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***Simulium (Nevermannia) khunklangense*, a new species of black fly (Diptera: Simuliidae) from Chiang Mai, Thailand**

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Abstract

Simulium (Nevermannia) khunklangense sp. nov. is described from females, males, pupae and larvae collected in Doi Inthanon National Park, Chiang Mai, Thailand. This new species is placed in the *vernum* species-group of the subgenus *Nevermannia* and is similar to *S. (N.) chomthongense* Takaoka & Srisuka described from Doi Inthanon National Park, Thailand, but is distinguished in the male by the number of enlarged upper-eye facets and the relative width of the hind basitarsus against the hind tibia and femur, and in the pupa by the short common basal stalk of the gill and the cocoon with an anterodorsal bulge or a short anterodorsal projection. Taxonomic notes are provided to separate this new species from five other known species of the *vernum* species-group, which share an accessory sclerite on the larval abdomen, a rare characteristic in this species-group.

Key words: Diptera, Simuliidae, new species

Introduction

The *Simulium (Nevermannia) vernum* species-group, which consists of about 130 species, is widely distributed in the Holarctic Region and extends its distribution southward into the Oriental Region where 24 species are recorded (Adler & Crosskey 2013). In Thailand, this species-group is represented by one species, *S. (N.) chomthongense* Takaoka & Srisuka (Takaoka *et al.* 2012), which was first reported as *Simulium (Eusimulium)* sp. A (Takaoka & Suzuki 1984), and later tentatively identified as *S. (N.) caudisclerum* Takaoka & Davies originally described from Peninsular Malaysia (Takaoka & Choochote 2004).

Females of the *vernum* species-group are considered ornithophilic, as judged by the claws with a large basal tooth (Adler *et al.* 2004). In Asian countries, species of this group have not been investigated for their role as a vector of human and animal disease agents except one species, *S. (N.) uchidai* Takahasi, a common species in Japan, which was reported to be a vector of an unnamed filarial species, probably of a bird (Fukuda *et al.* 2005) and a potential vector of *Leucocytozoon lovati*, a haematozoan parasite of an endangered rock ptarmigan (Sato *et al.* 2009).

Recently, we collected another species of the *vernum* species-group, which is similar to *S. (N.) chomthongense* in many characters including an accessory sclerite on the larval abdomen, a rare character in this species-group, but it is distinguished from the latter species in the male by the number of upper-eye facets and the relative width of the hind basitarsus against the hind tibia and femur, and in the pupa by the short common basal stalk of the gill filaments relative to the interspiracular trunk, and the cocoon with a triangular anterodorsal bulge or a short anterodorsal projection.

In this paper, this species is described as new to science based on females, males, pupae and mature larvae collected in Doi Inthanon National Park, Chiang Mai, Thailand. Taxonomic notes are provided to separate this new

species from five other known species of the *vernum* species-group, which share an accessory sclerite on the larval abdomen.

The methods of collection, description and illustration, as well as terms for morphological features used here, follow those of Takaoka (2003). The type specimens are deposited at the Entomology Section, Queen Sirikit Botanic Garden, Chiang Mai, Thailand.

***Simulium (Nevermannia) khunklangense* Takaoka & Srisuka sp. nov.**

Description. Female. Body length 2.6–2.8 mm. **Head.** Slightly narrower than width of thorax. Frons brownish-black, gray pruinose, not shiny, densely covered with whitish-yellow scale-like recumbent short hairs and lacking dark simple longer hairs along each lateral margin; frontal ratio 1.61–1.84:1.00:1.93–2.22; frons:head ratio 1.00:4.34–4.43. Fronto-ocular area well developed, narrow, directed dorsolaterally. Clypeus dark brown, gray pruinose, moderately covered with whitish-yellow hairs interspersed with few dark longer hairs near lower margin on each side (except medial portion of upper half bare). Labrum 0.79–0.86 times as long as clypeus. Antenna composed of scape, pedicel and 9 flagellomeres, dark brown to brownish-black except scape, pedicel and base of first flagellomere dark yellow to medium brown. Maxillary palp composed of 5 segments, light to medium brown, proportional lengths of third, fourth, and fifth segments 1.00:0.74–0.75:1.46–1.57; third segment (Fig. 1A) swollen; sensory vesicle (Fig. 1A) elongate (0.59 times as long as third segment), with medium-sized opening. Maxillary lacinia with 11–14 inner and 15 or 16 outer teeth. Mandible with 34–36 inner and 14 or 15 outer teeth. Cibarium without denticles. **Thorax.** Scutum brownish-black except anterolateral calli ochreous, slightly shiny when illuminated at certain angle, densely covered with yellow scale-like recumbent hairs interspersed with several yellow medium-long to long hairs as well as dark brown long upright hairs on prescutellar area. Scutellum medium brown, slightly shiny when illuminated at certain angle, moderately covered with yellow medium-long to long hairs mixed with several dark brown long upright hairs. Postnotum dark brown, slightly shiny when illuminated at certain angle and bare. Pleural membrane bare. Katepisternum dark brown to brownish-black, longer than deep, slightly shiny when illuminated at certain angle, and bare. **Legs.** Foreleg: coxa dark yellow; trochanter light brown except basal 1/2 of outer surface yellow; femur dark yellow except apical cap medium brown; tibia medium brown, with median large portion on outer surface grayish light brown; tarsus dark brown, with moderate dorsal hair crest; basitarsus moderately dilated, 8.13–8.25 times as long as its greatest width. Midleg: as in foreleg except coxa dark brown on anterolateral surface and brownish-black on posterolateral surface, and trochanter light brown. Hind leg: coxa medium brown; trochanter dark yellow except anterior surface light brown; femur dark yellow with apical cap medium brown and basal tip light brown on anterior surface; tibia medium to dark brown except extreme base yellow, and medial large portion on outer surface grayish light brown; tarsus dark brown except basitarsus light to medium brown (though base dark brown) and basal 1/2 of second tarsomere grayish; basitarsus (Fig. 1B) narrow, nearly parallel-sided, 6.77–6.85 times as long as wide, and 0.76 and 0.59 times as wide as greatest widths of tibia and femur, respectively; calcipala (Fig. 1B) nearly as long as width at base, and 0.46–0.5 times as wide as greatest width of basitarsus. Pedisulcus (Fig. 1B) well defined. Claw (Fig. 1C) with large basal tooth 0.48 times as long as claw. **Wing.** Length 3.0 mm. Costa with dark brown spinules and hairs except basal portion covered with patch of yellow hairs. Subcosta with light brown hairs except apical 1/4 bare. Hair tuft on stem vein light to dark brown. 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 stem darkened. **Abdomen.** Basal scale ochreous to light brown, with fringe of whitish-yellow hairs. Dorsal surface of abdomen dark brown to brownish-black except segments 2 and 7–9 light brown (though base of segment 2 ochreous), moderately covered with dark short to long hairs and yellow short hairs; tergites of segments 3–6 relatively narrow, those of segments 2 and 7–9 wide and all dull. Ventral surface of abdomen mostly pale ochreous; sternal plate on segment 7 developed medially. **Genitalia.** Sternite 8 (Fig. 1D) bare medially, with 12–17 short to long yellow hairs intermixed with 2 or 3 long dark brown hairs on each side. Ovipositor valves (Fig. 1D) triangular (though medioposterior corners rounded), thin, membranous, moderately covered with microsetae interspersed with 5 or 6 yellow short slender hairs (of which 1 hair usually medium-long) on each side; inner margins slightly concave medially, somewhat sclerotized, and somewhat separated from each other. Genital fork (Fig. 1E) of usual inverted-Y form, with slender stem; arms of moderate width medially; lateral plate of each arm strongly sclerotized along dorsolateral margin, and with thin lobe directed medioposteriorly. Paraproct in ventral

view (Fig. 1F) nearly pentagonal, with 4 or 5 sensilla on unpigmented anteromedial surface; paraproct in lateral view (Fig. 1G) somewhat produced ventrally, 0.74 times as long as wide, with 15 or 16 medium-long to long hairs on ventral and lateral surfaces. Cercus in lateral view (Fig. 1G) short, rounded posteriorly, 0.64 times as long as wide. Spermatheca (Fig. 1H) ovoidal, 1.16 times as long as its greatest width, well sclerotized except duct and small area near juncture with duct unsclerotized, and with hexagonal patterns (though not well defined) on surface; internal setae absent; both accessory ducts slender, subequal in diameter to major one.

Male. Body length 2.7 mm. **Head.** Wider than thorax. Upper eye medium brown, consisting of 18 vertical columns and 19 or 20 horizontal rows of large facets. Face dark brown, grayish-white pruinose. Clypeus brownish-black, whitish pruinose, moderately covered with golden yellow medium-long hairs (mostly directed upward) interspersed with few light brown longer hairs near lower margin. Antenna composed of scape, pedicel and 9 flagellomeres, dark brown except base of first flagellomere yellow; first flagellomere elongate, 2.18 times length of second one. Maxillary palp light to medium brown, with 5 segments, proportional lengths of 3rd, 4th, and 5th segments 1.00:0.86:1.83; third segment (Fig. 2A) not swollen; sensory vesicle (Fig. 2A) ellipsoidal, small, 0.28 times as long as third segment, and with small opening. **Thorax.** As in female. **Legs.** Color nearly as in female except following characteristics: fore coxa dark yellow to light brown, mid trochanter medium brown except anterior surface pale, and basal 1/4 of hind second tarsomere grayish. Fore basitarsus slightly dilated, 11.2 times as long as its greatest width. Hind basitarsus (Fig. 2B) enlarged, spindle-shaped, 4.35 times as long as wide, and 1.0 and 1.1 times as wide as greatest width of hind tibia and femur, respectively; calcipala (Fig. 2B) slightly shorter than basal width, and 0.3 times as wide as greatest width of basitarsus. Pedisulcus (Fig. 2B) well defined at basal 1/4 of second tarsomere. **Wing.** Length 2.6 mm; other characteristics as in female except subcosta without hairs and basal portion of costal vein covered with dark hairs mixed with yellow hairs. **Abdomen.** Basal scale medium brown, with fringe of yellow long hairs. Dorsal surface of abdomen dark brown to brownish-black, moderately covered with yellow short hairs intermixed with dark brown short to medium-long hairs. **Genitalia.** Coxite in ventral view (Fig. 2C) nearly rectangular, 1.90 times as long as its greatest width. Style in ventral view (Fig. 2C) bent inward, nearly parallel-sided, rounded apically and with apical spine; style in medial view (Fig. 2D) shorter than coxite (0.82 times as long as coxite), boot-shaped, with triangular apical lobe directed dorsomedially; style in ventrolateral view (Fig. 2E) straight up to apical 1/3, then curved inwardly, wide basally, narrowed to basal 2/5, nearly parallel-sided up to apical 1/5, and with rounded apex. Ventral plate in ventral view (Fig. 2C) with body transverse, 0.52 times as long as wide, with anterior margin slightly produced medioanteriorly, posterior margin with two shallow submedial notches, darkened along anterior margin, and nearly bare except posteromedian portion of ventral surface densely covered with microsetae; basal arms of moderate length, directed forward, somewhat divergent from base; ventral plate in lateral view (Fig. 2F) with ventral margin of body concave; ventral plate in caudal view (Fig. 2G) with body appearing as shallow inverted-V shape, having similar width, posteroventral margin roughly undulate, and densely covered with microsetae medially on posterior surface. Median sclerite (Fig. 2F, H) club-shaped, narrow, with forked apex, and with base located in front of anterior margin of ventral plate. Parameres (Fig. 2I) large, each with small apical appendix directed forwardly, and with 1 distinct long and stout hook. Aedeagal membrane (Fig. 2J) moderately setose, dorsal plate well defined, broadly produced ventrally with round apex, though constricted subbasally. Ventral surface of abdominal segment 10 (Fig. 2K) with 2–5 distinct hairs near each posterolateral margin. Cercus (Fig. 2K) small, rounded, encircled with 10–13 hairs.

Pupa. Body length 3.0–3.6 mm. **Head.** Integument light yellowish-brown, bare, though frons with few to several tubercles; antennal sheath without any protuberances; face with pair of simple long trichomes with coiled apices, and frons with 2 simple long trichomes with coiled or uncoiled apices on each side in 2 pupae, or with 1 simple long trichome on left side and 2 simple long trichomes on right side in 2 pupae, or with 1 simple long trichome on right side, and 1 simple long trichome and 1 bifid long trichome on left side in 1 pupa. **Thorax.** Integument light yellowish-brown, bare except dorsal and dorsolateral surfaces sparsely covered with small round tubercles, with 3 simple very long dorsomedial trichomes with coiled or uncoiled apices, 2 simple anterolateral trichomes (1 very long with coiled apex, 1 long with coiled or uncoiled apex), 1 simple medium-long mediolateral trichome with coiled or uncoiled apex, and 3 simple ventrolateral trichomes with uncoiled apices (1 medium-long, 2 short), on each side. Gill (Fig. 3A,B) composed of 4 slender thread-like filaments, arranged in pairs, with short common basal stalk having somewhat swollen transparent organ ventrally (often partially broken) at base; common basal stalk short, 0.50–0.65 times as long as interspiracular trunk; stalk of dorsal pair of filaments 0.56–1.32 times

as long as common basal stalk, and that of ventral pair of filaments 0.48–1.47 times as long as common basal stalk; all filaments subequal in length and thickness to one another, though their lengths including their own stalk and common basal stalk varying by individual pupae from 4.8 mm to 6.5 mm by pupae; cuticle of all filaments with well-defined annular ridges and furrows, and densely covered with minute tubercles. **Abdomen.** Dorsally, segments 1–4 dark grayish and without distinct tubercles; segment 1 with 1 medium-long simple slender hair-like seta on each side; segment 2 with 1 short simple slender hair-like seta and 5 short somewhat spinous setae submedially on each side; segments 3 and 4 each with 4 hooked spines and 1 short somewhat spinous seta on each side; segments 5–8 each with spine-combs in transverse row on each side, and segments 6–9 each with comb-like groups of minute spines on each side; segment 9 with pair of cone-like terminal hooks. Ventrally, segment 4 with 1 simple hook and few simple slender short setae on each side; segment 5 with pair of bifid hooks submedially and few short simple slender 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 simple slender setae on each side; segments 4–8 with comb-like groups of minute spines. Each side of segment 9 without grapnel-shaped hooklets. **Cocoon** (Fig. 3C). Wall pocket-shaped, moderately woven, widely extended ventrolaterally, appearing round when viewed dorsally; anterior margin thickly woven, with triangular medial bulge or a short mediodorsal projection (up to 0.7 mm long) (though 2 of 5 cocoons with anterior margin roughly woven and without anterodorsal bulge or projection—Fig. 3D); posterior 2/3 with floor roughly or moderately woven; individual threads invisible or partially visible; 4.3–5.0 mm long by 3.5–4.0 mm wide.

Mature larva. Body length 6.2–7.0 mm. Body creamy. Cephalic apotome whitish-yellow, though narrow area along posterior margin somewhat darkened; head spots moderately positive except anterior spot of posterolateral spots usually obscure. Lateral surface of head capsule whitish-yellow except eye-spot region whitish; eyebrow moderately positive; among spots in front of posterior margin, 2 relatively large spots moderately positive, and 2 small spots faintly positive; 1 or 2 small round spots below eye-spot region indistinct or faintly positive. Ventral surface of head capsule whitish-yellow to yellow except darkened area near posterior margin on each side of postgenal cleft; horizontal long spot and round spot on each side of postgenal cleft faintly or moderately positive. Antenna composed of 3 segments and apical sensillum, somewhat longer than stem of labral fan; proportional lengths of 1st, 2nd, and 3rd segments 1.00:1.07–1.11:0.68. Labral fan with 31–37 main rays. Mandible (Fig. 3E) with 3 comb-teeth, of which 1st tooth longest and 2nd tooth as long as or slightly longer than 3rd one; mandibular serration composed of 2 teeth (1 medium-sized and 1 very small); major tooth at obtuse angle against mandible on apical side; supernumerary serration usually absent. Hypostoma (Fig. 3F) with row of 9 apical teeth; median and each corner tooth prominent, subequal in length to one another and much longer than 3 intermediate teeth on each side; lateral margin weakly serrate; 5 or 6 hypostomal bristles per side lying parallel to lateral margin. Postgenal cleft (Fig. 3G) short, somewhat pointed apically (though rarely rounded apically as shown in Fig. 3H), 0.69–0.92 times as long as postgenal bridge. Cervical sclerites composed of 2 light to medium brown small oblong pieces, not fused to occiput, widely separated medially from each other. Thoracic cuticle bare. Abdominal cuticle almost bare except few posterior segments sparsely covered with colorless simple setae, and last segment densely covered with colorless simple setae on each side of anal sclerite. Rectal scales present. Rectal papillae compound, each of 3 lobes with 8–14 finger-like secondary lobules. Anal sclerite of usual X-form, with anterior arms 0.89–1.00 times as long as posterior ones, broadly sclerotized at base; accessory sclerite (Fig. 3I) composed of 3–5 sclerotized spots on each side. Last abdominal segment with pair of large conical ventral papillae. Posterior circlet with 84–94 rows of up to 14 hooklets per row.

Type specimens. Holotype male with associated pupal exuviae and cocoon (Thailand QSBG-2012-254-1) (preserved in 80% ethanol), reared from pupa, collected from a stream (width 50 cm, depth 5.5 cm, stream-bed sandy and muddy, water temperature 17.5°C, pH 6.3, partially shaded, altitude 1,611 m, 18°31'15.6" N, 98°29'59.5" E) moderately flowing in a forest (before Check Point 2), Chomthong District, Doi Inthanon National Park, Chiang Mai, Thailand, 27. XI. 2012, by W. Srisuka & S. Suriya. Paratypes: 2 females, with associated pupal exuviae and cocoons (QSBG-2012-265-1 & 2; same data as holotype except 14. XII. 2012, stream width 45 cm, water temperature 15.8 °C, PH 6.6, collected by W. Srisuka & R. Sawkord), 1 pharate female and 1 pharate male (QSBG-2012-265), same data as 2 female paratypes, and 8 mature larvae (QSBG-2012-254), same data and date as those of the holotype, all paratype specimens preserved in 80% ethanol.

Biological notes. The pupae and larvae of this new species were collected from dead tree leaves in the water. The only associated species was *Simulium (Gomphostilbia) inthanonense* Takaoka & Suzuki, 1984.

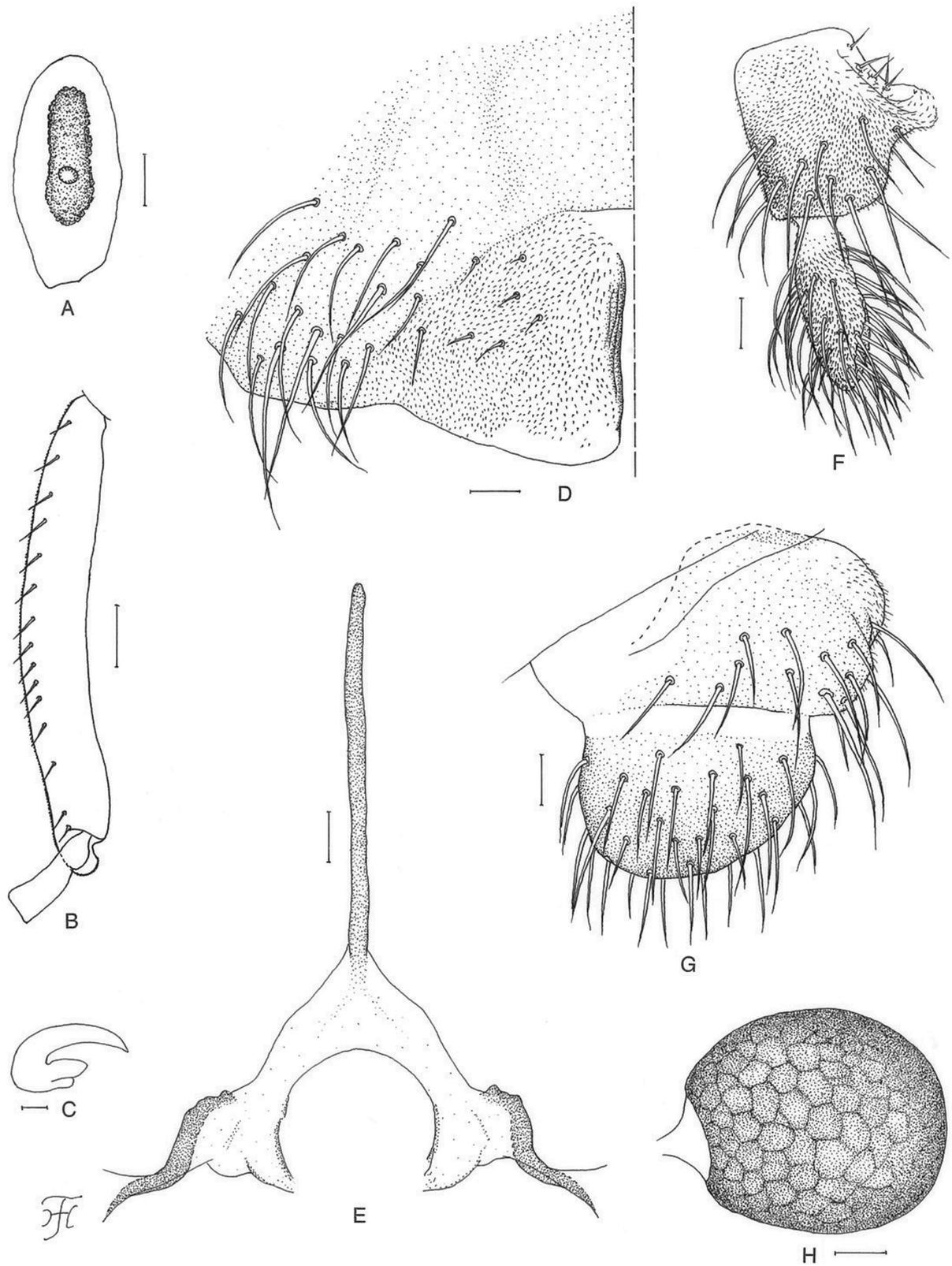


FIGURE 1. Female of *Simulium* (*Nevermannia*) *khunklangense* sp. nov. A, third segment of right maxillary palp with sensory vesicle (front view); B, basitarsus and second tarsomere of left hind leg showing calcipala and pedisulcus (outer view); C, claw (lateral view); D, sternite 8 and ovipositor valve (only right half shown) (ventral view); E, genital fork (ventral view); F & G, right paraprocts and cerci (F, ventral view; G, lateral view); H, spermatheca. Scale bars. 0.1 mm for B; 0.04 mm for A; 0.02 mm for D–H; 0.01 mm for C.

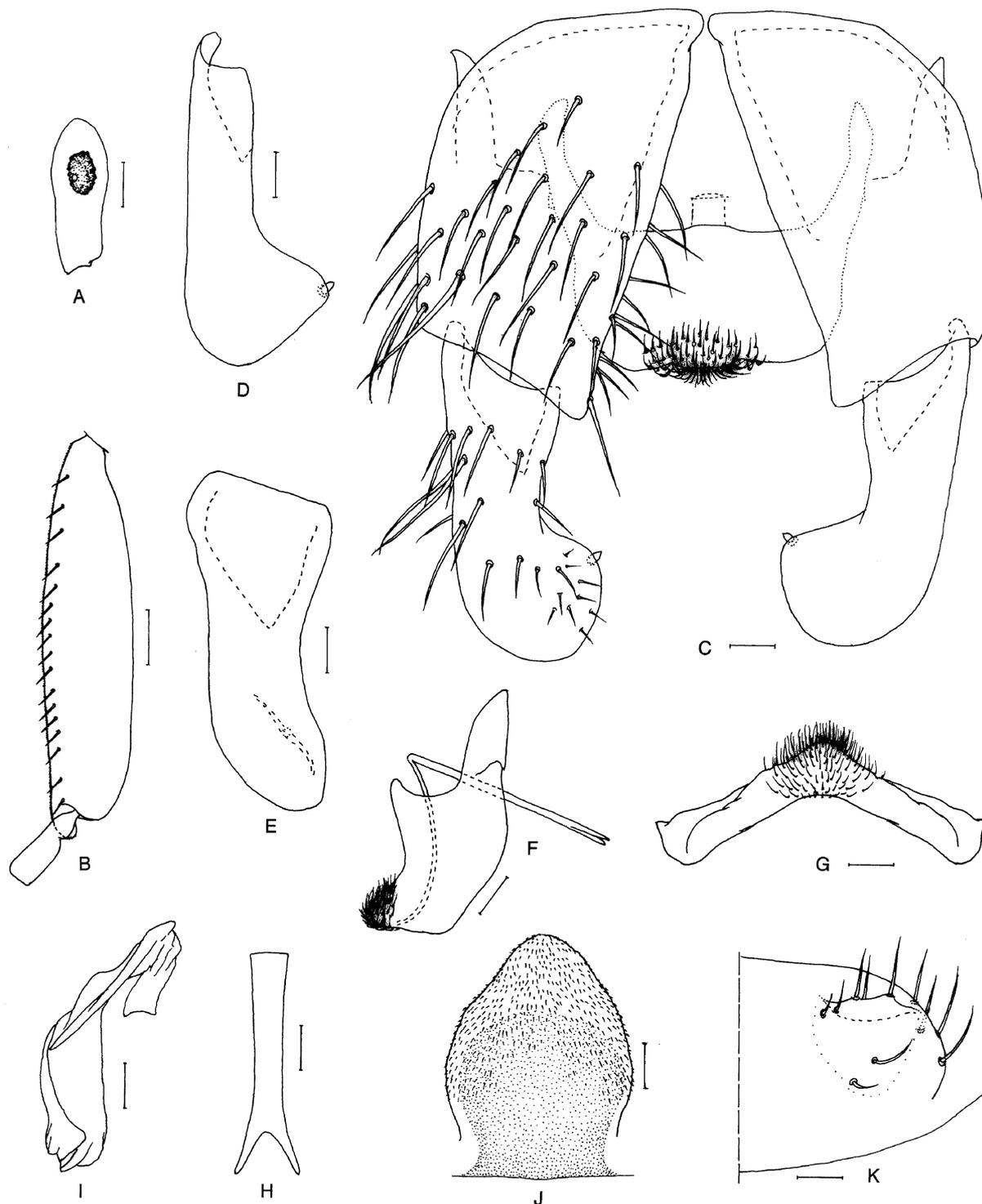


FIGURE 2. Male of *Simulium* (*Nevermannia*) *khunklangense* sp. nov. A, third segment of right maxillary palp with sensory vesicle (front view); B, basitarsus and second tarsomere of left hind leg showing calcipala and pedisulcus (outer view); C, coxites, styles and ventral plate (ventral view); D & E, right styles (D, medial view; E, ventrolateral view); F, ventral plate and median sclerite (lateral view); G, ventral plate (caudal view); H, median sclerite (caudal view); I, right paramere (caudal view); J, aedeagal membrane and dorsal plate (caudal view); K, abdominal segment 10 and cercus (left side; caudal view). Scale bars. 0.1 mm for B; 0.04 mm for A; 0.02 mm for C–K.

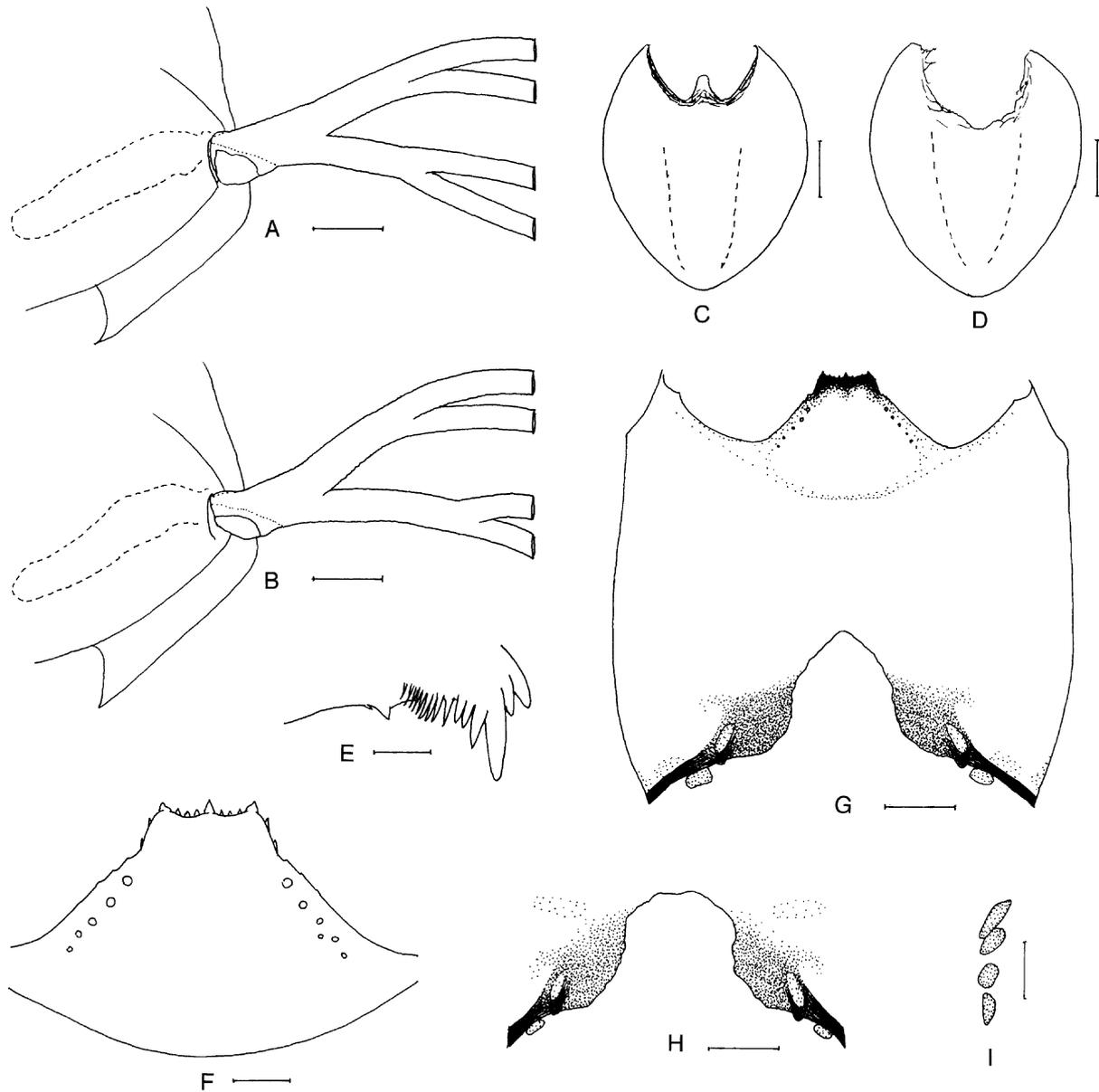


FIGURE 3. Pupa and larva of *Simulium (Nevermannia) khunklangense* sp. nov. A–D, pupa and E–I, larva. A & B, interspiracular trunk and basal portion of gill filaments showing different lengths of stalks of paired filaments relative to common basal stalk (right side; lateral view); C & D, cocoons (dorsal view; C, with short anterodorsal projection; D, without anterodorsal bulge or projection); E, mandible (lateral view); F, hypostoma (ventral view); G, head capsule showing postgenal cleft with pointed apex (ventral view); H, postgenal cleft with rounded anterior margin; I, accessory sclerite (left side; ventrolateral view). Scale bars. 1.0 mm for C & D; 0.1 mm for A, B, G & H; 0.04 mm for F; 0.02 mm for E & I.

Etymology. The species name *khunklangense* refers to the name of the village, Khunklang, in Doi Inthanon National Park, where this new species was collected.

Remarks. *Simulium (Nevermannia) khunklangense* sp. nov. is placed in the *vernum* species-group in the subgenus *Nevermannia*, mainly based on the shape of the male genitalia (Fig. 2C–K). Among the species of this group, this new species apparently is similar to the following six known species in bearing an accessory sclerite: *S. (N.) aberrans* Delfinado from the Philippines (Delfinado 1969; Takaoka 1983), *S. (N.) caudisclerum* from Peninsular Malaysia (Takaoka & Davies 1995), *S. (N.) chomthongense* from Thailand (Takaoka *et al.* 2012), *S. (N.) ludingense* Chen, Zhang & Huang from Sichuan, China (Chen *et al.* 2005), *S. (N.) yushangense* Takaoka from Taiwan (Takaoka 1979), and *S. (N.) zhangjiajiense* Chen, Zhang & Bi from Hunan, China (Chen *et al.* 2004).

This new species appears to be most closely related to *S. (N.) chomthongense* among these species by sharing many adult female and larval characters, but it differs in the adult male and pupa from the latter species by having the following characteristics (characteristics of *S. (N.) chomthongense* in parentheses): male upper-eye facets in 18 vertical columns and 19 or 20 horizontal rows (in 15 or 16 vertical columns and 17 or 18 horizontal rows), ratio of the width of the male hind basitarsus against those of the hind tibia and femur, 1.0 and 1.1 (1.09–1.13 and 1.33–1.35), ratio of the length of the common basal stalk of the pupal gill filaments against that of the interspiracular trunk 0.50–0.65 (0.76–0.79), and the cocoon with a triangular anterodorsal bulge or a short anterodorsal projection (with a long anterodorsal projection).

The close relationship between *S. (N.) khunklangense* **sp. nov.** and *S. (N.) chomthongense* shown by morphological characters is also revealed by comparing the sequences of the mitochondrial 16S rRNA gene (516 base pairs) of both species (Otsuka et al. unpublished data). The difference in the sequences of this gene between *S. (N.) khunklangense* **sp. nov.** (Accession number in GenBank: AB820367) and *S. (N.) chomthongense* (Accession number in GenBank: AB699899) was only two base pairs.

Simulium (N.) khunklangense **sp. nov.** might have adapted to middle altitudes (about 1,600 m above sea level) with moderate water temperatures (16–18°C), whereas *S. (N.) chomthongense* is confined to middle and higher altitudes (1,700–2,200 m above sea level) with low to moderate water temperatures (10–15°C) in Doi Inthanon National Park (Takaoka *et al.* 2012). The distance of the type localities of both species is approximately 20 km.

This new species is distinguished in the male from the other five known species by the number of upper-eye facets (cf. about 22 horizontal rows in *S. (N.) aberrans*, 14 vertical columns and 15 horizontal rows in *S. (N.) caudisclerum*, 16 vertical columns and 15 horizontal rows in *S. (N.) ludingense*, about 17 horizontal rows in *S. (N.) yushangense*, and 17 vertical columns and 13 horizontal rows in *S. (N.) zhangjiajiense*); and in the pupa from *S. (N.) ludingense*, *S. (N.) yushangense* and *S. (N.) zhangjiajiense* by the gill filaments of equal thickness (cf. the dorsalmost filament somewhat thicker than three other filaments in the latter three known species), and from *S. (N.) aberrans* by the head and thoracic integument almost bare (cf. densely covered with tubercles in *S. (N.) aberrans*), and from *S. (N.) caudisclerum* by the short common basal stalk and the cocoon with a triangular anterodorsal bulge or a short anterodorsal projection (cf. the common basal stalk is medium-long, and the cocoon bears a long anterodorsal projection in *S. (N.) caudisclerum*).

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