Zootaxa 5497 (3): 381–399
https://www.mapress.com/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5497.3.4
http://zoobank.org/urn:lsid:zoobank.org:pub:BFEC2071-EFCF-4489-93AD-18F3B0DCDBC6

Egg morphology of six East Palaearctic species of the genus *Ephemera* Linnaeus (Ephemeroptera: Ephemeridae)

TATIANA M. TIUNOVA
Federal Scientific Center of the East Asia Terrestrial Biodiversity, FEB, RAS, Prosp. 100-letiya Vladivostoka 159, Vladivostok 690022 Russia.
tiunova@biosoil.ru; https://orcid.org/0000-0002-9586-1502

Abstract

The eggs of six East Palaearctic species in the subgenera *Ephemera* Linnaeus s.s. and *Sinephemera* Kluge from the Russian Far East are investigated by scanning electron microscopy. Eggs of the subgenus *Ephemera* have a thick adhesive layer ranging from 1.3 to 3.0 µm. In species of the subgenus *Sinephemera*, the adhesive layer is thin, amorphous, and 0.5 µm in *E.* (*S.*) *shengmi* Hsu, while in the other two species it is presented in the form of a thin film. All studied species have micropyles of the "tagenoform type," with the exception of *E.* (*S.*) *shengmi*, in which the micropyle can be classified as linear. The shape of the sperm guide is species-specific and is clearly visible only on the adhesive layer. The structure of the chorion varies markedly among the species studied. So in *E.* (*S.*) *japonica* McLachlan, it is practically smooth; in *E.* (*E.*) *transbajkalica* Tshernova, it is reticulate; in *E.* (*E.*) *orientalis McLachlan* and *E.* (*S.*) *strigata* Eaton, it is finely wrinkled; and in *E.* (*E.*) *sachalinensis* Matsumura and *E.* (*S.*) *shengmi,* it is intermittently sinuous. The use of micropyle structure, size of eggs, along with chorion sculpturing, resulted useful in egg and species identification.

Key words: mayflies, egg, attachment structures, micropyle, chorionic sculpturing, *Ephemera*, East Palaearctic, SEM

Introduction

At the present time, in the Eastern Palaearctic, the genus *Ephemera* Linnaeus contains about 23 species, of which 6 species are also found in the Oriental Region (Hwang & Bae 2008; Hwang *et al.* 2008; Zhou 2013; Sartori *et al*. 2016). In the fauna of the Russian Far East, seven species of the genus *Ephemera* are recorded, belonging to two subgenera: *Ephemera* s.s. and *Sinephemera* Kluge, 2004. Subgenus *Ephemera* includes three East Palearctic species: *E. orientalis* McLachlan, 1875; *E. sachalinensis* Matsumura, 1931; and *E. transbajkalica* Tshernova, 1973, while subgenus *Sinephemera* includes four East Asian species: *E. japonica* McLachlan, 1875; *E. separigata* Bae, 1995; *E. shengmi* Hsu, 1937; and *E. strigata* Eaton, 1892 (Kluge, 2023).

Of the large number of East Palearctic and Oriental species, the structure of eggs has been described in only seven species: *E. (E.) formosana* Ulmer, 1920 (Kang & Yang 1994; Tojo & Machida 1998), *E. (S.) japonica* McLachlan (Koss & Edmunds 1974; Tojo & Machida 1998), *E. (E.) maoyangensis* Zhang, Gui & You, 1995 (Su & Zhu 1997), *E. (E.) orientalis* (Okazaki 1981, 1984; Tojo & Machida 1998), *E. (E.) sauteri* Ulmer, 1912 (Kang & Yang 1994), *E. (S.) strigata* (Okazaki 1981, 1984; Tojo & Machida 1998) and *E. (E.) vulgata* (Bauernfeind & Soldan 2012). In some cases, the authors did not consider the presence of a thick adhesive layer, mistaking it for the chorion (Balasubramanian *et al*. 1991; Kang & Yang 1994; Bauernfeind & Soldan 2012).

The study aimed to examine the egg structure of *Ephemera* species using SEM analysis and identify species-specific morphological features for taxonomic purposes, in addition to chorion sculpture.

Accepted by C. Molineri: 30 Jul. 2024; published: 26 Aug. 2024

Material and Methods

All samples used in this work were previously fixed in 80% ethanol. The eggs of each species for the scanning electron microscope study were extracted from female imagines. Eggs were cleaned using a Branson 3510 digital ultrasonic cleaner in two stages. For the micropyle study, eggs were cleaned for 35–45 seconds. To see the structure of the chorion, the duration of egg cleaning was 3–5 minutes (until the adhesive layer broke down). In some cases, when the adhesive layer was difficult to destroy, a mechanical method was used. For this purpose, a piece of an insect pin (2–3 mm long) was placed in a sample tube with eggs and cleaned for 3–5 minutes. After cleaning, eggs were placed in 99% alcohol for 24 hours, and then mounted on stubs using double sticky tape, coated with carbon, and examined on a Merlin 62-15 scanning electron microscope.

Egg size was determined, and the range and mean values for the populations used were determined. The mean values of egg sizes are given in parentheses. For other measurements (sperm guide, length of micropylar canal, and micropylar opening), the mean values of at least ten measurements of this characteristic are given. In describing the morphological structure of eggs, we used the terminology proposed by Koss & Edmunds (1974) and Ubero-Pascal & Puig (2007).

All material deposited in the collection of the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch, Russian Academy of Sciences, Vladivostok.

Results

Ephemera (*Ephemera*) *orientalis* McLachlan, 1875

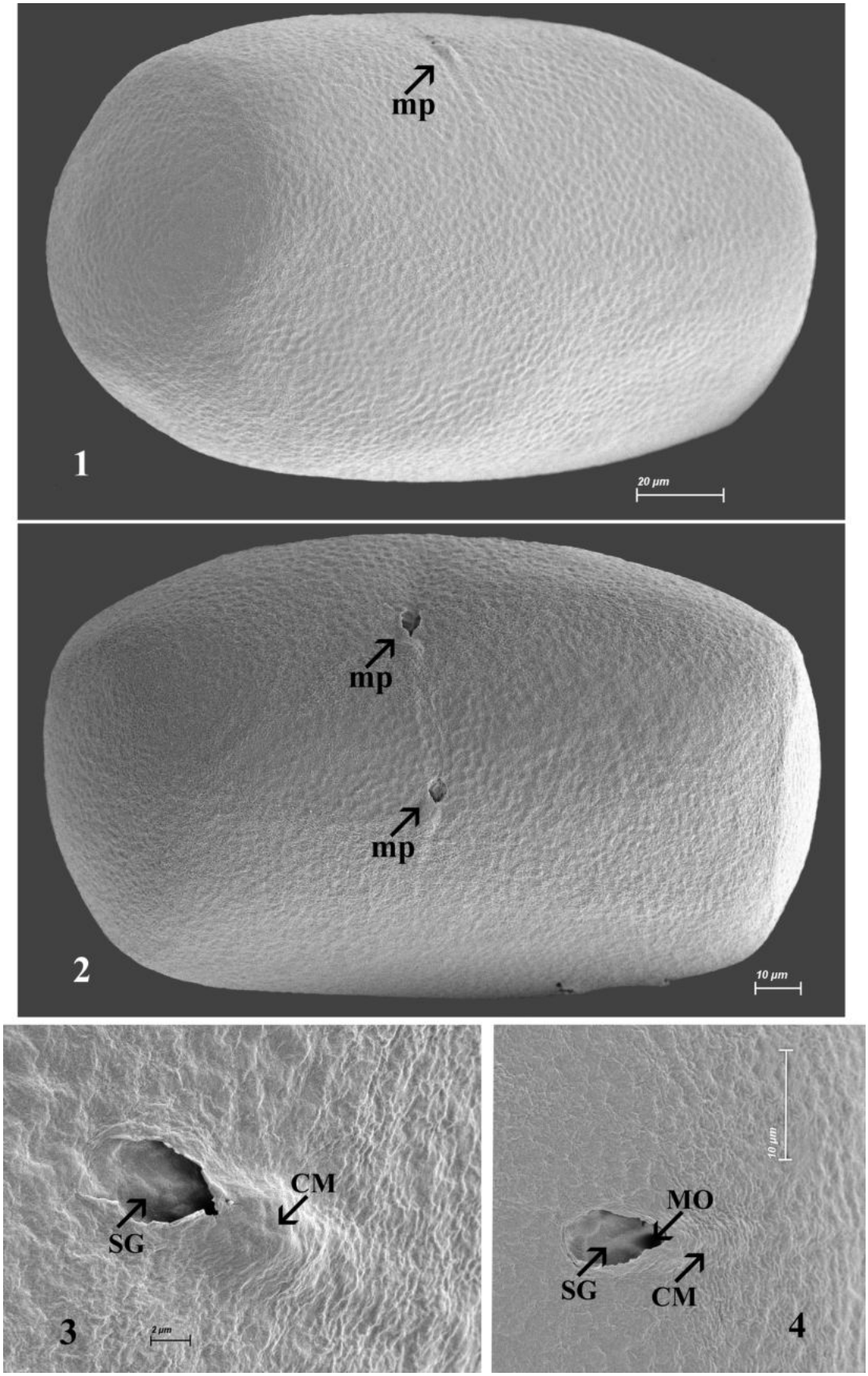
Figures 1–8

Material examined. Russia: Primorsky Krai, Ussuri River basin, Kabarga River, below the road bridge, Vladivostok – Khabarovsk highway, 17.06.2000, 3♀ adults, T. Tiunova; Ussuri River, near Stepanovka village, 15.06.2005, 2♀ adults, T. Tiunova; Khabarovsky Krai, Amur River, near Bystrinsk village, 30.06.2005, 1♀ adult, T. Tiunova.

Distribution. Siberia, Far-East Russia, Japan, Mongolia, Korea, N.E. China, Kazakhstan (Irtysh River basin).

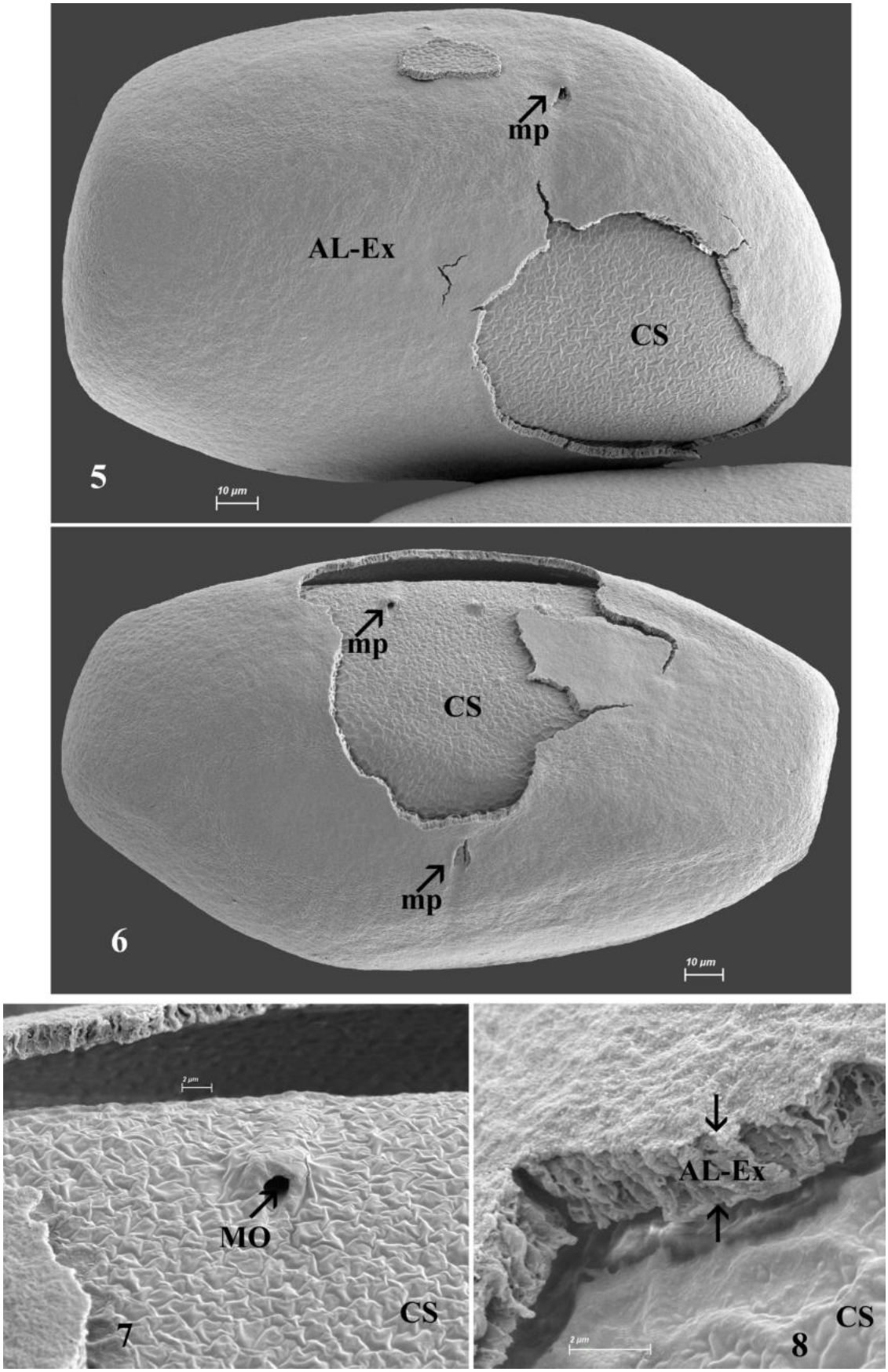
The egg has been described by Okazaki (1981, p. 9: fig. 5; 1984, p. 21: fig. 16), Su & Zhu (1997, p. 122: fig. 70), and Tojo & Machida (1998, p. 576: fig. 3). From the above descriptions, the following can be emphasized: the egg is rectangular in shape, with a length of 187.0 µm and a width of 120.8 µm. In the work of Tojo and Machida, the egg is ellipsoidal in shape, about 200x100 µm, the adhesive layer is thick (3–4 µm), smooth, the chorion has well-defined reticulation, the opening diameter is 1–1.5 µm, and the sperm guide is hood-like in shape.

According to our data, the egg has an oval shape close to quadrangular with rounded corners (Figs 1–2). Dimensions: 173.0–206.0 µm in length (191.3 µm) and 104.1–123.4 µm in width (111.0 µm). The attachment structure is a complex extrachorion-adhesive layer (AL-Ex) that covers the entire surface of the chorion (CS) (Figs 1–2, 5–6). Its thickness for *E. orientalis* is 1.6 –1.8 µm (Fig. 8). The surface of the adhesive layer (AL) is slightly bumpy (Figs 1–2). There are one or two micropyles (mp) per egg in the equatorial area (Figs 1–2, 5–6). The micropyles are "tagenoform-type," with a well-expressed sperm guide (SG) oval and relatively deep (Figs 3–4). Sperm guide is 5.8–10.4 µm long and 5.0–6.1 µm wide. It should be noted that the sperm guide is only clearly visible on the adhesive surface of the egg. Once the adhesive layer has been removed, the sperm guide is not visible on the chorion (Figs 3, 4, 6). The micropylar canal, 12–18 µm long and 4–6 µm wide, protrudes slightly above the adhesive layer (Figs 2–3, 5). As shown previously (Ubero-Pascal & Puig 2007), features of the chorion structure are visible on SEM only after removal of the adhesive layer. For *E. orientalis,* the surface of the chorion is finely wrinkled (Figs 5–6). The thickened proximal part of the tunnel-type micropylar canal and micropylar opening are clearly visible on the surface of the chorion. (Figs 6–7).

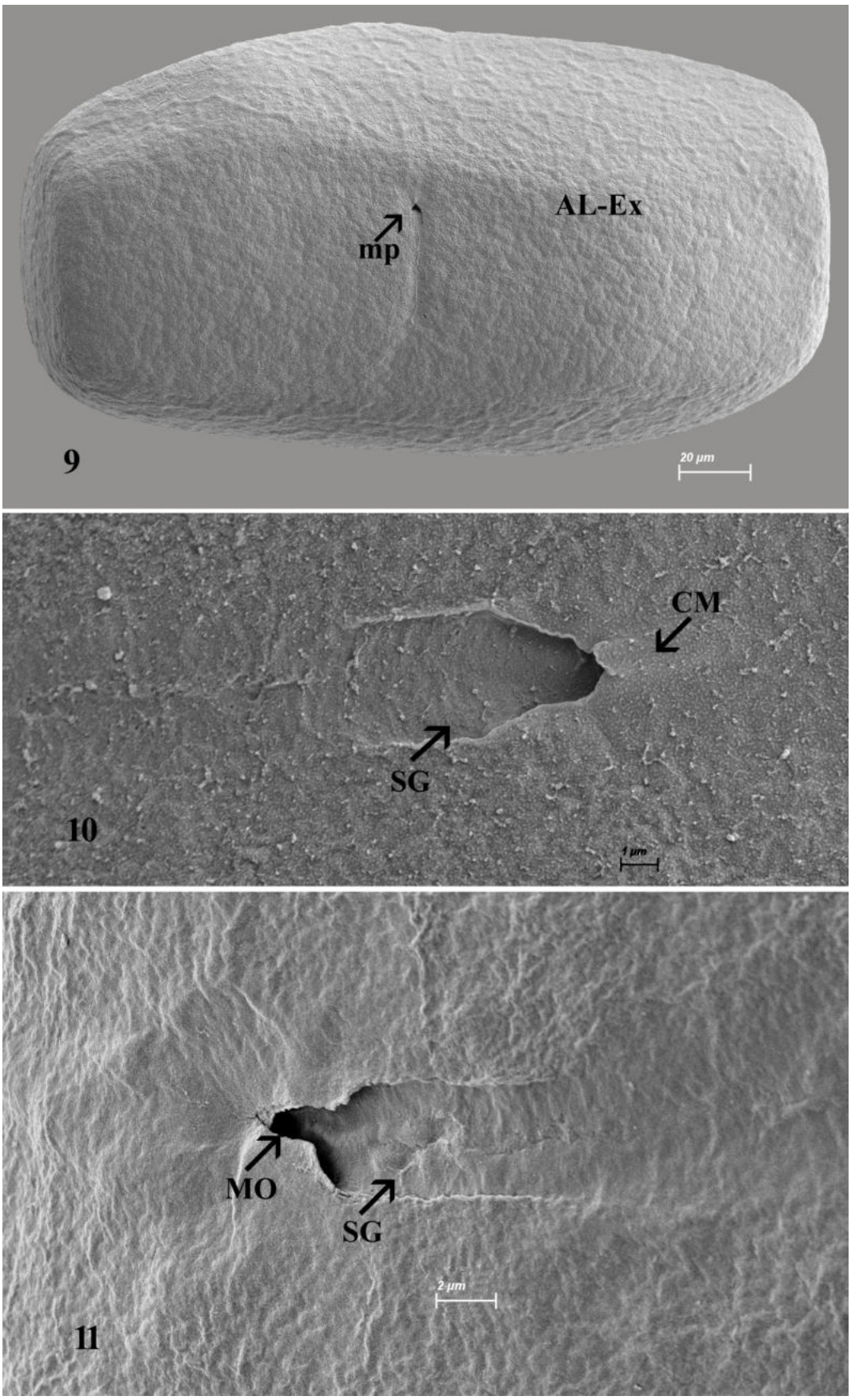


FIGURES 1–4. Egg morphology of *Ephemera* (*Ephemera*) *orientalis***:** 1–2, general shape; 3–4, micropyle of the complex extrachorion-adhesive layer.

CM—canal micropylar; MO—micropylar opening; mp—micropyle; SG—sperm guide.

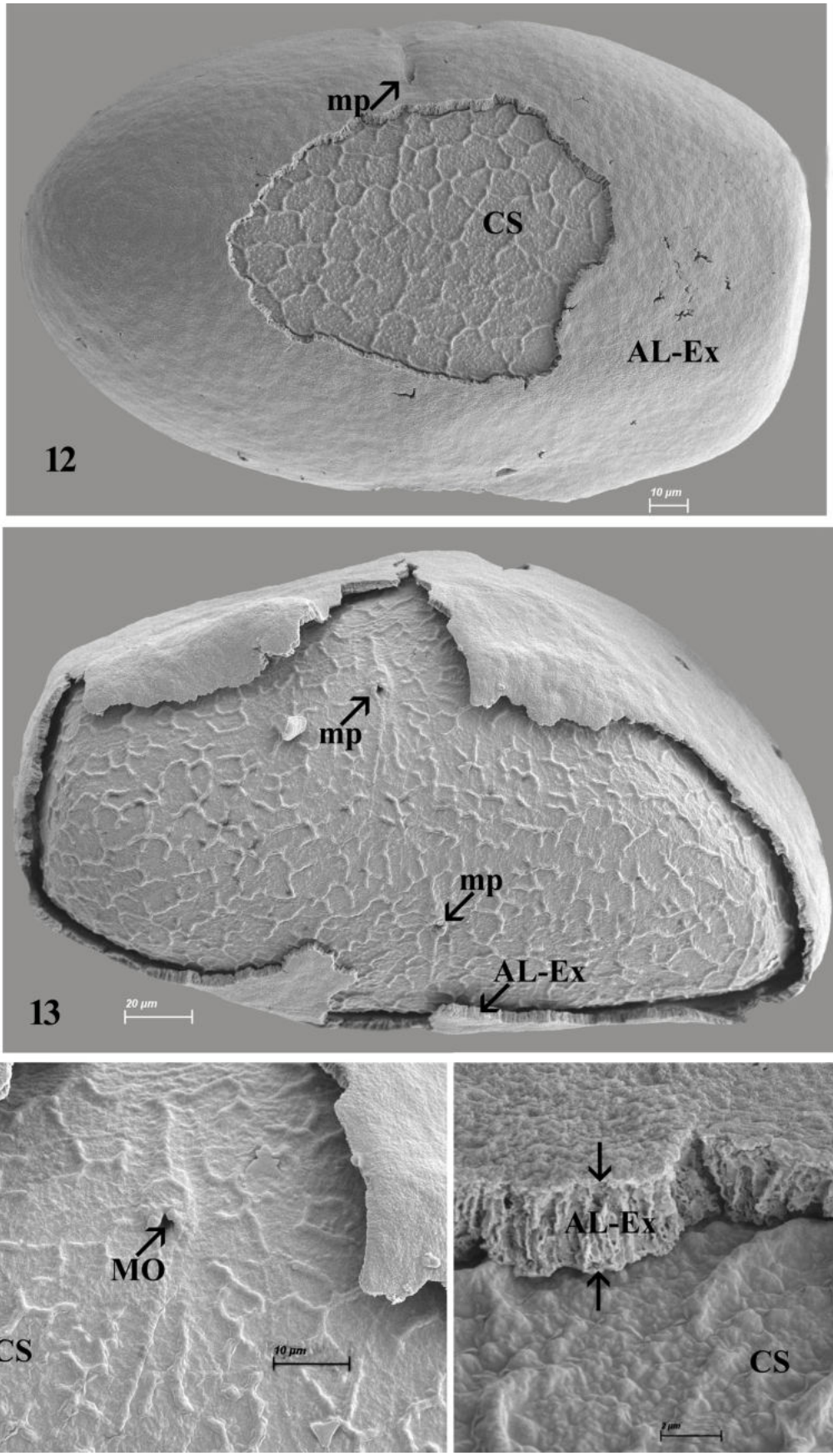


FIGURES 5–8. Egg morphology of *Ephemera* (*Ephemera*) *orientalis*: 5–6, egg surface without complex extrachorion-adhesive layer; 7, proximal part of micropylar canal on chorion surface; 8, thickness of the layer forming the eggshell. AL-Ex—complex adhesive-extrachorion layer; CS—chorion surface; MO—micropylar opening; mp—micropyle.



FIGURES 9–11. Egg morphology of *Ephemera* (*Ephemera*) *sachalinensis*: 9, general shape; 10–11, micropyle in the complex extrachorion-adhesive layer.

AL-Ex—complex adhesive-extrachorion layer; MO—micropylar opening; mp—micropyle; CM—canal micropylar; SG—sperm guide.



FIGURES 12–15. Egg morphology of *Ephemera* (*Ephemera*) *sachalinensis*: 12–13, egg surface without complex extrachorion-adhesive layer; 14, proximal part of micropylar canal on chorion surface; 15, thickness on the layer forming the eggshell. AL-Ex—complex adhesive-extrachorion layer; CS—chorion surface; MO—micropylar opening; mp—micropyle.

Ephemera (*Ephemera*) *sachalinensis* Matsumura, 1911

Figures 9–15

Material examined. Russia: Primorsky Krai, Khasansky District, Barabashevka River, below Fish Hatchery, 03.08.2007, 2♀ adults, T. Tiunova; Ryazanovka River, below Okhotbaza, 11.06.2003, 2♀ adults, T. Tiunova; Sakhalinskaya Oblast, Sakhalin Island, Lake Vavayskoye, eastern part, 18.07.2002, 3♀ adults, V. Teslenko; Amurskaya Oblast, Amur River basin, Bureya River, below Kulikovka village, 3♀ adults, T. Tiunova.

Distribution. East Siberia, Far East Russia, Mongolia, Korea, China, Kazakhstan (Irtysh River basin).

The egg has an oval shape close to quadrangular with rounded corners (Fig. 9). Dimensions: 204.0–230.0 μm in length (213.5 μm) and 118.0–132.0 μm in width (125.8 μm). The thickness of the extrachorion-adhesive layer is 1.9–3.0 μm (Fig. 15). The surface of the adhesive layer is shagreen or roughened (Fig. 9). There are one or two micropyles per egg in the equatorial area (Figs 9, 13). Micropyles are "tagenoform-type" (Figs 9–10), sperm guide weakly expressed (6.4–9.7 μm long, 3.0–5.0 μm wide), almost rectangular, elongated, distal margin not closed (Figs 10–11). The micropylar canal, about 5 μm long, does not protrude above the adhesive layer (Figs 9–10). Chorionic sculpturing consists of interrupted broken ridges, whose distribution and arrangement extend regularly over the whole chorion surface (Figs 12–13). The proximal part of the micropylar opening (MO) does not elevate above the chorion (Figs 13–14).

Ephemera (*Ephemera*) *transbajkalica* Tshernova, 1973

Figures 16–23

Material examined. Russia: Amur Oblast, Selemdzha River basin, Burunda River, mouth, tributary of Nora River, 16.06.2004, 2♀ adults, T. Tiunova; Republic of Sakha (Yakutia), Aldan River basin, Ungra River, base of Ungrinsky reserve "Yukhta," 01.08.2006, 2♀ adults (reared), T. Tiunova.

Distribution. East Siberia, Far East Russia, Mongolia.

The egg is oval-shaped (Figs 16–17). Dimensions: 220.0–226.0 μm in length (220.8 μm) and 123.0–129.0 μm in width (124.6 μm). The thickness of the extrachorion-adhesive layer is 1.3–1.7 μm (Figs 21, 23). The surface of the adhesive layer is slightly roughened; almost smooth (Figs 16, 18). There are one or two micropyles in the equatorial area (Figs16–17); micropyles are "tagenoform-type"; sperm guide well expressed (10.0–19.6 μm long, 4.2–7.2 μm wide), elongated, oval, with pointed distal margin, not deep (Figs 18–19). Micropylar canal: 4.0–6.0 μm long and 2.3–7.1 μm wide; weakly protrudes above the adhesive layer (Figs 16, 18). The chorionic surface is covered by the regular mesh unit's penta- or hexagonal cells, each with a flat, almost smooth bottom and a convex protuberance in the middle (Fig. 23). The proximal part of the micropylar opening elevates above the chorion (Figs 20, 22).

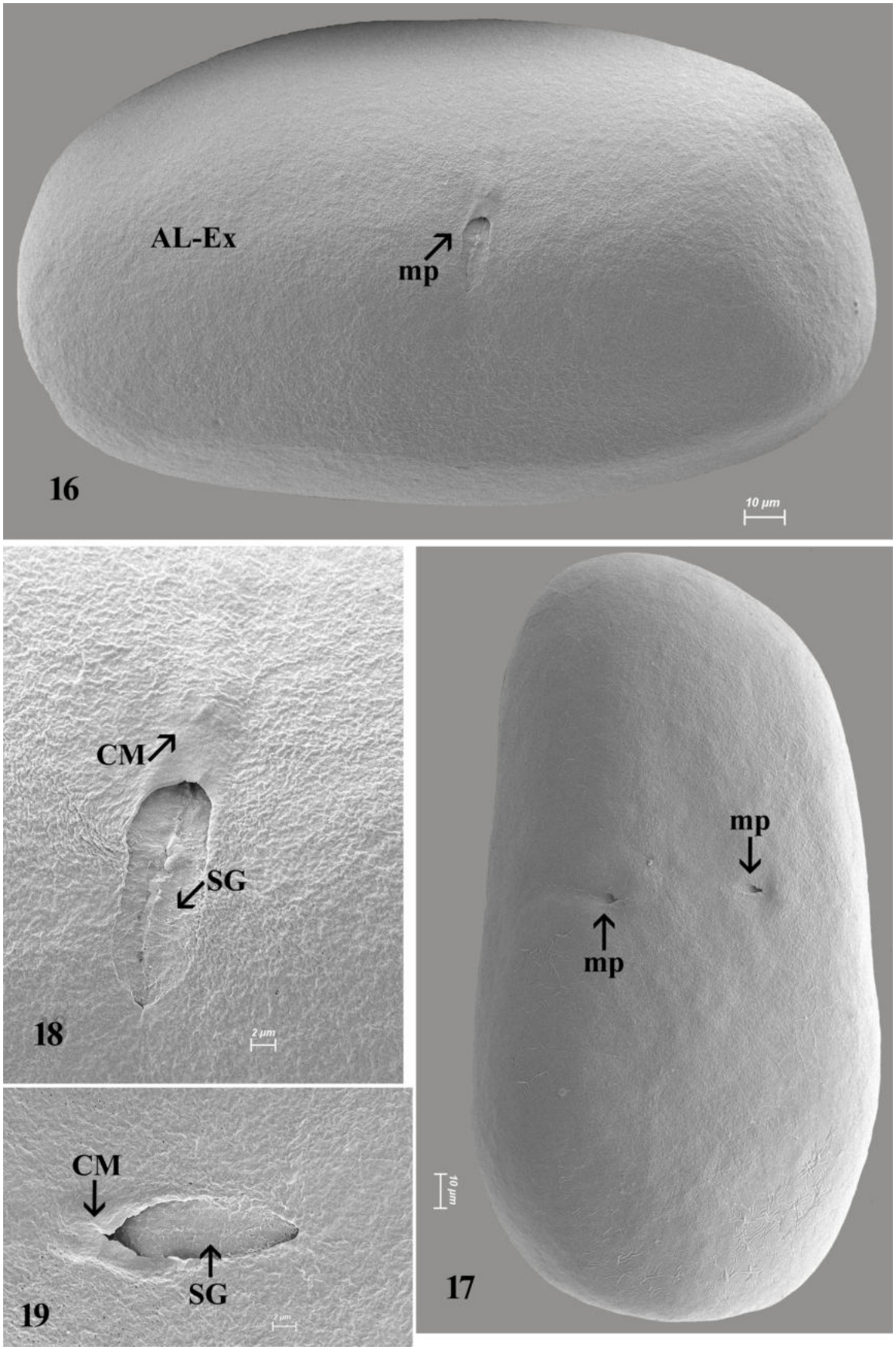
Ephemera (*Sinephemera*) *japonica* McLachlan, 1875

Figures 24–30

Material examined. Russia: Sakhalinskaya Oblast: Sakhalin Island, Belaya River, 2 km southeast of "Sokol" station, 21.07.2001, 1♀ adult, V. Teslenko; Sakhalin Island, unnamed stream, 25 km south of Vostochny village, 01.08.2001, 1♀ adult, V.Teslenko; Iturup Island, Ketoviy Bay, Podoshevka River, 1.5 km above Fish Hatchery, 29.07.1997, 4♀ adults, V. Teslenko; Kunashir Island, Lesnaya River, about 1 km above the mouth of Kislyy Stream, 04.08.1994, 2♀ adults, T. Tiunova.

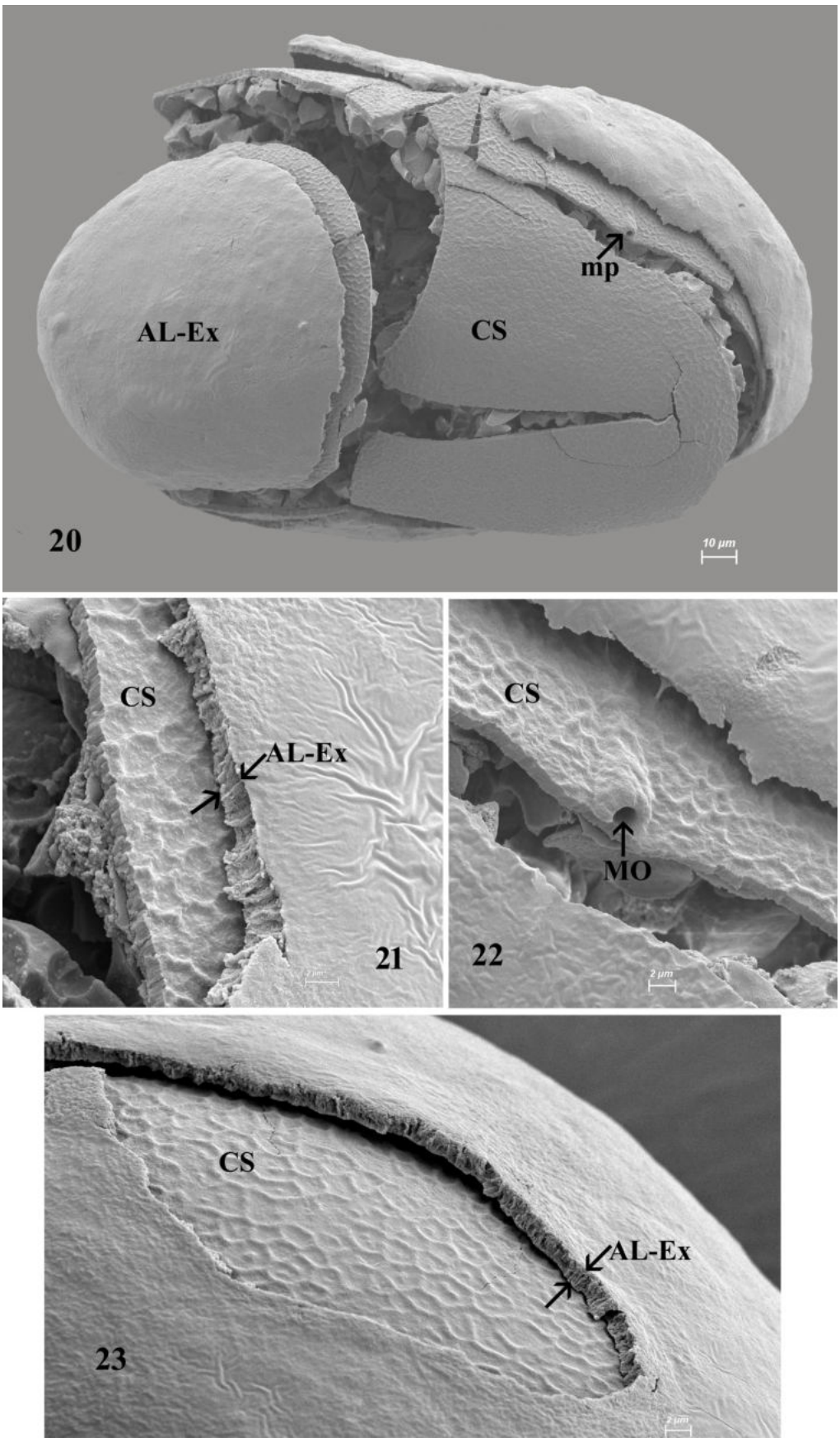
Distribution. Far East Russia, Japan, China.

The egg was described previously by Koss & Edmunds (1974) and Tojo & Machida (1998, p. 574: fig. 1). The description notes: the shape of the egg is ellipsoidal, about 200x100 μm; the adhesive layer is very thin (about 0.1 μm); the surface of the chorion has an ill-developed reticulation (by Tojo & Machida 1998) or smooth (by Koss & Edmunds 1974); micropyle, one per egg; the sperm guide is undeveloped; micropylar canal according to Koss and Edmunds (elongated, 42–55 μm long); according to Tojo and Machida (about 20 μm).



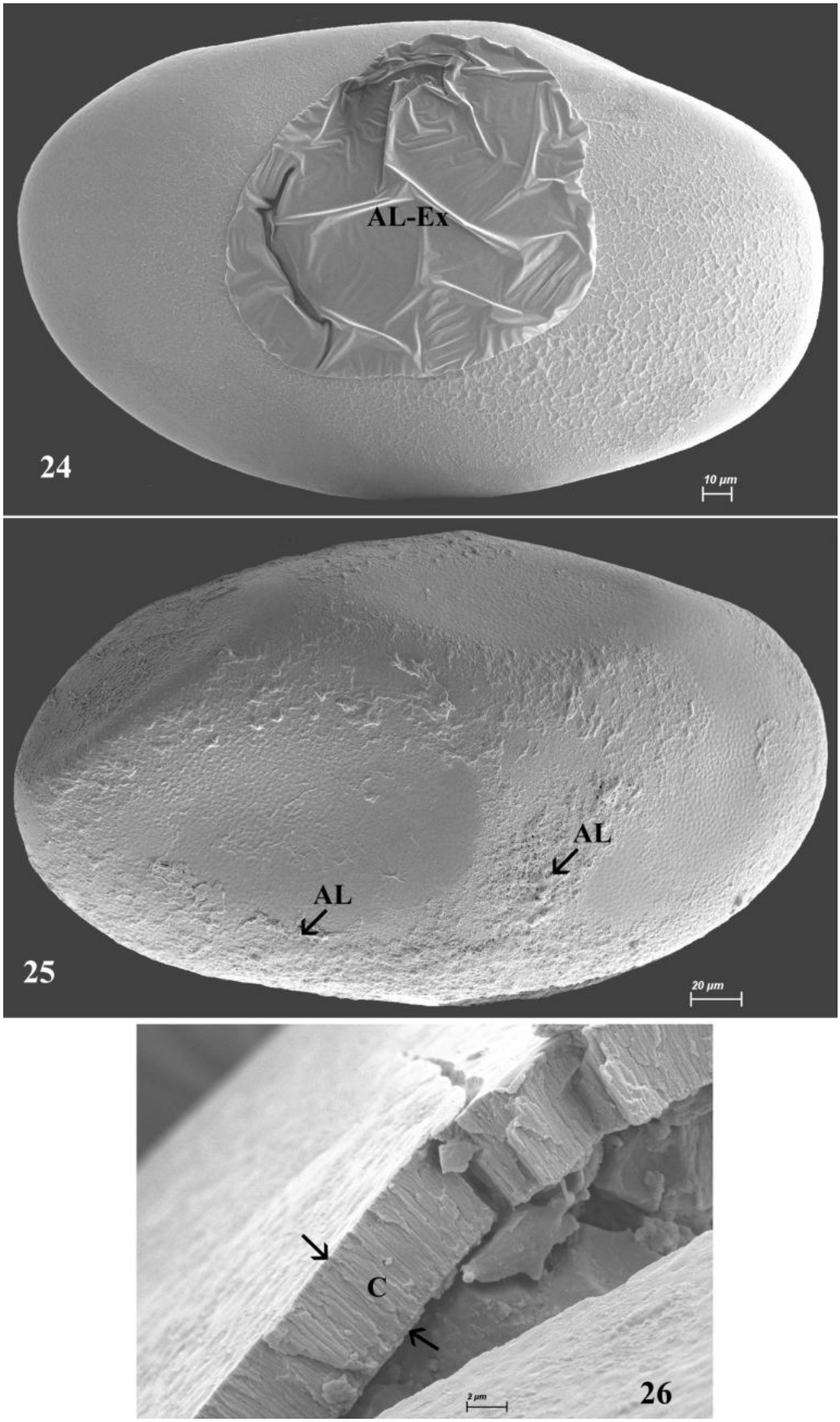
FIGURES 16–19. Egg morphology of *Ephemera* (*Ephemera*) *transbajkalica*: 16–17, general shape; 18–19, micropyle in the complex extrachorion-adhesive layer.

AL-Ex—complex adhesive-extrachorion layer; CM—canal micropylar; mp—micropyle; SG—sperm guide.



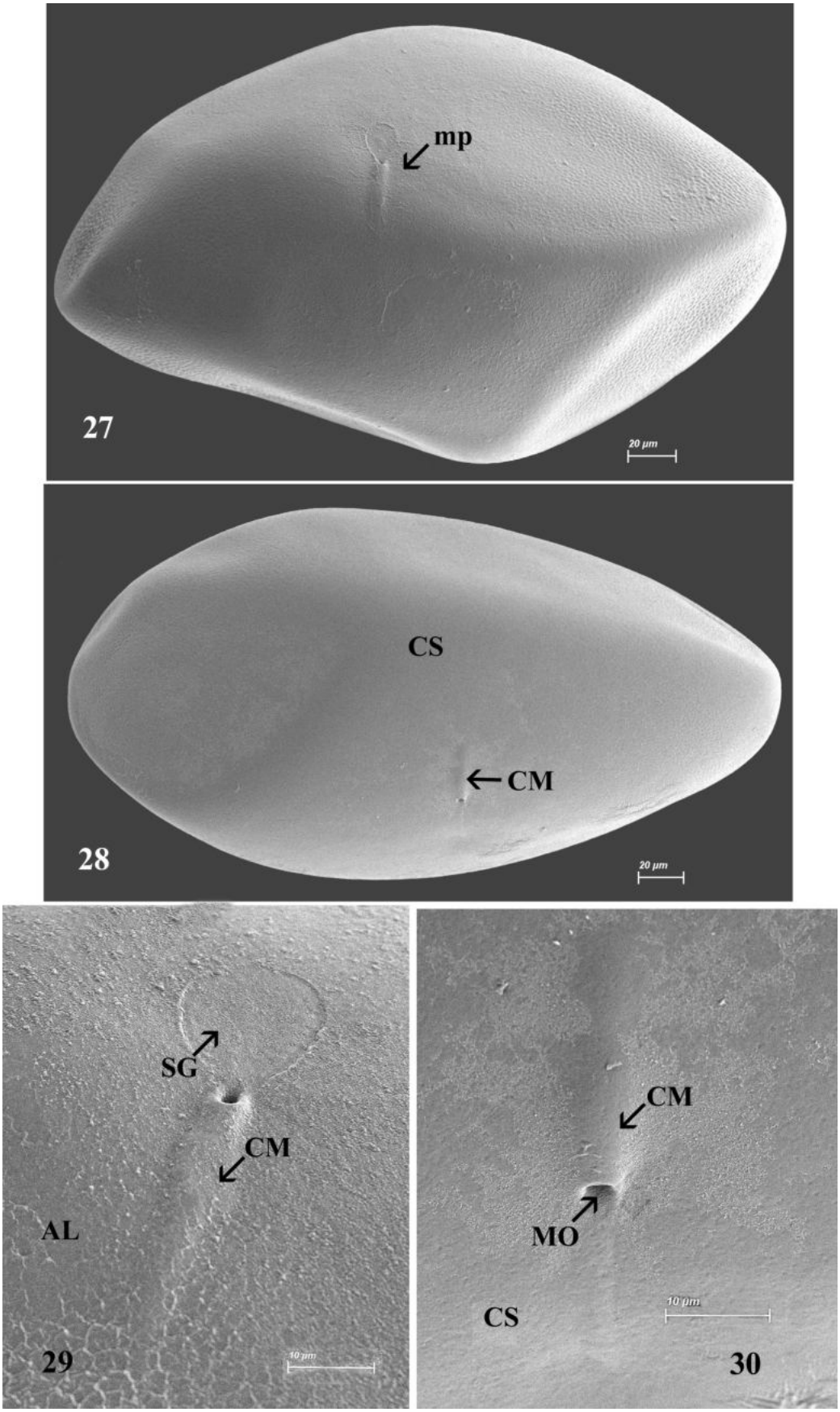
FIGURES 20–23. Egg morphology of *Ephemera* (*Ephemera*) *transbajkalica*: 20, egg surface without complex extrachorion-adhesive layer; 21, thickness on the layer forming the eggshell; 22, proximal part of micropylar canal on chorion surface; 23, chorionic sculpturing.

AL-Ex—complex adhesive-extrachorion layer; CS—chorion surface; MO—micropylar opening; mp—micropyle.



FIGURES 24–26. Egg morphology of *Ephemera* (*Sinephemera*) *japonica*: 24–25, remains of the adhesive layer on the egg surface; 26, chorion thickness.

AL-Ex—complex adhesive-extrachorion layer; AL—adhesive layer; C—chorion.



FIGURES 27–30. Egg morphology of *Ephemera* (*Sinephemera*) *japonica*: 27–28, egg shapes; 29, micropyle in the complex extrachorion-adhesive layer; 30, proximal part of micropylar canal on chorion surface.
AL—adhesive layer; CM—canal micropylar; MO—micropylar opening; CS—chorion surface; mp—micropyle; SG—sperm guide.

According to our data, the egg is oval, more often ovoid in shape (Figs 24–25, 27–28). Dimensions: 293.0–334.0 μm in length (315.4 μm) and 173–197 μm in width (180.1 μm). The extrachorion-adhesive layer covering the egg is thin, smoothly amorphous (Fig. 24–25). In the equatorial area, there is one micropyle per egg (Fig. 27). The micropyle is of the "tagenoform type," with a sharply expanding, rounded, poorly defined sperm guide, 14–16 μm long and 21–24 μm wide (Figs 27, 29). As in other species, the sperm guide is visible only on the adhesive surface of the egg. After the removal of the adhesive layer, only the micropylar canal is visible (Figs 28, 30). The micropylar canal is 16–30 μm long and 3–8 μm wide, protruding prominently above the adhesive layer and chorion (Figs 29–30). The surface of the chorion is smooth (Fig. 28), or with a weakly expressed very fine reticulation, and the proximal part of the tunnel-type micropylar canal and a micropylar opening 2–3 μm wide are clearly visible on the surface of the chorion (Figs 28, 30).

***Ephemera* (*Sinephemera*) *shengmi* Hsu, 1937**

Figures 31–37

Material examined. Russia: Primorsky Krai, Ussuriysky district, Razdolnaya River, above Zarechnoe village, 04.08.2007, 2 ♀ adults, T. Tiunova; Khankaisky district, Lake Khanka, Przhevalsky spit, 12.08.2002, 10♀ adults, T. Tiunova.

Distribution. South of the Russian Far East, China.

The egg is oval (Figs 31–32, 35–36). Dimensions: 224.0–255.0 μm in length (239.9 μm) and 130.0–142.0 μm in width (136.3 μm). The extrachorion-adhesive layer is thin, 0.5–1.2 μm; therefore, the surface of the adhesive layer partially follows the sculpture of the chorion (Figs 31–32, 35). In the equatorial area, there are one to two micropyles per egg (Figs 31–32, 35).

Eggs are characterized by a linear-type micropyle (Koss and Edmunds 1974). In this type of micropyle, there is no sperm guide, and the micropylar canal is relatively short and protrudes over both the adhesive and chorion surfaces (Figs 31–32). The micropylar canal, tunnel-shaped with thick walls, is relatively short, 8–14 μm long and 7–10 μm wide (Fig. 33). The micropylar opening is nearly round, 3.5 μm wide and 3.0 μm high (Figs 33, 37). The sculpture of the chorion consists of discontinuous long and short ridged, curved, and broken filaments, the distribution and arrangement of which regularly cover the entire surface of the chorion (Figs 35–37).

Ephemera* (*Sinephemera*) *strigata Eaton, 1892

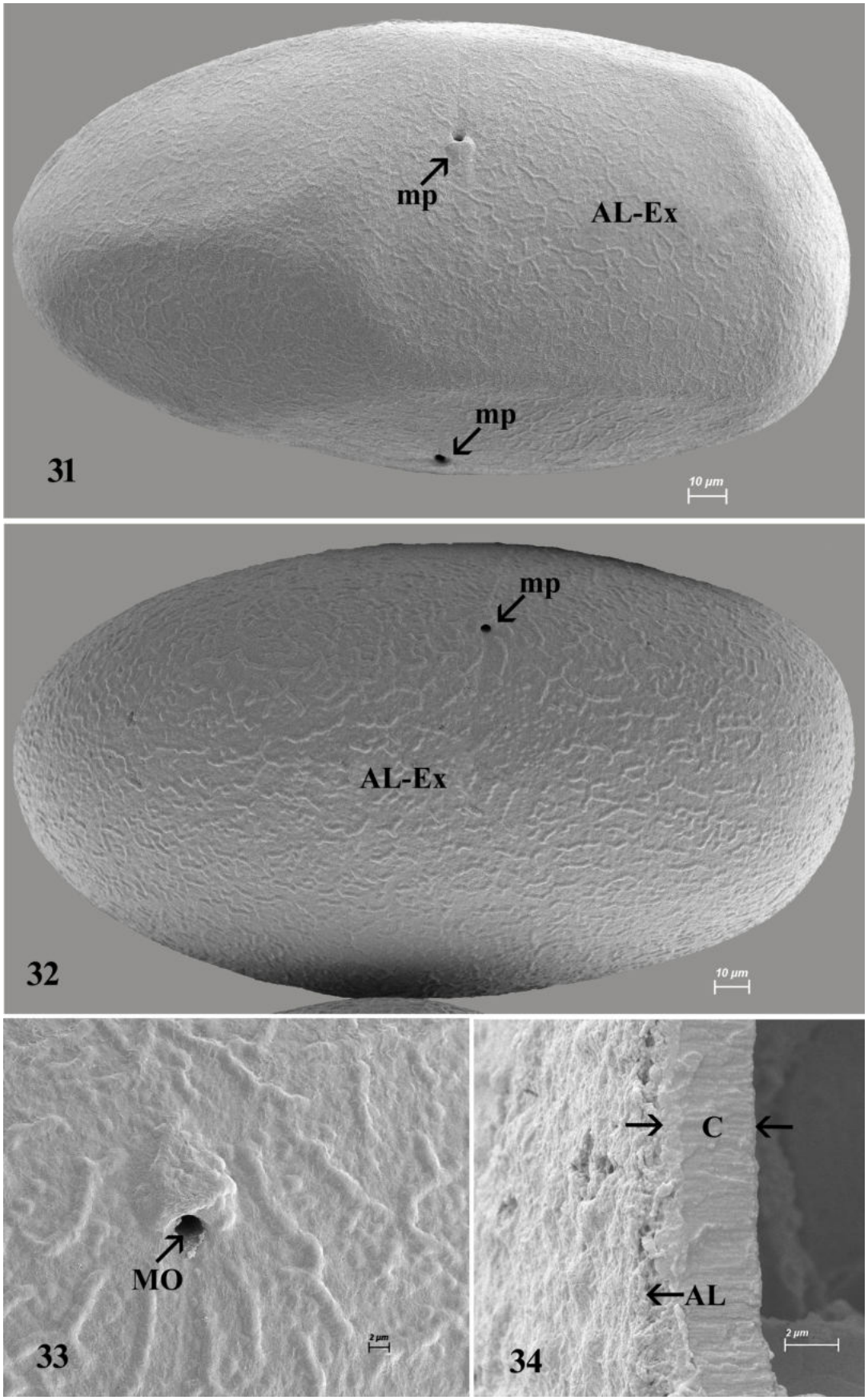
Figures 38–45

Material examined. Russia: Primorsky Krai, Khasansky district: Barabashevka River, below the Fish Hatchery, 4♀ adults, 10.06.2003, T. Tiunova; Barabashevka River, above the Fish Hatchery, 24.06.2021, 1♀ adult, T. Tiunova; Ryazanovka River, below the Okhotbaza, 11.06.2003, 3♀ adults, T. Tiunova.

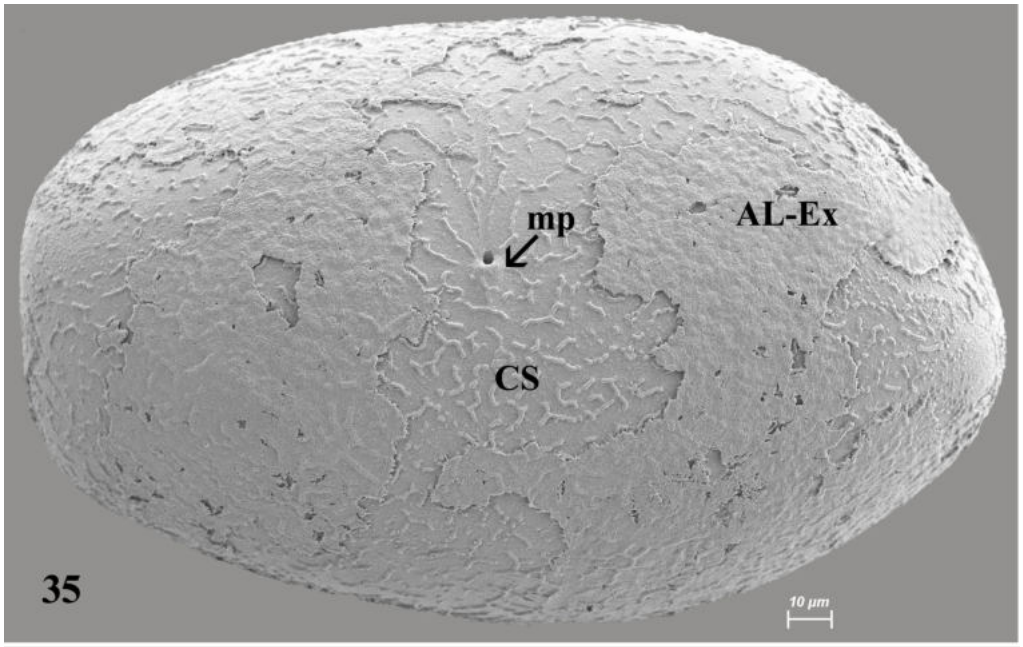
Distribution. East Siberia, Far East Russia, Japan, Mongolia, Korea, China.

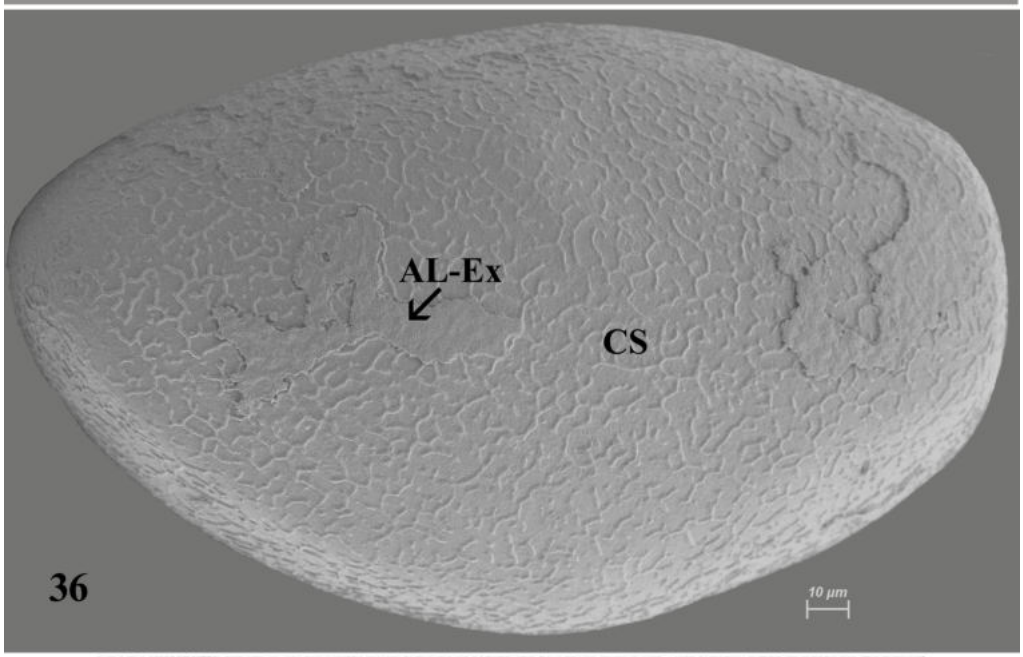
The egg has been described by Okazaki (1981, p. 9: fig. 6; 1984, p. 21: fig. 15) and Tojo & Machida (1998, p. 575: fig. 2). The authors write that the eggs of *E. strigata* and *E. japonica* are very similar in egg size and shape, in the thickness of the adhesive layer, in the structure of the chorion, and in the absence of micropyle.

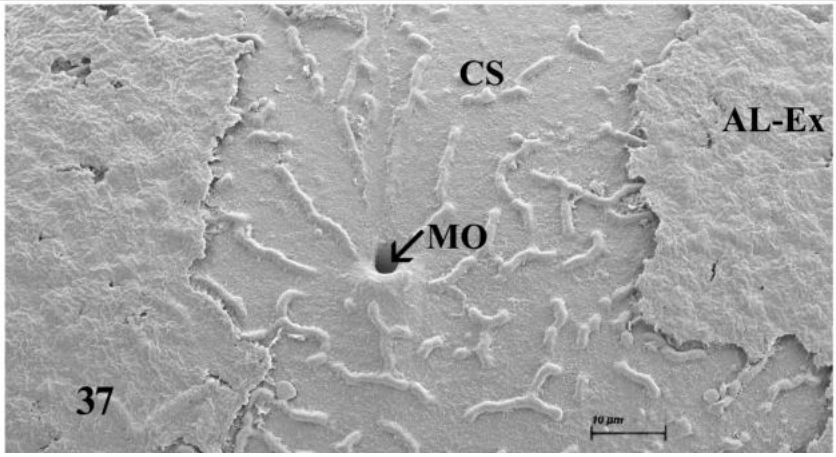
According to our data, the egg is oval (Figs 38–39). Dimensions: 197.0–239.0 μm in length (215.2 μm) and 116.0–139.0 μm in width (126.6 μm). The extrachorion-adhesive layer covering the egg is presented as a thin membrane (Fig. 42). The adhesive layer is almost smooth (Figs 38–39, 41). There are one to two micropyles per egg in the equatorial area (Figs 38–39). The micropyle is of the "tagenoform type," with a drop-shaped, poorly defined sperm guide, 14–18 μm long and 16–18 μm wide (Fig. 40). The micropylar canal is 9.8–15.6. μm long and 5.5–7.2 μm wide; the entrance to the micropylar canal is tunnel-shaped, with thick walls; it protrudes above the adhesive layer and chorion (Figs 40, 43). The micropylar opening is rounded to 2.5–3.0 μm wide and 2.5–2.9 μm high (Figs 43–44). The chorionic surface is finely wrinkled (Figs 43, 45).



FIGURES 31–34. Egg morphology of *Ephemera* (*Sinephemera*) *shengmi*: 31–32, general shape and arrangement of the micropyles; 33, proximal part of micropylar canal on chorion surface; 34, thickness on the layers forming the eggshell and chorion. AL-Ex—complex adhesive-extrachorion layer; AL—adhesive layer; C—chorion; MO—micropylar opening; mp—micropyle.







FIGURES 35–37. Egg morphology of *Ephemera* (*Sinephemera*) *shengmi*: 35–36, egg surface without complex extrachorion-adhesive layer; 37, proximal part of micropylar canal on chorion surface.
AL-Ex—complex adhesive-extrachorion layer; CS—chorion surface; MO—micropylar opening; mp—micropyle.

Key to six Ephemera species upon eggs

1. The egg size is 250 μm or more, and the surface of the chorion is smooth without or with a weakly expressed very fine reticulation (Figs 27–28) . . . . . *E.* (*S.*) *japonica*
- The egg size is less than 250 μm; surface of the chorion with reticulation (Figs 6, 12, 23, 36, 45) 2
2. Sperm guide well expressed (Figs 2, 9, 16, 27, 39) 3
- Sperm guide absent (Fig. 31–32) . . . . . *E. (E.) shengmi*
3. Sperm guide relatively deep (Figs 2, 9, 16) 4
- Sperm guide is shallow and almost flat (Figs 38–39) . . . . . *E. (E.) strigata*
4. Sperm guide is relatively oval and short; the micropylar canal is 12–18 μm (Figs 3–4). . . . . *E. (E.) orientalis*
- Sperm guide an elongated, micropylar canal less than 10 μm (Figs 10–11, 18–19) 5
5. Sperm guide is almost rectangular; the distal margin is weakly defined and almost open; the surface of the chorion is intermittently fractured (Figs 10, 11, 13) . . . . . *E. (E.) sachalinensis*
- Sperm guide oval, with pointed, close distal margin; surface of the chorion with small-mesh reticulation (Figs 18, 19, 23) . . . . . *E. (E.) transbajkalica*

Discussion

Seven species of the genus *Ephemera* from two subgenera, *Ephemera* and *Sinephemera*, are currently present in the Russian Far East. Detailed descriptions of egg morphology (chorion structure, micropyle, thickness of the adhesive layer) using scanning electron microscopy (SEM) are known for such East Asian species as *E*. (*S*.) *japonica*, *E*. (*S*.) *strigata*, *E*. (*E*.) *orientalis*, *E*. (*E*.) *formosana* (Koss & Edmunds 1974; Tojo & Machida 1998), one European species, *E*. (*E*.) *danica* (Ubero-Pascal & Puig 2007), and one Oriental species of the subgenus *Aethephemera* McCafferty & Edmunds, 1973 *E*. (*A*.) *nadinae* McCafferty & Edmunds, 1973 (Balasubramanian *et al*. 1991).

According to Koss (1968) and Koss & Edmunds (1974) there are three morphological characteristics of eggs useful for taxonomic purposes: 1—various attachment structures; 2—the sculpturing of the chorion surface; and 3—micropyle structures.

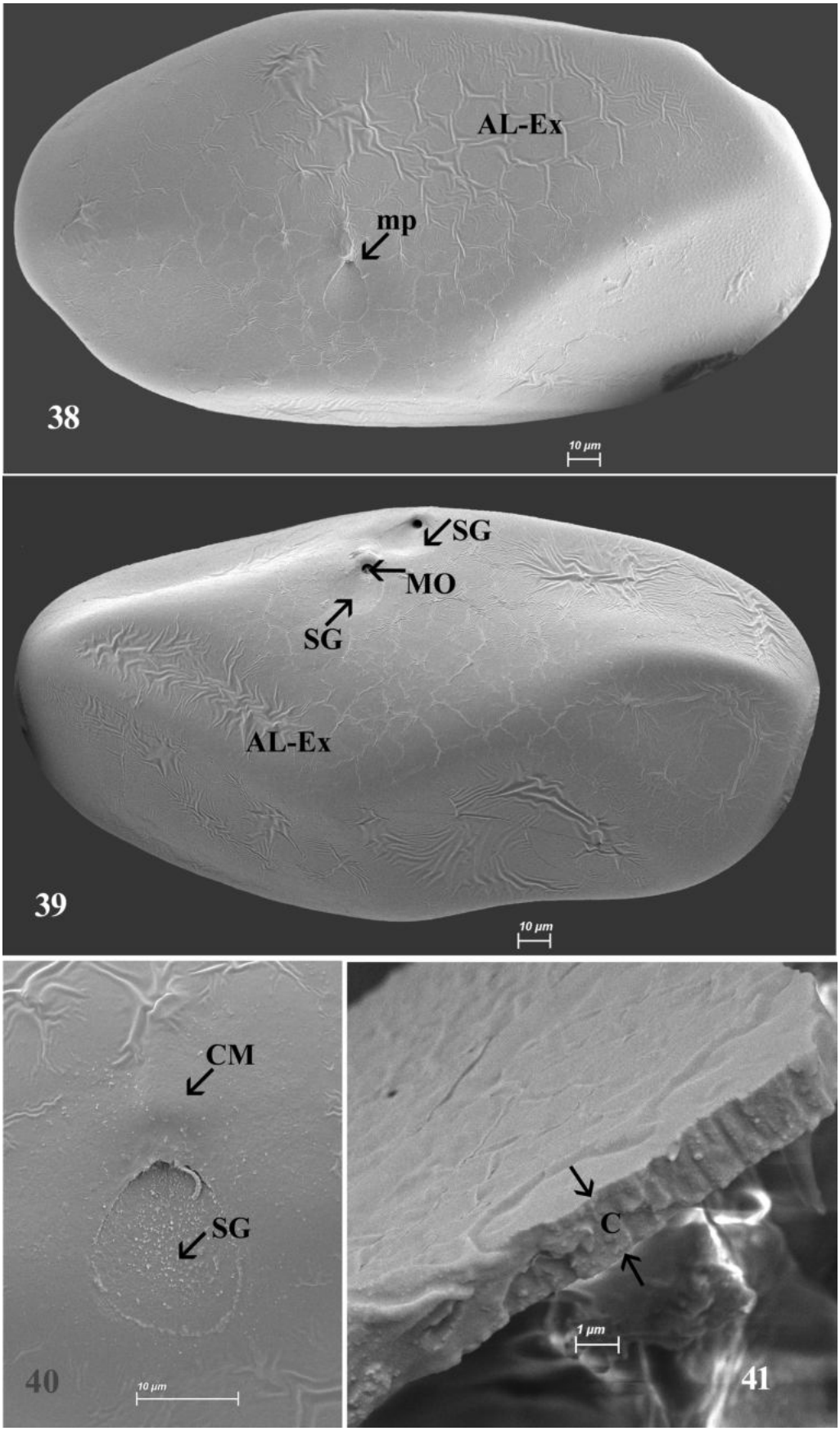
Let us consider the significance of these structures on the basis of the six species studied.

The attachment structure on eggs of species of the genus *Ephemera* is known to be a complex formed by an adhesive layer and an extrachorion (Degrange 1960; Koss 1968; Koss & Edmunds 1974; Tojo & Machida 1978; Ubero-Pascal & Puig 2007). Because the adhesive layer lacks any sculpture (has a granular or amorphous appearance), *Ephemera* species are usually similar to each other (Balasubramanian *et al*. 1991; Kang & Yang 1994; Bauernfeind & Soldan 2012).

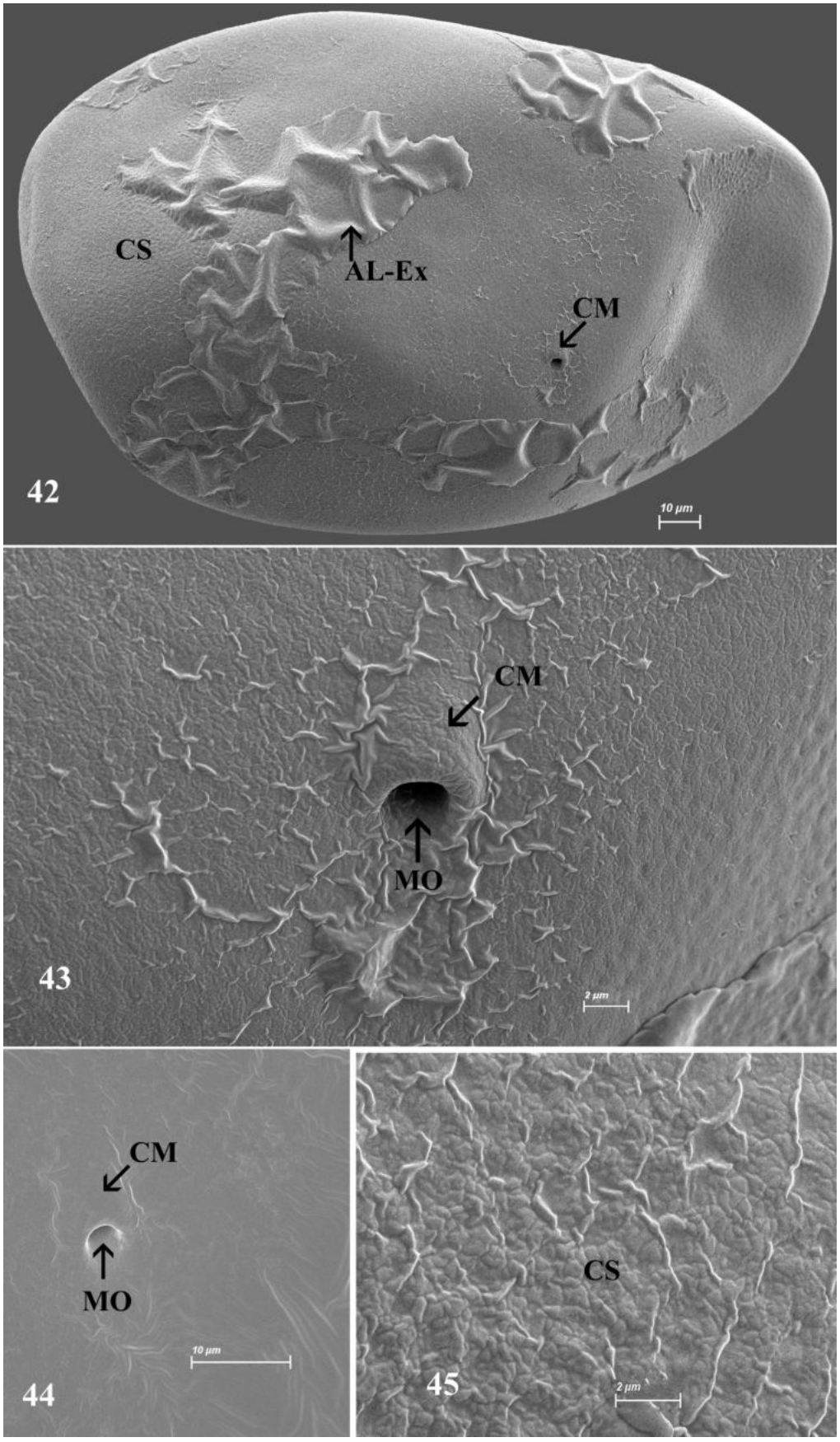
In the examined species of subgenus *Ephemera*, the adhesive layer is thick from 1.3 to 3.0 μm (Figs 8, 15, 23), which agrees with the data obtained earlier for *E. orientalis* and *E. danica* (Tojo & Machida 1998; Ubero-Pascal & Puig 2007). In species of the subgenus *Sinephemera*, the adhesive layer is thin and is 0.54–1.0 μm in *E. shengmi* (Fig. 34), and in *E. japonica* and *E. strigata* it is represented as a thin film (Figs 24, 42). According to Tojo & Machida (1998), the thickness of the adhesive layer in these species is about 0.1 μm.

The surface of the chorion of the *Ephemera* species examined varied markedly. The most common sculpture of the chorion of this genus is a fine mesh reticulate, and there is a wide range of sculptural patterns, including dots, granulations, and irregular ridges. In at least one species, the chorion is smooth (Koss & Edmunds 1974; Ubero-Pascal & Puig 2007). In the species we studied, the structure of the chorion was very diverse. For example, in *E.* (*S.*) *japonica*, at first glance, it is almost smooth (Fig. 28). However, upon closer examination of the chorion surface, one can see very fine and weakly pronounced reticulation (Fig. 27). Apparently, for this reason, Koss & Edmunds (1978) described the chorion surface as smooth and Tojo & Machida (1998) as ill-developed reticulation. The well-developed reticulation of the species we examined is well expressed only in *E. (E.) transbajkalica* (Fig. 23). In *E. (E.) orientalis* and *E. (S.) strigata*, the surface of the chorion is finely wrinkled (Figs. 7, 45). According to Tojo & Machida (1998), *E. (E.) orientalis* has a well-defined reticulation of the chorion surface, and *E. (S.) strigata* has an ill-developed reticulation. In *E. (E.) sachalinensis* and *E. (S.) shengmi*, the chorion sculpture is discontinuously fractured (Fig. 12, 36).

The micropyle in species of the genus *Ephemera* is of a "tagenoform type", with an oval-shaped sperm guide, sometimes almost elliptical, followed by a long intrachorionic tube, the micropylar canal (Degrange 1960; Koss 1968; Koss & Edmunds 1974; Ubero-Pascal & Puig 2007). Micropyles are represented by one or two, located in the equatorial area of the egg and are usually separated by distance, rarely located side by side (Fig. 39). Sometimes one



FIGURES 38–41. Egg morphology of *Ephemera* (*Sinephemera*) *strigata*: 38–39, general shape and arrangement of the micropyles; 40, micropyle in the complex extrachorion-adhesive layer; 41, chorion thickness.
AL-Ex—complex adhesive-extrachorion layer; C—chorion; CM—canal micropylar; MO—micropylar opening; SG—sperm guide.



FIGURES 42–45. Egg morphology of *Ephemera* (*Sinephemera*) *strigata*: 42, remains of the adhesive layer on the egg surface; 43–44, proximal part of micropylar canal on chorion surface; 45, chorionic sculpturing.
AL-Ex—complex adhesive-extrachorion layer; CS—chorion surface; CM—canal micropylar; MO—micropylar opening.

of the micropyle may shift to the subpolar region. In species examined, the micropyle is also of the "tagenoform type," except for *E. (S.) shengmi*, in which the micropyle can be attributed to the linear type (Fig. 31). In this type of micropyle, there is no sperm guide, and protrudes over both the adhesive and chorion surfaces (Figs 31–32).This distinguishes it from all investigated species of the genus *Ephemera*. It should be noted that in morphology, *E. (S.) shengmi* has a number of differences that are not characteristic of the East Palaearctic species of the genus *Ephemera*. This, first of all, refers to the structure of the genitalia. Thus, the first segment of the gonostylus is elongated (in contrast to other species having short segments), and its length is practically equal to the second segment.

The presence of a sperm guide was noted in all species studied, except *E. (S.) shengmi*. In contrast to our data, Tojo & Machida (1998) indicate the absence of it in *E. (S.) japonica* and *E. (S.) strigata*. We believe that this is an erroneous statement. As shown in our study, the sperm guide is clearly visible on the adhesive-extrachorion layer (27, 29, 38, 41), and after removing the adhesive layer, the sperm guide is not visible on the chorion (30, 42, 44). Because the adhesive layer in *E. (S.) japonica* and *E. (S.) strigata* is very thin, it is relatively easily destroyed, even by a short cleaning of the egg.

The shape and size of the sperm guide differed significantly in all species studied. This gave reason to believe that the structure of the micropyle, along with the sculpture of the chorion, can be very useful in species identification. For example, Tojo and Machida (1998) wrote that the eggs of *E. (S.) japonica* and *E. (S.) strigata* are very similar in shape, size, chorion structure, thickness of adhesive layer, and absence of sperm guide, making them difficult to distinguish. In our case, *E. (S.) japonica* has a micropyle with a sharply expanded and rounded sperm guide, 14–16 μm long and 21–24 μm wide (Figs. 27, 29), while *E. (S.) strigata* has a micropyle with a drop-shaped sperm guide, 14–18 μm long and 16–18 μm wide (Fig. 40).

It would be desirable to pay attention to the size of the eggs of the studied species. Thus, of the six species, the largest eggs were observed in *E. (S.) japonica* (average 315 μm), and the smallest eggs were observed in *E. (E.) orientalis* (average 191 μm). However, in the work of Tojo & Machida (1998), egg size was similar (200x100 μm) for all species they studied. Relatively large eggs were also noted for *E. (S.) shengmi* (239.9 μm on average).

Thus, the use of micropyle structure along with chorion sculpture can be very useful in identifying species of the genus *Ephemera*. The use of SEM to study eggs will certainly help to determine the usefulness of morphological structures for taxonomic purposes.

Acknowledgements

The scanning electron micrographs were prepared with the help of Vitaliy Kazarin (Federal Scientific Center of the East Asia Terrestrial Biodiversity Far Eastern Branch, Russian Academy of Sciences, Vladivostok). I express my sincere gratitude to the reviewers for their valuable advice and suggestions. The research was carried out within the state assignment of Ministry of Science and Higher Education of the Russian Federation (theme No. 124012400285-7).

References

Bae, Y.J. (1995) *Ephemera separigata*, a new species of Ephemeridae (Insecta: Ephemeroptera) from Korea. *Korean Journal of Systematic Zoology*, 11 (2), 159–166.

Balasubramanian, C., Venkataraman, K. & Sivaramakrishnan, K.G. (1991) Life stages of a South Indian burrowing mayfly, *Ephemera* (*Aethephemera*) *nadinae* McCafferty and Edmunds 1973 (Ephemeroptera: Ephemeridae). *Aquatic Insects*, 13, 223–228.
https://doi.org/10.1080/01650429109361449

Bauernfeind, E. & Soldan, T. (2012) *The mayflies of Europe* (*Ephemeroptera*). Apollo Books, Ollerup, 781 pp.
https://doi.org/10.1163/9789004260887

Degrange, C. (1960) Recherches sur la Reproduction des Éphéméroptères. *Travaux du Laboratoire d'Hydrobiologie et de Pisciculture de l'Université de Grenoble*, 51, 71–93.

Hsu, Y.-C. (1937) The mayflies of China. *Peking Natural History Bulletin*, 11 (3), 287–296.

Hwang, J.M. & Bae, Y.J. (2008) Review of the tropical Southeast Asian *Ephemera* (Ephemeroptera: Ephemeridae). *Aquatic Insects*, 30 (2), 105–126.
https://doi.org/10.1080/01650420701883333

Hwang, J.M., Bae, Y.J. & McCafferty, W.P. (2008) A checklist of the burrowing mayfly family Ephemeridae. *In*: Hauer, F.R.

& Stanford, J.A. & Newell, R.L. (Eds.), International advances in the ecology, zoogeography and systematics of mayflies and stoneflies. University of California Publications in Entomology 128. *Preceding of the 11 International. Conference on Ephemeroptera, Montana, USA*, 22–29 August 2004, pp. 159–172.
https://doi.org/10.1525/california/9780520098688.003.0012

Eaton, A.E. (1892) On two new and some other Japanese species of Ephemeridae. *Entomologist's Monthly Magazine*, Series 2, 3 (28), 302–303.

Kang, S.C. & Yang, C.T. (1994) Ephemeroidea of Taiwan (Ephemeroptera). *Chinese Journal of Entomology*, 14, 391–399.

Kluge, N.J. (2004) *The phylogenetic system of Ephemeroptera*. Kluwer Academic Publishers, 456 pp.
https://doi.org/10.1007/978-94-007-0872-3

Kluge, N.J. (2023) Ephemeroptera of the world. Available from: http://insecta.bio.spbu.ru/z/Eph-spp/index.htm (accessed 14 February 2023)

Koss, R.W. (1968) Morphology and taxonomic use of Ephemeroptera eggs. *Annals of the Entomological Society of America*, 61, 696–721.
https://doi.org/10.1093/aesa/61.3.696

Koss, R.W. & Edmunds, G.F. Jr. (1974) Ephemeroptera eggs and their contribution to phylogenetic studies of the order. *Zoological Journal of the Linnean Society*, 55, 267–349.
https://doi.org/10.1111/j.1096-3642.1974.tb01648.x

Matsumura, S. (1931) *6000 illustrated Insects of Japan-Empire*. *Ephemerida, 1465–1480*. Tokoshoin, Tokyo, 1497 pp.

McCafferty, W.P. & Edmunds, G.F. Jr. (1973) Subgeneric classification of *Ephemera* (Ephemeroptera: Ephemeridae). *Pan-Pacific Entomologist*, 49 (4), 300–307.

McLachlan, R. (1875) A sketch of our present knowledge of the neuropterous fauna of Japan (excluding Odonata and Trichoptera). *Transactions of the Entomological Society, London*, 167–190.
https://doi.org/10.1111/j.1365-2311.1875.tb01906.x

Okazaki, H. (1981) On the eggs of Japanese mayflies (1). *Biology of Inland Waters*, 2, 8–10.

Okazaki, H. (1984) Observation of the eggs of Japanese mayflies by scanning electron microscope. *Biology of Inland Waters*, 3, 19–27.

Su, C.-R. & Zhu, C.-D. (1997) Observation on the chorion structure of four species of mayfly by scanning electron microscope. *Journal of Nanjing Normal University (Nature Science)*, 20 (4), 121–123.

Tojo, K. & Machida, R. (1998) Egg Structures of Japanes Ephemeridae species (Ephemeroptera). *Entomological Science*, 1 (4), 573–579.

Tshernova, O.A. (1973) Palearctic mayfly species of the genus *Ephemera* (Ephemeroptera, Ephemeridae). *Revue d'Entomologie de l'URSS*, 52 (2), 324–339.

Ubero-Pascal, N.A. & Puig, M.A. (2007) Egg morphology update based on new chorionic data of *Potamanthus luteus* (Linnaeus), *Ephemera danica* Müller and *Oligoneuriella rhenana* (Imhoff) (Insecta, Ephemeroptera) obtained by scanning electron microscopy. *Zootaxa*, 1465 (1), 15–29.
https://doi.org/10.11646/zootaxa.1465.1.2

Ulmer, G. (1912) H. Sauter's Formosa-Ausbeute. Ephemeriden. *Entomologische Mitteilungen*, 1, 369–375.
https://doi.org/10.5962/bhl.part.25902

Ulmer, G. (1920) Neue Ephemeropteren. *Archiv für Naturgeschichte*, Series A, 85 (11), 1–80.

Zhang, J., Gui, H. & You, D.S. (1995) Studies on the Ephemeridae (Insecta: Ephemeroptera) of China. *Journal of Nanjing Normal University* (*Nature Science*), 18 (3), 68–76. [in Chinese]

Zhou, C.F. (2013) A species list of Chinese mayflies (Insecta: Ephemeroptera). *Biology of Inland waters*, 6 (2), 167–225.