



## รายงานวิจัยฉบับสมบูรณ์

### โครงการ

การจำแนกชนิดหนอนพยาธิฟิลาเรียและแมลงรืนดำพาหะ  
ในประเทศไทยโดยวิธีทางโมเลกุล

**Molecular Identification of Filariae Larvae and  
their Black Fly Vectors in Thailand**

โดย

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พฤษภาคม 2561

## รายงานวิจัยฉบับสมบูรณ์

โครงการ การจำแนกชนิดหนอนพยาธิฟิลาเรียและ  
แมลงร้นดำพาหะในประเทศไทยโดยวิธีทางโมเลกุล

**Molecular Identification of Filariae Larvae and  
their Black Fly Vectors in Thailand**

ผู้วิจัย

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ภาควิชาปรสิตวิทยา

คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

(ความเห็นในรายงานนี้เป็นของผู้วิจัย สกว. และ สกอ. ไม่จำเป็นต้องเห็นด้วยเสมอไป)

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## บทคัดย่อ

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ชื่อโครงการ: การจำแนกชนิดหนอนพยาธิฟิลาเรียและแมลงริ้นดำพาหะในประเทศไทยโดยวิธีทางโมเลกุล

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ระยะเวลาโครงการ: 24 เดือน (ตั้งแต่วันที่ 2 พฤษภาคม พ.ศ. 2559 ถึง วันที่ 1 พฤษภาคม พ.ศ. 2561)

**บทนำ:** แมลงริ้นดำเป็นแมลงขนาดเล็ก จัดอยู่ในวงศ์ซิมูลิอิดี อันดับดิพเทอร่า ที่มีความสำคัญด้านการแพทย์และสัตวแพทย์ การกัดของแมลงริ้นดำเพศเมียนั้น เป็นปัญหาอย่างมากแก่คนและสัตว์เลี้ยง แมลงริ้นดำบางชนิดเป็นพาหะนำหนอนพยาธิฟิลาเรียมาสู่คนและสัตว์ โรคโปรโตซัวในเลือดไวรัสและแบคทีเรียในสัตว์ นอกจากนี้ มีรายงานพบผู้ป่วยโรค zoonotic onchocerciasis ในหลายประเทศ ซึ่งเกิดจากการติดเชื้อหนอนพยาธิฟิลาเรียที่พบในสัตว์เลี้ยง และติดต่อมาสู่คนโดยมีแมลงริ้นดำเป็นพาหะนำโรค อย่างไรก็ตาม องค์ความรู้เกี่ยวกับการติดเชื้อหนอนพยาธิฟิลาเรียในแมลงริ้นดำในประเทศไทยยังมีอยู่น้อยมาก ทั้งยังไม่มีการศึกษาวิจัยอย่างเป็นระบบและจริงจัง

**วิธีการทดลอง:** ทำการเก็บตัวอย่างแมลงริ้นดำในระยะตัวหนอนวัยสุดท้าย และระยะดักแด้ จากแหล่งน้ำธรรมชาติจำนวน 14 แหล่ง ใน 10 จังหวัด ซึ่งเป็นตัวแทนของ 4 ภูมิภาคของประเทศไทย ในระหว่างเดือนพฤษภาคม พ.ศ. 2559 ถึง เดือนพฤษภาคม พ.ศ. 2561 และจับแมลงริ้นดำตัวเต็มวัยเพศเมียที่มีพฤติกรรมบินเข้ามาหาคน โดยใช้สวิงโฉบจับ ในช่วงเวลาตั้งแต่ 06.00 น. ถึง 18.00 น. ที่บ้านแม่กลองคี อำเภออุ้มผาง จังหวัดตาก ภาคตะวันตกของประเทศไทย (ระดับความสูง 1,237 เมตร จากระดับน้ำทะเลปานกลาง) จากนั้นทำการผ่าตัวอย่างแมลงริ้นดำที่จำแนกชนิดแล้ว เพื่อตรวจหาการติดเชื้อหนอนพยาธิฟิลาเรีย จำแนกชนิดและวัดขนาดหนอนพยาธิฟิลาเรียตามลักษณะทางสัณฐานวิทยา ร่วมกับการใช้เทคนิคทางชีววิทยาระดับโมเลกุล โดยการวิเคราะห์ลำดับนิวคลีโอไทด์ของไมโทคอนเดรียลดีเอ็นเอ (ยีน COI และ 12S rRNA) นอกจากนี้ยังได้ทำการศึกษาลักษณะทางสัณฐานวิทยาภายนอกของแมลงริ้นดำตัวเต็มวัยเพศเมียนชนิด *Simulium* (*Simulium*) *nigrogilvum* โดยใช้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด (SEM) และการใช้เทคนิคทางชีววิทยาระดับโมเลกุล (ยีน COI, COII และ ECP1) เพื่อทำการยืนยันความแตกต่างระดับชนิดของแมลงริ้นดำชนิดนี้กับชนิด *S. (S.) umphangense* ซึ่งเป็นชนิดที่มีความคล้ายคลึงกันเป็นอย่างมาก ทั้งในด้านลักษณะทางสัณฐานและนิสัยการเข้ากัดคน ทั้งนี้ยังได้ทำการศึกษาความสัมพันธ์ทางพันธุกรรมด้วยการสร้างแผนภูมิวิวัฒนาการรูปต้นไม้ของกลุ่มแมลงริ้นดำและหนอนพยาธิฟิลาเรียที่พบในการศึกษานี้กับชนิดอื่นๆ

**ผลการทดลอง:** ในช่วงระยะเวลา 2 ปี การศึกษาได้ค้นพบแมลงร้นดำชนิดใหม่ของโลก จำนวน 14 ชนิด ที่จัดอยู่ใน 3 สกุลย่อยของแมลงร้นดำสกุล *Simulium* นอกจากนี้ ยังได้เขียนอธิบาย ลักษณะตัวเต็มวัยเพศผู้ของแมลงร้นดำชนิด *Simulium (Simulium) suchariti* และตัวเต็มวัยเพศเมียและระยะตัวหนอนของ *Simulium (Gomphostibia) udomi* เป็นครั้งแรกอีกด้วย ผลการศึกษา อวัยวะรับรู้สีกบนหนวดแมลงร้นดำตัวเต็มวัยเพศเมีย *S. (S.) nigrogilvum* โดยใช้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด พบว่ามีจำนวน 4 แบบ ได้แก่ chaetica sensilla, trichoid sensilla, basiconic sensilla และ coeloconic sensilla ในการศึกษาพบการติดเชื้อหนอนพยาธิฟิลาเรียตัวอ่อนระยะที่ 3 (ระยะติดต่อ) ในทรวงอกของแมลงร้นดำ *S. (S.) nigrogilvum* จำนวน 4 ตัว จากแมลงร้นดำทั้งหมดที่จับได้จำนวน 494 ตัว (คิดเป็นร้อยละ 0.81) โดยพบตัวอ่อนหนอนพยาธิระยะที่ 3 จำนวน 6 ตัว มีลำตัวขนาดใหญ่ (ยาว 1,100-1,300  $\mu\text{m}$  กว้าง 24-26  $\mu\text{m}$ ) และหลุดอาหารเรียวยาวและมีขนาดสั้นกว่าครึ่งหนึ่งของความยาวของลำตัว ผลการจำแนกชนิดตัวอ่อนระยะที่ 3 ด้วยลักษณะทางสัณฐานวิทยาและการใช้เทคนิคทางชีววิทยาระดับโมเลกุล พบว่าตัวอ่อนพยาธิทั้งหมดเป็นชนิด *Onchocerca* sp. type A ซึ่งตรงกับพยาธิฟิลาเรียที่พบในโคของประเทศไทย

**สรุปผลการทดลอง:** ค้นพบและรายงานแมลงร้นดำชนิดใหม่ของโลกจากประเทศไทยแล้วจำนวน 14 ชนิด ซึ่งทำให้จำนวนชนิดของแมลงร้นดำในประเทศไทยมีเพิ่มมากขึ้น โดยมีรายงานการค้นพบแล้วขณะนี้จำนวนทั้งสิ้น 107 ชนิด และเป็นครั้งแรกที่มีการศึกษาลักษณะทางสัณฐานวิทยาภายนอกในระดับโครงสร้างที่มีความละเอียดมากยิ่งขึ้นในแมลงร้นดำตัวเต็มวัยเพศเมียชนิด *S. (S.) nigrogilvum* ทำให้เข้าใจชีววิทยาของแมลงร้นดำชนิดนี้ได้ดียิ่งขึ้น ทั้งยังทำให้ข้อมูลด้านอนุกรมวิธานของแมลงร้นดำชนิดนี้มีความสมบูรณ์มากขึ้น แมลงร้นดำ *S. (S.) nigrogilvum* มีพฤติกรรมชอบกัดกินเลือดคน และพบเป็นจำนวนมากที่สุดในพื้นที่ของหมู่บ้านแม่กลองดี อำเภออุ้มผาง จังหวัดตาก ซึ่งจากผลการตรวจพบการติดเชื้อหนอนพยาธิฟิลาเรียระยะติดต่อ ทำให้ประชาชนที่อาศัยในหมู่บ้านนี้ มีแนวโน้มที่จะมีความเสี่ยงต่อการติดเชื้อหนอนพยาธิฟิลาเรียจากสัตว์

**คำหลัก:** แมลงร้นดำ หนอนพยาธิฟิลาเรีย ไมโตคอนเดรียลดีเอ็นเอ

## Abstract

**Project Code:** MRG5980101

**Project Title:** Molecular identification of filariae larvae and their black fly vectors in Thailand

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**Project Period:** 24 months (From 2 May 2016 to 1 May 2018)

**Background:** Black flies belonging to the family Simuliidae of the order Diptera, are a group of small bloodsucking insects of medical and veterinary importance. Due to the biting habit, females of certain simuliid species can transmit viruses, protozoans and filarial parasites. Numerous cases of zoonotic onchocerciasis have been caused by *Onchocerca* spp. of animal origin, as reported from several countries, but little is known about the transmission of zoonotic filarial parasites by black flies in Thailand.

**Materials and Methods:** Black fly larvae and pupae were collected from 14 stream sites in 10 provinces in four regions across Thailand during May 2016 to May 2018. Female adult flies flying around a human were collected at Mae Klong Kee village, Um-phang District, Tak Province, western Thailand (elevation 1,237 m) during the daytime from 06.00 to 18.00 hours. Adult female black flies were dissected and examined for filarial larvae. The filarial larvae were identified based on morphological and morphometric characters and analysis of sequences of mitochondrial COI and 12S rRNA gene regions. Furthermore, the external morphology of the adult female *Simulium* (*Simulium*) *nigrogilvum* was examined using scanning electron microscopy (SEM) and DNA-based assays [multi-locus approaches: mitochondrial genes (COI, COII) and nuclear gene (elongation complex protein 1, ECP1)] for species identification of *S. (S.) nigrogilvum* and its closely *S. (S.) umphangense*. The phylogenetic trees were constructed in order to determine the relationships among these black fly species as well as *Onchocerca* species.

**Results:** Fourteen new species of black flies belonging to three subgenera of the genus *Simulium* were discovered and described during 2 year periods. In addition, this study was the first to describe the male of *S. (S.) suchariti* and the female and larva of *S. (G.) udomi*. Using SEM, four types of antennal sensilla, including chaetica sensilla, trichoid sensilla, basiconic sensilla and coeloconic sensilla, were observed on flagellum of *S. (S.) nigrogilvum*. Four of 494 *S. (S.) nigrogilvum* females dissected were naturally infected with

six third-stage larvae (infective stage) (0.81%). All these infective larvae are characterized by their large body size (1,100-1,300  $\mu\text{m}$  long by 24-26  $\mu\text{m}$  wide), and elongate esophagus, which was shorter than half of the body length. All L3 were morphologically and molecularly identified as type A of *Onchocerca* sp., a parasite of cattle in Japan.

**Conclusions:** Fourteen new black fly species were discovered in our recent surveys, which led to a total 107 black-fly species in Thailand. External morphological characters of adult female *S. (S.) nigrogilvum* were studied by SEM for the first time, providing a better understanding of biological behaviours of this species, and greatly assisting in species identification. *Simulium (S.) nigrogilvum* is a main human-biter in Mae Klong Kee village, Um-phang District, Tak Province. People in this village are likely to be exposed to the risk of infection with zoonotic filariae.

**Keywords:** Black fly, Filarial worm, Mitochondrial DNA

## EXECUTIVE SUMMARY

Black flies (Diptera: Simuliidae) are biting dipteran insects of medical and veterinary importance and widely distributed worldwide. The medical and socioeconomic impact associated with black flies include reduced levels of tourism, the death of domesticated birds and mammals, and the transmission of viral, protozoan and filarial diseases. More than 25 black fly species transmit the filarial nematode, *Onchocerca volvulus*, which is the causative agent of onchocerciasis or river blindness in humans in Africa, and Central and South America. Furthermore, at least 35 cases of zoonotic onchocerciasis caused by *Onchocerca* spp. of animal origin have been reported from several countries. A biting habit of adult females can cause serious problems for humans, since it inflicts pain, localized swelling, intense itching, inflammation accompanied by intense irritation that lasts for several days or weeks, and chronic allergic dermatitis, which is caused by IgE-mediated reactions.

Prior to studies of biology, ecology and behavior of simuliid vectors, and implementation of effective control programs, accurate species identification is an essential first step for success in obtaining robust information. However, the taxonomic identification of adult black flies is relatively complicated due to identical morphology or minimal morphological distinction of sibling or cryptic species. Imperfect taxonomy could lead to misidentification of certain vector species, and may jeopardize vector control programmes. Throughout the world, several approaches have been used for species identification of black flies, such as, morphotaxonomy, cytotaxonomy and molecular methods, but only one report used scanning electron microscopy (SEM) to examine the larva cuticular patterns of black flies.

*Simulium (Gomphostilbia) asakoe*, *Simulium (Simulium) (nigrogilvum)* and *Simulium (Simulium) nodosum*, were incriminated as natural vectors of three filarial species including the genus *Onchocera*, based on the recovery of infective stages (L<sub>3</sub> larvae) from wild-caught female black flies. At present, taxonomy classification is based on the morphometric measurement of filarial larvae at the infective stage, with this now being the principal method for species identification. Nonetheless, this method has a limitation, as it cannot distinguish isomorphic filarial species. Hence, an alternative method such as a molecular-based DNA technique is needed.

Nowadays, the number of tourists visiting at northern Thailand is increasing year by year, particularly for ecotourism in forest, waterfall and high mountainous areas, like Doi

Suthep-Pui and Doi Inthanon National Park. Hence, people and animals may be at risk of infection with zoonotic filarial parasites transmitted by black fly vectors. However, the vectorial status of these three vector species and other biting species in Chiang Mai Province, northern Thailand should be revised since adult females of most of these human biting species are a species complex composed of two or more related sibling species which are morphologically indistinguishable. In particular, adult females of *S. asakoe* are so similar to those of other members of the *S. asakoe* species-group.

As pointed out by the above information, this study used the novel morphological approach through scanning electron microscopy (SEM) and DNA-based assays (multi-locus approaches: 2 mitochondrial genes: COI, COII and nuclear gene: ECP1) for species identification of human- and animal-biting black flies and mitochondrial cytochrome c oxidase subunit I (COI) gene-based DNA barcoding and 12S rRNA gene regions for the filarial larvae of *Onchocerca* spp.

The results obtained from this study provided a new body of knowledge on the prevalence of zoonotic filarial parasites and their black fly vectors, and the application of novel approaches were useful for precise species identification of black flies in Thailand and/or other countries, where these diseases exist. Accurate information is an important key necessary in forming a reliable and effective strategy for vector control measures in future.

During 2 year's operation of this project: 12 papers were published in international journals that contributing to PubMed online. Two Ph.D. students were the dynamic research assistants of this project.

## OBJECTIVES

1. To determine the vectorial roles of human- and animal-biting black fly species in zoonotic filarial transmission.
2. To identify the human- and animal-biting black fly species using scanning electron microscopy and molecular approaches.

## วิธีการทดลอง (MATERIALS AND METHODS)

### 1. Ethical approval

All experiments were performed in accordance with animal guidelines and regulations of Institute of Animal for Scientific Purposes Development (IAD), The National Research Council of Thailand (NRCT). No specific permits were required for this study, which did not involve endangered or protected species.

### 2. Study sites

Simuliid larvae and pupae were collected from 14 stream-sites in 10 provinces of Thailand during May 2016 to May 2018 (Table 1).

### 3. Collection of black flies

Collected larvae were preserved in small glass vials (for 10 ml) with 80% ethanol, whereas each pupa attached to a substrate was maintained in a plastic tube (10 cm long and 1.7 cm diameter), which contained very little water for adequate moisture until the adult fly emerged, which usually takes about 1 to 3 days. The emerged adults were maintained while alive in the same plastic tube for at least 24 hours in order to secure hardening and coloring of the body and legs, and then they were killed and kept individually in small plastic vials (for 1.5 ml) with 80% ethanol, together with their associated pupal exuvia and cocoon.

Adult female black flies were caught by an insect net while flying around human at Mae Klong Kee village, Um-phang District (elevation 1,237 m), Tak Province, western Thailand during the daytime from 06.00 to 18.00 hours. They were kept in paper cup with a pad of cotton wool soaked with 5% sucrose solution placed on the top-screen, and stored in a humid chamber. After that, they were transported to Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand, and then were processed for species identification and further experiments.

**Table 1.** Names of sampling sites, geographical coordinates and elevation of black-fly collections at 14 sampling sites in 10 provinces of Thailand

| Province       | Names of sampling sites                                               | Regions* | Latitude/Longitude           | Elevation<br>(m) |
|----------------|-----------------------------------------------------------------------|----------|------------------------------|------------------|
| Chiang Rai     | Pang Khon village, Muang District                                     | N        | 19°53'55.4" N/99°35'59.4" E  | 1,321            |
| Chiang Mai     | Mae Klang Watershed Management Unit, Chomthong District               | N        | 18°31'01.9" N/98°28'17.3" E  | 1,314            |
|                | Huai Kaeo, Muang District                                             | N        | 18°48'44.2" N/98°56'21.3" E  | 503              |
|                | Kiew Mae Pan, Chomthong District                                      | N        | 18°33'29.4" N/98°22'51.7" E  | 2,210            |
| Lampang        | Pa Miang village, Chae Hom District                                   | N        | 18°50'03.7" N/99°22'32.2" E  | 1,097            |
| Phrae          | Mae Yom, Song District                                                | N        | 19°26'55.5" N/97°59'50.1" E  | 267              |
| Nan            | Khunsathan, Nanoi District                                            | N        | 18°16'44.5" N/100°30'14.2" E | 1,316            |
| Mae Hong Son   | Mae Suai Au village, Muang District                                   | N        | 19°15'59" N/97°51'12.7" E    | 605              |
|                | Doi Chang village, Pai District                                       | N        | 19°21'00.2" N/98°35'06.4" E  | 1,737            |
| Tak            | Mae Klong Kee village, Um-phang District                              | W        | 16°13'34.2" N/98°58'47.4" E  | 1,237            |
|                | Mae Klong Yai village, Um-phang District                              | W        | 16°14'39.2" N/98°59'56.5" E  | 1,241            |
| Kamphaeng Phet | Chong Yen, Pang-sila Thong District                                   | C        | 16°05'21.2" N/99°06'47.9" E  | 1,274            |
| Phetchabun     | Ton Nampasak Research Station, Khao Kho District, Phetchabun Province | C        | 16°32'42.9" N/101°02'17.8" E | 540              |
| Surin          | Along Korn waterfall, Sang kha, District                              | NE       | 14°23'26.4" N/103°51'50.8" E | 337              |

N = northern; W = western; C = central; NE: northeastern

#### 4. Morphological description and illustration under light microscope

Comparative morphological description and illustration of last-instar larva (matured), pupa and reared adult species followed those of published articles that identified black flies in Thailand (Takaoka and Davies 1995; Adler et al. 2004; Takaoka and Choochote 2004, 2006; Takaoka and Srisuka 2009; Takaoka 2012, 2017). Description was made from the best alcohol specimens of adults, pupal exuvia and mature larvae. Additional specimens of each stage were examined also for intraspecific variations. The head, wings, legs and genitalia of each adult specimen were removed, but only the head and genitalia were cleared in 10% NaOH at room temperature for 12-16 hours. The head was placed on a glass cavity slide with distilled water, and the genitalia on a similar glass slide with glycerin. For illustrations and measurements, the length of the female labrum being shown relative to that of the clypeus. The other body parts of the adults (e.g., legs and wing) and cocoons were illustrated and measured while held on a glass cavity slide with 80% ethanol. The pupal exuviae and mature larvae were placed on a glass cavity slide with glycerin and acetic alcohol, respectively, for illustrations and measurements, using a camera lucida (Olympus U-DA) attached to a compound microscope. The cryptic morphological identification of some black-fly species was processed for molecular identification.

#### 5. Fine structure of adult female black flies revealed by scanning electron microscopy (SEM)

The ultrastructure of adult females of *S. (S.) nigrogilvum* was investigated using SEM with minor modifications to the method described by Taai et al. (2017) and Hempolchom et al. (2017). Briefly, 30 specimens of adult females were immersed in phosphate buffer in order to remove surface debris and prefixed in 2.5% glutaraldehyde mixed with phosphate buffered at 4 °C for 24 hours. Head of each female black fly was excised under a stereo microscope. All samples were then dehydrated through an ethanol series of 35, 70, 80% (10 min, two changes) and 95% (15 min, two changes), followed by absolute ethanol (10 min, two changes). Following dehydration, they were dried in a critical point dryer. The antennae were carefully dissected from the head capsule under a stereomicroscope. Antennae were mounted on aluminum stubs with double-sided carbon adhesive tape and sputter-coated with gold. Sensilla were observed and photographed in a JEOL-JSM6610LV scanning electron microscope (JEOL, Japan). The terminology and nomenclatures used to identify antennal sensilla types in this study follow previously

reported (Zacharuk 1985; Sukontason et al. 2008; Tangtrakulwanich et al. 2011; Wang et al. 2014; Liu et al. 2016).

## 6. Molecular species identification of female black flies

### 6.1 DNA extraction

Genomic DNA was extracted from individual black fly using the PureLink<sup>®</sup> Genomic DNA Mini Kit (Invitrogen, USA) according to the manufacturer's instructions. DNA concentrations were measured using the Nanodrop 8000 Spectrophotometer (Thermo Scientific, USA).

### 6.2 PCR amplifications and sequencing

Amplifications of the mitochondria-encoded COI and COII, and nuclear-encoded ECP1 genes of *S. nigrogilvum* and *S. umphangense* were performed following the methods described previously (Conflitti et al. 2012; Low et al. 2014, 2016b). Primer pairs for amplifying each gene region are listed in Table 2. Briefly, PCR reactions were conducted in a total volume of 20 µl containing 1-2 µl of genomic DNA, 0.5 U of *Taq* DNA polymerase, 1.5-3 mM of MgCl<sub>2</sub>, 0.25 mM dNTPs and 0.2 pM of each primer. The PCR cycling parameters followed those of Conflitti (2012) for COI; Low et al. (2014) for COII; and Low et al. (2016b) for ECP1. To obtain the single banded products, the annealing temperature of the ECP1 gene was varied as necessary.

After PCR amplification, the amplified products were subjected to electrophoresis on 1.5% agarose gel stained with SYBR<sup>®</sup> Safe DNA gel stain (Invitrogen, USA), and 100 bps DNA marker was used as standard. Then, PCR products were purified using the PureLink<sup>®</sup> PCR Purification Kit (Invitrogen, USA). Sequencing were performed at Macrogen (Seoul, Korea) using the same primers as in the PCR. The sequences obtained from this study were also compared with deposited sequences available through GenBank.

### 6.3 Sequence alignment and DNA analyses

Both forward and reverse sequences were assembled and edited manually using MEGA Version 7.0 program (Kumar et al. 2016). All sequences were aligned using the CLUSTAL W multiple alignment programs (Thompson et al. 1994). Gap sites were excluded from the following analyses. To determine whether the separate genes should be analyzed in combination, a partition homogeneity test (Farris et al. 1995) was calculated using the PAUP 4.0b10 (Swofford 2002), with 100 replicates. The test revealed significant differences among the three gene regions, but no significant differences were found between the two mitochondria-encoded gene regions. Thus, the aligned sequences of single genes and the

concatenated dataset (COI + COII) were used for phylogenetic analyses. Accession numbers of sequences retrieved from GenBank for the analyses were shown in phylogenetic trees. Genetic distances were estimated from the Kimura two-parameter method (Kimura 1980). Phylogenetic trees were constructed using neighbor-joining (NJ) and maximum-likelihood (ML) methods in MEGA Version 7.0 program (Kumar et al. 2016). Branch supports for both methods were calculated using bootstrap test with 1,000 replications. Sequences of *Aedes aegypti* and *Simulium quinquestriatum* were used as outgroup in phylogenetic analyses.

**Table 2.** Primers for amplifying the mitochondrial genes and nuclear genes of black flies.

| Gene and primers     | Sequences (5'–3')          | References              |
|----------------------|----------------------------|-------------------------|
| <b>Mitochondrial</b> |                            |                         |
| <i>COI</i>           |                            |                         |
| LCO1490              | GGTCAACAAATCATAAAGATATTGG  | Folmer et al. (1994)    |
| HCO2198              | TAAACTTCAGGGTGACCAAAAAATCA |                         |
| <i>COII</i>          |                            |                         |
| TL2-J-3034           | ATTATGGCAGATTAGTGCA        | Conflitti et al. (2010) |
| TK-N-3785            | GTTTAAGAGACCAGTACTTG       |                         |
| <b>Nuclear</b>       |                            |                         |
| <i>ECP1</i>          |                            |                         |
| BECP1_F              | TGCCCTCAAATATCGTCACA       | Low et al. (2016b)      |
| BECP1_R              | GGCCTTCTTCAATGTCCAAA       |                         |

## 7. Dissections of adult black flies and study of recovered nematodes

### 7.1 Morphological species identification of nematodes

After classification to species, individual adult female black fly was microscopically dissected and examined for filarial larvae in a drop of 0.85% normal saline solution on a slide glass. The filarial larva found was measured under a compound microscope (Olympus BX53, Japan). Morphological generic identification of infective larvae (third-stage larvae) recovered followed Bain and Chabaud (1986). In addition, infective larvae of *Onchocerca* species were classified into two types (A with the body width over 23 µm and esophagus

shorter than half of the body length; B with the body width narrower than 23  $\mu\text{m}$  and esophagus longer than half of the body length) (Fukuda et al. 2008; 2010).

## **7.2 Molecular species identification of nematodes**

### **7.2.1 DNA extraction of infective larvae**

Total DNA of individual infective larvae was extracted with the PureLink<sup>®</sup> Genomic DNA Mini Kit (Invitrogen, USA) according to the manufacturer's protocol. The final volume of each extracted DNA was 30-50  $\mu\text{l}$ .

### **7.2.2 PCR amplifications and sequencing**

The partial mitochondrial COI gene was amplified using a primer set: COIintF (5'-TGATTGGTGGTTTTGGTAA-3') and COIintR (5'-ATAAGTACGAGTATCAATATC-3') (Casiraghi et al. 2001). PCR was performed in a final volume of 20  $\mu\text{l}$  comprised 1-2  $\mu\text{l}$  of genomic DNA, 0.5 U of *Taq* DNA polymerase, 2-3 mM of  $\text{MgCl}_2$ , 0.25 mM dNTPs and 0.2 pM of each primer under the following conditions: an initial denaturation at 94 °C for 3 min, followed by five cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min and 37 cycles of 94 °C for 30 s, 48 °C for 30 s, and 72 °C for 1 min. The mitochondrial 12S rRNA gene was also amplified with a primer set: 12SF (5'-GTTCCAGAATAATCGGCTA-3') and 12SR (5'-ATTGACGGATG(AG)TTTGTACC-3') Casiraghi et al. (2004). Amplifications of 12S rRNA gene were conducted using the same reaction mixture as described above. The thermal profile included 40 cycles of 94 °C for 45 s, 50 °C for 45 s, and 72 °C for 90 s (Fukuda et al. 2010).

The amplified fragments were electrophoresed on 1.5% agarose gels and stained with SYBR<sup>®</sup> Safe DNA gel stain (Invitrogen, USA). Then, PCR products were purified using the PureLink<sup>®</sup> PCR Purification Kit (Invitrogen, USA). Sequencing were performed at Macrogen (Seoul, Korea) using the same primers as in the PCR. The sequences obtained from this study were also compared with deposited sequences available through GenBank.

### **7.2.3. Sequence alignment and DNA analyses**

Sequence alignment and phylogenetic analyses were performed using similar procedures as mentioned above. However, phylogenetic trees were constructed separately for each gene (COI and 12S rRNA) using neighbor-joining (NJ) and maximum-likelihood methods. Accession numbers of sequences retrieved from GenBank for the analyses were shown in phylogenetic trees.

## ผลการทดลอง (RESULTS)

### 1. Discovery of new species

Since May 2016, 14 new species of black flies which belong to three subgenera of the genus *Simulium* have been discovered, including *S. (G.) maleewongae* Takaoka, Srisuka & Saeung, *S. (S.) umphangense* Takaoka, Srisuka & Saeung, *S. (S.) srisukai* Takaoka & Saeung, *S. (S.) kiewmaepanense* Takaoka, Srisuka & Saeung, *S. (G.) fukudae* Takaoka, Srisuka & Saeung, *S. (A.) saeungae* Takaoka & Srisuka, sp. nov., *S. (G.) chiangraiense* Takaoka, Srisuka & Saeung sp. nov., *S. (G.) pamiangense* Takaoka, Srisuka & Saeung sp. nov., *S. (G.) maeklangense* Takaoka, Srisuka & Saeung sp. nov., *S. (G.) khaokhoense* Takaoka, Srisuka & Low sp. nov., *S. (G.) huaikaeoense* Takaoka, Srisuka & Low sp. nov., *S. (G.) isanense* Takaoka, Srisuka & Saeung sp. nov., *S. (S.) phraense* Takaoka, Srisuka & Saeung sp. nov., *S. (G.) paiense* Takaoka, Srisuka & Saeung sp. nov., based on the systematic investigations (see appendix, article number 2-4, 6-12).

### 2. Description of unknown stage of black flies

#### 2.1 *S. (S.) suchariti* Takaoka & Choochote

The male of *S. (S.) suchariti* Takaoka & Choochote is described for the first time based on a unique male reared from a pupa collected from a stream at Ang Ka, Doi Inthanon National Park, Thailand. A key to identify males of all 16 species of the *Simulium griseifrons* species-group recorded from Thailand is provided (see appendix, article number 5).

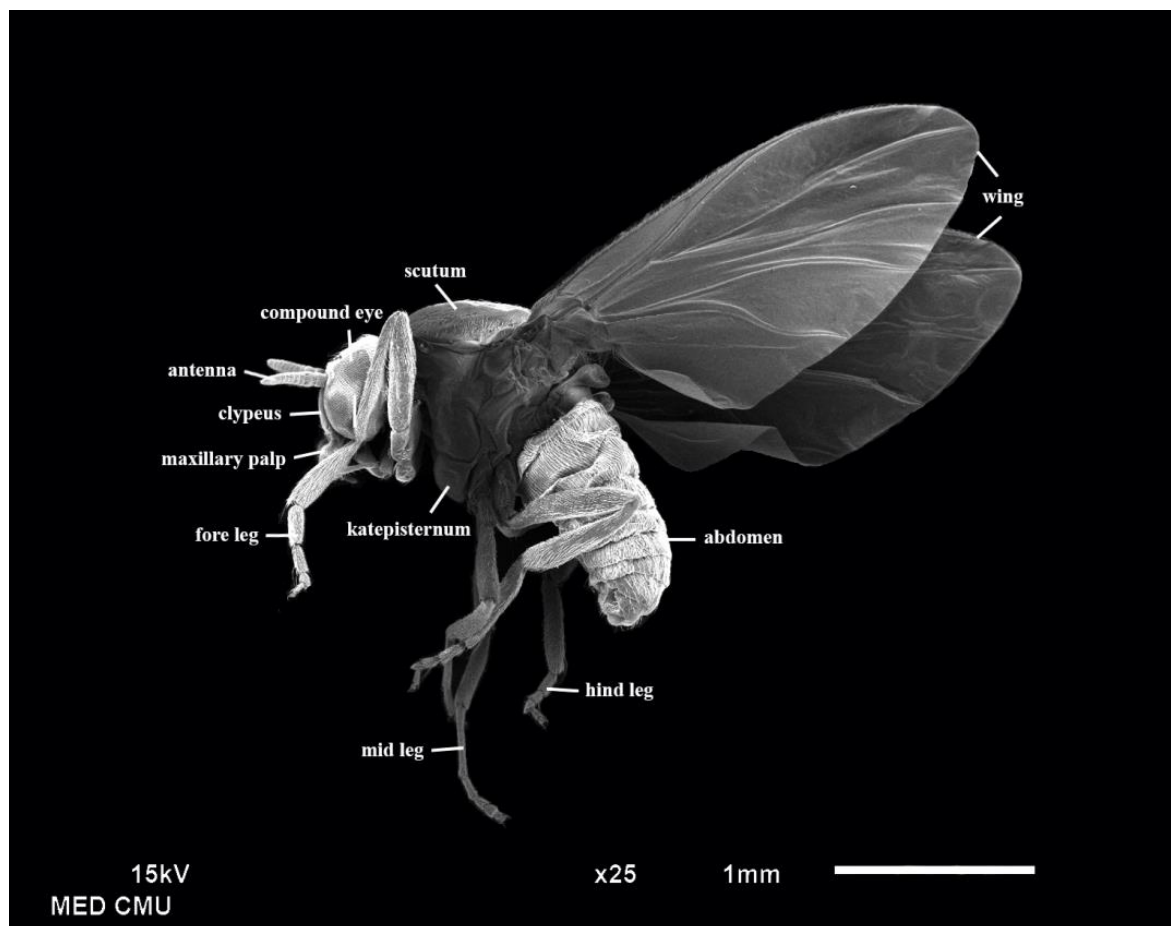
#### 2.2 *S. (G.) udomi* Takaoka & Choochote

The female and larva of *S. (G.) udomi* Takaoka & Choochote collected from Thab Tao village, Doi Chom Dao, Thoeng District, Chiang Rai Province, are described for the first time. The female of this species is similar to those of *S. (G.) asakoe* Takaoka & Davies from Peninsular Malaysia, Thailand, Hong Kong and Vietnam, and *S. (G.) chiangdaoense* Takaoka & Srisuka from Thailand. The larva of this species is similar to *S. (G.) curtatum* Jitklang et al. and *S. (G.)* nr. *asakoe* 2 from Thailand in having a medium-long postgenal cleft. Taxonomic notes are given to separate this species from these related species. The COI gene sequence of *S. (G.) udomi* is compared with those of eight species of the *S. asakoe* species-group and three species of the *S. ceylonicum* species-group. This species is transferred from the *S. ceylonicum* species-group to the *S. asakoe* species-group based on the adult female and male morphological characters, comparisons

of the genetic distances and phylogenetic relationships inferred from the COI gene sequences (see appendix, article number 1).

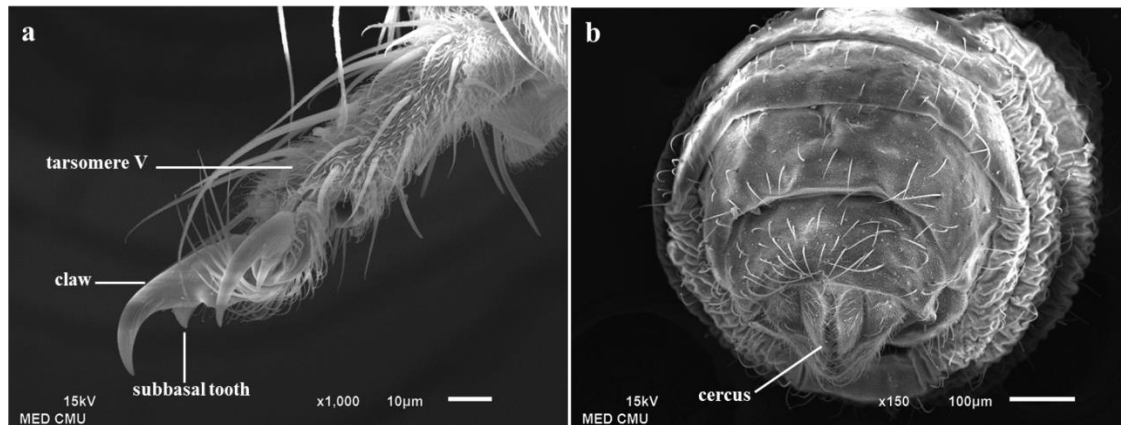
### 3. Description of morphology of black flies revealed by SEM

The external morphology of adult female of the human-biting black fly species, *S. (S.) nigrogilvum*, was examined using SEM. Adult female *S. (S.) nigrogilvum* are small, at about 2-3 mm long, with stout bodies and a humped thorax. The body is divided into three parts: head, thorax, and abdomen (Fig. 1). The thorax of *S. (S.) nigrogilvum* is comprised of three segments: scutum, scutellum and postnotum. A pair of short and broad wing is housed on scutellum, whereas a pair of halter is housed on postnotum. The wing includes three expressed veins: the costa, subcosta and radius. Each thorax segment composed of a pair of leg (forelegs, mid legs and hind legs) (Fig. 1).



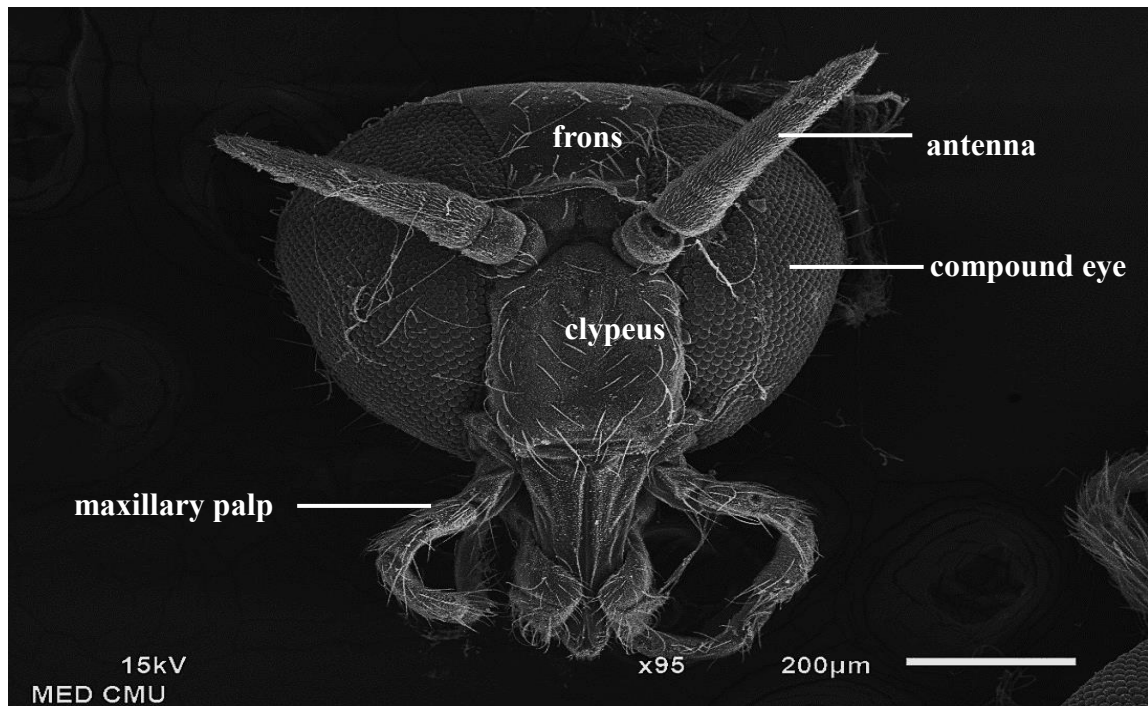
**Figure 1.** Scanning electron micrograph of adult female *S. (S.) nigrogilvum*.

The distal part of each leg consisted of a pair of claw with subbasal tooth which protrude from tarsomere V (Fig. 4). *Simulium* (S.) *nigrogilvum* has nine abdomen segments (tergite I-IX) which the cercus and ovipositor are housed on the last segment (Fig. 2).

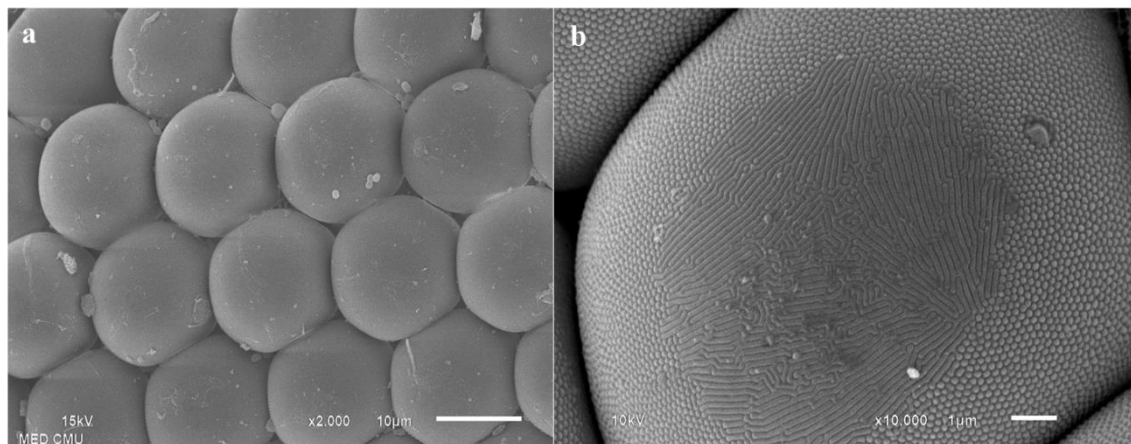


**Figure 2.** Scanning electron micrographs of (a) claw with subbasal tooth and (b) cercus of adult female *S. (S.) nigrogilvum*.

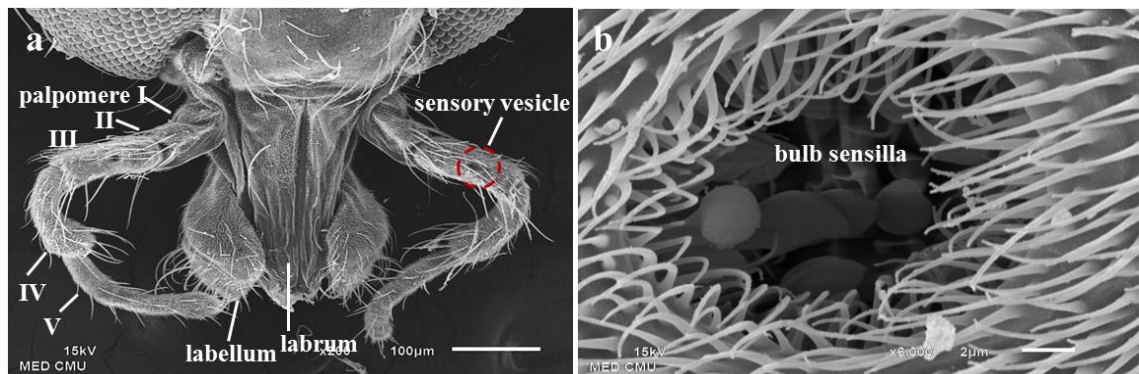
The head of this species is consisted of a pair of large compound eyes which almost completely in the male (holoptic), while incompletely, being divided medially by the frons in the female (dichoptic) (Fig. 3). The compound eyes were made up of dome-shaped facets or ommatidia. A close-up view of an ommatidium revealing surface ultrastructure of the corneal lens (Fig. 4). Maxillary palp consists of five segments, with ellipsoidal sensory vesicle (or pit) having medium-sized opening (Figs. 3 and 5). Group of bulb sensilla (club-like or spoon-like) were found in the sensory pit (Fig. 5b).



**Figure 3.** Scanning electron micrographs of head of adult female *S. (S.) nigrogilvum*.



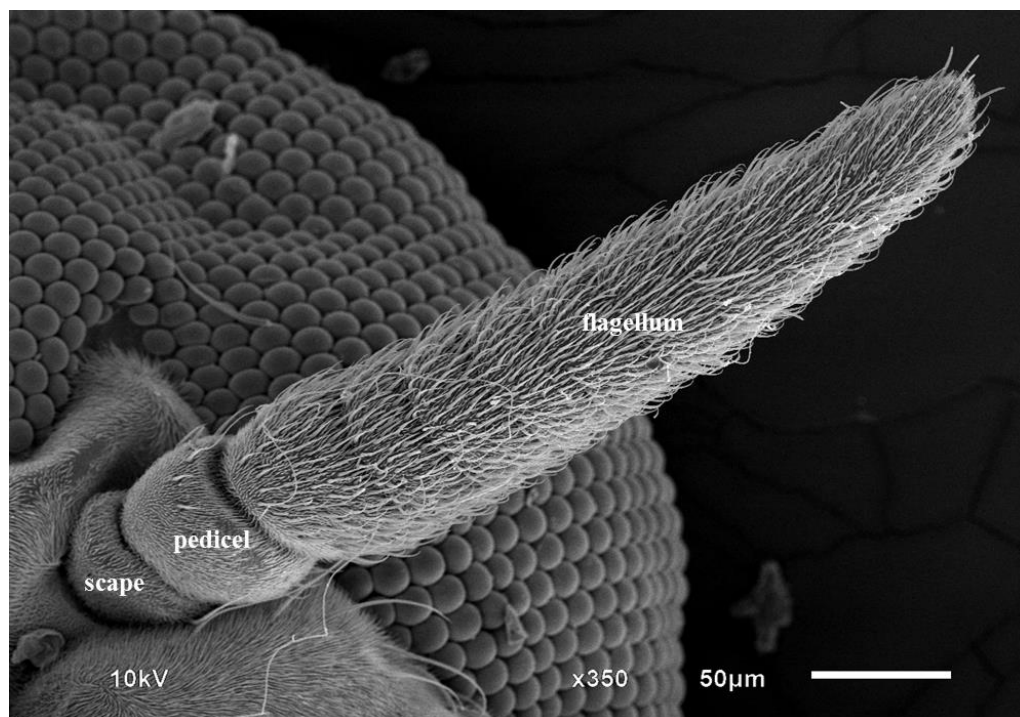
**Figure 4.** Scanning electron micrographs of compound eye of adult female *S. (S.) nigrogilvum*. (a) dome-shaped ommatidia, (b) higher magnification of an ommatidium revealing surface ultrastructure of the corneal lens.



**Figure 5.** Scanning electron micrographs of (a) mouthpart and maxillary palpi, (b) higher magnification of bulb sensilla inside the sensory vesicle (pit) observed on the palpomere III of maxillary palp of adult female *S. (S.) nigrogilvum*.

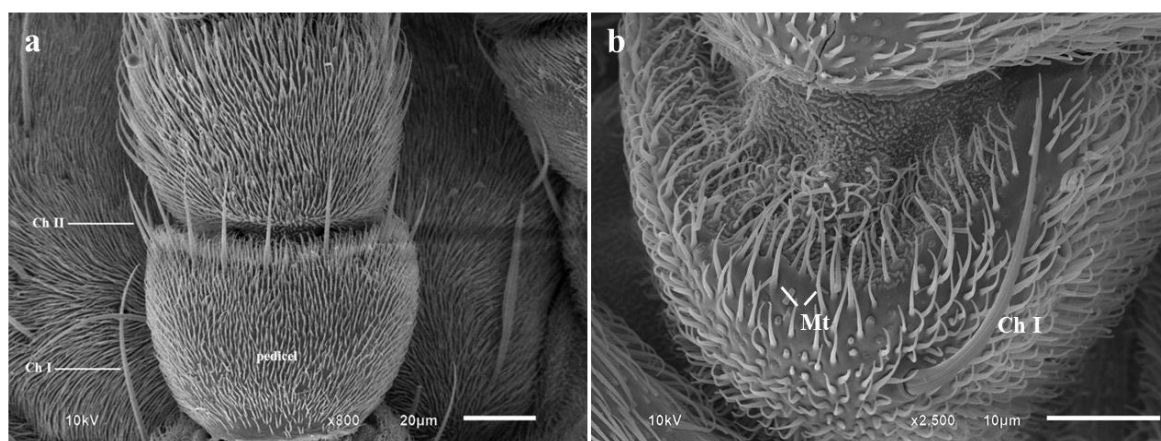
#### Characterization of antennal sensilla

The antenna of the black fly is short and cigar-like. Each antenna composed of scape, pedicel, and distal flagellum. The scape (Sc) is collar-shaped and hidden behind the pedicel (Pe), which is a bulbous cup shaped-segment and provides the attachment of the flagellum. Each flagellum consists of nine flagellomeres (Fig. 6).



**Figure 6.** Scanning electron micrographs of antenna of adult female *S. (S.) nigrogilvum*.

The surface of the scape, pedicel and flagellomere is covered densely with microtrichia (Mt) (Fig. 7). Only one type sensillum, chaetica sensilla (Ch) are found on the antennal scape and pedicel. Ch are arranged in a single row and characterized as long bristle-like structures with longitudinal grooved wall, whose bases are fitted in apparently elevated and smooth sockets. Three subtypes of Ch (Ch I, Ch II and Ch III) can be distinguished by their shape and size. Ch I and Ch II are observed on scape and pedicel while Ch III are found only on the flagellum. The longest Ch I are curved sensilla that gradually taper to an acute tip (Fig. 7a, b), whereas Ch II are straight sensilla and shorter than that of Ch I (Fig. 7a).



**Figure 7.** Scanning electron micrographs of scape and pedicel of adult female *S. (S.) nigrogilvum*. (a) chaetica sensilla (Ch) I and II on scape and pedicel, (b) higher magnification of microtrichia (Mt) and Ch I on scape.

Four major types of sensillum: chaetica (Ch); trichoid (Tr); basiconic (Ba) and coeloconic (Co) exist on the cuticular surface of flagellum (Fig. 8). The entire surfaces of nine flagellomeres are densely covered with microtrichia that taper to their acute apices.

#### *Chaetica sensilla*

Chaetica sensilla subtype III (Ch III) found on nine flagellomeres with a few numbers. They were characterized by having a deeply longitudinally grooved cuticular wall along the shaft with blunt tips and were set into a tight socket. They were the shortest among all chaetica subtypes (Fig. 8).

#### *Trichoid sensilla*

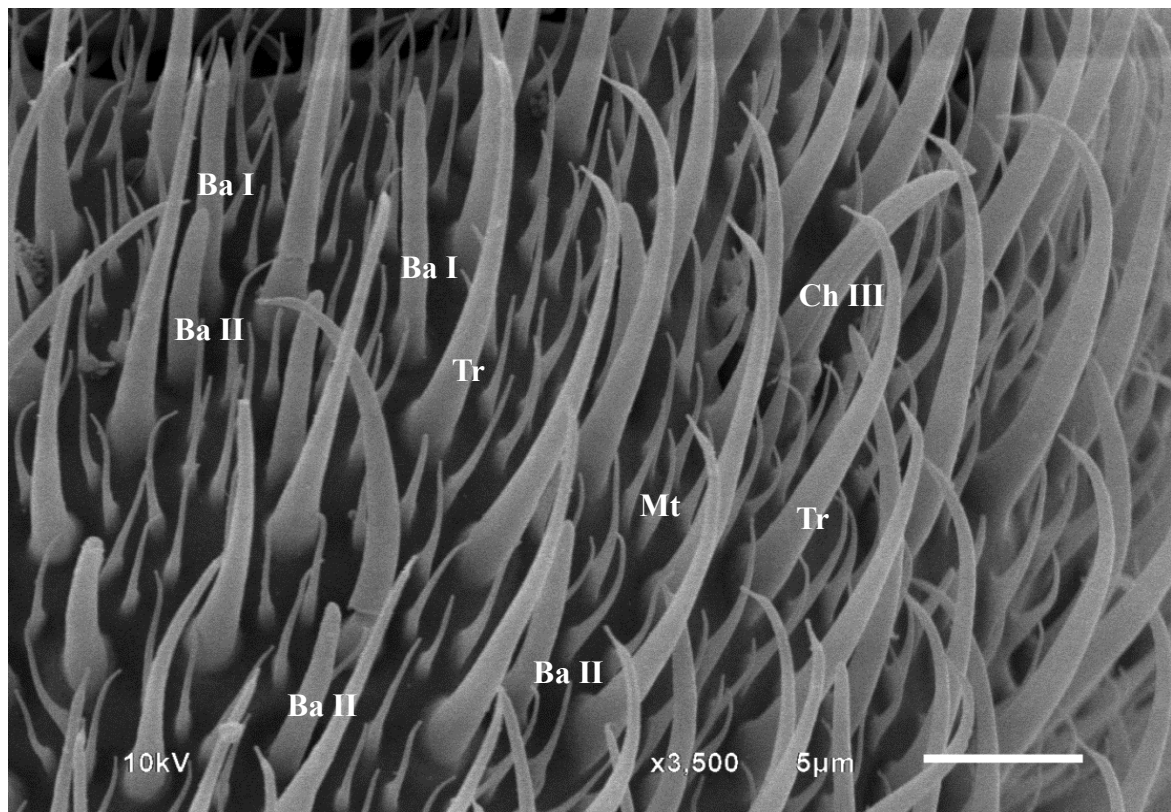
Trichoid sensilla (Tr) are elongated, slightly curved, and hair-like structures with smooth surface and a gradually tapers to an acute tip distally. They are slender and shorter in width and length than Ch I but longer than Ch III. Tr is the most abundant sensilla type found on antennal flagellum. No pores or grooves are found on their surface (Fig. 8).

#### *Basiconic sensilla*

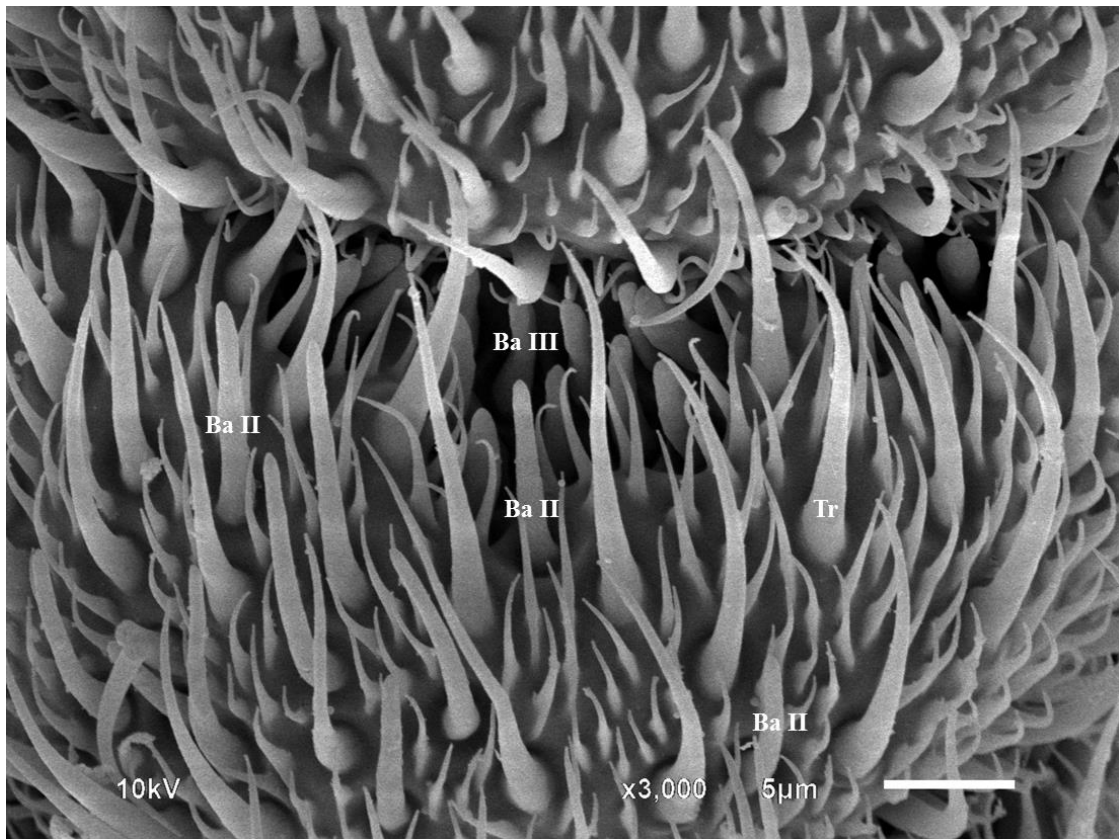
Multiporous basiconic sensilla can be classified into four subtypes: Ba I, Ba II, Ba III and Ba IV, according to their shape and size (Figs. 8-11). Their length is shorter than that of Tr. Sensilla basiconica subtype I (Ba I) have the appearance of long, straight or slightly curve and sharp tip (pen-like shape), and with or without a distinctive droplet shape (Fig. 8). Sensilla basiconica subtype II (Ba II) are straight or slightly curve with blunt tipped-sensilla (Figs. 8 and 9). Ba I and Ba II are widely distributed all over the flagellum surface of antennae. Sensilla basiconica subtype III (Ba III) are seated within the deeply sunken depressions or sacculus. Ba III are shorter than Ba I but broader than Ba I and Ba II (Figs. 9 and 10). Sensilla basiconica subtype IV (Ba IV) have morphologically resemble Ba III but with point tips (Fig. 11). Both subtypes are commonly found on the proximal region of each flagellomere either singularly or in groups (Figs. 9-11). Remarkably, Ba I and Ba II have a random pattern of pits in the cuticle whereas densely pitting of cuticle were observed on Ba III and Ba IV surface (Figs. 10 and 11).

#### *Coeloconic sensilla*

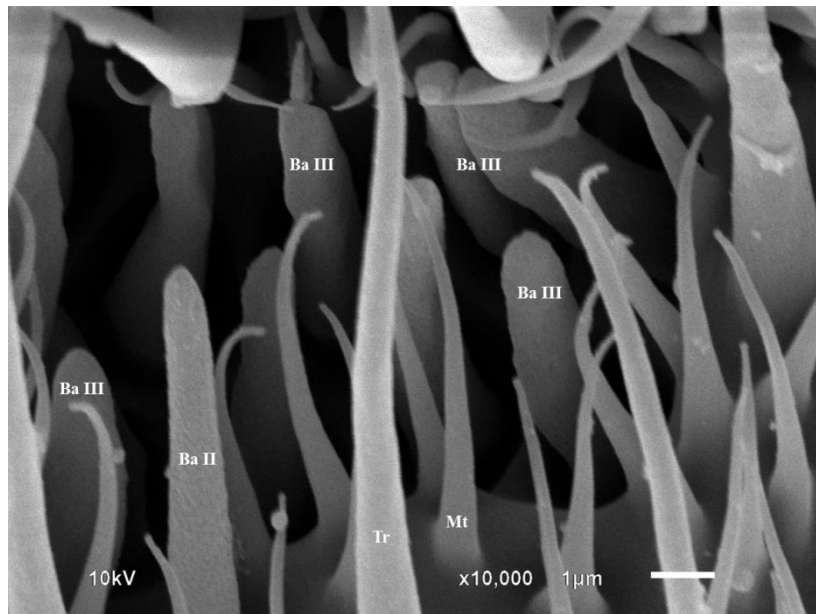
Coeloconic sensilla (Co) are short, cone-shaped peg with a relatively pointed tip sensilla and characterized by distinct longitudinal grooved on their cuticular walls. They situated centrally in a deep sacculus on the antennal flagellum, and stand up perpendicularly or slightly curve (Fig. 12)



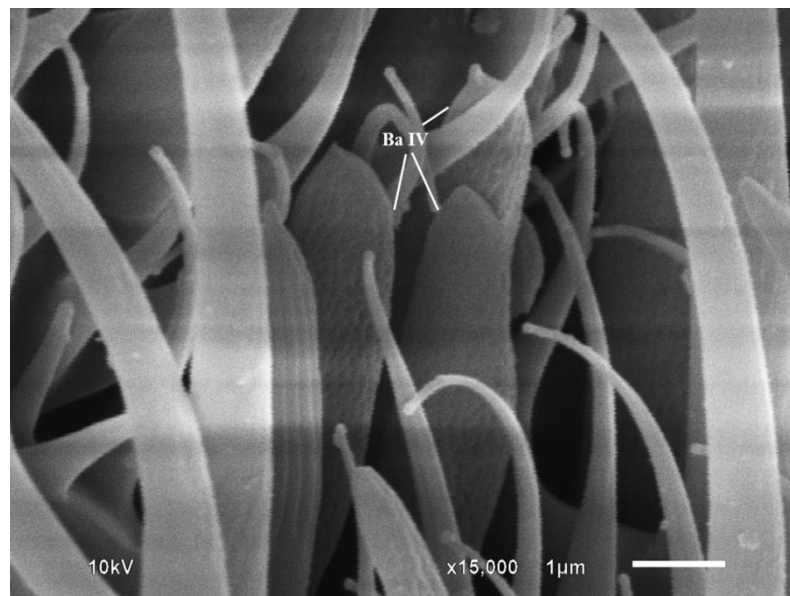
**Figure 8.** Scanning electron micrographs of antennal sensilla of adult female *S. (S.) nigrogilvum*. Distribution of different types of sensilla on the surface of antennal flagellum. Abbreviations: Ba I, subtype I basiconic sensilla; Ba II, subtype II basiconic sensilla; Ch III, subtype III chaetica sensilla; Mt, microtrichia; Tr, trichoid sensilla.



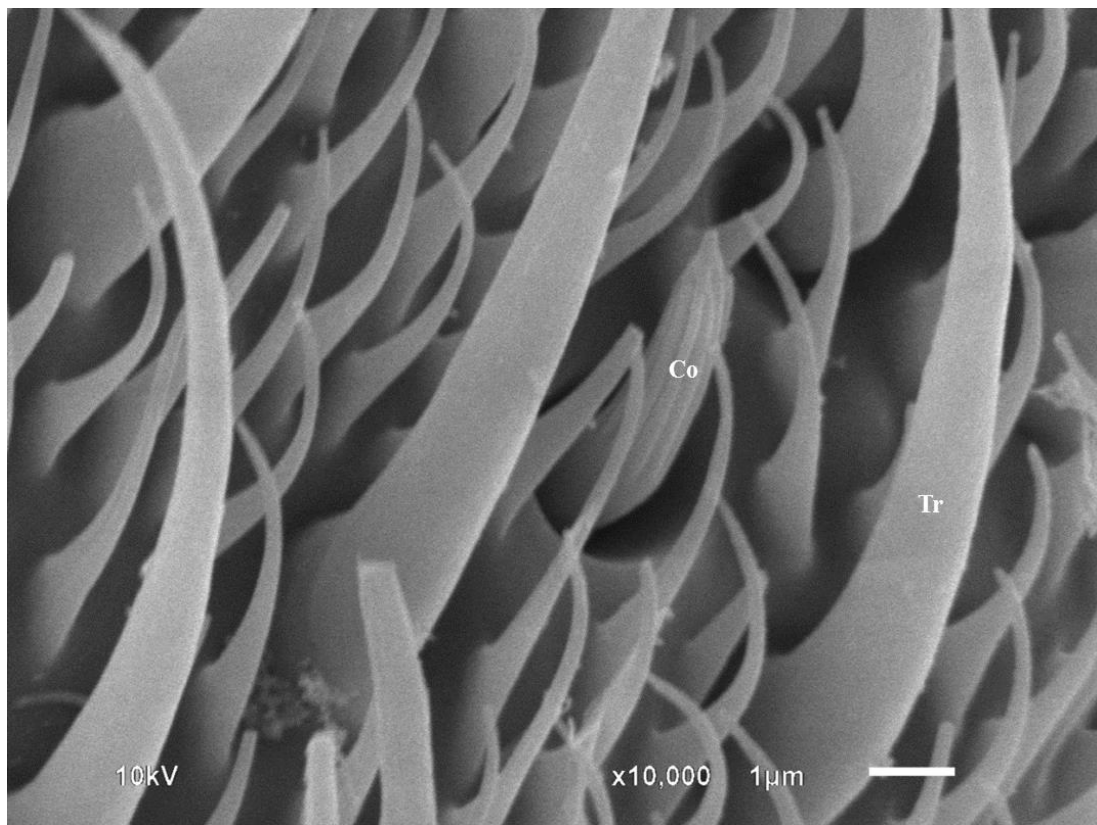
**Figure 9.** Scanning electron micrographs of basiconic sensilla on the surface of antennal flagellum of adult female *S. (S.) nigrogilvum*. Abbreviations: Ba II, subtype II basiconic sensilla; Ba III, subtype III basiconic sensilla; Tr, trichoid sensilla.



**Figure 10.** Scanning electron micrographs of basiconic sensilla situated on a deep and big sacculus of antennal flagellum of adult female *S. (S.) nigrogilvum*. Higher magnification revealed highly pitted cuticle of two subtypes (Ba II and Ba III). Abbreviations: Ba II, subtype II basiconic sensilla; Ba III, subtype III basiconic sensilla.



**Figure 11.** Scanning electron micrographs of subtype IV basiconic sensilla on the surface of antennal flagellum of adult female *S. (S.) nigrogilvum*. Abbreviations: Ba IV, subtype IV basiconic sensilla.



**Figure 12.** Scanning electron micrographs of basiconic sensilla on the surface of antennal flagellum of adult female *S. (S.) nigrogilvum*. Abbreviations: Co, coeloconic sensilla; Tr, trichoid sensilla.

#### 4. Dissections of adult black flies and study of recovered nematodes

##### 4.1 Morphological species identification of nematodes

Natural filarial infections of adult black flies were investigated. A total of 494 females were collected, and all were identified as *S. (S.) nigrogilvum* (Fig. 13). Four of 494 females dissected were infected with six infective third-stage larvae (L3) (0.81%). However, *S. (S.) nigrogilvum* is morphologically very similar to *S. (S.) umphangense*. Thus, the identity of the *Onchocerca*-positive *S. (S.) nigrogilvum* were confirmed their species identity using PCR. The measurements of all unknown infective larvae were 1,100-1,300  $\mu\text{m}$  long by 24-26  $\mu\text{m}$  wide, esophagus shorter than half of the body length (Fig. 14). All L3 were morphologically identified as *Onchocerca* sp. type A. In order to clarify its morphotaxonomic status, the PCR-based assay was further conducted for molecular species identification.



**Figure 13.** Adult female of the human-biting black fly, *S. (S.) nigrogilvum*. Scale bar = 2 mm.



**Figure 14.** Third-stage larva (Infective larva) found in thorax of *S. (S.) nigrogilvum* collected from Um-phang District, Tak Province. Scale bar = 100  $\mu$ m.

#### 4.2 Molecular species identification of black flies

The ranges of intraspecific genetic divergence among four *Onchocerca*-positive *S. (S.) nigrogilvum* sequences were 0.00-0.30% for COI, 0.60-0.90% for COII and 0.70-1.50% for ECP1. Intraspecific divergence for COI+COII dataset varied from 0.30 to 0.60%. The ranges of intraspecific genetic divergence among three sequences of *S. (S.) umphangense* were 0.20-0.30% for COI, 0.00-0.10% for COII and 0.00% for ECP1. Intraspecific divergence for COI+COII dataset varied from 0.10 to 0.20%. Average interspecific genetic divergence between *S. (S.) nigrogilvum* and *S. (S.) umphangense* was 5.40%, 7.53%, 3.83% and 6.45% for COI, COII, ECP1 and concatenated COI+COII sequences, respectively. Intraspecific and interspecific genetic distances of *S. (S.) nigrogilvum* and *S. (S.) umphangense* are summarized in Tables 3-4.

**Table 3.** Ranges of intraspecific and interspecific genetic distances based on concatenated COI and COII sequences

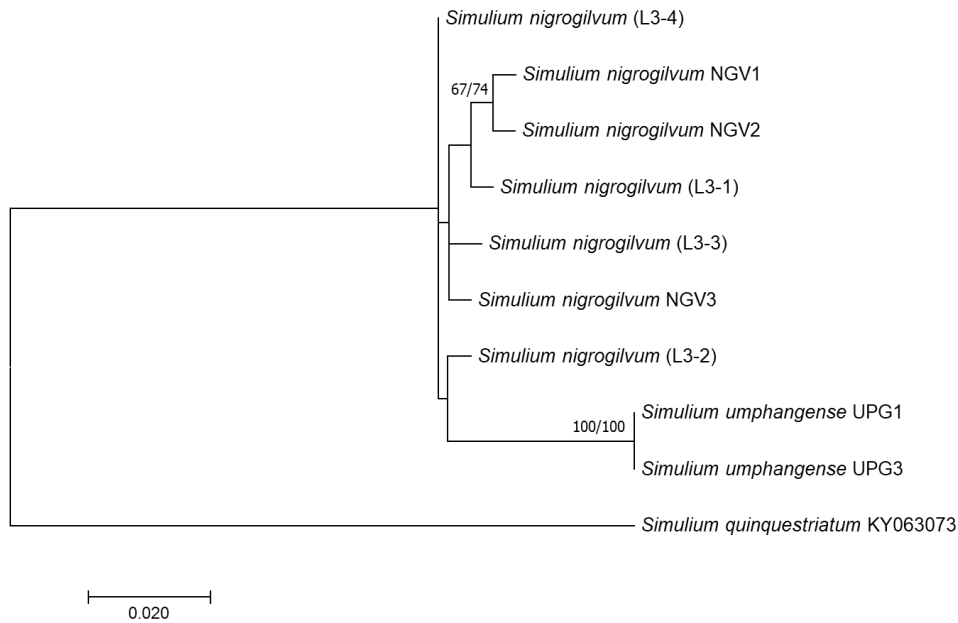
|                          | 1          | 2          |
|--------------------------|------------|------------|
| 1. <i>S. nigrogilvum</i> | 0.30-0.60% |            |
| 2. <i>S. umphangense</i> | 6.40-6.60% | 0.10-0.20% |

**Table 4.** Ranges of intraspecific and interspecific genetic distances based on ECP1 sequences

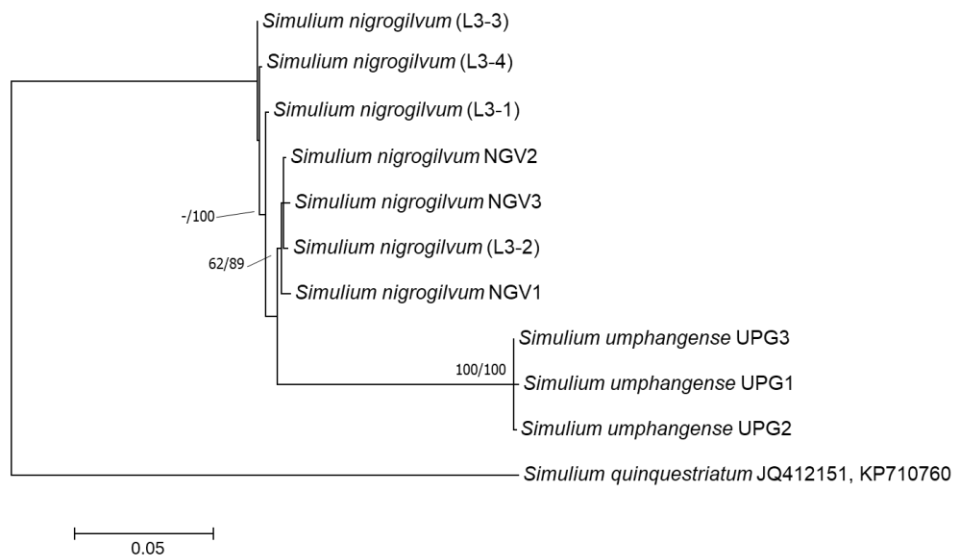
|                          | 1          | 2     |
|--------------------------|------------|-------|
| 1. <i>S. nigrogilvum</i> | 0.70-1.50% |       |
| 2. <i>S. umphangense</i> | 3.70-4.10% | 0.00% |

### Phylogenetic analyses

Phylogenetic trees based on single genes (COI, COII and ECP1) and concatenated dataset (COI+COII) revealed the similar tree topology in all phylogenetic analysis methods (NJ and ML); thus only ML trees are shown for ECP1 gene (Fig. 15) and for the combined dataset (Fig. 16). All trees comprised two main clusters with strong support. One cluster consisted of *S. (S.) nigrogilvum* and the other cluster consisted of *S. (S.) umphangense*. The level of genetic divergence and the phylogenetic analyses based on single genes and combined dataset confirmed that *S. (S.) nigrogilvum* identified by morphological characteristics was exact *S. nigrogilvum*. *Simulium quinquestriatum* was used as an outgroup.



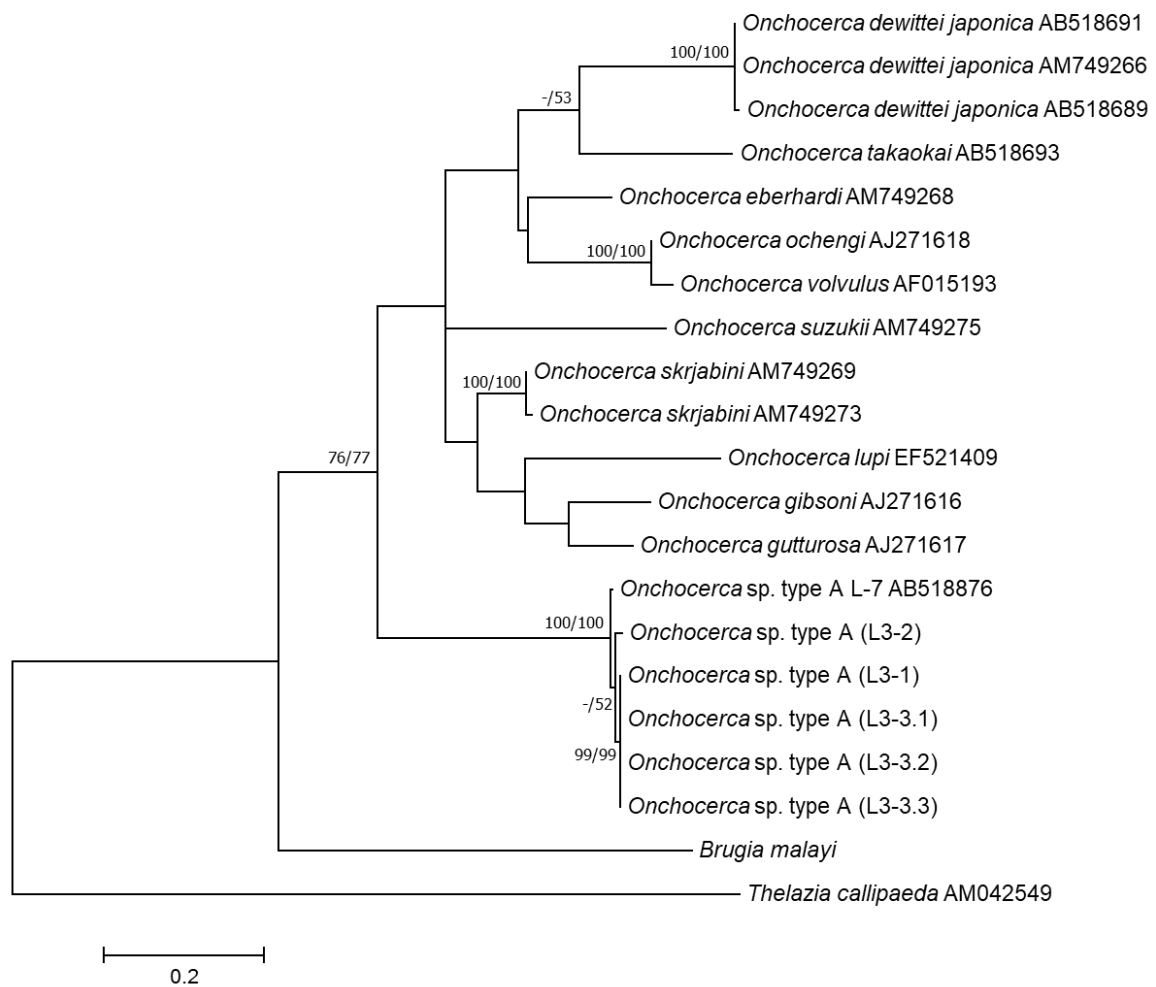
**Figure 15.** Maximum likelihood phylogenetic tree of (*S.*) *nigrogilvum* and *S. (S.) umphangense* from Thailand based on ECP1 gene. Bootstrap values [ML/NJ] are shown above or near the branches. The scale bar represents 0.02 substitutions per nucleotide position. ML = maximum likelihood; NJ = neighbor-joining.



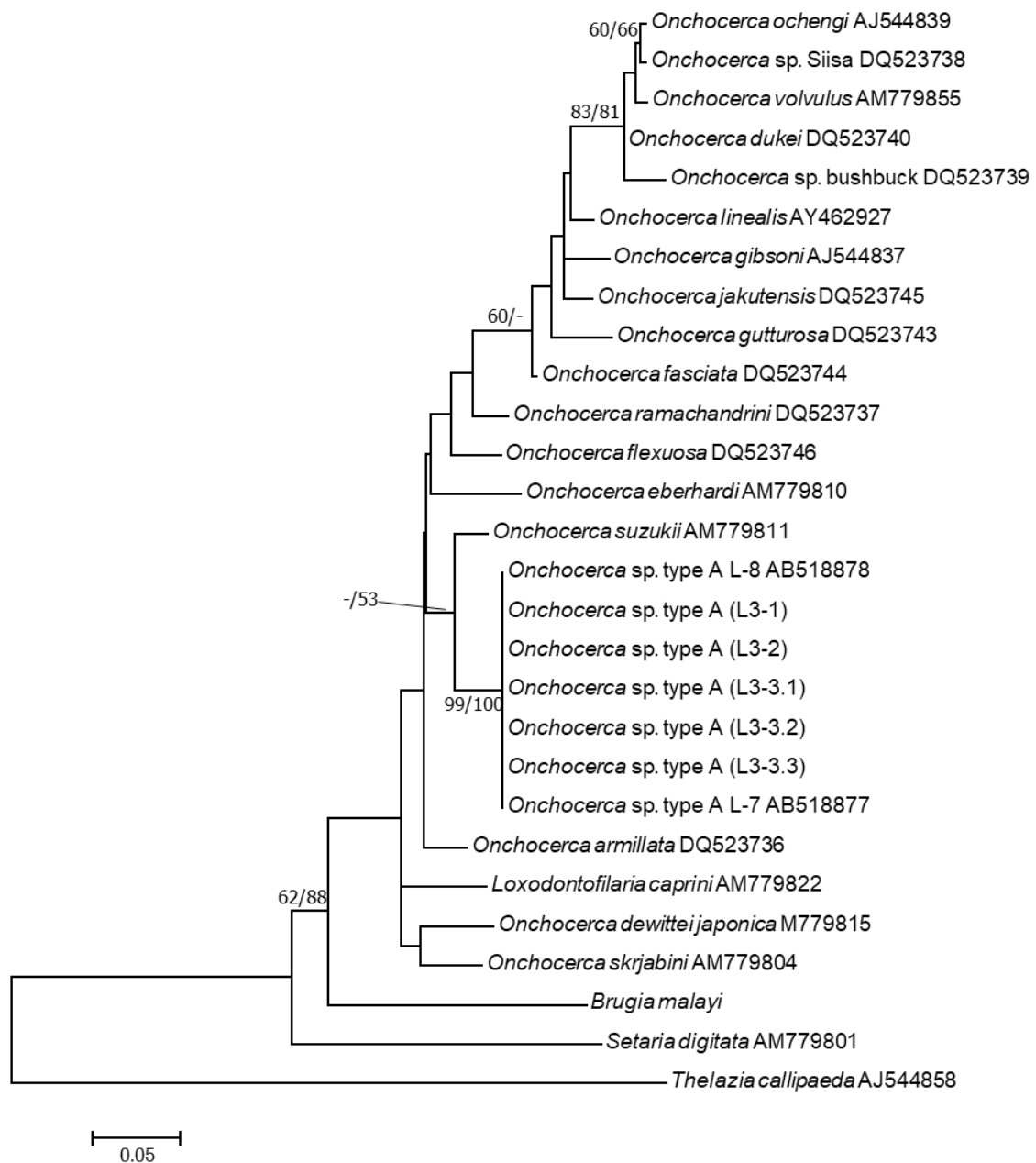
**Figure 16.** Maximum likelihood phylogenetic tree of (*S.*) *nigrogilvum* and *S. (S.) umphangense* from Thailand based on concatenated sequences of COI and COII genes. Bootstrap values [ML/NJ] are shown above or near the branches. The scale bar represents 0.02 substitutions per nucleotide position. ML = maximum likelihood; NJ = neighbor-joining.

### 4.3 Molecular identification of infective larvae from wild-caught black flies

All six infective larvae morphologically identified as *Onchocerca* sp. type A were used for determination of the mitochondrial COI and 12S rRNA gene regions. All the sequences were 649 bp long for COI gene and 471 bp long for 12S rRNA gene. Compared with other *Onchocerca* species available in GenBank databases, five of the six infective larvae successfully amplified were identical to *Onchocerca* sp. type A with nucleotide differences 1.00-1.20% and 0.00% for COI and 12S rRNA genes, respectively. *Onchocerca* sp. type A of the present study clustered with *Onchocerca* sp. type A infected *Simulium bidentatum* in Japan and formed a monophyletic clade with high bootstrap support in phylogenetic trees of both genes. Sequence analyses of the COI and 12S rRNA genes of *Onchocerca* sp. type A revealed 9.80-13.50% and 3.80-7.20% difference, respectively, when compared with those of all *Onchocerca* spp. The phylogenetic relationships based on COI and 12S rRNA genes between *Onchocerca* sp. type A and other *Onchocerca* spp. are shown in ML trees (Figs. 17 and 18).



**Figure 17.** Maximum likelihood phylogenetic tree based on COI gene sequences of *Onchocerca* spp. Numbers at the nodes are the bootstrap values [ML/NJ] after 1,000 replicates. Values more than 50% are shown. The scale bar represents 0.2 substitutions per nucleotide position. ML = maximum likelihood; NJ = neighbor-joining.



**Figure 18.** Maximum likelihood phylogenetic tree based on 12S rRNA gene sequences of *Onchocerca* spp. Numbers at or near the nodes are the bootstrap values [ML/NJ] after 1,000 replicates. Values more than 50% are shown. The scale bar represents 0.05 substitutions per nucleotide position. ML = maximum likelihood; NJ = neighbor-joining.

## สรุปและวิจารณ์ผลการทดลอง (DISCUSSION AND CONCLUSIONS)

In this study, 14 new species were discovered in Thailand. In addition, this study was the first to describe the male of *S. (S.) suchariti* and female and larval stage of *S. (G.) udomi*. Our study yielded the body of new knowledge for better understanding of the species diversity of the simuliid fauna in the country, which is necessary for the future studies of ecology, behavior, population genetics, and roles of simuliids as disease vectors and/or vicious biters to humans and animals.

Some black fly species has been misidentified because the overlapping of their morphological characters. Thus, we applied a multi-gene approach, recognized for resolving evolutionary relationships in the Simuliidae (Phayuhasena et al. 2010; Low et al. 2015). Our first attempt reveals that the mitochondria-encoded COI gene could clearly distinguish *S. (S.) nigrogilvum* from *S. (S.) umphangense*. In addition, the mitochondria-encoded COII gene and a fast-evolving nuclear gene, ECP1, which has been useful to resolve species complexes and species groups (Senatore et al. 2014, Low et al. 2016b) were used to separate these two closely related species. The COII and ECP1 genes also successfully differentiate *S. (S.) nigrogilvum* from *S. (S.) umphangense*, further supporting their morphological classification and molecular identification based on the COI gene. In this study, the two mitochondrial genes, COI and COII, can unequivocally produce higher phylogenetic signals than did ECP1 or the concatenated dataset. The large COI and COII genetic distances between *S. nigrogilvum* and *S. umphangense* would certainly serve as the barcoding gap for species delimitation (Low et al. 2016a). Furthermore, because cutoff of 3% divergence is the basic criterion to advocate species complex in insects (Low et al. 2016a; Pramual et al. 2016), we propose the use of COI and COII genes as the gold standard for identifying these two species.

In addition, fine structure of *S. (S.) nigrogilvum*, is presented for the first time using scanning electron microscopy (SEM) to contribute information on the morphology of the adult of this fly species and may eventually provide a basis for understanding the biological behavior of this species. Many types of sensilla examined on the antennae of *S. (S.) nigrogilvum* are similar to those of other insects species such as blow fly (*C. megacephala*), stable flies (*Stomoxys calcitrans*), cleg fly (*Haematopota pandazisi*), (Sukontason et al., 2008; Tangtrakulwanich et al. 2011; Wang et al. 2014; Liu et al. 2016; Pezzi et al. 2018). These sensilla include the chaetica sensilla (2 subtypes), trichoid sensilla, basiconic sensilla (4 subtypes) and coeloconic sensilla.

In this study, all infective larvae were morphologically identified as *Onchocerca* sp. type A, resemble *Onchocerca* sp. type A infected *Simulium bidentatum*, a natural vector of several *Onchocerca* spp. in Japan (Fukuda et al. 2010). Type A is presumed to be identical to type I from black flies collected in a cattle shed (Takaoka and Bain 1990; Takaoka 1994). Molecular identification of the infective larvae based on mitochondrial COI and 12S rRNA genes also indicated that they are *Onchocerca* sp. type A which similar to the previously reported (Fukuda et al. 2010). It is thus suggested that *S. (S.) nigrogilvum* is the predominant black fly species attacking human in the study area and one type of *Onchocerca* larvae are natural infecting this black fly species.

#### ข้อเสนอแนะ (SUGGESTION)

The whole mitochondrial and nuclear genome sequences of *Onchocerca* sp. type A need further investigation in order to obtain the genomic information.

## เอกสารอ้างอิง (REFERENCES)

- Adler PH, Currie DC, Wood DM. The Black Flies (Simuliidae) of North America. Cornell University Press, Ithaca, New York, USA, 2004.
- Bain O, Chabaud AG. Atlas des larves infestantes de Filaires. Trop Med Parasitol 1986;37:301-40.
- Casiraghi M, Anderson TJ, Bandi C, Bazzocchi C, Genchi C. A phylogenetic analysis of filarial nematodes: comparison with the phylogeny of *Wolbachia* endosymbionts. Parasitology 2001;122:93-103.
- Conflitti IM, Kratochvil MJ, Spironello M, Shields GF, Currie DC. Good species behaving badly: non-monophyly of black fly sibling species in the *Simulium arcticum* complex (Diptera: Simuliidae). Mol Phylo Evol 2010;57:245-57.
- Conflitti IM, Shields GF, Currie DC. A “complex” problem: delimiting sibling species boundaries in black flies (Diptera: Simuliidae). Can Entomol 2012;144:323-36.
- Farris JS, Kallersjo M, Kluge AG, Bult C. Testing significance of incongruence. Cladistics. 1995;10:315-9.
- Fukuda M, Otsuka Y, Uni S, Bain O, Takaoka H. Molecular identification of infective larvae of three species of *Onchocerca* found in wild-caught females of *Simulium bidentatum* in Japan. Parasite 2010;17:39-45.
- Fukuda M, Takaoka H, Uni S, Bain O. Infective larvae of five *Onchocerca* species from experimentally infected *Simulium* species in an area of zoonotic onchocerciasis in Japan. Parasite 2008;15:111-9.
- Hempolchom C, Yasanga T, Wijit A, Taai K, Dedkhad W, Srisuka W, Thongsahuan S, Otsuka Y, Takaoka H, Saeung A. Scanning electron microscopy of antennal sensilla of the eight *Anopheles* species of the Hyrcanus Group (Diptera: Culicidae) in Thailand. Parasitol Res 2017;116:143-53.
- Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 1980;16:111-20.
- Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 2016;33:1870-4.
- Liu XH, Liu JJ, Li XY, Zhang D. Antennal sensory organs of *Scathophaga stercoraria* (Linnaeus, 1758) (Diptera: Scathophagidae): ultramorphology and phylogenetic implications. Zootaxa 2016;4067:361-72.

- Low VL, Adler PH, Takaoka H, Ya'cob Z, Lim PE, Tan TK, Lim YA, Chen CD, Norma-Rashid Y, Sofian-Azirun M. Mitochondrial DNA markers reveal high genetic diversity but low genetic differentiation in the black fly *Simulium tani* Takaoka & Davies along an elevational gradient in Malaysia. PLoS One 2014;9:e100512.
- Low VL, Takaoka H, Adler PH, Ya'cob Z, Norma-Rashid Y, Chen CD, Sofian-Azirun M. A multi-locus approach resolves the phylogenetic relationships of the *Simulium asakoe* and *Simulium ceylonicum* species groups in Malaysia: evidence for distinct evolutionary lineages. Med Vet Entomol 2015;29:330-7.
- Low VL, Takaoka H, Pramual P, Adler PH, Ya'cob Z, Chen CD, Yotopranoto S, Zaid A, Hadi UK, Lardizabal ML, Nasruddin-Roshidi A, Sofian-Azirun M. Three Taxa in One: Cryptic Diversity in the Black Fly *Simulium nobile* (Diptera: Simuliidae) in Southeast Asia. J Med Entomol 2016a;53:972-6.
- Low VL, Takaoka H, Pramual P, Adler PH, Ya'cob Z, Huang YT, Da Pham X, Ramli R, Chen CD, Wannaket A, Sofian-Azirun M. Delineating taxonomic boundaries in the largest species complex of black flies (Simuliidae) in the Oriental Region. Sci Rep 2016b;6:20346.
- Phayahasena S, Colgan DJ, Kuvangkadilok C, Pramual P, Baimai V. Phylogenetic relationships among the black fly species (Diptera: Simuliidae) of Thailand based on multiple gene sequences. Genetica 2010;138:633-48.
- Pramual P, Thaijarern J, Wongpakam K. DNA barcoding of human-biting black flies (Diptera: Simuliidae) in Thailand. Acta Trop 2016;164:33-40.
- Sukontason K, Methanitikorn R, Kurahashi H, Vogtsberger RC, Sukontason KL. External morphology of *Chrysomya pinguis* (Walker) (Diptera: Calliphoridae) revealed by scanning electron microscopy. Micron 2008;39:190-7.
- Swafford D. PAUP\*. Phylogenetic analysis using parsimony (\* and other methods). Vol Sinauer Associates, Sunderland, MA. 2002.
- Taai K, Harbach RE, Aupalee K, Srisuka W, Yasanga T, Otsuka Y, Saeung A. An effective method for the identification and separation of *Anopheles minimus*, the primary malaria vector in Thailand and its sister species *Anopheles harrisoni*, with a comparison of their mating behaviors. Parasit Vectors 2017;10:97.
- Takaoka H. Natural vectors of three bovine *Onchocerca* species (Nematoda: Onchocercidae) and seasonal transmission by three blackfly species (Diptera: Simuliidae) in central Kyushu, Japan. J Med Entomol 1994;31:404-16.

- Takaoka H. Morphotaxonomic revision of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) in Oriental Region. Zootaxa 2012;3577:1-42.
- Takaoka H. Morphotaxonomic revision of species-groups of *Simulium* (*Simulium*) (Diptera: Simuliidae) in the Oriental Region. Zootaxa 2017;4353:425-46.
- Takaoka H, Bain O. Infections of blackflies (Diptera: Simuliidae) with three types of zoonotic *Onchocerca* larvae in Oita, Japan. Japanese J Trop Med Hyg 1990;18:1-10.
- Takaoka H, Davies DM. The black flies (Diptera: Simuliidae) of west Malaysia: Kyushu University Press, Fukuoka, Japan, 1995.
- Takaoka H, Choochote W. Two new species of *Simulium* (*Simulium*) (Diptera: Simuliidae) from Thailand. Trop Med Health. 2004a;32:31-6.
- Takaoka H, Choochote W. A new species of the subgenus *Simulium* (*Asiosimulium*) (Diptera: Simuliidae) from Thailand. Med Entomol Zool 2006;57:45-8.
- Takaoka H, Srisuka W. *Simulium* (*Gomphostilbia*) *chiangdaoense* sp. nov. (Diptera: Simuliidae) a new species from northern Thailand. Med Entomol Zool 2009;60:269-76.
- Tangtrakulwanich K, Chen H, Baxendale F, Brewer G, Zhu JJ. Characterization of olfactory sensilla of *Stomoxys calcitrans* and electrophysiological responses to odorant compounds associated with hosts and oviposition media. Med Vet Entomol 2011;25:327-36.
- Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994;22:4673-80.
- Wang QK, Liu XH, Lu PF, Zhang D. Ultrastructure of antennal sensilla in *Hydrotaea armipes* (Fallén) (Diptera: Muscidae): new evidence for taxonomy of the genus *Hydrotaea*. Zootaxa 2014;3790:577-86.
- Zacharuk RY. Antennal sensilla. In: Kerkut, G.A., Gilbert, L.I. (Eds.), Comparative Insect Physiology, Biochemistry and Pharmacology, vol. 6. Pergamon Press, Oxford, 1985, pp. 1-69.

OUTPUT จากโครงการวิจัย

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ (ระบุชื่อผู้แต่ง ชื่อเรื่อง ชื่อวารสาร ปี เล่มที่ เลขที่ และหน้า) หรือผลงานตามที่คาดไว้ในสัญญาโครงการ) จำนวน 12 เรื่อง

1.1 **Saeung A**, Srisuka W, Low VL, Maleewong W, Takaoka H. Descriptions of the female and larva of *Simulium (Gomphostilbia) udomi* (Diptera: Simuliidae) from Thailand, and its transfer to the *Simulium asakoe* species-group. Acta Trop 2017;172:14-9. **(Q2, ISI impact factor 2017 = 2.509)**

1.2 Srisuka W, Takaoka H, Otsuka Y, Fukuda M, Thongsahuan S, Taai K, **Saeung A\***. Biodiversity, seasonal abundance and distribution of black flies (Diptera: Simuliidae) in six different regions of Thailand. Parasit Vectors 2017;10:574. **(Q1, ISI impact factor 2017 = 3.163)**

1.3 Takaoka H, Srisuka W, **Saeung A**. *Simulium maleewongae*, a new species of *Simulium (Gomphostilbia)* (Diptera: Simuliidae) from Thailand. J Med Entomol 2017;54:91-9. **(Q1, ISI impact factor 2017 = 1.968)**

1.4 Takaoka H, Srisuka W, **Saeung A**. A new human-biting black fly species of *Simulium (Simulium)* (Diptera: Simuliidae) from Thailand. J Med Entomol 2017;54:945-8. **(Q1, ISI impact factor 2017 = 1.968)**

1.5 Takaoka H, Srisuka W, **Saeung A**. Description of the male of *Simulium (Simulium) suchariti* (Diptera: Simuliidae) from Thailand. Med Entomol Zool 2017;68:27-30.

1.6 Takaoka H, Srisuka W, **Saeung A**. Two new species of the *Simulium (Simulium) variegatum* species-group of black flies (Diptera: Simuliidae) from Thailand. J Med Entomol 2017;54:1213-23. **(Q1, ISI impact factor 2017 = 1.968)**

1.7 Takaoka H, Srisuka W, Low VL, **Saeung A**. A new black fly species of the *Simulium (Gomphostilbia) epistum* species-group (Diptera: Simuliidae) from Thailand. Acta Trop 2017;176:373-9. **(Q2, ISI impact factor 2017 = 2.509)**

1.8 Takaoka H, Srisuka W, **Saeung A**. A new black fly species of *Simulium (Gomphostilbia)* (Diptera: Simuliidae) From Thailand. J Med Entomol 2017;54:1552-9. **(Q1, ISI impact factor 2017 = 1.968)**

1.9 Takaoka H, Srisuka W, Low VL, **Saeung A**. A New Species of the *Simulium (Simulium) striatum* species group (Diptera: Simuliidae) from Thailand, and its differentiation from two related species based on a fast-evolving nuclear gene. J Med Entomol 2018;55:561-8. **(Q1, ISI impact factor 2017 = 1.968)**

1.10 Takaoka H, Srisuka W, **Saeung A**. A new species of *Simulium* (*Asiosimulium*) (Diptera: Simuliidae) from Thailand. J Med Entomol 2018;55:569-74. **(Q1, ISI impact factor 2017 = 1.968)**

1.11 Takaoka H, Srisuka W, Low VL, **Saeung A**. Five new species of the *Simulium decuplum* subgroup of the *Simulium* (*Gomphostilbia*) *batoense* species-group (Diptera: Simuliidae) from Thailand and their phylogenetic relationships. Acta Trop 2018;182:271-84. **(Q2, ISI impact factor 2017 = 2.509)**

1.12 Takaoka H, Srisuka W, Low VL, **Saeung A**. A new species and a new record of the *Simulium* (*Gomphostilbia*) *gombakense* species-group (Diptera: Simuliidae) from Thailand. Acta Trop. 2018;185:156-66. **(Q2, ISI impact factor 2017 = 2.509)**

## 2. การนำผลงานวิจัยไปใช้ประโยชน์

- เชิงสาธารณะ มีการเชื่อมโยงกับนักวิจัยและผู้เชี่ยวชาญในต่างประเทศ
  - Assoc. Prof. Dr. Yasushi Otsuka, an expert on molecular genetics of insect vectors from Research Center for the Pacific Islands, Kagoshima University, Japan
  - Dr. Masako Fukuda, an expert on zoonotic onchocerciasis from Research Promotion Institute, Oita University, Japan
  - Dr. Van Lun Low, an expert on molecular genetics of insect vectors of the Tropical Infectious Diseases Research and Education Centre (TIDREC), University of Malaya, Malaysia
- เชิงวิชาการ (มีการพัฒนาการเรียนการสอน/สร้างนักวิจัยใหม่)
  - ความรู้ที่ได้จากงานวิจัยนำไปใช้เพื่อการเรียนการสอน ในกระบวนวิชาที่ภาควิชาทางการแพทย์ แก่นักศึกษาระดับบัณฑิตศึกษา สาขาวิชาปรสิตวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
  - สร้างนักวิจัยรุ่นใหม่ระดับปริญญาเอก จำนวน 2 คน (นางสาวชฎานิส เหมพลชม และนายกิตติภัทร อุปาลี)

## 3. อื่นๆ (เช่น ผลงานตีพิมพ์ในวารสารวิชาการในประเทศ การเสนอผลงานในที่ประชุมวิชาการ หนังสือ การจดสิทธิบัตร)

- Poster Presentation in International Conference
  - Aupalee K, Srisuka W, Fukuda M, Otsuka Y, Hempolchom C, Takaoka H, **Saeung A\***. Natural infection with a filarial larva of a man-biting black fly, *Simulium nigrogilvum* (Diptera: Simuliidae), in Chiang Mai Province, northern

Thailand. Joint International Tropical Medicine Meeting 2017 “Tropical Medicine 4.0 Effective Collaboration for an Impact on Global Health”, 6-8 December 2017, Amari Watergate Hotel, Bangkok.

- Hempolchom C, Srisuka W, Reamtong O, Sookrung N, Sakolvaree Y, Chaicumpa W, Taai K, Dedkhad W, Takaoka H, **Saeung A\***. Protein profiles of female salivary glands of three human-biting black flies (Diptera: Simuliidae) in Thailand. Joint International Tropical Medicine Meeting 2017 “Tropical Medicine 4.0 Effective Collaboration for an Impact on Global Health”, 6-8 December 2017, Amari Watergate Hotel, Bangkok.

## ภาคผนวก

ผลงานวิจัยที่ได้รับการตีพิมพ์เผยแพร่ในวารสารวิชาการระดับนานาชาติจำนวน 12 เรื่อง

1. **Saeung A**, Srisuka W, Low VL, Maleewong W, Takaoka H. Descriptions of the female and larva of *Simulium (Gomphostilbia) udomi* (Diptera: Simuliidae) from Thailand, and its transfer to the *Simulium asakoe* species-group. Acta Trop 2017;172:14-9. **(Q2, ISI impact factor 2017 = 2.509)**
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3. Takaoka H, Srisuka W, **Saeung A**. *Simulium maleewongae*, a new species of *Simulium (Gomphostilbia)* (Diptera: Simuliidae) from Thailand. J Med Entomol 2017;54:91-9. **(Q1, ISI impact factor 2017 = 1.968)**
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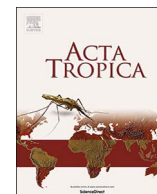
10. Takaoka H, Srisuka W, **Saeung A.** A new species of *Simulium* (*Asiosimulium*) (Diptera: Simuliidae) from Thailand. J Med Entomol 2018;55:569-74. **(Q1, ISI impact factor 2017 = 1.968)**
11. Takaoka H, Srisuka W, Low VL, **Saeung A.** Five new species of the *Simulium decuplum* subgroup of the *Simulium* (*Gomphostilbia*) *batoense* species-group (Diptera: Simuliidae) from Thailand and their phylogenetic relationships. Acta Trop 2018;182:271-84. **(Q2, ISI impact factor 2017 = 2.509)**
12. Takaoka H, Srisuka W, Low VL, **Saeung A.** A new species and a new record of the *Simulium* (*Gomphostilbia*) *gombakense* species-group (Diptera: Simuliidae) from Thailand. Acta Trop 2018;185:156-66. **(Q2, ISI impact factor 2017 = 2.509)**

## บทความสำหรับการเผยแพร่

## แมลงรืนดำและการเป็นพาหะนำโรคในประเทศไทย

รืนดำเป็นแมลงขนาดเล็กที่มีความสำคัญทางด้านการแพทย์และสัตวแพทย์ โดยเป็นพาหะนำโรคมาสู่คนและสัตว์เลี้ยง เช่น โรค Onchocerciasis (หรือโรค river blindness) ซึ่งเกิดจากหนอนพยาธิฟิลาเรียชนิด *Onchocerca volvulus* ซึ่งทำให้เกิดอาการตาบอดในประชากรของหลายประเทศในทวีปแอฟริกา ลาตินอเมริกา และประเทศเยเมน นอกจากนี้มีรายงานพบผู้ป่วยจากการติดเชื้อหนอนพยาธิฟิลาเรียในสกุล *Onchocerca* ในประเทศญี่ปุ่น และเยอรมัน ซึ่งเป็นพยาธิฟิลาเรียที่พบในสัตว์เลี้ยง เช่น โค กระบือ และหมูป่า ซึ่งติดต่อมาสู่คนโดยมีแมลงรืนดำเป็นพาหะ (zoonotic onchocerciasis) ในประเทศไทย มีรายงานว่านักท่องเที่ยวและประชาชนท้องถิ่นที่อาศัยในเขตป่าที่เป็นแหล่งเพาะพันธุ์ตามธรรมชาติของแมลงรืนดำ มีอาการภูมิแพ้จากการถูกแมลงรืนดำกัด เช่น ตุ่มบวมแดง ผื่นหนังอักเสบ ขอบวม และมีไข้ นอกจากนี้ มีรายงานพบหนอนพยาธิฟิลาเรียในสกุลนี้เช่นกันที่จังหวัดเชียงใหม่ โดยพบในแมลงรืนดำ 3 ชนิด คือ *Simulium nigrogilvum*, *Simulium nodosum* และ *Simulium asakoe* และข้อมูลล่าสุดจากงานวิจัยในระหว่างปี พ.ศ. 2559 ถึง พ.ศ. 2561 พบการติดเชื้อหนอนพยาธิฟิลาเรีย *Onchocerca* type A ในแมลงรืนดำชนิด *Simulium nigrogilvum* ที่จังหวัดตาก อย่างไรก็ตาม แม้ว่าขณะนี้ยังไม่มีรายงานพบผู้ป่วยโรค zoonotic onchocerciasis ในประเทศไทย แต่ควรมีการเฝ้าระวังการเจ็บป่วยด้วยโรคนี้อย่างใกล้ชิด

ในช่วงระหว่างปี พ.ศ. 2527 ถึง พ.ศ. 2557 มีรายงานการค้นพบแมลงรืนดำในประเทศไทยจำนวน 93 ชนิด ใน 6 สกุลย่อยของสกุล *Simulium* Latreille s.l. ในประเทศไทย ได้แก่ *Asiosimulium*, *Daviesellum*, *Gomphostilbia*, *Montisimulium*, *Nevermannia* และ *Simulium* s. str. จากการสำรวจความหลากหลายของชนิดแมลงรืนดำ ที่มีการกระจายตัวในภูมิภาคต่างๆ ของประเทศไทย โดยการศึกษาอนุกรมวิธานร่วมกับการประยุกต์ใช้เทคนิคทางชีววิทยาระดับโมเลกุลในการศึกษาระหว่างปี พ.ศ. 2559 ถึง พ.ศ. 2561 ได้รายงานการค้นพบแมลงรืนดำชนิดใหม่เพิ่มเติมอีกจำนวน 14 ชนิด ทำให้ขณะนี้พบแมลงรืนดำในประเทศไทยจำนวนทั้งสิ้น 107 ชนิด ซึ่งองค์ความรู้ใหม่ที่ได้จากงานวิจัย มีประโยชน์ต่อผู้ที่ศึกษาวิจัยแมลงรืนดำทั่วโลก สำหรับใช้จำแนกชนิดแมลงรืนดำ รวมทั้งนำไปใช้เป็นแนวทางในการวางแผนบริหารจัดการทรัพยากรชีวภาพในประเทศไทย และเพื่อการประยุกต์ใช้ประโยชน์ด้านการแพทย์ในอนาคต



# Descriptions of the female and larva of *Simulium (Gomphostilbia) udomi* (Diptera: Simuliidae) from Thailand, and its transfer to the *Simulium asakoe* species-group



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## ABSTRACT

The female and larva of *Simulium (Gomphostilbia) udomi* Takaoka & Choochote from Thailand are described for the first time. The female of this species is similar to those of *S. (G.) asakoe* Takaoka & Davies from Peninsular Malaysia, Thailand, Hong Kong and Vietnam, and *S. (G.) chiangdaoense* Takaoka & Srisuka from Thailand. The larva of this species is similar to *S. (G.) curtatum* Jitklang et al. and *S. (G.) nr. asakoe* 2 from Thailand in having a medium-long postgenal cleft. Taxonomic notes are given to separate this species from these related species. The COI gene sequence of *S. (G.) udomi* is compared with those of eight species of the *S. asakoe* species-group and three species of the *S. ceylonicum* species-group. This species is transferred from the *S. ceylonicum* species-group to the *S. asakoe* species-group based on the adult female and male morphological characters, comparisons of the genetic distances and phylogenetic relationships inferred from the COI gene sequences.

## 1. Introduction

*Gomphostilbia* Enderlein is the second largest among 37 subgenera of the genus *Simulium* Latreille (Diptera: Simuliidae), consisting of 234 species which are classified into 15 species-groups (Adler and Crosskey 2016; Takaoka 2012). It is characterized by a combination of certain morphological characters, such as the haired katapisternum, haired basal portion of the radius in the adults, ventral plate with hairs on the posterior surface in the male, abdominal segment 9 with grapnel-shaped hooklets in the pupa, hypostoma with smooth lateral margins and mandible with a major tooth at the acute angle against its ventral margin. It is mainly distributed in the Oriental Region although 13 and 26 species are recorded from the Palearctic and the Australasian Regions, respectively.

Among 10 species-groups of the subgenus *Gomphostilbia* in the Oriental Region, the *S. ceylonicum* species-group, redefined by Takaoka (2012), is unique in having four subgroups, which are characterized by 4, 6, 8 and 10 pupal gill filaments, respectively.

*Simulium (Gomphostilbia) udomi* Takaoka & Choochote was originally described based on a single male reared from a pupa from

Samorraphume, Thung Chang District, Nan Province, Thailand (Takaoka and Choochote, 2006). It is characterized by the enlarged male hind basitarsus, pupal gill with six long filaments and cocoon with an elongate anterodorsal projection. It was temporarily placed in the *Simulium ceylonicum* species-group, redefined by Takaoka (2012), mainly based on the ventral plate of the male genitalia. The female and larva remained unknown. The biting habit and other biological aspects of this species remain to be studied.

Recently, we collected pupae and larvae of this species from Chiang Rai Province, Thailand and reared four females and two males from pupae. The female and mature larva of *S. (G.) udomi* are described for the first time, and their COI gene segments are sequenced and compared with those of eight species of the *S. asakoe* species-group and those of three species of the *S. ceylonicum* species-group.

The relationships of *S. (G.) udomi* are investigated based on adult female and male morphological characters and by comparing the genetic distances and constructing a phylogenetic tree, using the COI gene sequences

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## 2. Material and methods

Materials examined in this study include four females, two males, all reared from pupae, and 10 mature larvae of *S. (G.) udomi*, collected from a stream (width 60 cm, depth 3 cm, bottom sandy, temperature 20.5°C, pH 5.92, partially shaded, elevation 1286 m, 19°44'17.4"N, 100°23'58.9"E), Thab Tao village, Doi Chom Dao, Thoeng District, Chiang Rai Province, Thailand, 28-X-2016, by W. Srisuka.

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

Two females and two larvae were used for sequencing of the COI genes and a subsequent phylogenetic analysis. The protocols for DNA extraction, PCR amplification, and sequencing follow those of Low et al. (2015). Briefly, DNA was extracted from each specimen using the i-genomic CTB DNA Extraction Mini Kit (iNtRON Biotechnology, Inc., Seongnam, South Korea). PCR amplification of the COI gene region was carried out in a final volume of 50 µL containing 50–100 ng of genomic DNA, 25 µL of ExPrime TaqMasterMix (GENETBIO, Inc., Daejeon, South Korea), and 10 pmol of each forward and reverse primer from Folmer et al. (1994). Purified PCR products were sent to a commercial company for DNA sequencing. The COI gene sequences of *S. udomi* were assembled and edited using ChromasPro Version 1.7.7 (Technelysium Pty Ltd., Australia and BioEdit 7.0.9.0 (Hall, 1999)). The representative sequence generated in this study was deposited in the NCBI GenBank database under accession number KY751928.

The sequences of *S. (G.) asakoe* Takaoka & Davies (Thai samples) (Pramual et al., 2011) and those of 10 related species (seven spp. of the *S. asakoe* species-group and three spp. of the *S. ceylonicum* species-group, all Malaysian samples) (Low et al., 2015) were used for calculation of genetic distances and phylogenetic tree construction. A maximum likelihood analysis was performed on an on-line web-based server PhyML 3.0 (Guindon et al., 2010). An automatic model selection was implemented based on the Akaike information criterion (AIC). The best-fit model was the general time-reversible (GTR) model with a proportion of invariable sites of 0.588 and with a gamma shape parameter of 0.819. To estimate the level of genetic divergence, uncorrected p pairwise genetic distances were estimated using PAUP 4.0b10. (Swofford, 2002).

## 3. Results

### 3.1. Descriptions of the female and larva of *S. (G.) udomi*

**Female.** Body length 2.1–2.2 mm **Head.** Slightly narrower than width of thorax. Frons brownish black, densely covered with yellowish-white scale-like recumbent short hairs interspersed with few dark longer hairs along lateral margins and near vertex; frontal ratio 1.8–1.9:1.0:2.3–2.5; frons:head ratio 1.0:4.2–4.5. Fronto-ocular area well developed, narrow, directed dorsolaterally. Clypeus brownish black, densely covered with yellowish-white scale-like hairs interspersed with six to eight dark longer hairs on each side. Labrum 0.6–0.7 times length of clypeus. Antenna composed of scape, pedicel and nine flagellomeres, medium brown except scape, pedicel and basal one-fourth to half of first flagellomere yellowish. Maxillary palp composed of five segments, light brown except third segment medium brown, proportional lengths of third, fourth, and fifth segments 1.0:1.2:2.3–2.7; third segment (Fig. 1A) slightly widened apically; sensory vesicle (Fig. 1A) medium sized, ellipsoidal (0.3 times length of third segment), with medium-sized opening. Maxillary lacinia with 10–12 inner and 14–16 outer teeth. Mandible (Fig. 1B) with 24 or 25 inner teeth and seven or eight outer teeth at some distance from tip; outer margin near tip undulated, appearing four or five vestigial teeth. Cibarium (Fig. 1C) medially forming sclerotized plate folded forward from posterior margin, with moderately sclerotized mediolongitudinal ridge with bifid apex. **Thorax.** Scutum medium brown except ante-

rolateral calli ochreous, and three blackish longitudinal vittae (one median, two submedian), thinly pruinose and shiny when illuminated at certain angles, densely covered with whitish-yellow to yellow scale-like recumbent short hairs except three vittae with dark recumbent short hairs. Scutellum ochreous, 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 bare. Katepisternum longer than deep, medium brown, shiny when illuminated at certain angles, moderately covered with fine short hairs. **Legs.** Foreleg: coxa whitish yellow; trochanter yellow; femur dark yellow with apical cap light brown (though extreme tip yellowish, and basal two-thirds of inner surface yellowish white); tibia white except apical one-fourth dark brown; tarsus brownish-black, with moderate dorsal hair crest; basitarsus moderately dilated, 6.4–6.6 times as long as its greatest width. Midleg: coxa medium brown; trochanter whitish yellow; femur light brown with basal one-fifth whitish yellow and apical cap medium brown (though extreme tip yellowish); tibia light brown except basal two-thirds yellowish white and apical cap dark brown, covered with yellowish-white fine hairs on posterior and inner surfaces of basal two-thirds; tarsus dark brown though basal one-third of basitarsus dark yellow to light brown. Hind leg: coxa light brown; trochanter whitish yellow; femur light brown with base whitish yellow and apical cap dark brown (though extreme tip yellowish white); tibia (Fig. 1D) yellowish white on basal two-thirds and brownish black on rest, covered with whitish fine hairs on outer and posterior surfaces of little more than basal three-fourths; tarsus medium black except basal two-thirds (though base light brown) and basal half of second tarsomere yellowish white; basitarsus (Fig. 1E) narrow, nearly parallel-sided though slightly narrowed apically, 6.2–6.5 times as long as wide, and 0.7 and 0.6 times as wide as greatest widths of tibia and femur, respectively; calcipala (Fig. 1E) slightly shorter than width at base, and 0.5–0.6 times as wide as greatest width of basitarsus; pedisulcus (Fig. 1E) well developed; claw (Fig. 1F) with large basal tooth 0.54 times length of claw. **Wing.** Length 2.1–2.3 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<sub>1</sub> with dark spinules and hairs; R<sub>2</sub> with hairs only. Basal cell absent. **Halter.** White except basal portion darkened. **Abdomen.** Basal scale grayish yellow, with fringe of whitish-yellow hairs. Dorsal surface of abdomen light to medium brown except segment 2 yellow, moderately covered with dark short to long hairs; tergites of segments 2 and 6–9 shiny when illuminated at certain angles. Ventral surface of segment 2 yellow, those of segments 3 and 4 dark yellow and those of other segments medium brown; sternal plate on segment 7 undeveloped. **Terminalia.** Sternite 8 (Fig. 1G) bare medially, with 18–22 medium-long to long hairs together with four or five slender short hairs on each side. Ovipositor valves (Fig. 1G) triangular (though posteromedial corners rounded), thin, membranous, moderately covered with microsetae interspersed with one or two short hairs; inner margins slightly sinuous, somewhat sclerotized, and moderately separated from each other. Genital fork (Fig. 1H) of usual inverted-Y form, with slender stem; arms of moderate width, moderately folded medially, without lobe directed posteromedially. Paraproct in ventral view (Fig. 1I) somewhat concave anterolaterally, with three or four sensilla on anteromedial surface; paraproct in lateral view (Fig. 1J) produced ventrally beyond ventral tip of cercus, 0.5 times as long as wide, with 17–20 medium-long to long hairs on ventral and lateral surfaces. Cercus in lateral view (Fig. 1J) short, rounded posteriorly, 0.4 times as long as wide. Spermatheca (Fig. 1K) ellipsoidal, 1.3–1.4 times as long as its greatest width, well sclerotized except duct and small area near juncture with duct unsclerotized, and with many fissures on outer surface; internal setae absent; both accessory ducts slender, subequal in diameter to major one.

**Mature larva.** Body length 5.3–5.8 mm. Body light ochreous except abdominal segments 1–3 light greenish and ventral surface of abdom-

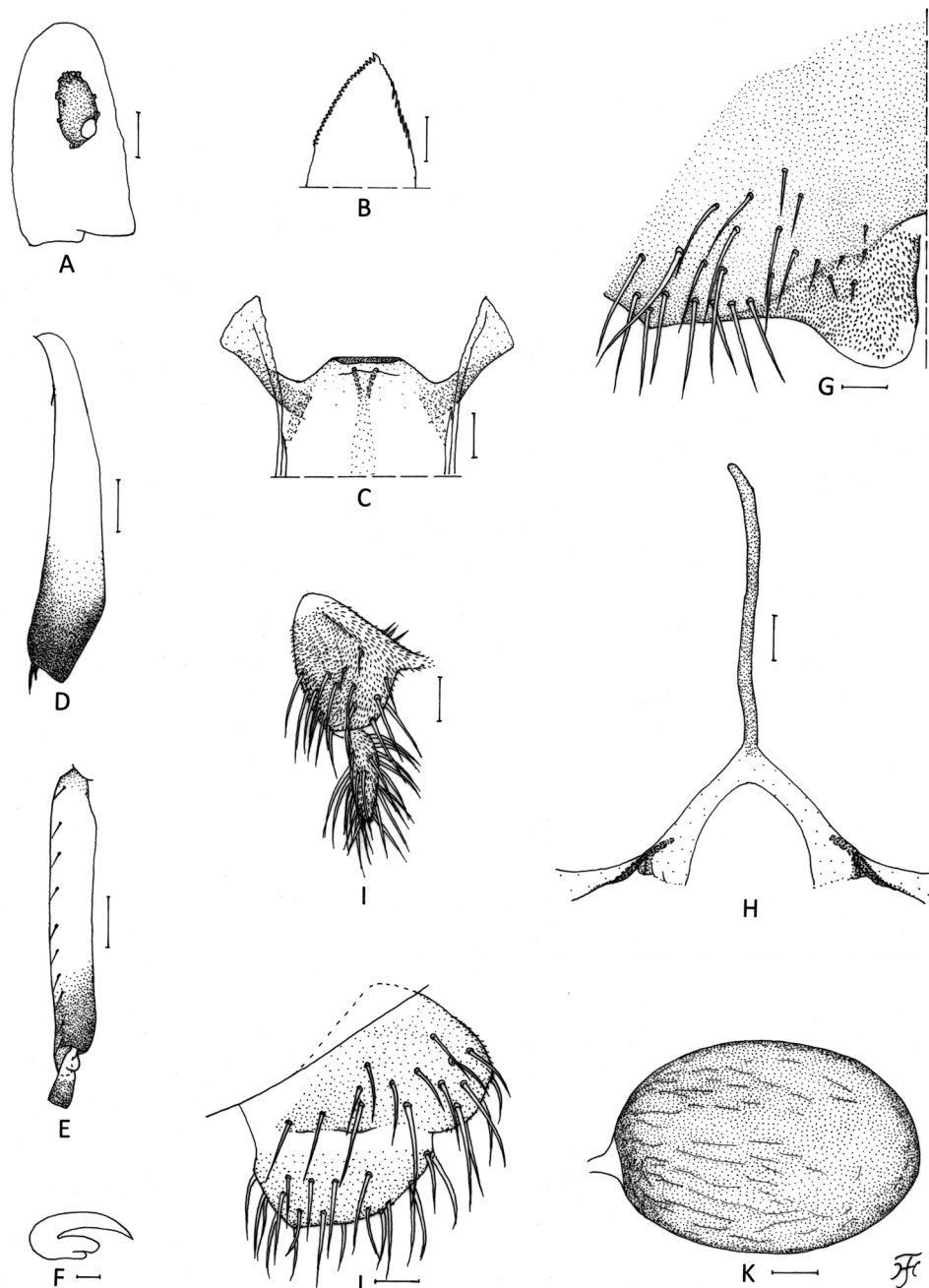


Fig. 1. Female of *Simulium* (*Gomphostilbia*) *udomi*. (A) Third segment of maxillary palp showing sensory vesicle (right side; front view). (B) Mandible. (C) Cibarium. (D) Hind tibia. (E) Basitarsus and tarsomere 2 of hind leg (left side; outer view). (F) Claw. (G) Sternite 8 and ovipositor valve (only right half shown; ventral view). (H) Genital fork (ventral view). (I) & (J) Paraprocts and cerci (right side; I, ventral view; J, lateral view). (K) Spermatheca. Scale bars. 0.1 mm for D and E; 0.02 mm for A–C, G–K; 0.01 mm for F.

inal segments 5–9 whitish, with following color markings: abdominal segments 5 and 6 each with reddish-brown transverse band having darkest medial portion on dorsal surface along anterior margin, though that on abdominal segment 6 often so faint, leaving medial portion as small spot dorsomedially; abdominal segment 4 rarely with faint reddish-brown transverse band; abdominal segments 7 and 8 rarely with faint reddish-brown markings dorsally, dorsolaterally and ventrally. **Head.** Head capsule yellow except eye-spot region whitish, sparsely covered with minute setae (though moderately on dorsal surface); head spots faintly positive though mediolateral spots and anterior spots of posterolateral spots on cephalic apotome usually indistinct; eyebrow faintly discernible. Antenna composed of three articles and apical sensillum, longer than stem of labral fan; proportional lengths of first, second, and third articles 1.0:0.6–0.8:0.7. Labral

fan with 30–36 primary rays. Mandible (Fig. 2A) 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 acute angle degrees against mandible on apical side; supernumerary serrations absent. Hypostoma (Fig. 2B) with row of nine apical teeth, of which median tooth is longer than each corner tooth; lateral margin smooth; five hypostomal bristles per side lying nearly parallel to lateral margin. Postgenal cleft (Fig. 2C) rounded, medium-long, 1.2–1.3 times length of postgenal bridge. Cervical sclerites composed of pair of small yellow rod-like pieces. **Thorax and Abdomen.** Histoblast of pharate pupal gill with six long thread-like filaments. Cuticle of thoracic and abdominal segments sparsely covered with unbranched colorless and dark minute setae on dorsal surface, though more setae on few posterior abdominal segments; last abdominal segment densely covered with

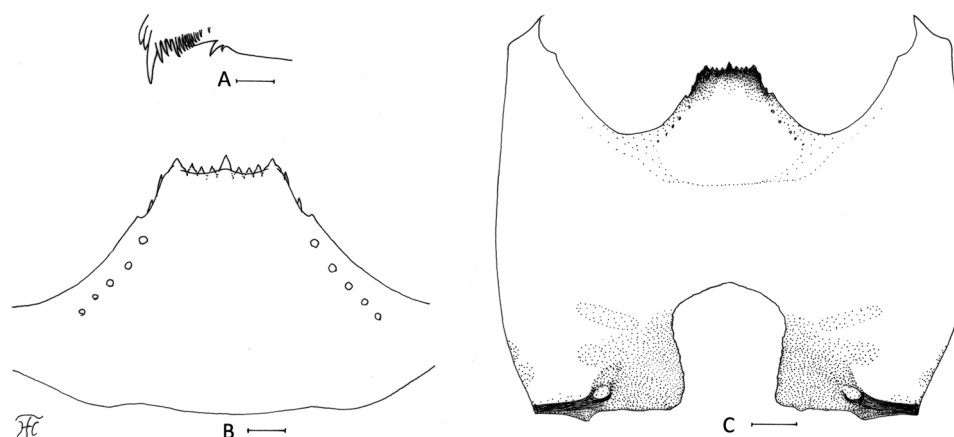


Fig. 2. Larva of *Simulium (Gomphostilbia) udomi*. (A) Mandible. (B) Hypostoma. (C) Head capsule showing postgenal cleft (ventral view). Scales. 0.05 mm for C; 0.02 mm for A and B.

Table 1

Ranges of interspecific uncorrected p distances (%) among members of the *Simulium asakoe* and *S. ceylonicum* species-groups based on COI sequences.

|                            | 1           | 2           | 3           | 4           | 5         | 6           | 7         | 8           | 9          | 10    | 11 |
|----------------------------|-------------|-------------|-------------|-------------|-----------|-------------|-----------|-------------|------------|-------|----|
| 1. <i>S. udomi</i>         | –           |             |             |             |           |             |           |             |            |       |    |
| 2. <i>S. asakoe</i>        | 6.83–7.68   | –           |             |             |           |             |           |             |            |       |    |
| 3. <i>S. izuae</i>         | 6.66–6.83   | 7.68–8.19   | –           |             |           |             |           |             |            |       |    |
| 4. <i>S. roslhashimi</i>   | 6.31–6.83   | 7.17–8.02   | 1.71–2.39   | –           |           |             |           |             |            |       |    |
| 5. <i>S. tanahrataense</i> | 7.51        | 6.83–8.00   | 4.27–4.44   | 4.44–4.61   | –         |             |           |             |            |       |    |
| 6. <i>S. brinchangense</i> | 6.83–7.00   | 6.14–7.17   | 5.97–6.31   | 5.63–6.41   | 5.29–5.63 | –           |           |             |            |       |    |
| 7. <i>S. sofioni</i>       | 8.36        | 8.02–8.70   | 8.19–8.36   | 7.85–8.02   | 6.66      | 7.00–7.17   | –         |             |            |       |    |
| 8. <i>S. lurauense</i>     | 8.02–8.53   | 7.85–9.04   | 7.85–8.36   | 7.51–8.02   | 6.31–6.66 | 6.66–7.17   | 0.34–0.68 | –           |            |       |    |
| 9. <i>S. trangense</i>     | 10.07–10.41 | 9.90–11.09  | 10.24–10.58 | 9.56–10.24  | 9.73–9.90 | 9.22–9.39   | 9.39      | 9.04–9.73   | –          |       |    |
| 10. <i>S. leparens</i>     | 10.58       | 10.41–11.26 | 10.92–11.43 | 10.92–11.09 | 9.56      | 10.41–10.75 | 10.41     | 10.24–10.75 | 11.95      | –     |    |
| 11. <i>S. sheilae</i>      | 9.90        | 9.56–9.90   | 10.41–10.58 | 9.73–9.90   | 9.73      | 10.24–10.58 | 9.56      | 9.56–9.90   | 9.73–10.07 | 10.92 | –  |

unbranched colorless minute setae on dorsolateral and lateral surfaces of each side of anal sclerite and on each lateral surface even down to near tip of ventral papilla; thorax and abdomen without dorsal protuberances. Rectal scales minute and colorless (almost overlooked). Rectal organ compound, each of three lobes with 14–16 finger-like secondary lobules. Anal sclerite of usual X-form, with anterior arms 1.1 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 101–103 rows of hooklets with up to 12–14 hooklets per row.

### 3.2. COI gene sequence-based analysis

Genetic distances between *S. (G.) udomi* and seven members of the *S. asakoe* species-group and three members of the *S. ceylonicum* species-group are 6.83–7.68% for *S. (G.) asakoe*, 6.66–6.83% for *S. (G.) izuae*, 6.31–6.83% for *S. (G.) roslhashimi*, 7.51% for *S. (G.) tanahrataense*, 6.83–7.00% for *S. (G.) brinchangense*, 8.36% for *S. (G.) sofioni* and 8.02–8.53% for *S. (G.) lurauense*, 10.07–10.41% for *S. (G.) trangense*, 10.58% for *S. (G.) leparens* and 9.90% for *S. (G.) sheilae* (Table 1).

The phylogenetic tree constructed based on the COI gene sequences is shown in Fig. 4. *Simulium (G.) udomi* is differentiated from all the related species by forming a highly supported monophyletic clade (100% bootstrap value). Its phylogenetic relationship is closest to *S. (G.) asakoe*, followed by six species of the *S. asakoe* species-group and most distant to three species of the *S. ceylonicum* species-group. Nevertheless, the numbers of *S. udomi* used in the present study might not sufficient to infer their intraspecific variation; and the specimens were only collected from a single location which did not allow us to evaluate their possible geographic variations. Further works with increasing

sampling efforts are needed to clarify these situations.

## 4. Discussion

### 4.1. Morphotaxonomic notes on the female and larva of *S. (G.) udomi*

The female and mature larva of *S. (G.) udomi* are described for the first time. The female of this species is characterized by having the medium-sized sensory vesicle (Fig. 1A), mandible with outer teeth (Fig. 1B), yellow hair tuft of the base of the radial vein, hind tibia whitish on the basal two-thirds (Fig. 1D), and yellow abdominal segment 2. This species is morphologically similar in the female to two species of the *S. asakoe* species-group of the subgenus *Gomphostilbia*: *S. (G.) asakoe* Takaoka & Davies, originally described from Peninsular Malaysia (Takaoka and Davies, 1995), and recorded from Thailand (Takaoka and Davies, 1995), Hong Kong (Takaoka et al., 1995) and Vietnam (Pham, 1998), and *S. (G.) chiangdaoense* Takaoka & Srisuka from Thailand (Takaoka and Srisuka, 2009). The female of this species is barely distinguished from that of *S. (G.) asakoe* by the narrower plate folded from the dorsal margin of the cibarium, which is one-third as wide as the width of the cibarium in *S. (G.) udomi* (Fig. 1C) but slightly more than half the width of the cibarium in *S. (G.) asakoe*, and from *S. (G.) chiangdaoense* by the color of the hind tibia, which is whitish on the basal two-thirds in *S. (G.) udomi* (Fig. 1D) but whitish on the little more than basal half in *S. (G.) chiangdaoense*.

The mature larva of this species is characterized by the medium-long postgenal cleft with an anterior margin rounded (Fig. 2C), by which it is distinguished from most species of the *S. asakoe* and *S. ceylonicum* species-groups, except two Thai species, *S. (G.) nr. asakoe* 2, of which the female and male are unknown, and *S. (G.) curtatum* Jitklang et al. (Jitklang et al., 2008). However, the mature larva of *S. (G.) udomi* is easily distinguished from those of the latter two species by

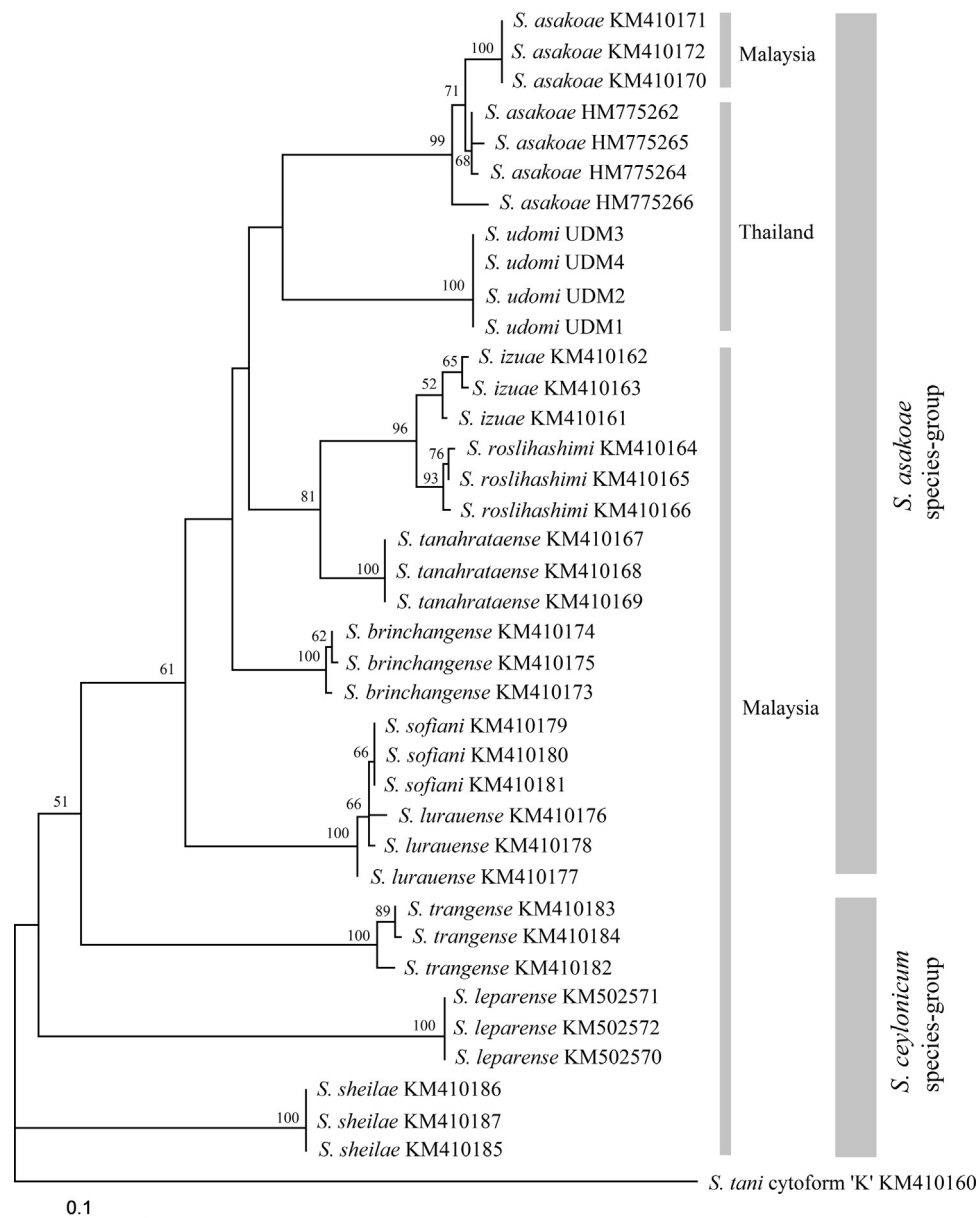


Fig. 3. Maximum likelihood phylogenetic tree of the *Simulium asakoe* and *S. ceylonicum* species-groups based on COI gene sequences.

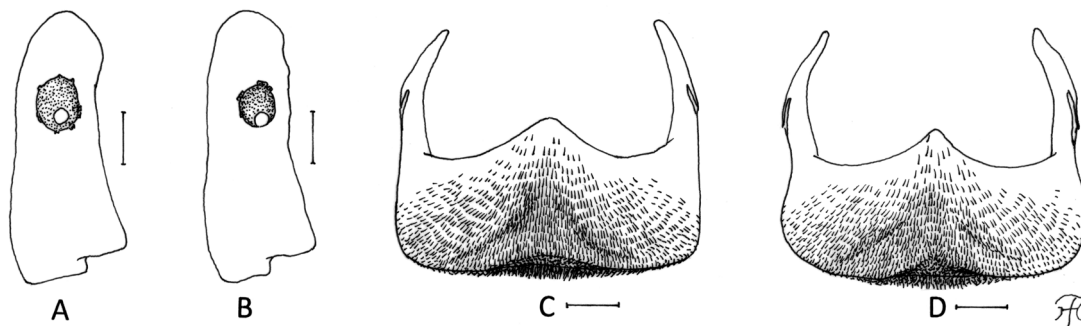


Fig. 4. Male of *Simulium (Gomphostilbia) udomi*. (A) & (B) Third segments of maxillary palp of two different males showing sensory vesicles (right side; front view). (C) & (D) Ventral plates of two different males (ventral view). Scales. 0.02 mm for A–D.

having a reddish-brown marking on the dorsal surface of abdominal segment 5 (without such a color marking in *S. (G.)* nr. *asakoe* 2) and pharate pupal gill with six filaments (eight filaments in *S. (G.)* nr. *asakoe* 2 and *S. (G.) curtatum*).

#### 4.2. Molecular identification of *S. (G.) udomi*

The close morphological similarities of the female of *S. (G.) udomi* to that of *S. (G.) asakoe* raise a challenging problem to identify adult female flies caught in the field for transmission studies of *S. (G.)*

*asakoe*, which is a human biter (Choochote et al., 2005) and a vector of an unknown filaria in Thailand (Fukuda et al., 2003; Ishii et al., 2008).

Our result of the COI gene sequence-based analysis (Table 1) clearly shows that *S. (G.) udomi* and *S. (G.) asakoe* are molecularly distinguished from each other, making it possible to investigate in the future whether females of *S. (G.) udomi* are included in wild-caught females morphologically identified as *S. (G.) asakoe* and also whether they are involved in the transmission of any pathogens and parasites.

#### 4.3. Transfer of *S. (G.) udomi* to the *S. asakoe* species-group

*Simulium (G.) udomi* was temporarily placed in the *S. ceylonicum* species-group, redefined by Takaoka (2012), based on the shape of the ventral plate of the male genitalia, despite the hair tuft of the base of the radial vein being yellow (not dark, as one of key characters of the group). The ventral plate of the male genitalia illustrated in the original description (Takaoka and Choochote, 2006) is, when viewed ventrally, widest subbasally, then gradually narrowed posteriorly, somewhat similar to the typical ventral plate of the *S. ceylonicum* species-group, which is, though, abruptly narrowed on the posterior half. However, the ventral plates are nearly parallel-sided (Fig. 4C) in one male examined in this study, fitting neither the definition of the *S. ceylonicum* species-group nor that of the *S. asakoe* species-group. On the other hand, the ventral plate of another male is widened from the base to the middle, then tapered posteriorly (Fig. 4D), being essentially similar in shape to a typical ventral plate of the *S. asakoe* species-group, which is characterized by its widest portion posteriorly and lateral margins depressed basally or subbasally (Takaoka, 2012).

Despite the variability of the shape of the ventral plate, we propose the transfer of this species from the *S. ceylonicum* species-group to the *S. asakoe* species-group based on the facts that both the female and male have yellow hair tufts of the base of the radius, the female hind tibiae are whitish on the basal two-thirds, and this species is closely related to *S. (G.) chiangdaoense* of the *S. asakoe* species-group in sharing many adult and pupal characters including the cocoon with an elongate anterodorsal projection, a rare character occurring only in these two species within the subgenus *Gomphostilbia*. Our phylogenetic analysis using the COI gene sequences (Fig. 3) shows that *S. (G.) udomi* is most closely related to *S. (G.) asakoe* of the *S. asakoe* species-group, followed by six other members of the *S. (G.) asakoe* species-group whereas it is relatively distantly related to *S. (G.) leparensae*, *S. (G.) sheilae* and *S. (G.) trangense*, all of the *S. ceylonicum* species-group, strongly supporting the transfer of *S. (G.) udomi* to the *S. asakoe* species-group. Although the *S. ceylonicum* species-group is not monophyletic based on the COI gene, our previous multi-locus sequence analysis has revealed two distinct evolutionary lineages, conforming to the morphotaxonomically recognized *S. asakoe* and *S. ceylonicum* species-groups (Low et al., 2015).

#### 5. Supplementary notes for the male of *S. (G.) udomi*

The holotype male of *S. (G.) udomi* has the different shape of the sensory vesicles on each side: an opening of the sensory vesicle is large in the right side and small in the left side (Takaoka and Choochote, 2006). Two males of this species newly obtained in this study show that sensory vesicles have a small opening (Fig. 4A, B). Since the right maxillary palp of the holotype male is aberrant, having the third and fourth segments unsegmented (Takaoka and Choochote, 2006), a large opening of its sensory vesicle is interpreted to be exceptional and the sensory vesicle with a small opening of the left sensory vesicle is normal, as in males of most species of the subgenus *Gomphostilbia*.

The enlarged upper-eye facets of the two males of this species examined are in 13 vertical columns and 14 or 15 horizontal rows (in 13 vertical columns and 16 horizontal rows in the original description).

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#### References

- Adler, P.H., Crosskey, R.W., (2016) World Blackflies (Diptera: Simuliidae): A Comprehensive Revision of the Taxonomic and Geographical Inventory [2016]. 126 pp. [Accessed on Feb. 10, 2017].
- Adler, P.H., Currie, D.C., Wood, D.M., 2004. The Black Flies (Simuliidae) of North America. Cornell University Press, Ithaca, New York, USA (xv + 941 pp.).
- Choochote, W., Takaoka, H., Fukuda, M., Otsuka, Y., Aoki, C., Eshima, N., 2005. Seasonal abundance and daily flying activity of black flies (Diptera: Simuliidae) attracted to human baits in Doi Inthanon National Park, northern Thailand. Med. Entomol. Zool. 56, 335–348.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.
- Fukuda, M., Choochote, W., Bain, O., Aoki, C., Takaoka, H., 2003. Natural infections with filarial larvae in two species of black flies (Diptera: Simuliidae) in northern Thailand. Jpn. J. Trop. Med. Hyg. 31, 99–102.
- Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321.
- Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.
- Ishii, Y., Choochote, W., Bain, O., Fukuda, M., Otsuka, Y., Takaoka, H., 2008. Seasonal and diurnal biting activities and zoonotic filarial infections of two *Simulium* species (Diptera: Simuliidae) in northern Thailand. Parasite 15, 121–129.
- Jitklang, S., Kuvangkadilok, C., Baimai, V., Takaoka, H., Adler, P.H., 2008. Cytogenetics and morphotaxonomy of the *Simulium (Gomphostilbia) ceylonicum* species group (Diptera: Simuliidae) in Thailand. Zootaxa 1917, 1–28.
- Low, V.L., Takaoka, H., Adler, P.H., Ya'cob, Z., Norma-Rashid, Y., Chen, C.D., Sofian-Azirun, M., 2015. A multi-locus approach resolves the phylogenetic relationships of the *Simulium asakoe* and *Simulium ceylonicum* species groups (Diptera: Simuliidae) in Malaysia: evidence for distinct evolutionary lineages. Med. Vet. Entomol. 29, 330–337.
- Pham, X.D., 1998. New records of six black fly species (Diptera: Simuliidae) from Vietnam. Med. Entomol. Zool. 49, 121–123.
- Pramual, P., Wongpakam, K., Adler, P.H., 2011. Cryptic biodiversity and phylogenetic relationships revealed by DNA barcoding of Oriental black flies in the subgenus *Gomphostilbia* (Diptera: Simuliidae). Genome 54, 1–9.
- Swofford, D.L., 2002. PAUP\* Phylogenetic Analysis Using Parsimony (\*and Other Methods). Sinauer Associates, USA.
- Takaoka, H., Choochote, W., 2006. A new species of *Simulium (Gomphostilbia)* (Diptera: Simuliidae) from northern Thailand. Med. Entomol. Zool. 57, 229–233.
- Takaoka, H., Davies, D.M., 1995. The Black Flies (Diptera: Simuliidae) of West Malaysia. Kyushu University Press, Fukuoka, Japan (viii + 175 pp.).
- Takaoka, H., Davies, D.M., Dudgeon, D., 1995. Black flies (Diptera: Simuliidae) from Hong Kong: taxonomic notes with descriptions of two new species. Jpn. J. Trop. Med. Hyg. 23, 189–196.
- Takaoka, H., 2003. The Black Flies (Diptera: Simuliidae) of Sulawesi, Maluku and Irian Jaya. Kyushu University Press, Fukuoka, Japan (xxii + 581 pp.).
- Takaoka, H., 2012. Morphotaxonomic revision of *Simulium (Gomphostilbia)* (Diptera: Simuliidae) in the Oriental Region. Zootaxa 3577, 1–42.
- Takaoka, H., Srisuka, W., 2009. *Simulium (Gomphostilbia) chiangdaoense* sp. nov. (Diptera: Simuliidae) from northern Thailand *Simulium (Gomphostilbia) chiangdaoense* sp. nov. (Diptera: Simuliidae) from northern Thailand. Med. Entomol. Zool. 60, 269–276.

RESEARCH

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# Biodiversity, seasonal abundance, and distribution of blackflies (Diptera: Simuliidae) in six different regions of Thailand

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## Abstract

**Background:** Blackflies are an important medical and veterinary group of small blood-sucking insects. Ninety-three blackfly species have been reported in Thailand. However, information on their biodiversity and population dynamics in each region is lacking. The main aim of this study was to assess the regional biodiversity, seasonal abundance and distribution of blackflies in six eco-geographically different regions in the country.

**Methods:** Blackfly larvae and pupae were sampled monthly from 58 sites between May 2011 and April 2013. Diversity parameters, seasonal abundance, regional distribution and frequency of species occurrence in stream sites were analyzed.

**Results:** A total of 19,456 mature larvae representing 57 species, and belonging to six subgenera in the genus *Simulium* Latreille (s.l.), were found. The five predominant taxa were *S. fenestratum* (8.6%), the *S. asakoe* complex (8.3%), *S. nakhonense* (7.5%), the *S. siamense* complex (7.4%) and the *S. doipuiense* complex (6.7%). The most frequent taxa at all sites were the *S. asakoe* complex (84.5%), followed by *S. fenestratum* (82.8%), the *S. siamense* complex (75.9%), *S. decuplum* (60.3%), *S. nakhonense* (58.6%) and the *S. tani* complex (48.3%). The richness of regional species was highest (40 species) in the north and predominated in the cold season. However, blackflies in the south predominated during the hot season. The highest numbers of blackflies collected from central, northeastern, eastern and western regions of the country were observed in the rainy season. Overall, the mean number of blackflies collected across the six regions during the rainy and cold season had no statistically significant difference, but it differed significantly in the hot season.

**Conclusions:** Blackflies in Thailand were surveyed in all three seasons across six geographical regions. These findings demonstrated that blackfly communities at each stream site varied with seasonality, and the regional relative abundance of blackflies differed markedly in the hot season. It was also found that the occurrence and distribution of blackflies in each region were associated strongly with elevation.

**Keywords:** Blackfly, *Simulium*, Biodiversity, Shannon diversity index, Regional distribution, Thailand

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## Background

Blackflies (Diptera: Simuliidae) are distributed widely in all zoogeographical regions and found almost everywhere with running water that is suitable as a habitat for their aquatic stages [1]. Larvae and pupae are aquatic, and attach themselves to various submerged objects in many types of lotic environments, ranging from large rivers to tiny spring-fed trickles, and from swift currents to water that barely moves [2]. The choice of habitat usually varies between species. Due to their blood-sucking habits, adult females of certain blackfly species are of a medical and veterinary importance. Blackflies have been considered as vectors of many pathogens, such as filarioid nematodes of the genus *Onchocerca* in humans, cattle and deer, the genus *Dirofilaria* in bears, the genus *Splendidofilaria* in ducks; blood protozoans of the genera *Leucocytozoon* and *Trypanosoma* in birds; and viruses (rift valley fever, vesicular stomatitis) in horses and cattle; as well as chlamydial bacteria that cause blindness in sheep and abortion in cattle [1–3]. Furthermore, blackfly bites can cause other severe problems in humans, since they frequently inflict pain, localized swelling, chronic dermatitis and inflammation accompanied by intense irritation that lasts for several days or even weeks [2].

In Thailand, a total of 93 blackfly species belonging to six subgenera, including *Asiosimulium*, *Daviesellum*, *Gomphostilbia*, *Montisimulium*, *Nevermannia* and *Simulium*, have been reported ([4], W. Srisuka, unpublished observations), with most new blackfly species being discovered in the northern part of the country. Remarkably, although the above information reflects rich species diversity, there are only a few reports of simuliids from other regions in Thailand, for instance, *S. otsukai*, *S. thongsahuani*, *S. datfaense* and *S. trangense*, in the south [5–7]; *S. vanellum* from the west [8], *S. atipornae* and *S. lomkaense* from central Thailand [9, 10]; and *S. kuvangkadilokae* from the northeast [11, 12]. Notably, there were no reports on regional biodiversity, seasonal abundance or distribution of blackflies in macro-scale areas of Thailand, apart from only the hotspot area in the tropical rainforest at Doi Pha Hom Pok National Park, in the northern region [13]. Additionally, human-biting blackfly species are found in large numbers, and cause irritation in domestic environments and to indigenous people and tourists [14], thus, most previous studies focused on the annual biting activity of adult females at Doi Inthanon and Doi Suthep-Pui National Park as well as in the village of Ban Pang Faen, Chiang Mai Province, northern Thailand [14–17].

Hence, the main aim of this study was to determine the seasonal abundance and dynamics of blackflies in six geographically and ecologically different regions of Thailand.

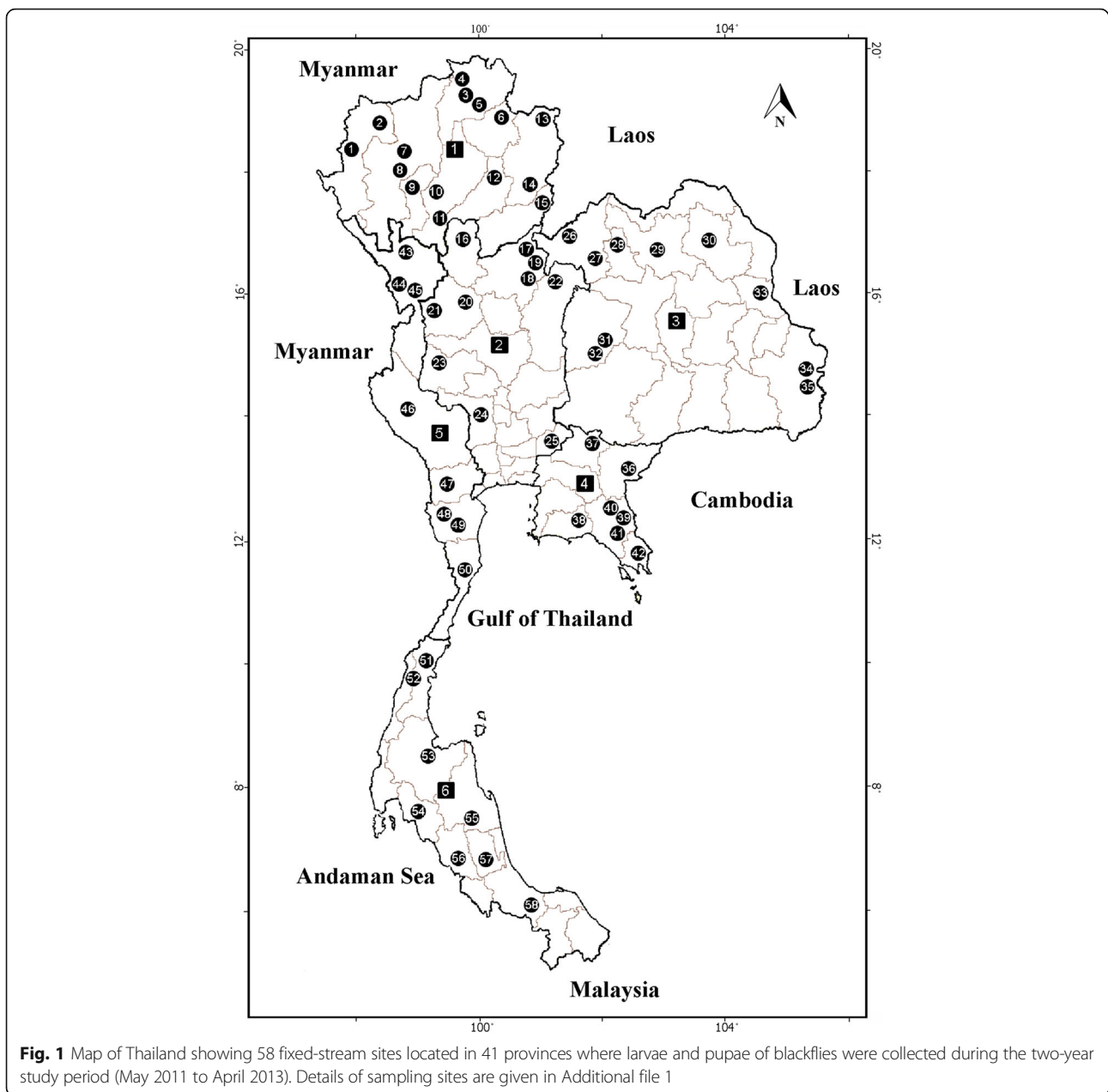
## Methods

### Study areas and sampling

This study was carried out at 58 fixed-stream sites in 41 provinces in six regions across Thailand, including 15, 10, 10, 7, 8 and 8 sites in the north (9 provinces), central (7 provinces), northeast (7 provinces), east (5 provinces), west (5 provinces) and south (8 provinces), respectively (Fig. 1, Additional file 1: Table S1). A total of 696 collections were made in this study (12 at each 58 fixed-stream sites at monthly intervals) from May 2011 to April 2013 which covered all seasons for each region. Larvae and pupae were hand sampled using fine forceps from available substrates in streams, such as fallen leaves, mud or rock surfaces, and trailing grasses. Forty-five minutes exactly were spent for the collection of larvae and pupae by the same person (one person) at each stream site. Larvae were preserved in 80% ethanol. The substrates were cut into pieces so that each part harboured a single pupa. Matured pupae were maintained individually in a plastic tube (10 cm long and 1.7 cm in diameter) with very little water at the bottom until adults emerged. After emergence, adult flies were kept alive in the same tube for at least 24 h, to secure hardening and colouring of their body and legs. Adult flies, associated with their pupal exuviae, were used to confirm the species identification of the larvae.

### Meteorology and regions

The classification of the season in each region follows the Thai Meteorological Department, which bases its records on rainfall and air temperature data. Thus, each year is divided climatically into three seasons in the following regions: North region, with hilly and mountainous areas ranging from 392 to 2210 m in height, total rainfall of approximate 1287 mm and an average air temperature of 25 °C; West region, with mostly mountainous areas in the range of 111–560 m in height, similar to the north, and total rainfall and average air temperature of 1243 mm and 27.13 °C, respectively; Central region, with largely low-level plains and a few mountains ranging from 167 to 1550 m high in the northern and western part of the region, with a total rainfall and average air temperature of 1377 mm and 26.1 °C, respectively; Northeast region, which is a naturally high-level plain called the northeast plateau; Northwest-southeast region, which has Phu Phan Ridge oriented in the northeastern portion that separates this area into two basins, the first one is a large high-level plain in the west and the other smaller and sloped towards the east with an elevation ranging from 110 to 1337 m; this region has a total rainfall and average air temperature of 1589 mm and 25.1 °C, respectively; East region, which is mountainous in the northeast, and its eastern area is close to the Gulf of Thailand with an



elevation ranging from 76 to 409 m, and total rainfall and an average air temperature of 2903 mm and 26.7 °C, respectively; South region, which has a peninsula mountain spine that is very steep. Its eastern area is close to the Gulf of Thailand and its western region near the Andaman Sea. It has an elevation ranging from 111 to 560 m, and total rainfall and an average air temperature of 2601 mm and 28.9 °C, respectively.

#### Species identification

Species identification was based on morphological characteristics of last-instar larvae (matured), pupae and reared adults by using the standard keys of Takaoka &

Choochote [18], which covered 45 blackfly species, and additional keys that dealt with blackflies in Thailand [5–12, 19–26]. When formally named species were known to consist of cryptic species, they were referred to as species complex [4]. All specimens of blackflies from this study were deposited at the Entomology Section, Queen Sirikit Botanic Garden (QSBGE), Chiang Mai Province, Thailand.

#### Data analysis

Species and relative abundance of mature larvae at each site were recorded. The frequency of blackfly species was calculated by the total number of species occurrence

divided by the total number of collections and presented in percentages. Stream occurrence (SO) (expressed in percentage) was obtained by calculating the number of sites where a species was taken and dividing it by the total number of sites sampled ( $n = 58$ ). Species diversity and richness (SDR) version 4 [27] and PAST version 3.11 were employed for statistical analyses [28]. Determination and comparison of diversity parameters between regions were calculated using the Shannon-Wiener index ( $H$ ), the expected value of  $H$  (Exp  $H$ ) and evenness ( $J'$ ). The species accumulation curves (rarefaction) for regions were also compared. In evaluating species richness, the first order jackknife was used to estimate the number of species presenting in all stream sites. Sample interpopulation was used to estimate the number of species from all regions (696 collections) [27]. Detrended correspondence analysis (DCA) was used to describe the regional distribution of blackfly larvae associated with sampling sites [28]. A ternary plot, based on data of blackfly species in each season and region, was used to interpret seasonal occurrence and abundance [28]. Regional and seasonal differences in blackflies were compared using non-parametric Kruskal-Wallis tests, and  $P$ -values were adjusted by the Bonferroni correction for *post-hoc* multiple comparison tests. In addition, the Mann-Whitney  $U$ -test was used to compare the mean number of blackflies collected from the southern region in two seasons. Statistical analyses were conducted using IBM SPSS statistics, version 24 for Windows (Chicago, SPSS Inc.). Statistical significance was set at  $P < 0.05$ .

## Results

### Species composition of blackflies

A total of 19,456 mature larvae, representing 57 blackfly species of six subgenera, were collected from 58 stream sites across six regions in Thailand (Table 1, Additional file 1: Table S1). At the subgenus level, *Simulium* was the most diverse (28 species), followed by *Gomphostilbia* (16 species), *Nevermannia* (7 species), *Asiosimulium* (3 species), *Montisimulium* (2 species) and *Daviesellum* (1 species). *Simulium fenestratum*, the *S. asakoe* complex, *S. nakhonense*, the *S. siamense* complex, and the *S. doipuiense* complex were the five predominant taxa, each representing 8.6% ( $n = 1681$ ), 8.3% ( $n = 1608$ ), 7.5% ( $n = 1451$ ), 7.4% ( $n = 1441$ ) and 6.7% ( $n = 1298$ ) of those collected. The most frequent taxa at all sites were the *S. asakoe* complex (84.5%, 49/58 sites), followed by *S. fenestratum* (82.8%, 48/58 sites), the *S. siamense* complex, (75.9%, 44/58 sites), *S. decuplum* (60.3%, 35/58 sites), *S. nakhonense* (58.6%, 34/58 sites) and the *S. tani* complex, (48.3%, 28/58 sites).

### Species diversity, richness and distribution pattern

Species diversity and richness of blackflies in each region are shown in Fig. 2, with the highest in the northern region ( $H = 3.1$ ,  $J' = 0.8$ ) and lowest in the southern region ( $H = 2.1$ ,  $J' = 0.5$ ). Of 58 stream sites, the Shannon diversity index ( $H$ ) was highest at Rom Klao (Phitsanulok Province), followed by Doi Phu Kha (Nan Province), Phu Ruea (Loei Province) and Mae Wong (Kamphaeng Phet Province), which represented 2.4, 2.4, 2.3 and 2.3, respectively (Additional file 2: Table S2).

The species accumulation (rarefaction) curves (Fig. 3) showed the expected and observed richness of species occurring in all sites, with a total from all collections being 71 and 57 species, respectively. The expected ( $\pm$  SE) and observed species richness were  $52 \pm 4.5$  and 40 spp. in northern,  $45 \pm 4.1$  and 34 spp. in the central,  $38 \pm 2.9$  and 28 spp. in western,  $31 \pm 2.8$  and 22 spp. in northeastern,  $25 \pm 4.3$  and 17 spp. in southern and  $24 \pm 1.8$  and 15 spp. in eastern regions, respectively (Fig. 4).

The detrended correspondence analysis (DCA) for the distribution of blackfly species associated with sampling sites (axis 1: eigenvalue 0.8; axis 2: eigenvalue 0.5) is presented in Fig. 5. Overall, the *S. asakoe* complex and *S. fenestratum* were common species found in all sites. The distribution of the *S. doipuiense* complex, *S. inthanonense*, *S. fruticosum*, *S. chiangdaoense*, *S. maeaiense*, *S. (Montisimulium) sp.*, *S. vessabutrae*, *S. atipornae* and *S. lomkaoense* were associated strongly with high elevation (site nos. 8, 13, 14, 17, 21 and 22) in the northern and central region, while *S. quinquestriatum*, *S. siamense* complex, *S. decuplum* and *S. dentistylum* were associated with low elevation (site nos. 20, 23, 24, 25, 29, 30, 31, 35, 38, 40, 41 and 42) in the central and northeastern regions. However, *S. lampangense*, *S. weji*, *S. prayongi* and *S. takense* were associated with calcareous waterfalls (site nos. 5, 12 and 44).

### Seasonal dynamics

Overall, *S. fenestratum* was the dominant species during all three seasons (Additional file 3: Table S3). Almost all of the species in the north were dominant in the cold season, and *S. chumpornense* and *S. phayaoense* were recorded only during that time, while *S. pahangense* was collected only during the rainy season (Fig. 6, Additional file 3: Table S3). Most species in the central region were discovered in the rainy season, and *S. oblongum*, *S. aureohirtum*, *S. bullatum*, *S. phukaense* and the *S. tani* complex were found only during that time, whereas *S. yongi* was only recorded in the cold season (Fig. 6, Additional file 3: Table S3). A greater number of blackflies were collected during the rainy season in the northeastern, eastern and western regions (Figs. 7, 8, Additional file 3: Table S3). *Simulium oblongum*, *S. aureohirtum* and *S. yuphae* were found only during the rainy season in the eastern region,

**Table 1** Total number, relative abundance (percentage), and stream occurrence (SO) of mature larvae of 57 blackfly species collected from 58 sampling sites in six regions in Thailand

| Species                                                                         | Total collected | %flies | %SO  |
|---------------------------------------------------------------------------------|-----------------|--------|------|
| <i>Simulium</i> ( <i>Asiosimulium</i> ) <i>furvum</i>                           | 34              | 0.2    | 1.7  |
| <i>Simulium</i> ( <i>Asiosimulium</i> ) <i>oblongum</i>                         | 487             | 2.5    | 17.2 |
| <i>Simulium</i> ( <i>Asiosimulium</i> ) <i>wanchaii</i>                         | 34              | 0.2    | 3.5  |
| <i>Simulium</i> ( <i>Daviesellum</i> ) <i>pahangense</i>                        | 9               | 0.1    | 5.2  |
| <i>Simulium</i> ( <i>Gomphostilbia</i> ) <i>angulistylum</i> complex            | 515             | 2.7    | 22.4 |
| <i>Simulium</i> ( <i>Gomphostilbia</i> ) <i>asakoe</i> complex <sup>a,b</sup>   | 1608            | 8.3    | 84.5 |
| <i>Simulium</i> ( <i>Gomphostilbia</i> ) <i>burtoni</i>                         | 523             | 2.7    | 24.1 |
| <i>Simulium</i> ( <i>Gomphostilbia</i> ) <i>chiangdaoense</i>                   | 515             | 2.7    | 10.3 |
| <i>Simulium</i> ( <i>Gomphostilbia</i> ) <i>chumpornense</i>                    | 182             | 0.9    | 27.6 |
| <i>Simulium</i> ( <i>Gomphostilbia</i> ) <i>curtatum</i>                        | 235             | 1.2    | 12.1 |
| <i>Simulium</i> ( <i>Gomphostilbia</i> ) <i>decuplum</i> <sup>a</sup>           | 1175            | 6      | 60.3 |
| <i>Simulium</i> ( <i>Gomphostilbia</i> ) <i>dentistylum</i>                     | 367             | 1.9    | 37.9 |
| <i>Simulium</i> ( <i>Gomphostilbia</i> ) <i>duolongum</i>                       | 450             | 2.3    | 22.4 |
| <i>Simulium</i> ( <i>Gomphostilbia</i> ) <i>gombakense</i>                      | 90              | 0.5    | 13.8 |
| <i>Simulium</i> ( <i>Gomphostilbia</i> ) <i>inthanonense</i>                    | 753             | 3.9    | 19   |
| <i>Simulium</i> ( <i>Gomphostilbia</i> ) <i>piroonae</i>                        | 52              | 0.3    | 1.7  |
| <i>Simulium</i> ( <i>Gomphostilbia</i> ) <i>kuvangkadilokae</i>                 | 98              | 0.5    | 3.5  |
| <i>Simulium</i> ( <i>Gomphostilbia</i> ) <i>parahiyangum</i>                    | 3               | 0      | 1.7  |
| <i>Simulium</i> ( <i>Gomphostilbia</i> ) <i>sheilae</i>                         | 556             | 2.9    | 44.8 |
| <i>Simulium</i> ( <i>Gomphostilbia</i> ) <i>siamense</i> complex <sup>a,b</sup> | 1441            | 7.4    | 75.9 |
| <i>Simulium</i> ( <i>Montisimulium</i> ) <i>nanense</i>                         | 51              | 0.3    | 1.7  |
| <i>Simulium</i> ( <i>Montisimulium</i> ) sp.                                    | 57              | 0.3    | 1.7  |
| <i>Simulium</i> ( <i>Nevermannia</i> ) <i>aureohirtum</i>                       | 408             | 2.1    | 22.4 |
| <i>Simulium</i> ( <i>Nevermannia</i> ) <i>fangense</i>                          | 22              | 0.1    | 1.7  |
| <i>Simulium</i> ( <i>Nevermannia</i> ) <i>feuerborni</i> complex                | 142             | 0.7    | 8.6  |
| <i>Simulium</i> ( <i>Nevermannia</i> ) <i>fruticosum</i>                        | 329             | 1.7    | 17.2 |
| <i>Simulium</i> ( <i>Nevermannia</i> ) <i>khunklangense</i>                     | 73              | 0.4    | 1.7  |
| <i>Simulium</i> ( <i>Nevermannia</i> ) <i>maeiense</i>                          | 241             | 1.2    | 8.6  |
| <i>Simulium</i> ( <i>Nevermannia</i> ) <i>vessabutrae</i>                       | 7               | 0      | 1.7  |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>atipornae</i>                            | 98              | 0.5    | 3.5  |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>baimaii</i>                              | 69              | 0.4    | 1.7  |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>brevipar</i>                             | 16              | 0.1    | 1.7  |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>bullatum</i>                             | 61              | 0.3    | 10.3 |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>chamlongi</i>                            | 217             | 1.1    | 20.7 |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>chiangmaiense</i>                        | 79              | 0.4    | 3.5  |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>doipuiense</i> complex <sup>b</sup>      | 1298            | 6.7    | 25.9 |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>fenestratum</i> <sup>a,b</sup>           | 1681            | 8.6    | 82.8 |

**Table 1** Total number, relative abundance (percentage), and stream occurrence (SO) of mature larvae of 57 blackfly species collected from 58 sampling sites in six regions in Thailand (Continued)

| Species                                                              | Total collected | %flies | %SO  |
|----------------------------------------------------------------------|-----------------|--------|------|
| <i>Simulium</i> ( <i>Simulium</i> ) <i>grossifilum</i>               | 15              | 0.1    | 3.5  |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>lampangense</i>               | 139             | 0.7    | 5.2  |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>lomkaoense</i>                | 98              | 0.5    | 3.5  |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>malayense</i>                 | 21              | 0.1    | 1.7  |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>manooni</i>                   | 196             | 1      | 8.6  |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>nakhonense</i> <sup>a,b</sup> | 1451            | 7.5    | 58.6 |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>nigrogilvum</i>               | 17              | 0.1    | 5.2  |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>nobile</i>                    | 843             | 4.3    | 17.2 |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>nodosum</i>                   | 301             | 1.5    | 15.5 |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>phayaoense</i>                | 54              | 0.3    | 6.9  |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>prayongi</i>                  | 35              | 0.2    | 1.7  |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>phukaense</i>                 | 37              | 0.2    | 6.9  |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>quinquestriatum</i>           | 614             | 3.2    | 34.1 |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>siripoomense</i>              | 19              | 0.1    | 1.7  |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>takense</i>                   | 69              | 0.4    | 1.7  |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>tani</i> complex <sup>a</sup> | 385             | 2      | 48.3 |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>thailandicum</i>              | 228             | 1.2    | 12.1 |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>weji</i>                      | 609             | 3.1    | 6.9  |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>yongi</i>                     | 38              | 0.2    | 3.5  |
| <i>Simulium</i> ( <i>Simulium</i> ) <i>yuphae</i>                    | 311             | 1.6    | 36.2 |
| Total                                                                | 19,456          | 100    |      |

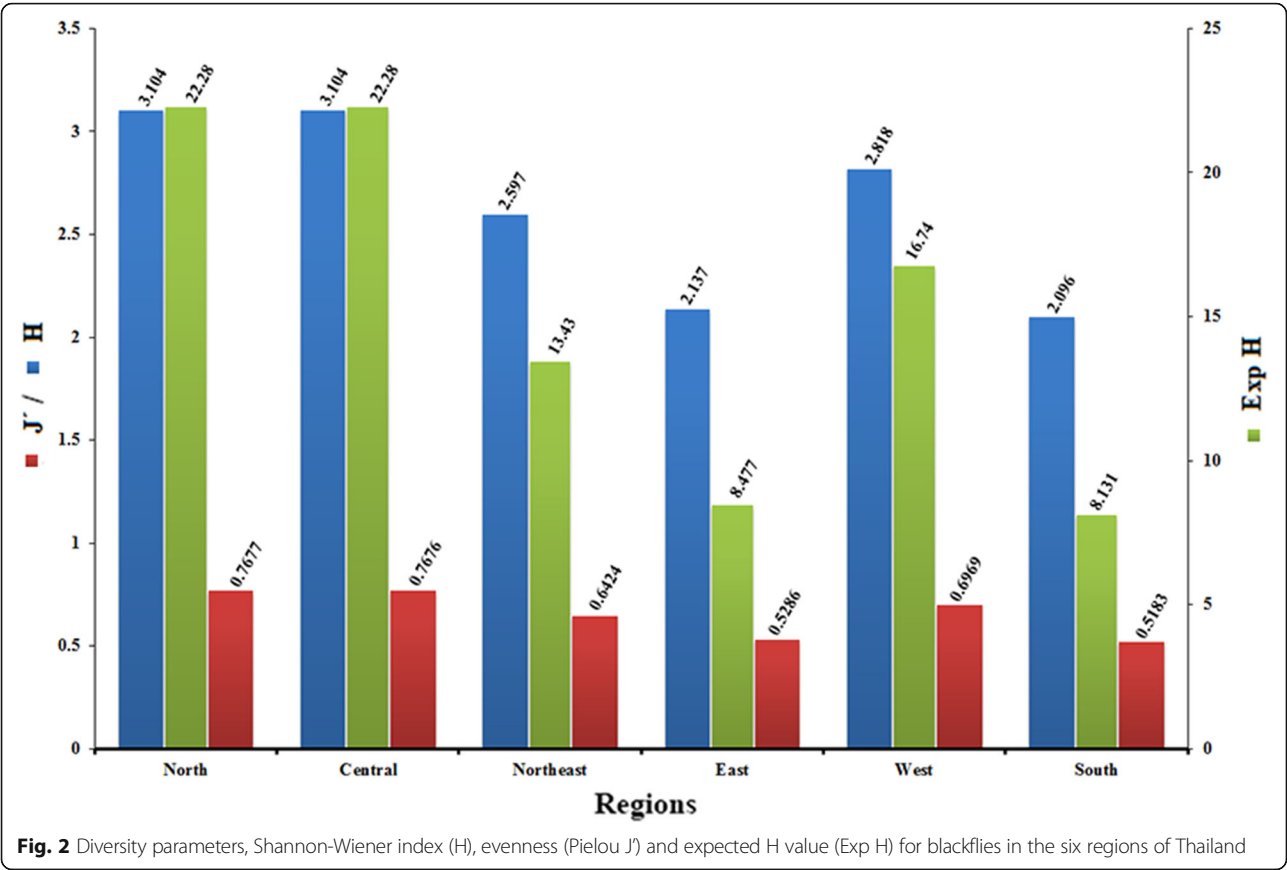
<sup>a</sup>The most frequent taxa at all sites<sup>b</sup>The most predominant taxa

similar to species found in the central region. Remarkably, blackflies in the southern region were more dominant during the hot season (Fig. 8, Additional file 3: Table S3). The mean number of blackflies collected across the six regions during the rainy (Kruskal-Wallis test,  $H = 6.242$ ,  $df = 5$ ,  $P = 0.283$ ) and cold season (Kruskal-Wallis test,  $H = 8.650$ ,  $df = 4$ ,  $P = 0.070$ ) had no statistically significant difference, but it differed significantly in the hot season (Kruskal-Wallis test,  $H = 26.589$ ,  $df = 5$ ,  $P < 0.0001$ ).

### Regional relative abundance

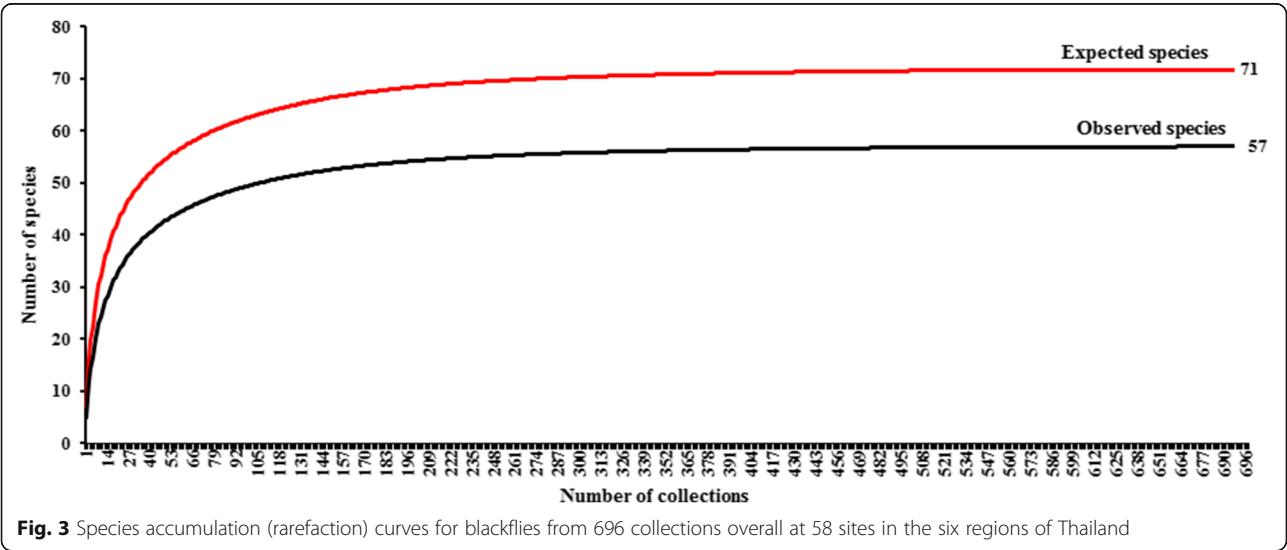
#### Northern region

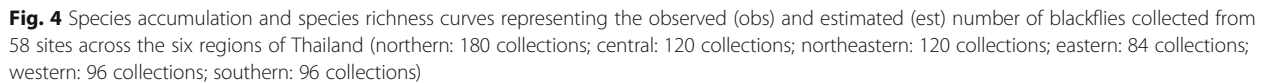
The most frequent taxa at all sites were *S. asakoe* complex (80%) and *S. fenestratum* (80%), followed by *S. yuphae* (73.3%) and the *S. siamense* complex, (66.7%). In addition, the *S. doipuiense* complex, *S. inthanonense*, the *S. asakoe* complex, *S. decuplum*/*S. fenestratum* and *S. chiangdaoense* were the five predominant taxa, representing 15.4% ( $n = 860$ ), 11.6% ( $n = 647$ ), 7.0% ( $n = 389$ ), 6.4% ( $n = 358$ ) and 5.3% ( $n = 298$ ), respectively (Additional file 4: Table S4). The hot season had significantly lower mean numbers when compared to the rainy (Kruskal-Wallis

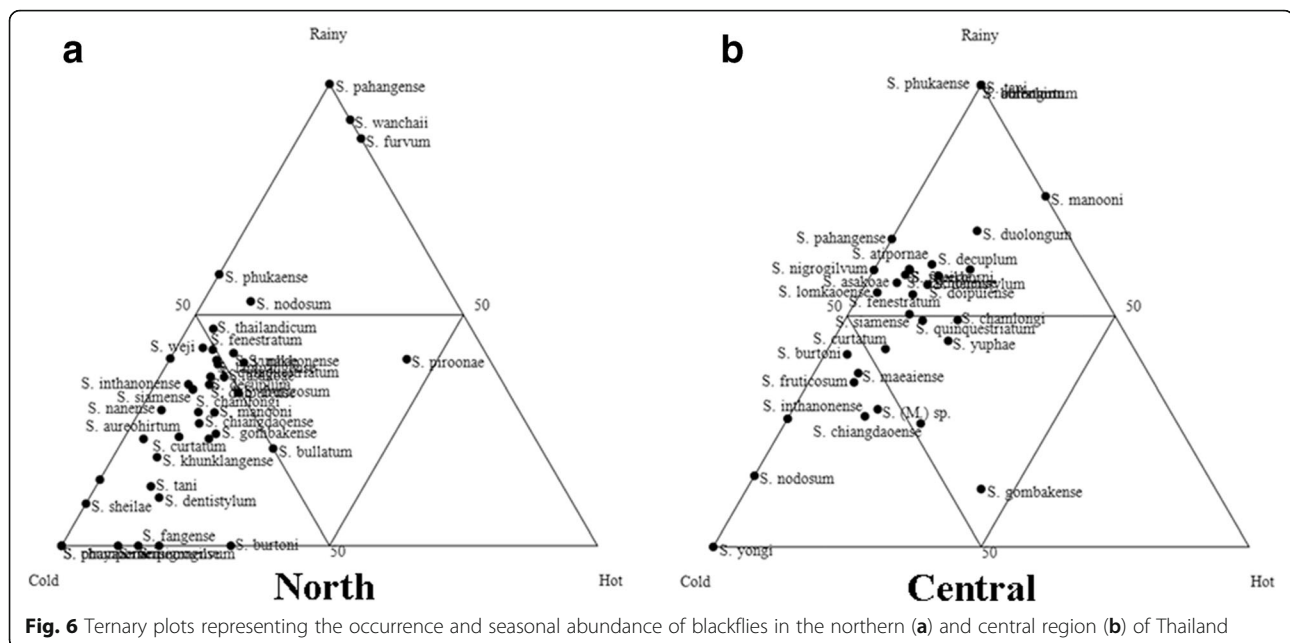


test,  $H = 21.195$ ,  $df = 2$ ,  $P = 0.021$ ) and cold seasons (Kruskal-Wallis test,  $H = 34.122$ ,  $df = 2$ ,  $P < 0.0001$ ).

**Central region**  
The most frequent taxa at all sites were *S. asakoe* complex (90%) and the *S. siamense* complex, (90%), followed by *S. decuplum* and *S. fenestratum* (80%). Additionally, the *S. asakoe* complex, the *S. doipuiense* complex, the *S. siamense* complex, *S. nakhonense* and *S. ducuplum*, were the five predominant taxa, representing 10.3% ( $n = 353$ ), 10% ( $n = 345$ ), 7.4% ( $n = 254$ ), 7.3% ( $n = 251$ ) and 6.3% ( $n = 218$ ), respectively (Additional file 5: Table S5).







There were significant differences in the mean number of blackflies captured in the central region during the hot season, when compared to the rainy (Kruskal-Wallis test,  $H = 30.265$ ,  $df = 2$ ,  $P < 0.0001$ ) and cold seasons (Kruskal-Wallis test,  $H = 19.456$ ,  $df = 2$ ,  $P = 0.020$ ).

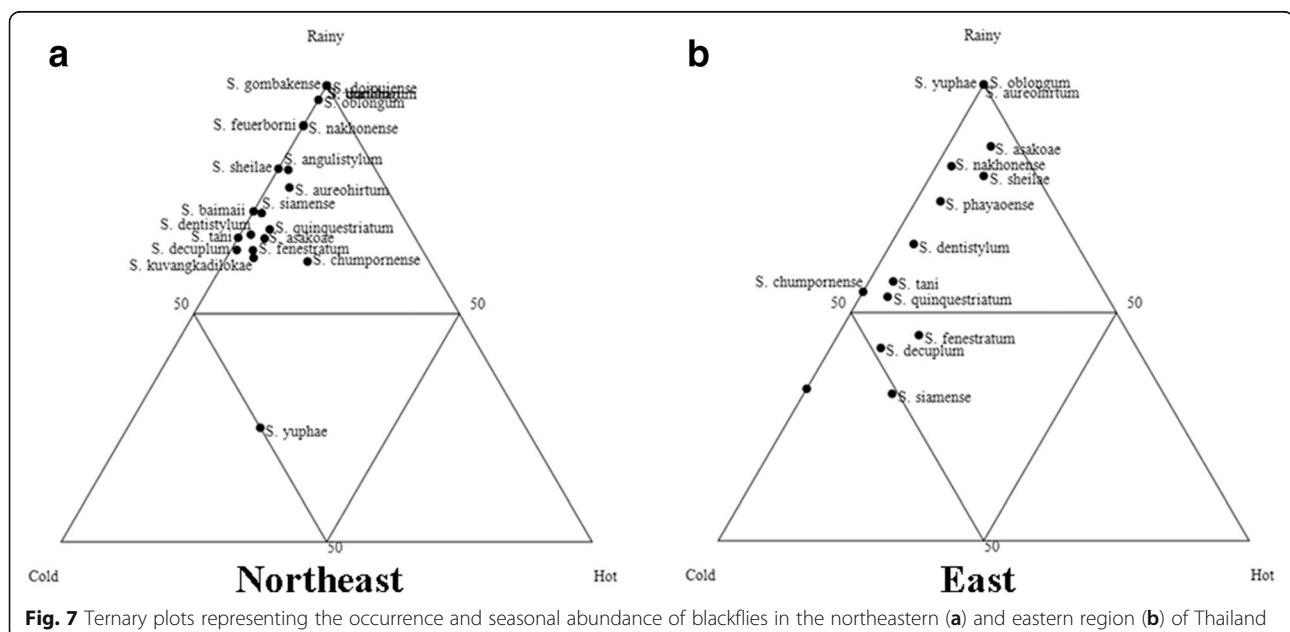
#### Northeastern region

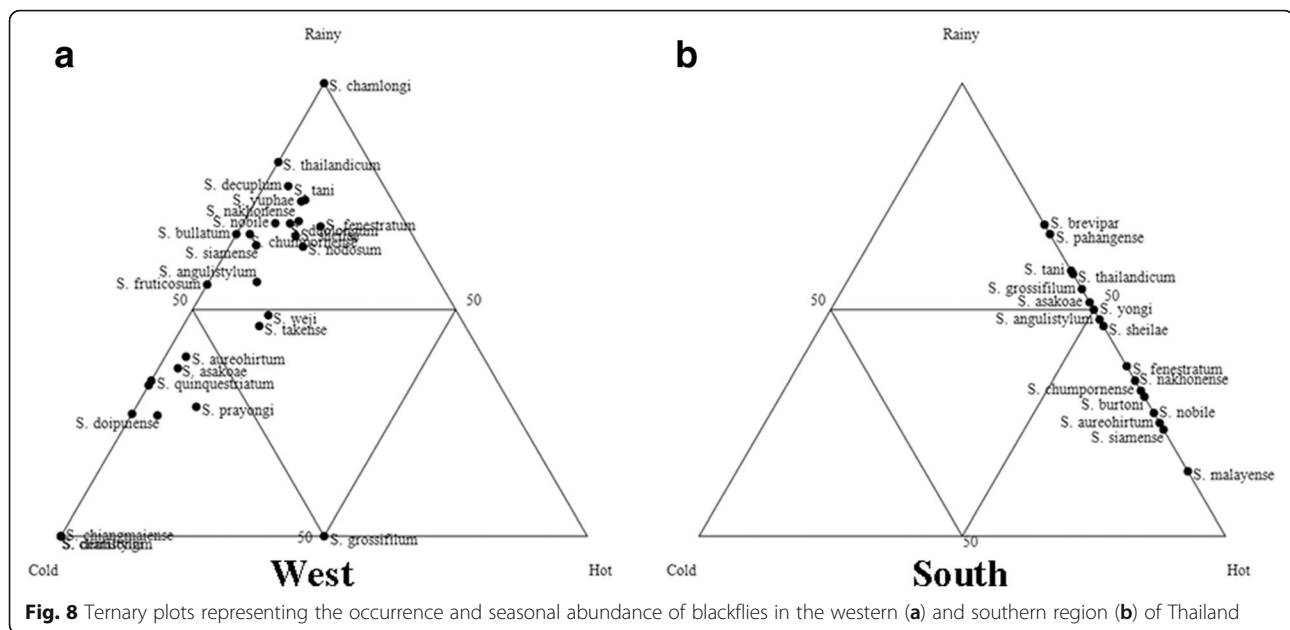
The most frequent taxa at all sites were *S. asakoe* complex and *S. siamense* complex (100%), followed by *S. decuplum*, *S. fenestratum* and *S. quinquestratum* (90%). In addition, the *S. asakoe* complex, *S. oblongum*, *S. fenestratum*, the *S. siamense* complex and *S. quinquestratum*

were the five predominant taxa, representing 15.7% ( $n = 415$ ), 13.4% ( $n = 353$ ), 12.8% ( $n = 338$ ), 11% ( $n = 290$ ) and 8.3% ( $n = 219$ ), respectively (Additional file 6: Table S6). There was a significant difference in the mean number of blackflies collected among the three seasons (Kruskal-Wallis test,  $H = 28.687$ ,  $df = 2$ ,  $P < 0.0001$ ).

#### Eastern region

The most frequent taxa at all sites were *S. decuplum*, *S. dentistylum*, the *S. siamense* complex, *S. fenestratum* and the *S. tani* complex (100%), followed by the *S.*





**Fig. 8** Ternary plots representing the occurrence and seasonal abundance of blackflies in the western (a) and southern region (b) of Thailand

*asakoe* complex and *S. nakhonense* (85.7%). Additionally, *S. fenestratum*, the *S. siamense* complex, *S. decuplum*, *S. dentistylum* and *S. nakhonense* were the five predominant taxa, representing 24% ( $n = 479$ ), 19.6% ( $n = 391$ ), 18.5% ( $n = 369$ ), 8.1% ( $n = 163$ ) and 7% ( $n = 140$ ), respectively (Additional file 7: Table S7). The mean number of blackflies captured in the hot season was lower than that in the rainy (Kruskal-Wallis test,  $H = 21.195$ ,  $df = 2$ ,  $P = 0.021$ ) and cold season (Kruskal-Wallis test,  $H = 34.122$ ,  $df = 2$ ,  $P < 0.0001$ ). There was a significant difference in a mean number of the blackflies collected in this region between the hot and rainy seasons (Kruskal-Wallis test,  $H = 15.033$ ,  $df = 2$ ,  $P < 0.005$ ).

#### Western region

The most frequent taxa at all sites were *S. nakhonense* and *S. tani* complex (75%), followed by the *S. angulistylum* complex, the *S. asakoe* complex, *S. sheilae*, the *S. siamense* complex and *S. fenestratum* (62.5%). In addition, *S. nakhonense*, *S. weji*, the *S. angulistylum* complex, *S. duolongum* and the *S. siamense* complex were the five predominant taxa, representing 16.1% ( $n = 548$ ), 12% ( $n = 406$ ), 9.3% ( $n = 317$ ), 7.5% ( $n = 255$ ) and 6.5% ( $n = 221$ ), respectively (Additional file 8: Table S8). The mean number of blackflies captured was lower during the hot season than that during the rainy (Kruskal-Wallis test,  $H = 27.071$ ,  $df = 2$ ,  $P < 0.0001$ ) and cold season (Kruskal-Wallis test,  $H = 22.589$ ,  $df = 2$ ,  $P = 0.002$ ).

#### Southern region

The most frequent taxa at all sites were *S. burtoni*, *S. sheilae*, *S. nakhonense* and *S. nobile* (100%), followed by the *S. asakoe* complex and *S. fenestratum* (87.5%).

Additionally, *S. nobile*, *S. burtoni*, *S. nakhonense*, *S. sheilae* and the *S. asakoe* complex were the five predominant taxa, representing 31.1% ( $n = 746$ ), 19.8% ( $n = 474$ ), 12.4% ( $n = 297$ ), 12.1% ( $n = 289$ ) and 5.2% ( $n = 124$ ), respectively (Additional file 9: Table S9). There were no significant differences in the mean number of blackflies captured between the hot and rainy seasons (Mann-Whitney  $U$ -test,  $U = 128.000$ ,  $P = 0.570$ ).

## Discussion

### Species composition, species richness, seasonal abundance and diversity

The number of blackflies reached its highest during the cold season, according to a previous report by Srisuka et al. [13], who studied the seasonal biodiversity of blackflies at Doi Pha Hom Pok, northern Thailand. The greatest number of blackflies in the southern region was in the hot season. This study found that seven of seventeen species identified from this region increased their populations approximately two to three times during this season. This observation agrees with the study of blackflies in northern Sweden, where they were higher in the summer than other seasons [29]. The highest number of blackflies collected from central, northeastern, eastern and western regions of Thailand peaked in the rainy season. The findings in this study were consistent with those in a previous report by Pramual & Wongpakam [30], who studied the seasonal variation of blackflies at Phu Phan mountain range in northeastern Thailand. They demonstrated that the species abundance was higher in rainy seasons than in others and blackfly communities at each stream site varied with seasonality, i.e. *S. nakhonense*, the *S. angulistylum* complex and *S.*

*kuvangkadilokae* were more dominant in the rainy season, whereas the *S. asakoe* complex, *S. aureohirtum* and *S. trangense* were dominant in the hot and cold season [30]. Likewise, blackflies were caught in higher numbers during the rainy season in Nigeria, Africa [31]. Takaoka [32] showed that seasonal abundance patterns of adult populations of *S. ochraceum*, the vector of onchocerciasis in Guatemala, Central America, differ by localities depending on the availability of permanent and temporary streams suitable for its immature stages.

In addition to seasons and geographical locations, elevation also can influence blackfly populations. The results of this study showed that the Shannon diversity index was highest in areas with high elevations, i.e. Rom Klao (1047 m), Doi Phu Kha (1629 m), Phu Ruea (1337 m) and Mae Wong (1274 m). It was found that 36 species manifested in optimal or unique environments that had suitable factors for their breeding habitats. For example, *S. baimaii* breeds at Phu Kradueng, Loei Province in only high mountains, with slow-flowing streams exposed to sunlight. Likewise, all species members in the subgenus *Montisimulium* are restricted to high elevations at Doi Inthanon National Park, Chiang Mai Province. In contrast, *S. gombakense* has a wide vertical distribution range from a height of 500 m in small streams in the foothills to 2100 m near the summit of Doi Pha Hom Pok National Park [13], and it is also found at an elevation of 412 m at Mae Klang Waterfall, Doi Inthanon National Park [33]. In addition, the *S. asakoe* complex, *S. fenestratum*, the *S. siamense* complex, *S. decuplum*, *S. nakhonense*, and the *S. tani* complex were the most common taxa found in this study, which is similar to previous reports by Pramual & Kuvangkadi-  
lok [34], and Pramual & Wongpakam [30].

#### Relationship of subgenera to elevation

The subgenus *Asiosimulium* is a small and endemic subgenus in the Oriental region. It is represented by four species, of which three, *S. oblongum*, *S. wanchaii* and *S. furvum*, have been reported in Thailand [35–37], and the remaining one, *S. suchitrae*, in Nepal [38]. The first three species were found in lowland streams, flowing slowly over rock surfaces exposed to the sun during the rainy season, while *S. suchitrae* was found at high elevation (1826 m) in a small stream flowing slowly over rocks [38]. Both *S. furvum* and *S. wanchaii* were restricted to their sites, but *S. oblongum* was distributed widely in and near the northeastern, central and eastern regions. The subgenus *Daviesellum* is represented by two species, *S. pahangense* and *S. courtneyi*, in Thailand [39]. Only *S. pahangense* was distributed at high elevation from northern to central regions along the boundary with Myanmar, and also in lowland streams in the southern region. Most species of *Gomphostilbia*, such as

those of the *S. batoense*, *S. ceylonicum*, *S. epistum*, *S. gombakense* and *S. varicorne* species-groups, are the second largest subgenus in Thailand and distributed at low elevations. This study found *S. sheilae*, the *S. siamense* complex and *S. chumpornense* in all six regions of Thailand, with their breeding habitats mostly in lowland streams as previously reported [7, 30]. In contrast, species of the *S. asakoe* and *S. darjeelingense* species-groups were found in highland streams, except for the *S. asakoe* complex, which was distributed widely from low elevations to 2500 m at the summit of Doi Inthanon National Park, Chiang Mai Province and in other Asian countries, such as Malaysia, China (Hong Kong) and Vietnam [7, 13, 21, 25, 40–42].

The subgenus *Montisimulium* is represented by six species in Thailand. Two of them, i.e. *S. nanense* and *S. (Montisimulium) sp.*, were discovered in this study at high elevations, as reported by Takaoka & Somboon [43] and Takaoka et al. [44], who collected three species of this subgenus at high elevations ranging from 2229 to 3720 m in Bhutan and 1750 m in Vietnam, respectively. The remaining species have been found only on Doi Inthanon and Doi Pha Hom Pok in Chiang Mai Province [45, 46]. Of ten species of the subgenus, *Nevermannia* reported in Thailand, seven were found in this study. Most species were collected at high elevations, for example, the high mountains of Chiang Mai Province, northern Thailand [13, 26, 47, 48]. Other reports from several other Asian countries, such as Malaysia, Myanmar, Vietnam, Indonesia and Bhutan, indicated that members of this subgenus were associated with high elevations ranging from 1000 to 2532 m [43, 49–53]. The subgenus *Simulium* is the largest subgenus in Thailand, including 45 described species, of which 28 (62% of total species) were collected. Most of the common taxa, such as *S. fenestratum*, *S. nakhonense*, *S. quinquestriatum*, and the *S. tani* complex, breed in lowland streams. The findings of this study were in accordance with those reported by Takaoka et al. [44], Srisuka et al. [13] and Pramual & Wongpakham [30]. Species of the *S. christophersi*, *S. malyschevi* and *S. variegatum* species-groups were distributed in middle to high elevations (1200–2200 m), as previously studied in Vietnam and Thailand [44, 54]. In contrast, most of the species within the *S. multistriatum*, *S. nobile* and *S. striatum* species-groups occurred in lowland streams, as reported by Srisuka et al. [13] and Ishii et al. [17]. Members of the *S. griseifrons* species-group colonized streams at low to high elevations (200–2210 m).

#### Conclusion

The findings of this study demonstrated that the richness and relative abundance of blackflies were different between regions, and blackfly communities at each

stream site varied with seasonality. Also, the elevation of sampling sites, which ranged from high mountainous to lowland streams as well as covering all mainland steams, influenced the distribution of blackflies in the country. Concurrent species, population dynamics and seasonal abundance in each area are important as useful information for pest species management and control programs, and especially for ecotourism in forests, by waterfalls and in high mountainous areas, where the number of tourists increases yearly.

## Additional files

**Additional file 1: Table S1.** Names of sampling sites, geographical coordinates, altitudes, and environmental variables for blackfly collections at 58 sampling sites in six regions of Thailand. (DOCX 38 kb)

**Additional file 2: Table S2.** Diversity parameters for blackflies at 58 sampling sites in the six regions of Thailand. (DOCX 19 kb)

**Additional file 3: Table S3.** Seasonal abundance and species richness of blackfly species at 58 sampling sites representing six regions in Thailand. (DOCX 44 kb)

**Additional file 4: Table S4.** Regional distribution and relative abundance of blackflies at 15 sampling sites in northern Thailand. (DOCX 30 kb)

**Additional file 5: Table S5.** Regional distribution and relative abundance of blackflies at 10 sampling sites in central Thailand. (DOCX 24 kb)

**Additional file 6: Table S6.** Regional distribution and relative abundance of blackflies at 10 sampling sites in northeastern Thailand. (DOCX 20 kb)

**Additional file 7: Table S7.** Regional distribution and relative abundance of blackflies at 7 sampling sites in eastern Thailand. (DOCX 18 kb)

**Additional file 8: Table S8.** Regional distribution and relative abundance of blackflies at 8 sampling sites in western Thailand. (DOCX 21 kb)

**Additional file 9: Table S9.** Regional distribution and relative abundance of blackflies at 8 sampling sites in southern Thailand. (DOCX 19 kb)

## Abbreviations

DCA: Detrended correspondence analysis; Exp H: Expected value of H; Pielou J': evenness; H: Shannon-Wiener index

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## Availability of data and materials

The data sets supporting the conclusions of this article are included within the article and its additional files.

## Authors' contributions

WS and AS conceived and designed the study. WS and AS performed field and laboratory experiments, analyzed the data, interpreted the findings and wrote the manuscript. HT participated in species identification, data analysis and revised the

manuscript. YO and MF helped to revise the manuscript. ST and KT participated in field experiments. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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## References

1. Takaoka H. Insecta: Diptera, Simuliidae. In: Yule CM, Yong HS, editors. Freshwater invertebrates of the Malaysian region. Kuala Lumpur, Malaysia: The Academy of Sciences Malaysia; 2004. p. 673–82.
2. Srisuka W. Species diversity of black flies in Thailand, and the evaluation of ecological factors influencing black-fly species diversity in Doi Phahompok National Park. PhD Thesis, Chiang Mai University; 2015.
3. Murdock CC, Adler PH, Frank J, Perkins SL. Molecular analyses on host-seeking blackflies (Diptera: Simuliidae) reveal a diverse assemblage of *Leucocytozoon* (Apicomplexa: Haemospororida) parasites in an alpine ecosystem. *Parasit Vectors*. 2015;8:343.
4. Adler PH, Crosskey RW. World blackflies (Diptera: Simuliidae): A comprehensive revision of the taxonomic and geographical inventory. Available at: <http://entweb.clemson.edu/biomia/pdfs/blackflyinventory.pdf>. Accessed 20 Feb 2017.
5. Takaoka H, Otsuka Y, Choochote W, Thongsahuan S. Two new and one newly recorded species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from southern Thailand. *Med Entomol Zool*. 2009;60:259–68.
6. Takaoka H, Otsuka Y, Choochote W, Thongsahuan S. A new species of *Simulium* (*Simulium*) (Diptera: Simuliidae) from southern Thailand. *Med Entomol Zool*. 2010;61:17–25.
7. Jitklang S, Kuvangkadilok C, Baimai V, Takaoka H, Adler PH. Cytogenetics and morphotaxonomy of the *Simulium* (*Gomphostilbia*) *ceylonicum* species group (Diptera: Simuliidae) in Thailand. *Zootaxa*. 2008;1917:1–28.
8. Huang YT, Phasuk J, Chanpaisaeng J, Adler PH. A new species of black fly in the subgenus *Simulium* (Diptera: Simuliidae) from Thailand. *Med Entomol Zool*. 2010;61:49–58.
9. Takaoka H, Srisuka W, Otsuka Y, Choochote W. A new species and species-group of *Simulium* (*Simulium*) (Diptera: Simuliidae) from Thailand. *J Med Entomol*. 2014; 51:725–32.
10. Takaoka H, Srisuka W, Saeung A, Otsuka Y, Choochote W. *Simulium* (*Simulium*) *lomkaoense*, a new species of black fly (Diptera: Simuliidae) from Thailand. *J Med Entomol*. 2014;51:1109–15.
11. Pramual P, Tangkawanit U. A new species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from northeastern Thailand. *Med Entomol Zool*. 2008; 59:297–303.
12. Takaoka H, Srisuka W. Description of the female of *Simulium* (*Gomphostilbia*) *kuvangkadilokae* (Diptera: Simuliidae) from Thailand. *Med Entomol Zool*. 2010;61:39–47.

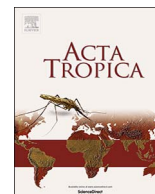
13. Srisuka W, Takaoka H, Otsuka Y, Fukuda M, Thongsahuan S, Taai K, et al. Seasonal biodiversity of black flies (Diptera: Simuliidae) and evaluation of ecological factors influencing species distribution at Doi Pha Hom Pok National Park, Thailand. *Acta Trop.* 2015;149:212–9.
14. Choochote W, Takaoka H, Fukuda M, Otsuka Y, Aoki C, Eshima N. Seasonal abundance and daily flying activity of black flies (Diptera: Simuliidae) attracted to human baits in Doi Inthanon National Park, northern Thailand. *Med Entomol Zool.* 2005;56:335–48.
15. Ittiponpanya N. A whole-year study of the flying activity of black fly attracted to humans in Doi Suthep-Pui National Park. MSc Thesis, Chiang Mai University; 2006.
16. Srisuka W. Life history and morphology of some black flies species at Mae Aeb Nai Village, ban Laung subdistrict, Chom thong district, Chiang Mai province, MSc Thesis, Chiang Mai University; 2007.
17. Ishii Y, Choochote W, Bain O, Fukuda M, Otsuka Y, Takaoka H. Seasonal and diurnal biting activities and zoonotic filarial infections of two species (Diptera: Simuliidae) in northern Thailand. *Parasite.* 2008;15:121–9.
18. Takaoka H, Choochote W. A list of and keys to black flies (Diptera: Simuliidae) in Thailand. *Trop Med Hlth.* 2004;32:189–97.
19. Edwards FW. The Simuliidae (Diptera) of Java and Sumatra. *Arch Hydrobiol.* 1934;13(Suppl. 5):92–138.
20. Takaoka H. The black flies of Taiwan (Diptera: Simuliidae). *Pacific Insects.* 1979;20:365–403.
21. Takaoka H, Davies DM. The black flies (Diptera: Simuliidae) of West Malaysia. Fukuoka, Japan: Kyushu University Press; 1995. 175 pp.
22. Takaoka H, Srisuka W. *Simulium (Nevermannia) vessabutrae* sp. nov. (Diptera: Simuliidae) from Thailand. *Med Entomol Zool.* 2010;61:97–104.
23. Takaoka H, Srisuka W. Description a new species of *Simulium (Montisimulium)* (Diptera: Simuliidae) from Thailand. *Med Entomol Zool.* 2010;61:261–4.
24. Takaoka H, Srisuka W. A new species of *Simulium (Nevermannia)* (Diptera: Simuliidae) from Thailand, with keys to members of the *Simulium feuerborni* species group in Thailand. *ZooKeys.* 2011;89:57–70.
25. Takaoka H, Suzuki H. The black flies (Diptera: Simuliidae) from Thailand. *Jpn J Sanit Zool.* 1984;35:7–45.
26. Takaoka H, Srisuka W, Saeung A, Otsuka Y, Choochote W. *Simulium (Nevermannia) chomthongense*, a new species of black fly (Diptera: Simuliidae) from Chiang Mai. *Thailand Trop Biomed.* 2012;29:381–90.
27. Seaby RM, Henderson PA. Species diversity and richness version 4. 2016. Pisces conservation Ltd., Lymington, England.
28. Hammer O, Harper DAT, Ryan PD. PAST: paleontological statistics software package for education and data analysis. *Palaeontol Electron.* 2001;4:1–9.
29. Malmqvist B, Zhang Y, Adler PH. Diversity, distribution and larval habitats of north Swedish blackflies (Diptera: Simuliidae). *Freshwat Biol.* 1999;42:301–14.
30. Pramual P, Wongpakam K. Seasonal variation of black fly (Diptera: Simuliidae) species diversity and community structure in tropical streams of Thailand. *Entomol Sci.* 2010;13:17–28.
31. Akpan SS, Alaribe AAA, Ejazie GC. The distribution of black flies (*Simulium* species) in Ugbem and Ukwepiyere communities of Biase local Govt, area of Cross River state. *Nigeria J Dental Med Sci.* 2012;1:24–8.
32. Takaoka H. Seasonal occurrence of *Simulium ochraceum*, the principal vector of *Onchocerca volvulus* in the southeastern endemic area of Guatemala. *Am J Trop Med Hyg.* 1981;30:1121–32.
33. Takaoka H, Srisuka W, Saeung A. Description of the female of *Simulium (Gomphostilbia) gombakense* (Diptera: Simuliidae) from Thailand. *Med Entomol Zool.* 2010;61:111–4.
34. Pramual P, Kuvangkadilok C. Agricultural land use and black fly (Diptera, Simuliidae) species richness and species assemblages in tropical streams. *Northeastern Thailand Hydrobiologia.* 2009;625:173–84.
35. Takaoka H, Choochote W. A new subgenus and a new species of *Simulium* s.l. (Diptera: Simuliidae) from Thailand. *Med Entomol Zool.* 2005;56:33–41.
36. Takaoka H, Choochote W. A new species of the subgenus *Simulium (Asiosimulium)* (Diptera: Simuliidae) from Thailand. *Med Entomol Zool.* 2006;57:45–8.
37. Takaoka H, Srisuka W, Saeung A, Choochote W. *Simulium (Asiosimulium) furvum*, a new species of black fly (Diptera: Simuliidae) from Thailand. *J Med Entomol.* 2013;50:493–500.
38. Takaoka H, Shrestha S. New species of black flies (Diptera: Simuliidae) from Nepal. *Zootaxa.* 2010;2731:1–62.
39. Takaoka H, Adler PH. A new subgenus, *Simulium (Daviesellum)*, and a new species, *S. (D.) courtneyi*, (Diptera: Simuliidae) from Thailand and Peninsular Malaysia. *Jpn J Trop Med Hyg.* 1997;25:17–27.
40. Takaoka H, Saito K. A new species and new records of black flies (Diptera: Simuliidae) from Thailand. *Jpn J Trop Med Hyg.* 1996;24:163–9.
41. Takaoka H. Morphotaxonomic revision of *Simulium (Gomphostilbia)* (Diptera: Simuliidae) in Oriental Region. *Zootaxa.* 2012;3577:1–42.
42. Low VL, Adler PH, Sofian-Azirun M, Srisuka W, Saeung A, Huang YT, et al. A multi-locus approach resolves the phylogenetic relationships of the *Simulium asakoeae* and *Simulium ceylonicum* species groups in Malaysia: evidence for distinct evolutionary lineages. *Med Vet Entomol.* 2015;29:330–7.
43. Takaoka H, Somboon P. Eleven new species and one new record of black flies (Diptera: Simuliidae) from Bhutan. *Med Entomol Zool.* 2008;59:213–62.
44. Takaoka H, Sofian-Azirun M, Ya'cob Z, Chen CD, Lau KW, Low VL, et al. The black flies (Diptera: Simuliidae) of Vietnam. *Zootaxa.* 2017;4261:1–165.
45. Takaoka H, Choochote W. Two new species of *Simulium (Montisimulium)* (Diptera: Simuliidae) from northern Thailand. *Med Entomol Zool.* 2005;56: 21–31.
46. Takaoka H, Choochote W. Discovery of two more new species of *Simulium (Montisimulium)* (Diptera: Simuliidae) in Doi Inthanon National Park, Chiang Mai, Thailand. *Trop Med Hlth.* 2005;33:209–15.
47. Takaoka H, Choochote W. Two new species of *Simulium (Nevermannia)* (Diptera: Simuliidae) from northern Thailand. *Trop Med Hlth.* 2005;33:133–41.
48. Takaoka H, Srisuka W. *Simulium (Nevermannia) wichaii*, a new black fly species (Diptera: Simuliidae) from northern Thailand. *Med Entomol Zool.* 2010;61:273–9.
49. Ya'cob Z, Takaoka H, Low VL, Sofian-Azirun M. Uncovering the mask of the *Simulium feuerborni* Complex (Diptera: Simuliidae): description of a new pseudocryptic species *Simulium pairoti* from Malaysia. *Acta Trop.* 2017;169: 133–41.
50. Ya'cob Z, Takaoka H, Sofian-Azirun M. *Simulium ledangense*, a new species of the *Simulium feuerborni* species-group of the subgenus *Nevermannia* (Diptera: Simuliidae) from Mount Ledang, Peninsular Malaysia. *Zootaxa.* 2014;3881(3):228–36.
51. Takaoka H. Notes on blackflies (Diptera: Simuliidae) from Myanmar (formerly Burma). *Japan. J Trop Med Hyg.* 1989;17:243–57.
52. Takaoka H, Sofian-Azirun M, Ya'cob Z, Chen CD, Lau KW, Pham XD. The black flies (Diptera: Simuliidae) from Thua Thien Hue and Lam Dong provinces, Vietnam. *Zootaxa.* 2015;3961:1–96.
53. Takaoka H, Davies MDA. New black flies species of *Simulium (Nevermannia)* from Sumatra, Indonesia. *Jpn J Trop Med Hyg.* 1995;23:127–31.
54. Takaoka H, Srisuka W, Saeung A. Two new species of the *Simulium (Simulium) variegatum* species-group of black flies (Diptera: Simuliidae) from Thailand. *J Med Entomol.* 2017;54:1213–23.

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# A new black fly species of the *Simulium* (*Gomphostilbia*) *epistum* species-group (Diptera: Simuliidae) from Thailand



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## ABSTRACT

A new species of black fly, *Simulium* (*Gomphostilbia*) *isanense*, is described based on females, males, pupae and mature larvae from Thailand. This new species is placed in the *Simulium epistum* species-group of the subgenus *Gomphostilbia* Enderlein. It is characterized by the pupal gill with eight filaments arranged as 3 + 3 + 2 from dorsal to ventral, of which an inner filament of the ventral pair is slightly longer than its counter filament. Taxonomic notes are provided to distinguish this new species from *S. (G.) angulistylum* Takaoka & Davies from Peninsular Malaysia, and three other related species. The difference between this new species and *S. (G.) angulistylum* is supported by genetic distances using the mitochondrial COI gene.

## 1. Introduction

The *Simulium epistum* species-group is one of 15 species-groups of the subgenus *Gomphostilbia*, the second largest of the 37 subgenera of the genus *Simulium* Latreille (Adler and Crosskey, 2017). This species-group consists of 30 species and is distributed only in the Oriental Region (Adler and Crosskey, 2017; Takaoka, 2012). In this species-group, little is known about the biting habits and other biological aspects, although *S. (Gomphostilbia) asakoe* Takaoka & Davies of the *S. asakoe* species-group is a vector of an unknown filaria parasite in northern Thailand (Fukuda et al., 2003; Ishii et al., 2008).

In Thailand, the *S. epistum* species-group is represented by five species, *S. (G.) adleri* Jitklang & Kuvangkadilok, *S. (G.) angulistylum* Takaoka & Davies, *S. (G.) cheongi* Takaoka & Davies, *S. (G.) datfaense* Takaoka & Otsuka, and *S. (G.) otsukai* Takaoka & Choochote (Takaoka and Davies, 1995; Kuvangkadilok and Takaoka, 2000; Jitklang and Kuvangkadilok, 2007; Takaoka et al., 2009). Pramual and Kuvangkadilok (2012) reported that *S. (G.) angulistylum* is a species complex consisting of three cytoforms in Thailand.

In a recent survey of pupae and larvae of black flies in Surin Province in northeastern Thailand, we collected one undescribed species of the *S. epistum* species-group, which is similar to *S. (G.) angulistylum* in having the male style wide and abruptly bent inward, a character differing from those of the four other Thai species of the same group.

The DNA sequence-based analysis using the COI gene supports the difference between this undescribed species and *S. (G.) angulistylum*.

This species is here described as new based on females, males, pupae and larvae.

## 2. Materials and methods

The methods of collection, description and illustration, and terms for morphological features follow those of Takaoka (2003) and partially those of Adler et al. (2004). The holotype and paratypes are deposited at the Entomology Section, Queen Sirikit Botanic Garden, Chiang Mai, Thailand.

*Simulium* (*G.*) *isanense* sp. nov. from Thailand, three Peninsular Malaysian species, *S. (G.) angulistylum* (from the type locality, Gombak), *S. (G.) whartoni* Takaoka & Davies and *S. (G.) cheongi* Takaoka & Davies, and one Indonesian species, *S. (G.) atratum* De Meijere, all members of the *S. epistum* species-group, were subjected to DNA isolation, COI gene amplification, and sequencing as described by Low et al. (2015). Thai *S. (G.) angulistylum* sequences (AY251483–AY251486, HM775234–HM775241, and JQ619907–JQ619931) were retrieved from the NCBI GenBank database and included for analysis. New sequences generated from the present study were deposited in the NCBI GenBank database under accession numbers MF476242–MF476257.

Uncorrected p pairwise genetic distances were computed using PAUP 4.0b10 (Swofford 2002). Bayesian inference (BI) analysis was

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performed using MrBayes (Huelsenbeck and Ronquist, 2001) with the following settings. The analysis model employed two substitution types (“nst = 2”), with rate variation across sites modelled using a gamma distribution (rates = “gamma”). The Markov chain Monte Carlo search was run with four chains for 500000 generations, with trees begin sampled every 100 generations. The first 1000 trees were discarded as burnin. Neighbor-joining (NJ) analysis was performed in MEGA7 (Kumar et al., 2016) with Kimura's two-parameter model of substitution (K2P distance), using 1000 bootstrap replicates.

### 3. Results

#### 3.1. *Simulium (Gomphostilbia) isanense* Takaoka, Srisuka & Saeung sp. nov.

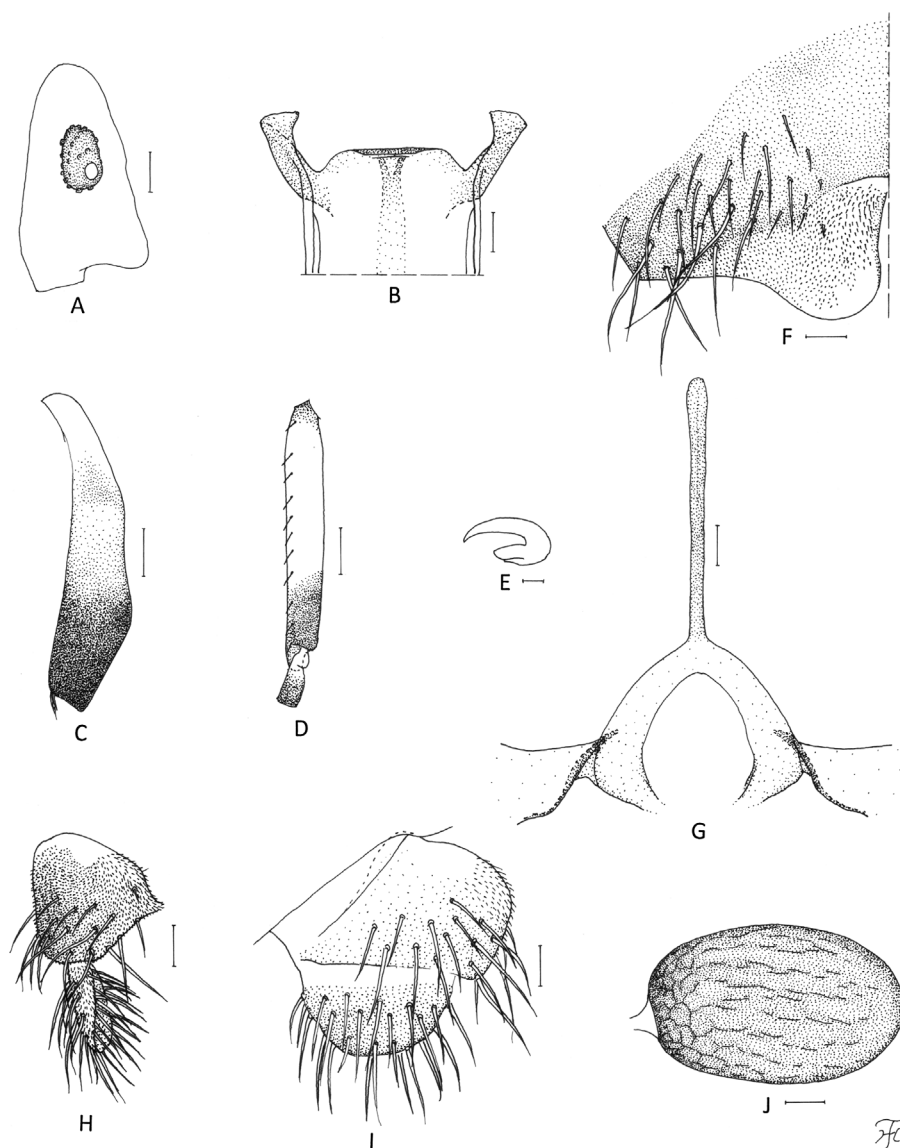
##### 3.1.1. Female

Body length 2.2–2.7 mm. **Head:** Slightly narrower than width of thorax. Frons brownish black, and moderately covered with yellowish-white scale-like recumbent short hairs interspersed with few dark longer hairs near vertex; frontal ratio 1.7:1.0:2.8–3.0; frons: head ratio 1.0:5.3–5.5. Fronto-ocular area well developed, directed dorsolaterally. Clypeus brownish black, densely covered with yellowish-white scale-like hairs interspersed with several dark longer hairs on each side of lower half. Labrum 0.7 times length of clypeus. Antenna composed of scape, pedicel and nine flagellomeres, medium to dark brown except scape, pedicel and basal half of first flagellomere yellow. Maxillary palp composed of five segments, light to medium brown, proportional lengths of third, fourth, and fifth segments 1.0:1.1–1.2:2.5–2.7; third segment (Fig. 1A) somewhat widened apically; sensory vesicle (Fig. 1A) medium sized, ellipsoidal (0.27–0.30 times length of third segment), with medium-sized opening. Maxillary lacinia with 10 or 11 inner and 13–15 outer teeth. Mandible with 21–24 inner teeth and 11–15 outer teeth. Cibarium (Fig. 1B) medially forming small sclerotized plate folded forward from posterior margin, with weakly sclerotized medio-longitudinal ridge having bifid apex. **Thorax:** Scutum brownish black except anterolateral calli dark brown, shiny and thinly white pruinose except medial and submedial longitudinal vittae nonpruinose, when illuminated dorsally and viewed anterodorsally, densely covered with whitish-yellow scale-like recumbent short hairs except on median and submedial longitudinal vittae. Scutellum dark brown, covered with yellow short hairs and dark long upright hairs. Postnotum dark brown, shiny when illuminated dorsally and viewed dorsolaterally, and bare. Pleural membrane bare. Katepisternum longer than deep, dark brown, shiny when illuminated ventrally and viewed laterally, moderately covered with fine short hairs. **Legs:** Foreleg: coxa yellow; trochanter light brown except base and inner surface yellow; femur light brown with apical cap medium brown (though extreme tip yellow); tibia medium brown except median large areas on outer surface light brown and base yellow; tarsus brownish black, with moderate dorsal hair crest; basitarsus moderately dilated, 7.3–7.4 times as long as its greatest width. Midleg: coxa medium brown except posterolateral surface dark brown; trochanter yellow except posterior surface light brown; femur light brown with inner surface widely yellow and apical cap medium brown (though extreme tip yellow); tibia white on base, yellow to light brown on basal one-third with medium brown subbasal spot, and medium to dark brown on apical two-thirds; tarsus dark brown except basal half of basitarsus dark yellow to light brown. Hind leg: coxa yellow except anterior and posterior surface medium brown; trochanter yellow; femur dark yellow to light brown except base yellow and apical cap dark brown (though extreme tip yellow); tibia (Fig. 1C) dark yellow to light brown on basal two-third except base yellowish white, subbasal spot and posterior surface medium brown, and apical one-third brownish black; 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, 6.4–6.5 times as long as wide, and 0.54 and 0.50 times

as wide as greatest widths of tibia and femur, respectively; calcipala (Fig. 1D) as long as width at base, and 0.53 times as wide as greatest width of basitarsus; pedisulcus (Fig. 1D) well developed; claw (Fig. 1E) with large basal tooth 0.51 times length of claw. **Wing:** Length 2.0–2.1 mm. Costa with dark spinules and hairs except basal patch of hairs yellow. Subcosta haired except near apex bare. Hair tuft on base of radius yellow. Basal portion of radius fully haired; R<sub>1</sub> with dark spinules and hairs; R<sub>2</sub> with hairs only. Basal cell absent. **Halter:** White except basal portion darkened. **Abdomen:** Basal scale light brown, with fringe of whitish-yellow hairs. Dorsal surface of abdomen light to dark brown except basal half yellow, moderately covered with dark short to long hairs and yellow short hairs; tergites of segments 2 and 6–8 shiny when illuminated at certain angles. Ventral surface of segment 2 white, those of other segments light to medium brown; sternal plate on segment 7 undeveloped. **Terminalia:** Sternite 8 (Fig. 1F) bare medially, with 18–25 medium-long to long hairs together with two to five slender short hairs on each side. Ovipositor valve (Fig. 1F) tongue-like, thin, membranous, moderately covered with microsetae interspersed with one or two short hairs; inner margins shallowly concave, 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 dorsally, with distinct short lobe directed posteromedially. Paraproct in ventral view (Fig. 1H) nearly triangular, with anterior surface bare, and anteromedial surface well sclerotized, and with three to five sensilla; paraproct in lateral view (Fig. 1I) slightly produced ventrally beyond ventral tip of cercus, 0.63 times as long as wide, with 17–20 medium-long to long hairs on ventral and lateral surfaces. Cercus in lateral view (Fig. 1I) short, rounded posteriorly, 0.6 times as long as wide. Spermatheca (Fig. 1J) ellipsoidal, 1.4–1.6 times as long as its greatest width, well sclerotized and with many fissures on outer surface; internal setae absent; both accessory ducts unpigmented, subequal in diameter to major one.

##### 3.1.2. Male

Body length 2.3–2.5 mm. **Head:** Somewhat wider than thorax. Upper eye medium brown, consisting of large facets in 11 (rarely 12) vertical columns and 12 or 13 horizontal rows. Face brownish black, white pruinose when illuminated dorsally and viewed anteriorly. Clypeus brownish black, white pruinose when illuminated dorsally and viewed anteriorly, densely covered with yellow hairs interspersed with several dark brown longer hairs on each side of lower half. Antenna as in female except following characters: light to medium brown except scape, pedicel and base of first flagellomere; first flagellomere elongate, 1.5–1.6 times length of second one. Maxillary palp light to medium brown, with five segments, proportional lengths of third, fourth, and fifth segments 1.0:1.2–1.3:2.8–2.9; third segment (Fig. 2A) widened apically; sensory vesicle (Fig. 2A) small, ellipsoidal (0.18–0.26 times length of third segment), and with small opening. **Thorax:** As in female. **Legs:** Color almost same as that of female. Fore basitarsus moderately dilated, 7.7–7.8 times as long as its greatest width. Hind basitarsus (Fig. 2B) nearly parallel-sided, 5.4–5.5 times as long as wide, and 0.53–0.54 and 0.57–0.59 times as wide as greatest widths of tibia and femur, respectively; calcipala (Fig. 2B) slightly longer than basal width, and 0.5 times as wide as greatest width of basitarsus. Pedisulcus (Fig. 2B) well developed. **Wing:** Length 1.9–2.0 mm. Other characters as in female except subcosta bare. **Halter:** Grayish except basal stem darkened. **Abdomen:** Basal scale light to medium brown, with fringe of yellow hairs. Dorsal surface of abdomen medium brown to brownish black except basal four-fifths of second segment yellow, covered with dark brown short to long hairs; segments 2 and 5–7 each with pair of shiny dorsolateral or lateral patches; ventral surface of segment 2 whitish, those of segments light to medium brown. **Genitalia:** Coxite in ventral view (Fig. 2C) nearly rectangular, 1.5 times as long as its greatest width. Style in ventral view (Fig. 2C) bent inward, tapered to apex, and with apical spine; style in ventrolateral view (Fig. 2D) 0.7 times length of coxite, 2.3 times as long as basal width, nearly parallel-



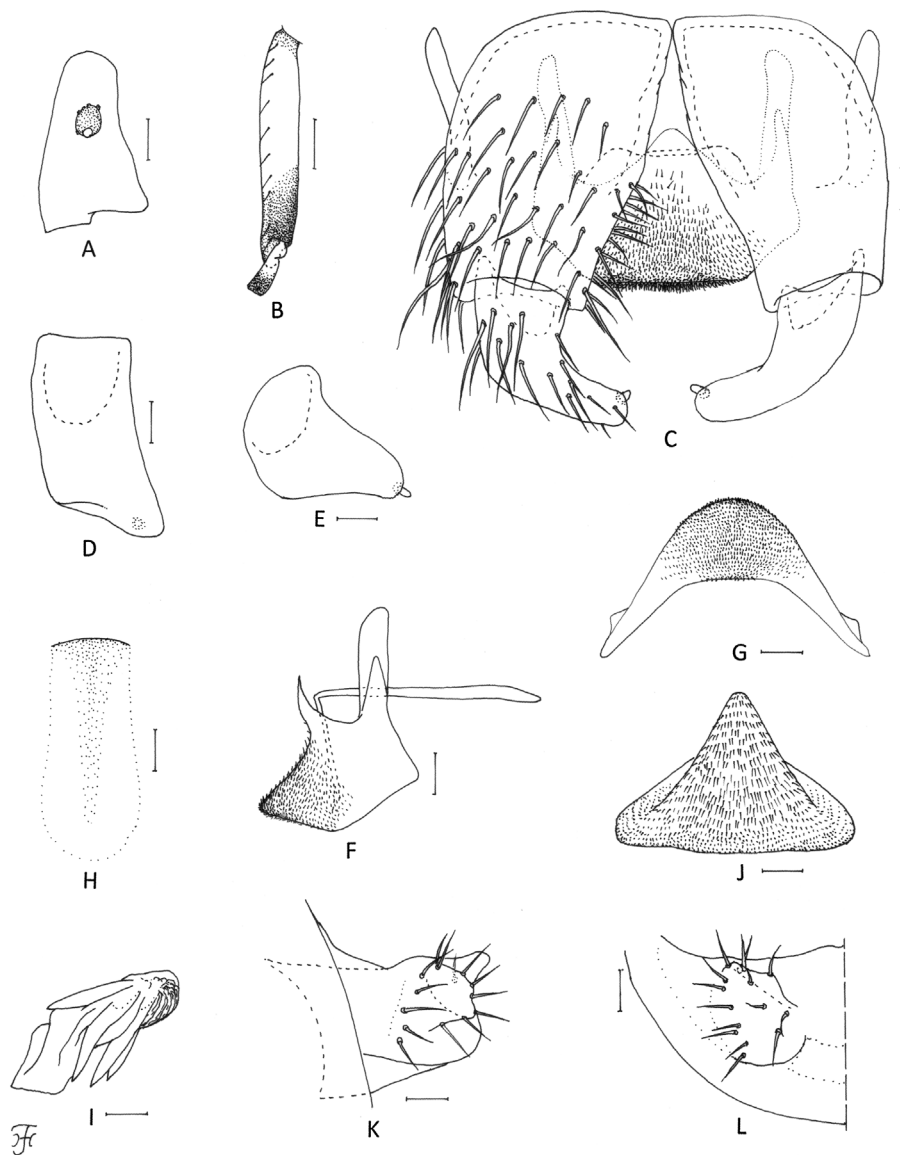
**Fig. 1.** Female of *Simulium (Gomphostilbia) isanense* sp. nov. (A) Third segment of maxillary palp with sensory vesicle (right side; front view). (B) Cibarium (front view); (C) Hind tibia (left side; outer view). (D) Hind basitarsus and second tarsomere (left side; outer view). (E) Claw. (F) Sternite 8 and ovipositor valve (right side only; ventral view). (G) Genital fork (ventral view). (H) and (I) Paraprocts and cerci (H, ventral view; I, lateral view). (J) Spermatheca. Scale bars: 0.1 mm for C and D; 0.02 mm for A, B and F–I; 0.01 mm for E.

sided from base to near apex, then abruptly tapered toward apex, with rounded apex; style in caudal view (Fig. 2E) abruptly bent inward and tapered toward 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 nearly straight, and lateral margins much narrowed posteriorly from middle, and densely covered with microsetae on ventral surface except anterolateral areas bare; basal arms of moderate length, parallel-sided or somewhat divergent; ventral plate in lateral view (Fig. 2F) moderately produced ventrally and also dorsally; ventral plate in caudal view (Fig. 2G) with ventral margin rounded, dorsal margin nearly straight medially, densely covered with microsetae on posterior surface except dorsolateral areas bare. Median sclerite (Fig. 2H) weakly sclerotized medially, and wide throughout its length. Parameres (Fig. 2I) of moderate size, each with four distinct long hooks and several short hooks. Aedeagal membrane (Fig. 2J) moderately covered with microsetae; dorsal plate absent. Ventral surface of abdominal segment 10 (Fig. 2K and L) without distinct hairs near posterolateral corners. Cercus (Fig. 2K and L) small, rounded, with 14–18 hairs.

### 3.1.3. Pupa

Body length 2.5–3.0 mm. **Head:** Integument ochreous, densely covered with small round tubercles except antennal sheaths and ventral

surface almost bare; antennal sheath without any protuberances; frons with three unbranched long trichomes with straight or coiled apices (Fig. 3A), arising close together on each side; face with one unbranched long trichome with straight or coiled apex (Fig. 3B) on each side. **Thorax:** Integument ochreous, densely covered with round tubercles, except posterior one-third of thorax moderately covered with tubercles on dorsal surface and sparsely on lateral surface, and with three long anterodorsal trichomes of different lengths (anterior trichome longest, and posterior one shortest) with coiled apices (Fig. 3C), two anterolateral trichomes (anterior trichome medium-long, with straight apex, and posterior one long, with coiled apex) (Fig. 3D), one medium-long mediolateral trichome with straight apex (Fig. 3E), and three ventrolateral trichomes (one short, two medium-long) with straight apices (Fig. 3F) on each side; all trichomes unbranched. Gill (Fig. 3G) composed of eight slender thread-like filaments, arranged as  $(1 + 2) + (1 + 2) + 2$  or  $3 + (1 + 2) + 2$  from dorsal to ventral; two triplets and one ventral pair arising close together from short common basal stalk, and each with short primary stalk; two triplets not sharing stalk; dorsal triplet composed of one individual and two paired filaments with extremely short stalk, or of three individual filaments arising at same level; middle triplet composed of one individual and two paired filaments with secondary stalk that is extremely short to medium-long (0.9 times length of interspiracular trunk); all filaments subequal in length



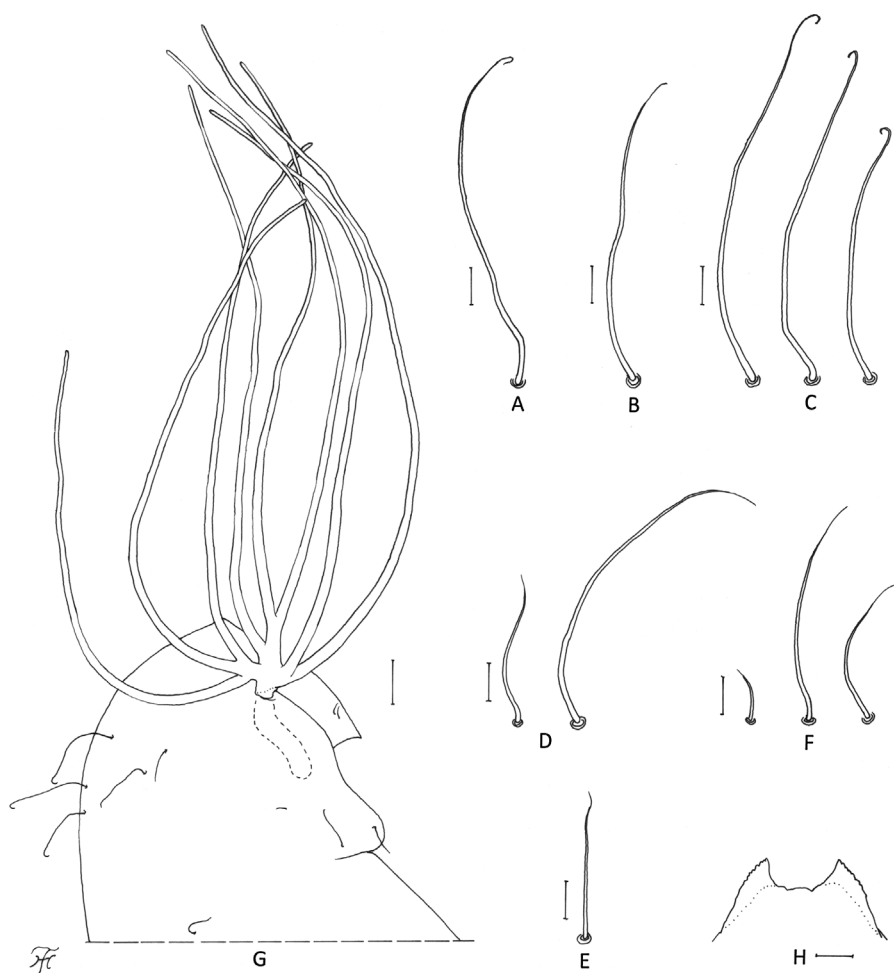
**Fig. 2.** Male of *Simulium* (*Gomphostilbia*) *isanense* sp. nov. (A) Third segment of maxillary palp with sensory vesicle (right side; front view). (B) Hind basitarsus and second tarsomere (left side; outer view). (C) Coxites, styles and ventral plate (ventral view). (D) and (E) Styles (right side; D, ventrolateral view; E, caudal view). (F) Ventral plate and median sclerite (lateral view). (G) Ventral plate (caudal view). (H) Median sclerite (caudal view). (I) Paramere (right side; caudal view). (J) Aedeagal membrane (caudal view). (K) & (L) Abdominal segment 10 and cerci (right side; K, lateral view; L, caudal view). Scale bars: 0.1 mm for B; 0.02 mm for A and C–L.

to one another (1.7–1.9 mm long) except three filaments of dorsal triplet slightly shorter; relative thickness of eight filaments from dorsal to ventral when measured basally 1.0:0.8–1.0:0.8–1.0:1.0–1.2:0.8–1.0:0.8–1.0:1.0–1.2:1.1–1.2; cuticle of all filaments ochreous, with annular ridges and furrows, though less distinct on apical half of filaments, and densely covered with minute tubercles. **Abdomen:** Dorsally, segments 1, 2 and 9 almost entirely light brown, segments 3, 4 and 5 each light brown narrowly along anterior margin, and other segments unpigmented; segment 2 weakly tuberculate anterolaterally or not tuberculate; segment 1 with one unbranched slender short hair-like seta on each side; segment 2 with one minute seta submedially near anterior margin, and one unbranched slender short hair-like seta and five minute setae near posterior margin, on each side; segments 3 and 4 each with one minute seta submedially near anterior margin, four hooked spines and one minute seta near posterior margin, on each side; segment 5 without spine-combs in transverse row, and with or without comb-like groups of minute spines on each side; segments 6–9 each with spine-combs in transverse row and comb-like groups of minute spines on each side; segment 5 with four minute setae near posterior margin on each side; segments 6–8 each with two minute setae near posterior margin on each side; segment 9 with pair of wide terminal hooks, of which outer margin 2.7–3.3 times length of inner margin and crenulated (Fig. 3K). Ventrally, segment 4 with one unbranched hook

(nearly as long as those on segments 5–7) and few minute setae on each side; segment 5 with pair of bifid or trifid hooks submedially and few minute setae on each side; segments 6 and 7 each with pair of bifid inner and unbranched or bifid outer hooks somewhat spaced from each other and few minute setae on each side; segments 4–8 each with comb-like groups of minute spines. Each side of segment 9 with three grapnel-shaped hooklets. **Cocoon:** Wall-pocket-shaped, densely woven, moderately extended ventrolaterally; anterior margin somewhat thickly woven, without anterodorsal projection; posterior three-fifths with floor roughly woven; individual threads clearly visible; 3.1–3.8 mm long by 1.6–2.5 mm wide.

#### 3.1.4. Mature larva

Body length 4.5–5.2 mm. Body grayish, mottled with reddish-brown pigments to varying extent. **Head:** Head capsule moderately covered with colorless unbranched minute setae dorsally (sparsely laterally and ventrally); cephalic apotome yellow to dark yellow except anterior two-fifths whitish yellow, head spots obscured, except anterior one of two mediolongitudinal spot groups faintly positive and anterior one of posterolateral spot groups negative; lateral surface of head capsule dark yellow except eye-spot region white and area along posterior margin somewhat darkened, with spots obscured; eyebrow well defined; ventral surface of head capsule yellow to dark yellow except each basal side



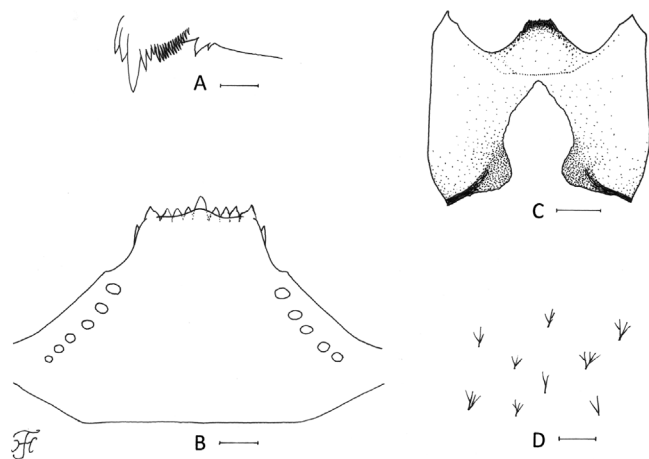
**Fig. 3.** Pupa of *Simulium (Gomphostilbia) isanense* sp. nov. (A) Frontal trichome. (B) Facial trichome. (C)–(F) Thoracic trichomes (C, anterodorsal; D anterolateral; E, mediolateral; F ventrolateral). (G) Anterior half of thorax and gill filaments (right side; outer view). (H) Terminal hooks (caudal view). Scale bars: 0.1 mm for G; 0.02 mm for A–F and H.

of postgenal cleft darkened. Antenna composed of three articles and apical sensillum, longer than stem of labral fan; proportional lengths of first, second, and third articles (apical sensillum not measured) 1.00:0.92:0.88. Labral fan with 37–41 primary rays. Mandible (Fig. 4A) 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 acute angle against mandible on apical side; supernumerary serrations absent. Hypostoma (Fig. 4B) with row of nine

apical teeth, of which median tooth is slightly longer than each corner tooth; three intermediate teeth on each side shorter than corner tooth; lateral margin smooth, with five or six hypostomal bristles per side, lying nearly parallel to lateral margin. Postgenal cleft (Fig. 4C) long, 11.8–12.9 times length of postgenal bridge, widened to middle, then narrowed to apex, and length:width ratio 1.0:0.7. Cervical sclerites composed of pair of small yellow rod-like pieces. **Thorax and Abdomen:** Thoracic and abdominal segments 1–4 sparsely covered with unbranched colorless minute setae dorsally; abdominal segments 5–8 moderately to densely covered with dark setae each with two to five branches (Fig. 4D) on dorsal and dorsolateral surfaces, and sparsely to moderately covered with unbranched colorless minute setae on lateral surface; last abdominal segment moderately 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 present, unpigmented. Rectal organ compound, each of three lobes with 9–14 finger-like secondary lobules. Anal sclerite of usual X-form, with anterior arms 0.9 times as long as posterior ones, broadly sclerotized at base; no sensilla on broad base or posterior to posterior arms; accessory sclerite absent. Last abdominal segment with pair of conical ventral papillae. Posterior circlet with 94–98 rows of hooklets with up to 15 or 16 hooklets per row.

### 3.1.5. Type material

**HOLOTYPE:** Female (with associated pupal exuviae and cocoon) reared from pupa, collected from a moderately flowing stream (width 3 m, depth 5 cm, bottom sandy and rocky, temperature 21.7° C, pH 4.71, partially shaded, elevation 337 m, 14°23' 26.4"N, 103°51'50.8"E), Along Korn waterfall, Sangkha District, Surin Province, Thailand, 2-I-



**Fig. 4.** Larva of *Simulium (Gomphostilbia) isanense* sp. nov. (A) Mandible. (B) Hypostoma. (C) Head capsule showing postgenal cleft (ventral view). (D) Dark multi-branched setae on dorsal surface of abdominal segment 8. Scale bars: 0.1 mm for C; 0.02 mm for A, B and D.

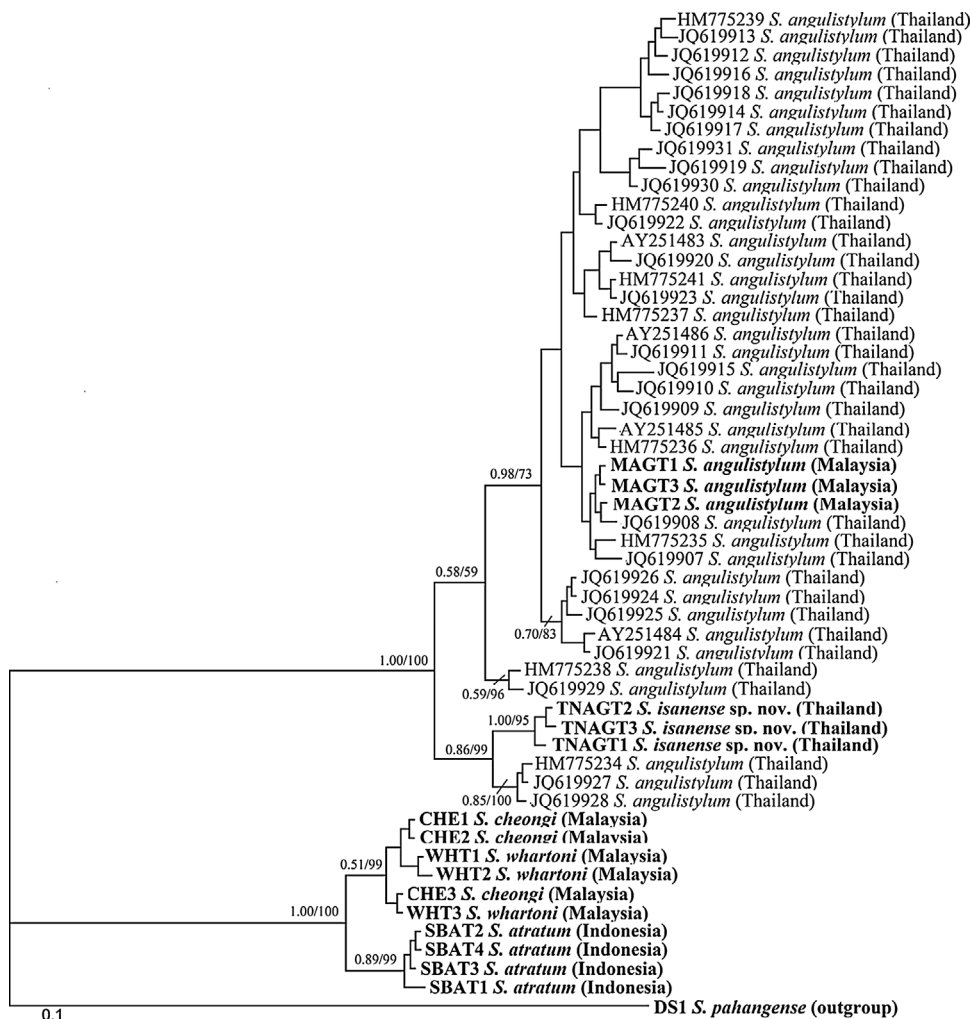


Fig. 5. Phylogenetic relationships among five members of the *S. epistum* species group. Posterior probabilities/bootstrap values (BI/NJ) are shown on the branches. The scale bar represents 0.1 substitutions per nucleotide position. Sequences generated in this study are in bold.

2017, by W. Srisuka. PARATYPES: Six females, eight males (all with associated pupal exuviae and cocoons), and 10 mature larvae, same data as those of the holotype; two pupae, one pupal exuviae and one mature larva, same data as those of the holotype except 29-XI-2016, by S. Suriya.

### 3.1.6. Biological notes

The pupae and larvae of this new species were collected from trailing grasses and fallen leaves in the water. A species collected in the same stream was *S. (G.) fukudae* Takaoka, Srisuka & Saeung.

### 3.1.7. Etymology

The species name *isanense* refers to the name of the region, Isan, including Surin Province, where this new species was collected.

## 4. Discussion

This new species is assigned to the *Simulium epistum* species-group of the subgenus *Gomphostilbia*, defined by Takaoka (2012), by having the antenna with nine flagellomeres, bare pleural membrane, yellow hair tuft on the base of the radius, tibiae of the female and male with a subbasal dark spot, and slender male hind basitarsus (Fig. 2B).

This new species is characterized in the female by the genital fork with a distinct posteromedial projection on each arm (Fig. 1G); in the male by the style wide, abruptly bent inward (Fig. 2C, E), and the ventral plate widest subbasally (Fig. 2C); and in the pupa by the gill with eight filaments arranged in three groups arising at the same level

from a short common basal stalk (Fig. 3G), dorsal surface of abdominal segments 1–5 darkened, and terminal hooks wide and with serrated outer margin (Fig. 3H).

This new species is similar to *S. (G.) angulistylum* described from Peninsular Malaysia (Takaoka and Davies, 1995), but is distinguished from the latter species by the following characters (those of *S. (G.) angulistylum* in parentheses): in the female by the length of the fore basitarsus relative to its greatest width, which is 7.3–7.4 (6.5); in the male by the length of the fore basitarsus relative to its greatest width, which is 7.7–7.8 (5.5); and in the pupa by abdominal segments 1–5 darkened on the dorsal surface (not darkened).

In sharing the wide, inwardly twisted style, this new species appears to be related to *S. (G.) auratum* Takaoka from Sarawak, Malaysia, *S. (G.) epistum* Delfinado from Palawan Island, the Philippines, and *S. (G.) otsukai* Takaoka & Choochote from southern Thailand (Takaoka, 1983, 2009; Takaoka et al., 2009). However, these three species are distinguished from this new species by the arrangement of the pupal gill filaments. An outer filament of the ventral pair is twice as long as the other filaments in *S. (G.) auratum* and *S. (G.) epistum*, and the gill filaments are arranged as 1 + 1 + 2 + [1 + (1 + 2)] from dorsal to ventral in *S. (G.) otsukai*.

In accordance with its morphological classification, *S. (G.) isanense* sp. nov. is genetically related to *S. (G.) angulistylum*, and clearly separated from *S. (G.) whartoni*, *S. (G.) cheongi* and *S. (G.) atratum*, although a non-monophyletic relationship is observed for *S. (G.) whartoni* and *S. (G.) cheongi* (Fig. 5). This new species is in its own genetic clade with high bootstraps support (BI = 1.00, NJ = 95%).

Pramual and Kuvangkadilok (2012) reported divergent mitochondrial lineages within cytoforms (A, B, and C) of *S. (G.) angulistylum* in Thailand. However, none of their Thai gene sequences of *S. (G.) angulistylum* was consistent with those of the new species, although three sequences of *S. (G.) angulistylum*: HM775234 (unknown cytoform), JQ619927 (cytoform B), and JQ619928 (cytoform B), all collected from Sisaket Province in the lower northeastern region, show a sister relationship with the new species (Fig. 5). The genetic distances between these two clades are relatively high (2.48–2.97%), and are comparable with the species boundaries among members of the subgenus *Gomphostilbia* in Southeast Asia (Low et al., 2015; Saeung et al., 2017; Takaoka et al., 2017).

Further chromosomal studies are needed to determine whether one of the three cytoforms of *S. (G.) angulistylum* is the same or not the same as *S. (G.) isanense* sp. nov. According to Pramual and Kuvangkadilok (2012), cytoform A is recorded from lowlands (110–330 m in elevation) in the northeast and south regions, cytoform B is distributed in the central, northeast and east regions, and cytoform C is restricted to a high elevation (1153 m) in the northeast region.

## Acknowledgements

We are grateful to Prof. Peter H. Adler (Clemson University, Clemson, SC, USA) for reading the current manuscript and providing valuable comments. Thanks are due to S. Suriya (Entomology Section, Queen Sirikit Botanic Garden, Chiang Mai, Thailand) for his kind help collecting in the field. This work was supported by a research grant from University of Malaya (RP021A/16SUS) to H. Takaoka, and the Thailand Research Fund and the Office of the Higher Education Commission through the Research Grant for New Scholar (grant MRG5980101) to A. Saeung. Finally, we thank the Research Administration Office of Chiang Mai University for providing the budget for our Excellence Center in Insect Vector Study.

## References

- Adler, P.H., Crosskey, R.W., 2017. World Blackflies (Diptera: Simuliidae): A Comprehensive Revision of the Taxonomic and Geographical Inventory [2017]. pp. 131. <http://entweb.clemson.edu/biomia/pdfs/blackflyinventory.pdf>. (Accessed 10

March 2017).

- Adler, P.H., Currie, D.C., Wood, D.M., 2004. The Black Flies (Simuliidae) of North America. Cornell University Press, Ithaca, New York, USA, pp. xv + 941.
- Fukuda, M., Choochote, W., Bain, O., Aoki, C., Takaoka, H., 2003. Natural infections with filarial larvae in two species of black flies (Diptera: Simuliidae) in northern Thailand. *Jpn. J. Trop. Med. Hyg.* 31, 99–102.
- Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17 (8), 754–755.
- Ishii, Y., Choochote, W., Bain, O., Fukuda, M., Otsuka, Y., Takaoka, H., 2008. Seasonal and diurnal biting activities and zoonotic filarial infections of two *Simulium* species (Diptera: Simuliidae) in northern Thailand. *Parasite* 15, 121–129.
- Jitklang, S., Kuvangkadilok, C., 2007. A new species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from southern Thailand, with description of its polytene chromosomes. *Stud. Dipterol.* 14, 1–7.
- Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33 (7), 1870–1874.
- Kuvangkadilok, C., Takaoka, H., 2000. Taxonomic notes on Simuliidae (Diptera) from Thailand: description of a new species and new distributional records of nine known species. *Jpn. J. Trop. Med. Hyg.* 28 (3), 167–175.
- Low, V.L., Takaoka, H., Adler, P.H., Ya, Z., cob, Norma-Rashid, Y., Chen, C.D., Sofian-Azirun, M., 2015. A multi-locus approach resolves the phylogenetic relationships of the *Simulium asakoe* and *Simulium ceylonicum* species groups (Diptera: Simuliidae) in Malaysia: evidence for distinct evolutionary lineages. *Med. Vet. Entomol.* 29 (3), 330–337.
- Pramual, P., Kuvangkadilok, C., 2012. Integrated cytogenetic, ecological, and DNA barcode study reveals cryptic diversity in *Simulium* (*Gomphostilbia*) *angulistylum* (Diptera: Simuliidae). *Genome* 55, 447–458.
- Saeung, A., Srisuka, W., Low, V.L., Maleewong, W., Takaoka, H., 2017. Descriptions of the female and larva of *Simulium* (*Gomphostilbia*) *udomi* (Diptera: Simuliidae) from Thailand, and its transfer to the *Simulium asakoe* species-group. *Acta Trop.* 172, 14–19.
- Swofford, D.L., 2002. PAUP\* Phylogenetic Analysis Using Parsimony (\*and Other Methods). Sinauer Associates, USA.
- Takaoka, H., Davies, D.M., 1995. The Black Flies (Diptera: Simuliidae) of West Malaysia. Kyushu University Press, Fukuoka, Japan, pp. viii + 175.
- Takaoka, H., Otsuka, Y., Choochote, W., Thongsuhuan, S., 2009. Two new and one newly recorded species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from southern Thailand. *Med. Entomol. Zool.* 60 (4), 259–268.
- Takaoka, H., Srisuka, W., Low, V.L., Maleewong, W., Saeung, A., 2017. Two new species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from Myanmar, and their phylogenetic relationships with related species in the *S. asakoe* species-group. *Acta Trop.* 176, 39–50.
- Takaoka, H., 1983. The blackflies (Diptera: Simuliidae) of the Philippines. Japan Society for the Promotion of Science, Tokyo, pp. xi + 119.
- Takaoka, H., 2003. The Black Flies (Diptera: Simuliidae) of Sulawesi, Maluku and Irian Jaya. Kyushu University Press, Fukuoka, Japan, pp. xxii + 581.
- Takaoka, H., 2009. Three new species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from Sabah and Sarawak, Malaysia. *Med. Entomol. Zool.* 60 (2), 97–112.
- Takaoka, H., 2012. Morphotaxonomic revision of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) in the Oriental Region. *Zootaxa* 3577, 1–42.

# *Simulium maleewongae*, a New Species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) From Thailand

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## Abstract

*Simulium* (*Gomphostilbia*) *maleewongae* sp. nov. is described based on the adult males and females, their pupal exuviae, and larvae from Thailand. This new species is placed in the *Simulium gombakense* species-group of *Simulium* (*Gomphostilbia*). It is characterized by the female cibarium with a cup-like appendage, male ventral plate deeply depressed ventromedially, pupal gill composed of an inflated structure and eight slender filaments, cone-shaped pupal terminal hooks, and cocoon with an anterodorsal projection. Taxonomic notes are given to separate this new species from 10 other species of the same species-group known from China, India, Malaysia, Thailand, Nepal, and Vietnam. Keys to identify all 11 species of the *S. gombakense* species-group are provided for females, males, pupae, and larvae.

**Key words:** black fly, *Gomphostilbia*, Simuliidae, Thailand, new species

The fauna of black flies (Diptera: Simuliidae) in Thailand is rich, consisting of 85 species (16.2% of the total number, 524, of species in the Oriental Region), all of which are in the genus *Simulium* Latreille and are further placed in six subgenera (three species in *Asiosimulium* Takaoka & Choochote, two species in *Daviesellum* Takaoka & Adler, six species in *Montisimulium* Rubtsov, 20 species in *Gomphostilbia* Enderlein, nine species in *Nevermannia* Enderlein, and 45 species in *Simulium*; Adler and Crosskey 2016). In Thailand, three species (*S. (G.) asakoe* Takaoka & Davies, *S. (S.) nigrogilvum* Summer, and *S. (S.) nodosum* Puri) are reported to be vectors of unidentified filarial parasites (Fukuda et al. 2003, Takaoka et al. 2003, Ishii et al. 2008).

In a recent survey of pupae and larvae of black flies in Mae Hong Son Province in northern Thailand, we collected one undescribed species, the pupa of which has the gill composed of an inflated structure and eight slender filaments. It is assignable to the *Simulium gombakense* species-group of subgenus *Gomphostilbia*, defined by Takaoka (2012), and differs from all 10 species of the same species-group including *S. (G.) gombakense* Takaoka & Davies known from Peninsular Malaysia and Thailand and *S. (G.) prayongi* Takaoka & Choochote from Thailand, by the configuration of the pupal gills or shape of the male ventral plate (Takaoka and Davies 1995, Kuvangkadilok and Takaoka 2000, Takaoka 2000, Takaoka and Choochote 2005, Takaoka et al. 2010a).

This species is described as new, based on adult female, male, pupal, and larval specimens, and taxonomic notes are given to separate

it from other related species from China, India, Malaysia, Nepal, Thailand, and Vietnam. Keys to identify all 11 species of the *S. gombakense* species-group are provided.

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

## Nomenclature

This paper and the nomenclatural act it contains have been registered in Zoobank (www.zoobank.org), the official register of the International Commission on Zoological Nomenclature. The LSID (Life Science Identifier) number of the publication is:

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

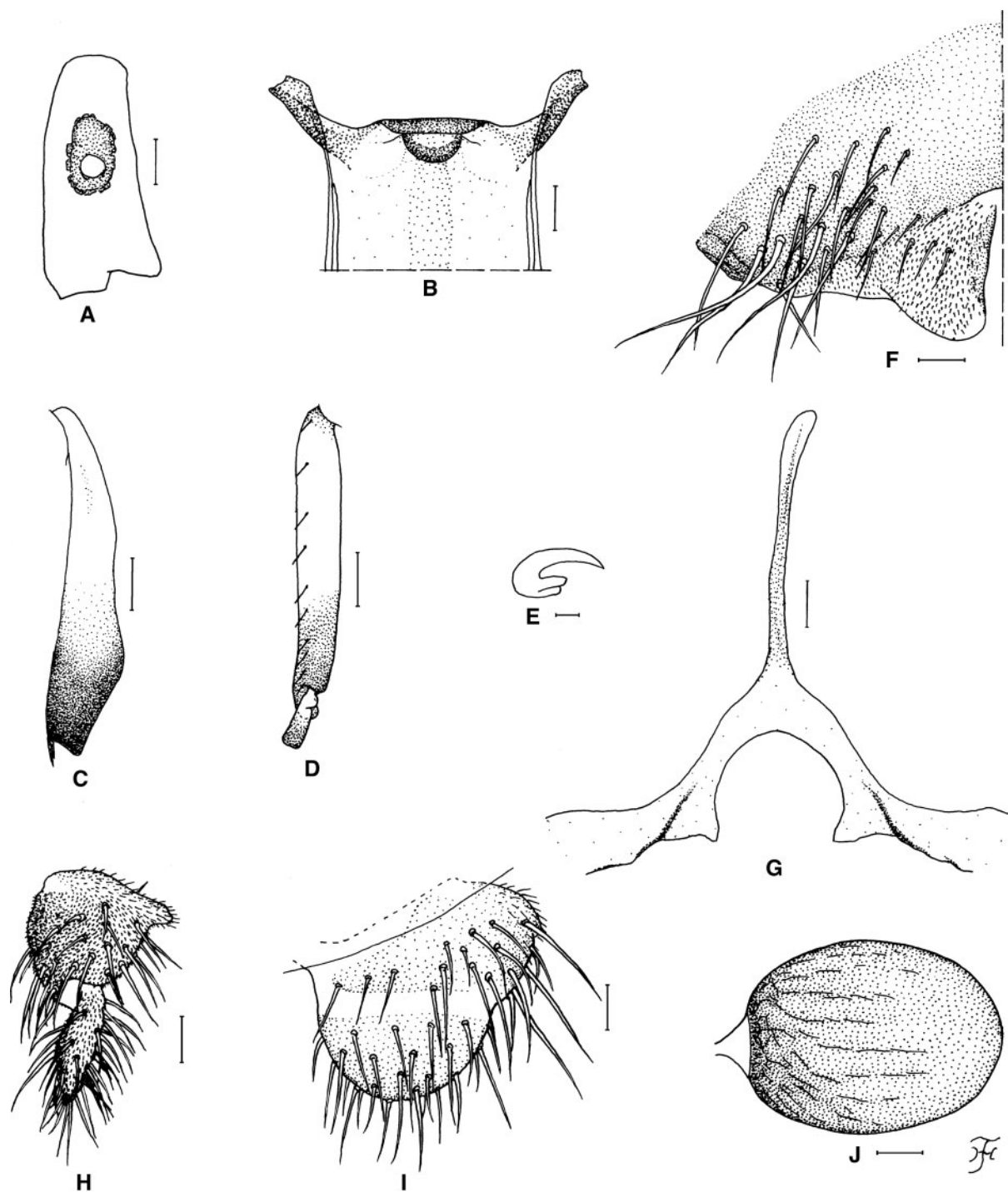
(urn:lsid:zoobank.org:act:2181C0AF-8D8F-4CA8-B4CE-1A946C11AC0D).

The female of this new species is almost identical to that of *S. (G.) thuathienense* (Takaoka et al. 2015) as “Female. Body length 2.0 mm. **Head.** As wide as thorax. Frons black, thinly, densely covered with whitish-yellow recumbent short hairs interspersed with several dark simple longer hairs along each lateral margin; frontal ratio 1.71–1.73:1.00:3.07–3.49; frons:head ratio 1.0:5.71–5.83.

Fronto-ocular area well developed, narrow, directed dorsolaterally. Clypeus black, densely covered with whitish yellow recumbent short hairs (though mediolongitudinal portion on posterior half narrowly bare) interspersed with seven to ten dark-brown longer hairs along each lateral margin. Labrum 0.61–0.66 times as long as clypeus. Antenna composed of scape, pedicel, and nine flagellomeres, medium to dark brown except scape, pedicel, and basal half of first flagellomere whitish-yellow, though first flagellomere entirely yellow when viewed anteriorly. Maxillary palp composed of five segments, light to medium brown, proportional lengths of third, fourth, and fifth segments 1.00:1.00–1.02:2.07–2.21; third segment (Fig. 1A) somewhat produced inward; sensory vesicle (Fig. 1A) of medium size, 0.29–0.32 times as long as third segment, with medium-sized opening. Maxillary lacinia with 10 inner and 14 outer teeth. Mandible with 26–28 inner and 11 outer teeth. Cibarium (Fig. 1B) medially forming short wide sclerotized plate folded forward from posterior margin, and with slightly sclerotized medial longitudinal ridge with well-sclerotized cup-like apex. **Thorax.** Scutum brownish black, slightly shiny, thinly grayish-white pruinose with three faint nonpruinose longitudinal vittae (one medial and two submedial) when illuminated anterodorsally and viewed dorsally, densely covered with whitish-yellow recumbent hairs (except three longitudinal vittae bare). Scutellum light brown, covered with whitish-yellow short hairs and with dark-brown long upright hairs along posterior margin. Postnotum dark brown to brownish black, bare. Pleural membrane bare. Katepisternum dark brown, longer than deep, moderately covered with whitish-yellow hairs interspersed with dark-brown hairs. **Legs.** Foreleg: coxa whitish yellow; trochanter light brown except anterodorsal portion whitish yellow; femur light brown with apical cap medium brown (though extreme tip whitish yellow); tibia light brown with apical cap medium brown; tarsus brownish black, with moderate dorsal hair crest; basitarsus moderately dilated, 6.60–6.78 times as long as its greatest width. Midleg: coxa medium brown except posterolateral surface dark brown; trochanter light brown except anterior portion whitish yellow; femur light brown with apical cap medium brown (though extreme apex whitish yellow); tibia light to medium brown except basal one-third whitish yellow; tarsus dark brown except basal half whitish yellow. Hind leg: coxa light brown; trochanter whitish yellow; femur light brown except base whitish yellow and apical cap dark brown (though extreme tip yellow); tibia (Fig. 1C) whitish yellow on basal one-half with light-brown narrow spot subbasally, then light brown on middle one-fourth and dark brown on apical one-fourth; tibia densely covered with yellowish-white fine hairs on posterior and outer surfaces of basal three-fourths; tarsus dark brown except basal three-fifths of basitarsus (though base light brown) and basal half of second tarsomere yellowish white; basitarsus (Fig. 1D) narrow, nearly parallel-sided, 6.67 times as long as wide, and 0.63–0.68 and 0.54–0.58 times as wide as greatest widths of tibia and femur, respectively; calcipala nearly as long as wide, and 0.53 times as wide as greatest width of basitarsus. Claw (Fig. 1E) with large basal tooth 0.46 times as long as claw. **Wing.** Length 2.0 mm. Costa with dark-brown spinules as well as light to medium brown hairs except basal portion with patch of yellow hairs. Subcosta with medium-brown hairs except near apex bare. Hair tuft of base of radius yellow. Basal portion of radius fully haired;  $R_1$  with dark-brown spinules and light to medium brown hairs;  $R_2$  with light to medium brown hairs only. Basal cell absent. **Halter.** White with dark base. **Abdomen.** Basal scale light brown, with fringe of whitish-yellow hairs. Dorsal surface of abdominal segments medium brown to brownish black except tergite of segment 2 light brown, moderately covered with dark brown hairs interspersed with yellow fine

hairs; tergites of segments 2 and 6–9 wide and shiny; ventral surface of segments 2 and 3 entirely pale whitish yellow and those of other segments medium brown; sternal plate on segment 7 undeveloped. **Terminalia.** Sternite 8 (Fig. 1F) bare medially, with 19–21 medium-long to long hairs together with four to six short setae on each side. Ovipositor valves (Fig. 1F) triangular, with round medioposterior corners, thin, membranous, moderately covered with microsetae interspersed with five or six short setae; inner margins slightly sinuous, moderately sclerotized and narrowly separated from each other. Genital fork (Fig. 1G) of usual inverted-Y form, with arms of moderate width; arm with triangular projection directed posteromedially and folded medially. Paraproct in ventral view (Fig. 1H) nearly quadrate, somewhat raised along anteromedial margin and with four or five sensilla on anteromedial surface; paraproct in lateral view (Fig. 1I) somewhat produced ventrally, with 22–26 medium-long to long hairs on ventral and lateral surfaces. Cercus in lateral view (Fig. 1I) short, rounded posteriorly, 0.48 times as long as wide. Spermatheca (Fig. 1J) ellipsoidal, 1.3–1.5 times as long as wide, well sclerotized except duct and small area at juncture with duct unsclerotized, and with many fissures on surface; internal setae absent; both accessory ducts slender, subequal in diameter to each other and slightly thicker than major duct.”

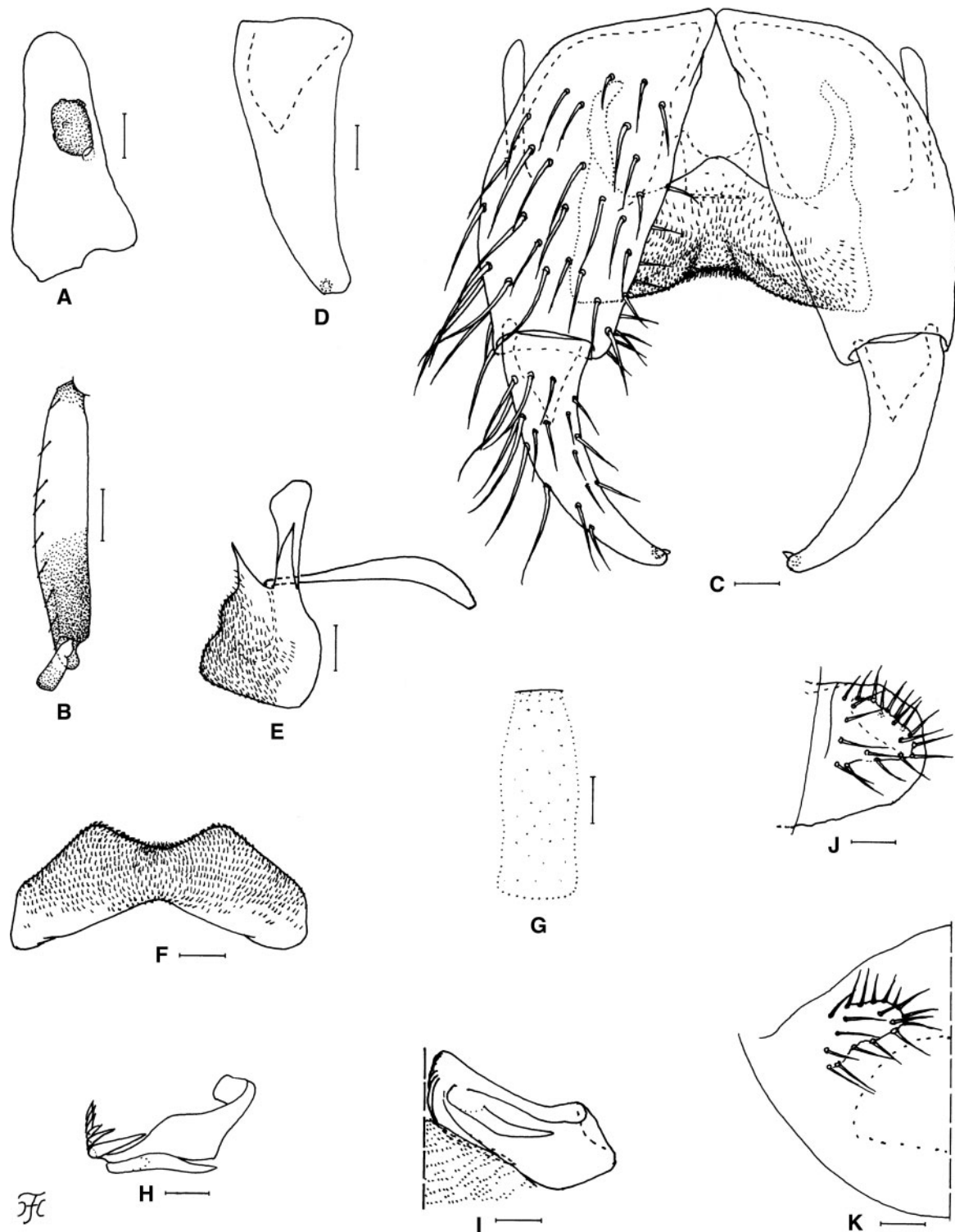
The male of this new species is almost identical to that of *S. nuwakotense* (Takaoka and Shrestha 2010) as “**Male.** Body length 2.6 mm. **Head.** Much wider than thorax. Holoptic, upper eye consisting of large facets in 15 vertical columns and in 15 horizontal rows. Face dark brown, white pruinose, bare. Clypeus dark brown, white pruinose, densely covered with golden-yellow scale-like short hairs interspersed with dark longer hairs. Antenna composed of scape, pedicel, and nine flagellomeres, medium to dark brown except scape, pedicel, and base of first flagellomere yellow; first flagellomere somewhat elongate, 1.5 times as long as second flagellomere. Maxillary palp composed of five segments, proportional lengths of third, fourth, and fifth segments 1.00:1.10:2.29; third segment (Fig. 2A) somewhat enlarged; sensory vesicle (Fig. 2A) ellipsoidal, 0.22–0.24 times as long as third segment, with small opening apically. **Thorax.** Scutum brownish black, slightly shiny when illuminated at certain angles, densely covered with golden-yellow scale-like recumbent hairs. Scutellum medium brown, with golden-yellow short hairs and dark upright long hairs. Postnotum dark brown, shiny when illuminated at certain angles, and bare. Pleural membrane bare. Katepisternum longer than deep, with dark hairs intermixed with yellow hairs. **Legs.** Coloration nearly as in female except basitarsus of midleg yellowish on basal one-third (though its border not well defined). Fore basitarsus, 8.2 times as long as its greatest width. Hind basitarsus (Fig. 2B) slightly widened to basal three-fifths, then narrowed to apex, 4.84 times as long as its greatest width, and 0.68 and 0.67 times as wide as hind tibia and femur, respectively; calcipala moderately developed, nearly as long as wide, and 0.42 times as wide as greatest width of basitarsus; pedisulcus moderately developed. **Wing.** Length 2.0 mm; other characters as in female except subcosta bare. **Halter.** Light gray with darkened base. **Abdomen.** Basal scale dark brown with fringe of dark long hairs laterally. Dorsal surface of abdomen medium to dark brown, moderately covered with dark short hairs; segments 2 and 5–7 each with pair of shiny dorsolateral patches, those on segment 2 connected in middle to each other. **Genitalia.** Coxites, styles, and ventral plate in ventral view as in Fig. 2C. Coxite in ventral view (Fig. 2C) subquadrate, 2.00 times as long as wide. Style in ventral view (Fig. 2C) slender, 0.79 times as long as coxite, gently curved inward, tapered toward apex and with one apical spine; style in ventrolateral view (Fig. 2D)



**Fig. 1.** Female of *Simulium* (*Gomphostilbia*) *maleewongae* sp. nov. (A) Third segment of right maxillary palp with sensory vesicle (front view). (B) Cibarium. (C) Left hind tibia (outer view). (D) Basitarsus and second tarsomere of left hind leg showing calcipala and pedisulcus (outer view). (E) Claw. (F) Sternite 8 and ovi-positor valve (right half; ventral view). (G) Genital fork (ventral view). (H, I) Right paraprocts and cerci (H, ventral view; I, lateral view). (J) Spermatheca. Scale bars = 0.1 mm for C and D; 0.02 mm for A, B, and F–J; 0.01 mm for E.

gradually tapered from base toward apex. Ventral plate in ventral view (Fig. 2C) transverse, much wider than long, somewhat widened posteriorly, with anterior margin produced medially, with posterior margin deeply concave in middle, and moderately covered with microsetae on ventral surface, though portions along anterior margin bare or sparsely covered with microsetae; basal arms nearly parallel-sided, though somewhat convergent apically; ventral plate in lateral view (Fig. 2E) with ventral margin

undulate; ventral plate in caudal view (Fig. 2F) deeply depressed medially so that ventral margin appearing flattened letter M, and densely covered with microsetae on most medial portion of posterior surface. Median sclerite (Fig. 2G) broad, plate-like, though lateral margins not well defined. Paramere (Fig. 2H) of moderate size, with several hooks decreasing in length from posterior to anterior. Aedeagal membrane (Fig. 2I) sparsely covered with microsetae. Ventral surface of abdominal segment 10 (Fig. 2J, K)



**Fig. 2.** Male of *Simulium* (*Gomphostilbia*) *maleewongae* sp. nov. (A) Third segment of right maxillary palp with sensory vesicle (front view). (B) Basitarsus and second tarsomere of left hind leg showing calcipala and pedisulcus (outer view). (C) Coxites, styles and ventral plate (ventral view). (D) Right style (ventrolateral view). (E) Ventral plate and median sclerite (lateral view). (F) Ventral plate (caudal view). (G) Median sclerite (caudal view). (H) Right paramere (dorsal view). (I) Left paramere and aedeagal membrane (caudal view). (J, K) Abdominal segment 10 and cerci (right half; J, lateral view; K, caudal view). Scale bars = 0.1 mm for B; 0.02 mm for A and C–K.

without distinct hairs. Cercus (Fig. 2J, K) small, encircled with 19 or 20 short hairs”.

The pupa and larva of this new species are similar to those of *S. aziruni* (Takaoka et al. 2013) as “Pupa. Body length 2.4–2.5 mm. **Head.** Integument yellow, moderately covered with round tubercles on frons and parts of each lateral surface; antennal sheath bare, and without projections. Frons with three unbranched long trichomes with straight apices (Fig. 3A) on each side; all trichomes subequal in length and stoutness to one another. Face with one unbranched long trichome with coiled or straight apex (Fig. 3B) on each side. **Thorax.** Integument yellow, sparsely to moderately covered with round tubercles except dorsal surface of posterior portion almost bare, with three long dorsomedial trichomes with coiled or straight apices (Fig. 3C), two anterolateral trichomes with straight apices (one medium-long, one long; Fig. 3D), one medium-long mediolateral trichome with straight apex (Fig. 3E), and three ventrolateral trichomes with straight apices (one medium-long, two short; Fig. 3F), on each side. Gill (Fig. 3G) of much inflated structure (1.0–1.2 mm long) arising from medium-long basal common stalk, and with eight slender filaments, of which one arising from anterior tip of inflated structure, one arising from ventral surface of basal portion of inflated structure, three arising close together as one individual and two paired filaments with medium-long stalk from inner dorsal surface near apex of common basal stalk and three individually arising close together from dorsal surface of middle of common basal stalk; inflated structure divided into two portions, one long, directed forward another short, directed upward, and both rounded apically; all filaments subequal in length (0.8–1.0 mm) and thickness to one another; surface of inflated portion and all filaments light yellow, with no pattern on inflated portion and no transverse ridges or furrows on filaments, and densely covered with minute tubercles. **Abdomen.** Dorsally, all segments unpigmented except segments 1, 2, and 9 light yellowish and without tubercles; segment 1 with one slender short seta (Fig. 3H) on each side; segment 2 with one slender short seta and five minute setae (Fig. 3I) on each side; segments 3 and 4 each with four hooked spines and one minute seta on each side; segment 5 with four minute setae on each side and lacking spine-combs and comb-like groups of minute spines; segment 6–9 each with spine-combs and comb-like groups of minute spines in transverse row (though spine-combs on segment 9 much smaller than those on other segments) on each side; segment 6 with three minute setae and segments 7 and 8 each with two minute setae; segment 9 with pair of distinct horn-like terminal hooks somewhat bent anteriorly (Fig. 3J). Ventrally, segment 4 with four minute setae (or one of which is unbranched hook in one pupa) on each side; segment 5 with pair of bifid hooks submedially and few minute 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 minute setae on each side; three grapple-shaped hooklets on each side of segment 9. **Cocoon** (Fig. 3K). Wall-pocket-shaped, pale yellow, thinly woven with no open spaces in weave, extended ventrolaterally, with short anterodorsal projection; floor moderately woven on posterior two-thirds of cocoon; individual threads not visible or slightly visible; 2.9–3.0 mm long by 2.2–2.8 mm wide.

**Mature larva.** Body length 5.0–6.0 mm. Body white except ventral surface of thoracic segments 2 and 3 ochreous (in addition, dorsal and lateral surfaces of these segments thinly ochreous in some larvae), and entire surface of abdominal segments 1 and 2 dark gray, with following reddish-brown markings: thoracic segment 1 encircled with distinct transverse band though narrowly disconnected mediodorsally and widely disconnected ventrally; abdominal segments 1–4 each encircled with transverse band, though narrowly

disconnected mediodorsally, and faintly connected or disconnected dorsolaterally, ventrolaterally, and ventromedially; abdominal segment 5 encircled with transverse band, though disconnected dorso-medially and ventromedially; dorsal and ventral ends of transverse band directed forward and backward, respectively in one larva, while only ventral end of this band directed backward in other larvae; abdominal segments 6–8 each with somewhat faint transverse band on dorsal surface, though disconnected in middle; abdominal segment 7 with pair of round spots ventrally. Head capsule whitish yellow except eye-spot region white, sparsely covered with simple minute setae. Head spots faintly to moderately positive; eyebrow indistinct. Cervical sclerites composed of two small light brown rod-like pieces, not fused to occiput, widely separated from each other. Antenna composed of three segments and apical sensillum, much longer than stem of labral fan; proportional lengths of first, second, and third segments 1.00:0.61–0.67:0.89–0.99. Labral fan with 33–40 primary rays. Mandible (Fig. 4A) with three comb-teeth decreasing in length from first to third; mandibular serration composed of two teeth (one large and one small); large tooth at acute angle to mandible on apical side; accessory serrations absent. Hypostoma (Fig. 4B) with row of nine apical teeth, of which median and corner teeth prominent, and intermediate teeth lowest; lateral margins smooth; five or six hypostomal bristles in row, nearly parallel to lateral margin on each side. Postgenal cleft (Fig. 4C, D) medium-sized, rounded or pointed apically, 0.74–1.17 times as long as postgenal bridge. Pharate pupal gill with wrinkled enlarged portion with eight slender thread-like filaments. Thoracic cuticle bare. Abdominal cuticle almost bare except both sides of anal sclerite (down to base of ventral papillae) moderately covered with colorless simple setae. Rectal scales present and unpigmented. Rectal organ compound, each of three lobes with 9–11 finger-like secondary lobules. Anal sclerite X-shaped, anterior arms nearly as long as posterior ones; accessory sclerites absent; sensilla absent. Ventral papillae well developed, conical. Posterior circlet with 83–94 rows of up to 14–16 hooks per row”.

### Type Material

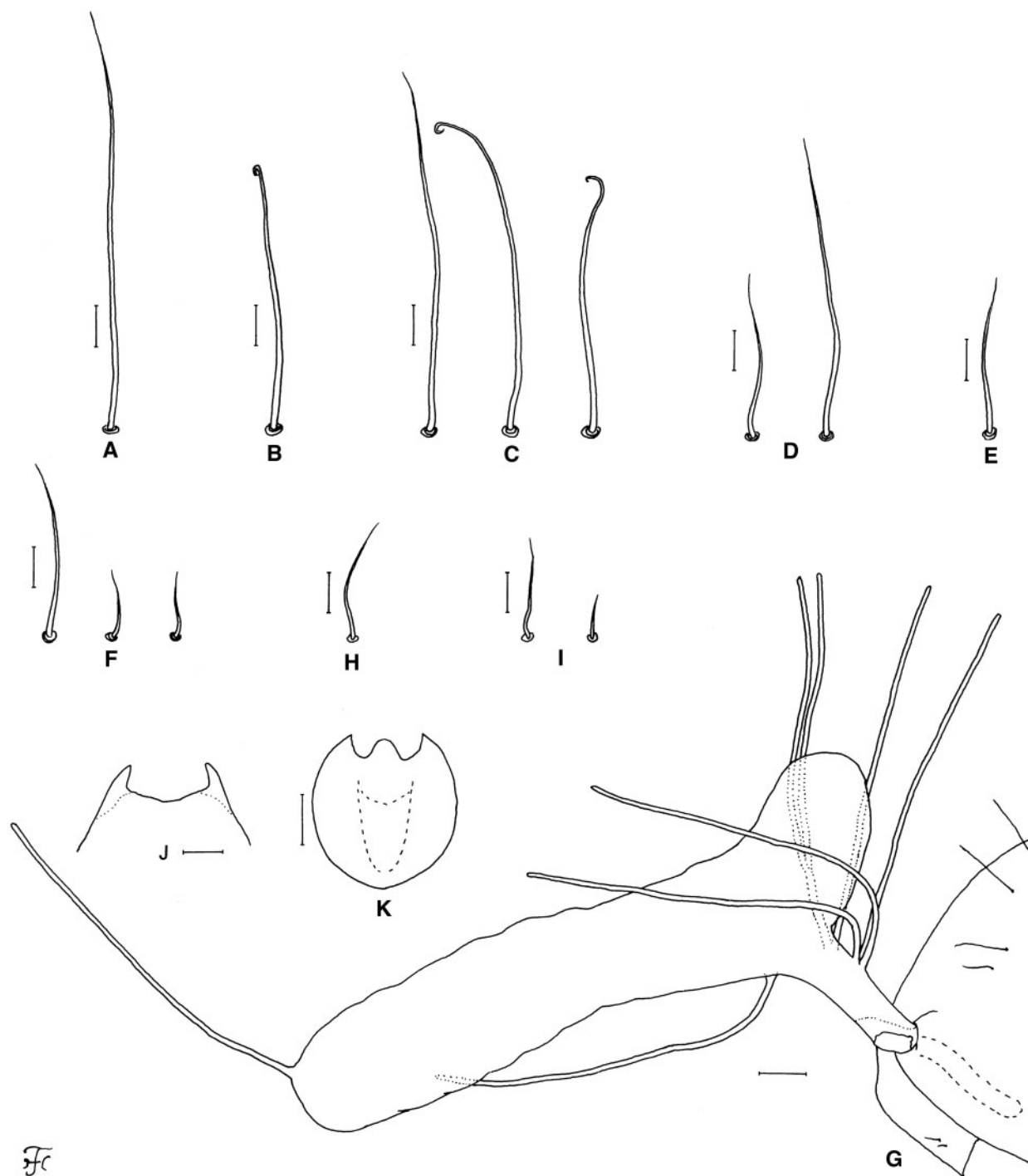
**HOLOTYPE.** Female, with associated pupal exuviae and cocoon (in 80% ethanol) reared from pupa (QSBG-2015-206), THAILAND: Mae Hong Son Province, Meung District, Mae Suai Au Village, Chong stream, 9-XII-2015, by W. Srisuka and A. Saeung. **PARATYPES:** Three females, one male (all with associated pupal exuviae and cocoons) reared from pupae, and seven mature larvae (all in 80% ethanol), same data as those of the holotype.

### Biological Notes

The pupae and larvae of this new species were collected from fallen leaves and pebbles in a small, slow-flowing stream (partially shaded by canopy, water temperature 21.9°C, pH 6.94, altitude 605 m, 19° 15' 59.1" N, 97° 51' 12.7" E),

### Etymology

The species name *maleewongae* is in honor of Prof. Pewpan Maleewong Intapan, Department of Parasitology and Research and Diagnostic Center for Emerging Infectious Diseases, Faculty of Medicine, Khon Kaen University, who kindly supported W. Srisuka to collect black flies in Myanmar and Laos.



**Fig. 3.** Pupa of *Simulium* (*Gomphostilbia*) *maleewongae* sp. nov. (A) Frontal trichome. (B) Facial trichome. (C–F) Thoracic trichomes (C, dorsomedial; D, anterolateral; E, mediolateral; F, ventrolateral). (G). Left gill (outer view). (H) Hair-like seta on dorsum of abdominal segment 1. (I) Hair-like seta and minute seta on dorsum of abdominal segment 2. (J) Terminal hooks (caudal view). (K) Cocoon (dorsal view). Scale bars = 1.0 mm for K; 0.1 mm for G; 0.02 mm for A–F and H–J.

## Discussion

*Simulium* (*G.*) *maleewongae* sp. nov. is placed in the *S. gombakense* species-group, defined by Takaoka (2012), by having the male hind basitarsus spindle-shaped, ventral plate gradually widened posteriorly when viewed ventrally, and pupal gill composed of an inflated structure with eight slender filaments. This new species is the third

member of the *S. gombakense* species-group recorded from Thailand.

Among 10 known species of this species-group (Adler and Crosskey 2016), it is similar to *S. (G.) aziruni* Takaoka, Hashim & Chen, originally described from a pupal exuviae and a mature larvae collected from Peninsular Malaysia (Takaoka et al. 2012) in having the pupal gill with an elongate inflated structure and eight slender

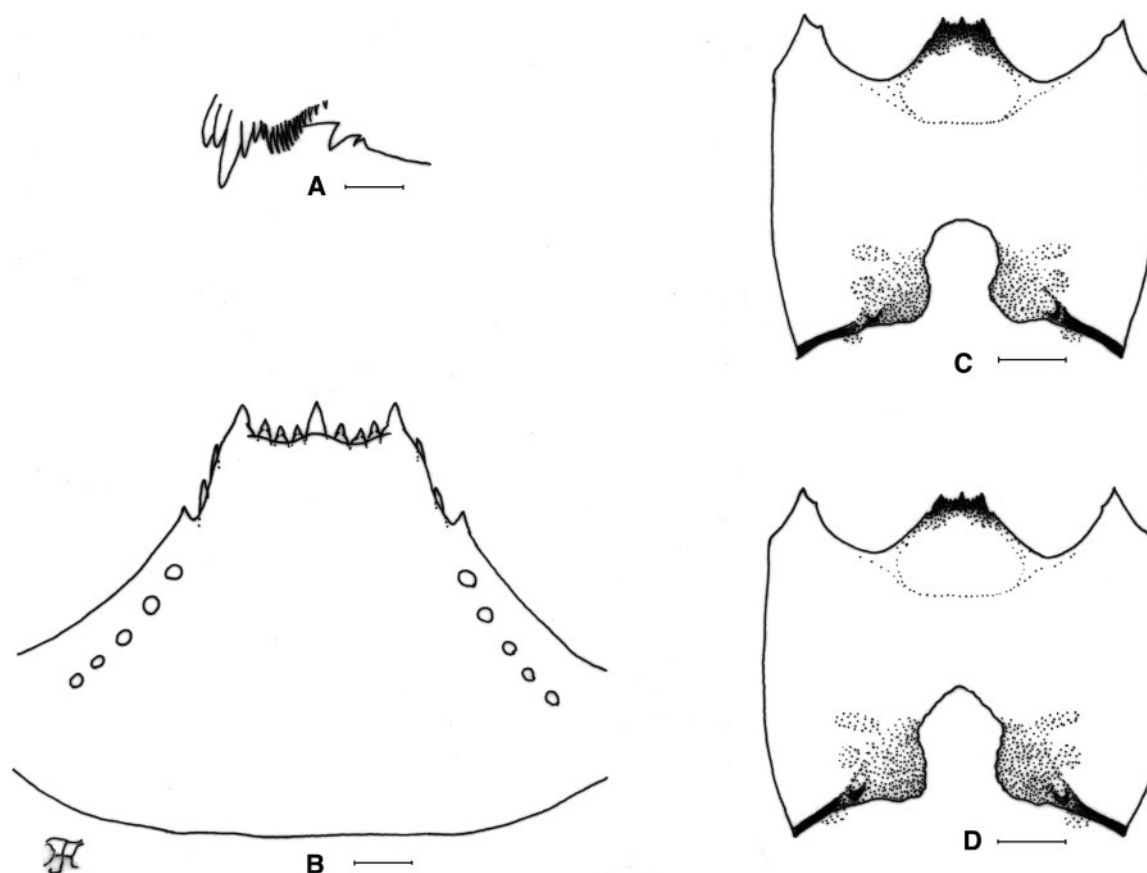


Fig. 4. Mature larva of *Simulium (Gomphostilbia) maleewongae* sp. nov. (A) Tip of mandible (lateral view). (B) Hypostoma (ventral view). (C, D) Head capsules showing postgenal clefts of different shape (ventral view). Scale bars = 0.1 mm for C and D; 0.02 mm for A and B.

filaments (Fig. 3G), simple pupal terminal hooks (Fig. 3J), and cocoon with an anterodorsal projection (Fig. 3K). However, the pupa of this new species is distinguished from that of *S. (G.) aziruni* by the following characters (those of *S. (G.) aziruni* in parentheses): the gill with two inflated portions (only one portion), forwardly directed inflated portion with same width up to the apex (tapered from the middle to the apex), cuticular surface of the inflated structure lacking any pattern (with finely defined reticulate pattern), thoracic integument sparsely covered with tubercles on the dorsal surface of the anterior half (almost bare). The pupa of *S. (G.) thuathienense* Takaoka & Sofian-Azirun from Vietnam has also simple, cone-shaped terminal hooks, but differs from this new species by the different configuration of the gill and simple cocoon (Takaoka et al. 2015).

The deeply depressed ventral margin of the ventral plate (Fig. 2F) in this new species, a rare character in the subgenus *Gomphostilbia*, is shared by *S. (G.) nuwakotense* Takaoka & Shrestha from Nepal and *S. (G.) sachini* Takaoka & Willie from India and Nepal (Takaoka and Shrestha 2010). However, the pupae of these two species differ from that of this new species by having the inflated gills of different configuration, wide terminal hooks with crenulated outer margins, and simple cocoons.

This new species is distinguished from *S. (G.) dudgeoni* Takaoka & Davies, described based on four males and one female from Hong Kong (Takaoka et al. 1995), by the male ventral plate deeply concave ventromedially when viewed posteriorly (Fig. 2F; convex in *S. (G.) dudgeoni*).

This new species is easily distinguished in the pupa from five other species of the same species-group (*S. (G.) antlerum* Chen from

Hainan Island, China, *S. (G.) gombakense*, *S. (G.) langkawiense* from Peninsular Malaysia, *S. (G.) prayongi*, and *S. (G.) willie* Takaoka & Thapa from India) by the different configuration of the gills, simple, cone-shaped terminal hooks, and cocoon with an anterodorsal projection (Takaoka 2000; Chen 2001; Takaoka and Choochote 2005; Takaoka et al. 2010b, 2013).

### Keys to Identify 11 Species of the *Simulium gombakense* Species-Group

#### Females\*

1. Hind tibia whitish yellow on basal one-fourth (at least on anterior surface) and darkened on rest. . . . . *S. dudgeoni*  
Hind tibia whitish yellow on basal half and darkened on apical half (Fig. 1C) . . . . . 2
2. Sensory vesicle elongate, over 0.52 times length of third maxillary palpal segment . . . . . 3  
Sensory vesicle medium-long, less than 0.5 times length of third maxillary palpal segment . . . . . 4
3. Sensory vesicle 0.52 times length of third maxillary palpal segment; claw tooth 0.46 times length of claw . . . *S. langkawiense*  
Sensory vesicle 0.61 times length of third maxillary palpal segment; claw tooth 0.53 times length of claw . . . *S. gombakense*
4. Fore basitarsus 6.60–6.78 times as long as its greatest width . . . . . *S. maleewongae* sp. nov.  
Fore basitarsus 5.58 times as long as its greatest width . . . . . *S. thuathienense*

## Males\*\*

1. Hind tibia whitish yellow on basal one-fourth and darkened on rest ..... *S. dudgeoni*  
Hind tibia whitish yellow on basal one-half or two-thirds and darkened on rest ..... 2
2. Ventral plate deeply concave ventromedially when viewed posteriorly (Fig. 2F) ..... 3  
Ventral plate not concave ventromedially when viewed posteriorly ..... 5
3. Upper eye with large facets in 15 vertical columns and 15 horizontal rows ..... *S. maleewongae* sp. nov.  
Upper eye with large facets in 17 vertical columns and 18 horizontal rows ..... 4
4. Ratio of width of hind basitarsus against that of hind femur equal to 0.75 ..... *S. nuwakotense*  
Ratio of width of hind basitarsus against that of hind femur equal to 0.80–0.83 ..... *S. sachini*
5. Upper-eye facets in 11 or 12 vertical columns and 12 or 13 horizontal rows ..... 6  
Upper-eye facets in 14 or 15 vertical columns and 15 or 16 horizontal rows ..... 7
6. Pleural membrane haired ..... *S. antlerum*  
Pleural membrane bare ..... *S. langkawiense*
7. Hind basitarsus 4.75 times as long as its greatest width .... *S. prayongi*  
Hind basitarsus 5.00 times as long as its greatest width ..... *S. gombakense*

## Pupae\*\*\*

1. Cocoon with anterodorsal projection (Fig. 3K) ..... 2  
Cocoon without anterodorsal projection ..... 3
2. Gill with one inflated portion tapered apically. .... *S. aziruni*  
Gill with two inflated portions, one long, forwardly directed, with same width, and one short, directed dorsally (Fig. 3G) ..... *S. maleewongae* sp. nov.
3. Gill with one inflated portion, without nipple-like or finger-like projections ..... *S. williei*  
Gill with two inflated portions, each with nipple-like or finger-like projections ..... 4
4. Gill with 10 slender filaments ..... *S. antlerum*  
Gill with 8 slender filaments ..... 5
5. Inflated portions of gill with only nipple-like short projections ..... *S. prayongi*  
Inflated portions of gill with only finger-like long projections or finger-like long and nipple-like short projections ..... 6
6. Dorsally directed inflated portion of gill without constriction basally ..... 7  
Dorsally directed inflated portion of gill with one constriction or two ..... 8
7. Ratio of width:length of inflated portion of gill equal to 0.245. *langkawiense*  
Ratio of width:length of inflated portion of gill equal to 0.30 ..... *S. gombakense*
8. Forwardly directed inflated portion of gill with one constriction; terminal hooks simple, cone-shaped (similar to Fig. 3J) ..... *S. thuathienense*  
Forwardly directed inflated portion of gill with two constrictions; terminal hooks wide, with crenulated outer margins. . 9
9. Dorsally directed inflated portion of gill with one constriction ..... *S. sachini*  
Dorsally directed inflated portion of gill with two constrictions ..... *S. nuwakotense*

## Larvae\*\*\*\*

1. Postgenal cleft medium-long, 0.7–1.2 times length of postgenal bridge ..... 2  
Postgenal cleft long, over 3.0 times length of postgenal bridge ..... 4
2. Abdominal segments 1 and 2 dark gray. . . *S. maleewongae* sp. nov.  
Abdominal segments 1 and 2 not gray ..... 3
3. Posterior circlet with 84–88 rows of hooklets .... *S. thuathienense*  
Posterior circlet with 72 rows of hooklets ..... *S. aziruni*
4. Postgenal cleft long, approaching posterior margin of hypostoma leaving narrow postgenal bridge; labral fan with 22 or 23 primary rays. .... *S. williei*  
Postgenal cleft long, but not approaching posterior margin of hypostoma, about 3.0 times length of postgenal bridge; labral fan with 25–34 primary rays. .... 5
5. Posterior circlet with 96–100 rows of hooks . *S. langkawiense*  
Posterior circlet with 74–88 rows of hooks ..... 6
6. Posterior circlet with 84–88 rows of hooks ..... *S. sachini*  
Posterior circlet with 74–82 rows of hooks ... *S. gombakense*  
\*The females of *S. antlerum*, *S. aziruni*, *S. nuwakotense*, *S. prayongi*, *S. sachini*, and *S. williei* are unknown.  
\*\*The males of *S. aziruni*, *S. thuathienense*, and *S. williei* are unknown.  
\*\*\*The pupa of *S. dudgeoni* is unknown.  
\*\*\*\*The larvae of *S. antlerum*, *S. dudgeoni*, *S. nuwakotense*, and *S. prayongi* are unknown.

## Acknowledgments

We are grateful to Peter H. Adler (Professor, Clemson University, Clemson, SC, USA) for reading the current manuscript and providing valuable comments. This work was supported by the research grant from University of Malaya (RP021A-16SUS) and the Thailand Research Fund through the TRF Senior Research Scholar (grant RTA5880001), and the Research Grant for New Scholar (grant MRG5980101) to A. Saeung. In addition, this study was partially supported by the Diamond Research Grant of the Faculty of Medicine to A. Saeung. Finally, we thank the research administration office of Chiang Mai University for providing the budget for our Excellence Center in Insect Vector Study.

## References Cited

- Adler, P. H., and R. W. Crosskey. 2016. World blackflies (Diptera: Simuliidae): A comprehensive revision of the taxonomic and geographical inventory. p. 126. (<http://entweb.clemson.edu/biomia/pdfs/blackflyinventory.pdf>) (accessed 10 May 2016)
- Chen, H. B. 2001. Studies on black flies of Hainan Island II. Record of subgenus *Morops* Enderlein in China with description of a new species (Diptera: Simuliidae). *Acta Zool. Sin.* 26: 565–568.
- Fukuda, M., W. Choochote, O. Bain, C. Aoki, and H. Takaoka. 2003. Natural infections with filarial larvae in two species of black flies (Diptera: Simuliidae) in northern Thailand. *Jpn. J. Trop. Med. Hyg.* 31: 99–102.
- Ishii, Y., W. Choochote, O. Bain, M. Fukuda, Y. Otsuka, and H. Takaoka. 2008. Seasonal and diurnal biting activities and zoonotic filarial infections of two *Simulium* species (Diptera: Simuliidae) in northern Thailand. *Parasite* 15: 121–129.
- Kuvangkadilok, C., and H. Takaoka. 2000. Taxonomic notes on Simuliidae (Diptera) from Thailand: Description of a new species and new distributional records of nine known species. *Jpn. J. Trop. Med. Hyg.* 28: 167–175.
- Takaoka, H. 2000. Taxonomic notes on *Simulium gombakense* (Diptera: Simuliidae) from Peninsular Malaysia: Descriptions of male and pupa, and subgeneric transfer from *Morops* to *Gomphostilbia*. *Jpn. J. Trop. Med. Hyg.* 28: 111–114.

- Takaoka, H. 2003. The black flies (Diptera: Simuliidae) of Sulawesi, Maluku and Irian Jaya. Kyushu University Press, Fukuoka, Japan, pp. xxii +581.
- Takaoka, H. 2012. Morphotaxonomic revision of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) in the Oriental Region. Zootaxa 3577: 1–42.
- Takaoka, H., W. Choochote, C. Aoki, M. Fukuda, and O. Bain. 2003. Black flies (Diptera: Simuliidae) attracted to humans and water buffalos and natural infections with filarial larvae, probably *Onchocerca* sp., in northern Thailand. Parasite 10: 3–8.
- Takaoka, H., and W. Choochote. 2005. Two new species of *Simulium* Latreille (Diptera: Simuliidae) from northwestern Thailand. Med. Entomol. Zool. 56: 123–133.
- Takaoka, H., and D. M. Davies. 1995. The Black Flies (Diptera: Simuliidae) of West Malaysia. Kyushu University Press, Fukuoka, Japan, pp. viii +175.
- Takaoka, H., D. M. Davies, and D. Dudgeon. 1995. Black flies (Diptera: Simuliidae) from Hong Kong: Taxonomic notes with descriptions of two new species. Jpn. J. Trop. Med. Hyg. 23: 189–196.
- Takaoka, H., and S. Shrestha. 2010. New species of black flies (Diptera: Simuliidae) from Nepal. Zootaxa (Monograph) 2731: 1–62.
- Takaoka, H., M. Sofian-Azirun, R. Hashim, Z. Ya'cob, and C. D. Chen. 2012. Two new species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from Peninsular Malaysia. J. Med. Entomol. 49: 803–812.
- Takaoka, H., M. Sofian-Azirun, and Z. Ya'cob. 2013. A new species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from Langkawi Island, Peninsular Malaysia. J. Med. Entomol. 50: 701–708.
- Takaoka, H., M. Sofian-Azirun, Z. Ya'cob, C. D. Chen, K. W. Lau, and X. D. Pham. 2015. The black flies (Diptera: Simuliidae) from Thua Thien Hue and Lam Dong Provinces, Vietnam. Zootaxa (Monograph) 3961: 1–96.
- Takaoka, H., W. Srisuka, and A. Saeng. 2010a. Description of the female of *Simulium* (*Gomphostilbia*) *gombakense* (Diptera: Simuliidae) from Thailand. Med. Entomol. Zool. 61: 111–114.
- Takaoka, H., S. Thapa, and W. Henry. 2010b. Description of two new species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from Darjeeling, India. Med. Entomol. Zool. 61: 105–110.

# A New Human-Biting Black Fly Species of *Simulium* (*Simulium*) (Diptera: Simuliidae) From Thailand

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## Abstract

*Simulium* (*Simulium*) *umphangense*, a new human-biting species of black fly, is described based on females captured while attacking humans in western and central Thailand. The female of this new species is similar to those of *S. (S.) indicum* Becher, *S. (S.) nigrogilvum* Summers, and *S. (S.) vanellum* Huang et al. in the *Simulium griseifrons* species-group of the subgenus *Simulium* Latreille in having the frons densely covered with golden-yellow short hairs, a character rarely found in the subgenus *Simulium*. This new species is readily distinguished from the three related species by having a darkened fore tibia, a bare subcosta (or rarely with a few hairs), and an ovipositor valve not protruded posteriorly. Additional diagnostic characters of this new species are noted. This is the seventh human-biting species of black fly in Thailand.

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

In Thailand, six species of black flies [*Simulium* (*Gomphostilbia*) *asakoe* Takaoka & Davies, *S. (Simulium) chamlongi* Takaoka & Suzuki, *S. (S.) doipuiense* Takaoka & Choochote (complex), *S. (S.) nigrogilvum* Summers, *S. (S.) nodosum* Puri, and *S. (S.) rufibasis* Brunetti (complex)] were reported to be human biters (Choochote et al. 2005, Pramual et al. 2016). Among these, *S. (G.) asakoe*, *S. (S.) nigrogilvum*, and *S. (S.) nodosum* are vectors of three different kinds of filariae (Fukuda et al. 2003, Takaoka et al. 2003, Ishii et al. 2008).

We collected 310 female black flies attacking humans at three sites (two sites in Tak Province, western Thailand, in March 2013 and November 2016, and one site in Kamphaeng Phet Province, central Thailand, in March 2016). We preliminarily identified 277 females as *S. (S.) nigrogilvum* and 33 as *S. (S.)* sp., both of which are similar to each other except in body size and leg color. We compared morphological characters of *S. (S.)* sp. with those of *S. (S.) nigrogilvum* from Thailand (Takaoka and Suzuki 1984), *S. (S.) indicum* Becher from India and Pakistan (Lewis 1974), and *S. (S.) vanellum* Huang et al. from Thailand (Huang et al. 2010), all of which are similar to one another and form a distinct subgroup in the *Simulium griseifrons* species-group. We found clear differences in several features, including the terminalia, between *S. (S.)* sp. and the three related species.

*Simulium* (*S.*) sp. is described as a new species based on females attacking humans in Thailand. The search of its pupae and larvae in nearby streams failed; thus, its male, pupa, and larva remain unknown.

The methods of description and illustration, and terms for morphological features used here, follow those of Takaoka (2003).

The holotype and paratypes are deposited at the Queen Sirikit Botanic Garden, Chiang Mai, Thailand.

## Nomenclature

This paper and the nomenclatural act it contains have been registered in Zoobank (www.zoobank.org), the official register of the International Commission on Zoological Nomenclature. The LSID (Life Science Identifier) number of the publication is: urn:lsid:zoobank.org:pub:0533B2D3-A677-45A0-8ED8-330C6079C9B2

## *Simulium* (*Simulium*) *umphangense* Takaoka, Srisuka & Saeung sp. nov.

(urn:lsid:zoobank.org:act:1D7F1432-14DF-492A-BDBF-1736F9B7F0C4)

## Female

Body length 3.5 mm.

## Head

Slightly narrower than thorax. Frons brownish black, white pruinose and slightly shiny when illuminated at certain angles, densely covered with golden-yellow scale-like recumbent short hairs except mediolongitudinal portion narrowly bare, and with several dark brown longer hairs along each lateral margin; frontal ratio 1.39:1.00:1.13; frons:head ratio 1.00:3.27. Fronto-ocular area well

developed, short, directed laterally, and pointed apically. Clypeus brownish black, slightly shiny, and white pruinose when illuminated at certain angles, sparsely covered with dark brown medium-long hairs and yellow short hairs except mediolongitudinal portion of upper four-fifths widely bare. Labrum 0.79 times length of clypeus. Antenna composed of scape, pedicel, and nine flagellomeres, medium to dark brown except scape, pedicel, and base of first flagellomere yellow. Maxillary palp with five segments, medium brown except first segment whitish yellow although posterior surface light brown, and second segment light brown; proportional lengths of third, fourth, and fifth segments 1.00:1.11:1.78; third segment (Fig. 1A) of normal size, with medium-sized ellipsoidal sensory vesicle (0.33–0.36 times length of third segment) having large or medium-sized opening. Maxillary lacinia with 14 or 15 inner and 18 or 19 outer teeth. Mandible (Fig. 1B) with 26 inner teeth and seven or eight outer teeth at some distance from tip. Cibarium (Fig. 1C) with triangular ragged median process directed posterodorsally, appearing to have few teeth at base of median process on each side.

### Thorax

Scutum black, densely covered with golden-yellow recumbent short hairs and sparsely with dark brown long upright hairs on prescutellar area. Scutellum black, densely covered with golden-yellow recumbent short hairs interspersed with dark-brown upright long hairs. Postnotum brownish black, slightly shiny, white pruinose when illuminated at certain angles, and bare. Pleural membrane bare. Katepisternum dark brown, longer than deep, shiny when illuminated at certain angles, and bare.

### Legs

Foreleg: coxa whitish yellow; trochanter whitish yellow except apical one-third light brown; femur yellow except apical cap dark brown; tibia medium to dark brown (though basal tip whitish yellow in some females); tarsus brownish black, with thick dorsal hair crest; basitarsus greatly dilated, 4.46 times as long as its greatest width. Midleg: coxa brownish black; trochanter whitish yellow except apical half medium brown; femur medium to dark brown except basal one-third (or little more) yellow; tibia dark brown except basal two-fifths yellow; tarsus dark brown except basal one-fourth of basitarsus dark yellow to light brown and basal tips of tarsomeres 2 and 3 whitish yellow. Hind leg: coxa medium brown; trochanter yellow; femur medium brown except basal two-fifths yellow and apical cap dark brown; tibia dark brown except basal half yellow; tarsus dark brown except little less than basal half of basitarsus yellow and basal two-fifths of tarsomere 2 whitish yellow; basitarsus (Fig. 1D) nearly parallel-sided, 6.19 times as long as wide, and 0.65 and 0.58 times as wide as greatest widths of hind tibia and femur, respectively; calcipala (Fig. 1D) moderately developed, nearly as long as width at base, and 0.38 times as wide as greatest width of basitarsus; pedisulcus (Fig. 1D) well developed. Claw (Fig. 1E) with small tooth.

### Wing

Length 3.0 mm. Costa with dark-brown spinules and medium-brown hairs; subcosta bare or rarely with one or two hairs; basal section of radius fully covered with medium-brown hairs;  $R_1$  with dark brown spinules and medium-brown hairs;  $R_2$  with medium-brown hairs; hair tuft on base of radius dark brown; basal cell absent.

### Halter

White except base light brown.

### Abdomen

Basal scale whitish yellow, with fringe of golden-yellow and light-brown hairs. Dorsal surface of abdomen medium brown except that of segment 2 entirely whitish yellow and that of segment 3 whitish yellow except medial portion and dorsolateral portion on each side light brown, and moderately or densely covered with yellow recumbent short hairs interspersed with dark-brown longer hairs; tergites dull (though dorsolateral portions of tergites 6–8 in some females somewhat shiny when illuminated at certain angles). Ventral surface of abdomen whitish yellow except that of segment 8 medium brown (though in some females, ventral surfaces of segments 4–7 grayish); ventral surface of segment 7 without sclerotized sternal plates.

### Terminalia

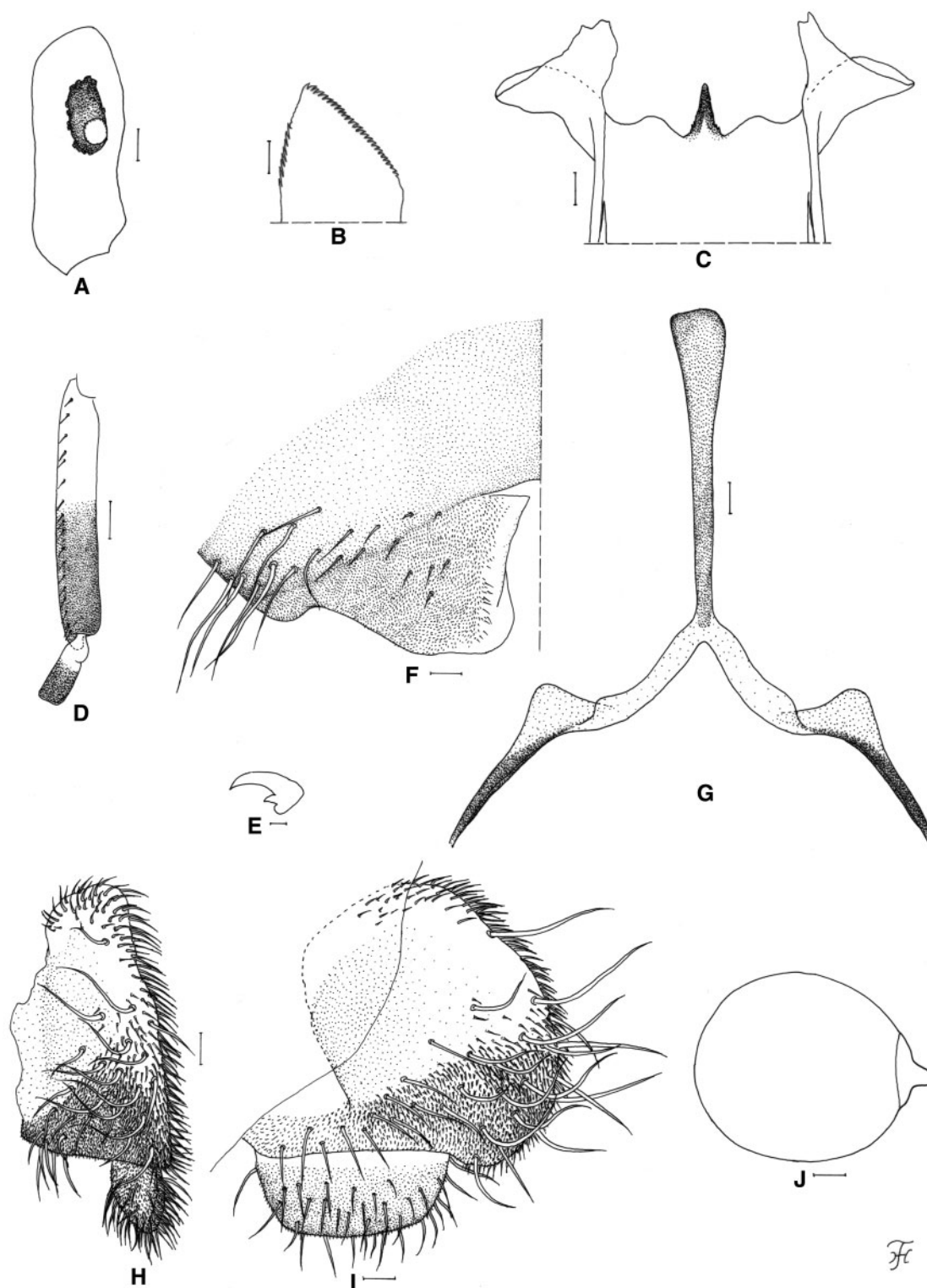
Sternite 8 (Fig. 1F) bare medially, with 10 or 11 dark brown medium-long to long stout hairs and three to six yellow short hairs on each lateral surface. Ovipositor valves (Fig. 1F) triangular, though posteromedial portion rounded, membranous except narrow area along inner margin slightly sclerotized, covered with three or four short yellow hairs and numerous microsetae except narrow portions along inner margin and near posteromedial tip bare; inner margins slightly sinuous, moderately separated from each other. Genital fork (Fig. 1G) of inverted-Y form, with long narrow well-sclerotized stem somewhat widened apically; arms of narrow width, each with short moderately sclerotized round projection directed dorsally. Paraproct in ventral view (Fig. 1H) subquadrate, narrowly produced ventrally along inner margin, moderately covered with unpigmented short setae on ventral surface of this produced portion, with outer surface widely concave and mostly bare, and medial surface unpigmented, with 20–22 sensilla; paraproct in lateral view (Fig. 1I) 0.91 times as long as wide, much protruded ventrally beyond ventral margin of cercus, with outer surface moderately sclerotized and widely bare except narrow areas covered with short or minute setae along ventral and posterior margins, intermixed with 29–32 long to medium-long stout hairs. Cercus in lateral view (Fig. 1I) short, 0.37 times as long as wide, with numerous medium-long hairs, with posterior margin slightly convex posteriorly. Spermatheca (Fig. 1J) nearly ovoid, 1.14 times as long as greatest width, well sclerotized except portion of junction with duct unsclerotized, with faint reticulate pattern on its surface near junction; internal setae present; accessory ducts subequal in thickness to each other, and slightly thicker than major duct.

### Male, Pupa, and Mature Larva

Unknown.

### Type Material

HOLOTYPE. Female, collected attacking a human by a hand net, at site (air temperature 21°C, elevation 1,237 m, 16° 13'34.2" N, 98° 58'47.4" E), Mae Klong Kee village, Um-phang district, Tak Province, western Thailand, 1-III-2013, by W. Srisuka, S. Suriya, R. Saokod, and S. Pilakantha. PARATYPES. Seven females, data same as those of the holotype; two females, same data as those of the holotype except air temperature 17°C, 21-XI-2016, by W. Srisuka, S.



**Fig. 1.** Female of *Simulium* (*Simulium*) *umphangense* sp. nov. (A) Third segment of maxillary palp showing sensory vesicle (right side; front view). (B) Mandible. (C) Cibarium. (D) Basitarsus and tarsomere 2 of hind leg (left side; outer view). (E) Claw. (F) Sternite 8 and ovipositor valve (only right half shown; 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 for D; 0.02 mm for A–C, F–J; 0.01 mm for E.

Suriya, R. Saokod, and C. Rangsan; 14 females, same village as noted under the holotype but different site (air temperature 19°C, elevation 785 m, 16° 17'16.4" N, 98° 59'45.9" E), 22-III-2016, by W. Srisuka, S. Suriya, R. Saokod, and T. Somboonchai; 10 females, at a

site (air temperature 17°C, elevation 1,274 m, 16° 05'21.2" N, 99° 06'47.9" E), Chong Yen, Pang-sila Thong district, Kamphaeng Phet Province, central Thailand, 23-III-2016, by W. Srisuka, S. Suriya, R. Saokod, and T. Somboonchai.

**Table 1.** Female morphological characters of four related species in the *Simulium griseifrons* species-group of the subgenus *Simulium*

|                   | <i>S. umphangense</i> sp. nov.   | <i>S. indicum</i> <sup>a</sup>               | <i>S. nigrogilvum</i> <sup>b</sup>           | <i>S. vanellum</i> <sup>c</sup>              |
|-------------------|----------------------------------|----------------------------------------------|----------------------------------------------|----------------------------------------------|
| Maxillary lacinia |                                  |                                              |                                              |                                              |
| Inner teeth       | 14 or 15                         | 11                                           | 14                                           | 11                                           |
| Outer teeth       | 18 or 19                         | 12                                           | 15                                           | 14 or 15                                     |
| Mandible          |                                  |                                              |                                              |                                              |
| Inner teeth       | 26                               | ca. 35                                       | ca. 30                                       | 34                                           |
| Outer teeth       | 6 or 7 distinct                  | ca. 9 vestigial                              | 5 or 6 distinct                              | 6 vestigial                                  |
| Cibarium          |                                  |                                              |                                              |                                              |
| Median process    | Pointed apically                 | Rounded apically                             | Pointed apically                             | Pointed apically                             |
| Fore tibia        | Almost entirely darkened         | Yellow on basal half and dark on apical half | Yellow on basal half and dark on apical half | Yellow on basal half and dark on apical half |
| Subcosta          | Bare or rarely with 1 or 2 hairs | With many hairs                              | With many hairs                              | With many hairs                              |
| Ovipositor valve  | Triangular                       | Tongue-like                                  | Tongue-like                                  | Tongue-like                                  |
| Paraproct         |                                  |                                              |                                              |                                              |
| Outer surface     | Widely bare                      | Widely bare                                  | Widely bare                                  | Covered with short hairs                     |
| Stout hairs       | 29–32                            | 13 or 14                                     | 14                                           | 18–20                                        |

<sup>a</sup> Based on Lewis (1974).<sup>b</sup> Based on Takaoka and Suzuki (1984).<sup>c</sup> Based on Huang et al. (2010).

## Biological Notes

The females of this new species bite humans. The aquatic habitats of this new species remain unknown.

## Remarks

The female of *S. (S.) umphangense* sp. nov. is similar to those of *S. (S.) indicum*, *S. (S.) nigrogilvum*, and *S. (S.) vanellum* in the *S. griseifrons* species-group of the subgenus *Simulium* in having the frons and scutum densely covered with yellow scale-like recumbent short hairs, cibarium with a triangular median process directed posterodorsally (Fig. 1C), fore tarsus with a thick hair crest, claw with a short sub-basal tooth (Fig. 1E), basal portion of the radius fully haired, and abdominal segments 2 and 3 lighter than other segments. However, *S. (S.) umphangense* sp. nov. is easily distinguished from these three related species by the fore tibia, which is almost entirely darkened in this new species but yellow on the basal half and darkened on the apical half in the three other species, and by the ovipositor valve, which is not protruded posteriorly (Fig. 1F) in this new species but protruded posteriorly, forming a tongue-like projection in the three other species. Additional diagnostic characters are shown in Table 1.

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## References

- Choochote, W., H. Takaoka, M. Fukuda, Y. Otsuka, C. Aoki, and N. Eshima. 2005. Seasonal abundance and daily flying activity of black flies (Diptera: Simuliidae) attracted to human baits in Doi Inthanon National Park, northern Thailand. *Med. Entomol. Zool.* 56: 335–348.
- Fukuda, M., W. Choochote, O. Bain, C. Aoki, and H. Takaoka. 2003. Natural infections with filarial larvae in two species of black flies (Diptera: Simuliidae) in northern Thailand. *Jpn. J. Trop. Med. Hyg.* 31: 99–102.
- Huang, Y. T., J. Phasuk, J. Chanpaisaeng, and P. H. Adler. 2010. A new species of black fly in the subgenus *Simulium* (Diptera: Simuliidae) from Thailand. *Med. Entomol. Zool.* 63: 49–58.
- Ishii, Y., W. Choochote, O. Bain, M. Fukuda, Y. Otsuka, and H. Takaoka. 2008. Seasonal and diurnal biting activities and zoonotic filarial infections of two *Simulium* species (Diptera: Simuliidae) in northern Thailand. *Parasite* 15: 121–129.
- Lewis, D. J. 1974. Man-biting Simuliidae (Diptera) of northern India. *Israel J. Entomol.* 9: 23–53.
- Pramual, P., J. Thairarern, and K. Wongpakam. 2016. Cryptic diversity in human-biting black flies (Diptera: Simuliidae) in Thailand. The 1st Asian Simuliidae Symposium Proceeding, 8.
- Takaoka, H. 2003. The Black Flies (Diptera: Simuliidae) of Sulawesi, Maluku and Irian Jaya. xxii + 581 pp., Kyushu University Press, Fukuoka, Japan.
- Takaoka, H., and H. Suzuki. 1984. The blackflies (Diptera: Simuliidae) from Thailand. *Jpn. J. Sanit. Zool.* 35: 7–45.
- Takaoka, H., W. Choochote, C. Aoki, M. Fukuda, and O. Bain. 2003. Black flies (Diptera: Simuliidae) attracted to humans and water buffalos and natural infections with filarial larvae, probably *Onchocerca* sp., in northern Thailand. *Parasite* 10: 3–8.

# A New Black Fly Species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) From Thailand

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## Abstract

*Simulium* (*Gomphostilbia*) *fukudae* sp. nov. is described based on females, males, pupae, and larvae from Thailand. This new species is placed in the *Simulium batoense* species-group of *Simulium* (*Gomphostilbia*). It is characterized by the female frons almost bare, male paramere covered with minute setae, pupal gill composed of six slender filaments, pupal terminal hooks cone-shaped, and cocoon with an anterodorsal projection. Taxonomic notes are given to separate this new species from the most similar species, *Simulium* (G.) *sextuplum* Takaoka & Davies and *Simulium* (G.) *paukatense* Takaoka, both from Malaysia.

**Key words:** batoense species-group, sextuplum subgroup, fauna, new species

The *Simulium batoense* species-group is the largest assemblage of heterogeneous lineages among 15 species-groups of the subgenus *Gomphostilbia* Enderlein. It consists of 59 species classified into eight subgroups (seven named and one unnamed) and is widely distributed in the Oriental Region, though five species are recorded from the Palaearctic Region (Takaoka 2012, Adler and Crosskey 2016). In this species-group, little is known about the biting habits and other biological aspects, although *Simulium* (G.) *asakoe* Takaoka & Davies of the *S. asakoe* species-group is a vector of an unidentified filarial parasite of animal origin in Thailand (Fukuda et al. 2003, Ishii et al. 2008).

In Thailand, the *S. batoense* species-group is represented by four species, of which one is placed in the *Simulium decuplum* subgroup, two in the *Simulium duolongum* subgroup, and one in the *Simulium parahiayangum* subgroup (Takaoka 2012).

In a recent survey of pupae and larvae of black flies in Surin Province in northeastern Thailand, we collected one undescribed species of the *Simulium sextuplum* subgroup of the *S. batoense* species-group of the subgenus *Gomphostilbia*. The *S. sextuplum* subgroup is characterized by the six slender long pupal gill filaments on each side (Takaoka 2012). This subgroup is small, containing only two species: *Simulium* (G.) *sextuplum* Takaoka & Davies from Peninsular Malaysia (Takaoka and Davies 1995) and *Simulium* (G.) *paukatense* Takaoka from Sarawak, Malaysia (Takaoka 2008).

This species is described as new based on females, males, pupae, and larvae, and taxonomic notes are given to separate it from the two morphologically similar species from Malaysia.

The methods of collection, description and illustration, and terms for morphological features follow those of Takaoka (2003) and partially those of Adler et al. (2004). The holotype and paratypes are deposited at the Queen Sirikit Botanic Garden, Chiang Mai, Thailand.

## Nomenclature

This paper and the nomenclatural act it contains have been registered in Zoobank ([www.zoobank.org](http://www.zoobank.org)), the official register of the International Commission on Zoological Nomenclature. The LSID (life science identifier) number of the publication is: urn:lsid:zoobank.org:pub:FF4624CF-9221-4F60-BC40-DA115FE8F264

***Simulium* (*Gomphostilbia*) *fukudae* sp. nov.**  
(urn:lsid:zoobank.org:act:81331958-5B98-40A7-88BA-61D1ACEBED21)

## Female

Body length 2.0–2.1 mm ( $n = 2$ ).

## Head

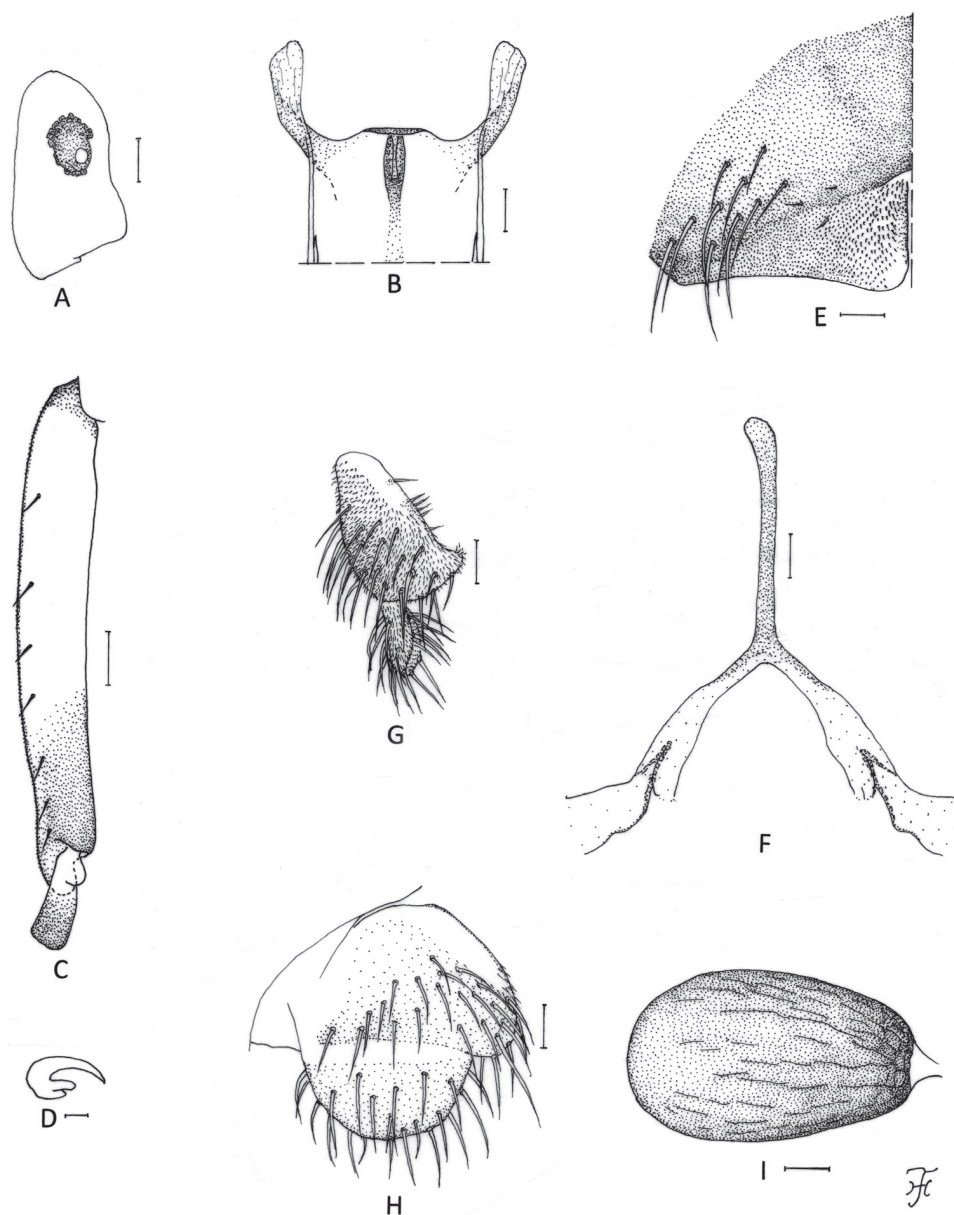
Slightly narrower than width of thorax. Frons brownish black, shiny, widely bare, with three to six yellow and dark short hairs along each lateral margin and one yellow hair on lower median

portion; frontal ratio 1.5:1.0:2.1 (1.5:1.0:3.1 in paratype female); frons:head ratio 1.0:5.4 (1.0:6.4 in paratype female). Fronto-ocular area well developed, narrow, directed dorsolaterally. Clypeus brownish black, grayish-white pruinose, shiny, moderately covered with yellow fine short hairs interspersed with several dark longer hairs on each side of lower portion. Labrum 0.6–0.7 times as long as clypeus. Antenna composed of scape, pedicel, and nine flagellomeres, medium to dark brown except scape, pedicel, and little less than basal half of first flagellomere yellow, and rest of first flagellomere light brown, when viewed dorsally (first flagellomere mostly yellow when viewed ventrally). Maxillary palp composed of five segments, light to medium brown except first and second segments dark yellow, proportional lengths of third, fourth, and fifth segments 1.0:1.2:2.5–2.6; third segment (Fig. 1A) somewhat swollen apically; sensory vesicle (Fig. 1A) ellipsoidal, 0.2–0.3 times as long

as third segment, with small opening apically. Maxillary lacinia with 9 or 10 inner and 11–13 outer teeth. Mandible with 19 inner and 10 or 11 outer teeth. Cibarium (Fig. 1B) medially forming sclerotized plate folded forward from posterior margin, with moderately sclerotized medial longitudinal ridge having split apex.

#### Thorax

Scutum brownish black, markedly shiny, thinly grayish-white pruinose when illuminated anteriorly, faintly with dark longitudinal vittae (one medial and two submedial), moderately covered with brassy recumbent short hairs interspersed with white recumbent hairs on shoulders and along lateral margins. Scutellum brownish black, covered with brassy short hairs and dark-brown long upright hairs along posterior margin. Postnotum black, shiny, grayish-white pruinose when viewed at certain angles, and bare. Pleural membrane



**Fig. 1.** Female of *S. (G.) fukudae* sp. nov. (A) Third segment of maxillary palp with sensory vesicle (right side; front view). (B) Cibarium (front view). (C) Hind basitarsus and second tarsomere (left side; outer view). (D) Claw. (E) Sternite 8 and ovipositor valve (right side only; ventral view). (F) Genital fork (ventral view). (G, H) Paraprocts and cerci (G, ventral view; H, lateral view). (I) Spermatheca. Scale bars—0.05 mm for C; 0.02 mm for A, B, and E–I; 0.01 mm for D.

bare. Katepisternum brownish black, with its length longer than depth, shiny, moderately covered with dark hairs.

### Legs

Foreleg: coxa yellow; trochanter light brown except base yellow; femur light brown with apical cap medium brown; tibia medium brown, with extreme base and large medial portion of outer surface light brown; tibia densely covered with white fine hairs (brightly shiny in light) on most of outer surface (except apical cap); tarsus brownish black, with moderate dorsal hair crest; basitarsus moderately dilated, 5.3–5.7 times as long as its greatest width. Midleg: coxa dark brown; trochanter light brown except base much lighter; femur light to medium brown, with apical cap medium to dark brown; tibia light to medium brown except base dark yellow and apical cap dark brown; tibia densely covered with white fine hairs (brightly shiny in light) on most of posterior surface (except apical cap); tarsus medium to dark brown. Hind leg: coxa medium brown; trochanter yellow; femur medium brown, with apical cap dark brown; tibia medium brown, with base yellow and apical cap dark brown to brownish black; tibia densely covered with white fine hairs (brightly shiny in light) on posterior surface of basal two-thirds; tarsus (Fig. 1C) dark brown except basal two-thirds of basitarsus (though base medium brown) and basal half of second tarsomere whitish; basitarsus narrow, nearly parallel-sided though slightly narrowed apically, 6.5 times as long as wide, and 0.6 and 0.55 times as wide as greatest widths of tibia and femur, respectively; calcipala as long as wide, and nearly half as wide as greatest width of basitarsus. Claw (Fig. 1D) with large basal tooth 0.48 times as long as claw.

### Wing

Length 1.6–1.7 mm ( $n=2$ ). Costa with dark spinules and hairs. Subcosta with dark hairs except near apex bare. Hair tuft on stem vein dark brown. Basal portion of radius fully haired;  $R_1$  with dark spinules and hairs;  $R_2$  with hairs only. Basal cell absent.

### Abdomen

Basal scale light to medium brown, with fringe of whitish-yellow hairs. Dorsal surface of abdomen dark brown to brownish black except basal half of segment 2 yellow (though tergal plates dark yellow), moderately covered with dark short to long hairs; tergites of segments 2 and 6–9 wide and shiny; ventral surface of segment 2 entirely yellowish, and those of other segments medium to dark brown; sternal plate on segment 7 undeveloped.

### Terminalia

Sternite 8 (Fig. 1E) bare medially, with 11–14 medium-long to long hairs together with two to five short slender hairs on each side. Ovipositor valve (Fig. 1E) triangular, with round posteromedial corners, thin, membranous, moderately covered with microsetae interspersed with one short seta; inner margins nearly straight, slightly sclerotized, and narrowly separated from each other. Genital fork (Fig. 1F) of usual inverted-Y form, with arms of moderate width; arm moderately folded medially. Paraproct in ventral view (Fig. 1G) bare narrowly along anteromedial margin; anteromedial surface darkened, with five or six sensilla; paraproct in lateral view (Fig. 1H) somewhat produced ventrally, 0.7 times as long as wide, with 27 or 28 short to long hairs on ventral and lateral surfaces. Cercus in lateral view (Fig. 1H) short, rounded posteriorly or subquadrate, 0.46 times as long as wide. Spermatheca (Fig. 1I) ellipsoidal, 1.7 times as long as its greatest width, well sclerotized except duct and small area near juncture with duct unsclerotized, and with many

fissures on outer surface; internal setae absent; both accessory ducts slender, unpigmented, subequal in diameter to major one.

### Male

Body length 2.1–2.2 mm ( $n=2$ ).

### Head

Wider than thorax. Upper eye consisting of large facets in 14 or 15 vertical columns and 15 horizontal rows. Face black, grayish-white pruinose. Clypeus brownish black, grayish-white pruinose, moderately covered with yellow short hairs interspersed with several dark-brown longer stout hairs on each side of 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, 1.8–2.0 times as long as second flagellomere. Maxillary palp with five segments, light brown except first and second segments dark yellow, proportional lengths of third, fourth, and fifth segments 1.0:1.3:2.9; third segment (Fig. 2A) widened apically; sensory vesicle (Fig. 2A) nearly globular or ellipsoidal, 0.2–0.3 times as long as third segment, and with small opening.

### Thorax

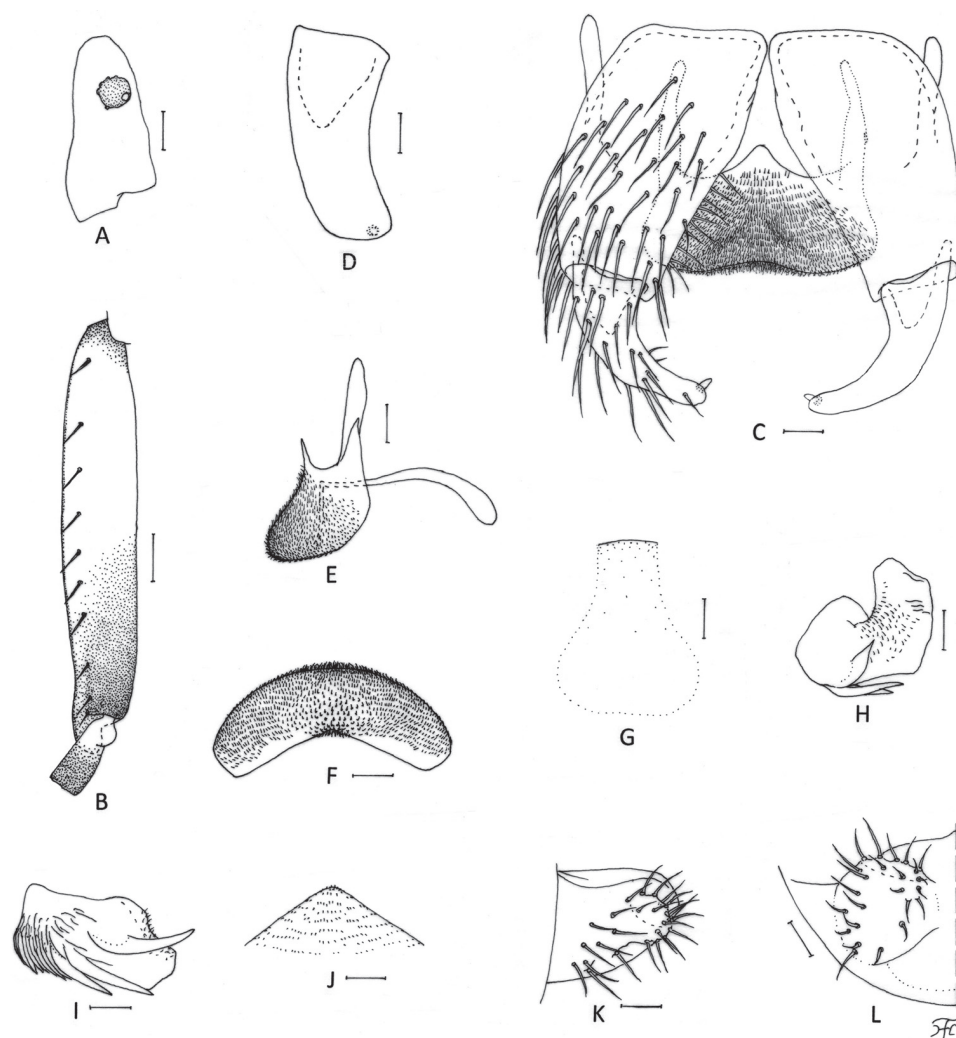
Scutum black, with shiny whitish-gray pruinose spot on each shoulder, which is connected through whitish-gray pruinose band along each lateral margin to large whitish-gray pruinose spot on prescutellar area; scutum moderately covered with brassy short hairs; scutellum brownish black, with dark-brown short hairs and dark-brown long upright hairs along posterior margin. Postnotum brownish black, whitish pruinose, shiny when illuminated at certain angles, and bare. Pleural membrane bare. Katepisternum brownish black, with its length longer than depth, moderately covered with dark short hairs.

### Legs

Foreleg: coxa yellow; trochanter light brown except base yellow; femur light brown, with apical cap medium brown; tibia medium brown, with large medial portion of outer surface light brown; tibia densely covered with white fine hairs (brightly shiny in light) on most of outer surface (except apical cap); tarsus brownish black, with moderate dorsal hair crest; basitarsus moderately dilated, 5.6–6.1 times as long as its greatest width. Midleg: coxa dark brown; trochanter medium brown except base light brown; femur, tibia, and tarsus dark brown; tibia without white hair on posterior surface. Hind leg: coxa dark brown; trochanter dark yellow; femur dark brown, with apical cap brownish black; tibia dark brown to brownish black; tibia without white hair on posterior surface; tarsus (Fig. 2B) dark brown except little more than basal half of basitarsus (though base medium brown) and basal half of second tarsal segment whitish; basitarsus narrow, nearly parallel-sided, though apical half slightly narrowed toward apex, 5.6–5.7 times as long as wide, and 0.5–0.6 and 0.6 times as wide as greatest widths of tibia and femur, respectively; calcipala (Fig. 2B) as long as wide, and 0.4 times as wide as greatest width of basitarsus; pedisulcus (Fig. 2B) well developed.

### Wing

Length 1.5–1.6 mm. Other features as in female except subcosta bare.



**Fig. 2.** Male of *S. (G.) fukudae* sp. nov. (A) Third segment of maxillary palp with sensory vesicle (right side; front view). (B) Hind basitarsus and second tarsomere (left side; outer 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, I) Parameres (left side; H, lateral view; I, caudal view). (J) Aedeagal membrane (caudal view). (K, L) Abdominal segment 10 and cerci (right side; K, lateral view; L, caudal view). Scale bars—0.05 mm for B; 0.02 mm for A and C–L.

### Abdomen

Basal scale black, with fringe of dark hairs. Dorsal surface of abdominal segments dark brown to brownish black except base of segment 2 light brown, moderately covered with dark short to long hairs; segments 2 and 5–7 each with pair of shiny whitish-gray dorsolateral patches, of which those on segment 2 broadly connected in middle to each other, and those on segments 5–7 narrowly connected in middle to each other; segment 8 with pair of similar lateral patches; ventral surface medium brown except most of segment 2 whitish.

### Genitalia

Coxite in ventral view (Fig. 2C) nearly rectangular, 1.8 times as long as its greatest width. Style in ventral view (Fig. 2C) slender, tapered toward apex, 0.8 times as long as coxite, gently bent inward, with apical spine; style in ventrolateral view (Fig. 2D) slightly narrowed from base to apex, with apex appearing truncate. Ventral plate in ventral view (Fig. 2C) with body transverse, 0.6 times as long as wide, widened posteriorly, with anterior

margin produced anteromedially, and posterior margin slightly concave medially, and densely covered with microsetae on ventral surface; basal arms of moderate length, nearly parallel-sided, though convergent apically; ventral plate in lateral view (Fig. 2E) moderately produced ventrally; ventral plate in end view (Fig. 2F) rounded ventrally, much wider than height and densely covered with microsetae on posterior surface. Median sclerite (Fig. 2E, G) thin, plate-like, wide, and connected to ventral plate far from anterior margin. Paramere (Fig. 2H, I) of moderate size, moderately covered with fine setae on outer surface near base, with three distinct long and stout hooks and several smaller ones close together near apex. Aedeagal membrane (Fig. 2J) moderately setose; dorsal plate not sclerotized. Abdominal segment 10 (Fig. 2K, L) slightly sclerotized near anterior margin of ventral surface, without distinct hairs near posterior margin. Cercus (Fig. 2K, L) small, with 20–24 hairs.

### Pupa

Body length 2.4–2.5 mm ( $n=4$ ).

### Head

Integument yellow, moderately covered with small round tubercles on frons and posterior part of each lateral surface, but bare on antennal sheath and on ventral surface of face; antennal sheath without any protuberances; frons with three unbranched long trichomes with straight or coiled apices on each side (Fig. 3A), all arising close together, subequal in length to one another; face with one

unbranched long trichome with straight or coiled apex on each side (Fig. 3B).

### Thorax

Integument yellow, moderately covered with round tubercles, with three long anterodorsal trichomes with coiled apices (though



**Fig. 3.** Pupa of *S. (G.) fukudae* sp. nov. (A) Frontal trichome. (B) Facial trichome. (C–F) Thoracic trichomes (C, anterodorsal; D anterolateral; E, mediolateral; F ventrolateral). (G) Gill filaments (right side; dorsal view). (H) Anterior part of thorax and basal portion of gill (right side; outer view). (I) Hair-like seta on dorsal surface of abdominal segment 1. (J) Minute seta near anterior margin of dorsal surface of abdominal segment 2. (K) Hair-like seta and minute seta near posterior margin of dorsal surface of abdominal segment 2. (L) Terminal hooks (caudal view). (M) Hooklet and minute seta on ventral surface of abdominal segment 4. (N, O) Cocoons (N, dorsal view; O, lateral view). Scale bars—1.0 mm for N and O; 0.1 mm for G and H; 0.02 mm for A–F and I, K and L; 0.01 mm for J and M.

posterior trichome much shorter; Fig. 3C), two long anterolateral trichomes (anterior trichome with straight apex, posterior one with straight or coiled apex; Fig. 3D), one medium-long mediolateral trichome with straight apex (Fig. 3E), and three ventrolateral trichomes with straight apices (one medium-long, two short; Fig. 3F) on each side; all trichomes unbranched. Gill (Fig. 3G, H) composed of six slender thread-like filaments, much longer than pupal body, arranged as  $(2 + 2) + 2$  from dorsal to ventral (one pair dorsal inner, one pair dorsal outer, and one pair ventral), with somewhat swollen transparent basal fenestra ventrally; common basal stalk short, 0.5–0.7 times length of interspiracular trunk; stalk of ventral pair medium-long, 0.9–1.0 times length of interspiracular trunk; common stalk of dorsal inner and dorsal outer pairs short, 0.3–0.9 times length of common basal stalk; stalks of dorsal inner and dorsal outer pairs nearly horizontal and short, 0.8–1.8 and 1.3–2.6 times length of their common stalk, respectively; common stalk of dorsal inner and dorsal outer pairs lying against that of ventral pair at angle of 40–60 degrees when viewed laterally; all filaments light brown, gradually tapered toward apex, subequal in length (lengths including their own stalks and common basal stalk 2.4–3.0 mm, though two filaments of ventral pair and one of dorsal inner pair slightly longer than others) and thickness to one another; cuticle of all filaments mostly smooth, though annular ridges and furrows present at irregular intervals on basal portions, and densely covered with minute tubercles.

### Abdomen

Dorsally, unpigmented except segments 1, 2, and 9 light yellow; segment 1 with one unbranched, slender medium-long hair-like seta (Fig. 3I) on each side; segment 2 with one minute seta (Fig. 3J) submedially near anterior margin, and one unbranched slender medium-long hair-like seta and five minute setae (Fig. 3K) near posterior margin, on each side; segments 3 and 4 each with one minute seta (similar to Fig. 3J) submedially near anterior margin, four hooked spines and one minute seta near posterior margin, on each side; segment 5 without spine-combs in transverse row 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 minute

spines on each side; segment 5 with four minute setae near posterior margin on each side; segments 6–8 each with one minute seta near posterior margin on each side; segment 9 with pair of conical terminal hooks (Fig. 3L). Ventrally, segment 4 with one unbranched hook (nearly as long as those on segments 5–7) and few minute setae (Fig. 3M) on each side; segment 5 with pair of bifid hooks submedially and few minute 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 minute setae on each side; segments 4–8 each with comb-like groups of minute spines. Each side of segment 9 with three grapnel-shaped hooklets.

### Cocoon (Fig. 3N, O)

Wall-pocket-shaped, moderately woven; anterodorsal margin widely produced as short projection; posterior two-thirds with floor moderately woven; individual threads visible; 3.0 mm long by 1.3 mm wide.

### Mature Larva

Body length 4.3–4.6 mm ( $n =$ ). Body reddish brown except most of lateral surface of thoracic segments 2 and 3, intersegmental areas from thoracic segment 3 to abdominal segment 5, and ventral surface of abdominal segments 8 and 9 unpigmented.

### Head

Cephalic apotome light brown, moderately covered with minute unpigmented setae; head spots obscure, though anterior and posterior mediolongitudinal spots faintly positive and mediolateral and posterolateral spots often faintly negative. Lateral surface of head capsule light brown except eye-spot region whitish and anterior one-third to one-fourth yellow; spots obscure, though one small round spot below eye-spot region faintly positive in two larvae. Ventral surface of head capsule (Fig. 4C) light brown though anterior one-fourth yellow; transverse spot on each side of postgenal cleft obscure. Antenna composed of three articles and apical sensillum, somewhat longer than stem of labral fan; proportional lengths of first, second, and third articles 1.0:0.8:0.9–1.0. Labral fan with 40–45 main rays. Mandible (Fig. 4A) with three comb-teeth decreasing in length from first to third; mandibular serration composed of two

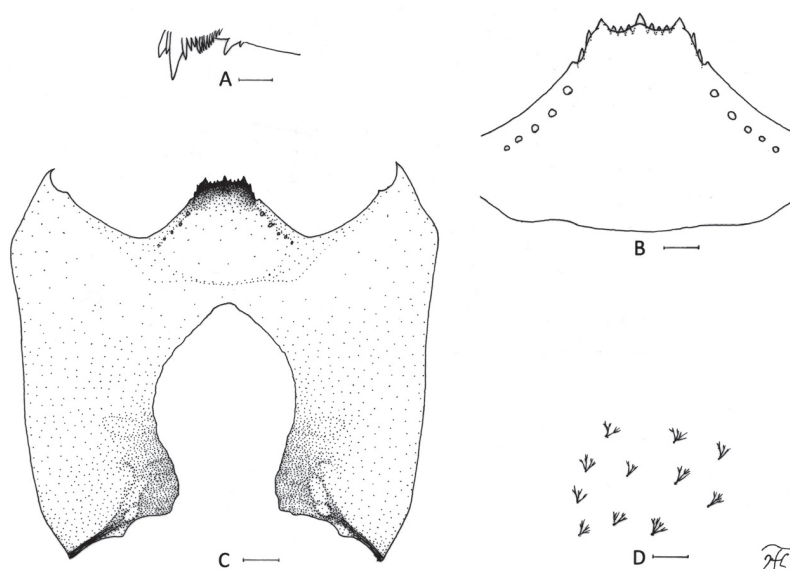


Fig. 4. Larva of *S. (G.) fukudae* sp. nov. (A) Mandible. (B) Hypostoma. (C) Head capsule showing postgenal cleft (ventral view). (D) Dark multibranched setae on dorsal surface of abdominal segment 8. Scale bars—0.05 mm for C; 0.02 mm for A, B, and D.

teeth (one medium-sized and one small); major tooth at acute angle against mandible on apical side; supernumerary serrations absent. Hypostoma (Fig. 4B) with row of nine apical teeth; median tooth most prominent, slightly longer than each corner tooth, and three intermediate teeth on each side shortest; lateral margin smooth; five hypostomal bristles per side, lying parallel to lateral margin. Postgenal cleft (Fig. 4C) deep, 11–12 times as long as postgenal bridge, moderately constricted basally, widest medially, and rounded apically. Cervical sclerite composed of two small light-brown rod-like pieces, not fused to occiput, widely separated medially from each other. Cuticle of thorax and abdominal segments 1–4 moderately covered with dark, minute branched setae each with four to seven branches, and cuticle of abdominal segments 5–9 moderately or densely covered with dark, branched minute setae each with 6–10 branches (Fig. 4D) on dorsal and dorsolateral surfaces; cuticle of abdominal segment 9 also densely covered with unbranched, colorless minute setae on each side of anal sclerite. Rectal scales absent. Rectal papillae compound, each of three lobes with 8–11 finger-like secondary lobules. Anal sclerite of usual X-form, with anterior arms 0.9 times length of posterior ones, broadly sclerotized at base; accessory sclerite absent; basal juncture area with no sensilla. Last abdominal segment expanded ventrolaterally forming double bulges on each side, visible as large conical ventral papilla when viewed from side. Posterior circlet with 95 rows of up to 15 or 16 hooklets per row.

### Type Material

Holotype female (with associated pupal exuviae and cocoon) reared from pupa, collected from a moderately flowing stream (width 3 m, depth 5 cm, bottom sandy and rocky, temperature 21.7°C, pH 4.71, partially shaded, elevation 337 m, 24° 23'26.4" N, 103° 51'50.8" E), Along Korn waterfall, Sang ka District, Surin Province, Thailand, 2-I-2017, by W. Srisuka. Paratypes: One female, two males (all with associated pupal exuviae and cocoons), and three mature larvae, same data as those of the holotype; nine mature larvae, same data as those of the holotype except 29-XI-2016, by S. Suriya.

### Biological Notes

The pupae and larvae of this new species were collected from grasses trailing in the water. Associated species was *S. (G.)* sp. (nr. *S. angulistylum* Takaoka & Davies).

### Etymology

The species name *fukudae* is in honor of Dr. Masako Fukuda, Oita University, Japan, for her excellent experimental and molecular studies of zoonotic *Onchocerca* species and vector black flies in relation to zoonotic onchocerciasis in Japan, and her pioneer studies of natural infections of black flies with filariae in Thailand.

### Remarks

*Simulium (G.) fukudae* sp. nov. is readily assigned to the *S. batoense* species-group within the subgenus *Gomphostilbia* by having the adult antenna with nine flagellomeres, pleural membrane bare, adult hind tibiae mostly dark, and male hind basitarsus not enlarged (Fig. 2B).

This new species is similar to *S. (G.) sextuplum* from Peninsular Malaysia (Takaoka and Davies 1995) and *S. (G.) paukatense* from Sarawak, Malaysia (Takaoka 2008), both of the *S. sextuplum* subgroup, in having the female frons almost bare, pupal gill with six slender filaments (Fig. 3G), cocoon with a short anterodorsal bulge or projection (Fig. 3N, O), larval body with reddish-brown bands

and covered with dark multibranched setae (Fig. 4D), and deep larval postgenal cleft (Fig. 4C).

However, this new species is distinguished from *S. (G.) sextuplum*, of which the male is unknown, by the following characters (those of *S. (G.) sextuplum* in parentheses): length ratio of the female sensory vesicle (Fig. 1A) against the third maxillary palpal segment 0.2–0.3 (0.4), stalks of the dorsal inner and dorsal outer pairs of filaments (Fig. 3H) 0.4–0.9 times as long as the interspiracular trunk (1.1 times), stalk of the ventral pair of filaments 0.9–1.0 times as long as the interspiracular trunk (1.5 times), and larval posterior circlet with 95 rows of hooklets (about 62 rows of hooklets).

This new species is indistinguishable in the female from *S. (G.) paukatense* but is distinguished in the male by the number of upper-eye large facets in 14 or 15 vertical columns and 15 horizontal rows (in 17 or 18 vertical columns and 16 or 17 horizontal rows in *S. (G.) paukatense*), and ventral plate with its body widened posteriorly (nearly parallel-sided in *S. (G.) paukatense*); in the pupa by the short stalks of the dorsal inner and dorsal outer pairs of filaments (Fig. 3H), which are 0.4–0.9 times as long as the interspiracular trunk (1.6–2.0 times in *S. (G.) paukatense*), and stalk of the ventral pair of filaments (Fig. 3H), which is 0.9–1.0 times as long as the interspiracular trunk (1.6 times in *S. (G.) paukatense*); and in the larva by the labral fan with 40–45 primary rays (34–38 primary rays in *S. (G.) paukatense*) and posterior circlet with 95 rows of hooklets (68–74 rows of hooklets in *S. (G.) paukatense*).

The males of this new species and *S. (G.) paukatense* are unique among the subgenus *Gomphostilbia* in that their parameres are moderately covered with minute setae on the outer surface (Fig. 2H). This rare character was reported for *Simulium (G.) tamdaoense* Takaoka, Sofian-Azirun & Ya'cob from Vietnam (Takaoka et al. 2014), *Simulium (Simulium) dumogaense* Takaoka and Roberts and *Simulium (S.) tumpaense* Takaoka and Roberts, both from Sulawesi, Indonesia (Takaoka 2003).

This new species is the first record of the *S. sextuplum* subgroup from Thailand.

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We are grateful to Peter H. Adler (Professor, Clemson University, Clemson, SC, United States) for reading the current manuscript and providing valuable comments. Thanks are due to S. Suriya, R. Saokod, S. Pilakantha, C. Rangsan, and T. Somboonchai (Entomology Section, Queen Sirikit Botanic Garden, Chiang Mai, Thailand) for their kind help in the field surveys and in the laboratory. This work was supported by a research grant from University of Malaya (RP021A/16SUS) to H. Takaoka, and the Thailand Research Fund (TRF), and the Office of the Higher Education Commission (OHEC) through the Research Grant for New Scholar (Grant MRG5980101), and the Diamond Research Grant of the Faculty of Medicine to A. Saeung. Finally, we thank the research administration office of Chiang Mai University for providing the budget for our Excellence Center in Insect Vector Study.

### References Cited

- Adler, P. H., and R. W. Crosskey. 2016. World blackflies (Diptera: Simuliidae): a comprehensive revision of the taxonomic and geographical inventory, p. 126. (<http://entweb.clemson.edu/biomia/pdfs/blackflyinventory.pdf>) (accessed 10 March 2017)
- Adler, P. H., D. C. Currie, and D. M. Wood. 2004. The black flies (Simuliidae) of North America, pp. xv+941. Cornell University Press, Ithaca, New York, NY.
- Fukuda, M., W. Choochote, O. Bain, C. Aoki, and H. Takaoka. 2003. Natural infections with filarial larvae in two species of black flies (Diptera: Simuliidae) in northern Thailand. *Jpn. J. Trop. Med. Hyg.* 31: 99–102.

- Ishii, Y., W. Choochote, O. Bain, M. Fukuda, Y. Otsuka, and H. Takaoka. 2008. Seasonal and diurnal biting activities and zoonotic filarial infections of two *Simulium* species (Diptera: Simuliidae) in northern Thailand. *Parasite* 15: 121–129.
- Takaoka, H. 2003. The black flies (Diptera: Simuliidae) of Sulawesi, Maluku and Irian Jaya, pp. xxii + 581. Kyushu University Press, Fukuoka, Japan.
- Takaoka, H. 2008. Four new species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from Sarawak, Malaysia. *Med. Entomol. Zool.* 59: 181–211.
- Takaoka, H. 2012. Morphotaxonomic revision of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) in the Oriental Region. *Zootaxa* 3577: 1–42.
- Takaoka, H., and D. M. Davies. 1995. The black flies (Diptera: Simuliidae) of West Malaysia, pp. viii + 175. Kyushu University Press, Fukuoka, Japan.
- Takaoka, H., M. Sofian-Azirun, Z. Ya'cob, C. D. Chen, K. W. Lau, and H. T. Pham. 2014. New species and records of blackflies (Diptera: Simuliidae) from Vinh Phuc Province, Vietnam. *Zootaxa* 3838: 347–366.

# Two New Species of the *Simulium* (*Simulium*) *variegatum* Species-Group of Black Flies (Diptera: Simuliidae) From Thailand

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## Abstract

Two new species of black flies, *Simulium* (*Simulium*) *srisukai* and *S.* (*S.*) *kiewmaepanense*, are described from specimens collected in Thailand. Both species are assigned to the *Simulium variegatum* species-group of the sub-genus *Simulium* (*Simulium*) Latreille. They are characterized by the darkened female femora and tibiae and six inflated pupal gill filaments, and the darkened female tibiae and six ordinary thread-like pupal gill filaments, respectively. Taxonomic notes are given to separate these new species from 10 related species among the group. These new species represent the third and fourth species of the *S. variegatum* species-group from Thailand.

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

The *Simulium variegatum* species-group is the largest taxon among 25 species-groups of *Simulium* (*Simulium*) Latreille, consisting of 52 species, of which 24 species are recorded only in the Palaearctic Region and 23 only in the Oriental Region, with five species in both regions (Adler and Crosskey 2016). *Simulium* (*S.*) *oitanum* (Shiraki), one of the common species of this group in Japan, was reported to be a natural vector of *Onchocerca* sp. of cattle (Takaoka et al. 1992), and to be a potential vector of four zoonotic *Onchocerca* species: *O. dewitiei japonica* Uni, Bain & Takaoka of wild boar (a causative parasite of zoonotic onchocerciasis in Japan), *O. takaokai* Uni, Fukuda & Bain of wild boar, *O. skrjabini* Rukhlyadev, and *O. eberhardi* Uni & Bain of deer (Fukuda et al. 2008).

In Thailand, 89 species of black flies are known, of which only two species, *S.* (*S.*) *chamlongi* Takaoka & Suzuki and *S.* (*S.*) *barnesi* Takaoka & Suzuki, are placed in the *S. variegatum* species-group (Adler and Crosskey 2016, Takaoka and Saeung 2016). *Simulium* (*S.*) *chamlongi* was reported to be a human biter (Choochote et al. 2005).

We surveyed pupae and larvae of black flies in Tak and Chiang Mai Provinces, Thailand, and collected two more species of this group. They are similar to each other by having the darkened female tibiae but are distinguished by the different form of the pupal gill filaments. These two species are described as new based on adults, pupae, and mature larvae.

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

The holotypes and paratypes are deposited at the Queen Sirikit Botanic Garden, Chiang Mai, Thailand.

## Nomenclature

This paper and the nomenclatural acts it contains have been registered in Zoobank (www.zoobank.org), the official register of the International Commission on Zoological Nomenclature. The LSID (Life Science Identifier) number of the publication is: urn:lsid:zoobank.org:pub:8CFE33DA-154C-44AC-A5D7-98D303E75BD2

## *Simulium* (*Simulium*) *srisukai* Takaoka & Saeung sp. nov.

(urn:lsid:zoobank.org:act:E063E91A-52ED-48C2-8A8C-4FFF2578560E)

## Female

Body length 3.1 mm.

## Head

Narrower than thorax. Frons black, shiny, with several dark long stout hairs along each lateral margin; frontal ratio 1.2–1.4:1.0:1.2–1.3; frons:head ratio 1.0:3.8–3.9. Fronto-ocular area well developed, short, directed laterally and slightly upward. Clypeus black,

white pruinose, slightly shiny when illuminated at certain angles, moderately covered with dark brown long stout hairs except upper area near dorsal margin somewhat widely bare. Labrum 0.6–0.7 times as long as clypeus. Antenna composed of scape, pedicel, and nine flagellomeres, medium to dark brown except scape, pedicel and base of first flagellomere yellow when viewed dorsally (first flagellomere dark yellow to light brown except base yellow when viewed ventrally). Maxillary palp with five segments, grayish brown except anterior surface of segment 1 yellow and segment 3 dark brown; proportional lengths of third, fourth, and fifth segments 1.0:1.1–1.3:2.2–2.7; third segment (Fig. 1A) of normal size; sensory vesicle (Fig. 1A) of moderate size, (0.3–0.4 times length of third segment) having opening of moderate size. Maxillary lacinia with 12–17 inner and 16 or 17 outer teeth. Mandible with 27–34 inner and 13 or 14 outer teeth. Cibarium (Fig. 1B) with blunt median process on posterior margin and with 40–60 minute tubercles near base of median process.

### Thorax

Scutum black, gray pruinose along lateral margins, shiny when illuminated at certain angles, densely covered with golden-yellow recumbent short hairs interspersed with dark brown short upright hairs near anterior margin, and several dark brown long upright hairs on prescutellar area. Scutellum brownish black to black, covered with dark brown upright long hairs and golden-yellow short hairs. Postnotum brownish black to dark, and bare. Pleural membrane bare. Katepisternum longer than deep, brownish black, shiny when illuminated at certain angles and bare.

### Legs

Foreleg: coxa and trochanter yellow; femur light brown (or yellow or dark yellow on basal one-fourth or little more in some females) except apical cap medium brown, though inner surface widely yellowish; tibia dark brown to brownish black with median portion widely white, with shiny sheen widely on outer surface when illuminated at certain angles; tarsus black, with moderate dorsal hair crest; basitarsus greatly dilated, 5.4 times as long as its greatest width. Midleg: coxa brownish black; trochanter yellow except outer surface of apical half light brown; femur yellow on basal one-third to half, medium brown to brownish black on rest on outer surface, and light to medium brown except basal one-fifth to one-third yellow and apical cap brownish black on inner surface; tibia light brown except base whitish yellow and apical cap dark brown, though whitish yellow on basal half on posterior surface; tibia with shiny sheen on posterior surface when illuminated at certain angles; tarsus dark brown except basal two-thirds of basitarsus yellow. Hind leg: coxa brownish black; trochanter yellow; femur medium to dark brown except basal two-fifths yellow and apical cap brownish black; tibia (Fig. 1C) medium to dark brown except base and narrow area on basal half along posterior margin yellow and apical cap brownish black, though basal half yellow on posterior surface, with shiny sheen on posterior surface when illuminated at certain angles; tarsus brownish black except basal two-thirds of basitarsus and basal half of second tarsomere yellow; basitarsus (Fig. 1D) nearly parallel-sided, 6.4 times as long as wide, and 0.6–0.7 and 0.5 times as wide as greatest widths of hind tibia and femur, respectively; calcipala (Fig. 1D) moderately developed, slightly shorter than basal width, and 0.5 times as wide as basitarsus; pedisulcus (Fig. 1D) well developed; claw (Fig. 1E) with small subbasal tooth.

### Wing

Length 2.9 mm. Costa with dark spinules and hairs; subcosta haired except near apex bare; basal section of radius bare;  $R_1$  with dark spinules and hairs;  $R_2$  with hairs; hair tuft on base of radial vein dark brown; basal cell absent.

### Halter

White with basal portion darkened.

### Abdomen

Basal scale light brown, with fringe of pale long hairs. Dorsal surface of abdomen dark brown except tergite of segment 2 light to medium brown, with short dark hairs; tergite 2 shiny, white iridescent when illuminated at certain angles, and tergites 6–9 shiny; ventral surface of segment 2 whitish, those of segments 3 and 4 yellowish and those of others light brown except sternites 7 and 8 dark brown; segment 7 with median large sternal plate having numerous hairs.

### Terminalia

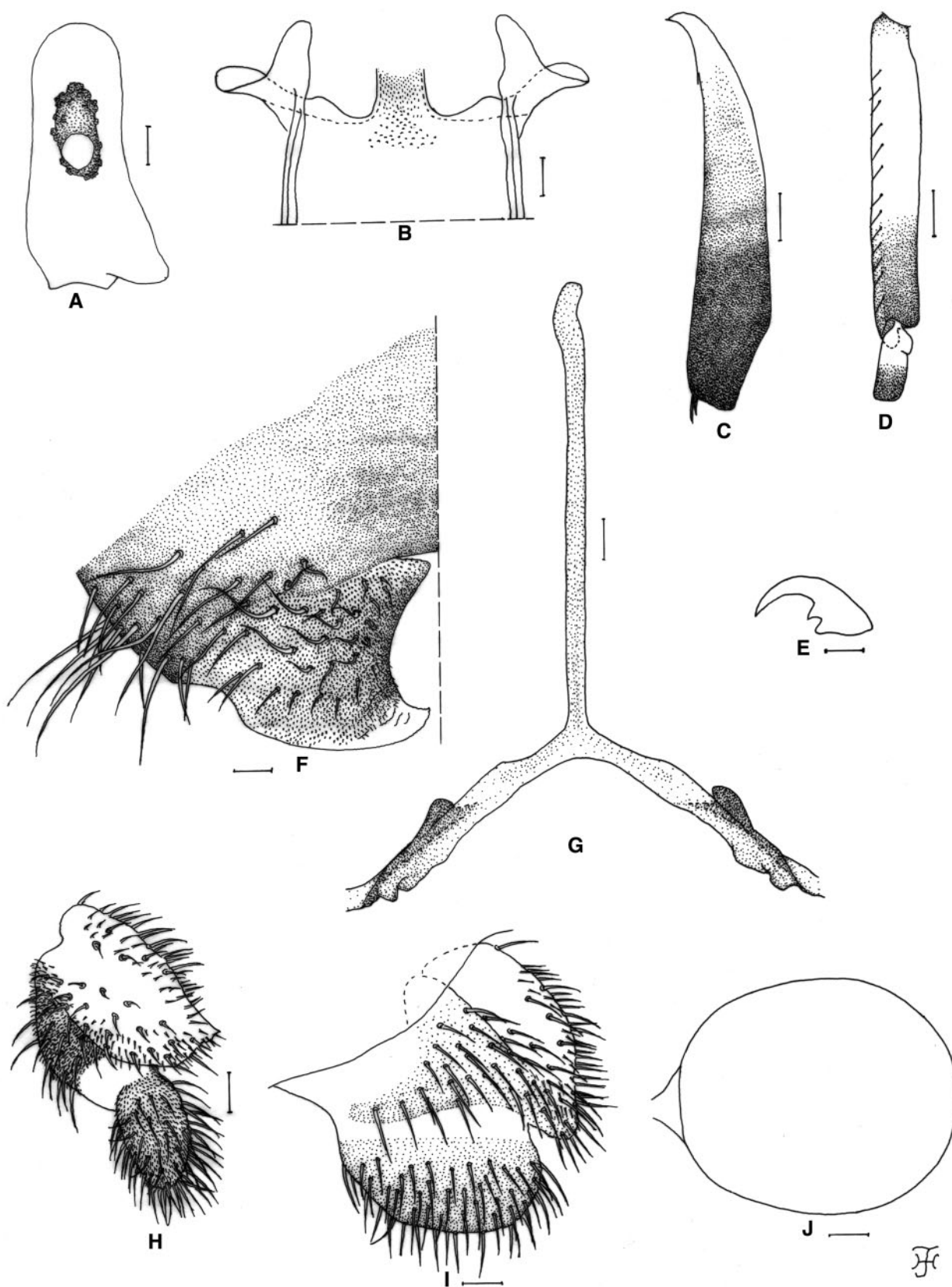
Sternite 8 (Fig. 1F) bare medially, with 18–26 dark medium-long to long stout hairs and two to five yellow short to medium-long hairs on each lateral surface. Ovipositor valves (Fig. 1F) wide, with posteromedial apices pointed anteromedially, each densely covered with microsetae and 23–29 dark brown short to medium-long hairs and three to eight yellow short hairs, although small area of posteromedial apex bare; inner margins widely concave and darkened except near apex unpigmented. Genital fork (Fig. 1G) of inverted-Y form, with narrow, well sclerotized stem; arms of moderate width, each with distinct short projection directed anterodorsally. Paraproct in ventral view (Fig. 1H) oblong, widely unpigmented and depressed on ventral surface; paraproct in lateral view (Fig. 1I) somewhat protruded ventrally beyond ventral margin of cercus, 0.9 times as long as wide, with 41–46 short to medium-long hairs on lateral and ventral surfaces; anteromedial surface somewhat darkened, with 10–12 short sensilla. Cercus in lateral view (Fig. 1I) short, rounded posteriorly, 0.5 times as long as wide, and with numerous short to medium-long hairs. Spermatheca (Fig. 1J) large, nearly ovoid, 1.2 times as long as wide, well sclerotized except portion of juncture with duct unsclerotized, with faintly defined reticulate patterns near base on outer surface, and with internal setae; accessory ducts subequal in thickness to each other, and to major duct.

### Male

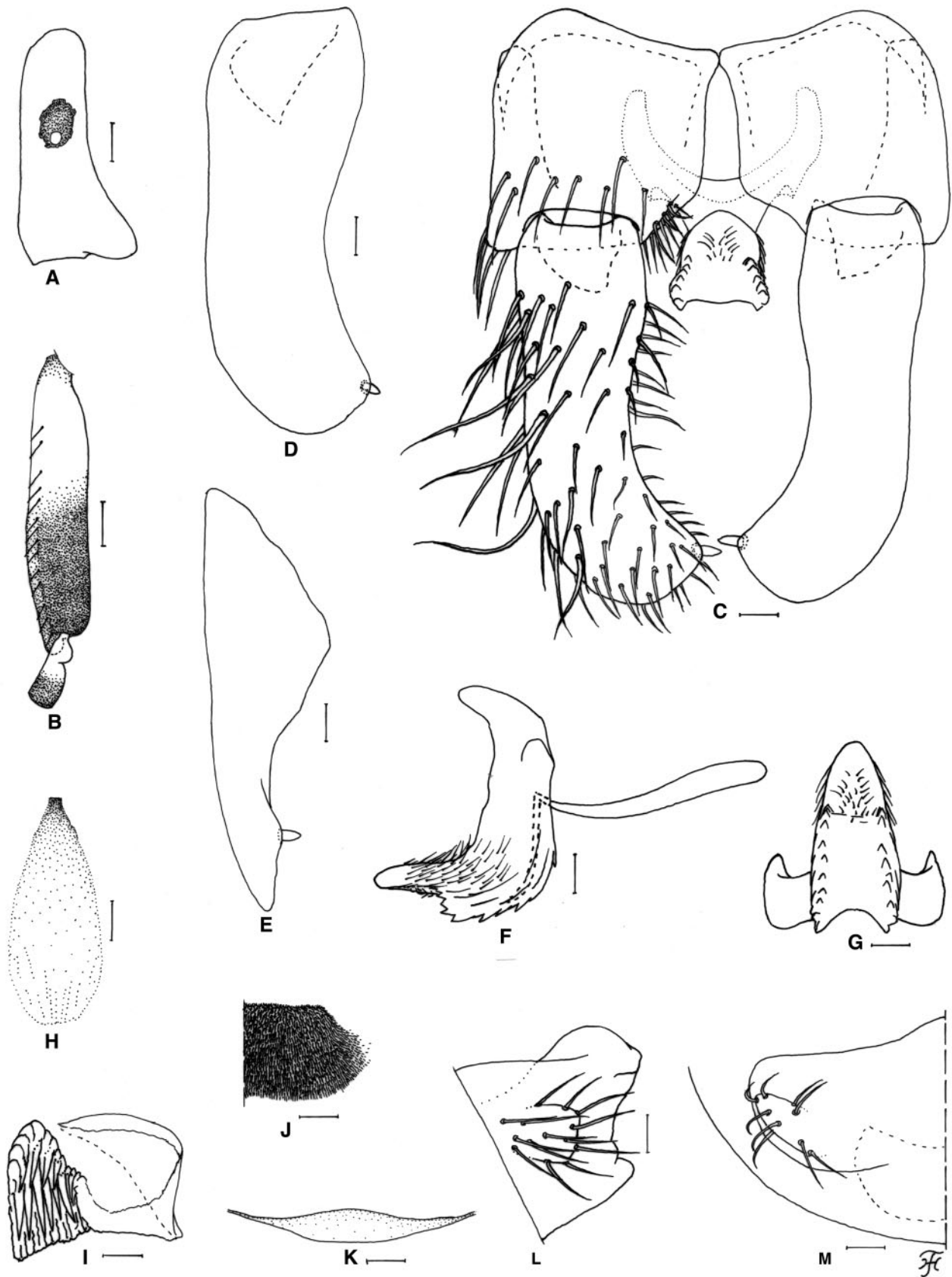
Body length 3.3–3.6 mm

### Head

Slightly wider than thorax. Upper eye medium brown, consisting of large facets in 24–26 vertical columns and in 24–26 horizontal rows. Clypeus black, thickly white pruinose, moderately covered with dark brown hairs. Antenna composed of scape, pedicel and nine flagellomeres, dark brown except base of first flagellomere light brown; first flagellomere elongate, 1.6 times as long as second one. Maxillary palp medium brown, composed of five segments with proportional lengths of third, fourth, and fifth segments 1.0:1.1–1.3:2.7–2.8; third segment (Fig. 2A) of moderate size, with apex somewhat produced inward; sensory vesicle (Fig. 2A) small (0.2–0.3 times length of third segment), ellipsoidal, and with small opening.



**Fig. 1.** Female of *Simulium* (*Simulium*) *srisukai* sp. nov. (A) Third segment of maxillary palp with sensory vesicle (right side; front view). (B) Cibarium (front view). (C) Hind tibia (left side; outer view). (D) Hind basitarsus and second tarsomere (left side; outer view). (E) Claw. (F) Sternite 8 and ovipositor valve (right side only; ventral view). (G) Genital fork (ventral view). (H) & (I) Paraprocts and cerci (H, ventral view; I, lateral view). (J) Spermatheca. Scale bars. 0.1 mm for C and D; 0.02 mm for A, B and E–J.



**Fig. 2.** Male of *Simulium* (*Simulium*) *srisukai* sp. nov. (A) Third segment of maxillary palp with sensory vesicle (right side; front view). (B) Hind basitarsus and second tarsomere (left side; outer view). (C) Coxites, styles, and ventral plate (ventral view). (D) & (E) Styles (right side; D, ventrolateral view; E, medial view). (F) Ventral plate and median sclerite (lateral view). (G) Ventral plate (caudal view). (H) Median sclerite (caudal view). (I) Paramere (left side; caudal view). (J) Aedeagal membrane (left half; caudal view). (K) Dorsal plate (caudal view). (L) & (M) Abdominal segment 10 and cerci (right side; L, lateral view; M, caudal view). Scale bars. 0.1 mm for B; 0.02 mm for A and C–M.

### Thorax

Scutum black, with white pruinose pattern, i.e., anterior pair of rectangular spots on shoulders extended posteriorly along lateral margins and connected to large transverse spot entirely covering prescutellar area; these pruinose areas shiny when illuminated at certain angles; scutum uniformly and densely covered with golden-yellow recumbent short hairs interspersed with dark brown long upright hairs on prescutellar area. Scutellum brownish black, with several dark long upright hairs and golden-yellow short hairs. Postnotum black, white pruinose when illuminated at certain angles and bare. Pleural membrane bare. Katepisternum longer than deep, brownish black to black, slightly shiny and white pruinose when illuminated at certain angles, and bare.

### Legs

Foreleg: coxa yellow; trochanter medium brown except base partially yellow; femur light to dark brown with apical cap brownish black; tibia dark brown to brownish black, though outer surface of middle large portion widely white; tarsus black, with moderate dorsal hair crest; basitarsus moderately dilated, 5.8–6.4 times as long as its greatest width. Midleg: dark brown to brownish black except base of trochanter dark yellow, extreme base of tibia white and basal half of basitarsus dark yellow to light brown; tibia with white sheen on posterior surface when illuminated at certain angles. Hind leg: coxa brownish black; trochanter dark yellow to light brown; femur dark brown except base dark yellow and apical cap brownish black; tibia dark brown to brownish black except basal tip white; tarsus (Fig. 2B) dark brown except basal two-fifths of basitarsus yellow (though base somewhat darkened) and little less than basal half of second tarsomere yellow; basitarsus (Fig. 2B) enlarged, slightly widened from base to apical one-third, then tapered to apex, 4.8–4.9 times as long as its greatest width, and 0.7 and 0.7 times as wide as greatest widths of hind tibia and femur, respectively; calcipala (Fig. 2B) small, slightly shorter than width at base, 0.4 times as wide as greatest width of basitarsus; pedisulcus (Fig. 2B) well developed.

### Wing

Length 2.5–2.6 mm. Other characters as in female except subcosta bare.

### Halter

White with basal portion darkened.

### Abdomen

Basal scale dark brown to brownish black, with fringe of dark long hairs. Dorsal surface of abdomen dark brown to brownish black, and covered with dark short hairs; segments 2 and 5–7 each with pair of white iridescent spots dorsolaterally, those on segment 2 broadly connected in middle.

### Genitalia

Coxites, styles, and ventral plate in ventral view as in Fig. 2C. Coxite in ventral view (Fig. 2C) nearly quadrate, 1.2 times as long as width. Style in ventrolateral view (Fig. 2D) elongate, 2.8 times as long as its greatest width near base, slightly tapered to middle, then nearly parallel-sided, with blunt apex having apical spine; style in medial view (Fig. 2E) 1.7 times as long as coxite, spatulate dorso-ventrally except basal two-thirds much produced dorsally in form of isosceles triangle. Ventral plate in ventral view (Fig. 2C) Y-shaped,

body with several teeth on each side of posterior surface, and with ventrally produced process covered with several minute setae on its posteroventral surface; arms widely divergent basally and directed forward apically; ventral plate in lateral view (Fig. 2F): body with serrated posterior margin, and sparsely covered with minute setae on each anterolateral surface, and having ventrally produced process covered with minute setae on anterior and anterolateral surfaces except near ventral tip bare; arm wide with apex curved ventrally; ventral plate in caudal view (Fig. 2G): body and ventrally produced process gradually tapered ventrally, with rounded apex, having several teeth in vertical row on each side of posterior surface, and several minute setae on posterior surface of ventrally produced process. Median sclerite (Fig. 2F, H) arising near anterior margin of ventral plate and directed dorsally, plate-like, widened from base toward apical one-third, then slightly narrowed toward apex, brown basally, but not so well sclerotized apically. Paramere (Fig. 2I) broad basally, with several medium-long and short hooks. Aedeagal membrane (Fig. 2J) densely covered with minute setae, and with partially sclerotized dorsal plate in form of horizontal bar (Fig. 2K). Abdominal segment 10 (Fig. 2L, M) without distinct hair on ventral and lateral surfaces. Cercus (Fig. 2L, M) small, rounded, with 9–14 distinct hairs.

### Pupa

Body length 3.5–4.0 mm.

### Head

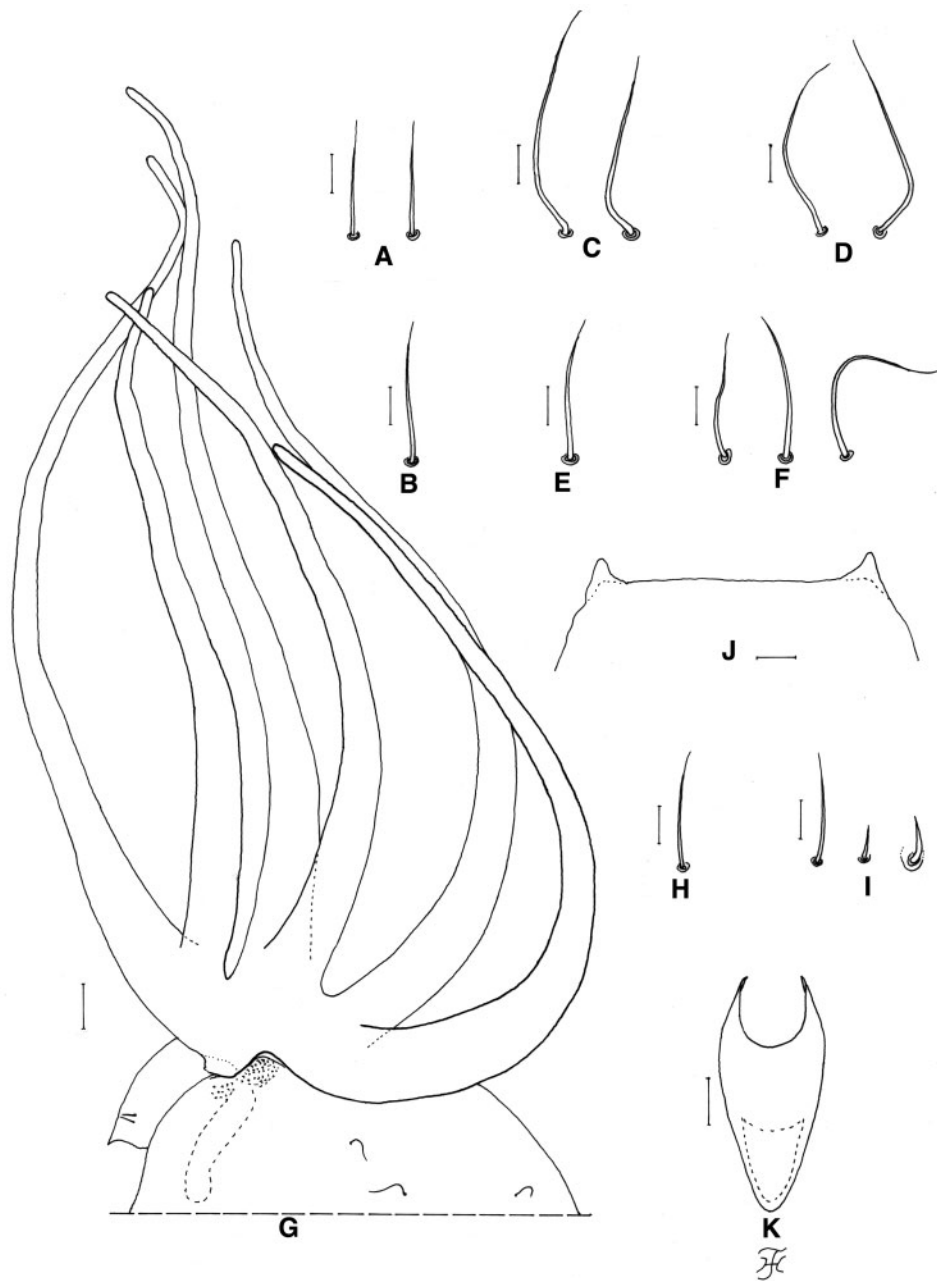
Integument yellow to ochreous, bare except small area on each side of ventral surface covered with small round tubercles; frons with pair of unbranched slender short trichomes (Fig. 3A) on each side; face with unbranched slender short trichome (Fig. 3B) on each side.

### Thorax

Integument yellow to ochreous, bare on anterior half (except small area at base of gill moderately covered with small round tubercles), and sparsely covered with small round tubercles on posterior half; thorax with two medium-long mediodorsal trichomes (Fig. 3C), two medium-long anterolateral trichomes (Fig. 3D), one short mediolateral trichome (Fig. 3E), and three short ventrolateral trichomes (Fig. 3F), on each side; all trichomes unbranched and with straight apices. Gill (Fig. 3G) with six somewhat inflated short filaments in three pairs; all pairs short-stalked, arising from base; gill filaments divergent basally, upper filament of dorsal pair lying at angle of 130 degrees against lower filament of ventral pair when viewed laterally; outer filament of each pair somewhat shorter than inner filament; outer filaments 2.1–2.4 mm long; basal portions of all filaments somewhat inflated, 1.7–2.7 times as thick as interspiracular trunk; relative thickness of six filaments from dorsal to ventral when basal portions were compared 1.0:0.8:0.8:0.9:0.7–0.8:0.9–1.0; all filaments light gray, basally somewhat inflated, tapered toward apex, with annular ridges and furrows only on apical portions, and densely covered with minute tubercles.

### Abdomen

Dorsally, segment 1 and basal two-thirds of segment 2 grayish and without minute tubercles, and other segments unpigmented except segment 9 and basal portions of spine-combs on segments 7 and 8 yellow; segment 1 with one unbranched slender short seta (Fig. 3H) on each side; segment 2 with one unbranched slender short seta and five unbranched minute setae, of which three are stout (Fig. 3I), on



**Fig. 3.** Pupa of *Simulium (Simulium) srisukai* sp. nov. (A) Frontal trichomes. (B) Facial trichome. (C)–(F) Thoracic trichomes (C, mediodorsal; D, anterolateral; E, mediolateral; F ventrolateral). (G) Anterior part of thorax and gill filaments (left side; outer view). (H) Hair-like seta on dorsal surface of abdominal segment 1. (I) Hair-like seta, minute seta, and stout seta on dorsal surface of abdominal segment 2. (J) Terminal hooks (caudal view). (K) Cocoon (dorsal view). Scale bars. 1.0 mm for K; 0.1 mm for G; 0.02 mm for A–F and H–J.

each side; segments 3 and 4 each with four distinct hooked spines and one unbranched minute seta on each side; segments 7–9 each with distinct spine-combs in transverse row (though those on segment 9 much smaller than those on segments 7 and 8) and comb-like groups of minute spines on each side; segment 5 bare; segment 6 with comb-like groups of minute spines on each side; segment 9 with cone-shaped terminal hooks (Fig. 3J). Ventrally, segments 3–9 unpigmented, each (except segment 9) with comb-like groups of minute spines; segment 4 with two unbranched slender minute setae and two unbranched hooklets on each side; segment 5 with pair of bifid stout hooks submedially and few unbranched short setae on each side; segments 6 and 7 each with pair of bifid inner and unbranched outer stout hooks somewhat separated from each other,

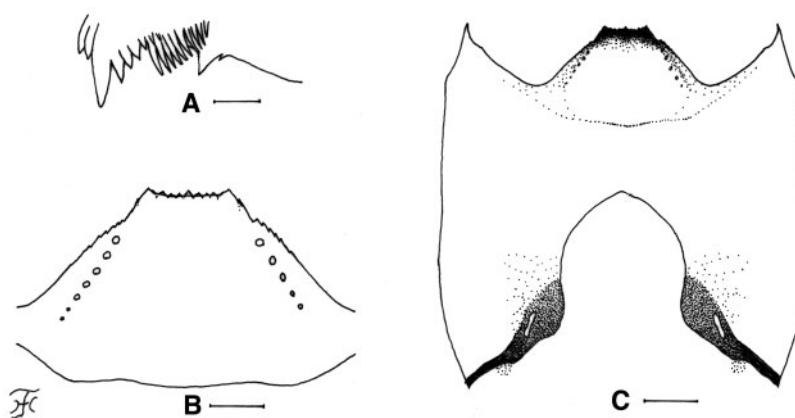
and few unbranched minute setae on each side. Grapnel-shaped hooklets absent on each side of segment 9.

#### Cocoon (Fig. 3K)

Wall-pocket-shaped, tightly and thickly woven, ochreous, not so extended ventrolaterally; individual threads not discernible; 4.5–5.0 mm long by 2.0–2.4 mm wide.

#### Mature Larva

Body length 8.0–9.0 mm. Body creamy white, though thoracic segment 1 encircled with pinkish transverse band disconnected ventromedially, and other thoracic segments and abdominal segments faintly pinkish dorsally to varying extent.



**Fig. 4.** Larva of *Simulium (Simulium) srisukai* sp. nov. (A) Mandible. (B) Hypostoma. (C) Head capsule showing postgenal cleft (ventral view). Scale bars. 0.1 mm for C; 0.05 mm for B; 0.02 mm for A.

### Head

Cephalic apotome yellowish white to yellow with somewhat darkened narrow area along posterior margin; head spots faintly positive though anterior spot of mediolongitudinal spots, anterior half of lateral spots and posterior spots of posterolateral spots indistinct; lateral surface of head capsule yellow except eye-spot region white, eyebrow darkened and area posterior to eye-spot region darkened; spots in front of posterior margin obscured or faintly negative; one small spot below eye-spot region indistinct; ventral surface of head capsule yellow, with spots on each side of postgenal cleft obscured or faintly negative. Head capsule almost bare except anterior portion of dorsal surface sparsely covered with minute setae. Antenna composed of three articles and apical sensillum, slightly longer than stem of labral fan; length ratio of three articles (from base to tip) 1.0:1.1:0.6. Labral fan with 44–49 primary rays. Mandible (Fig. 4A) with mandibular serration composed of two teeth (one medium-sized, one small); main tooth at obtuse angle against mandible on apical side; supernumerary serrations absent; comb-teeth decreasing in length from first to third. Hypostoma (Fig. 4B) with nine anterior teeth, of which corner teeth slightly longer than median tooth; three intermediate teeth on each side shortest; lateral margins moderately serrate apically; five to seven hypostomal bristles per side slightly divergent posteriorly from lateral margin. Postgenal cleft (Fig. 4C) bullet-shaped, 2.2–2.4 times as long as postgenal bridge; sheath of subesophageal ganglion unpigmented. Cervical sclerites on each side composed of anterior dark slender rod-like piece and posterior dark elliptical piece close together, and not fused to occiput.

### Thorax and Abdomen

Histoblast of pharate pupal gill with six somewhat inflated filaments. Thoracic and abdominal cuticle bare except last segment of abdomen moderately covered with short colorless setae on each side of anal sclerite. Rectal scales present. Rectal organ compound, each lobe with 13–15 finger-like secondary lobules. Anal sclerite X-shaped, with short broad anterior arms, each with forked apically, 0.7 times as long as posterior ones; no sensillum on base of anal sclerite; 10–12 sensilla posterior to posterior arms. Last abdominal segment lacking ventral papillae. Posterior circlet with 87–93 rows of hooklets with up to 16 hooklets per row.

### Type Material

**HOLOTYPE:** Female (with its associated pupal exuviae and cocoon in 80% ethanol), reared from a pupa collected from a stream (width

45 cm, depth 10 cm, bottom sandy, temperature 17.8°C, partially shaded, elevation 1,241 m, 16° 14'39.2" N, 98° 59'56.5" E) moderately flowing in a natural forest, Mae Klong Yai Village, Tak Province, Thailand, 24-XI-2016, by W. Srisuka, S. Suriya, R. Saokod, S. Pilakantha, C. Rangsang, and T. Somboonchai. **PARATYPES:** Four females, five males (all reared from pupae), and five mature larvae, same data as those of the holotype.

### Biological Notes

The pupae and larvae of *S. (S.) srisukai* sp. nov. were collected from grasses trailing in the current. Associated species were *S. (Gomphostilbia) Chiangdaoense* Takaoka & Srisuka, *S. (G.) inthanonense* Takaoka & Suzuki, *S. (S.) bullatum* Takaoka & Choochote, *S. (S.) Chamlongi*, *S. (S.) Choochotei* Takaoka, *S. (S.) doipuiense* Takaoka & Choochote (complex), and *S. (S.) yuphae* Takaoka & Choochote.

### Distribution

Thailand (Tak Province).

### Etymology

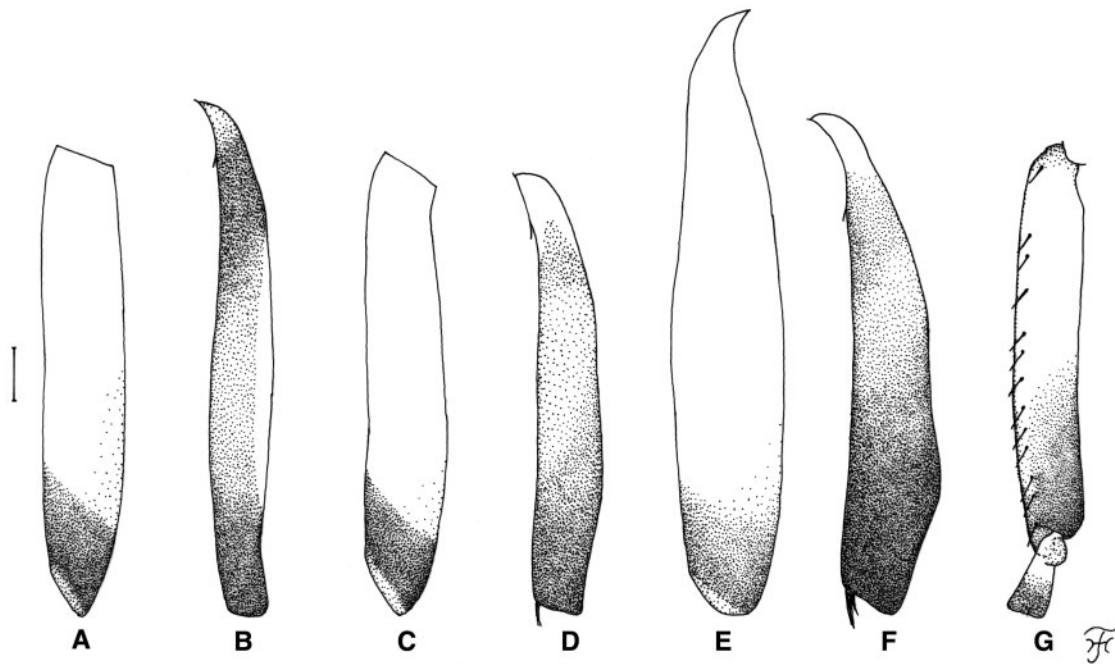
The species name *srisukai* is in honor of Dr. Wichai Srisuka, Queen Sirikit Botanic Garden, who collected this new species and greatly contributed to studies of black flies in Thailand.

### Remarks

*Simulium (S.) srisukai* sp. nov. is assigned to the *S. variegatum* species-group of the subgenus *Simulium*, redefined by Takaoka (2003), based on the pleural membrane bare, female claw with a small subbasal tooth (Fig. 1E), ovipositor valve with inner margins widely concave (Fig. 1F), male style without basal protuberance (Fig. 2E), ventral plate with teeth on the posterior surface (Fig. 2G), and pupal gill with six filaments (Fig. 3G).

This new species is characterized by darkened female legs, a greater number of the male upper-eye facets in 24–26 vertical columns and 24–26 horizontal rows, pupal integument with head and anterior half of the thorax bare, and somewhat inflated pupal gill filaments (Fig. 3G).

Among species of the *S. variegatum* species-group, 10 species have darkened female legs: *S. (S.) barnesi* from Thailand (Takaoka and Suzuki 1984), *S. (S.) hackeri* Edwards from Peninsular Malaysia (Takaoka and Davies 1995), *S. (S.) karenkoense* (Shiraki) from



**Fig. 5.** Female of *Simulium (Simulium) kiewmaepanense* sp. nov. (A) Fore femur. (B) Fore tibia. (C) Mid femur. (D) Mid tibia. (E) Hind femur. (F) Hind tibia. (G) Hind basitarsus and second tarsomere. (All left side and outer view.) Scale bar. 0.1 mm for A–G.

Taiwan (Shiraki 1935), *S. (S.) nilgircum* Puri from India (Puri 1932), *S. (S.) cruszi* Davies & Györkös, *S. (S.) nubis* Davies & Györkös and *S. (S.) paranubis* Davies & Györkös from Sri Lanka (Davies and Györkös 1987, 1992), *S. (S.) molliculum* Takaoka, *S. (S.) laxum* Takaoka, and *S. (S.) palapoense* Takaoka, all from Sulawesi, Indonesia (Takaoka 2003).

This new species is most similar to *S. (S.) hackeri*, of which the female and male are known, in having the female scutum covered with golden-yellow short hairs, greater number of male upper-eye facets, and similar shapes of female and male genitalia. In fact, it difficult to distinguish it in the female and male from *S. (S.) hackeri*, although there seem to be slight differences in numerical features: the length ratio of the female fore basitarsus against its greatest width, which is 5.4 in this new species but 6.6 in *S. (S.) hackeri*, and the length ratio of the male fore basitarsus against its greatest width, which is 5.8–6.4 in this new species but 7.3 in *S. (S.) hackeri*, and the width ratio of the male hind basitarsus against that of the hind tibia, which is 0.7 in this new species but 0.6 in *S. (S.) hackeri*. The pupa of this new species is also similar to that of *S. (S.) hackeri*, which was collected from Peninsular Malaysia (Takaoka, unpublished data), in having the integument with head and anterior half of the thorax bare and a similar arrangement of the gill filaments without a common basal stalk, but it is clearly distinguished by the somewhat inflated gill filaments (Fig. 3G) (gill filaments not inflated in *S. (S.) hackeri*).

This new species differs from *S. (S.) barnesi* and *S. (S.) karenkoseense*, both of which were described only from females, by the paraproct, which is depressed on the anteroventral surface (Fig. 1H) in this new species but is not depressed in *S. (S.) barnesi*, and the scutum, which is covered with golden-yellow short hairs in this new species but is covered with dark short hairs in *S. (S.) karenkoseense*.

This new species is distinguished from *S. (S.) palapoense* by six pupal gill filaments (the gill consists of three globes and two finger-like short filaments in *S. (S.) palapoense*).

The somewhat inflated pupal gill (Fig. 3G) separates this new species from all six other species, which have six ordinary thread-like gill filaments per side.

### ***Simulium (Simulium) kiewmaepanense* Takaoka, Srisuka & Saeung sp. nov.**

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#### **Female**

Similar to that of *S. (S.) srisukai* sp. nov. except in following characters. Body length 3.3 mm.

#### **Head**

Frontal ratio 1.2:1.0:1.1; frons:head ratio 1.0:3.8. Labrum 0.6 times as long as clypeus. Antenna medium to dark brown except scape, pedicel and base of first flagellomere yellow when viewed dorsally (first flagellomere dark yellow to light brown except base yellow when viewed ventrally). Maxillary palp: proportional lengths of third, fourth, and fifth segments 1.0:1.2:2.4; sensory vesicle of moderate size (0.3 times length of third segment) having large opening. Maxillary lacinia with 15–17 inner and 16 or 17 outer teeth. Mandible with 28–30 inner and 12 outer teeth. Cibarium with blunt median process on posterior margin and with 59 minute tubercles near base of median process.

#### **Legs**

Foreleg: coxa whitish yellow; trochanter yellow; femur (Fig. 5A) yellow (though dark yellow on apical half of outer surface) except apical cap dark brown; tibia (Fig. 5B) dark brown (though medial one-third light brown), with median portion widely white along outer margin, with shiny sheen widely on outer surface when illuminated at certain angles; tarsus brownish black except tarsomeres 4 and 5

medium brown, with moderate dorsal hair crest; basitarsus greatly dilated, 5.4 times as long as its greatest width. Midleg: coxa dark brown; trochanter yellow; femur (Fig. 5C) yellow (though dark yellow on outer surface near apical cap) except apical cap dark brown; tibia (Fig. 5D) light brown except base whitish yellow, subbasal portion medium brown, and posterior surface of apical half and apical cap dark brown; tibia with shiny sheen widely on posterior surface when illuminated at certain angles; tarsus medium to dark brown except basal three-fourths of basitarsus yellow to dark yellow. Hind leg: coxa dark brown; trochanter whitish yellow; femur (Fig. 5E) yellow (though dark yellow near apical cap) except apical cap dark brown; tibia (Fig. 5F) medium to dark brown (though median portion light brown) except base whitish yellow and apical cap brownish black, with shiny sheen on posterior surface when illuminated at certain angles; tarsus medium brown except little more than basal half of basitarsus and basal half of second tarsomere whitish yellow (though small rounded area light brown); basitarsus (Fig. 5G) nearly parallel-sided, 6.0 times as long as wide, and 0.7 and 0.6 times as wide as greatest widths of hind tibia and femur, respectively; calypala (Fig. 5G) moderately developed, 0.7 times as long as basal width, and 0.4 times as wide as basitarsus.

#### Wing

Length 3.0 mm.

#### Abdomen

Basal scale light brown, with fringe of light brown long hairs. Dorsal surface of abdomen medium brown except segment 2 light brown; ventral surface of segments 2–4 whitish and those of segments 5–7 light brown except sternal plates of segment 7 medium brown; segment 7 with pair of sternal plates having numerous hairs.

#### Terminalia

Sternite 8 bare medially, with 18–26 dark medium-long to long stout hairs and two to five yellow short to medium-long hairs on each lateral surface. Ovipositor valves each densely covered with microsetae and 28 or 29 dark brown short to medium-long hairs and six or seven yellow short hairs. Paraproct in lateral view somewhat protruded ventrally beyond ventral margin of cercus, 0.9 times as long as wide, with 34–38 short to medium-long hairs on lateral surface and four to six short hairs on ventral depressed surface; anteromedial surface slightly darkened, with seven to nine short sensilla. Cercus in lateral view short, rounded posteriorly, 0.5 times as long as wide, and with numerous short to medium-long hairs. Spermatheca large, nearly ovoid, 1.2 times as long as wide.

#### Pupa

Body length 3.8 mm.

#### Head

Integument yellow, moderately and uniformly covered with small tubercles except antennal sheaths bare; frons with pair of unbranched slender short trichomes (Fig. 6A) arising close together on each side; face with unbranched slender short trichome (Fig. 6B) on each side; facial trichome much longer than frontal ones.

#### Thorax

Integument yellow, moderately covered with small round tubercles; thorax with two mediodorsal trichomes (anterior trichome short,

posterior one medium-long; Fig. 6C), two medium-long anterolateral trichomes (Fig. 6D), one short mediolateral trichome (Fig. 6E), and three ventrolateral trichomes (two short, one medium-long; Fig. 6F), on each side; all trichomes unbranched and with straight apices. Gill (Fig. 6G) with six thread-like filaments in three pairs; all pairs short-stalked, arising from short common stalk; gill filaments divergent basally, stalk of dorsal pair lying at angle of 100 degrees against stalk of ventral pair when viewed laterally; all filaments subequal in length (2.3–2.4 mm); relative thickness of six filaments from dorsal to ventral when basal portions were compared 1.0:0.8:0.8:0.8:0.7:0.7; all filaments light gray, tapered toward apex, with annular ridges and furrows, and densely covered with minute tubercles.

#### Abdomen

Nearly as in *S. (S.) srisukai* sp. nov. except in following characters: dorsal surface of segment 1, basal two-thirds of segment 2 and basal half of segments 3 and 4 grayish and without minute tubercles, and those of other segments unpigmented except segment 9 and basal portions of spine-combs on segments 7 and 8 yellow.

#### Cocoon

Wall-pocket-shaped, tightly and thickly woven, ochreous, not so extended ventrolaterally; individual threads visible; 5.0 mm long by 2.0 mm wide.

#### Male and Larva

Unknown.

#### Type Material

HOLOTYPE: Female (with its associated pupal exuviae and cocoon in 80% ethanol), reared from a pupa collected from a stream (width 150 cm, depth 13 cm, bottom sandy, temperature 14.5°C, pH 5.4, partially shaded, elevation 2,210 m, 18° 33'29.4" N, 98° 28'51.7" E) fast flowing in a natural forest, Kiew Mae Pan, Chomthong District, Doi Inthanon National Park, Chiang Mai Province, Thailand, 29-IX-2014, by W. Srisuka, S. Suriya, R. Saokod, S. Pilakantha, C. Rangsan, and T. Somboonchai. PARATYPE: One female (with its associated pupal exuviae and cocoon in 80% ethanol), same data as those of the holotype except date (4-XI-2014).

#### Biological Notes

The pupae of *S. (S.) kiewmaepanense* sp. nov. were collected from grasses trailing in the current. Associated species were *S. (Nevermannia) chomthongense* Takaoka, Srisuka & Choochote, *S. (S.) crocinum* Takaoka & Choochote, and *S. (S.) suchariti* Takaoka & Choochote.

#### Distribution

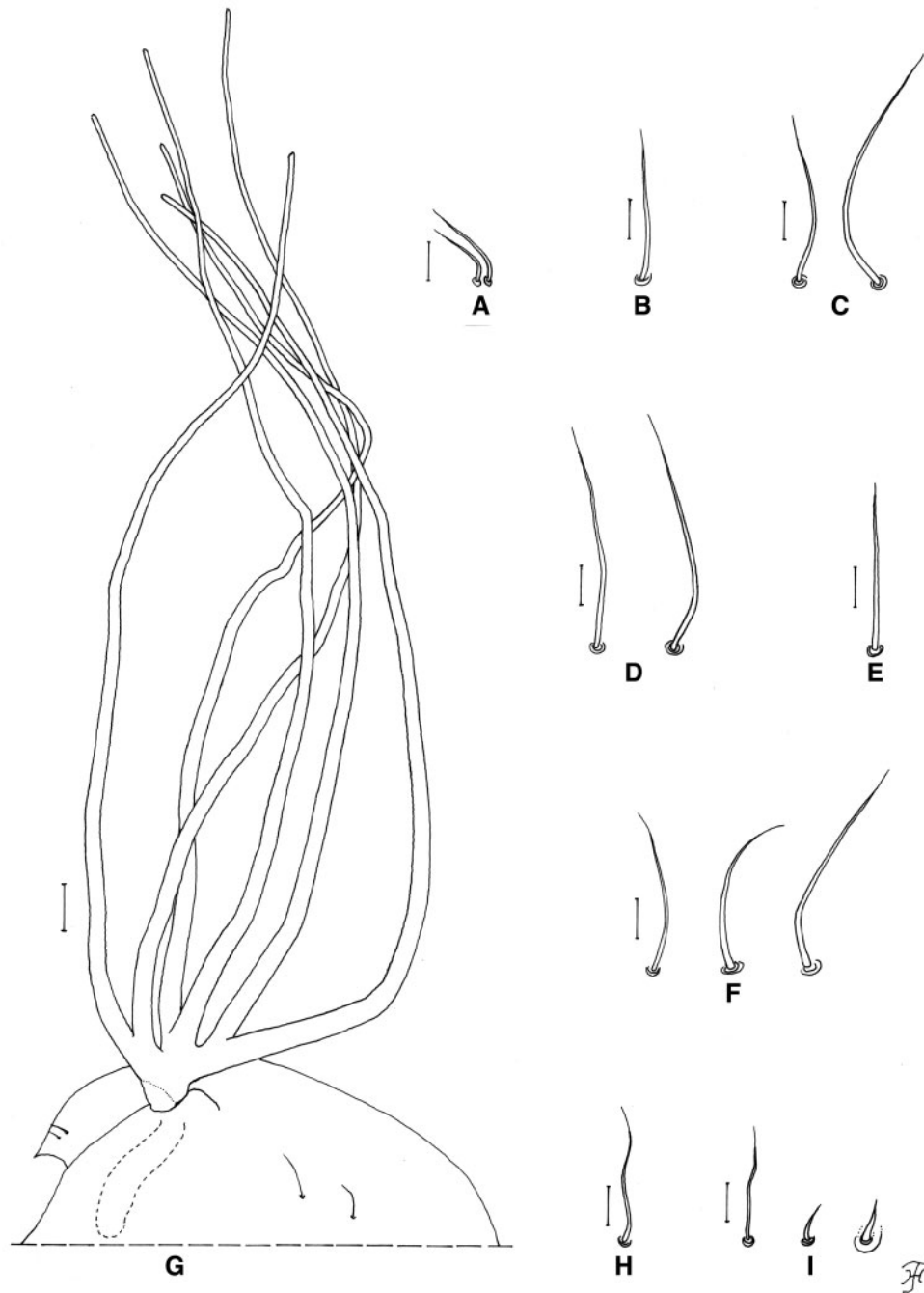
Thailand (Chiang Mai Province).

#### Etymology

The species name *kiewmaepanense* refers to the locality name, Kiew Mae Pan, where this new species was collected.

#### Remarks

*Simulium (S.) kiewmaepanense* sp. nov. is assigned to the *S. variegatum* species-group of the subgenus *Simulium*, redefined by Takaoka (2003), based on the pleural membrane bare, female claw with a small subbasal tooth, ovipositor valve with inner margins widely concave, and pupal gill with six filaments (Fig. 6G).



**Fig. 6.** Pupa of *Simulium* (*Simulium*) *kiewmaepanense* sp. nov. (A) Frontal trichomes. (B) Facial trichome. (C)–(F) Thoracic trichomes (C, mediodorsal; D, anterolateral; E, mediolateral; F ventrolateral). (G) Anterior part of thorax and gill filaments (left side; outer view). (H) Hair-like seta on dorsal surface of abdominal segment 1. (I) Hair-like seta, minute seta, and stout seta on dorsal surface of abdominal segment 2. Scale bars. 0.1 mm for G; 0.02 mm for A–F, H and I.

The female of this new species is similar to that of *S. (S.) srisukai* sp. nov. in many characters including the darkened tibiae (Fig. 5B, D, F) and terminalia but is distinguished from the latter species by the yellowish femora (Fig. 5A, C, E). The pupa of this new species is distinguished from that of *S. (S.) srisukai* sp. nov. by the integument of the head and anterior half of the thorax densely or moderately covered with round tubercles, thread-like pupal gill filaments (Fig. 6G) and pupal abdominal segment 9 lacking spine-combs.

As noted in the remarks of *S. (S.) srisukai* sp. nov., all 10 species of the *S. variegatum* species-group, which have darkened female tibiae, also have darkened female femora, a character differing from

that of *S. (S.) kiewmaepanense* sp. nov., which has yellowish female femora (Fig. 5A, C, E).

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## References Cited

- Adler, P. H., and R. W. Crosskey. 2016. World Blackflies (Diptera: Simuliidae): A Comprehensive Revision of the Taxonomic and Geographical Inventory [2016], p. 126. (<http://entweb.clemson.edu/biomia/pdfs/blackflyinventory.pdf>) (accessed 10 January 2017)
- Adler, P. H., D. C. Currie, and D. M. Wood. 2004. The Black Flies (Simuliidae) of North America, pp. xv + 941. Cornell University Press, Ithaca, New York.
- Choochote, W., H. Takaoka, M. Fukuda, Y. Otsuka, C. Aoki, and N. Eshima. 2005. Seasonal abundance and daily flying activity of black flies (Diptera: Simuliidae) attracted to human baits in Doi Inthanon National Park, northern Thailand. *Med. Entomol. Zool.* 56: 335–348.
- Davies, D. M., and H. Györkös. 1987. The Simuliidae (Diptera) of Sri Lanka. Descriptions of three new species of *Simulium* (*Simulium*). *Can. J. Zool.* 65: 2734–2746.
- Davies, D. M., and H. Györkös. 1992. The Simuliidae (Diptera) of Sri Lanka. Description of additional species of *Simulium* (*Simulium*), with a key for Sri Lankan species in the subgenus and a checklist for the country. *Can. J. Zool.* 70: 1029–1046.
- Fukuda, M., H. Takaoka, S. Uni, and O. Bain. 2008. Infective larvae of five *Onchocerca* species from experimentally infected *Simulium* species in an area of zoonotic onchocerciasis in Japan. *Parasite* 15: 111–119.
- Puri, I. M. 1932. Studies on Indian Simuliidae. Part I. *Simulium himalayense* sp. n.; *Simulium gurneyae* Senior-White; and *Simulium nilgircum* sp. n. *Ind. J. Med. Res.* 19: 883–898.
- Shiraki, T. 1935. Simuliidae of the Japanese Empire. *Mem. Fac. Sci. Agric. Taihoku Imp. Univ.* 16: 1–90.
- Takaoka, H. 2003. The Black Flies (Diptera: Simuliidae) of Sulawesi, Maluku and Irian Jaya, pp. xxii + 581. Kyushu University Press, Fukuoka, Japan.
- Takaoka, H., C. Aoki, and H. Hayakawa. 1992. Natural infections of blackflies with larvae of zoonotic *Onchocerca* spp. in northeast Japan. *Jpn. J. Trop. Med. Hyg.* 20: 1–9.
- Takaoka, H., and D. M. Davies. 1995. The Black Flies (Diptera: Simuliidae) of West Malaysia, pp. viii + 175. Kyushu University Press, Fukuoka.
- Takaoka, H., W. Srisuka, and A. Saeung. 2017. *Simulium maleewongae*, a new species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from Thailand. *J. Med. Entomol.* 54: 91–98. <https://doi.org/10.1093/jme/tjw208>.
- Takaoka, H., and H. Suzuki. 1984. The blackflies (Diptera: Simuliidae) from Thailand. *Jpn. J. Sanit. Zool.* 35: 7–45.

## Description of the male of *Simulium (Simulium) suchariti* (Diptera: Simuliidae) from Thailand

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**Abstract:** The male of *Simulium (Simulium) suchariti* Takaoka & Choochote is described for the first time based on a unique male reared from a pupa collected from a stream at Ang Ka, Doi Inthanon National Park, Thailand. It is characterized by abdominal segment 10 with about 15 distinct hairs on each side of the posteroventral surface. A key to identify males of all 16 species of the *Simulium griseifrons* species-group recorded from Thailand is provided.

Key words: black fly, *Simulium*, Simuliidae, Thailand, *griseifrons* species-group

### INTRODUCTION

*Simulium (Simulium) suchariti* Takaoka & Choochote was first described on the basis of two females attracted to a human at Ang Ka (elevation 2,465 m), Doi Inthanon National Park, Chiang Mai, Thailand and was placed in the *Simulium griseifrons* species-group (Takaoka and Choochote, 2004a). Its pupa and larva were soon described based on collections from a stream at the type locality of *S. (S.) suchariti* (Takaoka and Choochote, 2004b).

Recently, we collected two pupae of *S. (S.) suchariti* from a stream (elevation 2,210 m) near Ang Ka, Doi Inthanon National Park, and obtained one female and one male from these pupae. The male of *S. (S.) suchariti* is here described for the first time.

A key to identify males of all 16 species of the *S. griseifrons* species-group recorded from Thailand (Adler and Crosskey, 2016) is provided.

The methods of description and illustration, and terms for morphological features used here, follow those of Takaoka (2003).

### *Simulium (Simulium) suchariti* Takaoka & Choochote, 2004

**Male.** Body length 3.5 mm. **Head.** As wide as thorax. Upper eye medium brown, with large facets in 19 or 20 vertical columns and in 20 horizontal rows on each side. Clypeus black, thickly white pruinose, moderately covered with dark brown hairs. Antenna composed of scape, pedicel and nine flagellomeres, medium to dark brown except scape light brown, and base of pedicel and first flagellomere whitish yellow; first flagellomere elongate, 2.3 times as long as

second one. Maxillary palp light to medium brown, composed of five segments with proportional lengths of third, fourth, and fifth segments 1.0:1.3:2.8; third segment (Fig. 1A) of moderate size; sensory vesicle (Fig. 1A) small (0.3 times length of third segment), ellipsoidal, and with small opening. **Thorax.** Scutum black, with white pruinose pattern, i.e., anterior pair of rectangular spots on shoulders extended posteriorly along lateral margins and connected to large transverse spot entirely covering prescutellar area; these pruinose areas shiny when illuminated at certain angles; scutum uniformly and densely covered with golden-yellow recumbent short hairs interspersed with dark brown long upright hairs on prescutellar area. Scutellum dark brown, with several dark long upright hairs and golden-yellow short hairs. Postnotum dark brown, white pruinose when illuminated at certain angles and bare. Pleural membrane bare. Katepisternum longer than deep, brownish black, slightly shiny and white pruinose when illuminated at certain angles, and bare. **Legs.** Foreleg: coxa whitish yellow; trochanter medium brown except inner surface yellow; femur light brown (though inner surface yellowish) with apical cap brownish black; tibia dark brown except medial two-fifths light brown, though outer surface of middle portion widely white; tarsus brownish black, with moderate dorsal hair crest; basitarsus moderately dilated, 7.7 times as long as its greatest width. Midleg: coxa dark brown; trochanter medium brown except basal half of anterior surface dark yellow; femur dark yellow to light brown except apical cap medium brown; tibia medium to dark brown except basal two-fifths yellow; tarsus dark brown. Hind leg: coxa medium brown; trochanter yellow though anterior

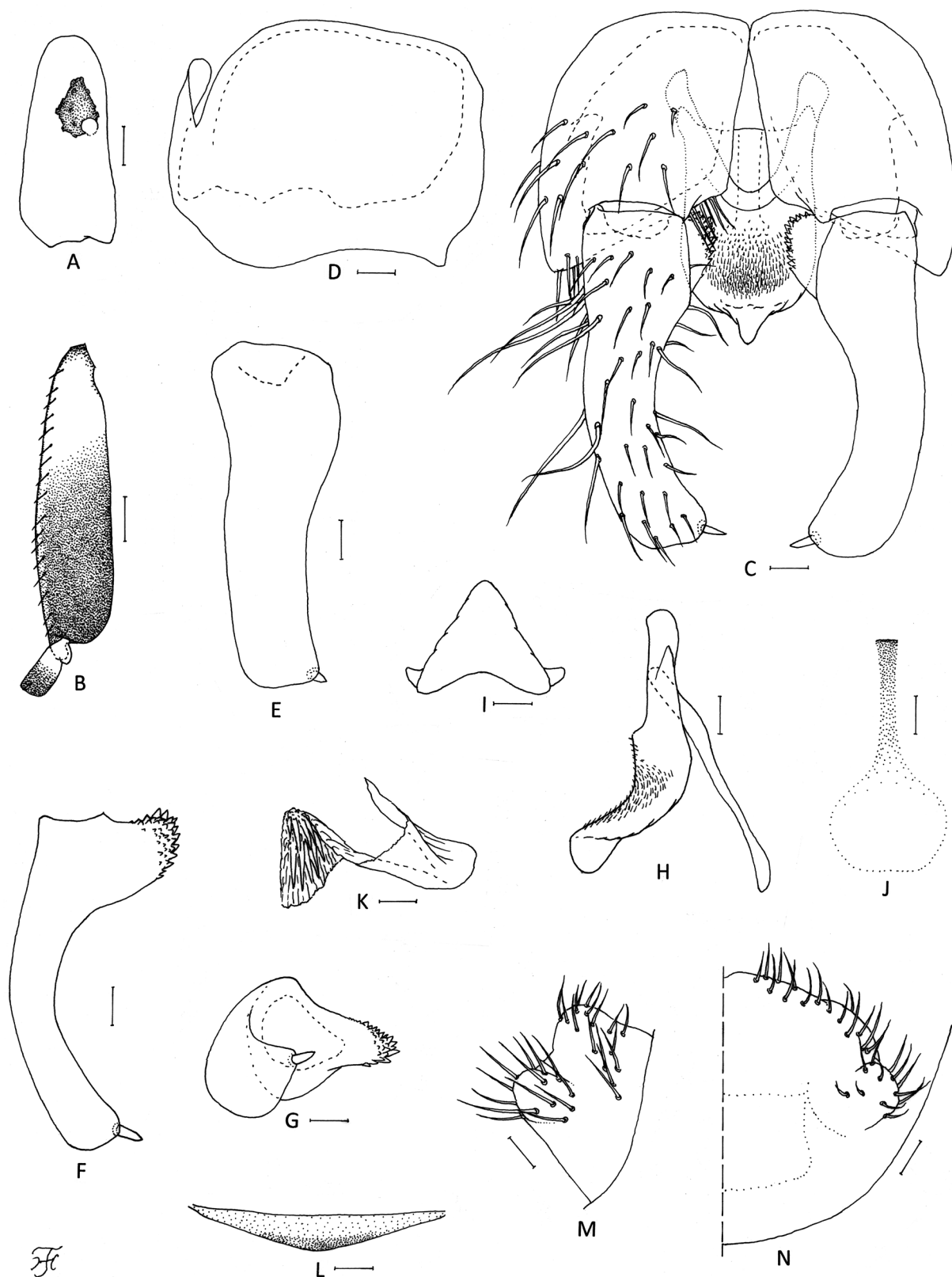


Fig. 1. Male of *Simulium* (*Simulium*) *suchariti*. A, Third segment of maxillary palp with sensory vesicle (right side; front view); B, Hind basitarsus and second tarsomere (left side; outer view); C, Coxites, styles and ventral plate (ventral view); D, Coxite (right side; ventrolateral view); E-G, Styles (right side; E, ventrolateral view; F, medial view; G, caudal view); H, Ventral plate and median sclerite (lateral view); I, Ventral plate (caudal view); J, Median sclerite (ventroposterior view); K, Paramere (left side; caudal view); L, Dorsal plate (caudal view); M & N, Abdominal segment 10 and cerci (left side; M, lateral view; N, caudal view). Scale bars. 0.1 mm for B; 0.02 mm for A and C-N.

surface slightly darkened; femur dark yellow to light brown except basal one-fourth or little less whitish yellow and apical cap brownish black; tibia dark brown to brownish black except basal tip whitish yellow; tarsus (Fig. 1B) dark brown except basal one-third of basitarsus yellow (though base somewhat darkened) and little less than basal half of second tarsomere dark yellow; basitarsus (Fig. 1B) enlarged, slightly widened from base to apical one-third, then slightly tapered to apex, 4.0 times as long as its greatest width, and 1.0 and 1.2 times as wide as greatest widths of hind tibia and femur, respectively; calcipala (Fig. 1B) small, slightly longer than width at base, 0.3 times as wide as greatest width of basitarsus; pedisulcus (Fig. 1B) well developed. **Wing.** Length 2.7 mm. Costa with dark spinules and hairs; subcosta bare; basal section of radius bare;  $R_1$  with dark spinules and hairs;  $R_2$  with hairs; hair tuft on base of radial vein dark brown; basal cell absent. **Halter.** White with basal portion darkened. **Abdomen.** Basal scale brownish black, with fringe of dark long hairs. Dorsal surface of abdomen dark brown to brownish black, and covered with dark short hairs; segments 2, 6 and 7 each with pair of white iridescent spots dorsolaterally, those on segment 2 broadly connected in middle. **Genitalia.** Coxites, styles and ventral plate in ventral view as Fig. 1C. Coxite in ventral view (Fig. 1C) nearly diamond-shaped, 1.4 times as long as width; coxite in ventrolateral view (Fig. 1D) subquadrate, 0.8 times as long as width. Style in ventral view (Fig. 1C) gently curved inward, nearly parallel-sided on basal one-fifth, tapered to middle, then nearly parallel-sided up to apex, with apical spine; style in ventrolateral view (Fig. 1E) elongate, 3.0 times as long as its greatest width near base, with blunt apex having apical spine; style in medial view (Fig. 1F) and in caudal view (Fig. 1G) with short basal protuberance having many small cone-shaped spines. Ventral plate in ventral view (Fig. 1C) with body nearly quadrate, having short round projection posteromedially, covered with minute setae on ventral and anteroventral surfaces; arms somewhat divergent anteriorly; ventral plate in lateral view (Fig. 1H) with body bent posteroventrally; ventral plate in caudal view (Fig. 1I) triangular though dorsal margin concave and bare on posterior surface. Median sclerite in ventral view (Fig. 1C) arising anterior to anterior margin of body of ventral plate and directed posterodorsally; median sclerite in posteroventral view (Fig. 1J), narrow on anterior half, then widened apically. Paramere (Fig. 1K) broad basally, with several medium-long and short hooks. Aedeagal membrane densely covered with minute setae, and with well sclerotized dorsal plate in form of horizontal bar (Fig. 1L). Abdominal segment 10 (Fig. 1M, N) with about 15 distinct hairs on posteroventral surface on each side. Cercus (Fig. 1M, N) small, rounded, with 12 distinct hairs.

**Material examined.** One male (QSBG-2014)

reared from a pupa, collected from a stream (width 150 cm, depth 13 cm, bottom sandy, temperature 14.5°C, pH 5.4, partially shaded, elevation 2,210 m, 18°33'29.4"N, 98°28'51.7"E) fast flowing in a natural forest, Kiew Mae Pan, Chomthong District, Doi Inthanon National Park, Chiang Mai Province, Thailand, 2-V-2014, by W. Srisuka, S. Suriya, R. Saokod and S. Pilakantha.

**Remarks.** The male of *S. (S.) suchariti* has the ventral plate without dentate posterolateral margins (Fig. 1I), one of the key characters of males in the *S. griseifrons* species-group (Takaoka and Davies, 1996). It is characterized by abdominal segment 10 with about 15 distinct hairs on each side of the posteroventral surface (Fig. 1M, N). The presence of distinct hairs on abdominal segment 10 is also reported in five of 15 other species of the *S. griseifrons* species-group recorded from Thailand (Adler and Crosskey, 2016): 13–16 hairs in *S. (S.) choochotei* Takaoka & Choochote and one to four hairs in *S. (S.) crocinum* Takaoka & Choochote, *S. (S.) digrammicum* Edwards, *S. (S.) maenoi* Takaoka & Choochote and *S. (S.) visuti* Takaoka & Choochote (Takaoka & Choochote, 2002, 2004b, 2006). The male of *S. (S.) suchariti* differs from the males of *S. (S.) choochotei*, *S. (S.) digrammicum* and *S. (S.) visuti* by the bare basal portion of the radial vein, and from *S. (S.) crocinum* and *S. (S.) maenoi* by abdominal segment 5 lacking a pair of shiny dorsolateral spots.

Apart from the presence of distinct hairs on abdominal segment 10, the male of *S. (S.) suchariti* is similar to that of *S. (S.) phukaense* Takaoka & Choochote (Takaoka and Choochote, 2005) by having the basal portion of the radial vein bare, abdominal segments 2, 6 and 7 each with a pair of pruinose spots, and the style with a short round basal protuberance having many spines (Fig. 1F, G). However, the two species differ from each other by the color of the hind basitarsus, which is yellow on the basal one-third and darkened on the rest (Fig. 1B) in *S. (S.) suchariti* but is entirely dark brown to brownish black in *S. (S.) phukaense*.

The males of all 16 Thai species of the *S. griseifrons* species-group can be identified by the following key.

#### Key to adult males of 16 species of the *Simulium griseifrons* species-group in Thailand

1. Basal portion of radial vein fully haired. . . . . 2  
Basal portion of radial vein bare or with few hairs. . . . . 7
2. Abdomen with pair of shiny whitish pruinose spots dorsally or dorsolaterally on segments 2, 5, 6 and 7. . . . . 3  
Abdomen with pair of shiny white pruinose spots dorsally or dorsolaterally on segments 2, 6 and 7. . . . . 4
3. Upper-eye with large facets in 17 vertical

- columns; hind basitarsus nearly parallel-sided.  
..... *S. choochotei*  
Upper-eye with large facets in 20 vertical columns;  
hind basitarsus spindle-shaped. .... *S. visuti*
4. Fore basitarsus with thick dorsal hair crest; hind  
basitarsus nearly parallel-sided. .... 5  
Fore basitarsus with moderate dorsal hair crest;  
hind basitarsus spindle-shaped. .... 6
5. Upper-eye with large facets in 20 vertical columns.  
..... *S. nigrogilvum*  
Upper-eye with large facets in 17 vertical columns.  
..... *S. vanellum*
6. Upper-eye with large facets in 19 vertical columns;  
ventral plate rectangular, nearly parallel-sided when  
viewed ventrally. .... *S. pukaengense*  
Upper-eye with large facets in 21 or 22 vertical  
columns; ventral plate gradually narrowed posteriorly  
when viewed ventrally. .... *S. digrammicum*
7. Scutum covered with dark brown recumbent hairs.  
..... *S. yongi*  
Scutum covered with golden-yellow recumbent  
hairs. .... 8
8. Mid femur almost entirely yellow. .... 9  
Mid femur almost entirely darkened. .... 10
9. Hind tibia brownish black except base narrowly  
yellow. .... *S. rudnicki*  
Hind tibia dark brown to brownish black except  
base widely yellow. .... *S. crocinum*
10. Hind basitarsus entirely light to dark brown. ... 11  
Hind basitarsus whitish on basal 1/2 to 1/3 and  
darkened on rest. .... 13
11. Abdomen with dorsolateral pair of silvery spots on  
segments 2, 6 and 7. .... *S. phukaense*  
Abdomen with dorsolateral pair of silvery spots on  
segments 2, 5, 6 and 7. .... 12
12. Style with apical spine. .... *S. maenoi*  
Style without apical spine. .... *S. thongsahuani*
13. Abdomen with dorsolateral pair of silvery spots on  
segments 2, 5, 6 and 7. .... 14  
Abdomen with dorsolateral pair of silvery spots on  
segments 2, 6 and 7. .... 15
14. Body of ventral plate nearly quadrate.  
..... *S. mediocoloratum*  
Body of ventral plate much wider than long.  
..... *S. grossifilum*
15. Body of ventral plate nearly quadrate (Fig. 1C);  
style with short round basal protuberance (Fig.  
1F). .... *S. suchariti*  
Body of ventral plate much wider than long; style

with long pointed basal protuberance.

..... *S. phayaoense*

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#### REFERENCES

- Adler, P. H. and Crosskey, R. W. 2016. *World Blackflies (Diptera: Simuliidae): A Comprehensive Revision of the Taxonomic and Geographical Inventory [2016]*. 126 pp. [accessed on Feb. 1, 2017]. Available from: [http://entweb.clemson.edu/biomia/pdfs/blackfly\\_inventory.pdf](http://entweb.clemson.edu/biomia/pdfs/blackfly_inventory.pdf).
- Takaoka, H. 2003. The Black Flies (Diptera: Simuliidae) of Sulawesi, Maluku and Irian Jaya. xxii +581 pp., Kyushu University Press, Fukuoka, Japan.
- Takaoka, H. and Choochote, W. 2002. Taxonomic notes on the *griseifrons* species-group in *Simulium* (*Simulium*) (Diptera: Simuliidae) from Thailand: Descriptions of two new species and description of the male, pupa and larva of *S. (S.) digrammicum* Edwards. *Jpn. J. Trop. Med. Hyg.* 30: 115–132.
- Takaoka, H. and Choochote, W. 2004a. Two new species of *Simulium* (*Simulium*) (Diptera: Simuliidae) from Thailand. *Trop. Med. Health*, 32: 31–36.
- Takaoka, H. and Choochote, W. 2004b. Taxonomic notes on the *griseifrons* species-group of *Simulium* (*Simulium*) (Diptera: Simuliidae) in Northern Thailand. *Trop. Med. Health*, 32: 311–327.
- Takaoka, H. and Choochote, W. 2005. A new species of *Simulium* (*Simulium*) from Northern Thailand (Diptera: Simuliidae). *Trop. Med. Health*, 33: 95–101.
- Takaoka, H. and Choochote, W. 2006. A new species of the *griseifrons* species-group of *Simulium* (*Simulium*) (Diptera: Simuliidae) in northern Thailand. *Med. Entomol. Zool.*, 57: 115–124.
- Takaoka, H. and Davies, D. M. 1996. The Black Flies (Diptera: Simuliidae) of Java, Indonesia. viii +81 pp., Bishop Museum Bulletin in Entomology 6, Bishop Museum Press, Honolulu, U.S.A.

# A New Species of the *Simulium* (*Simulium*) *striatum* Species Group (Diptera: Simuliidae) from Thailand, and Its Differentiation from Two Related Species Based on a Fast-Evolving Nuclear Gene

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## Abstract

*Simulium* (*Simulium*) *phraense* sp. nov. (Diptera: Simuliidae) is described from females, males, pupae, and larvae from Thailand. This new species is placed in the *Simulium striatum* species group and is most similar to *Simulium* (*Simulium*) *nakhonense* Takaoka & Suzuki (Diptera: Simuliidae) from Thailand among species of the same species group but is barely distinguished from the latter species by lacking annular ridges on the surface of the pupal gill filaments. The fast-evolving nuclear big zinc finger (BZF) gene has successfully differentiated this new species from its allies, *S. (S.) nakhonense* and *Simulium* (*Simulium*) *chiangmaiense* Takaoka & Suzuki (Diptera: Simuliidae) of the *S. striatum* species group. The BZF gene sequences show that this new species is more closely related to *S. (S.) nakhonense* than to *S. (S.) chiangmaiense*, further supporting its morphological classification.

**Key words:** black fly, big zinc finger gene, genetic distance, phylogenetics

The *Simulium striatum* species group is one of the 25 species groups of the subgenus *Simulium*, the largest of the 37 subgenera of the genus *Simulium* Latreille (Diptera: Simuliidae) (Adler and Crosskey 2017). It consists of 25 species (24 named and 1 unnamed) and is widely distributed in the Oriental Region, though four species are recorded from the Palearctic Region (Adler and Crosskey 2017; Takaoka et al. 2017a,b). In this species group, little is known about the biting habits and other biological aspects, although *Simulium* (*Simulium*) *quinquestriatum* (Shiraki) (Diptera: Simuliidae) is an experimental vector of *Onchocerca dewittei japonica* Uni, Bain & Takaoka, which is a parasite of wild boar and a causative agent of zoonotic onchocerciasis in Japan (Fukuda et al. 2008).

In Thailand, the *S. striatum* species group is represented by four species, *Simulium* (*Simulium*) *chiangmaiense* Takaoka & Suzuki (Diptera: Simuliidae), *Simulium* (*Simulium*) *nakhonense* Takaoka & Suzuki (Diptera: Simuliidae), *S. (S.) quinquestriatum*, and *Simulium* (*Simulium*) *thailandicum* Takaoka & Suzuki (Diptera: Simuliidae) (Takaoka and Suzuki 1984). All these species are morphologically similar in the adult female and male but are easily separable in the pupal stage by the arrangement and relative thickness of the gill

filaments (Takaoka and Suzuki 1984). Notably, *S. (S.) chiangmaiense* and *S. (S.) nakhonense* are chromosomally homosequential species and molecularly indistinguishable, based on the mitochondrial cytochrome c oxidase I (COI) and the nuclear elongation complex protein 1 (ECP1) genes (Pangjanda and Pramual 2017).

In a recent survey of pupae and larvae of black flies in Phrae Province in northern Thailand, we collected one undescribed species of the *S. striatum* species group, which is similar to *S. (S.) nakhonense* in the arrangement of the 10 pupal gill filaments but is distinguished from the latter species by lacking annular ridge on the surface of the gill filaments. This species is here described as new based on females, males, pupae, and larvae.

In the past few years, the fast-evolving nuclear big zinc finger (BZF) gene has been adopted for phylogenetic studies in several insects including the Dipterans (Puslednik et al. 2012, Senatore et al. 2014, Moulton, 2017). Strikingly, this gene has been found to produce promising results in resolving several structurally monomorphic species of the Simuliidae (Senatore et al. 2014). Accordingly, we also adopted the BZF gene to test whether it can differentiate this new species, and its congeners, *S. (S.) nakhonense* and *S. (S.) chiangmaiense*, from one another.

## Materials and Methods

Over all, 5 females and 6 males (all reared from pupae), 11 pupal exuviae and cocoons, and 5 mature larvae from Phrae Province, Thailand, were used for descriptions of the new species. Detailed information for the type localities is given under the 'Type material'.

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

Seven adults of the new species, reared from pupae, all from the type locality, five adults of *S. (S.) nakhonense*, all from Mae Klong River, elevation 785 m, 16°17'16.4"N/98°59'45.9"E, Mae Klong Watershed Management Unit, Um Phang District, Tak Province, Thailand, 25-XI-2016, by W. Srisuka, and six adults of *S. (S.) chiangmaiense*, all from Mae Sa-ngi stream, elevation 267 m, 19°26'55.5"N/97°59'50.1"E, Thung masan village, Muang District, Mae Hongson Province, Thailand, 29-III-2017, by W. Srisuka, were used for sequencing of the BZF gene and a subsequent phylogenetic analysis.

Genomic DNA was extracted from each specimen, using the i-genomic CTB DNA Extraction Mini Kit (iNtRON Biotechnology, Inc., Seongnam, South Korea). An approximately 930-bp fragment of the BZF gene was amplified using our newly designed primers: BBZF\_F (5'-CTCGCACGTCAAGGTGAGT-3') and BBZF\_R (5'-GATCCGAATGTGGATTGCT-3'). The cycling conditions included an initial denaturation at 94°C for 3 min; 35 cycles of 94°C for 30 s (denaturation), 55°C for 30 s (annealing), 72°C for 45 s (elongation); and a final elongation at 72°C for 10 min.

The BZF sequences generated from the present study were deposited in the National Center for Biotechnology Information (NCBI) GenBank database under accession numbers MF464025–MF464031 for *Simulium (Simulium) phraense* sp. nov. (Diptera: Simuliidae), MF464032–MF464036 for *S. (S.) nakhonense* and MF464037–MF464042 for *S. (S.) chiangmaiense*.

A maximum likelihood analysis was performed with PhyML v3.0 (Guindon et al. 2010), using GTR nucleotide substitution model. A neighbor-joining analysis based on Kimura two-parameter model was performed using MEGA7 (Kumar et al. 2016). To estimate the level of genetic divergence, uncorrected p pairwise genetic distances were estimated using PAUP 4.0b10 (Swofford 2002).

## Nomenclature

This paper and the nomenclatural act(s) it contains have been registered in Zoobank ([www.zoobank.org](http://www.zoobank.org)), the official register of the International Commission on Zoological Nomenclature. The LSID (Life Science Identifier) number of the publication is urn:lsid:zoobank.org:pub:AFC72BBD-E74B-4CBB-809A-877E9222B733

## Results

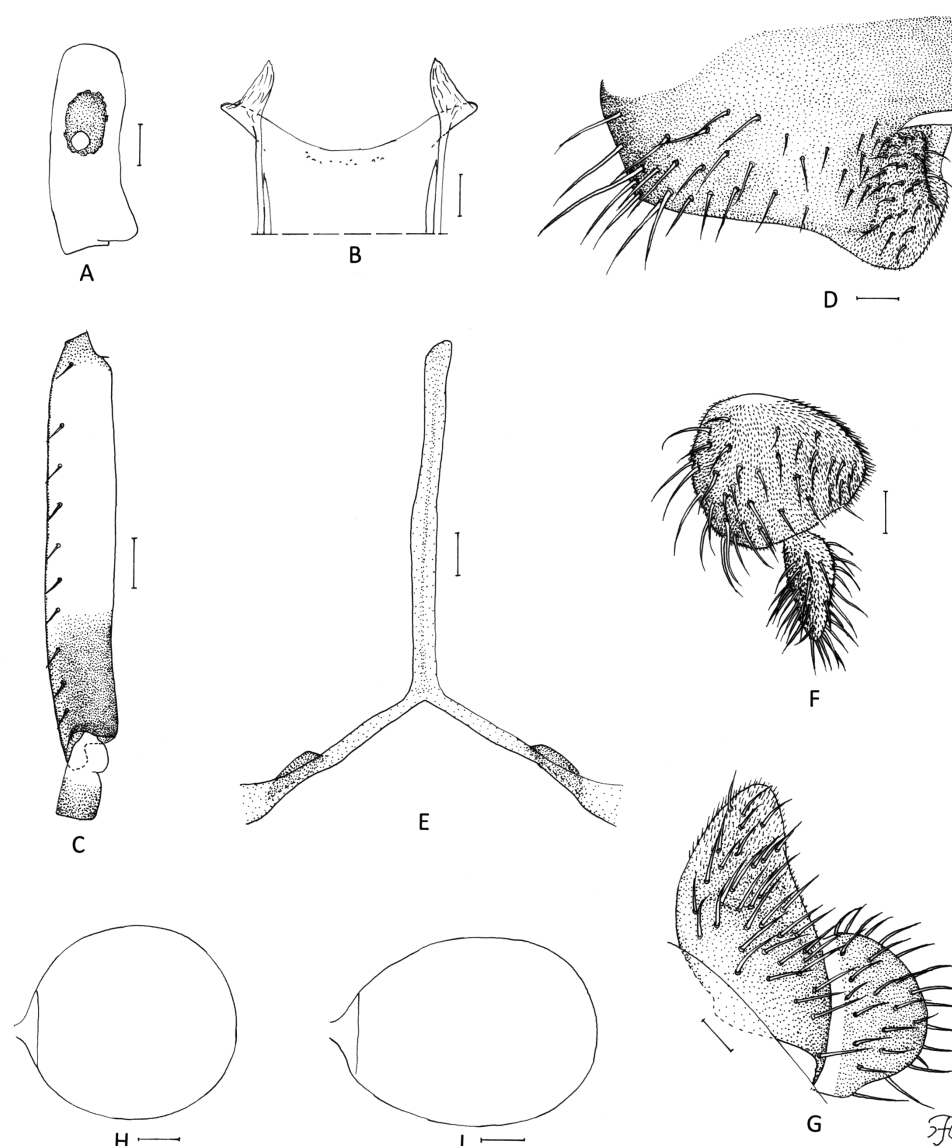
### Description of a New Species

*S. (S.) phraense* Takaoka, Srisuka & Sacung sp. nov.

(urn:lsid:zoobank.org:act:981024A1-C22F-4A11-B046-32B4A3AFC341)

**Female.** Body length 2.0–2.3 mm. **Head.** Slightly narrower than thorax. Frons black, shiny when illuminated at certain angles, with several dark stout hairs along lateral margins and several hairs just above lower margin; frontal ratio 1.3–1.4:1.0:1.5–1.8; frons:head ratio 1.0:4.0–4.2. Fronto-ocular area well developed, short, directed laterally, and rounded apically. Clypeus brownish-black, slightly shiny and gray pruinose when illuminated at certain angles, moderately covered with dark-brown medium-long hairs (though

mediolongitudinal portion of upper half widely bare). Labrum 0.67–0.71 times length of clypeus. Antenna composed of scape, pedicel, and nine flagellomeres; scape and pedicel yellow, flagellomeres dark yellow to light brown except apical two to four flagellomeres medium brown. Maxillary palp with five segments, medium brown except first and second segments ochreous or light brown, and third segment dark brown; proportional lengths of third, fourth, and fifth segments 1.0:1.1–1.2:1.8–1.9; third segment (Fig. 1A) of normal size, with medium-sized ellipsoidal sensory vesicle (0.3 times length of third segment) having opening of moderate size. Maxillary lacinia with 10–12 inner and 11 or 12 outer teeth. Mandible with 18–22 inner and 10–12 outer teeth. Cibarium (Fig. 1B) with several minute processes near posterodorsal margin. **Thorax.** Scutum brownish black to black, shiny, moderately covered with yellow recumbent short hairs intermixed with dark short hairs anteriorly and sparsely with several dark-brown long upright hairs on prescutellar area; scutum gray pruinose with five nonpruinose longitudinal vittae (one medial, two submedial, and two lateral), medial and submedial vittae well defined from anterior margin to posterior portion and submedial and lateral vittae united widely near anterior margin), all vittae united with transverse nonpruinose band on prescutellar area, when illuminated in front and viewed dorsally; scutum gray pruinose except four nonpruinose longitudinal vittae, when illuminated posteriorly and viewed dorsally. Scutellum brownish black, covered with dark-brown upright long hairs and yellow short hairs. Postnotum brownish black, shiny, white pruinose when illuminated at certain angles, and bare. Pleural membrane bare. Katepisternum dark brown to brownish black, longer than deep, shiny, white pruinose when illuminated at certain angles, and bare. **Legs.** Foreleg: coxa and trochanter yellowish white; femur dark yellow to light brown except inner surface widely yellow; tibia medium brown; tarsus brownish black, with moderate dorsal hair crest; basitarsus greatly dilated, 4.6–4.8 times as long as its greatest width. Midleg: coxa brownish black; trochanter dark yellow except base whitish; femur medium brown except apical cap dark brown; tibia light to medium brown with apical cap dark brown and base whitish, and with whitish sheen widely on posterior surface when illuminated at certain angles; basitarsus whitish except apical tip light brown, other tarsomers light brown except base of tarsomere 2 whitish. Hind leg: coxa brownish black; trochanter yellowish white; femur dark brown to brownish black except base yellowish white; tibia dark brown except base yellowish white and with whitish sheen on basal half or more of posterior surface when illuminated at certain angles; tarsus medium to dark brown except basal two-thirds of basitarsus and basal half of second tarsomere yellowish white; basitarsus (Fig. 1C) nearly parallel-sided, 5.5–5.7 times as long as wide, and 0.7 and 0.6 times as wide as greatest widths of hind tibia and femur, respectively; calcipala (Fig. 1C) moderately developed, slightly shorter than wide, and 0.33 times as wide as greatest width of basitarsus; pedisulcus (Fig. 1C) well developed. Claw simple, without tooth. **Wing.** Length 1.9–2.0 mm. Costa with dark spinules and hairs; subcosta haired except apical one-fourth to two-fifths bare; basal section of radius with hairs on apical three-fifths to four-fifths;  $R_1$  with dark brown spinules and hairs;  $R_2$  with dark-brown hairs; hair tuft on base of radius dark brown; basal cell absent. **Halter.** White except base somewhat darkened. **Abdomen.** Basal scale dark brown, with fringe of pale hairs. Dorsal surface of abdomen dark brown to brownish black, with dark-brown short hairs; tergite 2 shiny and silvery iridescent when illuminated at certain angles and tergites 6–9 shiny. Ventral surface of seventh segment with pair of weakly sclerotized submedian sternal plates. **Terminalia.** Sternite 8 (Fig. 1D) with posterior margin concave medially in form of reversed-U shape, bare

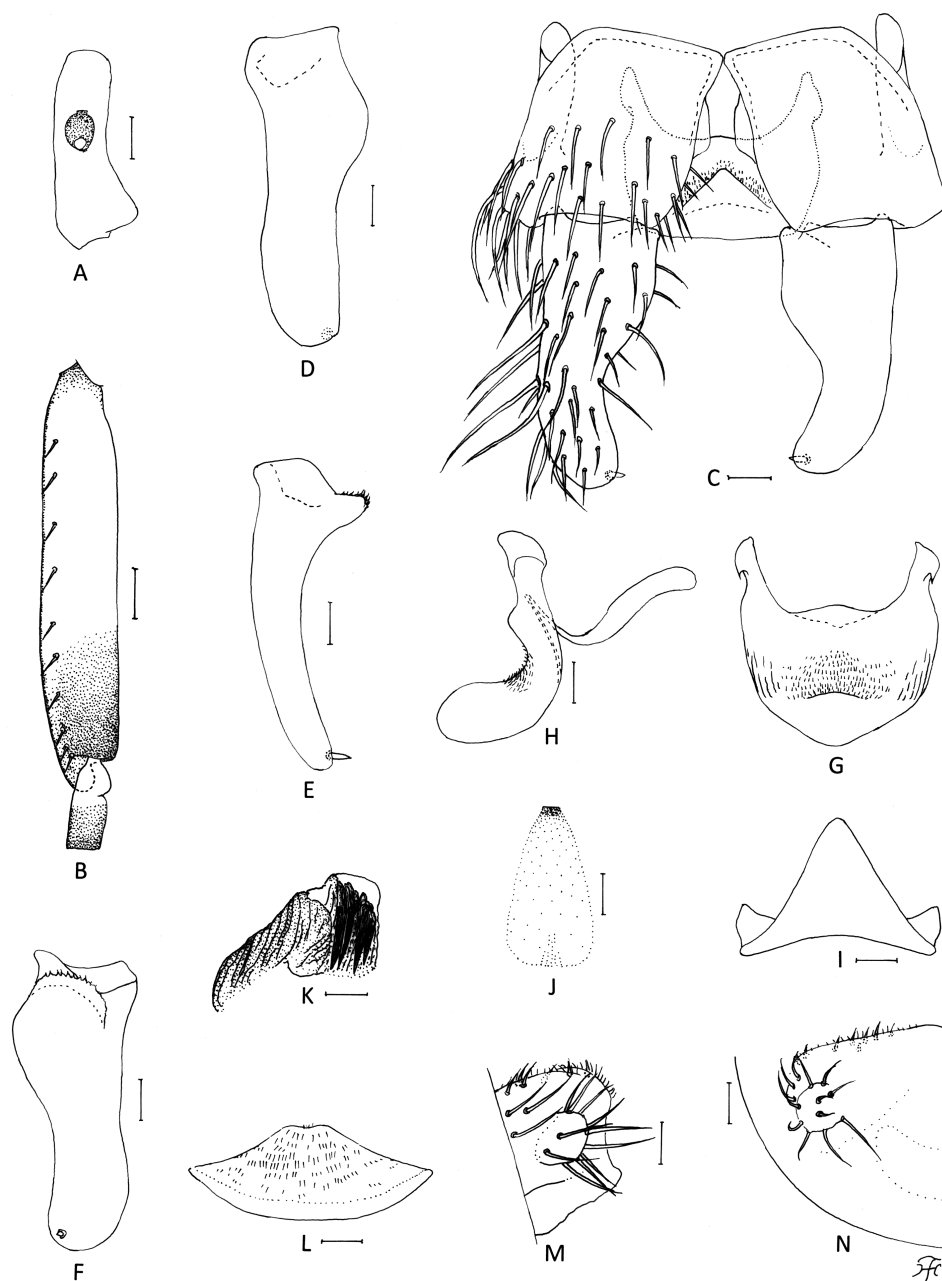


**Fig. 1.** Female of *S. (S.) phraense* sp. nov.: (A) third segment of maxillary palp with sensory vesicle (right side, front view); (B) cibarium (front view); (C) hind basitarsus and second tarsomere (left side, outer view); (D) sternite 8 and ovipositor valve (right side only, ventral view); (E) genital fork (ventral view); (F) and (G) paraprocts and cerci (F, ventral view; G, lateral view); and (H) and (I) spermatheca. Scale bars: 0.05 mm for C; 0.02 mm for A, B, and D–I.

medially, with 17–22 dark-brown medium-long to long stout hairs and 6–13 yellow short to medium-long hairs on each lateral surface. Ovipositor valve (Fig. 1D) triangular, with ventrally produced lobe near inner margin, membranous except narrow area along inner margin slightly sclerotized, covered with 18–26 short yellow hairs and numerous microsetae; inner margins slightly sinuous, somewhat separated from each other. Genital fork (Fig. 1E) of inverted-Y form, with narrow well-sclerotized stem; arms of moderate width, each with moderately sclerotized lateral portion. Paraproct in ventral view (Fig. 1F) rounded, subequal in length to greatest width, strongly pigmented on anterior surface, with 24–41 yellow and dark short to medium-long hairs on lateral and ventral surfaces; paraproct in lateral view (Fig. 1G) 0.53 times as long as wide, and much protruded ventrally beyond ventral margin of cercus. Cercus in lateral view (Fig. 1G) short, 0.5 times as long as wide, with numerous medium-long hairs, and rounded posteriorly. Spermatheca (Fig. 1H and I) nearly globular or ovoid, 1.1–1.3 times as long as greatest width, well sclerotized except portion of junction with duct widely

unpigmented, without definite reticulate patterns on its surface; internal setae present; accessory ducts unpigmented, subequal in thickness to each other, and slightly thicker than major duct.

**Male.** Body length 2.0–2.5 mm. **Head.** Slightly wider than thorax. Upper-eye large facets in 16 or 17 vertical columns and 16 or 17 (rarely 15) horizontal rows. Clypeus black, thickly white pruinose and iridescent when illuminated at certain angles, with dark brown hairs along and near lateral margins (most of central portion bare). Antenna composed of scape, pedicel and nine flagellomeres, yellow except apical three to six flagellomeres light to medium brown; first flagellomere elongate, 1.6–1.7 times length of second one. Maxillary palp with five segments, medium brown except first and second segments ochreous and fifth segment grayish light brown; proportional lengths of third, fourth, and fifth segments 1.0:1.2–1.5:2.2–2.7; third segment (Fig. 2A) of normal size; sensory vesicle (Fig. 2A) ellipsoidal, 0.21 times length of third segment, and with small opening. **Thorax.** Scutum brownish black to black, with whitish-pruinose pattern, i.e., anterior pair of large spots on shoulders extended posteriorly



**Fig. 2.** Male of *S. (S.) phraense* sp. nov.; (A) third segment of right maxillary palp with sensory vesicle (right side, front view); (B) hind basitarsus and second tarsomere showing calcupala and pedisulcus (left side, outer view); (C) coxites, styles, and ventral plate (ventral view); (D)–(F) styles (right side; D, ventrolateral view; E, medial view; F, dorsal view); (G) ventral plate (ventral view, little tilted posteriorly); (H) ventral plate and median sclerite (lateral view); (I) ventral plate (caudal view); (J) median sclerite (caudal view); (K) paramere (right side, dorsal view); (L) aedeagal membrane (caudal view); and (M) and (N) abdominal segment 10 and cerci (right half; M, lateral view; N, caudal view). Scale bars: 0.05 mm for B; 0.02 mm for A and C–N.

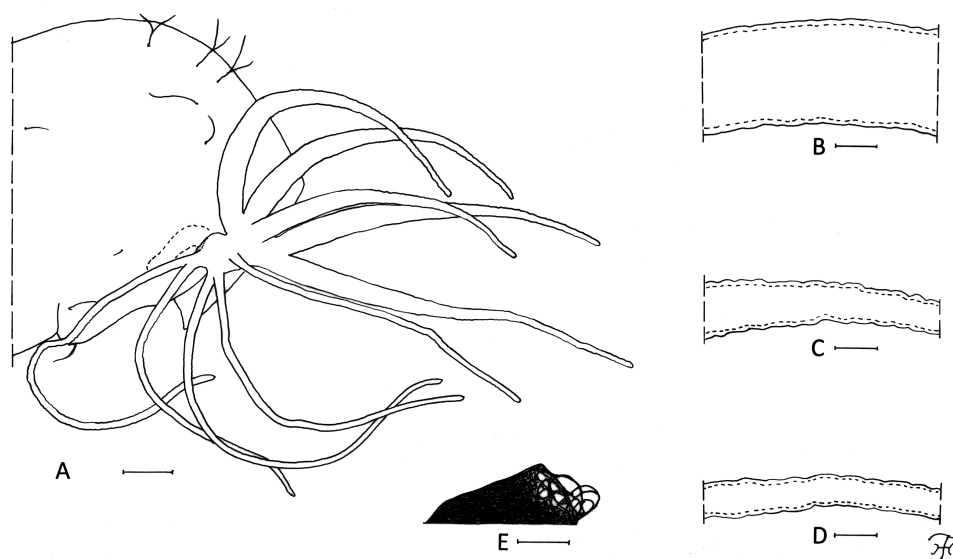
along lateral margins and narrowly connected to large transverse spot entirely covering prescutellar area, anterior pair of large spots divided into anterior half and posterior half, either of which disappears depending on direction of lights; all these spots brilliantly iridescent when illuminated at certain angles; scutum uniformly and moderately covered with brassy recumbent short hairs and with several dark brown long upright hairs on prescutellar area. Scutellum brownish black, with dark brown long upright hairs and brassy short hairs. Postnotum brownish black, shiny and whitish pruinose when illuminated at certain angles and bare. Pleural membrane bare. Katepisternum longer than deep, brownish black, and bare.

**Legs.** Similar in coloration to those of female except mid trochanter medium brown with basal half yellow, mid femur entirely medium brown, and hind basitarsus yellowish white on basal three-fifths or little more and medium brown on rest. Fore basitarsus greatly dilated, 5.8–5.9 times as long as its greatest width. Hind basitarsus (Fig. 2B) not enlarged, nearly parallel-sided, though slightly narrowed apically, 5.1–5.2 times as long as wide, and 0.7 and 0.6 times as wide as greatest widths of hind tibia and femur, respectively; calcupala (Fig. 2B) small, slightly shorter than its basal width, and 0.4 times as wide as greatest width of basitarsus; pedisulcus (Fig. 2B) well developed. **Wing.** Length 1.6–1.7 mm. Other characters as in

female except subcosta and basal portion of radius bare. **Abdomen.** Basal scale brownish black, with fringe of dark-brown long hairs. Dorsal surface of abdomen dark brown to brownish black, moderately covered with dark-brown short to medium long hairs; segments 2, 5, 6, and 7 each with pair of whitish pruinose spots (brilliantly iridescent when illuminated at certain angles) dorsolaterally, those on segment 2 broadly connected in middle to each other. **Genitalia.** Coxites, styles, and ventral plate in ventral view as in Fig. 2C; coxite in ventral view (Fig. 2C) rectangular, 1.2 times as long as wide; style in ventrolateral view (Fig. 2D) 1.5 times length of coxite, 3.1 times as long as greatest width at basal one-third, somewhat narrowed from basal one-third to middle, then nearly parallel-sided, with subapical spine; style in medial view (Fig. 2E) somewhat flattened dorsoventrally, with short basal protuberance directed dorsomedially, with several cone-like spines along its anterior margin; style in dorsal view (Fig. 2F) bearing basal protuberance with row of minute spines. Ventral plate in ventral view (Fig. 2C) with body broad, with lateral margins gently convex, anterior margin nearly straight, though anteromedial portion slightly produced, and posterior margin nearly straight; body bearing prominent median process sharply narrowed to round tip; body covered with minute setae medially; arms short, stout, divergent from base; ventral plate much produced posteriorly when slightly tilted posteriorly (Fig. 2G); ventral plate in lateral view (Fig. 2H) with median process abruptly bent ventrally at nearly right angle; ventral plate in caudal view (Fig. 2I) in form of equilateral triangle, and bare. Median sclerite in lateral view (Fig. 2H) arising slightly anterior to anteromedian portion of ventral plate, and curved dorsally at basal one-third; median sclerite in caudal view (Fig. 2J) wide, plate-like, slightly widened toward apex. Paramere in caudal view (Fig. 2K) enlarged basally, with several hooks apically. Aedeagal membrane in caudal view (Fig. 2L) sparsely covered with minute setae; dorsal plate weakly sclerotized and not pigmented. Abdominal segment 10 (Fig. 2M and N) with two to four hairs on each lateral surface, and several shorter hairs on ventral surface near posterior margin on each side; cercus (Fig. 2M and N) small, with 11–13 distinct hairs.

**Pupa.** Body length 2.5–3.0 mm. **Head.** Integument light ochreous, moderately and uniformly covered with round tubercles; frons with

two pairs of unbranched short slender trichomes with straight apices; face with pair of unbranched medium-long trichomes with straight apices, longer than frontal trichomes. **Thorax.** Integument light ochreous, moderately covered with round tubercles; thorax on each side with three unbranched, bifid and trifid medium long trichomes anterodorsally, two unbranched or bifid medium-long trichomes anterolaterally, one unbranched short to medium long trichome mediolaterally, and three unbranched trichomes with straight apices (two short, one medium-long) ventrolaterally. Gill (Fig. 3A) with 10 thread-like filaments arranged as 2 + 1 + 2 + 1 + 2 + 2 (rarely 3 + 2 + 1 + 2 + 2) from dorsal to ventral; dorsal, middle and two ventral pairs each with short stalk; all filaments slightly different in length (0.7–1.2 mm), and ventral five filaments always much thinner than dorsal five filaments, which are somewhat inflated on basal two-fifths (relative thickness of filaments from dorsal to ventral when compared basally 1.0:1.0:0.7–0.9:0.9–1.1:0.8–1.0:0.4–0.5:0.3–0.4:0.4–0.5:0.4:0.4); stalks of two ventral pairs directed ventrally and posteriorly, respectively, stalks of dorsal and posteroventral pairs at angle of 260–290 degrees; all filaments light whitish yellow, covered with annular furrows, lacking distinct annular ridges, thus no reticulate surface pattern (Fig. 3B–D), and densely covered with minute tubercles. **Abdomen.** Dorsally, all segments nearly transparent except segments 1 and 9 light yellow; segment 1 without tubercles, with one unbranched short seta on each side; segment 2 with one unbranched short seta and five minute setae, of which three or four are stout on each side; segments 3 and 4 each with four distinct hooks and one short spinous seta on each side; all setae and hooks unbranched; segments 5, 6, 7, and 9 lacking spine-combs; segment 8 with distinct spine-combs in transverse row; segments 5–9 each with comb-like groups of minute spines on each side; segment 9 with pair of small cone-like terminal hooks, though terminal hooks absent in one pupa, one of two terminal hooks absent in two pupae. Ventrally, all segments unpigmented except segment 9 yellowish; segments 4–8 each with comb-like groups of minute spines; segment 5 with pair of bifid stout hooklets submedially and few unbranched minute setae on each side; segments 6 and 7 each with pair of bifid inner and unbranched outer stout hooklets somewhat separated from each other and few unbranched minute setae



**Fig. 3.** Pupa of *S. (S.) phraense* sp. nov.; (A) anterior half of thorax and gill filaments (left side, outer view); (B) basal portion of dorsal filament; (C) middle portion of dorsal filament; (D) apical portion dorsal filament; and (E) cocoon (lateral view). Scale bars: 1.0 mm for E; 0.1 mm for A; 0.02 mm for B–D.

on each side. **Cocoon** (Fig. 3E). Light ochreous, shoe-shaped, with several small to large open spaces anterolaterally on each side; posterior half with floor; posterior half thickly woven and individual threads almost invisible; 2.7–3.6 mm long by 1.1–1.6 mm wide; height 0.4–0.7 mm.

**Mature larva.** Body length 4.5–5.3 mm. Body variable in color from light brown, brownish green to bluish green. Abdomen in lateral view gradually widened from segment 1 to segment 7, then narrowed to segment 9. **Head.** Head capsule variable in color from yellow to entirely dark brown; head spots faintly positive or negative or obscured. Antenna composed of three articles and apical sensillum, slightly longer than stem of labral fan; length ratio of three articles (from base to tip) 1.00:1.22–1.27:0.76–0.84. Labral fan with 42–48 primary rays. Mandible (Fig. 4A) with mandibular serration composed of two teeth (one medium-sized, one small); main tooth at right angle against mandible on apical side; comb-teeth gradually decreased in length from first to third; supernumerary serrations absent (though one minute tooth present between two mandibular teeth in left mandible of one larva). Hypostoma (Fig. 4B) with nine anterior teeth, of which median and corner teeth subequal in length to each other, followed by three intermediate teeth on each side; lateral margins serrate apically; four or five hypostomal bristles divergent posteriorly from lateral border on each side. Postgenal cleft (Fig. 4C) large, rounded, 5.7–7.5 times length of postgenal bridge, and 1.2 times as wide as long; sheath of subesophageal ganglion weakly or moderately pigmented. Cervical sclerites on each side composed of light brown elliptical piece, not fused to occiput. **Thorax and Abdomen.** Histoblast of pharate pupal gill with 10 filaments. Thoracic cuticle sparsely covered with minute colorless setae. Thoracic segment 3 and abdominal segments 1–8 each with pair of cone-like dorsolateral protuberances (Fig. 4D). Abdominal cuticle moderately covered with minute colorless setae on dorsal and dorsolateral surface of few posterior segments, and moderately covered with short colorless setae on each side of anal sclerite. Rectal scales present. Rectal organ of three lobes, each with 8–11 finger-like secondary lobules. Anal sclerite X-shaped, with short broad anterior arms 0.7 times length of posterior ones; base with deep unsclerotized incision posteriorly; one or two sensilla on base of anal sclerite; two to six sensilla posterior to posterior arms. Last abdominal segment not bulged laterally and lacking ventral papillae. Posterior circlet with 96–98 rows of hooklets with up to 15 or 16 hooklets per row.

**Type material.** HOLOTYPE: Male (with its associated pupal exuviae and cocoon, in 80% ethanol), collected from Mae Yom River (width 3.6 m; depth 15 cm; bottom rocky; water temperature 29.0°C; exposed to the sun; elevation 194 m; 18° 37'38.7"N, 100° 09'37.4"E), fast-flowing, Mae Yom National Park, Song District, Phrae Province, Thailand, 21-XII-2014, by W. Srisuka. PARATYPES. Five females, five males (with their associated pupal exuviae and cocoons) and five mature larvae, same data as those of the holotype. OTHER SPECIMENS EXAMINED. Three females and two males (with their associated pupal exuviae and cocoons), collected from a stream (18°40'35.9"N, 100°13'32.0"E; elevation 242 m), Sa-iab village, Song District, Phrae Province, Thailand, 4-VIII-2016, by W. Srisuka; four females, one male (with their associated pupal exuviae and cocoons) and one pupa, collected from a stream (17°30'47.8"N, 99°20'58.6"E; elevation 199 m), Den Maisung village, Thoen District, Lampang Province, Thailand, 23-X-2011, by W. Srisuka; one female and two males (with their associated pupal exuviae and cocoons), collected from Ban Huak stream (19°41'02.4"N, 100°24'02.0"E; elevation 489 m), Huak village, Phu Sang District, Phayao Province, Thailand, 5-VIII-2014, by W. Srisuka; one male (with its associated pupal exuviae and cocoon), collected from Mae Pert stream (16°02'40.3"N, 99°17'27.3"E; elevation 167 m), Khlong Lan District, Khamphaeng Phet Province, Thailand, 26-VI-2013, by W. Srisuka; three females and one male (with their associated pupal exuviae and cocoons), collected from a stream (16°45'35.6"N, 98°55'31.3"E; elevation 796 m), Mueang District, Tak Province, Thailand, 28-VI-2013, by W. Srisuka; one female (with its associated pupal exuviae and cocoon), collected from a stream (16°27'36.0"N, 99°07'03.2"E; elevation 352 m), Lo Kho village, Wang Chao District, Tak Province, Thailand, 21-VII-2016, by W. Srisuka.

**Biological Notes.** The pupae and larvae of this new species were collected from grass leaves trailing in the current. No other black fly species were found in five of seven streams where this new species was collected. In each of the two other streams, one in Khamphaeng Phet Province, and the other in Wang Chao District, Tak Province, only one pupa of this new species was collected together with many pupae of *S. (S.) nakhonense*.

**Distribution.** Thailand (Khamphaeng Phet, Lampang, Phayao, Phrae and Tak).

**Etymology.** The species name *phraense* refers to the name of the province, Phrae, where this new species was collected.

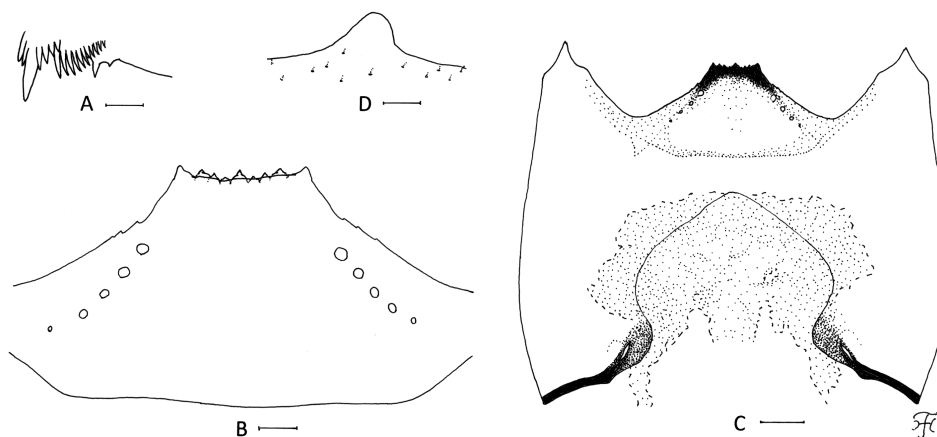


Fig. 4. Mature larva of *S. (S.) phraense* sp. nov.: (A) tip of mandible (lateral view); (B) hypostoma (ventral view); (C) head capsule showing hypostoma and postgenal cleft (ventral view); and (D) protuberance and microsetae on dorsum of abdominal segment 8. Scale bars: 0.05 mm for C; 0.02 mm for A, B, and D.

## DNA Analyses of a New Species and Two Related Species

Phylogenetic analyses reveal that the new species is differentiated from the closely related species by forming a highly supported monophyletic clade (ML = 100%/NJ = 99%; Fig. 5). This new species is distinctly separated from *S. (S.) nakhonense* and *S. (S.) chiangmaiense* with genetic distances of 3.37 and 3.86%, respectively.

## Discussion

*Simulium (S.) phraense* sp. nov. is assigned to the *S. striatum* species group, defined by Takaoka and Davies (1996), by the unique shape of the female terminalia (Fig. 1D and G) and male genitalia (Fig. 2C). This new species is characterized by having a haired basal portion of the radius of the female, female scutum covered with yellow short hairs, pupal gill with 10 short filaments arranged as 2 + 1 + 2 + 1 + 2 + 2 from the dorsum, of which the dorsal five filaments are somewhat inflated and about twice the thickness of the ventral five

filaments, all filaments lacking distinct annular ridges (Fig. 3A–D), and the larval body with paired protuberances (Fig. 4D).

This new species is similar to *S. (S.) nakhonense* from Thailand (Takaoka and Suzuki 1984) but is distinguished from the latter species by the pupal gill lacking distinct annular ridges on the surface of the filaments (Fig. 3B–D).

*Simulium (Simulium) palmatum* Puri (Diptera: Simuliidae) from India (Puri 1932) and *Simulium (Simulium) subpalmatum* Davies & Györkös (Diptera: Simuliidae) from Sri Lanka (Davies and Györkös 1992) have a similar arrangement and relative thickness of the pupal gill filaments but differ from this new species by having the cocoon without open weave anteriorly.

Over the past few years, there have been several attempts to resolve members of the *S. striatum* species group. The standard DNA barcoding COI region has been found ineffective to separate *S. (S.) chiangmaiense*, *S. (S.) nakhonense*, and *S. (S.) quinquestriatum* from one another (Pramual and Adler 2014). The nuclear ECP1 gene, which has been useful to resolve species complexes and species

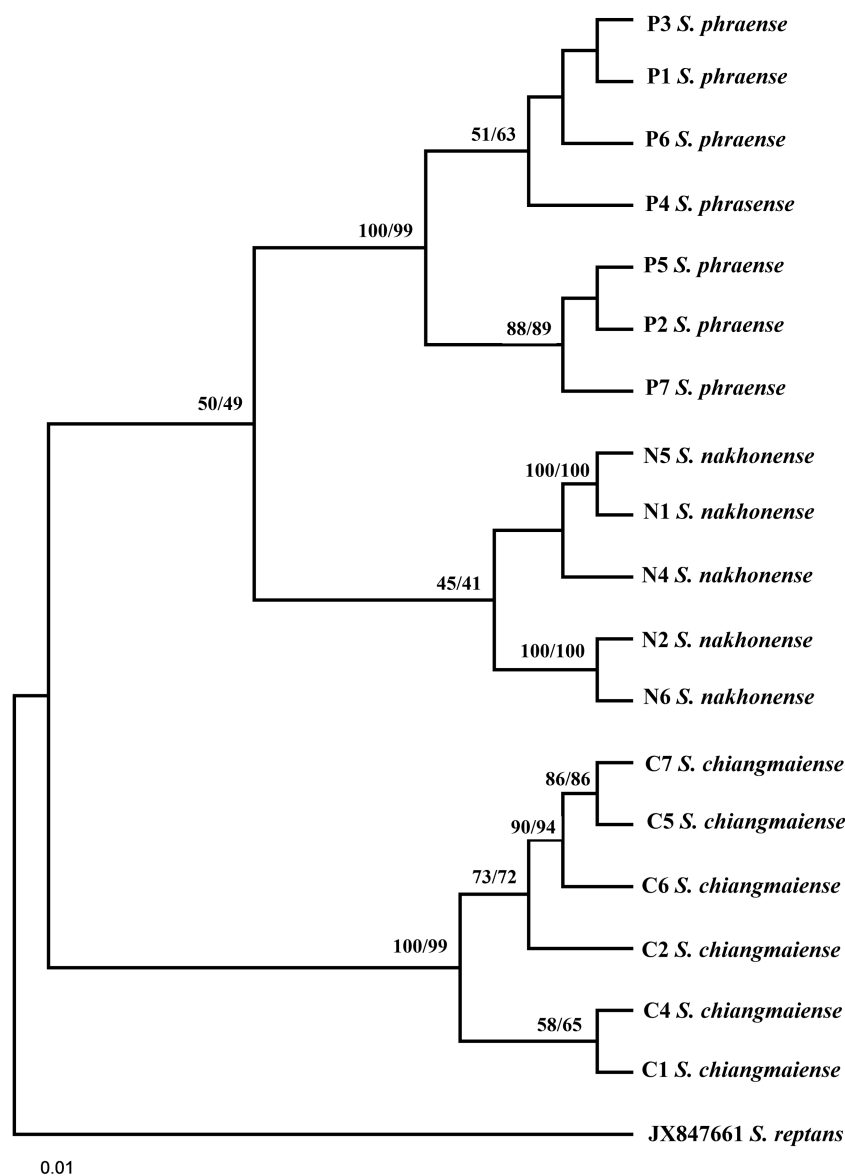


Fig. 5. Maximum likelihood phylogenetic tree of representatives of the *S. striatum* species group based on the BZF gene sequences. Bootstrap values (ML/NJ) are shown on the branches.

groups (Senatore et al. 2014, Low et al. 2016), also failed to distinguish *S. (S.) chiangmaiense* from *S. (S.) nakhonense* (Pangjanda and Pramual 2017). Nevertheless, the BZF gene used in the present study distinguishes *S. (S.) phraense* sp. nov., *S. (S.) chiangmaiense*, and *S. (S.) nakhonense* from one another. Low bootstrap values were observed for the clade of *S. (S.) nakhonense* (<50%); however, this observation is expected in closely related taxa (Keer et al. 2007, Low et al. 2017, Takaoka et al. 2017c). The BZF gene also demonstrates that the new species is more closely related to *S. (S.) nakhonense* than to *S. (S.) chiangmaiense*, further supporting its morphological classification.

Further studies are needed to determine to what extent the BZF gene sequences of these three related species vary by populations since two of them are widely distributed in Thailand, and even in neighboring countries.

## Acknowledgments

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## References Cited

- Adler, P. H., and R. W. Crosskey. 2017. World blackflies (Diptera: Simuliidae): a comprehensive revision of the taxonomic and geographical inventory [2017]. 131 pp. <https://biomia.sites.clemson.edu/pdfs/blackflyinventory.pdf> (accessed on 1 July 2017)
- Adler, P. H., D. C. Currie, and D. M. Wood. 2004. The black flies (Simuliidae) of North America. xv + 941 pp., Cornell University Press, Ithaca, NY.
- Davies, D. M., and H. Györkös. 1992. The simuliidae (Diptera) of Sri Lanka. Description of additional species of *Simulium* (*Simulium*), with a key for Sri Lankan species in the subgenus and a checklist for the country. Can. J. Zool. 70: 1029–1046.
- Fukuda, M., H. Takaoka, S. Uni, and O. Bain. 2008. Infective larvae of five *Onchocerca* species from experimentally infected *Simulium* species in an area of zoonotic onchocerciasis in Japan. Parasite 15: 111–119.
- Guindon, S., J. F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk, and O. Gascuel. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59: 307–321.
- Keer, K. C. R., M. Y. Stoeckle, C. J. Dove, L. A. Weigt, C. M. Francis, and P. D. Hebert. 2007. Comprehensive DNA barcode coverage of North American birds. Mol. Ecol. Notes. 7: 535–543.
- Kumar, S., G. Stecher, and K. Tamura. 2016. MEGA7: molecular evolutionary genetics analysis Version 7.0 for bigger datasets. Mol. Biol. Evol. 33: 1870–1874.
- Low, V. L., H. Takaoka, P. Pramual, P. H. Adler, Z. Ya'cob, Y. T. Huang, X. D. Pham, R. Ramli, C. D. Chen, A. Wannaket, et al. 2016. Delineating taxonomic boundaries in the largest species complex of black flies (Simuliidae) in the Oriental Region. Sci. Rep. 6: doi: 10.1038/srep20346.
- Low, V. L., T. K. Tan, B. K. Prakash, W. S. Vinnie-Siow, S. T. Tay, R. Masmeatathip, U. K. Hadi, Y. A. L. Lim, C. D. Chen, Y. Norma-Rashid, and M. Sofian-Azirun. 2017. Contrasting evolutionary patterns between two haplogroups of *Haematobia exigua* (Diptera: Muscidae) from the mainland and islands of Southeast Asia. Sci. Rep. 7: 5871.
- Moulton, J. K. 2017. The true identity of *Dixa modesta* Johannsen (Diptera: Dixidae) resolved: synonymy of *Dixa similis* Johannsen, designation of the *Dixa ubiquita* species group, and description of three new eastern Nearctic species. Zootaxa 4216: 247–260.
- Pangjanda, S., and P. Pramual. 2017. Tests of conspecificity for closely related black fly (Diptera: Simuliidae) species of the *Simulium striatum* group in Thailand. Zootaxa 4231: 421–430.
- Pramual, P., and P. H. Adler. 2014. DNA barcoding of tropical black flies (Diptera: Simuliidae) of Thailand. Mol. Ecol. Resour. 14: 262–271.
- Puslednik, L., R. C. Russell, and J. W. O. Ballard. 2012. Phylogeography of the medically important mosquito *Aedes (Ochlerotatus) vigilax* (Diptera: Culicidae) in Australasia. J. Biogeogr. 39: 1333–1346.
- Puri, I. M. 1932. Studies on Indian Simuliidae. Part V. Species and varieties of the *striatum* series. Indian J. Med. Res. 20: 515–532.
- Senatore, G. L., E. A. Alexander, P. H. Adler, and J. K. Moulton. 2014. Molecular systematics of the *Simulium jenningsi* species group (Diptera: Simuliidae), with three new fast-evolving nuclear genes for phylogenetic inference. Mol. Phylogenet. Evol. 75: 138–148.
- Swofford, D. L. 2002. PAUP\* phylogenetic analysis using parsimony (\* and other methods) Sinauer Associates, Sunderland, MA.
- Takaoka, H. 2003. The black flies (Diptera: Simuliidae) of Sulawesi, Maluku and Irian Jaya. xxii + 581 pp., Kyushu University Press, Fukuoka, Japan.
- Takaoka, H., and D. M. Davies. 1996. The black flies (Diptera: Simuliidae) of Java, Indonesia. viii + 81 pp., Bishop Museum Press, Honolulu, HI.
- Takaoka, H., M. Sofian-Azirun, Z. Ya'cob, C. D. Chen, K. W. Lau, V. L. Low, X. D. Pham, and P. H. Adler. 2017a. The black flies (Diptera: Simuliidae) of Vietnam. [Monograph] Zootaxa. 4261: 1–165.
- Takaoka, H., M. Sofian-Azirun, Z. Ya'cob, C. D. Chen, K. W. Lau, V. L. Low, and I. W. Suana. 2017b. The black flies (Diptera: Simuliidae) of the Lesser Sunda Archipelago, Indonesia. Acta Trop. 169: 170–186.
- Takaoka, H., W. Srisuka, V. L. Low, W. Maleewong, and A. Saeung. 2017c. Two new species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from Myanmar, and their phylogenetic relationships with related species in the *S. asakoe* species-group. Acta Trop. 176: 39–50.
- Takaoka, H., and H. Suzuki. 1984. The blackflies (Diptera: Simuliidae) from Thailand. Jpn. J. Sanit. Zool. 35: 7–45.

# A New Species of *Simulium* (*Asiosimulium*) (Diptera: Simuliidae) from Thailand

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## Abstract

*Simulium* (*Asiosimulium*) *saeungae* sp. nov. (Diptera: Simuliidae) is described based on females, males, pupae, and mature larvae collected from Nan Province, Northern Thailand. It is characterized by the medium-long cerci in the female, enlarged hind basitarsus, and broad ventral plate with its posterior margin not deeply concave in the male, arborescent pupal gill with 42–56 filaments in the pupa and smaller number of primary rays of the labral fan (30–33) in the larva. This is the fifth species of the subgenus *Asiosimulium*, the second smallest among 10 subgenera in the Oriental Region. Taxonomic notes are given to distinguish this new species from the three known species from Thailand and one from Nepal.

**Key words:** black fly, aquatic insect, fauna, taxonomy

*Simulium* (*Asiosimulium*) Takaoka & Choochote is the second smallest among the 10 simuliid subgenera of the genus *Simulium* Latreille (Diptera: Simuliidae) in the Oriental Region (Adler and Crosskey 2017). It is represented by four species, i.e., *S. furvum* Takaoka & Srisuka, *S. oblongum* Takaoka & Choochote, and *S. wanchaii* Takaoka & Choochote, all described from Thailand (Takaoka and Choochote 2005, 2006; Takaoka et al. 2013), and *S. suchitrae* Takaoka from Nepal (Takaoka and Shrestha 2010). The male of *S. suchitrae* is unknown.

The biological aspects of the subgenus *Asiosimulium* including the female biting habits remain to be studied, though females of *S. wanchaii* were caught on a human in Doi Suthep-Pui National Park, Chiang Mai, Northern Thailand (Takaoka and Choochote 2006).

In a recent survey of larvae and pupae of black flies in Khunsathan National Park, Nan Province, Northern Thailand, we collected pupae of the subgenus *Asiosimulium*, of which the pupal gill is arborescent, consisting of 42–56 thread-like filaments, a number of filaments much greater than those of four known species, which range from 19 to 33 (Srisuka et al. 2015).

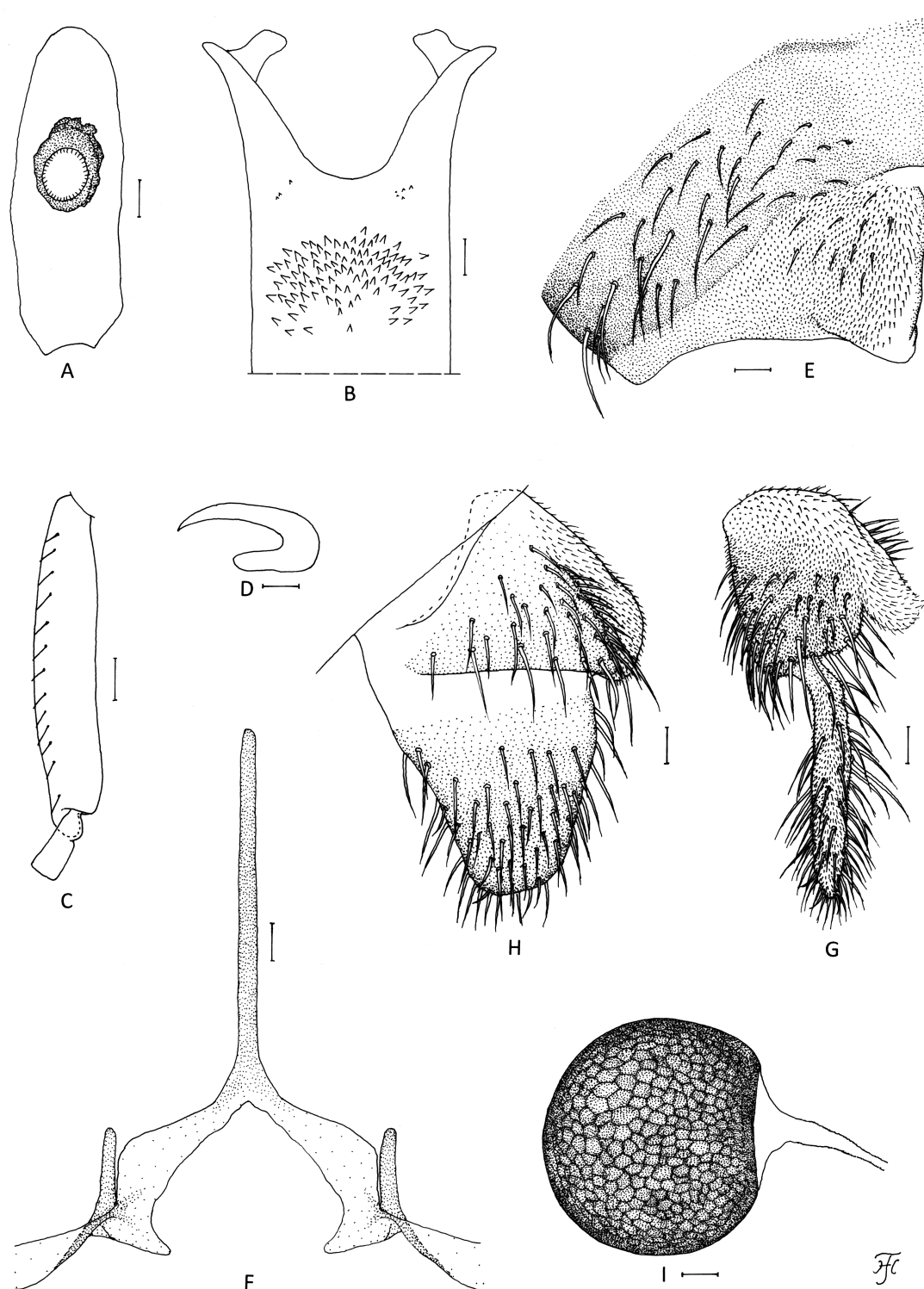
We describe this new species based on females, males, pupae, and mature larvae. The methods of collection, description, and illustration, and terms for morphological features used here follow those of Takaoka (2003) and partially those of Adler et al. (2004). The specimens used are deposited at the Entomology Section, Queen Sirikit Botanic Garden, Chiang Mai, Thailand.

## Nomenclature

This paper and the nomenclatural act(s) it contains have been registered in Zoobank ([www.zoobank.org](http://www.zoobank.org)), the official register of the International Commission on Zoological Nomenclature. The LSID (Life Science Identifier) number of the publication is urn:lsid:zoobank.org:pub:97173C2B-4BD9-43AD-8632-0CC98C26FD0B

*Simulium* (*Asiosimulium*) *saeungae* Takaoka & Srisuka, sp. nov. (urn:lsid:zoobank.org:act:ABAC9E36-9892-473A-B7B2-380A8498C3FB)

**Female** ( $n = 5$ ). Body length 3.0–3.5 mm. **Head.** Slightly narrower than thorax. Frons brownish black, densely covered with whitish-yellow hairs (except median longitudinal portion narrowly bare) interspersed with several dark longer and stouter hairs along each lateral margin; frontal ratio 1.32–1.36:1.00:1.57–1.68. Frons-head ratio 1.0:4.4–4.7. Fronto-ocular area well developed, triangular, directed laterally and somewhat upward. Clypeus brownish black, densely covered with whitish-yellow hairs intermixed with dark longer and stouter hairs. Labrum 0.71–0.78 times as long as clypeus. Antenna composed of scape, pedicel and nine flagellomeres, dark yellow to light brown, except scape, pedicel, and base of first flagellomere yellow and few apical flagellomeres darkened. Maxillary palp consisting of five segments, medium brown except third segment blackish brown, proportional lengths of third, fourth, and fifth segments 1.00:0.83–0.89:1.42–1.57; third segment (Fig. 1A) not enlarged; sensory vesicle (Fig. 1A) ellipsoidal, 0.25–0.29 times as



**Fig. 1.** Female of *Simulium* (*Asiosimulium*) *saeungae* sp. nov. (A) Third segment of maxillary palp with sensory vesicle (right side; front view). (B) Cibarium (posterior view). (C) Hind basitarsus and second tarsomere (left side; outer view). (D) Claw. (E) Sternite 8 and ovipositor valve (right side only; ventral view). (F) Genital fork (ventral view). (G) & (H) Paraprocts and cerci (G, ventral view; H, lateral view). (I) Spermatheca. Scale bars: 0.1 mm for C; 0.02 mm for A, B, and D–I.

long as third segment, with large opening apically. Lacinia with 13 or 14 inner and 12 or 13 outer teeth. Mandible with 25–27 inner and 15 or 16 outer teeth. Cibarium (Fig. 1B) weakly sclerotized except cornuae strongly sclerotized, moderately concave posterodorsally, and with 64–94 spinous processes elaborately arranged in narrow space between inner and outer walls, somewhat apart from

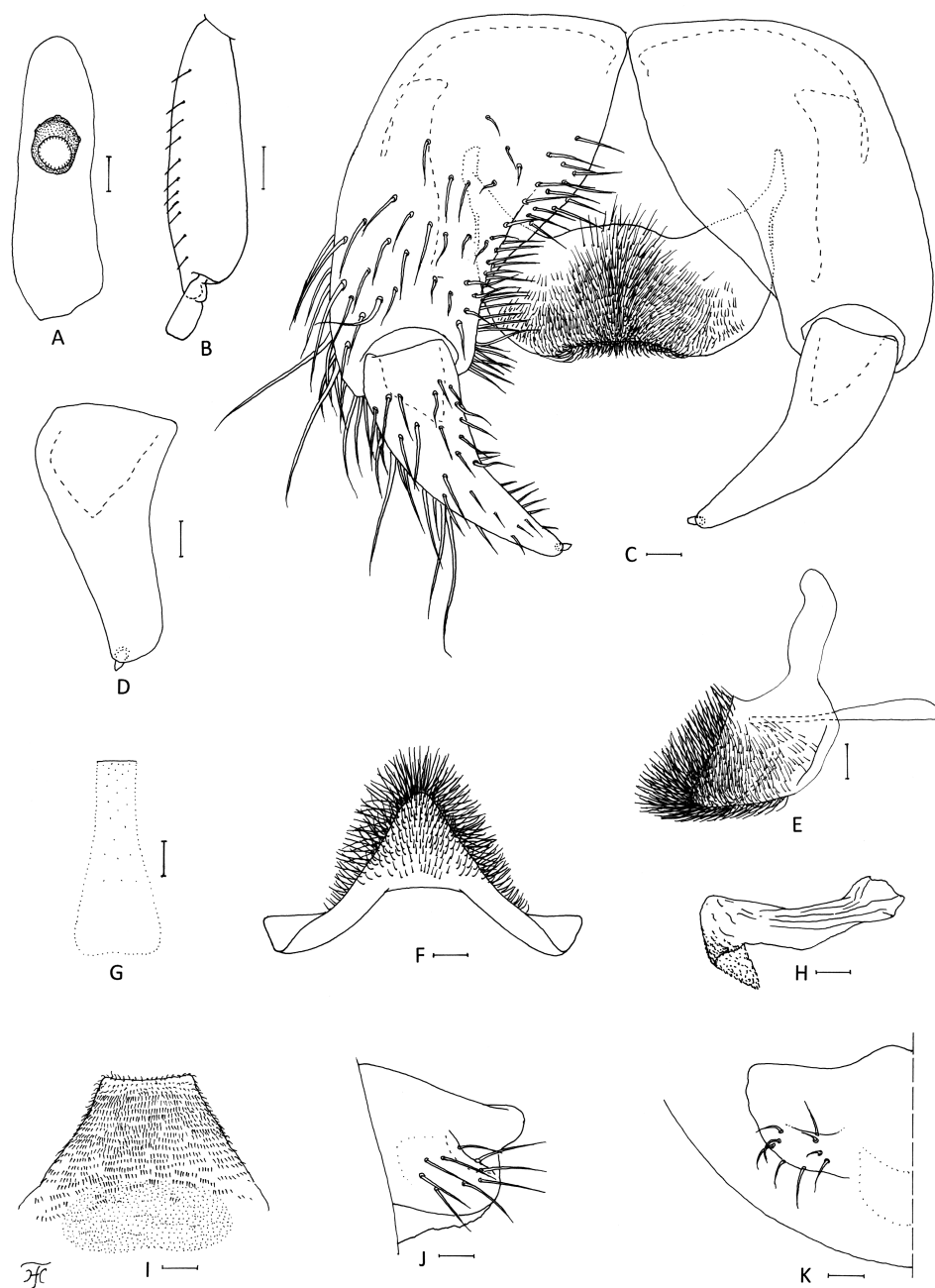
posterodorsal margin; these processes arising from inner wall (in one female, additional three and five smaller processes near posterodorsal margin). **Thorax.** Scutum medium brown except anterolateral calli light brown, with three dark brown narrow longitudinal vittae (one medial, two submedial) and two dark brown rather broad vittae along lateral margins; each of those lateral vittae anteriorly

curved inward and connected with anterior tip of submedian vitta; all these five vittae posteriorly connected with broad transverse dark brown prescutellar area; scutum densely covered with yellowish-white short hairs interspersed with several dark brown upright long hairs on prescutellar area. Scutellum medium to dark brown, with many dark-brown upright long hairs and yellowish-white short hairs. Postnotum medium to dark brown, and bare. Pleural membrane bare. Katepisternum longer than deep, brownish black, thinly white pruinose and without hairs. **Legs.** Medium brown to brownish black except hind trochanter light brown, base of mid trochanter, and extreme bases of all tibiae yellow to light brown. Fore basitarsus much dilated, 4.5–4.8 times as long as its greatest width, and with thick dorsal hair crest. Hind basitarsus (Fig. 1C) nearly parallel-sided though somewhat tapered near both ends, 5.3–6.0 times as long as its greatest width, 0.7 and 0.6–0.7 times as wide as greatest widths of tibia and femur, respectively; calcipala developed, short, 0.85 times as long as its width at base, and 0.42 times as wide as greatest width of basitarsus; pedisulcus well developed. All claws (Fig. 1D) with large basal tooth 0.43–0.45 times as long as claw. **Wing.** Length 2.9–3.0 mm. Costa with dark short spines and dark hairs intermixed with patch of white hairs near base. Subcosta with dark hairs except near apex bare. Hair tuft on stem vein dark brown. Basal portion of radius fully haired.  $R_1$  with dark spinules and hairs.  $R_2$  with dark hairs only. Basal cell and basal median cell absent. **Abdomen.** Basal scale dark brown, with fringe of yellowish-white long hairs intermixed with dark long hairs. Dorsal and lateral surfaces of abdomen dark brown to brownish black, moderately covered with dark short hairs, though densely covered with yellowish-white recumbent short hairs dorsolaterally and laterally on segments 2–4; tergites 6–8 shiny when illuminated dorsally and viewed laterally; ventral surface of abdomen dark brown except segment 2 grayish white to light brown; segment 7 with large sternal plate medially. **Terminalia.** Sternite 8 (Fig. 1E) wide, bare medially but furnished with 20–23 yellow short to medium-long and four or five dark long hairs together with two to seven minute hairs on each side. Ovipositor valve (Fig. 1E) nearly triangular, thin, membranous except inner margin narrowly sclerotized, densely covered with microsetae interspersed with 8–11 short hairs; inner margins nearly straight or slightly sinuous, moderately separated from each other. Genital fork (Fig. 1F) inverted Y-shaped, with well-sclerotized stem; each arm widest medially, with distinct long projection directed anterodorsally, and short triangular projection directed posteromedially. Paraproct in ventral view (Fig. 1G) subquadrate, with distinct process produced ventrally along anteromedial margin, with 8–10 colorless sensilla on darkened anteromedial surface; paraproct in lateral view (Fig. 1H) 0.75 times as long as wide, somewhat produced ventrally beyond ventral margin of cercus, and with numerous short to medium-long hairs on lateral and ventral surfaces. Cercus in ventral view (Fig. 1G) much produced posteriorly; cercus in lateral view (Fig. 1H) tapered posteriorly and rounded apically, nearly as long as its width at base. Spermatheca (Fig. 1I) globular, strongly sclerotized and pigmented except base of duct widely unpigmented, with distinct reticulate surface pattern; internal setae not discernible; accessory ducts unpigmented, subequal in diameter to each other and to main duct; main duct somewhat widened in diameter near junction with spermatheca.

**Male** ( $n = 5$ ). Body length 3.2–3.5 mm. **Head.** Slightly wider than thorax. Holoptic. Upper eye medium brown, consisting of large facets in 16 (rarely 17) vertical columns and 17 (rarely 18 or 19) horizontal rows. Clypeus brownish black, thinly white pruinose, moderately covered with dark brown long hairs interspersed with yellow fine hairs along lateral margins, and rather sparsely covered

with similar hairs on large median area. Antenna composed of scape, pedicel and nine flagellomeres, yellow except base of first flagellomere whitish yellow and apical flagellomere light brown; first flagellomere somewhat elongate, 1.7–1.8 times as long as second one. Maxillary palp composed of five segments, grayish brown except third segment dark brown; proportional lengths of third, fourth, and fifth segments 1.00:0.89:1.46–1.56; third segment (Fig. 2A) of moderate size; sensory vesicle (Fig. 2A) ellipsoidal, 0.19–0.21 times as long as third segment, with large opening apically. **Thorax.** Similar to that of female except short hairs on scutum and scutellum yellow. **Legs.** Color similar to that of female. Fore basitarsus 4.6–4.8 times as long as its greatest width, with thick hair crest. Hind basitarsus (Fig. 2B) enlarged, spindle-shaped, 3.32–3.67 times as long as its greatest width, 0.97–1.03 and 1.00 times as wide as greatest widths of hind tibia and femur, respectively; calcipala well developed, slightly shorter than its width at base, and 0.24 times as wide as greatest width of basitarsus; pedisulcus well developed. **Wing.** Length 2.5–2.8 mm. Other characters as in female except subcosta bare. **Abdomen.** Basal scale brownish black, with fringe of light brown long hairs. Dorsal surface of abdomen dark brown to brownish black, moderately covered with dark hairs; tergites 2 and 5–8 each with pair of dorsolateral or lateral shiny patches when illuminated anterodorsally; ventral surface of abdominal segments 2–4 grayish white to light brown, and those of other segments medium brown; sternites 3–8 medium brown and somewhat shiny when illuminated ventrally. Abdominal segment 9 in ventral view with sternite in form of transverse thick bar. **Genitalia.** Coxite in ventral view (Fig. 2C) rectangular, 1.7 times as long as its greatest width. Style in ventral view (Fig. 2C) short, 0.67 times as long as coxite, gradually tapered toward apex and with apical spine; style in ventrolateral view (Fig. 2D) wide basally, 0.48 times as wide as long, tapered toward middle, then slightly narrowed to apex, with blunt apex. Ventral plate in ventral view (Fig. 2C) with body wide, 0.41 as long as wide, with anterior margin slightly concave medially, with median portion produced ventrally, and densely covered with setae except both anterolateral portions bare; basal arms short, directed anteriorly, parallel-sided or slightly divergent apically; ventral plate in lateral view (Fig. 1E) moderately produced ventrally; ventral plate in caudal view (Fig. 1F) inverted V-shaped, produced ventrally, and covered with setae on median portion of posterior surface. Median sclerite in lateral view (Fig. 2E) arising near anterior margin of ventral plate and directed dorsally; median sclerite in caudal view (Fig. 2G) thin, plate-like, widened apically. Paramere (Fig. 2H) of moderate size, well sclerotized, without hook. Aedeagal membrane (Fig. 2I) moderately covered with minute setae; dorsal plate oval in shape, lightly pigmented. Ventral surface of 10th abdominal segment without distinct hairs laterally near cercus on each side. Cercus (Fig. 2J and K) in form of narrow lobe, covered with 8–15 hairs.

**Pupa** ( $n = 6$ ). Body length 3.5 mm. **Head.** Integument ochreous, without tubercles; frons with pair of unbranched short to medium-long trichomes arising close together on each side; face with one unbranched medium-long to long trichome on each side, which is longer than frontal trichomes; antennal sheath without any projection or tubercles. **Thorax.** Integument ochreous, bare except dorsal surface of posterior half sparsely or moderately covered with tubercles, and thorax on each side with two long trichomes anterodorsally, two trichomes (one long, one medium-long) anterolaterally, one medium-long trichome mediolaterally and three trichomes (one medium-long, two short) ventrolaterally; all trichomes unbranched. Gill (Fig. 3A) of arborescent type, composed of 42–56 short to medium-long slender thread-like filaments (some



**Fig. 2.** Male of *Simulium* (*Asiosimulium*) *saeungae* sp. nov. (A) Third segment of maxillary palp with sensory vesicle (right side; front view). (B) Hind basitarsus and second tarsomere (left side; outer 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 (left side; caudal view). (I) Aedeagal membrane and dorsal plate (caudal view). (J) and (K) Abdominal segment 10 and cerci (right side; J, lateral view; K, caudal view). Scale bars: 0.1 mm for B; 0.02 mm for A and C–K.

are extremely short and thumb or finger-like) arranged in five or six groups; all filaments light to medium brown, with longest filament 1.9–2.3 mm, with annular ridges and furrows, which are weakly developed and present irregularly) and covered densely with minute tubercles; gill with moderately developed transparent basal fenestra. **Abdomen.** Dorsally, all segments pale yellow except segments 1 and 2 yellow and segment 9 yellow to ochreous; segment 1 sparsely covered with minute tubercles, with one short slender setae on each side; segment 2 with one short seta and five short spinous setae on each side; segments 3 and 4 each with four stout hooks and one short spinous or minute seta on each side; segments 5–9 each

with comb-like groups of minute spines but lacking spine-combs on each side; segment 5 with five short setae on each side, segments 6–8 each with two short setae on each side; segment 9 with pair of small cone-shaped terminal hooks. Laterally, segment 9 with two grapple-shaped hooklets on each side. Ventrally, all segments nearly transparent except segment 9 yellow to ochreous; segment 4 with one unbranched hooklet and few short setae on each side; segments 5–7 each with pair of unbranched stout hooks on each side; segments 4–8 each with comb-like groups of minute spines on each side. **Cocoon.** Pale yellow, wall-pocket shaped, thinly woven, with anterodorsal margin somewhat thickly woven, and slightly

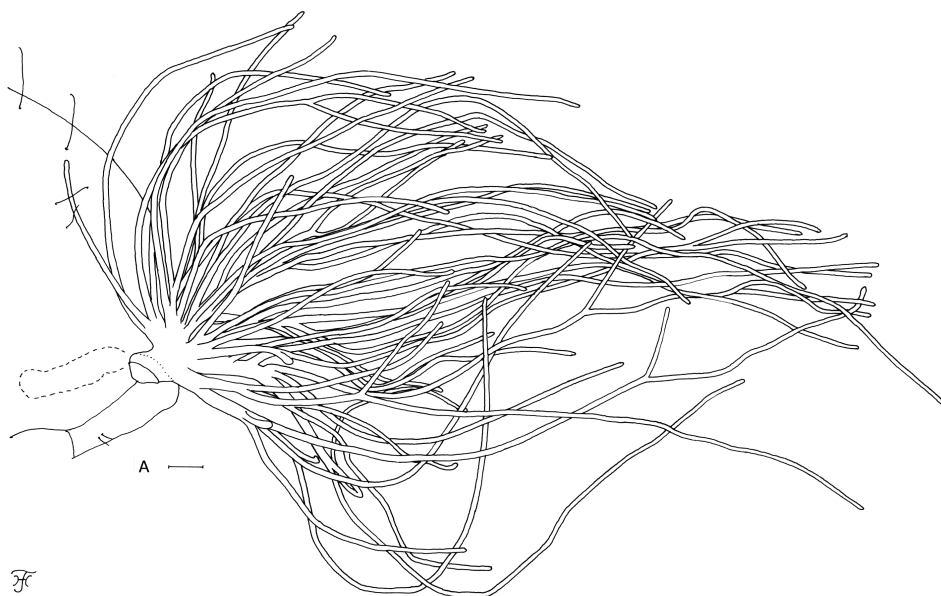


Fig. 3. Pupa of *Simulium (Asiosimulium) saeungae* sp. nov. (A) Anterior half of thorax and gill filaments (right side; outer view). Scale bar: 0.1 mm for A.

extended ventrolaterally; individual threads visible or invisible; 4.0–4.8 mm long by 1.9–2.5 mm wide.

**Mature larva** ( $n = 10$ ). Body length 7.0–8.0 mm. Body grayish, with reddish-brown markings as follows: thoracic segment 1 encircled with transverse band though disconnected ventromedially; abdominal segments 1–4 each with transverse band on each lateral surface, and abdominal segments 5–9 each covered with broad band dorsally and dorsolaterally though disconnected to varying extent dorsomedially. **Head.** Cephalic apotome whitish-yellow except wide area along posterior margin medium brown; head spots distinct, though posterolateral spots merged with darkened area and posterior spot of mediolongitudinal spots connected posteriorly to darkened area along posterior margin. Lateral surface of head capsule yellow except eye-spot region whitish and area above and posterior to eye-spot region light to medium brown, with distinct dark brown spots, i.e., two isolated small spots below eye-spot region (though one of these two often light brown and less distinct) and

two large spots and two small spots in front of posterior margin, all these spots merged with darkened area along posterior margin. Ventral surface of head capsule yellow except central portions along both sides of postgenal cleft and posterior portions along posterior margin widely light to medium brown; elongate and round spots on each side of postgenal cleft distinct, though merged with darkened portion. Cervical sclerites each composed of anterior light brown elongate piece fused to occiput and posterior elliptical light brown piece. Antenna consisting of three articles and apical sensillum, subequal to or little longer than stem of labral fan; proportional lengths of first, second, and third segments 1.0:1.2–1.3:0.7. Labral fan with 30–33 main rays. Mandible (Fig. 4A) with mandibular serration consisting of one large tooth and one small tooth; large tooth at right angle to mandible on apical side; comb-teeth composed of three teeth decreasing in size from first to third; supernumerary serrations absent, though one additional small tooth is present near small mandibular tooth in one mandible. Hypostoma (Fig. 4B) with

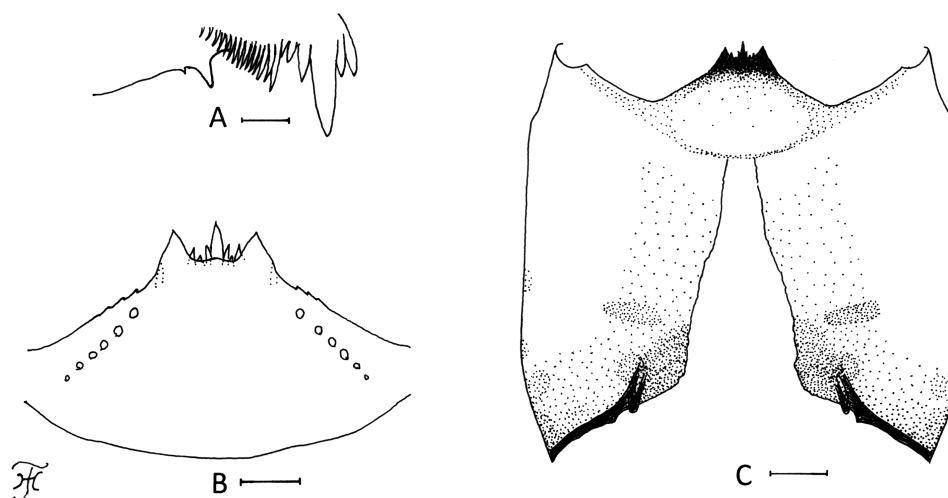


Fig. 4. Mature larva of *Simulium (Asiosimulium) saeungae* sp. nov. (A) Apical tip of mandible. (B) Hypostoma. (C) Head capsule showing postgenal cleft (ventral view). Scale bars: 0.1 mm for C; 0.05 mm for B; 0.02 mm for A.

nine apical teeth in row; median tooth much longer than corner teeth; lateral serration weakly developed anteriorly; six hypostomal bristles per side, lying slightly divergent posteriorly from lateral margin. Postgenal cleft (Fig. 4C) deep, reaching posterior margin of hypostoma. **Thorax and Abdomen.** Thoracic and abdominal cuticle almost bare except both sides of anal sclerite and ventral bulge moderately covered with simple colorless setae. Rectal scales not discernible. Rectal organ compound, with finger-like secondary lobules (number of secondary lobules not counted because rectal organ was withdrawn). Anal sclerite X-shaped, with anterior arms nearly as long as posterior ones; anterior arms broadened, and space between arms widely sclerotized basally; 8–13 sensilla just posterior to basal juncture; accessory sclerite absent. Last abdominal segment somewhat expanded ventrally forming ventral bulge, visible as small ventral papilla when viewed from side. Posterior circlet with 83 rows of up to 15 or 16 hooklets per row.

**Type material.** Holotype: female (with associated pupal exuviae and cocoon) (in 80% ethanol), reared from a pupa collected from a small stream (18°16'44.5"N, 100°30'14.2"E), Khunsathan National Park, Nan District, Nan Province, 27.IV.2017 by W. Srisuka. Paratypes: four females, five males (all with associated pupal exuviae and cocoons), and 10 mature larvae (all in 80% ethanol), same data as those of the holotype.

**Biological notes.** The pupae and larvae of this new species were collected from a stream slowly flowing on rocks (water temperature 19.6°C, partially shaded, elevation 1,316 m). Associated species are *S. (Gomphostilbia) asakoe* Takaoka & Davies complex, *S. (Nevermannia) vessabutrae* Takaoka & Srisuka, *S. (Simulium) doipuiense* Takaoka & Choochote complex and *S. (Simulium) yuphae* Takaoka & Choochote.

**Eymology.** The specific name *saeungae* is in honor of Dr. Atiporn Saeung, Department of Parasitology, Faculty of Medicine, Chiang Mai University, for her great contribution to studies of blackflies and mosquitoes in Thailand.

**Remarks.** This new species is assigned to the subgenus *Simulium* (*Asiosimulium*), defined by Takaoka and Choochote (2005), in that it has a combination of the following characteristics: numerous spinous processes on the cibarium (Fig. 1B), hairs on the basal portion of the radial vein, and a large basal tooth of the claw (Fig. 1D) in the female, paramere without hook (Fig. 2H) in the male, gill filaments of arborescent type (Fig. 3A), dorsal surface of abdominal segments 5–9 each without spine-combs, ventral surface of abdominal segments 5–7 each with pair of unbranched hooks on each side and lateral surface of abdominal segment 9 with grapnel-shaped hooklets in the pupa, and deep postgenal cleft (Fig. 4C) in the larva.

The female of *S. saeungae* sp. nov. is similar to those of *S. wanchaii* and *S. furvum* in sharing the medium-long cerci (Fig. 1G and H), but it is distinguished from *S. wanchaii* by the fore basitarsus much dilated (4.5–4.8 times as long as its greatest width) (5.4–5.6 times in *S. wanchaii*), and abdominal segment 5 not shiny (shiny in *S. wanchaii*), and from *S. furvum* by the globular spermatheca (Fig. 1I) (pear-shaped in *S. furvum*).

The male of this new species is similar to that of *S. wanchaii* in sharing the enlarged hind basitarsus (Fig. 2B) and broad ventral plate with its posterior margin not deeply concave (Fig. 2C), but it is distinguished from the latter species by the antenna yellow except

the apical flagellomere light brown (dark brown except the scape, pedicel and the base of the first flagellomere yellow in *S. wanchaii*), fore basitarsus much dilated (4.6–4.8 times as long as its greatest width) (7.5 times in *S. wanchaii*), and abdominal segments 5–8 each with a pair of shiny dorsolateral patches (without such shiny patches in *S. wanchaii*).

The pupa of this new species is similar to that of *S. suchitrae* in having the frons and anterior half of the thorax without tubercles, but it is distinguished from *S. suchitrae* by lacking an enlarged basal fenestra. The number of pupal gill filaments (42–56) (Fig. 3A) separates this new species from all the three other known species (22 in *S. furvum*, 31–33 in *S. oblongum*, 28 in *S. suchitrae*, and 19 in *S. wanchaii*).

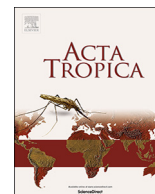
The larva of this new species is similar to those of the four known species in sharing the deep postgenal cleft (Fig. 4C), but it is barely distinguished from *S. furvum* by having an unpigmented sheath around the subesophageal ganglion, from *S. oblongum* and *S. wanchaii* by the smaller number of primary rays of the labral fan (30–33) (43–45 in *S. oblongum* and 38–40 in *S. wanchaii*), and from *S. suchitrae* by the smaller number of rows of the posterior circlet (83) (90 in *S. suchitrae*).

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## References Cited

- Adler, P. H., and R. W. Crosskey. 2017. World Blackflies (Diptera: Simuliidae): a Comprehensive Revision of the Taxonomic and Geographical Inventory [2017]. 131 pp., <http://entweb.clemson.edu/biomia/pdfs/blackflyinventory.pdf> (accessed on 1 August 2017).
- Adler, P. H., D. C. Currie, and D. M. Wood. 2004. The Black Flies (Simuliidae) of North America. xv+941 pp., Cornell University Press, Ithaca, NY.
- Srisuka, W., H. Takaoka, and A. Saeung. 2015. Description of the male, pupa and mature larva of *Simulium* (*Asiosimulium*) *wanchaii* Takaoka & Choochote (Diptera: Simuliidae) from Thailand, with keys to four species of the subgenus *Asiosimulium*. Trop. Biomed. 32: 504–510.
- Takaoka, H. 2003. The Black Flies (Diptera: Simuliidae) of Sulawesi, Maluku and Irian Jaya. xxii + 581 pp., Kyushu University Press, Fukuoka, Japan.
- Takaoka, H., and W. Choochote. 2005. A new subgenus and a new species of *Simulium* s. l. (Diptera: Simuliidae) from Thailand. Med. Entomol. Zool. 56: 33–41.
- Takaoka, H., and W. Choochote. 2006. A new species of the subgenus *Simulium* (*Asiosimulium*) (Diptera: Simuliidae) from Thailand. Med. Entomol. Zool. 57: 45–48.
- Takaoka, H., and S. Shrestha. 2010. New species of black flies (Diptera: Simuliidae) from Nepal. Zootaxa [Monograph]. 2731: 1–62.
- Takaoka, H., W. Srisuka, A. Saeung, and W. Choochote. 2013. *Simulium* (*Asiosimulium*) *furvum*, a new species of black fly (Diptera: Simuliidae) from Thailand. J. Med. Entomol. 50: 493–500.



# Five new species of the *Simulium decuplum* subgroup of the *Simulium* (*Gomphostilbia*) *batoense* species-group (Diptera: Simuliidae) from Thailand and their phylogenetic relationships

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## ABSTRACT

Five new species of black flies, *Simulium* (*Gomphostilbia*) *chiangraiense*, *S. (G.) huaikaeoense*, *S. (G.) khaokhoense*, *S. (G.) maeklangense*, and *S. (G.) pamiangense*, are described based on adults, pupae and mature larvae from Thailand. These five species are similar to one another and are placed in the *S. decuplum* subgroup of the *S. batoense* species-group by having the male fore coxae darkened, ventral plate flat and transverse; pupal gill with 10 short filaments; and larval postgenal cleft deep approaching or reaching the posterior margin of the hypostoma. *Simulium* (*G.*) *chiangraiense* sp. nov. and *S. (G.) pamiangense* sp. nov. differ from the three other new species by the much longer pupal gill filaments and darkened dorsal surface of abdominal segments 1 and 2. Taxonomic notes are provided to distinguish these five new species from five other related species. The phylogenetic positions of these new species in the *S. decuplum* subgroup are presented based on the mitochondrial COI gene. *Simulium* (*G.*) *pamiangense* sp. nov. and *S. (G.) huaikaeoense* sp. nov. are highly similar to each other genetically, showing a sister relationship, though they are clearly different morphologically. On the contrary, *S. (G.) khaokhoense* sp. nov. is distantly positioned from *S. (G.) huaikaeoense* sp. nov., though it is almost indistinguishable morphologically from the latter.

## 1. Introduction

*Gomphostilbia* Enderlein is the second largest of 37 subgenera in the genus *Simulium* Latreille (Diptera: Simuliidae) containing 240 species, of which most are distributed in the Oriental Region (Adler and Crosskey, 2017). They are further classified into 15 species-groups, of which the *Simulium batoense* species-group is the largest assemblage of heterogeneous lineages (Takaoka, 2012). This group consists of 60 species in eight subgroups (seven named and one unnamed) (Adler and Crosskey, 2017; Takaoka, 2012). In this species-group, little is known about the biting habits and other biological aspects, although *S. (G.) asakoe* Takaoka & Davies of the *S. asakoe* species-group is a vector of an unidentified filarial parasite of animal origin in Thailand (Fukuda et al., 2003; Ishii et al., 2008).

In Thailand, the *S. batoense* species-group is represented by six species, of which one is placed in the *S. decuplum* subgroup, two in the *S. duolongum* subgroup, two in the *S. parahiyangum* subgroup and one in the *S. sextplum* subgroup (Takaoka, 2012; Takaoka et al., 2017d).

In recent surveys of pupae and larvae of black flies in Chiang Rai, Lampang, Nan and Chiang Mai Provinces in northern Thailand, and Phetchabun Province in central Thailand, we collected five undescribed species of the *S. batoense* species-group, all of which are assignable to the *S. decuplum* subgroup, defined by Takaoka (2012), based on the male fore coxae darkened and pupal gill with 10 short filaments. This subgroup is small, containing only five species: *Simulium* (*G.*) *banmaense* Chen & Zhang from China (Chen and Zhang, 2003); *Simulium* (*G.*) *chongqingense* Zhu & Wang from China (Zhu and Wang, 1995); *Simulium* (*G.*) *jiulianshanense* Chen, Kang & Zhang described from pupae and larvae from China (Kang et al., 2007); *Simulium* (*G.*) *decuplum* Takaoka & Davies from Peninsular Malaysia, Thailand and India (Takaoka and Davies, 1995; Takaoka and Saito, 1996; Borah et al., 2012); and *S. (G.) lamdongense* Takaoka & Sofian-Azirun from Vietnam (Takaoka et al., 2015).

Five species are described herein as new, based on females, males, pupae and larvae, and taxonomic notes are given to separate them from related species.

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The phylogenetic relationships of these new species with two related species in the *S. decuplum* subgroup are also presented based on the mitochondrial COI gene.

## 2. Materials and methods

The methods of collection, description and illustration, and terms for morphological features, follow those of Takaoka (2003) and partially those of Adler et al. (2004). The holotype and paratypes are deposited at the Queen Sirikit Botanic Garden, Chiang Mai, Thailand.

*Simulium* (G.) *chiangraiense* sp. nov., *S.* (G.) *pamiangense* sp. nov., *S.* (G.) *maeklangense* sp. nov., *S.* (G.) *khaokhoense* sp. nov., and *S.* (G.) *hualikaoense* sp. nov., their congeners, *S.* (G.) *decuplum* collected from Peninsular Malaysia, and *S.* (G.) *lamdongense* collected from Vietnam, were subjected to DNA isolation, COI gene amplification, and sequencing as described by Low et al. (2015). New sequences of *S.* (G.) *chiangraiense* sp. nov. (MF968964–MF968965), *S.* (G.) *pamiangense* sp. nov. (MF968953–MF968954), *S.* (G.) *maeklangense* sp. nov. (MF968966–MF968967), *S.* (G.) *khaokhoense* sp. nov. (MF968968–MF968969), *S.* (G.) *hualikaoense* sp. nov. (MF968955–MF968958), *S.* (G.) *decuplum* (MF968959–MF968961), and *S.* (G.) *lamdongense* (MF968962–MF968963) generated from the present study were deposited in the National Center for Biotechnology Information (NCBI) GenBank database. For phylogenetic tree construction, all available members of the *S. batoense* species-group: *Simulium* (G.) *siamense* Takaoka & Suzuki, *S.* (G.) *johorensis* Takaoka, Sofian-Azirun & Ya'cob, *S.* (G.) *laosense* Takaoka, Srisuka & Saeung, *S.* (G.) *duolongum* Takaoka & Davies, *S.* (G.) *tahanense* Takaoka & Davies, and *S.* (G.) *parahiyangum* Takaoka & Sigit, were retrieved from the NCBI GenBank database.

The COI sequences were assembled and edited using ChromasPro Version 1.7.7 (Technelysium Pty Ltd., Australia) and BioEdit 7.0.9.0 (Hall, 1999). Uncorrected p pairwise genetic distances among seven members of the *S. decuplum* subgroup of the *S. batoense* species-group were computed using PAUP 4.0b10. (Swofford, 2002). Neighbor-joining (NJ) and maximum likelihood (ML) analyses were used to construct a phylogenetic tree of the *S. batoense* species-group. NJ analysis was performed in MEGA7 (Kumar et al., 2016) with Kimura's two-parameter model of substitution (K2P distance), using 1000 bootstrap replicates. ML analysis was performed on an on-line web-based server PhyML 3.0 (Guindon et al., 2010) using HKY85 substitution model.

This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the International Commission on Zoological Nomenclature (ICZN). The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix <http://zoobank.org/>. The LSID for this publication is: urn:lsid:zoobank.org:pub:00933071-FDA2-42EB-AA1D-B40A563D91DD.

## 3. Results and discussion

### 3.1. Descriptions of five new species

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

(urn:lsid:zoobank.org:act:661EB26E-8230-4C78-8AF6-CC6B0C4021DE)

**Female.** Body length 1.9–2.1 mm. **Head.** Slightly narrower than thorax. Frons brownish black, densely covered with yellowish-white scale-like recumbent short pubescence; frontal ratio 1.6–1.7:1.0:1.9–2.1; frons:head ratio 1.0:4.5–4.6. Fronto-ocular area well developed, narrow, directed dorsolaterally. Clypeus brownish black, densely covered with yellowish-white recumbent pubescence interspersed with few to several dark longer hairs on each side. Labrum 0.6 times as long as clypeus. Antenna composed of scape, pedicel and nine flagellomeres, dark brown except scape and pedicel yellow and

basal extreme of first flagellomere light brown. Maxillary palp composed of five segments, light brown except first and second segments ochreous and third segment dark brown, proportional lengths of third, fourth, and fifth segments 1.0:0.9:1.8–1.9; third segment (Fig. 1A) of moderate size; sensory vesicle (Fig. 1A) elongate, 0.61–0.65 times as long as third segment and with medium-sized opening. Maxillary lacinia with 12 or 13 inner and 16–18 outer teeth. Mandible with 27 or 28 inner teeth and five or six outer teeth at some distance from apex. Cibarium (Fig. 1B) with strongly sclerotized dorsal margin having short plate produced forwardly and posteriorly, and with well sclerotized medial longitudinal ridge with bifid apex. **Thorax.** Scutum brownish black except anterolateral calli medium brown, shiny when illuminated at certain angles, densely covered with yellow scale-like recumbent pubescence. Scutellum medium brown, covered with yellow short pubescence and dark-brown long upright hairs. Postnotum brownish black, bare. Pleural membrane bare. Katepisternum dark brown, longer than deep, shiny when illuminated at certain angles, moderately covered with pale and dark short hairs. **Legs.** Foreleg: coxa yellow; trochanter dark yellow except base pale yellow; femur light brown with apical cap medium brown (though extreme tip yellow); tibia light brown except base yellow, subbasal area medium brown and apical one-fourth dark brown, with whitish-yellow short pubescence (brightly shiny when illuminated) on outer surface of basal three-fourths; tarsus brownish black, with moderate dorsal hair crest; basitarsus somewhat dilated, 5.9–6.1 times as long as its greatest width. Midleg: coxa medium brown except posterolateral surface dark brown; trochanter light brown except base yellow; femur light brown except base yellow and apical cap medium brown (though extreme tip yellow); tibia light brown except base yellow, subbasal small area medium brown and little less than apical half dark brown, and with whitish-yellow short pubescence (brightly shiny when illuminated) on outer and posterior surfaces of basal three-fourths; tarsus dark brown except base of basitarsus light brown. Hind leg: coxa light brown; trochanter yellow; femur light brown except extreme base yellow and apical cap dark brown (though extreme tip yellow); tibia (Fig. 1C) light brown (gradually darkened toward apex) except base yellow, subbasal area medium brown and apical one-fourth dark brown; tibia moderately covered with whitish-yellow short pubescence (brightly shiny when illuminated) on outer and posterior surfaces of basal three-fourths; tarsus (Fig. 1D) brownish black except basal two-thirds of basitarsus (though base light brown) and basal half of second tarsomere whitish yellow; basitarsus (Fig. 1D) narrow, nearly parallel-sided, 5.7–6.5 times as long as wide, and 0.6 and 0.5 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 1D) well developed, nearly as long as wide, and 0.5 times as wide as greatest width of basitarsus; pedisulcus (Fig. 1D) well developed; claw (Fig. 1E) with large basal tooth 0.58 times length of claw. **Wing.** Length 1.8–1.9 mm. Costa with dark-brown spinules and light-brown hairs except basal patch of yellow hairs. Subcosta hairy except near apex bare. Hair tuft on base of radial vein dark brown. Basal portion of radius fully haired. Basal cell absent. **Halter.** Clear white except basal stem darkened. **Abdomen.** Basal scale dark yellow, with fringe of yellowish-white hairs. Dorsal surface of abdomen medium to dark brown except segment 2 light brown though middle portion of tergal plate 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 creamy, those of other segments light to dark brown; sternal plate on segment 7 undeveloped. **Terminalia.** Sternite 8 (Fig. 1F) bare medially, with 15–18 medium-long to long hairs together with two to four slender short hairs on each side. Ovipositor valve (Fig. 1F) triangular (though medioposterior corner rounded), tapered laterally, thin, membranous, moderately covered with microsetae interspersed with two to four short hairs; inner margins nearly straight or slightly sinuous, moderately sclerotized, and somewhat separated from each other. Genital fork (Fig. 1G) of usual inverted-Y form, with slender stem; arms of moderate width, moderately folded medially, with short projection directed anterodorsally.

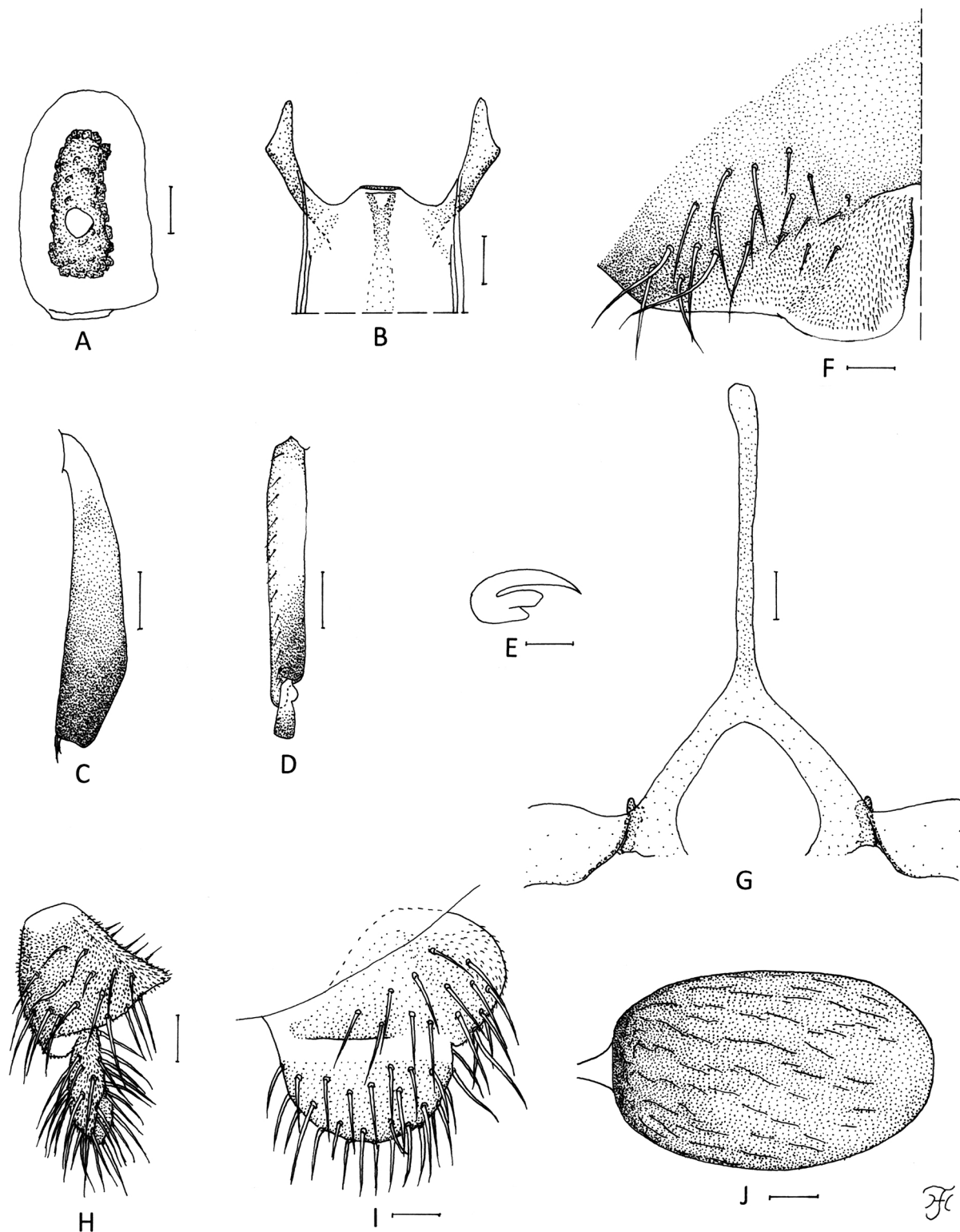


Fig. 1. Female of *Simulium* (*Gomphostilbia*) *chaingraiense* sp. nov. (A) Third segment of maxillary palp with sensory vesicle (right side; front view). (B) Cibarium (front view). (C) Hind tibia (left side; outer view). (D) Hind basitarsus and second tarsomere (left side; outer view). (E) Claw. (F) Sternite 8 and ovipositor valve (right side only; ventral view). (G) Genital fork (ventral view). (H) and (I) Paraprocts and cerci (H, ventral view; I, lateral view). (J) Spermatheca. Scale bars: 0.1 mm for C and D; 0.02 mm for A, B and E.

Paraproct in ventral view (Fig. 1H) nearly triangular, bare on anterior portion, with two or four sensilla on anteromedial surface; paraproct in lateral view (Fig. 1I) much produced ventrally, 0.61 times as long as wide, with 12–19 medium-long to long stout hairs on ventral and lateral surfaces. Cercus in lateral view (Fig. 1I) short, rounded posteriorly, 0.48 times as long as wide. Spermatheca (Fig. 1J) ellipsoidal, 1.6–1.7

times as long as greatest width, well sclerotized except duct and small area near juncture with duct unpigmented, and with many fissures on surface; internal setae absent; both accessory ducts slender, unpigmented, subequal in diameter to major one.

**Male.** Body length 2.2–2.5 mm. **Head.** Slightly wider than thorax. Upper eye medium brown, consisting of large facets in 16 vertical

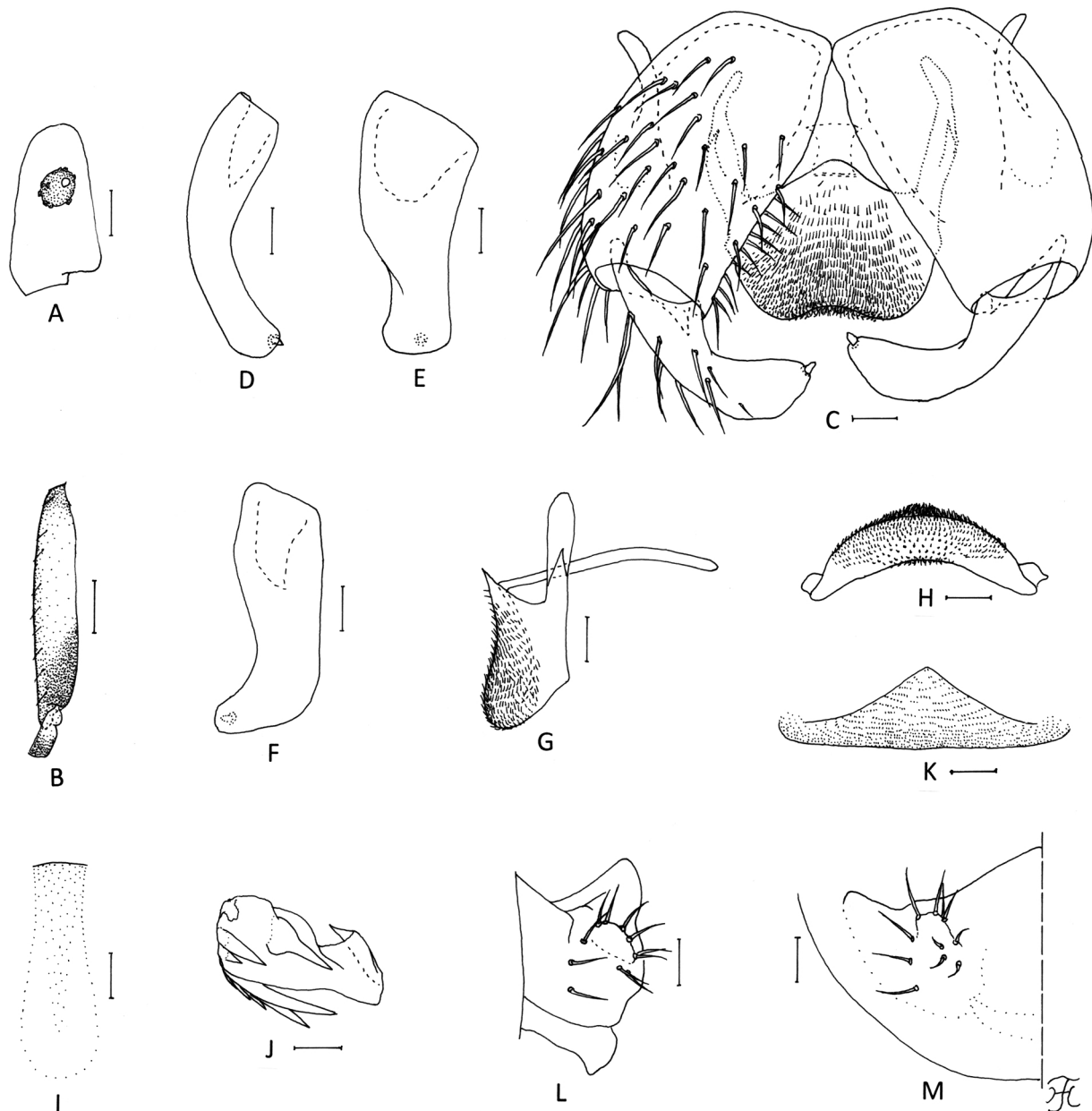


Fig. 2. Male of *Simulium* (*Gomphostilbia*) *chiangraiense* sp. nov. (A) Third segment of maxillary palp with sensory vesicle (right side; front view). (B) Hind basitarsus and second tarsomere (left side; outer view). (C) Coxites, styles and ventral plate (ventral view). (D)–(F) Styles (right side; D, medial view; E, ventrolateral view; F, lateral view). (G) Ventral plate and median sclerite (lateral view). (H) Ventral plate (caudal view). (I) Median sclerite (caudal view). (J) Paramere (left side; caudal view). (K) Aedeagal membrane (caudal view). (L) and (M) Abdominal segment 10 and cerci (right side; L, lateral view; M, caudal view). Scale bars: 0.1 mm for B; 0.02 mm for A and C–M.

columns and 16 horizontal rows. Face black, whitish-gray pruinose, shiny when illuminated at certain angles. Clypeus black, whitish-gray pruinose, shiny when illuminated at certain angles, and moderately covered with yellow short pubescence interspersed with several dark-brown longer hairs on surface of ventral half on each side. Antenna composed of scape, pedicel and nine flagellomeres, dark brown to brownish-black except scape, pedicel and base of first flagellomere yellow; first flagellomere elongate, 1.5–1.8 times as long as second one. Maxillary palp with five segments, light brown except third segment dark brown, proportional lengths of third, fourth, and fifth segments 1.0:1.1:2.4; third segment (Fig. 2A) somewhat widened apically; sensory vesicle (Fig. 2A) globular or ellipsoidal, small, 0.15–0.19 times length of third segment, and with small opening. **Thorax.** Scutum brownish black, shiny on shoulders, along lateral margins and on pre-scutellar area, when illuminated at certain angles, densely covered with golden-yellow short pubescence. Scutellum dark brown, with golden

yellow short pubescence and dark-brown long upright hairs. Postnotum brownish black, white pruinose and shiny when illuminated at certain angles, and bare. Pleural membrane bare. Katepisternum dark brown to brownish black, shiny when illuminated at certain angles, moderately covered with pale and dark short hairs. **Legs.** Foreleg: coxa and trochanter light to medium brown; femur light brown with apical cap dark brown; tibia dark brown except extreme base yellow and middle large portion light brown, and with yellow short pubescence (brightly shiny when illuminated at certain angles) on outer surface of basal five-sixths; tarsus brownish black; basitarsus slightly flattened, 7.6–7.8 times as long as greatest width. Midleg: coxa dark brown except posterolateral surface brownish black; trochanter light to medium brown; femur light brown with apical cap dark brown (though extreme tip somewhat paler); tibia medium to dark brown except extreme base yellow, and with golden yellow short pubescence on posterior surface of basal one-third; tarsus dark brown to brownish black. Hind leg: coxa medium

brown; trochanter dark yellow; femur medium brown with apical cap dark brown (though extreme tip yellow); tibia light brown on basal two-fifths except base yellow and subbasal area medium brown, and dark brown to brownish black on apical three-fifths, and covered with golden-yellow short pubescence on posterior surface of little more than basal half; tarsus dark brown except basal half to two-thirds of basitarsus and basal half of second tarsomere grayish light brown; basitarsus (Fig. 2B) narrow, nearly parallel-sided, 5.0–5.3 times as long as wide, and 0.62–0.67 and 0.65–0.70 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 2B) well developed, nearly as long as width at base, and 0.5 times as wide as greatest width of basitarsus; pedisulcus (Fig. 2B) well developed. **Wing.** Length 1.8 mm. As in female except subcosta bare. **Halder.** Grayish with basal portion darkened. **Abdomen.** Basal scale brownish black, with fringe of light to dark brown long fine hairs. Dorsal surface of abdomen medium brown to brownish black, covered with light-brown short to long stout hairs; segments 2, 6 and 7 each with pair of shiny dorsolateral patches, when illuminated at certain angles; ventral surface dark brown except segment 2 grayish white. **Genitalia.** Coxite in ventral view (Fig. 2C) nearly rectangular, 1.7 times as long as its greatest width. Style in ventral view (Fig. 2C) 0.8 times length of coxite, slender, curved inward, with apical spine; style in medial view (Fig. 2D) gently curved dorsally, gradually tapered to middle, then nearly parallel-side; style in ventrolateral view (Fig. 2E) gradually tapered to middle, abruptly narrowed to apical one-fifth, with narrowest part nearly half as wide as greatest width at base, then slightly widened toward apex, with truncated apex; style in lateral view (Fig. 2F) with apical portion twisted dorsally. Ventral plate in ventral view (Fig. 2C) transverse, 0.7 times as long as greatest width at base, with body nearly parallel-sided from base to middle, then tapered posteriorly, with anterior margin produced anteromedially, posterior margin shallowly concave, and densely covered with microsetae on ventral surface except anterolateral areas bare; basal arms of moderate length, somewhat divergent, then slightly convergent apically; ventral plate in lateral view (Fig. 2G) with posterior portion of body slightly produced posteroventrally; ventral plate in caudal view (Fig. 2H) rounded ventrally (width: height 1.0:0.4), moderately covered with microsetae on most of posterior surface. Median sclerite (Fig. 2G, I) plate-like, arising from near anterior tip of ventral plate, and directed dorsally. Paramere (Fig. 2J) with four relatively longer hooks and several shorter ones. Aedeagal membrane (Fig. 2K) moderately covered with microsetae, and with no sclerotized dorsal plate. Ventral surface of abdominal segment 10 (Fig. 2L, M) without distinct hair near posterior margin on each side. Cercus (Fig. 2L, M) rounded, somewhat produced ventrally, with 9–15 distinct hairs.

**Pupa.** Body length 2.3–2.4 mm. **Head.** Integument yellow, moderately covered with small round tubercles except ventral surface of face and antennal sheaths bare; antennal sheath without any protuberances; frons with three pairs of unbranched long trichomes with straight or coiled apices (Fig. 3A); face with pair of unbranched long trichomes with straight or coiled apices (Fig. 3B); three frontal trichomes on each side arising close together, subequal in length to one another and somewhat longer than facial one. **Thorax.** Integument yellow, sparsely covered with small round tubercles, with three long anterodorsal trichomes with coiled apices (Fig. 3C), two anterolateral trichomes (anterior one short with straight apex, posterior one long with coiled apex) (Fig. 3D), one short mediolateral trichome with straight apex (Fig. 3E), and three ventrolateral trichomes with straight apices (one medium-long, two short) on each side (Fig. 3F); all trichomes unbranched. Gill (Fig. 3G) composed of 10 slender thread-like filaments, arranged as [(1 + 2) + 1] + (1 + 1 + 2) + 2 or (3 + 1) + (1 + 3) + 2 from dorsal to ventral, with extremely short common basal stalk having basal fenestra at base; dorsal group with short stalk directed upward or backward, with two individual and two paired filaments or one individual and three filaments arising at same level; middle group with short stalk directed forward and little upward, consisting of two individual and two paired filaments or one individual and three filaments

arising at same level; ventral pair with short stalk directed downward and forward; all filaments yellow to ochreous, slightly tapered toward apices; slightly becoming longer from dorsal to ventral, with inner filament of ventral pair longest (1.5–1.6 mm), and subequal in thickness except inner filament of ventral pair 1.5 times as thick as others, when compared basally; cuticle of all filaments with weakly-defined annular ridges and furrows, and densely covered with minute tubercles. **Abdomen.** Dorsally, segments 1 and 2 entirely light brown, segments 3–5 each light brown on anterior half along anterior margin, and segment 9 yellow entirely; segment 1 with few to several microtubercles near anterior margin and one unbranched slender medium-long hair-like seta on each side; segment 2 with several to dozen microtubercles near anterior margin, one unbranched slender medium-long hair-like seta and five short somewhat spinous setae submedially near posterior margin on each side; segments 3 and 4 each with four hooked spines and one short somewhat spinous seta near posterior margin on each side; segment 5 with four short setae on each side; segments 6–8 each with two short setae near posterior margin on each side; segments 6–9 each spine-combs in transverse row, comb-like groups of minute spines near anterior margin; segment 9 with pair of triangular terminal hooks (Fig. 3H). Ventrally, segment 4 with one unbranched hook and few unbranched short setae on each side; segment 5 with pair of bifid hooks submedially and few unbranched 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 unbranched short slender setae on each side; segments 4–8 with comb-like groups of minute spines. Each side of segment 9 with three grapnel-shaped hooklets. **Cocoon.** Wall pocket-shaped, moderately woven, extended ventrolaterally; anterior margin somewhat thickly woven, without anterodorsal bulge or projection; posterior half with floor roughly or moderately woven; individual threads invisible; 3.0–3.1 mm long by 2.0–2.1 mm wide.

**Mature larva.** Body length 4.0–4.6 mm. Body light grayish. Cephalic apotome yellowish, and moderately covered with colorless and somewhat darkened minute setae; head spots indistinct or faintly positive. Lateral surface of head capsule yellowish except eye-spot region whitish; eyebrow faintly defined; two relatively large spots and one small spot near posterior margin faintly positive or indistinct; one or two small spots below eye-spot region indistinct. Ventral surface of head capsule yellowish except darkened area near posterior margin on each side of postgenal cleft; one elongate spot and one round spot on each side of postgenal cleft indistinct. Antenna composed of three segments and apical sensillum, somewhat longer than stem of labral fan; proportional lengths of first, second, and third segments 1.0:1.0–1.1:0.8–1.0. Labral fan with 24–27 main rays. Mandible (Fig. 4A) 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 acute angle against mandible on apical side; supernumerary serrations absent. Hypostoma (Fig. 4B) with row of nine apical teeth, of which median tooth slightly longer than each corner tooth; lateral margin smooth; four or five hypostomal bristles per side lying parallel to lateral margin. Postgenal cleft (Fig. 4C) deep, 6.3–6.6 times length of postgenal bridge. Cervical sclerite composed of two small yellow rod-like pieces, not fused to occiput, widely separated medially from each other. Cuticle of thoracic segments 1–3 and abdominal segments 1 and 2 sparsely covered with colorless unbranched setae; cuticle of abdominal segments 3 and 4 sparsely covered with colorless unbranched setae and dark branched setae dorsally, and cuticle of abdominal segments 5–8 densely covered with dark branched setae (divided into 3–7 branches) (Fig. 4D) interspersed with colorless unbranched setae dorsally and dorsolaterally; last abdominal segment moderately covered with colorless unbranched setae on each side of anal sclerite. Rectal scales undiscernible. Rectal papilla compound, each of three lobes with 9–14 finger-like secondary lobules. Anal sclerite of usual X-form, with anterior arms 0.8 times as long as posterior ones, broadly sclerotized at base; accessory sclerite absent. Last

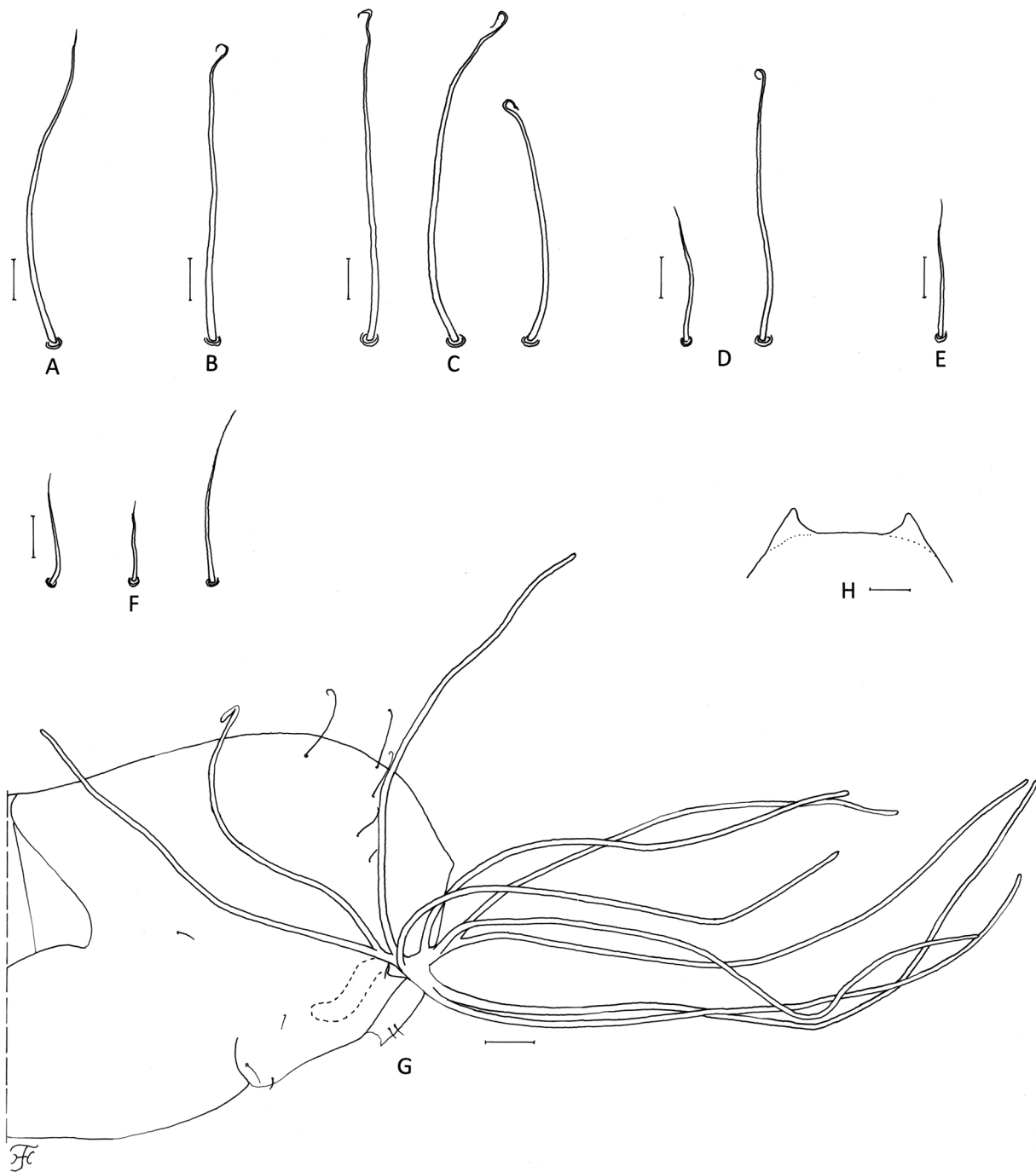


Fig. 3. Pupa of *Simulium (Gomphostilbia) chiangraiense* sp. nov. (A) Frontal trichomes. (B) Facial trichome. (C)–(F) Thoracic trichomes (C, anterodorsal; D, anterolateral; E, mediolateral; F, ventrolateral). (G) Thorax and gill filaments (right side; outer view). (H) Terminal hooks (caudal view). Scale bars: 0.1 mm for G; 0.02 mm for A–F and H.

abdominal segment expanded ventrolaterally forming double bulges on each side, visible as large conical ventral papilla when viewed from side. Posterior circlet with 77–79 rows of up to 14 or 15 hooklets per row.

**Type material.** HOLOTYPE. Female (with associated pupal exuviae and cocoon) (in 80% ethanol), reared from pupa, collected from a small stream (width 1 m, depth 2.5 cm, bottom sandy, water temperature 20.8°C, pH 6.5, exposed to the sun, elevation 1321 masl, 19°53'55.4" N, 99°35'59.4" E), moderately flowing, Pang Khon village, Muang District, Chiang Rai Province, northern Thailand (Fig. 8), 15–VII-2016, by W. Srisuka. PARATYPES: Three females, two males (with associated pupal exuviae and cocoon), all reared from pupae, and four mature larvae (in 80% ethanol), same data as those of the holotype.

**Biological notes.** The pupae and larvae of this new species were collected from grasses trailing in the water. Associated species were *S. (G.) asakoe*, *S. (Simulium) fenestratum* Edwards, and *S. (S.) nakhonense* Takaoka & Suzuki. The biting habits of the females remain unknown.

**Etymology.** The species name *chiangraiense* refers to the province name, Chiang Rai, where this new species was collected.

**Remarks.** This new species is assigned to the *S. batoense* species-group of the subgenus *Gomphostilbia*, redefined by Takaoka (2012), based on the antenna with nine flagellomeres, pleural membrane bare, hind tibiae mostly darkened (Fig. 1C), spermatheca without pigmented neck (Fig. 1J), male hind basitarsus slender and parallel-sided (Fig. 2C), ventral plate slightly produced ventrally (Fig. 2G) (its ratio of the height against the greatest width is 0.36).

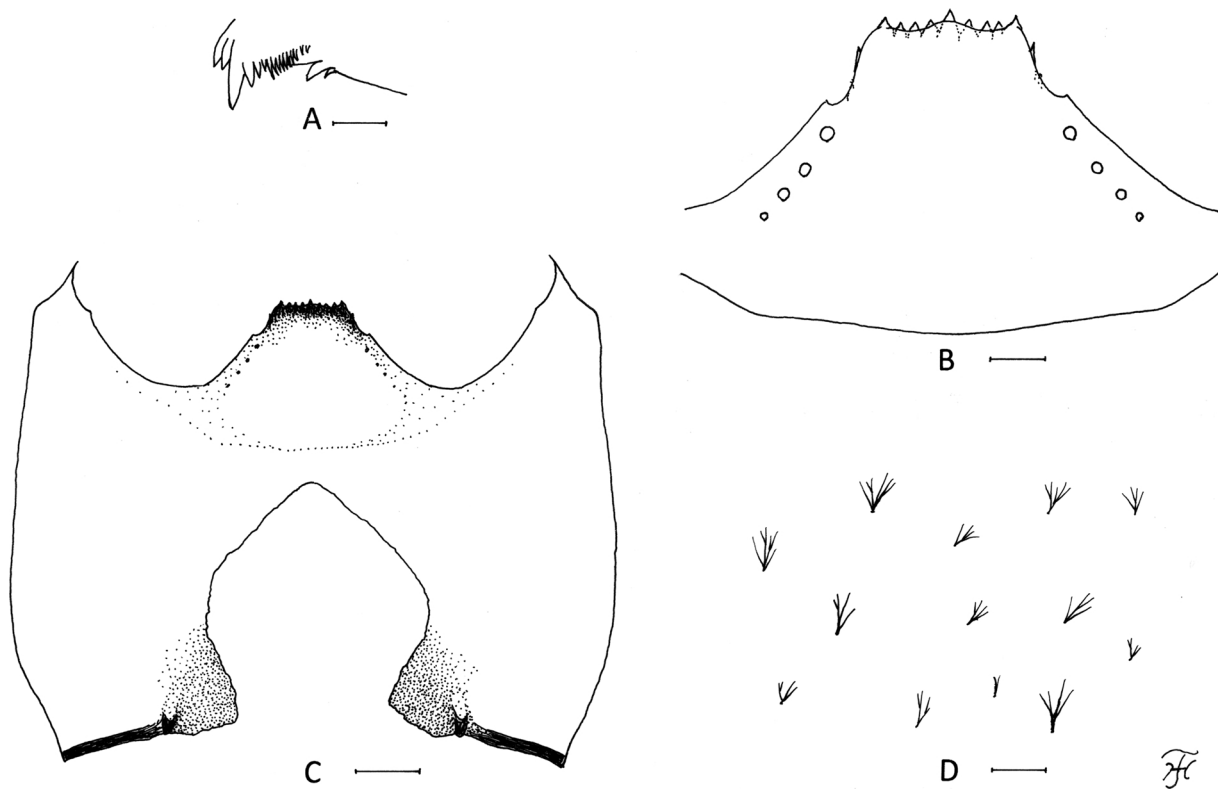


Fig. 4. Larva of *Simulium (Gomphostilbia) chiangraiense* sp. nov. (A) Apical tip of mandible. (B) Hypostoma. (C) Head capsule showing postgenal cleft (ventral view). (D) Dark minute setae on dorsum of abdominal segment 7. Scale bars: 0.1 mm for C; 0.02 mm for A, B and D.

Among six subgroups in the *S. batoense* species-group (Takaoka, 2012), *S. (G.) chiangraiense* sp. nov. is placed in the *S. decuplum* subgroup by having the pupal gill with 10 short slender filaments (Fig. 3G). All the five known species of this subgroup differ from this new species as follows: *Simulium (G.) bannaense* differs by having the female claw with a small subbasal tooth and the smaller number of male upper-eye facets in 12 vertical columns and 13 horizontal rows (Chen and Zhang, 2003); *Simulium (G.) chongqingense* differs by having the female genital fork with an inflated apex (Zhu and Wang, 1995); *Simulium (G.) jiulianshanense* is distinguished by lacking grapple-shaped hooklets on the pupal abdomen (Kang et al., 2007); *Simulium (G.) decuplum* differs by the shorter female sensory vesicle (0.54 times the length of the third maxillary palpal segment), the longer female claw tooth (0.61 times the length of the claw), the smaller number of male upper-eye facets in 14 or 15 vertical columns and 14 or 15 horizontal rows, the shorter gill filaments (ventral paired filaments ca. 0.7 mm), pale dorsal surface of pupal abdominal segments 1 and 2, and larval thorax moderately covered with dark multibranched setae (Takaoka and Davies 1995; Takaoka, unpublished data); *Simulium (G.) lamdongense* differs by the shorter female sensory vesicle (0.43–0.45 times as long as the third maxillary palpal segment), the slender male fore basitarsus (8.8 times as long as its greatest width), pale dorsal surface of pupal abdominal segments 1 and 2, larval thorax moderately covered with dark multibranched setae dorsally, and labral fan with 36–39 primary rays (Takaoka et al., 2015).

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

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This new species is similar to *S. (G.) chiangraiense* sp. nov. in many characters except the following characters:

**Female.** Body length 2.0 mm. **Head.** Frontal ratio 1.7:1.0:2.4; frons:head ratio 1.0:4:9. Clypeus densely covered with yellowish-white recumbent pubescence interspersed with four or five dark longer hairs

on each side. Labrum 0.5 times as long as clypeus. Maxillary palp: proportional lengths of third, fourth, and fifth segments 1.0:1.1:2.1; sensory vesicle elongate, 0.58 times as long as third segment. Maxillary lacinia with 14 or 15 inner and 17 outer teeth. Mandible with 25–28 inner teeth and four or five outer teeth at some distance from apex. **Legs.** Foreleg: basitarsus somewhat dilated, 6.3 times as long as its greatest width. Hind leg: basitarsus 6.7 times as long as wide, and 0.6 and 0.5 times as wide as greatest width of tibia and femur, respectively; calcipala slightly longer than wide, and 0.56 times as wide as greatest width of basitarsus; claw with large basal tooth 0.52 times length of claw. **Wing.** Length 1.8 mm. **Terminalia.** Sternite 8 with 15 or 16 medium-long to long stout hairs together with three slender short hairs on each side. Ovipositor valve with inner margin sinuous. Paraproct in ventral view with five sensilla on anteromedial surface; paraproct in lateral view much produced ventrally, 0.54 times as long as wide, with 18–21 medium-long to long stout hairs on ventral and lateral surfaces. Cercus in lateral view short, rounded posteriorly, 0.5 times as long as wide. Spermatheca ellipsoidal, 1.4 times as long as greatest width.

**Male.** Body length 2.3 mm. **Head.** Upper eye consisting of large facets in 17 vertical columns and 17 horizontal rows. Antenna dark brown to brownish black except scape, pedicel and base of first flagellomere yellow, though dorsal surface of pedicel light brown; first flagellomere elongate, 1.7 times as long as second one. Maxillary palp: proportional lengths of third, fourth, and fifth segments 1.0:1.1:2.3; sensory vesicle globular or ellipsoidal, small, 0.2 times length of third segment. **Legs.** Fore basitarsus 6.56 times as long as greatest width. Hind basitarsus 4.75 times as long as wide, and 0.62 and 0.66 times as wide as greatest width of hind tibia and femur, respectively; calcipala 0.4 times as wide as greatest width of basitarsus. **Genitalia.** Coxite in ventral view (Fig. 5A) nearly rectangular, 1.6 times as long as its greatest width. Style in ventral view (Fig. 5A) 0.85 times length of coxite. Ventral plate in ventral view (Fig. 5A) transverse, 0.6 times as long as greatest width at base, with body slightly narrowed from base to middle, suddenly narrowed in middle, then tapered posteriorly; basal

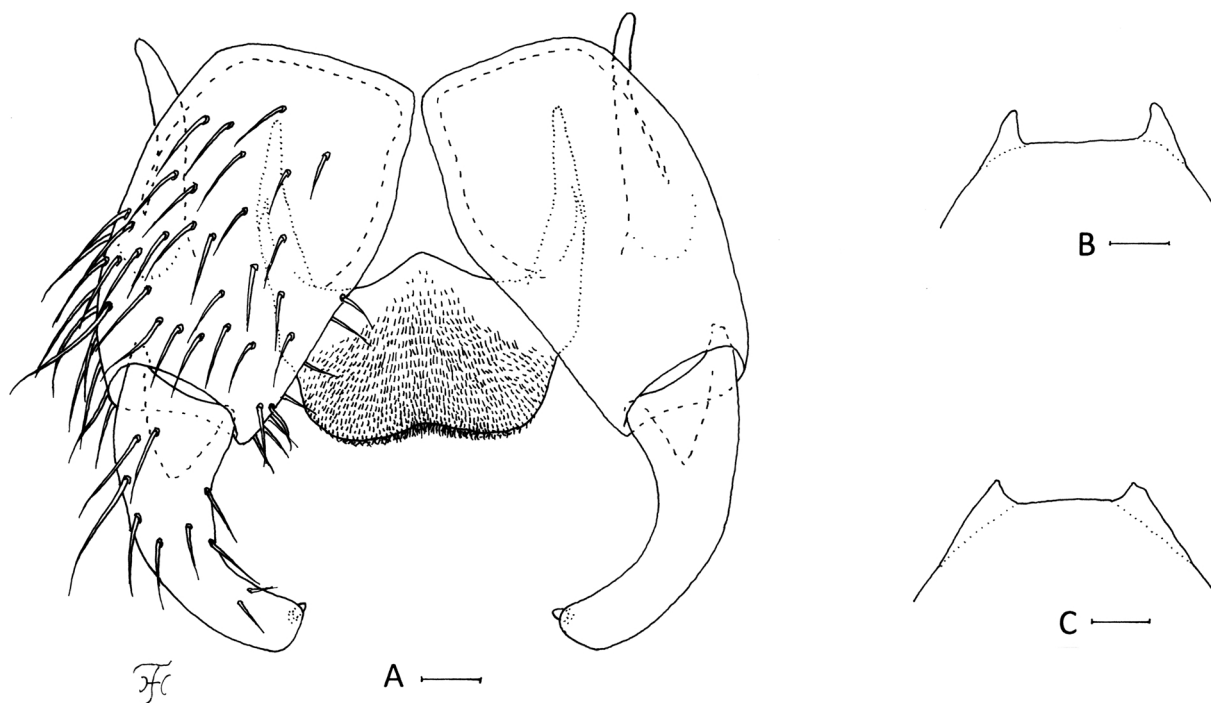


Fig. 5. Male and pupa of *Simulium (Gomphostilbia) pamiangense* sp. nov. (A) Male genitalia (ventral view). (B) and (C) Pupal terminal hooks (caudal view; B, triangular; C, broad, plate-like). Scale bars: 0.02 mm for A–C.

arms of moderate length, slightly divergent from base to middle, then nearly parallel-sided. Cercus rounded, somewhat produced ventrally, with 13 or 14 hairs.

**Pupa.** Body length 2.5 mm. Gill filaments arranged (2 + 1 + 1) + (1 + 1 + 2) + 2 or 3 + 1 + (1 + 1 + 2) + 2 from dorsal to ventral or from posterior to anterior. Terminal hooks are triangular (Fig. 5B), though they are wide, plate-like (Fig. 5C) in one of four pupae examined.

**Mature larva.** Body length 3.8–4.0 mm. Head spots obscured. Eyebrow indistinct. Proportional lengths of first, second, and third segments of antenna 1.00:0.83–1.90:0.88–0.96. Labral fan with 32–36 primary rays. Postgenal cleft deep, reaching posterior margin of hypostoma in three larvae (approaching but not reaching to posterior margin of hypostoma leaving narrow postgenal bridge in one larva, similar to Fig. 4C). Cuticle of thoracic segments 1–3 and abdominal segments 1 and 2 sparsely covered with colorless unbranched setae and dark multibranched setae dorsally; cuticle of abdominal segments 3 and 4 moderately covered with colorless unbranched setae and dark multibranched setae dorsally, and cuticle of abdominal segments 5–8 densely covered with dark branched setae (divided into 5–12 branches) interspersed with colorless unbranched setae dorsally and dorsolaterally. Rectal papilla compound, each of three lobes with 7–9 finger-like secondary lobules. Anal sclerite of usual X-form, with anterior arms 0.9–1.0 times as long as posterior ones. Posterior circlet with 77–85 rows of up to 14 hooklets per row.

**Type material.** HOLOTYPE. Female (with associated pupal exuviae and cocoon) (in 80% ethanol), reared from pupa, collected from a small stream (width 30 cm, depth 5 cm, bottom sandy, water temperature 22.6° C, pH 6.3, exposed to the sun, elevation 1097 masl, 18°50′03.7″ N, 99°22′32.2″ E), moderately flowing, Pa Miang village, Chae Hom District, Lampang Province, Thailand (Fig. 8), 9-VIII-2016, by W. Srisuka. PARATYPES: One male (with associated pupal exuviae and cocoon), reared from pupa, two pupae and five mature larvae (in 80% ethanol), same data as those of the holotype.

**Biological notes.** The pupae and larvae of this new species were collected from grasses trailing in the water. Associated species were *S. (G.) asakoae*, *S. (Nevermannia) fruticosum* Takaoka & Choochote, and *S.*

(*S.*) *fenestratum*. The biting habits of the females remain unknown.

**Etymology.** The species name *pamiangense* refers to the village name, Pa Miang, where this new species was collected.

**Remarks.** This new species is similar to *S. (G.) chiangraiense* sp. nov. but is distinguished from the latter species in the male by the length of the fore basitarsus relative to its greatest width (6.6 in this new species versus 7.6–7.8 in *S. (G.) chiangraiense* sp. nov.), and the shape of the ventral plate (anterior half of the body narrowed posteriorly in this new species versus parallel-sided in *S. (G.) chiangraiense* sp. nov.), and in the larva by the number of the primary rays of the labral fan (32–36 in this new species versus 24–27 in *S. (G.) chiangraiense* sp. nov.).

Of the five known species in the *S. decuplum* subgroup, *S. (G.) decuplum* differs from this new species by the longer female claw tooth (0.61 times the length of the claw), smaller number of the male upper-eye facets in 14 or 15 vertical columns and 14 or 15 horizontal rows, shorter gill filaments (ventral paired filaments ca. 0.7 mm), pale dorsal surface of pupal abdominal segments 1 and 2, and larval thorax moderately covered with dark multibranched setae (Takaoka and Davies, 1995; Takaoka, unpublished data), and *S. (G.) lamdongense* differs by the shorter female sensory vesicle (0.43–0.45 times as long as the third maxillary palpal segment), slender male fore basitarsus (8.8 times as long as its greatest width), pale dorsal surface of pupal abdominal segments 1 and 2, and larval thorax moderately covered with dark multibranched setae dorsally (Takaoka et al., 2015). All the three known Chinese species of this subgroup also differ from this new species by the characters as noted under *S. (S.) chiangraiense* sp. nov.

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

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This new species is similar to *S. (G.) chiangraiense* sp. nov. in many characters except the following characters:

**Female.** Body length 1.6–1.9 mm. **Head.** Frontal ratio 1.6:1.0:1.7–1.8; frons:head ratio 1.0:4.0–4.3. Labrum 0.5–0.6 times as long as clypeus. Antenna composed of scape, pedicel and nine flagellomeres, dark brown except scape and pedicel yellow and basal extreme of first flagellomere light brown. Maxillary palp: proportional

lengths of third, fourth, and fifth segments 1.0:1.1:1.8–1.9; sensory vesicle 0.56–0.61 times as long as third segment and with medium-sized opening. Maxillary lacinia with 12–16 inner and 17–20 outer teeth. Mandible with 31–33 inner teeth and five to seven outer teeth at some distance from apex. **Legs.** Fore basitarsus 5.6–6.8 times as long as its greatest width. Hind basitarsus 5.6–6.9 times as long as wide, and 0.6–0.7 and 0.5–0.7 times as wide as greatest width of hind tibia and femur, respectively; calypala well developed, slightly longer than wide, and 0.5 times as wide as greatest width of basitarsus; claw with large basal tooth 0.56 times length of claw. **Wing.** Length 1.6–1.8 mm. **Abdomen.** Basal scale yellow. Dorsal surface of abdomen medium to dark brown except segment 2 yellow though tergite somewhat darkened. **Terminalia.** Sternite 8 with 17–20 medium-long to long stout hairs together with one to four slender short setae on each side. Paraproct in ventral view with three or four sensilla on anteromedial surface; paraproct in lateral view 0.54–0.60 times as long as wide, with 15–18 medium-long to long stout hairs on ventral and lateral surfaces. Cercus in lateral view 0.51 times as long as wide. Spermatheca ellipsoidal, 1.5–1.6 times as long as greatest width.

**Male.** Body length 2.1 mm. **Head.** Upper-eye large facets in 16 or 17 vertical columns and 16 horizontal rows. Antenna: first flagellomere 1.8 times as long as second one. Maxillary palp: proportional lengths of third, fourth, and fifth segments 1.0:1.1:2.2; sensory vesicle ellipsoidal, 0.29 times length of third segment, and with small opening. **Legs.** Fore basitarsus 7.9–8.6 times as long as greatest width. Hind basitarsus 5.4–5.6 times as long as wide, and 0.6 and 0.6–0.7 times as wide as greatest width of tibia and femur, respectively. **Wing.** Length 1.6–1.7 mm. **Genitalia.** Style in ventral view 0.8 times length of coxite. Cercus with 13 hairs.

**Pupa.** Body length 2.0–2.3 mm. **Thorax.** Integument yellow, sparsely to moderately covered with small round tubercles, with three long anterodorsal trichomes with coiled apices (Fig. 6A), two anterolateral trichomes (anterior one medium-long with straight apex, posterior one long with coiled apex) (Fig. 6A), one medium-long mediolateral trichome with straight apex (Fig. 6A), and three ventrolateral trichomes with straight apices (one medium-long, two short) on each side (Fig. 6A); all trichomes unbranched. Gill (Fig. 6A) composed of 10 slender thread-like filaments, arranged in three groups as  $(1 + 2) + (1 + 1 + 1 + 2) + 2$  or rarely  $[(1 + 2) + 1] + (1 + 1 + 2) + 2$  from dorsal to ventral, with short common basal stalk having basal fenestra at base; dorsal group with short stalk directed upward, with

one individual and two paired filaments; middle group with extremely short stalk directed forward, consisting of three individual and two paired filaments; ventral pair with short stalk directed downward and forward; all filaments yellow to ochreous, slightly tapered toward apices; gradually becoming longer from dorsal to ventral, with inner filament of ventral pair longest (0.7–1.0 mm), and subequal in thickness except two filaments of ventral pair slightly thicker than others (outer filament of ventral pair somewhat thicker than counter filament, e.g. 1.3 times as thick as counter filament in some pupae), when compared basally; cuticle of all filaments nearly smooth although basal half of filaments with weakly-defined annular furrows, and densely covered with minute tubercles. **Abdomen.** Dorsally, segments 1, 2 and 9 pale yellow. **Cocoon.** 2.5–2.9 mm long by 1.8–2.0 mm wide.

**Mature larva.** Body length 3.6–4.2 mm. Body grayish with ochreous band on thoracic segment 1. Head capsule moderately to densely covered with minute unpigmented setae though sparsely on ventral surface. Head spots indistinct or faintly negative. Antenna composed of three segments and apical sensillum, somewhat longer than stem of labral fan; proportional lengths of first, second, and third segments 1.0:0.8–0.9:0.8. Labral fan with 27–31 main rays. Postgenal cleft deep, reaching posterior border of hypostoma. Cervical sclerite indistinct. Cuticle of thoracic segments 1–3 and abdominal segments 1–9 densely covered with black, branched setae (divided into 5–13 branches with apical portions paler) (Fig. 6B) dorsally and dorsolaterally; last abdominal segment densely covered with unbranched colorless setae on posterior half of each side of anal sclerite, and base of ventral papilla. Rectal scales minute, unpigmented. Rectal papilla compound, each of three lobes with 7 or 8 finger-like secondary lobules. Anal sclerite of usual X-form, with anterior arms nearly as long as posterior ones. Posterior circlet with 78–82 rows of up to 14 or 15 hooklets per row.

**Type material.** HOLOTYPE. Female (with associated pupal exuviae and cocoon) (in 80% ethanol), reared from pupa, collected from a small stream (width 45 cm, depth 24 cm, bottom sandy, water temperature 20.2°C, partially shaded, elevation 1314 masl, 18°31'01.9" N, 98°28'17.3" E), moderately flowing, Mae Klang Watershed Management Unit, Doi Inthanon National Park, Chiang Mai Province, northern Thailand (Fig. 8), 17–VII-2017, by W. Srisuka. PARATYPES: 11 females, six males (with associated pupal exuviae and cocoon), all reared from pupae, and eight mature larvae (in 80% ethanol), same data as those of the holotype.

**Biological notes.** The pupae and larvae of this new species were



Fig. 6. Pupa and larva of *Simulium (Gomphostilbia) maeklangense* sp. nov. (A) Head and anterior half of thorax showing gill and trichomes (right side; outer view). (B) and (C). Dark multi-branched minute setae on dorsum of abdominal segment 7 (B, *S. (G.) maeklangense* sp. nov.; C, *S. (G.) huaikaeoense* sp. nov.). Scale bars: 0.1 mm for A; 0.02 mm for B and C.

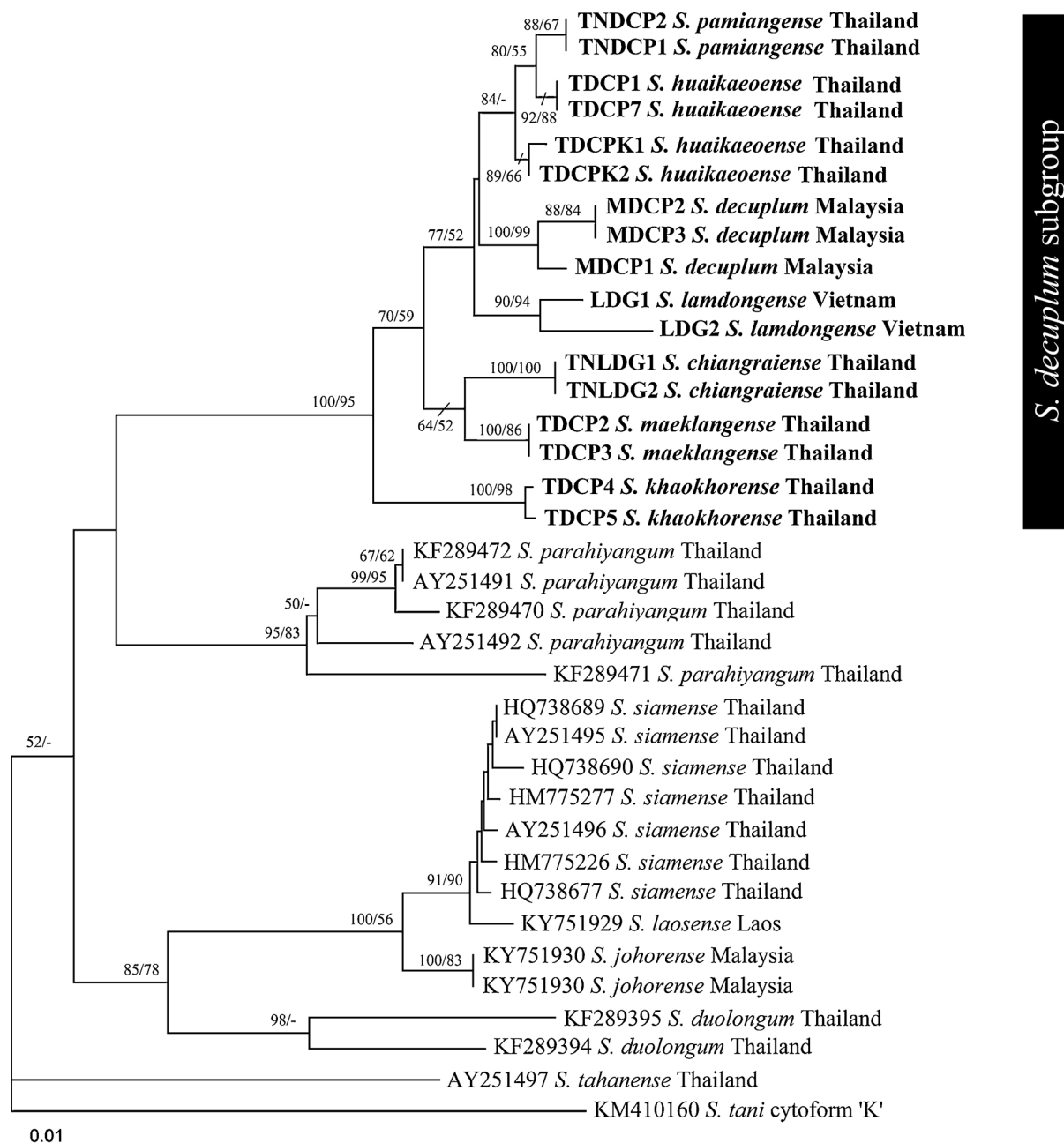


Fig. 7. Neighbor-joining phylogenetic tree of five new species and related species of the *Simulium batoense* species-group inferred from the mitochondrial-encoded COI gene sequences. Bootstrap values [NJ/ML] are shown on the branches. Sequences generated from this study are in bold.

collected from grasses trailing in the water. Associated species were *S. (G.) asakoe*, *S. (G.) chiangdaoense* Takaoka & Srisuka, *S. (G.) inthanonense* Takaoka & Suzuki, and *S. (N.) fruticosum*. The biting habits of the females remain unknown.

**Etymology.** The species name *maeklangense* refers to the locality name, Mae Klang, where this new species was collected.

**Remarks.** This new species is distinguished from the two new species, *S. (G.) chiangraiense* and *S. (G.) pamiangense*, by the shorter pupal gill filaments (0.7–1.0 mm long), pale dorsal surface of pupal abdominal segments 1 and 2, and larval thoracic and abdominal segments densely covered with dark multibranched setae dorsally and dorsolaterally.

Of the five known species in the *S. decuplum* subgroup, *S. (G.) decuplum* differs from *S. (G.) maeklangense* sp. nov. by the female mandible with about 24 inner teeth, female abdominal segment 2 with a yellow tergal plate, and smaller number of male upper-eye facets in 14

or 15 vertical columns and 14 or 15 horizontal rows (Takaoka, unpublished data), and *S. (G.) lamdongense* differs by the shorter female sensory vesicle (0.43–0.45 times as long as the third maxillary palpal segment) and larval labral fan with 36–39 primary rays (Takaoka et al., 2015). All three Chinese species, *S. (G.) bannaense*, *S. (G.) chongqingense* and *S. (G.) jiulianshanense*, differ from this new species as noted under *S. (G.) chiangraiense* sp. nov.

***Simulium (Gomphostilbia) khaokhoense* Takaoka, Srisuka & Low sp. nov.**

(urn:lsid:zoobank.org:act:F7E59FC3-610A-4B72-B10D-916401FAC3EB)

**Female.** Body length 1.5–1.6 mm. Nearly as in female of *S. (G.) chiangraiense* sp. nov. except the following characters: **Head.** Frontal ratio 1.7–1.9:1.0:2.2–2.5; frons:head ratio 1.0:4.4–4.7. Labrum 0.5–0.6 times as long as clypeus. Maxillary palp: proportional lengths of third, fourth, and fifth segments 1.0:1.1:2.1; sensory vesicle elongate, 0.5

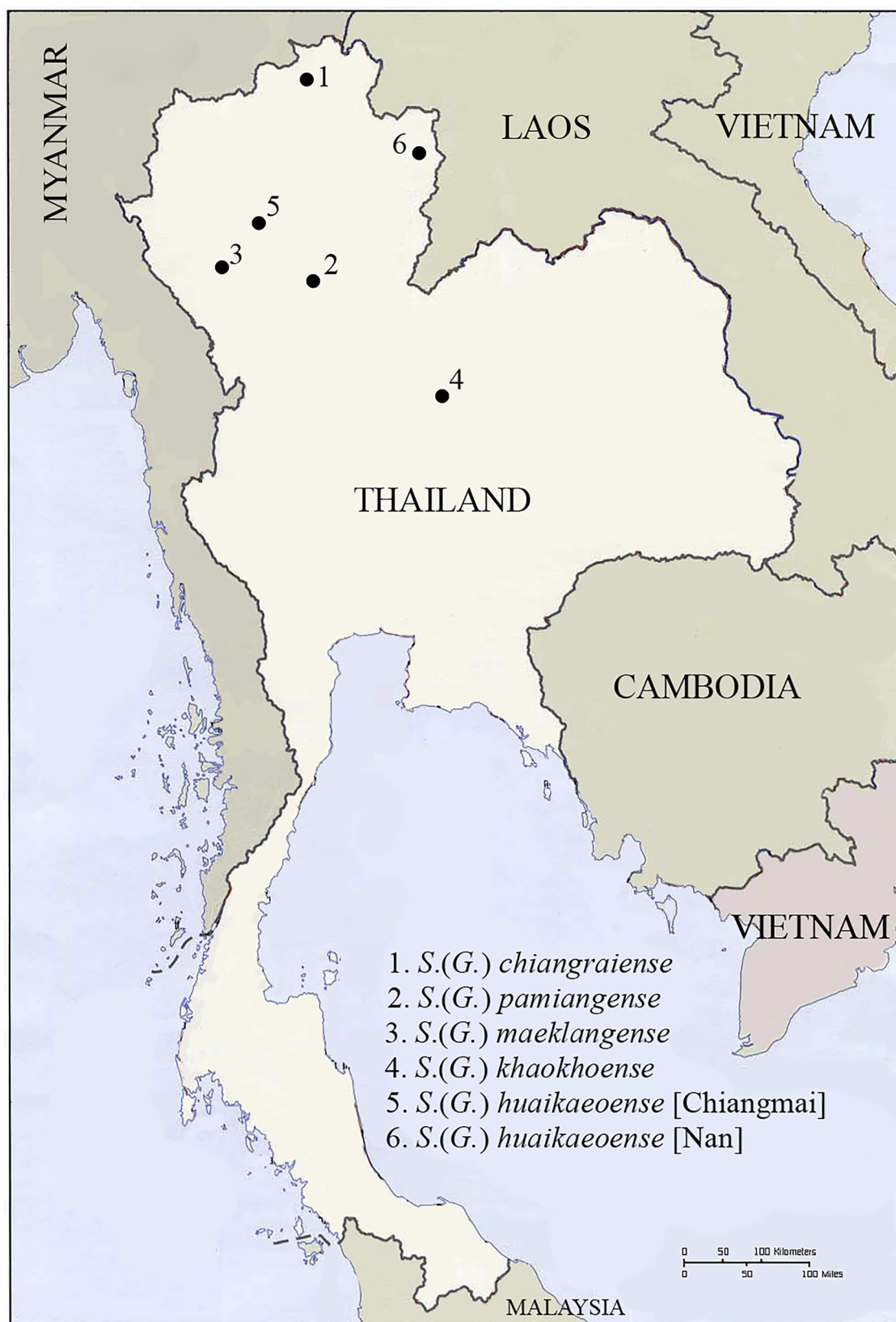


Fig. 8. Map of Thailand showing six geographical locations of five new species of the *Simulium decuplum* subgroup.

times as long as third segment and with medium-sized opening. Maxillary lacinia with 12–14 inner and 15–20 outer teeth. Mandible with 24 or 25 inner teeth and four or five outer teeth at some distance from apex. **Legs.** Foreleg: coxa and trochanter whitish yellow; femur

light brown except base whitish yellow and apical cap medium brown (though extreme tip yellow); basitarsus somewhat dilated, 5.8–5.9 times as long as its greatest width. Midleg: trochanter yellow; femur yellow to dark yellow except apical cap medium brown (though

Table 1

Uncorrected p distances (%) among seven members of the *Simulium decuplum* subgroup inferred from the mitochondrial-encoded COI gene sequences.

|                                     | 1         | 2         | 3         | 4         | 5         | 6         | 7 |
|-------------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|---|
| 1. <i>S. pamiangense</i> sp. nov.   | –         |           |           |           |           |           |   |
| 2. <i>S. huaikaeoense</i> sp. nov.  | 0.74–1.24 | –         |           |           |           |           |   |
| 3. <i>S. decuplum</i>               | 2.72–2.97 | 1.73–2.72 | –         |           |           |           |   |
| 4. <i>S. lamdongense</i>            | 2.97–3.71 | 2.48–3.47 | 2.72–4.46 | –         |           |           |   |
| 5. <i>S. chiangraiense</i> sp. nov. | 4.21      | 3.22–3.96 | 3.47–3.71 | 4.21–4.95 | –         |           |   |
| 6. <i>S. maeklangense</i> sp. nov.  | 2.97      | 2.97–3.71 | 3.71–4.46 | 3.47–5.20 | 2.23      | –         |   |
| 7. <i>S. khaokhoense</i> sp. nov.   | 3.96–4.21 | 4.46–4.95 | 5.45–6.44 | 4.95–6.68 | 5.45–5.69 | 3.47–3.71 | – |

extreme tip yellow); tibia light brown except base whitish yellow, and apical cap medium brown; tarsus medium brown except basal one-fourth of basitarsus yellow. Hind leg: femur yellow to dark yellow except apical cap dark brown (though extreme tip yellow); tarsus dark brown except basal two-thirds of basitarsus (though base light brown) and basal half of second tarsomere yellow; basitarsus 6.2–6.3 times as long as wide, and 0.7 and 0.5–0.6 times as wide as greatest width of tibia and femur, respectively; calcipala well developed, nearly as long as wide, and 0.55 times as wide as greatest width of basitarsus; claw with large basal tooth 0.53 times length of claw. **Wing.** Length 1.5–1.6 mm. **Abdomen.** Basal scale yellow, with fringe of yellowish-white fine hairs. Dorsal surface of abdomen light to medium brown except segment 2 yellow though tergal plate light brown. **Terminalia.** Sternite 8 bare medially, with 16–20 medium-long to long stout hairs together with three to five slender short hairs on each side. Ovipositor valves moderately covered with microsetae interspersed with zero to two short setae. Paraproct in ventral view with three or four sensilla on anteromedial surface; paraproct in lateral view much produced ventrally, 0.5 times as long as wide, with 15–17 medium-long to long stout hairs on ventral and lateral surfaces. Cercus in lateral view 0.47–0.55 times as long as wide. Spermatheca ellipsoidal, 1.71–1.75 times as long as greatest width.

**Male.** Body length 1.6–1.9 mm. Nearly as in male of *S. (G.) chiangraiense* sp. nov. except the following characters: **Head.** Upper-eye large facets in 14 vertical columns and 14 horizontal rows. Antenna: first flagellomere 1.8 times as long as second one. Maxillary palp: proportional lengths of third, fourth, and fifth segments 1.0:1.2:2.4; sensory vesicle globular, 0.26 times length of third segment, and with small opening. **Legs.** Foreleg: coxa and trochanter light brown; femur light brown with apical cap dark brown; tibia medium brown except extreme base yellow and middle large portion light brown; basitarsus 6.9–7.1 times as long as greatest width. Midleg: coxa dark brown except posterolateral surface brownish black; trochanter light brown; tibia medium brown except extreme base yellow; tarsus medium brown to dark brown. Hind leg: femur medium brown with base yellow and apical cap dark brown (though extreme tip yellow); tibia medium brown except base yellow and subbasal area dark brown, and apical one-third dark brown; basitarsus 5.7–5.8 times as long as wide, and 0.6 and 0.6 times as wide as greatest width of tibia and femur, respectively. **Wing.** Length 1.4–1.5 mm. **Genitalia.** Cercus with 9–12 distinct hairs.

**Pupa.** Body length 1.8–2.1 mm. Nearly as in *S. (G.) maeklangense* sp. nov. except the following characters: **Thorax.** Gill composed of 10 slender thread-like filaments, arranged in three groups as (2 + 1 + 1) + (1 + 1 + 2) + 2 or 3 + (1 + 1 + 1 + 2) + 2 or 3 + (1 + 1 + 3) + 2, or arranged in four groups as 3 + 1 + (1 + 1 + 2) + 2 or (1 + 2) + 1 + (1 + 1 + 2) + 2 from dorsal to ventral. **Cocoon.** 2.3–2.4 mm long by 2.0 mm wide.

**Mature larva.** Body length 3.4–3.5 mm. Nearly as in *S. (G.) maeklangense* sp. nov. except the following characters: **Head.** Antenna: proportional lengths of first, second, and third segments 1.00:0.78–0.83:0.90–1.00. Labral fan with 34–36 primary rays. **Abdomen.** Rectal papilla compound, each of three lobes with 6–10 finger-like secondary lobules. Anal sclerite of usual X-form, with anterior arms 0.80–0.85 as long as posterior ones. Posterior circlet with

71–75 rows of up to 13 or 14 hooklets per row.

**Type material.** HOLOTYPE. Female (with associated pupal exuviae and cocoon) (in 80% ethanol), reared from pupa, collected from a small stream (width 70 cm, depth 4 cm, bottom sandy, water temperature 24.2°C, partially shaded, elevation 540 masl, 16°32′42.9″ N, 101°02′17.8″ E), moderately flowing, Ton Nampasak Research Station, Khao Kho District, Phetchabun Province, central Thailand (Fig. 8), 27-VI-2017, by W. Srisuka. PARATYPES: Four females, five males (with associated pupal exuviae and cocoon), all reared from pupae, and four mature larvae (in 80% ethanol), same data as those of the holotype.

**Biological notes.** The pupae and larvae of this new species were collected from grasses trailing in the water. Associated species were *S. (S.) fenestratum*, *S. (S.) nakhonense* and *S. (S.) tani* Takaoka & Davies complex. The female biting habits remain unknown.

**Etymology.** The species name *khaokhoense* refers to the locality name, Khao Kho, where this new species was collected.

**Remarks.** This new species is distinguished from *S. (G.) chiangraiense* sp. nov. and *S. (G.) pamiangense* sp. nov. by the shorter wing length (1.4–1.6 mm), smaller number of male upper-eye large facets in 14 vertical columns and 14 horizontal rows, shorter pupal gill filaments (0.7–1.0 mm long), pale dorsal surface of pupal abdominal segments 1 and 2, and larval thorax and abdomen densely covered with black, multibranched setae dorsally and dorsolaterally.

This new species is similar to *S. (G.) maeklangense* sp. nov. but distinguished from it in the female by the lighter color of the mid and hind femora, and in the male by the smaller number of upper-eye facets in 14 vertical columns and 14 horizontal rows.

Of the five known species in the *S. decuplum* subgroup, *S. (G.) decuplum* is similar to this new species in many characters including the number of male upper-eye facets, but slightly differs in the female by the relative length of the height of the frons against its narrowest width (1.8), frons:head ratio (1.0:3.8), tergite 2 yellow, and larval labral fan with about 30 primary rays (Takaoka and Davies, 1995), and *S. (G.) lamdongense* differs in the female by the shorter sensory vesicle (0.43–0.45 times as long as the third maxillary palpal segment) and mid and hind femora mostly light brown, and in the male by the larger number of upper-eye facets in 16 or 17 vertical columns and 17 or 18 horizontal rows (Takaoka et al., 2015). All three known Chinese species, *S. (G.) bannaense*, *S. (G.) chongqingense* and *S. (G.) jiulianshanense*, differ from this new species as noted under *S. (G.) chiangraiense* sp. nov.

**Simulium (Gomphostilbia) huaikaeoense** Takaoka, Srisuka & Low sp. nov.

(urn:lsid:zoobank.org:act:14718B7F-A56A-4885-9002-E4BDA548672C)

This new species is similar to *S. (G.) khaokhoense* sp. nov. in many characters except the following characters:

**Female.** Body length 1.5–2.0 mm. **Head.** Frontal ratio 1.54–1.74:1.00:1.94–2.17; frons:head ratio 1.00:3.89–4.54. Labrum 0.56–0.59 times as long as clypeus. Maxillary palp: proportional lengths of third, fourth, and fifth segments 1.0: 1.0:1.7–1.9; sensory vesicle elongate, 0.52–0.63 times as long as third segment and with medium-sized opening. Maxillary lacinia with 13 inner and 16 outer teeth. Mandible with 25 inner teeth and one or two outer teeth at some distance from apex. **Legs.** Fore basitarsus somewhat dilated, 5.3 times as

long as its greatest width. Hind basitarsus 6.2 times as long as wide, and 0.61 and 0.55 times as wide as greatest width of tibia and femur, respectively; calcipala well developed, nearly as long as wide, and 0.51 times as wide as greatest width of basitarsus; claw with large basal tooth 0.55 times length of claw. **Wing.** Length 1.6–1.9 mm. Subcosta haired except near apex bare (bare on left side and with one hair medially on right side in one female). **Abdomen.** Dorsal surface of abdomen light to medium brown except segment 2 yellow, though tergal plate yellow or slightly darkened. **Terminalia.** Sternite 8 bare medially, with 11–15 medium-long to long stout hairs together with two or three slender short hairs on each side. Ovipositor valves moderately covered with microsetae interspersed with zero to two short hairs. Paraproct in ventral view with four to seven sensilla on anteromedial surface; paraproct in lateral view much produced ventrally, 0.48 times as long as wide, with 10–17 medium-long to long stout hairs on ventral and lateral surfaces. Cercus in lateral view 0.46 times as long as wide. Spermatheca ellipsoidal, 1.53 times as long as greatest width.

**Male.** Body length 1.8–2.0 mm. **Head.** Upper-eye large facets in 13 or 14 vertical columns and 14 or 15 horizontal rows. Antenna: first flagellomere 1.97 times as long as second one. Maxillary palp: proportional lengths of third, fourth, and fifth segments 1.0:1.3:2.5; sensory vesicle globular, 0.19–0.22 times length of third segment, and with small opening. **Legs.** Fore basitarsus 7.4 times as long as greatest width. Midleg: basitarsus medium brown with base pale. Hind basitarsus 5.4 times as long as wide, and 0.65 and 0.65 times as wide as greatest width of tibia and femur, respectively. **Wing.** Length 1.5–1.6 mm. **Genitalia.** Coxite in ventral view nearly rectangular, 1.53 times as long as its greatest width. Style in ventrolateral view 0.85 times length of coxite. Ventral plate in ventral view transverse, 0.55 times as long as greatest width at base, with body widest basally, slightly tapered posteriorly. Cercus with 12–14 distinct hairs.

**Pupa.** Body length 2.0 mm. Gill composed of 10 slender thread-like filaments, arranged in four groups as 3 + 1 + (3 + 1) + 2 or (1 + 2) + 1 + (1 + 1 + 2) + 2 from dorsal to ventral. **Cocoon.** 2.1–2.5 mm long by 1.7–2.2 mm wide.

**Mature larva.** Body length 3.2–3.6 mm. Antenna: proportional lengths of first, second, and third segments 1.00:0.86:0.88. Labral fan with 30 main rays. Cuticle of thoracic and abdominal segments densely covered with dark multibranched slender setae each with 6–10 branches (apical portions paler) dorsally and dorsolaterally, intermixed with dark multibranched thick setae with 6–12 branches (apical portions paler) (Fig. 6C) on abdominal segments 5–9. Rectal papilla compound, each of three lobes with 8 or 9 finger-like secondary lobules. Anal sclerite of usual X-form, with anterior arms nearly as long as posterior ones. Posterior circlet with 86 rows of up to 13 hooklets per row.

**Type material.** HOLOTYPE. Male (with associated pupal exuviae and cocoon) (in 80% ethanol), reared from pupa, collected from a stream (width 3.5 m, depth 35 cm, bottom sandy, water temperature 23.2°C, partially shaded, elevation 503 masl, 18°48'44.2" N, 98°56'21.3" E), moderately flowing, Huai Kaeo, Doi Suthep-Pui National Park, Chiang Mai Province, northern Thailand (Fig. 8), 18–VII-2017, by W. Srisuka. PARATYPES: One female, one male (both with associated pupal exuviae and cocoons), all reared from pupae, and three mature larvae (in 80% ethanol), same data as those of the holotype; three females, four males (all with associated pupal exuviae and cocoon), all reared from pupae, and five mature larvae (in 80% ethanol), collected from a stream (width 4 m, depth 30 cm, bottom sandy, water temperature 19.5°C, exposed to the sun, elevation 1192 masl, 19°11'18.3" N, 101°04'43.7" E), fast flowing, Namdan Village, Doi Phuoka National Park, Pua District, Nan Province, northern Thailand (Fig. 8), 25–VII-2017, by W. Srisuka.

**Biological notes.** The pupae and larvae of this new species were collected from grasses trailing in the water. Associated species was *S. (G.) parahiyangum* Takaoka & Sigit, *S. (S.) fenestratum*, *S. (S.) nakhoense* and *S. (S.) quinquestriatum* (Shiraki). The biting habits of the females remain unknown.

**Etymology.** The species name *huaikaeoense* refers to the locality name, Huai Kaeo, where this new species was collected.

**Remarks.** This new species is distinguished from *S. (G.) chiangraiense* sp. nov. and *S. (G.) pamiangense* sp. nov. by the smaller number of the male upper-eye large facets in 13 or 14 vertical columns and 14 or 15 horizontal rows, shorter pupal gill filaments (0.7–1.0 mm long), pale dorsal surface of pupal abdominal segments 1 and 2, and larval thorax and abdomen densely covered with black, multibranched setae dorsally and dorsolaterally.

This new species is distinguished from *S. (G.) maeklangense* sp. nov. in the female by the lighter color of the mid and hind femora, in the male by the smaller number of upper-eye facets in 13 or 14 vertical columns and 14 or 15 horizontal rows; and from *S. (G.) khaokhoense* sp. nov. in the female by the fore basitarsus 5.3 times as long as its greatest width, in the male by the fore basitarsus 7.4 times as long as its greatest width, and in the larva by the posterior circlet with 86 rows of hooklets.

*Simulium (G.) decuplum* is similar to this new species in many characters including the number of male upper-eye facets, but slightly differs in the larva by the posterior circlet with about 76 rows of hooks (Takaoka and Davies, 1995); and *S. (G.) lamdongense* differs in the female by the shorter sensory vesicle (0.43–0.45 times as long as the third maxillary palpal segment) and mid and hind femora mostly light brown, and in the male by the larger number of upper-eye facets in 16 or 17 vertical columns and 17 or 18 horizontal rows (Takaoka et al., 2015).

All three known Chinese species, *S. (G.) bannaense*, *S. (G.) chongqingense* and *S. (G.) jiulianshanense*, differ from this new species as noted under *S. (G.) chiangraiense* sp. nov.

Takaoka and Saito (1996) recorded *S. (G.) decuplum* from Thailand, based on one female and one male, both reared from pupae and two mature larvae collected from Huai Kaeo, Chiang Mai Province, where this new species was collected. The previous record of *S. (G.) decuplum* from Thailand might have been based on *S. (G.) huaikaeoense* sp. nov. Maybe true *S. (G.) decuplum* has not been collected near this area.

### 3.2. DNA analysis of five new species and related species

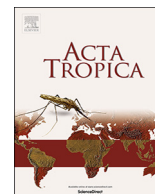
The phylogenetic positions of these new species and related species inferred from the mitochondrial COI gene are presented in Fig. 7. *Simulium (G.) decuplum*, *S. (G.) lamdongense* and three new species *S. (G.) chiangraiense*, *S. (G.) maeklangense*, and *S. (G.) khaokhoense* showed their own phylogenetic clades with high bootstrap supports (84–100%). Their genetic distances (Table 1) are comparable with species boundaries among nominal species of the subgenus *Gomphostilbia* in the Oriental Region (Low et al., 2015; Saeung et al., 2017; Takaoka et al., 2017a,b,c). *Simulium (G.) pamiangense* sp. nov. showed a sister relationship with *S. (G.) huaikaeoense* sp. nov. with low genetic distances (0.74–1.24%), though they are morphologically distinct. On the contrary, *S. (G.) khaokhoense* sp. nov. is distantly positioned from *S. (G.) huaikaeoense* sp. nov. (3.96–4.21%), though it is almost indistinguishable morphologically from the latter.

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## References

- Adler, P.H., Crosskey, R.W., 2017. World Blackflies (Diptera: Simuliidae): A Comprehensive Revision of the Taxonomic and Geographical Inventory [2017]. 131 pp., <http://entweb.clemson.edu/biomia/pdfs/blackflyinventory.pdf>. (Accessed 1 July 2017).
- Adler, P.H., Currie, D.C., Wood, D.M., 2004. The Black Flies (Simuliidae) of North America. Cornell University Press, Ithaca, New York, USA xv+941 pp.
- Borah, S., Rahman, I., Goswami, S., Manab Deka, M., Takaoka, H., 2012. Notes on black flies (Diptera: Simuliidae) from North-East India: New records of five species from Arunachal Pradesh and taxonomic reviews of two species from Assam. *Trop. Biomed.* 29, 92–97.
- Chen, H.B., Zhang, C.L., 2003. Review of taxonomy of blackflies from Xishuangbanna, China, with a description of a new species (Diptera: Simuliidae). *Acta Zootaxonomica Sinica* 28, 542–545.
- Fukuda, M., Choochote, W., Bain, O., Aoki, C., Takaoka, H., 2003. Natural infections with filarial larvae in two species of black flies (Diptera: Simuliidae) in northern Thailand. *Jpn. J. Trop. Med. Hyg.* 31 (2), 99–102.
- Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst. Biol.* 59 (3), 307–321.
- Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41, 95–98.
- Ishii, Y., Choochote, W., Bain, O., Fukuda, F., Otsuka, Y., Takaoka, H., 2008. Seasonal and diurnal biting activities and zoonotic filarial infections of two *Simulium* species (Diptera: Simuliidae) in northern Thailand. *Parasite* 15, 121–129.
- Kang, Z., Zhang, C.L., Chen, H.B., 2007. A new species of *Simulium* (*Gomphostilbia*) from Jiangxi Province, China (Diptera: Simuliidae). *Acta Parasitol. Med. Entomol. Sinica* 14, 185–187.
- Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33 (7), 1870–1874.
- Low, V.L., Takaoka, H., Adler, P.H., Ya'cob, Z., Norma-Rashid, Y., Chen, C.D., Sofian-Azirun, M., 2015. A multi-locus approach resolves the phylogenetic relationships of the *Simulium asakoeae* and *Simulium ceylonicum* species groups (Diptera: Simuliidae) in Malaysia: evidence for distinct evolutionary lineages. *Med. Vet. Entomol.* 29 (3), 330–337.
- Saeung, A., Srisuka, W., Low, V.L., Maleewong, W., Takaoka, H., 2017. Descriptions of the female and larva of *Simulium* (*Gomphostilbia*) *udomi* (Diptera: Simuliidae) from Thailand: and its transfer to the *Simulium asakoeae* species-group. *Acta Trop.* 172, 14–19.
- Swofford, D.L., 2002. PAUP\* Phylogenetic Analysis Using Parsimony (\*and Other Methods). Sinauer Associates, USA.
- Takaoka, H., Davies, D.M., 1995. The Black Flies (Diptera: Simuliidae) of West Malaysia. Kyushu University Press, Fukuoka, Japan viii + 175 pp.
- Takaoka, H., Saito, K., 1996. A new species and new records of black flies (Diptera: Simuliidae) from Thailand. *Jpn. J. Trop. Med. Hyg.* 24 (3), 163–169.
- Takaoka, H., Sofian-Azirun, M., Ya'cob, Z., Chen, C.D., Lau, K.W., Pham, X.D., 2015. The black flies (Diptera: Simuliidae) from Thua Thien Hue and Lam Dong Provinces, Vietnam. *Zootaxa* (Monograph) 1–96.
- Takaoka, H., Srisuka, W., Low, V.L., Maleewong, W., Saeung, A., 2017a. Two new species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from Myanmar: and their phylogenetic relationships with related species in the *S. asakoeae* species-group. *Acta Trop.* 176, 39–50.
- Takaoka, H., Srisuka, W., Low, V.L., Maleewong, W., Saeung, A., 2017b. A new black fly species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from Laos. *J. Med. Entomol.* 54, 1543–1551.
- Takaoka, H., Srisuka, W., Low, V.L., Saeung, A., 2017c. A new black fly species of the *Simulium* (*Gomphostilbia*) *epistum* species-group (Diptera: Simuliidae) from Thailand. *Acta Trop.* 176, 373–379.
- Takaoka, H., Srisuka, W., Saeung, A., 2017d. A new black fly species of *Simulium* (*Gomphostilbia*) from Thailand. *J. Med. Entomol.* 54, 1552–1559.
- Takaoka, H., 2003. The Black Flies (Diptera: Simuliidae) of Sulawesi, Maluku and Irian Jaya. Kyushu University Press, Fukuoka, Japan xxii + 581 pp.
- Takaoka, H., 2012. Morphotaxonomic revision of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) in the Oriental Region. *Zootaxa* 3577, 1–42.
- Zhu, J.Z., Wang, S.P., 1995. A new species of *Simulium* from China (Diptera: Simuliidae). *Sichuan J. Zool.* 14, 13–15.



# A new species and a new record of the *Simulium* (*Gomphostilbia*) *gombakense* species-group (Diptera: Simuliidae) from Thailand

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## ABSTRACT

Larvae and adults reared from pupae of *Simulium* (*Gomphostilbia*) *gombakense* Takaoka & Davies from eight streams in five provinces of Thailand were genetically analyzed by using cytochrome c oxidase subunit 1 sequences. The material was composed of four lineages, of which lineages 2, 3 and 4 are genetically closely related to one another, but more distantly related to lineage 1, which is equivalent to typical *S. (G.) gombakense* from Peninsular Malaysia. Lineages 2, 3 and 4, which are morphologically indistinguishable, are designated as genoforms A, B and C within a species complex of *S. (G.) paiense* sp. nov., which is described based on specimens of lineage 2 (= genoform A). *Simulium* (*G.*) *paiense* sp. nov. is similar to *S. (G.) gombakense* from Peninsular Malaysia but appears to be barely distinguished by the relative length of the female fore basitarsus. *Simulium* (*G.*) *thuathienense* Takaoka & Sofian-Azirun is newly recorded from Thailand and its male is described for the first time. The female of *S. (G.) gombakense* is briefly described based on females reared from pupae collected from Peninsular Malaysia.

## 1. Introduction

Black flies (Simuliidae) are one of dipteran insects and are widely distributed in many parts of the world, where clean, running streams and rivers, as the sites for the breeding of their immature stages and for the oviposition of the gravid females, are available.

Due to the biting habit of their adult females, black flies are of medical and veterinary importance. Females of certain simuliid species can transmit viruses, protozoans and filarial parasites. In Asia, *Simulium* (*Simulium*) *bidentatum* (Shiraki) is a vector of zoonotic onchocerciasis caused by *Onchocerca dewittei japonica*, a parasite of wild boar, recently discovered in Japan (Takaoka et al., 2012), and three other black fly species, *Simulium* (*Gomphostilbia*) *asakoe* Takaoka & Davies, *Simulium* (*Simulium*) *nigrogilvum* Summers and *Simulium* (*Simulium*) *nodosum* Puri, are reported to transmit three different filarial species, all of which are of possible public health importance, from Thailand (Fukuda et al., 2003; Takaoka et al., 2003).

Investigations of black flies' role in the transmission of disease pathogens require the reliable identification of species in question. For this, recent chromosomal and DNA sequence-based analyses have proved to be good tools to disclose cryptic taxa or lineages within a single morphospecies (e.g., Adler et al., 2016; Low et al., 2015).

*Simulium* (*Gomphostilbia*) is one of two predominant subgenera in South-East Asia, consisting of about 230 species (including *S. (G.) asakoe* as a filarial vector), which are placed in 10 species-groups (Adler and Crosskey, 2017). Adult females of species in each species-group of this subgenus are usually morphologically similar and difficult to differentiate from one another (Takaoka, 2012).

We aim to study whether *S. (G.) gombakense* Takaoka & Davies in Thailand is a single or multiple species. This species belongs to the *Simulium gombakense* species-group, defined by Takaoka (2012), which is a small taxon consisting of 11 species, and is characterized by the male ventral plate widened posteriorly and pupal gill composed of an inflated structure and eight or 10 slender filaments.

In Thailand, this species-group is represented by three species: *Simulium* (*G.*) *gombakense* Takaoka & Davies (originally described from Peninsular Malaysia), *S. (G.) maleewongae* Takaoka, Srisuka & Saeung, and *S. (G.) prayongi* Takaoka & Choochote (Takaoka and Davies, 1995, Takaoka and Choochote, 2005, Takaoka et al., 2017). *Simulium* (*G.*) *gombakense* in Thailand includes two lineages with high genetic divergence (7.35%), one lineage from Chongyen, Nakhonsawan Province, and the other from Chiang Mai and Loei Provinces, according to cytochrome c oxidase subunit 1 (COI) sequences analyzed by Pramual et al. (2011).

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We molecularly examined larvae and adults reared from pupae, which are morphologically identifiable as *S. (G.) gombakense*, collected from eight streams in five provinces of Thailand. We found that Thai populations of *S. (G.) gombakense* are composed of at least four lineages including two previously reported: lineage 1 (same as a lineage by Pramual et al., 2011), lineage 2, lineage 3 and lineage 4 (same as another lineage by Pramual et al., 2011). Two new lineages, 2 and 3, are closely related to lineage 4 but distantly to lineage 1, which is equivalent to typical *S. (G.) gombakense* from Peninsular Malaysia.

Lineages 2, 3 and 4 are so distantly separated from lineage 1 that they are treated as genoforms A, B and C within a complex of a new species, since all these three lineages are morphologically indistinguishable.

This new species is here described, and possible differing characters between this new species and *S. (G.) gombakense* from Peninsular Malaysia, are discussed.

In this survey, pupae and larvae *S. (G.) thuathienense* Takaoka & Sofian-Azirun originally described from Vietnam (Takaoka et al., 2015), were collected for the first time in Thailand. The male of this species is described. In addition, the female of *S. (G.) gombakense* is briefly described on the basis of specimens reared from pupae collected from Peninsular Malaysia.

## 2. Material and methods

The larvae and adults reared from pupae that were used for genetic analysis were collected from eight streams in five provinces of Thailand (sites 1–8 in Table 1). In addition, *S. (G.) thuathienense* and *S. (G.) maleewongae*, both collected from Thailand, *S. (G.) gombakense* from Peninsular Malaysia, and five sequences registered in the NCBI GenBank (HM775247, HM775249, HM77250, HM77252, HM77253, all under *S. (G.) gombakense* from Thailand), were included in our genetic analysis. Specimens were used for sequencing of the COI gene and a subsequent phylogenetic analysis. The protocols for DNA extraction, PCR amplification, and sequencing followed those of Low et al. (2015). A maximum likelihood analysis was performed on an on-line web-based server PhyML 3.0 (Guindon et al., 2010). An automatic model selection was implemented based on the Akaike information criterion (AIC). The best-fit model was the general time-reversible (GTR) model with a proportion of invariable sites of 0.633 and with a gamma shape parameter of 1.591. Neighbor-joining analysis was performed in PAUP 4.0b10. (Swofford, 2002), with Kimura's two-parameter model of substitution (K2P distance), using 1000 bootstrap replicates. *Simulium (G.) laosense* was used as an outgroup. To estimate the level of genetic divergence, uncorrected p pairwise genetic distances were estimated using PAUP 4.0b10. The representative sequences generated in this study were deposited in the NCBI GenBank database under accession numbers MG958560–MG958562 for *S. (G.) gombakense*, MG958563–MG958580 for *S. (G.) paiense* sp. nov., MG958581–MG958582 for *S. (G.) thuathienense* and MG958583–MG958584 for *S. (G.) maleewongae*.

A new species was described based on females, males, pupae and

mature larvae collected from Pai District, Mae Hong Son Province (site 1 in Table 1), where only lineage 2 (genoform A) was recognized. It was morphologically compared with three other lineages (i.e., typical *S. (G.) gombakense* and genoforms B and C of the new species). The methods of description and illustration, as well as terms for morphological features, followed those of Takaoka (2003).

The holotype and paratypes are deposited at the Queen Sirikit Botanic Garden, Chiang Mai, Thailand.

This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix <http://zoobank.org/>. The LSID for this publication is: urn:lsid:zoobank.org:pub:048CE051-DC9D-4076-A766-218CBEE9F740.

## 3. Results

### 3.1. Genetic analysis using COI gene

The result (Fig. 1) reveals that Thai populations of *S. (G.) gombakense* are represented by at least four lineages including two previously reported lineages: lineage 1 [same as lineage (HM775252) from Nakhonsawan Province reported by Pramual et al., 2011], lineage 2, lineage 3, and lineage 4 [same as lineage (HM775247, HM775249, HM775250, HM775253) from Chiang Mai and Loei Provinces reported by Pramual et al., 2011]. Lineage 1 is distinctly separated from lineages 2, 3 and 4 with genetic distances of 7.35–8.55%, 7.86–8.89% and 6.50–8.72%, respectively.

Two new lineages 2 and 3 are geographically restricted to one site (site 1) in Mae Hong Son Province and three sites (sites 2, 3 and 4) in Chiang Mai Province, respectively, whereas lineage 4 is widely distributed in five provinces [two previously reported provinces plus Mae Hong Son, Tak and Nan Provinces (sites 5, 6 and 7) in our survey]. On the other hand, lineage 1, which includes samples from Trang Province (site 8) and from Nakhonsawan Province (HM775252), reported by Pramual et al. (2011), forms a cluster with Peninsular Malaysian samples of *S. (G.) gombakense*. There are no sites supporting two or more lineages except site 2 (Mae Klang Waterfall, Chiang Mai Province) where both lineages 3 and 4 coexist.

Genetically, lineages 2, 3 and 4 are so distantly separated from lineage 1 (=typical *S. (G.) gombakense*) that they can be treated as genoforms A, B and C within a complex of a new species described here, rather than as genoforms B, C and D within the *S. gombakense* species complex, since all three lineages are morphologically indistinguishable. The genetic distances within the three lineages of *S. (G.) paiense* sp. nov. are 0–4.44%; of which the genoform A is separated from genoform B by 4.10–4.44%, and genoform C by 2.73–3.93%. The genoform B differs from genoform C by 3.08–4.10%.

Two other members of the *S. gombakense* species-group from Thailand, *S. (G.) maleewongae* and *S. (G.) thuathienense*, were genetically

**Table 1**  
Collection data for black flies used in morphological and genetic analyses.

| Site no. | District  | Province     | Latitude      | Longitude      | Elevation (masl) | Collection date | No. of samples*   |
|----------|-----------|--------------|---------------|----------------|------------------|-----------------|-------------------|
| 1        | Pai       | Mae Hong Son | 19°21' 00"N   | 98°35' 06.4"E  | 1737             | 28 ix. 2016     | 6F, 6M, 15PE, 20L |
| 2        | Chomthong | Chiang Mai   | 18°29' 57.0"N | 98°40' 06.2"E  | 412              | 3 iv. 2017      | 2F, 2M, 4PE, 7L   |
| 3        | Muang     | Chiang Mai   | 18°48' 44.2"N | 98°56' 21.3"E  | 503              | 18 vii. 2017    | 5M, 5PE           |
| 4        | Maecham   | Chiang Mai   | 18°45' 21.3"N | 98°08' 00.3"E  | 1,434            | 7 xii. 2015     | 2F, 1M, 3PE, 8L   |
| 5        | Muang     | Mae Hong Son | 19°36' 57.9"N | 97°59' 48.2"E  | 1,007            | 12 vii. 2017    | 2F, 3M, 5PE, 8L   |
| 6        | Umphang   | Tak          | 16°18' 00.5"N | 99°01' 22.6"E  | 1,095            | 22 viii. 2017   | 2F, 1M, 2PE, 2L   |
| 7        | Wiang Sa  | Nan          | 18°25' 39.9"N | 100°57' 47.6"E | 551              | 26 vii. 2017    | 4L                |
| 8        | Muang     | Trang        | 07°22' 20.0"N | 99°49' 16.0"E  | 401              | 31 iii. 2012    | 1M, 2PE, 2L       |

\*F, female(s); M, male(s); PE, pupal exuviae; L, larva(e).

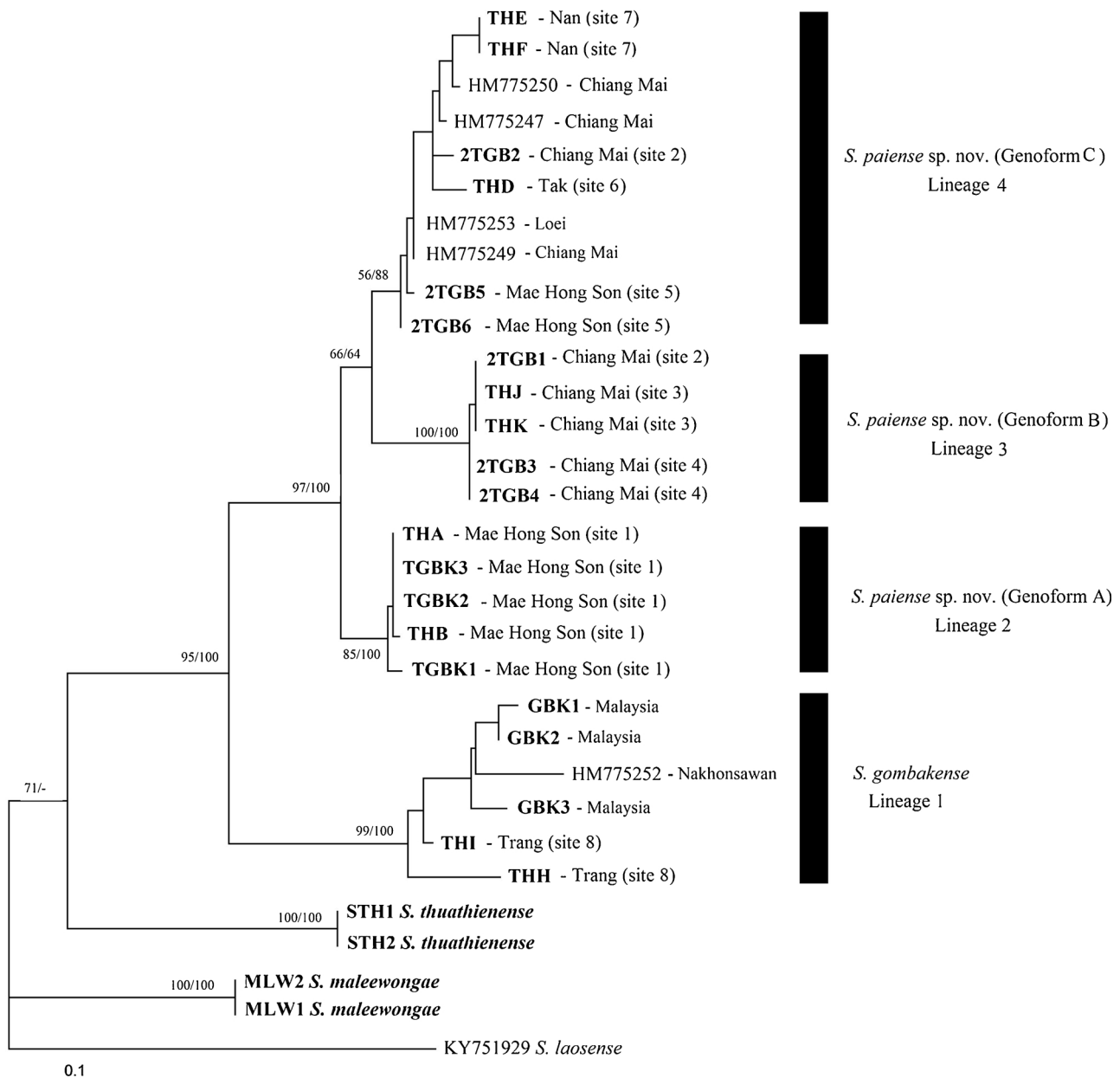


Fig. 1. Maximum likelihood phylogenetic tree of the *S. gombakense* species-group inferred from the mitochondrial-encoded COI gene sequences. Posterior probability/bootstrap and values (ML/NJ) are shown on the branches. Newly generated sequences are in bold.

analyzed for the first time. The results show that both species are distinctly separated from each other and from all four lineages of *S. (G.) gombakense* (Fig. 1).

### 3.2. Morphological analysis

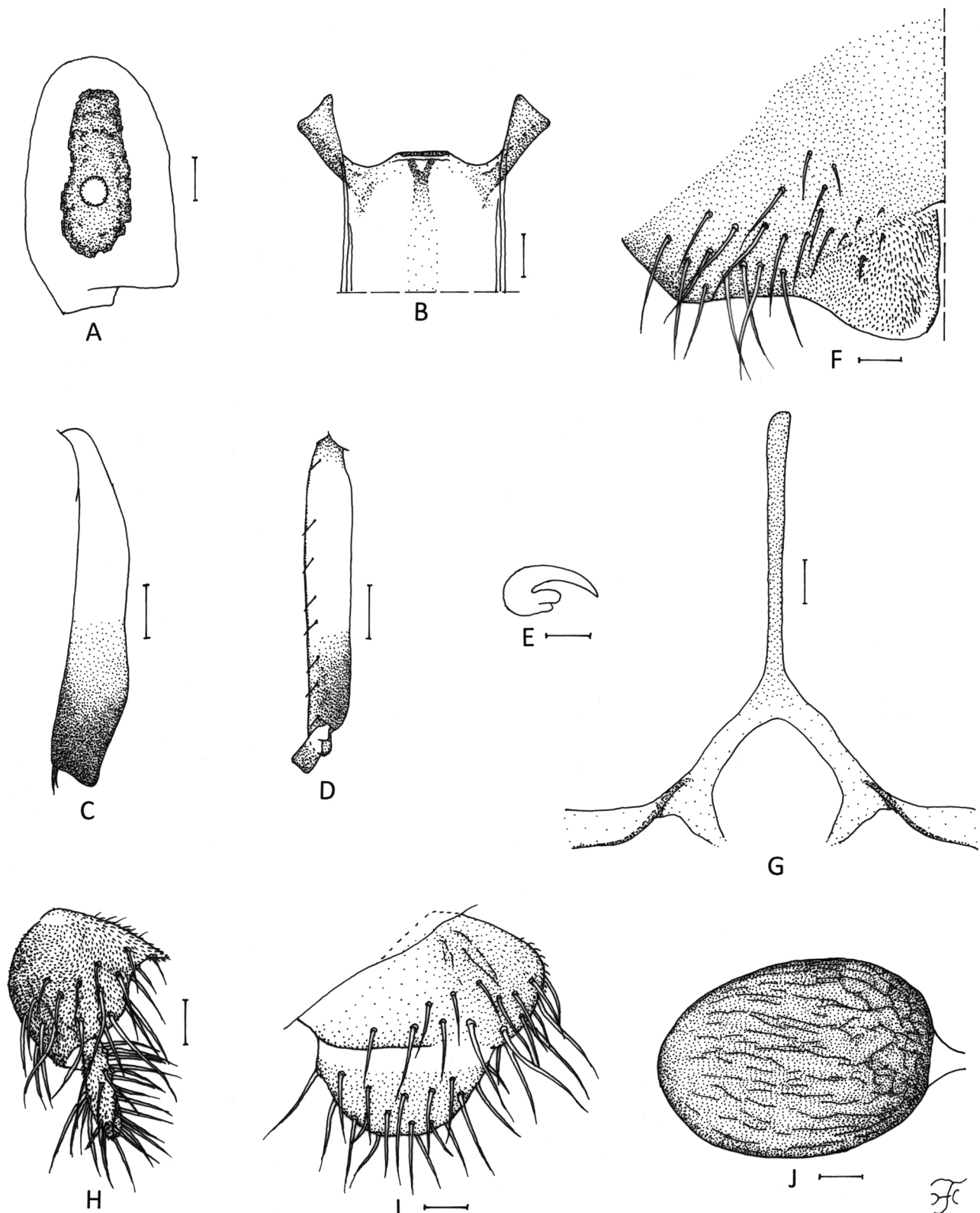
#### 3.2.1. Description of a new species

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

(urn: lsid:zoobank.org:act:F20966CE-8E86-4 ACE-9C9B-D0862A 2E6F83)

**Female** (n = 6). Body length 2.0–2.2 mm. **Head.** Slightly narrower than width of thorax. Frons brownish black, slightly shiny when illuminated at certain angles, densely covered with yellowish-white recumbent short hairs interspersed with several dark unbranched longer hairs along each lateral margin; frontal ratio 1.7–1.8:1.0:2.4–2.7; frons-head ratio 1.0:4.4–4.8. Fronto-ocular area well developed, narrow, directed dorsolaterally. Clypeus brownish black, densely covered with

yellowish-white recumbent short hairs interspersed with several dark-brown longer hairs on each side of lower two-thirds. Labrum 0.6–0.7 times as long as clypeus. Antenna composed of scape, pedicel and nine flagellomeres, dark brown except scape, pedicel and base of first flagellomere yellow. Maxillary palp composed of five segments, light to medium brown, proportional lengths of third, fourth, and fifth segments 1.0:0.8:2.1; third segment (Fig. 2A) swollen; sensory vesicle (Fig. 2A) oblong, 0.7–0.8 times as long as third segment, with medium-sized opening. Maxillary lacinia with 12 or 13 inner and 15 or 16 outer teeth. Mandible with 21–24 inner and 10–12 outer teeth. Cibarium (Fig. 2B) medially forming short wide sclerotized plate folded forward from posterior margin, and with moderately sclerotized medial longitudinal ridge with forked apex. **Thorax.** Scutum brownish-black to black (except anterior calli medium brown), shiny, thinly grayish-white pruinose with three faint non-pruinose longitudinal vittae (one medial and two submedial) when illuminated dorsally and viewed anteriorly, densely covered with whitish-yellow recumbent hairs. Scutellum dark brown, covered with whitish-yellow short hairs and dark-brown long upright



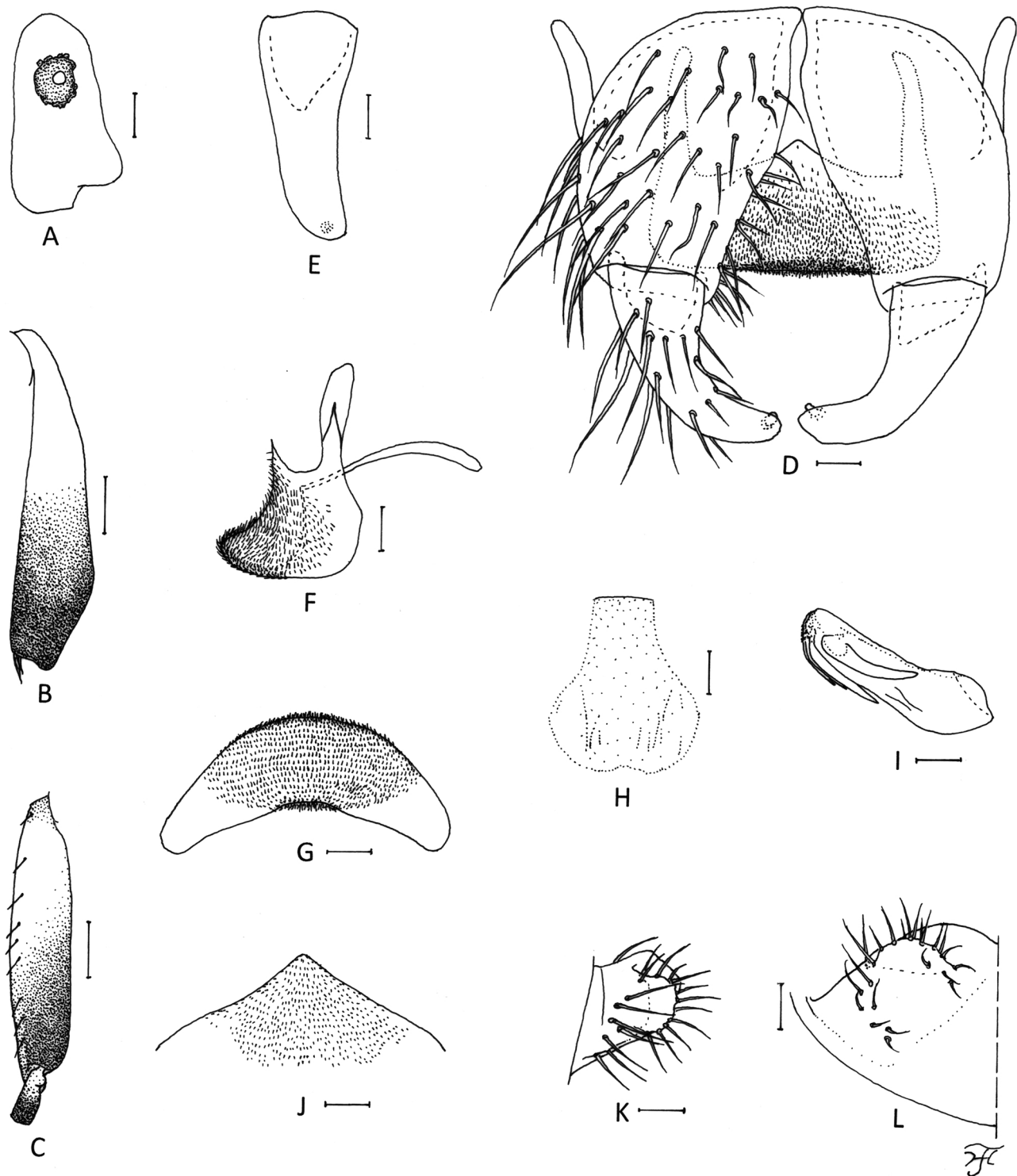
**Fig. 2.** Female of *Simulium* (*Gomphostilbia*) *paiense* sp. nov. (A) Third segment of right maxillary palp with sensory vesicle (front view). (B) Cibarium. (C) Hind tibia (left side; outer view). (D) Basitarsus and second tarsomere of hind leg showing calcipala and pedisulcus (left side; outer view). (E) Claw. (F) Sternite 8 and ovipositor valve (right half; ventral view). (G) Genital fork (ventral view). (H, I) Right paraprocts and cerci (H, ventral view; I, lateral view). (J) Spermatheca. Scale bars = 0.1 mm for C and D; 0.02 mm for A, B and E–J.

hairs along posterior margin. Postnotum dark brown to brownish-black, bare. Pleural membrane bare. Katepisternum medium to dark brown, longer than deep, moderately covered with short hairs. **Legs.** Foreleg: coxa whitish yellow; trochanter light brown except base whitish yellow; femur light brown with apical cap medium brown though extreme tip whitish yellow; tibia yellowish white except apical one-fourth dark brown and inner surface light brown; tibia densely covered with yellow hairs on outer surface of basal four-fifths; tarsus brownish black, with moderate dorsal hair crest; basitarsus moderately dilated, 6.6–7.4 times as long as its greatest width. Midleg: coxa light brown except posterolateral surface medium brown; trochanter light brown except base whitish yellow; femur light brown with apical cap medium brown (though extreme apex whitish yellow); tibia light to medium brown except little more than basal one-third whitish yellow; tibia densely covered with whitish hairs on posterior surface of basal five-sixths; tarsus medium to dark brown except basal half yellow. Hind leg: coxa light brown; trochanter whitish yellow; femur light brown except base whitish yellow and apical cap medium brown (though extreme tip whitish yellow); tibia (Fig. 2C) yellowish white on basal half or little more, light brown to dark brown on rest; tibia densely covered with white fine hairs on posterior and outer surfaces of basal three-fourths or little more; tarsus medium to dark brown except little more than basal two-thirds of basitarsus (though base light brown) and basal half of second tarsomere yellowish white; basitarsus (Fig. 2D) narrow, nearly parallel-sided, 6.1–6.5 times as long as wide, and 0.68–0.74 and 0.57–0.61 times as wide as greatest widths of tibia and femur, respectively; calcipala nearly as long as wide, and 0.5–0.6 times as wide as greatest width of basitarsus. Claw (Fig. 2E) with large basal tooth 0.47 times as long as claw. **Wing.** Length 1.9–2.0 mm. Costa with dark-brown spinules and hairs except basal short portion with patch of whitish-yellow hairs. Subcosta with medium-brown hairs except apical one-third to one-fourth bare. Hair tuft on stem vein whitish yellow. Basal portion of radius fully haired;  $R_1$  with dark brown spinules and light to medium brown hairs;  $R_2$  with light to medium brown hairs only. Basal cell absent. **Halder.** Whitish with darkened base. **Abdomen.** Basal scale light brown, with fringe of whitish-yellow hairs. Dorsal surface of abdominal segments dark brown to brownish black except tergite of segment 2 light brown, moderately covered with dark brown hairs; tergites of segments 2 and 6–8 wide and shiny; ventral surface of segment 2 entirely pale whitish-yellow and those of other segments light to medium brown; sternal plate on segment 7 undeveloped. **Terminalia.** Sternite 8 (Fig. 2F) bare medially, with 18–22 medium-long to long hairs and two to four short hairs on each side. Ovipositor valve (Fig. 2F) triangular, with round posteromedial corner, thin, membranous, moderately covered with microsetae interspersed with one to three short hairs; inner margins slightly sinuous, moderately sclerotized and narrowly separated from each other. Genital fork (Fig. 2G) of usual inverted-Y form, with arms of moderate width; arm moderately folded medially and with prominent projection directed posteromedially. Paraproct in ventral view (Fig. 2H) nearly triangular, and with three or four sensilla on anteromedial surface; paraproct in lateral view (Fig. 2I) somewhat produced ventrally, 0.6 times as long as wide, with 16–20 short to long hairs on ventral and lateral surface. Cercus in lateral view (Fig. 2I) short, 0.4 times as long as wide, and rounded posteriorly. Spermatheca (Fig. 2J) ellipsoidal, 1.4 times as long as wide, well sclerotized except duct and small area at juncture with duct unsclerotized, and with many fissures on surface; internal setae absent; both accessory ducts slender, unsclerotized, subequal in diameter to major one.

**Male** ( $n = 6$ ). Body length 2.2–2.4 mm. **Head.** Much wider than thorax. Holoptic, upper eye consisting of large facets in 14 vertical columns and in 14 horizontal rows. Face dark brown, white pruinose, bare. Clypeus dark brown, white pruinose, densely covered with golden yellow scale-like short hairs interspersed with dark longer hairs. Antenna composed of scape, pedicel and nine flagellomeres, dark brown except scape, pedicel and base of first flagellomere yellow; first

flagellomere somewhat elongate, 1.6–1.8 times as long as second flagellomere. Maxillary palp composed of five segments, proportional lengths of third, fourth and fifth segments 1.0:1.1:2.7–2.8; third segment (Fig. 3A) somewhat widened apically; sensory vesicle (Fig. 3A) ellipsoidal, 0.2–0.3 times as long as third segment, with small opening apically. **Thorax.** Scutum brownish black, slightly shiny on shoulders, along lateral margins and prescutellar area when illuminated at certain angles; scutum slightly gray pruinose with three non-pruinose longitudinal vittae (one medial and two submedial) when illuminated dorsally and viewed anterodorsally, densely covered with golden-yellow scale-like recumbent hairs. Scutellum dark brown, with golden-yellow short hairs and dark upright long hairs. Postnotum dark brown and bare. Pleural membrane bare. Katepisternum longer than deep, medium to dark brown with short hairs. **Legs.** Foreleg: coxa yellow; trochanter light brown except base yellow; femur light brown with apical cap medium brown; tibia light brown except apical one-third medium brown; tarsus brownish-black, with moderate dorsal hair crest; basitarsus moderately dilated, 7.6–8.5 times as long as its greatest width. Midleg: coxa light brown except posterolateral surface medium to dark brown; trochanter light brown except base yellow; femur light brown with apical cap medium brown (though extreme apex yellow); tibia medium to dark brown except basal one-third yellow; tarsus dark brown except basal half of basitarsus light to medium brown. Hind leg: coxa medium brown; trochanter yellow; femur medium brown except base yellow and apical cap dark brown (though extreme tip yellow); tibia (Fig. 3B) yellowish-white on little less than basal half, dark brown on rest except apical cap brownish black; tarsus medium to dark brown except basal half of basitarsus (though base light brown) and little less than basal half of second tarsomere yellow; basitarsus (Fig. 3C) narrow, nearly parallel-sided, 4.3–4.6 times as long as wide, and 0.78–0.80 and 0.82–0.83 times as wide as greatest widths of tibia and femur, respectively; calcipala nearly as long as wide, and 0.44 times as wide as greatest width of basitarsus. **Wing.** Length 1.9–2.0 mm; other characters as in female except subcosta bare. **Halder.** Light gray with darkened base. **Abdomen.** Basal scale dark brown with fringe of dark long hairs laterally. Dorsal surface of abdomen medium to dark brown, moderately covered with dark short hairs; segments 2 and 5–8 each with pair of shiny dorsolateral patches, those on segment 2 connected in middle to each other. **Genitalia.** Coxites, styles and ventral plate in ventral view as in Fig. 3D. Coxite in ventral view (Fig. 3D) subquadrate, 1.7 times as long as wide. Style in ventral view (Fig. 3D) slender, 0.8 times as long as coxite, gently curved inward, tapered toward apex and with one apical spine; style in ventrolateral view (Fig. 3E) 2.3 times as long as its greatest width at base, gradually tapered from base toward apex. Ventral plate in ventral view (Fig. 3D) transverse, much wider than long, somewhat widened posteriorly, with anterior margin produced medially, with posterior margin nearly straight, and moderately covered with microsetae on ventral surface; basal arms nearly parallel-sided, though somewhat convergent apically; ventral plate in lateral view (Fig. 3F) much produced ventrally; ventral plate in caudal view (Fig. 3G) gently rounded ventrally, and densely covered with microsetae on most of posterior surface except lateral portions bare. Median sclerite (Fig. 3H) broad, plate-like, though lateral margins not well defined. Paramere (Fig. 3I) of moderate size, with two long hooks and few shorter ones. Aedeagal membrane (Fig. 3J) moderately covered with microsetae. Ventral surface of abdominal segment 10 (Fig. 3K, L) without distinct hairs (though one hair on right side in one male). Cercus (Fig. 3K, L) small, with 10–22 short hairs.

**Pupa** ( $n = 15$ ). Body length 2.4–2.6 mm. **Head.** Integument yellow, moderately or densely covered with round tubercles (some appearing to have tiny secondary projections) on frons and each lateral surface; antennal sheath bare, and without any projections. Frons with three unbranched or bifid long trichomes (Fig. 4A) on each side; all trichomes subequal in length and thickness to one another. Face with one unbranched or bifid long trichome (Fig. 4B) on each side. **Thorax.** Integument yellow, moderately or densely covered with round tubercles



**Fig. 3.** Male of *Simulium* (*Gomphostilbia*) *paiense* sp. nov. (A) Third segment of right maxillary palp with sensory vesicle (front view). (B) Hind tibia (left side; outer view). (C) Basitarsus and second tarsomere of hind leg showing calcipala and pedisulcus (left side; outer view). (D) Coxites, styles and ventral plate (ventral view). (E) Right style (ventrolateral view). (F) Ventral plate and median sclerite (lateral view). (G) Ventral plate (caudal view). (H) Median sclerite (caudal view). (I) Right paramere (caudal view). (J) Aedeagal membrane (caudal view). (K, L) Abdominal segment 10 and cerci (right half; K, lateral view; L, caudal view). Scale bars = 0.1 mm for B and C; 0.02 mm for A and D–L.

(some appearing to have tiny secondary projections) except dorsal surface of posterior one-fifth almost bare, with two unbranched or bifid slender short or medium-long trichomes, or rarely three unbranched or bifid trichomes (two medium-long and one short, or one medium-long and two short trichomes) (Fig. 4C) dorsomedially, two unbranched or bifid slender medium-long trichomes (Fig. 4D) anterolaterally, one

unbranched or bifid slender medium-long trichome (Fig. 4E) medio-laterally, and three unbranched or bifid trichomes (two medium-long, one short) (Fig. 4F) ventrolaterally, on each side. Gill (Fig. 4G) of much inflated structure (0.5 mm long), which is 2.6–2.8 times as long as greatest width, and divided into three portions (basal, middle and apical) by two constrictions; second constriction 1.0–1.4 times as wide

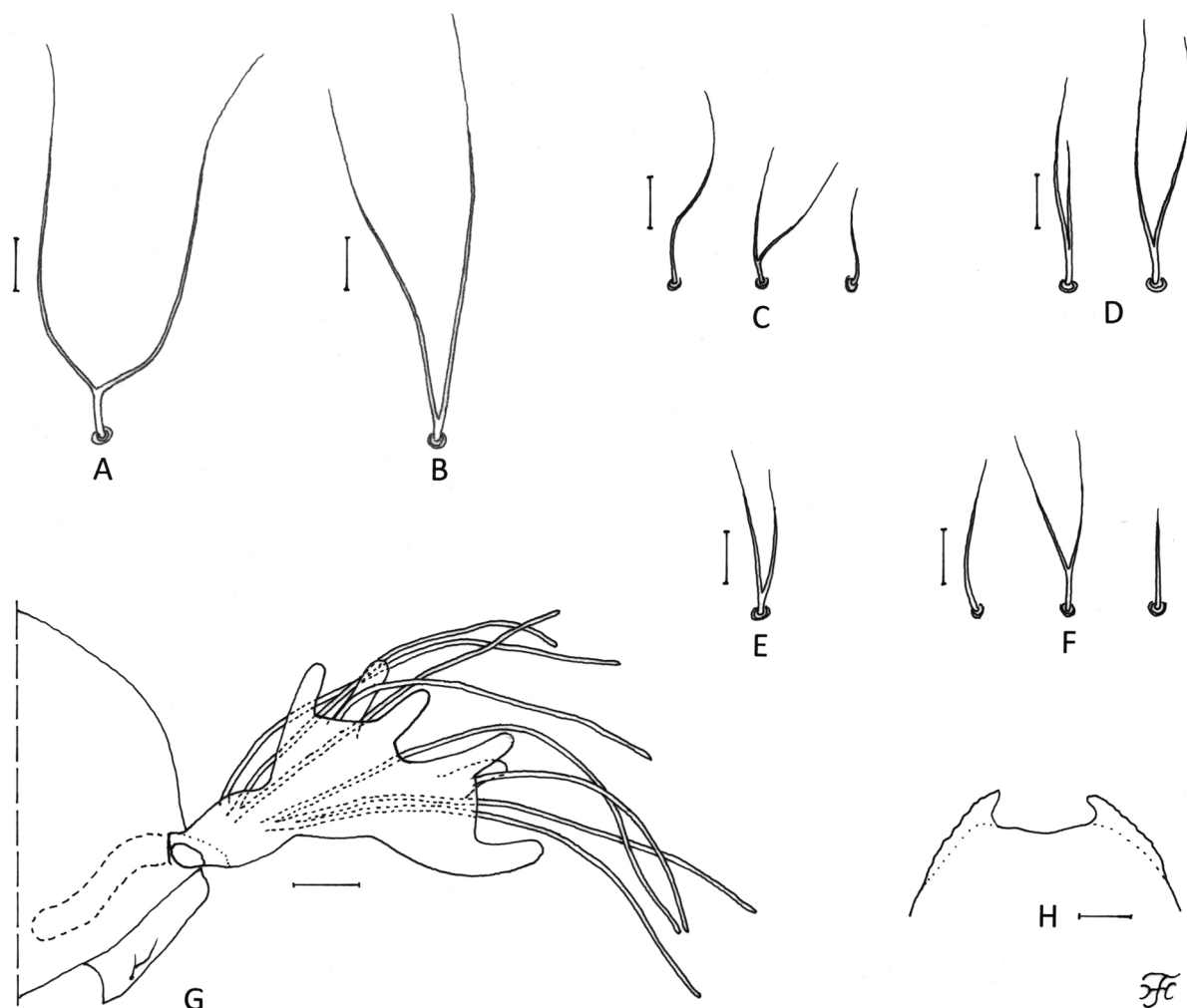


Fig. 4. Pupa of *Simulium* (*Gomphostilbia*) *paiense* sp. nov. (A) Frontal trichome. (B) Facial trichome. (C–F) Thoracic trichomes (C, dorsomedial; D, anterolateral; E, mediolateral; F, ventrolateral). (G). Right gill (outer view). (H) Terminal hooks (caudal view). Scale bars = 0.1 mm for G; 0.02 mm for A–F and H.

as first constriction; middle and apical portions each with three thumb-like or finger-like projections; longest projection of middle inflated portion 0.7–0.8 times as long as greatest width of apical inflated portion; gill with eight slender thread-like filaments, of which six filaments arise in three groups of one, two and three filaments from dorsal, dorsal-inner and inner surfaces of basal portion of inflated structure, respectively, and all subequal in length (0.7–0.9 mm) and thickness to one another, and remaining two filaments isolated, subequal in length (0.5 mm), each arising from dorsal surface of middle and apical portions of inflated structure; surface of inflated portion and all filaments light brown, without any pattern on inflated portion or any transverse ridges or furrows on filaments; surface of inflated portion densely covered with microtubercles and that of filaments smooth, though appearing to be densely covered with microtubercles on inner layer. **Abdomen.** Dorsally, all segments pale whitish yellow, without tubercles, with one unbranched slender short seta on each side; segment 2 with one unbranched slender short seta and five minute setae on each side; segments 3 and 4 each with four hooked spines and one unbranched minute seta on each side; segment 5 with comb-like groups of minute spines and four minute setae, but lacking spine-combs on each side; segments 6–9 each with spine-combs (though those on segment 9 somewhat smaller than those on other segments) and comb-like groups of minute spines in transverse row on each side; segments 6–8 each with two minute setae on each side; segment 9 with pair of wide plate-like terminal hooks with weakly crenulated outer margin (Fig. 4H). Ventrally, segment 4 with one unbranched short hook (somewhat

smaller in size than those on segments 5–7) and few minute setae on each side; segment 5 with pair of bifid hooks submedially and few short setae on each side; segments 6 and 7 each with pair of bifid inner and simple outer hooks somewhat spaced from each other, and few short setae on each side; segments 4–8 each with comb-like groups of minute spines on each side; each side of segment 9 with three grapnel-shaped hooklets. **Cocoon.** Wall-pocket-shaped, thinly and compactly woven with no open spaces in weave, somewhat extended ventrolaterally, covering entire abdomen and posterior one-third of thorax; anterior margin somewhat thickened or not; floor loosely woven on posterior one half of cocoon; individual threads invisible; 2.1–2.8 mm long by 1.2–2.0 mm wide.

**Mature larva** (n = 20). Body length 4.5–5.6 mm. Body entirely gray with reddish-brown pigment on dorsal and dorsolateral surfaces of abdominal segments 6–9 (mostly segment 8). Head capsule sparsely covered with minute colorless setae. Cephalic apotome yellow to dark yellow, with faint to moderate positive head spots, or somewhat darkened along posterior margin, obscuring posterolateral spots. Lateral surface of head capsule yellow except eye-spot region white, and portions above and posterior to eye-spot region darkened, thus two large spots in front of posterior margin faintly positive or merged; eyebrow distinct; one round spot below eye-spot region and two round spots in front of posterior margin faintly positive or obscured; lateral surface entirely light brown except eye-spot region white in three larvae. Ventral surface of head capsule yellow except medial portion widely darkened along both sides of postgenal cleft, obscuring elongate spots

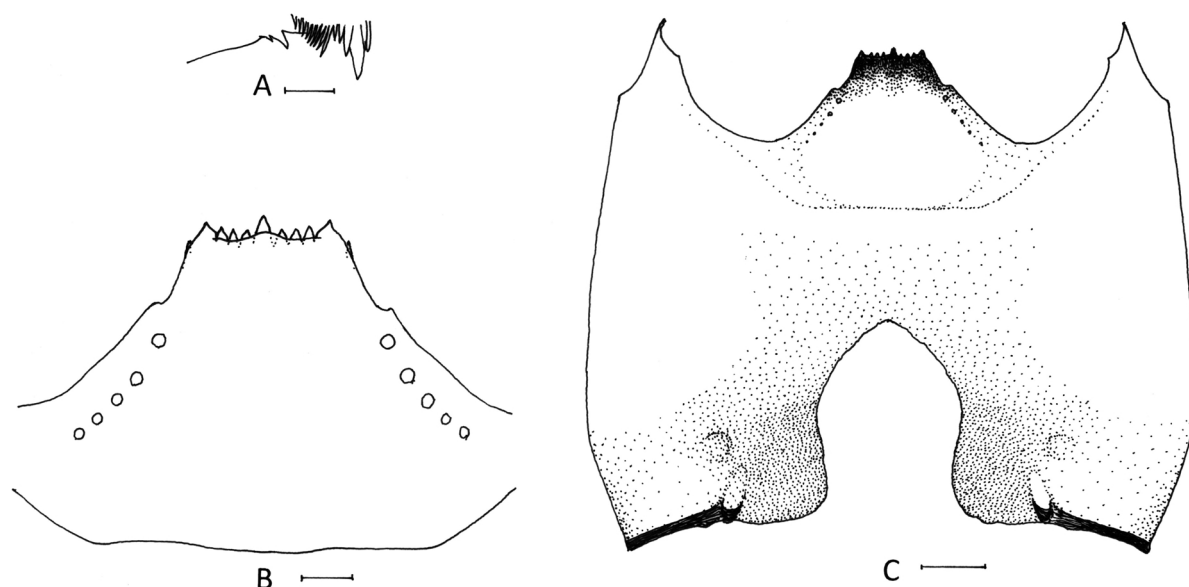


Fig. 5. Mature larva of *Simulium* (*Gomphostilbia*) *paiense* sp. nov. (A) Tip of mandible (lateral view). (B) Hypostoma (ventral view). (C) Head capsule showing postgenal cleft (ventral view). Scale bars = 0.05 mm for C; 0.02 mm for A and B.

on each side of postgenal cleft; ventral surface of head entirely light-brown except narrow portion along posterior margin yellow in three larvae. Cervical sclerites composed of two small light brown rod-like pieces, not fused to occiput, widely separated from each other. Antenna composed of three segments and apical sensillum, much longer than stem of labral fan; antenna pale except dorsal surface of first segment somewhat darkened toward base; proportional lengths of first, second, and third segments 1.00:0.75–0.86:0.82–0.86. Labral fan with 24–28 primary rays. Mandible (Fig. 5A) with three comb-teeth decreasing in length from first to third; mandibular serrations composed of two teeth (one large and one small); large tooth at acute angle to mandible on apical side. Hypostoma (Fig. 5B) with row of nine apical teeth, of which median tooth most prominent, always longer than corner teeth, and intermediate teeth shortest; lateral margins smooth; five hypostomal bristles in row, nearly parallel to lateral margin on each side. Postgenal cleft (Fig. 5C) nearly parallel-sided or slightly widened from base to middle, then convergent, forming pointed apex, and 1.7–2.5 times as long as postgenal bridge (though postgenal clefts of two larvae are 1.3 and 3.0 times as long as postgenal bridge, respectively). Pharate pupal gill with wrinkled enlarged body with six thumb-like or finger-like projections and eight slender thread-like filaments. Thoracic cuticle bare. Abdominal cuticle bare except few posterior segments sparsely covered with minute colorless setae and both sides of anal sclerite (down to base of ventral papillae) densely covered with minute colorless setae. Rectal scales absent. Rectal organ compound, each of three lobes with 8–11 finger-like secondary lobules. Anal sclerite X-shaped, anterior arms 0.9 times as long as posterior ones; accessory sclerites absent; sensillum absent. Ventral papillae well developed, conical. Posterior circlet with 83–88 rows of up to 15 or 16 hooks per row.

**Type material.** HOLOTYPE. Female, with associated pupal exuviae and cocoon (in 80% ethanol), reared from a pupa collected from site 1 in Table 1, THAILAND: Mae Hong Son Province, Pai District, Doi Chang Village, 28-IX-2016, by W. Srisuka and A. Saeung. PARATYPES. Five females, six males (all with associated pupal exuviae and cocoons) reared from pupae, three pupae and 20 mature larvae (all in 80% ethanol), same data as those of the holotype.

**Biological notes.** The pupae and larvae of this new species were collected from fallen leaves in a small seasonal stream (width 20 cm, depth 3 cm, exposed to the sun, water temperature 13.8 °C, elevation 1737 m above sea level, 19°21'00.2"N, 98°35'06.4"E).

**Etymology.** The species name *paiense* refers to the district name,

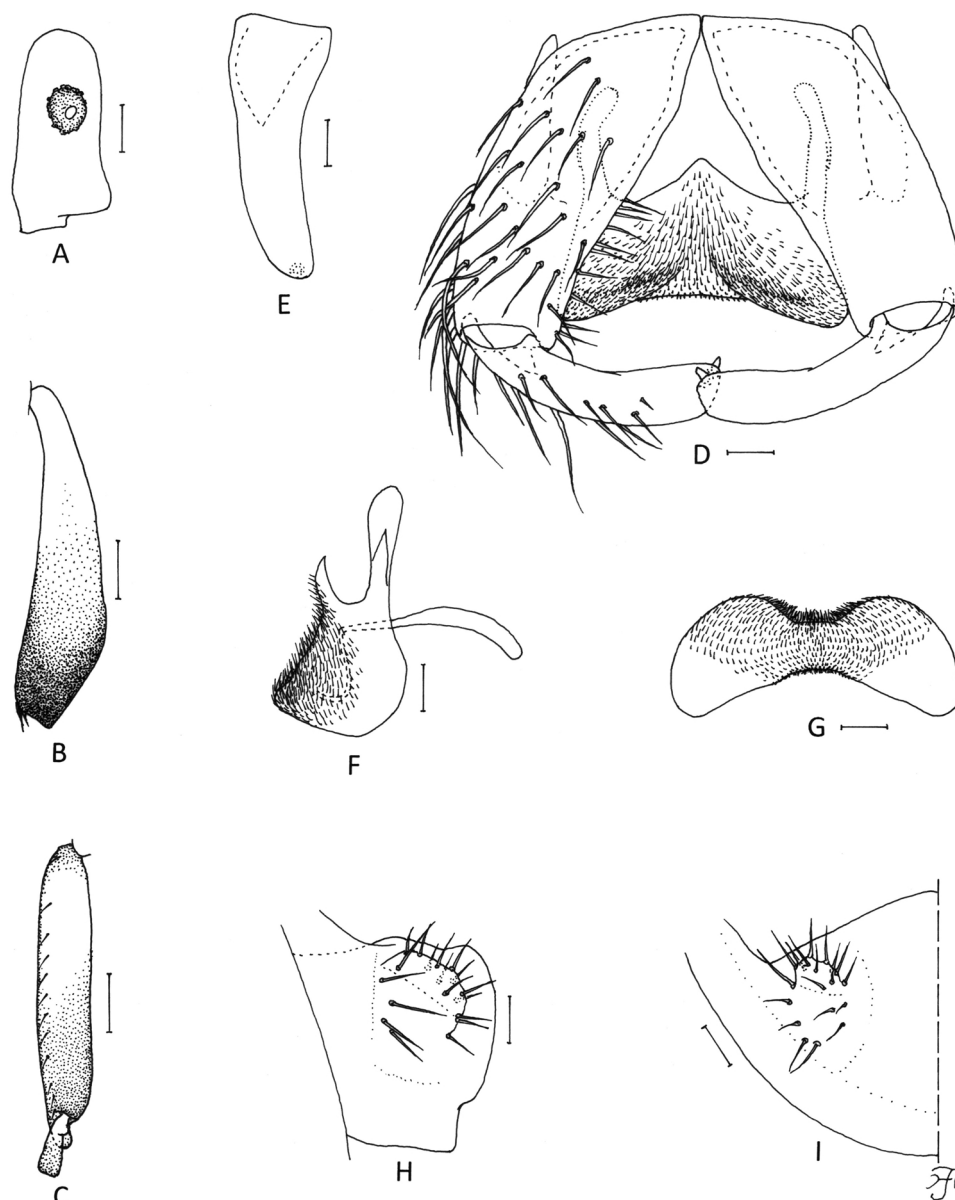
Pai, where this new species was collected.

**Remarks.** *Simulium* (*G.*) *paiense* sp. nov. is placed in the *S. gombakense* species-group by having the male ventral plate gradually widened posteriorly when viewed ventrally (Fig. 3D), and pupal gill composed of an inflated structure with eight slender filaments (Fig. 4G). This new species is the fourth member of the *S. gombakense* species-group recorded from Thailand.

This new species is similar to *S. (G.) gombakense*, originally described from a mature larvae collected from Gombak, Peninsular Malaysia (Takaoka and Davies, 1995), and from a male reared from a pupa, collected from Fraser's Hill, Peninsular Malaysia (Takaoka, 2000) in having the pupal gill composed of an elongated inflated structure divided into three portions by two constrictions, with six thumb-like or finger-like projections and eight slender filaments (Fig. 4G), and wide pupal terminal hooks (Fig. 4H). This new species is barely distinguished from *S. (G.) gombakense* by the following characters (those of *S. (G.) gombakense* in parentheses): in the female: the length ratio of the fore basitarsus against its greatest width 6.6–7.1 (5.8–6.0); in the male: the length ratio of the fore basitarsus against its greatest width 7.6–8.5 (6.8–7.1); in the pupa: the ratio of the width of the second constriction against that of the first one 1.0–1.4 (0.8–0.9), and ratio of the longest finger-like projection on the middle inflated portion against the greatest width of the apical inflated portion 0.7–0.8 (1.0–1.5).

Adults, pupae and larvae of genoforms B and C of the *S. (G.) paiense* sp. nov. complex are almost indistinguishable from those of genoform A, except a few characters including the fore basitarsi of five males of genoform B from site 3, which are 7.0–7.9 times as long as their greatest width, overlapping the range for *S. (G.) gombakense*, and labral fans of some larvae of genoform B or C from site 2, which have as many as 36 primary rays, showing a wider range of intraspecific variation (24–36 primary rays).

Overall, there seem to be only a few morphological features, which may be used to separate genoforms A, B and C of the *S. (G.) paiense* sp. nov. complex from *S. (G.) gombakense*: in the female by the length ratio of the fore basitarsus against its greatest width: 6.2–7.2 ( $n = 14$ ) versus 5.8–6.0 ( $n = 3$ ); in the pupa by the ratio of the longest finger-like projection on the middle inflated portion against the greatest width of the apical inflated portion: 0.7–0.8 ( $n = 34$ ) versus 1.0–1.5 ( $n = 5$ ), and relative size of the second constriction against the first one: 1.0–1.4 ( $n = 34$ ) versus 0.8–0.9 ( $n = 5$ ). Further studies are needed to confirm the differing morphological characters between *S. (G.) paiense* sp. nov.



**Fig. 6.** Male of *Simulium (Gomphostilbia) thuathienense*. (A) Third segment of right maxillary palp with sensory vesicle (front view). (B) Hind tibia (left side; outer view). (C) Basitarsus and second tarsomere of hind leg showing calcipala and pedisulcus (left side; outer view). (D) Coxites, styles and ventral plate (ventral view). (E) Right style (ventrolateral view). (F) Ventral plate and median sclerite (lateral view). (G) Ventral plate (caudal view). (H, I) Abdominal segment 10 and cerci (right half; H, lateral view; I, caudal view). Scale bars = 0.1 mm for B and C; 0.02 mm for A and D–I.

(complex) and *S. (G.) gombakense* since the number of specimens of *S. (G.) gombakense* used in this study was limited.

### 3.3. Description of the male of *S. (G.) thuathienense*

#### *Simulium (Gomphostilbia) thuathienense* Takaoka & Sofian-Azirun, 2015

*Simulium (Gomphostilbia) thuathienense* Takaoka & Sofian-Azirun, in Takaoka et al., 2015: 48–52 (Female, pupa and larva).

This species was described from a pharate female dissected from a pupa and mature larvae collected in Thua Thien Hue Province, central Vietnam (Takaoka et al., 2015). The pupa of this species is characterized by the terminal hooks cone-like (not broad and plate-like), and gill with an inflated Y-shaped structure (when viewed laterally) and eight slender filaments. The inflated structure is composed of a basal portion, and dorsal and forward branches, each branch with three finger-like projections. Among eight slender filaments, six arise from the basal

portion in four groups of one, two, one and two filaments from dorsal to inner-ventral, and each of the remaining two filaments from the apex of one of three projections on each branch. There are three constrictions on the inflated structure, one near the apex of the basal portion and one basally on each branch. The larva of this species is characterized by the postgenal cleft medium-long, nearly as long as the postgenal bridge. The pupa and mature larvae from Thailand agree morphologically with those of *S. (G.) thuathienense* except that pupal abdominal segment 9 has a transverse row of spine-combs on each side.

The male of this species is here described based on two males reared from pupae collected in Thailand.

This is the first record of *S. (G.) thuathienense* from Thailand.

**Male.** Similar to that of *S. (G.) paiense* sp. nov. except following characters. Body length 2.5 mm. **Head.** Much wider than thorax. Holoptic, upper eye consisting of large facets in 14 vertical columns and in 15 or 16 horizontal rows. Face dark brown, bare. Clypeus dark brown, white pruinose, densely covered with golden-yellow scale-like

short hairs interspersed with dark-brown longer hairs except central portion bare. Antenna composed of scape, pedicel and nine flagellomeres, dark brown except base of first flagellomere slightly paler; first flagellomere somewhat elongate, 1.6 times as long as second flagellomere. Maxillary palp: proportional lengths of third, fourth and fifth segments 1.0:1.1:1.9; third segment (Fig. 6A) somewhat widened apically; sensory vesicle (Fig. 6A) ellipsoidal, 0.2–0.3 times as long as third segment, with small opening apically. **Thorax.** Scutum dark brown, slightly shiny on shoulders, along lateral margins and pre-scutellar area when illuminated at certain angles; scutum slightly gray pruinose, densely covered with golden-yellow scale-like recumbent hairs. Scutellum medium brown, with golden-yellow short hairs and dark upright long hairs. Postnotum dark brown and bare. Pleural membrane bare. Katepisternum longer than deep, medium to dark brown with short hairs. **Legs.** Foreleg: coxa dark yellow; trochanter medium brown except base yellow; femur light brown with apical cap medium brown; tibia medium brown except medial large area pale; tarsus dark brown, with moderate dorsal hair crest; basitarsus slightly dilated, 8.9 times as long as its greatest width (7.6 times as long as its greatest width in one male from Chiang Mai Province). Midleg: coxa dark brown; trochanter medium brown; femur light brown with apical cap medium brown; tibia light to medium brown except basal one-third yellow and medial outer surface pale; tarsus medium brown except basal one-third of basitarsus dark yellow. Hind leg: coxa medium brown; trochanter yellow; femur light brown with basal extreme yellow and apical cap dark brown; tibia (Fig. 6B) yellow on basal two-fifths, with light-brown narrow area subbasally, and light to medium brown on rest except apical cap dark brown; tarsus medium brown except basal half of basitarsus (though base light brown) and basal half of second tarsomere yellow; basitarsus (Fig. 6C) nearly parallel-sided, 5.0 times as long as greatest width, and 0.70 and 0.69 times as wide as greatest width of tibia and femur, respectively, (or slightly enlarged, spindle-shaped, 4.3 times as long as wide, and 0.9 and 0.9 times as wide as greatest widths of tibia and femur, respectively in one male from Chiang Mai Province); calipala nearly as long as wide, and 0.36 times as wide as greatest width of basitarsus. **Wing.** Length 2.0 mm. **Genitalia.** Coxites, styles and ventral plate in ventral view as in Fig. 6D. Coxite in ventral view (Fig. 6D) subquadrate, 2.4 times as long as wide. Style in ventral view (Fig. 6D) slender, 0.7 times as long as coxite, slightly curved inward, nearly parallel-sided and with one apical spine; style in ventrolateral view (Fig. 6E) 2.8 times as long as its greatest width at base, tapered from base to basal one-third, nearly parallel-sided to apical one-fourth, then slightly tapered toward round apex. Ventral plate in ventral view (Fig. 6D) transverse, much wider than long, widened posteriorly, with anterior margin produced medially, with posterior margin widely concave, and moderately covered with microsetae on ventral surface; basal arms nearly parallel-sided, though somewhat convergent apically; ventral plate in lateral view (Fig. 6F) somewhat produced ventrally; ventral plate in caudal view (Fig. 6G) deeply concave ventromedially, rounded laterally, and densely covered with microsetae on most of posterior surface except lateral portions bare. Median sclerite broad, plate-like, though lateral margins not well defined (similar to Fig. 3H). Paramere of moderate size, with several long to medium-long hooks. Aedeagal membrane moderately covered with microsetae (similar to Fig. 3J). Ventral surface of abdominal segment 10 (Fig. 6H, I) without distinct hairs. Cercus (Fig. 6H, I) small, with 17 or 18 short hairs.

**Specimens examined.** One male and one female (both with their associated pupal exuviae and cocoons), reared from pupae, and five mature larvae collected a small seasonal stream (elevation 1316 m above sea level, 18°16'44.5"N, 100°30'14.2"E), Khun Sathan village, near Khun Sathan National Park, Nanai District, Nan Province, Thailand, 5-VIII-2017, by W. Srisuka; one male (with its associated pupal exuviae and cocoon), reared from a pupa, and two mature larvae collected a small seasonal stream (elevation 1972 m above sea level, 20°02'12.9"N, 99°08'40.4"E), Banlek, near Doi Phahompok National

Park, Fang District, Chiang Mai Province, Thailand, 23-XI-2011, by W. Srisuka.

**Remarks.** The male of *S. (G.) thuathienense* has the ventral margin of the ventral plate deeply concave medially (Fig. 6G) when viewed posteriorly, a rare character in the subgenus *Gomphostilbia*. This character has, however, been reported in *S. (G.) malewongae* from Thailand, *S. (G.) nuwakotense* Takaoka & Shrestha from Nepal, and *S. (G.) sachini* Takaoka & Willie from India and Nepal (Takaoka et al., 2017; Takaoka and Shrestha, 2010), all in the *S. gombakense* species-group, although the pupae of these three species differ from that of this species by having the inflated gills of different configuration.

In the original description of the female of *S. (G.) thuathienense*, color observations and measurements of legs were limited because only one pharate female dissected from a pupa was available (Takaoka et al., 2015). The following characters, based on a female of this species reared from a pupa from Nan Province, Thailand, augment the original description: fore tibia light brown except the apical cap medium brown and a large median area on the outer surface yellowish; fore basitarsus 6.6 times as long as its greatest width; mid basitarsus yellow on the basal half; hind tibia whitish yellow on the little less than the basal three-fifths; hind basitarsus parallel-sided, 6.5 times as long as wide, and 0.67 and 0.57 times as wide as the greatest widths of the hind tibia and femur, respectively.

#### 3.4. Description of the female of *S. (G.) gombakense*

##### *Simulium (Gomphostilbia) gombakense* Takaoka & Davies, 1995

*Simulium (Morops) gombakense* Takaoka & Davies, 1995: 82–84 (Larva).

*Simulium (Gomphostilbia) gombakense*: Takaoka, 2000: 111–114 (Male and pupa)

This species was described from a mature larva under the subgenus *Morops* from Peninsular Malaysia (Takaoka and Davies, 1995). It was transferred to the subgenus *Gomphostilbia* when its male and pupa were described by Takaoka (2000). The female of this species was later described based on females reared from pupae collected from Mae Klang Waterfall, Chiang Mai Province, Thailand (site 2 in Table 1) (Takaoka et al., 2010). However, our genetic analysis shows that the female thought to be *S. (G.) gombakense* probably is that of genotype B or C of the *S. (G.) paiense* sp. nov. complex, described above. The females and pupae from Mae Klang Waterfall agree morphologically with those of *S. (G.) paiense* sp. nov., although the frons:head ratio (1.0:5.2–5.3) of the female is different from that (1.0:4.4–4.8) of *S. (G.) paiense* sp. nov.

The female of *S. (G.) gombakense* is here described based on two females reared from pupae collected from Gombak (type locality) and one female reared from a pupa from Fraser's Hill, Peninsular Malaysia.

**Female** (n = 3). Similar to female of *S. (G.) paiense* sp. nov. except the following characters. Body length 2.0–2.3 mm. **Head.** Frontal ratio 1.7–1.8:1.0:2.6–3.0; frons:head ratio 1.0:5.0–5.4. Maxillary palp: proportional lengths of third, fourth, and fifth segments 1.0:1.0:2.3–2.4; third segment swollen; sensory vesicle oblong, 0.6–0.7 times as long as third segment. Maxillary lacinia with 8 inner and 10 or 11 outer teeth. Mandible with 20–22 inner and 8–10 outer teeth. **Legs.** Foreleg: basitarsus moderately dilated, 5.8–6.0 times as long as its greatest width. Hind leg: basitarsus 5.9–6.7 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. Claw with large basal tooth 0.46 times as long as claw. **Wing.** Length 2.0–2.1 mm. **Terminalia.** Sternite 8 with 15–18 medium-long to long hairs and three to five short hairs on each side. Ovipositor valve moderately covered with microsetae interspersed with one or two short hairs. Paraproct in lateral view somewhat produced ventrally, 0.5 times as long as wide, with 16–20 short to long hairs on ventral and lateral surface. Cercus in lateral view 0.5 times as long as wide. Spermatheca 1.4–1.5 times as long as wide.

**Specimens examined.** Two females reared from pupae collected from Gombak, Peninsular Malaysia, 17-III-2011, by H. Takaoka, and

one female reared from a pupa collected from Fraser's Hill, Peninsular Malaysia, 12-IV-2011, by H. Takaoka.

**Distribution.** Peninsular Malaysia and Thailand.

**Remark.** The slight difference in the females of *S. (G.) gombakense* and *S. (G.) paiense* sp. nov. is recognized, as noted above.

#### 4. Discussion

Our genetic analysis using COI sequences reveals that Thai populations of the so-called *S. (G.) gombakense* are composed of at least four lineages including two new lineages. Lineage 1 is equivalent to typical *S. (G.) gombakense*, and lineages 2, 3 and 4 are treated as genoforms A, B and C within a species complex of *S. (G.) paiense* sp. nov. described based on genoform A. The current data show that in Thailand, *S. (G.) gombakense* is distributed in the central and southern regions, whereas all three genoforms of *S. (G.) paiense* sp. nov. have a limited distribution in the northern region, though genoform C is also distributed in the northeastern and western regions. More extensive surveys are needed to determine the extent of genetic divergence and geographical distribution of each genoform of the *S. (G.) paiense* sp. nov. complex.

#### 5. Conclusion

Our molecular and morphological analyses show that *S. (G.) gombakense* in Thailand is composed of two morphologically distinguishable species, *S. (G.) gombakense* and *S. (G.) paiense* sp. nov., and the latter is represented by three genetically distinct lineages. The present survey increases the number of black fly species in the *S. (G.) gombakense* species-group in Thailand from three to five, by adding *S. (G.) thuathienense* and *S. (G.) paiense* sp. nov. All but one species, *S. (G.) gombakense*, are distributed in the northern region of Thailand, suggesting that this region is a hot spot of biodiversity for this species-group.

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#### References

- Adler, P.H., Crosskey, R.W., 2017. World Blackflies (Diptera: Simuliidae): A Comprehensive Revision of the Taxonomic and Geographical Inventory [2017]. 131 pp. (Accessed 10 January 2018). <http://entweb.clemson.edu/biomia/pdfs/blackflyinventory.pdf>.
- Adler, P.H., Takaoka, H., Sofian-Azirun, M., Low, V.L., Ya'cob, Z., Chen, C.D., Lau, K.W., Pham, Z.D., 2016. Vietnam, a hotspot for chromosomal diversity and cryptic species in black flies (Diptera: Simuliidae). *PLoS One* 11 (10), e0163881.
- Fukuda, M., Choochote, W., Bain, O., Aoki, C., Takaoka, H., 2003. Natural infections with filarial larvae in two species of black flies (Diptera: Simuliidae) in northern Thailand. *Jpn. J. Trop. Med. Hyg.* 31 (2), 99–102.
- Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst. Biol.* 59 (3), 307–321.
- Low, V.L., Takaoka, H., Adler, P.H., Ya'cob, Z., Norma-Rashid, Y., Chen, C.D., Sofian-Azirun, M., 2015. A multi-locus approach resolves the phylogenetic relationships of the *Simulium asakoe* and *Simulium ceylonicum* species groups (Diptera: Simuliidae) in Malaysia: evidence for distinct evolutionary lineages. *Med. Vet. Entomol.* 29, 330–337.
- Pramual, P., Wongpakam, K., Adler, P.H., 2011. Cryptic biodiversity and phylogenetic relationships revealed by DNA barcoding of Oriental black flies in the subgenus *Gomphostilbia* (Diptera: Simuliidae). *Genome* 54, 1–9.
- Swofford, D.L., 2002. PAUP\* Phylogenetic Analysis Using Parsimony (\*and Other Methods). Sinauer Associates, United States.
- Takaoka, H., 2000. Taxonomic notes on *Simulium gombakense* (Diptera: Simuliidae) from Peninsular Malaysia: descriptions of male and pupa, and subgeneric transfer from *Morops* to *Gomphostilbia*. *Jpn. J. Trop. Med. Hyg.* 28 (2), 111–114.
- Takaoka, H., 2003. The Black Flies (Diptera: Simuliidae) of Sulawesi, Maluku and Irian Jaya. xxii + 581 pp. Kyushu University Press, Fukuoka.
- Takaoka, H., 2012. Morphotaxonomic revision of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) in the Oriental Region. *Zootaxa* 3577, 1–42.
- Takaoka, H., Choochote, W., 2005. Two new species of black flies (Diptera: Simuliidae) from northern Thailand. *Med. Entomol. Zool.* 56, 319–334.
- Takaoka, H., Choochote, W., Aoki, C., Fukuda, M., Bain, O., 2003. Black flies (Diptera: Simuliidae) attracted to humans and water buffalos and natural infections with filarial larvae, probably *Onchocerca* sp., in northern Thailand. *Parasite* 10 (1), 3–8.
- Takaoka, H., Davies, D.M., 1995. The Black Flies (Diptera: Simuliidae) of West Malaysia. viii + 175 pp. Kyushu University Press, Fukuoka.
- Takaoka, H., Fukuda, M., Otsuka, Y., Aoki, C., Uni, S., Bain, O., 2012. Blackfly vectors of zoonotic onchocerciasis in Japan. *Med. Vet. Entomol.* 26, 372–378.
- Takaoka, H., Shrestha, S., 2010. New species of black flies (Diptera: Simuliidae) from Nepal. *Zootaxa* 2731, 1–62.
- Takaoka, H., Sofian-Azirun, M., Ya'cob, Z., Chen, C.D., Lau, K.W., Pham, X.D., 2015. The black flies (Diptera: Simuliidae) from Thua Thien Hue and Lam Dong Provinces, Vietnam. *Zootaxa* 3961, 1–96.
- Takaoka, H., Srisuka, W., Saeung, A., 2010. Description of the female of *Simulium* (*Gomphostilbia*) *gombakense* (Diptera: Simuliidae) from Thailand. *Med. Entomol. Zool.* 61 (2), 111–114.
- Takaoka, H., Srisuka, W., Saeung, A., 2017. *Simulium malewongae*, a new species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from Thailand. *J. Med. Entomol.* 54, 91–99.