Tropical Biomedicine 39(1): 60-65 (2022)
https://doi.org/10.47665/tb.39.1.010

RESEARCH ARTICLE

Description of the male of *Simulium triglobus* Takaoka & Kuvangkadilok (Diptera: Simuliidae) from Thailand

Pramual, P.[1*], Jomkumsing, P.[1], Tangkawanit, U.[2], Wongpakam, K.[3], Bunchom, N.[4], Takaoka, H.[5]

[1]Department of Biology, Faculty of Science, Mahasarakham University, Kantharawichai District, Maha Sarakham 44150, Thailand
[2]Department of Entomology and Plant Pathology, Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand
[3]Walai Rukhavej Botanical Research Institute, Mahasarakham University, Kantharawichai District, Maha Sarakham, 44150, Thailand
[4]School of Biotechnology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand, 30000, Thailand
[5]Higher Institution of Centre of Excellence (HICoE), Tropical Infectious Diseases Research and Education Centre (TIDREC), Universiti Malaya, 50603, Kuala Lumpur, Malaysia
*Corresponding author: pairot.p@msu.ac.th

ARTICLE HISTORY

Received: 31 January 2022
Revised: 22 February 2022
Accepted: 22 February 2022
Published: 31 March 2022

ABSTRACT

Simulium triglobus Takaoka & Kuvangkadilok from Thailand, in the *Simulium* (*Simulium*) *multistriatum* species-group, is unique among species in the family Simuliidae in having the female terminalia with three spermathecae (rather than one spermatheca). This species was described from Nan province, northern Thailand based on larvae, pupae and females but its male has remained unknown. In this study, the male of *S. triglobus* is described for the first time based on adult males reared from pupae collected from the type locality. The most distinctive characteristic of the male of *S. triglobus* is the shape of the ventral plate, which is hexagonal when viewed ventrally. No other members of *S. multistriatum* species group known thus far have such a unique ventral plate. In addition, the number of upper-eye (large) facets and color patterns of the legs can be used to differentiate this species in the male from other members of the *S. multistriatum* species-group. Mitochondrial cytochrome *c* oxidase I sequences enabled association of adult male specimens of *S. triglobus* with previously known life stages. Phylogenetic analysis based on these sequences revealed that specimens of *S. triglobus* formed a strongly supported monophyletic clade, being genetically distinct from other members of *S. multistriatum* species-group in Thailand.

Keywords: Black fly; ventral plate; spermathecae; morphology; *S. multistriatum* species-group.

INTRODUCTION

Black flies (Diptera: Simuliidae) are medically and veterinary important blood-sucking insects. Many species are known as vectors of disease-causing agents transmitted to humans and other animals. The most well recognized disease is human onchocerciasis or river blindness which is caused by the filarial parasite, *Onchocerca volvulus*, which is transmitted by at least 27 black fly species/species complexes (Adler & McCreadie, 2019). In addition to *O. volvulus* causing disease in humans, there are at least 11 other *Onchocerca* species that are found in other animals which might potentially cause zoonotic disease (Takaoka *et al*., 2012). Black flies are also vectors of haemosporidian parasites of the genus *Leucocytozoon* and *Trypanosoma* that cause disease in many avian species (Adler & McCreadie, 2019). Even without disease agent transmission, biting of black flies can cause nuisance and irritation (Adler & McCreadie, 2019).

There are 2,401 black fly species recorded globally, comprising 2,384 extant and 17 fossil taxa (Adler, 2021). In Thailand, 138 black fly species have been recorded (Srisuka *et al*., 2019, 2021; Thaijarern *et al*., 2019; Takaoka *et al*., 2019a, 2019b, 2020, 2021; Aupalee *et al*., 2020).

Simulium triglobus Takaoka & Kuvangkadilok from Thailand is remarkable in having the female terminalia with three spermathecae (rather than one spermatheca) (Takaoka & Kuvangkadilok, 1999). It belongs to the *S. multistriatum* species-group, in which 36 species are recorded globally. Of these, 12 (*S. bullatum* Takaoka & Choochote, *S. chainarongi* Kuvangkadilok & Takaoka, *S. chaliowae* Takaoka & Boonkemtong, *S. chanyae* Takaoka & Choochote, *S. daoense* Takaoka & Adler, *S. fenestratum* Edwards complex, *S. lampangense* Takaoka & Choochote, *S. malayense* Takaoka & Davies complex, *S. takense* Takaoka & Choochote, *S. triglobus* Takaoka & Kuvangkadilok, *S. ubonae* Thaijarern, Wongpakam, Kangrang & Pramual, *S. undecimum* Takaoka, Srisuka & Saeung) have been reported in Thailand (Adler, 2021). Among the members of the *S. multistriatum* species-group found in Thailand, *S. fenestratum* and *S. malayense* were considered as species complexes based on cytogenetic and molecular investigations (Thaijarern *et al*., 2018; Takaoka *et al*., 2019a). One chromosomally distinct member (cytoform C) of the *S.*

malayense complex was recognized as *S. malayense* sensu stricto, another (cytoform B) was subsequently described as *S. undecimum*, and a third (cytoform A) remains formally undescribed (Thaijarern *et al*., 2018; Adler *et al*., 2019; Takaoka *et al*., 2019b). The *S. fenestratum* complex remains taxonomically unresolved.

Simulium triglobus was described based on females, pupae and larvae collected from highly calcareous stream (Tontong waterfall) in Nan province, northern Thailand (Takaoka & Kuvangkadilok, 1999). However, its male remained unknown (Takaoka *et al*., 2019a).

In the present study, the male of *S. triglobus* is described for the first time based on specimens reared from pupae collected from the type locality. The species identity of the males is confirmed morphologically by the characteristics of their associated pupal exuviae and cocoons and genetically by the mitochondrial cytochrome *c* oxidase I (COI) gene sequence-based analysis. Morphological and molecular characteristics of adult males of *S. triglobus* are compared with other species of the *S. multistriatum* species-group.

MATERIALS AND METHODS

Sample collection

Black fly specimens were sampled from Tontong waterfall, Nan Province (19° 12' 39" N /101° 04' 04" E, 1,034 m above sea level) the type locality of *S. triglobus* (Takaoka & Kuvangkadilok, 1999) on 5 October 2018. Pupae were collected from a stream using fine forceps and preserved in 80% ethanol. Some pupae were reared to adults in plastic bottles. Adult specimens were fixed in 80% ethanol for further morphological examination.

Material examined

In total, seven adult males of *S. triglobus* were successfully reared from the pupae, three were used for morphological examination and description and four were used for molecular analysis.

Morphological examination and descriptions

Morphological characteristics of three males were examined under a stereomicroscope. Heads and abdomens dissected were cleared in hot 85% lactic acid, transferred to glycerin and then examined under a compound microscope. Morphological characteristics were measured using a microscopic micrometer. Illustrations were drawn using a camera lucida, attached to a Leica DM 750 compound microscope. Descriptions of morphological characteristics followed the terminology of Takaoka and Suzuki (1984) and Adler *et al*. (2004). Specimens were deposited in the Department of Biology, Mahasarakam University, Maha Sarakham Province, Thailand.

DNA extraction, polymerase chain reaction (PCR), and sequencing

DNA was extracted from the whole individual using the GF-1 Nucleic Acid Extraction Kit (Vivantis Technologies Sdn. Bhd, Malaysia). Polymerase chain reaction (PCR) of the COI gene barcoding region was amplified using the primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al*., 1994). PCR reaction conditions followed those of Tangkawanit *et al.* (2018). PCR products were checked with 1% agarose gel electrophoresis and purified using a PureDireX PCR Clean-Up & Gel Extraction Kit (Bio-Helix, Taiwan). DNA sequencing was performed at ATCG Company Limited (Thailand Science Park (TSP), Pathumthani, Thailand) using the same primers as for PCR.

DNA sequence analysis

A total of 146 COI sequences were obtained for analyses of genetic variation and genetic relationship among 11 species members of *S. multistriatum*. Of these sequences, four were obtained from male specimens of *S. triglobus* described in this study (GenBank accession no. OM421807 – OM421810) and the remainder were from previous studies (Thaijarern *et al*., 2018, 2019; Pramual *et al*., 2021). Intraspecific and interspecific genetic divergences were calculated based on Kimura 2-parameter in TaxonDNA (Meier *et al*., 2006). Phylogenetic relationships were inferred based on COI sequences using neighbor-joining (NJ) estimated in MEGA X (Kumar *et al*., 2018) and branch support was calculated using the bootstrapping method with 1,000 replicates. Two sequences of *S. siripoomense* (GenBank accession no. MZ508659 - 60) were used as outgroups for phylogenetic analysis The output trees were visualized and graphically prepared with FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

RESULTS

Description of the male of* Simulium triglobus *Takaoka & Kuvangkadilok

Male (n=3). Body length 2.5–2.8 mm. ***Head.*** Slightly wider than thorax. Upper eye medium brown, with large facets in 17 or 18 vertical columns and 18 or 19 horizontal rows. Clypeus black, thickly white pruinose, sparsely or moderately covered with dark-brown hairs. Antenna with scape, pedicel and nine flagellomeres, dark brown except base of first and second flagellomeres yellow; first flagellomere elongate, twice as long as second one. Maxillary palpus grayish to dark brown, composed of five segments with proportional lengths 1.0:1.3:2.2:2.8:6.0; third segment of moderate size, with apex somewhat produced inward; sensory vesicle (Figure 1A) short, 0.26 times length of third segment, ellipsoidal and with opening of moderate size. ***Thorax***. Scutum black, densely covered with white pruinosity that is shiny and iridescent when illuminated in front and viewed dorsally; scutum uniformly and densely covered with golden-yellow recumbent short hairs interspersed with dark-brown long upright hairs on prescutellar area. Scutellum brownish black, covered with golden-yellow short hairs and several dark long upright hairs. Postnotum black, white pruinose when illuminated in front and viewed dorsally. Pleural membrane bare. Katepisternum longer than deep, brownish black to black, shiny and bare. ***Legs***. Foreleg (Figure 1B): coxa, trochanter and femur whitish-yellow; tibia medium brown, gradually darkened apically with apical 1/4 brownish-black except outer surface widely white medially; tarsus entirely black, with moderate dorsal hair crest; basitarsus greatly dilated, 4.4 times as long as its greatest width. Midleg (Figure 1C): coxa brownish-black; trochanter light brown on basal 1/2 and medium brown on rest; femur medium brown; tibia light brown on basal 1/3, gradually darkened apically, tarsus brownish-black except basal 1/3 of basitarsus light brown; Hind leg (Figure 1D) coxa brownish-black; trochanter whitish-yellow, femur light brown, gradually darkened apically with apical 1/5 brownish-black; tibia brownish-black with large white sheen area on posterior surface of the basal portion; tarsus brownish-black with basal 1/2 of basitarsus light brown; basitarsus much enlarged, 4.5 times as long as its greatest width and 0.83 times and 0.67 times as wide as greatest width of hind tibia and femur, respectively; calcipala small, slightly shorter than width at base, 0.28 times as wide as greatest width of basitarsus; pedisulcus well developed.

Wing. Length 2.4–2.5 mm. Costa with dark spinules and hairs; subcosta haired except near apex bare; basal section of radius entirely bare, R_1 with dark spinules and hairs, R_2 with hairs; hair tuft on base of radius dark brown; basal cell absent. ***Halter.*** Light yellow. ***Abdomen.*** Basal scale dark brown to brownish black, with fringe of dark long hairs. Dorsal surface of abdomen brownish black, covered with dark short hairs, segments 2 and 5–7 each with pair of silvery or bluish iridescent dorsolateral spots, those on segment 2 broadly connected in middle to each other.

Genitalia. Coxite in ventral view (Figure 1E) nearly quadrate, 0.8 times as long as wide, covered with many stout hairs on posterior half. Style in ventral view (Figure 1E) elongate, 3.85 times as long as its greatest width at apical quarter, nearly parallel-sided and with subapical spine; style in dorsolateral view (Figure 1F), 1.5 times as long as coxite, with short horn-like basal protuberance having numerous small cone-like spines on anterior surface; ventral plate in ventral view (Figure 1G) hexagonal in shape, much widened on the middle part, with several setae on ventral surface;

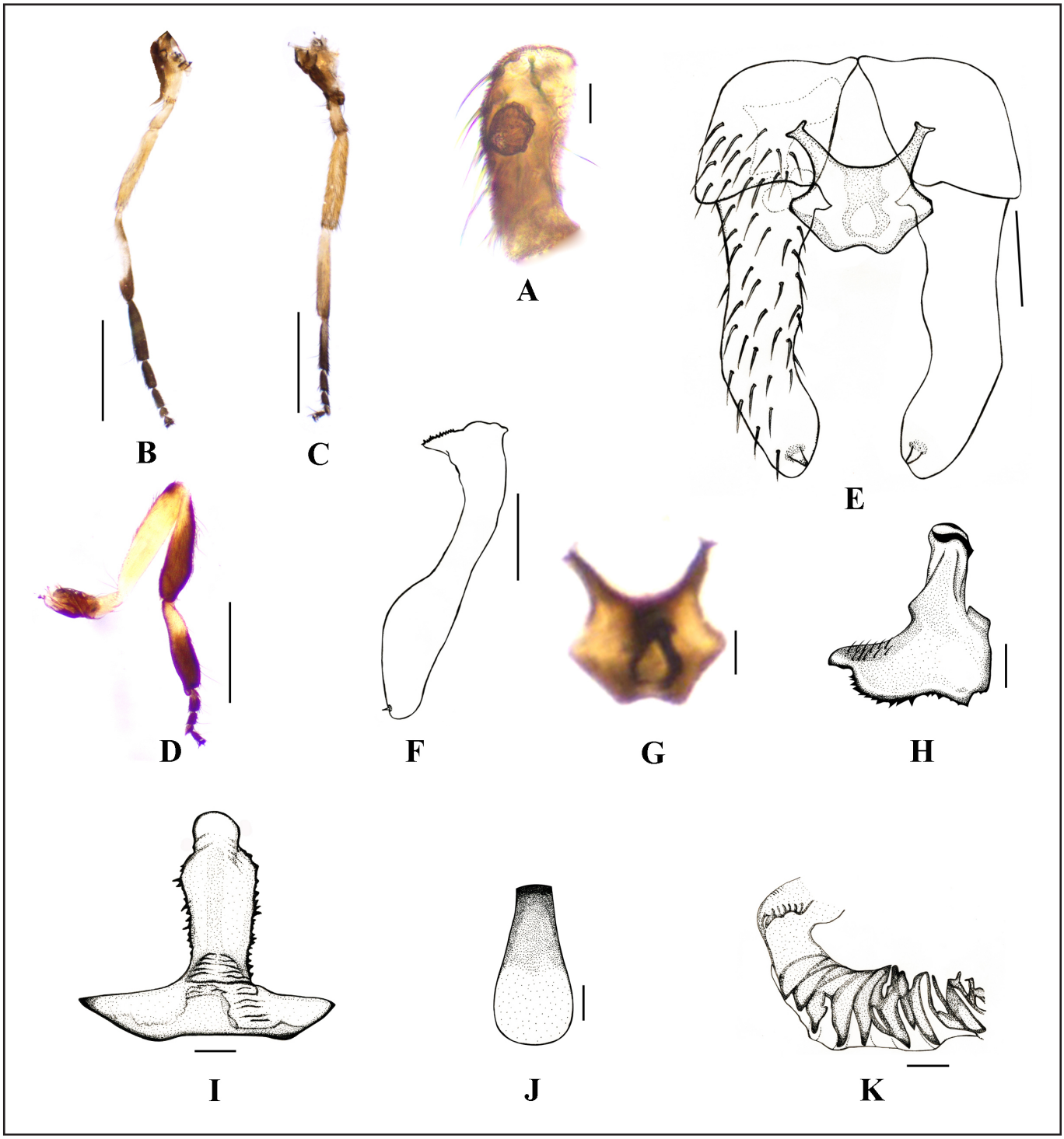

Figure 1. Male of *Simulium triglobus* (A) Third segment of maxillary palp with sensory vesicle (right side; side view) (B) Fore leg (C) Mid leg (D) Hind leg (E) Coxites, styles and ventral plate (ventral view) (F) Style (right side; dorsolateral view) (G) Ventral plate (ventral view) (H) Ventral plate (lateral view) (I) ventral plate (caudal view) (J) Median sclerite (caudal view) (K) paramere (right side; caudal view). Scale bars: 0.5 mm for B – D; 0.1 mm for E, F; 0.02 mm for A, G – K.

arms directed forward and outward diverging from each other at angle of 70–80°; ventral plate in lateral view (Figure 1H), with serrated posterior margin; ventral plate in end view (Figure 1I) having several teeth in irregular rows on posterior surface except apical part of elliptical process smooth. Median sclerite (Figure 1J) plate-like, with apical tip widened, with round apex. Paramere (Figure 1K) composed of 8 distinct large hooks and several smaller ones. Aedeagal membrane moderately covered with minute setae, with weakly sclerotized dorsal plate in form of curved bar. Abdominal segment 10 without distinct hairs on ventral and lateral surfaces.

Biological notes. The biting behavior of adult of *S. triglobus* is unknown. The pupae and larvae of this species were collected from fallen leaves or attached to the streambed in a highly calcareous, completely shaded stream at 1,034 m above sea level, stream width of 1.67 m, depth of 0.027 m, current velocity of 1.08 ms^{-1}, water conductivity of 300 μScm^{-1} and water temperature of 17.7°C. Associated black fly species were *Simulium weji* Takaoka and *S. chaliowae* Takaoka and Boonkemtong.

DNA sequence variation and DNA barcode tree

The COI sequences of males of *S. triglobus* in this study diverged from previously reported data from conspecifics (range between 0.16% and 1.29% with an average of 0.58%). Minimum interspecific genetic distance compared between *S. triglobus* and other members of *S. multistriatum* was between 7.59% (*S. malayense* complex) and 9.69% (*S. daoense*, *S. takense*, *S. undecimum*) (Table 1). The NJ tree (Figure 2) revealed that all specimens of *S. triglobus*, including those from adult males described in this study, were clustered and formed a monophyletic clade with strong (100%) bootstrap support.

DISCUSSION

Males of *S. triglobus* possess a unique morphological characteristic of the ventral plate (hexagonal-shape in ventral view) among the known species of *S. multistriatum* species-group. The ventral plates of most species of this species-group are nearly rectangular or subquadrate although varying in degrees of roundness on the posterolateral corner. Thus, this morphological characteristic could readily distinguish *S. triglobus* in the male from other known species of the *S. multistriatum* species-group.

In addition, the male of *S. triglobus* is morphologically differentiated from other known species of *S. multistriatum* species-group recorded in Thailand (except *S. bullatum*, of

Table 1. Range and mean of genetic distances between *Simulium triglobus* (n = 11) and other members of *Simulium multistriatum* species-group in Thailand based on COI sequences using Kimura 2-parameter model

Species (n)	Minimum (%)	Maximum (%)	Mean (%)
S. bullatum (12)	7.92	9.05	8.25
S. chainarongi (14)	8.24	9.21	8.7
S. chaliowae (23)	8.24	10.66	9.37
S. daoense (8)	9.69	14.54	11.41
S. fenestratum complex (41)	7.75	10.18	8.7
S. lampangense (8)	7.75	9.21	8.62
S. malayense complex (11)	7.59	15.19	9.72
S. takense (6)	9.69	11.31	10.53
S. ubonae (9)	8.72	10.02	9.28
S. undecimum (3)	9.69	10.34	9.92

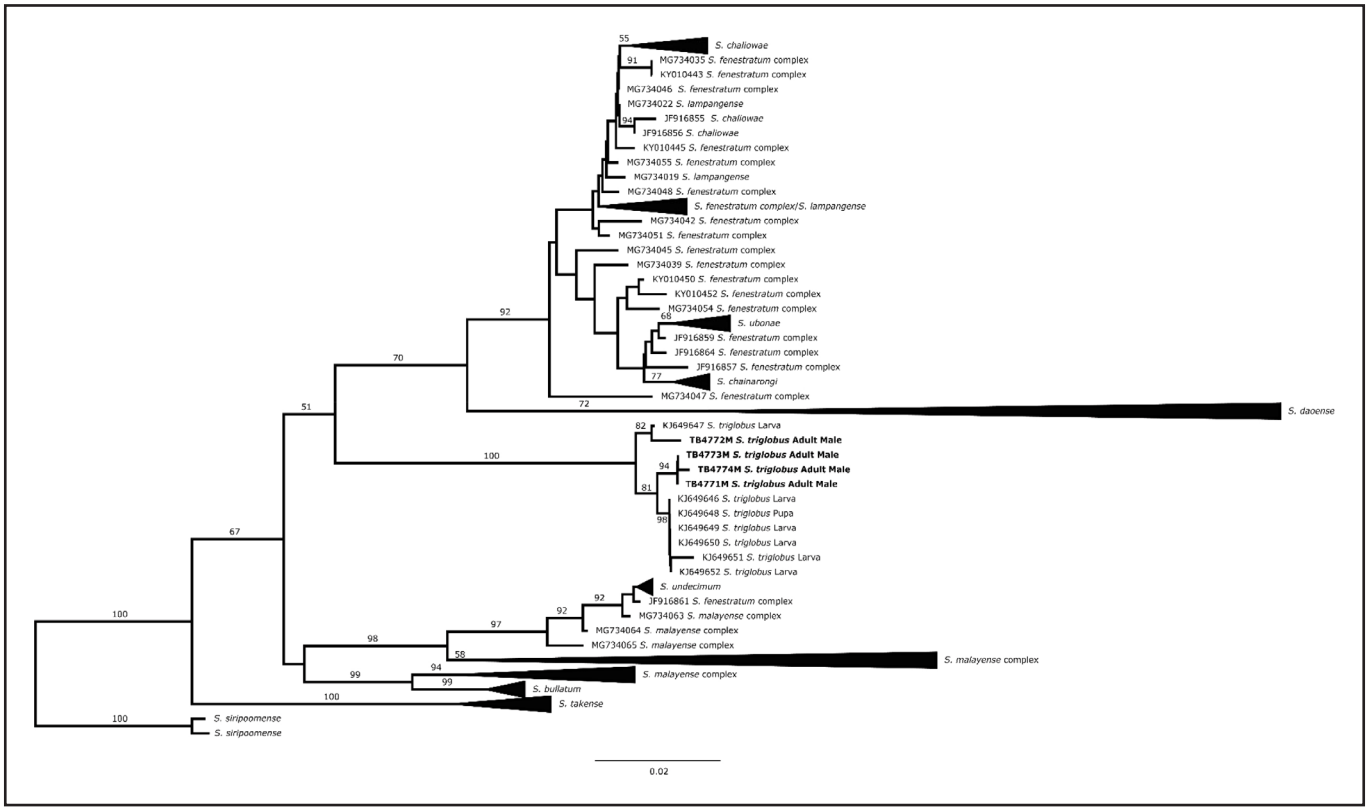

Figure 2. Neighbor-joining tree based on 146 mitochondrial cytochrome *c* subunit I haplotypes of 11 species of the black flies of the *Simulium multistriatum* species-group in Thailand. Bootstrap values are shown above or near the branches. Bold characters indicates specimens obtained in this study.

which the male is unknown) by the following characteristics (those of the known species in parentheses): from *S. chainarongi*, *S. takense*, *S. undecimum* and *S. malayense* by the number of the upper-eye (large) facets in 17 or 18 vertical columns and 18 or 19 horizontal rows (15–16 vertical columns and 17 horizontal rows in *S. malayense,* and 20 or 21 vertical columns and 20 or 21 horizontal rows in the three other species) (Takaoka & Kuvangkadilok, 1999; Takaoka & Choochote, 2005; Takaoka *et al*., 2018, 2019b); from *S. chaliowae* and *S. fenestratum* by coloration of the hind basitarsus, which is light brown on the basal ½ and brownish-black on the rest (entirely light brown) (Takaoka & Kuvangkadilok, 1999), from *S. daoense* by coloration of the mid tibia without a white sheen on posterior surface (with a white sheen on posterior surface) (Takaoka *et al*., 2017), from *S. chanyae* also by coloration of mid tibia, which is brown on the basal 1/3 and gradually darkened toward the apex (whitish except the apical cap darkened) (Takaoka *et al*., 2018).

Among the known species of *S. multistriatum* species-group in Thailand, the male of *S. triglobus* is most similar to *S. lampangense* in sharing several morphological characteristics (except the shape of the ventral plate in ventral view) including the number of the upper-eye (large) facets in 17 or 18 vertical columns and 18 or 19 horizontal rows, and small sensory vesicle (0.26 times the length of the third segment of the maxillary palpus).

Molecular information based on COI barcoding sequences confirmed the species identity of the male specimens collected from the type locality of *S. triglobus*. Maximum genetic distance of 1.29% between adult males compared with the previously reported data for the larva and pupa of *S. triglobus* falls in the range of within-species genetic variation for tropical black fly species (Pramual *et al*., 2021). However, DNA barcodes were unsuccessful for identification of some members of the *S. multistriatum* species-group in Thailand such as the *S. fenestratum* complex, *S. chainarongi*, *S. chalioawae* and *S. lampangense* (Pramual *et al*., 2021). All specimens of *S. triglobus* are molecularly clearly differentiated from other closely related species. The results from COI barcoding sequences thus support the morphological uniqueness of *S. triglobus*.

Females of this species possess three spermathecae, a unique characteristic among all black fly species known thus far (Takaoka & Kuvangkadilok, 1999). The adult male described in the present study also shows a unique morphological characteristic with the hexagonal-shaped ventral plate.

ACKNOWNLEDGEMENTS

This research project was financially supported by Thailand Science Research and Innovation (TSRI) and Mahasarakham University. We would like to thank Adrian Plant for valuable comments on a previous version of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

Adler, P.H. (2021). World blackflies (Diptera: Simuliidae): a comprehensive revision of the taxonomic and geographical inventory [2021]. https://biomia.sites.clemson.edu/pdfs/blackflyinventory.pdf. Accessed 30 May 2021.

Adler, P.H., Currie, D.C. & Wood, D.M. (2004). The Black Flies (Simuliidae) of North America. New York: Cornell University Press. pp. xv+941.

Adler, P.H. & McCreadie, J.W. (2019). Black flies (Simuliidae). In: Medical and Veterinary Entomology, Mullen, G.R. & Durden, L. A. (editors), 3rd edition. San Diego, CA: Elsevier, pp. 237-259. https://doi.org/10.1016/B978-0-12-814043-7.00014-5

Aupalee, K., Saeung, A., Srisuka, W., Fukuda, M., Junkum, A., Pitasawat, B. & Takaoka, H. (2020). Three new species of the *Simulium* (*Simulium*) *striatum* species-group (Diptera: Simuliidae) from Thailand, with their phylogenetic relationships. *Acta Tropica* **211**: 1-14. https://doi.org/10.1016/j.actatropica.2020.105625

Adler, P.H., Srisuka, W., Low, V.L., Takaoka, H. & Saeung, A. (2019). High-elevation chromosomal diversity of black flies (Diptera: Simuliidae) in Thailand. *Insect Systematics and Diversity* **3**: 1-10. https://doi.org/10.1093/isd/ixz004

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. *Molecular Marine Biology and Biotechnology* **3**: 294-299.

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35**: 1547-1549. https://doi.org/10.1093%2Fmolbev%2Fmsy096

Meier, R., Shiyang, K., Vaidya, G. & Ng, P.K. (2006). DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. *Systematic Biology* **55**: 715-728. https://doi.org/10.1080/10635150600969864

Pramual, P., Jomkumsing, P., Wongpakam, K. & Wongwian, P. (2021). DNA barcoding of tropical black flies (Diptera: Simuliidae) in Thailand: One decade of progress. *Acta Tropica* **224**: 106116. https://doi.org/10.1016/j.actatropica.2021.106116

Srisuka, W., Takaoka, H., Fukuda, M., Otsuka, Y. & Saeung, A. (2019). A new species of the *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from Thailand, with its phylogenetic relationships with related species in the *Simulium asakoae* species-group. *Acta Tropica* **197**: 105043. https://doi.org/10.1016/j.actatropica.2019.105043

Srisuka, W., Aupalee, K., Low, V.L., Yacob, ´ Z., Fukuda, M., Saeung, A. & Takaoka, H. (2021). A new species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from Northern Thailand, with its genetic relationship in the *S. asakoae* species-group. *Acta Tropica* **218**: 105889. https://doi.org/10.1016/j.actatropica.2021.105889

Takaoka, H. & Choochote, W. (2005). Two new species of *Simulium* Latreille (Diptera: Simuliidae) from northwestern Thailand. *Medical Entomology and Zoology* **56**: 123-133. https://doi.org/10.7601/mez.56.123

Takaoka, H., Fukuda, M., Otsuka, Y., Aoki, C., Uni, S. & Bain, O. (2012). Blackfly vectors of zoonotic onchocerciasis in Japan. *Medical and Veterinary Entomology* **26**: 372-378. https://doi.org/10.1111/j.1365-2915.2012.01023.x

Takaoka, H. & Kuvangkadilok, C. (1999). Four new species of black flies (Diptera: Simuliidae) from Thailand. *Japanese Journal of Tropical Medicine and Hygiene* **27**: 497-509. https://doi.org/10.2149/tmh1973.27.497

Takaoka, H., Sato, H. & Higashihara, J. (2018). New records of five species of black flies (Diptera: Simuliidae) from Myanmar, with descriptions of the male and pupa of *Simulium chanyae*. *Tropical Biomedicine* **35**: 804-814.

Takaoka, H., Sofian-Azirun, M., Ya'Cob, Z., Chen, C.D., Lau, K.W., Low, V.L., Pham, X.D. & Adler, P.H. (2017). The black flies (Diptera: Simuliidae) of Vietnam. *Zootaxa* **4261**: 1-165. https://doi.org/10.11646/zootaxa.4261.1.1

Takaoka, H., Srisuka, W., Fukuda, M. & Saeung, A. (2020). Twenty-one species of the *Simulium* (*Gomphostilbia*) *asakoae* species-group (Diptera: Simuliidae) in Thailand. *ZooKeys* **950**: 51-152. https://doi.org/10.3897%2Fzookeys.950.51298

Takaoka, H., Srisuka, W., Low, V.L. & Saeung, A. (2019b). A new species of the *Simulium* (*Simulium*) *multistriatum* species group (Diptera: Simuliidae) from Thailand and its phylogenetic relationships with related species. *Journal of Medical Entomology* **56**: 86-94. https://doi.org/10.1093/jme/tjy178

Takaoka, H., Srisuka, W. & Saeung, A. (2019a). Checklist and keys for the black flies (Diptera: Simuliidae) in Thailand. *Medical Entomology and Zoology* **70**: 53-77. https://doi.org/10.7601/mez.70.53

Takaoka, H., Srisuka, W., Ya'cob, Z., Low, V.L. & Saeung, A. (2021). A new species of the *Simulium* (*Gomphostilbia*) *ceylonicum* species-group (Diptera: Simuliidae) from Thailand. *Acta Tropica* **217**: 105865. https://doi.org/10.1016/j.actatropica.2021.105865

Takaoka, H. & Suzuki, H. (1984). The blackflies (Diptera: Simuliidae) from Thailand. *Medical Entomology and Zoology* **35**: 7-45. https://doi.org/10.7601/mez.35.7

Tangkawanit, U., Wongpakam, K. & Pramual, P. (2018). A new black fly (Diptera: Simuliidae) species of the subgenus *Asiosimulium* Takaoka Choochote from Thailand. *Zootaxa*, **4388**: 111-122. https://doi.org/10.11646/zootaxa.4388.1.8

Thaijarern, J., Adler, P.H. & Pramual, P. (2018). Limited differentiation among black flies in the *Simulium multistriatum* species group (Diptera: Simuliidae) in Thailand: cryptic species, homosequential species and homosequential cryptic species. *Zoological Journal of the Linnean Society* **184**: 1024-1054. https://doi.org/10.1093/zoolinnean/zly023

Thaijarern, J., Wongpakam, K. & Pramual, P. (2019). A new species of black fly (Diptera: Simuliidae) in the *Simulium* (*Simulium*) *multistriatum* species group from Thailand. *Zootaxa* **4586**: 461-474. https://doi.org/10.11646/zootaxa.4586.3.4