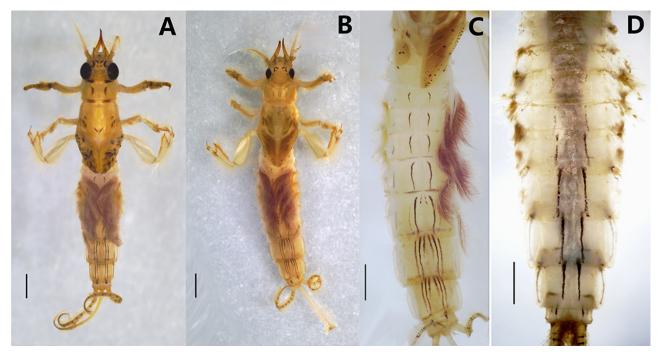


FIGURE 1. Nymphal habitus of *Ephemera pieli*. A: Male (dorsal view); B: Female (dorsal view); C: Female abdomen (left fore wingpad and gills removed, showing the hind wingpad, abdominal stripes and posterolateral projections; dorsal view); D: Female abdomen (ventral view). Scale bar= 1 mm

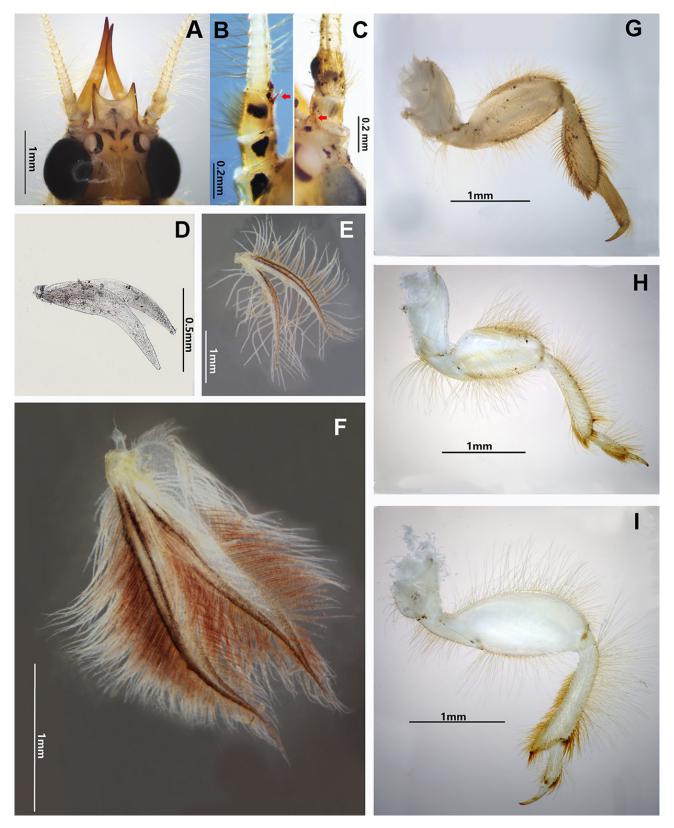


FIGURE 2. Nymphal structures of *Ephemera pieli*. A: Male head (dorsal view); B: Antennal base (ventral view, red arrow indicating the two bristles); C: Antennal base (dorsal view, red arrow indicating the projection); D: Gill I; E: Gill VII; F: Gill II; G: Foreleg (dorsal view); H: Mideleg (dorsal view); I: Hindleg (dorsal view).

Mouthparts: labrum with hair-like setae on both dorsal and ventral surfaces; anterior margin with shallow median emargination, two rows of bristles besides it (Fig. 3A). Left mandible: outer incisor divided into four apical denticles, inner one with three denticles; prostheca fused with inner incisor basally, stick-like but apex divided into several spines; tusk with golden to brown apex, outer base with some spine-like and hair-like setae (Fig. 3B). Right mandible similar to left one but tusk slightly shorter, outer and inner incisor divided into three and two denticles respectively; prostheca shorter than left one; molar with an projection near prostheca (Fig. 3C). Maxilla: dorsal and ventral surface with a row of hair-like setae, mesal margin with a row of setae too; three canine present, two distal dentisetae clearly visible (Figs. 3D–E); segment I of maxillary palpi with hair-like setae on outer margin, segments II and III with setae on whole surface; length ratio of segments = 1.00: 0.64: 0.72; cardo with sparse hair-like setae; superlinguae with notch on outer margin, both surface with setae (Fig. 3G). Labium (Fig. 3F) with glossae heart-like in shape, paraglossae slightly longer than glossae, both sides of them with dense hair-like setae; segment I: II: III of labial palpi = 1.00: 0.39: 0.75; segment I with two rows of long hair-like setae, one on inner margin, one on middle ventral surface; segments II and III with setae on whole surface; segment III expanded progressively from base to apex, making it with a straight apex, with some spine-like setae (Fig. 3F).

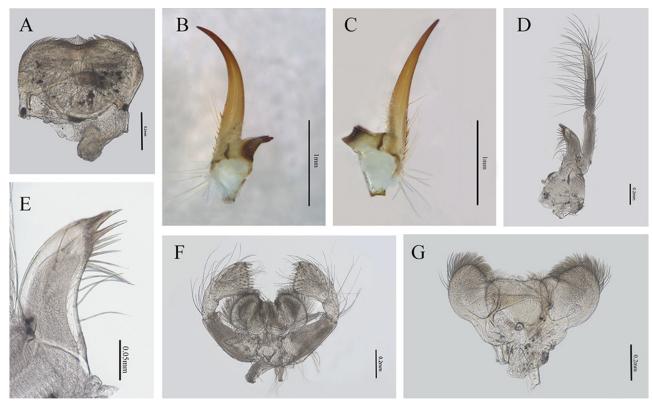


FIGURE 3. Mouthparts of *Ephemera pieli* nymph. A: Labrum (dorsal view); B: Left mandible; C: Right mandible; D: Maxilla (dorsal view); E: galea-lacinia of maxilla enlarged (dorsal view); F: Labium (ventral view); G: Hypopharynx (ventral view).

Thorax: Nota yellow to amber, pronotum with a pair of brown median longitudinal stripes, mesonotum with a pair of oblique brown stripes near midline; base of wingpads browner than other places (Figs. 1A–B); forewingpads with distinct dark dots (Figs. 1A–C), hind-wingpads with two dark median dots (Fig. 1C); median parts of mesonotum expanded posteriorly into lobe (Fig. 1C). Margins of nota with rows of hair-like setae; coxa and trochanter with setae on inner margin (Figs. 2G–I). Dorsal surface of forefemora with three longitudinal rows of golden hair-like setae: one on middle, two on margins; inner apex with some spine-like setae (Fig. 2G); dorsal surface of foretibiae with dense golden hair-like and spine-like setae (Fig. 2G); foretarsi with hair-like setae on whole surface, spine-like setae on inner margin only (Fig. 2G); forefemora wider than tibiae, foretibiae wider than tarsi and with expanded apical 2/3. Length ratio of forefemora: tibiae: tarsi = 1.00: 1.03: 0.64 (Fig. 2G). Midleg similar to foreleg in color and setal pattern but shorter than foreleg (Fig. 2H), midtibiae with sparser but stronger spine-like setae than counterparts of forelegs (Fig. 2H); length ratio of midfemora: tibiae: tarsi = 1.00: 1.02: 0.63 (Fig. 2H). Hindfemora with hair-like setae on both margins; projection of hindtibiae ca. 0.7x hindtarsi, tibiae with

more and longer hair-like and spine-like setae (Fig. 2I). Length ratio of hindfemora: tibiae: tarsi = 1.0: 0.72: 0.33 (Fig. 2I). Claw apex of all legs hooked (Figs. 2G–I).

Lateral margins of abdomen with hair-like setae, gill locations of tergites II–VII also surrounded by golden hair-like setae, those of tergites III–VI denser than others (Fig. 1C). Gills I much smaller than others, bifurcated into two branches, progressively thinner from base to apex, margins with very sparse and tiny hair-like setae (Fig. 2D). Gills II–VI similar to each other, progressively smaller from anterior to posterior, with reddish to brown median trachea, maginal fringes pale to reddish; ventral lamella with broader base than dorsal one (Fig. 2F). Gills VII with two similar thin lamellae and pale fringes (Fig. 2E). Tergites III–VI and X with one pair of dark longitudinal stripes, tergites VII–IX each with three pairs of stripes (Figs. 1A–C). Sternites III–X with one pair of dark stripes (Fig. 1D). All posterior margins of tergites and sternites dark but sometimes indistinct (Fig. 1C–D). Cerci and terminal filament with hair-like setae on both lateral sides, articulations of cerci usually dark (Figs. 1A–B).

Male imago (in alcohol): body length 15.0–16.0 mm, cerci 21.0–22.0 mm, terminal filament 18.0–20.0 mm; body general pale to cream, with chocolate brown to dark stripes, dots on head and thorax (Figs. 4A, 5D–F). Upper portion of compound eyes grey, lower portion dark, eyes spherical (Figs. 4A, 5C–F); upper portions of ocelli pale to grey too; base of all ocelli and compound eyes dark; pedicel of antennae with dark dots to bands on apex, other parts pale to yellowish (Fig. 5C). Pronotum and anterior half of mesonotum with a pair of lateral longitudinal stripes, three additional brown to dark dots between them on mesonotum, two pairs of them grey to chocolate, the other bigger pair brown (Fig. 5D); scutellum of mesonotum with a pair of dark dots too (Fig. 5D); forewings with brown dots, especially at bulla points and bases of MA, MP (Figs. 4A, 5A); almost all crossveins pigmented with amber cloud, those of costal and subcostal regions more distinct (Fig. 5A); MP₂ fused with CuA at base shorter or longer but its base clear (Fig. 5A); three to four pairs of intercalaries between CuA and CuP (Fig. 5A). Hindwings with cream leading and outer margins, forking point of Rs pigmented with brown to dark dots, two other crossveins between MA and MP, MP₁ and MP₂ also washed with dark dots (Fig. 5B), they look like two big dots at median area of hindwing; almost all crossveins also pigmented more or less; MA single, MP forked asymmetrically (Fig. 5B).

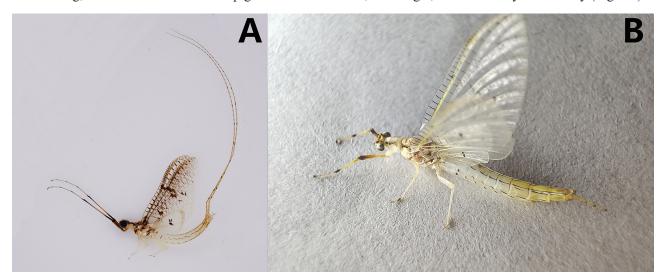


FIGURE 4. Imaginal habitus of Ephemera pieli. A: Male imago; B: Female imago.

Forelegs: coxa and trochanter pale to cream, with dorsal brown stripes; other part of foreleg amber to brown, apexes of all segments and basal tibiae washed with chocolate to dark; length ratio of forefemur: tibia: tarsus= 1.0: 2.6: 3.5; that ratio of five segments of foretarsus = 1.0: 8.4: 7.0: 5.0: 3.0; foreleg with two similar blunt claws (Fig. 5G). Coxa and trochanter of midleg with reddish to brown stripes, other parts pale to cream except tibia with a dot on apex; length ratio of midfemur: tibia: tarsus = 1.0: 1.2: 0.6, length ratio of four segments of midtarsus = 1.0: 0.9: 0.7: 3.1 (Fig. 5H). Hindleg with one to two dark dots on coxa, other part pale; hindfemur: tibia: tarsus = 1.0: 0.9: 0.4, three basal segments of tarsus subequal, apical segment about 3.0x each of them (Fig. 5I). Mid- and hindleg each with a pair of dissimilar claws, one blunt, one acute (Figs. 5H–I).

Abdominal tergites and sternites I–IX each with a black posterior margin, those lines of segment I–III sometimes unclear or divided into two (Figs. 5D–E); tergites I–X with dark lines on lateral margins (Fig. 5F); tergite I pale to cream (Fig. 5D); tergite II with a pair of dark dots near lateral margins (Figs. 5D, 5F); tergites III–VI, X each with

a pair of longitudinal stripes (Fig. 5D); tergites VII–IX with three pairs of dark median lines, the inner or outer pair sometimes indistinct (Fig. 5D); sternites III–X each with a pair of dark stripes (Fig. 5E).

Genitalia (Figs. 5J–L): posterior margin of subgenital plate smoothly concave (Fig. 5K); forceps amber but apexes of segments 2–4 dark (Figs. 5K–L); length ratio of all segment from base to apex = 1.0: 3.5: 0.85: 0.85; basal segment broader than others, other three segments subequal in width (Figs. 5K–L). Lateral margin of penis slightly concave, apex round, narrowed smoothly; two titillators distinct (Fig. 5J).

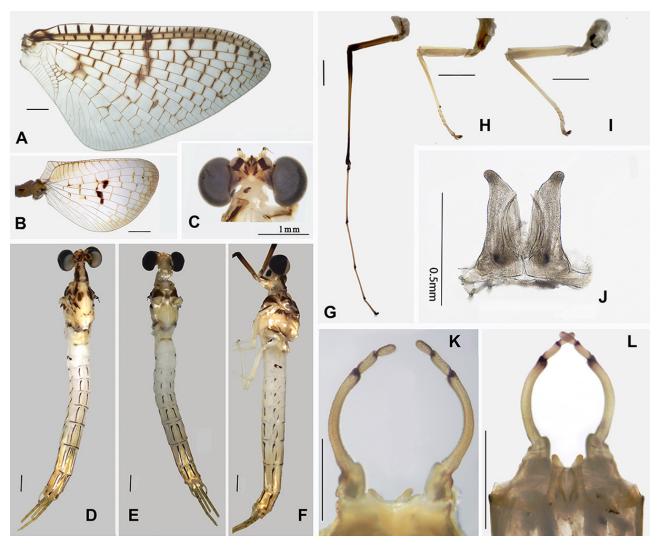


FIGURE 5. Structures of *Ephemera pieli* male imago. A: Forewing; B: Hindwing; C: Head (dorsal view); D: Habitus (dorsal view); E: Habitus (ventral view); F: Habitus (lateral view); G: Foreleg; H: Midleg; I: Hindleg; J: Penes (ventral view); K: Genitalia (ventral view); L: Genitalia (dorsal view) (scale bar=1 mm except in J= 0.5 mm).

Terminal filament cream to deep yellow, joints of it with chocolate to dark ring (Fig. 4A); cerci slightly deeper than terminal filament in color, amber to deep amber, besides dark joints, every four segments with dark bands (Fig. 4A).

Male subimago (in alcohol): body 15.0–16.0 mm, cerci 14.0–15.0 mm, terminal filament 10.0 mm. body color similar to male imago but a little bit duller or paler, especially ventral thorax and legs, dot and stripe pattern on body similar to male imago (Figs. 6A, 6C–D). Forecoxae and trochanters pale, apexes of all other segments and basal forefemora dark, all claws dark; midleg pale, hindleg pale too except coxae with a dark dot (Fig. 6A). Length ratio of forefemur: tibia: tarsus = 1.0: 1.5: 1.6, length ratio of five segments of foretarsus = 1.0: 4.4: 3.8: 2.8: 1.5 (Fig. 6A). Length ratio of midfemur: tibia: tarsus = 1.0: 1.5: 0.6, length ratio of four segments of midtarsus = 1.0: 1.0: 1.1: 2.4 (Fig. 6A). Length ratio of hindfemur: tibia: tarsus = 1.0: 1.2: 0.4, length ratio of four segments of hindtarsus = 1.0: 1.0: 0.8: 3.8 (Fig. 6A).

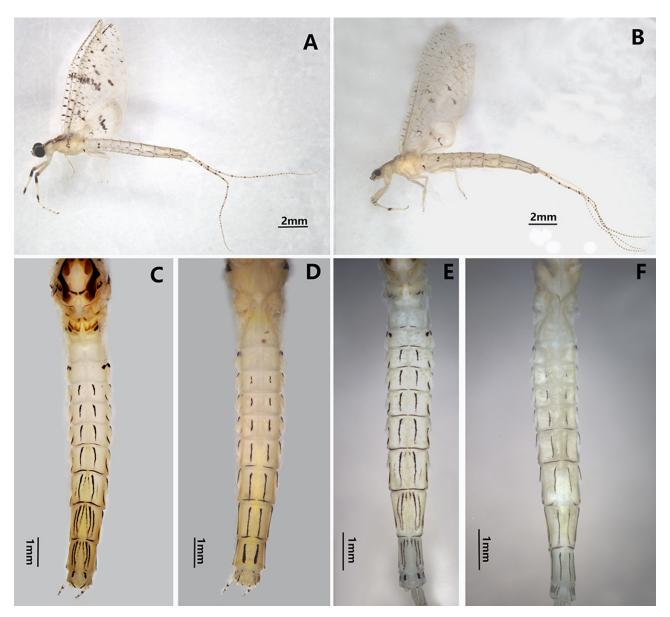


FIGURE 6. Morphology of *Ephemera pieli* subimago. A: Male (lateral view); B: Female (lateral view); C: Male abdomen (dorsal view); D: Male abdomen (ventral view); E: Female abdomen (dorsal view); F: Female abdomen (ventral view).

Female imago (in alcohol): body length 14.0–15.0 mm, cerci 16.0–17.0 mm. Body color pattern similar to male imago but dark dots and stripes on wings and body smaller (Figs. 4B, 7A, 7F–H); forecoxae and trochanters pale, apical half of forefemora golden, apexes of forefemora and tibiae with dark apex (Fig. 7C); midleg pale (Fig. 7D), hindcoxa with one or two dark dots (Figs. 7E, 7H). Length ratio of forefemur: tibia: tarsus = 1.0: 1.1: 0.7, length ratio of five segments of foretarsus = 1.0: 4.5: 3.5: 2.3: 4.0 (Fig. 7C). Length ratio of midfemur: tibia: tarsus = 1.0: 1.5: 0.6, length ratio of four segments of midtarsus = 1.0: 1.0: 1.1: 2.6 (Fig. 7D). Length ratio of hindfemur: tibia: tarsus = 1.0: 0.9: 0.4, length ratio of four segments of hindtarsus = 1.0: 1.0: 1.0: 3.4 (Fig. 7E). Margins of hindwings without clear cloud but median dots clear at middle (Fig. 7B). Subgenital plate of sternum VII extended slightly, with a waved posterior margin (Fig. 7G).

Female subimago (in alcohol): body length 9.0–10.0 mm, cerci 11.0–12.0 mm, terminal filament 10.0–11.0 mm. color pattern similar to female imago (Figs. 6B, 6E–F). Length ratio of forefemur: tibia: tarsus = 1.0: 1.3: 0.9, length ratio of five segments of foretarsus = 1.0: 2.1: 1.9: 1.3: 2.3 (Fig. 6B). Length ratio of midfemur: tibia: tarsus = 1.0: 1.3: 0.5, length ratio of four segments of midtarsus = 1.0: 1.2: 1.1: 3.0 (Fig. 6B). Length ratio of hindfemur: tibia: tarsus = 1.0: 0.9: 0.4, length ratio of four segments of hindtarsus = 1.0: 1.0: 1.0: 2.8 (Fig. 6B).

Egg: whole surface with very shallow ridges, without clear shape or pattern; micropyle near equator (Fig. 8).

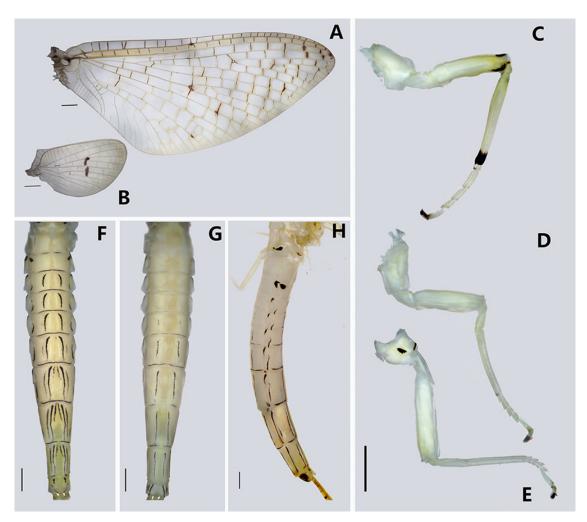


FIGURE 7. Structures of *Ephemera pieli* female imago. A: Forewing; B: Hindwing; C: Foreleg; D: Midleg; E: Hindleg; F: Abdomen (dorsal view); G: Abdomen (ventral view); H: Abdomen (lateral view) (scale bar=1 mm).

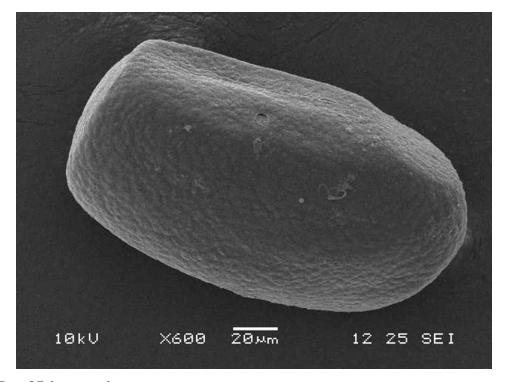


FIGURE 8. Egg of *Ephemera pieli*.



FIGURE 9. One habitat of Ephemera pieli nymph (Putian, Fujian Province, China)

Diagnosis

All stages of the species *Ephemera pieli* (mature nymph, subimago and imago) can be identified easily by their dark dots on hindwings or hind wingpads (Figs. 1C, 4A–B, 5B, 6A–B, 7B). In addition, the other color pattern is also very helpful: the fore- and midcoxae of adults of this species have brown markings (Figs. 4A–B, 5G–H), the hindcoxae have dark dots (one or two, Figs. 4A–B, 5F, 5I, 7E), the abdominal stripes on tergites (tergite I without any dark marking, tergite II with a pair of dots, tergites III–VI with one pair of stripes, tergites VII–IX with three pairs, tergite X with one or two pairs, Figs. 5D, 6C, 6E, 7F); sternites III–X with one pair of lines (Figs. 5E, 6D, 6F, 7G), and all segments of the abdomen with dark transverse lines on posterior margins (Figs. 4A–B, 5D–F, 6A–F, 7F–H). For male imago, besides color pattern, its genitalia is also somewhat unique: forceps with subequal segments III and IV, penes with somehow tapered apex, and its subgenital plate with smoothly concave free margin (Figs. 5J–L).

There are several Chinese *Ephemera* species, like *E. wuchowensis* Hsu (1937) and *E. serica* Eaton (1871), having similar abdominal stripes but their hindwings never have distinct large dots. The species *E. rufomaculata* Zhou & Zheng (2003) and *E. purpurata* Ulmer (1919) have dots on hindwings but their tergites I–II have dark markings. Some other species whose hindwings have clear markings, like *E. pictipennis* Ulmer (1924) and *E. nigroptera* Zhou *et al.* (1998), do not have titillator on their penes. Additionally, their longitudinal veins of wings are dark, the stripes on the abdomen are oblique.

The egg of *Ephemera pieli* is similar to those of the Chinese *E. lota* Navás, 1933 or European *E. vulgata* Linnaeus, 1758 and *E. danica* Müller, 1764 (Bauernfeind and Soldán, 2012).

Discussion

The nymphs of *Ephemera pieli* have two mandibular tusks subequal in length. Its male imagoes have titillators on penes, its abdomen has longitudinal stripes, and MP₂ fuses with CuA at base of forewing. Those characters indicate this species is a member of the subgenus *Ephemera* (*Ephemera*) in both systems of McCafferty & Edmunds (1973) and Kluge (2004).

The streams or rivers where *Ephemera pieli* nymphs live are usually with stony bottoms, sand and mud. The nymphs can be found in lentic sections of water (Fig. 9).

Acknowledgments

This work was supported by the National Natural Science Foundation of China, grant numbers 31750002 and 32070475; the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD); the key projects of science-technology basic condition platform from The Ministry of Science and Technology of the People's Republic of China, grant number 2005DKA21402; Project of Biological Resources Survey in Wuyishan National Park; the Biodiversity Survey and Assessment Project of the Ministry of Ecology and Environment of China, grant number 2019HJ2096001006. Mr. Zhi-Ming Lei help us take and edit the photos.

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