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Overlooked but not forgotten: the first new extant species of Hawaiian land snail described in 60 years, *Auriculella gagneorum* sp. nov. (Achatinellidae, Auriculellinae)

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Abstract

Recent surveys of Oahu's Waianae Mountains uncovered a small, previously undescribed species of *Auriculella* that is conchologically similar to the three members of the *A. perpusilla* group all of which are endemic to the Koolau Mountain Range. However, sequence data demonstrate that the *perpusilla* group is not monophyletic. Moreover, the new species is not closely related to *A. perpusilla* or *A. perversa*, the only extant members of the group, but instead is sister to *A. tenella*, a species from the high spired *A. castanea* group. A neotype is designated for *A. auricula*, the type species of *Auriculella*; all members of the conchologically similar *perpusilla* group are anatomically redescribed; and lectotypes designated for *A. minuta*, *A. perversa*, and *A. tenella*. The new species is described and compared to the type of the genus, members of the *perpusilla* group, and the genetically similar species *A. tenella*.

Keywords

gastropod, island, Oahu, Pacific, systematics

Introduction

Pacific Island land snails are among the most threatened faunas in the world, with more recorded extinctions since 1600 than any other group of animals (Régnier et al. 2009). Of the more than 25,000 islands spread across the Pacific, few have been extensively surveyed in modern times for their invertebrate fauna, and the estimates of extinction are probably a vast underestimate. Of the few islands and archipelagos that have been studied, like Hawaii, extinctions have been shown to be extensive (Régnier et al. 2015; Yeung and Hayes 2018). For example, as much as 93% of the endemic family Amastridae has been lost, and the other 12 families of land snails represented in Hawaii are not fairing much better (Yeung and Hayes 2018). Critical to understanding and slowing the rate of extinction is accurate and updated systematics and biogeography of land snails, and other understudied groups (Cardoso et al. 2011).

The Pacific Island family Achatinellidae is the second most diverse land snail family in the Hawaiian Islands with 209 species divided into five subfamilies, two of which, the Achatinellinae Gulick, 1873 and Auriculellinae Odhner, 1922, are endemic (Cooke and Kondo 1960). Historically the large and colorful Achatinellinae have garnered much attention and the lion's share of molluscan conservation attention in Hawaii (Gulick 1872; Hadfield et al. 1993; Thacker and Hadfield 2000; Holland and Hadfield 2002, 2004, 2007; Erickson and Hadfield 2008; Hadfield and Saufler 2009; O'Rorke et al. 2015; Price et al. 2015, 2016a, b, 2018; Sischo et al. 2016), and include the only Hawaiian land snail species protected under the US Endangered Species Act (1981, 2013). However, the smaller, less colorful Auriculellinae, comprising 31 species in the genus *Auriculella* Pfeiffer, 1854 and one species in the genus *Gulickia* Cooke in Pilsbry & Cooke, 1915 have remained understudied and unprotected since the last revisions more than a century ago (Pilsbry and Cooke 1914–1916). Although fossils (Solem 1977; Severns 2009) and extinct species (Severns 2011) of Hawaiian land snails have continued to be described, no new extant species of native Hawaiian land snails have been described in more than 60 years. The last described extant Hawaiian land snail species was an achatinellid in the subfamily Tornatellidinae Cooke & Kondo, 1960, *Philopoa singularis* Cooke & Kondo, 1960 and the most recently described *Auriculella* species is *A. lanaiensis* Cooke in Pilsbry & Cooke, 1915.

Cooke and Kondo (1960) arranged *Auriculella* into four conchologically distinct groups: the *cerea* group from the southeastern islands of Hawaii, Lanai, Maui, and Molokai; and the *auricula*, *castanea*, and *perpusilla* groups which are all endemic to Oahu (Pilsbry and Cooke 1914–1916; Cooke and Kondo 1960). The *perpusilla* group (*A. perpusilla* Smith, 1873, *A. minuta* Cooke & Pilsbry in Pilsbry & Cooke, 1915, and *A. perversa* Cooke in Pilsbry & Cooke, 1915) contains the smallest species; all 6 mm or less in adult shell height. In addition to their small size these species have thin shells with 5 strongly convex whorls with low spires and weakly reflected apertures distinguishing them from the many-whorled, high-spired *castanea* group and the larger, thicker shelled *auricula* and *cerea* groups (Pilsbry and Cooke 1914–1916).

In addition to their morphological similarity, the three species in the *perpusilla* group are all endemic to Oahu's eastern Koolau range (Fig. 1B–D). Recent collecting

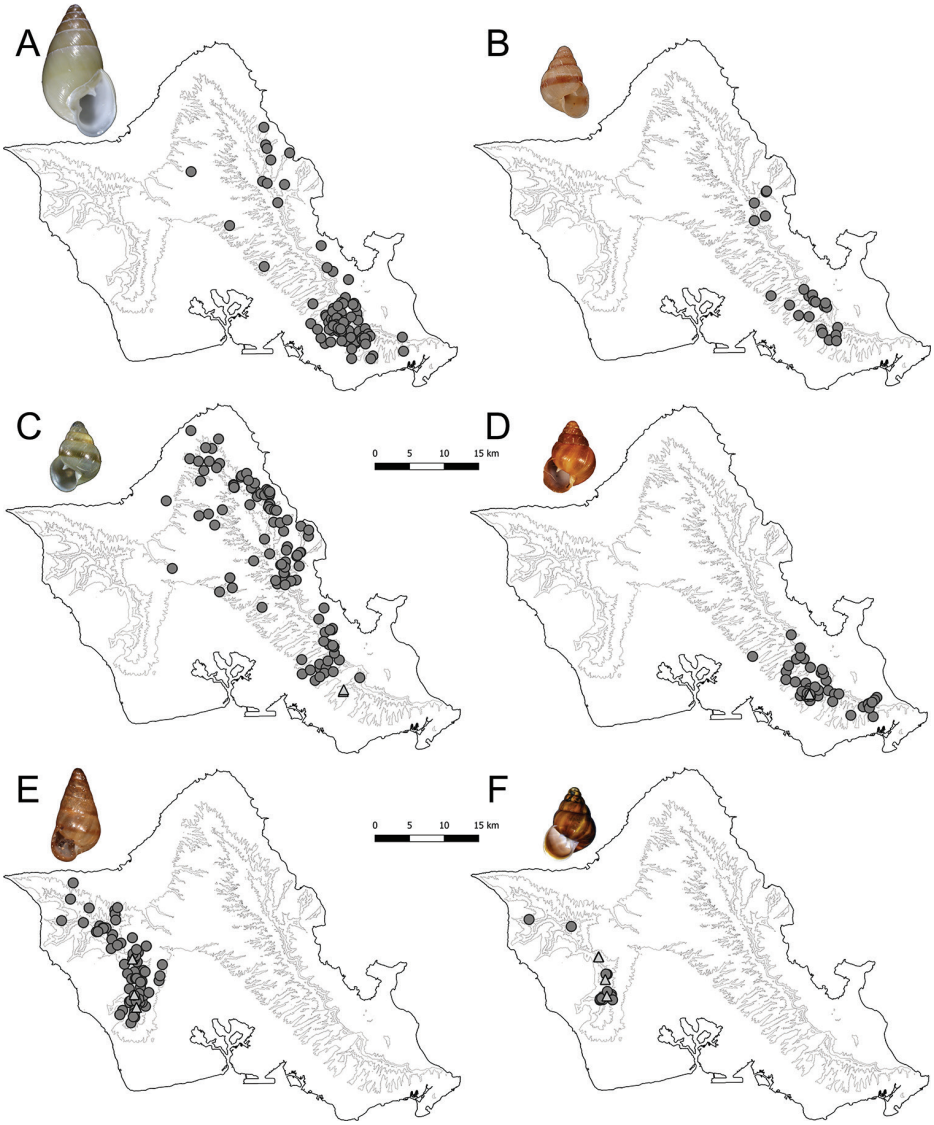

Figure 1. Distributional map of *Auriculella* spp. **A** *Auriculella auricula* **B** *Auriculella minuta* **C** *Auriculella perpusilla* **D** *Auriculella perversa* **E** *Auriculella tenella* and **F** *Auriculella gagneorum* sp. nov. Dark grey circle = historical; light grey triangle = since 2010.

in the island's western Waianae range uncovered a previously undescribed species with features of shell size and shape that would place it in this group. The two mountain ranges are separated by a relatively dry, low elevation saddle 22 km long and 8 km wide and few land snail species have distributions in both ranges (Pilsbry and Cooke 1914–1916; Cowie et al. 1995). Specimens of the undescribed species were also found in samples collected prior to 1940, which were housed in the Bishop Museum (BPBM) and labelled by Y. Kondo as a potentially new species.

Within *Auriculella*, intraspecific shell morphology varies and may often overlap interspecifically, making species delineation based on conchology alone difficult (Pilsbry and Cooke 1914–1916). As such, additional morphological and molecular data (e.g., DNA and RNA sequences) are necessary to distinguish among closely related species. The reproductive anatomy of only a few *Auriculella* species is known. Pilsbry and Cooke (1915 on plate 22) figured *A. pulchra* Pease, 1868 (figs 1, 2); *A. cerea* (Pfeiffer, 1855) (fig. 3); and *A. armata* (Mighels, 1845) as *A. westerlundiana* Ancey, 1889 (fig. 6). The reproductive anatomy of the type species of the genus, *Auriculella auricula* (Férussac, 1821), was figured and described by Cooke and Kondo (1960: figs 113a–d, 114a–c) who also dissected 22 other species but figured only *A. castanea* (Pfeiffer, 1853) (Cooke and Kondo 1960: fig. 114d). The reproductive anatomy of the other species has never been figured or described, including all members of the *perpusilla* group. As part of a broader project whose aim is to fully revise the systematics of the Achatinellidae, we use an integrative approach using data from conchology, radula, reproductive system, and DNA sequences, to redescribe *A. auricula*, the type species of the genus and all members of the *perpusilla* group (*A. perpusilla*, *A. minuta*, *and A. perversa*). We also describe a new species, *A. gagneorum* sp. nov., based on recently collected material and from lots housed in the Bishop Museum. Relationships of the taxa traditionally relegated to the *perpusilla* group, and of the conchologically similar *A. gagneorum* sp. nov., are explored with a mitochondrial and nuclear gene dataset. To enhance the stability of the nomenclature, we designate a neotype for *A. auricula* and lectotypes for members of the *perpusilla* group.

Materials and methods

As part of a long-term study of extant Hawaiian land snails, our team has surveyed more than 1000 sites across the six largest Hawaiian Islands (Kauai, Oahu, Maui, Molokai, Lanai, Hawaii). The targeted locations were those that historically supported snail populations, as well as more remote areas with remnant native vegetation that were often accessible only by helicopter. Surveys followed Durkan et al. (2013) and consisted of leaf litter sampling and hand collecting for at least one-person-hour by a minimum of two experienced malacologists in quadrats of at least 10 m^2, but up to 100 m^2, terrain permitting. GPS coordinates were collected at every survey site and coordinates were estimated for historical BPBM specimen records using locality, field notes, maps, and other descriptions. The precise locations (e.g., GPS coordinates) for material listed are not provided here for conservation purposes but are kept in the State of Hawaii Department of Land and Natural Resources Snail Extinction Prevention Program and Bishop Museum Malacology databases. Distributional maps were created using QGIS v3.8.2 (QGIS 2019) and used to show historical and current distributions of the species treated herein.

Newly collected material was photographed, flash boiled (Fukuda et al. 2008), and then fixed in 95% ethanol, after which a small piece of foot tissue was removed for DNA extraction. The remaining soft tissues were preserved in 80% ethanol, and dissections

were performed on preserved specimens submerged in 75% ethanol. Shells and reproductive anatomy were photographed with digital single-lens reflex cameras (e.g., Cannon EOS 7D) attached to a dissecting microscope. Photographs of reproductive anatomy were traced in Photoshop to produce line drawings. Shell measurements were made using an ocular micrometer and each measurement was repeated three times and averaged for 50 specimens per species. Shell measurements, shell height **(H)**, shell width **(W)**, aperture height **(AH)**, aperture width **(AW)**, and number of whorls **(WH)** were made following Slapcinsky and Kraus (2016: 30, fig. 1). All pertinent type and comparative material were examined and photographed. Locality and collector information of materials examined were listed as verbatim. Materials examined for the new species is provided in the text and all others can be found in Suppl. material 1. Museum collections are abbreviated:

ANSP Academy of Natural Sciences, Philadelphia;
BPBM Bernice P. Bishop Museum, Honolulu;
MCZ Museum of Comparative Zoology, Cambridge;
MNHN Muséum national d'Histoire naturelle, Paris;
NMW National Museum of Wales, Cardiff;
RBINS Royal Belgian Institute of Natural Sciences, Brussels;
SMF Naturmuseum Senckenberg, Frankfurt.

Radulae were tissue-digested in 180 µL of T1 lysis buffer (Macherey-Nagel) containing 20 mg/mL of Proteinase-K and rinsed in de-ionized water. Cleaned radulae were mounted directly on carbon adhesive tabs attached to aluminum stubs, which were then coated with 25–30 nm gold/palladium (60/40) and photographed using an Apreo scanning electron microscope (FEI Company) at the National Museum of Natural History, Washington.

Total genomic DNA (gDNA) was extracted from an approximately 1 mm^3 piece of foot tissue using the Macherey-Nagel NucleoSpin Tissue Kit following the manufacturer's instructions, with the exception that elution was with 60 µl of elution buffer supplied with the kit, and gDNA stored at -20 °C prior to amplification via the polymerase chain reaction (PCR).

Portions of two mitochondrial genes, 16S ribosomal DNA (rDNA) and cytochrome *c* oxidase subunit I (COI), and the nuclear encoded 28S rDNA were amplified using primers listed in Table 1. Reactions were carried out in 25 µl volumes containing 1–2 µl template DNA and a final concentration of 1 U of MangoTaq DNA polymerase (Bioline), 1X reaction buffer, 0.2 mM each dNTP, 2.5 mM $MgCl_2$ and 0.75 µM of each primer, 10 µg BSA, and 0.5% DMSO. Cycling parameters were one cycle of 5 min at 95 °C, 1 min at 44–48 °C, 2 min at 72 °C, followed by 34 cycles of 95 °C, 46–50 °C,

Table 1. Primers and PCR annealing temperatures.

Locus	T_A °C	Primers F/R
COI	44-46	LCO1490/HCO2198 (Folmer et al. 1994)
16S	48-50	16Sar/16S2 (Palumbi 1996; Garey et al. 1998)
28S	46-48	LSU2/LSU5 (Wade et al. 2006)

and 72 °C for 30 sec each, and a final extension of 5 min at 72 °C. A final 4 °C incubation of 30 min terminated each reaction (Table 1). The amount and specificity of amplifications were verified via agarose electrophoresis and single product amplicons were cycle sequenced using the ABI BigDye terminator kits (Perkin-Elmer Applied Biosystems, Inc.). Sequences were electrophoresed and analyzed on an ABI 3730XL (Perkin-Elmer Applied Biosystems, Inc.) at either the University of Hawaii's Advanced Studies in Genomics, Proteomics, and Bioinformatics facility or Eurofins Genomics, LLC. All loci were initially sequenced in one direction, and any unique haplotypes sequenced in both directions. The COI fragment was sequenced for all individuals, and subsets of these were selected based on unique COI haplotypes and sequenced for 16S and 28S. Due to lower variability in the other two loci, not all individuals with a unique COI haplotype were sequenced for all other loci. All sequences have been uploaded to the Barcode of Life Data System (BoLD; https://doi.org/10.5883/DS-AURICOI) and to GenBank (Accession numbers MT519807–MT519913; Table 2)

Electropherograms were checked for errors, edited, and assembled using Geneious Prime 2019 (http://www.geneious.com/). Sequences of COI were unambiguously aligned using MAFFT ver. 7.388 with the iterative refinement method E-INS-I (Katoh and Standley 2013) implemented in Geneious Prime 2019. Alignments where checked against amino acid sequences as references. Ribosomal genes were aligned using MAFFT and refined using Gblocks ver. 0.91b (Castresana 2000). Refinement of the 16S and 28S alignments in Gblocks removed regions of ambiguous homology created by the addition of gaps during initial alignment and the hypervariable nature of some regions. Phylogenetic analyses were done with and without these regions to evaluate their impact. Sequence alignments were concatenated in Geneious Prime and exported as phylip files for phylogenetic analysis.

Phylogenetic reconstruction was conducted using maximum likelihood (ML) in IQ-TREE ver. 1.6.12 (Nguyen et al. 2015). The best-fit partitioning scheme and the most appropriate substitution model for each partition were estimated using the integrated ModelFinder algorithm (Kalyaanamoorthy et al. 2017) and partition models (Chernomor et al. 2016). Nodal support was estimated with 5,000 ultra-fast bootstrap replicates (Hoang et al. 2018).

To corroborate species delineation based on conchological and anatomical analyses and phylogenetic reconstruction, we used the DNA barcode-based species identification method implemented in SpeciesIdentifier ver. 1.8 (Meier et al. 2006).

Museum catalog numbers for specimens used in DNA analysis with numbers of specimens from which shell measurements, reproductive anatomy, and radular morphology were obtained, are listed in Table 2.

Results

Recent surveys recorded extant populations of two of the three species within the *perpusilla* group: *A. perpusilla* and *A. perversa* (Fig. 1C, D, respectively) and a new spe-

Table 2. Museum catalog numbers for specimens used in genetic analysis with numbers of specimens from which shell measurements, reproductive anatomy and radular morphology were obtained. Catalog numbers (BPBM) are for lots from which specimens were sequenced (N = number of individuals sequenced from each lot).

Genus	Species	Island	BPBM	COI	16S	28S	Shell measurements	Reproductive system	Radula
Auriculella	*ambusta*	Oahu	BPBM 285779 (1)	MT519807	–	MT519879	–	–	–
		Oahu	BPBM 285779 (1)	MT519811	MT519861	MT519880	–	–	–
		Oahu	BPBM 285780 (1)	MT519808	MT519860	–	–	–	–
		Oahu	BPBM 285781 (1)	MT519809	–	–	–	–	–
		Oahu	BPBM 285782 (2)	MT519810	–	–	–	–	–
	auricula	Oahu	BPBM 119141	–	–	–	–	1	–
		Oahu	BPBM 119157	–	–	–	–	–	1
		Oahu	BPBM 119172	–	–	–	–	–	2
		Oahu	BPBM 119202	–	–	–	–	1	–
		Oahu	BPBM 12651	–	–	–	21	–	–
		Oahu	BPBM 12666	–	–	–	26	–	–
		Oahu	BPBM 164138	–	–	–	–	–	1
		Oahu	BPBM 164143	–	–	–	–	–	1
		Oahu	BPBM 189709	–	–	–	–	–	1
		Oahu	BPBM 189710	–	–	–	–	–	1
		Oahu	BPBM 190854	–	–	–	–	–	1
		Oahu	BPBM 285783	–	–	–	3	–	–
	crassula	Maui	BPBM 285784 (1)	MT519819	–	MT519888	–	–	–
		Maui	BPBM 285785 (1)	MT519814	–	MT519883	–	–	–
		Maui	BPBM 285786 (1)	MT519813	MT519863	MT519882	–	–	–
		Maui	BPBM 285787 (3) BPBM 285793 (3) BPBM 285791 (5) BPBM 285792 (1)	MT519816	MT519865	MT519885	–	–	–
		Maui	BPBM 285788 (1)	MT519815	MT519864	MT519884	–	–	–
		Maui	BPBM 285788 (2)	MT519817		MT519886	–	–	–
		Maui	BPBM 285789 (4) BPBM 285788 (2)	MT519818	–	MT519887	–	–	–
		Maui	BPBM 285790 (3)	MT519812	MT519862	MT519881	–	–	–
	gagneorum sp. nov.	Oahu	BPBM 174233	–	–	–	7	–	–
		Oahu	BPBM 21823	–	–	–	40	–	–
		Oahu	BPBM 285794 (1) BPBM 285844 (1) BPBM 285796 (1)	MT519823	–	MT519891	–	–	–
		Oahu	BPBM 285795 (1)	MT519826	–	–	–	–	–
		Oahu	BPBM 285796 (1) BPBM 285794 (1)	MT519820	MT519866	MT519889	–	–	–
		Oahu	BPBM 285797 (1)	MT519821	MT519867	MT519890	–	1	–
		Oahu	BPBM 285797 (1)	MT519824	–	MT519892	–	–	–
		Oahu	BPBM 285798 (1)	MT519825	–	–	–	1	1
		Oahu	BPBM 285799 (1) BPBM 285800 (2) BPBM 285843 (1)	MT519822	MT519868	–	–	–	–
	malleata	Oahu	BPBM 285801 (1)	MT519830	–	MT519894	–	–	–
		Oahu	BPBM 285801 (1)	MT519831	–	MT519895	–	–	–
		Oahu	BPBM 285802 (1)	MT519829	MT519869	MT519893	–	–	–
		Oahu	BPBM 285803 (3)	MT519828	–	–	–	–	–
		Oahu	BPBM 285804 (1)	MT519832	MT519870	MT519896	–	–	–
		Oahu	BPBM 285804 (1)	MT519827	–	–	–	–	–
	minuta	Oahu	BPBM 12799	–	–	–	14	–	–
		Oahu	BPBM 12804	–	–	–	25	–	–
		Oahu	BPBM 170304	–	–	–	10	–	–
		Oahu	BPBM 98043	–	–	–	–	–	2
		Oahu	BPBM 99164	–	–	–	–	2	–

Genus	Species	Island	BPBM	COI	16S	28S	Shell measurements	Reproductive system	Radula
Auriculella	*minuta*	Oahu	BPBM 99164	–	–	–	–	–	3
	montana	Oahu	BPBM 285805 (1)	MT519833	–	–	–	–	–
	perpusilla	Oahu	BPBM 122643	–	–	–	15	–	–
		Oahu	BPBM 134280	–	–	–	15	–	–
		Oahu	BPBM 134431	–	–	–	15	–	–
		Oahu	BPBM 15048	–	–	–	4	–	–
		Oahu	BPBM 285806 (1)	MT519837	MT519872	MT519898	–	–	1
		Oahu	BPBM 285807 (1) BPBM 285808 (1)	MT519835	MT519871	MT519897	–	–	–
		Oahu	BPBM 285808 (1)	MT519834	–	–	–	–	–
		Oahu	BPBM 285808 (1)	MT519836	–	–	–	–	–
		Oahu	BPBM 90853	–	–	–	–	–	2
		Oahu	BPBM 93626	–	–	–	–	3	2
	perversa	Oahu	BPBM 12798	–	–	–	15	–	–
		Oahu	BPBM 164180	–	–	–	–	–	2
		Oahu	BPBM 22767	–	–	–	34	–	–
		Oahu	BPBM 285809 (1)	MT519839	–	–	–	–	–
		Oahu	BPBM 285810 (2)	MT519838	–	–	–	1	–
		Oahu	BPBM 97904	–	–	–	–	1	2
	tenella	Oahu	BPBM 125606	–	–	–	7	–	–
		Oahu	BPBM 162827	–	–	–	–	1	–
		Oahu	BPBM 18943	–	–	–	1	–	–
		Oahu	BPBM 211034	–	–	–	–	1	1
		Oahu	BPBM 285811	–	–	–	2	–	–
		Oahu	BPBM 285812 (1)	MT519841	MT519874	MT519900	–	–	–
		Oahu	BPBM 285812 (1) BPBM 285813 (1) BPBM 285814 (1)	MT519842	MT519875	–	–	–	–
		Oahu	BPBM 285815 (1)	MT519843	–	–	–	–	1
		Oahu	BPBM 285816 (1)	MT519840	MT519873	MT519899	–	–	–
		Oahu	BPBM 285817 (1)	MT519844	MT519876	MT519901	–	–	–
		Oahu	BPBM 33194	–	–	–	42	–	–
	turritella	Oahu	BPBM 285818 (1)	MT519845	–	MT519902	–	–	–
		Oahu	BPBM 285818 (1) BPBM 285819 (1) BPBM 285829 (2) BPBM 285832 (1) BPBM 285833 (1)	MT519850	–	MT519904	–	–	–
		Oahu	BPBM 285821 (1)	MT519847	–	–	–	–	–
		Oahu	BPBM 285823 (1) BPBM 285824 (3) BPBM 285822 (1) BPBM 285820 (1) BPBM 285826 (1) BPBM 285827 (1) BPBM 285828 (1)	MT519849	–	MT519903	–	–	–
		Oahu	BPBM 285825 (1) BPBM 285830 (1) BPBM 285831 (2) BPBM 285834 (1)	MT519848	–	–	–	–	–
		Oahu	BPBM 285832 (1) BPBM 285835 (1)	MT519846	–	–	–	–	–
	uniplicata	Maui	BPBM 285836 (1)	MT519851	–	MT519905	–	–	–
		Maui	BPBM 285836 (1)	MT519852	–	MT519906	–	–	–
		Maui	BPBM 285837 (1)	MT519853		MT519907	–	–	–
		Maui	BPBM 285837 (1)	MT519856	–	MT519910	–	–	–
		Maui	BPBM 285838 (2)	MT519855	–	MT519909	–	–	–
		Maui	BPBM 285839 (1)	MT519857	–	MT519911	–	–	–
		Maui	BPBM 285840 (2)	MT519854	–	MT519908	–	–	–
Tornatellaria	sp.	Maui	BPBM 285841 (1)	MT519858	MT519877	MT519912	–	–	–
Tornatellides	sp.	Molokai	BPBM 285842 (1)	MT519859	MT519878	MT519913	–	–	–

cies with similar shell morphology, *A. gagneorum* sp. nov. (Fig. 1F). No populations of *A. auricula* (type species of the genus, Fig. 1A) or *A. minuta* (Fig. 1B) were recorded in our surveys and both species may be extinct.

The 104 snails representing ten *Auricullela* species and two outgroup taxa (*Tornatellaria* sp. and *Tornatellides* sp.) sequenced for this study produced 53 COI haplotypes, 19 and 35 sequences for 16S and 28S, respectively. Alignments for each locus were 654 bp for COI, 464 bp for 16S and 539 bp for 28S, making the concatenated dataset of 53 individuals 1657 bp with 223 parsimony informative sites. Sixteen individuals were represented by all three loci, while three individuals had only COI and 16S, 19 with COI and 28S, and 15 with only COI. The best-fit partitioning scheme used distinct models for each locus with the best-fit models being K3Pu+F+I+G4, TPM2u+F+G4, and TIM3+F for COI, 16S, and 28S respectively.

The ML tree constructed from the concatenated dataset produced a well-resolved tree with all conchologically defined taxa recovered in strongly supported clades (Fig. 2). None of the groupings suggested by Cooke and Kondo (1960) based on gross shell morphology were recovered in the ML tree. As such, *A. perpusilla* and *A. perversa*, previously referred to the *perpusilla* group were recovered in unrelated clades with each as sister to much larger shelled species, *A. ambusta* and *A. montana*, respectively. Similarly, the new species *Auriculella gagneorum* sp. nov. was recovered as sister to *A. tenella* and not close to *A. perpusilla* or *A. perversa* with which it was previously confused.

The best match/best close match criteria (Meier et al. 2006) applied to all 53 COI haplotypes successfully matched all sequences in the correct conspecific clusters within a 3–4% threshold consistent with conchologically and phylogenetically recognized clades. Correct identifications with both approaches was 94.33%, with the other 5.66% (three sequences) lacking any conspecific sequences with which to cluster. These included the two outgroup taxa and *A. montana*, all of which were represented by a single sequence.

Systematics

Class Gastropoda Cuvier, 1795
Subclass Heterobranchia Burmeister, 1837
Order Stylommatophora A. Schmidt, 1855
Superfamily Pupilloidea W. Turton, 1831
Family Achatinellidae Gulick, 1873
Subfamily Auriculellinae Odhner, 1921

Genus *Auriculella* Pfeiffer, 1854

Type species. *Partula auricula* Férussac, 1821 by subsequent designation (Gulick 1873).

Diagnosis. Small to moderately sized Achatinellidae, 4 to 12 mm in adult shell height. Shells either dextral or sinistral, taller than wide, with a strong parietal la-

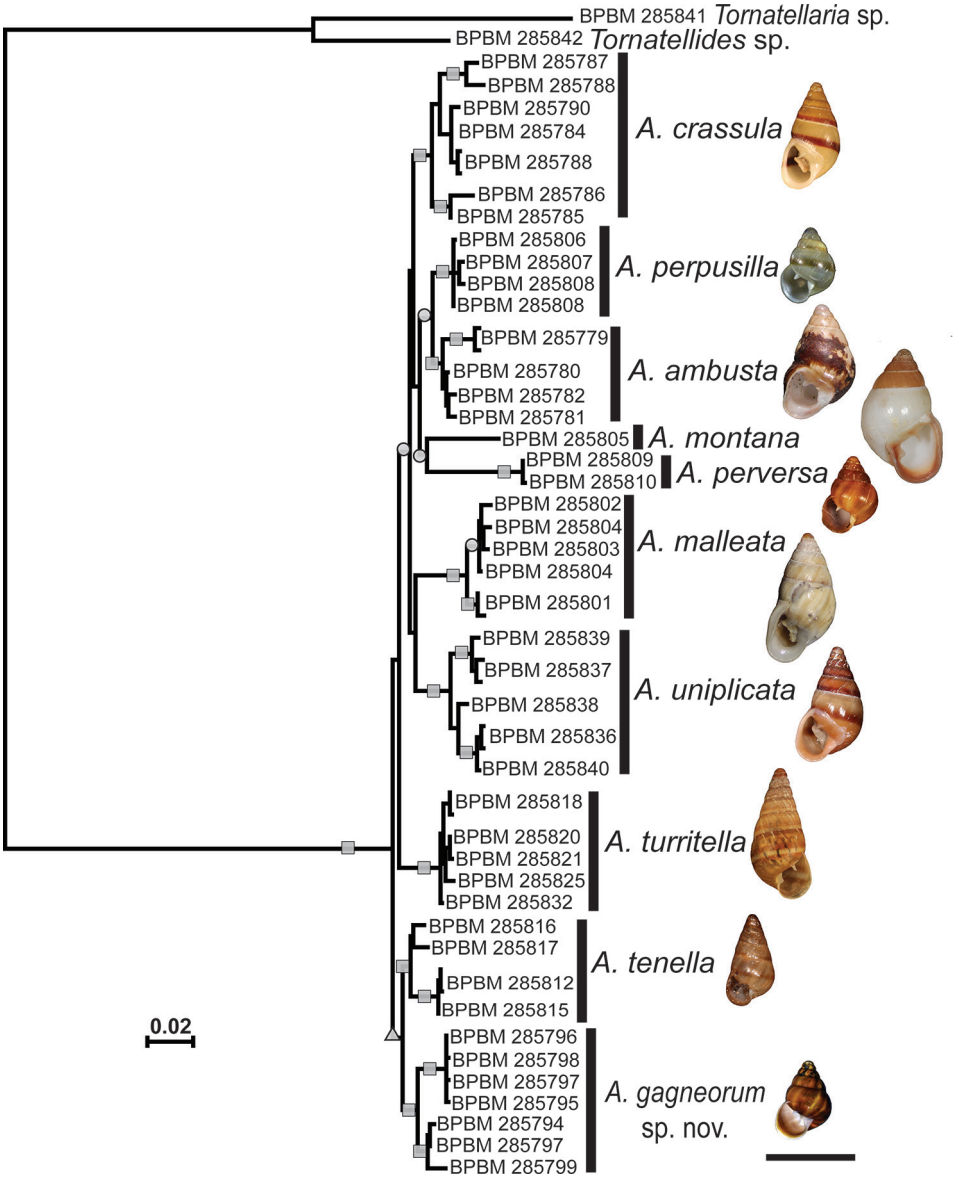

Figure 2. Phylogenetic tree of ten *Auriculella* spp. produced via Maximum Likelihood using a concatenated matrix composed of partial sequences of COI, 16S and 28S. Shapes on the nodes correspond to ML bootstrap values of 70–79 (triangle), 80–89 (circle), and 90–100 (square).

mella. Juvenile shells have two columellar lamellae, one or both of which are lacking in adults. Phallus with an epiphallus and a nearly apical appendix. Phallus retractor muscle inserted apically on the epiphallus and not secondarily attached to the appendix. Members of *Auriculella* are the only achatinellids known to have an epiphallus. All *Auriculella* species are oviparous (Pilsbry and Cooke 1914–1916).

***Auriculella auricula* (Férussac, 1821)**
Figures 1A, 3A, B, 4A, 5A

Partula auricula Férussac, 1821: 66.

Auriculella auricula – Gulick 1872: 222; Gulick 1873: 91; Pilsbry and Cooke 1915: 78–80, pl. 24, figs 1–10; Cooke and Kondo 1960: 270–272, figs 113a–e, 114a–c; Cowie et al. 1995: 75; Severns 2011: 206, pl. 80, fig. 2.

Type material. ***Neotype*:** USA • 1; H = 8.7 mm, W = 4.2 mm, AH = 4.6 mm, AW = 3.3 mm, with 6.4 WH; Honolulu County, Oahu, Koolau Mountains, Tantalus; 09 Jun 1943; Y. Tanada leg.; BPBM 189709.

Type locality. "Sans doute les îles de la mer du Sud?" [without doubt the south sea islands?]; colloquially "sans doute" means probably; here restricted to Tantalus.

Diagnosis. ***Shell.*** Shell dextral or sinistral with flat-sided whorls and an obtuse apex, H = 8.0 ± 0.4 mm, W = 4.3 ± 0.2 mm, WH 6.0 ± 0.2, AH = 4.1 ± 0.2 mm, AW = 3.1 ± 0.2 mm (*N* = 50; Table 2). Columella with a single strong lamella and without an axial ridge. Parietal lamella is strong and smooth and not undulate, extending 0.3 to 0.7 whorls into the aperture. Shell color is tan, brown, or yellowish, often with a single narrow brown or white band (Fig. 3B). White bands are sometimes bordered by two darker brown bands and apical whorls are often darker brown. Lip reflected, thickened, white or brown in color.

Reproductive system. Phallus retractor muscle relatively long, attached apically to a short but well-defined epiphallus (Fig. 4A). Appendix is longer than the phallus and about ⅔ the diameter of the phallus at its attachment. The appendix narrows abruptly at ⅕ its length and remains narrow to its terminus. Phallus is broad, narrowing only slightly apically and basally. Atrium is relatively short and broad. Vagina is about ⅓ the length of the phallus.

Radula. Radula with an irregular rachidian flanked on either side by rastriform marginal teeth, as diagnostic of the family (Fig. 5A). Each tooth has a long narrow base that expands slowly for ¾ of the length of the tooth before reaching the forward curving cusps, which comprise the remaining ¼ of the tooth. There are three long cusps at mesocone, endocone, and ectocone positions with two or more alternating larger and smaller cusps intercalated between them. Number of teeth per row range from 177 to 183 (*N* = 6; Table 2).

Distribution and ecology. *Auriculella auricula* is endemic to Oahu's Koolau Mountains (Fig. 1A), historically found across the range at elevations from 61 m to 305 m. The species is arboreal and found on vegetation including: *Cordyline* sp. *Freycinetia arborea*, *Metrosideros polymorpha*, *Canna* sp. (BPBM 34025, 49056, 51405), *Aleurites moluccanus*, *Psychotria* sp., *Zingiber* sp., *Psidium cattleyanum*, *Musa* sp., *Asplenium* sp., and unspecified ferns and shrubs. The species has also been recorded on the ground under stones, logs and dead leaves. Live specimens recorded in the BPBM collection were last collected by Y. Kondo in 1946 from Palolo Valley; the species has not been recorded in recent surveys and is considered here possibly extinct.

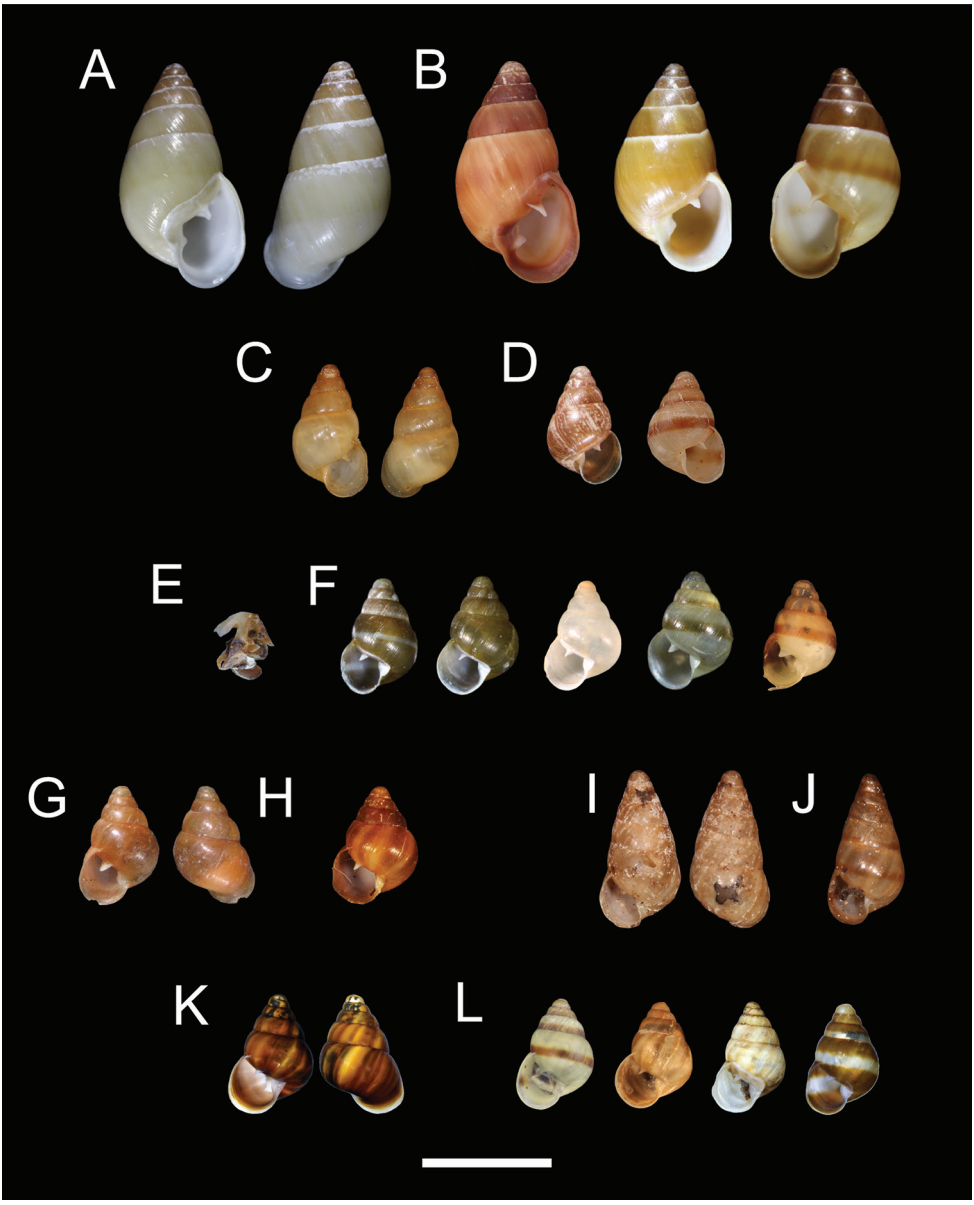

Figure 3. Comparative shell morphology of **A** *Auriculella auricula* neotype BPBM 18709 **B** *Auriculella auricula* shell variation (left to right) BPBM 12651, BPBM 12666 **C** *Auriculella minuta* lectotype BPBM 42377 **D** *Auriculella minuta* shell variation (left to right) BPBM12804, paralectotype MCZ 73037 **E** *Auriculella perpusilla* holotype MCZ 39912 **F** *Auriculella perpusilla* (left to right) BPBM 285806, BPBM 134341 (2 spm), BPBM 134280 white one, BPBM 122643, BPBM 13443 **G** *Auriculella perversa* lectotype BPBM 42384, 3 shells **H** *Auriculella perversa* shell variation (left to right) paralectotype ANSP 91817 **I** *Auriculella tenella* lectotype BPBM 18943 **J** *Auriculella tenella* shell variation paralectotype BPBM 109679 **K** *Auriculella gagneorum* sp. nov. holotype (BPBM 285843) and **L** *Auriculella gagneorum* sp. nov. paratypes, left to right (BPBM 285797, 285794, 285795, 285798). Scale bar: 5 mm.

Remarks. In the original description, Férussac (1821) provided measurements for a single shell of three lines (6.8 mm) in height and 1¾ lines (4.0 mm) in width. His collection is housed in MNHN where there are two lots labelled *A. auricula* that are attributed to Férussac. The first (MNHN IM-2000-34306, 34307, 34308) is from Férussac's collection but does not contain original labels. The three dextral shells are identified as *A. auricula* from the Mariana Islands, but they are not *A. auricula* and instead appear to be a gerontic adult and two juveniles similar to *Auriculella ambusta*, a species not found on the same mountain range as *A. auricula*. The other lot (MNHN IM-2014-7009) is from the Deshayes collection. Its source is unknown but probably came from Férussac whose specimens Deshayes used to complete Férussac's "Histoire naturelle des mollusques terrestres et fluviatiles" after Férussac's death in 1836. The lot contains six specimens of *Auriculella pulchra*, two of which are sinistral and all of which are larger than 6.8 mm. The two lots are not consistent with Férussac's description, and we exclude these lots as possible syntypes of *A. auricula*. We have not located any other type material of *A. auricula* and we consider the types to be lost. Stabilizing the nomenclature of this species is important because it is the type species of the genus *Auriculella*, a genus with many similar but conchologically variable and poorly resolved species, nearly all of which are highly endangered. We designate BPBM 18709 (Fig. 3A) from Tantalus, Oahu as neotype of *Auriculella auricula* to stabilize the taxonomic status and type locality of the species as well as the genus *Auriculella*. The neotype matches Férussac's original description in having an acute ovoid shell with an obtuse apex, strong parietal lamella, and single columellar lamella. The color of the neotype is more tan than yellowish as described in the original description but the species is known to be polymorphic for shell color and pattern as well as chirality. The shell used in Férussac's description was sinistral while the neotype is dextral. We chose a dextral specimen with slightly different coloration because it was used by Cooke and Kondo (1961) to describe the nervous system and reproductive anatomy of *Auriculella auricula* thus clearly defining the species as well as the genus. The other four specimens from BPBM 189709 are re-cataloged as BPBM 285783. One of these is a broken shell presumably corresponding to the animal dissected by Cooke and Kondo (1961).

Auriculella minuta Cooke & Pilsbry, 1915

Figures 1B, 3C, D, 4B, 5B

Auriculella minuta Cooke & Pilsbry in Pilsbry & Cooke, 1915: 90, pl. 25, figs 5–9; Cowie et al. 1995: 76; Johnson 1996: 190; Severns 2011: 210, pl. 82, fig. 1.

Type material. ***Lectotype*:** USA • 1, H = 4.9 mm, W = 2.8 mm, AH = 2.2 mm, AW = 1.7 mm, WH = 5.7.; Honolulu County, Oahu, Koolau Mountains, Nuuanu; Nuuanu Valley Ridge 7, east, on ti, lehua, *Passiflora foetida*; Cooke leg.; BPBM 42377, here designated

***Paralectotypes*:** USA – Honolulu County, Oahu, Koolau Mountains • 1; Nuuanu Valley; Cooke leg.; BPBM 42377 • 1; Nuuanu Ridge; BPBM 13034 • 2; Nuuanu;

BPBM 42379 • 1; Nuuanu Ridge; Cooke leg.; BPBM 42380 • 33; Nuuanu Ridge; Cooke leg.; BPBM 4238 • 1; Nuuanu Valley; Cooke leg.; BPBM 42382 • 82; Nuuanu Valley; Cooke leg.; BPBM 42383 • 5, Palolo Valley; Lyman leg.; BPBM 12808

Paralectotypes not examined. ANSP 91816 (11 spm), ANSP 113294 (10 spm), MCZ 73037 (5 spm), SMF 7127 (4 spm), BPBM 12808 (5 spm).

Possible paralectotype. USA – Honolulu County, Oahu, Koolau Mountains • 6; Palolo Valley; BPBM 16435.

Type locality. Hawaiian Islands, Oahu, Nuuanu. See Remarks.

Diagnosis. ***Shell.*** Shell dextral, H = 4.4 ± 0.18 mm, W = 2.7 ± 0.11 mm, WH = 5.1 ± 0.08, AH = 1.9 ± 0.11 mm, AW = 1.3 ± 0.08 mm (*N* = 50; Table 2). Whorls inflated. Columella in juveniles with a strong lamella that is reduced and covered by a thickening of the inner edge of the lip in adults. Some adults show a short projection or angulation where the columellar lamella was located. Adult columella reflected, without an axial ridge. Parietal lamella is smooth and not undulate, extending 0.2 to 0.5 whorls into the aperture. Shell color is pale tan or dark brown, with or without a single peripheral color band of pale tan or dark brown (Fig. 3D, MCZ 73037).

Reproductive system. Phallus retractor muscle relatively long, attached apically to a short but well-defined epiphallus (Fig. 4B). Appendix is nearly equal in length to the phallus. Appendix the diameter of the phallus at its attachment, narrowing abruptly at ⅓ its length and remaining narrow to its terminus. Apical ¾ of the phallus is broad, basal ¼ narrows abruptly remaining narrow to the junction with the moderately long atrium. Vagina is long and nearly half the length of the phallus.

Radula. Radula with an irregular rachidian flanked on either side by rastriform marginal teeth, as diagnostic of the family (Fig. 5B). Each tooth has a long narrow base that expands slowly for ¾ of the length of the tooth before reaching the forward curving cusps, which comprise the remaining ¼ of the tooth. There are three long cusps at mesocone, endocone, and ectocone positions with two or more alternating larger and smaller cusps intercalated between them. There are roughly 105 teeth per row (*N* = 5; Table 2).

Distribution and ecology. *Auriculella minuta* is endemic to Oahu's Koolau Mountain Range (Fig. 1B), found predominantly in the southern portion of the range with a few historical records from the southern edge of the northern Koolau Mountains. No elevational range information is available with these historical specimen records. The species is arboreal and found on vegetation, including *Cordyline fruticosa*, *Dioscorea alata*, *Freycinetia arborea*, *Kadua affinis*, *Lobelia* sp., *Psidium guajava*, and *Touchardia latifolia*. Live specimens recorded in the BPBM collection were last collected by Olaf Oswald in Waiahole in 1931 and is considered herein extinct.

Remarks. A holotype was not designated in the original description and the type series came from two different localities: Nuuanu collected by Cooke, and Palolo collected by both Cooke and Lyman (Pilsbry and Cooke 1915: 90). Five figures were provided with the original description (Pilsbry and Cooke 1915: pl. 25, figs 5–9) from Nuuanu, which according to the figure caption were based on specimens from BPBM and ANSP. The figure caption did not indicate which museum lots the figured specimens came from but the BPBM ledger in Cooke's handwriting lists: BPBM 42377 "holotype", figs 5, 9 (see note for ANSP 91816 below); BPBM 42378, "cotype",

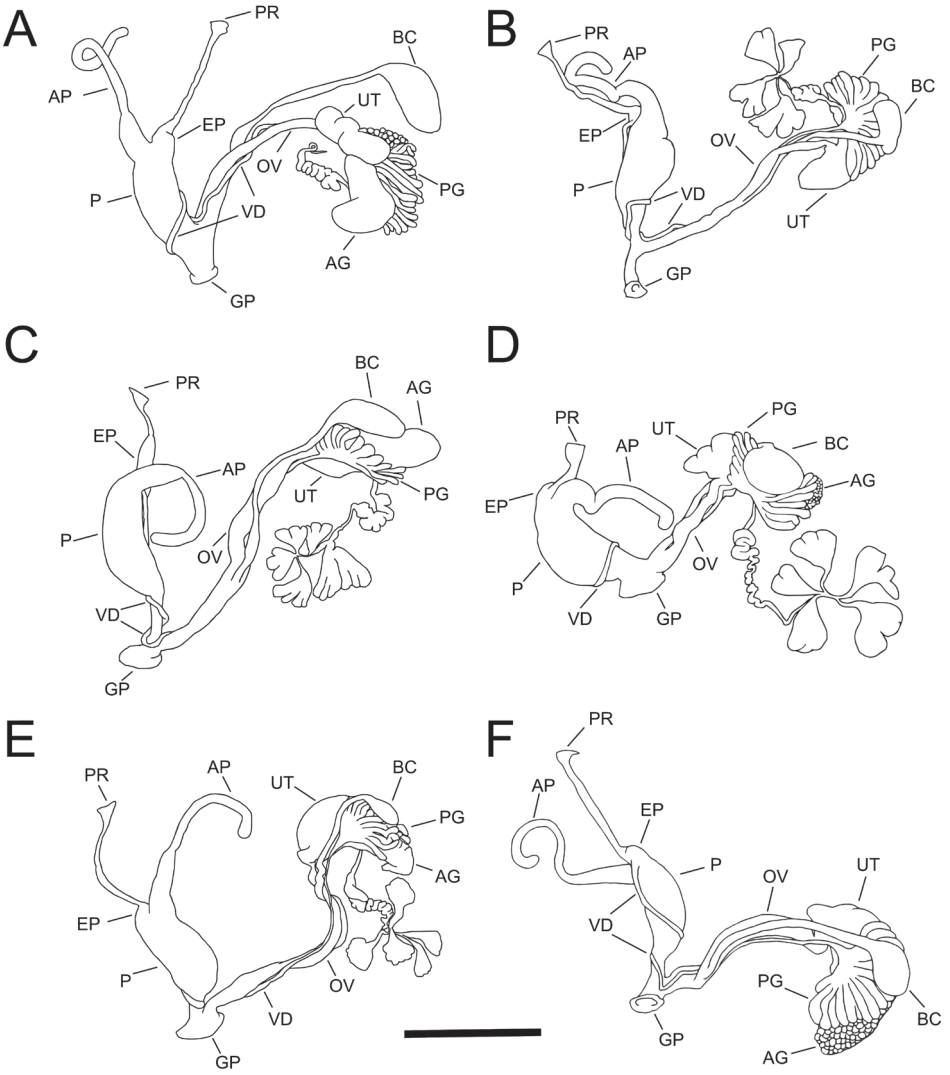

Figure 4. Comparative reproductive anatomy of **A** *Auriculella auricula* BPBM 119141 **B** *Auriculella minuta* BPBM 99146 **C** *Auriculella perpusilla* BPBM 93626 **D** *Auriculella perversa* BPBM 97904 **E** *Auriculella tenella* BPBM 211034 **F** *Auriculella gagneorum* sp. nov. paratype BPBM 285800. Abbreviatons for reproductive structures are: AG = albumen gland; AP = penial appendix; BC = bursa copulatrix; EP = epiphallus; GP = gonopore; P = penis; OV = free oviduct; PG = prostate gland; PR = penial retractor muscle; UT = uterus; VD = vas deferens. Scale bar: 1mm.

fig. 8 (not ANSP 113294 as stated in Severns, 2011: 210); BPBM 42379 “paratypes”; BPBM 42380, “cotype”, fig. 7; BPBM 42381, “paracotypes”; 42382, “cotype”, fig. 6; BPBM 42383, “paracotypes”. The BPBM ledger documents that BPBM lots were the source of other type material: BPBM 42379 – 83 were the source for SMF 7127 (Zilch 1962: 78) and BPBM 42379, split from BPBM 13034, was the source lot for MCZ

73037. The ledger also indicated that two specimens were given to Dautzenberg whose collections were obtained by RBINS. Two ANSP lots 91816 and 113294 were received by Pilsbry from Cooke. The original label for ANSP 91816 is marked "cotype" and the source for fig. 9 in the description. Because the caption for figs 5–9 states that at least one of the figured specimens is from ANSP we believe this to be the source for fig. 9 rather than BPBM 42377 as stated in the BPBM ledger, although we do believe BPBM 42377 is the source for fig. 5. Johnson (1996) lists lot BPBM 42377 as the holotype citing the original BPBM specimen labelling. However, the species description is clearly based on multiple specimens all of which should be considered syntypes. In addition to the specimens from Nuuanu, the material from Palolo collected by both Lyman and Cooke are also part of the type series. There is only one lot of *Auriculella minuta* (BPBM 12808) collected by Lyman from Palolo and although it is not labelled as being part of the type series it is likely the lot collected by Lyman that was mentioned in the species description. A second lot, BPBM 16435, lacks information on the collector but may be the lot collected by Cooke. We here designate lot BPBM 42377 as the lectotype, restricting the type locality to Nuuanu.

Unlike the other species traditionally placed in the *perpusilla* group, the shell of *A. minuta* is dextral rather than sinistral. The columella does not bear an axially oriented ridge like the one found in *A. perversa*. The palatal lamella is smooth and not undulate unlike that of *A. gagneorum* sp. nov. The epiphallus is short and well defined similar to *A. gagneorum* sp. nov., but unlike the long epiphallus of *A. perpusilla* or the poorly defined epiphallus of *A. perversa*. The appendix narrows abruptly at approximately ⅓ its length unlike the gently tapered appendix of *A. gagneorum* sp. nov.

Auriculella perpusilla E. Smith, 1873

Figures 1C, 3E, F, 4C, 5C

Auriculella perpusilla E. Smith in Gulick & Smith, 1873: 87, pl. 10, fig. 26; Pilsbry and Cooke 1915: 91–92, pl. 25, figs 1, 2; Cowie et al. 1995: 77; Johnson 1996: 193; Severns 2011: 210, pl. 82, fig. 3.

Type material. ***Holotype*:** USA • 1; shell crushed; H = 4 mm, W = 2 ⅔ mm (according to original description); Honolulu County, Oahu, Koolau Mountains; 1918; John T. Gulick leg.; MCZ 39912.

Type locality. "Kohalu" (*sic*, Kahaluu) on Oahu.

Diagnosis. ***Shell.*** Shell sinistral with inflated whorls, H = 4.4 ± 0.26 mm, W = 3.0 ± 0.15 mm, WH = 5.0 ± 0.14, AH = 2.1 ± 0.14 mm, AW = 1.5 ± 0.11 mm (*N* = 50; Table 2f). Columella in juveniles with a strong lamella that is reduced and covered by a thickening of the inner edge of the lip in adults. Some adults show a short projection or angulation where the columellar lamella was located. Parietal lamella is smooth and not undulate, extending 0.3 whorls into the aperture, and sometimes bears a weak angulation at mid-point. Shell color is pale tan or dark brown, with or without a single peripheral color band of pale tan or dark brown (Fig. 3F).

Reproductive system. Phallus retractor muscle relatively short, attached apically to a long epiphallus, which is nearly ⅓ the length of the phallus (Fig. 4C). Appendix is nearly equal in length to the phallus. Appendix slightly over half the diameter of the phallus at its attachment, narrowing abruptly at ⅓ its length and remaining narrow to its terminus. Apical ⅔ of the phallus is broad, basal ⅓ narrows abruptly and remains narrow to the junction with the short atrium. Vagina is long and nearly half the length of the phallus.

Radula. Radula with an irregular rachidian flanked on either side by rastriform marginal teeth, as diagnostic of the family (Fig. 5C). Each tooth has a long narrow base that expands slowly for ¾ of the length of the tooth before reaching the forward curving cusps, which comprise the remaining ¼ of the tooth. There are three long cusps at mesocone, endocone, and ectocone positions with two or more alternating larger and smaller cusps intercalated between them. There are roughly 127 teeth per row (N = 5; Table 2).

Distribution and ecology. *Auriculella perpusilla* is endemic to Oahu's Koolau Mountain Range (Fig. 1C), recorded from across the range at elevations of 61 m to 1066 m. The species is arboreal and found on vegetation, including: *Antidesma pulvinatum*, *Cordyline fruticosa*, *Freycinetia arborea*, *Kadua affinis*, *Lobelia* sp., *Metrosideros polymorpha*, *Myrsine* sp., *Psidium guajava*, *Psychotria kaduana*, *Syzygium sandwicense*, *Touchardia latifolia*, and on unspecified ferns, tree trunks, and dead leaves. Recent observations are restricted to Tantalus (southern Koolau Mountains; Fig. 1C).

Remarks. No holotype was designated in the original description which included a single figure and provided a single set of measurements: height 4 mm width 2 ⅔ mm. The shell donated by Gulick is MCZ 39912 and is labeled holotype. Pilsbry and Cooke (1915: 91) indicated that only a single shell existed; "The single specimen collected by Mr. Gulick and described by Mr. Smith, is unfortunately broken." Consequently, MCZ 39912 is the holotype by monotypy.

Unlike *A. minuta*, *A. perpusilla* is sinistral and the columella does not bear an axially oriented ridge like the one found in *A. perversa*. The palatal lamella is smooth and not undulate like *A. gagneorum* sp. nov. The epiphallus is long unlike the poorly defined epiphallus of *A. perversa* or the short but well-defined epiphallus of *A. minuta* and *A. gagneorum* sp. nov. The appendix narrows abruptly at approximately ⅓ its length unlike *A. gagneorum* sp. nov.

Auriculella perversa Cooke, 1915

Figures 1D, 3G, H, 4D, 5D

Auriculella perversa Cooke in Pilsbry & Cooke, 1915: 90–91, pl. 25, figs 3, 4; Cowie et al. 1995; 77; Johnson 1996: 193; Severns 2011: 210, pl. 82, fig. 2.

Type material. ***Lectotype*:** USA • 1; H = 4.7 mm, W = 3.3 mm, AH = 2.2 mm, AW = 2.0 mm, WH = 5.1; Honolulu County, Oahu, Koolau Mountains, Nuuanu; Ridge 9, east side, on *Passiflora foetida*; Cooke leg.; BPBM 42384, here designated.

***Paralectotypes*:** USA • 1; Honolulu County, Oahu, Koolau Mountains, Nuuanu; Ridge 9, east side, on *Passiflora foetida*; Cooke leg.; BPBM 42385.

***Paralectotypes not examined*:** ANSP 91817 (6 spm), ANSP 108272 (13 spm), ANSP 163399 (1 spm), ANSP 163411 (5 spm), MCZ 73044 (2 spm), SMF 7090 (1 spm).

Type locality. Oahu: Nuuanu. See Remarks.

Diagnosis. *Shell*. Shell sinistral with inflated whorls, H = 4.4 ± 0.26 mm, W = 3.0 ± 0.23 mm, WH = 5.2 ± 0.08, AH = 2.0 ± 0.18 mm, AW = 1.4 ± 0.08 mm (Table 2). Columella in juveniles with a strong lamella that is reduced and covered by a thickening of the inner edge of the lip in adults. The columellar thickening usually bears an axially oriented ridge. Adults do not show a short projection or angulation where the columellar lamella was located. Parietal lamella is smooth and not undulate, extending 0.3 to 0.5 whorls into the aperture. Shell color is solid brown to dark brown with darker brown axial bands (Fig. 3H).

***Reproductive system*.** Phallus retractor muscle relatively short attached apically to a short and poorly defined epiphallus (Fig. 4D). Appendix is as long as the phallus and a bit over half the diameter of the phallus at its attachment, narrowing abruptly at ⅓ its length and remaining narrow to its terminus. Phallus is broad, narrowing only slightly at the junction with the short atrium. Vagina is short.

***Radula*.** Radula with an irregular rachidian flanked on either side by rastriform marginal teeth, as diagnostic of the family (Fig. 5D). Each tooth has a long narrow base that expands slowly for ¾ of the length of the tooth before reaching the forward curving cusps, which comprise the remaining ¼ of the tooth. There are three long cusps at mesocone, endocone, and ectocone positions with two or more alternating larger and smaller cusps intercalated between them. There are roughly 127 teeth per row (N = 4; Table 2).

Distribution and ecology. *Auriculella perversa* is endemic to Oahu's southern Koolau Mountain Range (Fig. 1D), recorded from 61 m to 914 m elevation. *Auriculella perversa* is arboreal and found on *Clermontia* sp., *Cordyline fruticosa*, *Dubautia laxa*, *Freycinetia arborea*, *Metrosideros polymorpha*, *Musa* sp., *Pritchardia* sp., *Psidium guajava*, and unspecified ferns, tree trunks, and dead leaves. Prior to our recent surveys the last live specimens were collected in 1939 by O.H. Emerson, E.H. Bryan Jr., and D. Anderson on Kulepeamoa Ridge in the southern Koolau Mountain Range, and the only known extant population recorded occurs in Tantalus.

Remarks. A holotype was not designated in the original description and the type series came from two different localities: Nuuanu collected by Cooke, and Kuliouou collected by Thaanum. Two figures were provided with the original description (Pilsbry and Cooke 1915: pl. 25, figs 3, 4) for material from Nuuanu at BPBM. However, the figure caption does not indicate type status or lot numbers. The BPBM ledger in Cooke's handwriting lists: BPBM 42384 "holotype", figs 3, 4; BPBM 42385, "paratypes". The BPBM ledger documents that BPBM 42385 was also the source of MCZ 7034 and SMF 7090 (Zilch, 1962: 78). The ANSP online catalog list additional specimens from BPBM and labeled as syntypes: ANSP 163411, 91817, Nuuanu; ANSP 163399 Kuliousu [*sic*]. ANSP 108272 Kuliouou was collected by D. Thaanum. Johnson (1996: 193) stated that the "holotype" was BPBM 42384 based on its specimen label. However, it is clear that the original description was based on multiple specimens which should be considered syntypes. We here designate BPBM 42384 as the lectotype. As a result of this lectotype designation the type locality is restricted to Nuuanu.

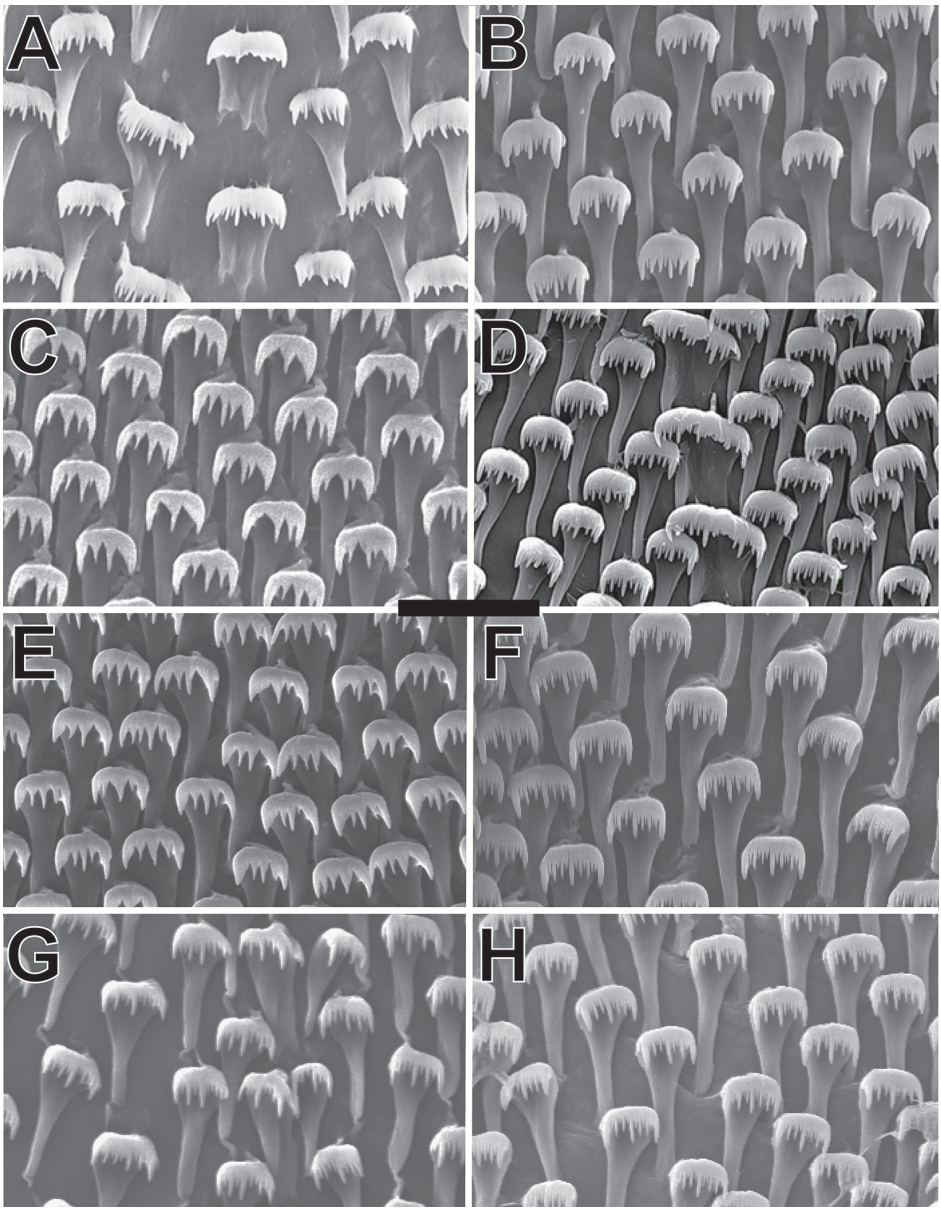

Figure 5. Comparative radular morphology of **A** *A. auricula* (irregular rachidian and rastriform marginal teeth) **B** *A. auricula* (rastriform marginal teeth) **C** *A. minuta* (rastriform marginal teeth) **D** *A. perpusilla* (irregular rachidian and rastriform marginal teeth) **E** *A. perversa* (rastriform marginal teeth) **F** *A. tenella* (rastriform marginal teeth) **G** *A. gagneorum* sp. nov. (irregular rachidian and rastriform marginal teeth) **H** *A. gagneorum* sp. nov. (rastriform marginal teeth). Scale bar: 10 μm.

Unlike *A. minuta*, the shell of *A. perversa* is sinistral. The columella bears an axially oriented ridge unlike all other species in the *perpusilla* group. The palatal lamella is smooth and not undulate like *A. gagneorum* sp. nov. The reproductive system includes a short and poorly defined epiphallus and an appendix that narrows abruptly at ap-

proximately ⅓ its length. The epiphallus is short and poorly defined unlike the long epiphallus of *A. perpusilla* or the short but well-defined epiphallus of *A. minuta* and *A. gagneorum* sp. nov.

Auriculella tenella Ancey, 1889

Figures 1E, 3I, J, 4E, 5E

Auriculella tenella Ancey, 1889: 232–233; Pilsbry and Cooke 1915: 99–100, pl. 19, figs 7, 8; Cowie et al. 1995:77; Wood and Gallichan 2008: 88, pl. 2, fig. 8, ix; Severns 2011: 204, pl. 79, fig. 5.

Type material. ***Lectotype*:** USA • 1; H = 6.2 mm, W = 3.5 mm, AH = 2.3 mm, AW = 1.6 mm, WH = 6.6 whorls; Honolulu County, Oahu, Waianae Mountains; Baldwin leg.; BPBM 18943, here designated.

***Paralectotypes*:** USA • 2; Honolulu County, Oahu, Waianae Mountains; Baldwin leg.; BPBM 285811.

***Paralectotypes not examined*:** NMW 1955.158.24126 (1 spm); RBINS 10591 (accession, 1 spm).

Type locality. "Waianae, dans la partie occidentale de l'île d'Oahu." [Waianae, western part of Oahu Island].

Diagnosis. ***Shell*.** Shell sinistral with inflated whorls, H = 5.6 ± 0.8 mm, W = 3.0 ± 0.4 mm, WH = 6.5 ± 0.3, AH = 2.0 ± 0.3 mm, AW = 1.9 ± 0.3 mm (*N* = 50; Table 2). Columella in juveniles with two lamellae that are reduced and visible only deep within the aperture of adults. Columellar reflection lacks an axially oriented ridge. Parietal lamella is smooth and not undulate, extending 0.3 to 0.5 whorls into the aperture. Shell color straw to brown, indistinctly streaked with red, with or without a single darker brown marginal spiral band.

***Reproductive system*.** Phallus retractor muscle relatively long attached apically to a short but well-defined epiphallus (Fig. 4E). Appendix ⅓ longer and about half the diameter of the phallus at its attachment, narrowing abruptly at ⅓ its length and remaining narrow to its terminus. Phallus is broad, narrowing by half at the junction with the short atrium. Vagina is of moderate length.

***Radula*.** Radula with an irregular rachidian flanked on either side by rastriform marginal teeth, as diagnostic of the family (Fig. 5E). Each tooth has a long narrow base that expands slowly for ¾ of the length of the tooth before reaching the forward curving cusps, which comprise the remaining ¼ of the tooth. There are three long cusps at mesocone, endocone, and ectocone positions with two or more alternating larger and smaller cusps intercalated between them. There are roughly 129 teeth per row (*N* = 3; Table 2).

Distribution and ecology. *Auriculella tenella* is endemic to Oahu's Waianae Mountains, historically found throughout the range between 518 and 1227 m in elevation (Fig. 1E). This species is arboreal and found on *Broussaisia* sp., *Cordyline* sp., *Freycinetia arborea*, *Lantana* sp., *Pelea* sp., *Sadleria cyatheoides*, *Bidens* sp., *Coprosma* sp.,

Euphorbia sp., *Metrosideros* sp., *Psychotria* sp., *Ilex* sp., *Philodendron* sp., and unspecified ferns, grasses, tree trunks, and small plants on stream banks. Occasionally, this species has been recorded on the ground on stones, dead leaves, and bark. The last live specimens in the BPBM collection were recorded in 1948. Our recent surveys documented the species in only three locations in the southern Waianae range.

Remarks. A holotype was not designated in the original description, however, the type locality is listed as "Waianae" and collected by Baldwin. Ancey provided measurements in the original description, "Long., 6; diam., 3; alt. ap., 2 2/3 millim.", which agree well with the designated lectotype. The ledger entry for BPBM 18943 lists four "types" collected by Baldwin from Waialae [*sic*]. However, only three specimens were found. The material probably came from Paul Geret who acquired Ancey's collection after his death and subsequently sold it. Much of Ancey's Hawaiian land and freshwater material was purchased by BPBM in 1908 (Johnson, 1996) but some was sold to other buyers. Both NMW 1955.158.24126 and RBINS 10591 (accession number) have Geret "cotype" labels (Wood and Gallichan 2008:88). Tomlin, the source of the NMW lot, had a sales list confirming purchase from the Ancey collection.

The shell of *A. tenella* has approximately seven nearly flat-sided whorls unlike *A. auricula*, *A. minuta*, *A. perpusilla* and *A. perversa*, which have approximately five whorls, and are inflated in all but *A. auricula*. *Auriculella tenella* is sinistral unlike *A. minuta* and does not bear an axially oriented columellar ridge like *A. perversa* or an undulating palatal lamella like *A. gagneorum* sp. nov. The epiphallus is short and well defined unlike the long epiphallus of *A. perpusilla* or the poorly defined epiphallus of *A. perversa*. The appendix narrows abruptly at approximately ⅓ its length unlike *A. gagneorum* sp. nov.

***Auriculella gagneorum* sp. nov.**
http://zoobank.org/25f68bf8-12f1-461e-be17-263982427bb0
Figures 1F, 3K, L, 4F, 5F, 6A-C

Material examined. *Holotype*: USA • 1, H = 4.7 mm, W = 3.4 mm, AH = 2.3 mm, AW = 1.8 mm, WH = 5.3 whorls; Honolulu County, Oahu, Waianae Mountains, Palawai Gulch; 710 m; 9 Feb. 2018; K. A. Hayes, N. W. Yeung, J. Slapcinsky; hand collected on *Pisonia umbellifera*; GenBank: MT519824-MT519826, MT519866-MT519868, MT519889-MT519592; BPBM 285843.

***Paratypes*:** USA – Honolulu County, Oahu, Waianae Mountains • 1; Puu Hapapa; 23 Jan 2013; D.T.A. Gary, K. Leung, D. R. Sischo, V. J. Costello; BPBM 285794 • 8; Puu Hapapa; 23 Jan 2013; D.T.A. Gary, K. Leung, D. R. Sischo, V. J. Costello; BPBM 285795 • 1; Palawai; 24 Dec 2014; D. R. Sischo and SEPP crew; BPBM 285799 • 3; Puu Hapapa; 24 Jan 2013; D.T.A. Gary, K. Leung, D. R. Sischo, V. J. Costello; BPBM 285796 • 2; Ekahanui; 17 Feb 2013; D.T.A. Gary, K. Leung, D. T. B. Ressler, V. J. Costello; BPBM 285797 • 1; Palawai; 24 Dec 2014; D. R. Sischo and SEPP crew; BPBM 285798 • 2; Palawai; 24 Dec 2014; D. R. Sischo and SEPP crew; BPBM 285800.

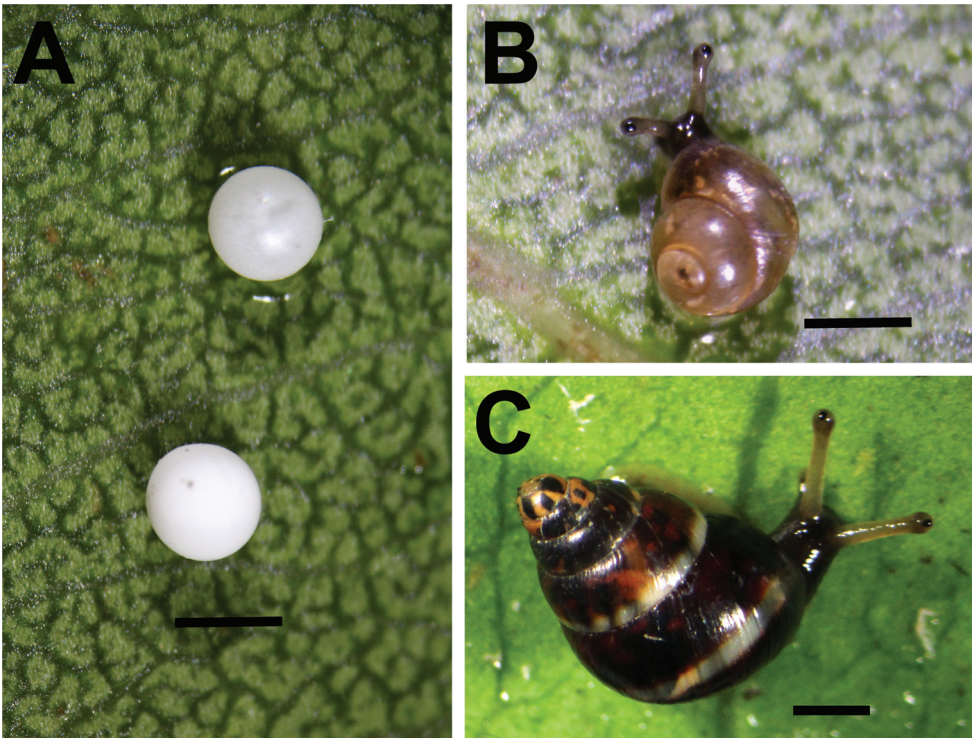

Figure 6. Photographs of live animals of *Auriculella gagneorum* sp. nov. **A** eggs **B** 1-day old juveniles **C** adult. Scale bars: 1 mm.

***Other material*:** USA – Honolulu County, Oahu, Waianae Mountains • 37; Palikea Ridge; 12 October 1912; R. von Holt, Cooke; BPBM 24989 • 44; Palikea Ridge; 12 October 1912; von Holt, Cooke; BPBM 33011 • 27; Palikea Ridge; 12 October 1912; von Holt, Cooke; BPBM 33018 • 10; Palikea Ridge; 12 October 1912; von Holt, Cooke; BPBM 33006 • 3; Makua; 16 November 1913; Spalding; BPBM 34847 • 3; Palikea Ridge; 27 December 1914; Alice T. Cooke, C.M. Cooke; BPBM 38031 • 79; Palikea Ridge; 24 August 1922; R. von Holt, C.M. Cooke Jr., M.C. Neal; BPBM 59612 • 11; Napepeiauolelo; 25 March 1934; Meinecke, William H.; BPBM 127221 • 1; Palawai Gulch; 30 August 1935; D'Alte A. Welch, Glen W. Russ; BPBM 174037 • 15; Palawai Gulch; 30 August 1935; Glen W. Russ, D'Alte A. Welch; BPBM 174233 • 2; Palawai Gulch; 30 August 1935; D'Alte A. Welch, Glen W. Russ; BPBM 174141 • 3; Palawai Gulch; 30 August 1935; D'Alte A. Welch, Glen W. Russ; BPBM 174081 • 1; Manuwaikaalae Gulch; 28 March 1936; J. Winne, D'Alte A. Welch; BPBM 176456 • 2; Pohakea Gulch; 30 March 1936; J. Winne, D'Alte A. Welch; BPBM 176596 • 3; Pualii Gulch; 30 March 1936; J. Winne, D'Alte A. Welch; BPBM 176651 • 21; Pualii Gulch; 30 March 1936; J. Winne, D'Alte A. Welch; BPBM 176766 • 1; Kaaikukai Gulch; 03 April 1936; B. Bowen, D'Alte A. Welch; BPBM 176916 • 11; Kaaikukai; 03 April 1936; B. Bowen, D'Alte A. Welch; BPBM 176973 • 15; Palawai Gulch; 19

April 1936; J. Winne, D'Alte A. Welch; BPBM 177217 • 9; Palawai Gulch; 19 April 1936; J. Winne, D'Alte A. Welch; BPBM 177278 • 1; Kaaikukai Gulch; 05 May 1936; R. Yamaguchi, D'Alte A. Welch; BPBM 177468 • 1; Mount Kaala; 27 March 1937; F. Raymond Fosberg; BPBM 162712 • 11; Napepeiauolelo; 03 April 1938; William H. Meinecke, E. Meadows, Donald Anderson; BPBM 173979 • 9; Napepeiauolelo; 03 April 1938; William H. Meinecke, E. Meadows, Donald Anderson; BPBM 173980 • 2; Pualii Gulch; 03 April 1938; William H. Meinecke, E. Meadows,, Donald Anderson; BPBM 184885 • 5; Ekahanui Gulch; 16 September 1941; Rokuro Yamaguchi, Yoshio Kondo; BPBM 211563 • 2; Ekahanui Gulch; 16 September 1941; Rokuro Yamaguchi, Yoshio Kondo; BPBM 211678 • 6; Ekahanui Gulch; 16 September 1941; Rokuro Yamaguchi, Yoshio Kondo; BPBM 211723 • 5; Napepeiauolelo-Pualii Ridge; 15 October 1960; Yoshio Kondo, T.M. {T. Maa?}, George F. Arnemann, P.C. {Peter Char?}; BPBM 216123 • 2; Palawai Gulch; BPBM 183862 • 135; Palikea Ridge; R. von Holt, Cooke; BPBM 21823 • 17; Palikea Ridge; R. von Holt, Cooke; BPBM 21824 • 74; Palikea Ridge; Spalding; BPBM 22739 • 6; Palikea Ridge; Spalding; BPBM 19891 • 1; Palikea Ridge; Cooke; BPBM 16884.

Type locality. Palawai Gulch, Waianae Mountains, Honolulu County, Oahu

Diagnosis. ***Shell.*** Shell sinistral with inflated whorls, H = 4.8 ± 0.3 mm, W = 3.2 ± 0.2 mm, WH = 5.4 ± 0.4, AH = 2.3 ± 0.1 mm, AW = 1.7 ± 0.1 mm (Table 2). Columella in juveniles with a strong lamella that is reduced and covered by a thickening of the inner edge of the lip in adults. Adults do not show a short projection or angular edge where the columellar lamella was located. Parietal lamella is often undulate, usually with three peaks, extending 0.2 to 0.5 whorls into the aperture. Shell color is white, pale tan or dark brown, with or without irregularly placed axial bands of brown, or with a single peripheral band of pale tan or dark brown. Specimens occasionally pale tan with two poorly defined dark bands on either side of a pale tan peripheral band.

Reproductive system. Phallus retractor muscle long, attached apically to a short but well-defined epiphallus (Fig. 4F). Appendix slightly longer than the phallus. Appendix ⅔ the diameter of the phallus at its attachment, tapering gently to ⅓ its length, then remaining narrow to its terminus. Apical ¾ of the phallus is broad, tapering slightly both apically and basally, basal ¼ narrows slightly above junction with the short atrium. Vagina is short.

Radula. Radula with an irregular rachidian flanked on either side by rastriform marginal teeth, as diagnostic of the family (Fig. 5F). Each tooth has a long narrow base that expands slowly for ¾ of the length of the tooth before reaching the forward curving cusps, which comprise the remaining ¼ of the tooth. There are three long cusps at mesocone, endocone, and ectocone positions with two or more alternating larger and smaller cusps intercalated between them. Number of teeth per row range from 135 to 153 (N = 3; Table 2).

Distribution and ecology. *Auriculella gagneorum* sp. nov. is endemic to Oahu's Waianae Mountain Range and was recorded as a potentially new species primarily from the southern Waianae Mountain Range, with several populations in the northern

part of the range (Fig. 1F). The species is arboreal and has been found on *Antidesma platyphyllum*, *Broussaisia arguta*, *Lantana* sp., *Melicope anisate*, *Myrsine lessertiana*, and occasionally on unspecified ferns and dead leaves. The last known record of this species prior to recent surveys was by Yoshio Kondo, T. Maa, George F. Arnemann, and Peter Char in 1960. From 2013 to 2018 we recorded extant populations of this species from three locations in the southern Waianae Mountains.

Remarks. The shell is sinistral unlike *A. minuta* and the columella does not bear an axially oriented ridge like the one found in *A. perversa*. The palatal lamella is often undulate unlike all other members of the *A. perpusilla* group. The epiphallus is short but well defined similar to *A. minuta* but unlike the long epiphallus of *A. perpusilla* or the poorly defined epiphallus of *A. perversa.* The appendix tapers gently unlike the appendices of *A. auricula*, *A. minuta*, *A. perpusilla*, *A. perversa*, and *A. tenella* which all narrow abruptly.

Etymology. Named in honor of Betsy and Wayne Gagne for their indefatigable efforts advocating for the conservation of Hawaii's unique and highly endangered biota.

Discussion

The *Auriculella perpusilla* species group (*A. perpusilla*, *A. perversa*, *A. minuta*) was defined as having species with small, thin, relatively low spired shells of approximately five inflated whorls. *Auriculella gagneorum* sp. nov., shares these shell characteristics. These four species can be distinguished from one another using a suite of morphological features including shell chirality (only *A. minuta* is dextral); presence of axially oriented ridge of the columella (only present in *A. perversa*); appearance of the palatal lamella (undulated only in *Auriculella gagneorum* sp. nov.); length of the epiphallus (those of both *Auriculella gagneorum* sp. nov. and *minuta* are short and well-defined); and development of the appendix (tapers gently in *Auriculella gagneorum* sp. nov. and narrow abruptly in others). The DNA data corroborate the difference seen in anatomy and conchology. In contrast to expectations based on shell morphology alone, the *perpusilla* group is not monophyletic and *Auriculella gagneorum* sp. nov. is not closely related to either *A. perpusilla* or *A. perversa*, the only other extant members of the group for which DNA data are available (Fig. 2). Instead, *A. gagneorum* sp. nov. clusters with *A. tenella*, a high spired and tightly coiled species from the *castanea* group, which also occurs in the Waianae Mountains. Similarly, *A. perpusilla* and *A. perversa* are more closely related to species with highly dissimilar shell morphologies, *A. ambusta* and *A. montana*, respectively (Fig. 2). The latter two have large, thick shells and are usually placed in the *auricula* group with other robust species. Patterns of relatedness recovered in our phylogenetic analyses indicate these gross shell characters, which are unlikely to be independent of one another, are insufficient for delineating taxa or characterizing relationships within the genus. Multiple instances of convergence in shell morphology across the genus may be explained by adaptation to similar microhabitats, or non-adaptive diversification combined with constraints on shell morphospace (Gittenberger 1991; Cowie 1995; Rundell and Price 2009; Chiba and Cowie 2016;

Gillespie et al. 2018). Disentangling the processes responsible for these patterns will require additional studies of the functional morphology, ecology, and behavior of *Auriculella* species.

Historically, all four species treated here once had much larger geographic ranges, with multiple populations recorded in the last century (Fig. 1). Like nearly all land snail species across Hawaii, *Auriculella* spp. numbers have declined dramatically with an estimated 45% of the species considered extinct, and many historical populations extirpated as a result of habitat destruction, invasive species, and possibly climate change. Despite the grim statistics, there remain a number of species that can yet be saved from extinction, but only with a clear understanding of their systematics, biogeography and ecology. For example, *A. tenella*, *A. gagneorum* sp. nov., *A. perversa*, and *A. perpusilla*, are now known from only three locations for each of these species. These data combined with knowledge of reproduction and population growth rates can be used to better manage these imperiled species.

Low reproductive and growth rates are often characteristic of species that have evolved on isolated oceanic islands (MacArthur and Wilson 2001; Covas 2011), and *Auriculella* spp. are probably no exception. Two laboratory reared adults of *Auriculella gagneorum* sp. nov. produced 33 eggs in 250 days between 17 May 2018 and 23 January 2019 (Fig. 6A–C). The delicate nature of the eggs of this imperiled species permitted the measurement of only three eggs, which had an average diameter of 0.99 ± 0.05 mm. These large eggs, relative to the size of the animal, take approximately 58 days to hatch (Lindsay Renshaw, pers comm.). Such low fecundity in combination with extreme range reduction decreases the chances of long-term species and population persistence (Bick et al. 2018), particularly in the face of predation by introduced predators (Chiba and Cowie 2016).

Updated and comprehensive assessments of the systematics, biogeography, and ecology of taxa are necessary for effective management and development of long-term recovery plans. Additional surveys to locate remaining species and persisting populations are needed now, while there is still an opportunity to prevent or slow the rate of species loss (Solem 1990; Yeung and Hayes 2018). These surveys provide important opportunities to study and preserve species and develop populations for captive rearing, which in turn can be repatriated to protective enclosures in natural habitats with the goal of ultimately reintroducing species back into the wild (Natural Area Reserves Program 2016; Yeung and Hayes 2018). Our surveys have recovered species not recorded alive since the 1950s (e.g., *Auriculella perpusilla*, *A. perversa*, *A. tenella*) and others feared extinct (Yeung et al. 2015, 2018). They have also uncovered several previously undescribed species, indicating that there is still much to learn about this highly imperiled fauna, and still hope that we might save some of it for future generations (Solem 1990).

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Supplementary material 1

Non-type material examined for *Auriculella auricula*, *A. minuta*, *A. perpusilla*, *A. perversa*, and *A. tenella*

Authors: Norine W. Yeung, John Slapcinsky, Ellen E. Strong, Jaynee R. Kim, Kenneth A. Hayes

Data type: species data

Explanation note: All material examined for *A. gagneorum* sp. nov. is provided in the body of the manuscript.

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RESEARCH ARTICLE

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A new *Atrococcus* species (Hemiptera, Coccomorpha, Pseudococcidae) from China, with a key to Chinese species

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Abstract

A new mealybug species *Atrococcus rushuiensis* Zhang, **sp. nov.**, collected under the leaf sheath of *Sporobolus fertilis* (Poaceae) in Fuzhou City, Jiangxi Province, China, is described and illustrated. A new combination is introduced, transferring *Allotrionymus shanxiensis* Wu to the genus *Atrococcus* as *A. shanxiensis* (Wu), **comb. nov.** A key is presented for the species of *Atrococcus* recorded from China.

Keywords

Atrococcus rushuiensis, Jiangxi, mealybug, new combination, *Sporobolus fertilis*, taxonomy

Introduction

The genus *Atrococcus* Goux, 1941 (Pseudococcidae, Pseudococcinae) was established with *Atrococcus melanovirens* Goux as its type species. With oral rim ducts present, *Atrococcus* is morphologically similar to *Allotrionymus* Takahashi, *Chorizococcus* McKenzie, *Spilococcus* Ferris, and *Vryburgia* De Lotto (Wu 2000). Due to the inadequate definition, different authors have applied different combinations of characters for generic separation (e.g., Williams 1962; Tang 1992; Danzig and Gavrilov-Zimin 2015).

Based on a study of mealybugs from Russia and neighbouring countries, Danzig and Gavrilov-Zimin (2015) found that there is no difference between *Allotrionymus* and *Atrococcus*, and treated *Allotrionymus* as a junior synonym of *Atrococcus*. Hence, we follow Danzig and Gavrilov-Zimin (2015) in regarding *Allotrionymus* as a junior synonym of *Atrococcus*.

In China, Tang and Li (1988) first recorded *Atrococcus* from Inner Mongolia and reported three species: *A. achilleae* (Kiritchenko) (on the root of *Bassia scoparia*), *A. innermongolicus* Tang in Tang and Li (on the root of *Artemisia apiacea*) and *A. paludinus* (Green) (on *Leppula intermedia*). Later, Tang (1992) recorded two *Allotrionymus* species: *Al. elongatus* Takahashi (on *Heteropappus altaicus*) and *Al. multipori* Kawai (on *Chloris radiata*) from Inner Mongolia; the former was a misidentification of *Chorizococcus scorzonerae* Tang found by Wu (2000). Meanwhile, Tang (1992) also transferred *Spilococcus pacificus* (Borchsenius) to *Atrococcus* and *Trionymus plurostiolatus* Borchsenius to *Allotrionymus*. The above-mentioned four species were placed under *Atrococcus* by Danzig and Gavrilov-Zimin (2015). Subsequently, Wu (1999) described a new species *Allotrionymus calamagrostis* Wu (under the leaf sheath of *Calamagrostis* sp.) from Henan; this species was placed in the genus *Atrococcus* by Danzig and Gavrilov-Zimin (2015). Recently, Wu (2000) provided a study of *Atrococcus* and its related genera, reporting a new species, *Allotrionymus shanxiensis* Wu (under the leaf sheath of *Melica scabrosa*) from Shanxi, which is transferred to *Atrococcus* as *A. shanxiensis* comb. nov. following the present generic diagnosis. Wu (2000) also reported a new Chinese record, *A. cracens* Williams, collected from Inner Mongolia (on *Artemisia halodendron*?) and Shanxi (on *Artemisia* sp. and *Heteropappus altaicus*). Including the new species described here, there are ten *Atrococcus* species recorded in China: *A. achilleae*, *A. calamagrostis*, *A. cracens*, *A. innermongolicus*, *A. multipori*, *A. pacificus*, *A. paludinus*, *A. plurostiolatus*, *A. rushuiensis* sp. nov., and *A. shanxiensis* comb. nov.

In this study, a new species, *A. rushuiensis* Zhang, sp. nov., is described from China, and a key to the Chinese *Atrococcus* species is also provided.

Materials and methods

All mealybug specimens were collected from under the leaf sheaths and transferred into 75% alcohol, then prepared and mounted mainly according to the method of Borchsenius (1950). The terminology for the morphological features used in the description are mainly explained by Williams (1962, 2004). Photograph was taken with a Nikon D7500 camera. The descriptions and measurements were made using a light microscope (SOPTOP BH200) fitted with an ocular micrometre, and six slide-mounted specimens were studied for measurements. Measurements are in micrometres (μm) except the lengths and widths of the bodies are given in millimetres (mm); all measurements are given as minimum and maximum. Drawings are presented as is usual for Coccomorpha, with the central drawing showing the outline of the body and the dis-

tribution of characters (dorsum on the left side, venter on the right), with the enlarged details (not to scale) showing the structure of important characters around the margin.

All specimens examined are deposited in the College of Forestry, Jiangxi Agricultural University, Jiangxi, China.

Taxonomy

Atrococcus Goux, 1941

Pseudococcus (*Atrococcus*) Goux, 1941: 69. Type species: *Atrococcus melanovirens* Goux by original designation.

Generic diagnosis. Body in life usually greenish or white, females of some species show black coloration after being placed in ethanol or potash. Body of adult female on slide oval to elongate oval. Antennae seven- or eight- segmented. Circulus present or absent. Legs well developed, claw without a denticle. Both pairs of ostioles well developed. Anal ring usually situated at apex of abdomen, bearing six setae. Anal lobes moderately developed, each bearing a normal apical seta. Cerarii numbering 1–17 pairs, each cerarius usually bearing two conical setae. Trilocular pores numerous, evenly scattered on all body surface. Multilocular disc pores usually present on venter, more rarely present on dorsum. Oral rim ducts present, forming transverse rows on dorsum and sometimes present on venter. Oral collar tubular ducts present, at least on venter, sometimes present on dorsum. Most species have group of oral collar tubular ducts on prothorax in front of anterior spiracles, often accompanied by a group of multilocular disc pores. Flagellate setae of different sizes present on both body surfaces (adapted from Tang 1992, Danzig and Gavrilov-Zimin 2015).

Atrococcus rushuiensis Zhang, sp. nov.

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Figures 1, 2

Material studied. ***Holotype.*** ♀ (mounted singly on a slide), China, Jiangxi Province, Fuzhou City, Rushui Forest Park [27°58'N, 116°22'E], under the leaf sheath of *Sporobolus fertilis* (Poaceae), 2.x.2019, coll. Jiang-Tao Zhang. ***Paratypes.*** 8 ♀♀ (mounted on 8 slides), same data as holotype.

Etymology. The species name is based on the collection locality, Rushui Forest Park.

Description. Alive: body elongate, dark reddish, with thin covering of white mealy wax, and only caudal filaments present (Fig. 1).

Slide-mounted specimens (N = 6): body of adult female (Fig. 2) elongate, 2.5–3.1 mm long, 0.9–1.4 mm wide. Anal lobes moderately developed, ventral surface of each lobe bearing an apical seta, each 100–122.5 μm long. Antennae eight-segmented,

Figure 1. Habitus photograph of *Atrococcus rushuiensis* sp. nov. under the leaf sheath of *Sporobolus fertilis* (Poaceae).

267.5–289 μm long, lengths of each segment: I 42.5–52.5, II 37.5–38.8, III 23.8–27.5, IV 18.8–22.5, V 17.5–23.8, VI 20–26.3, VII 28.8–32.5 and VIII 65–76.3 μm. Eye spot located at body margin lateral to antennal base. Legs well developed; hind coxa 52.5–65 μm long, hind trochanter + femur 185–215 μm long, hind tibia + tarsus 198.8–240 μm long; claw 17.5–22.5 μm long, both tarsal digitules and claw digitules knobbed, longer than claw. Ratio of lengths of hind tibia + tarsus to hind trochanter + femur 1.07–1.14:1. Ratio of lengths of hind tibia to tarsus 1.49–1.69:1. Translucent pores present, minute duct-like, present on anterior and posterior surface of hind coxa. Circulus absent. Clypeolabral shield 125–145 μm long. Labium with three segments, 62.5–70 μm long. Ostioles moderately developed, each lip with 4–13 trilocular pores and 0–2 short setae. Anal ring normal, 61.3–70 μm in diameter, bearing six long setae, each seta 82.5–103.8 μm long. Cerarii numbering a single pair on anal lobes only. Anal lobe cerarii (C_{18}) each containing two slender conical setae, each seta 18–21 μm long, with 2–3 auxiliary setae, and 4–5 trilocular pores near conical setae base, all situated on a membranous area.

Dorsum. Setae short and slender, each 15–25 μm long. Trilocular pores each 3.5 μm in diameter, evenly distributed. Oral rim ducts each 9–10 μm long, 6 μm wide, in more or less single transverse rows on most segments. Oral collar tubular ducts absent or present, if present, each 6–7 μm long, 3 μm wide, having fewer numbers marginally on abdominal segments VI or VII. Multilocular disc pores each 7–8 μm in diameter, forming transverse rows or scattered on medial abdominal segments V–VII (V has 0–7 pores, VI has 1–7 pores, VII has 3–12 pores), occasionally few present on margin of abdominal segments V–VII. Discoidal pores minute, scattered.

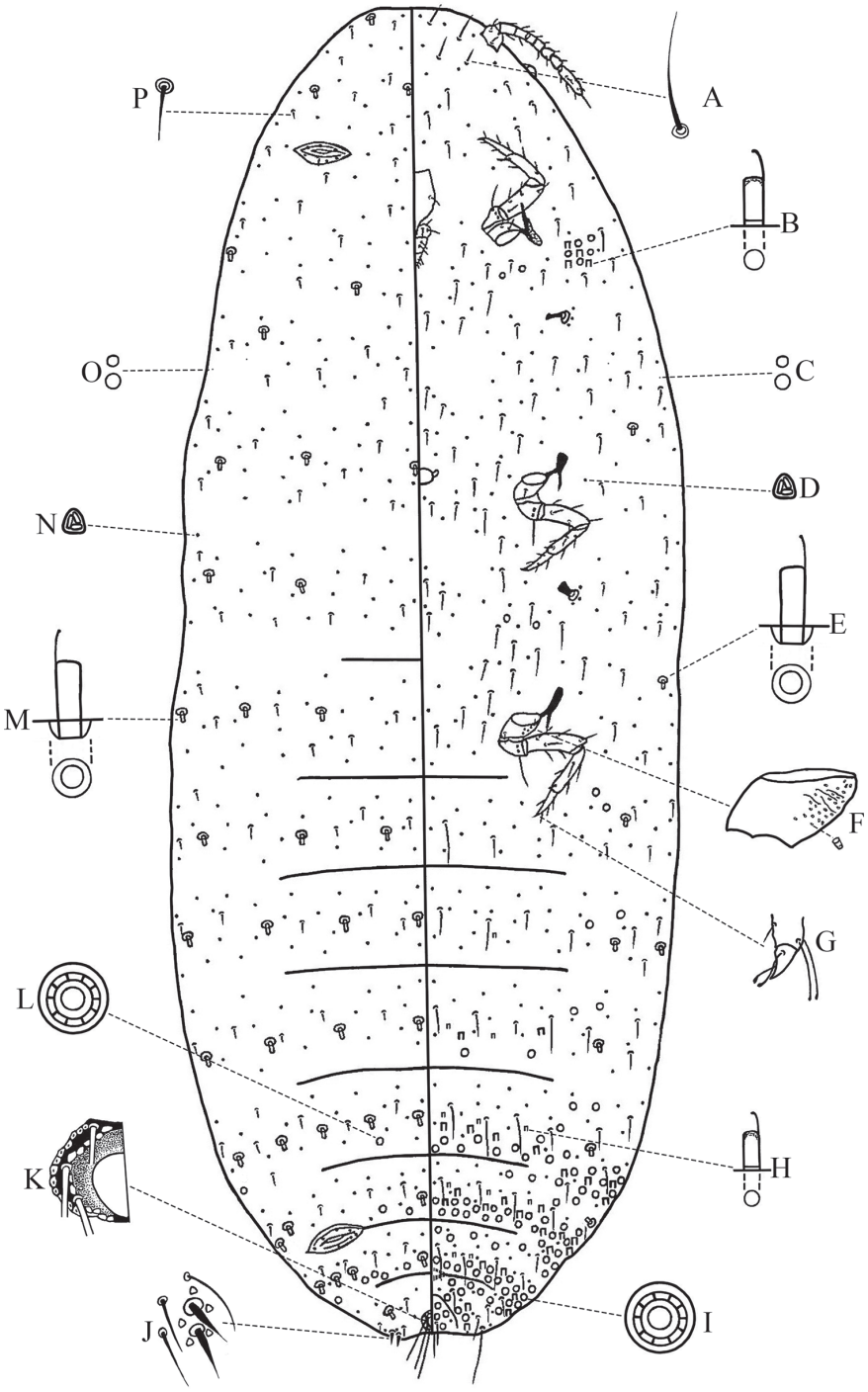

Figure 2. Adult female of *Atrococcus rushuiensis* sp. nov. Venter (**A–I**) **A** flagellate seta **B** large type of oral collar tubular duct **C** disc pore **D** trilocular pore **E** oral rim duct **F** hind coxa **G** claw **H** small type of oral collar tubular duct **I** multilocular disc pore. Dorsum (**J–P**) **J** anal lobe cerarius **K** anal ring **L** multilocular disc pore **M** oral rim duct **N** trilocular pore **O** disc pore **P** dorsal seta.

***Venter*.** Setae slender, longer than those on dorsum, each 36.3–80 μm long. Trilocular pores similar to those on dorsum, evenly distributed. Oral rim ducts same as those on dorsum, present on margin and submargin areas of thoracic and abdominal segments. Oral collar tubular ducts of two types: a large type, similar to those on dorsum, present in transverse rows across abdominal segments III–VIII or IV–VIII, also in marginal groups on abdominal segments V–VIII or VI–VIII, and a small group (together with multilocular disc pores) present on prothorax in front of anterior spiracles (4–11 ducts and 6–17 pores); a small type, each 5 μm long, 2 μm wide, mainly distributed across middle areas of abdominal segments III–VIII or IV–VIII, a few also present on margin with large ducts. Multilocular disc pores same as those on dorsum, numerous, present posterior to vulva, in transverse rows at posterior edges of abdominal segments IV–VII, in transverse rows at anterior edges of abdominal segments VI–VII, a few occurring on submargin areas of abdominal segments II–IV near oral rim ducts, also forming groups along margin of abdominal segments V–VIII or VI–VIII. Discoidal pores minute, scattered.

Host plant. Poaceae: *Sporobolus fertilis*.

Distribution. China: Jiangxi (Fuzhou).

Biology. Living under the leaf sheath of its host plant.

Remarks. *Atrococcus rushuiensis* sp. nov. is very similar to *A. luffi* (Newstead) in the number of cerarii and multilocular disc pores present on both body sides, but it differs from the latter by the following features (condition of *A. luffi* given in parenthesis): (i) dorsal margin oral collar tubular ducts absent or few (numerous, with multilocular disc pores in submarginal groups up to segment III); (ii) ventral oral rim ducts absent in median areas of prothorax and mesothorax (present in these areas); (iii) translucent pores duct-like (normal, not duct-like) [The morphology of *A. luffi* is mainly based on Williams (1962)].

The new species also resembles *A. paludinus* in possessing fewer than 20 oral rim ducts on each segment, which is different from *A. luffi* in having about 20 oral rim ducts on each segment, but differs from the latter by the following features (condition of *A. paludinus* given in parentheses): (i) cerarii numbering one pair only (cerarii numbering 6–7 pairs); (ii) Translucent pores duct-like (normal, not duct-like) [The morphology of *A. paludinus* is also mainly based on Williams (1962)].

In *A. rushuiensis* sp. nov., the number of ducts and pores vary among individuals, which belong to intraspecific variation. Some specimens have only a small number of ducts and pores, but in other specimens those ducts and pores are much more numerous.

Key to adult females of *Atrococcus* known from China

1 Multilocular disc pores present on venter and dorsum **2**

– Multilocular disc pores present on venter only **5**

2 Dorsal tubular ducts absent or present in compact groups along abdominal margin only **3**

– Dorsal tubular ducts present and forming transverse rows..... ..... ***A. achilleae*** **(Kiritchenko)**

3 Cerarii present in 3–7 pairs **4**
– Only one pair of cerarii present ..... ***A. rushuiensis* sp. nov.**
4 Cerarii 6–7 pairs, dorsal multilocular disc pores on margins only of posterior abdominal segments ..... ***A. paludinus* (Green)**
– Cerarii 3–4 pairs, noticeable groups of multilocular disc pores in submedian areas of dorsum ..... ***A. cracens* Williams**
5 Circuli present **6**
– Circuli absent ..... ***A. innermongolicus* Tang in Tang and Li**
6 Oral rim duct present on venter and dorsum **7**
– Oral rim duct present on dorsum only **8**
7 Circuli 2–5 in number ..... ***A. plurostiolatus* (Borchsenius)**
– One circulus present ..... ***A. multipori* (Kawai)**
8 Prothoracic group of tubular ducts absent **9**
– Prothoracic group of tubular ducts present ..... ***A. pacificus* (Borchsenius)**
9 Translucent pores normal, also extend to metathorax cuticle near hind coxae ..... ***A. calamagrostis* (Wu)**
– Translucent pores duct-like, only present on the cuticle of hind coxae ..... ***A. shanxiensis* (Wu) comb. nov.**

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RESEARCH ARTICLE

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A new species of *Oomyzus* Rondani (Hymenoptera, Eulophidae) and first record of *O. gallerucae* (Fonscolombe) from China, with a key to Chinese species

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Abstract

Oomyzus flavotibialis **sp. nov.** is described from Liaoning and Shandong provinces, China. *Oomyzus gallerucae* (Fonscolombe) is reported for the first time from China. A key to Chinese species of *Oomyzus* is provided.

Keywords

Chalcidoidea, new species, taxonomy, Tetrastichinae

Introduction

The genus *Oomyzus* currently contains 26 valid species worldwide (Noyes 2019), with only four species known from China: *O. scaposus* (Thomson), *O. sokolowskii* (Kurdjumov), *O. sinensis* Sheng & Zhu, and *O. hubeiensis* Sheng & Zhu (Li 1984; Sheng and Zhu 1998; Wang et al. 1998). Most species of the genus are parasitoids of Coleoptera, Neuroptera, Diptera, and Lepidoptera. They attack larvae or pupae, and even eggs of their hosts, and several species were widely used in classical biological control (Yaseen 1978; Alam 1982; LaSalle 1994; Liu et al. 2000).

Oomyzus can be recognized by the following combination of characteristics (Graham 1991; LaSalle 1994): female antenna usually with funicle segments not or slightly

longer than wide (rarely funicle segments relatively longer); mesoscutum with 2–5 adnotaular setae on each side; submedian grooves usually weak or absent, rarely strong; propodeum with or without paraspiracular carinae (but not as in *Tetrastichus*); meso- and metabasitarsus often shorter than second tarsomere; forewing with one rarely two, dorsal seta on submarginal vein; metasoma subcircular to ovate, from shorter than mesosoma to about as long as head and mesosoma combined.

In the present paper, a new species of *Oomyzus* from China is described, *O. gallerucae* is newly reported from China, and a key to Chinese species is provided.

Materials and methods

Specimens were collected by sweep net and most were dissected and mounted dorsally in Canada balsam on steps following the method of Noyes (1982). Photographs were taken with a digital CCD camera attached to an Olympus BX51 compound microscope, and most measurements were made from slide-mounted specimens using an eye-piece reticle with an Olympus CX21 microscope. Terminology follows the Hymenoptera Anatomy Consortium (2019), and the following abbreviations are used: F1–4 (flagellomeres 1–4), POL (minimum distance between lateral ocelli), OOL (minimum distance between lateral ocellus and eye margin), OD (longest diameter of a lateral ocellus), MV (marginal vein), STV (stigmal vein), SMV (submarginal vein). All the specimens listed below are deposited in Northeast Forestry University, Harbin (**NEFU**), China.

Key to species of *Oomyzus* Rondani from China. Females.

1 Mid lobe of mesoscutum without median line (Fig. 3) **2**

– Mid lobe of mesoscutum with median line present although sometimes weak **3**

2 MV 2.1–2.6× as long as STV (Fig. 4); propodeum with median carina poorly defined; reticulation absent (Fig. 3) ..... ***O. flavotibialis* sp. nov.**

– MV 3.0–3.7× as long as STV; propodeum with median carina sharply defined; reticulation present ..... ***O. scaposus* (Thomson)**

3 Callus with 5–8 setae; F1 as long as pedicel **4**

– Callus with 2 setae; F1 shorter than pedicel **5**

4 Propodeum with median carina (see Sheng and Zhu 1998: fig. 7) .... ***O. sinensis* Sheng & Zhu**

– Propodeum without median carina (see Sheng and Zhu 1998: fig. 4) .... ***O. hubeiensis* Sheng & Zhu**

5 Clava 2.0–2.25× as long as broad; meso- and metabasitarsus hardly shorter than corresponding second tarsomeres ..... ***O. sokolowskii* (Kurdjumov)**

– Clava 2.7× as long as broad (Fig. 10); meso- and metabasitarsus distinctly shorter than corresponding second tarsomeres (Fig. 14) .... ***O. gallerucae* (Fonscolombe)**

***Oomyzus flavotibialis* sp. nov.**
http://zoobank.org/A6DC6119-AF2F-4A2E-AF3D-35BBFA2EFC91
Figures 1–8

Type material. ***Holotype*:** female [on slide], China, Liaoning Province, Anshan City, Mount Qian Shan, 23.VI.2015, Hui Geng, Yan Gao, Zhi-Guang Wu, and Si-Zhu Liu, by sweeping. Deposited in NEFU.

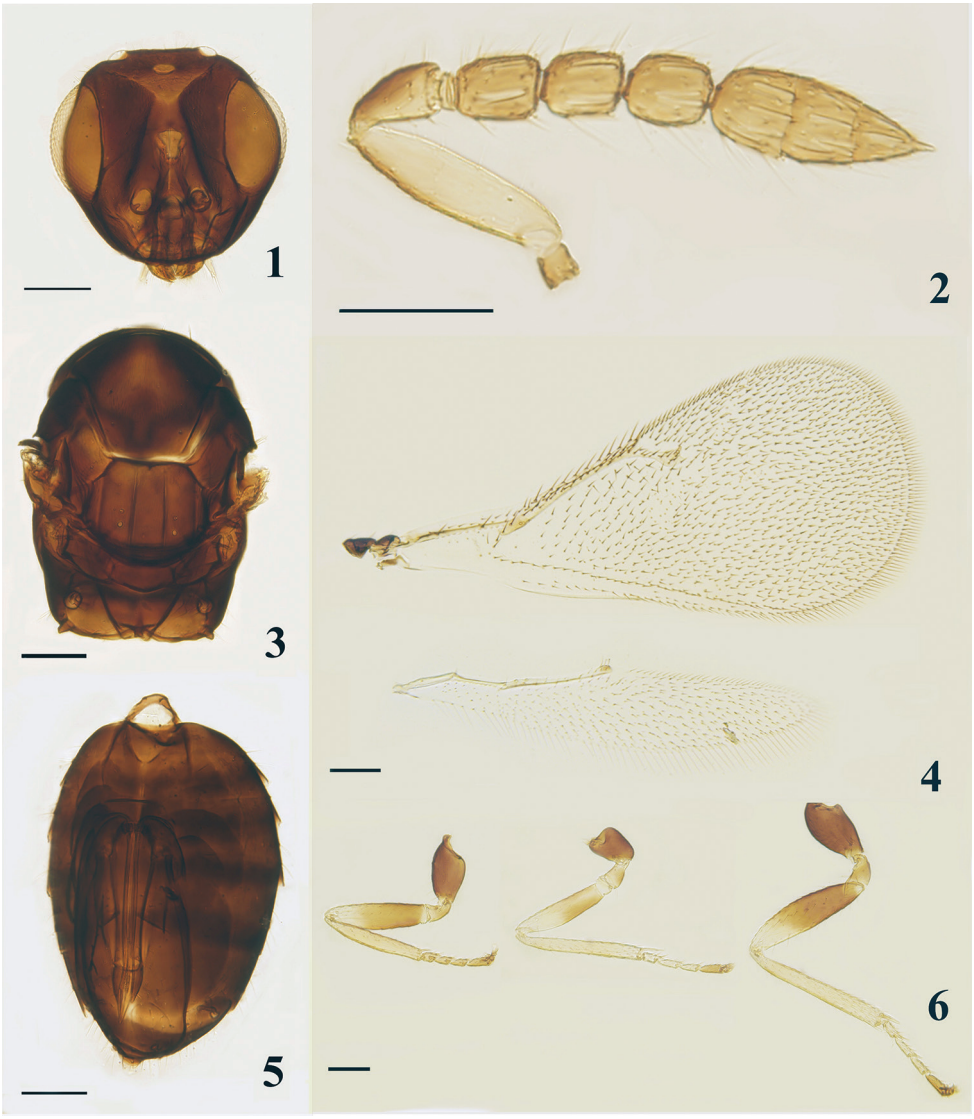
***Paratypes*:** 8 females, 1 male. China. **Liaoning Province**, same data as holotype, [1 female on card]; Anshan City, Mount Qian Shan, 25.VI.2015, Hui Geng, Yan Gao, Si-Zhu Liu, and Zhi-Guang Wu, sweeping, [2 females on slides]; Anshan City, Mount Qian Shan, 20.IX.2015, Hui Geng, Yan Gao, and Xin-Yu Zhang, sweeping, [2 females on slides]; Fushun City, 18.VI.2012, Hui Geng, Xiang-Xiang Jin, and Jiang Liu, sweeping, [1 female on slide]; **Shandong Province**, Pingdu City, Mount Daze, 18.VII.2014, Hui Geng, Yan Gao, Si-Zhu Liu, and Zhi-Guang Wu, sweeping, [2 females, 1 male on slides]. All deposited in NEFU.

Diagnosis. Female. Body black with all tibiae yellow; propodeum with median carina poorly defined, spiracle circular, partly exposed, and separated by about its diameter from metanotum; propodeum reticulation absent; MV 2.1–2.6× as long as STV; SMV with two dorsal setae. *Male.* Antenna with plaque 0.67× as long as scape. Forewing with costal cell 1.4× as long as MV, MV 2.5× as long as STV, SMV with two dorsal setae.

The new species belongs to the *incertus*-group (Graham 1991). Among the species recorded from China, *O. flavotibialis* is similar to *O. scaposus*, but can be separated from the latter by the following combination of characteristics: propodeum with median carina poorly defined, (vs sharply defined), spiracle circular, partly exposed (vs suboval, fully exposed) and separated by about its diameter from metanotum (vs about 0.5× its diameter); propodeum reticulation absent (vs present but very fine); MV 2.1–2.6× as long as STV (vs 3.0–3.7×); SMV with two dorsal setae (vs usually with only one); tibiae completely yellow (vs mainly brown to blackish). The new species is also similar to the extralimital species *O. incertus* (see Graham 1991 for description), but can be separated from the latter by characteristics: POL 2.1–2.5× OOL (vs 1.5–1.65×), OOL 1.5–1.8× OD (vs 2.3–2.5×); propodeum medially distinctly longer than dorsellum (vs hardly longer than).

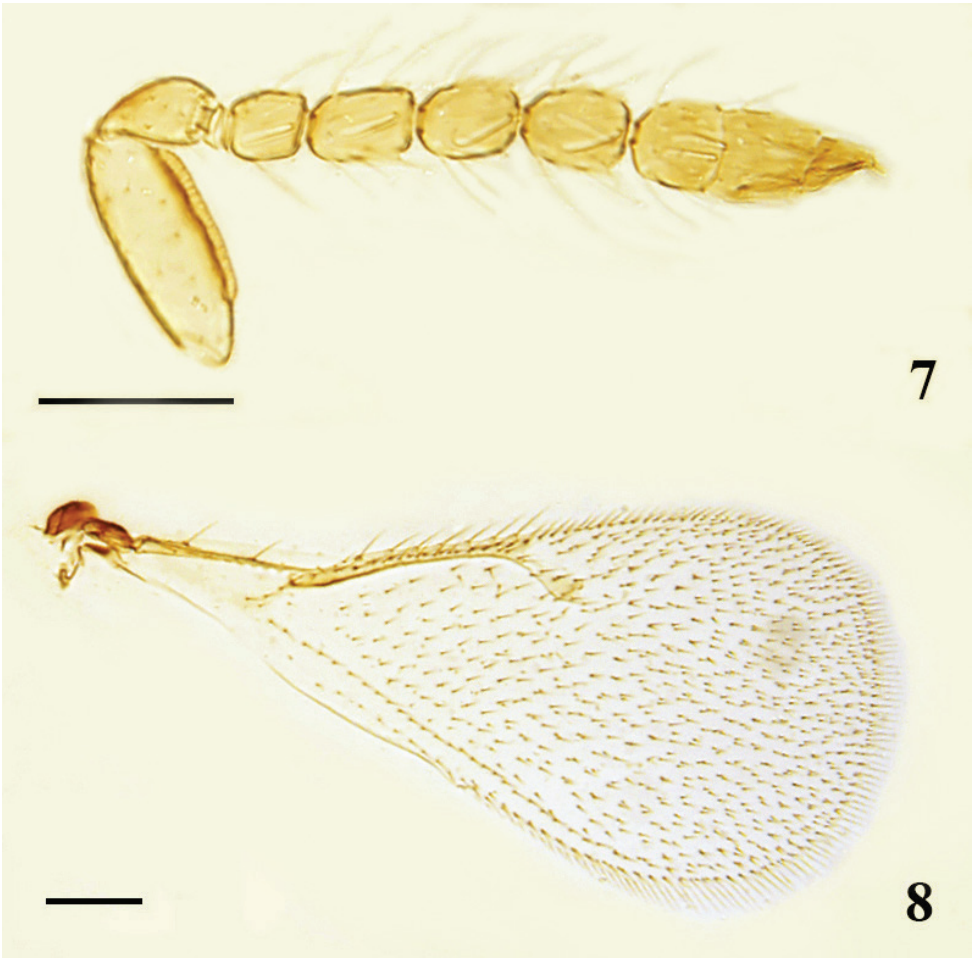
Description. Female. ***Body length*** 1.0–1.2 mm (1.1 mm), black with dark-green metallic reflection. Antenna with radicle dark brown, scape mostly yellow, brown along dorsal edge, pedicel with dorsal half brown, ventral half yellow, flagellum yellowish brown. Metasoma oval, smooth, with weak bronze and bluish tint like mesosoma. Wings hyaline, venation yellowish brown. Legs with coxae dark brown, trochanters yellowish brown; basal 2/3 of pro- and metafemora brown with distal 1/3 yellow, a little more than half of mesofemora yellowish brown basally, remaining distal part yellow; tibiae and basal three tarsomeres yellow, last tarsomeres brown to dark brown.

Head (Fig. 1) in dorsal view, slightly broader than mesosoma with slightly raised reticulation, 3.0–3.4× (3.2×) as broad as long; POL 2.1–2.5× (2.2×) OOL, OOL 1.5–1.8× (1.8×) OD. Malar space 0.53× as long as eye, malar sulcus straight; mouth



Figures 1–6. *Oomyzus flavotibialis* sp. nov. holotype, female **1** head, frontal view **2** antenna, lateral view **3** mesosoma, dorsal view **4** fore- and hind wings, dorsal view **5** metasoma, ventral view **6** legs, lateral view. Scale bars: 100 μm.

cavity 1.45× as wide as malar space. Clypeus with anterior margin weakly bidentate. Facial depression moderate, with weak but slightly raised reticulation. Vertex with setae slightly shorter than OD. Torulus with lower edge a little below the ventral edge of eyes. Antenna (Fig. 2) with scape 3.8× as long as broad, shorter than an eye length and not reaching vertex; pedicel longer than F1, 1.7–2.0× (1.9×) as long as broad; three anelli; F1–F3 1.1–1.3× (1.2×), 1.0–1.2× (1.2×), and 1.0–1.2× (1.2×) as long as broad



Figures 7, 8. *Oomyzus flavotibialis* sp. nov., paratype male **7** antenna, lateral view **8** forewing, dorsal view. Scale bars: 100 μm.

respectively; clava broader than F3, 2.0–2.7× (2.7×) as long as broad, longer than F2 and F3 combined, sensilla moderately numerous, relatively long.

Mesosoma (Fig. 3) 1.3–1.7× (1.4×) as long as broad. Pronotum short, arched. Mid-lobe of mesoscutum 1.2× as broad as long, without median line, with two or three adnotaular setae on each side in one row. Scutellum 1.2× as broad as long; anterior pair of setae in middle, submedian grooves and sublateral grooves distinct, distance between submedian grooves equal to submedian grooves to sublateral grooves. Mesoscutum with extremely fine reticulation, scutellum with similar but finer sculpture. Dorsellum about 2.5× as broad as long. Propodeum medially distinctly longer than dorsellum, smooth and reticulation absent; median carina poorly defined, not distinct; without plicae or traces of plicae at hind margin; spiracle circular, moderate in size and partly

exposed, separated from metanotum by about its diameter; callus with four or five setae arranged irregularly. Forewing (Fig. 4) 2.0–2.1× (2.1×) as long as broad, with costal cell 1.1–1.5× (1.5×) as long as MV, MV 2.1–2.6× (2.1×) as long as STV; SMV with two dorsal setae; speculum medium-sized, closed posteriorly. Hind wing (Fig. 4) 4.5–4.8× (4.8×) as long as broad. Legs (Fig. 6) of medium length and thickness, meso- and metabasitarsus distinctly shorter than (0.6–0.7×) corresponding second tarsomeres.

Metasoma (Fig. 5) ovate, slightly depressed dorsally, as long as or slightly longer than mesosoma, 1.5–1.7× (1.6×) as long as broad; cercal setae subequal in length. Ovipositor originated from about basal third of gaster, about 0.6× as long as gaster and not exerted at apex, third valvula 0.22× as long as second valvifer.

Male. Similar to female. Antenna (Fig. 7) with scape robust, shorter than an eye, 2.62× as long as broad; plaque 0.67× as long as scape; pedicel 1.71× as long as broad; F1 quadrate, shorter than other funicular segments, F2–F4 similar in shape, 1.40× as long as broad; clava broader than funicle, 2.74× as long as broad. Forewing (Fig. 8) with costal cell 1.4× as long as MV, MV 2.5× as long as STV.

Host. Unknown.

Distribution. China (Liaoning, Shandong).

Etymology. *Flavus*, Latin for yellow, golden; in reference to the completely yellow tibiae.

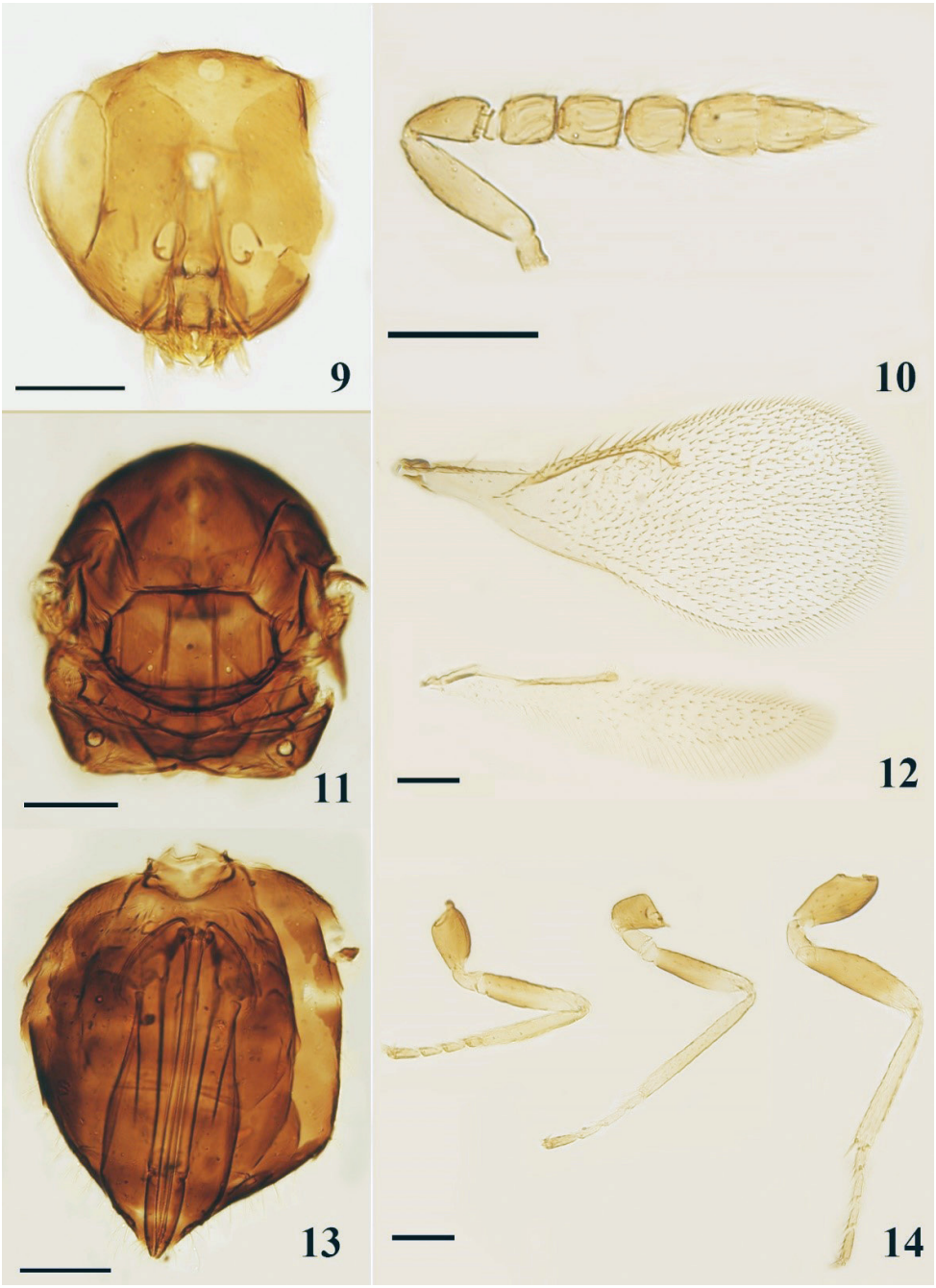
Oomyzus gallerucae **(Fonscolombe)**
Figures 9–14

Material examined. China, **Liaoning Province**, Fushun City, 18.VI.2012, Hui Geng, Xiang-Xiang Jin, and Jiang Liu, sweeping [2 females on slides, NEFU].

Diagnosis. Female. Head (Fig. 9) with POL about 2× OOL. Antenna (Fig. 10) with funicle segments quadrate or only very slightly longer than wide; clava as long as funicle, 2.7× as long as broad. Mesosoma (Fig. 11) 1.20–1.25× as long as broad. Mesoscutum midlobe with three adnotaular setae on each side in one row, median line present. Propodeum medially as long as or slightly shorter than dorsellum, median carina raised, narrower in front but broadening posteriorly. Forewing (Fig. 12) costal cell as long as or slightly longer than MV; SMV with one dorsal seta; MV 2.8–3.2× STV. **Male.** Unknown for Chinese material.

Comments. Our specimens agree well with the description by Graham (1985). For a redescription and taxonomic history, see Graham (1985).

Hosts. Unknown from China. Outside records include: *Cassida rubiginosa* (Thompson, 1955), *Galerucella lineola* (Herting, 1973), *Galerucella singhara* (Husain & Khan, 1986), *Galerucella xanthomelaena* (Peck, 1963), *Xanthogaleruca luteola* (Meiners & Hilker, 1997) =*Galerucella luteola* (Hesami et al., 2010) = *Pyrrhalta luteola* (Graham, 1985) (Coleoptera: Chrysomelidae)



Figures 9–14. *Oomyzus gallerucae*, female **9** head, frontal view (partly destroyed) **10** antenna, lateral view **11** mesosoma, dorsal view **12** fore- and hind wings, dorsal view **13** metasoma, ventral view **14** legs, lateral view. Scale bars: 100 μm.

Distribution. China (Liaoning) [new record], India, Iran, Russia, many countries in Europe, USA, Argentina, Australia.

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RESEARCH ARTICLE

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Twenty-one new species of the *Simulium* (*Gomphostilbia*) *asakoae* species group (Diptera, Simuliidae) in Thailand, with their genetic relationships

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Abstract

Females and males reared from pupae, their pupal exuviae and cocoons, and mature larvae of the *Simulium* (*Gomphostilbia*) *asakoae* species group from various localities in Thailand were morphologically examined. A total of 25 species was identified, including two of four known species (*Simulium asakoae* Takaoka & Davies and *S. chiangdaoense* Takaoka & Srisuka), one newly confirmed species (*S. myanmarense* Takaoka, Srisuka & Saeung, originally described from Myanmar), one newly transferred species (*S. inthanonense* Takaoka & Suzuki formerly of the *S. ceylonicum* species group), and 21 new species. Descriptions of all 21 new species are given, and the first full description of the male of *S. inthanonense*, together with the revised descriptions of its female, pupa, and larva, is also provided. Keys to identify all 27 members of this species group from Thailand are given for females, males, pupae, and larvae. The genetic relationships of all but one species were resolved using COI gene sequence-based analysis. All 26 species were divided into nine subgroups, I–IX, each consisting of two, one, four, nine, one, three, two, one and three species, respectively.

Keywords

Aquatic insects, biodiversity, blackflies, Oriental Region, taxonomy

Table of contents

Introduction

The *Simulium asakoae* species group, as defined by Takaoka (2012), is the second largest among the 14 species groups in *Simulium* (*Gomphostilbia*) (Adler 2019). It includes 36 species from various countries in the Oriental Region, of which 13 species have been recorded from Vietnam, eight species from Peninsular Malaysia, and four species from Thailand (Takaoka et al. 2017a, 2018, 2019; Srisuka et al. 2019). *Simulium asakoae* Takaoka & Davies, the first-described member of this species group, is a natural vector of a filarial species in Thailand (Fukuda et al. 2003).

The number of species in this species group in Thailand is expected to be much greater because a high rate of radiation in this species group was suggested by Jitklang et al. (2008), who described one new species (*S. doisaketense* Jitklang et al.) and recorded four unnamed species (*S.* sp. nr. *asakoae*-2, *S.* sp. nr. *asakoae*-3, *S.* sp. nr. *asakoae*-4, and *S.* sp. nr. *sheilae*-3) based on larval salivary gland chromosome studies. All but *S.* sp. nr. *asakoae*-4 are likely to be members of the *S. asakoae* species group, although their adult female and male are unknown. Similarly, Jomkumsing et al. (2019), based on an analysis of COI gene sequences, divided human-biting females of the *S. asakoae* species group collected in Thailand into seven groups, of which five groups included known and unknown haplotypes and two other groups included only unknown haplotypes, suggesting that more described species known in neighboring countries (e.g., Myanmar and Peninsular Malaysia) and more undescribed cryptic species are distributed in Thailand (pers. obs.).

The *S. asakoae* species group is separated from the other species groups in the subgenus *Gomphostilbia* by a combination of the yellowish hair tuft on the base of the radial vein and yellowish forecoxae in the female and male, hind basitarsus enlarged, and ventral plate emarginated on both sides when viewed ventrally in the male (Takaoka 2012).

In general, females of most members of the *S. asakoae* species group are morphologically similar to one another and are often difficult to identify to species, although certain morphological features, such as the width ratio of the frons against the head, relative length of the sensory vesicle against the length of the third palpal segment, presence or absence of the outer mandibular teeth, relative length of the fore- and hind basitarsi against their greatest width, and relative length of the claw tooth against the claw, are used for species identification (Takaoka et al. 2013, 2014b).

The possible involvement of undescribed cryptic species, as well as the difficulty of morphological identification of the females, is a serious problem for studies of the vectorial roles in the transmission of parasites and pathogens among species of the *S. asakoae* species group.

In this study, we first aimed to explore the fauna of the *S. asakoae* species group in Thailand by morphologically examining numerous females and males reared from pupae, their associated pupal exuviae and cocoons, and mature larvae collected in various provinces in this country, and secondly to molecularly resolve the genetic relationships of Thai members of the *S. asakoae* species group by a COI gene sequence-based analysis.

Materials and methods

Morphological analysis

The material examined in this study consisted of females and males, their associated pupal exuviae and cocoons, and mature larvae of the *S. asakoae* species group collected in various localities in Thailand. All specimens were fixed in 80% ethanol.

Methods of morphological observation, terms of features, descriptions, and illustrations followed Takaoka (2003) and partly followed Adler et al. (2004). The morphological identification at the species level was carried out by using certain female, male, pupal and larval features, such as the relative length of the female sensory vesicle, number of male upper-eye (large) facets, presence or absence of an anterodorsal projection on the cocoon, and color pattern of the larval abdomen. Diagnostic characters of each new species are provided in the "Diagnosis" to distinguish the species from all others in the *S. asakoae* species group.

Due to close morphological similarities of all new species, the description of the first new species was fully made based on as many morphological characters as possible, whereas those of the other new species were made only for morphological characters differing from those of the first new species.

The holotypes and paratypes of the new species are deposited in the Entomology Section of the Queen Sirikit Botanic Garden, Chiang Mai province, Thailand.

Genetic analysis

Thoraces of females and males reared from pupae, and whole bodies of mature larvae were used for genetic analysis. The procedures for DNA extraction, COI gene amplification, sequencing, and data analysis followed those of Srisuka et al. (2019). The sequences were deposited in DDBJ/EMBL/GenBank under accession numbers in Figure 26. The following COI gene sequences registered in GenBank of eleven known species of the *S. asakoae* species group were used for comparison: *S. asakoae*, *S. brinchangense* Takaoka, Sofian-Azirun & Hashim, *S. izuae* Takaoka, Sofian-Azirun & Hashim, *S. lurauense* Takaoka, Sofian-Azirun & Hashim, *S. monglaense* Takaoka, Srisuka & Saeung, *S. myanmarense* Takaoka, Srisuka & Saeung, *S. rampae* Takaoka, Srisuka & Saeung, *S. roslihashimi* Takaoka & Sofian-Azirun, *S. tanahrataense* Takaoka, Sofian-Azirun & Ya'cob, *S. sofiani* Takaoka & Hashim, and *S. udomi* Takaoka & Choochote. Those of *S. leparense* Takaoka, Sofian-Azirun & Ya'cob, *S. sheilae* Takaoka & Davies, and *S. trangense* Jitklang et al., which are members of the *S. ceylonicum* species group, were also used for reference.

Nomenclature

This paper and the nomenclatural acts have been registered in ZooBank (www.zoobank.org), the official register of the International Commission on Zoological Nomenclature. The Life Science Identifier (LSID) numbers are noted under each of the 21 new species of black flies.

Results

Morphological analysis

A total of 25 species was identified morphologically, comprising two known species (*S. asakoae* and *S. chiangdaoense* Takaoka & Srisuka), one newly confirmed species (*S. myanmarense* originally described from Myanmar), one newly transferred species (*S. inthanonense* Takaoka & Suzuki), and 21 new species. *Simulium inthanonense*, one of three Thai members of the *S. ceylonicum* species group, is here transferred to the *S. asakoae* species group based on the male ventral plate, which is emarginated on both sides (Fig. 24F) when viewed ventrally, one of the key characters of the *S. asakoae* species group (Takaoka 2012).

Simulium asakoae, originally described from Peninsular Malaysia (Takaoka and Davies 1995), was collected from various localities in many provinces (data not shown), indicating that it is the most common species among 27 species of this species group in Thailand.

Five taxa (*S. doisaketense*, *S.* sp. nr. *asakoae*-2, *S.* sp. nr. *asakoae*-3, *S.* sp. nr. *asakoae*-4, and *S.* sp. nr. *sheilae*-3) reported based on larval salivary gland chromosome studies by Jitklang et al. (2008), which are morphologically known only as pupae and larvae, were not recovered in this study.

The possibility of the distribution in Thailand of two Myanmar species (*S. myanmarense* and *S. monglaense*) was noted by Jomkumsing et al. (2019) judging from a COI gene sequence-based analysis. In our study, the distribution of *S. myanmarense* was confirmed based on morphological evidence (and also on molecular evidence), but that of *S. monglaense* was not confirmed by morphological examination.

All 21 new species are here described, and the male of *S. inthanonense* is fully described for the first time. Keys to identify all 27 members of the *S. asakoae* species group from Thailand are given for females, males, pupae, and larvae.

Descriptions of new species

***Simulium* (*Gomphostilbia*) *thungchangense* Takaoka, Srisuka & Saeung, sp. nov.**
http://zoobank.org/9A667B98-3B35-4F7C-9F30-097D662CC2DB
Figs 1–3, 25Q

Material examined. *Holotype*: Male (with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium thungchangense* male, QSBG col. no. 8, Thailand, 24-I-2019, by W. Srisuka", collected from a stream (width 60 cm, depth 10 cm, bed sandy, moderate flow, pH 6.9, 17.9 °C, exposed to the sun, elevation 1,376 m, 18°32'44.4"N, 98°30'53.0"E), at Siribhum waterfall, Chom thong District, Chiang Mai Province, Thailand, 24-I-2019, by W. Srisuka (Coll. No. 8).

***Paratypes*:** 10 females, 10 males (one male for DNA analysis) (with their associated pupal exuviae and cocoons), and 20 mature larvae (two mature larvae for DNA analy-

sis) (in 80% ethanol), same data as for holotype; one male (with its associated pupal exuviae and cocoon) and one pharate male (with its associated pupal exuviae and cocoon) (in 80% ethanol), collected from a stream (18 °C, elevation 1,470 m) from Phu Kha, Samorrophum, Thung Chang District, Nan Province, 2-XII-2004, by W. Choochote.

Diagnosis. Female: mandible with several teeth on the outer margin and hind tibia yellowish on little more than the basal half (Fig. 1E). Male: small number of upper-eye facets in nine vertical columns and 12 horizontal rows, presence of many hairs on the subcosta and much widened hind basitarsus 1.5–1.7 times as wide as the hind femur. Pupa: dorsal surface of abdominal segments 1 and 2 sparsely covered with minute tubercles. Larva: postgenal cleft as long as or little longer than the postgenal bridge (Fig. 3F) and abdominal segments 1–4 light greenish or greenish grey (Fig. 25Q).

Description. Female (*N* = 10). Body length 2.5–2.6 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 1.8–1.9:1.0:2.5–2.6; frons:head ratio 1.0:4.2–4.7. Fronto-ocular area well developed, narrow, directed dorsolaterally. Clypeus dark brown, densely covered with yellowish white scale-like hairs interspersed with several dark longer hairs on each side. Labrum 0.62–0.68 times length of clypeus. Antenna composed of scape, pedicel and nine flagellomeres, dark brown except scape, pedicel and base of first flagellomere yellow. Maxillary palpus composed of five palpomeres, light to medium brown, proportional lengths of third, fourth, and fifth palpal segments 1.0:1.1:2.6; sensory vesicle (Fig. 1A) ellipsoidal, medium-long (0.33–0.38 times length of third palpal segment), with medium-sized opening. Lacinia with 10–12 inner and 12–16 outer teeth. Mandible with 23–26 inner teeth and five to eight outer teeth at some distance from tip. Cibarium (Fig. 1B) medially forming sclerotized plate folded forward from posterior margin, with weakly sclerotized mediolongitudinal ridge with dark bifid apex.

Thorax. Scutum dark brown except anterolateral calli ochreous, and three blackish longitudinal vittae (one median, two submedian) faintly visible, thinly pruinose and shiny when illuminated at certain angles, densely covered with yellow scale-like recumbent short hairs. Scutellum medium brown, 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 ochreous and bare. Katepisternum longer than deep, medium to dark brown, shiny when illuminated at certain angles, moderately covered with fine yellow and brown short hairs.

Legs. Foreleg: coxa and trochanter whitish yellow; femur dark yellow to light brown with apical cap medium brown (though extreme tip yellowish); tibia yellowish white except apical one-fourth brownish black and covered with white fine hairs on basal four-fifths; tarsus brownish black, with moderate dorsal hair crest; basitarsus moderately dilated, 6.6–6.9 times as long as its greatest width. Midleg: coxa medium brown except posterolateral surface dark brown; trochanter whitish yellow; femur light to medium brown with basal one-fourth whitish yellow and apical cap medium brown (though extreme tip yellowish); tibia whitish yellow on basal two-fifths and light to dark brown on rest (though whitish yellow on basal half or little more on posterior surface in some females), and covered with yellowish fine hairs on posterior and inner

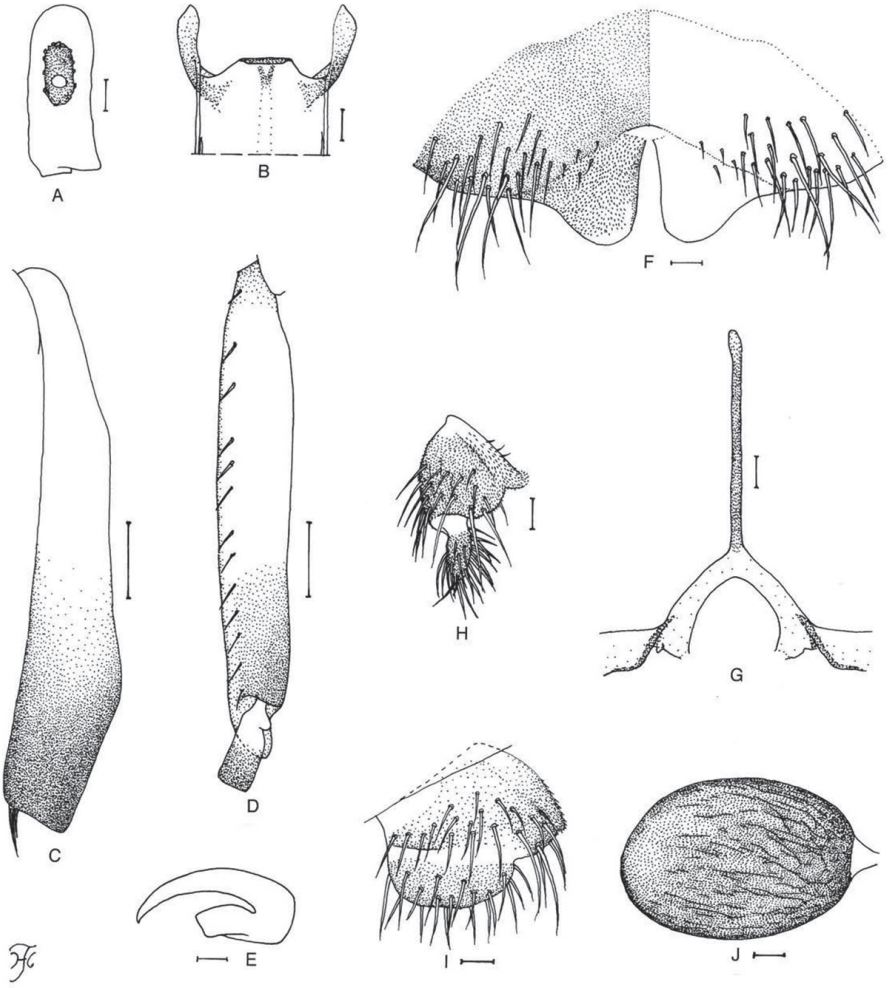

Figure 1. Female of *S. thungchangense* sp. nov. **A** third palpal segment with sensory vesicle (right side; anterior view) **B** cibarium (anterior view) **C** hind tibia (left side; lateral view) **D** hind basitarsus and second tarsomere (left side; lateral view) **E** claw **F** sternite 8 and ovipositor valves (ventral view) **G** genital fork (ventral view) **H, I** paraprocts and cerci (right side **H** ventral view **I** lateral view) **J** spermatheca. Scale bars: 0.1 mm (**C, D**); 0.02 mm (**A, B, F–J**); 0.01 mm (**E**).

surfaces of basal two-thirds; tarsus dark brown to brownish black though basal one-third of basitarsus dark yellow (its border not well defined). Hind leg: coxa medium brown with apical one-third yellow; trochanter whitish yellow; femur medium brown with base whitish yellow and apical cap dark brown (though extreme tip yellowish white); tibia (Fig. 1C) yellowish white on basal half and light brown to brownish black on rest, covered with whitish fine hairs on outer and posterior surfaces of little more than basal three-fourths; tarsus brownish black except basal two-thirds (though base light brown) and basal half of second tarsomere yellowish white; basitarsus (Fig. 1D) narrow, nearly parallel-sided, though slightly narrowed apically, 5.9–6.4 times as long

as wide, and 0.7–0.8 and 0.6 times as wide as greatest widths of tibia and femur, respectively; calcipala (Fig. 1D) nearly as long as width at base, and 0.5 times as wide as greatest width of basitarsus; pedisulcus (Fig. 1D) well developed; claw (Fig. 1E) with large basal tooth 0.5 times length of claw.

Wing. Length 2.2–2.4 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 darkened.

Abdomen. Basal scale ochreous, with fringe of whitish yellow hairs. Dorsal surface of abdomen medium to dark brown except anterior half of segment 2 ochreous, moderately covered with dark short to long hairs; tergites of segments 2 and 6–9 shiny when illuminated at certain angles. Ventral surface of segments 2–4 yellow and those of other segments medium to dark brown; sternal plate on segment 7 undeveloped.

Terminalia. Sternite 8 (Fig. 1F) bare medially, with 19 or 20 medium-long to long hairs together with three or four slender short hairs on each side. Ovipositor valves (Fig. 1F) tongue-like, thin, membranous, each moderately covered with microsetae interspersed with one or two short hairs; inner margins slightly concave medially, somewhat sclerotized, and moderately separated from each other. Genital fork (Fig. 1G) of usual inverted-Y form, with slender stem; arms of moderate width, moderately folded medially. Paraproct in ventral view (Fig. 1H) somewhat concave anterolaterally, with five or six sensilla on anteromedial surface; paraproct in lateral view (Fig. 1I) somewhat produced ventrally beyond ventral tip of cercus, 0.6–0.7 times as long as wide, with 22–25 medium-long to long hairs on ventral and lateral surfaces. Cercus in lateral view (Fig. 1I) short, slightly rounded posteriorly, 0.4 times as long as wide. Spermatheca (Fig. 1J) ellipsoidal, 1.4 times as long as its greatest width, well sclerotized and darkened except duct and small area near juncture with duct unpigmented, and with many fissures on outer surface; internal setae absent; both accessory ducts slender, subequal in diameter to major one.

Male (N = 14). Body length 2.6–3.1 mm.

Head. Nearly as wide as thorax. Upper eye vermilion, consisting of large facets in 9 (rarely 10) vertical columns and 12 horizontal rows on each side. Clypeus brownish black, whitish pruinose, densely covered with golden-yellow scale-like medium-long hairs (mostly directed upward) interspersed with several dark-brown simple longer hairs near lower margin. Antenna composed of scape, pedicel and nine flagellomeres, medium to dark brown except scape, pedicel, and base of first flagellomere yellow; first flagellomere elongate, 1.8 times length of second. Maxillary palpus light brown, with five palpal segments, proportional lengths of third, fourth, and fifth palpomeres 1.0:1.1–1.2:2.7–3.0; third palpomere (Fig. 2A) slender; sensory vesicle (Fig. 2A) small, ellipsoidal (0.18–0.24 times length of third palpal segment), and with small opening.

Thorax. Scutum dark brown to brownish black except anterolateral calli ochreous, shiny and white pruinose when illuminated at certain angles, and densely covered with whitish yellow scale-like recumbent short hairs. Scutellum dark brown, covered with

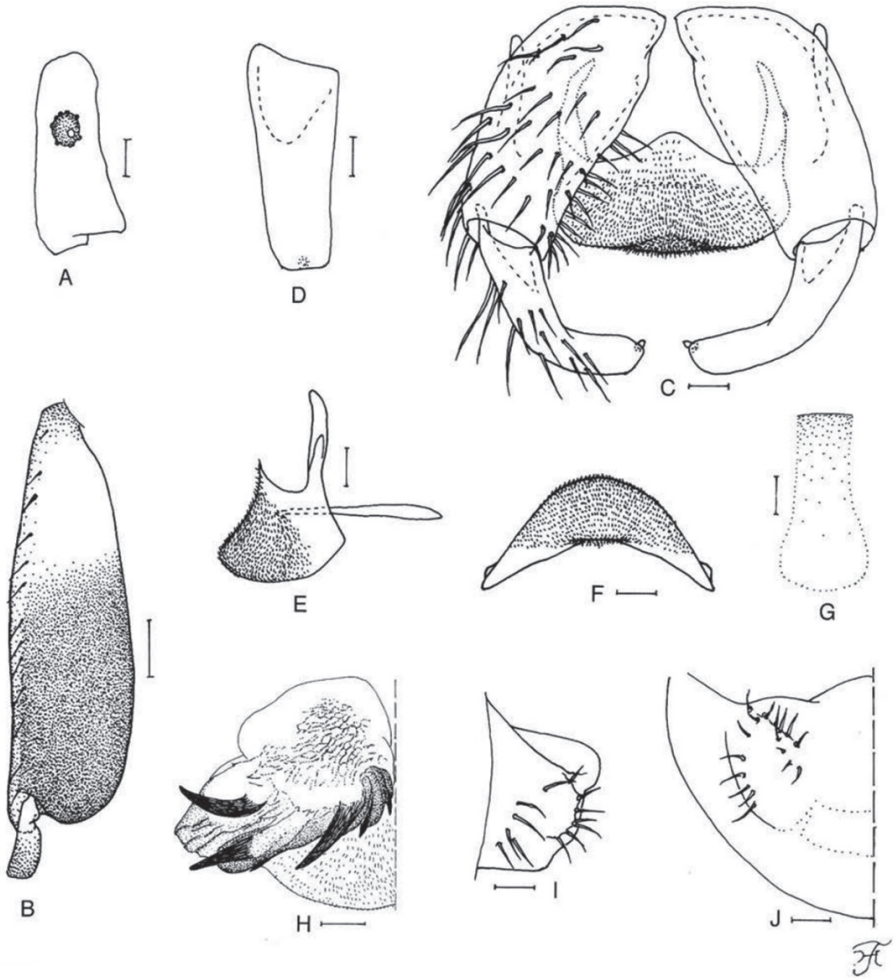

Figure 2. Male of *S. thungchangense* sp. nov. **A** sensory vesicle (right side; anterior view) **B** hind basitarsus and second tarsomere (left side; lateral view) **C** coxites, styles and ventral plate (ventral view) **D** style (right side; ventrolateral view) **E** ventral plate and median sclerite (lateral view) **F** ventral plate (caudal view) **G** median sclerite (caudal view) **H** paramere and aedeagal membrane (right half; caudal view) **I, J** tenth abdominal segments and cerci (right side **I** lateral view **J** caudal view). Scale bars: 0.1 mm (**B**); 0.02 mm (**A, C–J**).

yellow short hairs and dark-brown long upright hairs along posterior margin. Postnotum brownish black, slightly shiny and white pruinose when illuminated at certain angles, and bare. Pleural membrane ochreous and bare. Katepisternum dark brown, longer than deep, shiny and white pruinose when illuminated at certain angles, moderately covered with yellow and brown fine short hairs.

Legs. Foreleg: coxa whitish yellow; trochanter light brown; femur light brown except apical tip yellowish; tibia whitish yellow except little more than apical one-third dark brown, and covered with white hairs on whitish yellow portion; tarsus brownish

black; basitarsus slightly dilated, 8.4–8.7 times as long as its greatest width. Midleg: coxa dark brown except posterolateral surface brownish black; trochanter dark yellow to light brown except base yellow; femur light to medium brown with base yellowish and apical cap dark brown (though apical tip yellow); tibia dark brown except basal one-third (or little more on posterior surface) whitish yellow; tarsus dark brown except basal one-fourth or less of basitarsus dark yellow to light brown (border not well defined). Hind leg: coxa dark brown; trochanter yellowish; femur medium to dark brown with base yellow and apical cap brownish black (though apical tip yellow); tibia dark brown to brownish black except little more than basal two-fifths whitish yellow; tarsus (Fig. 2B) brownish black except basal two-fifths of basitarsus and little less than basal half of second tarsomere whitish yellow; basitarsus (Fig. 2B) enlarged, 3.3–3.7 times as long as wide, and 1.1–1.2 and 1.5–1.7 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 2B) slightly shorter than basal width, and 0.23 times as wide as greatest width of basitarsus; pedisulcus (Fig. 2B) well developed.

Wing. Length 2.2–2.4 mm. Other characters as in female except subcosta with 9–13 hairs. ***Halter.*** Dull white except basal stem darkened.

Abdomen. Basal scale dark brown, with fringe of light-brown hairs. Dorsal surface of abdomen medium brown to brownish black, covered with dark brown short to long hairs except segment 2 with yellowish hairs; segments 2 and 5–8 each with pair of shiny dorsolateral or lateral patches; ventral surface of segment 2 yellow, those of segments 3 and 4 yellow though sternal plates light brown, and those of other segments medium to dark brown.

Genitalia. Coxite in ventral view (Fig. 2C) nearly rectangular, 1.7 times as long as its greatest width. Style in ventral view (Fig. 2C) bent inward, with triangular apex having single spine; style in ventrolateral view (Fig. 2D) slightly tapered toward apex, with truncated apex. Ventral plate in ventral view (Fig. 2C) with body transverse, 0.6 times as long as wide, with anterior margin produced anteromedially, posterior margin somewhat concave medially, and lateral margin emarginated medially, and densely covered with microsetae on ventral surface; basal arms of moderate length, slightly divergent, then convergent apically; ventral plate in lateral view (Fig. 2E) moderately produced ventrally; ventral plate in caudal view (Fig. 2F) rounded ventrally, densely covered with microsetae on posterior surface. Median sclerite (Fig. 2G) plate-like, wide. Parameres (Fig. 2H) of moderate size, each with four distinct long and medium-long stout hooks, and without minute setae on outer surface of basal arm. Aedeagal membrane (Fig. 2H) moderately setose; dorsal plate not defined. Ventral surface of abdominal segment 10 (Fig. 2I, J) slightly sclerotized along anterior margin and without distinct hairs near posterolateral corners. Cercus (Fig. 2I, J) small, rounded, with 12–17 hairs.

Pupa (*N* = 24). Body length 2.5–3.3 mm.

Head. Integument deep yellow, moderately covered with small round tubercles except antennal sheaths and ventral surface almost bare; antennal sheath without any protuberances; frons with three pairs of unbranched long trichomes with or without coiled apices; face with pair of unbranched (rarely bifid) long trichomes with straight

apices; three frontal trichomes on each side arising close together, subequal in length to one another and slightly longer than facial one.

Thorax. Integument deep yellow, moderately covered with round tubercles, and with three long dorsomedial trichomes with coiled apices, two long anterolateral trichomes (anterior trichome more slender and shorter with straight or coiled apex, posterior one with coiled apex), one medium-long mediolateral trichome with straight apex, and three ventrolateral trichomes (one medium-long, two short) with straight apices, on each side; all trichomes unbranched. Gill (Fig. 3A) composed of eight slender thread-like filaments, arranged as [3+(1+2)]+2 (or rarely [(2+1)+(1+2)]+2 or [(2+1)+3]+2) from dorsal to ventral, with medium-long common basal stalk having somewhat swollen transparent basal fenestra at base; common basal stalk 0.7–0.8 times length of interspiracular trunk; dorsal and middle triplets sharing short stalk, and dorsal triplet mostly composed of three individual filaments arising at same level, middle triplet typically composed of one individual and two paired filaments with extremely short secondary stalk; stalk of ventral pair of filaments variable in length, 0.7–1.6 times length of common basal stalk, and 0.6–1.1 times length of interspiracular trunk, and 0.9 times as thick as common stalk of middle and dorsal triplets; primary stalk of dorsal triplet lying against that of lower pair at angle of 60–90° when viewed laterally; filaments of dorsal and middle triplets subequal in length (2.2–2.7 mm) and thickness to one another; two filaments of ventral pair subequal in length (3.0–3.4 mm) and thickness to each other and 1.3 times as thick as six other filaments of dorsal and middle triplets when compared basally; all filaments yellow to light brown, gradually tapered toward apex; cuticle of all filaments with well-defined annular ridges and furrows though becoming less marked apically, densely covered with minute tubercles.

Abdomen. Dorsally, all segments unpigmented or light yellowish except segment 9 and bases of spine-combs of segments 6–8 yellow; segments 1 and 2 sparsely covered with minute tubercles; segment 1 with one unbranched slender short hair-like seta on each side; segment 2 with one unbranched slender short hair-like seta and five somewhat spinous minute setae submedially on each side; segments 3 and 4 each with four hooked spines and one somewhat spinous minute seta on each side; segment 5 lacking spine-combs and comb-like groups of minute spines on each side; segments 6–9 each with spine-combs in transverse row and comb-like groups of micro-spines on each side; segment 5 with four minute setae on each side; segments 6–8 each with two minute setae on each side; segment 9 with pair of wide flat terminal hooks (Fig. 3B), of which outer margin 2.7 times length of inner margin and crenulated when viewed caudally. Ventrally, segment 4 with one unbranched hook (subequal in size to those on segments 5–7) and few slender short setae, of which one is much longer and stouter, on each side; segment 5 with pair of bifid or trifid hooks submedially and few short slender setae on each side; segments 6 and 7 each with pair of bifid inner and unbranched outer hooks somewhat spaced from each other and few short slender setae on each side; segments 4–8 each with comb-like groups of micro-spines. Each side of segment 9 with three grapnel-shaped hooklets.

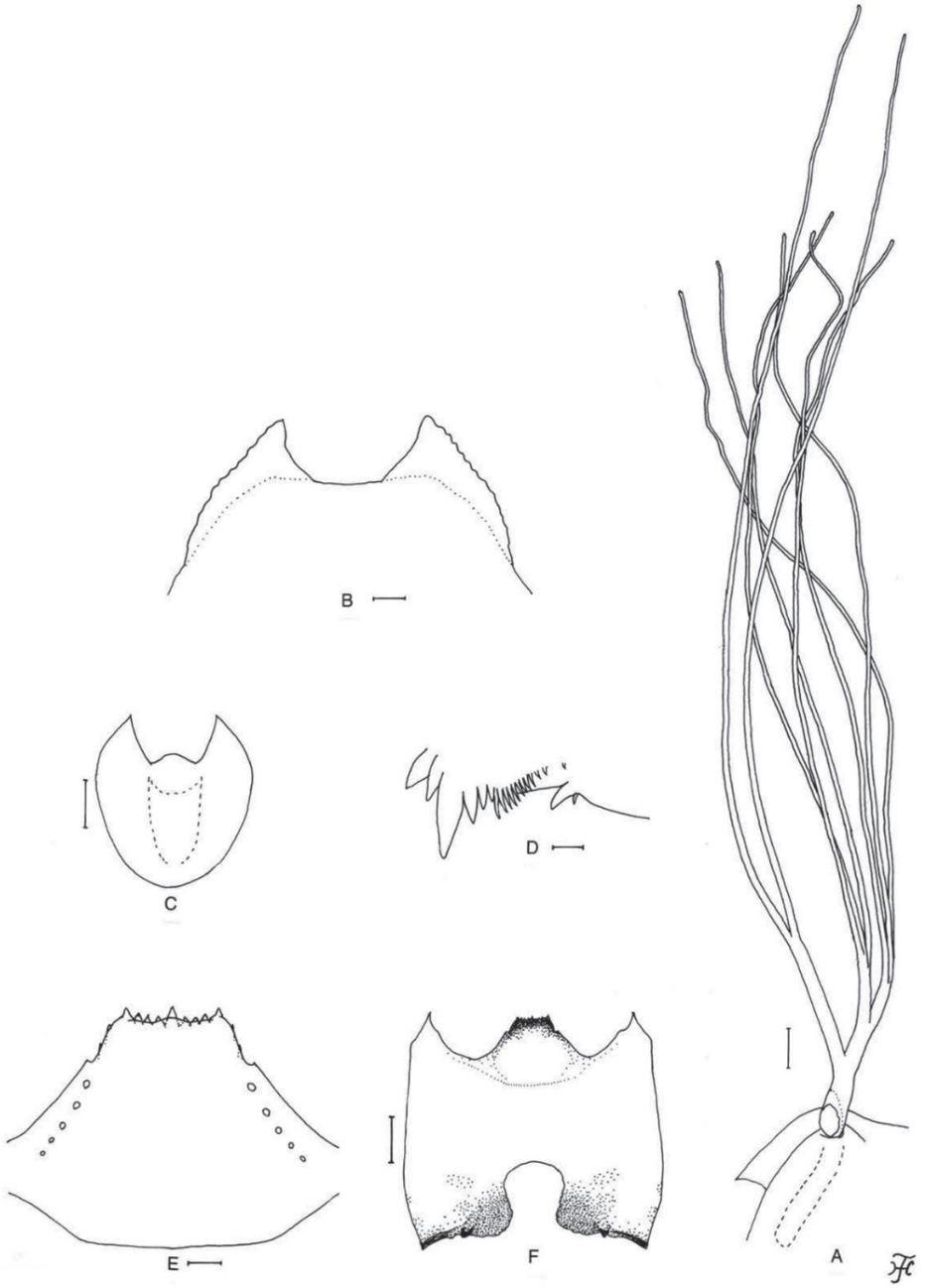

Figure 3. Pupa and larva of *S. thungchangense* sp. nov. **A–C** pupa **D–F** larva. **A** gill filaments (left side; lateral view) **B** terminal hooks (caudal view) **C** cocoon (dorsal view) **D** mandible **E** hypostoma **F** head capsule (ventral view). Scale bars: 1.0 mm (**C**); 0.1 mm (**A, F**); 0.02 mm (**E**); 0.01 mm for (**B, D**).

Cocoon (Fig. 3C). Slipper-shaped, roughly to moderately woven, widely extended ventrolaterally; anterior margin thickly woven medially, often with bulge or short projection; posterior three-fifths with floor roughly woven; individual threads visible; 3.4–4.2 mm long by 2.2–3.5 mm wide.

Mature larva (*N* = 20). Body length 5.5–6.5 mm. Body creamy white to light ochreous with following color markings: thoracic segment 1 encircled with distinct ochreous band (though disconnected ventromedially), thoracic segments 2 and 3 ochreous on ventral surface; abdominal segments 1–4 entirely light green or greenish grey, abdominal segment 4 with reddish brown transverse band (though often partially faded, leaving narrow band or small spot(s) dorsally), abdominal segment 5 with distinct reddish brown, W-shaped, transverse band dorsally, abdominal segment 6 often with three distinct, reddish brown spots (one round dorsomedial spot and two lateral spots of various size and shape), dorsal and dorsolateral surface of abdominal segments 5–8 faintly to distinctly covered with pinkish pigment (Fig. 25Q).

Head. Head capsule yellow to dark yellow except eye-spot region whitish, sparsely covered with minute setae (though moderately on dorsal surface); head spots faintly positive or indistinct. Antenna composed of three articles and apical sensillum, longer than stem of labral fan; proportional lengths of first, second, and third articles 1.0:0.8:0.7. Labral fan with 29–31 primary rays. Mandible (Fig. 3D) with three comb-teeth decreasing in length from first tooth to third; mandibular serration composed of two teeth (one medium sized, one small); major tooth at angle of little less than 90° against mandible on apical side; supernumerary serrations absent. Hypostoma (Fig. 3E) with row of nine apical teeth, of which median tooth little longer than each corner tooth; lateral margin smooth; four or five hypostomal bristles per side lying nearly parallel to lateral margin. Postgenal cleft (Fig. 3F) small, rounded, 1.0–1.2 times length of postgenal bridge. Cervical sclerites composed of pair of small yellow rod-like pieces.

Thorax and ***abdomen***. Cuticle sparsely covered with unpigmented minute setae (though few posterior abdominal segments sparsely covered also with dark minute unbranched setae) dorsally; last abdominal segment densely covered with unbranched colorless minute setae on dorsolateral and lateral surfaces of each side of anal sclerite and on each lateral surface even down to base of ventral papilla. Rectal scales minute, unpigmented. Rectal organ compound, each of three lobes with seven or eight finger-like secondary lobules. Anal sclerite of usual X-form, with anterior arms 1.2 times as long as posterior ones, broadly sclerotized at base; no sensilla on broad base and posterior to posterior arms; accessory sclerite absent. Last abdominal segment with pair of large conical ventral papillae. Posterior circlet with 80–86 rows of hooklets with up to 14 or 15 hooklets per row.

Etymology. The species name, *thungchangense*, refers to the district, Thung Chang, one of the two localities where this species was collected.

Distribution. Thailand (Chiang Mai and Nan).

Discussion. This new species is similar to *S. chaudinhense* Takaoka & Sofian-Azirun described from Vietnam (Takaoka et al. 2017a) in many characters including the presence of teeth on the outer margin of the female mandible, small number of male upper-eye facets, and pupal abdominal segments 1 and 2 each with small tubercles on the

dorsal surface. However, it is distinguished from the latter species by the male hind basitarsus 1.5–1.7 times as wide as the hind femur, and larval abdominal segments 1–4 light green or greenish grey (Fig. 25Q) (in *S. chaudinhense*, the male hind basitarsus is 1.2–1.3 times as wide as the hind femur and larval abdominal segments 1 and 2 are greyish).

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

http://zoobank.org/9B827DD8-0B95-41C4-B6AF-D5C403A7810C
Fig. 4

Material examined. ***Holotype*****:** Male (together with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium puaense* male, QSBG col. no. 60, Thailand, 25-VII-2017, by W. Srisuka" reared from a pupa collected from a small waterfall (width 80 cm, depth 3 cm, flast flow, pH 7.2, 20.1 °C, exposed to the sun, elevation 1,157 m, 19°11'10.3"N, 101°04'41.7"E), Nam Dan Village, Pua District, Nan Province, northern Thailand, 25-VII-2017, by W. Srisuka (Coll. No.60).

Paratypes**:** One female (thorax for DNA analysis) (together with its associated pupal exuviae and cocoon) (in 80% ethanol), reared from a pupa collected from a stream (width 30 cm, depth 5 cm, bed sandy, moderate running, pH 6.3, 22.6 °C, exposed to the sun, elevation 1,097 m, 18°50'03.7"N, 99°22'32.2"E), at Pa Meing Village, Muang Pan District, Lampang Province, northern Thailand, 9-VIII-2016, by W. Srisuka (Coll. No. 86); three males (thorax of one male for DNA analysis) (together with their associated pupal exuviae and cocoons) (in 80% ethanol), same data as for holotype.

Diagnosis. Female: small sensory vesicle 0.24 times as long as the third palpal segment (Fig. 4A) and relatively shorter labrum against the clypeus. Male: small number of upper-eye (large) facets in nine or ten vertical columns and 12 horizontal rows, and hind basitarsus 3.2–3.6 times as long as its greatest width and 0.9–1.1 and 1.1–1.2 times as wide as the hind tibia and femur, respectively.

Description. Female (*N* = 1). Body length 2.1 mm.

Head. Frontal ratio 1.9:1.0:2.4 frons:head ratio 1.0:4.4. Labrum 0.56 times length of clypeus. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.1:2.0; sensory vesicle (Fig. 4A) 0.24 times length of third palpal segment. Lacinia with ten or eleven inner and 12 or 13 outer teeth. Mandible (Fig. 4B) with 20 inner teeth and two or three outer teeth at some distance from tip.

Legs. Foreleg: coxa and trochanter whitish yellow; femur dark yellow to light brown with apical cap medium brown (though extreme tip yellowish); tibia yellowish white except little more than apical one-fourth brownish black; basitarsus moderately dilated, 5.8 times as long as its greatest width. Midleg: tarsus dark brown to brownish black though basal half of basitarsus dark yellow (its border not well defined). Hind leg: coxa light brown; tibia yellowish white on little more than basal half and light brown to brownish black on rest; basitarsus 5.9 times as long as wide, and 0.7 and 0.5 times as wide as greatest widths of tibia and femur, respectively; claw with large basal tooth 0.47 times length of claw.

Wing. Length 2.0 mm.

Figure 4. Female, male and pupa of *S. puaense* sp. nov. **A, B** female **C–H** male **I–L** pupa. **A** sensory vesicle (left side; anterior view) **B** mandible (left side) **C** hind basitarsus and second tarsomere (left side; lateral view) **D** coxites, styles and ventral plate (ventral view) **E, F** styles (right side; ventrolateral view **E** gradually tapered toward apex **F** nearly parallel-sided) **G** ventral plate and median sclerite (lateral view) **H** ventral plate (caudal view) **I** gill filaments (left side; lateral view) **J, K** terminal hooks (caudal view **J** normal **K** right hook abnormal) **L** cocoon (dorsal view). Scale bars: 1.0 mm (**L**); 0.1 mm (**C, I**); 0.02 mm (**A, D–H**); 0.01 mm (**B, J, K**).

Abdomen. Dorsal surface of abdomen medium to dark brown except anterior two-thirds of segment 2 ochreous.

Terminalia. Sternite 8 with 20 medium-long to long hairs together with two or three slender short hairs on each side. Paraproct in ventral view with four or five sensilla on anteromedial surface; paraproct in lateral view 0.6 times as long as wide, with 21–23 medium-long to long hairs on ventral and lateral surfaces.

Male (*N* = 4). Body length 2.1–2.4 mm.

Head. Slightly wider than thorax. Upper eye dark brown, consisting of large facets in nine or ten vertical columns and 12 horizontal rows on each side. Antenna: first flagellomere 1.5–1.6 times length of second. Maxillary palpus light brown, with five palpal segments, proportional lengths of third, fourth, and fifth palpal segments 1.0:1.0:2.0; sensory vesicle 0.13–0.18 times length of third palpal segment.

Legs. Foreleg: basitarsus moderately dilated, 6.6–7.2 times as long as its greatest width. Hind leg: tibia dark brown to brownish black except basal half whitish yellow; tarsus (Fig. 4C) brownish black except basal half or little less of basitarsus and basal one-third of second tarsomere whitish yellow; basitarsus (Fig. 4C) enlarged, 3.2–3.6 times as long as wide, and 0.9–1.1 and 1.0–1.2 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 4C) slightly shorter than basal width, and 0.28 times as wide as greatest width of basitarsus.

Wing. Length 2.0–2.1 mm. Subcosta with two to five hairs (though subcosta bare in one male).

Genitalia. Coxite in ventral view (Fig. 4D) nearly rectangular, 1.7–2.2 times as long as its greatest width. Style in ventrolateral view (Fig. 4E, F) gradually tapered toward apex or nearly parallel-sided from basal one-third to apical one-fourth, with truncated apex. Ventral plate in ventral view (Fig. 4D) with basal arms of moderate length, nearly parallel-sided; ventral plate in caudal view (Fig. 4H) with ventral margin nearly straight. Cercus with 11–14 hairs.

Pupa (*N* = 5). Body length 2.6–3.0 mm.

Thorax. Gill (Fig. 4I) composed of eight slender thread-like filaments, arranged as [3+(1+2)]+2 from dorsal to ventral; common basal stalk 0.6–0.7 times length of interspiracular trunk; dorsal and middle triplets sharing short stalk, and dorsal triplet mostly composed of three individual filaments arising at same level from extremely short stalk, middle triplet mostly composed of one individual and two paired filaments with extremely short secondary stalk; stalk of ventral pair of filaments variable in length, 0.8–1.3 times length of common basal stalk, and 0.6–0.8 times length of interspiracular trunk; primary stalk of dorsal triplet lying against that of lower pair at angle of 40–60° when viewed laterally; filaments of dorsal triplet subequal in length (1.5–1.8 mm) and thickness to one another; filaments of middle triplet subequal in length (1.7–2.0 mm); two filaments of ventral pair subequal in length (2.3–2.6 mm) and thickness to each other and 1.5 times as thick as six other filaments of dorsal and middle triplets when compared basally; all filaments light brown.

Abdomen. Dorsally, all segments light yellowish; segments 1 and 2 without minute tubercles; segment 9 with pair of wide flat terminal hooks (Fig. 4J), of which outer

margin is 2.0 times length of inner margin and crenulated when viewed caudally (terminal hooks abnormally formed in one pupa, Fig. 4K).

Cocoon (Fig. 4L). Slipper-shaped, light yellow, moderately woven, moderately extended ventrolaterally; anterior margin thickly woven medially, rarely with small bulge; individual threads not visible; 3.0–3.5 mm long by 2.2–2.5 mm wide.

Mature larva. Unknown.

Etymology. The species name, *puaense*, refers to the district, Pua, one of the two localities where this species was collected.

Distribution. Thailand (Lampang and Nan)

Discussion. This new species is similar to *S. vinhphucense* Takaoka & Low from Vietnam (Takaoka et al. 2017a) by having a small number of brown male upper-eye (large) facets, but is distinguished from the latter species by the relative length of the male fore basitarsus against its greatest width (6.6–7.2 in this new species versus 8.2 in *S. vinhphucense*) and relative width of the male hind basitarsus against the hind femur, which is 1.1–1.2 in this new species versus 1.5 in *S. vinhphucense*), and ventral plate in caudal view trapezoidal (Fig. 4H) (rounded ventrally in *S. vinhphucense*) .

This new species is also similar to *S. thungchangense* sp. nov. and *S. chaudinhense* from Vietnam (Takaoka et al. 2017a) in having a small number of male upper-eye (large) facets, but is barely distinguished from the two latter species by the upper-eye (large) facets medium brown (vermilion in the two latter species) and dorsum of pupal abdominal segments 1 and 2 bare (with minute tubercles in in the two latter species).

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

http://zoobank.org/10B68AD2-5E0C-4E3C-9E9F-040769084BE6

Figs 5, 25C

Material examined. *Holotype*: Male (with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium sutheppuiense* male, QSBG col. no. 92, Thailand, 7-IX-2017, by W. Srisuka", collected from a small stream (width 60 cm, depth 15 cm, bed sandy, moderate flow, pH 6.9, 20 °C, partially shaded, elevation 1,395 m, 18°49'09.5"N, 98°53'14.3"E), at Doi Pui Temple, Doi Suthep Pui, Muang District, Chiang Mai Province, Thailand, 7-IX-2017, by W. Srisuka (Coll. No. 92).

***Paratypes*:** One female (thorax for DNA analysis), three males (thorax of one male for DNA analysis) (with their associated pupal exuviae and cocoons), and five mature larvae (one mature larva for DNA analysis) (in 80% ethanol), same data as for holotype;

Diagnosis. Female: mandible with three teeth on the outer margin (Fig. 5A). Male: small number of upper-eye facets in eleven vertical columns and 13 horizontal rows. Pupa: dorsal triplet of the gill filaments with an extremely short stalk (Fig. 5G). Larva: postgenal cleft 1.2–2.4 as long as the postgenal bridge (Fig. 5J) and abdominal segments 1–4 light ochreous (Fig. 25C).

Description. Female (N = 1). Body length 2.0 mm.

Head. Frontal ratio 2.0:1.0:2.5; frons:head ratio 1.0:4.6. Labrum 0.57 times length of clypeus. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.2:2.7; sensory vesicle 0.29–0.32 times length of third palpal segment. Lacinia with ten or eleven inner and 13 or 14 outer teeth. Mandible (Fig. 5A) with 23 inner teeth and three outer teeth at some distance from tip.

Legs. Foreleg: basitarsus moderately dilated, 6.3 times as long as its greatest width. Midleg: tarsus dark brown to brownish black though basal half of basitarsus yellow (its border not well defined). Hind leg: coxa light brown; tibia yellowish white on basal three-fifths and light brown to brownish black on rest; basitarsus 5.3 times as long as wide, and 0.8 and 0.6 times as wide as greatest widths of tibia and femur, respectively.

Wing. Length 2.0 mm.

Abdomen. Dorsal surface of abdomen medium to dark brown except most of segment 2 ochreous.

Terminalia. Sternite 8 with 25 or 26 medium-long to long hairs together with three or four slender short hairs on each side. Ovipositor valves each moderately covered with microsetae interspersed with two or three short hairs. Paraproct in ventral view with three or four sensilla on anteromedial surface; paraproct in lateral view 0.5 times as long as wide, and with 24 medium-long to long hairs on ventral and lateral surfaces. Cercus in lateral view 0.5 times as long as wide. Spermatheca 1.5 times as long as its greatest width.

Male (N = 4). Body length 2.0–2.2 mm.

Head. Slightly wider than thorax. Upper eye dark brown, consisting of large facets in eleven vertical columns and 13 horizontal rows. Antenna light to medium brown except scape, pedicel, and base of first flagellomere yellow; first flagellomere elongate, 1.8–1.9 times length of second one. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.1:2.6; sensory vesicle 0.15 times length of third palpal segment.

Legs. Foreleg: tibia whitish yellow except apical three-tenths dark brown; basitarsus slightly dilated, 7.5–7.9 times as long as its greatest width. Hind leg: coxa medium brown; tibia dark brown to brownish black except little less than basal half whitish yellow; tarsus (Fig. 5B) brownish black except basal two-fifths of basitarsus and basal one-third of second tarsomere whitish yellow; basitarsus (Fig. 5B) enlarged, 3.6–3.8 times as long as wide, and 1.0 and 1.1 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 5B) 0.3 times as wide as greatest width of basitarsus.

Wing. Length 1.9–2.0 mm. Subcosta with 1–11 hairs (though no hairs in one male).

Genitalia. Coxite in ventral view (Fig. 5C) nearly rectangular, 1.8 times as long as its greatest width. Style in ventral view (Fig. 5C) with round or truncate apex; style in ventrolateral view (Fig. 5D) slightly tapered toward apex, with truncated apex, and 0.8 times as long as coxite. Ventral plate in caudal view (Fig. 5F) with ventral margin nearly straight. Cercus with 17 or 18 hairs.

Pupa (N = 5). Body length 2.5–2.7 mm.

Head. Integument yellow.

Thorax. Integument yellow, moderately covered with round tubercles except dorsolateral surface of posterior half sparsely covered with tubercles. Gill (Fig. 5G) composed of eight slender thread-like filaments, arranged as (3+3)+2 or [3+(1+2)]+2 from

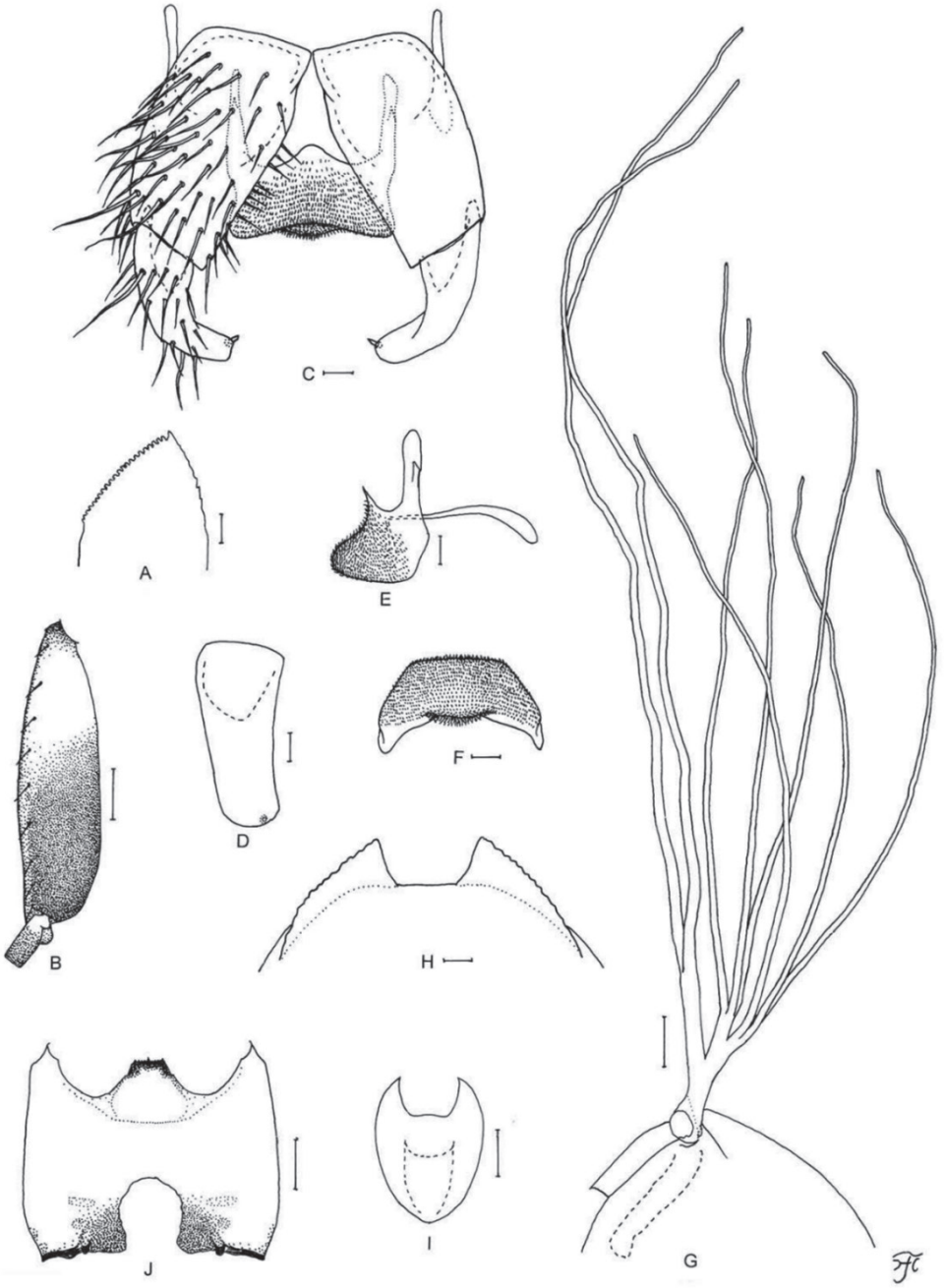

Figure 5. Female, male, pupa and larva of *S. sutheppuiense* sp. nov. **A** female **B–F** male **G–I** pupa **J** larva. **A** mandible (right side) **B** hind basitarsus and second tarsomere (left side; lateral view) **C** coxites, styles and ventral plate (ventral view) **D** style (right side; ventrolateral view) **E** ventral plate and median sclerite (lateral view) **F** ventral plate (caudal view) **G** gill filaments (left side; lateral view) **H** terminal hooks (caudal view) **I** cocoon (dorsal view) **J** head capsule (ventral view). Scale bars: 1.0 mm (**I**); 0.1 mm (**B, G, J**); 0.02 mm (**C–F**); 0.01 mm (**A, H**).

dorsal to ventral; common basal stalk 0.6–0.7 times length of interspiracular trunk; dorsal and middle triplets sharing short stalk, and each composed of three individual filaments arising at same level except middle triplet rarely composed of one individual and two paired filaments with extremely short secondary stalk; stalk of ventral pair of filaments 1.0–1.3 times length of common basal stalk, and 0.6–0.8 times length of interspiracular trunk; primary stalk of dorsal triplet lying against that of lower pair at angle of 40–70° when viewed laterally; filaments of dorsal and middle triplets subequal in length (1.5–1.8 mm) and thickness to one another; two filaments of ventral pair subequal in length (2.3–2.5 mm) and thickness to each other and 1.3–1.5 times as thick as six other filaments of dorsal and middle triplets when compared basally.

Abdomen. Dorsally, all segments unpigmented except segments 1, 2, and 9 light yellowish; segments 1 and 2 without minute tubercles; segment 9 with pair of wide flat terminal hooks (Fig. 5H), of which outer margin 2.8–2.9 times length of inner margin and crenulated when viewed caudally.

Cocoon (Fig. 5I). Light yellow to light brown, slipper-shaped, moderately woven, widely extended ventrolaterally; anterior margin not thickly woven medially, without bulge or short projection; individual threads visible; 3.2–3.6 mm long by 1.9–2.5 mm wide.

Mature larva (N = 4). Body length 4.7–5.5 mm. Body light ochreous with following color markings: thoracic segment 1 encircled with light to dark brown band (though disconnected ventrally), thoracic segments 2 and 3 ochreous on ventral surface; abdominal segment 4 faintly with reddish brown transverse band (though often entirely faded), abdominal segments 5 and 6 each with distinct reddish brown, W-shaped, transverse band dorsally along posterior margin, though often partially faded leaving one round dorsomedial spot and two lateral spots of various size and shape, dorsal and dorsolateral surface of abdominal segments 5–8 faintly to moderately covered with pinkish or reddish brown pigment (Fig. 25C).

Head. Head capsule yellow except eye-spot region whitish; head spots moderately positive or indistinct. Antenna: proportional lengths of first, second, and third articles 1.0:0.7–0.8:0.8. Labral fan with 29 or 30 primary rays. Postgenal cleft (Fig. 5J) small to medium sized, rounded or arrow-headed, 1.2–2.4 times length of postgenal bridge.

Thorax and ***Abdomen.*** Thoracic and abdominal cuticle sparsely covered with unpigmented minute setae, though few posterior abdominal segments sparsely covered with dark minute unbranched setae dorsally. Rectal organ compound, each of three lobes with nine or ten finger-like secondary lobules. Posterior circlet with 86–89 rows of hooklets with up to 14 or 15 hooklets per row.

Etymology. The species name, *sutheppuiense*, refers to the locality name, Doi Suthep Pui, where this species was collected.

Distribution. Thailand (Chiang Mai).

Discussion. This new species is similar to *S. asakoae* in many characters including the presence of teeth on the outer margin of the female mandible, and small number of male upper-eye facets. However, it is distinguished from the latter species in the female by the shorter sensory vesicle relative to the third palpal segment, in the male by the dark brown upper-eye (large) facets, in the pupa by the light yellow dorsum of abdominal segments 1–3, and in the larva by abdominal segments 1–4 light ochreous (Fig. 25C).

***Simulium* (*Gomphostilbia*) *teerachanense* Takaoka, Srisuka & Fukuda, sp. nov.**
http://zoobank.org/40239302-BA1E-4A02-A968-0C4AA624FF15
Figs 6, 25A

Material examined. *Holotype*: Male (with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium teerachanense* male, QSBG col. no. 105, Thailand, 14-VII-2017, by W. Srisuka", collected from a small stream (width 25 cm, depth 2.5 cm, bed sandy, moderate flow, pH 6.8, 21.9 °C, partially shaded, elevation 974 m, 18°20'35.8"N, 98°01'23.8"E), at Tee Ra Chan Waterfall, Mae La-noi, Mae Hong Son Province, Thailand, 14-VII-2017, by W. Srisuka (Coll. No. 105).

***Paratypes*:** Eight males (thoraces of two males for DNA analysis) (with their associated pupal exuviae and cocoons) and four mature larvae (in 80% ethanol), same data as for holotype. One mature larva (for DNA analysis) collected from a stream (elevation 817m, 19°11'22.0"N, 98°04'12.1"E) , Huai Hee Village, Mae Sarieng, Mae Hong Song Province, northern Thailand, 13-VII-2017, by W. Srisuka (Coll. No. 106).

Diagnosis. Male: small number of upper-eye facets in eleven vertical columns and 13 horizontal rows and antenna almost entirely yellow. Larva: postgenal cleft long, 2.8–3.3 times as long as the postgenal bridge (Fig. 6I) and abdominal segments 1–4 dull ochreous (Fig. 25A).

Description. Male (*N* = 9). Body length 2.0–2.2 mm.

Head. Somewhat wider than thorax. Upper eye dark brown, consisting of large facets in eleven vertical columns and 13 horizontal rows. Antenna entirely yellow, though few apical flagellomeres slightly darkened in some males; first flagellomere elongate, 1.8 times length of second. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.2:2.5; sensory vesicle small, globular or ellipsoidal (0.16–0.17 times length of third palpal segment).

Legs. Foreleg: tibia whitish yellow except apical three-tenths dark brown; basitarsus slightly dilated, 7.3–7.5 times as long as its greatest width. Hind leg: coxa light brown; tibia dark brown to brownish black except little more than basal two-fifths whitish yellow; tarsus (Fig. 6A) brownish black except little less than basal half of basitarsus and basal one-third of second tarsomere whitish yellow; basitarsus (Fig. 6A) enlarged, wedge-shaped, 3.6–3.8 times as long as wide, and 0.9–1.0 and 1.0 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 6A) slightly shorter than basal width, and 0.35 times as wide as greatest width of basitarsus.

Wing. Length 1.9–2.0 mm. Subcosta with one to four hairs, though no hairs in three males.

Abdomen. Dorsal surface of abdomen medium brown to brownish black, except most of segment 2 yellow to light ochreous.

Genitalia. Ventral plate in ventral view (Fig. 6B) with basal arms nearly parallel-sided, then convergent apically; ventral plate in caudal view (Fig. 6E) with ventral margin nearly straight. Cercus with 12 or 13 hairs.

Pupa (*N* = 9). Body length 2.4–2.6 mm.

Head. Integument yellow.

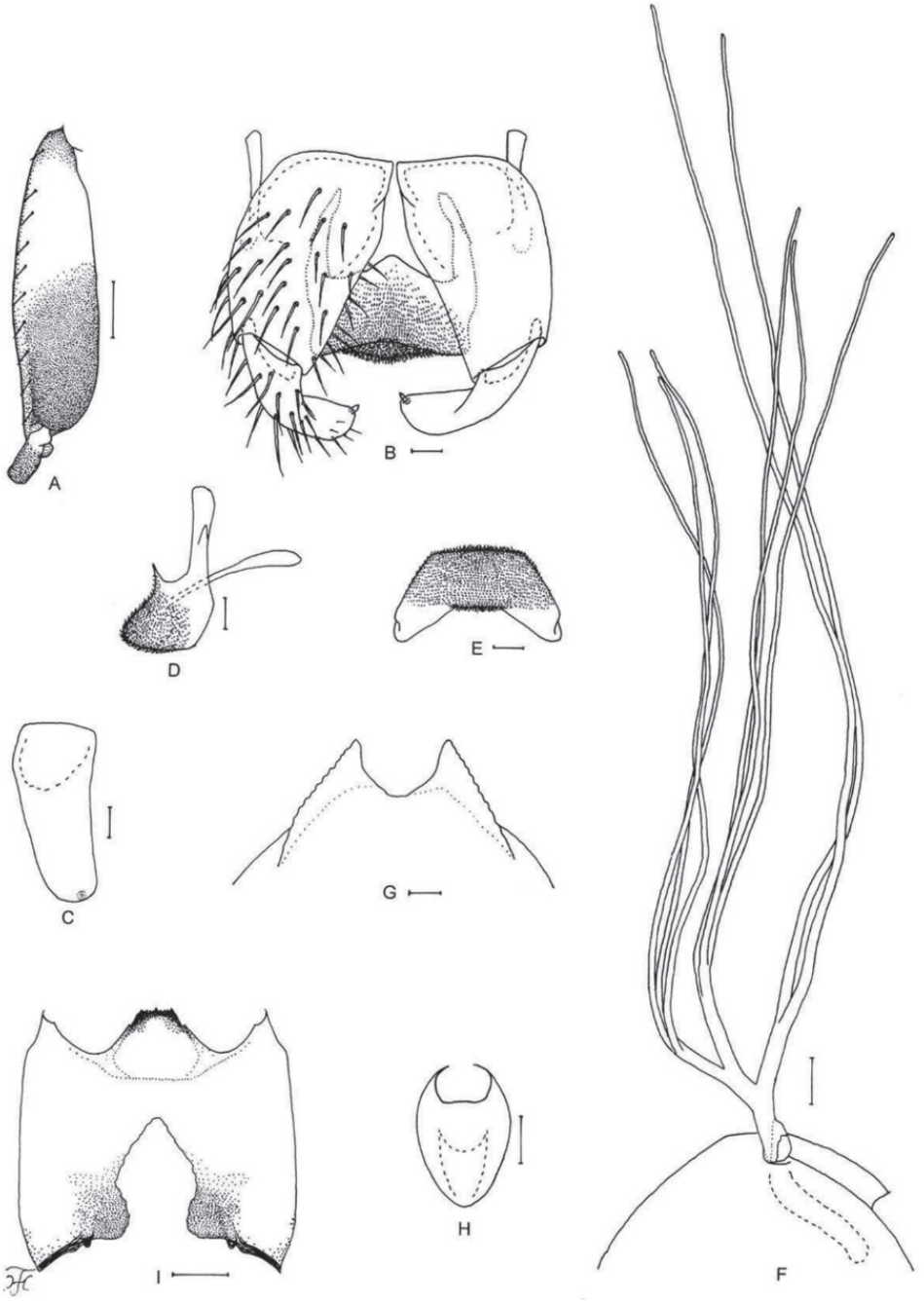

Figure 6. Male, pupa and larva of *S. teerachanense* sp. nov. **A–E** male **F–H** pupa **I** larva. **A** hind basitarsus and second tarsomere (left side; lateral view) **B** coxites, styles and ventral plate (ventral view) **C** style (right side; ventrolateral view) **D** ventral plate and median sclerite (lateral view) **E** ventral plate (caudal view) **F** gill filaments (right side; lateral view) **G** terminal hooks (caudal view) **H** cocoon (dorsal view) **I** head capsule (ventral view). Scale bars: 1.0 mm (**H**); 0.1 mm (**A, F, I**); 0.02 mm (**B–E**); 0.01 mm (**G**).

Thorax. Integument yellow, moderately covered with round tubercles, except dorsolateral surface of posterior half sparsely covered with tubercles. Gill (Fig. 6F) composed of eight slender thread-like filaments, arranged as [3+(1+2)]+2 or (3+3)+2 from dorsal to ventral; common basal stalk 0.6–0.7 times length of interspiracular trunk; stalk of ventral pair of filaments variable in length, 0.8–1.3 times length of common basal stalk, and 0.5–0.8 times length of interspiracular trunk; primary stalk of dorsal triplet lying against that of lower pair at angle of 70–90° when viewed laterally; filaments of dorsal and middle triplet subequal in length (1.5–2.1 mm) and thickness to one another; two filaments of ventral pair subequal in length (2.5–3.0 mm) and thickness to each other and 1.4–1.7 times as thick as six other filaments of dorsal and middle triplets when compared basally.

Abdomen. Dorsally, all segments unpigmented except segments 1, 2, and 9 and bases of spine-combs of segments 6–8 light yellow; segments 1 and 2 without minute tubercles; segment 9 with pair of wide flat terminal hooks (Fig. 6G), of which outer margin 2.6–2.9 times length of inner margin and crenulated when viewed caudally.

Cocoon (Fig. 6H). Yellow to dark brown, slipper-shaped, moderately woven, extended ventrolaterally; anterior margin thickly woven medially, without bulge or short projection; individual threads visible; 2.9–3.3 mm long by 2.0–2.1 mm wide.

Mature larva (*N* = 4). Body length 4.5–5.5 mm. Body dull ochreous with following color markings: thoracic segment 1 encircled with dark brown band (though disconnected ventrally), abdominal segments 5 and 6 each with distinct reddish brown, W-shaped, transverse band dorsally along posterior margin, though that on segment 6 often partially faded leaving one round dorsomedial spot and two lateral spots of various size and shape), dorsal and dorsolateral surface of abdominal segments 5–8 faintly covered with pinkish or reddish brown pigments (Fig. 25A).

Head. Head capsule yellow except eye-spot region whitish; head spots indistinct except those on lateral and ventral surfaces faintly positive. Antenna: proportional lengths of first, second, and third articles 1.0:0.7–0.8:0.8–1.0. Labral fan with 28–30 primary rays. Postgenal cleft (Fig. 6I) long, rounded or arrow-headed, 2.8–3.3 times length of postgenal bridge.

Thorax and ***Abdomen.*** Thoracic and abdominal cuticle sparsely covered with unpigmented minute setae, though few posterior abdominal segments sparsely covered with dark minute unbranched setae dorsally. Rectal organ compound, each of three lobes with 7–11 finger-like secondary lobules. Posterior circlet with 90–95 rows of hooklets with up to 14 hooklets per row.

Female. Unknown.

Etymology. The species name, *teerachanense*, refers to the name of the waterfall, Tee Ra Chan, where this species was collected.

Distribution. Thailand (Mae Hong Son).

Discussion. This new species is similar to *S. roslihashimi* described from Peninsular Malaysia (Takaoka et al. 2011b) in many characters including the small number of the male upper-eye large facets and male antenna almost entirely yellow. However, it is distinguished from the latter species by the male fore basitarsus 7.3–7.5 times as

long as its greatest width (6.6–6.8 times in *S. roslihashimi*) and ventral plate with its ventral margin nearly straight (Fig. 6E) when viewed posteriorly (ventral plate rounded ventrally in *S. roslihashimi*).

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

http://zoobank.org/232C34A2-71F4-4F44-B1C9-E0E8D405219F
Figs 7, 25M

Material examined. ***Holotype*:** Male (with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium maewongense* male, QSBG col. no. 80, Thailand, 12-VII-2018, by W. Srisuka", collected from a small stream (width 20 cm, depth 2 cm bed sandy, slow flow, 19.5 °C, partially shaded, elevation 1,322 m, 16°06'02.9"N, 99°06'23.8"E, 98°30'53.0"E), at Chon Yen, Mae Wong National Park, Klong Lan District, Kham Phaeng Phet Province, Thailand, 12-VII-2018, by W. Srisuka (Coll. No. 80).

***Paratypes*:** Three females, four males (thorax of one male for DNA analysis) (with their associated pupal exuviae and cocoons), and 14 mature larvae (two mature larvae for DNA analysis) (in 80% ethanol), same data as for holotype; one female (with its associated pupal exuviae and cocoon). (in 80% ethanol), collected from a stream of Klong Nam Lai (width 1.4 m, depth 13 cm, bed sandy, moderate flow, pH 6.23, 25.8 °C, exposed to the sun, elevation 196 m, 16°12'28.3"N, 99°15'47.8"E), at Klong Lan District, Kham Phaeng Phet Province, Thailand, 27-VI-2013, by W. Srisuka (Coll. No. 144).

Diagnosis. Female: mandible lacking teeth on the outer margin. Male: upper-eye (large) facets in eleven vertical columns and 13 or 14 horizontal rows. Pupa: thorax bare except the anterior two-fifths or half and small area of the dorsal surface near the posterior margin moderately covered with tubercles, and cocoon with a short anterodorsal projection or bulge (Fig. 7G). Larva: abdominal segments 1 and 2 greyish (Fig. 25M), and the postgenal cleft medium-long, 0.9–1.2 times as long as the postgenal bridge.

Description. Female (*N* = 4). Body length 2.1–2.2 mm.

Head. Frontal ratio 1.8–2.0:1.0:2.3–3.3. Frons: head ratio 1.0:4.3–5.4. Labrum 0.68 times length of clypeus. Maxillary palpus: proportional length of third, fourth and fifth palpal segments 1.0:1.0–1.1:2.2–2.3; sensory vesicle medium-long, 0.36 times as long as third palpal segment. Lacinia with 8–11 inner and 12 or 13 outer teeth. Mandible with 21–23 teeth on inner margin and lacking teeth on outer margin (though outer margin undulated).

Legs. Fore basitarsus 6.3–6.5 times as long as its greatest width. Hind basitarsus 5.9–6.1 times as long as its greatest width and 0.7 and 0.6 times as wide as greatest width of hind tibia and femur, respectively. Calcipala nearly as long as wide and 0.56 times as wide as greatest width of hind basitarsus.

Wing. Length 2.2–2.3 mm.

Terminalia. Sternite 8 with 17–22 medium to long stout hairs and three to five short slender hairs. Ovipositor valve with one to four short hairs. Paraproct 0.5 times as

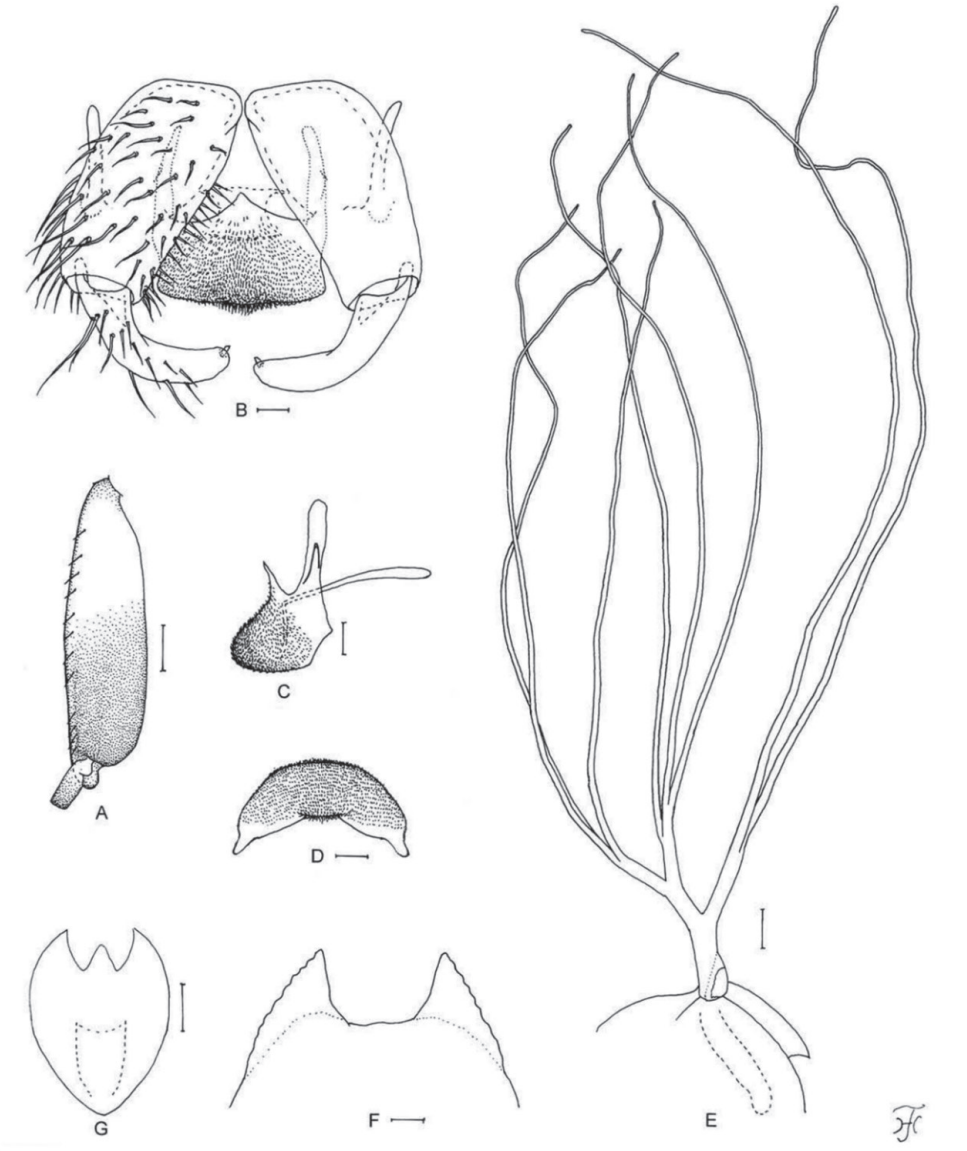

Figure 7. Male and pupa of *S. maewongense* sp. nov. **A–D** male **E–G** pupa. **A** hind basitarsus and second tarsomere (left side; lateral view) **B** coxites, styles and ventral plate (ventral view) **C** ventral plate and median sclerite (lateral view) **D** ventral plate (caudal view) **E** gill filaments (right side; lateral view) **F** terminal hooks (caudal view) **G** cocoon (dorsal view). Scale bars: 1.0 mm (**G**); 0.1 mm (**A, E**); 0.02 mm (**B–D**); 0.01 mm (**F**).

long as wide, and with 22–25 short to medium-long hairs on outer surface. Cercus 0.4 times as long as wide. Spermatheca ellipsoidal, 1.4 times as long as its greatest width.

Male (*N* = 5). Body length 2.6 mm.

Head. Upper eye dark brown, consisting of large facets in eleven vertical columns and 13 or 14 horizontal rows on each side. Antenna: first flagellomere 1.6 times as long as second. Maxillary palpus: proportional length of third, fourth and fifth palpal segments 1.0:1.2:2.8; sensory vesicle small, globular or ellipsoidal, 0.19–0.21 times as long as palpal segment 3.

Legs. Foreleg: basitarsus 7.7 times as long as its greatest width. Hind leg: basitarsus (Fig. 7A) dark brown except little less than basal half yellowish white, and enlarged, 3.5 times as long as its greatest width, and 1.0 and 1.2–1.3 times as wide as greatest width of hind tibia and femur, respectively. Calcipala (Fig. 7A) as long as wide and 0.3 times as wide as greatest width of hind basitarsus.

Wing. Length 2.2 mm. Subcosta haired except near apex bare.

Genitalia. Ventral plate in ventral view (Fig. 7B) somewhat emarginated on each lateral margin; ventral plate in caudal view (Fig. 7D) rounded ventrally. Cercus with 15–17 hairs.

Pupa (*N* = 9). Body length 3.0 mm.

Thorax. Integument almost bare except anterior two-fifths to half moderately covered with round tubercles and small dorsal area near posterior margin sparsely covered with tubercles. Gill (Fig. 7E) composed of eight slender thread-like filaments, arranged as [3+(1+2)]+2or [(2+1)+(1+2)]+2 or [(2+1)+3]+2 from dorsal to ventral, with medium-long common basal stalk having somewhat swollen transparent basal fenestra; common basal stalk 0.6–0.7 times length of interspiracular trunk; dorsal and middle triplets sharing short stalk, and dorsal triplet mostly composed of three individual filaments arising at same level, middle triplet mostly composed of one individual and two paired filaments with extremely short secondary stalk; stalk of ventral pair of filaments, 0.9–1.3 times length of common basal stalk, and 0.6–0.9 times length of interspiracular trunk, and 0.9 times as thick as common stalk of middle and dorsal triplets; primary stalk of dorsal triplet lying against that of lower pair at angle of 60–90° when viewed laterally; filaments of dorsal and middle triplets subequal in length (1.8–2.2 mm) and thickness to one another; two filaments of ventral pair subequal in length (2.8–3.2 mm) and thickness to each other and 1.6 times as thick as six other filaments of dorsal and middle triplets when compared basally.

Abdomen. Dorsally, segments 1 and 2 bare. Terminal hooks (Fig. 7F) with outer margin 2.0 times as long as inner margin.

Cocoon (Fig. 7G). Slipper-shaped, moderately woven, widely extended ventrolaterally; anterior margin thickly woven medially, with bulge or short projection; 3.0–4.0 mm long by 2.5–2.8 mm wide.

Mature larva (*N* = 12). Body length 5.5–6.5 mm. Body creamy white to light ochreous with following color markings: thoracic segment 1 encircled with ochreous or reddish brown band (though disconnected ventromedially), thoracic segments 2 and 3 ochreous on ventral surface; abdominal segments 1 and 2 entirely grey, dorsal and dorsolateral surface of abdominal segment 4 with faint reddish brown band or spot (though completely faded in some larvae), abdominal segments 5 and 6 each with reddish brown, W-shaped, transverse band near posterior margin of dorsal and dorsolateral surface, which is always distinct on abdominal segment 5 but is faded to varying extent on abdominal segment 6 leaving one small round dorsomedial spot and two distinct lat-

eral areas, abdominal segments 7 and 8 distinctly covered with reddish brown pigment on each dorsolateral surface (Fig. 25M), and ventral surface of abdominal segments 6 and 7 each with two light reddish brown spots (though absent in some larvae).

Head. Head spots faintly (or rarely moderately) positive or indistinct. Antenna: proportional lengths of first, second, and third articles 1.0:0.8:0.7–0.8. Labral fan with 27–29 primary rays. Hypostoma: median tooth little longer than each corner tooth. Postgenal cleft small, rounded, 0.9–1.2 times length of postgenal bridge.

Abdomen. Rectal organ compound, each of three lobes with 6–8 finger-like secondary lobules. Anal sclerite with anterior arms 1.1–1.2 times as long as posterior ones. Posterior circlet with 91–96 rows of hooklets with up to 14 or 15 hooklets per row.

Etymology. The species name, *maewongense*, refers to the name of the national park, Mae Wong, where this species was collected.

Distribution. Thailand (Kham Phaeng Phet).

Discussion. Among 36 species of the *S. asakoae* species group, *S. gyorkosae* Takaoka & Davies from Indonesia (Takaoka and Davies 1996), *S. jianfengense* Long et al. from Hainan Island, China (Long et al. 1994), *S. myanmarense* from Myanmar (Takaoka et al. 2017b), *S. yunnanense* Chen & Zhang from Yunnan, China (Chen and Zhang 2004), and *S. phulocense* Takaoka & Chen and *S. unii* Takaoka & Pham, both from Vietnam (Takaoka et al. 2015, 2017a), have a similar cocoon with a short anterodorsal projection. However, *S. maewongense* sp. nov. is distinguished from these six known species by the following characters (those of each related species in parentheses): from *S. gyorkosae* by the female mandible lacking outer teeth (four or five outer teeth); from *myanmarense* by the number of male upper-eye facets in eleven vertical columns (15 or rarely 14 vertical columns); from *S. jianfengense* by the ventral plate produced anteromedially when viewed ventrally (Fig. 7B) (not produced), and with its ventral margin rounded when viewed posteriorly (Fig. 7D) (pointed ventrally); from *S. yunnanense* by the yellow hair tuft of the base of the radial vein (black hair tuft); from *S. phulocense* by the relative length of the female sensory vesicle against the third palpal segment 0.36 (0.26–0.27) and male upper-eye facets in eleven vertical columns (12 or 13 vertical columns); from *S. unii* by upper-eye facets in eleven vertical columns (13 or 14 vertical columns), and the ventral plate with its lateral margins emarginated medially (Fig. 7B) (ventral plate narrowed posteriorly).

The larval body color pattern (Fig. 25M) of this new species is similar to that of *S. tuenense* Takaoka from Taiwan (Takaoka 1979), although *S. tuenense* differs from this new species by the number of male upper-eye facets in 15 vertical columns and 15 or 16 horizontal rows and cocoon without an anterodorsal projection (Huang et. al. 2011).

***Simulium* (*Gomphostilbia*) *loeiense* Takaoka, Srisuka & Fukuda, sp. nov.**

http://zoobank.org/E183734D-DE58-48AA-BE1F-731ECC7B3861
Figs 8, 25H

Material examined. *Holotype*: Male (with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium loeiense* male, QSBG col. no. 43, Thai-

land, 6-IX-2018, by W. Srisuka", collected from a small stream (width 40 cm, depth 2 cm, bed sandy, slow flow, pH 5.8, 19.5 °C, partially shaded, elevation 1,525 m, 17°16'51.7"N, 101°31'02.5"E), at Khok Nok Kra Ba, Phu Luang, Phu Ruea District, Loei Province, Thailand, 6-IX-2018, by W. Srisuka (Coll. No. 43).

***Paratypes*:** Five females, five males (thorax of one male for DNA analysis) (with their associated pupal exuviae and cocoons), and 15 mature larvae (two mature larvae for DNA analysis) (in 80% ethanol), same data as for holotype.

Diagnosis. Female: mandible with several teeth on the outer margin. Male: small number of upper-eye facets in eleven vertical columns and 13 horizontal rows, presence of many hairs on subcosta and much widened hind basitarsus (Fig. 8B) 1.2–1.3 times as wide as the hind femur. Pupa: dorsal surface of abdominal segments 1 and 2 with several minute tubercles on each side. Larva: postgenal cleft 1.2–1.3 times as long as the postgenal bridge (Fig. 8I) and abdominal segments 1–5, 7 and 8 grey (Fig. 25H).

Description. Female (*N* = 5). Body length 1.9–2.2 mm.

Head. Frontal ratio 1.9:1.0:2.7–2.9; frons:head ratio 1.0:4.7–4.8. Labrum 0.7 times length of clypeus. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.1–1.2:2.6; sensory vesicle 0.26–0.32 times length of third palpal segment. Lacinia with 10–12 inner and 12–16 outer teeth. Mandible with 19–21 inner teeth and three to four outer teeth at some distance from tip.

Legs. Foreleg: trochanter yellow to dark yellow; femur light brown with apical cap medium brown (though extreme tip yellowish); tibia white except apical one-fourth brownish black; basitarsus moderately dilated, 6.1–6.7 times as long as its greatest width. Midleg: trochanter dark yellow; femur light brown except base and extreme apical tip yellowish); tibia whitish on basal one-third, greyish on middle one-third and dark brown on apical one-third (though whitish on basal two-thirds on posterior surface); tarsus dark brown to brownish black except basal one-fourth to half yellow (its border not well defined). Hind leg: femur light to medium brown with base whitish yellow and apical cap dark brown (though extreme tip yellowish); tibia white to yellowish white on basal two-thirds or little less and brownish black on rest; tarsus brownish black except little more than basal two-thirds (though base light brown) and basal half of second tarsomere white; basitarsus 5.6–6.3 times as long as wide, and 0.7–0.8 and 0.6 times as wide as greatest widths of tibia and femur, respectively; calcipala nearly as long as width at base, and 0.53–0.57 times as wide as greatest width of basitarsus.

Wing. Length 2.0–2.2 mm.

Abdomen. Dorsal surface of abdomen medium to dark brown except anterior four-fifths of segment 2 ochreous.

Terminalia. Sternite 8 with 21–26 medium-long to long hairs together with three or four slender short hairs on each side. Ovipositor valve moderately covered with microsetae interspersed with two to four short hairs. Genital fork (Fig. 8A) with inner margins of arms divergent apically. Paraproct with four to six sensilla on anteromedial surface; paraproct in lateral view 0.6 times as long as wide, with 23–29 medium-long to long hairs on ventral and lateral surfaces. Cercus in lateral view 0.5 times as long as wide. Spermatheca 1.3–1.4 times as long as its greatest width; both accessory ducts slender, subequal in diameter to each other and slightly thicker than major one.

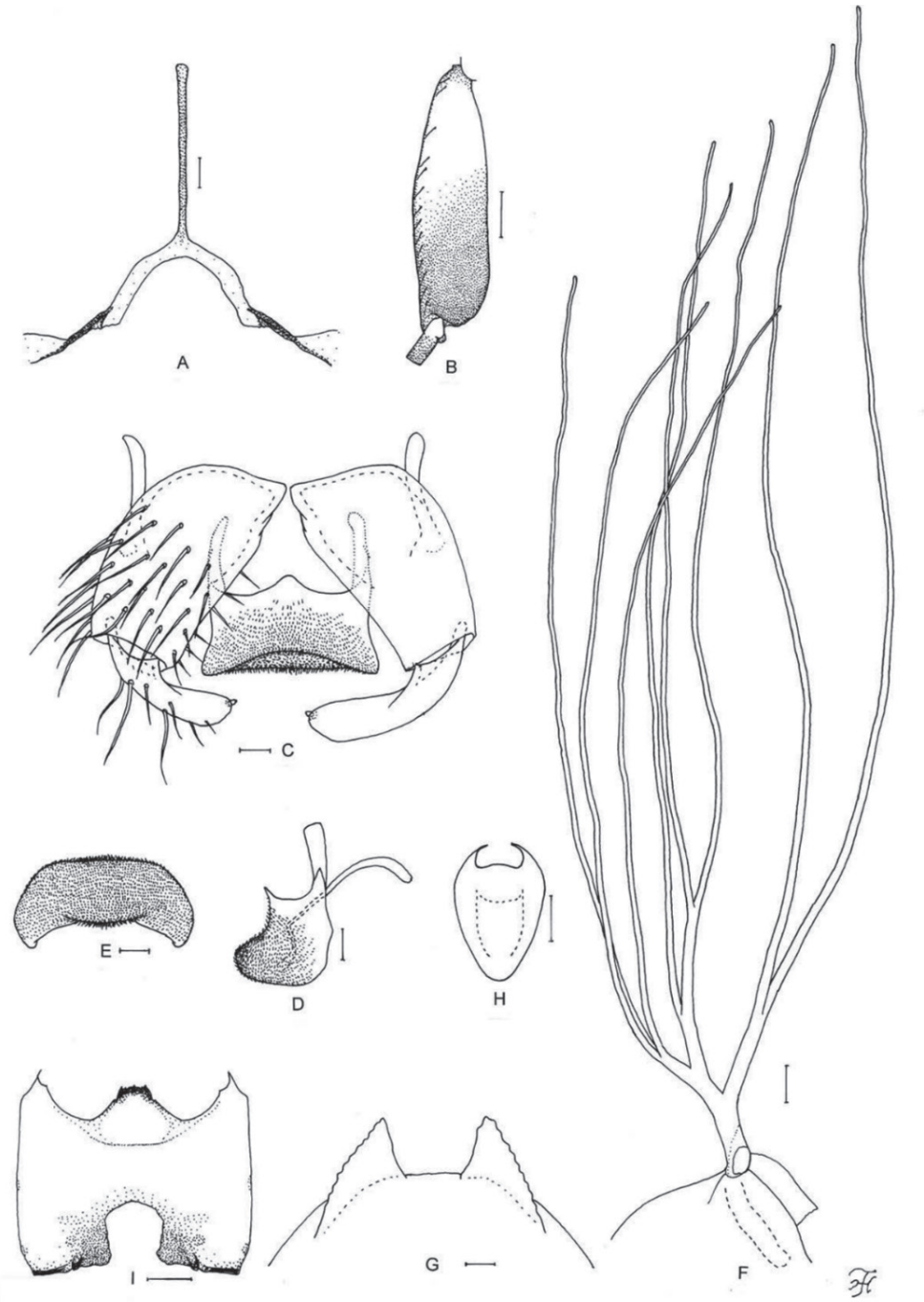

Figure 8. Female, male, pupa and larva of *S. loeiense* sp. nov. **A** female **B–E** male **F–H** pupa **I** larva. **A** genital fork (ventral view) **B** hind basitarsus and second tarsomere (left side; lateral view) **C** coxites, styles and ventral plate (ventral view) **D** ventral plate and median sclerite (lateral view) **E** ventral plate (caudal view) **F** gill filaments (right side; lateral view) **G** terminal hooks (caudal view) **H** cocoon (dorsal view) **I** head capsule (ventral view). Scale bars: 1.0 mm (**H**); 0.1 mm (**B, F, I**); 0.02 mm (**A, C–E**); 0.01 mm (**G**).

Male (*N* = 6). Body length 2.3–2.6 mm.

Head. Upper eye bright medium brown, consisting of (large) facets in eleven vertical columns and 13 horizontal rows. Antenna light to medium brown except scape, pedicel, and base of first flagellomere yellow; first flagellomere elongate, 1.5 times length of second. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.1:2.4–2.5; sensory vesicle globular or ellipsoidal (0.14–0.19 times length of third palpal segment).

Legs. Foreleg: trochanter dark yellow; femur light brown except apical tip yellowish; tibia whitish except apical one-third dark brown and inner margin dark yellow to light brown, and covered with white hairs on whitish yellow portion; tarsus brownish black; basitarsus somewhat dilated, 7.1–7.6 times as long as its greatest width. Midleg: trochanter yellow; femur light brown with base yellowish and apical cap medium brown (though apical tip yellow); tibia light to medium brown except basal one-third (or little more on posterior surface) whitish; tarsus dark brown except basal one-fourth to one-third of basitarsus dark yellow (border not well defined). Hind leg: coxa light brown; trochanter yellowish; femur light to medium brown with base yellow and apical cap dark brown (though apical tip yellow); tibia medium to dark brown except little less than basal half yellowish white; tarsus (Fig. 8B) dark brown to brownish black except basal two-fifths of basitarsus (though basal half or little more of narrow portion along anterior margin) and little less than basal half of second tarsomere whitish yellow; basitarsus (Fig. 8B) 3.3–3.7 times as long as wide, and 1.0 and 1.2–1.3 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 8B) slightly shorter than basal width, and 0.24 times as wide as greatest width of basitarsus.

Wing. Length 2.0–2.1 mm. Subcosta with three to six hairs.

Genitalia. Coxite in ventral view (Fig. 8C) 1.8 times as long as its greatest width. Style in ventral view (Fig. 8C) bent inward, with round apex having single spine; style in ventrolateral view slightly tapered toward apex, or nearly parallel-sided from middle to apex, with round or truncated apex, and 0.8 times length of coxite. Ventral plate in ventral view (Fig. 8C) with body transverse, 0.54 times as long as wide, with lateral margin emarginated medially; basal arms of moderate length, nearly parallel-sided, then convergent apically; ventral plate in lateral view (Fig. 8D) moderately produced ventrally; ventral plate in caudal view (Fig. 8E) trapezoidal ventrally, with ventral margin nearly straight, densely covered with microsetae on posterior surface. Cercus with 15–19 hairs.

Pupa (*N* = 11). Body length 2.5–2.8 mm.

Thorax. Gill (Fig. 8F) composed of eight slender thread-like filaments, arranged as [(1+2)+(1+2)]+2 from dorsal to ventral; common basal stalk 0.7–0.9 times length of interspiracular trunk; primary and second stalks of dorsal triplet short, those of middle triplet mostly short (though medium-long in few pupae); stalk of ventral pair of filaments variable in length, 1.0–1.7 times length of common basal stalk, and 0.8–1.2 times length of interspiracular trunk; primary stalk of dorsal triplet lying against that of lower pair at angle of 70–80° when viewed laterally; filaments of dorsal triplet subequal in length (2.4–2.5 mm) and thickness to one another; filaments of middle triplet subequal in length (2.8–2.9 mm) and thickness to one another; two filaments of ventral pair subequal in length (3.1–3.2 mm) and thickness to each other and 1.3 times as

thick as six other filaments of dorsal and middle triplets according to measurement of intact right gill of one pupa.

Abdomen. Segment 9 with pair of wide flat terminal hooks (Fig. 8G), of which outer margin 2.2 times length of inner margin and crenulated when viewed caudally.

Cocoon (Fig. 8H). Slipper-shaped, moderately woven, somewhat extended ventrolaterally; anterior margin thickly woven medially, often with bulge; individual threads visible or not; 2.6–3.2 mm long by 1.3–2.2 mm wide.

Mature larva (*N* = 13). Body length 5.0–5.5 mm. Body creamy white to light ochreous with following color markings: thoracic segment 1 encircled with distinct ochreous band (though disconnected ventromedially and dorsomedially), thoracic segments 2 and 3 ochreous or dark grey on ventral surface; abdominal segments 1–4 entirely greenish grey, abdominal segments 5, 7, and 8 dark grey on dorsal and dorsolateral surface; abdominal segments 5 and 6 each with distinct reddish brown, W-shaped, transverse band along posterior margin dorsally, though that on abdominal segment 6 often partially faded, leaving three distinct, reddish brown spots (one round dorsomedial spot and two lateral spots of various size and shape), dorsal and dorsolateral surface of abdominal segments 7 and 8 faintly to distinctly covered with reddish brown pigment (Fig. 25H); ventral surface of abdominal segments 5–7 each with pair of reddish brown small spots.

Head. Head capsule yellow except narrow areas along posterior margin and surrounding areas of postgenal cleft somewhat darkened, and eye-spot region whitish; head spots distinctively positive. Antenna: proportional lengths of first, second, and third articles 1.0:0.8:0.9. Labral fan with 35 or 36 primary rays. Hypostoma: four hypostomal bristles per side lying nearly parallel to lateral margin. Postgenal cleft (Fig. 8I) medium sized, rounded, 1.3–1.4 times length of postgenal bridge.

Abdomen. Rectal organ compound, each of three lobes with 10–15 finger-like secondary lobules. Anal sclerite of usual X-form, with anterior arms 0.9 times as long as posterior ones. Posterior circlet with 76–86 rows of hooklets with up to 14 or 15 hooklets per row.

Etymology. The species name, *loeiense*, refers to the name of the province, Loei, where this species was collected.

Distribution. Thailand (Loei).

Discussion. This new species is similar to *S. asakoae* in many characters including the presence of teeth on the outer margin of the female mandible, and small number of male upper-eye facets. However, it is distinguished from the latter species by the dorsum of pupal abdominal segments 1 and 2 yellowish (the dorsum of pupal abdominal segments 1 and 2 darkened in *S. asakoae*).

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

http://zoobank.org/5776AAC8-0AE0-45E1-8873-B482DD48F13E
Fig. 9

Material examined. *Holotype*: Male (with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium maelanoiense* male, QSBG col. no. 105,

Thailand, 14-VII-2017, by W. Srisuka", collected from a small stream (width 25 cm, depth 2.5 cm, bed sandy, moderate flow, pH 6.8, 21.9 °C, partially shaded, elevation 974 m, 18°20'35.8"N, 98°01'23.8"E), at Tee La Chan Waterfall, Mae La Noi District, Mae Hong Son Province, Thailand, 14-VII-2017, by W. Srisuka (Coll. No. 105).

***Paratypes*:** Two females (thoraces for DNA analysis) (with its associated pupal exuviae and cocoon) (in 80% ethanol), collected from a stream (width 110 cm, depth 12 cm, bed sandy, moderate flow, pH 6.9, 18.3 °C, partially shaded, elevation 1,446 m, 18°51'38.8"N, 99°22'15.2"E), at Kiew Fin, Muang Pan District, Lampang Province, Thailand, 6-IV-2018, by W. Srisuka (Coll. No. 36); two males (thorax of one male for DNA analysis) (with their associated pupal exuviae and cocoons) (in 80% ethanol), same data as for holotype.

Diagnosis. Female: mandible with one tooth on the outer margin (Fig. 9A). Male: small number of upper-eye facets in eleven vertical columns and 13 horizontal rows, and hind basitarsus (Fig. 9B) 1.0–1.1 times as wide as the hind femur.

Description. Female (*N* = 2). Body length 2.0 mm.

Head. Frontal ratio 1.9:1.0:2.7; frons:head ratio 1.0:4.9. Labrum 0.70 times length of clypeus. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.1:2.1; sensory vesicle 0.26–0.32 times length of third palpal segment. Lacinia with ten or eleven inner and 12 or 13 outer teeth. Mandible (Fig. 9A) with 20 inner teeth and one outer tooth at some distance from tip.

Legs. Foreleg: basitarsus moderately dilated, 6.6 times as long as its greatest width. Midleg: tarsus dark brown to brownish black though basal half of basitarsus dark yellow (its border not well defined). Hind leg: coxa light brown; tibia yellowish white on basal three-fifths and light brown to brownish black on rest; tarsus brownish black except basal three-fifths (though base light brown) and basal half of second tarsomere yellowish white; basitarsus 6.9 times as long as wide, and 0.7 and 0.5 times as wide as greatest widths of tibia and femur, respectively; calcipala 0.6 times as wide as greatest width of basitarsus; claw with large basal tooth 0.44 times length of claw.

Wing. Length 2.0 mm.

Abdomen. Dorsal surface of abdomen medium to dark brown except anterior four-fifths of segment 2 ochreous.

Terminalia. Sternite 8 with 27 medium-long to long hairs together with two to four slender short hairs on each side. Ovipositor valves each moderately covered with microsetae interspersed with one to three short hairs. Paraproct in ventral view with four sensilla on anteromedial surface; paraproct in lateral view 0.6 times as long as wide, with 19 or 20 medium-long to long hairs on ventral and lateral surfaces.

Male (*N* = 3). Body length 2.3–2.4 mm.

Head. Somewhat wider than thorax. Upper eye medium brown, consisting of large facets in eleven (rarely ten) vertical columns and 13 horizontal rows on each side. Antenna light to medium brown except scape, pedicel, and base of first flagellomere whitish yellow; first flagellomere elongate, 1.9 times length of second. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.2:2.6; sensory vesicle 0.16–0.21 times length of third palpal segment.

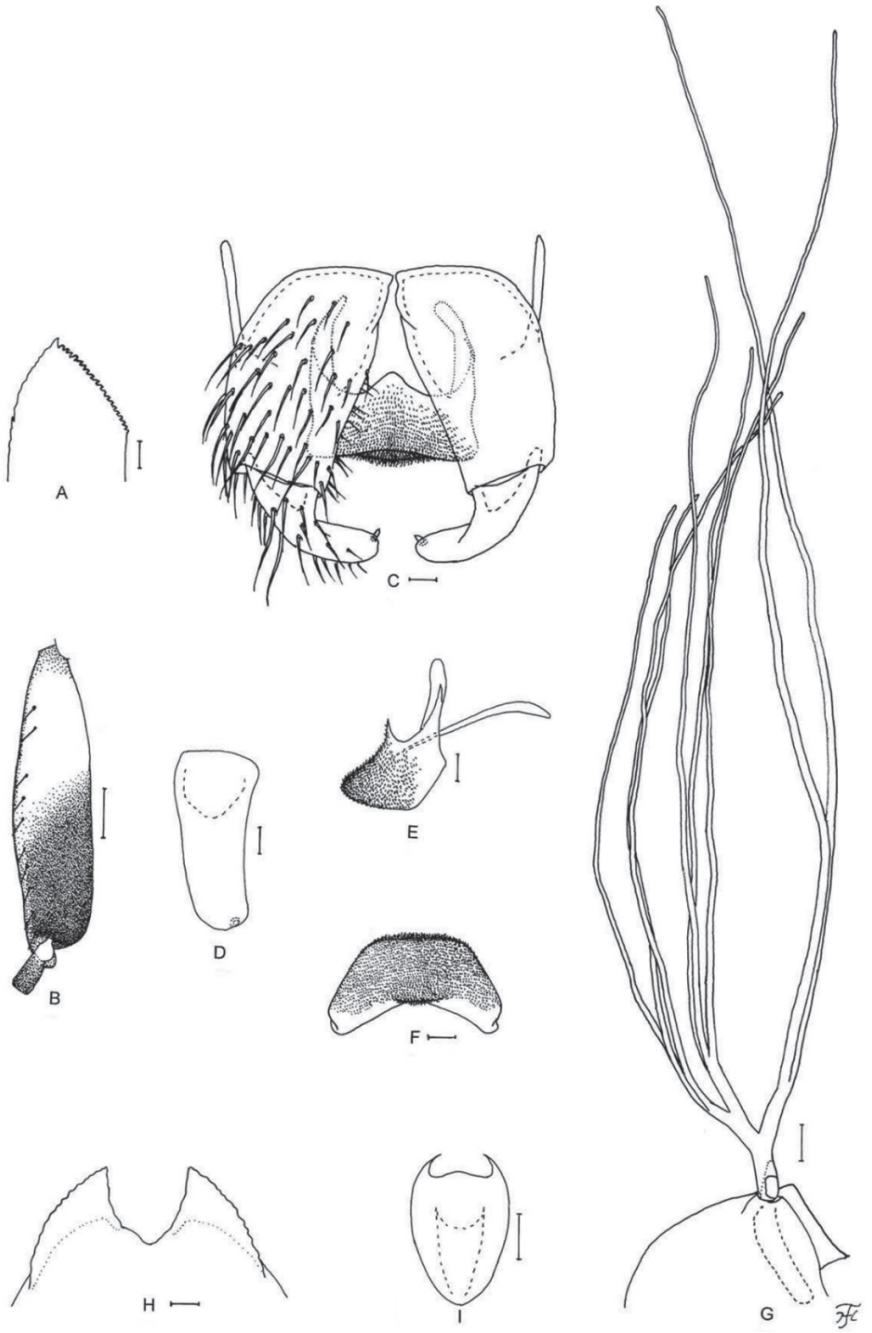

Figure 9. Female, male and pupa of *S. maelanoiense* sp. nov. **A** female **B–F** male **G–I** pupa. **A** mandible (left side) **B** hind basitarsus and second tarsomere (left side; lateral view) **C** coxites, styles and ventral plate (ventral view) **D** style (right side; ventrolateral view) **E** ventral plate and median sclerite (lateral view) **F** ventral plate (caudal view) **G** gill filaments (right side; lateral view) **H** terminal hooks (caudal view) **I** cocoon (dorsal view). Scale bars: 1.0 mm (**I**); 0.1 mm (**B, G**), 0.02 mm (**C–F**); 0.01 mm (**A, H**).

Legs. Foreleg: tibia yellowish white except apical three-tenths dark brown; basitarsus moderately dilated, 6.6–7.8 times as long as its greatest width. Hind leg: coxa light brown; tibia dark brown to brownish black except little less than basal half yellowish white; tarsus (Fig. 9B) brownish black except little more than basal two-fifths of basitarsus and basal one-third of second tarsomere yellowish white; basitarsus (Fig. 9B) 3.6–3.8 times as long as wide, and 1.0 and 1.0–1.1 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 9B) slightly shorter than basal width, and 0.28 times as wide as greatest width of basitarsus.

Wing. Length 2.0–2.1 mm. Subcosta with 2–13 hairs, though rarely without hair.

Genitalia. Style in ventrolateral view (Fig. 9D) slightly tapered toward apex, with round apex. Ventral plate in ventral view (Fig. 9C) with basal arms of moderate length, nearly parallel-sided, then convergent apically; ventral plate in caudal view (Fig. 9F) with ventral margin nearly straight. Cercus with 13–15 hairs.

Pupa (*N* = 5). Body length 2.5–3.0 mm.

Head. Integument yellow.

Thorax. Integument yellow, moderately covered with round tubercles except dorsal surface of posterior half sparsely covered with tubercles, and dorsolateral surface of posterior half almost bare. Gill (Fig. 9G) composed of eight slender thread-like filaments, arranged as (3+3)+2 (or rarely [3+(1+2)]+2) from dorsal to ventral; common basal stalk 0.6–0.7 times length of interspiracular trunk; dorsal triplet composed of three individual filaments arising at same level, and with extremely short stalk in some pupae, middle triplet mostly composed of three individual filaments arising at same level, or rarely composed of one individual and two paired filaments with extremely short secondary stalk; stalk of ventral pair of filaments variable in length, 0.6–1.3 times length of common basal stalk, and 0.5–1.0 times length of interspiracular trunk; primary stalk of dorsal triplet lying against that of lower pair at angle of 60–80° when viewed laterally; filaments of dorsal triplet subequal in length (1.5–2.0 mm) and thickness to one another; filaments of middle triplet subequal in length (1.7–2.5 mm) and thickness, and two filaments of ventral pair subequal in length (2.5–3.6 mm) and thickness to each other and 1.4–1.8 times as thick as six other filaments of dorsal and middle triplets when compared basally.

Abdomen. Dorsally, segments 1 and 2 without minute tubercles; segment 9 with pair of wide flat terminal hooks (Fig. 9H), of which outer margin 2.1–2.3 times length of inner margin and crenulated when viewed caudally.

Cocoon (Fig. 9I). Dark brown, slipper-shaped, moderately woven, moderately extended ventrolaterally; anterior margin thickly woven medially, often with bulge; 3.0–3.8 mm long by 2.0–2.5 mm wide.

Mature larva. Unknown.

Etymology. The species name, *maelanoiense*, refers to the district, Mae La Noi, where this species was collected.

Distribution. Thailand (Lampang and Mae Hong Son).

Discussion. *Simulium maelanoiense* sp. nov. is most similar in the male and pupa to *S. sutheppuiense* sp. nov. in many characters including the number of male upper-

eye facets, relative width of the hind basitarsus compared to the hind tibia and femur, shape of the ventral plate when viewed caudally, and arrangement of the pupal gill. However, this new species is barely distinguished in the female from *S. sutheppuiense* sp. nov. by the number of the outer teeth of the mandible (one tooth in this new species versus three teeth in *S. sutheppuiense* sp. nov.) and the length ratio of the labrum against the clypeus (0.7 in this new species versus 0.6 in *S. sutheppuiense* sp. nov.).

***Simulium* (*Gomphostilbia*) *phapeungense* Takaoka, Srisuka & Fukuda sp. nov.**
http://zoobank.org/25D1EED4-BFCB-4080-9122-9D511575114C
Figs 10, 25N

Material examined. *Holotype*: Male (thorax for DNA analysis) (with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium phapeungense* male, QSBG col. no. 107, Thailand, 12-VII-2017, by W. Srisuka", collected from a stream (width 120 cm, depth 2 cm, bed sandy, fast flow, pH 7.6, 21.2 °C, partially shade, elevation 1,034 m, 19°36'58.5"N, 97°59'48.2"E), at Pha Peung, Muang District, Mae Hong Son Province, Thailand, 12-VII-2017, by W. Srisuka (Coll. No.107).

***Paratype*:** One male (thorax for DNA analysis) (with its associated pupal exuviae and cocoon) (in 80% ethanol), same data as for holotype; five mature larvae (one mature larva for DNA analysis) (in 80% ethanol), collected from a stream (width 80 cm, depth 2.5 cm, moderate flow, pH 7.2, 20.1 °C, partially shaded, elevation 1,157 m, 19°11'10.3"N, 101°04'41.7"E), Nam Dan Village, Pua District, Nan Province, Thailand, 25-VII-2017, by W. Srisuka (Coll. No. 60).

Diagnosis. Male: small number of brown upper-eye (large) facets in eleven vertical columns and 13 horizontal rows, and widened hind basitarsus (Fig. 10A) 1.2 times as wide as the hind femur. Pupa: dorsum of pupal abdominal segments 1 and 2 without minute tubercles. Larva: abdominal segments 1, 3, 4, and 5 light grey (Fig. 25N).

Description. Male (*N* = 2). Body length 2.2 mm.

Head. Somewhat wider than thorax. Upper eye dark brown, consisting of large facets in eleven vertical columns and 13 horizontal rows on each side. Antenna: first flagellomere elongate, 1.5 times length of second. Maxillary palpus: proportional lengths of third, fourth, and fifth palpomeres 1.0:1.3:2.7; sensory vesicle ellipsoidal, 0.18–0.21 times length of third palpomere.

Legs. Foreleg: tibia whitish except apical three-tenths dark brown; basitarsus somewhat dilated, 6.9 times as long as its greatest width. Midleg: tarsus dark brown except basal one-third of basitarsus dark yellow (border not well defined). Hind leg: coxa light brown; tibia light to dark brown except little less than basal half whitish; tarsus (Fig. 10A) dark brown except basal half of basitarsus and basal one-third of second tarsomere whitish to yellowish white; basitarsus (Fig. 10A) enlarged, 3.5 times as long as wide, and 1.0 and 1.2 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 10A) 0.27 times as wide as greatest width of basitarsus.

Wing. Length 2.0 mm. Subcosta with five hairs.

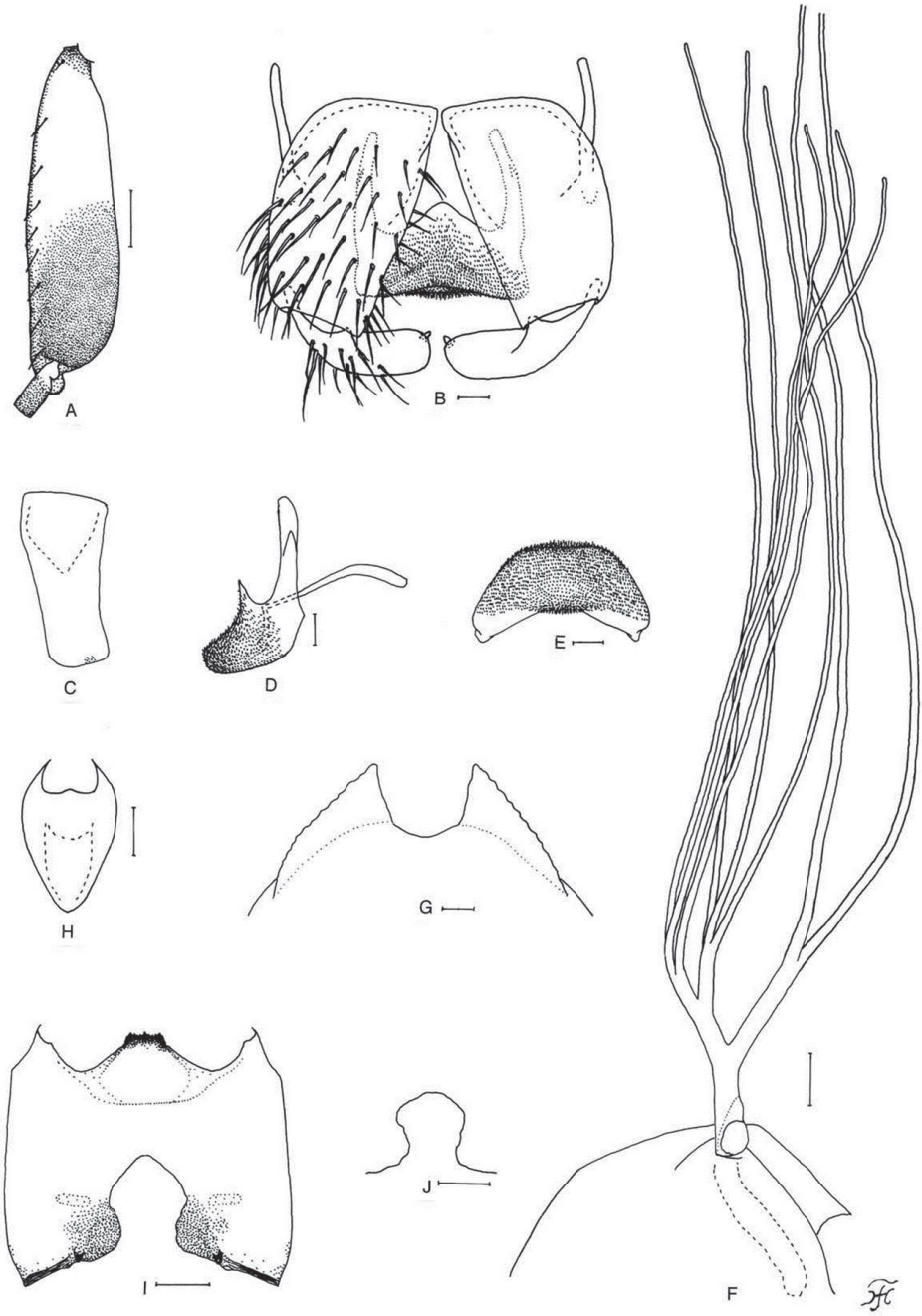

Figure 10. Male, pupa and larva of *S. phapeungense* sp. nov. **A–E** male **F–H** pupa **I, J** larva **A** hind basitarsus and second tarsomere (left side; lateral view) **B** coxites, styles and ventral plate (ventral view) **C** style (right side; ventrolateral view) **D** ventral plate and median sclerite (lateral view) **E** ventral plate (caudal view) **F** gill filaments (right side; lateral view) **G** terminal hooks (caudal view) **H** cocoon (dorsal view) **I** head capsule showing postgenal cleft (ventral view) **J** postgenal cleft (ventral view). Scale bars: 1.0 mm (**H**); 0.1 mm (**F, I, J**); 0.02 mm (**A–E**); 0.01 mm (**G**).

Genitalia. Style in ventral view (Fig. 10B) bent inward, with round apex. Ventral plate in ventral view (Fig. 10B) with basal arms nearly parallel-sided, then convergent apically; ventral plate in caudal view (Fig. 10E) with ventral margin nearly straight or slightly concave. Cercus with 12 or 13 hairs.

Pupa (*N* = 2). Body length 2.5 mm.

Head. Integument yellow.

Thorax. Integument yellow, moderately covered with round tubercles except dorsal surface of posterior two-thirds sparsely covered with tubercles and dorsolateral surfaces of posterior two-thirds almost bare. Gill (Fig. 10F) composed of eight slender thread-like filaments, arranged as (3+3)+2 from dorsal to ventral; common basal stalk 0.7–0.8 times length of interspiracular trunk; dorsal and middle triplets each composed of three individual filaments arising at same level; stalk of ventral pair of filaments variable in length, 0.9–1.3 times length of common basal stalk, and 0.7–1.0 times length of interspiracular trunk; primary stalk of dorsal triplet lying against that of lower pair at angle of 50–60° when viewed laterally; filaments of dorsal triplet subequal in length (1.9–2.0 mm) and thickness to one another; filaments of middle triplet subequal in length (2.0–2.2 mm) and thickness to one another; two filaments of ventral pair, of which apical tips were lost, probably ca. 2.5 mm long and 1.3–1.8 times as thick as six other filaments of dorsal and middle triplets when compared basally.

Abdomen. Dorsally, all segments light yellowish except bases of spine-combs of segments 6–8 yellow; segments 1 and 2 without minute tubercles; segment 9 with pair of wide flat terminal hooks (Fig. 10G), of which outer margin 2.5–2.6 times length of inner margin and crenulated when viewed caudally.

Cocoon (Fig. 10H). Pale yellow or medium brown, slipper-shaped, roughly or moderately woven, somewhat extended ventrolaterally; anterior margin thickly woven medially, often with small bulge; individual threads visible; 2.8–3.3 mm long by 2.0 mm wide.

Mature larva (*N* = 4). Body length 4.8–5.2 mm. Body creamy white to light ochreous with following color markings: thoracic segment 1 encircled with distinct grey band (though disconnected ventromedially), thoracic segments 2 and 3 grey on ventral surface; abdominal segments 1, 3, 4, and 5 light grey, abdominal segment 4 with reddish brown small spots dorsolaterally in one larva, abdominal segments 5 and 6 each with distinct reddish brown, W-shaped, transverse band dorsally (though that on abdominal segment 6 partially faded, leaving one round dorsomedial spot and two lateral spots of various size and shape), dorsal and dorsolateral surface of abdominal segments 7 and 8 faintly greyish partially, overlaid by reddish brown pigment (Fig. 25N).

Head. Head capsule yellow except narrow portion along posterior margin moderately darkened connected to dark posterolateral spots; head spots faintly to moderately positive. Antenna: proportional lengths of first, second, and third articles 1.0:0.8:0.8. Labral fan with 30–33 primary rays. Hypostoma: lateral margin with four to six hypostomal bristles per side. Postgenal cleft (Fig. 10I, J) rounded (in three larvae) or somewhat pointed (in two larvae), 1.0–2.0 times length of postgenal bridge.

Abdomen. Rectal organ compound, each of three lobes with 11–14 finger-like secondary lobules. Posterior circlet with 85–87 rows of hooklets with up to 14 or 15 hooklets per row.

Female. Unknown.

Etymology. The species name, *phapeungense*, refers to the name of the village, Pha Peung, where this species was collected.

Distribution. Thailand (Mae Hong Son and Nan).

Discussion. *Simulium phapeungense* sp. nov. is similar to *S. loeiense* sp. nov. described above by having the small number of brown upper-eye (large) facets in eleven vertical columns and 13 horizontal rows and a widened hind basitarsus (Fig. 10A) 1.2 times as wide as the hind femur. However, this new species is readily distinguished from the latter species by the dorsum of pupal abdominal segments 1 and 2 without minute tubercles and larval abdominal segments 1, 3, 4, and 5 light grey (Fig. 25N).

***Simulium* (*Gomphostilbia*) *nanthaburiense* Takaoka, Srisuka & Fukuda, sp. nov.**

http://zoobank.org/BDE6F4E0-EAE2-4806-AA76-016012E171A0

Fig. 11

Material examined. *Holotype*: Male (with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium nanthaburiense* male, QSBG col. no. 60, Thailand, 25-VII-2017, by W. Srisuka", collected from a stream (width 80 cm, depth 2.5 cm, moderate flow, pH 7.2, 20.1 °C, partially shaded, elevation 1,157 m, 19°11'10.3"N, 101°04'41.7"E), Nam Dan Village, Pua District, Nan Province, Thailand, 25-VII-2017, by W. Srisuka (Coll. No. 60).

***Paratypes*:** One female (thorax for DNA analysis) and 10 males (thorax of one male for DNA analysis) (with their associated pupal exuviae) (in 80% ethanol), same date and data as the holotype; one female (thorax for DNA analysis) (with their associated pupal exuviae) (in 80% ethanol) collected from a stream (width 120 cm, depth 10 cm, bed sandy, moderate flow, pH 6.63, 17.1 °C, partially shaded, elevation 1,154 m, 19°03'36.8"N, 99°19'15.7"E), Huai Mor Nuea Village, Doi Saket, Chiang Mai Province, northern Thailand, 2-II-2019, by W. Srisuka and A. Saeung (Coll. No. 49).

Diagnosis. Female: mandible with two distinct teeth on the outer margin (11A). Male: small number of upper-eye facets in 12 vertical columns and 13 or 14 horizontal rows.

Description. Female (*N* = 2). Body length 2.0 mm.

Head. Frontal ratio 1.7:1.0:2.1; frons:head ratio 1.0:4.3. Labrum 0.64 times length of clypeus. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.1:2.4; sensory vesicle 0.26–0.29 times length of third palpal segment. Maxillary lacinia with ten or eleven inner and 13 or 14 outer teeth. Mandible (Fig. 11A) with 22 inner teeth and two outer teeth at some distance from tip.

Legs. Foreleg: coxa and trochanter whitish yellow; femur dark yellow to light brown with apical cap medium brown (though extreme tip yellowish); tibia yellowish white except little less than apical three-tenths brownish black; basitarsus moderately dilated, 6.0 times as long as its greatest width. Midleg: tarsus dark brown to brownish black though basal half or little less of basitarsus dark yellow (its border not well defined). Hind leg: coxa light brown with apical one-third yellow; tibia yellowish white

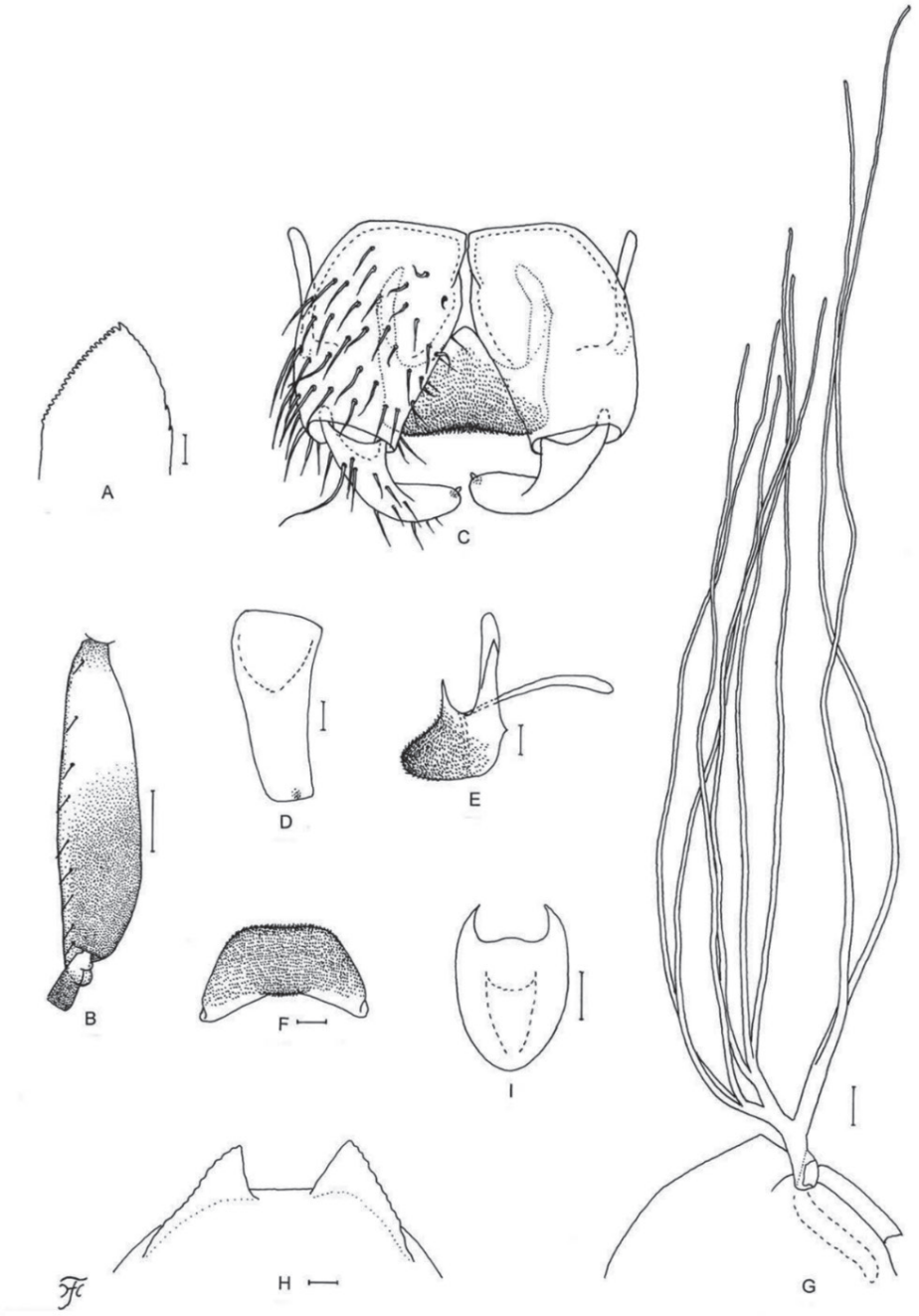

Figure 11. Female, male and pupa of *S. nanthaburiense* sp. nov. **A** female **B–F** male **G–I** pupa. **A** mandible (right side) **B** hind basitarsus and second tarsomere (left side; lateral view) **C** coxites, styles and ventral plate (ventral view) **D** style (right side; ventrolateral view) **E** ventral plate and median sclerite (lateral view) **F** ventral plate (caudal view) **G** gill filaments (right side; lateral view) **H** terminal hooks (caudal view) **I** cocoon (dorsal view). Scale bars: 1.0 mm (**I**); 0.1 mm (**B, G**); 0.02 mm (**C–F**); 0.01 mm (**A, H**).

on basal two-thirds and brownish black on rest; basitarsus 6.2 times as long as wide, and 0.65 and 0.53 times as wide as greatest widths of tibia and femur, respectively; calcipala slightly longer than width at base, and 0.56 times as wide as greatest width of basitarsus; claw with large basal tooth 0.45 times length of claw.

Wing. Length 2.0 mm.

Abdomen. Dorsal surface of abdomen medium to dark brown except most of segment 2 ochreous.

Terminalia. Sternite 8 with 22 or 23 medium-long to long hairs together with three to six slender short hairs on each side. Paraproct in ventral view with three or four sensilla on anteromedial surface; paraproct in lateral view 0.6 times as long as wide, with 27–29 medium-long to long hairs on ventral and lateral surfaces. Cercus in lateral view 0.5 times as long as wide. Spermatheca 1.6 times as long as its greatest width.

Male (*N* = 11). Body length 2.0–2.3 mm.

Head. Somewhat wider than thorax. Upper eye dark brown, consisting of large facets in 12 vertical columns and 13 or 14 horizontal rows. Antenna light to medium brown except scape, pedicel and base of first flagellomere yellowish white; first flagellomere elongate, 1.8 times length of second. Maxillary palp light brown, with five palpal segments, proportional lengths of third, fourth, and fifth palpal segments 1.0:1.1.2:2.3; sensory vesicle 0.19 times length of third palpal segment.

Legs. Foreleg: trochanter yellow to dark yellow; tibia whitish except apical three-tenths dark brown and subbasal portion dark yellow to light brown (though outer surface whitish); basitarsus moderately dilated, 6.8–7.4 times as long as its greatest width. Midleg: tarsus dark brown except basal one-fourth to one-third of basitarsus dark yellow to light brown (border not well defined). Hind leg: coxa light brown; tarsus (Fig. 11B) brownish black except basal half to two-fifths of basitarsus and basal one-third of second tarsomere yellow; basitarsus (Fig. 11B) 3.8–4.1 times as long as wide, and 0.9 and 1.0 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 11B) slightly shorter than basal width, and 0.33 times as wide as greatest width of basitarsus.

Wing. Length 1.9–2.0 mm. Subcosta with 1–10 hairs, though no hair in two males.

Genitalia. Coxite in ventral view (Fig. 11C) 1.6 times as long as its greatest width. Style in ventral view (Fig. 11C) with round apex. Ventral plate in caudal view (Fig. 11F) with ventral margin nearly straight. Cercus small, rounded, with 12 or 13 hairs.

Pupa (*N* = 13). Body length 2.5–2.7 mm.

Head. Integument yellow. Thorax. Integument yellow, moderately covered with round tubercles except dorsolateral surface of posterior half sparsely covered with tubercles. Gill (Fig. 11G) composed of eight slender thread-like filaments, arranged as (3+3)+2 or [3+(1+2)]+2 or [(2+1)+(1+2)]+2 or [(2+1)+3]+2) from dorsal to ventral; common basal stalk 0.6–0.8 times length of interspiracular trunk; stalk of ventral pair of filaments short, 0.6–0.9 times length of common basal stalk, and 0.4–0.7 times length of interspiracular trunk; primary stalk of dorsal triplet lying against that of lower pair at angle of 70–90° when viewed laterally; filaments of dorsal triplet subequal

in length (1.9–2.0 mm) and thickness to one another; filaments of middle triplet subequal in length (2.0–2.3 mm) and thickness to one another; two filaments of ventral pair subequal in length (2.9–3.0 mm) and thickness to each other and 1.5–1.7 times as thick as six other filaments of dorsal and middle triplets when compared basally; all filaments light brown (rarely dark brown).

Abdomen. Dorsally, all segments light yellowish; segments 1 and 2 without minute tubercles; segment 9 with pair of wide flat terminal hooks (Fig. 11H), of which outer margin 1.9–2.1 times length of inner margin and crenulated when viewed caudally.

Cocoon (Fig. 11I). Pale yellow, slipper-shaped, moderately woven, moderately extended ventrolaterally; anterior margin thickly woven medially, often with bulge; individual threads visible or not; 3.0–3.3 mm long by 1.8–2.5 mm wide.

Mature larva. Unknown.

Etymology. The species name, *nanthaburiense*, refers to the historical name of Nan Province, Nanthaburi, where this species was collected.

Distribution. Thailand (Nan).

Discussion. This new species is similar to *S. brinchangense* described from Peninsular Malaysia (Takaoka et al. 2014b), in having a similar number of male upper-eye facets, but is distinguished by the relative length of the stalk of the ventral pair of filaments against the common basal stalk, which is 0.6–0.9 in this new species but 1.1–1.2 in *S. brinchangense*, and relative length of the outer margin against the inner margin of the pupal terminal hooks, which is 1.9–2.1 in this new species but 3.0–3.6 in *S. brinchangense*.

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

http://zoobank.org/2DE34247-A149-4F4D-BF0C-AD6A8EBC6D64
Figs 12, 25J

Material examined. ***Holotype*****:** Male (with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium nanoiense* male, QSBG col. no. 66, Thailand, 5-VIII-2017, by W. Srisuka", collected from a stream (width 1 m, depth 3 cm, bed sandy, moderate flow, pH 6.2, 19.6 °C, partially shaded, elevation 1,349 m, 18°16'40.4"N, 100°30'19.0"E), Khun Sathan Village, Na Noi District, Nan Province, Thailand, 5-VIII-2017, by W. Srisuka (Coll. No. 66).

Paratypes**:** One female, nine males (thoraces of two males for DNA analysis) (with their associated pupal exuviae and cocoons), and 10 mature larvae (one mature larva for DNA analysis) (in 80% ethanol), same data as for holotype.

Diagnosis. Female: small sensory vesicle 0.22–0.24 times as long as the third palpal segment (Fig. 12A) and mandible with two teeth on the outer margin (Fig. 12B). Male: medium number of upper-eye (large) facets in 12 or 13 vertical columns and 14 (rarely 13) horizontal rows. Larva: postgenal cleft as long as or little longer than the postgenal bridge (Fig. 12K) and abdominal segments 1–3, 7, and 8 greyish (Fig. 25J).

Description. Female (*N* = 1). Body length 2.0 mm.

Head. Frontal ratio 1.8:1.0:2.7; frons:head ratio 1.0:5.0. Labrum 0.66 times length of clypeus. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.1:2.4; sensory vesicle (Fig. 12A) small, ellipsoidal (0.22–0.24 times length of third palpal segment). Lacinia with ten or eleven inner and 13 outer teeth. Mandible (Fig. 12B) with 21 inner teeth and two outer teeth at some distance from tip.

Legs. Foreleg: tibia yellowish white except apical three-tenths brownish black; basitarsus moderately dilated, 7.1 times as long as its greatest width. Hind leg: coxa light brown; tibia yellowish white on little more than basal half and light brown to brownish black on rest; tarsus brownish black except basal three-tenths (though base light brown) and basal half of second tarsomere yellowish white; basitarsus 6.2 times as long as wide, and 0.7 and 0.6 times as wide as greatest widths of tibia and femur, respectively; calcipala nearly as long as width at base, and 0.6 times as wide as greatest width of basitarsus.

Wing. Length 2.2 mm.

Abdomen. Basal scale ochreous, with fringe of whitish yellow hairs. Dorsal surface of abdomen medium to dark brown except anterior four-fifths of segment 2 ochreous.

Terminalia. Sternite 8 bare medially, with 27 or 28 medium-long to long hairs together with two to four slender short hairs on each side. Ovipositor valves each moderately covered with microsetae interspersed with two short hairs. Paraproct in ventral view with four or five sensilla on anteromedial surface; paraproct in lateral view 0.6 times as long as wide, with 19–21 medium-long to long hairs on ventral and lateral surfaces. Cercus in lateral view 0.5 times as long as wide. Spermatheca 1.5 times as long as its greatest width.

Male (N = 10). Body length 2.1–2.3 mm.

Head. Slightly wider than thorax. Upper eye medium brown, consisting of large facets in 12 or 13 vertical columns and 14 (rarely 13) horizontal rows. Antenna: first flagellomere elongate, 1.7 times length of second. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.3:2.6; sensory vesicle 0.14–0.17 times length of third palpal segment.

Legs. Foreleg: tibia whitish yellow except little more than apical one-third dark brown, and small subbasal area on inner and lateral surfaces light brown; basitarsus somewhat dilated, 7.0–7.1 times as long as its greatest width. Midleg: tarsus dark brown except basal one-fourth to one-third of basitarsus dark yellow (border not well defined). Hind leg: coxa light brown; femur light to medium brown with base yellow and apical cap brownish black (though apical tip yellow); tibia dark brown to brownish black except basal half or little less whitish yellow; tarsus (Fig. 12C) brownish black except basal two-fifths of basitarsus and basal one-third of second tarsomere whitish yellow; basitarsus (Fig. 12C) 3.8–4.0 times as long as wide, and 0.9–1.0 and 1.1 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 12C) slightly shorter than basal width, and 0.32 times as wide as greatest width of basitarsus.

Wing. Length 1.9–2.0 mm. Subcosta bare in three males but with one to seven hairs in six males.

Genitalia. Style in ventrolateral view (Fig. 12E) slightly tapered from base to middle, then nearly parallel-sided toward apex, with truncated apex, and 0.8 times as long

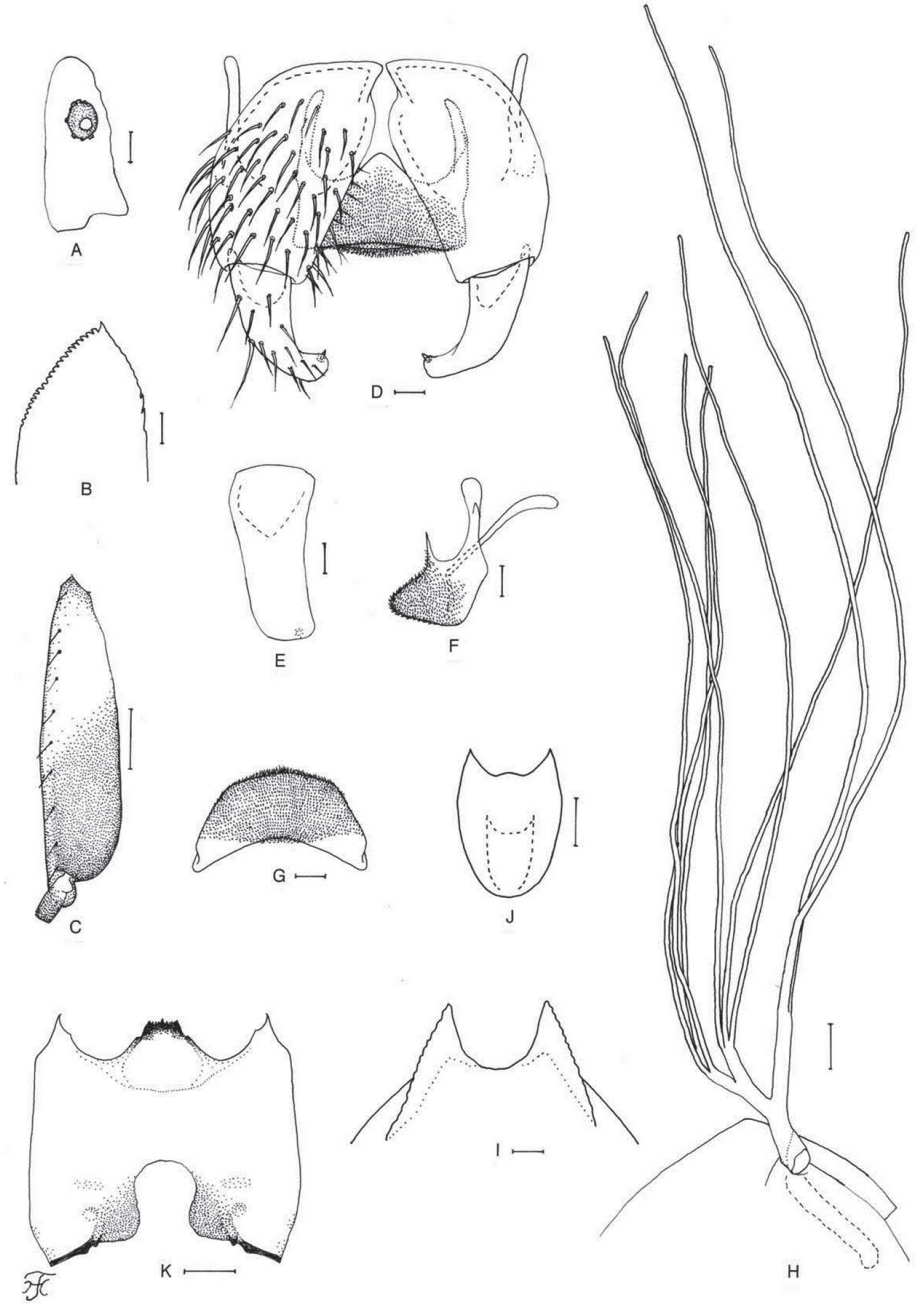

Figure 12. Female, male, pupa and larva of *S. nanoiense* sp. nov. **A, B** female **C–G** male **H–J** pupa **K** larva. **A** sensory vesicle (right side; anterior view) **B** mandible (right side) **C** hind basitarsus and second tarsomere (left side; lateral view) **D** coxites, styles and ventral plate (ventral view) **E** style (right side; ventrolateral view) **F** ventral plate and median sclerite (lateral view) **G** ventral plate (caudal view) **H** gill filaments (right side; lateral view) **I** terminal hooks (caudal view) **J** cocoon (dorsal view) **K** head capsule (ventral view). Scale bars: 1.0 mm (**J**); 0.1 mm (**C, H, K**); 0.02 mm (**A, D–G**); 0.01 mm(**B, I**).

as coxite. Ventral plate in ventral view (Fig. 12D) with basal arms slightly convergent apically; ventral plate in caudal view (Fig. 12G) rounded ventrally. Cercus small, rounded, with 12 or 13 hairs.

Pupa (*N* = 11). Body length 2.4–2.6 mm.

Head. Integument yellow. Thorax. Integument yellow, moderately covered with round tubercles except dorsal and dorsolateral surfaces of posterior half almost bare or sparsely covered with tubercles, though small dorsal area near posterior margin sparsely to moderately covered with tubercles.

Thorax. Gill (Fig. 12H) composed of eight slender thread-like filaments, arranged as (3+3)+2 or [(2+1)+3]+2) or [3+(1+2)]+2 from dorsal to ventral; common basal stalk 0.7–0.8 times length of interspiracular trunk; dorsal and middle triplets sharing short stalk, and mostly composed of three individual filaments arising at same level; stalk of ventral pair of filaments variable in length, 0.9–1.1 times length of common basal stalk, and 0.7–0.8 times length of interspiracular trunk; primary stalk of dorsal triplet lying against that of lower pair at angle of 70–80° when viewed laterally; filaments of dorsal triplet subequal in length (1.6–1.9 mm) and thickness to one another; filaments of middle triplets subequal in length (2.0–2.3 mm) and thickness to one another; two filaments of ventral pair subequal in length (2.5–3.0 mm) and thickness to each other and 1.4 times as thick as six other filaments of dorsal and middle triplets when compared basally.

Abdomen. Dorsally, all segments light yellowish; segments 1 and 2 without tubercles; segment 9 with pair of wide flat terminal hooks (Fig. 12I), of which outer margin 2.0–2.1 times length of inner margin and crenulated when viewed caudally.

Cocoon (Fig. 12J). Light yellow, slipper-shaped, roughly to moderately woven, widely extended ventrolaterally; anterior margin not thickly woven medially, without bulge or short projection; individual threads visible or not; 2.9–3.2 mm long by 2.0–2.8 mm wide.

Mature larva (*N* = 9). Body length 5.0–5.6 mm. Body creamy white with following color markings: thoracic segment 1 encircled with dark grey band (though disconnected ventromedially), dorsal surface of thoracic segments 1–3 often light grey except posterior half of segment 3 light ochreous; ventral surface of thoracic segment 2 dark grey; ventral surface of thoracic segment 3 light grey on anterior one-third and light ochreous on posterior two-thirds; abdominal segments 1–3 entirely grey, abdominal segments 7 and 8 light grey dorsally, abdominal segment 4 with purplish transverse band (though often partially faded, leaving narrow band or small spot(s) dorsomedially), abdominal segments 5 and 6 each with distinct, dark purplish, W-shaped, transverse band dorsally (though that on segment 6 often faded partially, leaving one distinct round dorsomedial spot and two dorsolateral spots), dorsal and dorsolateral surfaces of abdominal segments 7 and 8 each covered with dark purplish pigment, though dorsomedial portions usually faded (Fig. 25J).

Head. Head spots faintly or moderately positive. Antenna: proportional lengths of first, second, and third articles 1.0:0.7:0.8. Labral fan with 33–38 primary rays. Postgenal cleft (Fig. 12K) small, rounded, 1.0–1.1 times length of postgenal bridge.

Thorax and ***Abdomen***. Thoracic and abdominal cuticle sparsely covered with unpigmented minute setae dorsally except last abdominal segment densely covered with unbranched colorless minute setae on dorsolateral and lateral surfaces of each side of anal sclerite and on each lateral surface even down to base of ventral papilla. Rectal organ compound, each of three lobes with ten or eleven finger-like secondary lobules. Anal sclerite with anterior arms slightly longer than posterior ones. Posterior circlet with 85–88 rows of hooklets with up to 14 hooklets per row.

Etymology. The species name, *nanoiense*, refers to the district, Na Noi, where this species was collected.

Distribution. Thailand (Nan).

Discussion. This new species is similar to *S. brinchangense* described from Peninsular Malaysia (Takaoka et al. 2014b), in having a similar number of male upper-eye facets but is distinguished by the male ventral plate with its ventral margin rounded (Fig. 12G) when viewed posteriorly (ventral margin is nearly straight or slightly concave in *S. brinchangense*).

This new species is similar in larval body color to *S. junkumae* sp. nov. but is distinguished from the latter species by the dorsal and dorsolateral surfaces of larval abdominal segments 7 and 8 light grey (Fig. 25J).

***Simulium* (*Gomphostilbia*) *muangpanense* Takaoka, Srisuka & Fukuda, sp. nov.**
http://zoobank.org/637B60FF-279B-4E5C-988D-BDDB11C4A469
Figs 13, 25O

Material examined. *Holotype*: Male (with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium muangpanense* male, QSBG col. no. 86, Thailand, 9-VIII-2016, by W. Srisuka", collected from a stream (width 30 cm, depth 5 cm, bed sandy, moderate running, pH 6.3, 22.6 °C, exposed to the sun, elevation 1,097 m, 18°50'03.7"N, 99°22'32.2"E), at Pa Meing Village, Muang Pan District, Lampang Province, northern Thailand, 9-VIII-2016, by W. Srisuka (Coll. No. 86).

***Paratypes*:** Three females (thorax of one female for DNA analysis), four males (thorax of one male for DNA analysis) (with their associated pupal exuviae and cocoons), and five mature larvae (one mature larva for DNA analysis) (in 80% ethanol), same data as for holotype.

Diagnosis. Female: mandible with two or three outer teeth (Fig. 13A). Male: number of upper-eye facets in 13 vertical columns and 14 horizontal rows, hind basitarsus (Fig. 13B) 0.9 times as wide as the hind femur. Larva: abdominal segments 1, 4, 5, 7, and 8 grey at least on dorsal and dorsolateral surface (Fig. 25O).

Description. Female (*N* = 3). Body length 2.0–2.1 mm.

Head. Frontal ratio 1.8:1.0:2.1; frons:head ratio 1.0:4.0. Labrum 0.61 times length of clypeus. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.0:2.5; sensory vesicle medium sized, ellipsoidal (0.29–0.31 times length of third palpal segment), with small opening. Lacinia with nine or ten inner and

14 outer teeth. Mandible (Fig. 13A) with 20 inner teeth and two or three outer teeth at some distance from tip.

Legs. Foreleg: basitarsus moderately dilated, 6.4 times as long as its greatest width. Midleg: femur light brown with basal one-fourth whitish yellow and apical cap medium brown (though extreme tip yellowish); tarsus dark brown to brownish black though little less than basal half of basitarsus yellow (its border not well defined). Hind leg: coxa light brown with apical one-third yellowish white; tibia white to yellowish white on basal two-thirds and light brown to brownish black on rest; basitarsus 6.04 times as long as wide, and 0.76 and 0.58 times as wide as greatest widths of tibia and femur, respectively; calcipala nearly as long as or lightly longer than width at base, and 0.5–0.6 times as wide as greatest width of basitarsus.

Wing. Length 2.0 mm.

Terminalia. Sternite 8 with 18–21 medium-long to long hairs together with four slender short hairs on each side. Ovipositor valve moderately covered with microsetae interspersed with two or three short hairs. Paraproct in ventral view with four or five or six sensilla on anteromedial surface; paraproct in lateral view 0.6 times as long as wide, with 24–28 medium-long to long hairs on ventral and lateral surfaces. Cercus in lateral view 0.46 times as long as wide.

Male (*N* = 5). Body length 2.1–2.5 mm.

Head. Slightly wider than thorax. Upper eye dark brown, consisting of large facets in 13 vertical columns and 14 horizontal rows on each side. Antenna: first flagellomere 1.5–1.7 times length of second. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments1.0:1.2:2.9–3.1; sensory vesicle globular or ellipsoidal, 0.15–0.18 times length of third palpal segment.

Legs. Foreleg: femur light brown except apical cap medium brown (though apical tip yellowish); tibia light brown on basal one-third (though basal tip yellow and outer surface narrowly yellowish white), yellowish white on middle one-third, and brownish black on apical one-third; basitarsus moderately dilated, 6.7–7.3 times as long as its greatest width. Midleg: femur light brown with base yellowish on inner surface and apical cap medium brown (though apical tip yellowish); tibia medium brown except basal one-third (or little more on posterior surface) yellowish white; tarsus dark brown except base of basitarsus dark yellow to light brown (border not well defined). Hind leg: coxa light brown with apical one-third yellowish; femur light to medium brown with base whitish yellow and apical cap brownish black (though apical tip yellow); tibia dark brown to brownish black except basal half or little less whitish yellow; tarsus dark brown except basal two-fifths of basitarsus and little less than basal half of second tarsomere whitish yellow; basitarsus (Fig. 13B) 4.0–4.2 times as long as wide, and 0.9 and 0.9 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 13B) slightly shorter than basal width, and 0.33 times as wide as greatest width of basitarsus.

Wing. Length 1.6–1.9 mm. Other characters as in female except subcosta with 0–6 hairs.

Genitalia. Coxite in ventral view (Fig. 13C) 1.9 times as long as its greatest width. Style in ventral view (Fig. 13D) with round apex, and in ventrolateral view (Fig. 13D) slightly tapered toward apex or nearly parallel-sided from middle to apex, and 0.8 times

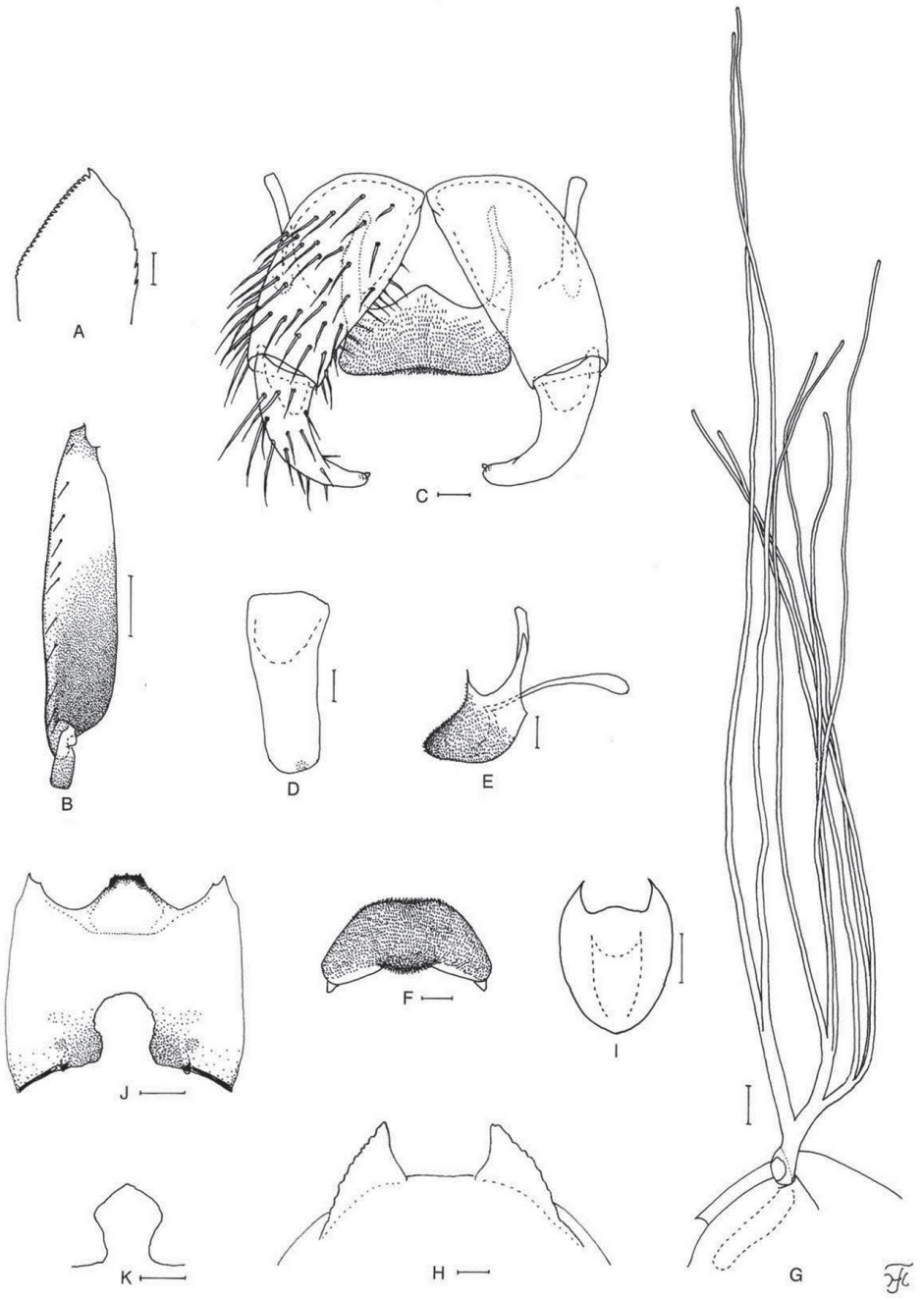

Figure 13. Female, male, pupa and larva of *S. muangpanense* sp. nov. **A** female **B–F** male **G–I** pupa **J, K** larva. **A** mandible (right side) **B** hind basitarsus and second tarsomere (left side; lateral view) **C** coxites, styles and ventral plate (ventral view) **D** style (right side; ventrolateral view) **E** ventral plate and median sclerite (lateral view) **F** ventral plate (caudal view) **G** gill filaments (left side; lateral view) **H** terminal hooks (caudal view) **I** cocoon (dorsal view) **J** head capsule (ventral view) **K** postgenal cleft. Scale bars: 1.0 mm (**I**); 0.1 mm (**B, G, J, K**); 0.02 mm (**C–F**); 0.01 mm (**A, H**).

length of coxite. Ventral plate in ventral view (Fig. 13C) with basal arms nearly parallel-sided, then convergent apically; ventral plate in caudal view (Fig. 13F) rounded ventrally, though ventral margin slightly concave medially. Ventral surface of abdominal segment 10 moderately sclerotized along anterior margin. Cercus with 13–16 hairs.

Pupa (*N* = 8). Body length 2.3–2.6 mm.

Thorax. Gill (Fig. 13G) composed of eight slender thread-like filaments, arranged as [(2+1)+(1+2)]+2 or [3+(1+2)]+2 from dorsal to ventral; common basal stalk 0.6–0.8 times length of interspiracular trunk; stalk of ventral pair of filaments variable in length, 1.0–1.5 times length of common basal stalk, and 0.7–1.0 times length of interspiracular trunk; filaments of dorsal triplet subequal in length to one another (2.0–2.2 mm) and those of middle triplet subequal in length to one another (2.4–2.5); two filaments of ventral pair subequal in length to each other (3.0–3.2 mm) and thickness to each other and 1.6 times as thick as six other filaments of dorsal and middle triplets when compared basally.

Abdomen. Dorsally, all segments pale yellowish; segments 1 and 2 almost bare or sparsely covered with minute tubercles; segment 9 with pair of flat terminal hooks (Fig. 13H), of which outer margin 2.8–3.1 times length of inner margin and crenulated when viewed caudally.

Cocoon (Fig. 13I). Slipper-shaped, moderately woven, moderately extended ventrolaterally; anterior margin thickly woven medially, rarely with bulge; 3.0–3.4 mm long by 2.0–2.5 mm wide.

Mature larva (*N* = 4). Body length 4.8–5.0 mm. Body ochreous with following color markings: thoracic segment 1 encircled with grey band (though disconnected ventromedially), thoracic segments 2 and 3 grey on ventral surface (though often faded on segment 3); abdominal segments 1 and 4 entirely grey, abdominal segments 5, 7, and 8 grey on dorsal and dorsolateral surface; abdominal segment 4 with faint narrow reddish brown transverse band on dorsal and dorsolateral surface in one larva; abdominal segment 5 with distinct reddish brown, W-shaped, transverse band dorsally, abdominal segment 6 often with three reddish brown spots (one round dorsomedial spot and two lateral spots of various size and shape), and abdominal segments 7 and 8 distinctly covered with reddish brown pigment usually on dorsolateral surface (Fig. 25O).

Head. Head capsule yellow with narrow area along posterior margin darkened; head spots faintly or moderately positive. Antenna: proportional lengths of first, second, and third articles 1.0:0.7–0.8:0.8–0.9. Labral fan with 31 or 32 primary rays. Hypostoma with five hypostomal bristles per side lying nearly parallel to lateral margin. Postgenal cleft (Fig. 13J, K) medium-long, 1.2–2.1 times length of postgenal bridge, usually with apical margin round (though angulated in one larva).

Thorax and ***Abdomen.*** Thoracic and abdominal cuticle almost bare except abdominal segments 5–8 moderately covered with unbranched dark minute setae (though setae rarely bifid) on dorsal and dorsolateral surface. Rectal organ compound, each of three lobes with seven to nine finger-like secondary lobules. Anal sclerite with anterior arms 1.1–1.2 times as long as posterior ones. Posterior circlet with 76–80 rows of hooklets with up to 14 or 15 hooklets per row.

Etymology. The species name, *muangpanense*, refers to the district, Muang Pan, where this species was collected.

Distribution. Thailand (Lampang).

Discussion. The male of this new species is similar to *S. phulocense* from Vietnam (Takaoka et al. 2015) by having the same number of upper-eye (large) facets, but is distinguished in the female by the fore basitarsus 5.8 times as long as its greatest width (6.9 times in *S. phulocense*), in the male by the fore basitarsus 6.7–7.3 times as long as its greatest width (8.3 times in *S. phulocense*), and ventral plate trapezoidal ventrally when viewed posteriorly (rounded ventrally in *S. phulocense*) and in the pupa by the cocoon without an anterodorsal projection (with an anterodorsal projection in *S. phulocense*).

Simulium tanahrataense from Peninsular Malaysia (Takaoka et al. 2014b) and *S. confertum* Takaoka & Sofian-Azirun from Vietnam (Takaoka et al. 2015) have a similar number of male upper-eye facets (in 14 vertical columns and 15 horizontal rows) and a similar shape of the ventral plate (trapezoidal in caudal view), but are barely distinguished from this new species by the male hind basitarsi as wide as the hind femora (0.9 times as wide as the hind femur in this new species) and the length of the ventral paired filaments of the pupa 2.6–2.7 mm (3.0–3.2 mm in this new species).

The larva of this new species is almost the same in body color pattern as *S. monglaense* described from Myanmar (Takaoka et al. 2017b) but is barely distinguished from the latter species by the posterior circlet with 76–80 rows (85–93 rows in *S. monglaense*). This new species is distinguished from *S. monglaense* in the female by the mandible with distinct outer teeth and in the male by the relative length of the fore basitarsus against its greatest width (6.7–7.3 in this new species versus 8.0 in in *S. monglaense*).

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

http://zoobank.org/BD4BF904-907D-460F-9276-168D189BC582

Figs 14, 25P

Material examined. *Holotype*: Male (with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium maehongsonense* male, QSBG col. no. 107, Thailand, 12-VII-2017, by W. Srisuka", collected from a stream (width 120 cm, depth 2 cm, bed sandy, fast flow, pH 7.6, 21.2 °C, partially shade, elevation 1,034 m, 19°36'58.5"N, 97°59'48.2"E), at Pha Peung, Muang District, Mae Hong Son Province, Thailand, 12-VII-2017, by W. Srisuka (Coll. No. 107).

***Paratypes*:** Two females (thorax of one female for DNA analysis), seven males (thorax of one male for DNA analysis) (with their associated pupal exuviae and cocoons), and six mature larvae (one mature larva for DNA analysis) (in 80% ethanol), same data as for holotype.

Diagnosis. Female: short sensory vesicle and mandible lacking distinct outer teeth (Fig. 14A). Male: number of upper-eye facets in 13 vertical columns and 14 horizontal rows. Pupa: relatively shorter gill filaments (longest 2.1–2.3 mm long) (Fig. 14G) and

relatively wider terminal hooks (Fig. 14H). Larva: postgenal cleft 1.4–2.2 times as long as the postgenal bridge (Fig. 14J) and abdominal segment 1 light grey (Fig. 25P).

Description. Female (*N* = 2). Body length 1.9 mm.

Head. Frontal ratio 1.9:1.0:2.1; frons:head ratio 1.0:4.2. Labrum 0.52 times length of clypeus. Maxillary palpus: proportional length of third, fourth and fifth palpal segments 1:1.1:2.2; sensory vesicle medium-long (0.26 times length of third palpal segment. Lacinia with 9–11 inner and 11–13 outer teeth. Mandible (Fig. 14A) with 23 inner teeth and lacking distinct outer teeth (though three vestigial teeth present at some distance from tip).

Legs. Foreleg: tibia yellowish white except apical three-tenths brownish black; basitarsus moderately dilated, 5.5 times as long as its greatest width. Midleg: tarsus dark brown though basal half of basitarsus yellow (its border not well defined). Hind leg: coxa light brown; tibia yellowish white on basal two-thirds and light brown to brownish black on rest; basitarsus 6.7 times as long as wide, and 0.6 and 0.5 times as wide as greatest widths of tibia and femur, respectively; calcipala 0.5 times as wide as greatest width of basitarsus; claw with large basal tooth 0.46 times length of claw.

Wing. Length 1.9 mm.

Abdomen. Dorsal surface of abdomen medium to dark brown except most of segment 2 ochreous (though narrow portion along posterior margin somewhat darkened).

Terminalia. Sternite 8 bare medially, with 20–22 medium-long to long hairs together with two or three slender short hairs on each side. Ovipositor valves each moderately covered with microsetae interspersed with two or three short hairs. Paraproct in lateral view 0.6 times as long as wide, with 18 medium-long to long hairs on ventral and lateral surfaces. Spermatheca ellipsoid, 1.3 times as long as its greatest width.

Male (*N* = 8). Body length 2.0–2.4 mm.

Head. Slightly wider than thorax. Upper eye dark brown, consisting of large facets in 13 vertical columns and 14 horizontal rows on each side. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.2:2.8; sensory vesicle 0.17 times length of third palpal segment.

Legs. Foreleg: tibia yellowish white except l apical one-third dark brown, and inner and lateral surfaces of subbasal portion light brown; basitarsus moderately dilated, 6.5–7.3 times as long as its greatest width. Hind leg: coxa light brown; tibia dark brown to brownish black except little less than basal half yellowish white; tarsus dark brown except little less than basal half of basitarsus and basal one-third of second tarsomere yellowish; basitarsus (Fig. 14B) 3.8–3.9 times as long as wide, and 1.0 and 1.0 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 14B) 0.32 times as wide as greatest width of basitarsus.

Wing. Length 1.9–2.0 mm. Subcosta with four to nine hairs in four males but bare in four males.

Genitalia. Coxite in ventral view (Fig. 14C) 1.8 times as long as its greatest width. Style in ventral view (Fig. 14C) with round apex; style in ventrolateral view (Fig. 14D) slightly tapered toward apex, with round apex. Ventral plate in ventral view (Fig. 14C) with basal arms of moderate length, nearly parallel- sided, then convergent apically; ventral plate in caudal view (Fig. 14F) with ventral margin nearly straight or slightly concave. Cercus with 14 hairs.

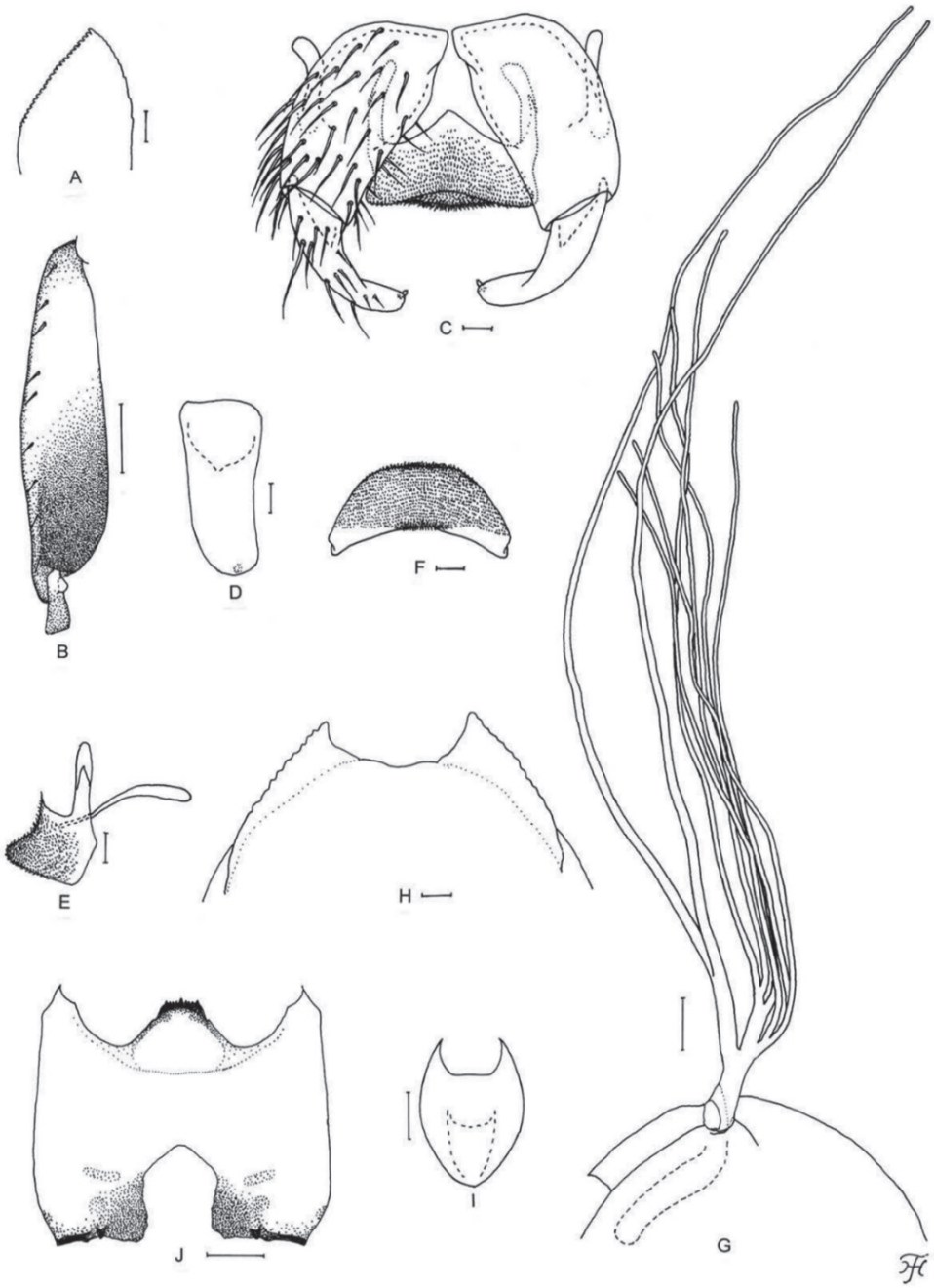

Figure 14. Female, male, pupa and larva of *S. maehongsonense* sp. nov. **A** female **B–F** male **G–I** pupa **J** larva. **A** mandible (right side) **B** hind basitarsus and second tarsomere (left side; lateral view) **C** coxites, styles and ventral plate (ventral view) **D** style (right side; ventrolateral view) **E** ventral plate and median sclerite (lateral view) **F** ventral plate (caudal view) **G** gill filaments (left side; lateral view) **H** terminal hooks (caudal view) **I** cocoon (dorsal view) **J** head capsule (ventral view). Scale bars: 1.0 mm (**I**); 0.1 mm (**B, G, J**); 0.02 mm (**C–F**); 0.01 mm (**A, H**).

Pupa (*N* = 10). Body length 2.5–2.7 mm.

Head. Integument yellow. Thorax. Integument yellow.

Thorax. Gill (Fig. 14G) composed of eight slender thread-like filaments, arranged as (3+3) +2 or [3+(1+2)]+2 from dorsal to ventral; common basal stalk 0.5–0.7 times length of interspiracular trunk; dorsal triplet with extremely short stalk; stalk of ventral pair of filaments 0.9–1.5 times length of common basal stalk, and 0.5–0.8 times length of interspiracular trunk; primary stalk of dorsal triplet lying against that of lower pair at angle of 70° when viewed laterally; filaments of dorsal triplet subequal in length (1.1–1.3 mm) and thickness to one another; filaments of middle triplet subequal in length (1.5–1.7 mm) and thickness to one another; two filaments of ventral pair subequal in length (2.1–2.3 mm) and thickness to each other and 1.6 times as thick as six other filaments of dorsal and middle triplets when compared basally.

Abdomen. Dorsally, all segments light yellowish; segments 1 and 2 without minute tubercles; segment 9 with pair of wide flat terminal hooks (Fig. 14H), of which outer margin 3.3–4.0 times length of inner margin and crenulated when viewed caudally.

Cocoon (Fig. 14I). Pale yellow, slipper-shaped, roughly to moderately woven, moderately extended ventrolaterally; anterior margin thickly woven medially, without bulge or short projection; 3.0–3.5 mm long by 2.0–2.9 mm wide.

Mature larva (*N* = 5). Body length 4.5–5.5 mm. Body light ochreous with following color markings: thoracic segment 1 encircled with distinct greyish band (though disconnected ventromedially), thoracic segment 2 greyish and thoracic segment 3 ochreous on ventral surface; abdominal segment 1 light grey, abdominal segments 5 and 6 each with distinct reddish brown spot of various size and shape dorsomedially, and dorsal and dorsolateral surface of abdominal segments 4–8 faintly to moderately covered with pinkish to reddish brown pigments (Fig. 25P).

Head. Head spots moderately positive. Antenna: proportional lengths of first, second, and third articles 1.0:0.8:0.7–0.9. Labral fan with 38–40 primary rays. Hypostoma with four to six hypostomal bristles per side lying nearly parallel to lateral margin. Postgenal cleft (Fig. 14J) medium sized, 1.4–2.2 times length of postgenal bridge.

Abdomen. Posterior circlet with 88–96 rows of hooklets with up to 14 or 15 hooklets per row.

Etymology. The species name, *maehongsonense*, refers to the province, Mae Hong Son, where this species was collected.

Distribution. Thailand (Mae Hong Son).

Discussion. The male of this new species is similar to *S. phulocense* from Vietnam (Takaoka et al. 2015) by having the same number of upper-eye large facets, but is distinguished by the fore basitarsus 6.5–7.3 times as long as its greatest width (8.3 times in *S. phulocense*), and ventral plate trapezoidal ventrally (Fig. 14F) when viewed posteriorly (rounded ventrally in *S. phulocense*) and in the pupa by the cocoon without an anterodorsal projection (Fig. 14I) (with an anterodorsal projection in in *S. phulocense*).

***Simulium* (*Gomphostilbia*) *chaowaense* Takaoka, Srisuka & Saeung, sp. nov.**
http://zoobank.org/20CF4BE9-8A76-48F6-A1A8-C37CA48336D7
Figs 15, 25G

Material examined. *Holotype*: Male (with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium chaowaense* male, QSBG col. no. 164, Thailand, 16-III-2017, by W. Srisuka", collected from a small stream (width 40 cm, depth 3.5 cm, bed sandy, moderate flow, pH 7.3, 21.9 °C, partially, elevation 582 m, 18°45'30.2"N, 100°20'11.4"E), Chao Wa Waterfall, Song District, Phrae Province, Thailand, 16-III-2017, by W. Srisuka (Coll. No. 164).

***Paratypes*:** Three males (thorax of one male for DNA analysis) (with their associated pupal exuviae and cocoons), and six mature larvae (one mature larva for DNA analysis) (in 80% ethanol), same data as in the holotype

Diagnosis. Male: number of upper-eye facets in 14 or 15 vertical columns and 14 or 15 horizontal rows, and moderately widened hind basitarsus (Fig. 15A) 0.9 times as wide as hind femur. Larva: medium-long postgenal cleft (Fig. 15H) 1.2–1.3 times as long as the postgenal bridge and all abdominal segments greyish (Fig. 25G).

Description. Male (*N* = 4). Body length 2.0–2.3 mm.

Head. Distinctly wider than thorax. Upper eye dark brown, consisting of large facets in 14 or 15 vertical columns and 14 or 15 horizontal rows on each side. Antenna light to medium brown except scape, pedicel, and base of first flagellomere yellow; first flagellomere elongate, 1.8 times length of second. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.0–1.2:2.5–2.8; sensory vesicle globular or ellipsoidal, 0.16–0.17 times length of third palpal segment.

Thorax. Scutum with faint longitudinal vittae (one median and two submedian).

Legs. Foreleg: tibia whitish yellow except basal one-fourth light brown and apical one-third dark brown; basitarsus moderately dilated, 7.3–7.4 times as long as its greatest width. Hind leg: coxa light brown; femur light to medium brown with base yellow and apical cap dark brown (though apical tip yellow); tibia dark brown except little less than basal half yellow; tarsus (Fig. 15A) brownish black except little less than basal half of basitarsus and little less than basal half of second tarsomere whitish yellow; basitarsus (Fig. 15A) 3.8–4.0 times as long as wide, and 0.8–0.9 and 0.9 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 15A) slightly shorter than basal width, and 0.36 times as wide as greatest width of basitarsus.

Wing. Length 1.6–1.7 mm. Subcosta with 2–8 hairs.

Genitalia. Coxite in ventral view (Fig. 15B) nearly rectangular, 1.8 times as long as its greatest width. Style in ventrolateral view slightly tapered toward apex, with truncated apex, and 0.8 times as long as coxite. Ventral plate in ventral view (Fig. 15B) with body transverse, 0.7 times as long as wide, posteroventral margin somewhat concave medially (though slightly convex medially on posterodorsal margin), and lateral margin emarginated medially; basal arms of moderate length, slightly divergent, then convergent apically; ventral plate in caudal view (Fig. 15D) rounded ventrally. Cercus with 15 or 16 hairs.

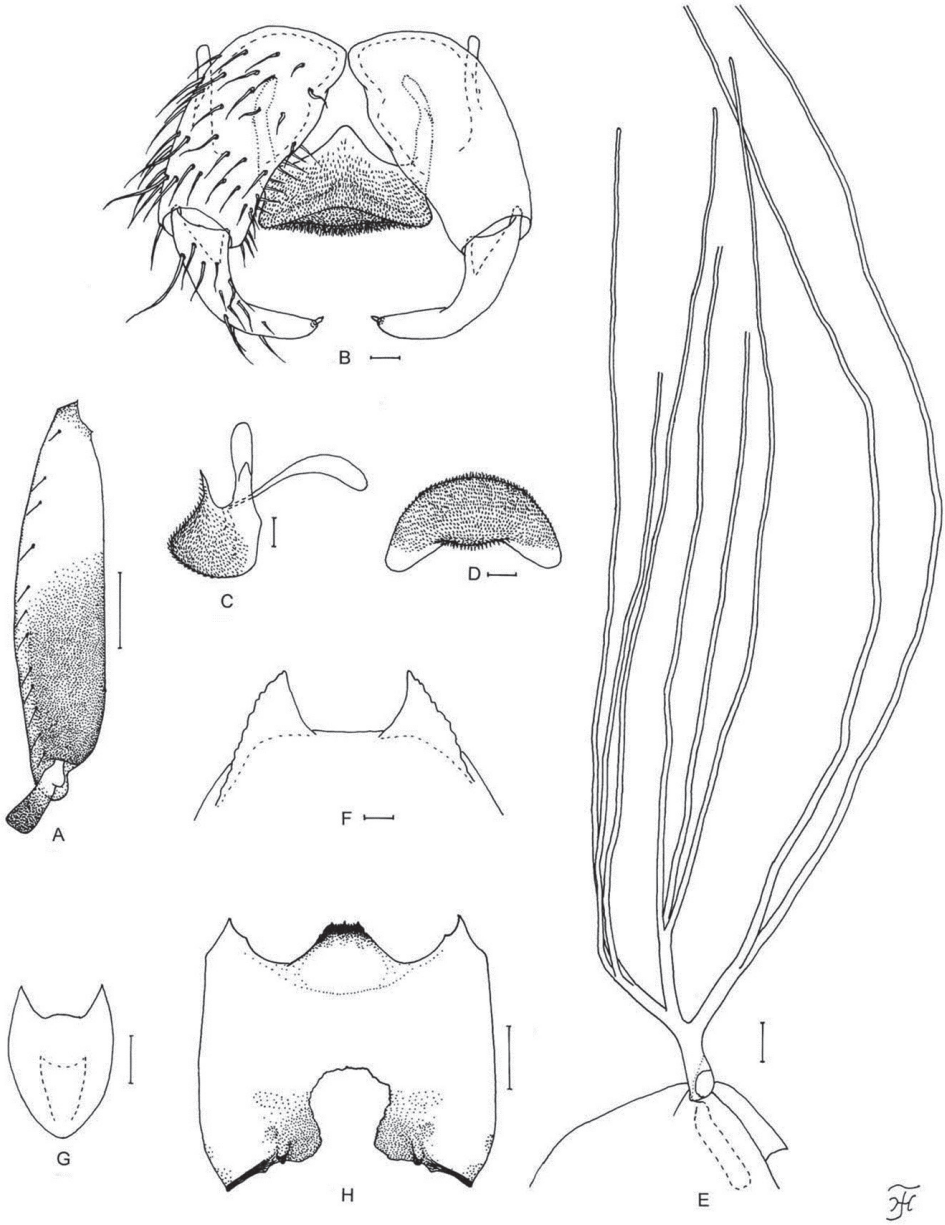

Figure 15. Male, pupa and larva of *S. chaowaense* sp. nov. **A–D** male **E–G** pupa **H** larva. **A** hind basitarsus and second tarsomere (left side; lateral view) **B** coxites, styles and ventral plate (ventral view) **C** ventral plate and median sclerite (lateral view) **D** ventral plate (caudal view) **E** gill filaments (right side; lateral view) **F** terminal hooks (caudal view) **G** cocoon (dorsal view) **H** head capsule (ventral view). Scale bars: 1.0 mm (**G**); 0.1 mm (**A, E, H**); 0.02 mm (**B–D**); 0.01 mm (**F**).

Pupa (*N* = 4). Body length 2.3–2.5 mm.

Head. Integument yellow.

Thorax. Integument yellow, moderately covered with round tubercles except posterior half sparsely covered with small tubercles on dorsolateral and lateral surfaces. Gill (Fig. 15E) composed of eight slender thread-like filaments, arranged asr [(2+1)+3]+2 from dorsal to ventral; common basal stalk 0.7–0.8 times length of interspiracular trunk; dorsal and middle triplets sharing short stalk, and dorsal and middle triplets mostly composed of one individual and two paired filaments with extremely short secondary stalk; stalk of ventral pair of filaments 1.0–1.2 times length of common basal stalk, and 0.7–0.9 times length of interspiracular trunk; primary stalk of dorsal triplet lying against that of lower pair at angle of 70–80° when viewed laterally; filaments of dorsal and middle triplets subequal in length (2.5–2.6 mm) and thickness to one another; two filaments of ventral pair subequal in length (3.0 mm) and thickness to each other (though inner filaments slightly thicker than inner one) and 1.5 times as thick as six other filaments of dorsal and middle triplets when compared basally.

Abdomen. Dorsally, all segments light yellowish; segments 1 and 2 without minute tubercles; segment 9 with pair of wide flat terminal hooks (Fig. 15F), of which outer margin is 1.7–2.4 times length of inner margin and crenulated when viewed caudally.

Cocoon (Fig. 15G). Slipper-shaped, moderately woven, moderately extended ventrolaterally; anterior margin without bulge or projection; individual threads visible; 3.2–3.6 mm long by 2.0–2.4 mm wide.

Mature larva (*N* = 5). Body length 4.1–4.5 mm. Body with following color markings: thoracic segment 1 encircled with greyish band (though disconnected ventromedially), ventral surface of thoracic segment 2 grey and that of thoracic segment 3 ochreous; abdominal segments 1–4 encircled with grey transverse band, and abdominal segments 5–8 greyish dorsally and dorsolaterally; abdominal segments 5 and 6 each overlaid with light reddish brown transverse band, appearing W-shaped, on dorsal and dorsolateral surfaces (though often faded out to varying extent, leaving small round medial spot and dorsolateral spots, or only small medial spot), and also overlaid with pair of round spots on ventral surface; abdominal segments 7 and 8 each overlaid with light reddish brown pigments to varying extent on dorsal and dorsolateral surfaces (completely faded out in one larva) (Fig. 25G); abdominal segment 7 with light reddish brown transverse band on ventral surface (often faded out, leaving pair of round spots).

Head. Head capsule yellow to dark yellow except eye-spot region whitish, sparsely covered with minute setae (though moderately on dorsal surface); head spots faintly to moderately positive. Antenna: proportional lengths of first, second, and third articles 1.00:0.65–0.75:0.81–0.95. Labral fan with 34–38 primary rays. Hypostoma with row of nine apical teeth, of which median tooth little longer than each corner tooth; lateral margin smooth; four hypostomal bristles per side lying nearly parallel to lateral margin. Postgenal cleft (Fig. 15H) rounded, 1.2–1.3 times length of postgenal bridge.

Thorax and ***Abdomen.*** Thoracic and abdominal cuticle very sparsely covered with unpigmented minute setae (though few posterior abdominal segments sparsely to moderately covered with dark minute unbranched or bifid setae (rarely trifid setae)

on dorsal and dorsolateral surfaces; last abdominal segment densely covered with unbranched colorless minute setae on dorsolateral and lateral surfaces of each side of anal sclerite and on each lateral surface even down to base of ventral papilla. Rectal scales minute, unpigmented. Rectal organ compound, each of three lobes with seven to nine finger-like secondary lobules. Anal sclerite of usual X-form, with anterior arms 1.1 times as long as posterior ones. Posterior circlet with 73–78 rows of hooklets with up to 13 or 14 hooklets per row.

Female. Unknown.

Etymology. The species name, *chaowaense*, refers to the name of the waterfall, Chao Wa, where this species was collected.

Distribution. Thailand (Phrae).

Discussion. This new species is similar to *S. tanahrataense* described from males and their associated pupal exuviae collected from Peninsular Malaysia (Takaoka et al. 2014b) in many characters including the number of male upper-eye facets. However, it is barely distinguished in the male from the latter species by the first flagellomere of the antenna 1.8 times as long as the second (2.1 times in *S. tanahrataense*), male hind basitarsus 0.9 times as wide as the hind femur (1.0 time in *S. tanahrataense*), and ventral plate with the ventral margin rounded (Fig. 15D) when viewed posteriorly (straight or slightly concave in *S. tanahrataense*). The pupa of this new species is almost indistinguishable from that of *S. tanahrataense*, although there is a slight difference in the length of filaments of the dorsal and middle triplets including their stalks and the common basal stalk (2.5–2.6 mm in this new species versus 1.6–2.2 mm in *S. tanahrataense*).

This new species is similar to *S. confertum* from Vietnam (Takaoka et al. 2017a) in having the similar number of male upper-eye large facets but is distinguished from the latter species by the ventral plate with the ventral margin rounded when viewed posteriorly (nearly straight in *S. confertum*).

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

http://zoobank.org/D35AD402-A33C-4560-B98A-5718CDA4B683
Figs 16, 25B

Material examined. ***Holotype*:** Male (with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium pitasawatae* male, QSBG col. no. 49, Thailand, 2-II-2019, by W. Srisuka", collected from a stream (width 120 cm, depth 10 cm, bed sandy, moderate flow, pH 6.6, 17.1 °C, partially shaded, elevation 1,154 m, 19°03'36.8"N, 99°19'15.7"E), Huai Mor Nuea Village, Doi Saket, Chiang Mai, Thailand, 2-II-2019, by W. Srisuka and A. Saeung (Coll. No. 49).

***Paratypes*:** Three females, eight males (thorax of one male for DNA analysis) (with their associated pupal exuviae and cocoons), and 10 mature larvae (one mature larva for DNA analysis) (in 80% ethanol), same data as for holotype.

Diagnosis. Female: small sensory vesicle and mandible with three to five distinct teeth on outer margin (Fig. 16A). Male: number of upper-eye facets in 14 vertical columns and

15 horizontal rows. Pupa: dorsal triplet of the gill filaments without their stalk or with an extremely short stalk (Fig. 16G, H). Larva: postgenal cleft as long as or little longer than the postgenal bridge (Fig. 16K) and abdominal segments 1–4 light ochreous (Fig. 25B).

Description. Female (*N* = 3). Body length 2.0 mm.

Head. Frontal ratio 1.7–1.8:1.0:2.3–2.5; frons:head ratio 1.0:4.2–4.9. Labrum 0.61–0.64 times length of clypeus. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.1–1.2:2.0–2.3; sensory vesicle medium sized, ellipsoidal (0.32–0.35 times length of third palpal segment). Lacinia with nine or ten inner and 12–14 outer teeth. Mandible (Fig. 16A) with 20–23 inner teeth and three to five outer teeth at some distance from tip.

Legs. Foreleg: basitarsus moderately dilated, 6.3–6.7 times as long as its greatest width. Hind leg: coxa medium brown; tibia yellowish white on basal three-fifths and light brown to brownish black on rest; basitarsus 5.7–6.0 times as long as wide, and 0.7–0.8 and 0.6 times as wide as greatest widths of tibia and femur, respectively; claw with large basal tooth 0.43 times length of claw.

Wing. Length 2.0–2.1 mm.

Abdomen. Dorsal surface of abdomen medium to dark brown except anterior one-fifths of segment 2 ochreous.

Terminalia. Sternite 8 bare medially, with 19–23 medium-long to long hairs together with two to four slender short hairs on each side. Paraproct in ventral view with four sensilla on anteromedial surface; paraproct in lateral view 0.6–0.7 times as long as wide, with 18–20 medium-long to long hairs on ventral and lateral surfaces. Cercus in lateral view 0.5 times as long as wide.

Male (*N* = 9). Body length 2.1–2.3 mm.

Head. Somewhat wide than thorax. Upper eye dark brown, consisting of large facets in 14 (rarely 13) vertical columns and 15 horizontal rows on each side. Antenna: first flagellomere elongate, 1.8 times length of second. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.1:2.4; sensory vesicle small, ellipsoidal (0.22–0.25 times length of third palpal segment).

Legs. Foreleg: tibia light grey to light brown except basal tip and outer surface of basal two-thirds whitish, and apical one-third dark brown; basitarsus 7.9–8.3 times as long as its greatest width. Hind leg: coxa medium brown; tarsus (Fig. 16B) 3.7–4.0 times as long as wide, and 0.9–1.0 and 1.0–1.1 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 16B) slightly shorter than basal width, and 0.3 times as wide as greatest width of basitarsus.

Wing. Length 2.0–2.1 mm. Subcosta bare in three males but with one to five hairs in six males.

Genitalia. Coxite in ventral view (Fig. 16C) nearly rectangular, 1.6 times as long as its greatest width. Style in ventrolateral view (Fig. 16D) 0.8 times length of coxite. Ventral plate in ventral view with basal arms slightly divergent, then convergent apically; ventral plate in caudal view (Fig. 16F) with ventral margin nearly straight or slightly concave medially. Paramere with basal arm bare or rarely with few minute setae on outer surface. Cercus with 15–18 hairs.

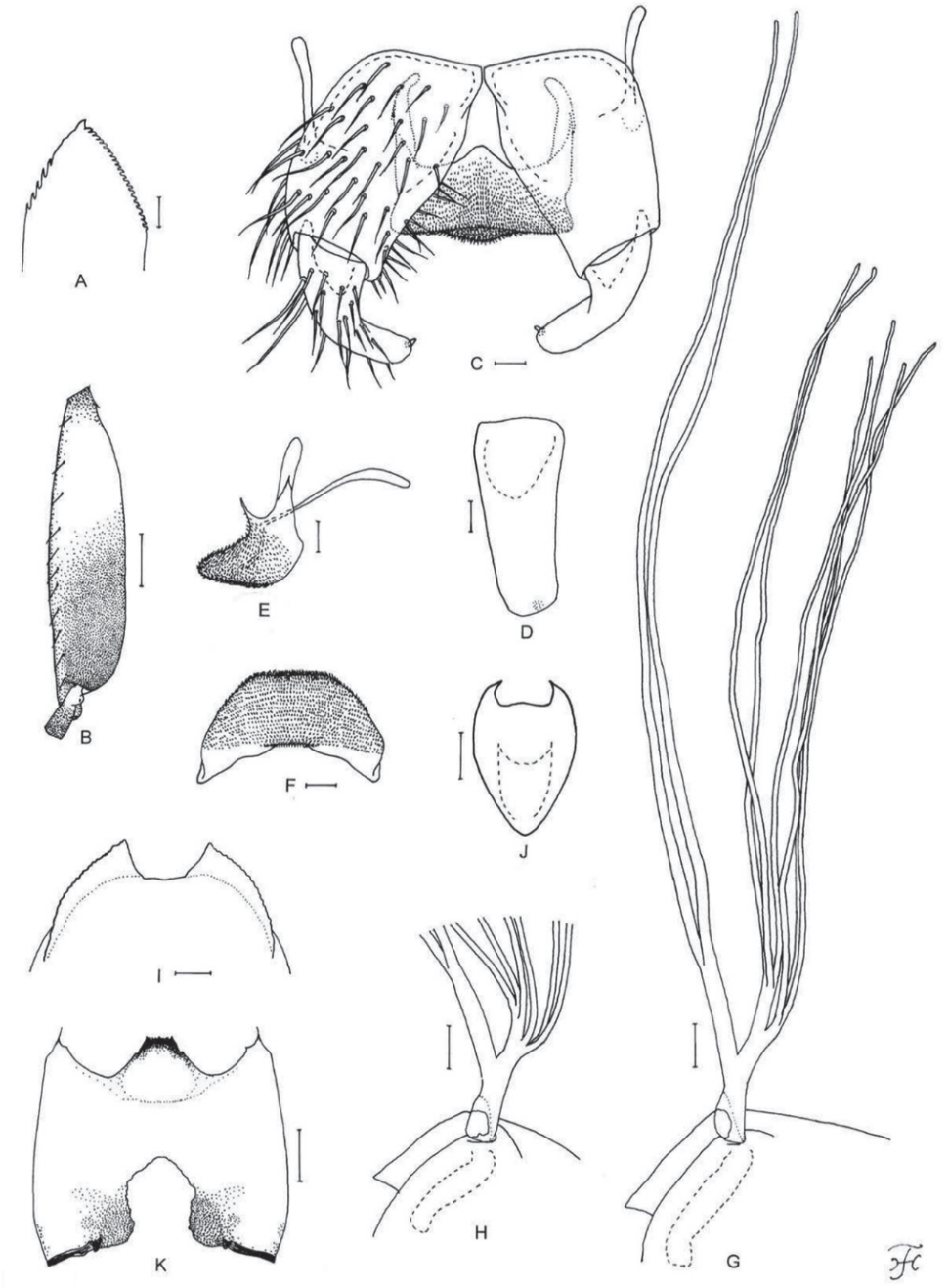

Figure 16. Female, male, pupa and larva of *S. pitasawatae* sp. nov. **A** female **B–F** male **G–J** pupa **K** larva. **A** mandible (left side) **B** hind basitarsus and second tarsomere (left side; lateral view) **C** coxites, styles and ventral plate (ventral view) **D** style (right side; ventrolateral view) **E** ventral plate and median sclerite (lateral view) **F** ventral plate (caudal view) **G, H** gill filaments (left side; lateral view) **I** terminal hooks (caudal view) **J** cocoon (dorsal view) **K** head capsule (ventral view). Scale bars: 1.0 mm (**J**); 0.1 mm (**B, G, H, K**); 0.02 mm (**C–F**); 0.01 mm (**A, I**).

Pupa (*N* = 12). Body length 2.4–2.7 mm.

Head. Integument yellow, Thorax. Integument yellow, moderately covered with round tubercles except dorsal and dorsolateral surfaces of posterior half sparsely covered with tubercles.

Thorax. Gill (Fig. 16G, H) composed of eight slender thread-like filaments, arranged as [3+(1+2)]+2 or (3+3)+2 or (2+1+3)+2 from dorsal to ventral; common basal stalk 0.6–0.8 times length of interspiracular trunk; dorsal and middle triplets sharing short stalk, and dorsal triplet mostly composed of three individual filaments arising at same level from extremely short stalk or directly from stalk of middle triplet, middle triplet mostly composed of three individual filaments arising at same level or one individual and two paired filaments with extremely short secondary stalk; stalk of ventral pair of filaments 1.1–1.3 times length of common basal stalk, and 0.7–0.9 times length of interspiracular trunk; primary stalk of dorsal triplet lying against that of lower pair at angle of 50–60° when viewed laterally; filaments of dorsal and middle triplets subequal in length (1.7–2.0 mm) and thickness to one another; two filaments of ventral pair subequal in length (2.5–3.0 mm) and thickness to each other and 1.5 times as thick as six other filaments of dorsal and middle triplets when compared basally.

Abdomen. Dorsally, all segments light yellowish; segments 1 and 2 without tubercles; segment 5 with one spine (same size as those on segments 6–9) on one side in two pupae; segment 9 with pair of wide flat terminal hooks (Fig. 16I), of which outer margin 3.6–3.9 times length of inner margin and crenulated when viewed caudally.

Cocoon (Fig. 16J). Whitish yellow, slipper-shaped, roughly to moderately woven, widely extended ventrolaterally; anterior margin moderately woven medially, without bulge or short projection; individual threads invisible; 3.0–3.5 mm long by 1.7–2.4 mm wide.

Mature larva (*N* = 9). Body length 4.3–5.4 mm. Body light ochreous with following color markings: thoracic segment 1 encircled with light to dark brown (rarely reddish brown) band (though disconnected ventrally), thoracic segments 2 and 3 ochreous on ventral surface; abdominal segment 4 with reddish brown transverse band (though often entirely faded out), abdominal segments 5 and 6 each with distinct reddish brown, W-shaped, transverse band dorsally along posterior margin, though often partially faded out leaving one round dorsomedial spot and two lateral spots of various size and shape), dorsal and dorsolateral surface of abdominal segments 5–8 faintly to moderately covered with pinkish or reddish brown pigments (Fig. 25B).

Head. Head capsule yellow except eye-spot region whitish; head spots moderately positive. Antenna: proportional lengths of first, second, and third articles 1.0:0.7–0.8:0.8–1.0. Labral fan with 23 or 24 primary rays. Postgenal cleft (Fig. 16K) rounded or pentagonal, 1.1–1.4 times length of postgenal bridge.

Thorax and ***Abdomen.*** Thoracic and abdominal cuticle sparsely covered with unpigmented minute setae, though few posterior abdominal segments sparsely covered also with dark minute unbranched setae dorsally. Rectal organ compound, each of three lobes with 7–11 finger-like secondary lobules. Anal sclerite of usual X-form, with anterior arms 1.1 times as long as posterior ones. Posterior circlet with 81–85 rows of hooklets with up to 14 or 15 hooklets per row.

Etymology. The species name, *pitasawatae*, is in honor of Associate Prof. Dr. Benjawan Pitasawat, Head of Department of Parasitology, Faculty of Medicine, Chiang Mai University, Thailand, who kindly supported A. Saeung in collections of black flies.

Distribution. Thailand (Chiang Mai).

Discussion. This new species is similar to *S. tamdaoense* Takaoka, Sofian-Azirun & Ya'cob described from Vietnam (Takaoka et al. 2014a) and *S. tanahrataense* described from Peninsular Malaysia (Takaoka et al. 2014b) in many characters including the number of male upper-eye facets and shape of the ventral plate in caudal view. However, it is distinguished from the latter two species by the dorsal triplet of the pupal gill filaments without their stalk or with an extremely short stalk (with their stalk nearly as long as the common stalk of the dorsal and middle triplet in the latter two species), from *S. tamdaoense* by the male fore basitarsus 7.9–8.3 times as long as its greatest width (6.8 times in *S. tamdaoense*) and the outer margin of the pupal terminal hook 3.7–3.9 times as long as the inner margin (1.8–1.9 times in *S. tamdaoense*), and from *S. tanahrataense* by the male first flagellomere of the antenna 1.8 times as long as the second (2.1 times in *S. tanahrataense*), and male sensory vesicle 0.22–0.25 times length of third palpal segment (0.16–0.18 time in *S. tanahrataense*).

The larva of this new species is similar in the body color pattern to *S.* (*G.*) *sutheppuiense* sp. nov. but is barely distinguished from the latter species by the labral fan with 23 or 24 primary rays (29 or 30 primary rays in *S.* (*G.*) *sutheppuiense* sp. nov.).

Simulium (*Gomphostilbia*) *banluangense* Takaoka, Srisuka & Fukuda sp. nov.

http://zoobank.org/A5D4E739-20B2-43F0-95F8-D43A76A51A62

Fig. 17

Material examined. *Holotype*: Male (with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium banluangense* male, Thailand, 12-VI-2001, by W. Choochote & H. Takaoka", reared from a pupa collected from a stream (width 1.0 m, 25 °C, exposed to the sun, elevation 804 m, 18°32'33.3"N, 98°35'32.5"E), at Ban Luang, Chiang Mai Province, Thailand, 12-VI-2001, by W. Choochote and H. Takaoka.

Diagnosis. Male: upper-eye (large) facets in 14 vertical columns and 14 or 15 horizontal rows on each side, and hind basitarsus (Fig. 17A) spindle-shaped. Pupa: gill with a short common basal stalk and a short stalk of the ventral pair of filaments, which is thicker than the interspiracular trunk (Fig. 17E, F), and terminal hooks triangular (Fig. 17G).

Description. Male (*N* = 1). Body length 2.4 mm.

Head. Slightly wider than thorax. Upper eye medium brown, consisting of 14 vertical columns and 14 or 15 horizontal rows of large facets on each side. Antenna: first flagellomere elongate, 1.9 times length of second. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.2:3.0; sensory vesicle 0.21–0.24 times length of third palpal segment.

Figure 17. Male and pupa of *S. banluangense* sp. nov. **A–D** male **E–H** pupa. **A** hind basitarsus and second tarsomere (left side; lateral view) **B** coxites, styles and ventral plate (ventral view) **C** ventral plate and median sclerite (lateral view) **D** ventral plate (caudal view) **E, F** gill filaments (lateral view **E** right side **F** left side) **G** terminal hooks (caudal view) **H** cocoon (dorsal view). Scale bars: 1.0 mm (**H**); 0.1 mm (**A, E, F**); 0.02 mm (**B–D**); 0.01 mm (**G**).

Legs. Foreleg: tibia light brown except median large area of outer surface of basal three-fourths whitish and apical one-fourth dark brown; tarsus brownish black; basitarsus moderately dilated, 6.4 times as long as its greatest width. Midleg: tarsus dark brown except basal one-fourth to two-fifths of basitarsus dark yellow to light brown (border not well defined). Hind leg: tibia dark brown to brownish black except little less than basal half whitish yellow; tarsus (Fig. 17A) brownish black except basal half of basitarsus and basal one-third of second tarsomere whitish yellow; basitarsus (Fig. 17A) 3.8 times as long as wide, and 0.9 and 0.9 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 17A) slightly shorter than basal width, and 0.34 times as wide as greatest width of basitarsus.

Wing. Length 2.1 mm. Subcosta with seven hairs.

Genitalia. Coxite in ventral view (Fig. 17B) nearly rectangular, 1.6 times as long as its greatest width. Style in ventrolateral view slightly tapered toward apex, with truncated apex, and 0.8 times as long as coxite. Ventral plate in ventral view (Fig. 17B) with body transverse, 0.5 times as long as wide; ventral plate in caudal view (Fig. 17D) rounded ventrally, with ventral margin slightly raised medially. Cercus small, rounded, with 15–17 hairs.

Pupa (N = 1). Body length 3.0 mm.

Thorax. Gill (Fig. 17E, F) composed of eight slender thread-like filaments, arranged as [(2+1)+(1+2)]+2 or [3+(1+2)]+2 from dorsal to ventral; common basal stalk short, 0.5–0.6 times length of interspiracular trunk; dorsal and middle triplets sharing short stalk, stalk of ventral pair of filaments short, 0.8 times length of common basal stalk, and 0.4–0.5 times length of interspiracular trunk, and thicker than interspiracular trunk; primary stalk of dorsal triplet lying against that of lower pair at angle of 90° when viewed laterally; stalk of middle triplet directed inward; filaments of dorsal and middle triplets subequal in length (2.5–2.9 mm) and thickness to one another; two filaments of ventral pair subequal in length (3.2–3.5 mm) and thickness to each other and 1.6 times as thick as six other filaments of dorsal and middle triplets when compared basally.

Abdomen. Dorsally, segments 1 and 2 without minute tubercles; terminal hooks (Fig. 17G) triangular, with outer margin slightly undulate, and little less than twice length of inner margin.

Cocoon (Fig. 17H). Slipper-shaped, roughly to moderately woven, widely extended ventrolaterally; anterior margin thickly woven medially, with bulge; individual threads visible; 3.5 mm long by 3.0 mm wide.

Female and **Larva.** Unknown.

Etymology. The species name, *banluangense*, refers to the name of the locality, Ban Luang, where this species was collected.

Distribution. Thailand (Chiang Mai).

Discussion. *Simulium doisaketense* described based on pupal and larval specimens from Thailand (Jitklang et al. 2008), has the pupal gill with a short common basal stalk, like this new species, but the stalk of the ventral pair of filaments is medium-long, 1.3 times as long as the common basal stalk, and thinner than the interspiracular trunk, based on observations of the type specimen.

***Simulium* (*Gomphostilbia*) *junkumae* Takaoka, Srisuka & Saeung, sp. nov.**
http://zoobank.org/327F3EB9-C555-4B08-A691-789310BB612F
Figs 18, 25K

Material examined. *Holotype*: Male (with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium junkumae* male, QSBG col. no. 92, Thailand, 7-IV-2017, by W. Srisuka", collected from a small stream (width 40 cm, depth 3 cm, bed sandy, moderate flow, pH 6.2, 19 °C, partially shaded, elevation 1,395 m, 18°49'09.5"N, 98°53'14.3"E), at Doi Pui Temple, Doi Suthep Pui, Muang District, Chiang Mai Province, Thailand, 7-IV-2017, by W. Srisuka (Coll. No. 92).

***Paratypes*:** Five females, five males (thorax of one male for DNA analysis) (with their associated pupal exuviae and cocoons), and six mature larvae (two mature larvae for DNA analysis) (in 80% ethanol), same data as for holotype.

Diagnosis. Female: relatively narrower frons against the head width (1.0:4.7–5.5), and mandible without teeth on the outer margin (Fig. 18A). Male: greater number of upper-eye facets in 15–17 vertical columns and 15–17 horizontal rows on each side. Larva: small number of the primary rays of the labral fan (24 or 25), postgenal cleft as long as or little shorter than the postgenal bridge (Fig. 18M) and abdominal segments 1–3 entirely grey (Fig. 25K).

Description. Female (*N* = 5). Body length 2.0–2.3 mm.

Head. Frontal ratio 1.8–1.9:1.0:2.5–3.2; frons:head ratio 1.0:4.7–5.5. Labrum 0.64–0.67 times length of clypeus. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.0–1.1:2.0–2.1; sensory vesicle medium sized, ellipsoidal (0.27–0.32 times length of third palpal segment), with medium opening. Lacinia with ten or eleven inner and 14 or 15 outer teeth. Mandible (Fig. 18A) with 22–26 inner teeth and lacking distinct outer teeth (though outer margin undulated from tip for some short distance appearing to have ca. ten vestigial teeth).

Legs. Foreleg: trochanter dark yellow to light brown; femur light brown with apical cap medium brown (though extreme tip yellowish); basitarsus moderately dilated, 6.7–7.0 times as long as its greatest width. Midleg: trochanter dark yellow except basal half yellow; tarsus dark brown to brownish black except basal one-fourth of basitarsus dark yellow (its border not well defined). Hind leg: tibia yellowish white on little more than basal half and light brown to brownish black on rest; basitarsus (Fig. 18B) 5.9 times as long as wide, and 0.7 and 0.6 times as wide as greatest widths of tibia and femur, respectively; calcipala (Fig. 18B) nearly as long as width at base, and 0.56 times as wide as greatest width of basitarsus.

Wing. Length 2.1–2.3 mm.

Abdomen. Dorsal surface of abdomen medium to dark brown except anterior four-fifths of segment 2 whitish. Ventral surface of segment 2 whitish and those of other segments light to dark brown; sternal plate on segment 7 undeveloped.

Terminalia. Sternite 8 bare medially, with 25–28 medium-long to long hairs together with two or six slender short hairs on each side. Ovipositor valve triangular, thin, membranous, moderately covered with microsetae interspersed with two to four

short hairs; inner margins nearly straight or slightly sinuous, somewhat sclerotized, and moderately separated from each other. Paraproct in ventral view with five or six sensilla on anteromedial surface; paraproct in lateral view somewhat produced ventrally beyond ventral tip of cercus, 0.6 times as long as wide, with 25–28 medium-long to long hairs on ventral and lateral surfaces. Cercus in lateral view 0.4–0.5 times as long as wide. Spermatheca ellipsoidal, 1.3 times as long as its greatest width.

Male (*N* = 6). Body length 2.2–2.3 mm.

Head. Slightly wider than thorax. Upper eye dark brown, consisting of large facets in 15–17 vertical columns and 15–17 horizontal rows on each side. Antenna: first flagellomere elongate, 1.6–1.8 times length of second. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.0:2.4–2.6; sensory vesicle ellipsoidal, 0.17–0.23 times length of third palpal segment.

Legs. Foreleg: tibia light grey to light brown except basal tip and outer surface of basal two-thirds whitish, and apical one-third dark brown; basitarsus moderately dilated, 7.8–8.1 times as long as its greatest width. Midleg: basitarsus dark brown except basal tip yellow. Hind leg: coxa medium brown; tibia dark brown to brownish black except little more than basal one-third whitish yellow; tarsus (Fig. 18C) brownish black except little less than basal half of basitarsus and basal one-third of second tarsomere yellow; basitarsus (Fig. 18C) 3.7–4.0 times as long as wide, and 0.9 and 1.0 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 18C) slightly shorter than basal width, and 0.33 times as wide as greatest width of basitarsus.

Wing. Length 2.2–2.4 mm. Subcosta with 7–12 hairs.

Genitalia. Coxite in ventral view (Fig. 18D) nearly rectangular, 1.7 times as long as its greatest width. Style in ventral view (Fig. 18D) with triangular apex; style in ventrolateral view (Fig. 18E, F) slightly tapered toward apex or nearly parallel-sided from basal one-third to apex, and 0.8 times length of coxite. Ventral plate in ventral view (Fig. 18D) with body transverse, 0.46 times as long as wide, with anterior margin produced anteromedially, posterior margin nearly straight or somewhat concave medially, and lateral margin emarginated medially; basal arms of moderate length, nearly parallel-sided, then convergent apically; ventral plate in caudal view (Fig. 18I) trapezoidal, with ventral margin nearly straight or slightly concave ventrally. Cercus with 14 or 15 hairs.

Pupa (*N* = 11). Body length 2.4–2.8. mm.

Thorax. Integument moderately covered with round tubercles except dorsal and dorsolateral surfaces of posterior half sparsely covered with tubercles. Gill (Fig. 18J) composed of eight slender thread-like filaments, arranged as [3+(1+2)]+2 or [3+(2+1)]+2 from dorsal to ventral; common basal stalk 0.7–0.8 times length of interspiracular trunk; stalk of ventral pair of filaments 0.7–1.2 times length of common basal stalk, and 0.5–0.9 times length of interspiracular trunk; primary stalk of dorsal triplet lying against that of lower pair at angle of 60° when viewed laterally; filaments of dorsal and middle triplets subequal in length (2.0–2.6 mm) and thickness to one another; two filaments of ventral pair subequal in length (3.0–3.9 mm) and thickness to each other and 1.6–1.8 times as thick as six other filaments of dorsal and middle triplets when compared basally.

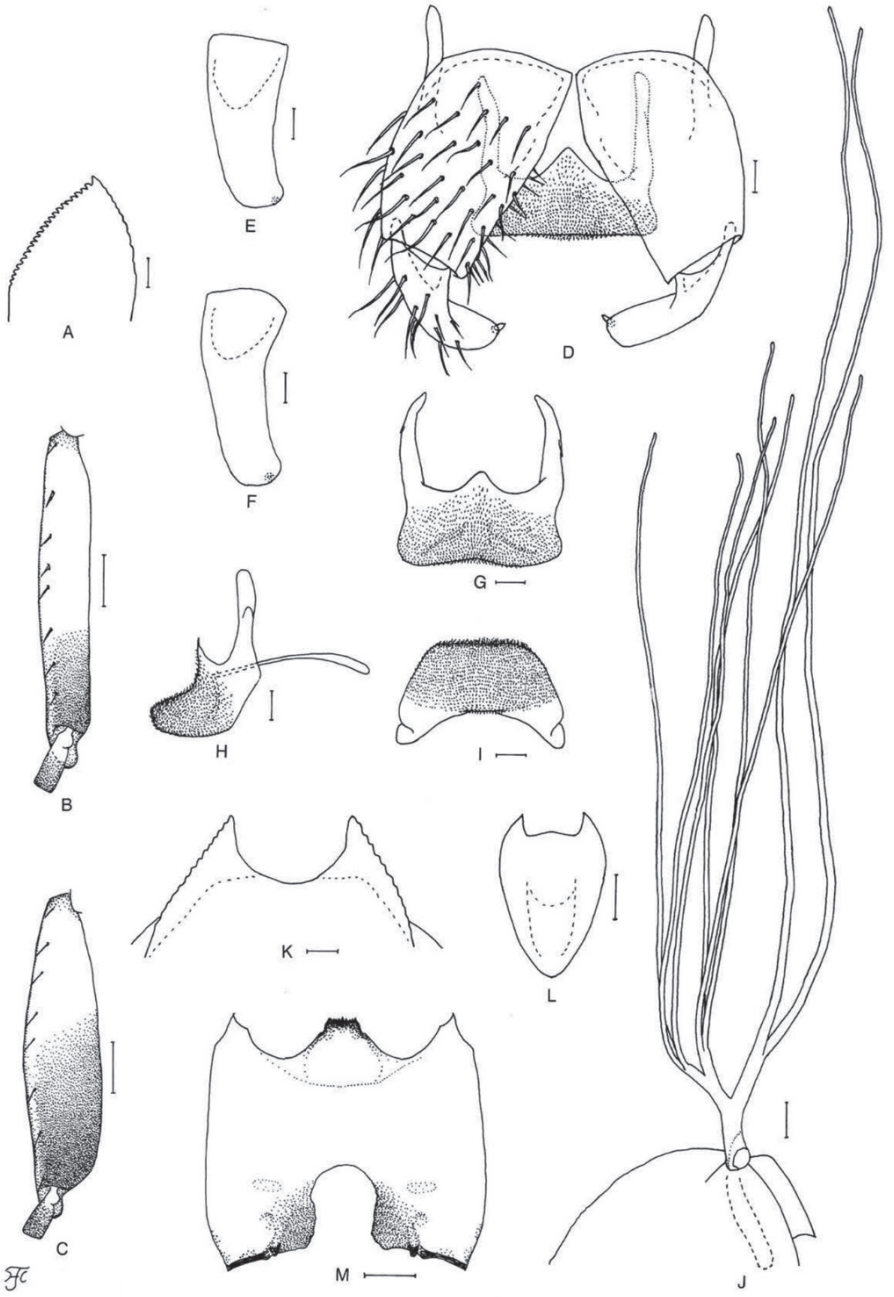

Figure 18. Female, male, pupa and larva of *S. junkumae* sp. nov. **A, B** female **C–I** male **J–L** pupa **M** larva. **A** mandible (right side) **B, C** hind basitarsus and second tarsomere (left side; lateral view **B** female **C** male) **D** coxites, styles and ventral plate (ventral view) **E, F** styles (right side; ventrolateral view) **G** ventral plate (ventral view) **H** ventral plate and median sclerite (lateral view) **I** ventral plate (caudal view) **J** gill filaments (right side; lateral view) **K** terminal hooks (caudal view) **L** cocoon (dorsal view) **M** head capsule (ventral view). Scale bars: 1.0 mm (**L**); 0.1 mm (**B, C, J, M**); 0.02 mm (**D–I**); 0.01 mm (**A, K**).

Abdomen. Dorsally, all segments light yellow; segments 1 and 2 without tubercles; segment 9 with pair of wide flat terminal hooks (Fig. 18K), of which outer margin is 2.2–2.6 times length of inner margin and crenulated when viewed caudally.

Cocoon (Fig. 18L). Wall-pocket-shaped, moderately woven, moderately extended ventrolaterally; anterior margin moderately woven medially, rarely with bulge; individual threads visible; 3.3–3.9 mm long by 2.0–2.5 mm wide.

Mature larva (*N* = 4). Body length 4.9–5.4 mm. Body creamy white with following color markings: thoracic segment 1 encircled with distinct reddish brown band (though disconnected ventromedially), thoracic segments 2 and 3 ochreous on ventral surface; abdominal segments 1–3 entirely grey, abdominal segment 4 with reddish brown transverse band dorsally (though often partially to completely faded, leaving narrow band or small spot(s) dorsally), abdominal segments 5 and 6 each with distinct reddish brown, W-shaped, transverse band (of which central and dorsolateral parts marked) along posterior margin on dorsal and dorsolateral surfaces (though band on abdominal segment 6 often partially faded, leaving one round dorsomedial spot and two larger dorsolateral lateral spots); abdominal segments 7 and 8 covered with reddish brown pigments on dorsal and dorsolateral surfaces (though central portion often faded to varying extent) (Fig. 25K).

Head. Head capsule yellow except eye-spot region whitish, sparsely covered with minute setae (though moderately on dorsal surface); head spots faintly to moderately positive or indistinct. Antenna: proportional lengths of first, second, and third articles 1.0:0.7–0.8:0.7–0.8. Labral fan with 24 or 25 primary rays. Postgenal cleft (Fig. 18M) rounded or quadrate, 0.9–1.0 times length of postgenal bridge.

Abdomen. Rectal organ compound, each of three lobes with 8–11 finger-like secondary lobules. Anal sclerite: anterior arms 1.0–1.2 times as long as posterior ones. Posterior circlet with 81–84 rows of hooklets with up to 14 hooklets per row.

Etymology. The species name *junkumae* is in honor of Assistant Prof. Dr. Anuluck Junkum, Department of Parasitology, Faculty of Medicine, Chiang Mai University, Thailand, for her kind help in the field and laboratory works of black flies.

Distribution. Thailand (Chiang Mai).

Discussion. This new species is similar to *S. hongthaii* Takaoka, Sofian-Azirun & Ya'cob described from Vietnam (Takaoka et al. 2014a) in having a greater number of male upper-eye facets. However, it is distinguished from the latter species in the female by the narrower frons (frons:head ratio 1:4.7–5.5 in the new species versus 1:4.1–4.2 in *S. hongthaii*), in the male by the subcosta with hairs (subcosta bare in *S. hongthaii*) and in the larva by abdominal segments 1–3 grey (Fig. 25K) (abdominal segments 1–4 greyish in *S. hongthaii*).

The larva of *S.* sp. nr. *asakoae*-2 reported from Thailand by Jitklang et al. (2008) has a medium-sized postgenal cleft and abdominal segments 1–3 each with a greenish transverse band, both characters resembling those of this new species, but it differs by lacking distinct color markings on abdominal segments 5–8.

***Simulium* (*Gomphostilbia*) *kiewfinense* Takaoka, Srisuka & Fukuda, sp. nov.**
http://zoobank.org/79DF8917-1F47-47C3-AC8A-6B26E78EAA83
Fig. 19

Material examined. *Holotype*: Male (with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium kiewfinense* male, QSBG col. no. 36, Thailand, 6-IV-2018, by W. Srisuka", collected from a stream (width 1.1 m, depth 12 cm, bed sandy, moderate flow, pH 6.9, 18.3 °C, partially shaded, elevation 1,446 m, 18°51'38.8"N, 99°22'15.2"E), at Kiew Fin, Muang Pan District, Lampang Province, Thailand, 6-IV-2018, by W. Srisuka (Coll. No. 36).

***Paratypes*:** Two males (thorax of one male for DNA analysis) (with their associated pupal exuviae and cocoons) (in 80% ethanol), same data as for holotype.

Diagnosis. Male: greater number of upper-eye facets in 16 or 17 vertical columns and 16 or 17 horizontal rows one each side.

Description. Male (*N* = 3). Body length 2.0–2.1 mm.

Head. Somewhat wider than thorax. Upper eye medium brown, consisting of large facets in 16 or 17 vertical columns and 16 or 17 horizontal rows. Antenna composed of scape, pedicel and nine flagellomeres, medium to dark brown except scape, pedicel, and base of first flagellomere yellow; first flagellomere elongate, 1.8 times length of second. Maxillary palpus light brown, with five palpal segments, proportional lengths of third, fourth, and fifth palpomeres 1.0:1.0:2.6; sensory vesicle 0.19 times length of third palpal segment.

Legs. Foreleg: tibia whitish except apical three-tenths dark brown and subbasal portion light brown; basitarsus moderately dilated, 6.6–7.1 times as long as its greatest width. Midleg: tarsus dark brown except basal one-third of basitarsus dark yellow to light brown (border not well defined). Hind leg: coxa light brown; tarsus (Fig. 19A) brownish black except basal two-fifths to little less than basal half of basitarsus and basal one-third of second tarsomere yellow; basitarsus (Fig. 19A) 3.7–3.8 times as long as wide, and 0.9 and 1.0 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 19A) slightly shorter than basal width, and 0.33 times as wide as greatest width of basitarsus.

Wing. Length 1.9–2.0 mm. Subcosta bare.

Genitalia. Coxite in ventral view (Fig. 19B) nearly rectangular, 1.8 times as long as its greatest width. Style in ventral view (Fig. 19B) with round apex. Ventral plate in ventral view (Fig. 19B) with basal arms nearly parallel-sided, then convergent apically; ventral plate in caudal view (Fig. 19E) with ventral margin nearly straight. Cercus with 14 or 15 hairs.

Pupa (*N* = 3). Body length 2.5–2.7 mm.

Head. Integument yellow.

Thorax. Integument yellow, moderately covered with round tubercles except dorsolateral surface of posterior half almost bare. Gill (Fig. 19F) composed of eight slender

Figure 19. Male and pupa of *S. kiewfinense* sp. nov. **A–E** male **F–H** pupa. **A** hind basitarsus and second tarsomere (left side; lateral view) **B** coxites, styles and ventral plate (ventral view) **C** style (right side; ventrolateral view) **D** ventral plate and median sclerite (lateral view) **E** ventral plate (caudal view) **F** gill filaments (left side; lateral view) **G** terminal hooks (caudal view) **H** cocoon (dorsal view). Scale bars: 1.0 mm (**H**); 0.1 mm (**A, F**); 0.02 mm (**B–E**); 0.01 mm (**G**).

thread-like filaments, arranged as [3+(1+2)]+2 from dorsal to ventral; common basal stalk 0.7–0.8 times length of interspiracular trunk; stalk of ventral pair of filaments 0.9–1.2 times length of common basal stalk, and 0.7–0.9 times length of interspiracular trunk; primary stalk of dorsal triplet lying against that of lower pair at angle of 70–80° when viewed laterally; filaments of dorsal triplet subequal in length (1.5–2.1 mm) and thickness to one another; filaments of middle triplet subequal in length (2.0–2.5 mm) and thickness to one another; two filaments of ventral pair subequal in length (3.0–3.5 mm) and thickness to each other and 1.3–1.5 times as thick as six other filaments of dorsal and middle triplets when compared basally.

Abdomen. Dorsally, all segments light yellowish; segments 1 and 2 without minute tubercles; segment 9 with pair of wide flat terminal hooks (Fig. 19G), of which outer margin 2.3–2.7 times length of inner margin and crenulated when viewed caudally.

Cocoon (Fig. 19H). Pale yellow, slipper-shaped, moderately woven, moderately extended ventrolaterally; anterior margin thickly woven medially, often with bulge; 3.0–3.3 mm long by 2.2 mm wide.

Female and **mature larva**. Unknown.

Etymology. The species name, *kiewfinense*, refers to the local name, Kiew Fin, where this species was collected.

Distribution. Thailand (Lampang).

Discussion. This new species is similar to *S. junkumae* sp. nov. described above and *S. hongthaii* from Vietnam (Takaoka et al. 2014a) in having the greater number of male upper-eye facets. However, it is distinguished from both species by the relative length of the fore basitarsus against its greatest width (6.6–7.1 in this new species versus 7.8–8.1 in *S. junkumae* sp. nov. and 7.5–8.4 in *S. hongthaii*), also from *S. junkumae* sp. nov. by the male subcosta bare (subcosta with hairs in *S. junkumae* sp. nov.) and from *S.* (*G.*) *hongthaii* by the relative length of the coxite against its greatest width (1.8 in this new species and 2.2 in *S. hongthaii*).

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

http://zoobank.org/2B1BBBD1-655B-4567-B20E-F96954413D2D
Figs 20, 25I

Material examined. ***Holotype.*** Male (with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium huaimorense* male, QSBG col. no. 49, Thailand, 2-II-2019, by W. Srisuka", collected from a stream (width 1.2 m, depth 10 cm, bed sandy, moderate flow, pH 6.6, 17.1 °C, exposed to the sun, elevation 1,154 m, 19°03'36.8"N, 99°19'15.7"E), at a coffee plantation, Huai Mor, Doi Saket, Chiang Mai Province, Thailand, 2-II-2019, by W. Srisuka (Coll. No. 49).

Paratypes. One female, two males (thorax of one male for DNA analysis) (with its associated pupal exuviae and cocoon) (in 80% ethanol), and three mature larvae (in 80% ethanol), collected from a small stream (width 30 cm, depth 2 cm, bed sandy, moderate flow, pH 7.3, 19.8 °C, partially shaded, elevation 1,440 m, 19°54'04.1"N,

99°34'26.6"E), at coffee plantation, Pha Lung Village, Muang District, Chiang Rai Province, northern Thailand, 30-VIII-2018, by W. Srisuka (Coll. No. 122).

Diagnosis. Male: upper-eye large facets in 16 vertical columns and 17 horizontal rows. Pupa: extremely short primary stalks of the dorsal and middle triplets of filaments (Fig. 20G) and cocoon with a short anterodorsal projection. Larva: abdominal segments 1–4 grey.

Description. Female (*N* = 1). Body length 2.0 mm.

Head. Frontal ratio 1.9:1.0:2.6; frons:head ratio 1.0:4.8. Labrum 0.67 times length of clypeus. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.2:2.3; sensory vesicle ellipsoidal, medium long (0.27–0.28 times length of third palpal segment). Lacinia with nine or ten inner and 14 or 15 outer teeth. Mandible (Fig. 20A) with 20 inner teeth and one or two outer teeth at some distance from tip.

Legs. Foreleg: Basitarsus moderately dilated, 6.5 times as long as its greatest width. Hind leg: coxa light brown; tibia yellowish white on basal two-thirds and light brown to brownish black on rest; basitarsus 6.2 times as long as wide, and 0.7 and 0.6 times as wide as greatest widths of tibia and femur, respectively; calcipala nearly as long as width at base, and 0.6 times as wide as greatest width of basitarsus; claw with large basal tooth 0.46 times length of claw.

Wing. Length 2.0 mm.

Abdomen. Dorsal surface of abdomen medium to dark brown except anterior five-sixths of segment 2 ochreous

Terminalia. Sternite 8 bare medially, with 23 or 24 medium-long to long hairs together with three or four slender short hairs on each side. Ovipositor valve moderately covered with microsetae interspersed with two or three short hairs; inner margins nearly straight or slightly sinuous, Paraproct with four sensilla on anteromedial surface; paraproct in lateral view 0.6 times as long as wide, with 24 or 25 medium-long to long hairs on ventral and lateral surfaces. Cercus in lateral view 0.5 times as long as wide. Spermatheca ellipsoidal, 1.3 times as long as its greatest width.

Male (*N* = 3). Body length 2.0 mm.

Head. Slightly wider than thorax. Upper eye dark brown, consisting of large facets in 16 vertical columns and 17 horizontal rows on each side. Antenna: first flagellomere elongate, 1.9 times length of second. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.2:2.2; sensory vesicle small, ellipsoidal, 0.22 times length of third palpal segment.

Legs. Foreleg: coxa whitish yellow; trochanter light brown; femur light brown except apical tip yellowish; tibia whitish yellow except inner and lateral surface of basal one-fifth light brown and little more than apical one-third dark brown; basitarsus 7.0–8.0 times as long as its greatest width. Midleg: trochanter light brown except base yellow; femur light to medium brown with base yellowish and apical cap dark brown (though apical tip yellow). Hind leg: coxa light brown except apical one-third whitish yellow; femur medium to dark brown with basal tip yellow and apical cap brownish black (though apical tip yellow); tibia dark brown to brownish black except little

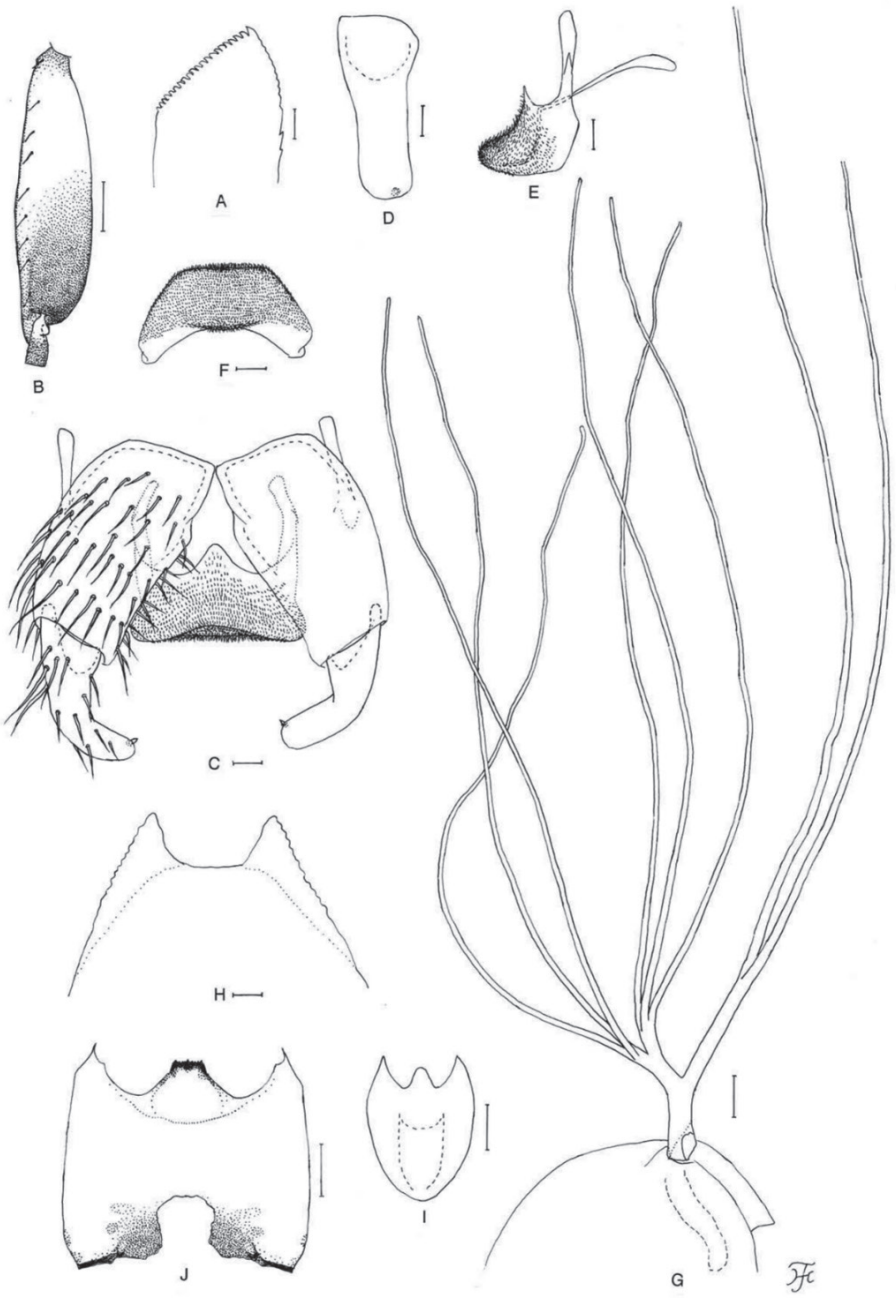

Figure 20. Female, male, pupa and larva of *S. huaimorense* sp. nov. **A** female **B–F** male **G–I** pupa **J** larva. **A** mandible (right side) **B** hind basitarsus and second tarsomere (left side; lateral view) **C** coxites, styles and ventral plate (ventral view) **D** style (right side; ventrolateral view) **E** ventral plate and median sclerite (lateral view) **F** ventral plate (caudal view) **G** gill filaments (right side; lateral view) **H** terminal hooks (caudal view) **I** cocoon (dorsal view) **J** head capsule (ventral view). Scale bars: 1.0 mm (**I**); 0.1 mm (**B, G, J**); 0.02 mm (**C–F**); 0.01 mm (**A, H**).

less than basal half whitish yellow and subbasal small portion somewhat dark yellow; tarsus (Fig. 20B) brownish black except little less than basal half of basitarsus (though its border not well defined) and basal one-third of second tarsomere whitish yellow; basitarsus (Fig. 20B) 3.6–4.0 times as long as wide, and 0.9–1.1 and 1.0–1.1 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 20B) 0.31 times as wide as greatest width of basitarsus.

Wing. Length 2.0 mm. Subcosta bare or with two hairs.

Abdomen. Ventral surface of segment 2 white, those of segments 3 and 4 light brown although sternal plates medial brown, and those of other segments medium to dark brown.

Genitalia. Coxite in ventral view (Fig. 20C) nearly rectangular, 1.8 times as long as its greatest width. Style in ventral view (Fig. 20C) with round apex; and in ventrolateral view (Fig. 20D) tapered from base to basal two-fifths, then nearly parallel-sided, with round apex. Ventral plate in ventral view (Fig. 20C) with posterior margin somewhat concave medially, and lateral margin emarginated medially; basal arms of moderate length, slightly divergent, then convergent apically; ventral plate in caudal view (Fig. 20F) trapezoidal, with ventral margin nearly straight medially. Cercus with 12–16 hairs.

Pupa (*N* = 4). Body length 2.5 mm.

Thorax. Integument yellow, moderately covered with round tubercles except dorsal and dorsolateral surface of posterior half sparsely covered with tubercles. Gill (Fig. 20G) composed of eight slender thread-like filaments, arranged as [3+(1+2)]+2from dorsal to ventral; common basal stalk 0.7 times length of interspiracular trunk; dorsal and middle triplets sharing short stalk, and dorsal triplet composed of three individual filaments arising at same level, middle triplet composed of one individual and two paired filaments with extremely short secondary stalk; stalk of ventral pair of filaments medium-long, 1.4 times length of common basal stalk, and nearly as long as interspiracular trunk; primary stalks of dorsal and middle triplets extremely short, 0.3 and 0.6 times length of their common stalk, respectively; primary stalk of dorsal triplet lying against that of lower pair at angle of 90° when viewed laterally; filaments of dorsal and middle triplets subequal in length (2.1–2.3 mm) and thickness to one another; two filaments of ventral pair subequal in length (3.0–3.5 mm) and thickness to each other, and 1.3–1.4 times as thick as six other filaments of dorsal and middle triplets when compared basally; all filaments light brown.

Abdomen. Dorsally, all segments light yellowish; segments 1 and 2 without minute tubercles; segment 9 with pair of wide flat terminal hooks (Fig. 20H), of which outer margin 2.9 times length of inner margin and crenulated when viewed caudally.

Cocoon (Fig. 20I). Light yellow, slipper-shaped, moderately woven, widely extended ventrolaterally; anterior margin thickly woven medially, with short projection; individual threads not visible; 3.5 mm long by 2.5 mm wide.

Mature larva (*N* = 3). Body length 4.5–5.3 mm. Body creamy white with following color markings: thoracic segment 1 encircled with distinct reddish brown band (though disconnected ventromedially), thoracic segments 2 and 3 ochreous on ventral surface; abdominal segments 1–4 entirely grey, abdominal segments 7 and 8 light grey

on dorsal and dorsolateral surfaces; abdominal segments 5 and 6 each with distinct reddish brown, W-shaped, transverse band (of which central and dorsolateral parts marked) along posterior margin of dorsal and dorsolateral surfaces (though band on abdominal segment 6 often partially faded, leaving one round dorsomedial spot and two larger dorsolateral lateral spots); abdominal segments 7 and 8 covered with reddish brown pigments on dorsal and dorsolateral surfaces (though central portion often faded out to varying extent) (Fig. 25I); abdominal segments 5–7 each with pair of reddish brown spots ventrally (though often faded).

Head. Head capsule yellow except eye-spot region whitish, sparsely covered with minute setae (though moderately on dorsal surface); head spots faintly to moderately positive. Antenna: proportional lengths of first, second, and third articles 1.00:0.72–0.75:0.75–0.82. Labral fan with 24–26 primary rays. Postgenal cleft (Fig. 20J) rounded or slightly triangular anteriorly, 0.8–1.0 times length of postgenal bridge.

Abdomen. Rectal organ compound, each of three lobes with 11–13 finger-like secondary lobules. Anal sclerite: anterior arms nearly as long as or slightly longer than posterior ones. Posterior circlet with 86–92 rows of hooklets with up to 14 hooklets per row.

Etymology. The species name, *huaimorense*, refers to the locality name, Huai Mor, where this species was collected.

Distribution. Thailand (Chiang Mai and Chiang Rai).

Discussion. *Simulium huaimorense* sp. nov. is similar to *S. myanmarense* described from Myanmar (Takaoka et al. 2017b) in that both species share the high number of male upper-eye large facets and cocoon with a short anterodorsal projection (Fig. 20I). However, this new species is distinguished in the male by the upper-eye large facets in 16 vertical columns and 17 horizontal rows (14 or 15 vertical columns and 15 or 16 horizontal rows in *S. myanmarense*), relative length of the hind basitarsus against its greatest width, which is 3.6–4.0 in this new species versus 4.3–4.4 in *S. myanmarense*, in the pupa by the extremely short primary stalks of the dorsal and middle triplets of filaments (Fig. 20G), which are 0.1–0.3 and 0.6–0.7 times as long as their common stalk (the dorsal and middle primary stalks are nearly as long as their common stalk in *S. myanmarense*), and in the larva by abdominal segments 1–4 grey (abdominal segments 1 and 2 grey in *S. myanmarense*).

This new species is distinguished from *S. maewongense* sp. nov. and four other related species (noted under *S. maewongense* sp. nov.), which have a cocoon with a short anterodorsal projection (Fig. 20I), by the higher number of the male upper-eye large facets.

This new species is similar in the higher number of male upper-eye facets and the arrangement of the pupal gill filaments to *S. kiewfinense* sp. nov., and *S. junkumae* sp. nov. from Thailand and *S. hongthaii* from Vietnam (Takaoka et al. 2014a) but is distinguished from the latter three species by the cocoon with a short anterodorsal projection, and from *S. junkumae* sp. nov. by larval abdominal segments 1–4 being grey (Fig. 25I) (larval abdominal segments 1–3 grey in *S. junkumae* sp. nov.).

This new species is distinguished from *S. doisaketense* from Doi Saket, Chiang Mai Province (Jitklang et al. 2008), by the primary stalks of the dorsal and middle triplets of gill filaments much shorter than their common stalk (much longer in *S. doisaketense*).

***Simulium* (*Gomphostilbia*) *songense* Takaoka, Srisuka & Fukuda, sp. nov.**
http://zoobank.org/C45AE92B-5925-45D9-B64F-E3DC2BF7D170
Fig. 21

Material examined. *Holotype*: Female (thorax for DNA analysis) (with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium songense* male, QSBG col. no. 60, Thailand, 25-VII-2017, by W. Srisuka", collected from a small stream (width 80 cm, depth 2.5, fast flow, pH 7.2, 20.1 °C, partially shaded, elevation 1,157 m, 19°11'10.3"N, 101°04'41.7"E), at Nam Dan Village, Pua District, Nan Province, northern Thailand, 25-VII-2017, by W. Srisuka (Coll. No. 60).

***Paratype*:** One female (thorax for DNA analysis) (with its associated pupal exuviae and cocoons) (in 80% ethanol), collected from a small stream (width 40 cm, depth 3.5 cm, bed sandy, moderate flow, pH 7.36, 21.9 °C, partially shaded, elevation 582 m, 18°45'30.2"N, 100°20'11.4"E), at Chao Wa Waterfall, Song District, Phrae Province, Thailand, 16-III-2017, by W. Srisuka (Coll. No.164).

Diagnosis. Female: mandible without distinct teeth on the outer margin (Fig. 21A). Pupa: short stalk of the ventral pair of gill filaments being half the length of the interspiracular trunk (Fig. 21B).

Description. Female (*N* = 2). Body length 1.9 mm.

Head. Frontal ratio 1.9:1.0:2.6; frons:head ratio 1.0:4.9. Labrum 0.65 times length of clypeus. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.1:2.6; sensory vesicle 0.26–0.29 times length of third palpal segment. Lacinia with nine or ten inner and 13 outer teeth. Mandible (Fig. 21A) with 19 or 20 inner teeth and lacking distinct teeth, though outer margin undulated, appearing to have three weakly developed teeth at some distance from tip.

Legs. Foreleg: basitarsus moderately dilated, 5.5 times as long as its greatest width. Midleg: tarsus dark brown to brownish black though basal half of basitarsus dark yellow or light brown (its border not well defined). Hind leg: coxa light brown); tibia yellowish white on basal two-thirds and light brown to brownish black on rest; tarsus brownish black except basal seven-tenths (though base light brown) and basal half of second tarsomere yellowish white; basitarsus 6.2 times as long as wide, and 0.7 and 0.5 times as wide as greatest widths of tibia and femur, respectively; calcipala nearly as long as width at base, and 0.6 times as wide as greatest width of basitarsus; claw with large basal tooth 0.46 times length of claw.

Wing. Length 2.0 mm.

Abdomen. Dorsal surface of abdomen medium to dark brown except most of segment 2 ochreous (though narrowly darkened along posterior margin).

Terminalia. Sternite 8 bare medially, with 28– 31 medium-long to long hairs together with three or four slender short hairs on each side. Ovipositor valves each moderately covered with microsetae interspersed with three to four short hairs. Paraproct in ventral view with four or five sensilla on anteromedial surface; paraproct in lateral view 0.5 times as long as wide, with 29–32 medium-long to long hairs on ventral and lateral surfaces. Cercus in lateral view 0.5 times as long as wide.

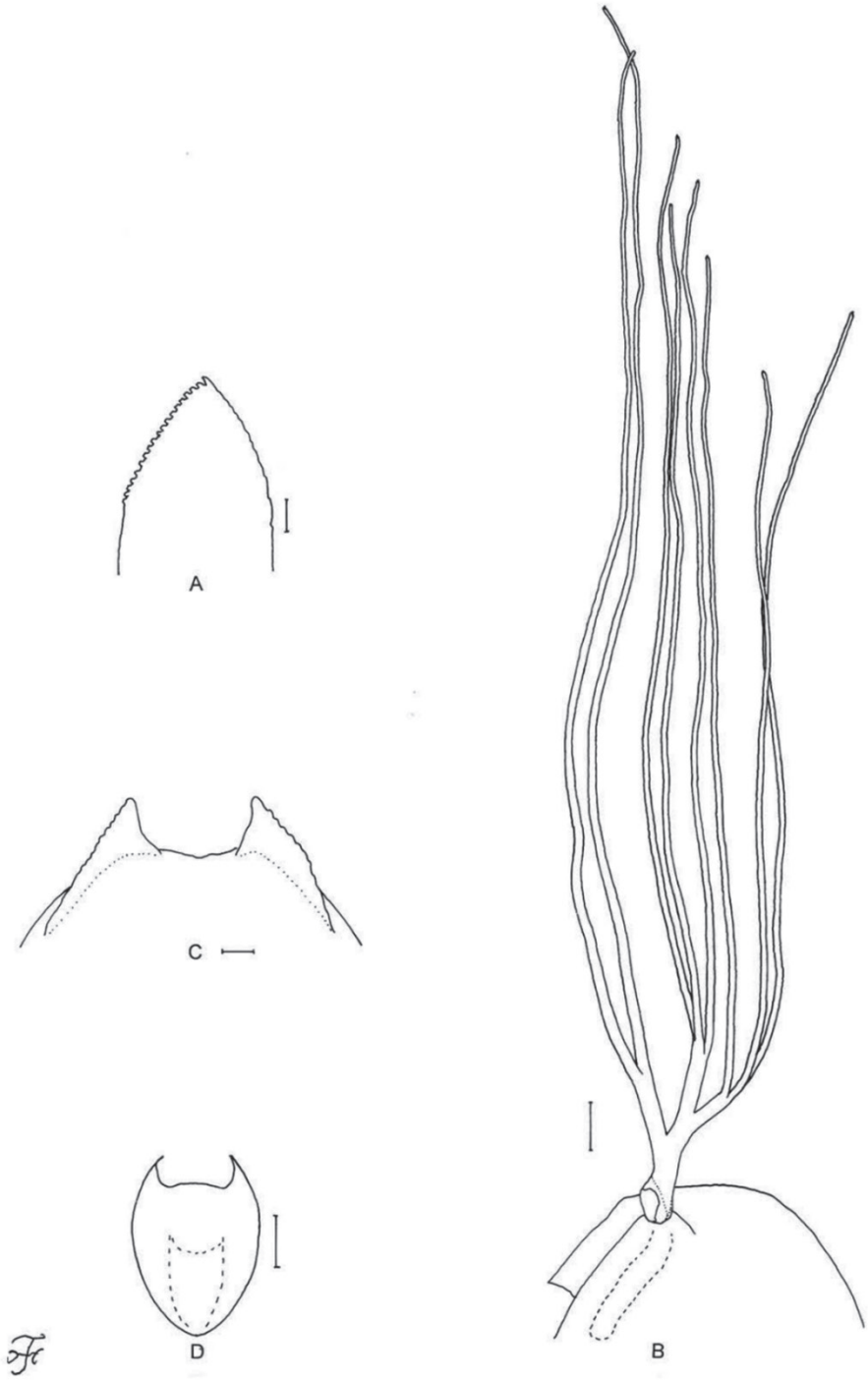

Figure 21. Female and pupa of *S. songense* sp. nov. **A** female **B–D** pupa. **A** mandible (right side) **B** gill filaments (left side; lateral view) **C** terminal hooks (caudal view) **D** cocoon (dorsal view). Scale bars: 1.0 mm (**D**); 0.1 mm (**B**); 0.01 mm (**A, C**).

Pupa (*N* = 2). Body length 3.0 mm.

Head. Integument yellow.

Thorax. Integument yellow, moderately covered with round tubercles except dorsolateral surface of posterior half sparsely covered with tubercles. Gill (Fig. 21B) composed of eight slender thread-like filaments, arranged as [3+(1+2)]+2 from dorsal to ventral; common basal stalk 0.7–0.8 times length of interspiracular trunk; stalk of ventral pair of filaments 0.6–0.8 times length of common basal stalk, and 0.5 times length of interspiracular trunk; primary stalk of dorsal triplet lying against that of lower pair at angle of 70° when viewed laterally; filaments of dorsal triplet subequal in length (1.8–2.0 mm) and thickness to one another; filaments of middle triplet subequal in length (2.1–2.2 mm) and thickness to one another; two filaments of ventral pair subequal in length (2.4–2.5 mm) and thickness to each other and 1.4–1.7 times as thick as six other filaments of dorsal and middle triplets when compared basally; all filaments yellow to light brown, gradually tapered toward apex; cuticle of all filaments with well-defined annular ridges and furrows though becoming less marked apically, densely covered with minute tubercles.

Abdomen. Dorsally, all segments light yellowish; segments 1 and 2 without tubercles; segment 9 with pair of wide flat terminal hooks (Fig. 21C), of which outer margin 2.5–2.6 times length of inner margin and crenulated when viewed caudally.

Cocoon (Fig. 21D). Pale whitish yellow, slipper-shaped, moderately woven, widely extended ventrolaterally; anterior margin thickly woven medially, without bulge or short projection; individual threads visible only on peripheral portions; 3.4 mm long by 2.4 mm wide.

Male and **mature larva.** Unknown.

Etymology. The species name, *songense*, refers to the district, Song, one of the two localities where this species was collected.

Distribution. Thailand (Nan and Phrae).

Discussion. Lacking teeth on the outer margin of the mandible, the female of this new species seems to be most similar to *S. myanmarense* and *S. monglaense* from Myanmar (Takaoka et al. 2017b), but is distinguished from the latter two species by the relative length of the fore basitarsus against its greatest width (5.5 in this new species versus 6.0 in *S. myanmarense* and 6.7 in *S. monglaense*).

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

http://zoobank.org/1D6E82E6-C730-4C82-9809-6F526A9851FB

Figs 22, 25E

Material examined. *Holotype*: Male (with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium klonglanense* male, QSBG col. no. 144, Thailand, 27-VI-2013, by W. Srisuka", collected from a stream of Klong Nam Lai (width 1.4 m, depth 13 cm, bed sandy, moderate flow, pH 6.2, 25.8 °C, exposed to the sun, elevation 196 m, 16°12'28.3"N, 99°15'47.8"E), at Klong Lan District, Kham Phaeng Phet Province, Thailand, 27-VI-2013, by W. Srisuka (Coll. No. 144).

***Paratypes*:** Three females, one male (thorax for DNA analysis) (with their associated pupal exuviae and cocoons), and eight mature larvae (in 80% ethanol), same data as for holotype.

Diagnosis. Female: sensory vesicle elongated (Fig. 22A) and mandible with four distinct teeth on the outer margin (Fig. 22B). Male: upper-eye (large) facets in 13 or 14 vertical columns and 14 or 15 horizontal rows on each side, and moderately widened hind basitarsus 0.9 times as wide as the hind tibia and femur. Pupa: small terminal hooks (Fig. 22K). Larva: postgenal cleft 3.7–4.0 times as long as the postgenal bridge (Fig. 22M) and abdominal segments 1–4 grey (Fig. 25E).

Description. Female (*N* = 3). Body length 1.8 mm.

Head. Frontal ratio 1.8:1.0:2.2; frons:head ratio 1.0:4.2. Labrum 0.59 times length of clypeus. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.0:2.1; sensory vesicle (Fig. 22A) elongated (0.65–0.68 times length of third palpal segment). Lacinia with 10 inner and 14–16 outer teeth. Mandible (Fig. 22B) with 27 inner teeth and four outer teeth at some distance from tip.

Legs. Foreleg: basitarsus moderately dilated, 5.2–5.4 times as long as its greatest width. Midleg: tarsus light to medium brown though little less than basal half of basitarsus yellow (its border not well defined). Hind leg: coxa light brown; tibia yellowish white on basal two-thirds and light to dark brown on rest; tarsus medium brown except basal two-thirds (though base light brown) and basal half of second tarsomere yellowish white; basitarsus 5.8–6.6 times as long as wide, and 0.7 and 0.6 times as wide as greatest widths of tibia and femur, respectively.

Wing. Length 1.6 mm.

Abdomen. Dorsal surface of abdomen medium to dark brown except most of segment 2 ochreous (though narrow portion along posterior margin somewhat darkened).

Terminalia. Sternite 8 with 17 medium-long to long hairs together with four slender short hairs on each side. Genital fork (Fig. 22C) of usual inverted-Y form, with slender stem; inner margins of arms divergent from each other. Paraproct in ventral view with 3–5 sensilla on anteromedial surface; paraproct in lateral view 0.6 times as long as wide, with 16 medium-long to long hairs on ventral and lateral surfaces. Cercus in lateral view short, rounded posteriorly, 0.5 times as long as wide. Spermatheca 1.44 times as long as its greatest width; both accessory ducts slender, subequal in diameter to each other and slightly wider than major one.

Male (*N* = 2). Body length 2.0 mm.

Head. Somewhat wider than thorax. Upper eye medium brown, consisting of large facets in 13 or 14 vertical columns and 14 or 15 horizontal rows on each side. Antenna: first flagellomere elongate, 1.7 times length of second. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.2:2.7; sensory vesicle (Fig. 22D) small, ellipsoidal (0.25–0.27 times length of third palpal segment).

Thorax. Scutum medium to dark brown except anterolateral calli ochreous, with three dark-brown longitudinal vittae (one medial and two submedial), white pruinose except three longitudinal vittae non-pruinose when illuminated anterodorsally and viewed dorsally. Scutellum medium brown. Postnotum medium to dark brown.

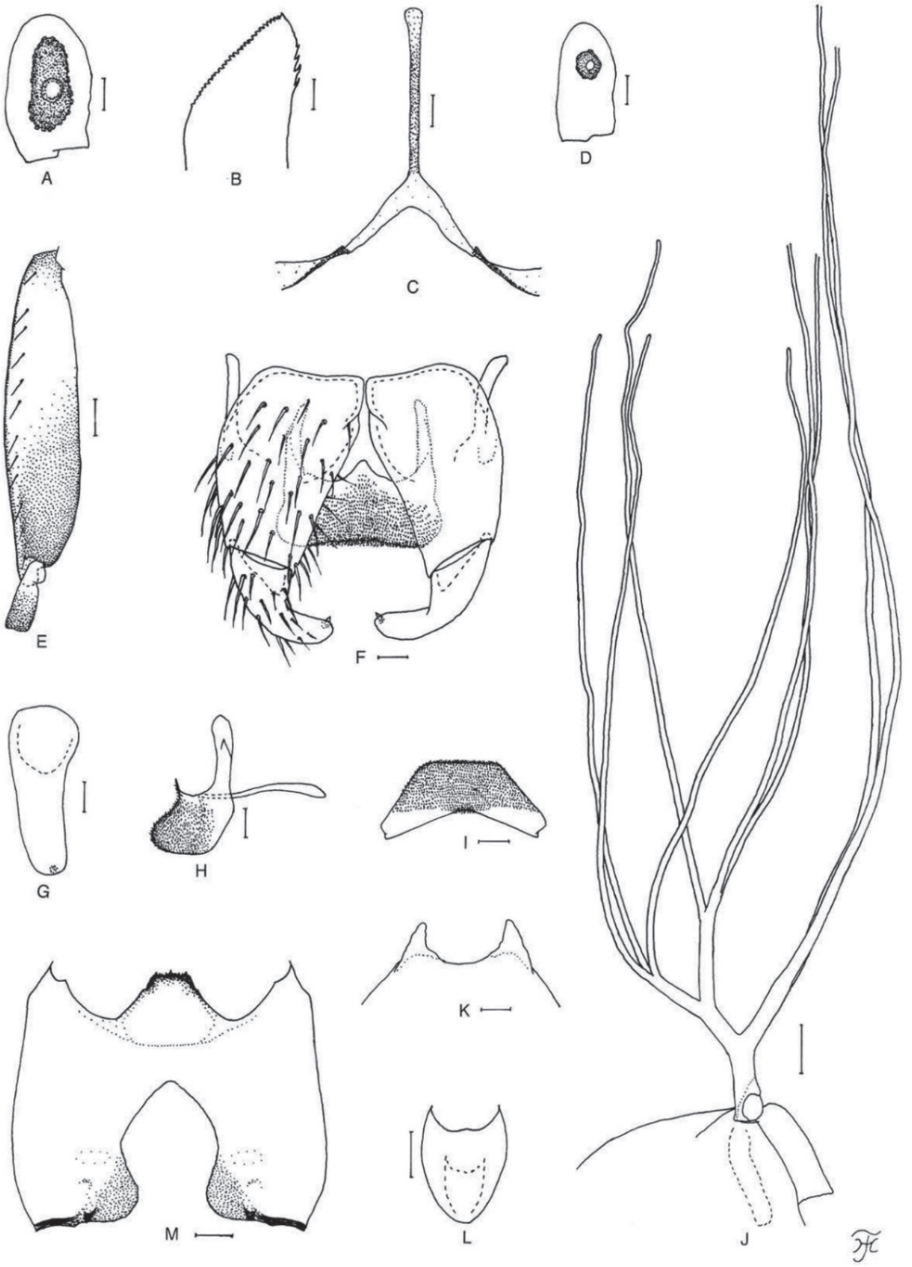

Figure 22. Female, male, pupa and larva of *S. klonglanense* sp. nov. **A–C** female **D–I** male **J–L** pupa **M** larva. **A, D** sensory vesicles (right side; anterior view **A** female **D** male) **B** mandible (right side) **C** genital fork **E** hind basitarsus and second tarsomere (left side; lateral view) **F** coxites, styles and ventral plate (ventral view) **G** style (right side; ventrolateral view) **H** ventral plate and median sclerite (lateral view) **I** ventral plate (caudal view) **J** gill filaments (right side; lateral view) **K** terminal hooks (caudal view) **L** cocoon (dorsal view) **M** head capsule (ventral view). Scale bars: 1.0 mm (**L**); 0.1 mm (**E, J, M**); 0.02 mm (**A, C, D, F–I**); 0.01 mm (**B, K**).

Legs. Foreleg: basitarsus slightly dilated, 6.8 times as long as its greatest width. Hind leg: coxa light brown; tibia dark brown except little less than basal half whitish yellow; tarsus (Fig. 22E) medium brown except little less than basal half of basitarsus and little less than basal half of second tarsomere whitish yellow; basitarsus (Fig. 22E) enlarged, 4.2 times as long as wide, and 0.9 and 0.9 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 22E) slightly shorter than basal width, and 0.21 times as wide as greatest width of basitarsus.

Wing. Length 1.5 mm. Subcosta with 0–2 hairs.

Genitalia. Style in ventral view (Fig. 22F) bent inward, with round apex having single spine; style in ventrolateral view (Fig. 22G) slightly tapered toward apex, with round apex. Ventral plate in ventral view (Fig. 22F): basal arms of moderate length, nearly parallel-sided, then slightly convergent apically; ventral plate in caudal view (Fig. 22I) trapezoidal, with ventral margin nearly straight. Cercus with 12 or 13 hairs.

Pupa (N = 5). Body length 2.0–2.4 mm.

Head. Integument light yellow.

Thorax. Integument light yellow, moderately covered with round tubercles except dorsolateral surface of posterior one-third nearly bare. Gill (Fig. 22J) composed of eight slender thread-like filaments, arranged as [3+(1+2)]+2 or [(2+1)+(1+2)]+2 or [(2+1)+3]+2) from dorsal to ventral; common basal stalk 0.7–0.8 times length of interspiracular trunk; stalk of ventral pair of filaments variable in length, 0.6–1.2 times length of common basal stalk, and 0.5–0.9 times length of interspiracular trunk; primary stalk of dorsal triplet lying against that of lower pair at angle of 80–90° when viewed laterally; filaments of dorsal triplet subequal in length (1.8 mm) and thickness to one another; filaments of dorsal triplet subequal in length (1.9–2.1 mm) and thickness to one another; two filaments of ventral pair subequal in length (tips of filaments of ventral pair lost, thus not possible to measure their lengths, which are probably little more than 2.4 mm) and thickness to each other and 1.6–1.8 times as thick as six other filaments of dorsal and middle triplets when compared basally; all filaments pale yellow.

Abdomen. Dorsally, all segments unpigmented except segment 9 light yellow; segments 1 and 2 without minute tubercles; segment 9 with pair of small flat terminal hooks (Fig. 22K) when viewed caudally.

Cocoon (Fig. 22L). Whitish yellow to medium brown, slipper-shaped, moderately woven, moderately extended ventrolaterally; anterior margin moderately woven medially, with or without bulge; individual threads visible or not visible; 2.4–3.0 mm long by 1.7–2.1 mm wide.

Mature larva (N = 8). Body length 3.3–4.0 mm. Body with following color markings: thoracic segment 1 encircled with grey (or greyish ochreous) band (though disconnected ventromedially), thoracic segments 2 and 3 grey or ochreous on ventral surface; abdominal segments 1–4 each encircled with greyish band, abdominal segments 5–8 greyish on dorsal and dorsolateral surfaces; abdominal segment 4 with or without reddish purplish transverse band (though often partially faded medially and ventrally), abdominal segments 5–8 each faintly to moderately overlaid with reddish purplish pigments dorsally and dorsolaterally (though faded medially to various extent), abdominal segments 5 and

6 each with pair of small grey or reddish purplish spots ventrally, and abdominal segment 7 with grey transverse band (overlaid with reddish purplish pigment) ventrally (Fig. 25E).

Head. Head capsule yellow except eye-spot region whitish; head spots indistinct. Antenna: proportional lengths of first, second, and third articles 1.0:0.8:0.8–1.0. Labral fan with 30–34 primary rays. Postgenal cleft (Fig. 22M) long, arrowhead shaped, 3.7–4.0 times length of postgenal bridge.

Abdomen. Rectal organ compound, each of three lobes with six to eight finger-like secondary lobules. Anal sclerite of usual X-form, with anterior arms nearly as long as posterior ones. Posterior circlet with 71–74 rows of hooklets with up to 14 hooklets per row.

Etymology. The species name, *klonglanense*, refers to the district, Klong Lan, where this species was collected.

Distribution. Thailand (Kham Phaeng Phet).

Discussion. This new species is similar to *S. lurauense* described from Peninsular Malaysia (Takaoka et al. 2011b) in many characters including the elongate female sensory vesicle, presence of teeth on the outer margin of the female mandible, and similar number of male upper-eye facets. However, it is distinguished from the latter species by the relative length of the female sensory vesicle against the third palpal segment (0.65–0.68 in this new species versus 0.50–0.54 in *S. lurauense*), and male hind basitarsus (0.9 times as wide as the hind tibia and femur in this new species versus 0.8 times as wide as the hind tibia and 0.7–0.8 times as wide as the hind femur in *S. lurauense*).

This new species is similar to *S. quychauense* Takaoka & Chen from Vietnam, which is known only for the male and pupa (Takaoka et al. 2017a) in having a similar number of male upper-eye (large) facets and small pupal terminal hooks. However, it is barely distinguished from the latter species by the ventral margin of the ventral plate nearly straight when viewed posteriorly (somewhat convex ventrally in *S. quychauense*) and pupal abdominal segment 9 with spine-combs (without spine-combs in *S. quychauense*).

This new species is similar to *S. thituyenae* Takaoka & Pham from Vietnam, which is known only for the female and pupa (Takaoka et al. 2015), but is barely distinguished from the latter species by the wing length (1.6 mm in this new species versus 2.0 mm in *S. thituyenae*), number of the outer teeth of the female mandible (four in this new species versus eight in *S. thituyenae*), and the angle of the stalk of the dorsal triplet of the pupal gill against that of the ventral pair of filaments (80–90° in this new species versus 60° in *S. thituyenae*).

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

http://zoobank.org/DA8262B8-729B-4579-B4FC-218F4A476899
Figs 23, 25F

Material examined. ***Holotype*:** Male (with its associated pupal exuviae and cocoon) (in 80% ethanol) labeled as "Holotype: *Simulium namdanense* male, QSBG col. no. 59, Thailand, 25-VII-2017, by W. Srisuka", collected from a medium-sized stream of Nam Khwang (width 4 m, depth 30 cm, bed sandy, fast flow, pH 7.2, 19.5 °C, par-

tially shaded, elevation 1,192 m, 19°11'18.3"N, 101°04'43.7"E), at Nam Dan Village, Pua District, Nan Province, Thailand, 25-VII-2017, by W. Srisuka (Coll. No. 59).

***Paratypes*:** Three females, two males (with their associated pupal exuviae and cocoons), and five mature larvae (in 80% ethanol), same data as for holotype; two females, four males (thorax of one male for DNA analysis) (with their associated pupal exuviae and cocoons), and two mature larvae (one mature larva for DNA analysis) (in 80% ethanol), collected from a medium-sized stream (width 3 m, depth 32 cm, bed sandy and rocky, moderate flow, pH 6.5, 23.2 °C, partially shaded, elevation 503 m, 18°48'44.2"N, 98°56'21.3"E), at Huai Kaew Waterfall, Doi Suthep, Muang District, Chiang Mai Province, Thailand, 30-VII-2017, by W. Srisuka (Coll. No. 94).

Diagnosis. Female: elongate sensory vesicle (Fig. 23A). Male: higher number of upper-eye facets in 15 or 16 vertical columns and 16 or 17 horizontal rows, and hind basitarsus (Fig. 23D) spindle-shaped, narrower than the hind tibia and femur. Pupa: small terminal hooks (Fig. 23K). Larva: postgenal cleft 2.5 times as long as the postgenal bridge (Fig. 23L) and abdominal segments 1–4 each encircled with a light greyish band (Fig. 25F).

Description. Female (*N* = 5). Body length 1.8–2.0 mm.

Head. Frons dark brown, densely covered with yellowish white scale-like recumbent short hairs (no dark longer hairs); frontal ratio 1.8–1.9:1.0:2.2–2.4; frons:head ratio 1.0:4.0–4.1. Labrum 0.59–0.63 times length of clypeus. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:0.9:1.9–2.0; sensory vesicle (Fig. 23A) elongate, (0.64–0.68 times length of third palpal segment), with medium-sized opening. Lacinia with 13 or 14 inner and 16–21 outer teeth. Mandible with 30–32 inner teeth and three to five outer teeth at some distance from tip.

Legs. Foreleg: trochanter light brown; basitarsus moderately dilated, 5.7–5.8 times as long as its greatest width. Midleg: trochanter dark yellow to light brown. Hind leg: coxa light brown; tibia yellowish white on basal half or little more and light brown to brownish black on rest; basitarsus yellowish white except base and little less than apical one-third dark brown, 6.1–6.4 times as long as wide, and 0.7 and 0.5–0.6 times as wide as greatest widths of tibia and femur, respectively; calcipala nearly as long as width at base, and 0.45–0.56 times as wide as greatest width of basitarsus; claw with large basal tooth 0.47–0.52 times length of claw.

Wing. Length 2.0 mm.

Abdomen. Dorsal surface of abdomen medium to dark brown except most of segment 2 (except narrow portion along posterior margin darkened) ochreous.

Terminalia. Sternite 8 with 18–26 medium-long to long hairs together with two or three slender short hairs on each side. Ovipositor valve moderately covered with microsetae interspersed with two to four short hairs. Genital fork (Fig. 23C) with inner margins of arms divergent from each other (not convergent posteriorly). Paraproct with four or five sensilla on anteromedial surface, 0.5 times as long as wide when viewed laterally, and with 20–23 short long hairs on ventral and lateral surfaces. Cercus in lateral view 0.44 times as long as wide. Spermatheca 1.55 times as long as its greatest width; both accessory ducts slender, subequal in diameter to each other and slightly thicker than major one.

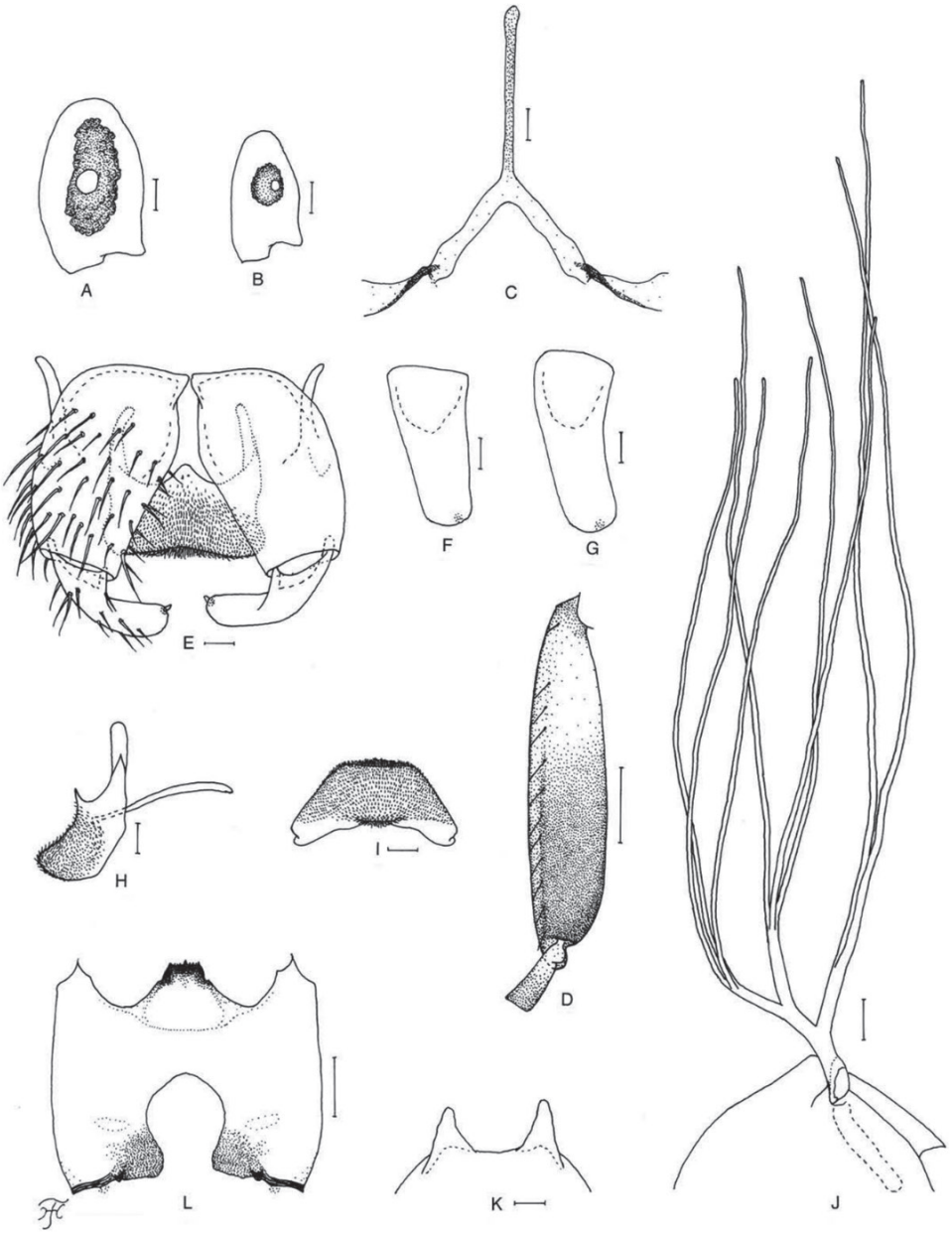

Figure 23. Female, male, pupa and larva of *S. namdanense* sp. nov. **A, C** female **B, D–I** male **J, K** pupa **L** larva. **A, B** sensory vesicles (right side; anterior view **A** female **B** male) **C** genital fork **D** hind basitarsus and second tarsomere (left side; lateral view) **E** coxites, styles and ventral plate (ventral view) **F, G** styles (right side; ventrolateral view) **H** ventral plate and median sclerite (lateral view) **I** ventral plate (caudal view) **J** gill filaments (right side; lateral view) **K** terminal hooks (caudal view) **L** head capsule (ventral view). Scale bars: 0.1 mm (**D, J, L**); 0.02 mm (**A–C, E–I**); 0.01 mm (**K**).

Male (*N* = 7). Body length 2.0 mm.

Head. Slightly wider than thorax. Upper eye medium brown, consisting of large facets in 15 or 16 vertical columns and 16 or 17 horizontal rows on each side. Antenna: first flagellomere elongate, 1.6–1.8 times length of second. Maxillary palpus light brown, with five palpal segments, proportional lengths of third, fourth, and fifth palpal segments 1.0:1.1–1.3:2.7; third palpomere (Fig. 23B) somewhat enlarged; sensory vesicle (Fig. 23B) medium sized, ellipsoidal (0.33 times length of third palpal segment).

Legs. Foreleg: femur light brown except apical cap medium brown (though apical tip yellowish); tibia light brown except median large portion of outer surface whitish and little less than apical one-third dark brown; basitarsus moderately dilated, 6.5–6.9 times as long as its greatest width. Hind leg: coxa light to medium brown; trochanter whitish yellow; femur light to medium brown with base whitish yellow and apical cap dark brown (though apical tip yellow); tibia dark brown to brownish black except little less than basal half yellow; tarsus (Fig. 23D) dark brown except little less than basal half of basitarsus (though its border not well defined due to covering of dark hairs throughout its length) greyish white and basal half of second tarsomere yellow; basitarsus (Fig. 23D) 4.2–4.4 times as long as wide, and 0.8 and 0.8–0.9 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 23D) as long as basal width, and 0.32 times as wide as greatest width of basitarsus.

Wing. Length 1.8–1.9 mm. Subcosta bare or with one to three hairs.

Genitalia. Coxite in ventral view (Fig. 23E) 1.6 times as long as its greatest width. Style in ventral view (Fig. 23E) with round apex; style in ventrolateral view (Fig. 23F, G) slightly tapered or parallel-sided from middle toward apex, with round apex. Ventral plate in ventral view (Fig. 23E) 0.54 times as long as wide; basal arms nearly parallel-sided, then convergent apically; ventral plate in caudal view (Fig. 23I) trapezoidal, though ventral margin slightly concave medially. Parameres each with three distinct long stout hooks and one short hook. Cercus with 13–16 hairs.

Pupa (*N* = 12). Body length 2.2–2.5 mm.

Head. Integument yellow. Thorax. Integument yellow, moderately covered with round tubercles except posterior half sparsely covered with minute tubercles on each dorsolateral and lateral surface.

Thorax. Gill (Fig. 23J) composed of eight slender thread-like filaments, arranged as [3+(1+2)]+2 (or rarely [(2+1)+(1+2)]+2 or [(2+1)+3]+2 or (3+3)+2) from dorsal to ventral, with medium-long common basal stalk; common basal stalk 0.8 times length of interspiracular trunk; stalk of ventral pair of filaments 0.7–1.1 times length of common basal stalk, and 0.5–0.8 times length of interspiracular trunk; primary stalk of dorsal triplet lying against that of lower pair at angle of 70–90° when viewed laterally; in one pupal exuviae with intact gill filaments measured, filaments of dorsal triplets subequal in length (1.8 mm) and thickness, those of middle triplet subequal in length (2.0 mm) and thickness, and two filaments of ventral pair subequal in length (2.3–2.5 mm) and thickness to each other; two filaments of ventral pair 1.6–2.0 times as thick as six other filaments of dorsal and middle triplets when compared basally; all filaments light brown.

Abdomen. Dorsally, all segments light yellow, or unpigmented except segments 1, 2 and 9 light yellow; segments 1 and 2 bare; segment 9 with pair of conical terminal hooks (Fig. 23K).

Cocoon. Whitish yellow, slipper-shaped, thinly woven, moderately extended ventrolaterally; anterior margin not thickly woven medially, with no bulge; 2.5–3.0 mm long by 1.5–2.0 mm wide.

Mature larva (*N* = 6). Body length 3.9–4.2 mm. Body with following color markings: thoracic segments 1–3 ochreous; abdominal segments 1–4 each encircled with light grey band, abdominal segments 5–8 light grey, overlaid with reddish brown pigment on dorsal and dorsolateral surfaces (though often irregularly faded to varying extent dorsomedially) (Fig. 25F); abdominal segments 5 and 6 each with pair of small round light grey or reddish brown spots ventrally, and abdominal segment 7 with faint light grey or reddish brown transverse band ventrally.

Head. Head capsule yellow, moderately covered with unpigmented minute setae (though sparsely on lateral and ventral surfaces); head spots faintly positive (rarely negative or indistinct). Antenna: proportional lengths of first, second, and third articles 1.0:0.7–0.8:0.8–0.9. Labral fan with 32–36 primary rays. Hypostoma: anterior row of nine teeth, of which median tooth somewhat longer than each lateral tooth, with four or five hypostomal bristles per side lying nearly parallel to lateral margin. Postgenal cleft (Fig. 23L) arrow-head-shaped, medium-long, 2.5 times length of postgenal bridge, usually with apical margin round.

Thorax and ***Abdomen.*** Thoracic and abdominal cuticle almost bare except abdominal segments 5–8 moderately covered with unpigmented and slightly-darkened unbranched minute setae on dorsal and dorsolateral surface; last abdominal segment moderately covered with unpigmented minute setae on each side of anal sclerite to base of ventral papillae. Rectal organ compound, each of three lobes with 8–10 finger-like secondary lobules. Anal sclerite with anterior arms 1.1 times as long as posterior ones. Posterior circlet with 72–84 rows of hooklets with up to 13 or 14 hooklets per row.

Etymology. The species name, *namdanense*, refers to the name of the locality, Nam Dan, where this species was collected.

Distribution. Thailand (Chiang Mai and Nan).

Discussion. This new species is similar to *S. lurauense* described from Peninsular Malaysia (Takaoka et al., 2011b) in many characters including the elongate female sensory vesicle and presence of teeth on the outer margin of the female mandible, male hind basitarsus spindle-shaped, and pupal abdominal segment 9 with a pair of small terminal hooks. However, it is barely distinguished from the latter species in the female by the sensory vesicle 0.64–0.68 times the length of the third maxillary palpal segment (0.50–0.54 times in *S. lurauense*) and in the male by the upper-eye facets in 15 or 16 vertical columns and 16 or 17 horizontal rows (14 or 15 vertical columns and 14 or 15 horizontal rows in *S. lurauense*) and the sensory vesicle 0.33 times the length of the third maxillary palpomere (0.25–0.29 times in *S. lurauense*), and in the pupa by the stalk of the ventral pair of filaments 0.5–0.8 times as long as the interspiracular trunk (0.9–1.0 times in. *S. lurauense*).

This new species is almost indistinguishable from *S. thituyenae* from Vietnam (Takaoka et al. 2015), though there appears to be a slight difference in the number of the outer teeth of the female mandible, which is three to five in this new species and eight in *S. thituyenae*. Further comparison is needed when the male and mature larva of *S. thituyenae* become available.

This new species is distinguished from *S. klonglanense* sp. nov. by the female wing length (2.0 mm in this new species versus 1.6 mm in *S. klonglanense* sp. nov.), and number of male upper-eye facets in 15 or 16 vertical columns and 16 or 17 horizontal rows (13 or 14 vertical columns and 14 or 15 horizontal rows in *S. klonglanense* sp. nov.).

Simulium (*Gomphostilbia*) *myanmarense* Takaoka, Srisuka & Saeung, 2017

Simulium (*Gomphostilbia*) *myanmarense* Takaoka, Srisuka & Saeung, in Takaoka et al. 2017b: 40–45 (female, male, pupa, and larva).

Specimens examined. 8 females, 6 males (with their associated pupal exuviae and cocoons), and 20 mature larvae (one mature larva for DNA analysis) (in 80% ethanol), collected from a small stream (width 55 cm, depth 3 cm, bed sandy, moderate flow, pH 7.2, 18.4 °C, partially shaded, elevation 901 m, 19°28'18.3"N, 100°28'00.8"E), at Pha Dang Village, Chiang Kham District, Pha Yao Province, Thailand, 20-III-2018, by W. Srisuka (Coll. No. 75); one female, one male (with their associated pupal exuviae and cocoons), and two mature larvae (in 80% ethanol), collected from a small stream (width 30 cm, depth 5 cm, bed sandy, moderate flow, pH 6.3, 23.6 °C, exposed to the sun, elevation 1,097 m, 18°50'03.7"N, 99°22'32.2"E), at Pa Miang Village, Muang Pan District, Lampang Province, Thailand, 9-VIII-2016, by W. Srisuka (Coll. no. 86).

Diagnosis. Female: mandible lacking outer teeth. Male: upper-eye facets in 15 (rarely 14) vertical columns and 15 (rarely 16) horizontal rows on each side. Pupa: cocoon with a short anterodorsal projection. Larva: medium-sized postgenal cleft and abdominal segments 1 and 2 each encircled with a grey transverse band (Fig. 25L).

Distribution. Myanmar and Thailand (Phayao and Lampang).

Discussion. Jomkumsing et al. (2019) suggested the presence in Thailand of *S. myanmarense* and *S. monglaense*, both originally described from Myanmar (Takaoka et al. 2017b), based on the close similarity of the COI gene sequences among females of '*S. asakoae* complex' caught using a human attractant. Our study confirmed the distribution of *S. myanmarense* in Thailand, based on morphological and molecular evidence.

Simulium (*Gomphostilbia*) *inthanonense* Takaoka & Suzuki, 1984

Figs 24, 25S, T

Simulium (*Gomphostilbia*) *inthanonense* Takaoka & Suzuki, 1984: 18–21 (female, pharate male, pupa, and larva).

Remarks. This species was described from females, pharate males, pupae and larvae collected from Doi Inthanon National park, Chiang Mai Province (Takaoka and Suzuki 1984), and was placed in the *S. ceylonicum* species group (Takaoka 2012).

This species is here transferred from the *S. ceylonicum* species group to the *S. asakoae* species group, based on the male ventral plate emarginated on both sides when viewed ventrally (Fig. 24F), though the dark tuft hairs on the base of the radial vein of the female and the male depart from the definition of the species group (Takaoka 2012). An analysis of the COI gene sequences supports this transfer (Fig. 26). The descriptions of the female, pupa and larva are revised, and the male is fully described for the first time.

Specimens examined. Ten females, 10 males (with their associated pupal exuviae and cocoons) (in 80% ethanol), collected from a small stream (width 40 cm, depth 10 cm, bed sandy, moderate flow, pH 7.2, 15.3 °C, partially shaded, elevation 1,314 m, 18°31'01.9"N, 98°28'17.3"E), at Mae Klang Watershed, Doi Inthanon National Park, Mae Cham District, Chiang Mai Province, Thailand, 21-XII-2018, by W. Srisuka (Coll. No. 25); five females and five males and five mature larvae (two mature larvae for DNA analysis) (in 80% ethanol) collected in a stream (width 1.0 m, depth 20 cm, bed sandy, flow moderate, partially shaded, pH 6.8, 14.0 °C, elevation 1,589 m, 18°30'29.8"N, 98°30'37.4"E), at Mae Aum, Doi Inthanon National Park, Chom Thong District, Chiang Mai Province, Thailand, 25-I-2019, by W. Srisuka (Coll. No. 20). One male (thorax for DNA analysis) (with its associated pupal exuviae and cocoon) (in 80% ethanol), collected from a stream (width 70 cm, depth 10 cm, pH 6.5, 13.8 °C, moderate flow, bed sandy, partially shaded, elevation 1,685 m, 18°31'15.4"N, 98°29'59.4"E), before check point 2, Doi Inthanon National parks, Chiang Mai Province, northern Thailand, 20-III-2018, by W. Srisuka (Coll. No. 6).

Diagnosis. Female and male: darkened hair tuft at the base of the radial vein and relatively long sensory vesicle (Fig. 24A, D). Female: mandible with several distinct teeth on the outer margin (Fig. 24B), and hind tibia yellowish on the basal half. Male: greater number of large upper-eye facets in 17 vertical columns and 17 (rarely 18) horizontal rows on each side, subcosta without hairs, and hind basitarsus (Fig. 24E) narrower than the hind tibia and femur. Pupa: dorsal surface of abdominal segments 1–5 light greyish brown. Larva: postgenal cleft (Fig. 24L) as long as or little shorter than the postgenal bridge and abdominal segments 1 and 3 (or 1–3) greenish grey (Fig. 25S, T). Two forms of this species are designated, based on larvae: morph 1 has abdominal segments 1–3 greenish grey (Fig. 25S) and morph 2 has abdominal segments 1 and 3 greenish grey (Fig. 25T).

Description. Female (*N* = 15). Body length 2.0–2.1 mm.

Head. Frontal ratio 1.6–1.8:1.0:2.3–2.8; frons:head ratio 1.0:4.7–5.1. Labrum 0.55–0.61 times length of clypeus. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:0.9–1.0:2.1–2.2; sensory vesicle (Fig. 24A) elongate, 0.58–0.61 times length of third palpal segment. Lacinia with 10–13 inner and 15–17 outer teeth. Mandible (Fig. 24B) with 28–30 inner teeth and four to six outer teeth at some distance from tip.

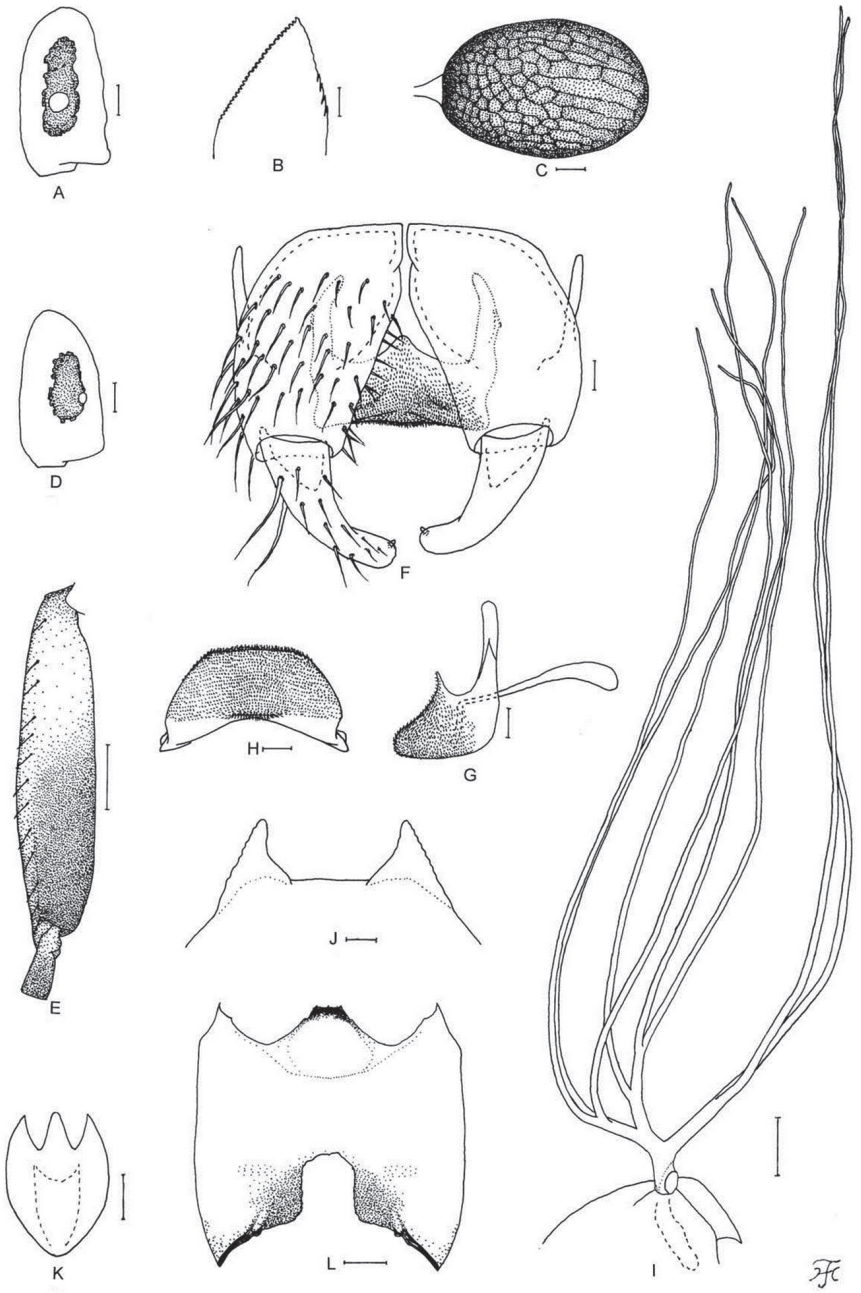

Figure 24. Female, male, pupa and larva of *S. inthanonense*. **A–C** female **D–H** male **I–K** pupa **L** larva. **A, D** sensory vesicles (right side; anterior view **A** female **D** male) **B** mandible (right side) **C** spermatheca **E** hind basitarsus and second tarsomere (left side; lateral view) **F** coxites, styles and ventral plate (ventral view) **G** ventral plate and median sclerite (lateral view) **H** ventral plate (caudal view) **I** gill filaments (right side; lateral view) **J** terminal hooks (caudal view) **K** cocoon (dorsal view) **L** head capsule (ventral view). Scale bars: 1.0 mm (**K**); 0.1 mm (**E, I, L**); 0.02 mm (**A, C, D, F–H**); 0.01 mm (**B, J**).

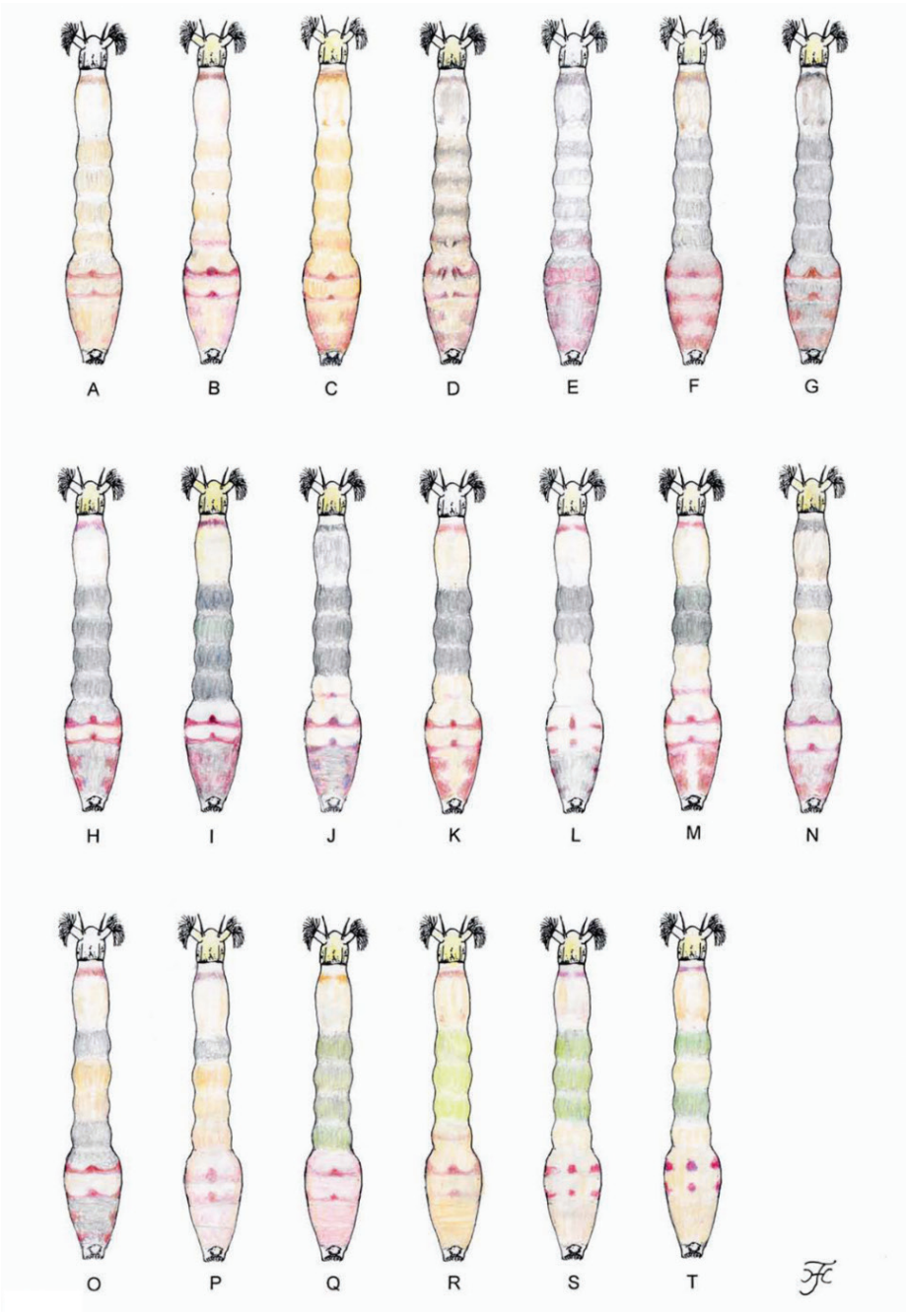

Figure 25. Schematic illustrations of larval body color patterns (dorsal view). **A** *S. teerachanense* sp. nov. **B** *S. pitasawatae* sp. nov. **C** *S. sutheppuiense* sp. nov. **D** *S. asakoae* **E** *S. klonglanense* sp. nov. **F** *S. namdanense* sp. nov. **G** *S. chaowaense* sp. nov. **H** *S. loeiense* sp. nov. **I** *S. huaimorense* sp. nov. **J** *S. nanoiense* sp. nov. **K** *S. junkumae* sp. nov. **L** *S. myanmarense* **M** *S. maewongense* sp. nov. **N** *S. phapeungense* sp. nov. **O** *S. muangpanense* sp. nov. **P** *S. maehongsonense* sp. nov. **Q** *S. thungchangense* sp. nov. **R** *S. chiangdaoense* **S** *S. inthanonense* (morph 1) **T** *S. inthanonense* (morph 2).

Legs. Foreleg: basitarsus moderately dilated, 6.2–6.4 times as long as its greatest width. Hind leg: coxa medium brown; basitarsus 7.1–7.2 times as long as wide, and 0.6–0.7 and 0.5–0.6 times as wide as greatest widths of tibia and femur, respectively; calcipala nearly as long as width at base, and 0.6 times as wide as greatest width of basitarsus; claw with large basal tooth 0.43 times length of claw.

Wing. Length 2.1–2.4 mm. Costa with dark spinules and hairs. Hair tuft on base of radius dark brown.

Abdomen. Dorsal surface of abdomen medium to dark brown except most of segment 2 whitish yellow.

Terminalia. Sternite 8 with 16–25 medium-long to long hairs together with three or four slender short hairs on each side. Ovipositor valves each covered with microsetae interspersed with three or four short hairs. Paraproct with five to eight sensilla on anteromedial surface; paraproct in lateral view with 26–32 medium-long to long hairs on ventral and lateral surfaces. Cercus in lateral view short, slightly rounded posteriorly, 0.5 times as long as wide. Spermatheca (Fig. 24C) ellipsoidal, 1.4–1.5 times as long as its greatest width, and with many fissures nearly entirely on outer surface in some females.

Male (*N* = 16). Body length 2.3–2.6 mm.

Head. Slightly wider than thorax. Upper eye medium brown, consisting of large facets in 17 vertical columns and 17 (rarely 18) horizontal rows on each side. Antenna medium to dark brown except scape, pedicel and base of first flagellomere yellow, though apical half or two-thirds of pedicel light brown in some males; first flagellomere 1.7 times length of second. Maxillary palpus: proportional lengths of third, fourth, and fifth palpal segments 1.0:1.2:2.6; third palpomere (Fig. 24D) somewhat enlarged; sensory vesicle (Fig. 24D) medium-long, 0.40–0.47 times length of third palpal segment.

Legs. Foreleg: coxa yellow; trochanter light brown; femur medium brown; light brown except apical one-third dark brown and median large portion on outer surface white and shiny; basitarsus moderately dilated, 6.9–7.7 times as long as its greatest width. Midleg: trochanter light brown except base yellow; femur medium brown with base yellowish and apical cap dark brown (though apical tip yellow); tarsus light to dark brown. Hind leg: coxa medium brown; trochanter yellow to dark yellow; tarsus (Fig. 24E) brownish black except basal two-fifths of basitarsus and basal one-third of second tarsomere dark yellow to light brown; basitarsus (Fig. 24E) 4.2–4.4 times as long as wide, and 0.8–0.9 and 0.8–0.9 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 24E) slightly shorter than basal width, and 0.35 times as wide as greatest width of basitarsus.

Wing. Length 2.1–2.3 mm. Other characters as in female except subcosta bare. *Halter.* Light to medium brown except lower portion whitish.

Genitalia. Coxite in ventral view (Fig. 24F) nearly rectangular, 1.7–1.8 times as long as its greatest width. Style in ventrolateral view slightly tapered toward apex, with round apex. Ventral plate in ventral view (Fig. 24F) with basal arms nearly parallel-sided, then convergent apically; ventral plate in caudal view (Fig. 24H) with ventral margin nearly straight. Cercus with 12–14 hairs.

Pupa (*N* = 31). Body length 2.5–3.0 mm.

Head. Integument yellow.

Thorax. Integument yellow, with two somewhat darkened areas in tandem on dorsal surface of posterior half. Gill (Fig. 24I) composed of eight slender thread-like filaments, arranged as [(2+1)+(1+2)]+2 from dorsal to ventral; common basal stalk 0.6–0.7 times length of interspiracular trunk; dorsal and middle triplets sharing short stalk, and composed of one individual and two paired filaments; stalk of ventral pair of filaments 1.3–1.7 times length of common basal stalk, and 0.9–1.1 times length of interspiracular trunk; primary stalk of dorsal triplet lying against that of ventral pair at angle of 80–120° when viewed laterally; filaments of dorsal triplet subequal in length (2.2–3.2 mm) and thickness to one another; those of middle triplets subequal in length (2.8–3.8) and thickness to one another, two filaments of ventral pair subequal in length (3.5–4.2 mm) and thickness to each other and 1.1–1.5 times as thick as six other filaments of dorsal and middle triplets when compared basally.

Abdomen. Dorsally, segments 1–5 light greyish brown, segment 9 and bases of spine-combs of segments 6–8 light yellow; segments 1 and 2 without minute tubercles; segment 9 with pair of triangular terminal hooks (Fig. 24J), of which outer margin 1.3–1.7 times length of the inner margin and weakly crenulated when viewed caudally.

Cocoon (Fig. 24K). Yellow to dark brown, slipper-shaped, moderately woven, widely extended ventrolaterally; anterior margin thickly woven medially and with short projection (its length usually 1.0 mm or slightly more); 3.2–3.9 mm long by 1.6–3.0 mm wide.

Mature larva (*N* = 3). Body length 5.6–6.0 mm. Body light ochreous with following color markings: thoracic segment 1 encircled with distinct reddish brown band (though disconnected ventromedially), thoracic segments 2 and 3 dark ochreous on ventral surface; abdominal segments 1 and 3 (or abdominal segments 1–3) greenish grey; abdominal segments 5 and 6 each with three distinct, reddish brown spots (one round dorsomedial spot and two dorsolateral spots) (Fig. 25S, T).

Head. Head spots faintly to moderately positive. Antenna: proportional lengths of first, second, and third articles 1.0:1.0–1.1:0.8–0.9. Labral fan with 40 primary rays. Hypostoma with four to six hypostomal bristles per side lying nearly parallel to or slightly diverginf from lateral margin. Postgenal cleft (Fig. 24L) short, nearly quadrate, as long as or little shorter than postgenal bridge.

Thorax and ***Abdomen.*** Thoracic and abdominal cuticle sparsely covered with unpigmented minute setae. Rectal organ compound, each of three lobes with 9–13 finger-like secondary lobules. Anal sclerite with anterior arms nearly as long as posterior ones. Posterior circlet with 82–87 rows of hooklets with up to 15 hooklets per row.

Distribution. Thailand (Chiang Mai), China and Vietnam.

Simulium (*Gomphostilbia*) *chiangdaoense* Takaoka & Srisuka, 2009

Simulium (*Gomphostilbia*) *chiangdaoense* Takaoka and Srisuka 2009: 269–276 (female, male, and pupa) (Takaoka and Srisuka 2009).

Specimens used for DNA analysis. One male and two mature larvae, collected from a small stream (width 55 cm, depth 3 cm, bed sandy, moderate flow, pH 7.2, 18.4 °C,

partially shaded, elevation 901 m, 19°28'18.3"N, 100°28'00.8"E), at Pha Tea Do mountain, Mae Cham, Chiang Mai Province, northern Thailand, 27-VI-2018, by W. Srisuka (Coll. No. 82).

***Simulium* (*Gomphostilbia*) *asakoae* Takaoka & Davies, 1995**

Simulium (*Gomphostilbia*) *asakoae* Takaoka and Davies 1995: 55–60 (female, male pupa, and larva).

Specimens used for DNA analysis. One female, reared from a pupa collected from a stream (width 30 cm, depth 5 cm, bed sandy, moderate flow, pH 6.3, 23.6 °C, exposed to the sun, elevation 1,097 m, 18°50'03.7"N, 99°22'32.2"E), at Pa Miang Village, Chae Hom, Lampang Province, Thailand, 9-VIII-2016, by W. Srisuka (Coll. No. 86); one male, reared from a pupa collected from a stream (width 5 m, depth 25 cm, pH 6.9, 22.1 °C, moderate flow, bed sandy, partially shade, elevation 759 m, 16°19'01.2"N, 99°06'19.9"E) near Tao Dam Waterfall, Klong Lan, Kam Phaeng Phet Province, Thailand, 20-XII-2016, by W. Srisuka (Coll. No. 145); one female and one male, reared from pupae collected from a stream (width 2.5 m, depth 30 cm, moderate flow, bed sandy, partially shaded, pH 5.9, 19 °C, elevation 1,293 m, 16°58'49.5"N, 101°03'29.3"E) at Rangkla Village, Nakhon Thai District, Phitsanulok Province, Thailand, 18-VI-2019, by W. Srisuka (Coll. No. 200); one female reared from a pupa and one mature larva collected from a stream (width 50 cm, depth 16 cm, moderate flow, bed sandy, pH 7.3, 21 °C, elevation 1,231 m, 18°32'16.3"N, 98°31'30.5"E) at Khun Klang Village, Doi Inthanon National Park, Chiang Mai Province, northern Thailand, 24-VII-2019, by W. Srisuka (Coll. No. 201).

Keys to identify the 27 Thai species of the *S. asakoae* species group

Female[*]

1	Hair tuft of base of radial vein brownish	***S. inthanonense***
–	Hair tuft of base of radial vein yellow	**2**
2	Sensory vesicle elongate, more than 0.5 times length of third palpal segment (Fig. 22A)	**3**
–	Sensory vesicle short to medium-long, less than 0.4 times length of third palpal segment (Fig. 1A)	**4**
3	Wing length 1.6 mm	***S. klonglanense* sp. nov.**
–	Wing length 2.0 mm	***S. namdanense* sp. nov.**
4	Mandible with distinct teeth on outer margin (Fig. 22B)	**5**
–	Mandible without distinct teeth on outer margin (Fig. 14A)	**16**

* The females of *S. banluangense* sp. nov., *S. kiewfinense* sp. nov., *S. phapeungense* sp. nov., *S. rampae* and *S. teerachanense* sp. nov. are unknown.

5 Body length 1.9–2.2 mm **6**
– Body length 2.4–2.6 mm **14**
6 Claw tooth 0.50–0.53 times length of claw (Fig. 1E) **7**
– Claw tooth 0.43–0.47 times length of claw **11**
7 Claw tooth 0.53 times length of claw ..... ***S. udomi***
– Claw tooth 0.50 times length of claw **8**
8 Sensory vesicle 0.22–0.24 times length of third palpal segment (Fig. 12A) .... ***S. nanoiense* sp. nov.**
– Sensory vesicle 0.26–0.32 times length of third palpal segment **9**
9 Labrum 0.6 times as long as clypeus ..... ***S. sutheppuiense* sp. nov.**
– Labrum 0.7 times as long as clypeus **10**
10 Fifth palpal segment 2.6 times as long as third ..... ***S. loeiense* sp. nov.**
– Fifth palpal segment 2.1 times as long as third ..... ***S. maelanoiense* sp. nov.**
11 Sensory vesicle 0.24 times length of third palpal segment ... ***S. puaense* sp. nov.**
– Sensory vesicle 0.26–0.35 times as long as third palpal segment **12**
12 Sensory vesicle 0.32–0.35 times as long as third palpal segment ..... ***S. pitasawatae* sp. nov.**
– Sensory vesicle 0.26–0.29 times length of third palpal segment **13**
13 Spermatheca 1.3 times as long as wide ..... ***S. huaimorense* sp. nov.**
– Spermatheca 1.6 times as long as wide ..... ***S. nanthaburiense* sp. nov.**
14 Labrum 0.54–0.56 times length of clypeus ..... ***S. asakoae***
– Labrum 0.61–0.68 times length of clypeus **15**
15 Claw tooth 0.50 times length of claw (Fig. 1E) ... ***S. thungchangense* sp. nov.**
– Claw tooth 0.47 times length of claw ..... ***S. chiangdaoense***
16 Sensory vesicle 0.36 times as long as third palpal segment; claw tooth 0.56 times as long as claw ..... ***S. maewongense* sp. nov.**
– Sensory vesicle 0.25–0.32 times as long as third palpal segment; claw tooth 0.46–0.50 times as long as claw **17**
17 Fore basitarsus 5.5 times as long as its greatest width **18**
– Fore basitarsus 5.9–6.5 times as long as its greatest width **19**
18 Labrum 0.52 times as long as clypeus ..... ***S. maehongsonense* sp. nov.**
– Labrum 0.65 times as long as clypeus ..... ***S. songense* sp. nov.**
19 Hind basitarsus 6.5 times as long as its greatest width ..... ***S. myanmarense***
– Hind basitarsus 5.9–6.2 times as long as its greatest width **20**
20 Frons: head ratio 1.0:4.7–5.5 ..... ***S. junkumae* sp. nov.**
– Frons: head ratio 1.0:4.0 ..... ***S. muangpanense* sp. nov.**

Males[*]

1 Hair tuft of base of radial vein brownish ..... ***S. inthanonense***
– Hair tuft of base of radial vein yellow **2**

* The male of *S. songense* sp. nov. is unknown.

2 Upper-eye (large) facets vermilion **3**
– Upper-eye (large) facets medium to dark brown **4**
3 Upper-eye (large) facets in 9 or 10 vertical columns and 12 horizontal rows; fore basitarsus 8.4–8.7 times as long as its greatest width ..... ***S. thungchangense* sp. nov.**
– Upper-eye (large) facets in 11 or 12 vertical columns and 13 or 14 horizontal rows; fore basitarsus 7.1–7.4 times as long as its greatest width .... ***S. asakoae***
4 Antenna yellowish except few apical flagellomeres slightly to somewhat greyish ..... ***S. teerachanense* sp. nov.**
– Antenna brownish except scape, pedicel and base of first flagellomere yellowish **5**
5 Hind basitarsus 0.9 time as wide as hind femur (Fig. 7A) **6**
– Hind basitarsus 1.0–1.3 times as wide as hind femur (Fig. 4C) **11**
6 Ventral plate with ventral margin rounded when viewed caudally (Fig. 17D) ... **7**
– Ventral plate with ventral margin nearly straight medially when viewed caudally (Fig. 20F) **9**
7 Fore basitarsus 6.4 times as long as its greatest width ..... ***S. banluangense* sp. nov.**
– Fore basitarsus 7.4–8.8 times as long as its greatest width **8**
8 Wing length 1.6–1.7 mm ..... ***S. chaowaense* sp. nov.**
– Wing length 2.0 mm ..... ***S. myanmarense***
9 Upper-eye (large) facets in 13 vertical columns ... ***S. muangpanense* sp. nov.**
– Upper-eye(large) facets in 14–16 vertical columns **10**
10 Upper-eye (large) facets in 14 vertical columns ..... ***S. klonglanense* sp. nov.**
– Upper-eye (large) facets in 15 or 16 vertical columns... ***S. namdangense* sp. nov.**
11 Upper-eye (large) facets in 9 or 10 vertical columns ..... ***S. puaense* sp. nov.**
– Upper-eye (large) facets in 11 or more vertical columns **12**
12. Upper-eye (large) facets in 11 vertical columns **13**
– Upper-eye (large) facets in 12–17 vertical columns **16**
13 Head nearly as wide as thorax **14**
– Head slightly wider than thorax **15**
14 Ventral plate rounded ventrally when viewed caudally (Fig. 7D) ..... ***S. maewongense* sp. nov.**
– Ventral plate with ventral margin nearly straight when viewed caudally (Fig. 8E) ..... ***S. loeiense* sp. nov.**
15 First antennal flagellomere 1.8–1.9 times as long as second ..... ***S. sutheppuiense* sp. nov. and *S. maelanoiense* sp. nov.**
– First antennal flagellomere 1.5–1.6 times as long as second ..... ***S. phapeungense* sp. nov.**
16 Upper-eye (large) facets in 16 or 17 vertical columns **17**
– Upper-eye (large) facets in 12–14 vertical columns **19**
17 Subcosta bare or with two hairs; wing length 1.9–2.0 mm ..... ***S. huaimorense* sp. nov. and *S. kiewfinense* sp. nov.**
– Subcosta with 7–12 hairs; wing length 2.2–2.5 mm **18**

18 Ventral plate with ventral margin nearly straight when viewed caudally (Fig. 18I) ..... ***S. junkumae* sp. nov.**
– Ventral plate with ventral margin rounded when viewed caudally... ***S. rampae***
19 Upper-eye (large) facets in 13 or 14 horizontal rows **20**
– Upper-eye (large) facets in 15 or 16 horizontal rows **22**
20 Ventral plate with ventral margin rounded when viewed caudally (Fig. 12G) ..... ***S. nanoiense* sp. nov.**
– Ventral plate with ventral margin nearly straight when viewed caudally (Fig. 11F)..... **21**
21 Upper-eye (large) facets in 12 vertical columns.... ***S. nanthaburiense* sp. nov.**
– Upper-eye (large) facets in 13 vertical columns... ***S. maehongsonense* sp. nov.**
22 Ventral plate with ventral margin nearly straight when viewed caudally (Fig. 16F)..... ***S. pitasawatae* sp. nov.**
– Ventral plate with ventral margin rounded when viewed caudally .... ***S. chiangdaoense* and *S. udomi***

Pupae

1 Cocoon with elongate anterodorsal projection (2.0 mm or little longer) extended far beyond anteroventral tips of cocoon..... **2**
– Cocoon with or without anterodorsal bulge or short anterodorsal projection (up to ca. 1.0 mm long) extended at most up to anteroventral tips of cocoon..... **3**
2 Gill with six filaments..... ***S. udomi***
– Gill with eight filaments ..... ***S. chiangdaoense***
3 Dorsum of abdominal segments 1 and 2 or 1–3 or 1–5 light to medium brown or light greyish brown..... **4**
– Dorsum of abdominal segments 1 and 2 unpigmented or light yellowish ... **5**
4 Dorsum of abdominal segments 1 and 2 or 1–3 light to medium brown; cocoon with short bulge..... ***S. asakoae***
– Dorsum of abdominal segments 1–5 light greyish brown; cocoon with short projection (Fig. 24K) ..... ***S. inthanonense***
5 Dorsum of abdominal segment 1 sparsely covered with minute tubercles ... **6**
– Dorsum of abdominal segment 1 without minute tubercles..... **7**
6 Outer margin of terminal hook 2.2 times length of inner margin (Fig. 8G).. ..... ***S. loeiense* sp. nov.**
– Outer margin of terminal hook 2.7 times length of inner margin (Fig. 3B) .. .. ***S. thungchangense* sp. nov.**
7 Cocoon with short anterodorsal projection (Figs 7G, 20I)..... **8**
– Cocoon without anterodorsal projection..... **9**
8 Primary stalks of dorsal and middle triplets of gill filaments much shorter than their common stalk (Fig. 20G) ..... ***S. huaimorense* sp. nov.**
– Primary stalks of dorsal and middle triplets of gill filaments nearly as long as their common stalk (Fig. 7E) ..... ***S. maewongense* sp. nov. and *S. myanmarense***

9 Terminal hooks conical, with outer margin as long as or slightly longer than inner margin and not crenulated (Figs 22K, 23K)..... ..... ***S. klonglanense* sp. nov. and *S. namdanense* sp. nov.**

– Terminal hooks widened, plate-like, with outer margin over 1.5 times length of inner margin, and crenulated..... **10**

10 Stalk of ventral pair thicker than interspiracular trunk (Fig. 17E, F) .... ***S. banluangense* sp. nov.**

– Stalk of ventral pair thinner than interspiracular trunk **11**

11 Gill arranged as 3+2+(2+1) or 2+2+(2+1) from dorsal to ventral, with long common basal stalk 1.3–1.5 times length of interspiracular trunk ...***S. rampae***

– Gill arranged as (3+3)+2 from dorsal to ventral, with medium-long common basal stalk shorter than interspiracular rrunk..... **12**

12 Outer margin of terminal hook 2.0–2.9 times as long as inner margin .... ***S. chaowaense*, sp. nov., *S. junkumae* sp. nov., *S. maelanoiense* sp. nov., *S. muangpanense* sp. nov., *S. nanoiesne* sp. nov., *S. phapeungense* sp. nov., *S. puaense* sp. nov., *S. songense* sp. nov., *S. sutheppuiense* sp. nov. and *S. teerachanense* sp. nov.**

– Outer margin of terminal hook 3.3–4.0 times as long as inner margin **13**

13 Filaments of ventral pair 2.1–2.3 mm long (Fig. 14G)..... ..... ***S. maehongsonense* sp. nov.**

– Filaments of ventral pair 2.5–3.0 mm long (Fig. 16G) ...***S. pitasawatae* sp. nov.**

Larvae[*]

1 Abdominal segments 1–4 unicolorous **2**

– Abdominal segments 1–4 not unicolorous **12**

2 Abdominal segments 1–4 light ochreous (Fig. 25A, B, C)..... **3**

– Abdominal segments 1–4 greyish or greenish grey or greenish **5**

3 Postgenal cleft 2.8–3.3 times as long as postgenal bridge (Fig. 6I)..... ..... ***S. teerachanense* sp. nov.**

– Postgenal cleft 1.2–2.4 times as long as postgenal bridge (Fig. 16K)..... **4**

4 Labral fan with 23 or 24 primary rays..... ***S. pitasawatae* sp. nov.**

– Labral fan with 29 or 30 primary rays..... ***S. sutheppuiense* sp. nov.**

5 Pharate pupal gill with elongate common basal stalk..... ***S. rampae***

– Pharate pupal gill with medium-long common basal stalk **6**

6 Postgenal cleft 1.5–4.0 times length of postgenal bridge 7

– Postgenal cleft 0.8–1.4 times length of postgenal bridge **9**

7 Head spots moderately positive..... ***S. asakoae***

– Head spots indistinct or faintly positive **8**

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* The larvae of *S. banluangense* sp. nov. *S. nanthaburiense* sp. nov., *S. kiewfinense* sp. nov., *S. maelanoiense* sp. nov., *S. puaense* sp. nov. and *S. songense* sp. nov. are unknown.

8 Postgenal cleft 2.5 times as long as postgenal bridge (Fig. 23L) ..... ***S. namdanense* sp. nov.**
– Postgenal cleft 3.7–4.0 times as long as postgenal bridge (Fig. 22M)..... ***S. klonglanense* sp. nov.**
9 Abdominal segments 5–8 pinkish (Fig. 25Q)..... ***S. thungchangense* sp. nov.**
– Abdominal color pattern otherwise **10**
10 Abdominal segments 7 and 8 light grey but abdominal segments 5 and 6 unpigmented (Fig. 25I)..... ***S. huaimorense* sp. nov.**
– Abdominal color pattern otherwise **11**
11 Abdominal segments 5, 7 and 8 grey but abdominal segment 6 unpigmented (Fig. 25H) ..... ***S. loeiense* sp. nov.**
– Abdominal segments 5, 6, 7 and 8 greyish (Fig. 25G) ... ***S. chaowaense* sp. nov.**
12 Abdominal segment 1 grey but abdominal segments 2–4 ochreous (Fig. 25P)..... ***S. maehongsonense* sp. nov.**
– Abdominal color pattern otherwise **13**
13 Abdominal segments 1 and 2 grey or green but abdominal segments 3 and 4 unpigmented **14**
– Abdominal color pattern otherwise **15**
14 Abdominal segments 7 and 8 grey with faint reddish brown pigment (Fig. 25L)..... ***S. myanmarense***
– Abdominal segments 7 and 8 not grey with distinct reddish brown pigment (Fig. 25M) ..... ***S. maewongense* sp. nov.**
15 Abdominal segments 1, 2 and 3 grey or greenish grey but abdominal segment 4 unpigmented or light ochreous (Fig. 25J, K, S)..... **16**
– Abdominal color pattern otherwise **18**
16 Abdominal segments 7 and 8 grey with reddish brown pigment (Fig. 25J).... ***S. nanoiense* sp. nov.**
– Abdominal segments 7 and 8 light ochreous (Fig. 25K, S) **17**
17 Abdominal segments 1, 2 and 3 greyish, and segments 7 and 8 with reddish brown pigment (Fig. 25K) ..... ***S. junkumae* sp. nov.**
– Abdominal segments 1, 2 and 3 greenish grey but segments 7 and 8 lacking reddish brown pigment (Fig. 25S) ..... ***S. inthanonense* (morph 1)**
18 Abdominal segments 1 and 3 greenish grey (Fig. 25T)..... ***S. inthanonense* (morph 2)**
– Abdominal color pattern otherwise **19**
19 Abdominal segments 1, 3 and 4 grey but abdominal segment 2 light ochreous (Fig. 25N)..... ***S. phapeungense* sp. nov.**
– Abdominal color pattern otherwise **20**
20 Abdominal segments 1 and 4 greyish but abdominal segments 2 and 3 light ochreous (Fig. 25O)..... ***S. muangpanense* sp. nov.**
– Abdominal segments 1–3 light green but abdominal segment 4 light ochreous (Fig. 25R)..... **21**
21 Pharate pupal gill with six filament ..... ***S. udomi***
– Pharate pupal gill with eight filaments ..... ***S. chiangdaoense***

Genetic analysis

The genetic relationships of all 26 species (exclusive of *S. banluangense* sp. nov.) of the *S. asakoae* species group in Thailand are shown in Fig. 26. All 26 species are divided into nine subgroups, I–IX, each consisting of two, one, four, nine, one, three, two, one, and three species. Genetic distances between the nine subgroups are shown in Table 1.

In subgroup I, two new species, *S. klonglanense* and *S. namdanense*, and two known species, *S. lurauense* and *S. sofiani* (Takaoka et al. 2011a, b), from Peninsular Malaysia are included. *Simulium klonglanense* sp. nov. and *S. namdanense* sp. nov. are close in their positions to each other but are distinguished by a nucleotide difference of 1.54 %. The nucleotide differences between these new species and *S. lurauense* were 2.90–3.41 % and 2.73–3.24 %, respectively.

Subgroup I is an assemblage of species that is morphologically characterized by the combination of an elongate female sensory vesicle and small pupal terminal hooks.

Subgroup II is represented only by *S. asakoae*. A similar distinct genetic status of this species within the species group was also shown by Jomkumsing et al. (2019).

None of six samples from four localities of Thailand was identical to those of *S. asakoae* from Peninsular Malaysia. The differences between six samples from Thailand and *S. asakoae* from Peninsular Malaysia were 1.22–1.98 % and 1.37–2.22 %, based on the sequences of 685 bp and 586 bp, respectively. These differences are regarded as intraspecific variation because four Thai populations examined in this study are morphologically indistinguishable from *S. asakoae* from Peninsular Malaysia. Relatively high intraspecific variation is likely to reflect a wide range of its aquatic habitats from low to high elevations (196 m to 1,610 m) in Thailand.

In subgroup III, one new species, *S. thungchangense*, and three known species, *S. chiangdaoense* (of which the COI gene sequences were newly added in this study), *S. rampae*, and *S. udomi* (Srisuka et al. 2019, Takaoka et al. 2009, Takaoka et al. 2006), are included. *Simulium thungchangense* sp. nov. and *S. udomi* have nearly identical COI sequences, although both species are morphologically different from each other by the number of pupal gill filaments (eight versus six).

In subgroup IV, nine new species and one known species, *S. brinchangense* from Peninsular Malaysia, are included. A high similarity of COI gene sequences of four new species, *S. huaimorense*, *S. junkumae*, *S. kiewfinense*, and *S. maewongense*, is observed. Notably, the first three of these new species are morphologically similar in sharing a higher number of male upper-eye large facets, suggesting that they have recently been derived from a common ancestor.

Subgroup V is represented only by *S. inthanonense*, which was formerly treated as a member of the *S. ceylonicum* species group (Takaoka 2012). The result that its genetic relationship is closer to members of the *S. asakoae* species group than to those of the *S. ceylonicum* species group supports the transfer of this species to the *S. asakoae* species group based on morphological characters.

In subgroup VI, *S. sutheppuiense* sp. nov. forms a close relationship with *S. tanahrataense* from Peninsular Malaysia, although both species differ morphologically from each other.

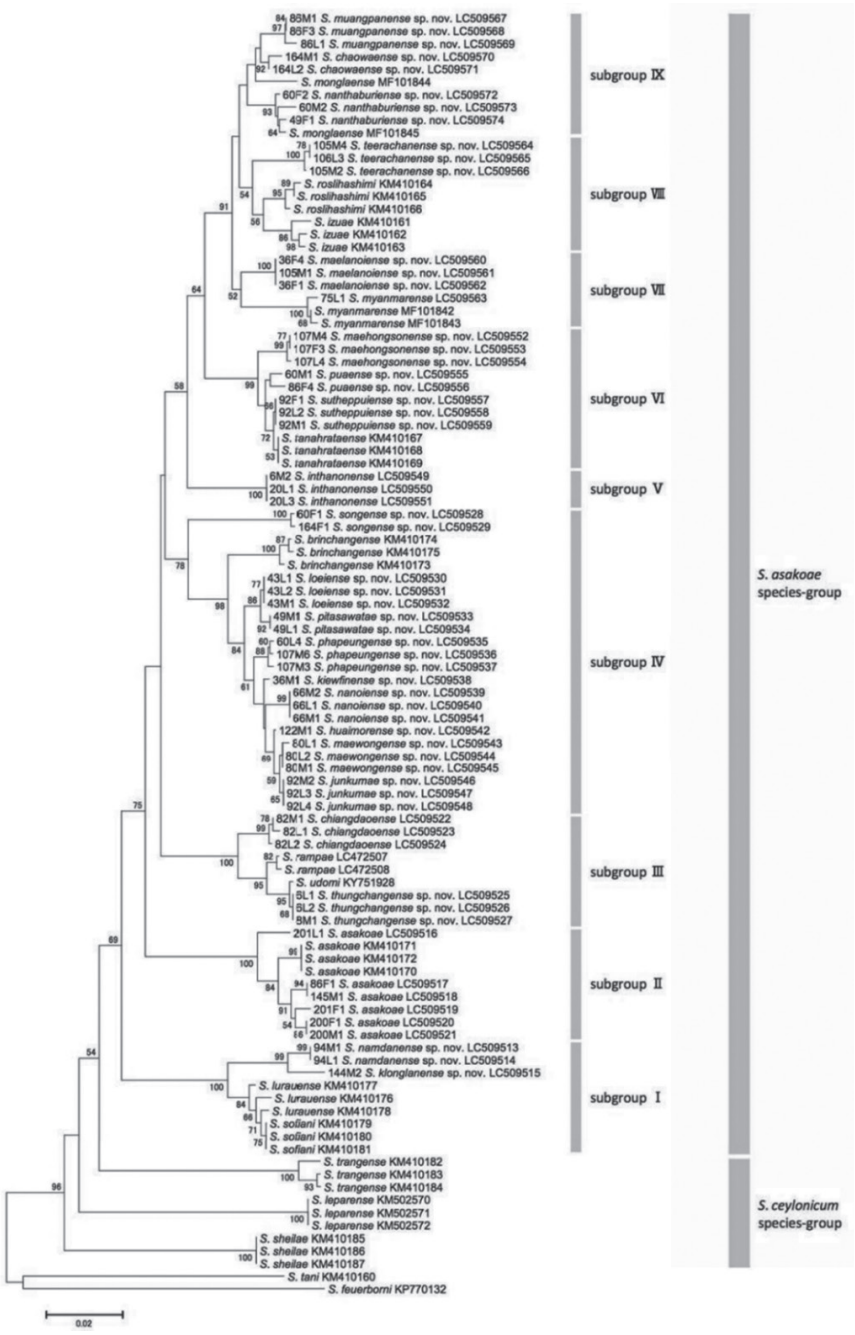

Figure 26. Neighbor-joining tree of the 26 Thai species of the *Simulium asakoae* species group based on COI gene sequences (586 positions). The numbers at the nodes mean the bootstrap confidence values after 500 replicates. The bootstrap values above 50% are shown. The scale bar indicates the distance in substitutions per nucleotide. The symbols in front of the species names are each the collection number (corresponding to those in the text), developmental stages (F, female; M, male; L, larva), and sample number (if two or more samples were examined) in this order.

Table 1. Nucleotide differences (%) among subgroups of the *Simulium asakoae* species group based on COI sequences.

	I	II	III	IV	V	VI	VII	VIII	IX
I	0–3.41								
II	7.68–9.04	0–2.22							
III	7.34–9.39	5.97–7.68	0–2.39						
IV	6.66–9.22	6.31–7.85	5.46–7.17	0–5.29					
V	6.66–7.85	5.63–6.31	5.12–5.97	5.12–6.14	0				
VI	6.31–8.87	6.48–7.34	5.80–7.85	4.95–6.66	3.92–4.78	0–4.61			
VII	7.51–9.73	7.34–8.36	5.46–7.00	5.46–7.51	4.27–4.78	3.41–5.12	0–2.90		
VIII	7.51–9.73	6.48–8.87	5.80–6.83	5.29–6.83	4.27–5.12	4.10–5.12	2.90–4.27	0–3.41	
IX	7.17–9.22	6.83–8.02	5.46–7.00	5.12–6.66	4.27–5.12	3.24–4.61	2.05–3.92	1.88–3.58	0–2.56

Subgroup VII is formed by two taxa, one being *S. maelanoiense* sp. nov. and the other being identified morphologically and genetically as *S. myanmarense*, originally described from Myanmar. *Simulium myanmarense* was suggested to exist in Thailand based on COI gene sequences of adult females (Jomkumsing et al. 2019). Here we confirmed the distribution of this species in Thailand by morphological and genetic evidence.

In subgroup VIII, one new species, *S. teerachanense*, is included together with two known species from Peninsular Malaysia, *S. roslihashimi* and *S. izuae*. S*imulium teerachanense* sp. nov. is morphologically similar to *S. roslihashimi* in sharing the yellowish male antennae. It is demonstrated that both species are genetically close to each other, with a difference of 2.05–2.56%.

In subgroup IX, three new species, *S. nanthaburiense*, *S. chaowaense*, and *S. muangpanense*, and one known species, *S. monglaense* from Myanmar, are included. *Simulium nanthaburiense* sp. nov. is almost identical to one of two haplotypes (MF101845) of *S. monglaense*, although both species are morphologically distinguished from each other by the number of male upper-eye (large) facets that are in 12 vertical columns and 13 or 14 horizontal rows in *S. nanthaburiense* sp. nov. but in 15 vertical columns and 15 horizontal rows in *S. monglaense* (Takaoka et al. 2017b). *Simulium monglaense* was suggested to exist in Thailand based on COI gene sequences of adult females (Jomkumsing et al. 2019). However, its distribution in Thailand has not been confirmed by the morphological evidence in our study. The possibility cannot be excluded that the COI gene sequences suspected as being those of *S. monglaense* (Jomkumsing et al. 2019) were actually those of *S. nanthaburiense* sp. nov.

Conclusions

Our morphological study reveals a high rate of species radiation of the *S. asakoae* species group in Thailand, as evidenced by the increased species number from four to 27. Most of these species are morphologically distinguished from one another as females, males, and larvae but not pupae, as shown in the keys. Ten of 27 species were indistinguishable in the pupal stage due to similarities of key characters such as the gill, terminal hooks and cocoon, the phenomenon being contrary to other species groups of the

subgenus *Gomphostilbia*, in which species are usually identified in the pupal stage by their distinct expression of the number and arrangement of gill filaments and/or shape of the inflated structure of the gill (Takaoka 2015).

A COI gene sequence-based analysis shows that all (but one species) in the *S. asakoae* species group in Thailand are divided into nine subgroups, of which subgroups II, V and VIII are represented by *S. asakoae*, *S. inthanonense*, and *S. teerachanense* sp. nov., respectively, and the other six subgroups are represented by two to nine species, suggesting a relatively recent radiation, accompanied by some morphological differentiation but few COI gene changes.

In the latter six subgroups, genetic differences between certain new species and known species are too small or even unrecognized, as in the cases of *S. thungchangense* sp. nov. versus *S. udomi*, *S. nanthaburiense* sp. nov. versus *S. monglaense*, and *S. sutheppuiense* sp. nov. versus *S. tanahrataense*. Certain members of the *S. asakoae* species group were previously reported to share the same or closely similar COI gene sequences, e.g., *S. udomi* versus *S. rampae* (Saeung et al. 2017, Srisuka et al. 2019), and *S. lurauense* versus *S. sofiani* (Low et al. 2015). These data indicate that certain morphospecies of the *S. asakoae* species group, such as species within subgroups I, III, IV, VI, VII, and IX, are not separable genetically from one another, at least by a COI gene sequence-based analysis.

DNA barcoding is generally useful for resolving the phylogenetic relationships among species of most species groups of the subgenus *Gomphostilbia* and for uncovering cryptic species. However, caution is required when members of the *S. asakoae* species group are identified based only on similarities of the COI gene sequences.

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