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Two new species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from Myanmar, and their phylogenetic relationships with related species in the *S. asakoae* species-group

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ABSTRACT

Two new species of *Simulium* (*Gomphostilbia*), *S.* (*G.*) *myanmarense* and *S.* (*G.*) *monglaense*, are described from females, males, pupae and larvae from Myanmar. The two new species are placed in the *S. asakoae* species-group, and are similar to each other in the female and male but distinguished in the pupa by the presence or absence of an anterodorsal projection of the cocoon, and in the larva by a unique pattern of colored markings on the abdomen. Taxonomic notes are given to separate these species from related species. The COI gene sequences of both species are compared with those of eight species of the *S. asakoae* species-group and three species of the *S. ceylonicum* species-group. Both new species are most closely related to each other, further supporting their morphological classification in the *S. asakoae* species-group.

1. Introduction

The *Simulium asakoae* species-group, defined by Takaoka (2012), is the second largest assemblage among 15 species-groups of the subgenus *Gomphostilbia* Enderlein (Takaoka, 2017). It consists of 33 species, of which 30 species are distributed in the Oriental Region and three in the Palaearctic Region (Takaoka, 2012; Adler and Crosskey, 2016; Takaoka et al., 2017a; Takaoka et al., 2017b). In this species-group, *S.* (*G.*) *asakoae* Takaoka & Davies is a human biter (Choochote et al., 2005) and a vector of an unidentified filarial parasite in Thailand (Fukuda et al., 2003; Ishii et al., 2008).

In Myanmar, where eight species of the genus *Simulium* are recorded, the subgenus *Gomphostilbia* is represented by one species of the *S. asakoae* species-group: *S.* (*G.*) *namense* Takaoka, described from females and males (Takaoka, 1989).

In a recent survey of pupae and larvae of black flies in Shan State in northeastern Myanmar, we collected three species of the *S. asakoae* species-group, of which one is identified as *S.* (*G.*) *asakoae*, and the remaining two are new species. These two new species are described from females, males, pupae and larvae. The COI gene segments of these two new species are sequenced and compared with the relevant and available up-to-date sequences of eight species of the *S. asakoae* species-group and three species of the *S. ceylonicum* species-group.

The relationships of these two new species are investigated by comparing the genetic distances and constructing a phylogenetic tree, using the COI gene sequences.

2. Material and methods

Four females and seven males, all reared from pupae, and nine mature larvae from Shan State, Myanmar were used for descriptions of two new species. Detailed information for the type localities are given under the '**Type material**' of each new species.

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).

One female and two larvae of each new species, and two larvae of *S.* (*G.*) *asakoae*, all collected from the type locality of *S.* (*G.*) *myanmarense* sp. nov., were used for sequencing the COI gene and a subsequent phylogenetic analysis.

The protocols for DNA extraction, PCR amplification, and sequencing follow those of Low et al. (2015). Briefly, the DNA was extracted

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from each specimen, using the i-genomic CTB DNA Extraction Mini Kit (iNtRON Biotechnology, Inc., Seongnam, South Korea). PCR amplification of the COI gene region was carried out in a final volume of 50 μL containing 50–100 ng of genomicDNA, 25 μL of ExPrime TaqMasterMix (GENETBIO, Inc., Daejeon, South Korea), and 10 pmol of each forward and reverse primer from Folmer et al. (1994). Purified PCR products were sent to a commercial company for DNA sequencing. The COI gene sequences were assembled and edited using ChromasPro Version 1.7.7 (Technelysium Pty Ltd., Australia and BioEdit 7.0.9.0 (Hall, 1999)). Representative sequences generated in this study were deposited in the NCBI GenBank database under accession numbers MF101842-MF101843 for *S.* (*G.*) *myanmarense* sp. nov., MF101844-MF101845 for *S.* (*G.*) *monglaense* sp. nov. and MF101846 for *S.* (*G.*) *asakoae*.

Sequences of *S.* (*G.*) *asakoae* (Thai samples) (Pramual et al., 2011) and *S.* (*G.*) *udomi* Takaoka & Srisuka (Thai samples) (Saeung et al., 2017) and those of 10 related species (seven spp. of the *S. asakoae* species-group and three spp. of the *S. ceylonicum* species-group, all Malaysian samples) (Low et al., 2015) were used for calculation of genetic distances and phylogenetic tree construction. A maximum likelihood analysis, with 100 bootstrap replicates, was performed on an on-line web-based server PhyML 3.0 (Guindon et al., 2010). 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 0.556 and with a gamma shape parameter of 0.618. To estimate the level of genetic divergence, uncorrected p pairwise genetic distances were estimated using PAUP 4.0b10. (Swofford, 2002).

3. Results and discussion

3.1. Descriptions of two new species

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

Female. Body length 2.0 mm. ***Head***. Slightly narrower than width of thorax. Frons dark brown, densely covered with yellowish-white scale-like recumbent short hairs interspersed with few dark longer hairs near vertex; frontal ratio 1.5:1.0:1.9; frons:head ratio 1.0:4.5. Frontoocular area well developed, narrow, directed dorsolaterally. Clypeus dark brown, densely covered with yellowish-white scale-like hairs interspersed with eight or nine dark longer hairs on each side. Labrum 0.6 times length of clypeus. Antenna composed of scape, pedicel and nine flagellomeres, light to medium brown except scape, pedicel and basal half of first flagellomere whitish yellow. Maxillary palp composed of five segments, light to medium brown, proportional lengths of third, fourth, and fifth segments 1.0:1.2:2.8; third segment (Fig. 1A) widened apically; sensory vesicle (Fig. 1A) small, ellipsoidal (0.26–0.28 times length of third segment), with medium-sized opening. Maxillary lacinia with 11 inner and 13 or 14 outer teeth. Mandible (Fig. 1B) with 23 inner teeth and no distinct outer teeth, though outer margin indented near apex and appearing to have few to several vestigial teeth. Cibarium (Fig. 1C) medially forming sclerotized plate folded forward from posterior margin, with moderately sclerotized mediolongitudinal ridge with bifid apex. ***Thorax***. Scutum bright medium brown except anterolateral calli ochreous, and three dark-brown longitudinal vittae (one median, two submedian), thinly pruinose, shiny when illuminated at certain angles, densely covered with whitish-yellow or yellow scale-like recumbent short hairs. Scutellum ochreous, covered with yellow short hairs and dark-brown long upright hairs along posterior margin. Postnotum dark brown, slightly shiny when illuminated at certain angles, and bare. Pleural membrane bare. Katepisternum longer than deep, medium to dark brown, shiny when illuminated at certain angles, moderately covered with fine short hairs. ***Legs***. Foreleg: coxa yellowish white; trochanter dark yellow; femur dark yellow with apical cap light brown (though extreme tip yellowish, and most of inner surface yellowish white); tibia white except apical one-fourth medium brown; tarsus dark brown, with moderate dorsal hair crest; basitarsus moderately dilated, 6.0 times as long as its greatest width. Midleg: coxa light brown except posterolateral surface medium brown; trochanter whitish yellow; femur dark yellow to light brown with basal one-fifth whitish yellow and apical cap medium brown (though extreme tip yellowish); tibia grayish except basal two-fifths yellowish white and apical cap dark brown (though basal four-fifths of inner surface yellowish white); tarsus dark brown except basal half of basitarsus yellowish white (though its border not well defined). Hind leg: coxa light brown except apical half yellowish; trochanter whitish yellow; femur light brown with base whitish yellow and apical cap dark brown (though extreme tip yellowish white); tibia (Fig. 1D) yellowish white on basal two-thirds and dark brown on rest; tarsus dark brown except basal three-fifths (though base light brown) and basal half of second tarsomere yellowish white; basitarsus (Fig. 1E) narrow, nearly parallel-sided though slightly narrowed apically, 6.5 times as long as wide, and 0.6 and 0.5 times as wide as greatest widths of tibia and femur, respectively; calcipala (Fig. 1E) slightly longer than width at base, and 0.6 times as wide as greatest width of basitarsus; pedisulcus (Fig. 1E) well developed; claw (Fig. 1F) with large basal tooth 0.48 times length of claw. ***Wing***. Length 2.0 mm. Costa with dark spinules and hairs except basal patch of hairs yellow. Subcosta with dark hairs except near apex bare. Hair tuft on base of radius yellow. Basal portion of radius fully haired; R_1 with dark spinules and hairs; R_2 with hairs only. Basal cell absent. ***Halter***. White except basal portion slightly darkened. ***Abdomen***. Basal scale ochreous, with fringe of whitish-yellow hairs. Dorsal surface of abdomen medium to dark brown except anterior three-fifths of segment 2 whitish, moderately covered with dark short to long hairs; tergites of segments 2 and 6–9 shiny when illuminated at certain angles. Ventral surface of segment 2 white, those of segments 3 and 4 ochreous and those of other segments medium brown; sternal plate on segment 7 undeveloped. ***Terminalia***. Sternite 8 (Fig. 1G) bare medially, with 20–24 medium-long to long hairs together with three or four slender short hairs on each side. Ovipositor valve (Fig. 1G) triangular (though posteromedial corner rounded), thin, membranous, moderately covered with microsetae interspersed with one or two short hairs; inner margins slightly sinuous, somewhat sclerotized, and moderately separated from each other. Genital fork (Fig. 1H) of usual inverted-Y form, with slender stem; arms of moderate width, moderately folded medially, with short unpigmented lobe directed posteromedially. Paraproct in ventral view (Fig. 1I) ellipsoidal, unpigmented anterolaterally, with three sensilla on anteromedial surface; paraproct in lateral view (Fig. 1J) slightly produced ventrally beyond ventral tip of cercus, 0.7 times as long as wide, with 26–29 medium-long to long hairs on ventral and lateral surfaces. Cercus in lateral view (Fig. 1J) short, rounded posteriorly, 0.4 times as long as wide. Spermatheca (Fig. 1K) ellipsoidal, 1.4 times as long as its greatest width, well sclerotized except duct and small area near juncture with duct unsclerotized, and with many fissures on outer surface; internal setae absent; both accessory ducts slender, subequal in diameter to major one.

Male. Body length 2.5–2.6 mm. ***Head***. Somewhat wider than thorax. Upper eye bright medium brown, consisting of large facets in 15 (rarely 14) vertical columns and 15 (rarely 16) horizontal rows. Face brownish black, white pruinose. Clypeus brownish black, whitish pruinose, densely covered with golden-yellow scale-like medium-long hairs (mostly directed upward) interspersed with six or seven dark-brown unbranched longer hairs on each side. Antenna composed of scape, pedicel and nine flagellomeres, light brown except scape, pedicel and base of first flagellomere whitish yellow; first flagellomere elongate, 1.7 times length of second one. Maxillary palp light to medium brown, with five segments, proportional lengths of third, fourth, and fifth segments 1.0:1.1–1.3:2.4–3.0; third segment (Fig. 2A) slender; sensory vesicle (Fig. 2A) small, globular or ellipsoidal (0.18–0.23 times length of third segment), and with small opening. ***Thorax***. Scutum medium to dark brown except anterolateral calli ochreous, with dark longitudinal vittae (one median, two submedian, only visible if scutum