

A new record of the subgenus *Eusimulium* Roubaud from Korea with an updated key to genera and subgenera of Korean black flies (Diptera: Simuliidae)

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Larvae and pupae of *Simulium* (*Eusimulium*) *satsumense* were collected and recorded from Korea for the first time. Detailed descriptions and photographs of both immatures are provided. Currently, 42 species are known from the subgenus *Eusimulium*, which is found globally, mainly in the Holarctic Region, but unknown from Korea. Members of the subgenus *Eusimulium* are morphologically homogeneous in male, female, and pupa, making them extremely difficult to distinguish from one another. *S. (E.) satsumense* can be distinguished from other Korean black flies by the following combination of the characteristics: Pupa: Gill of four slender filaments, dorsalmost filament strongly divergent from other filaments; cocoon slipper shaped without anterodorsal projection; Larva: Head spots distinctly positive; postgenal cleft short, apically rounded or squared; antenna with proximal segment brown; abdominal segments IX with prominent ventral tubercles; and rectal papillae of three simple lobes. In addition to descriptions for larvae and pupae of the species, a key to genera and subgenera of *Simulium* of Korean black flies are also provided.

Keywords: black fly, distribution, *Eusimulium*, Korea, taxonomy

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INTRODUCTION

Although black flies (Diptera: Simuliidae) are nuisance pests of medical and veterinary concern in many parts of the world (Adler *et al.*, 2004), little attention has been given to Korean black flies since no serious medical and veterinary issues have been reported so far, except a single biting case (Kim *et al.*, 2006). Korean black flies were first reported by Bentinck (1955), then Chow (1973), Takaoka (1974), and Yoon & Song (1989; 1990) conducted researches and provided lists and new species from Korea. Kim (2015a; 2015b; 2020a; 2020b) conducted extensive researches on Korean black flies and reported a total of four genera with six subgenera, and 24 species including single informal species (i.e., *Simulium* (*Gomphostilbia*) sp. J-12 Bentinck).

The subgenus *Eusimulium* Roubaud was first reported from Korea by Takaoka (1974) by describing new species, *Simulium* (*Eusimulium*) *subcostatum chejuense*, currently treated as *Simulium* (*Nevermannia*) *chejuense*. Then Yoon & Song (1990) revised the subgenus *Eusimulium*, which is now correctly treated as *Nevermannia*. Hence, there is

no record showing occurrence of *Eusimulium* in Korea to date. Currently, 42 species of the subgenus *Eusimulium* are recorded from the world, mainly in the Holarctic Region (Adler, 2019). Members of the subgenus *Eusimulium* are morphologically homogeneous in male, female and pupa (Adler *et al.*, 2004; Takaoka & Shrestha, 2010), making them extremely difficult to distinguish from one another. Though three species of the subgenus are recorded from Japan, *viz.* *Simulium* (*Eusimulium*) *aureum* Fries, *S. (E.) erimoense* (Ono), and *S. (E.) satsumense* Takaoka, one of the Japanese records, *S. aureum*, is in doubt (Adler, 2019).

In this study, detailed descriptions of larvae and pupae of *S. (E.) satsumense* and habitat information is provided for the first time in Korea. Additionally, an updated key to genera and subgenera of the genus *Simulium* of Korean black flies is provided.

MATERIALS AND METHODS

The classification and morphological terminology used

in the text follow those of Adler *et al.* (2004). Larvae and pupae 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). Heads and end of abdomens of ultimate instar larvae were removed from the body with two sets of fine forceps in 80% EtOH. Detached heads and abdomens were placed in a vial of 10% potassium hydroxide (KOH) and boiled for 10 min to facilitate examination of larval hypostoma and hooks of posterior proleg with phase-contrast microscopy. Cleared heads and part of abdomens then were neutralized by placing them in a vial containing 3% acetic EtOH for three minutes. Larval hypostoma and posterior prolegs 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 finger nail 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 macrocope 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.

Key to genera and subgenera of *Simulium* of Korean black flies

Pupa

1. Cocoon shoe, slipper or boot shaped 3
Cocoon lacking definite shape (amorphous) 2
2. Gill of 16 filaments in 3 short main trunk
..... *Prosimulium*
Gill of 16 filaments in 3 distinctly long and thick main trunk *Twinnia*
3. Gill of about 150–200 filaments arising from 3 main trunks *Helodon*
Gill of less than 16 filaments (Genus *Simulium*) 4
4. Gill of 4 filaments 5
Gill of 6 or more filaments 7
5. Dorsalmost filament strongly divergent from other filaments, angle between them more than 90°
..... *Eusimulium*
Dorsalmost filament not strongly divergent from other filaments, angle between them much less than 90° 6
6. Gill filaments longer than pupal body length. Gill fil-

aments close together, the angle between dorsalmost filament and ventralmost filament about 45°
..... *Nevermannia* (part)

Gill filaments shorter than pupal body length. Gill filaments separated, the angle between dorsalmost filament and ventralmost filament about 80°
..... *Boreosimulium*

7. Gill with ventralmost trunk distinctly longer than other trunk *Nevermannia* (part)
Gill with all trunks subequal to equal in length 8
8. Head with frontal trichomes 3 pairs *Gomphostilbia*
Head with frontal trichomes 2 pairs 9
9. Gill filament broad, thin-walled, ventralmost trunk distinctly longer than other trunk *Wilhelmia*
Gill filament slender, thick-walled, all trunks subequal to equal in length *Simulium*

Larva

1. Labral fan absent *Twinnia*
Labral fan present 2
2. Antenna with proximal and medial article transparent, colorless, contrasting with dark brown distal article 3
Antenna with proximal, medial or both article opaque, pigmented (Genus *Simulium*) 4
3. Prothoracic proleg with lateral sclerite a narrow bar lying parallel to base of hooks, extended at most one third distance to base of apical article *Helodon*
Prothoracic proleg with lateral sclerite better developed, extended half or more distance to base of apical article *Prosimulium*
4. Abdomen with ventral tubercle present 5
Abdomen with ventral tubercle absent 8
5. Abdomen covered with black spinous setae dorsally and laterally 6
Abdomen without black spinous setae dorsally and laterally 7
6. Antenna with 3 hyaline bands; Gill histoblast of 4 filaments *Boreosimulium*
Antenna without hyaline bands; Gill histoblast of 6 filaments *Gomphostilbia*
7. Gill histoblast with proximal stem bent perpendicularly *Eusimulium*
Gill histoblast with proximal stem bent roundly
..... *Nevermannia*
8. Rectal papillae of 3 simple lobes, postgenal cleft deep *Wilhelmia*
Rectal papillae of 3 compound lobes, if 3 simple lobes then postgenal cleft shallow *Simulium*

TAXONOMIC ACCOUNTS

Family Simuliidae Newman, 1834

Subfamily Simuliinae Newman, 1834

Tribe Simuliini Newman, 1834

Genus *Simulium* Latreille, 1802

Simulium Latreille, 1802: 426 (as genus) Type species: *Oestrus columbacensis* Scopoli, 1780: 133, by monotypy.

Subgenus *Eusimulium* Roubaud, 1906

Eusimulium Roubaud, 1906: 521 (as subgenus of *Simulium*) Type species: *Simulia aurea* Fires, 1824: 16, by monotypy.

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

Pupa. Gill of 4 slender filaments; dorsalmost filament strongly divergent from other filaments. Cocoon slipper shaped, without anterodorsal projection.

Larva. Antenna without hyaline bands. Hypostoma with lateral and median teeth rather prominent, extended anteriorly to about same level. Postgenal cleft extended about one third or less distance to hypostomal groove, with anterior margin rounded, squared, or biarcuate. Abdominal segment IX with prominent ventral tubercles. Rectal papillae of 3 simple lobes.

Simulium (Eusimulium) satsumense

Takaoka, 1976 (Figs. 1–3)

Simulium (Eusimulium) satsumense Takaoka, 1976: 171.

Type locality: Nansei Island, Japan.

Simulium (Eusimulium) satsumense: Takaoka, 1977: 219 (Japanese key); Ono, 1980: 350 (remark); Takaoka, 2002 (Japanese key); Takaoka & Shrestha, 2010 (remark); Saito, 2015: 15 (Japanese list and names); Adler, 2019: 45 (world checklist).

Diagnosis. This species can be distinguished from other Korean black flies by the following combination of characteristics: pupa with cocoon slipper-shaped; gill of 4 slender filaments, dorsalmost filament strongly divergent from other filaments, longer than pupal body; head and thorax densely covered with numerous minute tubercles; abdominal segments VI–VIII with small stout spines; terminal hook small; larva with head spot distinctly positive, anteromedial spots as long as posteromedial spots; antenna with proximal article brown; postgenal cleft small, apically rounded, squared or dull pointed; gill histoblast of 4 filaments, inverted P-shaped; ventral tubercle prominent, conical shaped; rectal papil-

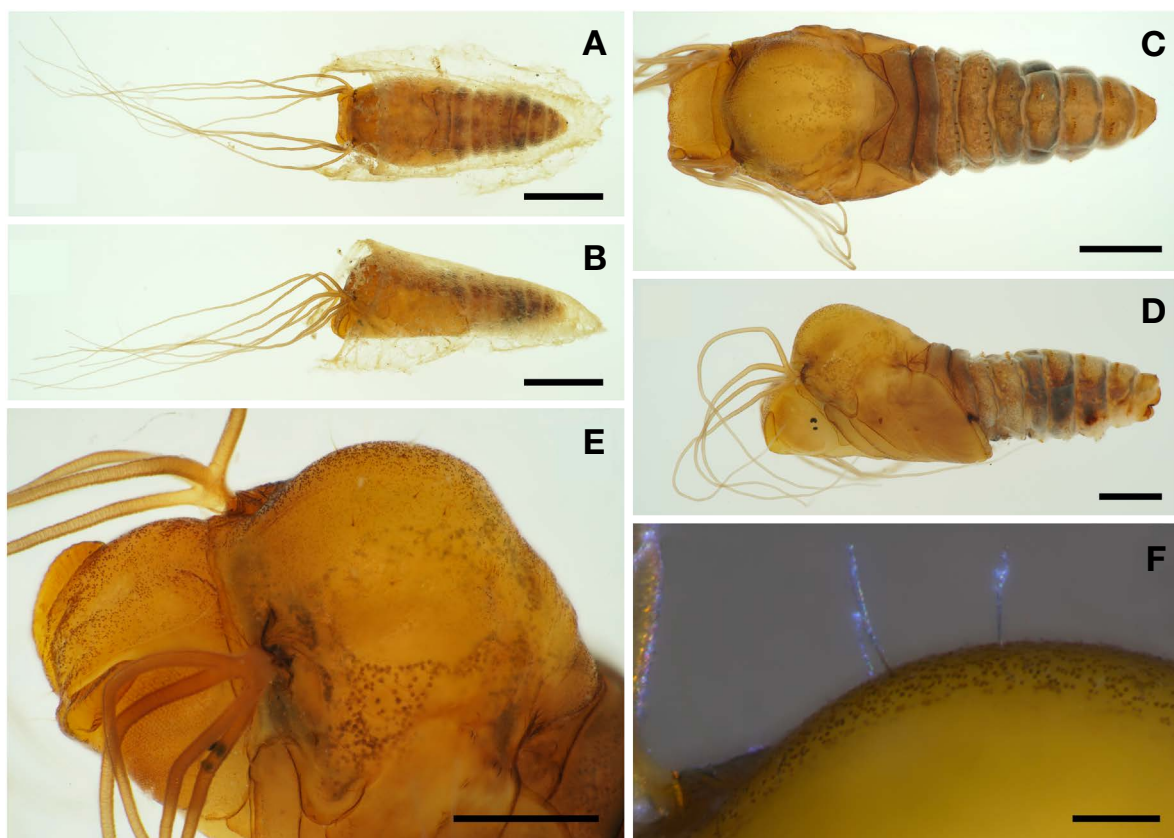


Fig. 1. Pupa of *Simulium (Eusimulium) satsumense* Takaoka. (A) Pupa with cocoon, dorsal view; (B) Pupa with cocoon, anterolateral view; (C) Pupa, cocoon removed, dorsal view; (D) Pupa, cocoon removed, lateral view; (E) Pupa, cocoon removed, head and thorax, oblique view; (F) Thoracic trichomes, lateral view. Scale bars, 1 mm (A, B); 0.5 mm (C–E); 100 μ m (F).

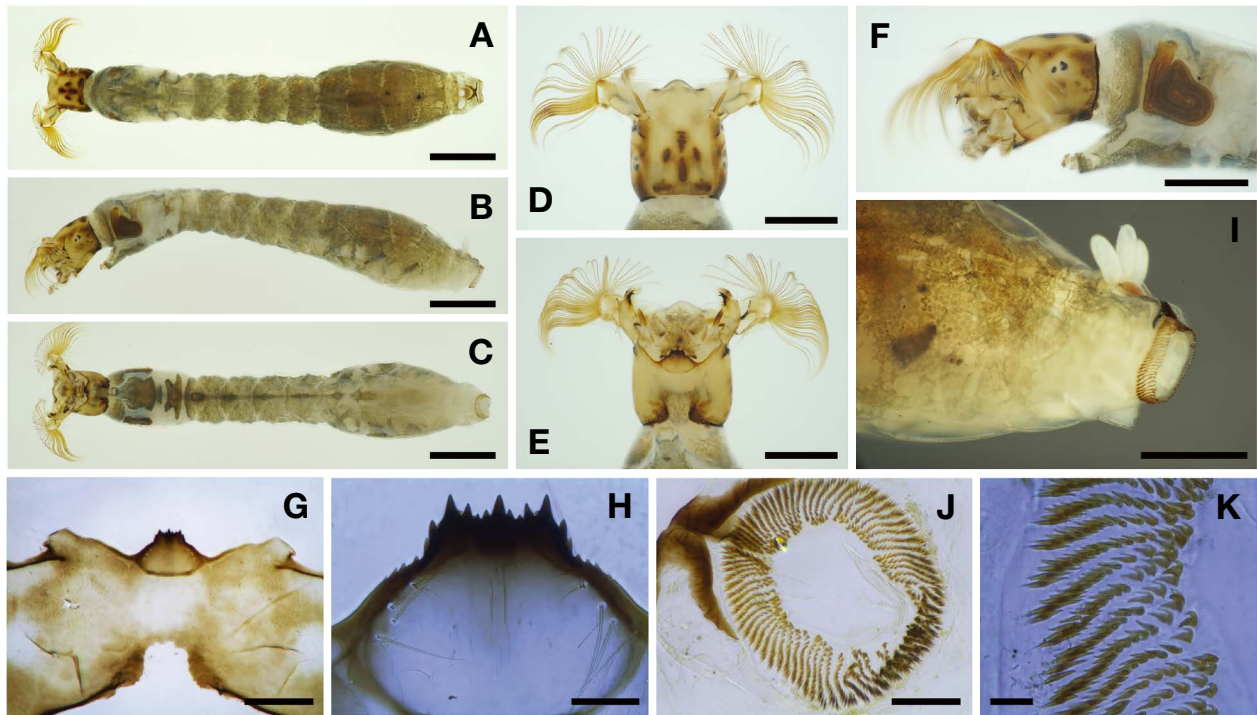


Fig. 2. Larva of *Simulium (Eusimulium) satsumense* Takaoka. (A) Larva, dorsal view; (B) Larva, lateral view; (C) Larva, ventral view; (D) Head, dorsal view; (E) Head, ventral view; (F) Head and anterior portion of thorax, lateral view; (G) Postgenal cleft and hypostoma, (slide mounted); (H) Hypostoma (slide mounted); (I) Posterior end of abdomen, lateral view; (J, K) Posterior circler (slide mounted). Scale bars, 1mm (A–C); 0.5 mm (D–F, I); 200 μ m (G); 100 μ m (J); 50 μ m (H); 20 μ m (K).

lae of 3 simple lobes.

Description. Pupa (Fig. 1). Body length: 3.2–4.0 mm ($n=9$). Body brown to reddish brown ground color. Gill (Fig. 1A, B, D) of 4 slender filaments in 2 + 2 arrangement, longer than pupal body length (4.5–4.9 mm); upper pair with short stalk, lower pair sessile; dorsalmost filament directed upward then perpendicularly bending forward, angle between uppermost and lowermost filaments about 90°; upper filament of lower pair (3rd filament from dorsal to ventral) directed outwardly then forwardly. Cephalic plate and vertex (Fig. 1E) densely covered with small tubercles. Frons with 2 pairs of medium long trichomes (0.08–0.1 mm), simple, as long as or little shorter than facial trichome; face with 1 medium long trichome (0.1–0.12 mm), simple. Thorax (Fig. 1F) densely covered with small tubercles; thoracic trichome (Fig. 1) in 6 pairs, simple (unbranched), long (0.12–0.17 mm). Abdomen (Fig. 1C). Abdominal tergites III and IV with 4 anteriorly directed spin hooks on posterior margin. Tergites VI–VIII with small spines or spine combs, spines on tergite VI the smallest, tergite VIII the largest. Tergite IX with small spine combs. Terminal hooks distinct, small. Cocoon (Fig. 1A, B) slipper shaped, tightly woven, longer than pupa, completely covered pupal body (3.3–4.4 mm).

Larva (Fig. 2). Body length 6.3–6.7 mm ($n=9$). Body (Fig. 2A–C) grayish brown ground color. Gill histoblast (Fig. 2F) 4 filaments, proximal portion of the uppermost filament bent perpendicularly, inverted P-shaped. Head (Fig. 2D, E) with head spots distinctly positive; antero-medial spots 6–7, close together, lying in straight line, as long as posteromedial spots, distinct; first and second anterolateral spots obliquely elongated, close together, distinct; posteromedial spots narrow, lying in straight line, distinct; first and second posterolateral spots separated, distinct. Single spot under ocellus, two spots posterolaterally. Antenna (Fig. 2D) pale yellow with proximal and distal article brown, 1.25X as long as labral fan stem, proportional ratio of antennal segments with 1 : 1.3–1.4 : 0.76–0.9. Labral fan with 53–55 primary rays. Postgenal cleft (Fig. 2G) small, apically rounded, squared or dull pointed, sometime apical portion not well defined, little wider than long, laterally with elongate spot and round spot. Hypostoma (Fig. 2H) with 9 teeth, lateral and median teeth prominent, subequal in length, basally with 4–5 hypostomal setae. Subesophageal ganglion darkly pigmented. Thorax and abdomen almost bare, with very minute whitish setae posterodorsally. Rectal papillae (Fig. 2I) of 3 simple lobes. Ventral tubercle prominent, conical shaped (Fig. 2I). Posterior

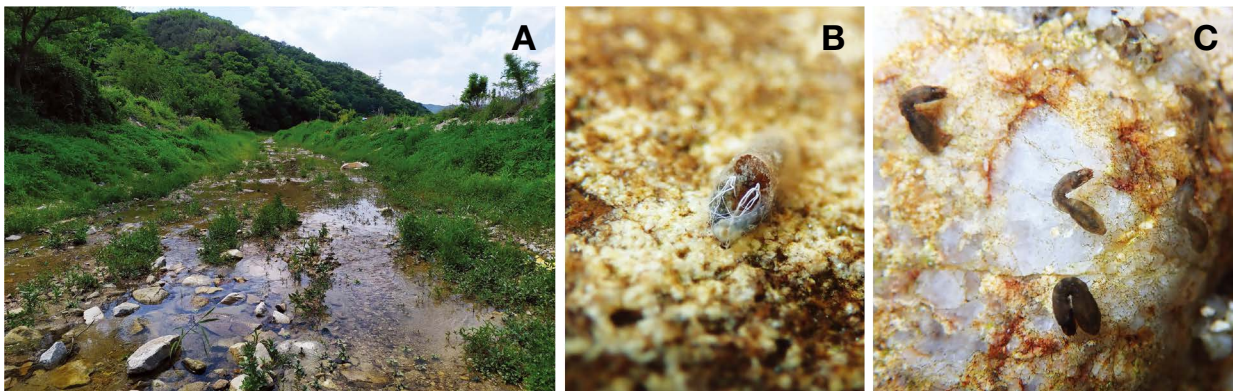


Fig. 3. (A) A stream where larvae and pupae of *Simulium (Eusimulium) satsumense* Takaoka were collected; (B) Pupa, attached to small stone under the water; (C) Larvae, attached to small stone under the water.

proleg (Fig. 2J, K) with 12–14 hooks (posterior circlet) in 68–72 rows.

Specimens examined. Korea: Gyeonggi-do, Yeosu-si, Gangcheon-myeon, Dojeon-ri, 649-1. 37°20'42"N, 127°44'54"E, altitude 149 m. 31.v.2019, SK Kim (15 pupae, 9 ultimate, 7 penultimate, 5 early instar larvae).

Distribution. Korea (new record), China (Liaoning), Japan (Nansei Islands).

Stream information. The stream (Fig. 3A), the only locality where the larvae and pupae of *S. (E.) satsumense* were collected so far, adjacent to agricultural fields and small residence area, was medium-sized stream with less than 10 m wide and 15 cm deep with very slow flowing with relatively clean water. The streambed was mainly consisted of small stones and rubble with sandy bottom along with many submerged broad-leaved vegetations where the larvae and pupae were attached themselves to those various substrates. The stream was totally exposed to the sun with partly shaded by concrete overhead bridge.

Remarks. All members of the subgenus are multivoltine and spend a portion of the winter in the egg stage (Adler *et al.*, 2004). Pupa of *S. (E.) satsumense* is morphologically very similar to that of *S. (E.) jomsomense* Takaoka & Shrestha from Nepal in many ways, making it impractical to distinguish them from one another. They can be separated by female ovipositor and male legs characteristics (Takaoka & Shrestha, 2010).

According to Takaoka (1976), larvae and pupae of the species were collected from trailing grasses in a very sluggish ditch for rice fields on 15 Jan. 1974 in Nansei Island (= Ryukyu Island, Okinawa), with normal temperature of January around 14.7°C, assuming its year-round occurrence in Japan, at least in southern part of the Japan. For Korean species, early to ultimate instar larvae along with many pupae were collected only once from a single stream with very slow flowing on 31 May 2019 with

average temperature of 16°C. It is premature to assume its multiple occurrences in Korea. More detailed study is warranted to determine its voltinism in Korea. Larvae and pupae were mainly attached themselves to small stones, rubbles or submerged vegetation (Fig. 3B, C) and collected along with other black flies such as, *S. (S.) arakawae*, *S. (S.) bidentatum*, *S. (S.) iwataense*, *S. (S.) suzukii*, and *S. (N.) uchidai*. Among the collected specimens, two penultimate instar larvae were patently infected with an unidentified microsporidian fungus.

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