Journal of Species Research 9(1):11-18, 2020

Two new genera of black flies (Diptera: Simuliidae) of Korea

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Larva and pupae of *Prosimulium kiotoense* and larvae of *Twinnia japonensis* were collected and reported from Korea for the first time. The genus *Prosimulium*, previously unknown from Korea, contains 80 species worldwide and 8 species are known in Japan. Species are separated into 3 species groups; *hirtipes* species-group (47 spp.), *macropyga* species-group (14 spp.) and *magnum* species-group (19 spp.), with which *P. kiotoense* belongs to the *hirtipes* species-group. The genus *Twinnia*, also previously unknown from Korea, contains only 10 species worldwide. In this study, I provide detailed descriptions and photographs of larvae and pupae *P. kiotoense* and larvae *T. japonensis*. *Prosimulium kiotoense* is characterized by pupa with gill of 16 filaments, cocoon amorphous and entirely covers the pupal body, head and thorax densely covered with microtubercles, larva with postgenal cleft apically straight, and hypostomal teeth with median tooth prominent. On the other hand, larva of *T. japonensis* can be characterized by labral fan absent, gill histoblast with 16 filaments, and hypostomal teeth with median tooth distinctly shorter than other teeth. In addition to descriptions for each species, a key to Korean *Prosimulium* and *Twinnia* is provided, with reference to the Japanese species and habitat information.

Keywords: black fly, Korea, *Prosimulium*, taxonomy, *Twinnia*

DOI:10.12651/JSR.2020.9.1.011

Introduction

Black flies are small, nuisance pests of many continents and are important vectors of many diseases such as leucocytozoonosis and onchocerciasis (river blindness) in many parts of the world (Adler *et al*., 2004). A total of 20 species in 2 genera of black flies are known to occur in Korea (Adler, 2019). The genus *Prosimulium*, previously unknown from Korea, contains 80 species worldwide (Adler, 2019). Species are separated into 3 species groups; *hirtipes* species-group (47 spp.), *macropyga* species-group (14 spp.), and *magnum* species-group (19 spp.). Eight species are known to occur in Japan, of which five species are assigned to the *magnum* species-group (*P. apoina*, *P. karibaense*, *P. kazukii*, *P. sarurense*, and *P. yezoense*) and the other three species belongs to the *hirtipes* species-group (*P. jezonicum*, *P. kanii*, and *P. kiotoense*). The genus *Twinnia*, also previously unknown from Korea, contains only 10 species worldwide (Adler, 2019). Among them, three species (*Twinnia cannibora*, *T. japonensis*, and *T. subtibbelesi*) are recorded from Japan.

In this study, I provide detailed descriptions and photographs of larva and pupae of *P. kiotoense* and larvae of *T. japonensis*. A key to Korean *Prosimulium* and *Twinnia* is provided, with reference to the Japanese species and habitat information.

Materials and Methods

The classification and morphological terminology used in the text follow those of Adler *et al*. (2004). Larvae and pupae of *P. kiotoense* were hand collected from streams and fixed in 100% EtOH. External morphology was examined under a Leica M165C dissecting microscope or Leica Z16 APO macroscope (Leica, Wetzlar, Germany). Head and posterior end of abdomen of penultimate instar larvae were removed from the body with two sets of fine forceps in 80% EtOH. Detached head and abdominal end were placed in a vial of 10% potassium hydroxide (KOH) and boiled for 10 min to facilitate examination of larval hypostoma and posterior circlet with phase-contrast microscopy. Cleared head and abdominal end were then neutralized by placing them in a vial containing 3% acetic EtOH for 3 min. Larval hypostoma and posterior circlet were recovered with fine needles and placed in a drop

of glycerin on a microscope slide or concave slide to examine them at different angles. A cover slip was applied and sealed with clear fingernail polish. The preparations were examined with phase-contrast microscopy (Leica DM 2500). Z-stacked digital images were taken with a digital camera (Leica DFC 295, Leica, Wetzlar, Germany) attached to the microscope, accompanied with Z-builder software (Leica Application Suite, Leica, Wetzlar, Germany). Consecutive digital images in different focal planes were taken with a Sony A6500 digital camera (Sony, Tokyo, Japan) attached to the Leica macroscope and the images were Z-stacked using Helicon Focus® software (Helicon Soft Ltd., Ukraine).

Voucher materials, including slide preparations, were deposited in the Applied Biology Program, Division of Bio-resource Sciences, Kangwon National University, Chuncheon, Korea.

Checklist of *Prosimulium* and *Twinnia* from Korea (bold) and Japan.

Genus *Prosimulium* Roubaud
Magnum species-group
1. *Prosimulium apoina* Ono, 1977
2. *Prosimulium karibaense* Ono, 1980
3. *Prosimulium kazukii* Takaoka, Saito, Adler & Baba, 2018
4. *Prosimulium sarurense* Ono, 1976
5. *Prosimulium yezoense* Shiraki, 1935

Hirtipes species-group
6. *Prosimulium jezonicum* (Matsumura, 1931)
7. *Prosimulium kanii* Uemoto, Onichi & Orii, 1973
8. *Prosimulium kiotoense* Shiraki, 1935

Genus *Twinnia* Stone & Jamnback
1. *Twinnia cannibora* Ono, 1977
2. *Twinnia subtibbelesi* Ono, 1980
3. *Twinnia japonensis* Rubtsov, 1960

Key to immatures of Korean species of *Prosimulium* and *Twinnia* (with reference to Japanese species)

1. Larva with labral fan absent ····· 2 (*Twinnia*)
Larva with labral fan well developed ····· 4 (*Prosimulium*, *Simulium*)
2. Antenna with basal segment dilated (swollen) ····· *T. cannibora*
Antenna with basal segment not dilated ····· 3
3. Each abdominal segment with two large dorsolateral dark spots ····· *T. subtibbelesi*
Each abdominal segment without dark spots ····· ***T. japonensis***
4. Larva with postgenal cleft truncated; prothoracic proleg with lateral sclerite broad and vertical portion well developed; pupa with cocoon amorphous ····· 5 (*Prosimulium*)
Larva with postgenal cleft pointed or round, not truncated; prothoracic proleg with lateral sclerite narrow or broad but vertical portion not well developed; pupa with cocoon shoe, boot, or slipper shaped ····· (*Simulium*)
5. Pupa with gill 16 filaments ·· 6 (*hirtipes* species-group)
Pupa with gill more than 16 filaments ····· 8 (*magnum* species-group)
6. Dorsal trunk of gill strongly divergent from ventral trunk ····· 7
Dorsal trunk of gill not divergent from ventral trunk ····· ***P. kiotoense***
7. Four pairs of gill filaments rising from ventral trunks with a short petiole; larval hypostoma with median tooth prominent ····· *P. jezonicum*
One of four pairs of gill filaments rising from ventral trunks sessile; larval hypostoma with median tooth as high as or lower than lateral tooth ····· *P. kanii*
8. Larval antenna with basally swollen abruptly; gill with 38–40 filaments ····· *P. apoina*
Larval antenna gradually tapered toward apex; gill with various filaments ····· 9
9. Gill with 46–48 filemants ····· *P. sarurense*
Gill with at most 40 filaments ····· 10
10. Pupa with frons and dorsal surface of the thorax with distinct tubercles ····· 11
Pupa with frons and dorsal surface of the thorax without tubercles ····· *P. kazukii*
11. Larval hypostoma with median and lateral teeth equal in length; gill with 26–30 filaments ····· *P. karibaense*
Larval hyposotm with median tooth distinctly longer and higher than lateral tooth; gill with 22–25 filaments ·· *P. yezoense*

Taxonomic Accounts

Family Simuliidae Latreille, 1802
Subfamily Simuliinae Newman, 1834
Tribe Prosimuliini Enderlein, 1921

Genus *Prosimulium* Roubaud

Prosimulium Roubaud, 1906: 519–521 (as subgenus of *Simulium*). Type species: *Simulia hirtipes* Fries, 1824: 17–18, designated by Malloch 1914: 16.

Hellichia Enderlein, 1925: 203–204 (as genus). Type species: *Hellichia latifrons* Enderlein, 1925: 204 (= *Melusina macropyga* Lundstrom, 1911: 20–21), by original designation.

Taeniopterna Enderlein, 1925: 203 (as genus). Type species: *Melusina macropyga* Lundstrom, 1911: 20–21, by

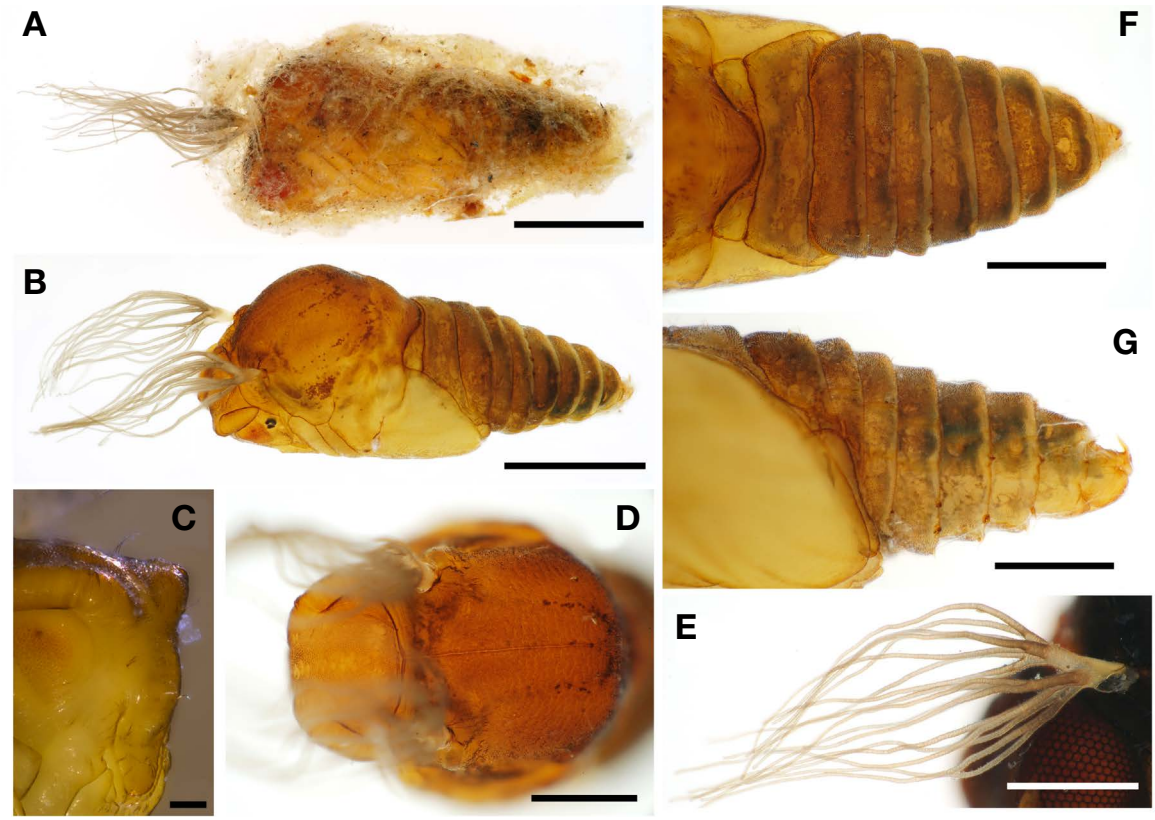

Fig. 1. Pupa of *Prosimulium kiotoense* Shiraki. (A) Pupa with cocoon, lateral view; (B) Pupa (cocoon removed), dorsolateral view; (C) Head, anterolateral view; (D) Head and thorax, dorsal view; (E) Gill filament, lateral view; (F) Abdomen, dorsal view; (G) Abdomen, lateral view. Scale bars, 1 mm (A, B); 100 μm (C); 500 μm (D–G).

original designation.

Mallochella Enderlein, 1930: 84 (preoccupied) (as genus). Type species: *Mallochella sibirica* Enderlein, 1930: 84 (=*Simulia hirtipes* Fries, 1824: 17–18), by original designation.

Mallochianella Vargas & Diaz Najera, 1948: 67 (substitute name for *Mallochella* Enderlein, same type species).

Piezosimulium Peterson, 1989: 317–318 (as genus). Type species: *Piezosimulium jeanninae* Peterson, 1989: 317–330 (=*Prosimulium neomacropyga* Peterson, 1970: 134–139), by original designation.

Diagnosis for genus (modified from Uemoto *et al*., 1973 and Adler *et al*., 2004)

Pupa: Gill of 9 or more filaments. Thoracic trichomes unbranched. Abdominal segment IX with a dorsal pair of long terminal spines. Cocoon with loose to densely woven sleeve, lacking definite shape (amorphous).

Larva: Basal two segments of antenna pale, distal segment darkly pigmented. Postgenal cleft variable, typically truncated or rounded apically. Median tooth of hypostoma trifid. Prothoracic proleg with each lateral sclerite broad, vertical portion well developed. Rectal papillae with 3 simple lobes.

***Prosimulium kiotoense* Shiraki, 1935 (Figs. 1, 2)**

Prosimulium kiotoense Shiraki, 1935: 6. Type locality: Kyoto, Japan.

Prosimulium kiotoense: Saito & Kajihara, 1975 (Japanese record); Saito *et al*., 1983 (Japanese record); Baba & Takaoka, 1985 (Japanese record and ecology); Baba & Takaoka, 1988 (ecology); Saito *et al*., 1988 (Japanese record); Baba & Takaoka, 1991a (ecology); Baba & Takaoka, 1991b (ecology); Baba & Takaoka, 1992 (ecology); Saito, 2015 (Japanese list and name); Adler, 2019 (world checklist).

Prosimulium (*Prosimulium*) *kiotoense*: Uemoto *et al*., 1973 (revision); Matsuo & Uemoto, 1975 (ultrastucture); Saito & Sato, 1984 (Japanese record); Saito *et al*., 1985 (Japanese record); Saito *et al*., 1990 (Japanese record); Saito *et al*., 1993 (Japanese record); Saito *et al*., 1996a (Japanese record); Saito *et al*., 1996b (Japanese record); Saito & Kanayama, 2002 (Japanese record); Uemoto, 2005 (Japanese key and illustrations).

Prosimulium hirtipes, not Fries: Ogata & Sasa, 1954 (Japanese list); Bentinck, 1955 (key and illustrations); Ogata & Sasa, 1955 (Japanese key and illustration); Shogaki, 1956 (Japanese list); Ogata & Fukui, 1957 (ecology).

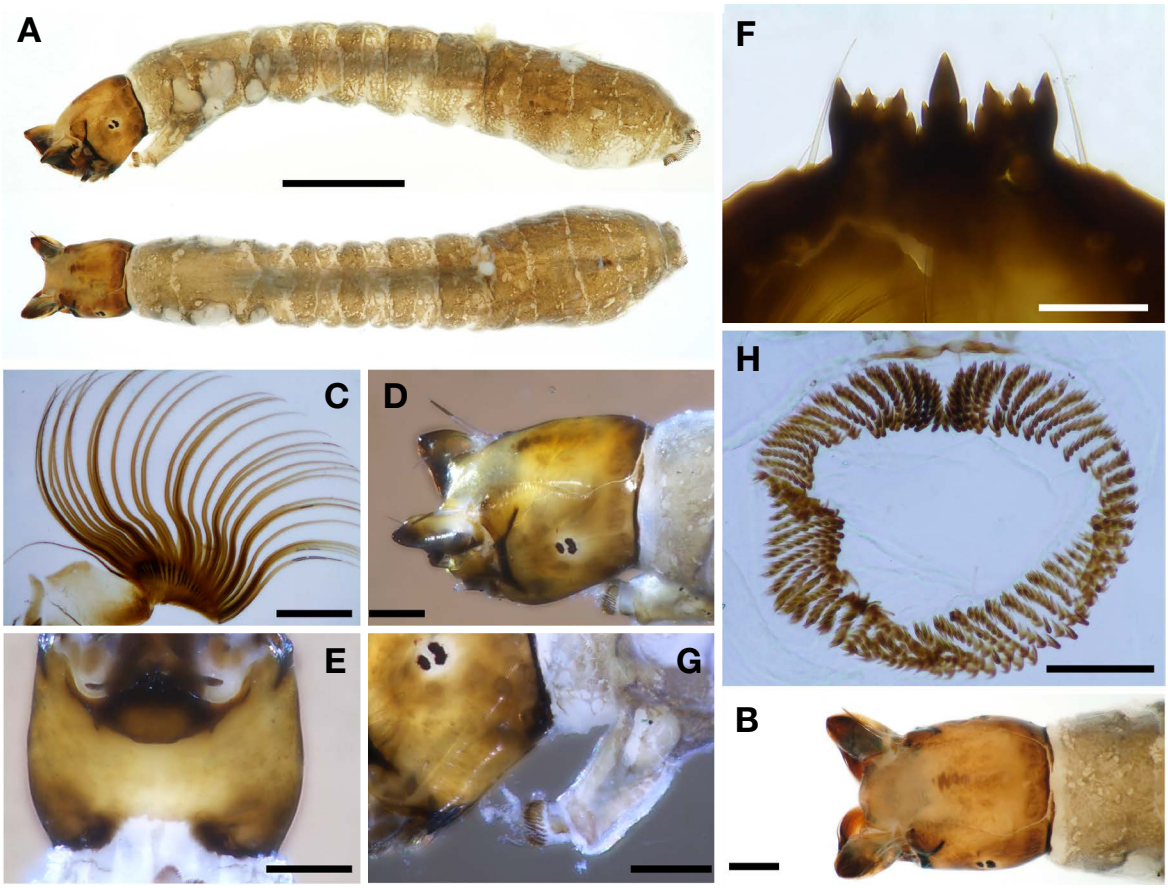

Fig. 2. Larva of *Prosimulium kiotoense* Shiraki. (A) Larva, lateral and dorsal view; (B) Head, dorsal view; (C) Labral fan (detached from the head); (D) Head, dorsolateral view; (E) Head, ventral view; (F) Hypostoma, phase contrast photomicrograph; (G) Prothoracic proleg, lateral view; (H) Posterior circlet, phase contrast photomicrograph. Scale bars, 1 mm (A); 200 μm (B–E, G); 100 μm (H); 50 μm (F).

Diagnosis. *Prosimulium kiotoense* can be easily distinguished from other Korean black flies by the following characteristics: cocoon amorphous, entirely cover the pupa; gill of 16 filaments; larval antenna with proximal and medial article pale, distal article brown; labral fan with 32 rays; hypostoma with median tooth prominent, trifid; postgenal cleft shallow, apex straight.

Description. Pupa. Body length 2.7–3 mm (n = 7). Pupa (Fig. 1A, 1B) brown to light brown ground color. Gill (Fig. 1B, 1E) with 16 filaments in 3 main stems; uppermost stem with 3 stalks, give rise to 8 filaments arranged in 2 + (1 + 2) + (1 + 2); middle and lowermost stems each with 2 stalks, give rise to 8 filaments in (2 + 2) arrangement, 0.6× as long as pupal body length. Head (Fig. 1C, 1D) with cephalic apotome light brown, densely covered with small tubercles; frontal trichome 3 pairs, simple; facial trichome 1 pair, simple. Thorax (Fig. 1B, 1D) brown, densely covered with small tubercles; thoracic trichome 5–6 pairs, long, simple. Abdomen (Fig. 1F, 1G) densely covered with small tubercles; abdominal tergite II with 4 pairs of subapical setae; tergites III and VI apically with 4 pairs of anterior directed hooks; tergites V–IX anteriorly with distinct spine combs; tergites VI–VIII with spin at posterolateral margin of each tergites; tergite VIII with 3 pairs of long golden setae. Abdominal sternum (Fig. 1G) with sternite IV with 2 pairs of small hooks; sternite V with 2 pairs of large hooks; sternites VI and VII with 1 pair of hook. Terminal spine (Fig. 1G) well developed, long, directed anteriorly. Cocoon (Fig. 1A) amorphous, loosely woven, entirely cover the pupa and base of gill filaments.

Larva (penultimate instar). Body length 5.3 mm (n = 1). Body (Fig. 2A) brownish grey ground color. Head capsule (Fig. 1B) brown to light brown. Head spots (Fig. 2B) with anteromedial spots distinct, other spots indistinct. Labral fan (Fig. 2C) with 32 primary rays. Antenna (Fig. 2D) with proximal and medial article transparent, distal article brown, as long as labral fan stem, gradually tapered toward apex; proportional ratio from proximal to distal article as 0.8 : 1 : 1.2. Postgenal cleft (Fig. 2E) shallow, apex straight or quadrate, laterally with elongate lateral spot. Hypostoma (Fig. 2F) with 7 teeth,

apically pointed; median tooth prominent, trifid, lateral teeth longer and larger than sublateral teeth, intermediate teeth well developed. Hypostomal seta 3 pairs, simple. Subesophageal ganglion (Fig. 2E) not pigmented. Prothoracic proleg (Fig. 2G) with well developed lateral sclerite, vertical portion well developed. Rectal papillae with 3 simple lobes. Posterior proleg (Fig. 2H) with 10–13 hooks in 68 rows. Anal sclerite X shaped with anterodorsal arms clearly longer than the posteroventral arms.

Specimens examined. Korea: Gyeonggi-do, Gapyeong-gun, Buk-myeon, Jeokmok-ri, Gapyeongcheon stream, 37°57′48″N, 127°26′58″E, altitude 290 m. 10.v.2019, SK Kim (1 pupa); ditto, 22.v.2019, SK Kim (2 pupae); Gyeonggi-do, Gapyeong-gun, Buk-myeon, Dodae-ri, Myeongjicheon stream, 37°56′07″N, 127°29′18″E, altitude 210 m, 10.v.2019, SK Kim (4 pupae, 1 penultimate instar larva).

Distribution. Korea (Gyeonggi-do, new record), Japan (Honshu, Kyushu).

Stream information. Two streams, the only localities where larvae and pupae of *P. kiotoense* were collected so far, are separated by Mt. Myeongjisan (1,267 m) by a distance of 4.6 km. Both were medium to large-sized streams with moderate to rapid flow and totally exposed to the sun. Stream beds consisted of boulders and rubble. Edges of the streams were lined with trailing vegetations including reeds. The streams were 10–20 m wide and 15–20 cm deep, but the streams were partially dried up due to spring drought.

Remarks. Japanese species assigned to *magnum* species-groups have 22–48 gill filaments in pupa, while species in *hirtipes* species-group, which *P. kiotoense* belongs to, have 16 gill filaments in pupa. Morphologically similar species, *P. jezonium* (Matsumura, 1931) and *P. kanii* Uemoto, Onichi & Orii, 1973, can be distinguished from *P. kiotoense* by the shape of gill filaments and diverging condition between dorsal and ventral stem of gill filaments (Uemoto *et al*., 1973). General description of *P. kiotoense* larva and pupa from Korea fits well with that of Uemoto *et al*. (1973). However, a great difference exists in pupal and larval size between Japanese (Uemoto *et al*., 1973) and Korean specimens: pupal body length 5.7–6.8 (Japan) vs. 2.7–3 (Korea) / larval body length 6.8–8.1 (Japan) vs. 5.3 (Korea). For larval body size, however, the studied material might be insufficient since only single penultimate instar larva was available for Korea. Although *P. kiotoense* is known as a univoltine and widespread in Japan (Uemoto *et al*., 1973; Baba & Takaoka, 1988), they were found only from two streams within the same district (Gyeonggi-do, Gapyeong-gun) in Korea. Since the immature stages are coldwater life forms, larval period is from autumn to early spring and has seven larval instars (Uemoto *et al*., 1973). First instar larvae were appeared on November and 7th instar larvae were first appeared early April (Baba & Takaoka, 1991a). It is believed that *P. kiotoense* can attack cattle and occasionally humans (Uemoto *et al*., 1973). All larvae and pupae were collected only twice in May 2019 from two streams, and I failed to collect *P. kiotoense* in subsequent attempts in other months at the same streams. This species was collected along with *Simulium* (*Boreosimulium*) *konoi*, *Simulium* (*Nevermannia*) *uchidai*, *Simulium* (*Simulium*) *suzukii*, *Simulium* (*Simulium*) *yamatoense*, and *Simulium* (*Simulium*) *japonicum*.

Genus *Twinnia* Stone & Jamnback

Twinnia Stone & Jamnback, 1955: 18–19 (as genus). Type species: *Twinnia tibblesi* Stone & Jamnback 1955: 19–21, by original designation.

Diagnosis for genus (modified from Adler *et al*., 2004) Larva: Cephalic apotome (frontoclypeal apotome) with median head spot extended anteriorly to level of anteriormost eyespot. Labral fans absent. Hypostoma weakly sclerotized, with broad, apically rounded teeth. Postgenal cleft absent. Anal sclerite Y shaped.

***Twinnia japonensis* Rubtsov, 1960 (Fig. 3)**

Twinnia nova japonensis Rubtsov, 1960: Die Fliegen der palaearktischen Region, 14 Simuliidae: 128. Type locality: Japan (Honshu).

Prosimulium novum: Bentinck, 1955, not Dyar & Shannon.

Prosimulium (*Twinnia*) *japonense*: Uemoto, 1980 (revision); Saito *et al*., 1996 (Japanese record); Uemoto, 2005 (Japanese key and illustrations).

Twinnia japonensis: Saito, 2015 (Japanese list and names); Adler, 2019 (world checklist).

Diagnosis. Larva of *T. japonensis* can be easily distinguished from other black flies by the following characteristics: labral fan absent; gill histoblast with 16 filaments in [(6 + 2) + (4 + 4)] arrangement; postgenal cleft absent; head with anteromedial and posterolateral head spots absent; posteromedial head spots distinct, extended to the level of eye spot; 7 spots at posterolateral and ventral margin of head capsule; hypostoma with teeth broad, apically rounded; median tooth trifid, shorter than lateral and sublateral teeth; rectal papillae with 3 simple, long, slender lobes.

Description. Larva. Body length 6.6 mm (n = 10). Body (Fig. 3A) light brownish grey ground color. Head capsule (Fig. 3B) light brown, longer than wide, triangular shaped in dorsal view; moderately covered with pale hairs with dark base (Fig. 3D). Cephalic apotome (frontoclypeal apotome) strongly contracted posteriorly (Fig. 3B). Head spots (Fig. 3B–3D) with first and second anterolateral head spots distinct, well separated; anteromedial and posterolateral spots absent; postero-

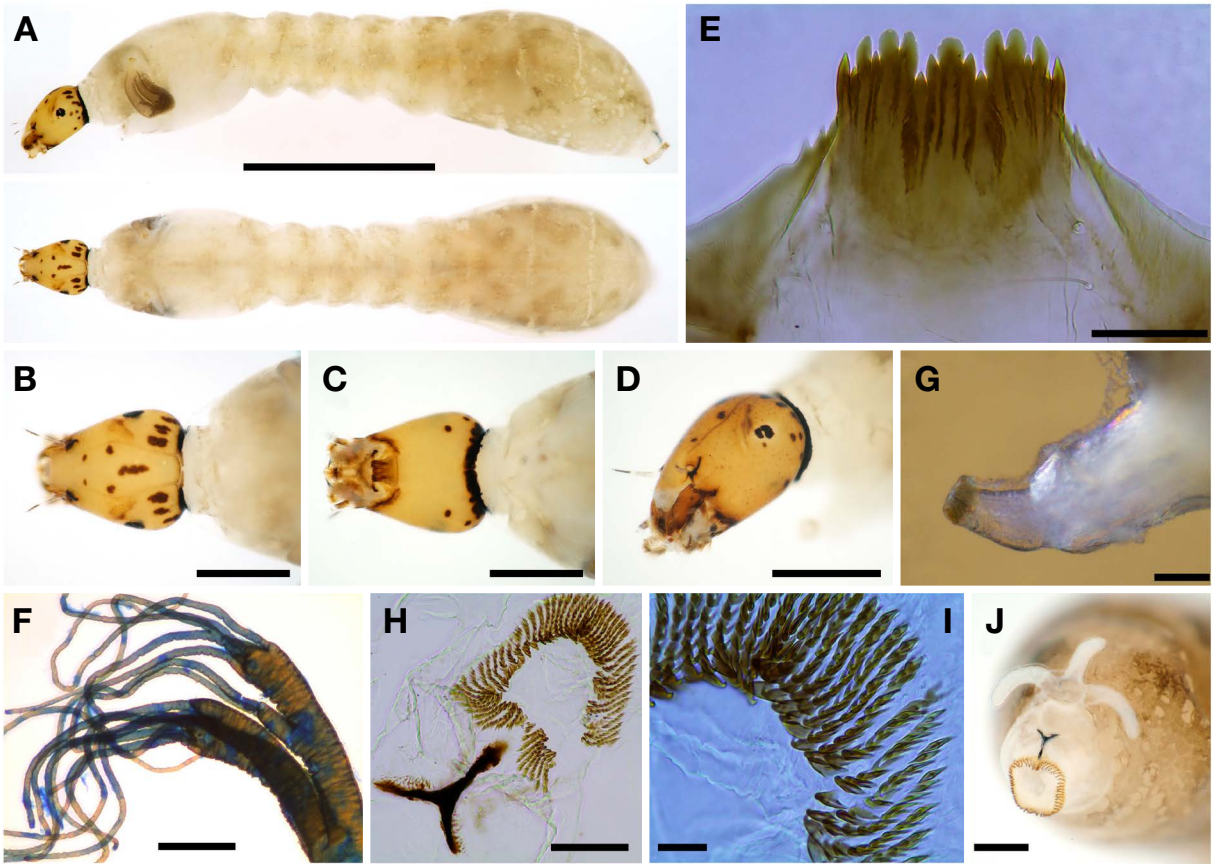

Fig. 3. Larva of *Twinnia japonensis* Rubtsov. (A) Larva, lateral and dorsal view; (B) Head, dorsal view; (C) Head, ventral view; (D) Head, anterodorsal view; (E) Hypostoma, phase contrast photomicrograph; (F) Gill histoblast, phase contrast photomicrograph; (G) Prothoracic proleg, lateral view; (H, I) Posterior circlet, phase contrast photomicrograph; (J) Rectal papillae, posterior view. Scale bars, 2 mm (A); 500 μm (B–D); 200 μm (F, J); 100 μm (G, H); 50 μm (E, I).

medial spots distinct, extended anteriorly to the level of eye spot; posterolateral margin of head capsule with 7 spots, 3 large groups of spots and 3 round spots at the area between eye spots and postocciput, single round spot ventrally (Fig. 3A, 3C). Labral fan absent. Antenna (Fig. 3B, 3D) with proximal and medial article transparent, distal article brown, gradually tapered toward apex; proportional ratio from proximal to distal article as 0.8 : 1 : 1. Postgenal cleft (Fig. 3C) absent. Hypostoma (Fig. 3E) with 7 broad, apically rounded teeth; sublateral teeth prominent, bifid; median tooth round, trifid, shortest; lateral tooth longer than median tooth, slender, curved, dull pointed; paralateral tooth well developed, apically pointed; intermediate teeth and lateral serration well developed; Hypostomal seta 3–4 pairs, simple. Subesophageal ganglion (Fig. 3C) not pigmented. Prothoracic proleg (Fig. 3G) without lateral sclerite. Gill histoblast (Fig. 3F) with 16 filaments, arranged as [(6 + 2) + (4 + 4)] from dorsal to ventral, with long stems. Posterior proleg (Fig. 3H, 3I) with 10–12 hooks in 58–64 rows. Rectal papillae (Fig. 3J) with 3 simple, long, slender lobes. Anal sclerite Y shaped.

Pupa. Unknown from Korea.

Specimens examined. Korea: Gangwon-do, Chuncheon-si, Namsan-myeon, Banghari, 37°47′16″N, 127° 32′28″E, altitude 75 m, 19.iv.2019, SK Kim (4 larvae with 2 ultimate, 2 penultimate); ditto, 24.iv.2019 (28 larvae with 3 ultimate, 11 penultimate, 14 early instars); Gangwon-do, Hongcheon-gun, Bukbang-myeon, Wonso-ri, 37°41′54″N, 127°43′30″E, altitude 118 m, 19.iv.2019 (3 larvae with 1 ultimate, 2 early instars); ditto, 23.iv.2019 (8 larvae with 3 ultimate, 3 penultimate, 2 early instars).

Distribution. Korea (Gangwon-do, new record), Japan (Honshu).

Stream information. The two streams, which are the only localities where larvae of *T. japonensis* were collected so far, were small mountainous stream with rubble and sandy bottom, leading to Bukhangang and Hongchengang River, respectively. There were agricultural fields and houses adjacent to the streams, but the streams were maintained relatively clean. The streams were 3–5 m wide and 5–20 cm deep. The streams were lined with trailing vegetations including reeds. The streams where larvae

were collected were partially dried up after May 2019 due to spring drought.

Remarks. Uemoto (1980) erroneously designated the holotype of this species from the female of Bentinck (1955). This species is known as a univoltine and overwinters as an egg and hatches in the late April or early May, so the larvae were mainly collected from middle to late April (Uemoto, 1980). According to Japanese records (Bentinck, 1955; Uemoto, 1980), this species was found infrequently from rapidly flowing small streams fed by melting snow at altitudes above 1,200−1,500 m. Larvae lacked labral fans and used their modified mouthparts to graze food from stones and submerged litter (Adler *et al*., 2004), so that the larvae and pupae usually attached themselves to the lower surface of stones (Bentinck, 1955; Uemoto, 1980), but could be found from various aquatic substances including submerged twigs. Korean specimens were collected from streams with moderate to rapid flow with well-developed submerged reed blades. Many larvae were collected from submerged reed roots at artificial waterways with concrete bottoms. No larvae were collected from stones. Unfortunately, no pupae and adults were collected from Korea. Adults can be seen from early June to early July and possibly bite humans (Bentinck, 1955; Shogaki *et al*., 1978; Uemoto, 1980). This species were collected along with commonly collected species such as, *Simulium* (*Nevermannia*) *uchidai*, *Simulium* (*Simulium*) *suzukii*, *Simulium* (*Simulium*) *yamatoense*, *Simulium* (*Simulium*) *japonicum*, and *Simulium* (*Simulium*) *oitanum*.

Distribution. Korea (Gangwon-do, new record), Japan (Honshu).

Acknowledgements

The author would like to express the greatest thanks to P.H. Adler (Clemson Univ., SC, USA) for valuable comments and information on species mentioned in the article. The author also thanks anonymous reviewers for reviewing the manuscript.

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Submitted: January 10, 2020
Revised: February 3, 2020
Accepted: February 3, 2020