A new species and a new record of the Simulium (Gomphostilbia) gombakense species-group (Diptera: Simuliidae) from Thailand

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ABSTRACT

Larvae and adults reared from pupae of Simulium (Gomphostilbia) gombakense Takaoka & Davies from eight streams in five provinces of Thailand were genetically analyzed by using cytochrome c oxidase subunit 1 sequences. The material was composed of four lineages, of which lineages 2, 3 and 4 are genetically closely related to one another, but more distantly related to lineage 1, which is equivalent to typical S. (G.) gombakense from Peninsular Malaysia. Lineages 2, 3 and 4, which are morphologically indistinguishable, are designated as genoforms A, B and C within a species complex of S. (G.) paiense sp. nov., which is described based on specimens of lineage 2 (=genoform A). Simulium (G.) paiense sp. nov. is similar to S. (G.) gombakense from Peninsular Malaysia but appears to be barely distinguished by the relative length of the female fore basitarsus. Simulium (G.) thuathaienthienense Takaoka & Sofian-Azirun is newly recorded from Thailand and its male is described for the first time. The female of S. (G.) gombakense is briefly described based on females reared from pupae collected from Peninsular Malaysia.

1. Introduction

Black flies (Simuliidae) are one of dipteran insects and are widely distributed in many parts of the world, where clean, running streams and rivers, as the sites for the breeding of their immature stages and for the oviposition of the gravid females, are available.

Due to the biting habit of their adult females, black flies are of medical and veterinary importance. Females of certain simulid species can transmit viruses, protozoans and filarial parasites. In Asia, Simulium (Simulium) bidentatum (Shiraki) is a vector of zoonotic onchocerciasis caused by Onchocerca dewittei japonica, a parasite of wild boar, recently discovered in Japan (Takaoka et al., 2012), and three other black fly species, Simulium (Gomphostilbia) asakoe Takaoka & Davies, Simulium (Simulium) nigroglum Summers and Simulium (Simulium) nodosum Purī, are reported to transmit three different filarial species, all of which are of possible public health importance, from Thailand (Fukuda et al., 2003; Takaoka et al., 2003).

Investigations of black flies’ role in the transmission of disease pathogens require the reliable identification of species in question. For this, recent chromosomal and DNA sequence-based analyses have proved to be good tools to disclose cryptic taxa or lineages within a single morphospecies (e.g., Adler et al., 2016; Low et al., 2015).

Simulium (Gomphostilbia) is one of two predominant subgenera in South-East Asia, consisting of about 230 species (including S. (G.) asakoe as a filarial vector), which are placed in 10 species-groups (Adler and Crosskey, 2017). Adult females of species in each species-group of this subgenus are usually morphologically similar and difficult to differentiate from one another (Takaoka, 2012).

We aim to study whether S. (G.) gombakense Takaoka & Davies in Thailand is a single or multiple species. This species belongs to the Simulium gombakense species-group, defined by Takaoka (2012), which is a small taxon consisting of 11 species, and is characterized by the male ventral plate widened posteriorly and pupal gill composed of an inflated structure and eight to 10 slender filaments.

In Thailand, this species-group is represented by three species: Simulium (G.) gombakense Takaoka & Davies (originally described from Peninsular Malaysia), S. (G.) maleewongae Takaoka, Srisuka & Saeung, and S. (G.) prayongi Takaoka & Choochote (Takaoka and Davies, 1995, Takaoka and Choochote, 2005, Takaoka et al., 2017). Simulium (G.) gombakense in Thailand includes two lineages with high genetic divergence (7.35%), one lineage from Chongyen, Nakhonsawan Province, and the other from Chiang Mai and Loei Provinces, according to cytochrome c oxidase subunit 1 (COI) sequences analyzed by Pramual et al. (2011).
We molecularly examined larvae and adults reared from pupae, which are morphologically identifiable as *S. (G.) gombakense*, collected from eight streams in five provinces of Thailand. We found that Thai populations of *S. (G.) gombakense* are composed of at least four lineages including two previously reported: lineage 1 (same as a lineage by Pramual et al., 2011), lineage 2, lineage 3 and lineage 4 (same as another lineage by Pramual et al., 2011). Two new lineages, 2 and 3, are closely related to lineage 4 but distantly to lineage 1, which is equivalent to typical *S. (G.) gombakense* from Peninsular Malaysia.

Lineages 2, 3 and 4 are so distinctly separated from lineage 1 that they are treated as genoforms A, B and C within a complex of a new species, since all these three lineages are morphologically indistinguishable.

This new species is here described, and possible differing characters between this new species and *S. (G.) gombakense* from Peninsular Malaysia, are discussed.

In this survey, pupae and larvae *S. (G.) thuathiencens* Takaoka & Sofian-Azirun originally described from Vietnam (Takaoka et al., 2015), were collected for the first time in Thailand. The male of this species is described. In addition, the female of *S. (G.) gombakense* is briefly described on the basis of specimens reared from pupae collected from Peninsular Malaysia.

### 2. Material and methods

The larvae and adults reared from pupae that were used for genetic analysis were collected from eight streams in five provinces of Thailand (sites 1–8 in Table 1). In addition, *S. (G.) thuathiencens* and *S. (G.) maleewongae*, both collected from Thailand, *S. (G.) gombakense* from Peninsular Malaysia, and five sequences registered in the NCBI GenBank (HM775247, HM775249, HM775250, HM775252, HM775253, all under *S. (G.) gombakense* from Thailand), were included in our genetic analysis. Specimens were used for sequencing of the COI gene and a subsequent phylogenetic analysis. The protocols for DNA extraction, PCR amplification, and sequencing followed those of Low et al. (2015). An automatic model selection was implemented based on the Akaike information criterion (AIC). The best-fit model was the general time-reversible (GTR) model with a proportion of invariable sites of 0.633 and with a gamma shape parameter of 1.591. Neighbor-joining analysis was performed using PAUP 4.0b10 (Swofford, 2002), with Kimura’s two-parameter model of substitution (K2P distance), using 1000 bootstrap replicates. *Simulium (G.) laosense* was used as an outgroup. To estimate the level of genetic divergence, uncorrected p pairwise genetic distances were estimated using PAUP 4.0b10. The representative sequences generated in this study were deposited in the NCBI GenBank database under accession numbers MG958560-MG958562 for *S. (G.) gombakense*, MG958563-MG958580 for *S. (G.) paiense* sp. nov., MG958581-MG958582 for *S. (G.) thuathiencens* and MG958583-MG958584 for *S. (G.) maleewongae*.

A new species was described based on females, males, pupae and mature larvae collected from Pai District, Mae Hong Son Province (site 1 in Table 1), where only lineage 2 (genoform A) was recognized. It was morphologically compared with three other lineages (i.e., typical *S. (G.) gombakense* and genoforms B and C of the new species). The methods of description and illustration, as well as terms for morphological features, followed those of Takaoka (2003).

The holotype and paratypes are deposited at the Queen Sirikit Botanic Garden, Chiang Mai, Thailand.

This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/.

### Table 1

<table>
<thead>
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<th>Site no.</th>
<th>District</th>
<th>Province</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Elevation (masl)</th>
<th>Collection date</th>
<th>No. of samples*</th>
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<tr>
<td>1</td>
<td>Pai</td>
<td>Mae Hong</td>
<td>19°21’ 00.0”N</td>
<td>98°35’ 06.4”E</td>
<td>1737</td>
<td>28. iv. 2016</td>
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<tr>
<td>2</td>
<td>Chomthong</td>
<td>Chiang Mai</td>
<td>18°29’ 57.0”N</td>
<td>98°40’ 06.2”E</td>
<td>412</td>
<td>3 iv. 2017</td>
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<tr>
<td>3</td>
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<td>Chiang Mai</td>
<td>18°44’ 42.2”N</td>
<td>98°56’ 21.3”E</td>
<td>503</td>
<td>18 vii. 2017</td>
<td>5M, 5PE</td>
</tr>
<tr>
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<td>Chiang Mai</td>
<td>18°45’ 21.3”N</td>
<td>98°08’ 00.3”E</td>
<td>1,434</td>
<td>7 xii. 2015</td>
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<tr>
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<td>Mae Hong Son</td>
<td>19°36’ 57.9”N</td>
<td>97°59’ 48.2”E</td>
<td>1,007</td>
<td>12 vii. 2017</td>
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<tr>
<td>6</td>
<td>Umphang</td>
<td>Tak</td>
<td>16°18’ 00.5”N</td>
<td>99°01’ 22.6”E</td>
<td>1,095</td>
<td>22 viii. 2017</td>
<td>2F, 1M, 2PE, 2L</td>
</tr>
<tr>
<td>7</td>
<td>Wiang Sa</td>
<td>Nan</td>
<td>07°22’ 39.9”N</td>
<td>105°57’ 47.6”E</td>
<td>551</td>
<td>26 vii. 2017</td>
<td>4L</td>
</tr>
<tr>
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<td>Muang</td>
<td>Trang</td>
<td>07°22’ 20.0”N</td>
<td>99°49’ 16.0”E</td>
<td>401</td>
<td>31 iii. 2012</td>
<td>1M, 2PE, 2L</td>
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</table>

*F, female(s); M, male(s); PE, pupal exuviae; L, larva(e).
analyzed for the first time. The results show that both species are distinctly separated from each other and from all four lineages of *S. (G.) gombakense* (Fig. 1).

### 3.2. Morphological analysis

#### 3.2.1. Description of a new species

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

(urn: lsid:zoobank.org:act:F20966CE-8E86-4ACE-9C9B-D0862A2E6F83)

**Female** (n = 6). Body length 2.0–2.2 mm. **Head.** Slightly narrower than width of thorax. Frons brownish black, slightly shiny when illuminated at certain angles, densely covered with yellowish-white recumbent short hairs interspersed with several dark unbranched longer hairs along each lateral margin; frontal ratio 1.7–1.8:1.0:2.4–2.7; frons-head ratio 1.0:4.4–4.8. Fronto-ocular area well developed, narrow, directed dorsolaterally. Clypeus brownish black, densely covered with yellowish-white recumbent short hairs interspersed with several dark-brown longer hairs on each side of lower two-thirds. Labrum 0.6–0.7 times as long as clypeus. Antenna composed of scape, pedicel and nine flagellomeres, dark brown except scape, pedicel and base of first flagellomere yellow. Maxillary palp composed of five segments, light to medium brown, proportional lengths of third, fourth, and fifth segments 1.0:0.8:2.1; third segment (Fig. 2A) swollen; sensory vesicle (Fig. 2A) oblong, 0.7–0.8 times as long as third segment, with medium-sized opening. Maxillary lacinia with 12 or 13 inner and 15 or 16 outer teeth. Mandible with 21–24 inner and 10–12 outer teeth. Cibarium (Fig. 2B) medially forming short wide sclerotized plate folded forward from posterior margin, and with moderately sclerotized medial longitudinal ridge with forked apex. **Thorax.** Scutum brownish-black to black (except anterior calli medium brown), shiny, thinly grayish-white pruinose with three faint non-pruinose longitudinal vittae (one medial and two submedial) when illuminated dorsally and viewed anteriorly, densely covered with whitish-yellow recumbent hairs. Scutellum dark brown, covered with whitish-yellow short hairs and dark-brown long upright
Fig. 2. Female of Simulium (Gomphostilbia) paiense sp. nov. (A) Third segment of right maxillary palp with sensory vesicle (front view). (B) Cibarium. (C) Hind tibia (left side; outer view). (D) Basitarsus and second tarsomere of hind leg showing calcipala and pedisulcus (left side; outer view). (E) Claw. (F) Sternite 8 and ovipositor valve (right half; ventral view). (G) Genital fork (ventral view). (H, I) Right paraprocts and cerci (H, ventral view; I, lateral view). (J) Spermatheca. Scale bars = 0.1 mm for C and D; 0.02 mm for A, B and E–J.
hairs along posterior margin. Postnotum dark brown to brownish-black. Male. Pleural membrane bare.Katepisternum medium to dark brown, longer than deep, moderately covered with short hairs. Legs. Foreleg: coxa whitish yellow; trochanter light brown except base whitish yellow; femur light brown with apical cap medium brown though extreme tip whitish yellow; tibia yellowish white except apical one-fourth dark brown and inner surface light brown; tibia densely covered with yellow hairs on outer surface of basal four-fifths; tarsus brownish black, with moderate dorsal hair crest; basitarsus moderately dilated, 6.6–7.4 times as long as its greatest width. Midleg: coxa light brown except posterolateral surface medium brown; trochanter light brown except base whitish yellow; femur light brown with apical cap medium brown (though extreme apex whitish yellow); tibia light to medium brown except little more than basal one-third whitish yellow; tibia densely covered with whitish hairs on posterior surface of basal five-sixths; tarsus medium to dark brown except basal half yellow. Hind leg: coxa light brown; trochanter whitish yellow; femur light brown except base whitish yellow and apical cap medium brown (though extreme tip whitish yellow); tibia (Fig. 2C) yellowish white on basal half or little more, light brown to dark brown on rest; tibia densely covered with white fine hairs on posterior and outer surfaces of basal three-fourths or little more; tarsus medium to dark brown except little more than basal two-thirds of basitarsus (though base light brown) and basal half of second tarsomere yellowish white; basitarsus (Fig. 2D) narrow, nearly parallel-sided, 6.1–6.5 times as long as wide, and 0.68–0.74 and 0.57–0.61 times as wide as greatest widths of tibia and femur, respectively; calciptala nearly as long as wide, and 0.5–0.6 times as wide as greatest width of basitarsus. Claw (Fig. 2E) with large basal tooth 0.47 times as long as claw. Wing. Length 1.9–2.0 mm. Costa with dark-brown spinules and hairs except basal short portion with patch of whitish-yellow hairs. Subcosta with medium-brown hairs except apical one-third to one-fourth bare. Hair tuft on stem vein whitish yellow. Basal portion of radius fully haired; R1 with dark brown spinules and hairs except basal short portion with patch of hairs on outer surface of basal four-fourths; all trichomes (Fig. 4A) on each side; all trichomes with distinct hairs (though one hair on right side in one male). Paramere (Fig. 3I) of moderate size, with two long hooks and without distinct hairs (though one hair on right side in one male). Aedeagal membrane (Fig. 3J) moderately covered with microsetae on ventral surface; basal arms nearly parallel-sided, though somewhat convergent apically; ventral plate in ventral view as in Fig. 3D. Coxite in ventral view (Fig. 3D) subquadrate, 1.7 times as long as wide. Style in ventral view (Fig. 3D) slender, 0.8 times as long as coxite, gently curved inward, tapered toward apex and with one apical spine; style in ventral lateral view (Fig. 3E) 2.3 times as long as its greatest width at base, gradually tapered from base toward apex. Ventral plate in ventral view (Fig. 3D) transverse, much wider than long, somewhat widened posteriorly, with anterior margin produced medially, with posterior margin nearly straight, and moderately covered with microsetae on ventral surface; basal arms nearly parallel-sided, though somewhat convergent apically; ventral plate in lateral view (Fig. 3F) much produced ventrally; ventral plate in caudal view (Fig. 3G) gently rounded ventrally, and densely covered with microsetae on most of posterior surface except lateral portions bare. Median sclerite (Fig. 3H) broad, plate-like, though lateral margins not well defined. Paramere (Fig. 3I) of moderate size, with two long hooks and a few shorter ones. Aedeagal membrane (Fig. 3J) moderately covered with microsetae. Ventral surface of abdominal segment 10 (Fig. 3K, L) without distinct hairs (though one hair on right side in one male). Cercus (Fig. 3K, L) small, with 10–22 short hairs. Pupa (n = 15). Body length 2.4–2.6 mm. Head. Integument yellow, moderately or densely covered with round tubercles (some appearing to have tiny secondary projections) on frons and each lateral surface; antennal sheath bare, and without any projections. Frons with three unbranched or bifid long trichomes (Fig. 4A) on each side; all trichomes subequal in length and thickness to one another. Face with one unbranched or bifid long trichome (Fig. 4B) on each side. Thorax. Integument yellow, moderately or densely covered with round tubercles
(some appearing to have tiny secondary projections) except dorsal surface of posterior one-fifth almost bare, with two unbranched or bifid slender short or medium-long trichomes, or rarely three unbranched or bifid trichomes (two medium-long and one short, or one medium-long and two short trichomes) (Fig. 4C) dorsomedially, two unbranched or bifid slender medium-long trichomes (Fig. 4D) anterolaterally, one unbranched or bifid slender medium-long trichome (Fig. 4E) mediolaterally, and three unbranched or bifid trichomes (two medium-long, one short) (Fig. 4F) ventrolaterally, on each side. Gill (Fig. 4G) of much inflated structure (0.5 mm long), which is 2.6–2.8 times as long as greatest width, and divided into three portions (basal, middle and apical) by two constrictions; second constriction 1.0–1.4 times as wide
as first constriction; middle and apical portions each with three thumb-like or finger-like projections; longest projection of middle inflated portion 0.7–0.8 times as long as greatest width of apical inflated portion; gill with eight slender thread-like filaments, of which six filaments arise in three groups of one, two and three filaments from dorsal, dorsal-inner and inner surfaces of basal portion of inflated structure, respectively, and all subequal in length (0.7–0.9 mm) and thickness to one another, and remaining two filaments isolated, subequal in length (0.5 mm), each arising from dorsal surface of middle and apical portions of inflated structure; surface of inflated portion and all filaments light brown, without any pattern on inflated portion or any transverse ridges or furrows on filaments; surface of inflated portion densely covered with microtubercles and that of filaments smooth, though appearing to be densely covered with microtubercles on inner layer. Abdomen. Dorsally, all segments pale whitish yellow, without tubercles, with one unbranched slender short seta on each side; segment 2 with one unbranched slender short seta and five minute setae on each side; segments 3 and 4 each with four hooked spines and one unbranched minute seta on each side; segment 5 with comb-like groups of minute spines and four minute setae, but lacking spine-combs on each side; segments 6–9 each with spine-combs (though those on segment 9 somewhat smaller than those on other segments) and comb-like groups of minute spines in transverse row on each side; segments 6–8 each with two minute setae on each side; segment 9 with pair of wide plate-like terminal hooks with weakly crenulated outer margin (Fig. 4H). Ventrally, segment 4 with one unbranched short hook (somewhat smaller in size than those on segments 5–7) and few minute setae on each side; segment 5 with pair of bifid hooks submedially and few short setae on each side; segments 6 and 7 each with pair of bifid inner and simple outer hooks somewhat spaced from each other, and few short setae on each side; segments 4–8 each with comb-like groups of minute spines on each side; each side of segment 9 with three grapnel-shaped hooklets. Cocoon. Wall-pocket-shaped, thinly and compactly woven with no open spaces in weave, somewhat extended ventrolaterally, covering entire abdomen and posterior one-third of thorax; anterior margin somewhat thickened or not; floor loosely woven on posterior one half of cocoon; individual threads invisible; 2.1–2.8 mm long by 1.2–2.0 mm wide.

Mature larva (n = 20). Body length 4.5–5.6 mm. Body entirely gray with reddish-brown pigment on dorsal and dorsolateral surfaces of abdominal segments 6–9 (mostly segment 8). Head capsule sparsely covered with minute colorless setae. Cephalic apotome yellow to dark yellow, with faint to moderate positive head spots, or somewhat darkened along posterior margin, obscuring posterolateral spots. Lateral surface of head capsule yellow except eye-spot region white, and portions above and posterior to eye-spot region darkened, thus two large spots in front of posterior margin faintly positive or merged; eyebrow distinct; one round spot below eye-spot region and two round spots in front of posterior margin faintly positive or obscured; lateral surface entirely light brown except eye-spot region white in three larvae. Ventral surface of head capsule yellow except medial portion widely darkened along both sides of postgenal cleft, obscuring elongate spots...
on each side of postgenal cleft; ventral surface of head entirely light-brown except narrow portion along posterior margin yellow in three larvae. Cervical sclerites composed of two small light brown rod-like pieces, not fused to occiput, widely separated from each other. Antenna composed of three segments and apical sensillum, much longer than stem of labral fan; antenna pale except dorsal surface of first segment somewhat darkened toward base; proportional lengths of first, second, and third segments 1.00:0.75–0.86:0.82–0.86. Labral fan with 24–28 primary rays. Mandible (Fig. 5A) with three comb-teeth decreasing in length from first to third; mandibular serrations composed of two teeth (one large and one small); large tooth at acute angle to mandible on intermediate teeth shortest; lateral margins smooth; and 3.0 times as long as postgenal bridge, respectively. Pharate pupal long as postgenal bridge (though postgenal clefts of two larvae are 1.3 times as long as postgenal bridge, respectively). Pharate pupal terminal hooks (Fig. 4H). This new species is barely distinguished from S. (G.) gombakense by the following characters (those of S. (G.) gombakense in parentheses): in the female: the length ratio of the fore basitarsus against its greatest width 6.8–7.1 (5.8–6.0); in the male: the length ratio of the fore basitarsus against its greatest width 7.6–8.5 (6.8–7.1); in the pupa: the ratio of the width of the second constriction against the greatest width of the apical in 0.7–0.8 (1.0–1.5). Remarks. S. (G.) paiense sp. nov. complex are almost indistinguishable from those of genoform A, except a few characters including the fore basitarsi of genoform B from site 3, which are 7.0–7.9 times as long as their greatest width, overlapping the range for S. (G.) gombakense, and labral fans of some larvae of genoform B from site 2, which have as many as 36 primary rays, showing a wider range of intraspecific variation (24–36 primary rays). Overall, there seem to be only a few morphological features, which may be used to separate genoforms A, B and C of the S. (G.) paiense sp. nov., complex from S. (G.) gombakense: in the female by the length ratio of the fore basitarsus against its greatest width: 6.2–7.2 (n = 14) versus 5.8–6.0 (n = 3); in the pupa by the ratio of the longest finger-like projection on the middle inflated portion against the greatest width of the apical inflated portion: 0.7–0.8 (n = 34) versus 1.0–1.5 (n = 5), and relative size of the second constriction against the first one: 1.0–1.4 (n = 34) versus 0.8–0.9 (n = 5). Further studies are needed to confirm the differing morphological characters between S. (G.) paiense sp. nov.
3.3. Description of the male of S. (G.) thuathienense

_Simulium_ (Gomphostilbia) _thuathienense_ Takaoka & Sofian-Azirun, 2015

_Simulium_ (Gomphostilbia) _thuathienense_ Takaoka & Sofian-Azirun, in Takaoka et al., 2015: 48–52 (Female, pupa and larva).

This species was described from a pharate female dissected from a pupa and mature larvae collected in Thua Thien Hue Province, central Vietnam (Takaoka et al., 2015). The pupa of this species is characterized by the terminal hooks cone-like (not broad and plate-like), and gill with an inflated Y-shaped structure (when viewed laterally) and eight slender filaments. The inflated structure is composed of a basal portion, and dorsal and forward branches, each branch with three finger-like projections. Among eight slender filaments, six arise from the basal portion in four groups of one, two, one and two filaments from dorsal to inner-ventral, and each of the remaining two filaments from the apex of one of three projections on each branch. There are three constrictions on the inflated structure, one near the apex of the basal portion and one basally on each branch. The larva of this species is characterized by the postgenal cleft medium-long, nearly as long as the postgenal bridge. The pupa and mature larvae from Thailand agree morphologically with those of _S. (G.)_ _thuathienense_ except that pupal abdominal segment 9 has a transverse row of spine-combs on each side.

The male of this species is here described based on two males reared from pupae collected in Thailand.

This is the first record of _S. (G.)_ _thuathienense_ from Thailand.

**Male.** Similar to that of _S. (G.)_ _paiense_ sp. nov. except following characters. Body length 2.5 mm. **Head.** Much wider than thorax. Holoptic, upper eye consisting of large facets in 14 vertical columns and in 15 or 16 horizontal rows. Face dark brown, bare. Clypeus dark brown, white pruinose, densely covered with golden-yellow scale-like
short hairs interspersed with dark-brown longer hairs except central portion bare. Antenna composed of scape, pedicel and nine flagello-
meres, dark brown except base of first flagellomere slightly paler; first flagellomere somewhat elongate, 1.6 times as long as second flagellomere. Maxillary palps: proportional lengths of third, fourth and fifth segments 1.0:1.1:1.9; third segment (Fig. 6A) somewhat widened apically; sensory vesicle (Fig. 6A) ellipsoid, 0.2–0.3 times as long as third segment, with small opening apically. Thorax. Scutum dark brown, slightly shiny on shoulders, along lateral margins and pre-
scutellar area when illuminated at certain angles; scutum slightly gray pruinose, densely covered with golden-yellow scale-like recumbent hairs. Scutellum medium brown, with golden-yellow short hairs and dark upright long hairs. Postnotum dark brown and bare. Pleural membrane bare. Kepisternum longer than deep, medium to dark brown with short hairs. Legs. Foreleg: coxa dark yellow; trochanter medium brown except base yellow; femur light brown with apical cap medium brown; tibia medium brown except medial large area pale; tarsus dark brown, with moderate dorsal hair crest; basitarsus slightly dilated, 8.9 times as long as its greatest width (7.6 times as long as its greatest width in one male from Chiang Mai Province). Midleg: coxa dark brown; trochanter medium brown; femur light brown with apical cap medium brown; tibia light to medium brown except basal one-
third yellow and medial outer surface pale; tarsus medium brown except basal one-third of basitarsus dark yellow. Hind leg: coxa medium brown; trochanter yellow; femur light brown with basal extreme yellow and apical cap dark brown; tibia (Fig. 6B) yellow on basal two-fifths, with light-brown narrow area subbasally, and light to medium brown on rest except apical cap dark brown; tarsus medium brown except basal half of basitarsus (though base light brown) and basal half of second tarsomere yellow; basitarsus (Fig. 6C) nearly parallel-sided, 5.0 times as long as greatest width, and 0.70 and 0.69 times as wide as greatest width of tibia and femur, respectively, (or slightly enlarged, spindle-shaped, 4.3 times as long as wide, and 0.9 and 0.9 times as wide as greatest widths of tibia and femur, respec-
tively in one male from Chiang Mai Province); calcipala nearly as long as wide, and 0.36 times as wide as greatest width of basitarsus. Wing. Length 2.0 mm. Genitalia. Coxites, styles and ventral plate in ventral view as in Fig. 6D. Coxite in ventral view (Fig. 6D) subquadrate, 2.4 times as long as wide. Style in ventral view (Fig. 6D) slender, 0.7 times as long as coxite, slightly curved inward, nearly parallel-sided and with one apical spine; style in ventrolateral view (Fig. 6E) 2.8 times as long as its greatest width at base, tapered from base to basal one-third, nearly parallel-sided to apical one-fourth, then slightly tapered toward round apex. Ventral plate in ventral view (Fig. 6D) transverse, much wider than long, widened posteriorly, with anterior margin produced medially, with posterior margin widely concave, and moderately covered with microsetae on ventral surface; basal arms nearly parallel-
sided, though somewhat convergent apically; ventral plate in lateral view (Fig. 6F) somewhat produced ventrally; ventral plate in caudal view (Fig. 6G) deeply concave ventromedially, rounded laterally, and densely covered with microsetae on most of posterior surface except lateral portions bare. Median sclerite broad, plate-like, though lateral margins not well defined (similar to Fig. 3H). Paramere of moderate size, with several to long-medium hooks. Aedeagal membrane moderately covered with microsetae (similar to Fig. 3J). Ventral sur-
face of abdominal segment 10 (Fig. 6H, I) without distinct hairs. Cercus (Fig. 6H, I) small, with 17 or 18 short hairs.

Specimens examined. One male and one female (both with their associated pupal exuviae and cocoons), reared from pupae, and five mature larvae collected a small seasonal stream (elevation 1316 m above sea level, 18°16.44.5′N, 100°30.14.2″E), Khun Nathan village, near Khun Nathan National Park, Naini District, Nan Province, Thailand, S-VIII-2017, by W. Srisuka; one male (with its associated pupal exuviae and cocoon), reared from a pupa, and two mature larvae collected a small seasonal stream (elevation 1972 m above sea level, 20°02′12.9″N, 99°08′40.4″E), Banlek, near Doi Phahomtop National Park, Fang District, Chiang Mai Province, Thailand, 23-XI-2011, by W. Srisuka.

Remarks. The male of S. (G.) thaithiense has the ventral margin of the ventral plate deeply concave medially (Fig. 6G) when viewed posteriorly, a rare character in the subgenus Gomphostilbia. This char-
acter has, however, been reported in S. (G.) malewongae from Thai-
lnd, S. (G.) nutukotense Takaoka & Shrestha from Nepal, and S. (G.) sachini Takaoka & Willie from India and Nepal (Takaoka et al., 2017; Takaoka and Shrestha, 2010), all in the S. gombakense species-group, although the pupae of these three species differ from that of this species by having the inflated gills of different configuration.

In the original description of the female of S. (G.) thaithiense, color observations and measurements of legs were limited because only one pharate female dissected from a pupa was available (Takaoka et al., 2015). The following characters, based on a female of this species reared from a pupa from Nan Province, Thailand, augment the original description: fore tibia light brown except the apical cap medium brown and a large median area on the outer surface yellowish; fore basitarsus 6.6 times as long as its greatest width; mid basitarsus yellow on the basal half; hind tibia whitish yellow on the little less than the basal three-fifths; hind basitarsus parallel-sided, 6.5 times as long as wide, and 0.67 and 0.57 times as wide as the greatest widths of the hind tibia and femur, respectively.

3.4. Description of the female of S. (G.) gombakense

Simulium (Gomphostilbia) gombakense Takaoka & Davies, 1995

Simulium (Morops) gombakense Takaoka & Davies, 1995: 82–84

(Larva).

Simulium (Gomphostilbia) gombakense: Takaoka, 2000: 111–114

(Male and pupa)

This species was described from a mature larva under the subgenus Morops from Peninsular Malaysia (Takaoka and Davies, 1995). It was transferred to the subgenus Gomphostilbia when its male and pupa were described by Takaoka (2000). The female of this species was later de-
scribed based on females reared from pupae collected from Mae Klang Waterfall, Chiang Mai Province, Thailand (site 2 in Table 1) (Takaoka et al., 2010). However, our genetic analysis shows that the female thought to be S. (G.) gombakense probably is that of genotype B or C of the S. (G.) paiense sp. nov. complex, described above. The females and pupae from Mae Klang Waterfall agree morphologically with those of S. (G.) paiense sp. nov., although the fronshead ratio (1:0.5:2–5.3) of the female is different from that (1:0.4:4–4.8) of S. (G.) paiense sp. nov.

The female of S. (G.) gombakense is here described based on two females reared from pupae collected from Gombak (type locality) and one female reared from a pupa from Fraser’s Hill, Peninsular Malaysia.

Female (n = 3). Similar to female of S. (G.) paiense sp. nov. except the following characters. Body length 2.0–2.3 mm. Head. Frontal ratio 1.7–1.8:1.0:2.6–3.0; frons-head ratio 1.0:5.0–5.4. Maxillary palp: propor-
tional lengths of third, fourth, and fifth segments 1.0:1.0:2.3–2.4; third segment swollen; sensory vesicle oblone, 0.6–0.7 times as long as third segment. Maxillary lacina with 8 inner and 10 outer teeth. Mandible with 20–22 inner and 8–10 outer teeth. Legs. Foreleg: basi-
tarsus moderately dilated, 5.8–6.0 times as long as its greatest width. Hind leg: basitarsus 5.9–6.7 times as long as wide, and 0.6–0.7 and 0.5–0.6 times as wide as greatest widths of tibia and femur, respec-
tively. Claw with large basal tooth 0.46 times as long as claw. Wing. Length 2.0–2.1 mm. Terminalia. Sternite 8 with 15–18 medium-long to long hairs and three to five short hairs on each side. Ovipositor valve moderately covered with microsetae interspersed with one or two short hairs. Paraproct in lateral view somewhat produced ventrally, 0.5 times as long as wide, with 16–20 short to long hairs on ventral and lateral surface. Cercus in lateral view 0.5 times as long as wide. Spermathea 1.4–1.5 times as long as wide.

Specimens examined. Two females reared from pupae collected from Gombak, Peninsular Malaysia, 17-III-2011, by H. Takaoka, and
Distribution. Peninsular Malaysia and Thailand.

Remark. The slight difference in the females of S. (G.) gombakense and S. (G.) paiense sp. nov. is recognized, as noted above.

4. Discussion

Our genetic analysis using COI sequences reveals that Thai popula-
tions of the so-called S. (G.) gombakense are composed of at least four lineages including two new lineages. Lineage 1 is equivalent to typical S. (G.) gombakense, and lineages 2, 3 and 4 are treated as genoforms A, B and C within a species complex of S. (G.) paiense sp. nov. described based on genofrom A. The current data show that in Thailand, S. (G.) gombakense is distributed in the central and southern regions, whereas all three genoforms of S. (G.) paiense sp. nov. have a limited distribution in the northern region, though genoform C is also distributed in the northeastern and western regions. More extensive surveys are needed to determine the extent of genetic divergence and geographical distribution of each genoform of the S. (G.) paiense sp. nov. complex.

5. Conclusion

Our molecular and morphological analyses show that S. (G.) gombakense in Thailand is composed of two morphologically distinguish-
able species, S. (G.) gombakense and S. (G.) paiense sp. nov., and the latter is represented by three genetically distinct lineages. The present survey increases the number of black fly species in the S. (G.) gombakense species-group in Thailand from three to five, by adding S. (G.) thaiathienense and S. (G.) paiense sp. nov. All but one species, S. (G.) gombakense, are distributed in the northern region of Thailand, sug-
gestng that this region is a hot spot of biodiversity for this species-
group.

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