

***Simulium bakalalanense*, a new species of the subgenus *Gomphostilbia* and three new records of black flies (Diptera: Simuliidae) from Mount Murud, Sarawak, Malaysia**

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Abstract. *Simulium* (*G.*) *bakalalanense*, is described based on male, pupa and larvae collected in Mount Murud, Sarawak, Malaysia. *S. (G.) bakalalanense* sp. nov. is placed in the *S. batoense* species-group and is distinguished from most of known species of the group by the pupal gill with an inflated basal fenestra, shoe-shaped cocoon, and lack of grapnel-shaped hooklets on the pupal abdominal segment 9. Three known species, *S. (G.) terengganuense* Takaoka, Sofian-Azirun & Ya'cob from Peninsular Malaysia, *S. (Simulium) alberti* Takaoka from Sabah, and *S. (S.) beludense* Takaoka from Sabah, are newly recorded from Sarawak.

INTRODUCTION

Adult black flies (Diptera: Simuliidae) are small (1.5–6.0 mm long), two-winged insects, and are well known as one of the biting flies of medical and veterinary importance. Females of certain species, when they bite and take a blood, not only cause severe skin diseases to human and cattle but also play as vectors of viral, protozoan and filarial diseases in humans and animals (Adler *et al.*, 2004, Takaoka *et al.*, 2012). In the Oriental Region, no such problems caused by black flies have been known except a few studies that reported the role of black flies in the transmission of zoonotic filariae in Thailand (Takaoka *et al.*, 2003, Fukuda *et al.*, 2003; Choochote *et al.*, 2005; Ishii *et al.*, 2008).

Black flies are suitable for studies on biodiversity and phylogenetic relationships through analyses of larval salivary gland chromosomes and of DNA sequences of certain genes. All these researches on medical, ecological and genetic aspects of black flies could not be reliably proceeded

without sufficient basic knowledge of the fauna of black flies established by traditional but still important morphotaxonomy.

Overall, Sarawak holds 17 species of the genus *Simulium* Latreille, of which 12 are in the subgenus *Gomphostilbia* Enderlein, one in the subgenus *Nevermannia* Enderlein and four in the subgenus *Simulium* (Takaoka 2001, 2008a, b, 2009, 2012; Takaoka & Leh, 2009).

In 2013, we conducted surveys across Bakalalan and Mount Murud and collected four new species (two of the subgenus *Gomphostilbia* and the other two of the subgenus *Simulium*), of which one *Gomphostilbia* species are described here based on male, pupa and mature larvae. Further, notes are provided for the three species, *S. (G.) terengganuense* Takaoka, Sofian-Azirun & Ya'cob from Peninsular Malaysia (Takaoka *et al.*, 2012), *S. (Simulium) alberti* Takaoka and *S. (Simulium) beludense* Takaoka, both from Sabah (Takaoka 1996, 2008a), all of which are newly recorded from Sarawak.

The methods of collection, description and illustration, and terms for morphological features used here, follow those of Takaoka (2003) and partially those of Adler *et al.* (2004).

The holotypes and paratypes of these new species are deposited in the Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.

***Simulium (Gomphostilbia) bakalanense* Ya'cob, Takaoka and Sofian-Azirun, sp. nov.**

Male (n=1). Body length 2.6 mm. **Head.** Slightly wider than thorax. Upper eye consist of 11 vertical columns and 13 horizontal rows of large facets. Clypeus brownish black, whitish pruinose covered with golden yellow short hairs interspersed with medium brown hairs. Face white pruinose. Antenna composed of scape, pedicle and nine flagellomeres, dark brown except scape, pedicel and first flagellomere yellowish; first flagellomere elongate, 1.9 times length of second flagellomere. Maxillary palp with five segments; proportional lengths of third, fourth, and fifth segments 1.0:1.2:3.0, sensory vesicle small, 0.13 times as long as third segment, with small opening medially (Fig. 1A). **Thorax.** Scutum dark brown, thinly white pruinose peripherally, covered with bright golden-yellow recumbent short hairs, somewhat shiny when illuminated at certain angles; medium longitudinal vittae dark brown, with golden yellow recumbent short hairs. Scutellum light brown, with golden yellow short recumbent hairs and several long marginal hairs. Postnotum dark brown, thinly white pruinose and bare. Pleural membrane bare. Katepisternum brownish-black, moderately covered with dark short hairs. **Legs.** *Foreleg:* coxa dark yellow; trochanter medium brown except base of posterior surface yellow; femur medium brown except apical cap dark brown; tibia medium brown except apical cap dark brown and basal tip dark yellow; tarsus dark brown; basitarsus slender slightly dilated, 8.4 times as long as its greatest width. *Midleg:* coxa medium brown on ventral surface and dark brown on posterolateral surface; trochanter

light brown except base yellow; femur medium brown except apical cap dark brown; tibia dark brown except basal one-fourth yellow; tarsus brownish black except base of basitarsus somewhat lighter or paler. *Hindleg:* coxa light brown; trochanter yellow; femur light brown except basal tip yellow and apical cap dark brown; tibia (Fig. 1B) dark yellow to light brown on basal half though base whitish, and medium brown on rest though apical cap brownish black (posterior surface darkened on apical two-thirds; tibia covered with golden yellow hairs on basal two-thirds; basitarsus slender, almost parallel-sided, whitish except apical half brownish black; basitarsus 6.33 times as long as its greatest width; and 0.56 and 1.94 times as wide as greatest width of tibia and femur, respectively; calcipala well-developed, nearly as long as wide, pedisulcus well developed. **Wing.** Length 2.2 mm. Similar to that of male of *S. hiroyukii* sp. nov. except costa with patch of golden-yellow hairs basally. **Abdomen.** Basal scale light brown, with fringe of dark hairs. Dorsal surface of abdomen light to dark brown except basal half of segment 2 or little less yellow; segments 5–8 each with pair of dorsolateral shiny, thinly whitish pruinose spots. **Genitalia.** Coxite in ventral view (Fig. 1D) nearly rectangular, 1.9 times as long as wide. Style in ventrolateral view (Fig. 1E) nearly parallel-sided from base to apical one-third, then tapered toward apex; style in medial view (Fig. 1F) 0.96 times length of coxite, gently curved inward, with apical spine. Ventral plate in ventral view (Fig. 1D) transverse, subquadrate, 0.42 times as long as wide, with anterior margin produced anteriorly, covered with microsetae almost entirely on ventral and posterior surface; basal arm slightly convergent; ventral plate in lateral view (Fig. 1G) much produced ventrally; ventral plate in caudal view (Fig. 1H) pointed medioventrally, densely covered with microsetae on middle of posterior surface. Paramere (Fig. 1I) with three long parameral hooks and several small, indistinct ones. Median sclerite weakly sclerotized, plate-like, widened toward tip, with round, unpigmented tip. Cercus in lateral view (Fig. 1J) encircled by 11–13 simple hairs.

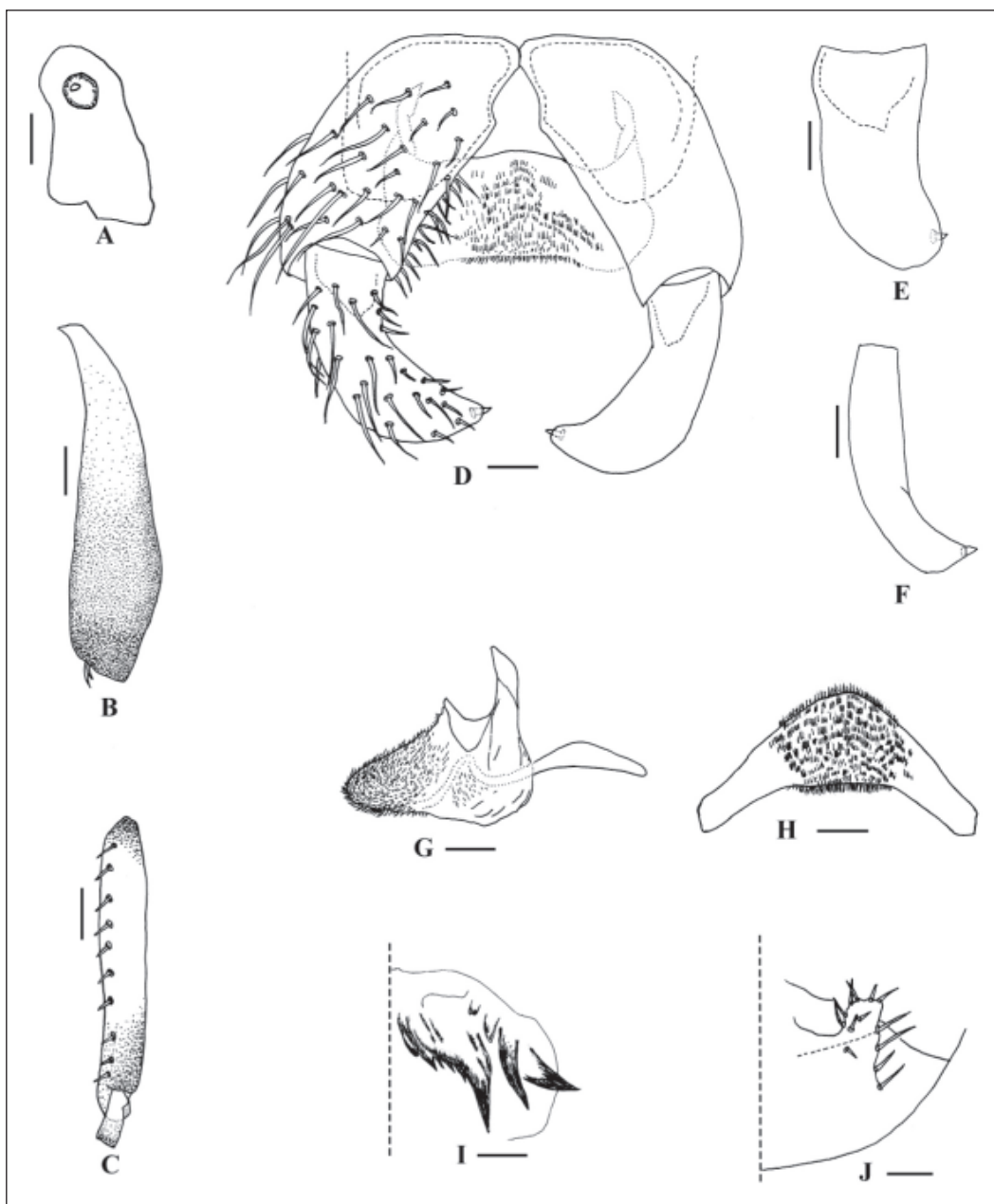


Figure 1. Male of *S. bakalalanense* sp. nov. (A) Third segment of right maxillary palp with sensory vesicle (front view). (B) Tibia of left hind leg. (C) Basitarsus and second tarsomere of left hind leg showing calcipala and pedisulcus (outer view). (D) Coxites, styles and ventral plate (ventral view). (E) Left style (ventrolateral view). (F) Left style (side view). (G) Ventral plate (caudal view). (H) Ventral plate and median sclerite (lateral view). (I) Paramere with 6 hooks in at both sides (end view). (J) Right half of 10th abdominal segments with cercus (lateral view). Scale bars = 0.02 mm (A, D-J) and 0.1 mm (B-C).

Pupa (n=1). Body length 2.8 mm. **Head.** Integument yellow and bare; antennal sheath without any tubercles; frons with three pairs of unbranched long trichomes with coiled and

uncoiled apices; face with pair of unbranched long trichomes with coiled apices; three frontal trichomes on each side arising close together, subequal in length to one another.

Thorax. Integument yellow and bare except dorsal surface of posterior one-third moderately covered with small round tubercles; thorax with three unbranched long mediodorsal trichomes with coiled apices, two unbranched long anterolateral trichomes with coiled apices, one unbranched medium-long mediolateral trichome with coiled

apex, and three unbranched ventrolateral trichomes (one medium-long with coiled apex, two short with uncoiled apices), on each side. Gill (Fig. 2A–C) composed of eight slender filaments, arranged as [2+1+(1+2)]+2 filaments from dorsal to ventral, with swollen transparent basal fenestra at base; common basal stalk medium-long

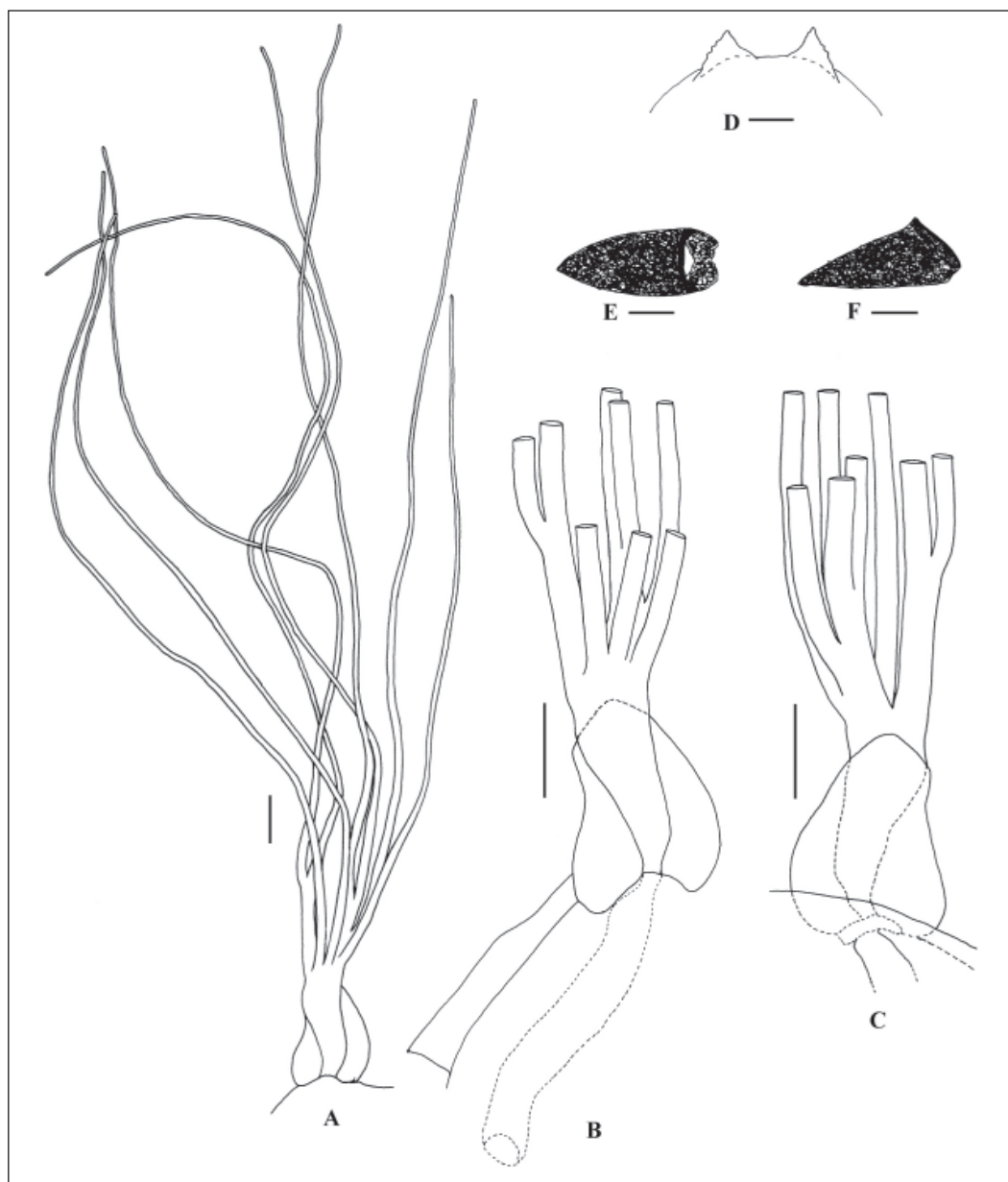


Figure 2. Pupa of *S. bakalalanense* sp. nov. (A) Gill filaments of a female pupa (right side, dorsal view). (B) Gill filament (dorsal view). (C) Gill filament (ventral view). (D) Terminal hooks of the ninth abdominal segment. (E) Cocoon (dorsal view). (F) Cocoon (lateral view). Scale bars = 0.1 mm (A–C), 0.02mm (D) and 1.0 mm (E and F).

(0.2 mm) 0.6 times as long as interspiracular trunk; dorsal individual and paired filaments arising basally from dorsal surface of primary stalk of inner ventral triplet; dorsal paired filaments almost sessile, arising close to, but slightly apart from individual filament. Ventral inner triplet (Fig. 2C) with short primary and secondary stalks; ventral outer paired filaments (Fig. 2C) with medium-long stalk, 0.9 times the length of common basal stalk. All filament brownish, tapered toward apex and subequal in length to one another. **Abdomen.** Dorsally segments 1 and 2 not pigmented and without tubercles; segment 1 with slender medium-long hair-like seta on each side; segment 2 with one slender medium-long hair-like seta and five short somewhat spinous setae submedially on each side; all setae unbranched; segments 3 and 4 each with four hooked spines and one unbranched short somewhat spinous seta on each side; segment 5 lacking spine-combs; segments 6–9 each with spine-combs in transverse row and comb-like groups of minute spines on each side; segment 9 with pair of widened triangular terminal hooks (Fig. 2D), of which outer margin is 1.55 times length of inner margin and partially crenulated. Ventrally, segment 4 with one unbranched somewhat dark pigmented hooklet and few unbranched slender short setae on each side; segment 5 with pair of bifid or trifid hooks submedially and few unbranched slender short setae on each side; segments 6 and 7 each with one bifid inner

hook (outer hook replaced by unbranched and unpigmented seta on left side of segment 8 in one pupa) and few unbranched slender short setae on each side; segments 4–8 each with comb-like groups of minute spines. Each side of segment 9 one with unbranched colorless seta but no grapnel-shaped hooklets. **Cocoon.** Shoe-shaped (Fig. 2E, F), tightly woven, individual threads visible; anterior margin thickly woven; posterior half with floor tightly woven; 3.1 mm long by 1.2 mm wide.

Mature larva (n=2). Body length 5.0 mm. Body dark gray, though intersegmental areas from abdominal segment 1 to segment 5 narrowly lighter. Cephalic apotome yellowish except narrow area along posterior margin somewhat darkened in one larva; head spots moderately positive in one larva but indistinct except anterior and posterior longitudinal spots positive in one larva. Lateral surface of head capsule yellow except eye-spot region white and posterior half darkened so that two large spots and one small spot near posterior margin obscured (though these spots are positive in another larva); eyebrow distinct. Ventral surface of head capsule (Fig. 3A) yellow except medial large portion darkened, and basal area on each side of postgenal cleft dark brown; one elongate and one round spot on each side of postgenal cleft markedly positive. Cervical sclerite composed of two faint small elliptical pieces, not fused to occiput, widely separated

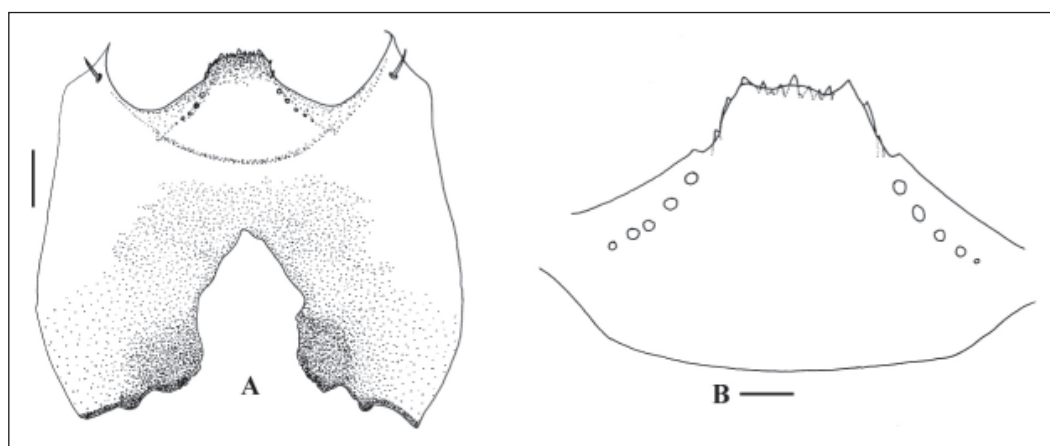


Figure 3. Larva of *S. bakalalanense* sp. nov. (A) Larva head capsule. (B) Hypostoma. Scale bars = 0.02 mm (A and B).

medially from each other. Antenna consisting of three articles and apical sensillum, much longer than stem of labran fan; proportional lengths of first, second, and third articles 1.00:0.74:1.04. Labran fan with 42 or 43 main rays. Mandible with serrations consisting of two teeth (one large and one small); large tooth at acute angle with mandible on apical side; comb-teeth composed of three teeth shortened from first to third; supernumerary serration absent. Hypostoma (Fig. 3B) with nine apical teeth in row; median and corner teeth well developed; lateral margin nearly smooth; five hypostomal bristles lying slightly divergent posteriorly from lateral margin on each side. Postgenal cleft (Fig. 3A) medium-long, 2.7 times length of postgenal bridge. Thoracic cuticle bare. Histoblast of pharate pupal gill with eight slender thread-like filaments. Abdominal cuticle bare except both sides of anal sclerite moderately covered with unbranched colorless setae and each lateral surface just above ventral papilla also sparsely covered with unbranched colorless setae. Rectal scales minute, colorless and secondary lobules are uncountable. Anal sclerite of usual X-form, with anterior arms nearly as long as posterior ones, broadly sclerotized at basal juncture; sensilla absent just posterior to basal juncture area; accessory sclerite absent. Last abdominal segment much expanded ventrolaterally forming large ventral papilla on each side. Posterior cirlet with 71 rows of up to 13 hooklets per row.

Female. Unknown.

Type specimens. Holotype male (with associated pupal exuviae and cocoon) (preserved in 80% ethanol), reared from pupa, collected at Church Camp, Mount Murud, Sarawak, at altitude 2,115 m (03°55'36.5"N, 115°30'50.6"E), 13. VI. 2013, by Z. Ya'cob. Paratypes: two mature larvae, same data as holotype.

Ecological notes. As noted under *S. (G.) hiroyukii* sp. nov.

Etymology. The species name *bakalalanense* refers to the Bakalalan Province, where this new species was collected.

Discussion. This new species is assigned in the *S. batoense* species-group of the subgenus *Gomphostilbia* redefined by Takaoka (2013) in having nine flagellomeres, pleural membrane bare, male hind basitarsus slender and almost parallel-sided (Fig. 1C), dark hair tuft at the base of the radial vein, hind tibia darkened on the apical two-thirds of the posterior surface (Fig. 1B), and pupal gill with eight filaments (Fig. 2A). In the key to subgroups of this group based on pupal characters (Takaoka 2012), this new species is placed to the *S. parahiyangum* subgroup.

The pupa of the *S. (G.) bakalalanense* sp. nov. is most striking in having the inflated basal fenestra, a rare character among species of the subgenus *Gomphostilbia*. Similarly, histoblast of the mature larvae with 8 slender gill filaments with an inflated basal fenestra arranged as that of pupa gill. *S. (G.) baglungense* Takaoka & Shrestha from Nepal was reported to have a similar basal fenestra (Takaoka and Shrestha 2010). However the pupa of this new species can be distinguished from that of *S. (G.) baglungense* by having an oblong inflated basal fenestra and a moderate length of common basal stalk, which is almost equal to the length of the interspiracular trunk. Whereas *S. (G.) baglungense* is characterized by having a smaller round inflated basal fenestra and a very short common basal stalk (Takaoka and Shrestha 2010). The pupa of this new species is also remarkable in lacking grapnel-shaped hooklets on the abdominal segment 9 and having a shoe-shaped cocoon, both characters very rarely reported in the subgenus *Gomphostilbia*.

Notes on newly recorded species

***Simulium (Gomphostilbia) terenggauense* Takaoka, Sofian-Azirun & Ya'cob, 2012**

Simulium (Gomphostilbia) terenggauense Takaoka, Sofian-Azirun & Ya'cob, 2012: 804–810 (female, male, pupa and larva).

Specimens examined. Two males reared from pupae collected from two small waterfalls (alt/location: 1,385 m/ N03°57'38.4"E115°34'19.6" and 1,316 m/ N03°57'47.7"E115°34'00.5") located near

the logging road along Bakalalan to the mount Murud, 10. VI. 2013.

Ecological Notes. Pupae were collected from two small, moderate to slow running streams (width/depth/water temperature/shade/vegetation: (i) 0.5–1.0 m/ 2–3 cm/ 21.8°C/ half shaded/natural forest, (ii) 0.8–1.0 m/ 7–11 cm/ 20.6 °C/ shaded/ natural forest. This species was collected together with *Simulium* (*G.*) *auratum* Takaoka 2009 and *Simulium* (*G.*) *paukatense* Takaoka 2008.

Distribution. Peninsular Malaysia (Terengganu) and Sarawak (**new record**)

Remarks. This species was assigned to the *S. batoense* species-group and further placed in the *terengganuense* subgroup defined by Takaoka (2012) by having the mushroom-like terminal hooks.

***Simulium* (*Simulium*) *beludense* Takaoka, 1996**

Simulium (*Simulium*) *beludense* Takaoka, 1996:157–161 (male and female)

Specimens examined. Four males, three females reared from pupae were collected from three streams in Bakalalan village (altitude/location/ date: (i) 928 m/ N03°50'10.4" E115°36'52.1"/ 9. IV.2013, (ii) 932 m/ N03°57'51.1" E115°37'03.6"/ 16.IV.2013, (iii) 938 m/ N03°50'20.8" E115°37'14.2"/ 16.IV.2013).

Ecological Notes. Pupae were collected from stream plant vegetation, fallen leaves and twigs in three moderately-flowing streams (width/depth/water temperature/shade/vegetation: (i) 3.5–4 m/ 10–15 m/ 23.3°C/ open/ secondary growth, (ii) 0.9–1.3 m/10–13 cm/25.3°C/ shaded/ natural forest, (iii) 1.5–1.6 m/10–14 cm/26.6°C/ shaded/ natural forest. This species was collected together with *Simulium* (*S.*) *keningauense* Takaoka and *Simulium* (*G.*) *parahiyangum* Takaoka and Sigit in all the three streams while *Simulium* (*G.*) *kelabitense* Takaoka and *Simulium* (*N.*) *aureohirtum* Brunetti were collected separately from different streams.

Distribution. Sabah and Sarawak (**new record**)

Remarks. This species was assigned in *S. argentipes* species-group within the subgenus *Simulium* (Takaoka 1996).

***Simulium* (*Simulium*) *alberti* Takaoka, 2008**

Simulium (*Simulium*) *alberti* Takaoka, 2008: 56–62 (male, female, pupa and larva)

Specimens examined. Two females reared from pupa, five pupae and six pupal skin collected from a stream (alt. 2,115 m) located at Church Camp station (N03°55'36.5" E115°30'50.6"), 13.IV.2013. One female reared from pupa collected in a stream (alt.1,385 m) located along the road from Pa'Rabata to Lepo Bunga (N03°57'27.7" E115°33'30.8"), 11.IV.2013. One male reared from a pupa collected from a stream (alt. 1,379 m) between Bakalalan and Pa'Rabata (N03°57'02.1" E115°34'13.5"), 10.IV.2013.

Ecological Notes. The pupae of this species were collected from fallen leaves, twigs and trailing aquatic plants in three streams of moderate to slow-flowing (width/depth/ temperature/shade/vegetation: (1) 0.3 m/ 4–15.5 cm/ 14.4°C/half shaded/natural forest, (2) 0.5 m/ 2–3 cm/ 20.1°C/ shaded/ natural forest, (3) 0.7–1.0 m/ 15–20 cm/ 20.2°C/ shaded/ natural forest. This species was collected together with *Simulium* (*G.*) *hiroyukii* sp. nov., *S.* (*G.*) *bakalalanense* sp. nov., *S.* (*G.*) *capillatum*, *S.* (*G.*) sp-1 and *S.* (*S.*) sp-2.

Distribution. Sabah (Mesilau), Sarawak (**new record**)

Remarks. This species was assigned to the *S. tuberosum* species-group within the subgenus *Simulium* (Takaoka 2008).

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