

DISCOVERY OF TWO MORE NEW SPECIES OF *SIMULIUM* (*MONTISIMULIUM*) (DIPTERA: SIMULIIDAE) IN DOI INTHANON NATIONAL PARK, CHIANG MAI, THAILAND

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Abstract: Two more new black-fly species of the rare subgenus *Simulium* (*Montisimulium*) were discovered in Doi Inthanon National Park, Chiang Mai, Thailand, where *S. (M.) merga* Takaoka and Choochote was known as the only named species. These two new species, *S. (M.) angkaense* sp. nov. and *S. (M.) laoleense* sp. nov., are described on the basis of the pupal and/or mature larvae. Both new species are easily distinguished from *S. (M.) merga* by the pupal gill with 12 slender filaments, and from all the 16 known species with 12 pupal gill filaments in other countries by the long and very long common basal stalk of the gill, respectively.

Key words: black fly, Simuliidae, *Simulium*, Thailand, new species, *Montisimulium*

Simulium (*Montisimulium*) Rubtsov is a small subgenus comprising 46 species [1], of which most species are morphologically very similar in the adult stage and their identification depends upon the differences in the shape and arrangement of the pupal gills [2]. This subgenus is very rarely collected because it often breeds in small temporary watercourses in high mountains and emerges only in certain months of the year. Most *Montisimulium* species are distributed in the Palaearctic Region and only five species have been reported from the Oriental Region [1]. In Thailand, this subgenus was thus far represented by only one unnamed species, *S. (M.)* sp. G, known only by larvae collected at Ang Ka (2,460 m in altitude) in Doi Inthanon National Park, Chiang Mai [3]. In 2004, we were able to collect a few pupae, probably of *S. (M.)* sp. G, at Ang Ka, and we described it as *S. (M.) merga* Takaoka and Choochote from reared adults, pupae and mature larvae, together with *S. (M.) surachaii* Takaoka and Choochote from a single female caught by a hand-net at the same locality [4].

Recently, we discovered several pupae and mature larvae of two more species of this subgenus in Doi Inthanon National Park, bringing the total number to three (so far as immature stages are concerned) in the same mountainous area. Coexistence of more than two species rarely occurs among the subgenus *Montisimulium*. Interestingly, it seems likely that these three species of *Montisimulium*, of which two utilize the same small streams, emerge at different times of the year, i.e., *S. (M.) laoleense* sp. nov. in Febru-

ary and *S. (M.) angkaense* sp. nov. in March and April at Siribhume Waterfall (1,400–1,500 m in altitude), and *S. (M.) angkaense* sp. nov. in February and *S. (M.) merga* in September through December at Ang Ka (2,460 m in altitude) according to our yearly surveys (unpublished data).

In this paper, we describe these two species as new to science on the basis of the pupal and/or larval specimens.

The terms for morphological features used here follow those of Takaoka [5]. Holotype and paratype specimens of the new species are deposited at the Department of Infectious Disease Control, Oita University.

Simulium (*Montisimulium*) *angkaense* sp. nov.

DESCRIPTION. Female and Male. Unknown.

Pupa. Body length 3.2–3.5 mm. **Head** (Fig. 1A). Integument yellowish-brown, moderately covered with large tubercles of various shapes each having several very minute nodule-like secondary projections on surface (Fig. 1B); antennal sheath moderately covered with smaller tubercles; frons with 2 short stout simple dark trichomes on each side, face with 1 long stout simple trichome with coiled apex (a little over twice as long as frontal trichomes) on each side. **Thorax.** Integument yellowish-brown, moderately covered with tubercles similar to those on head except lateral and posterior surfaces with smaller simple tubercles, with 3 long stout simple trichomes with coiled apex mediodorsally, 2 long simple trichomes (1 stout with coiled apex, 1 slender

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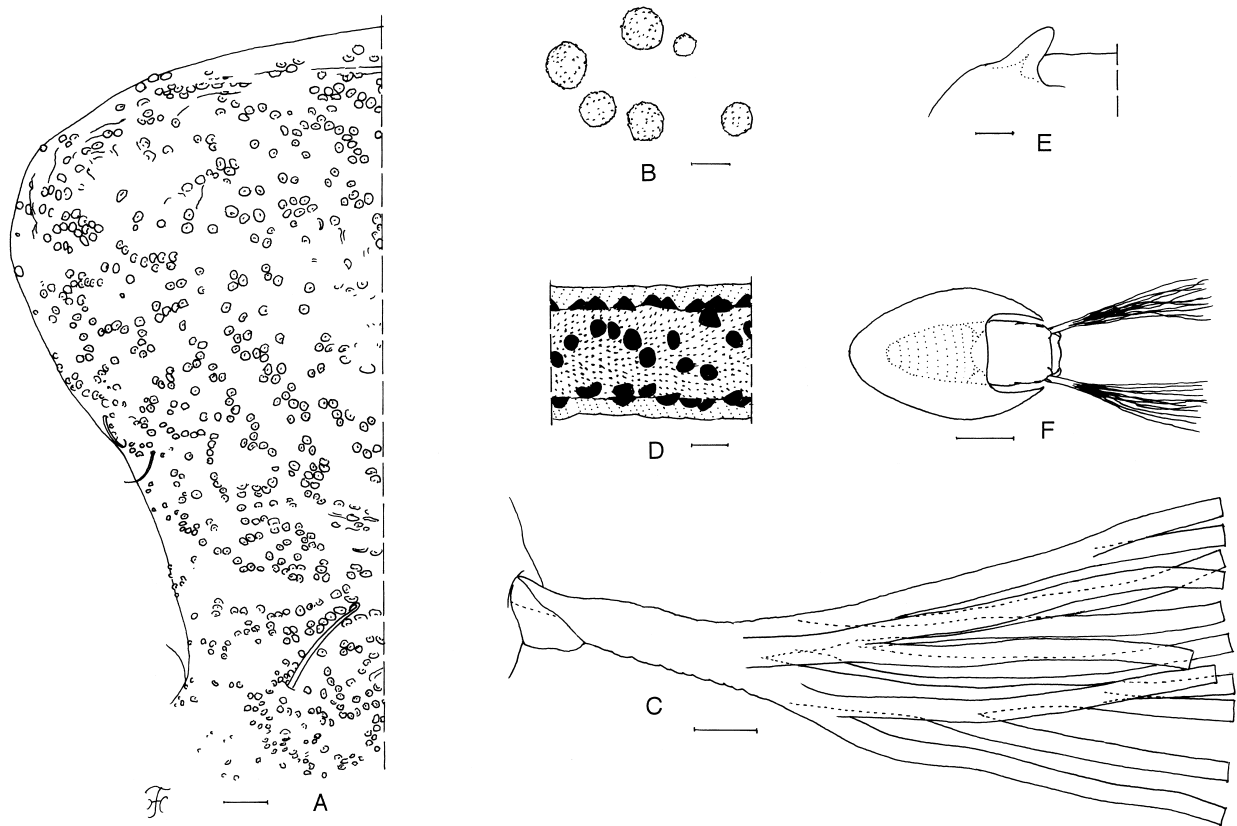


Fig. 1. Pupa of *Simulium (Montisimulium) angkaense* sp. nov. A, head integument with 2 frontal and 1 facial trichomes and with tubercles (right half, front view); B, large tubercles with secondary projections; C, basal portion of gill showing long common basal stalk and arrangement of 12 filaments (right side, outer view); D, enlargement of basal portion of gill filament showing many dark dots in outer cuticular layer; E, terminal hook (left side, end view); F, cocoon and pupa (dorsal view). Scale bars. 1.0 mm for F; 0.1 mm for C; 0.04 mm for A; 0.01 mm for B, D and E.

with uncoiled apex) mediolaterally, 1 long stout simple trichome with uncoiled apex posterolaterally, and 3 stout simple trichomes with uncoiled apex (1 long, 1 medium-long, 1 short) ventrolaterally, on each side. Gill (Fig. 1C) composed of 12 slender thread-like filaments closely arranged in 2 4 groups of filaments: e.g., $2+[2+(1+2)+2+(1+2)]$ or $[2+(1+2)]+2+[2+(1+2)]$ or $[1+1+(1+2)]+2+2+(1+2)$ or $2+2+[(1+2)+2]+(1+2)$, each group arising nearly at same level from long common basal stalk; all filaments light to medium brown, subequal in thickness to one another but somewhat different in length (2.5–3.5 mm long including common basal stalk) and, with numerous brownish-black to black small dots in surface cuticular layer at least on basal 3/4 (Fig. 1D), without annular ridges though annular furrows present irregularly. **Abdomen.** Dorsally, segments 1 and 2 weakly sclerotized and yellowish or yellowish-brown; segments 1 and 2 sparsely tuberculate; segment 1 with 1 long slender or stout simple dark hair with coiled or uncoiled apex on each side; segment 2 with 1 medium-long

slender dark hair and 5 short dark spines on each side; segments 3 and 4 light yellow, each with 4 dark hooks and 1 short dark spine on each side; segments 5–9 covered with comb-like groups of many minute spines on each side; segments 5 and 6 bare; segments 7 and 8 each with distinct spine-combs directed backward in transverse row on each side; segment 9 light yellow, with distinct horn-shaped terminal hooks (Fig. 1E). Laterally, segments 2–4 each with 3 short dark spines on each side; segment 9 without grapnel-like hooklets on each side. Ventrally, segments 3–8 nearly transparent and segment 9 weakly sclerotized and yellow; segments 3–8 with comb-like groups of minute spines; segment 4 with 1 simple or bifid dark hooklet submedially and a few slender dark setae on each side; segment 5 with pair of bifid dark hooks submedially and a few slender setae on each side; segments 6 and 7 each with pair of bifid inner and outer dark hooks and a few slender setae on each side. **Cocoon** (Fig. 1F). Wall-pocket-shaped, thin, moderately woven with no open spaces in webs, without anterodorsal

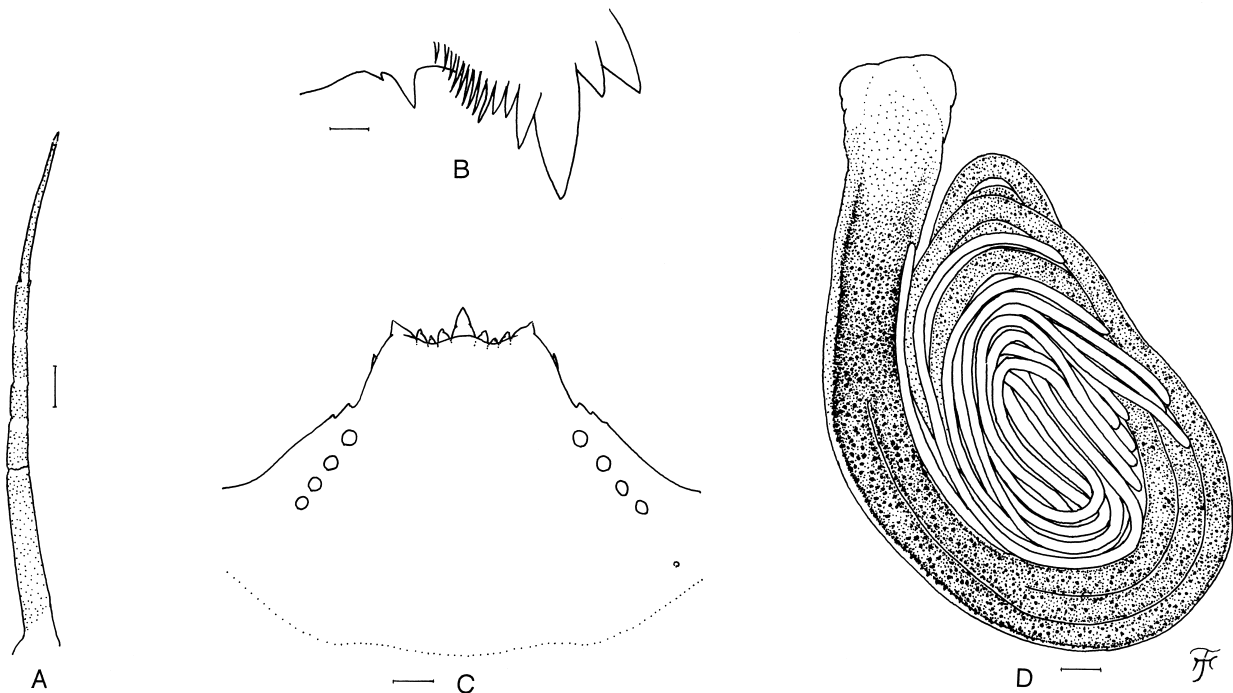


Fig. 2. Mature larva of *Simulium (Montisimulium) angkaense* sp. nov. A, antenna showing 3 thin unpigmented annular bands on segment 2; B, mandible; C, hypostomium; D, Pharate pupal gill (left side, outer view). Scale bars. 0.04 mm for A and D; 0.02 mm for C; 0.01 mm for B.

projection, and slightly extending ventrolaterally; individual threads invisible; 3.0–3.5 mm long by 1.8–2.4 mm wide.

Mature larva. Body length 6.5–6.8 mm. Body greyish, mottled with reddish-brown markings dorsally and laterally on segments 5–9 (Fig. 4A); abdomen, when viewed dorsally, equally narrow from segment 1 to segment 4, abruptly widened posteriorly from anterior margin of segment 5 to anterior margin of segment 6, then gradually narrowed toward segment 9; maximum width near border of segments 5 and 6 (though maximum width on segment 6 when viewed laterally). Cephalic apotome (Fig. 4D) yellow, with well-defined positive head-spots, or dark yellow to light brown (except narrow portions along both lateral margins light yellow), with positive head spots, of which posterolateral spots connected posteriorly to dark areas just anterior to posterior margin; lateral surface of head capsule dark yellow to light brown except eye-spot region clear yellowish-white, with dark broad eyebrow and dark area widely posterior to eye-spot region; 2 large and 3 small spots near posterior margin and 1 small spot just below eye-spot region positive, usually 2 large spots (and also all or 1 or 2 of 3 small spots) merged into dark background color; ventral surface of head capsule (Fig. 4E) dark yellow to medium brown (though narrow area along anterior margin and/or large area medially somewhat lighter in some larvae),

with dark brown basal area on each side of postgenal cleft; horizontal and round spots on each side of postgenal cleft distinctively positive (these spots seemed to merge into dark background color in a few larvae). Cervical sclerites composed of 2 small elliptical pieces, not fused to occiput, very widely separated medially from each other. Antenna (Fig. 2A) consisting of 3 segments and apical sensillum, much longer than stem of labral fan; proportional lengths of 1st, 2nd, and 3rd segments 1.0 : 1.0 : 0.8; all segments light yellow, with 3 thin unpigmented annular bands on segment 2. Labral fan with ca. 34 main rays. Mandible (Fig. 2B) with mandibular serrations consisting of 2 teeth (1 large, 1 small); large tooth nearly at right angle to mandible on apical side; comb-teeth composed of 3 teeth, of which 1st tooth longest, 2nd tooth subequal to, or slightly longer than, 3rd one; supernumerary serrations absent. Hypostomium (Fig. 2C) with 9 apical teeth in row; median and corner teeth well developed; median tooth of 3 intermediate teeth on each side smallest; lateral serrations weakly developed anteriorly; 4–6 hypostomal bristles per side, lying slightly divergent posteriorly from lateral margin. Postgenal cleft (Fig. 4E) very small, vestigial. Pharate pupal gill (Fig. 2D) with 12 thread-like filaments arising from long common basal stalk; each filament without transverse ridges but with numerous brownish-black to black small dots in surface cu-

ticular layer. Abdominal cuticle bare except both sides of anal sclerite moderately covered with simple colorless setae. Rectal scales present but scarcely visible. Rectal organ compound, each of 3 lobes with 11–16 finger-like (except apical 2 or 3 thumb- or nodule-like) secondary lobules. Anal sclerite X-shaped, with anterior arms 0.8 times as long as posterior ones; sensilla absent on and just posterior to basal juncture area; accessory sclerite absent. Last abdominal segment much expanded ventrally forming double bulges on each side, visible as a large ventral papilla when viewed from side. Posterior circling with ca. 76 rows of up to 14 hooklets per row.

TYPE SPECIMENS. Holotype pupal exuviae, collected from a small seasonal stream (water temperature 18.0 °C, shaded, altitude ca. 1,400 m), Siribhume Waterfall, Doi Inthanon National Park, Chiang Mai, Thailand, 16. III. 2005, by W. Choochote. Paratypes: 2 pupae, 2 pupal exuviae and 8 mature larvae, same data and date as those of holotype; 1 pupa, 1 pupal exuviae and 1 mature larva, same data as those of holotype except date, 28. IV. 2005, and water temperature 24.0 °C; 1 pupal exuviae (only right gill), 1 mature larva and 6 immature larvae, same data as those of holotype except date, 13. III. 2004; 1 immature larva, same locality as that of holotype but 100 m upstream (same data and date as those of holotype of *S. (M.) laoleense* sp. nov.); 10 mature larvae and 60 immature larvae, Ang Ka (altitude 2,460 m), Doi Inthanon National Park, Chiang Mai, Thailand, 28. II. 2004, by W. Choochote.

ECOLOGICAL NOTES. The pupae of this new species were found in small depressions formed on the surface of rocks in a small forest stream of Siribhume Waterfall, while larvae of this new species were collected from fallen leaves as well as the surface of rocks at both Siribhume Waterfall and Ang Ka. The pupae and/or mature larvae of this new species were collected only in March and April at Siribhume Waterfall, and in February at Ang Ka. Associated species were *S. (M.) laoleense* sp. nov., *S. (Simulium) doipuiense* at Siribhume Waterfall, and *S. (Gomphostilbia) inthanonense*, *S. (Nevermannia) caudisclerum*, *S. (S.) setukoae* and *S. (S.) suchariti* at Ang Ka.

DISTRIBUTION. Thailand.

ETYMOLOGY. The species *angkaense* refers to Ang Ka, where this new species was collected for the first time.

REMARKS. This new species is assigned to the subgenus *Simulium (Montisimulium)* by the pupal gill with 12 thread-like filaments (Fig. 1C) and the larval postgenal cleft absent

or very small (Fig. 4E). This species is characterized by the pupal gill with 12 thread-like filaments arising from the long common basal stalk. The arrangement of gill filaments separates this new species from all the 16 known species of this subgenus which have 12 filaments on the pupal gill [2, 6–8]. The mature larva of this new species is readily distinguished from *S. merga* by the greyish body with reddish-brown markings dorsally on the abdominal segments 5–9 (Fig. 4A), the head capsule almost entirely dark between the eye-spot region and the posterior margin (this dark area connected to the marked dark eyebrow) (Fig. 4D), and the antenna with unpigmented annular bands on the second segment (Fig. 2A).

It should be noted that there is a possibility that this new species is conspecific to *S. (M.) surachaii* described from a single female captured by a hand-net at Ang Ka, although this female was caught in July [4], different from the possible emergence time (February) of *S. (M.) angkaense* sp. nov. at Ang Ka.

***Simulium (Montisimulium) laoleense* sp. nov.**

DESCRIPTION. Female, Male and Pupa. Unknown.

Mature larva. Body length 5.5–6.1 mm. Body light yellow to greyish yellow (Fig. 4B); abdomen (Fig. 4B) shaped as in *S. (M.) angkaense* sp. nov. Cephalic apotome (Fig. 4F) clear yellow except narrow portion along posterior margin always darkened, with well-defined positive head-spots, of which posterolateral spots usually connected posteriorly to dark narrow area in front of posterior margin; lateral surface of head capsule clear yellow, with no dark well-defined eyebrow (somewhat darkened at most near both ends if present); 2 large and 2 (or 3) small spots near posterior margin distinctively positive, and 1 small spot just below eye-spot region faintly or moderately positive; ventral surface of head capsule (Fig. 4G) yellow, with dark basal area on each side of postgenal cleft; horizontal and round spots on each side of postgenal cleft distinctively positive. Cervical sclerites composed of 2 small elliptical pieces, not fused to occiput, very widely separated medially from each other. Antenna consisting of 3 segments and apical sensillum, much longer than stem of labral fan; proportional lengths of 1st, 2nd, and 3rd segments 1.0 : 0.8 : 0.8; all segments not or only slightly pigmented, then pale annular bands not visible, if any. Labral fan with ca. 32 main rays. Mandible (Fig. 3A) with mandibular serrations consisting of 2 teeth (1 large, 1 small); large tooth nearly at right angle to mandible on apical side; comb-teeth composed of 3 teeth, shortened from 1st to 3rd; supernumerary serrations absent. Hypostomium (Fig. 3B) with 9 apical teeth in row; median and corner teeth well developed; median tooth of 3 interme-

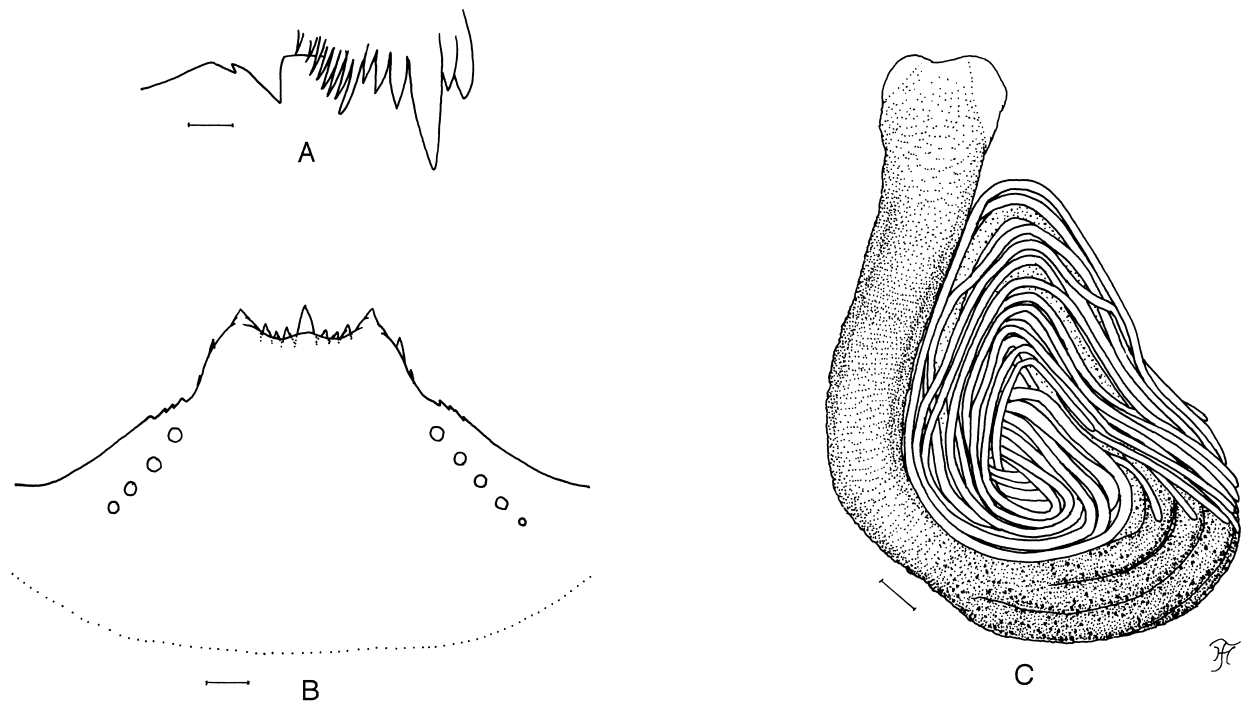


Fig. 3. Mature larva of *Simulium (Montisimulium) laoleense* sp. nov. A, mandible; B, hypostomium; C, Pharate pupal gill (left side, outer view). Scale bars. 0.04 mm for C; 0.02 mm for B; 0.01 mm for A.

diate teeth on each side smallest; lateral serrations weakly developed anteriorly; 4 or 5 hypostomal bristles per side, lying slightly divergent posteriorly from lateral margin. Post-genal cleft (Fig. 4G) very small, vestigial. Pharate pupal gill (Fig. 3C) with 12 thread-like filaments arising from very long common basal stalk about 1.6 times as long as that of *S. (M.) angkaense* sp. nov.; each filament with sharp transverse ridges and with numerous brownish-black to black small dots in surface cuticular layer. Abdominal cuticle bare except both sides of anal sclerite moderately covered with simple colorless setae. Rectal scales present. Rectal organ compound, each of 3 lobes with 13 or 14 finger-like secondary lobules. Anal sclerite X-shaped, with anterior arms 0.9 times as long as posterior ones; sensilla absent on and just posterior to basal juncture area; accessory sclerite absent. Last abdominal segment much expanded ventrally forming double bulges on each side, visible as a large ventral papilla when viewed from side. Posterior cirlet with ca. 78 rows of up to 14 hooklets per row.

TYPE SPECIMENS. Holotype mature larva, collected from a small seasonal stream (width 0.5 m, water temperature 18.5 °C, shaded, altitude ca. 1,500 m) very slowly flowing in a forest, Siribhume Waterfall, Doi Inthanon National Park, Chiang Mai, Thailand, 28. II. 2004, by W. Choochote. Paratypes: 4 mature larvae and 20 immature larvae, same

data as those of holotype; 5 immature larvae, same locality as that of holotype but 100 m downstream (same data and date as those of holotype of *S. (M.) angkaense* sp. nov.).

ECOLOGICAL NOTES. The larvae of this new species were found on fallen leaves in a small stream. Associated species were *S. (G.) inthanonense*, *S. (M.) angkaense* sp. nov., *S. (S.) crocinum* and *S. (S.) doipuiense*.

DISTRIBUTION. Thailand.

ETYMOLOGY. The species *laoleense* refers to Laolee, the Mong's name for Siribhume Waterfall, where this new species was collected.

REMARKS. This new species is also assigned to the subgenus *Simulium (Montisimulium)* as in the preceding species.

This new species is remarkable in having the pupal gill composed of 12 thread-like filaments arising from a very long common basal stalk (Fig. 3C). Within this subgenus, none of the known species has such a long common basal stalk except one unnamed species, i.e., *S. (M.)* sp. C reported from India [9], which has, though, 14 pupal gill filaments per side. The mature larva of this species is somewhat similar in the body and antennal colors to *S. (M.)*

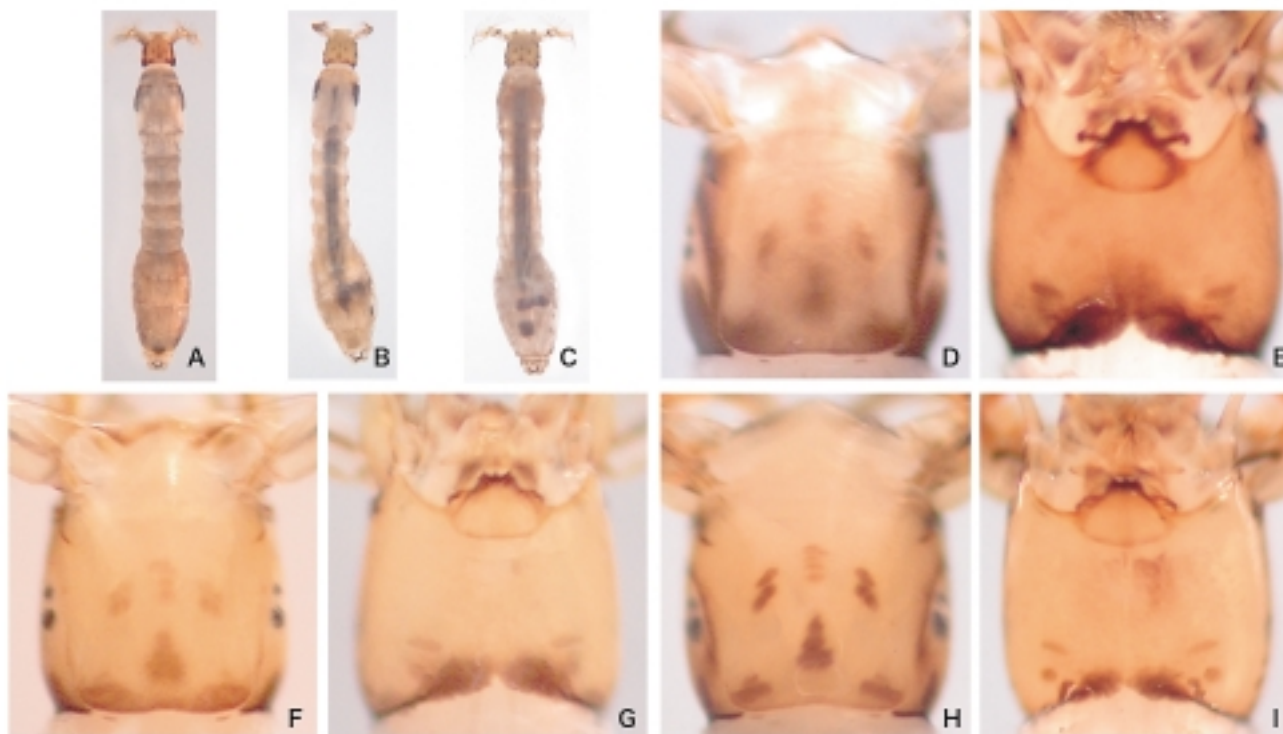


Fig. 4. Whole bodies and head capsules of mature larvae of three species of *Simulium* (*Montisimulium*) in Doi Inthanon National Park. A C, whole bodies (dorsal view); D I, head capsules, D, F and H, dorsal view; E, G and I, ventral view; A, D and E, *S. (M.) angkaense* sp. nov.; B, F and G, *S. (M.) laoleense* sp. nov.; C, H and I, *S. (M.) merga*.

merga (Fig. 4C), but differs from the latter species by the ill-defined eyebrow, the cephalic apotome with dark area just before the posterior margin which is connected to the posterolateral spots (Fig. 4F), and the fifth abdominal segment lacking a pair of dark small markings dorsally (Fig. 4B). *Simulium (M.) laoleense* sp. nov. seems to be related to *S. (M.) angkaense* sp. nov. in having the 12 pupal gill filaments but is easily distinguished from it by the light yellow body color (Fig. 4B), the antenna without distinctive hyaline annular bands on the second segment, the ill-defined faint eyebrow (Fig. 4F), and the pharate pupal gill with a very long common basal stalk (Fig. 3C).

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