



RESEARCH ARTICLE

Morphological redescription of *Simulium takahasii* (Rubtsov), the first species of the subgenus *Wilhelmia* Enderlein (Diptera: Simuliidae) recognized in East Asia

Takaoka, H.¹, Otsuka, Y.^{2*}, Fukuda, M.³, Low, V.L.^{1*}, Ya'cob, Z.¹

¹Higher Institution of Centre of Excellence (HICoE), Tropical Infectious Diseases Research and Education Centre (TIDREC), Universiti Malaya, 50603, Kuala Lumpur, Malaysia

²International Center for Island Studies, Kagoshima University, Korimoto 1-21-24, Kagoshima, 890-8580 Japan

³Institute for Research Management, Oita University, Idaigaoka 1-1, Hasama, Yufu City, Oita, 879-5593, Japan

*Corresponding authors: vanlun_low@um.edu.my; yotsuka@cpi.kagoshima-u.ac.jp

ARTICLE HISTORY

Received: 10 May 2023

Revised: 31 May 2023

Accepted: 31 May 2023

Published: 30 June 2023

ABSTRACT

Simulium takahasii (Rubtsov), which was originally described from Japan, and recorded from Korea and China, is the first among the 19 species of the subgenus *Wilhelmia* Enderlein recorded from East Asia. It is striking in mating, blood-feeding and ovipositing in captivity and in experimentally transmitting *Dirofilaria immitis* (Leidy) and *Brugia pahangi* (Buckley & Edeson), and it is a severe biter of cattle and horses, rarely of humans. Nevertheless, updated information about its morphological characteristics was lacking, making comparisons with related species described from China difficult, since species of the subgenus *Wilhelmia* are almost indistinguishable from one another, in particular, in their female terminalia, male genitalia and most of larval features. In this study, as many morphological characteristics as possible of *S. takahasii* based on specimens from Japan are redescribed. New information about many features of this species including the length of the female sensory vesicle against the third palpal segment, number of male upper-eye (large) facets, arrangement of the eight pupal gill filaments, presence or absence of tiny dark setae on the dorsum of the larval abdomen and the number of rows and hooklets of the larval posterior circler will be useful in evaluating the species status of several *Wilhelmia* species in China including the species regarded as *S. takahasii*.

Keywords: Black fly; morphotaxonomy; biodiversity; biting fly.

INTRODUCTION

Wilhelmia, one of the 38 subgenera of the genus *Simulium* Latreille, is a small homogeneous taxon, represented by 31 species, of which 30 species are placed in the *S. equinum* species-group and the remaining one species is in the *S. buettikeri* species-group (Adler, 2022). The subgenus is characterized in the female and male by the pleural membrane with hairs and hind basitarsi slender; in the female by the claw simple, abdomen silvery scaled and ovipositor valve forming a slender curled acuminate process posteromedially; in the male by the style much shorter than the coxite, ventral plate subtriangular, median sclerite furnished with numerous spines (and hooks); in the pupa by the gill composed of two inflated tubular filaments oriented dorso-ventrally, then inwardly, and two to six tubular filaments arising in between and cocoon shoe-shaped; and in the larva by the hypostoma with intermediate teeth as long as or longer than the corner teeth, postgenal cleft medium-long and ventral papillae absent (Crosskey, 1969).

The majority of species in the subgenus *Wilhelmia* are distributed in the Palearctic Region, although one species, *S. xingyiense* Chen & Zhang, is distributed in the Oriental Region, and one species, *S. takahasii* (Rubtsov), is distributed in both regions (Adler, 2022).

Simulium takahasii, which was described in the genus *Wilhelmia* by Rubtsov (1962), was the first among the 19 nominal species of the subgenus *Wilhelmia* recognized in East Asia (China, Japan and Korea) (Adler, 2022). It was formerly reported from Japan as *S. equinum* Linnaeus by Shiraki (1935) and Kani (1944) and as *S. salopiense* Edwards by Bentinck (1955) and Ogata *et al.* (1956). This species was also recorded from China and Korea (Bentinck, 1955; Adler, 2022).

This species is striking in mating, blood-feeding and ovipositing in captivity, enabling laboratory colonization (Takaoka, 1985), these characteristics being reported also for *S. (W.) lineatum* (Meigen) (Ham & Bianco, 1984). Its females are a severe biter of cattle, horses and rarely of humans (Bentinck, 1955; Ogata *et al.*, 1956), and an experimental vector of *Dirofilaria immitis* (Leidy) and *Brugia pahangi* (Buckley & Edeson) (Takaoka *et al.*, 1986; Takaoka & Baba, 1987). The larval salivary gland chromosomes of this species were studied by Adler *et al.* (2015), who found that it is chromosomally most similar to *S. lineatum* and *S. turgaicum* Rubtsov. This species has not been genetically studied, although several European species of the subgenus *Wilhelmia* were analyzed by using the mitochondrial cytochrome *c* oxidase subunit I (COI) gene sequences (Iuknit *et al.*, 2019; Inci *et al.*, 2017).

Beside its characteristic adult behaviors and implications for human and animal health, *S. takahasii* has received special attention as an important reference species when morphologically similar species, that is, 12 of the 19 species of the subgenus *Wilhelmia* in China, were described since 1997. However, a comparison of these new species with *S. takahasii* was not necessarily sufficient since no detailed information on morphological characteristics of this species was available, although the old description of its female and male by Shiraki (1935), the brief descriptions (in Japanese) and illustrations of its female, male, pupa and larva by Ogata *et al.* (1956), and illustrations of its female and male genitalia, pupal gill and abdomen, and cocoon by Bentinck (1955) were used.

Of the 19 species of the subgenus *Wilhelmia* in China, eight species including *S. takahasii* are morphologically almost indistinguishable from one another in the female terminalia, male genitalia and the arrangement of the pupal gill filaments (Chen, 2016). To evaluate the species status of these species including the species regarded as *S. takahasii* in China, updated morphological information of *S. takahasii* from Japan is needed.

We here redescribe the female, male, pupa and mature larva of *S. takahasii* in detail based on specimens from Japan.

MATERIAL AND METHODS

Specimens used in this study were one female, eight males (all reared from pupae), together with their pupal exuviae and cocoons, and 10 mature larvae, collected from an irrigation channel (ca. 2 m wide, 20 cm deep, and exposed to the sun) moderately running in a rice field, Yufuin, Oita, Japan, 13-VI-2011, by Y. Otsuka. All were fixed in 80% ethanol. The methods of collection, description and illustration, and terms for morphological features, followed those of Takaoka (2003) and partially those of Adler *et al.* (2004).

RESULTS AND DISCUSSION

Redescription of *Simulium (Wilhelmia) takahasii* (Rubtsov)

Diagnosis. Female and Male: Pleural membrane and part of upper mesopleuron region haired. Basal portion of radius fully haired. Hind basitarsi slender, nearly parallel-sided (Figure 1C & Figure 2B). Female: Frons densely covered with hairs. Sensory vesicle medium-long (Figure 1A). Mandible with both inner and outer teeth. Cibarium unarmed (Figure 1B). Scutum with three dark vittae. Claw simple (Figure 1D). Ovipositor valve with curled acuminate process posteromedially (Figure 1E, 1F). Spermatheca enlarged, egg-plant-shaped (Figure 1J). Male: Upper-eye (large) facets in 18 or 19 vertical columns and 19 horizontal rows. Abdominal segments 2 and 5-7 each with pair of shiny dorsolateral patches. Ventral plate subtriangular, flat, with long basal arms divergent (Figure 2C). Median sclerite rounded, with numerous short hooks and spines (Figure 2G). Paramere with numerous distinct hooks (Figure 2H). Cercus enlarged, with numerous hairs (Figure 2K, 2L). Pupa: Frons bare. Gill composed of two inflated tubular filaments oriented dorsoventrally, then inwardly, and six short tubular filaments in between (Figure 3A, 3B). Abdomen lacking spine-combs dorsally. Abdominal segments 5-7 each with pair of unbranched hooklets on each side. Last abdominal segment with pair of conical terminal hooks (Figure 3C). Cocoon shoe-shaped (Figure 3D, 3E). Mature larva: Postgenal cleft medium-long, 1.8-2.0 times as long as postgenal bridge (Figure 4C). Abdomen covered with dark tiny bifid or trifid setae dorsally (Figure 4D). Ventral papillae absent. Rectal organ simple. Posterior circler with 94-96 rows of hooklets with up to 28-30 hooklets per row.

Female (n=1). Body length 2.5 mm. **Head.** Slightly narrower than width of thorax. Back of head densely covered with yellowish white fine hairs intermixed with several dark hairs along dorsal margin. Frons brownish black, gray-pruinose, densely covered with yellowish white scale-like recumbent short hairs; frontal ratio 1.52:1.00:1.04; frons:head ratio 1.00:2.97. Fronto-ocular area well

developed, triangular, rounded dorsolaterally. Clypeus brownish black, gray-pruinose, moderately covered with yellowish white short hairs. Labrum 0.89 times as long as clypeus. Antenna composed of scape, pedicel and nine flagellomeres, brownish black except scape, pedicel, and base of first flagellomere yellow. Maxillary palpus composed of five segments, light brown except third segment medium to dark brown (though its apical tip yellow), proportional lengths of third, fourth, and fifth segments 1.0:1.1:2.3; third segment (Figure 1A) of moderate size; sensory vesicle (Figure 1A) medium-long (0.49 times as long as third segment), with large opening. Maxillary lacinia with 9 inner and 10 or 11 outer teeth. Mandible with 16 inner and 14 outer teeth. Cibarium (Figure 1B) with dorsal margin deeply depressed and unarmed. **Thorax.** Scutum brownish black, gray-pruinose, with three black longitudinal vittae (one median and two submedian), densely covered with yellowish white scale-like recumbent short hairs. Scutellum dark brown, covered with white short fine hairs and white longer upright hairs. Postnotum brownish black, bare. Pleural membrane dark brown, moderately covered with yellowish white fine short hairs usually on anterior half. Upper mesopleuron with several yellowish white fine short hairs near anterior margin of pleural membrane. Katepisternum dark brown, longer than deep, bare, and gray-pruinose. **Legs.** Foreleg: coxa dark brown; trochanter medium brown; femur medium brown with apex dark brown (though extreme tip yellow); tibia medium brown except base yellow and apical cap dark brown; tarsus brownish black, with moderate dorsal hair crest; basitarsus moderately dilated, 6.4 times as long as its greatest width. Midleg: coxa brownish black; trochanter medium brown; femur medium brown with apex dark brown (though extreme tip yellow); tibia light brown except base yellow and apical cap dark brown; tarsus dark brown except median large portion of basitarsus and basal half of second tarsomere yellow; basitarsus (Figure 1C) narrow, nearly parallel-sided, 6.8 times as long as wide, and 0.6 and 0.5 times as wide as greatest widths of tibia and femur, respectively; calcipala (Figure 1C) as long as wide, and 0.36 times as wide as greatest width of basitarsus. Pedisulcus (Figure 1C) well defined. Claw (Figure 1D) simple. **Wing.** Length 2.0 mm. Costa with dark spinules and light brown hairs except basal tuft of whitish yellow hairs. Subcosta bare or with one hair. Base of radial vein with tuft of yellowish white hairs. Basal portion of radius fully haired; R₁ with dark spinules and hairs; R₂ with hairs only. Basal cell absent. **Halder.** White except basal stem darkened. **Abdomen.** Basal scale dark brown, with fringe of whitish yellow hairs. Abdomen grayish brown, densely covered with yellowish white short to long hairs; tergites of segments 2 and 6-8 somewhat shiny when illuminated at certain angles. Sternal plate on segment 7 undeveloped. **Terminalia.** Sternite 8 (Figure 1E) markedly depressed medially on posterior one-fourth, with posterior margin deeply concave medially, widely bare medially, with 26-30 short to medium-long hairs on each side. Ovipositor valves (Figure 1E, 1F) thin, membranous, uniformly covered with setae, each forming slender curled acuminate process posteromedially; inner margins concave and moderately separated from each other. Genital fork (Figure 1G) of usual inverted-Y form, with slender stem; arms slender, with wide lateral plate. Paraproct in ventral view (Figure 1H) wide, nearly quadrate, and with relatively wide portion along inner margin unpigmented; paraproct in lateral view (Figure 1I) somewhat produced ventrally, 0.37 times as long as wide, with dozen of short to medium-long hairs on ventral and lateral surfaces. Cercus in lateral view (Figure 1I) short, rounded posteriorly, and 0.43 times as long as wide. Spermatheca (Figure 1J) enlarged, egg-plant-shaped, 1.85 times as long as its greatest width, dark brown except duct and wide portion near neck unpigmented, and without fissures on surface; internal setae absent; both accessory ducts unpigmented, slender, subequal in diameter to each other and to major one.

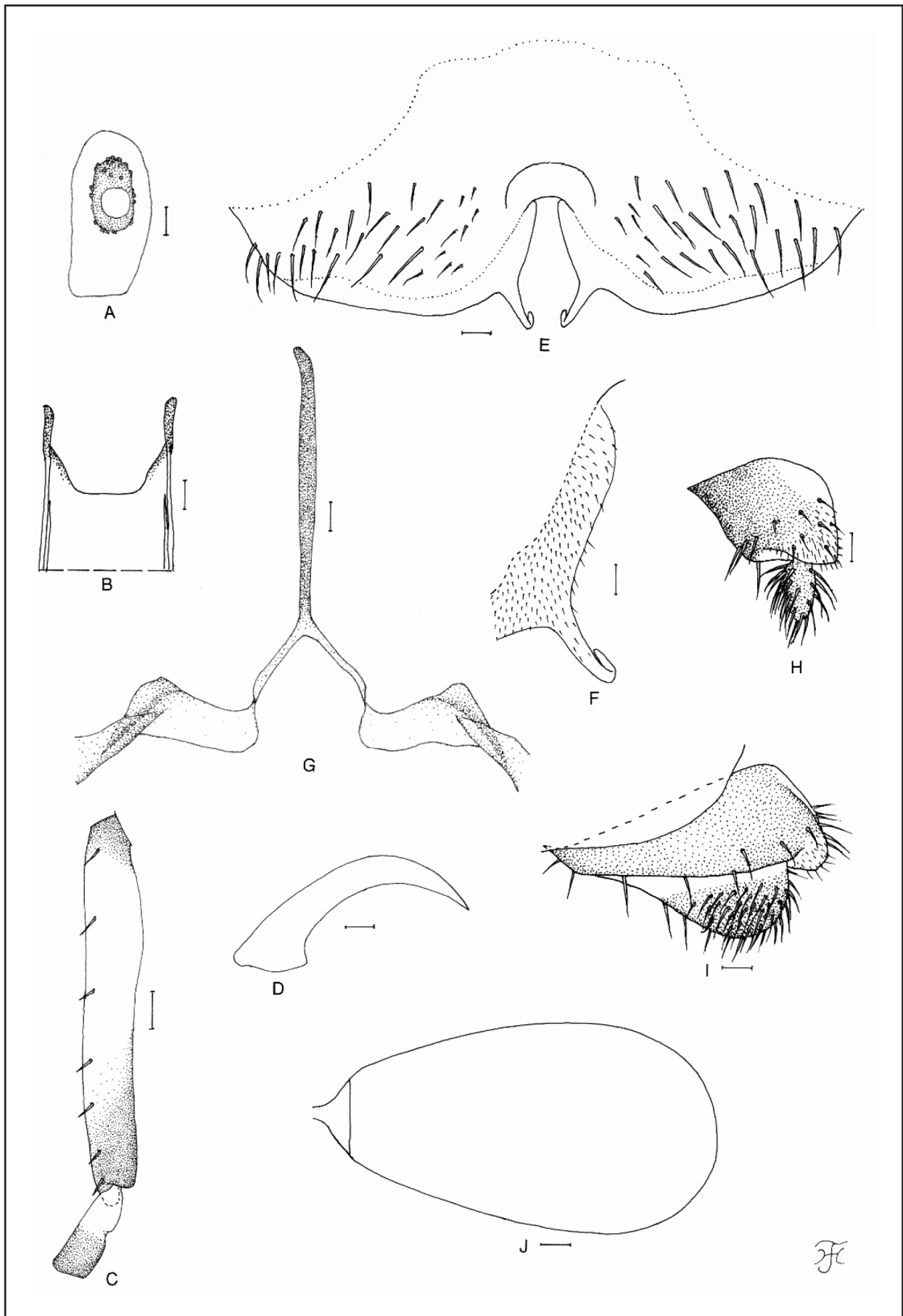


Figure 1. Female of *S. takahsii*. A, third palpal segment with sensory vesicle (front view); B, cibarium (front view); C, hind basitarsus and second tarsomere (left side; outer view); D, claw (lateral view); E, sternite 8 and ovipositor valves (ventral view); F, ovipositor valve (right side); G, genital fork (ventral view); H & I, paraprocts and cerci (right side; H, ventral view; I, lateral view); J, spermatheca (lateral view). Scale bars. 0.05 mm for C; 0.02 mm for A, B, E, G–J; 0.01 mm for D & F.

Male (n=8). Body length 2.5-2.7 mm. **Head.** Slightly wider than thorax. Upper eye bright medium brown, consisting of large facets in 19 (rarely 18) vertical columns and 19 horizontal rows. Clypeus brownish black, white-pruinose, moderately covered with dark medium-long hairs though mediolongitudinal area bare. Antenna as in female; first flagellomere elongate, 1.3-1.7 times length of second. Maxillary palpus light to medium brown, with five segments, proportional lengths of third, fourth, and fifth segments 1.0:1.3:2.6-2.7; third segment (Figure 2A) not enlarged; sensory vesicle (Figure 2A) globular or ellipsoidal, small, 0.28-0.33 times length of third segment, and with small opening. **Thorax.** Scutum brownish black, somewhat shiny on shoulders, along lateral margins and prescutellar area when illuminated at certain angles, and densely covered with whitish yellow short hairs intermixed with dark short hairs near anterior margin. Scutellum dark brown, with dark upright hairs and whitish short hairs. Postnotum brownish black, bare. Pleural membrane, upper mesopleuron and katepisternum as in female. **Legs.** Color almost as in female. Fore basitarsus moderately dilated, 8.0-8.1 times as long as its greatest width. Hind basitarsus in lateral view (Figure 2B) nearly parallel-sided, though slightly narrowed toward apex, 6.5-6.8 times as long as wide, and 0.6 and 0.5 times as wide as greatest width of tibia and femur, respectively; calcipala (Figure 2B) slightly shorter than basal width, and 0.36 times as wide as greatest width of basitarsus. Pedisulcus (Figure 2B) well defined. **Wing.** Length 2.0-2.1 mm; other characteristics as in female except subcosta without hairs, and base of radius with tuft of dark brown hairs. **Halter.** White except basal stem darkened. **Abdomen.** Basal scale dark brown, with fringe of light brown hairs. Dorsal surface of abdomen medium brown to brownish black except basal one-third of segment 2 dark yellow, moderately covered with dark short to long hairs and yellow short hairs; segments 2 and 5-7 each with pair of shiny dorsolateral patches. **Genitalia.** Coxite in ventral view (Figure 2C) nearly rectangular, 1.6 times as long as its greatest width. Style in ventral view (Figure 2C) gently bent dorsally, with its base connected to coxite at apical one-third of inner surface of coxite; style in caudal view (Figure 2D) 0.47 times length of coxite, gradually narrowed from base to apical one-third, then nearly parallel-sided to apex and with apical spine. Ventral plate in ventral view (Figure 2C) with body subtriangular, transverse, rounded posteriorly, with anterior margin nearly straight, densely covered with micro-setae even on most of arms and on wide anterior area between arms beyond anterior margin of body; arms long, widely divergent from each other; ventral plate in caudal view (Figure 2E) flat, with narrow median portion of body covered with micro-setae on posterior surface; ventral plate in lateral view (Figure 2F) narrow, nearly flat, though apex of arm directed ventrally;. Median sclerite (Figure 2G) thin, weakly dark-pigmented, plate-like, rounded, depressed dorsally, with ventral surface covered with numerous dark hooks directed ventrally on each side and numerous sharply pointed spines directed posteriorly on anterior and middle portions. Parameres (Figure 2H) with narrow basal arms, each with numerous dark hooks of various sizes, moderately covered with sharply-pointed spines on ventrolateral surface, densely covered with micro-setae on ventral surface near base and moderately covered with dark cone-like tiny spines on anteroventral surface. Aedeagal membrane (Figure 2I) moderately covered with micro-setae. Dorsal plate (Figure 2J) wide, strap-like and slightly darkened. Abdominal segment 10 without distinct hairs near posterolateral surface. Cerci (Figure 2K, 2L) relatively large, each with over 30 hairs.

Pupa (n=9). Body length 2.8-3.1 mm. **Head.** Integument yellow, bare except anterior portion of face moderately covered with small round tubercles. Frons with two unbranched slender short trichomes with uncoiled apices on each side; face with one unbranched slender short trichome with uncoiled apex on each side. **Thorax.** Integument yellow, bare except dorsal and dorsolateral portions of posterior half moderately covered with cone-like tubercles, with two dorsomedial trichomes, two anterolateral trichomes, one mediolateral trichome,

and three ventrolateral trichomes; all trichomes unbranched slender and short, with uncoiled apices. Gill (Figure 3A,B) composed of two inflated tubular filaments oriented dorso-ventrally, then inwardly, and six tubular filaments arising in between, of which two ventral filaments much longer and thicker than the four other filaments; cuticle of all filaments light brown and smooth. **Abdomen.** Dorsally, all segments transparent except segments 1, 2 and 9 light yellow; segments 1-4 moderately covered with tiny spines; segment 1 with one slender short seta on each side; segment 2 with one slender short seta and four short spinous setae submedially on each side; all setae unbranched; segments 3 and 4 each with four hooked spines and one unbranched short seta on each side; segments 5-9 without spine-combs; segment 9 with pair of small cone-like terminal hooks (Figure 3C). Ventrally, segments 5-7 each with pair of unbranched hooks submedially and few unbranched slender short setae on each side; segments 4-8 with comb-like groups of micro-spines. **Cocoon** (Figure 3D, 3E). Shoe-shaped, with low anteroventral neck, tightly woven, slightly extended ventrolaterally; individual threads invisible; 3.0-3.4 mm long by 1.5-1.7 mm wide.

Mature larva (n=10). Body length 4.3-5.5 mm. Body dark gray. **Head.** Head capsule whitish yellow except eye-spot region whitish, and bare except anterodorsal portion sparsely covered with unpigmented fine setae; head spots distinctively positive. Antenna composed of three articles and apical sensillum, nearly as long as stem of labral fan; proportional lengths of first, second, and third articles 1.0:1.4-1.7:0.8-0.9. Labral fan with 34-41 primary rays. Mandible (Figure 4A) with three comb-teeth slightly decreasing in length from first tooth to third; mandibular serration composed of two teeth (one medium-sized, one small); major tooth at obtuse angle against mandible on apical side; supernumerary serrations absent. Hypostoma (Figure 4B) with row of nine apical teeth, subequal in length to one another, except median tooth slightly longer than others; lateral margin serrated apically; three or four hypostomal bristles per side lying somewhat divergent posteriorly from lateral margin. Postgenal cleft (Figure 4C) medium-long, rounded apically, 1.8-2.0 times as long as postgenal bridge; sheath of subesophageal ganglion dark gray-pigmented. Cervical sclerites composed of pair of small medium brown ellipsoidal pieces. **Thorax and Abdomen.** Thoracic cuticle bare. Abdominal cuticle covered with dark tiny bifid or trifid setae (Figure 4D) dorsally, sparsely on segments 1 and 2, moderately to densely on segments 3-8; last abdominal segment sparsely covered with unbranched colorless minute setae on dorsolateral surfaces of each side of anal sclerite. Rectal organ simple. Anal sclerite of usual X-form, with anterior arms 0.7-0.8 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 lacking ventral papillae. Posterior circler with 94-96 rows of hooklets with up to 28-30 hooklets per row.

Remarks. Species in the currently recognized *S. equinum* group of the subgenus *Wilhelmia* were formerly divided into two groups, *S. equinum* species-group and *S. lineatum* species-group (at that time as the *S. salopiense* species-group), by the difference in the site where the style is connected to the coxite, and the shape of the median sclerite (Rubtsov, 1956). *Simulium takahasii* is related to the *S. lineatum* species-group, together with seven other Chinese species: *S. eurybrachium* Chen, Wen & Wei, *S. pekingense* Sun, *S. pinnatum* Chen, Zhang & Jiang, *S. qinghaiense* Liu et al., *S. qingxilingense* Chen & An, *S. tongbaishanense* Chen & Luo and *S. xingyiense* Chen & Zhang (Chen, 2016). These species are morphologically almost indistinguishable from one another in the female terminalia and male genitalia, and have a similar arrangement of the eight pupal gill filaments. Thus, species identification depends on other features such as the length of the sensory vesicle relative to the third palpal segment, and the number of male upper-eye (large) facets.

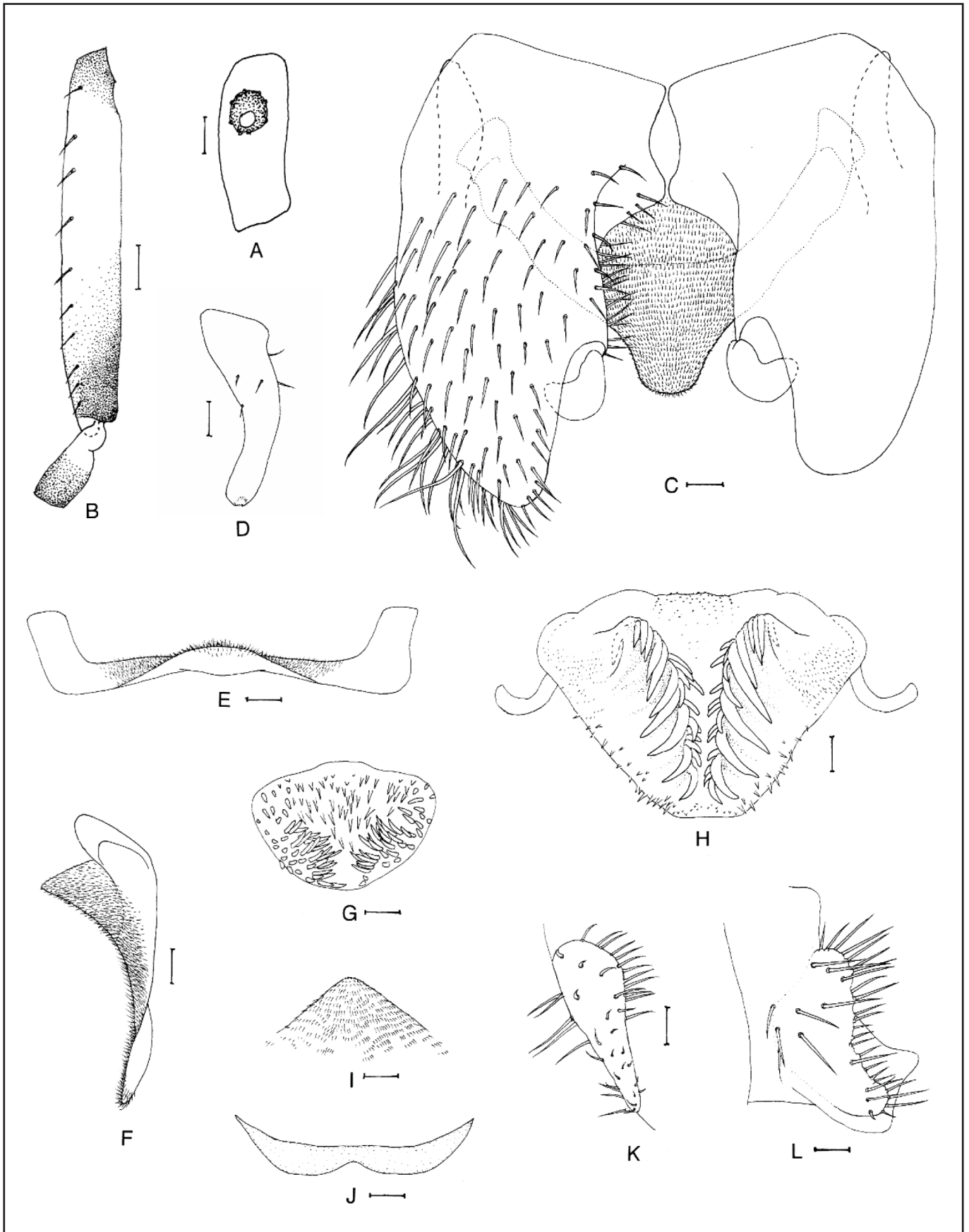


Figure 2. Male of *S. takahashii*. A, third palpal segment (front view); B, hind basitarsus and second tarsomere (right side; outer view); C, coxites, styles and ventral plate (ventral view); D, style (right side; caudal view); E & F, ventral plate (E, caudal view; F, lateral view); G, median sclerite (ventral view); H, parameres (caudal view); I, aedeagal membrane (caudal view); J, dorsal plate (ventral view); K & L, cerci (right side; K, caudal view; L, lateral view). Scale bars. 0.05 mm for B; 0.02 mm for A, C–L.

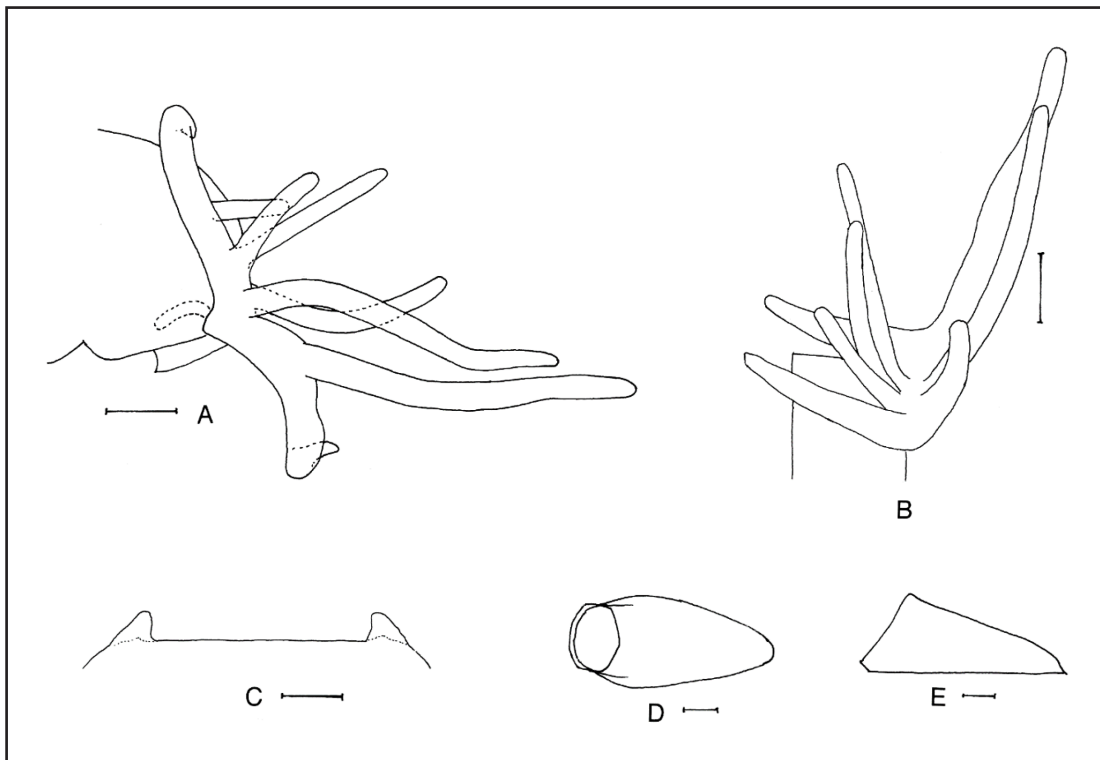


Figure 3. Pupa of *S. takahasii*. A & B, gills (right side; A, lateral view; B, dorsal view); C, terminal hooks (caudal view); D & E, cocoons (D, dorsal view; E, lateral view). Scale bars. 0.5 mm for D & E; 0.2 mm for A & B; 0.02 mm for C.

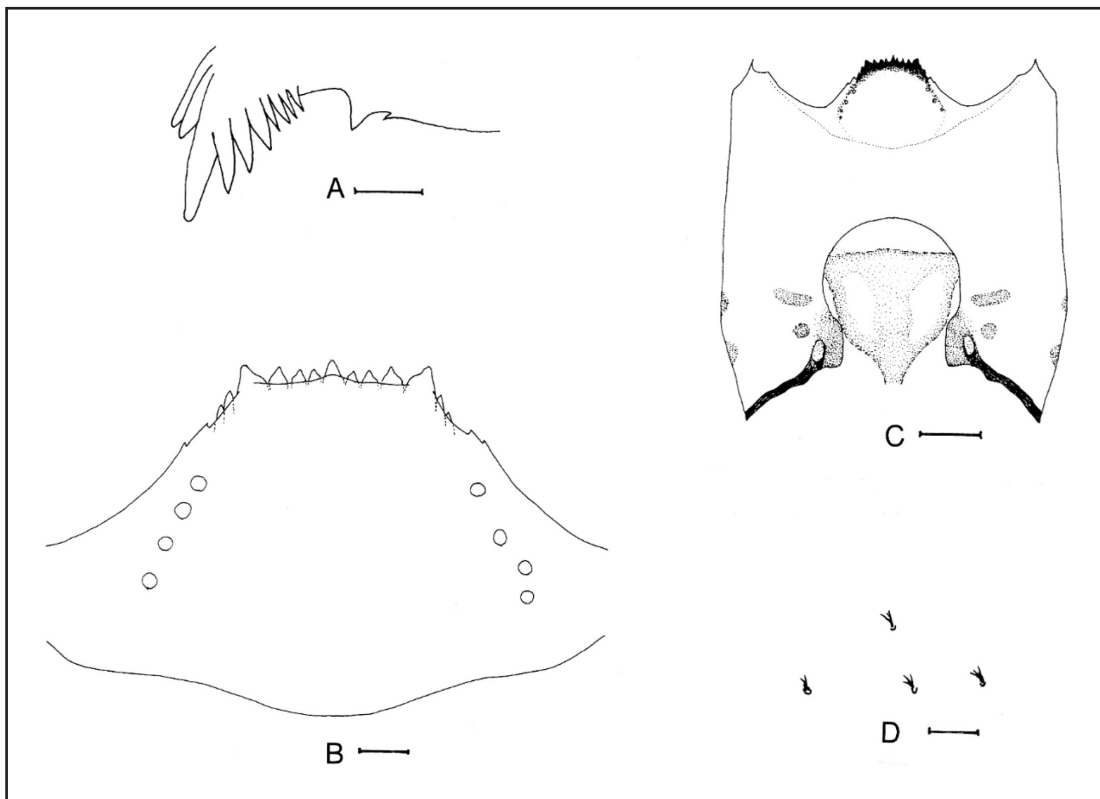


Figure 4. Mature larva of *S. takahasii*. A, mandible; B, hypostoma; C, head capsule (ventral view); D, dark tiny setae on dorsum of abdominal segment 8. Scale bars. 0.1 mm for C; 0.02 mm for A & B; 0.01 mm for D.

In this study, the following features of *S. takahasii* were newly described: frontal ratio, frons:head ratio, labrum:clypeus ratio, maxillary lacinia, mandible, cibarium, length:width ratios of the fore and hind basitarsi, and wing, in the female; number of upper-eye (large) facets, length ratio of the palpal segments 3-5, length:width ratios of the fore and hind basitarsi, wing, number of parameral hooks, and shape of the cercus, in the male; integument of the head and thorax in the pupa; mandible, hypostoma, number of primary rays of the labral fan, length ratio of the antennal articles 1-3, cuticle of abdomen, and numbers of rows and hooklets per row, in the larva. These new morphological data will be useful in future taxonomic studies to evaluate the species status of these similar species of the subgenus *Wilhelmia* in China.

Integrated analyses using morphology, chromosomes and DNA gene sequences are required for accurate identification of species of the subgenus *Wilhelmia* in East Asia.

ACKNOWLEDGEMENTS

We are grateful to Dr. Peter H. Adler (Professor Emeritus, Clemson University, Clemson, SC, USA) for reading the current manuscript and providing valuable comments. We acknowledge funding from the Ministry of Higher Education, Malaysia, under the Higher Institution Centre of Excellence (HiCoE) niche area vector and vector-borne diseases (project no. MO002-2019).

Declaration of Competing Interest

We declare that this is our original work. It has not been published elsewhere and we have no conflicts of interest concerning the work reported in this paper. All authors have contributed to this study throughout the study design, field work, data collection, data analyses and data interpretation. The authors have read and approved the manuscript.

REFERENCES

- Adler, P.H. (2022). World blackflies (Diptera: Simuliidae): A comprehensive revision of the taxonomic and geographical inventory [2022]. <http://biomia.sites.clemson.edu/pdfs/blackflyinventory.pdf>
- Adler, P.H., Currie, D.C. & Wood, D.M. (2004). The black flies (Simuliidae) of North America. New York: Cornell University Press, Ithaca, pp. xv+941.
- Adler, P.H., Inci, A., Yildirim, A., Duzlu, O., McCreadie, J.W., Kudela, M., Khazeni, A., Bruderova, T., Setiz, G., Takaoka, H. et al. (2015). Are black flies of the subgenus *Wilhelmia* (Diptera: Simuliidae) multiple species or a single geographical generalist? Insights from the macrogenome. *Biological Journal of the Linnean Society* **114**: 163-183. <https://doi.org/10.1111/bij.12403>
- Bentnick, W.C. (1955). The black flies of Japan and Korea (Diptera: Simuliidae). 406 Medical General Laboratory U.S.A. Army, Tokyo, pp. 23.
- Chen, H.B. (2016). Chinese blackflies (Diptera: Simuliidae). Guizhou Province Science and Technology Publication, Guiyang, China (In Chinese except the descriptions of new species written also in English).
- Crosskey, R.W. (1969). A re-classification of the Simuliidae (Diptera) in Africa and its islands. *Bulletin of the British Museum (Natural History) Entomology* **14**: 1-194.
- Đuknić, J., Jovanović, V.M., Popović, N., Živić, I, Raković, M., Čerba, D. & Paunović, M. (2019). Phylogeography of *Simulium* subgenus *Wilhelmia* (Diptera: Simuliidae) - insights from Balkan populations. *Journal of Medical Entomology* **56**: 967-978. <https://doi.org/10.1093/jme/tjz034>
- Ham, P.J. & Bianco, A.E. (1984). Maintenance of *Simulium* (*Wilhelmia*) *lineatum* (Maigen) and *Simulium* (*Boophthora*) *erythrocephalum* de Geer through successive generations in the laboratory. *Canadian Journal of Zoology* **62**: 870-877. <https://doi.org/10.1139/z84-127>
- Inci, A., Yildirim, A., Duzlu, O., Onder, Z., Ciloglu, A., Seitz, G. & Adler, P.H. (2017). Genetic diversity and identification of Palearctic black flies in the subgenus *Wilhelmia* (Diptera: Simuliidae). *Journal of Medical Entomology* **54**: 888-894. <https://doi.org/10.1093/jme/tjw246>
- Kani, T. (1944). On pupae of 11 species of the family Simuliidae in Kamogawa River, Kyoto. *Physiology and Ecology Research, Ohtsu Lake laboratory, Department of Zoology, Faculty of Science, Kyoto University* **11**: 1-8. (In Japanese).
- Ogata, K., Sasa, M. & Suzuki, T. (1956). The Japanese black flies and their control. Tokyo: DDT Kyokai, pp. 126. (In Japanese).
- Rubtsov, I.A. (1956). Blackflies (fam. Simuliidae). *Fauna of the USSR*. Moscow & Leningrad: Academia Nauka SSSR, pp. 859. [In Russian; translated to English and republished in 1989 by Amerind Publishing Co., New Delhi, 1,042 pp.].
- Rubtsov, I.A. (1962). Simuliidae (Melusinidae). In: Die Fliegen der Palaearktischen Region, Linder, E. (editor). **14**: 369-400.
- Shiraki, T. (1935). Simuliidae of the Japanese Empire. *Memoirs of the Faculty of Science and Agriculture, Taihoku Imperial University* **16**: 1-90.
- Takaoka, H. (1985). Observations on the mating, blood feeding and oviposition of *Simulium takahasii* (Rubtsov) (Simuliidae, Diptera) in the laboratory. *Japanese Journal of Sanitary Zoology* **36**: 211-217.
- Takaoka, H. (2003). The black flies (Diptera: Simuliidae) of Sulawesi, Maluku and Irian Jaya. Fukuoka: Kyushu University Press, pp. xxii + 581.
- Takaoka, H. & Baba, M. (1987). Experimental infection of *Simulium takahasii* with a canine filaria *Dirofilaria immitis*. *Japanese Journal of Sanitary Zoology* **38**: 1-6.
- Takaoka, H., Baba, M. & Aoki, Y. (1986). Experimental infection of the blackfly *Simulium takahasii* with *Brugia pahangi*. *Japanese Journal of Parasitology* **35**: 237-241.