

# Taxonomic review of Korean *Ephemera* Linnaeus with lectotype designation of *E. sachalinensis* Matsumura (Insecta: Ephemeroptera: Ephemeridae)

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The common mayfly genus *Ephemera* Linnaeus from South Korea was taxonomically reviewed, with emphasis on the sympatric *E. orientalis* McLachlan and *E. sachalinensis* Matsumura. A taxonomic key to South Korean *Ephemera* species is provided for larval and adult stages. The thick and continued pair of dark stripes on mesonotum and presence of pigmentation on abdominal tergum I–II are the diagnostic characters to distinguish *E. orientalis* from *E. sachalinensis* in both male and female adults and larvae. In the larvae, the shape and length of foreleg claws, and the shape of posterior margin of tergum X are useful diagnostic characters to distinguish between these two species. In addition, a female specimen deposited in the Hokkaido University Museum, Sapporo, Japan, that was used in the original description of *E. sachalinensis* by Matsumura in 1911 is designated as the lectotype of the species. A DNA barcoding analysis on the East Asian *Ephemera* species with paired multi-stripes on the abdominal terga well substantiated their species status.

Keywords: DNA barcoding, *Ephemera sachalinensis*, Ephemeridae, Korean *Ephemera*, lectotype, taxonomic review

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## INTRODUCTION

The burrowing mayfly family Ephemeridae has a world-wide distribution with exception in the poles and Oceania, and the family consists of four extant genera. The genus *Ephemera* Linnaeus is represented by approximately 60 nominal species; majority of them occur in China (30 species) (Yoon and Bae, 1985; Hwang and Bae, 2008; Hwang *et al.*, 2008). Despite the *Ephemera* species are relatively large in body size among mayflies and easily sampled from diverse freshwater habitats, morphological identifications of some closely related species still are challenging (Hwang *et al.*, 2009; Lee *et al.*, 2011). Since the early classical studies by Eaton in the late nineteenth century (Eaton, 1871; 1883-1888; 1892), East Asian *Ephemera* were studied by Tshernova (1973), Gose (1981), and Hwang and Bae (2008).

Historically, the *Ephemera* species have been identified by taxonomical keys by original descriptions and type information (Tshernova, 1973; Hwang and Bae, 2008).

Paired multi-stripes on the abdominal terga as one of important taxonomical keys to differentiate them used in closely related *Ephemera* species (Tshernova, 1973; Hwang and Bae, 2008). Some of *Ephemera* species often exhibit a wide range of intraspecific variations in all the life stages that make accurate species identification difficult (Hwang *et al.*, 2003; Hwang and Bae, 2008). For example, accurate species identification of widely distributed and sympatric *E. orientalis* McLachlan, 1875 and *E. sachalinensis* Matsumura, 1911 have frequently been occurred in ecological or biomonitoring studies in South Korea (Bae *et al.*, 1994; Hwang *et al.*, 2003). Particularly, the repeated mass emergence of *E. orientalis* with *E. sachalinensis* in the lower reaches of streams and large rivers in South Korea requires detailed taxonomic identification of these two species. Thus, the purpose of this study is to taxonomically review Korean *Ephemera* species with emphasis on distinct species delimitation of the two sympatric species, *E. orientalis* and *E. sachalinensis*. In addition, we designate a female *Ephemera* specimen

preserved in the Matsumura's collection in the Hokkaido University Museum as a lectotype of *E. sachalinensis*. We also provided DNA barcoding information using mitochondrial *COI* gene sequences to support identification of the East Asian *Ephemera* species.

## MATERIALS AND METHODS

All specimens used in this study are deposited in the Korea University Entomological Museum, Seoul, Republic of Korea (KUEM). Approximately 150 specimens (larvae, subimagos, and adults) from different localities in South Korea were examined and identified using the original description and taxonomic keys (McLachlan, 1875; Eaton, 1892; Matsumura, 1911; Demoulin, 1965; Tshernova, 1973; Bae, 1995; Hwang *et al.*, 2003).

For *COI* gene sequencing of the *Ephemera* species, genomic DNA was extracted from the leg or coxa muscle following the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following instructions. Mitochondrial *COI* genes were amplified and sequenced using the PCR conditions and primers used in previous studies (Hwang *et al.*, 2013; Suh *et al.*, 2019). Phylogenetic analyses were conducted using the T92 + G + I model determined as the best-fit evolutionary substitution model by AICc from jModeltest 2.1.7 (Darriba *et al.*, 2012) and subsequent phylogenetic analyses using the maximum likelihood (ML) with 1,000 bootstrap repeats, implemented in MEGA X (Kumar *et al.*, 2016).

## TAXONOMIC ACCOUNTS

***Ephemera orientalis* McLachlan, 1875** (Figs. 1A, 1B, 1C, 2A, 2C, 2E, 3A, 3B, 4A, 4E, 4I, 5A)

*Ephemera orientalis* McLachlan, 1875: 167; Demoulin, 1965: 215; Tshernova, 1973: 225; Gose, 1981: 13; Bae *et al.*, 1994: 70; Bae and Yoon, 1997: 148; Hwang *et al.*, 2003: 430.

**Material examined. South Korea: Seoul:** 6 males & 23 female subimagos, Gwangnaru, Hangang (R.), 2018-V-14, Park SH, Seok SW. **Gyeonggi-do:** 10 male subimagos & 19 female subimagos, Gapyeong, 2011-V-2; 4 males & 13 female subimagos, Gapyeong, 2011-VI-2; 17 males & 23 females, Gapyeong, Yeopgwang-gyo (Br.), 2017-V-8; 16 males & 19 females, Namyangju-si, Deokso, 2018-V-13; 12 males & 20 females, Pocheon-si, Hwanhyunmeon, 1991-VII-18; 3 males & 14 females, Yangpyeong, 2002-IV-29. **Gangwon-do:** 25 males & 37 females, Jeongsun-gun, Hwaam-myeon, 2018-V-7, Park SH, Kim JS; 5 larvae, Jeongseon, 2018-VI-2, Park SH, Kim JS; 4 larvae, Jeongseon-gun, Bungyang-ri (E 128°40'02.11" N 37°22'

41.04"), 2018-IX-14, Park SH, Kim JS, Mayorga A; 6 larvae, Wonju-si, Cheondungsan (Mt.) (E 127°56'52.88" N 37°04'31.31"), 2016-IX-11, Park SH, Seok SW. **Gyeongsangnam-do:** 9 larvae, Sancheong-gun, Jirisan National Park, 2017-V-31, Bae YJ, Park SH.

**Diagnosis.** Body length 16.4 ± 0.3 mm in female adult and 14.1 ± 0.1 mm in male adult (Fig. 1A, 1B). Pronotum with lateral dark stripes and yellowish pale median band (Fig. 2A, 2D, 2E). Abdomen with paired multi-striped markings; segments I–II of abdominal tergum with dark pigment covering all or almost all the segments; pigmentation can be as dots, bars or irregular-shaped (Figs. 3A, 3B, 4A).

**Adult.** Forewing transparent with dark brown marks on crossveins; costal area dark brown; MP1–MP2 with a dark spot; CuA and MP2 fused (Fig. 1C). Forelegs anterior half of fore femur pale brown and posterior half dark brown; fore tibia and tarsus dark brown (Fig. 1A). Male genital lobes square-shaped, sclerotized and without prominent apical inner concavity; penis with distinct titillators triangular-shaped (Fig. 5A).

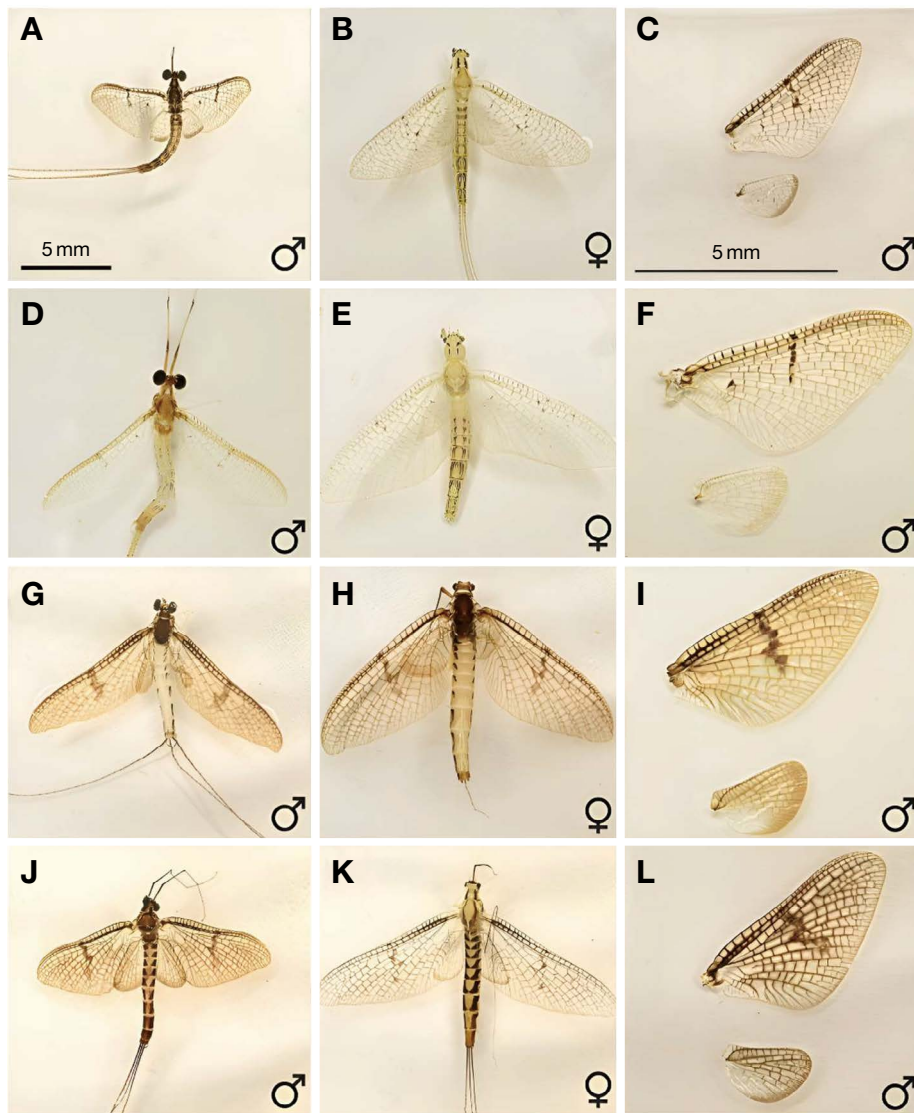
**Mature larva.** Claw pointed and elongated (Fig. 4I). Posterior margin of tergum X trapezoid-shaped and pronounced (Fig. 4E).

**Distribution.** China, Japan [type locality], Korea, Mongolia, Russia.

**Remarks.** Among the *Ephemera* species with paired multi-striped markings on abdominal segments, this species is one of the most widely distributed species in Far East Asia, and is well studied especially in Japan and South Korea in terms of life history (Gose, 1970; Lee *et al.*, 1995; 1999; 2008; Hwang *et al.*, 2009; Lee *et al.*, 2011; Seok *et al.*, 2019), the level of genetic diversity and its ecology (Kuroda *et al.*, 1984; Lee *et al.*, 1995; Kang *et al.*, 2005; Hwang *et al.*, 2009; Takenaka *et al.*, 2023).

This species was redescribed based on the holotype (Demoulin, 1965), although the redescription lacked details of larva and male genitalia. Because of its wide distribution in a variety of habitats and geographical regions, morphological variations between local populations or cohorts are still poorly understood. Based on a close examination of adult and larval materials, we found that the shape of the pigmentation on the abdominal segment I–II (Fig. 3A, 3B; also illustrated by Gose, 1981) could be a useful character to distinguish *E. orientalis* from its sympatric *E. sachalinensis*.

In addition, despite its wide distribution across various habitats and countries, studies on the morphological variability among populations and cohorts remain incomplete. For instance, this study observed some morphological variations. Specimens from different sites in South Korea exhibited certain variations in the pigmentation patterns on tergal segments I–II (Fig. 3A, 3B), such as complete dark pigmentation, or incomplete dark pigmentation (Fig.



**Fig. 1.** Adults of South Korean *Ephemera* Linnaeus: A. male *E. orientalis* McLachlan; B. female *E. orientalis*; C. male wings of *E. orientalis*; D. male *E. sachalinensis* Matsumura; E. female *E. sachalinensis*; F. male wings *E. sachalinensis*; G. male *E. separigata* Bae; H. female *E. separigata* Eaton; I. male wings *E. separigata*; J. male *E. strigata* Bae; K. female *E. strigata*; L. male wings *E. strigata*.

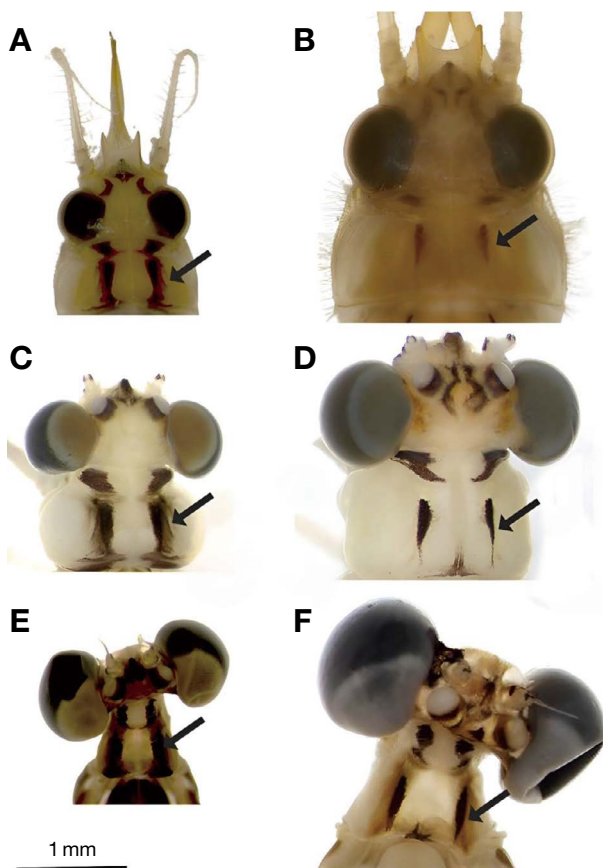
3A). However, other distinctive morphological features proposed in this study remained consistent across sex, both larval and adult stages.

***Ephemera sachalinensis* Matsumura, 1911** (Figs. 1D, 1E, 1F, 2B, 2D, 2F, 3C, 4B, 4F, 4J, 5B, 6)

*Ephemera sachalinensis* Matsumura, 1911: 7; Tshernova, 1973: 329; Hwang *et al.*, 2003: 430.

**Lectotype (New designation).** Female subimago (pinned; Fig. 6A), カラフト (Karafuto = Sakhalin), 三宅 (Miyake: Collector's name), Matsum (= Matsumura); ス川ヤ山 - 7/1907, deposited at the Hokkaido University Museum, Sapporo, Japan.

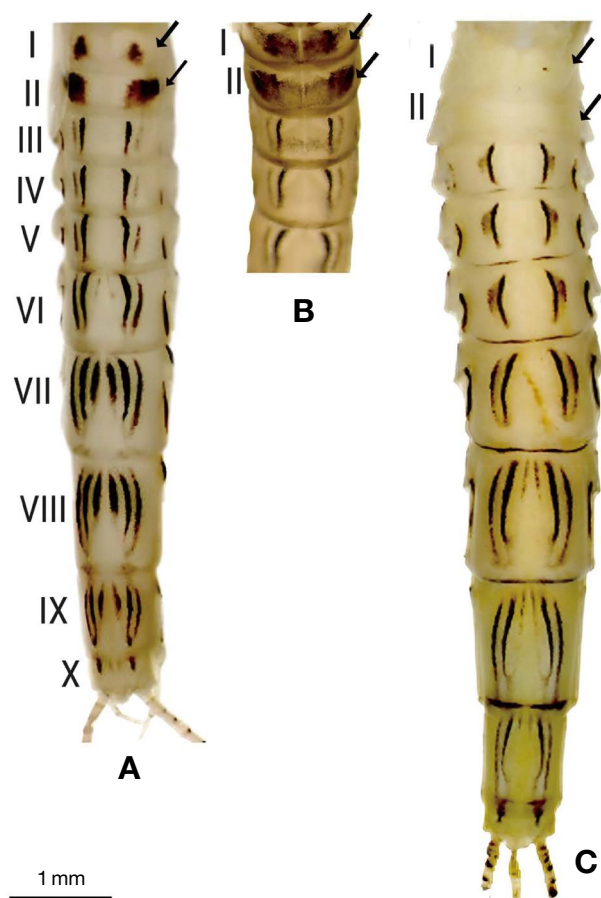
**Other material examined. South Korea: Seoul:** 34 female subimagos, Amsa-dong, Hangang (R.), 2017-VI-1, Kang JH, Seok SW, Lim CS; 1 male imago & 14 female subimagos, Seoul, Gwangnaru, Hangang (R.), 2018-V-14, Park SH, Seok SW. **Gyeonggi-do:** 1 male, Namyangju-si, Gu-am-dong-san, 1994-VII-20, Bae YJ; 3 male imagos, Gapyeong, 2005-VIII-21; 12 female imagos, Namyangju-si, Deokso, 2017-VI-29, Kang JH, Park SH, Lim CS; 4 larvae, Gapyeong, 1997-V-30, Bae YJ; 5 male subimagos & 12 female subimagos, Gapyeong, 2017-V-8; 8 male subimagos, Gapyeong, 2017-V-20, Park SH, Uy CJ. **Gangwon-do:** 3 larvae, Jeongseon-gun, Jeongseon-eup (E 128°42'50.5" N 37°23'15.3"), 2018-VI-2, Park SH, Kim JS; 37 larvae, Jeongseon-



**Fig. 2.** Head of *Ephemera orientalis* McLachlan and *Ephemera sachalinensis* Matsumura: A. larval head of *E. orientalis*; B. larval head of *E. sachalinensis*; C. subimago head of *E. orientalis*; D. subimago head of *E. sachalinensis*; E. imago head of *E. orientalis*; F. imago head of *E. sachalinensis*.

gun, Hwaam-myeon, 2018-VI-2, 2018-V-7, Park SH, Kim J. **Jeollanam-do:** 2 male subimagoes & 3 female subimagoes, Yeongam-gun, Hoemun-ri (N 34°46'46.1" E 126°40'47.6"), 2018-VII-18, Mayorga A, Uy CJ, Jung SW. **North Korea:** 1 male imago, Hwanghae Province (=Hwanghae-do): Haeju, 1986-VI-8, Kwang S. **Mongolia:** 3 female imagoes, Bulgan Aimag, Teshing Soum (wetland), 4.3 km SSW of Teshing (N 49°90'72.5" E 102°65'66.5"), 970 m a.s.l., 2005-VII-10-11, Samaa. **Russia:** 1 male imago, Primosky Krai, Nakhodka City, Westshore of Rivadia Lake (N 42°51'46.43" E 103°38'53.44"), 2013-VIII-17, Bae YJ, Kang JM, Lee JM, Vshivkova T.

**Redescription (adult female lectotype).** Specimen is pinned. Overall body yellowish and brownish (Fig. 6B). Pronotum with two lateral dark stripes becoming narrower basally and a yellowish pale median band (Fig. 6D). Mesonotum with a thick dark median band and lateral borders with dark pigmentation (Fig. 6C). Abdomen with paired multi-striped markings; abdominal terga I–II pale without



**Fig. 3.** Abdominal terga of *Ephemera orientalis* McLachlan and *Ephemera sachalinensis* Matsumura: A. variation I of abdominal terga of *E. orientalis*; B. variation II of abdominal terga of *E. orientalis*; C. abdominal terga of *E. sachalinensis*. Arrows indicate the key diagnostic characters between *E. orientalis* and *E. sachalinensis* at abdominal terga I and II.

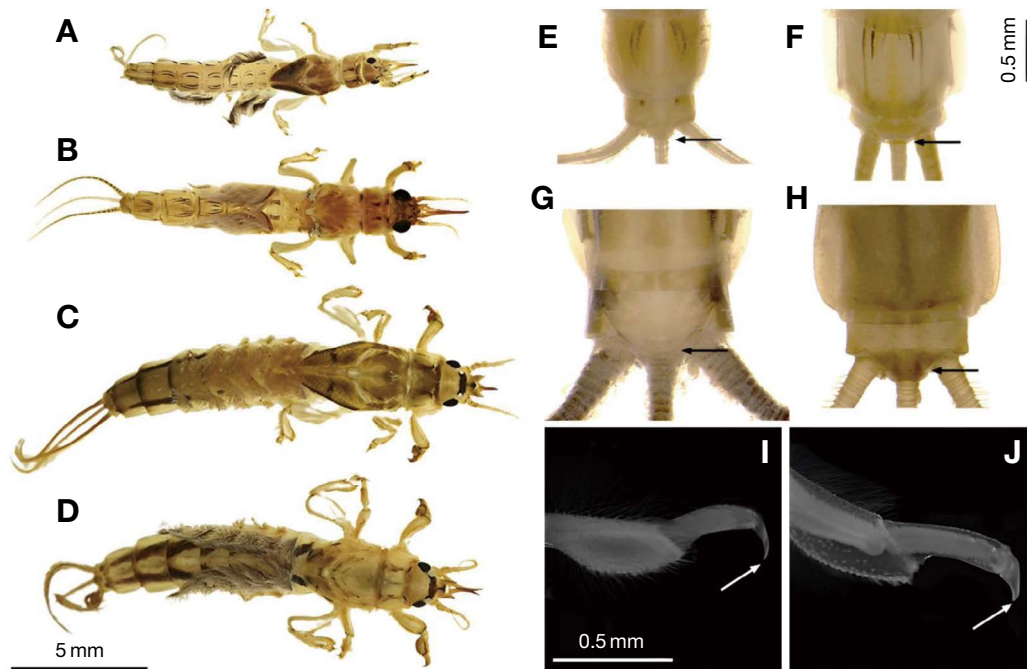
dark pigmentation; terga III–VI with apparently dark multiple stripes but becoming unclear at last segments (Fig. 6C, 6B). Forewings and hindwings completely damaged (Fig. 6B). Three yellowish to brownish caudal filaments present. Forelegs broken; middle legs and hind legs completely yellowish (Fig. 6B).

**Diagnosis.** Body length  $21.80 \pm 0.50$  mm in female adult and  $18.50 \pm 0.10$  in male adult (Fig. 1D, 1E). Pronotum with lateral dark stripes becoming narrower basally and a yellowish median band (Fig. 2B, 2D, 2F). Abdominal terga I–II pale without dark pigmentation (Fig. 3C).

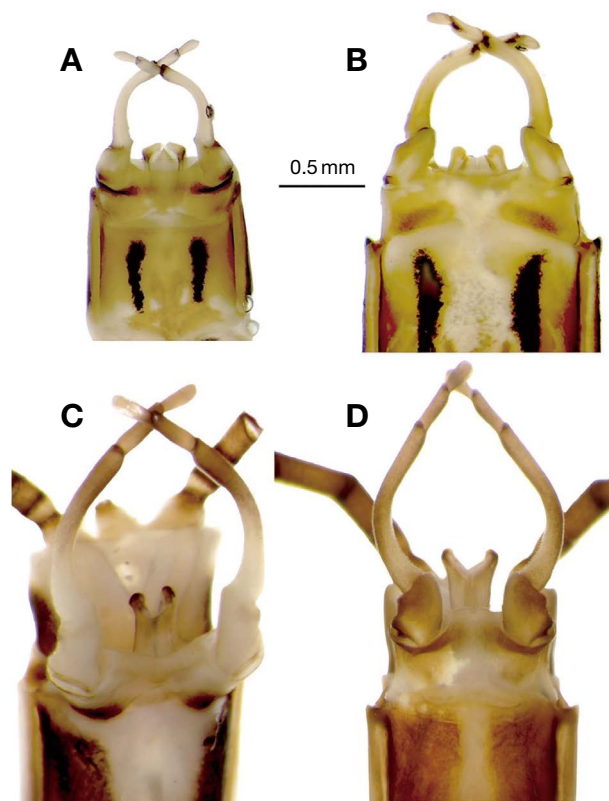
**Adult.** Forewings with dark brown marks on crossveins; costal area dark brown; CuA and MP2 fused (Fig. 1C). Foreleg trochanter, tibia-femur, tibia-tarsus junctions with dark pigmentation (Fig. 1D). Male genital lobes non-sclerotized, rounded at apex and elongated with inner concavity; penis with distinct thin titillators (Fig. 5B).

**Mature larva.** Claw neither elongated nor sharp (Fig. 4J).





**Fig. 4.** South Korean *Ephemera* Linnaeus larvae: A. *E. orientalis* McLachlan; B. *E. sachalinensis* Matsumura; C. *E. separigata* Bae; D. *E. strigata* Eaton. Posterior margin of tergum X of South Korean *Ephemera* larvae: E. *E. orientalis*; F. *E. sachalinensis*; G. *E. separigata*; H. *E. strigata*. Claw of foreleg of the larvae: I. *E. orientalis*; J. *E. sachalinensis*.

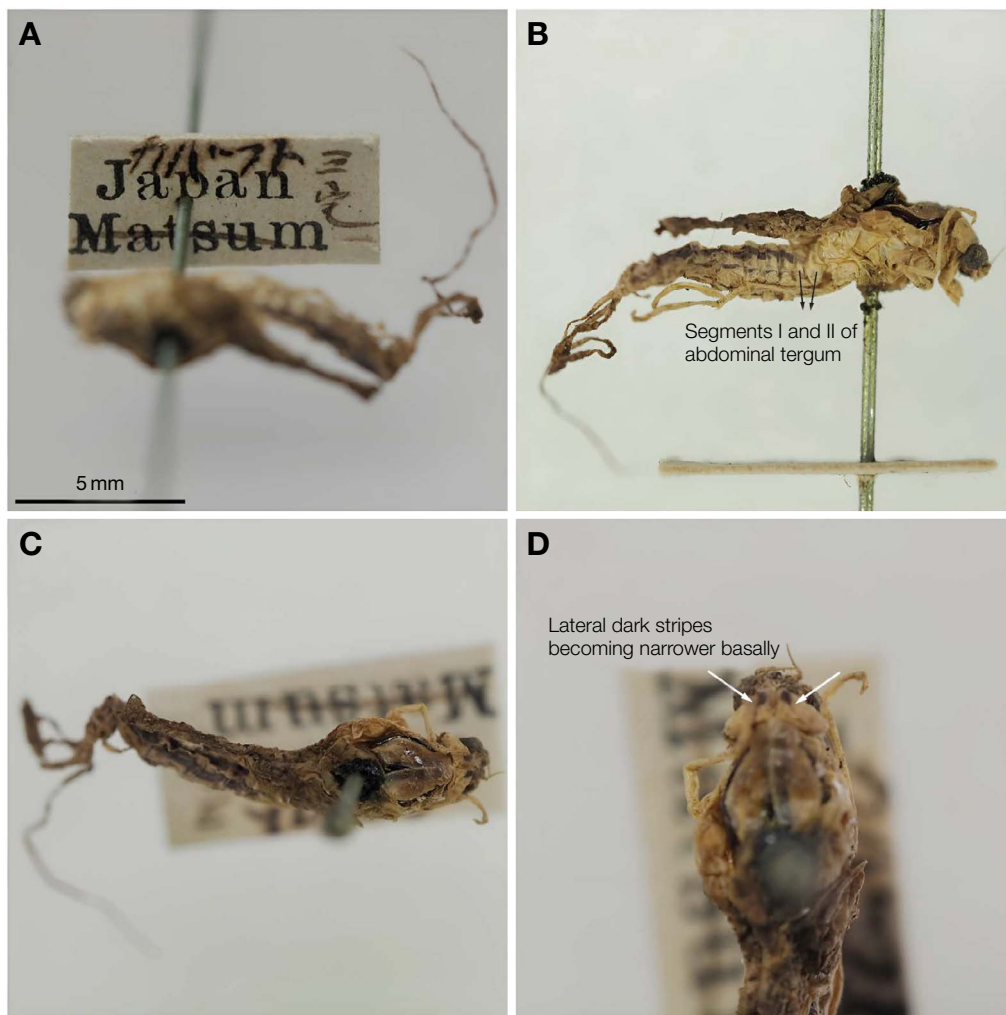


**Fig. 5.** Male genitalia of South Korean *Ephemera* Linnaeus males: A. *E. orientalis* McLachlan; B. *E. sachalinensis* Matsumura; C. *E. separigata* Bae; D. *E. strigata* Eaton.

Posterior margin of tergum X trapezoid-shaped and not pronounced, almost straight (Fig. 4F).

**Distribution.** China, Kazakhstan, Korea, Mongolia, Russia Far East [type locality].

**Remarks.** This species has not been well defined in terms of taxonomy and relations with other sympatric species in the same genus. Because the type information was lacked in the Matsumura's (1911) original description of this species, mayfly taxonomists like Tshernova (1973) were unable to locate any type specimens that were used in Matsumura's original description of this species. While the first author of this study had searched Matsumura's collection in the Hokkaido University Museum in Sapporo, a female subimago specimen (Fig. 6A) that was most probably labeled and used by Matsumura in the original description of *Ephemera sachalinensis* Matsumura, 1911 was found. The locality (Karafuto = Sakhalin) and collector (Miyake) also coincide with the information in the Matsumura's brief description of the species: "Fundorte: Chippsani und Suzuya, gesammelt in 2 females. Exemplaren von Herrn B. Miyake [= Locations: Chippsani and Suzuya, collected in 2 females. Specimens by Mr. B. Miyake]" (Fig. 6A). Although the condition of this Matsumura's specimen is not complete, some preserved characters such as the lateral dark stripes becoming narrower basally from pronotum and the lack of pigmentation at the abdominal terga I and II (Fig. 6B) well substantiate Matsumura's (1911) species concept of *E. sachalinensis*. The lectotype



**Fig. 6.** Lectotype of *Ephemera sachalinensis* Matsumura (female) deposited in the Hokkaido University Museum: A. label; B. lateral view; C. dorsal view; D. head close-up.

designation must involve one of the original specimens used by the author for the description, which is the case in our study. Further revision of fresh specimens from the type locality (Sakhalin, Russia) will be beneficial to complement both Matsumura's original description (1911) and ours. The discovery of this historical specimen has significant scientific and historical relevance.

*Ephemera sachalinensis* is morphologically similar to *E. pieli* Navás based on the observation data of Chinese specimens provided by Li *et al.* (2023). Both *E. sachalinensis* and *E. pieli* lack dark pigmentation on the abdominal terga I–II, but *E. pieli* possesses two lateral dark spots on the abdominal tergum II. In addition, *E. sachalinensis* can be distinguished by different shape of male genitalia (Li *et al.*, 2023).

*Ephemera sachalinensis* is widely distributed in North-east Asia and largely overlaps in distribution with its sym-

patric *E. orientalis* (Tshernova, 1973; Bae and Söldan, 1997; Hwang *et al.*, 2003; 2008). Further investigations on life history, distribution and habitat, and other ecological features are needed to understand adaptation and evolution of these two closely related species. Compared to *E. orientalis*, in specimens from different sites around South Korea and other countries, it was not found important variability in the diagnostic characters for this species.

***Ephemera separigata* Bae, 1995** (Figs. 1G, 1H, 1I, 4C, 4G, 5C)

*Ephemera separigata* Bae, 1995: 160; Tiunova and Semenchenko, 2022: 94.

**Specimens examined. South Korea: Gyeonggi-do:** 37 larvae, Gapyeong-gun, Buk-myon (E 127°26'35.1" N 38°01'31.3"), 2019-VI-4, Kang JH, Park SH, Lim C, Kim J.

**Gangwon-do:** 1 male subimago & 3 female subimagoes, Wonju-si, Chiaksan, 2002-VI-5, 2002-VI-15, Bae YJ.

**Gyeongsangbuk-do:** 3 larvae & 1 male subimago, Gimcheon, Hwangaksan, 1996-IV-20, 2003-V-20; 11 larvae, Yeongju, Sobaeksan National Park, 2007-VI-5; 7 larvae, Yeongju-si, Dansan-myeon, 2007-IX-15. **Jeollanam-do:** 1 male imago, Jirisan, Dalgung, 1986-VI-25, Bae YJ, Hwang MJ.

**Diagnosis.** Abdominal tergum I pale with a pair of dark thick lateral bars; terga V–IX with a pair of dark narrow lateral bars; lateral bars marginally oriented, not reaching the median area (Fig. 1G, 1H).

**Adult.** Forewings whitish color with dark brown marks on crossveins; costal area dark brown; CuA–MP2 not fused (Fig. 1I). Male genital lobes narrow, Y-shaped with inner concavity; forks apex curved inertly and slightly rounded penis without distinct titillators (Fig. 5C).

**Mature larva.** Generally similar to adults in abdominal markings. Posterior margin of tergum X round-shaped (Fig. 4G).

**Distribution.** South Korea [type locality], Russian Far East.

**Remarks.** This species is not as common as the other *Ephemera* species in South Korea because of its exclusive distribution in mountain streams at relatively high altitude (Hwang *et al.*, 2013). Monophyly of this species was well supported by previous molecular data (Hwang *et al.*, 2013; Tiunova and Semenchenko, 2022).

***Ephemera strigata* Eaton, 1892** (Figs. 1J, 1K, 1L, 4D, 4H, 5D)

*Ephemera strigata* Eaton 1892: 302; Tshernova, 1973: 332; Gose, 1981: 16.

**Specimens examined. South Korea: Gyeonggi-do:** 4 male imagoes & 4 female imagoes, Gapyeong, 2003-V-18, 2013-VI-9. **Gangwon-do:** 36 larvae, 2 male subimagoes, 15 male imagoes & 26 female imagoes, Jeongseon, 2018-V-6-7, 2018-VI-2, Park SH, Kim JS, Mayorga A. **Chungcheongbuk-do:** 12 larvae, Danyang, 2007-VIII-25, Bae YJ.

**Diagnosis.** Abdominal tergum I pale with a pair of dark thick lateral bars. Abdominal terga V–IX with a pair of dark thick lateral bars reaching the median area basally (Fig. 1J, 1K). Adult: Forewings transparent with dark brown marks on crossveins; costal area dark brown; CuA–MP2 not fused (Fig. 1L). Male genital lobes wide Y-shaped with a slight inner concavity; forks apex curved inertly; penis without distinct titillators (Fig. 5D).

**Mature larva.** Generally similar to adults in abdominal markings. Posterior margin of tergum X quadrilateral-shaped (Fig. 4H).

**Distribution.** China, Japan [type locality], Korea, Mongolia, Russia.

**Remarks.** This species has been frequently treated as an ecological model species of life history studies, intermediate host of nematodes, and population analysis using machine-learning techniques (Gose, 1970; Kuroda *et al.*, 1984; Takemon, 1990; Hirasawa and Urabe, 2003; Li *et al.*, 2020).

In some immature larval specimens (7–12 mm in body length), abdominal terga VIII–IX are completely dark with a pair of pale median stripes, but this pigmentation changes to typical larval markings in the mature larvae.

### Key to Korean *Ephemera* species

#### Mature larvae

1. Abdominal terga VII–IX with paired multi-striped markings (Fig. 4A, 4B)..... 2
  - Abdominal terga VII–IX with paired single-striped markings (Fig. 4C, 4D)..... 3
2. Mesonotum yellowish pale with a continuous pair of dark stripes (Fig. 2A, 2C, 2E). Segments I–II of tergum with a dark pigment covering all or most of the segment (Fig. 3A). Fore claw pointed and elongated; posterior margin of tergum X trapezoid-shaped and pronounced (Fig. 3E) ..... *E. orientalis*
  - Mesonotum pale with narrow discontinued pair of stripes becoming narrower at base (Fig. 3B). Segments I–II of tergum without dark pigment (Fig. 3B). Fore claws not elongated (Fig. 4J); posterior margin of tergum X almost square and not pronounced (Fig. 4F) ..... *E. sachalinensis*
3. Lateral dark bars of terga VII–IX narrow and only at borders, not reaching the median area of segments (Fig. 4C). Posterior margin of tergum X rounded (Fig. 4G) ..... *E. separigata*
  - Lateral dark bars of terga VII–IX thick and reaching median area of segments terminally (Fig. 4D). Posterior margin of tergum X angled (Fig. 4H)..... *E. strigata*

#### Adults

1. Abdominal terga with paired multi-striped markings (Fig. 3A, 3C)..... 2
  - Abdominal terga with paired single-striped markings (Fig. 1G, 1H, 1J, 1K)..... 3
2. Mesonotum yellowish pale with thick and continued pair of dark stripes (Fig. 2C, 2E). Segments Terga I–II with a dark pigment covering all or most of segment (Fig. 3A, 3B). Male genitalia sclerotized genital lobes without inner concavity (Fig. 5A). Foreleg dark brown, anterior half of femur pale..... *E. orientalis*
  - Mesonotum pale with narrow and discontinued pair of narrow stripes (Fig. 2D, 2F). Terga I–II without dark pigment (Fig. 3C). Male genitalia with non-sclerotized and rounded apical genital lobes with inner concavity at apex (Fig. 5B)..... *E. sachalinensis*

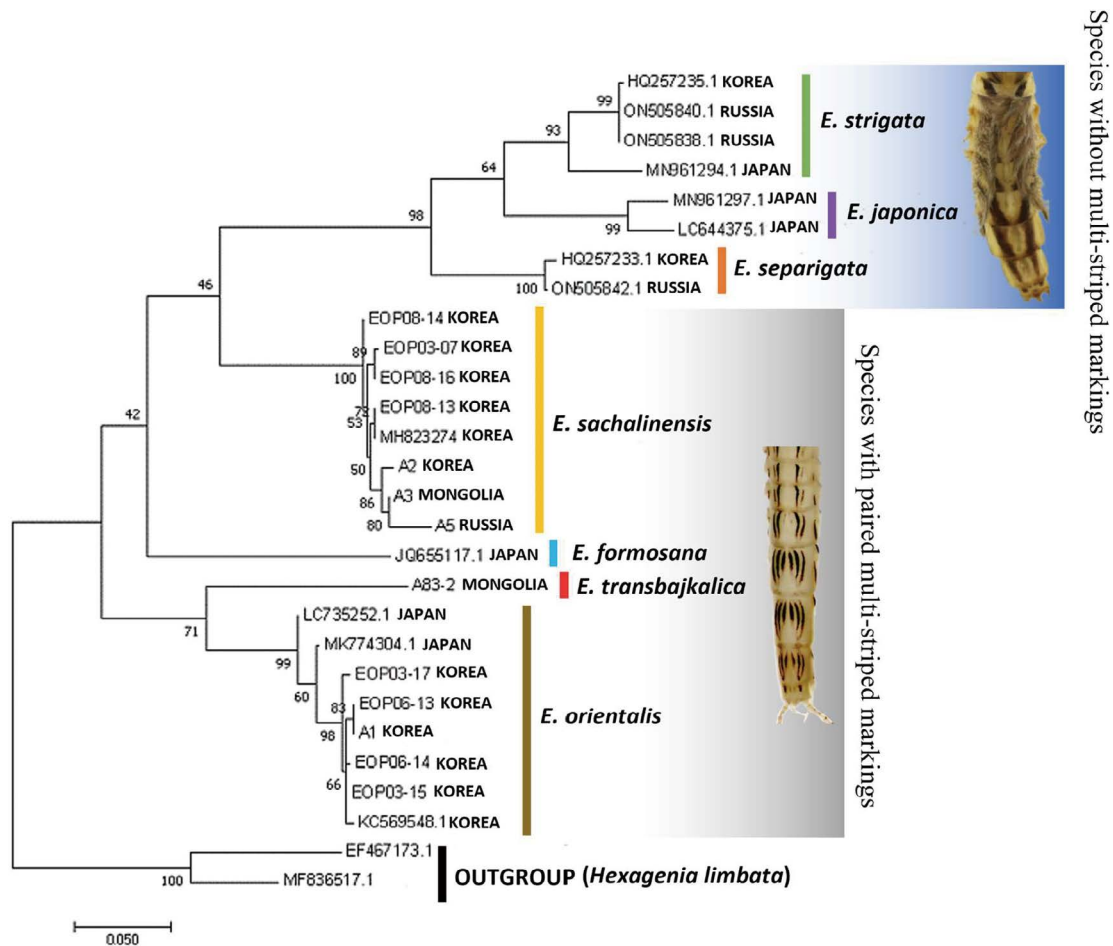
- 3. Mesonotum with pale wide band on disc and dark lateral bands (Fig. 1G, 1H). Lateral dark bars of tergum narrow and only at borders not reaching median area of segment (Fig. 1G, 1H). Male genital lobes narrow Y-shaped with inner concavity; forks apex curved inertly and slightly rounded (Fig. 5C).....*E. separigata*
- Mesonotum with pale wide band on disc and dark lateral bands (Fig. 1J, 1K). Lateral dark bars thick rhomboid-shaped and reaching median area of segment basally (Fig. 1J, 1K). Male genital lobes wide Y-shaped with a slight inner concavity; forks apex curved inertly (Fig. 5D)..... *E. strigata*

### DISCUSSION

Here, we reviewed the taxonomy of Korean *Ephemera* species, with an emphasis on clearly delimiting the two sympatric species, *E. orientalis* and *E. sachalinensis*. De-

tailed taxonomic examination is important since these two species are often confused in the field due to their similar morphology and shared habitat. Particularly, clear species identification keys will be very useful for the biomonitoring and the investigation of the recent mass emergence of *E. orientalis* in urban areas in Korea.

In addition, DNA barcodes using *COI* gene sequences clearly identified seven *Ephemera* species from Far East Asian region (Fig. 7). The intraspecific sequence divergence of the three *Ephemera* species (*E. orientalis*, *E. sachalinensis*, *E. separigata*) lies within the generally accepted species delimitation level of approximately 2% (Ratnasingham and Hebert, 2007; Suh *et al.*, 2019) (Table 1). Although the genetic distance of *E. sachalinensis* from Korea and Russia shows a slightly higher species delimitation level of 3%, the morphological re-examination in this study confirmed that they belong to the same species, with no significant morphological differences. A high level of intraspecific divergence (5.80%) was observed in *E.*



**Fig. 7.** Phylogenetic tree based on 26 mitochondrial *COI* gene sequences of *Ephemera* species from four countries from Far East Asia. The values on the branch indicate maximum likelihood (ML) bootstrap support values. Blue shade: non-multi-striped markings in the abdominal terga species. Gray shade: multi-striped markings in the abdominal terga species.



**Table 1.** Interspecific and intraspecific genetic diversity among *Ephemera* species based on the mean *p*-distance (%).

Interspecific diversity	Mean <i>p</i> -distance (%)	S.E. (%)
<i>E. formosana</i> vs. <i>E. japonica</i>	16.9	1.50
<i>E. formosana</i> vs. <i>E. orientalis</i>	14.5	1.02
<i>E. formosana</i> vs. <i>E. sachalinensis</i>	13.0	0.89
<i>E. formosana</i> vs. <i>E. separigata</i>	16.4	0.56
<i>E. formosana</i> vs. <i>E. strigata</i>	17.4	1.13
<i>E. formosana</i> vs. <i>E. transbajkalica</i>	14.2	1.10
<i>E. japonica</i> vs. <i>E. sachalinensis</i>	15.6	0.87
<i>E. japonica</i> vs. <i>E. separigata</i>	11.8	1.24
<i>E. japonica</i> vs. <i>E. strigata</i>	10.0	0.41
<i>E. japonica</i> vs. <i>E. transbajkalica</i>	16.7	0.65
<i>E. orientalis</i> vs. <i>E. sachalinensis</i>	12.7	1.37
<i>E. orientalis</i> vs. <i>E. separigata</i>	16.6	0.68
<i>E. orientalis</i> vs. <i>E. strigata</i>	15.0	0.74
<i>E. orientalis</i> vs. <i>E. transbajkalica</i>	10.8	1.49
<i>E. sachalinensis</i> vs. <i>E. separigata</i>	14.2	2.61
<i>E. sachalinensis</i> vs. <i>E. strigata</i>	15.0	1.06
<i>E. sachalinensis</i> vs. <i>E. transbajkalica</i>	15.3	0.93
<i>E. separigata</i> vs. <i>E. strigata</i>	11.1	0.92
<i>E. separigata</i> vs. <i>E. transbajkalica</i>	17.3	0.81
<i>E. strigata</i> vs. <i>E. transbajkalica</i>	16.6	0.75
<i>E. formosana</i> vs. <i>Hexagenia limbata</i> (Outgroup species)	16.9	2.80
<i>E. japonica</i> vs. <i>H. limbata</i>	17.0	0.88
<i>E. orientalis</i> vs. <i>H. limbata</i>	14.2	1.10
<i>E. sachalinensis</i> vs. <i>H. limbata</i>	15.8	2.52
<i>E. separigata</i> vs. <i>H. limbata</i>	17.3	1.80
<i>E. strigata</i> vs. <i>H. limbata</i>	16.9	0.70
<i>E. transbajkalica</i> vs. <i>H. limbata</i>	16.5	0.40
Intraspecific diversity	Mean <i>p</i> -distance (%)	S.E. (%)
<i>E. japonica</i>	3.9	0.76
<i>E. orientalis</i>	1.2	1.03
<i>E. sachalinensis</i>	1.4	0.30
<i>E. separigata</i>	0.7	0.50
<i>E. strigata</i>	2.9	1.10

S.E.: standard error

*strigata* individuals from Korea and Japan suggesting the possibility of cryptic species for this species in these countries, as reported by Suh *et al.* (2019) (see Table 2). This level of genetic divergence between the two regions suggests the need for a detailed morphological examination of specimens from Korea and Japan to clarify the taxonomic status of *E. strigata*.

More importantly, the species with multi-stripped markings in the abdominal terga are well-distinguished and congruent with the taxonomic data presented herein; nonetheless, this species group is not monophyletic as expected, but it is clearly differentiated from the species with non-

multi-stripped markings (*E. separigata* - *E. japonica* - *E. strigata* clade) (Fig. 7). Multi-stripped abdominal markings are neither synapomorphies nor phylogenetically informative characters in *Ephemera* species and, therefore, do not support the formation of a monophyletic group. Paired stripe patterns are likely homoplastic traits, indicating they may have evolved independently in different lineages rather than being inherited from a common ancestor.

In conclusion, we provided a detailed taxonomic review of Korean *Ephemera* species, along with the lectotype designation of *E. sachalinensis*. The DNA barcoding information of the East Asian *Ephemera* species provided in this

**Table 2.** Intraspecific genetic diversity of *Ephemera* species from Far East Asian countries based on the mean *p*-distance (%).

Comparisons between countries	Mean <i>p</i> -distance (%)	S.E. (%)
<b><i>E. orientalis</i></b>		
Japanese vs. Korean	2.10	0.70
<b><i>E. sachalinensis</i></b>		
Korean vs. Mongolian	1.20	0.45
Korean vs. Russian	3.00	1.12
Mongolian vs. Russian	1.90	0.81
<b><i>E. separigata</i></b>		
Korean vs. Russian	0.70	0.40
<b><i>E. strigata</i></b>		
Japanese vs. Korean	5.80	2.10
Japanese vs. Russian	5.60	1.50
Korean vs. Russian	0.75	0.64

S.E.: standard error

study will support clear species identification, contributing diverse ecological and evolutionary studies of this mayfly species group.

## CONFLICTS OF INTEREST

The author of this paper has no affiliation with any interests and is solely responsible for the paper.

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