Descriptions of the male, pupa and larva of Simulium (Gomphostilbia) novemarticulatum (Diptera: Simuliidae) from Peninsular Malaysia and Thailand

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Abstract: The male, pupa, and larva of Simulium (Gomphostilbia) novemarticulatum Takaoka and Davies, 1995 are described morphologically on the basis of specimens collected from Peninsular Malaysia (type country) and southern Thailand. This species is very similar to S. (G.) charlesi Takaoka, 2008 from Sarawak but readily distinguished in the male by the slender style, in the pupa by the ventral paired filaments of the gill of almost the same size, and in the larva by the bifid dark thick setae on the dorsal and dorsolateral surfaces of abdominal segments 5-8. The difference between S. (G.) novemarticulatum and S. (G.) charlesi, as well as the identification of Thai specimens as S. (G.) novemarticulatum, is confirmed molecularly.

Key words: black fly, Simulium, Simuliidae, Malaysia, Thailand

Simulium (Gomphostilbia) novemarticulatum was originally described from female specimens collected near Bentong, Pahang Province, Malaysia and assigned to the varicorne species-group (Takaoka and Davies, 1995), and later recorded from Thailand on the basis of female specimens reared from pupae collected from Khao Yai National Park, Nakhon Ratchasima Province (Phasuk et al., 2005). This species is distinctive among this species-group in that the antenna of the female is composed of the scape, pedicel, and seven flagellomeres (in place of eight flagellomeres). The male, pupa and mature larva of S. (G.) novemarticulatum remained to be described although Phasuk et al. (2005) gave photographs of the pupal gill filaments and setae on the larval abdomen and briefly described the larval postgenal cleft based on specimens collected from Khao Yai National Park, Thailand.

In this paper, we describe the male, pupa and mature larva of S. (G.) novemarticulatum on the basis of specimens collected from Gua Musang, Kelantan Province, Malaysia and Khao Sok National Park, Surat Thani Province, southern Thailand.

The terms for morphological features used here follow those of Takaoka (2003). All the specimens examined are deposited at the Department of Infectious Disease Control, Faculty of Medicine, Oita University, Oita, Japan.

Simulium (Gomphostilbia) novemarticulatum
Takaoka and Davies, 1995

DESCRIPTION. Male. Body length 1.9–2.0 mm. Head. Wider than thorax. Upper eye consisting of 11 (rarely 10 or 12) vertical col-
umns and 12 horizontal rows of large facets. Face dark brown, whitish-gray pruinose. Clypeus dark brown, densely covered with yellow scale-like short to medium-long hairs (mostly directed upwards) interspersed with several dark brown simple longer hairs on each side of lower 1/2. Antenna (Fig. 1A) composed of scape, pedicel and 7 flagellomeres, yellow except flagellomeres 3 and 5–7 entirely medium brown, and apical 3/4 of flagellomere 1 light brown; 1st flagellomere elongate, 1.63 times as long as 2nd flagellomere. Maxillary palp with 5 segments, light brown, proportional lengths of 3rd, 4th, and 5th segments 1:00 : 1.2 : 2.62; 3rd segment (Fig. 1B) somewhat widened apically; sensory vesicle (Fig. 1B) nearly ellipsoidal, 0.25 times as long as 3rd segment, and with medium-sized opening. Thorax. Scutum dark brown to brownish-black except anterior calli light brown, 5 faint longitudinal vitteae (1 medial, 2 submedial and 2 lateral) and prescutellar area black, shiny, thinly grayish-white pruinose, densely covered with yellowish scale-like recumbent hairs. Scutellum dark brown, covered with yellow short hairs and dark brown long upright hairs along posterior margin. Postnotum dark brown, slightly shiny, thinly grayish-white pruinose when viewed at certain angle of light, and bare. Pleural membrane bare. Katepisternum dark brown, longer than deep, shiny, moderately covered with dark hairs interspersed with yellow ones. Legs. Foreleg: coxa yellow; trochanter, femur and tibia light to medium brown with apex of femur yellowish; tarsus brownish-black, with moderate dorsal hair crest; basitarsus moderately dilated, 5.63–6.20 times as long as its greatest width. Midleg: coxa medium brown except posterior surface brownish-black; trochanter light brown except base yellow; femur light to medium brown with apex narrowly yellow; tibia medium brown except basal 1/3 yellow; tarsus light brown except basal 3/5 of basitarsus, basal 1/4 of 2nd tarsomere and base of 3rd tarsomere yellow. Hind leg: coxa medium brown; trochanter yellow; femur medium brown with apical cap dark brown, base and apex narrowly yellow; tibia (Fig. 1C) whitish-yellow to yellow except apical 1/2 light to dark brown; tarsus (Fig. 1D) light to medium brown except basal 2/3 of basitarsus (though base light brown) and basal 1/2 of 2nd tarsomere whitish-yellow; basitarsus narrow, nearly parallel-sided, 5.90–6.43 times as long as wide, and 0.57–0.61 and 0.51–0.57 times as wide as greatest widths of tibia and femur, respectively; calcipala (Fig. 1D) well developed, slightly longer than width and 0.45 as wide as greatest width of basitarsus; pedisculus (Fig. 1D) well developed. Wing. Length 1.5 mm. Costa with dark spinules and hairs except basal portion with yellow hairs. Subcosta bare. Hair tuft on stem vein mostly yellow. Basal portion of radius fully haired; R1 with dark spinules and hairs; R2 with hairs only. Basal cell absent. Abdomen. Basal scale dark brown, with fringe of yellow hairs. Dorsal surface of 2nd segment yellow to light brown except narrow area along posterior margin medium brown, those of other segments medium brown to brownish-black, moderately covered with dark brown hairs; segments 2 and 5–7 each with pair of shiny whitish-gray pruinose dorsolateral patches, of which those on segment 2 broadly connected in middle to each other, and those on segments 5–7 not connected to each other. Genitalia. Coxite in ventral view (Fig. 1E) nearly rectangular, 1.75 times as long as its greatest width. Style in ventral view (Fig. 1E) short, 0.77 times as long as coxite, gradually tapered from base to apex, gently bent inward, with apical spine; style in ventrolateral view (Fig. 1F) gradually narrowed from base to near middle, nearly parallel-sided, and then tapered toward apex. Ventral plate in ventral view (Fig. 1E) with body transverse, 0.64 times as long as wide, slightly narrowed posteriorly, with anterior margin slightly produced anteromedially and posterodorsal margin nearly straight though slightly rounded medially, and densely covered with microsetae on ventral surface except portion along anterior margin bare; basal arms of moderate length, diverged basally, then directed forward; ventral plate in lateral view (Fig. 1G) much produced ventrally; ventral plate in end view (Fig. 1H) triangular ventrally, and moderately covered with microsetae on medial portion of posterior surface. Median sclerite (Fig. 1G, I) thin, plate-like, narrow, and connected to ventral plate apart from anterior margin. Paramere (Fig. 1J) with body strongly sclerotized, with 4 distinct long and stout hooks and several weakly developed ones. Aedeagal membrane (Fig. 1K) moderately setose. Abdominal segment 10 with ventral surface weakly sclerotized anteriorly, and without distinct hair near posterior margin on
Fig. 1. Male of Simulium (Gomphostilbia) novemarticulatum. A, antenna (right side and dorsal view); B, 3rd segment of maxillary palp with sensory vesicle (right side and front view); C, hind tibia (left side and outer view); D, basitarsus and 2nd tarsomere of hind leg showing calcipala and pedisulcus (left side and outer view); E, coxites, styles, ventral plate and median sclerite (ventral view); F, style (right side and ventrolateral view); G, ventral plate and median sclerite (lateral view); H, ventral plate (end view); I, median sclerite (end view); J, paramere (right side and dorsal view); K, paramere and aedeagal membrane (right side and end view); L and M, 10th abdominal segments and cerci (L, right side and lateral view; M, right side and end view). Scale bars. 0.04 mm for A–M.
ventral surface. Cercus (Fig. 1L, M) round, encircled with 9–13 hairs.

**Pupa.** Body length 2.0–2.4 mm. **Head.** Integument yellow to yellowish-brown, moderately covered with small round and conical tubercles on frons and area along posterior margin of each lateral surface but bare on antennal sheath, most of lateral surface and underside of face; antennal sheath without any protuberance; face with pair of simple very long trichomes with uncoiled apices, and frons with 3 pairs of simple very long trichomes with uncoiled apices; 3 frontal trichomes on each side arising close together, subequal in length to one another and slightly longer than facial one. **Thorax.** Integument yellow to yellowish-brown, moderately covered with round and conical tubercles, with 3 simple very long trichomes with coiled or uncoiled apices dorsomedially, 2 simple very long trichomes with uncoiled apices anterolaterally, 1 simple medium-long trichome with uncoiled apex posterolaterally, and 3 simple trichomes with uncoiled apices (1 long, 2 medium-long) ventrolaterally on each side. Gill (Fig. 2A) composed of 8 slender thread-like filaments arranged as $(1+1+1)+(1+2)+2$ or $(2+1)+(1+2)+2$ from dorsal to ventral (though right gill of 1 pupa from Malaysia arranged as $2+(2+2)+2$ as shown in Fig. 2B), with somewhat swollen transparent organ ventrally (partially broken) at base; dorsal triplet composed of 3 individual filaments or 1 individual and 2 paired filaments arising from short stalk directed upward from short common basal stalk (stalk of dorsal triplet very short in some pupae collected from Thailand–Fig. 2C); middle triplet composed of 1 individual and 2 paired filaments with short stalk directed forward and somewhat inward (middle triplet rarely composed of 3 individual filaments); filaments of dorsal and middle triplets subequal in length (0.8–1.5 mm long) and thickness to one another, though filaments of middle triplet always longer than those of dorsal triplet; filaments of ventral pair longest and thickest of all, subequal in length (1.8–2.5 mm) and thickness to each other or inner filament slightly longer and thicker than outer filament, and 1.5–2.0 times as long as 6 other filaments of dorsal and middle triplets; stalk of ventral paired filaments long, much longer than that of middle triplet, and directed forward; middle triplet and ventral pair rarely sharing very short stalk; stalk of upper triplet lying against that of lower pair at angle of 90–110 degrees when viewed laterally; all filaments light brown, gradually tapered toward apex; cuticle of all filaments with annular ridges and furrows though becoming less marked apically, densely covered with minute tubercles. **Abdomen.** Dorsally, segments 1 and 2 light yellow, very weakly tuberculate; other segments almost transparent; segment 1 with 1 medium-long simple seta on each side; segment 2 with 1 medium-long simple seta and 5 very short setae submedially on each side; segments 3 and 4 each with 4 hooked spines and 1 very short seta on each side; segment 5 lacking spine-combs; segments 6–8 each with spine-combs in transverse row and comb-like groups of minute spines on each side; segment 9 with pair of distinct conical terminal hooks (Fig. 2D) and comb-like groups of minute spines (though spine-combs developed on segment 9 in some pupae but much smaller than those on segment 8). Ventrally, segment 4 with 1 simple hook and few very short setae on each side; segment 5 with pair of bifid hooks submedially and few simple slender very 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 simple slender very short setae on each side; segments 4–8 with comb-like groups of minute spines. Each side of segment 9 with 3 grapnel-shaped hooklets. **Cocoon.** Wall-pocket shaped, moderately and neatly woven, extending ventrolaterally; anterior margin somewhat thickly woven; floor roughly or moderately woven; individual threads visible; 2.5–2.8 mm long by 1.3–1.5 mm wide.

**Mature larva.** Body length 3.3–4.0 mm. Body whitish-yellow, with brownish markings (Fig. 3A, B); thorax light brown except lateral surface of segments 2 and 3 whitish; abdominal segment 1 encircled with medium to dark brown broad band, abdominal segments 2 and 3 each encircled by light to medium brown band though widely disconnected dorsomedially and narrowly connected or disconnected ventromedially, abdominal segment 4 encircled by medium to dark brown band though widely disconnected dorsomedially, abdominal segment 5 encircled by medium to dark brown broad band (though much narrowed ventrolaterally) which is fused dorsally and ventrally with similar band on abdominal seg-
Fig. 2. Pupa of *Simulium* (*Gomphostilbia*) *novemarticulatum*. A, gill filaments of a pupa collected from Malaysia (left side and outer view); B, basal portion of gill filaments (abnormal form) of the same pupa which has normally arranged gill filaments on left side (A) (right side and outer view); C, basal portion of gill filaments of a pupa collected from Thailand showing a very short stalk of the dorsal triplet (right side and outer view); D, terminal hooks (end view). Scale bars. 0.1 mm for A–C; 0.01 mm for D.
ment 6, abdominal segment 7 encircled by medium to dark brown band which is disconnected dorsomedially and ventrolaterally and connected on each dorsolateral surface with those on abdominal segments 6 and 8, and abdominal segment 8 with medium to dark brown broad dorsal band extending dorsolaterally; (color markings of larval body reddish-brown and less distinct in Thai specimens, i.e., segment 1 encircled almost completely by broad band, segments 2 and 3 each colored area on each dorsolateral surface, segment 4 often encircled by broad band though disconnected dorsomedially, segment 5 encircled with broad band though widely disconnected ventrally and also disconnected to varying extent dorsomedially, segment 5 also bearing another transverse band lying posterior to major band on ventral surface, segment 6 with colored area on each lateral surface, segments 7 and 8 each with colored area on each dorsolateral surface though these colored areas on segment 8 often connected dorsomedially; the colored band on segment 1 always most marked, while other colored band or areas on other segments less marked and almost indistinct in some larvae; thoracic segment 3 and abdominal segments 3–5 each with pair of small conical dorsolateral protuberances and abdominal segments 1 and 2 each with 2 pairs of small conical or round dorsolateral protuberances (Fig. 3A, B). Cephalic apotome whitish-yellow to yellow, sparsely covered with simple minute setae; head spots very faintly positive. Lateral surface of head capsule whitish-yellow except eye-spot region whitish; eyebrow indistinct. Ventral surface of head capsule (Fig. 3C) whitish-yellow except narrow area along each lateral margin of postgenal cleft darkened; transverse spot on each side of postgenal cleft indistinct or faintly negative. Antenna composed of 3 segments and apical sensillum, all pale, somewhat longer than stem of labral fan; proportional lengths of 1st, 2nd, and 3rd segments 1.00 : 0.76–0.80 : 0.98–1.04. Labral fan with about 40 main rays. Mandible (Fig. 3D) with 3 comb-teeth, of which 1st tooth longest, 2nd tooth as long as or slightly longer than 3rd one; mandibular serration composed of 2 teeth (1 medium-sized and 1 small); major tooth at acute angle against mandible on apical side; supernumerary serrations absent. Hypostoma (Fig. 3E) with row of 9 apical teeth; median and each corner tooth prominent, subequal in length to each other, and much longer than 3 intermediate teeth on each side; lateral margin mostly smooth except apical portion weakly serrated; 3 or 4 hypostomal bristles per side, lying parallel to lateral margin. Postgenal cleft (Fig. 3C) moderately constricted basally, widest medially, very deep, reaching posterior border of hypostoma. Cervical sclerite not discernible. Thorax and abdominal segments 1–4 very sparsely covered with very minute simple and branched (bifid or trifid) almost colorless setae on dorsal and dorsolateral surface; abdominal segments 5–8 moderately or densely covered with very minute colorless setae each with 2–4 branches interspersed with dark thick bifid setae with apices transparent (thus making it difficult to observe exact shape of apical portion) (Fig. 3F) dorsally and dorsolaterally; last segment moderately covered with simple and branched (2–4 branches) colorless minute setae on each side of anal sclerite. Rectal scales absent. Rectal papilla compound, each of 3 lobes with 7 or 8 finger-like secondary lobules. Anal sclerite of usual X-form, with anterior arms nearly as long as posterior ones, broadly sclerotized at base; accessory sclerite absent; basal juncture area with no sensilla. Last abdominal segment expanded ventrolaterally forming double bulges on each side, visible as large conical ventral papilla when viewed from side. Posterior circlet with 62–64 rows of up to 12 hooklets per row.

SPECIMENS EXAMINED. Six females and 6 males with associated pupal exuviae and cocoon, all reared from pupae, preserved in 80% ethanol, 3 pupae and 8 mature larvae, collected from a moderately flowing stream (width 5–8 m, depth 0.3–0.5 m, water temperature 26.0°C, shaded, altitude 220 m), Gua Musang, Kelantan Province, Malaysia, 23. III. 1998, by H. Takaoka and H. Hayakawa; 2 females, 7 males (with associated pupal exuviae and cocoon, all reared from pupae), 1 pupa and 7 mature larvae collected from a slowly flowing river (width about 20 m, depth 10–30 cm, water temperature 26.5°C, exposed to sun, altitude 60 m), Khao Sok National Park, Surat Thani Province, southern Thailand, 12. V. 2009, by H. Takaoka, Y. Otsuka, W. Choochote, S.
Fig. 3. Mature larva of Simulium (Gomphostilbia) novemarticulatum. A and B, whole body showing dorsolateral protuberances and color markings on thorax and abdomen (A, dorsal view; B, lateral view); C, head capsule showing postgenal cleft (ventral view); D, mandible; E, hypostoma; F, bifid dark thick setae and branched colorless delicate setae on dorsal surface of abdominal segment 7. Scale bars. 0.5 mm for A and B; 0.04 mm for C; 0.02 mm for E; 0.01 mm for D and F.
E ECOLOGICAL NOTES. The pupae and larvae of this new species were collected from dead tree leaves in the water. Associated species were S. (G.) tahanense Takaoka and Davies and S. (Simulium) nobile De Meijere in Malaysia, and S. (G.) burtoni Takaoka and Davies, S. (G.) duolongum Takaoka and Davies and S. (G.) parahiyangum Takaoka and Sigit in southern Thailand.

REMARKS. The females, males, pupae and mature larvae collected from Gua Musang, Kelantan Province, Peninsular Malaysia are identified as S. (G.) novemarticulatum because several main morphological characteristics of the females are in good agreement with those of the original description (Takaoka and Davies, 1995) including the antenna which is composed of the scape, pedicel and seven flagellomeres. However, it should be noted that there are several differences in the following features (characteristics of the present female specimens versus those of type specimens in the original description): length ratio of the sensory vesicle against the third maxillary palpal segment (0.21–0.24 versus 0.25), numbers of inner and outer teeth of the maxillary lacinia (8 or 9 and 10–12 versus 10 and 13 or 14), number of the outer teeth of the mandible (8 versus 10), ratio of the greatest width of the frons against that of the head (1 : 9.24 versus 1 : 10), length ratio of the hind basitarsus against its greatest width (6.52–6.61 versus 7.7). In addition, the present female specimens have the clypeus moderately covered with dark brown long hairs intermixed with few to several whitish-yellow shorter hairs except the mediolongitudinal region widely bare (though it was described as “densely covered with whitish-yellow pubescence and dark hairs” in the original description), and the subcosta has hairs only on the basal 1/2 (though it was noted briefly as “subcosta haired” in the original description).

The females, males, pupae and mature larvae collected from Surat Thani Province, southern Thailand, and examined in this study agree morphologically with those of S. (G.) novemarticulatum collected from Peninsular Malaysia. The mature larvae from southern Thailand, though, have reddish-brown (in place of dark brown) markings on the abdominal segments, which are less distinct but essentially the same color pattern as that of the larvae from Malaysia. The morphological species identification of the specimens from southern Thailand, despite some differences in larval body color, is supported by comparing the sequences of the mitochondrial 16S rRNA gene. Among four larval specimens examined, three (two from Thailand, one from Malaysia) had the identical gene sequences of the 517 base pairs (Gene Bank accession number: AB535133) and one larva from Malaysia had almost the same sequence but one different base pair (Gene Bank accession number: AB535132).

Simulium (G.) novemarticulatum is very similar to S. (G.) charlesi described from Bario, Sarawak, Malaysia, in many characteristics including the adult antenna with nine segments (Takaoka, 2008).

The female and pupa of S. (G.) novemarticulatum are almost indistinguishable from those of S. (G.) charlesi, although there seem to be slight differences between the two species in the following features: length ratio of the sensory vesicle against the third maxillary palpal segment (0.21–0.25 versus 0.29–0.31), length ratio of the labrum against the clypeus (0.61–0.63 versus 0.53), length of the wing (1.5–1.7 mm versus 2.1–2.2 mm) in the female; and relative length and thickness of the inner filament against the outer filament of the ventral pair (subequal or slightly longer and thicker versus much longer and thicker) and the basal stalks of the middle triplet and the ventral paired filaments (usually not sharing the common stalk versus always sharing the
very short common stalk) in the pupa. On the other hand, the male and mature larva of *S. (G.) novemarticulatum* are easily distinguished from those of *S. (G.) charlesi* by the usual slender style (Fig. 1F) and the bifid thick dark setae on the dorsal and dorsolateral surfaces of abdominal segments 5–8 (Fig. 3F), respectively. The style is wide and angulated, and the thick dark setae on the larval abdomen are simple in *S. (G.) charlesi* (Takaoka, 2008). The sequence of the mitochondrial 16S rRNA gene of *S. (G.) charlesi* from the type locality (Accession number in Gene Bank: AB535134) differs from those of *S. (G.) novemarticulatum* by seven or eight base pairs, which are much greater than the maximum difference of three base pairs usually seen as an intraspecific variation (Unpublished data).

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**References**


