



RESEARCH ARTICLE

A new species and four newly recorded species of black flies (Diptera: Simuliidae) from Bhutan

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ABSTRACT

Simulium (Simulium) thimphuense sp. nov. is described from a pupa and a mature larva in Bhutan. This new species is placed in the *S. multistriatum* species-group, and is characterized by the pupal gill with eight thread-like filaments divergent basally at an acute angle when viewed laterally and the cocoon slipper-shaped with several small openings anterolaterally. Four species of *Simulium (Simulium)* are newly recorded from Bhutan: *S. barraudi* Puri in the *S. multistriatum* species-group, *S. nodosum* Puri in the *S. nobile* species-group, *S. chiangmaiense* Takaoka & Suzuki in the *S. striatum* species-group and *S. himalayense* Puri in the *S. variegatum* species-group. Our study increases the number of black fly species known from Bhutan from 18 to 23.

Keywords: *Simulium*, blackfly, taxonomy, new species, Bhutan.

INTRODUCTION

The fauna of black flies in Bhutan is represented by 18 species, which are classified in four subgenera of the genus *Simulium* Latreille: two species in *Gomphostilbia* Enderlein, three species in *Montisimulium* Rubtsov, four species in *Nevermannia* Enderlein and nine species in *Simulium* Latreille (Lewis, 1974; Datta, 1991; Takaoka & Somboon, 2008). Of the nine species in the subgenus *Simulium*, one is in the *S. griseifrons* species-group, one is in the *S. indicum* species-group, two are in the *S. multistriatum* species-group, three are in the *S. striatum* species-group and two are in the *S. tuberosum* species-group.

We examined adults reared from pupae, pupal exuviae and larvae collected in 2007, 2016 and 2017 from Bhutan and found one new species and four newly recorded species. The new species is described based on a pupa and a mature larva and is placed in the *S. multistriatum* species-group. The four newly recorded species are each in the *S. multistriatum* species-group, *S. nobile* species-group, *S. striatum* species-group, and *S. variegatum* species-group.

Methods of morphological observation, terms of features, descriptions, and illustrations, followed those of Takaoka (2003) and partially those of Adler *et al.* (2004).

The holotype and paratype of the new species are deposited in the Entomology Section of the Queen Sirikit Botanic Garden, Chiang Mai, Thailand.

Simulium thimphuense sp. nov.

Pupa (n=1). Body length 2.5 mm. **Head.** Integument light

ochreous, densely and uniformly covered with small tubercles (Figure 1A) except most of antennal sheath bare; frons with two unbranched slender short trichomes (Figure 1B) on each side; face with one unbranched slender long trichome (Figure 1C) on each side. **Thorax.** Integument light ochreous, densely and uniformly covered with small tubercles; thorax with three bifid or trifid long mediodorsal trichomes (Figure 1D), two unbranched anterolateral trichomes (anterior trichome medium-long and posterior one long) (Figure 1E), one unbranched medium-long mediolateral trichome (Figure 1F), and three unbranched ventrolateral trichomes (one medium-long and two others long) (Figure 1G), on each side. Gill (Figure 1H, I) with eight slender thread-like short filaments arranged as 2+(2+2)+2 from dorsal to ventral; middle two pairs sharing short stalk, and lying side by side; all pairs short-stalked; common basal stalk short; stalk of dorsal pair forming an angle of 40–80 degrees against stalk of ventral pair when viewed laterally; filaments decreasing in length and thickness from dorsal to ventral, with upper filament of dorsal pair longest (about 1.5 mm) and lower filament of ventral pair shortest (about 0.8 mm); relative basal thickness of eight filaments from dorsal to ventral 2.0:1.6:1.4:1.4:1.2:1.2:1.0:1.0; all filaments dark brown, tapered toward apex (though nearly parallel-sided from base for short distance), with distinct annular ridges and furrows, though annular ridges becoming less distinct apically, and densely covered with minute tubercles. **Abdomen.** Dorsally, all segments unpigmented and without microtubercles except segment 1 yellowish; segment 1 with one hair-like seta, though broken, on each side; segment 2 with one hair-like

short seta, two slender minute setae, and three stout minute setae (Figure 1J), on each side; segments 3 and 4 each with four distinct hooked spines and one unbranched short seta on each side; segment 8 with distinct spine-combs and comb-like groups of microspines in transverse rows on each side; segments 5, 6, 7 and 9 lacking spine-combs; segment 9 without terminal hooks. Ventrally, segments 3–9 unpigmented, each (except segment 9) with comb-like groups of microspines; segment 4 with few unbranched slender minute setae on each side; segment 5 with a pair of bifid stout hooks submedially and few unbranched short setae on each side; segments 6 and 7 each with a pair of bifid inner and unbranched outer stout hooks somewhat separated from each other, and few unbranched short setae on each side. **Cocoon** (Figure 1K). Slipper-shaped, roughly woven, dark brown, with several small open spaces near anterior margin on each side, and not extended ventrolaterally; individual threads visible; 2.6 mm long by 1.2 mm wide.

Mature larva (n=1). Body length 4.5 mm. Body dark gray. **Head.** Cephalic apotome medium brown with narrow portions along both lateral margins and along posterior margin dark brown, with indistinct head spots; lateral surface of head capsule medium brown except eyespot region light ochreous and narrow portion above eyespot region dark brown, and one small dark spot below eyespot region and one or two small dark spots near posterior margin somewhat distinct; ventral surface of head capsule medium to dark brown except narrow portion along posterior margin of hypostoma light ochreous. Labral fan with 48 primary rays. Antenna composed of three articles and apical sensillum, slightly longer than stem of labral fan; length ratio of three articles (from base to tip) 1.0:1.2:0.6. Mandible (Figure 2A) with mandibular serration composed of two teeth (one medium-sized, one small); main tooth at obtuse angle against mandible on apical side; comb-teeth gradually reduced in length from first to third; supernumerary serrations absent. Hypostoma (Figure 2B) with nine anterior teeth, of which median tooth slightly longer than corner teeth, followed by three intermediate teeth on each side; lateral margins serrate apically; six or seven hypostomal bristles divergent posteriorly from lateral border on each side. Postgenal cleft (Figure 2C) large, mitre-shaped; sheath of subesophageal ganglion moderately pigmented. Cervical sclerites on each side composed of light brown elliptical piece, not fused to occiput. **Thorax and Abdomen.** Histoblast of pharate pupal gill (Figure 2D) with eight thread-like slender filaments arranged in four pairs, of which two middle pairs overlapped. Thoracic and abdominal cuticle almost bare except last segment moderately covered with short colorless setae on each side of anal sclerite. Rectal scales distinct. Rectal organ of three lobes, each with 13 to 14 finger-like secondary lobules. Anal sclerite X-shaped, with short broad anterior arms about 0.6 times length of posterior arms and with forked apices. Last abdominal segment not bulged laterally and lacking ventral papillae. Posterior circling with 86 rows of hooklets with up to 16 hooklets per row.

Type specimens. Holotype. Pupa, collected from grass in a fast running stream (partially shaded, elevation 2,391 m, 27°31'09" N, 89°38'40" E), at Thimphu District, the capital of Bhutan, 17-IX-2016, by P. Somboon. Paratype. One mature larva, same data and date as for the holotype.

Etimology. The species *thimphuense* refers to the locality, Thimphu, where this new species was collected.

Remarks. *Simulium thimphuense* sp. nov. is placed in the *S. multistriatum* species-group of the subgenus *Simulium*, defined by Takaoka and Davies (1996), by having a com-

ination of the pupal gill with eight filaments (Figure 1H, I) and pupal abdomen without terminal hooks.

This new species is distinguished from all 34 species of this species-group by the pupal gill with an acute angle between the stalks of the dorsal and ventral pairs of filaments when viewed laterally (Figure 1H, I) and cocoon slipper-shaped, with several small open spaces anterolaterally (Figure 1K). The known species of this species-group have the gill with a right or obtuse angle between the stalks of the dorsal and ventral pairs of filaments, and the cocoon slipper-shaped, with or without a relatively large open space, or shoe-shaped (Takaoka, 2017).

***Simulium barraudi* Puri, 1932**

Simulium (Simulium) barraudi Puri, 1932b: 1130–1132 (Female, male and pupa); Takaoka et al., 2020 (Male and pupa).

Simulium sp. B Lewis, 1964: 293–294 (Female).

Specimen examined. One male reared from a pupa, collected from a moderately flowing stream (width 2 m, 22.0°C, exposed to the sun, elevation 2,570 m, 27°32'20" N, 90°48'26" E), Dungmithang, Bumthang District, Bhutan, 5-IX-2007, by P. Somboon.

Distribution. Bhutan (**New record**), India, Myanmar, Nepal, Pakistan, Tibet.

Remarks. This species was originally described from India by Puri (1932b). It belongs to the *S. multistriatum* species-group (Adler, 2020). The male and pupa of this species were redescribed by Takaoka et al. (2020).

The male of this species is characterized by the upper-eye (large) facets in 19 vertical columns and 19 horizontal rows, scutum with fine yellow hairs, mid-femur entirely whitish yellow, ventral plate lacking ridges or teeth on the posterior surface and style broadest near its apex when viewed ventrolaterally.

The male examined is in good agreement with that in the redescription by Takaoka et al. (2020).

This species is recorded for the first time from Bhutan.

***Simulium nodosum* Puri, 1933**

Simulium (Simulium) nodosum Puri, 1933: 813–816 (Female, pupa and larva); Lewis, 1974: 34–37 (Female, male, pupa and larva).

Specimens examined. Two males reared from pupae, and 11 pupae collected from grasses in a small slow running stream (exposed to the sun, elevation 220 m, 26°51'09" N, 90°31'59" E), at Umling, Sarpang District, Bhutan, 31-X-2017, by P. Somboon.

Distribution. Bhutan (**New record**), China (Fujian, Hainan, Hong Kong, Yunnan), India, Myanmar, Taiwan, Thailand, Tibet, and Vietnam.

Remarks. This species was originally described from India by Puri (1933). It belongs to the *S. nobile* species-group (Adler, 2020).

This species is characterized by the pupal gill with three inflated tubal filaments. The males and pupae examined agree with those in the original description.

This species is recorded for the first time from Bhutan.

***Simulium chiangmaiense* Takaoka & Suzuki, 1984**

Simulium (Simulium) chiangmaiense Takaoka & Suzuki, 1984: 38–39 (Female, male, pupa); Takaoka et al., 2018: 285–288 (Pupa).

Specimen examined. One pupa collected from the same stream, from where *S. nodosum* was collected, at Umling, Sarpang District, Bhutan, 31-X-2017, by P. Somboon.

Distribution. Bhutan (**New record**), China (Yunnan), Myanmar, Thailand, and Vietnam.

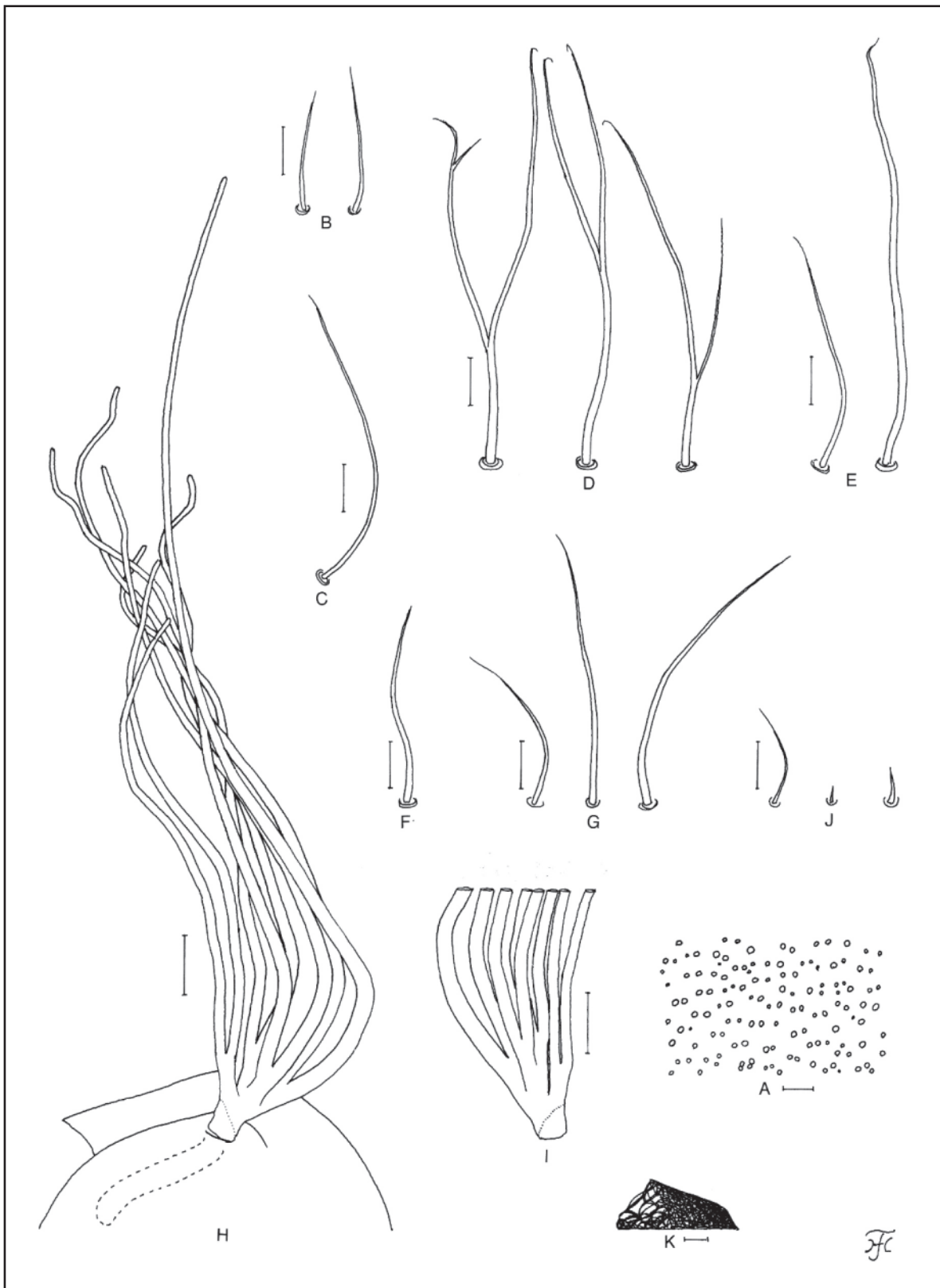


Figure 1. Pupa of *Simulium thimphuense* sp. nov. (A) Tubercles on frons. (B) Trichomes on frons. (C) Trichome on face. (D)–(G) Thoracic trichomes (D, mediodorsal; E, anterolateral; F, mediolateral; G, ventrolateral). (H) Anterior part of thorax and gill filaments (left side; lateral view). (I) Basal portion of gill filaments (right side; lateral view). (J) Hair-like seta, slender minute seta and stout minute seta on dorsum of abdominal segment 2. (K) Cocoon (lateral view). Scale bars. 0.5 mm for K; 0.1 mm for H and I; 0.02 mm for B–G and J; 0.01 mm for A.

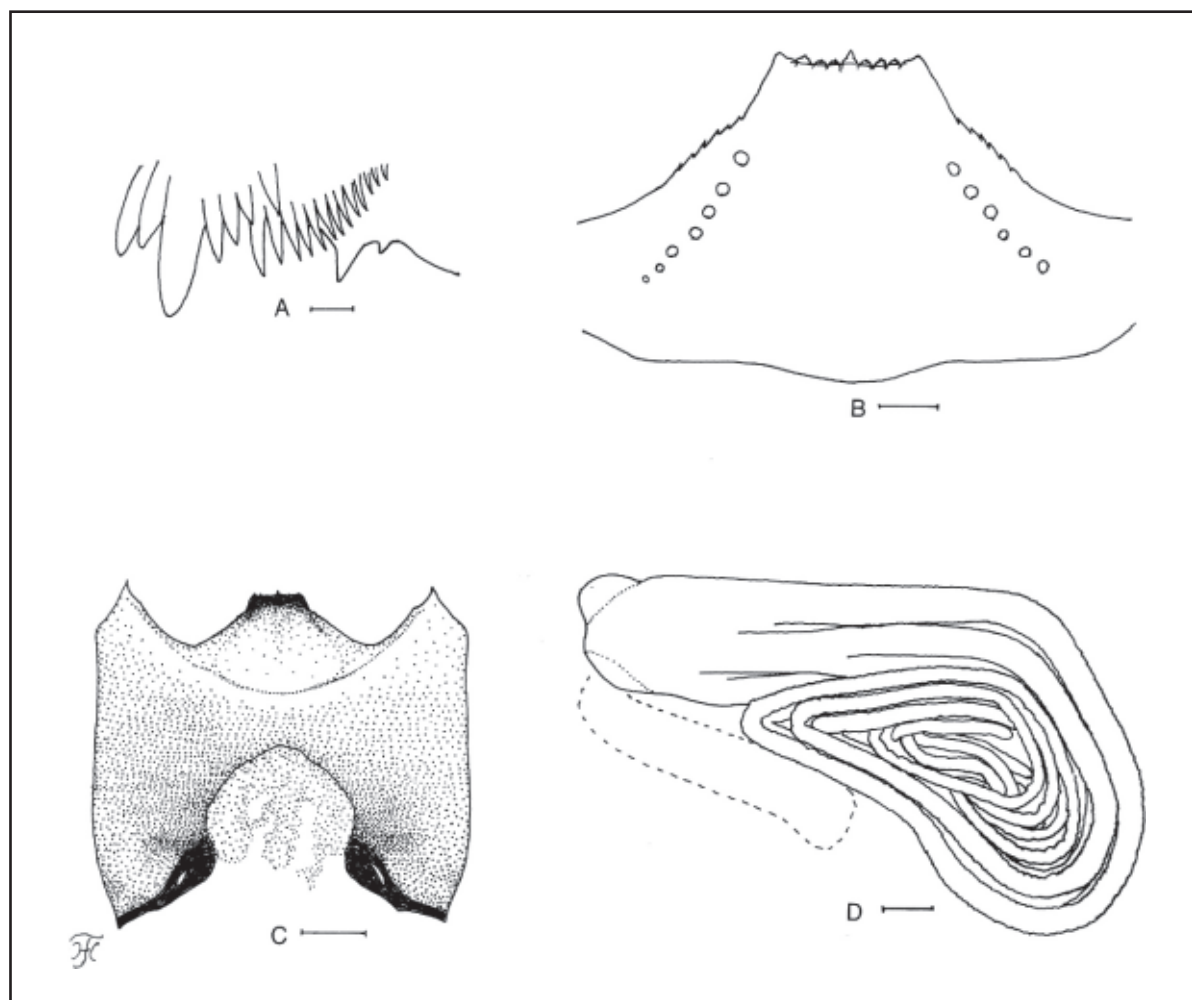


Figure 2. Mature larva of *Simulium thimphuense* sp. nov. (A) Mandible. (B) Hypostoma. (C) Head capsule (ventral view). (D) Histoblast of pupal gill filaments. Scale bars. 0.1 mm for C; 0.04 mm for D; 0.03 mm for B; 0.01 mm for A.

Remarks. This species was originally described from Thailand by Takaoka and Suzuki (1984). It belongs to the *S. striatum* species-group (Adler, 2020).

The pupa of this species is characterized by the head and thoracic integument densely covered with relatively large tubercles having secondary projections, and gill with eight filaments, of which six are relatively thicker than the remaining two filaments.

The pupa examined agrees with that in the original description. The pupa of *S. kapuri* in the *S. striatum* species-group from Assam, India, has the gill with eight filaments but differs from that of *S. Chiangmaiense* by lacking terminal hooks (Datta, 1975).

This species is recorded for the first time from Bhutan.

***Simulium himalayense* Puri, 1932**

Simulium (Simulium) himalayense Puri, 1932a: 885–890 (Female, male and pupa); Datta, 1974: 21 (Larva).

Specimens examined. One female reared from a pupa, collected from grass in a fast running stream (elevation 2,307 m, 27°23'07" N, 89°25'02" E), above Bonde, at Paro District, Bhutan, 25-X-2017, by P. Somboon; one female reared from a pupa, collected from grass in a small irrigation ditch in rice fields (elevation 1,298 m, 27°31'18" N, 89°52'15" E), at Punakha District, Bhutan, 24-X-2017, by P. Somboon; two pupae collected from grasses in a narrow concrete ditch (elevation 2,894 m, 27°29'18" N, 89°43'26" E) at Honsa checkpoint, Thimphu District, Bhutan, 18-IX-2016, by P. Somboon.

Distribution. Bhutan (**New record**), India, Myanmar, Nepal, Pakistan, Tibet.

Remarks. This species was originally described from India by Puri (1932a). It belongs to the *S. variegatum* species-group (Adler, 2020).

This species is characterized by yellowish female femora, integument of the pupal head and thorax moderately covered with tubercles, and pupal gill with six slender filaments divergent basally at an obtuse angle when viewed laterally.

The females and pupae examined agree with those in the original description.

This species is recorded for the first time from Bhutan.

Conflict of Interest:

The authors declare that they have no conflict of interest.

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