



A new black fly species of the *Simulium* (*Gomphostilbia*) *epistum* species-group (Diptera: Simuliidae) from Thailand



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ABSTRACT

A new species of black fly, *Simulium* (*Gomphostilbia*) *isanense*, is described based on females, males, pupae and mature larvae from Thailand. This new species is placed in the *Simulium epistum* species-group of the subgenus *Gomphostilbia* Enderlein. It is characterized by the pupal gill with eight filaments arranged as 3 + 3 + 2 from dorsal to ventral, of which an inner filament of the ventral pair is slightly longer than its counter filament. Taxonomic notes are provided to distinguish this new species from *S. (G.) angulistylum* Takaoka & Davies from Peninsular Malaysia, and three other related species. The difference between this new species and *S. (G.) angulistylum* is supported by genetic distances using the mitochondrial COI gene.

1. Introduction

The *Simulium epistum* species-group is one of 15 species-groups of the subgenus *Gomphostilbia*, the second largest of the 37 subgenera of the genus *Simulium* Latreille (Adler and Crosskey, 2017). This species-group consists of 30 species and is distributed only in the Oriental Region (Adler and Crosskey, 2017; Takaoka, 2012). In this species-group, little is known about the biting habits and other biological aspects, although *S. (Gomphostilbia) asakoeae* Takaoka & Davies of the *S. asakoeae* species-group is a vector of an unknown filaria parasite in northern Thailand (Fukuda et al., 2003; Ishii et al., 2008).

In Thailand, the *S. epistum* species-group is represented by five species, *S. (G.) adleri* Jitklang & Kuvangkadilok, *S. (G.) angulistylum* Takaoka & Davies, *S. (G.) cheongi* Takaoka & Davies, *S. (G.) datfaense* Takaoka & Otsuka, and *S. (G.) otsukai* Takaoka & Choochote (Takaoka and Davies, 1995; Kuvangkadilok and Takaoka, 2000; Jitklang and Kuvangkadilok, 2007; Takaoka et al., 2009). Pramual and Kuvangkadilok (2012) reported that *S. (G.) angulistylum* is a species complex consisting of three cytoforms in Thailand.

In a recent survey of pupae and larvae of black flies in Surin Province in northeastern Thailand, we collected one undescribed species of the *S. epistum* species-group, which is similar to *S. (G.) angulistylum* in having the male style wide and abruptly bent inward, a character differing from those of the four other Thai species of the same group.

The DNA sequence-based analysis using the COI gene supports the difference between this undescribed species and *S. (G.) angulistylum*.

This species is here described as new based on females, males, pupae and larvae.

2. Materials and methods

The methods of collection, description and illustration, and terms for morphological features follow those of Takaoka (2003) and partially those of Adler et al. (2004). The holotype and paratypes are deposited at the Entomology Section, Queen Sirikit Botanic Garden, Chiang Mai, Thailand.

Simulium (*G.*) *isanense* sp. nov. from Thailand, three Peninsular Malaysian species, *S. (G.) angulistylum* (from the type locality, Gombak), *S. (G.) whartoni* Takaoka & Davies and *S. (G.) cheongi* Takaoka & Davies, and one Indonesian species, *S. (G.) atratum* De Meijere, all members of the *S. epistum* species-group, were subjected to DNA isolation, COI gene amplification, and sequencing as described by Low et al. (2015). Thai *S. (G.) angulistylum* sequences (AY251483–AY251486, HM775234–HM775241, and JQ619907–JQ619931) were retrieved from the NCBI GenBank database and included for analysis. New sequences generated from the present study were deposited in the NCBI GenBank database under accession numbers MF476242–MF476257.

Uncorrected *p* pairwise genetic distances were computed using PAUP 4.0b10 (Swofford 2002). Bayesian inference (BI) analysis was

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performed using MrBayes (Huelsenbeck and Ronquist, 2001) with the following settings. The analysis model employed two substitution types (“nst = 2”), with rate variation across sites modelled using a gamma distribution (rates = “gamma”). The Markov chain Monte Carlo search was run with four chains for 500000 generations, with trees begin sampled every 100 generations. The first 1000 trees were discarded as burnin. Neighbor-joining (NJ) analysis was performed in MEGA7 (Kumar et al., 2016) with Kimura's two-parameter model of substitution (K2P distance), using 1000 bootstrap replicates.

3. Results

3.1. *Simulium (Gomphostilbia) isanense* Takaoka, Srisuka & Saeung sp. nov.

3.1.1. Female

Body length 2.2–2.7 mm. **Head:** Slightly narrower than width of thorax. Frons brownish black, and moderately covered with yellowish-white scale-like recumbent short hairs interspersed with few dark longer hairs near vertex; frontal ratio 1.7:1.0:2.8–3.0; frons: head ratio 1.0:5.3–5.5. Fronto-ocular area well developed, directed dorsolaterally. Clypeus brownish black, densely covered with yellowish-white scale-like hairs interspersed with several dark longer hairs on each side of lower half. Labrum 0.7 times length of clypeus. Antenna composed of scape, pedicel and nine flagellomeres, medium to dark brown except scape, pedicel and basal half of first flagellomere yellow. Maxillary palp composed of five segments, light to medium brown, proportional lengths of third, fourth, and fifth segments 1.0:1.1–1.2:2.5–2.7; third segment (Fig. 1A) somewhat widened apically; sensory vesicle (Fig. 1A) medium sized, ellipsoidal (0.27–0.30 times length of third segment), with medium-sized opening. Maxillary lacinia with 10 or 11 inner and 13–15 outer teeth. Mandible with 21–24 inner teeth and 11–15 outer teeth. Cibarium (Fig. 1B) medially forming small sclerotized plate folded forward from posterior margin, with weakly sclerotized medio-longitudinal ridge having bifid apex. **Thorax:** Scutum brownish black except anterolateral calli dark brown, shiny and thinly white pruinose except medial and submedial longitudinal vittae nonpruinose, when illuminated dorsally and viewed anterodorsally, densely covered with whitish-yellow scale-like recumbent short hairs except on median and submedial longitudinal vittae. Scutellum dark brown, covered with yellow short hairs and dark long upright hairs. Postnotum dark brown, shiny when illuminated dorsally and viewed dorsolaterally, and bare. Pleural membrane bare. Katepisternum longer than deep, dark brown, shiny when illuminated ventrally and viewed laterally, moderately covered with fine short hairs. **Legs:** Foreleg: coxa yellow; trochanter light brown except base and inner surface yellow; femur light brown with apical cap medium brown (though extreme tip yellow); tibia medium brown except median large areas on outer surface light brown and base yellow; tarsus brownish black, with moderate dorsal hair crest; basitarsus moderately dilated, 7.3–7.4 times as long as its greatest width. Midleg: coxa medium brown except posterolateral surface dark brown; trochanter yellow except posterior surface light brown; femur light brown with inner surface widely yellow and apical cap medium brown (though extreme tip yellow); tibia white on base, yellow to light brown on basal one-third with medium brown subbasal spot, and medium to dark brown on apical two-thirds; tarsus dark brown except basal half of basitarsus dark yellow to light brown. Hind leg: coxa yellow except anterior and posterior surface medium brown; trochanter yellow; femur dark yellow to light brown except base yellow and apical cap dark brown (though extreme tip yellow); tibia (Fig. 1C) dark yellow to light brown on basal two-third except base yellowish white, subbasal spot and posterior surface medium brown, and apical one-third brownish black; tarsus brownish black except basal two-thirds (though base light brown) and basal half of second tarsomere yellowish white; basitarsus (Fig. 1D) narrow, nearly parallel-sided though slightly narrowed apically, 6.4–6.5 times as long as wide, and 0.54 and 0.50 times

as wide as greatest widths of tibia and femur, respectively; calcipala (Fig. 1D) as long as width at base, and 0.53 times as wide as greatest width of basitarsus; pedisulcus (Fig. 1D) well developed; claw (Fig. 1E) with large basal tooth 0.51 times length of claw. **Wing:** Length 2.0–2.1 mm. Costa with dark spinules and hairs except basal patch of hairs yellow. Subcosta haired except near apex bare. Hair tuft on base of radius yellow. Basal portion of radius fully haired; R₁ with dark spinules and hairs; R₂ with hairs only. Basal cell absent. **Halter:** White except basal portion darkened. **Abdomen:** Basal scale light brown, with fringe of whitish-yellow hairs. Dorsal surface of abdomen light to dark brown except basal half yellow, moderately covered with dark short to long hairs and yellow short hairs; tergites of segments 2 and 6–8 shiny when illuminated at certain angles. Ventral surface of segment 2 white, those of other segments light to medium brown; sternal plate on segment 7 undeveloped. **Terminalia:** Sternite 8 (Fig. 1F) bare medially, with 18–25 medium-long to long hairs together with two to five slender short hairs on each side. Ovipositor valve (Fig. 1F) tongue-like, thin, membranous, moderately covered with microsetae interspersed with one or two short hairs; inner margins shallowly concave, somewhat sclerotized, and moderately separated from each other. Genital fork (Fig. 1G) of usual inverted-Y form, with slender stem; arms of moderate width, moderately folded dorsally, with distinct short lobe directed posteromedially. Paraproct in ventral view (Fig. 1H) nearly triangular, with anterior surface bare, and anteromedial surface well sclerotized, and with three to five sensilla; paraproct in lateral view (Fig. 1I) slightly produced ventrally beyond ventral tip of cercus, 0.63 times as long as wide, with 17–20 medium-long to long hairs on ventral and lateral surfaces. Cercus in lateral view (Fig. 1I) short, rounded posteriorly, 0.6 times as long as wide. Spermatheca (Fig. 1J) ellipsoidal, 1.4–1.6 times as long as its greatest width, well sclerotized and with many fissures on outer surface; internal setae absent; both accessory ducts unpigmented, subequal in diameter to major one.

3.1.2. Male

Body length 2.3–2.5 mm. **Head:** Somewhat wider than thorax. Upper eye medium brown, consisting of large facets in 11 (rarely 12) vertical columns and 12 or 13 horizontal rows. Face brownish black, white pruinose when illuminated dorsally and viewed anteriorly. Clypeus brownish black, white pruinose when illuminated dorsally and viewed anteriorly, densely covered with yellow hairs interspersed with several dark brown longer hairs on each side of lower half. Antenna as in female except following characters: light to medium brown except scape, pedicel and base of first flagellomere; first flagellomere elongate, 1.5–1.6 times length of second one. Maxillary palp light to medium brown, with five segments, proportional lengths of third, fourth, and fifth segments 1.0:1.2–1.3:2.8–2.9; third segment (Fig. 2A) widened apically; sensory vesicle (Fig. 2A) small, ellipsoidal (0.18–0.26 times length of third segment), and with small opening. **Thorax:** As in female. **Legs:** Color almost same as that of female. Fore basitarsus moderately dilated, 7.7–7.8 times as long as its greatest width. Hind basitarsus (Fig. 2B) nearly parallel-sided, 5.4–5.5 times as long as wide, and 0.53–0.54 and 0.57–0.59 times as wide as greatest widths of tibia and femur, respectively; calcipala (Fig. 2B) slightly longer than basal width, and 0.5 times as wide as greatest width of basitarsus. Pedisulcus (Fig. 2B) well developed. **Wing:** Length 1.9–2.0 mm. Other characters as in female except subcosta bare. **Halter:** Grayish except basal stem darkened. **Abdomen:** Basal scale light to medium brown, with fringe of yellow hairs. Dorsal surface of abdomen medium brown to brownish black except basal four-fifths of second segment yellow, covered with dark brown short to long hairs; segments 2 and 5–7 each with pair of shiny dorsolateral or lateral patches; ventral surface of segment 2 whitish, those of segments light to medium brown. **Genitalia:** Coxite in ventral view (Fig. 2C) nearly rectangular, 1.5 times as long as its greatest width. Style in ventral view (Fig. 2C) bent inward, tapered to apex, and with apical spine; style in ventrolateral view (Fig. 2D) 0.7 times length of coxite, 2.3 times as long as basal width, nearly parallel-

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