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Unrecorded subgenus of black fly (Diptera: Simuliidae) from Korea

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Larvae, pupae, and adults reared from the pupae of *Simulium* (*Boreosimulium*) *konoi* were collected and recorded from Korea for the first time. I provide detailed descriptions of both immatures and adults accompanied with photographs. The subgenus *Boreosimulium*, previously unknown from Korea, contains 19 species in 3 species-groups worldwide and only 2 species, *viz*, *S*. (*B*.) *konoi* and *S*. (*B*.) *tokachiense*, are recorded from Japan. *Simulium konoi* is unique among the members of the subgenus by having water drop-shaped denticles on the anterior portion of the cibarium in females, which is considered as an autapomorphic character so far not found in other black flies, W or inverted V-shaped ventral plate of male genitalia, and larval thorax and abdominal segments covered with short black setae. Korean specimens of *S. konoi* collected from two adjacent localities can readily be recognized by denticles on the cibarium in females and well-developed ventral plate of male genitalia but showed geographical variation, e.g., lacking ventral tubercle on larval abdominal segment IX. Due to the autapomorphy and other characters found in *S. konoi*, the current placement of the *S. konoi* in the subgenus *Boreosimulium* or species-group assignment is tentative.

Keywords: black fly, *Boreosimulium*, distribution, new to Korea, taxonomy

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Introduction

The subgenus *Boreosimulium*, previously unknown from Korea, contains 19 species in 3 species-groups (*annulus*, *baffinense*, and *johannseni* species-groups) worldwide, and only 2 species, *viz*, *Simulium* (*Boreosimulium*) *konoi* and *S*. (*B*.) *tokachiense*, are recorded from Japan (Adler, 2019). All members of the subgenus are recorded from the Holarctic region. According to Yankovsky & Yankovsky (2006), diagnostic characters of the subgenus *Boreosimulium* (as genus in the article) are: male ventral plate flat, female claw with very small basal tooth or without tooth, gill with 3−4 filaments with basally swollen. However, diagnostic characters given by Yankovsky & Yankovsky (2006) are not sufficient to define the subgenus, as they only made comparisons between *Boreosimulium* and *Hellichiella* (Yankovsky & Yankovsky, 2006).

In this study, I report the presence of *S*. (*B*.) *konoi* for the first time from Korea, and provide detailed descriptions of larvae, pupae, and adults reared from the pupae.

Materials and Methods

The classification and morphological terminology used in the text follow those of Adler *et al*. (2004). Larvae and pupae of *S. konoi* were hand collected from the streams and fixed in 100% EtOH. External morphology was examined under a Leica M165C dissecting microscope or Leica Z16 APO macroscope (Leica, Wetzlar, Germany). Adults were reared from pupae by placing pupae on moist filter paper in insect rearing dishes. Emerged adults were fixed in 100% EtOH. Heads of ultimate instar larvae and post abdomens of both males and females were removed from the body with two sets of fine forceps and preserved in 80% EtOH. Detached heads and post abdomens were placed in a vial of 10% potassium hydroxide (KOH) and boiled for 10 min to facilitate examination of larval hypostoma and genitalia of males and females with phase-contrast microscopy. Cleared head and post abdomens then were neutralized by placing them in a vial containing 3% acetic EtOH for 3 min. Larval hypostoma and genitalia were recovered with fine needles and placed in a drop of glycerin on a microscope slide or concave slide to examine them in 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 cam-

era (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.

Systematic Accounts

Family Simuliidae Latreille, 1802

Genus *Simulium* Latreille, 1802

Simulium Latreille, 1802: 426 (as genus) Type species: *Oestrus columbacensis* Scopoli by monotypy.

Subgenus *Boreosimulium* Rubtsov & Yankovsky

Boreosimulium Rubtsov & Yankovsky 1982: 183–184 (as genus). Type species: *Melusina annulus* Lundstrom 1911: 17–18, by original designation.

Diagnosis (Modified from Adler *et al*., 2004).
Adults: Radius with hair dorsobasally, scutum typically unpatterned. Female: claws each with basal thumb-like lobe. Cibarium with or without oblique rows of denticles. Spermatheca unpigmented proximally, wall with polygonal pattern. Male: Gonostylus slender, tapered to pointed apex, with 1 apical spinule. Ventral plate with median keel typically weak or well developed. Paramere with 0, 1 or multiple apical spines.
Pupa: Gill with 4 filaments. Cocoon slipper shaped.
Larva: Antenna with 3 or more hyaline bands or spots. Abdominal segments IX with or without prominent ventral tubercles. Abdominal setae unbranched, translucent, sparse. Rectal papillae of 3 compound lobes.

***Simulium* (*Boreosimulium*) *konoi* (Takahasi, 1950) (Figs. 1–4)**

Nevermannia konoi Takahasi, 1950: 1556. Type locality: Tottori, Honshu, Japan.
Simulium (*Nevermannia*) *yamayaense* Ogata & Sasa, 1954: 326–327. Type locality: Honshu, Japan; Takaoka & Okazawa, 1988: 99 (Japanese list); Uemoto, 1991: 191 (synonymization).
Simulium (*Nevermannia*) *liaodongense* Sun, 2012: 46–49. Type locality: Liaoning, China; Adler & Crosskey, 2014 (synonymization).
Simulium sp. J-9 Bentinck, 1955: 6; Takahasi, 1971 (synonymization).
Simulium (*Eusimulium*) sp. Ogata & Sasa, 1955: 14 (Japanese key).
Simulium (*Boreosimulium*) *konoi*: Sato *et al*., 2004 (taxonomy, redescription); Adler, 2019 (world list).
Simulium (*Nevermannia*) *konoi*: Ogata & Sasa, 1954: 328 (Japanese list and key); Ogata & Sasa, 1955: 11 (Japanese list and key); Shogaki, 1956: 275 (Japanese list); Ogata *et al*., 1956: 76–77 (description); Takahasi, 1971: 80 (taxonomic notes); Takaoka & Okazawa, 1988: 98 (Japanese list).
Eusimulium konoi: Orii *et al*., 1969: 3 (Japanese key); Uemoto, 1991: 191 (taxonomic note); Uemoto, 2005: 1017 (Japanese key, illustration).
Simulium (*Eusimulium*) *konoi*: Ogata & Uemoto, 1971: 80 (illustration); Matsuo & Uemoto, 1975: 130–133 (morphology).
Simulium (*Gomphostilbia*) *konoi*: Uemoto, 1985: 330 (Japanese key).
Simulium (*Eusimulium*) *yamayaense*: Ogata *et al*., 1956: 74 (morphology); Shogaki & Shimizu, 1956: 373 (illustration); Okamoto, 1958: 583 (taxonomic notes, illustration); Ogata & Uemoto, 1971: 77 (illustration); Uemoto, 1985: 330 (Japanese key).
Eusimulium yamayaense: Orii *et al*., 1969: 9–11 (Japanese key).
Cnetha konoi: Yankovsky, 2001: 305 (Russian key).
Cnetha konoi: Ono, 1979 (misidentification of *Simulium oligotuberculatum* (Knoz, 1965)).

Diagnosis. This species can be distinguished from other black flies by the following combination of characteristics: female cibarium equipped with oblique rows of denticles; male ventral plate well-developed W or inverted V-shaped; pupa with 4 gill filaments; larva with thorax and abdomen densely covered with short branched dark setae; larval antenna with 3 hyaline bands.
Description. Female (Fig. 1). Body length (Fig. 1A): 2.6–2.8 mm (n = 10). Head (Fig. 1B). As wide as thorax. Eye dichoptic. Antenna 11 segmented, terminal segment with 3–4 apical setae. Frons dark brown to black, dull densely covered with whitish golden hairs. Clypeus dorsally and laterally with dark brown, medially brown with whitish hairs, pruinosity. Maxillary palp (Fig. 1C) 5 segmented, proportional ratio of 3rd to 5th segments 1 : 1.2–1.3 : 2.4–2.6; sensory vesicle 0.4× as long as 3rd segment. Mandible with ca. 25 serrations. Maxillary lacinia (Fig. 1C) with 23–27 teeth. Cibarium (Fig. 1D, E) with ca. 70–80 water drop-shaped oblique rows of denticles anteriorly. Hypopharynx with 20–30 spine-like serrations. Thorax (Fig. 1F). Wing length: 2.5–2.7 mm. Basal section of radius with dorsal hairs. Scutum dark brown to black with densely covered with whitish golden hairs. Scutellum light brown with long golden hairs, apically with long

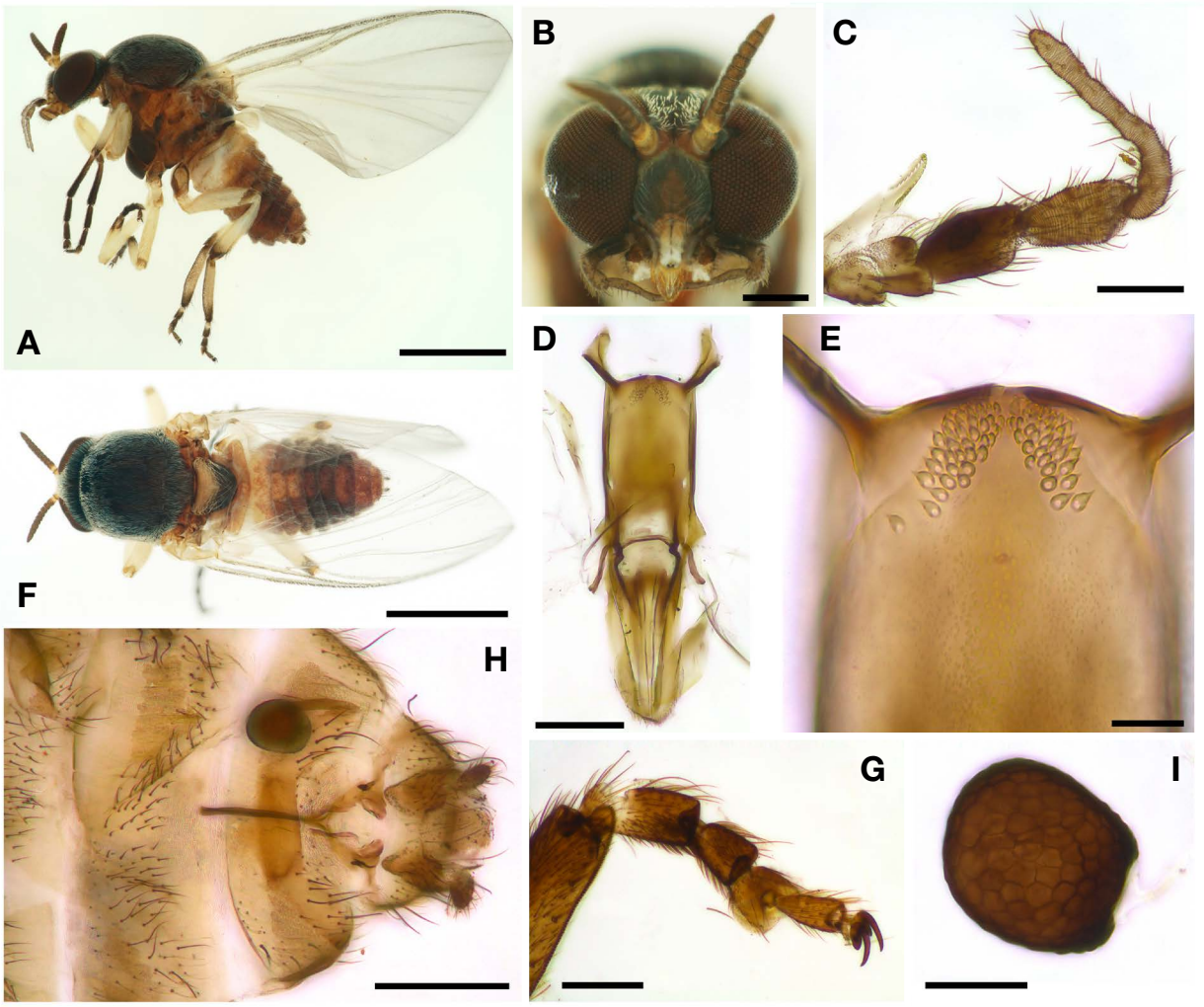

Fig. 1. Female of *Simulium* (*Boreosimulium*) *konoi*. (A) Body, lateral view; (B) Head, frontal view; (C) Maxillary palp with lacinia; (D) Cibarium with hypopharynx; (E) Cibarium; (F) Body, dorsal view; (G) Hind leg with basitarsus and tarsomeres; (H) Abdomen and genitalia, ventral view; (I) Spermatheca. Scale bars, 1 mm (A, F); 200 μm (B, H); 100 μm (C, D, G); 50 μm (I); 20 μm (E).

black hairs. Pleural membrane light brown, bare. Katepisternum brown, bare. Legs. All femora and tibiae brownish yellow with apex brown. Tarsi dark brown. Hind leg with basitarsus (Fig. 1G) with calcipala distinct, 1.6× as long as tarsomere 1–4 combined. Pedisulcus distinct. Tarsal claw with well-developed thumblike lobe, 0.64× as long as claw. Abdomen. Brown to dark brown, moderately covered with whitish hairs except abdominal segments 3–7 with dark long hairs near each spiracles. Genitalia (Fig. 1H). Genital fork with space between each arms semi-round. Anal lobe moderately produced ventrally, rounded, 13–17 long hairs. Cercus small, rounded posteriorly, setose. Spermatheca (Fig. 1I) slightly longer than wide, with distinct reticulate pattern.

Male (Fig. 2). Body length (Fig. 2A): 2.4–2.7 mm (n = 10). Head (Fig. 2B). Wider than thorax. Eye holoptic. Upper corneal facets consisting of 15–17 vertical rows and 20–22 horizontal rows. Antenna (Fig. 2C) 11 segmented, terminal segment with 3–5 apical setae. Clypeus dark brown, pruinosity, moderately covered with yellowish hairs. Maxillary palp (Fig. 2D) 5 segmented, proportional ratio of 3rd to 5th segments 1 : 1.1–1.3 : 2.4–2.6; sensory vesicle 0.2× as long as 3rd segment. Maxillary lacinia with 15–18 short setae, not well serrated. Cibarium (Fig. 2E) without denticles. Hypopharynx with 15–20 apical setae. Thorax (Fig. 2F). Wing length: 2.2–2.5 mm. Basal section of radius with dorsal hairs. Scutum brownish black, densely covered with golden hairs, posteriorly with long reclinate hairs. Scutellum yellowish brown with long black hairs and sparse golden hairs. Pleural membrane brown, bare. Katepisternum brown, bare. Legs. All femora and tibiae yellowish brown with apex brown. Tarsi brown to dark brown. Hind leg with basitarsus (Fig. 2G) with calcipala distinct, 1.4× as long as tarsomere 1–4

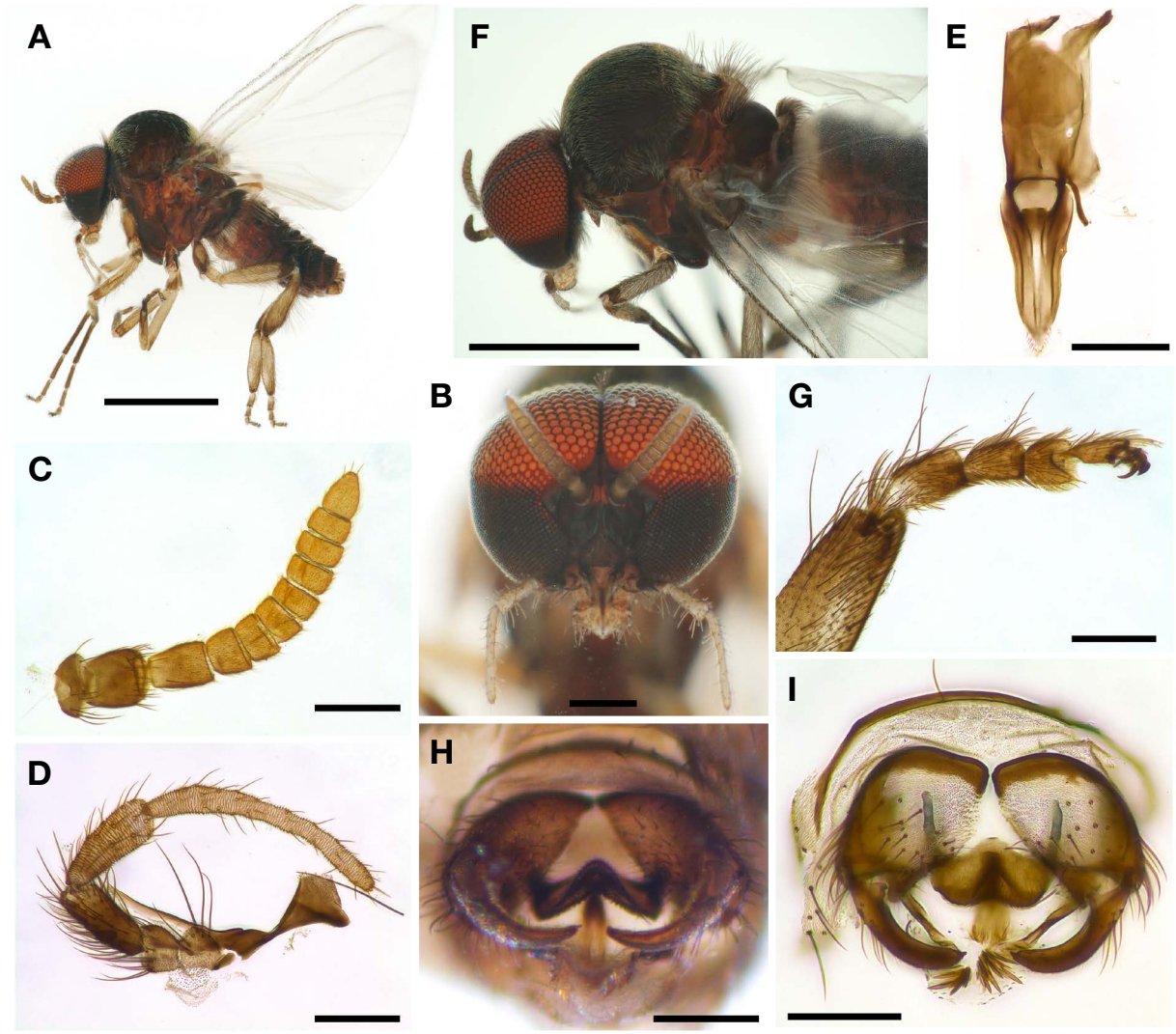

Fig. 2. Male of *Simulium* (*Boreosimulium*) *konoi*. (A) Body, lateral view; (B) Head, frontal view; (C) Antenna; (D) Maxillary palp with lacinia; (E) Cibarium with hypopharynx; (F) Body, dorsolateral view; (G) Hind leg with basitarsus and tarsomeres; (H) Genitalia, end view; (I) Genitalia, ventral view (slide mounted). Scale bars, 1 mm (A, F); 200 μm (B); 100 μm (C–E, G–I).

combined. Pedisulcus distinct. Tarsal claw small without thumb-like lobe. Abdomen brown to dark brown with all hairs brown to dark brown. Genitalia (Fig. 2H, I). Gonostylus slender, tapered to pointed apex, gently curved inwardly, with single small spinule. Ventral plate with well developed median keel, W or inverted V-shaped in ventral view. Median sclerite elongate, thin. Cercus small, rounded. Paramere moderate sized, 8–11 hooks, each with 10–15 short setae.

Pupa (Fig. 3). Body length (Fig. 3A, B): 2.7–3.0 mm (n = 10). Body (Fig. 3B, C) brown to reddish-brown ground color. Gill (Fig. 4B) with 4 filaments, equal in size, 0.8 × as long as pupal body length (2.2–2.5 mm), angle between uppermost and lowermost filaments less than 90°. Cephalic plate and vertex (Fig. 3D) bare, without tubercles. Frons with 3 pairs of short trichomes, simple, less than half the length of facial trichome, only visible in high magnification; face with 1 medium long trichome, simple, about half the length of thoracic trichomes. Thorax (Fig. 3D) bare anteriorly, moderately covered with small tubercles posteriorly; thoracic trichome (Fig. 3E) in 6 pairs, simple (unbranched), long. Abdomen (Fig. 3C). Abdominal tergites III and IV with 4 anteriorly directed spin hooks on posterior margin. Tergites V–IX with spine combs, spines on tergite V smallest, tergite VIII largest. Terminal hooks well developed. Cocoon (Fig. 3A) slipper-shaped, tightly woven, as long as pupa.

Larva (Fig. 4). Body length (Fig. 4A, B): 5.3–5.7 mm (n = 10). Body (Fig. 4A, B) reddish-brown or brownish-grey ground color. Gill histoblast (Fig. 4A, B) 4 filaments.

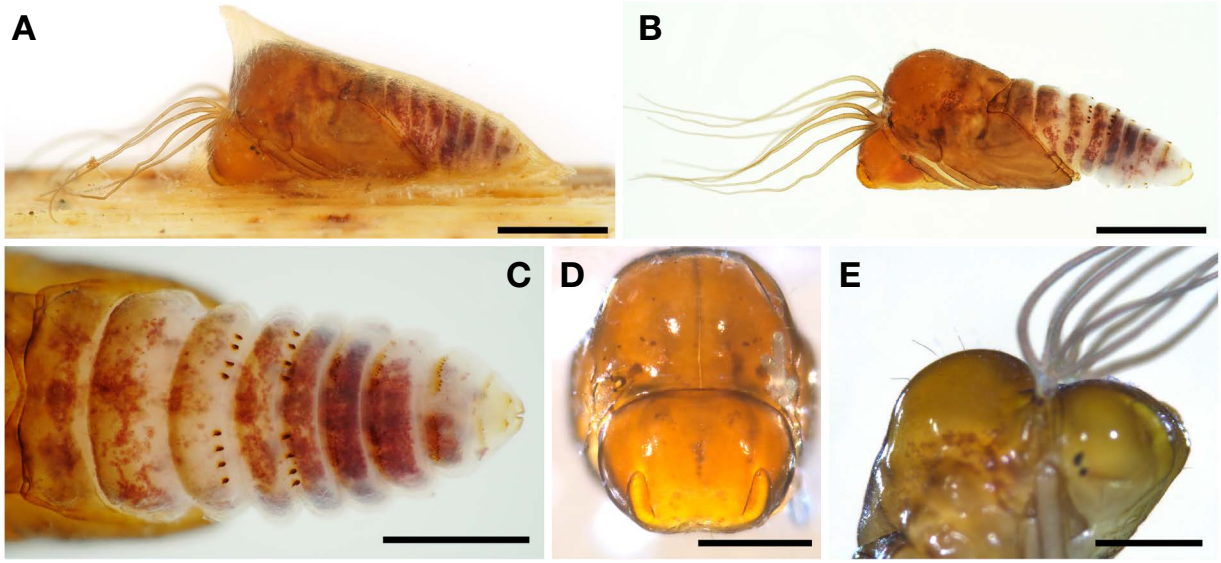

Fig. 3. Pupa of *Simulium* (*Boreosimulium*) *konoi*. (A) Pupa with cocoon, lateral view; (B) Pupa, lateral view; (C) Abdomen, dorsal view; (D) Head and thorax (gill filaments removed), frontal view; (E) Head and thorax, ventrolateral view. Scale bars, 1 mm (A, B); 500 μm (C–E).

Head (Fig. 4C) with head spots positive; anteromedial spots 4, separated, distinct; first and second anterolateral spots obliquely elongated, separated, parallel with each other; posteromedial spots narrow, distinct, 1.3× as long as anteromedial spots; first and second posterolateral spots separated, distinct. Single spot under ocellus. Antenna (Fig. 4D) brown with 3 hyaline bands, 1.2× as long as labral fan stem, proportional ratio of antennal segments 1 : 1.4 : 0.8. Labral fan with 35–38 primary rays. Postgenal cleft (Fig. 4E) not clearly defined, round, as long as wide, laterally with elongate spot. Hypostoma (Fig. 4F) with 9 teeth, lateral and median teeth subequal in length, basally with 3–4 hypostomal setae. Subesophageal ganglion darkly pigmented. Thorax and abdomen densely covered with short branched black setae dorsally and laterally (Fig. 4G, H). Rectal papillae (Fig. 4H, I) of 3 compound lobe with 6–7 secondary lobules. Ventral tubercle absent (Fig. 4I). Posterior proleg (Fig. 4J) with 10–12 hooks (posterior circlet), 60–65 rows.

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 (159 ultimate, 187 penultimate, 72 early instar larvae, 50 pupae (except for rearing), 16♂, 32♀; Gyeonggi-do, Gapyeong-gun, Buk-myeon, Dodae-ri, Myeongjicheon stream, 37°56′07″N, 127°29′18″E, altitude 210 m, 22.v.2019, SK Kim (1 ultimate, 6 penultimate instar larvae).

Distribution. Korea (Gyeonggi-do, new record), China (Liaoning), Japan (Hokkaido, Honshu, Kyushu, Shikoku), Siberia (Far East).

Stream information. Two streams, the only localities where larvae and pupae of *S. konoi* were collected so far, were separated by Mt. Myeongjisan (1,267 m) by a distance of 4.6 km. Both streams were medium to large-sized streams with moderate to rapid flow and stream beds consisting of boulders and rubble, and totally exposed to the sun. 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.

Biological notes. Larvae were collected from trailing vegetation, submerged twigs, and dead leaves. Body colors of fresh larvae were brownish red or brownish grey when fully matured. Specimens were collected along with *Prosimulium kiotoense*, *Simulium japonicum*, *Simulium malyschevi*, *Simulium suzukii*, and *Simulium yamatoense* (the latter was previously misidentified as *S. rufibasis*). All larvae and pupae were collected only twice in May 2019 from two streams, and I failed to collect *S. konoi* in subsequent attempts in other months at the same streams, suggesting *S. konoi* is univoltine as Sato *et al*. (2004) suggested. In Japan as well, larvae and pupae of *S. konoi* were collected only in April and had not been found in other months (Sato *et al*., 2004).

Remarks. Subgenus *Boreosimulium* contains three species-groups: *annulus* species-group where *S. konoi* belongs to, *baffinense* species-group, and *johannseni* species-group (Adler, 2019). The most distinctive characteristic of *S. konoi* is several rows of denticles on the female cibarium as Bentinck (1955) and Sato *et al*. (2004) recognized. Placement of *S. konoi* in the subgenus *Boreosimulium* is somewhat problematic since *S. konoi* possess many characteristics that do not match the diagnosis for *Boreosim-*

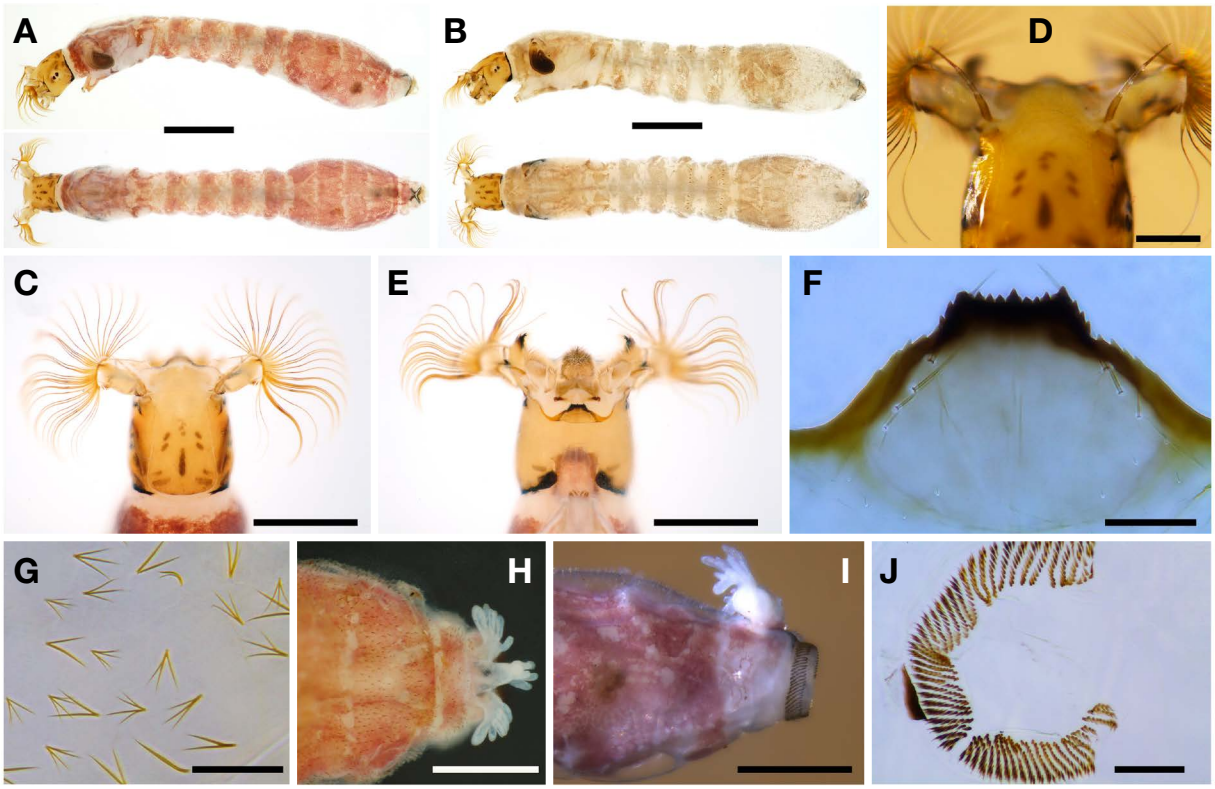

Fig. 4. Larva of *Simulium* (*Boreosimulium*) *konoi*. (A, B) Larva, lateral and dorsal view; (C, D) Head, dorsal view; (E) Head, ventral view; (F) Hypostoma (slide mounted); (G) Abdominal setae (slide mounted); (H) Posterior end of abdomen, dorsal view; (I) Posterior end of abdomen, lateral view; (J) Posterior circlet (slide mounted). Scale bars, 1 mm (A, B); 500 μm (C, E, H, I); 200 μm (D); 100 μm (F, J); 50 μm (G).

ulium, such as denticles on the cibarium of the female, the bi-colored legs of the female, and short black setae on the larval abdomen. Cibarial armature are present in species of *Psilopelmia*, *Psilozia* and *Hemicnetha* (Adler *et al*., 2004), but the shape and position of armature is different in *S. konoi*. Furthermore, chromosomal analysis showed that the chromosomes of *S. konoi* were very different and it did not match the typical chromosomes of *Boreosimulium* (e.g., *annulus* species-group) (Peter H. Adler, pers. comm.). Features of Korean *S. konoi* specimens matched those of Japanese specimens in almost all details, with some degree of geographical variation, such as ventral tubercle on larval abdominal segment IX and shape of larval head spots. Due to the autapomorphy and other characters found in *S. konoi*, the current placement of *S. konoi* in the subgenus *Boreosimulium* or species-group assignment is tentative, and future research is warranted to clarify the placement of the *S. konoi* in the subgenus or species-group.

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