



RESEARCH ARTICLE

A male black fly of *Simulium (Simulium) iwatense* (Shiraki) (Diptera: Simuliidae) with genitalia-like appendages on abdominal segment 8

Takaoka, H.^{1*}, Fukuda, M.², Otsuka, Y.³, Iwasa, M.⁴

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

²Institute for Research Promotion, Oita University, Idaigaoka 1-1, Hasama, Yufu, Oita 879-5593, Japan

³Research Center for the Pacific Islands, Kagoshima University, Korimoto 1-21-24, Kagoshima, Kagoshima 890-8580, Japan

⁴Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan.

*Corresponding author: takaoka@oita-u.ac.jp

ARTICLE HISTORY

Received: 10 February 2021

Revised: 4 March 2021

Accepted: 4 March 2021

Published: 30 April 2021

ABSTRACT

A rare non-sex mosaic abnormality represented by genitalia-like appendages on the ventral surface of abdominal segment 8 of a male black fly collected in Hokkaido, Japan, is reported. The appendages consist of a pair of style-like projections each arising from a coxite-like base, inverted-Y shaped ventral plate-like structure, and isolated round structure. This male was morphologically and molecularly identified as an abnormal form of *S. (S.) iwatense* (Shiraki), the only species in the *Simulium (Simulium) ornatum* species-group in Japan, although certain morphological characteristics of this male including the reduced number of upper-eye (large) facets and elongate cerci are different from those of *S. (S.) iwatense*.

Keywords: black fly; taxonomy; Simuliidae; biodiversity.

INTRODUCTION

Morphological abnormalities of adult black flies (Diptera: Simuliidae) are usually categorized as sex mosaics and non-sex mosaics. Examples of abnormalities recognized as non-sex mosaics include a right antenna of a female of *Simulium (Gomphostilbia) torautense* Takaoka & Roberts from Sulawesi, Indonesia, which is much shortened and has only a few flagellomeres (in place of nine flagellomeres) (Takaoka, 2003) and a right coxite of a male of the same species, which has two additional projections, each protruding posteriorly and anteriorly (the posterior projection is similar in size and shape to the normal style) (Takaoka, 2003).

We collected an adult male belonging to the *S. (S.) ornatum* species-group in Hokkaido, Japan by sweeping around the udder of a cow, and found that it has a non-sex mosaic abnormality represented by a pair of distinct projections directed ventrally from sternite 8.

This male was morphologically and molecularly identified as *S. (S.) iwatense* (Shiraki), which is a potential vector of *Onchocerca* sp., probably a bovine filaria, in Japan (Takaoka *et al.*, 1992).

Our aim is to describe and illustrate this rare morphological abnormality on sternite 8 of this male, and to highlight certain morphological characteristics deviated from normal males of *S. (S.) iwatense*.

MATERIALS AND METHODS

Morphological observation

The material studied included an abnormal form of the male collected by sweeping around the udder of a cow, in Obihiro, Hokkaido, Japan, 24-VI-2014, by M. Iwasa, and five males of *S. (S.) iwatense* (Shiraki) reared from pupae collected from Shihoro-cho, Hokkaido, Japan, 5-VIII-2011, by Y. Otsuka.

Methods of morphological observation, terms of features, descriptions, and illustrations, followed those of Takaoka (2003) and partially those of Adler *et al.* (2004).

The abnormal male examined is deposited in Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan.

Molecular analysis

The following three adult black flies were used for molecular analyses: an abnormal form of the male, a male of *S. (S.) iwatense* reared from a pupa collected from Shihoro-cho, Hokkaido, Japan, 5-VIII-2011, by Y. Otsuka, and a female of *S. (S.) iwatense* collected by a net from Shizukuishi-cho, Iwate, Honshu, Japan, 4-VIII-2017, by M. Fukuda. Thoraxes of these three flies were used for 16S rRNA and COI gene-based analyses. Genbank data (AB093117) on 16S rRNA gene sequence of *S. (S.) iwatense* from Kanagawa, Honshu, Japan, were also used for comparison.

The procedures for DNA extraction, PCR amplification, and sequencing followed those of Srisuka *et al.* (2019) with slight modification. The primers and the thermal cycling conditions for the 16S rRNA gene followed those of Otsuka *et al.* (2003).

RESULTS

Morphological observation

Description of an abnormal form of the male. Male. Body length 3.8 mm. **Head.** Slightly narrower than thorax. Upper eye medium brown, with large facets in 17 vertical columns and 16 horizontal rows. Clypeus brownish black, thickly white-pruinose, covered with dark brown hairs along lateral margins and near ventral margin and medial portion bare. Antenna composed of scape, pedicel and nine flagellomeres, dark brown; first flagellomere elongate, 1.71 times as long as second one. Maxillary palpus light brown except third and fourth palpomeres dark brown, composed of five palpomeres, with proportional lengths of third, fourth, and fifth palpomeres 1.00:1.30:3.02; third palpomere (Figure 1A) widened apically; sensory vesicle (Figure 1A) small, ellipsoidal, 0.23–0.27 times as long as third palpomere, and with small opening. **Thorax.** Scutum black, with white-pruinose pattern, i.e., anterior pair of triangular spots on shoulders extended posteriorly along lateral margins and connected near base of wings to large transverse posterior spot entirely covering prescutellar area; these pruinose areas silvery or bluish iridescent when illuminated at certain angles; scutum uniformly and moderately covered with yellow recumbent short hairs interspersed with several dark brown long upright hairs on prescutellar area. Scutellum black, with several dark brown long upright hairs and yellow short hairs. Postnotum brownish black, white-pruinose when illuminated at certain angles and bare. Pleural membrane with eight to ten fine yellow hairs on upper portion. Katepisternum longer than deep, brownish black, white-pruinose when illuminated at certain angles, and bare. **Legs.** Foreleg: coxa dark yellow; trochanter and femur dark brown; tibia brownish black except median large portion white on outer surface; tarsus black, with moderate dorsal hair crest; basitarsus greatly dilated, 5.15 times as long as its greatest width. Midleg: coxa brownish black; trochanter and femur dark brown; tibia medium to dark brown except basal one-third whitish (though basal half whitish on posterior surface); tarsus dark brown except base of basitarsus dark yellow. Hind leg: coxa dark brown; trochanter light brown; femur dark brown except base yellow and apical cap brownish black; tibia brownish black except basal one-third yellowish white; tarsus (Figure 1B) dark brown except basal half of basitarsus (though extreme base light brown) yellowish and basal one-third of second tarsomere medium brown; basitarsus (Figure 1B) moderately enlarged, nearly parallel-sided, 5.35 times as long as its greatest width, 0.79 and 0.74 times as wide as greatest widths of hind tibia and femur, respectively; calcipala (Figure 1B) small, much shorter than wide, 0.35 times as wide as greatest width of basitarsus; pedisulcus (Figure 1B) well developed. **Wing.** Length 2.5 mm. Costa with dark spinules and hairs except basal patch of hairs yellow; subcosta bare; basal section of radius bare; R_1 with dark brown spinules and hairs; R_2 with dark brown hairs; hair tuft on base of radius yellow intermixed with several dark brown hairs; basal cell absent. **Halter.** White except basal portion darkened. **Abdomen.** Basal scale brownish black, with fringe of dark long hairs. Dorsal surface of abdomen brownish black, with dark short hairs; segments 2, 6, 7 and 8 each with a pair of white large spots dorsolaterally or laterally (though segment 5 appearing to

have a pair of white small spots dorsolaterally along anterior margin), those on segment 2 broadly connected in middle to each other. Sternite of segment 8 abnormal, greatly modified as if it were miniature genitalia consisting of a pair of short, style-like submedial projections, each arising from coxite-like base (Figure 1 C, D), ventral plate-like structure (Figure 1C, F, G) medially, and isolated round structure (Figure 1C) posteromedially; ventral plate-like structure of inverted Y-shape, well sclerotized and darkly pigmented, composed of pear-like round body and two short arms (Figure 1F, G). Sternite of segment 9 in form of well sclerotized and darkly pigmented horizontal bar with small opening medially (Figure 1C). **Genitalia.** Coxite in ventral view (Figure 1C) nearly quadrate. Style in ventral view (Figure 1C) elongate, much longer than coxite, with subterminal spine; style in ventrolateral view (Figure 1H) widest at basal one-fourth, slightly widened from base to basal one-fourth, somewhat tapered toward middle, and slightly tapered to apex, and with round apex; style in medial view (Figure 1I) somewhat produced dorsally at basal one-tenth. Ventral plate in anteroventral view (Figure 1J) Y-shaped, well sclerotized and darkly pigmented, with body subquadrate (though slightly narrowed basally) having median process moderately covered with setae (except posterior surface with several setae); arms divergent at right angle from each other; ventral plate in lateral view (Figure 1K) with body having serrated posterior margin and distinct median process directed ventrally; ventral plate in caudal view (Figure 1L) with body short and slightly narrowed ventrally, having about ten teeth in two vertical and parallel rows, and round median process having several setae on posterior surface. Median sclerite in lateral view (Figure 1K) arising near anterior tip of body of ventral plate, directed posteriorly and dorsally; median sclerite in caudal view (Figure 1M) plate-like, gradually widened from base to apex, with round apex. Paramere in ventral view (Figure 1C) broad basally, with several distinct hooks. Aedeagal membrane (Figure 1C) densely covered with microsetae, with dorsal plate indistinct. Abdominal segment 10 with three distinct hairs on ventral surface on each side (Figure 1N). Cercus somewhat elongate, about twice as long as basal width (when viewed laterally), with numerous hairs (Figure 1N).

Comparison of an abnormal form of the male with males of *S. (S.) iwatense*. The abnormal form of the male examined in this study is assigned to the *S. ornatum* species-group in the subgenus *Simulium* by having the pleural membrane with hairs, and style much longer than the coxite.

Beside unusual appendages on the sternite 8 (Figure 1C–F), this form of the male is morphologically similar to the male of *S. (S.) iwatense*, the only species in the *S. (S.) ornatum* species-group known from Hokkaido and Honshu, Japan, in many characteristics including the color of legs and genitalia (Shiraki, 1935; Takaoka *et al.*, 2021). However, certain morphological characteristics are different from those of normal males of *S. (S.) iwatense* including the head slightly narrower than the thorax (as wide as the thorax in *S. (S.) iwatense*), upper-eye (large) facets in 17 vertical columns and 16 horizontal rows (in 24 vertical columns and 24 or 25 horizontal rows in *S. (S.) iwatense*), fore basitarsus 5.15 times as long as its greatest width (5.76–5.88 times in *S. (S.) iwatense*), and cercus longer than its basal width when viewed laterally (Figure 1N) (shorter than its basal width in *S. (S.) iwatense*).

Molecular analysis

No difference in the sequences (517bp) of 16S rRNA gene was shown among the abnormal male (LC604892), the male

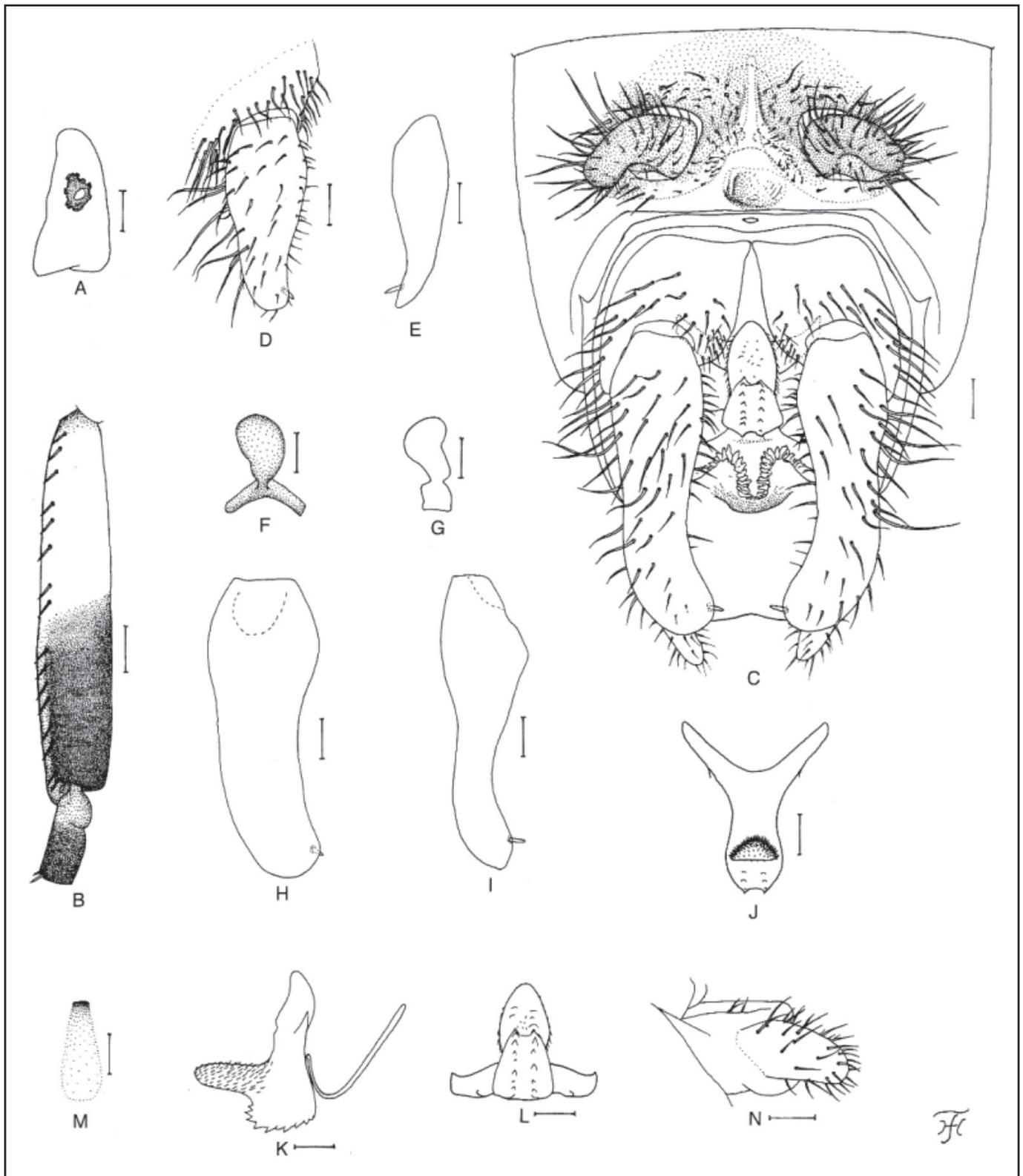


Figure 1. Abnormal male of *Simulium* (*Simulium*) *iwatense*. A, third palpomere (left side; anterior view). B, basitarsus and second tarsomere of hind leg (left side; outer view). C, eighth and ninth abdominal segments and genitalia showing abnormally formed appendages on sternite 8 (ventral view). D, style-like projection and coxite-like base (right side; anterior view). E, style-like projection (right side; lateral view). F and G, ventral plate-like structures (F, ventral view; G, lateral view). H and I, styles (right side; H, ventrolateral view; I, medial view). J, ventral plate (anteroventral view). K, ventral plate and median sclerite (lateral view). L, ventral plate (caudal view). M, median sclerite (caudal view). N, tenth abdominal segment and cercus (right side; lateral view). Scale bars. 0.1 mm for B; 0.04 mm for A and C–N.

of *S. (S.) iwatense* from Hokkaido, Japan (LC604893), the female of *S. (S.) iwatense* from Iwate, Honshu, Japan (LC604894), and larva of *S. (S.) iwatense* from Kanagawa, Honshu, Japan (AB093117).

Only one of 658 base pairs of COI gene sequences was different between the abnormal form of the male (LC604895) and the female of *S. (S.) iwatense* from Iwate, Honshu, Japan (LC604896). The COI gene sequence of the male of *S. (S.) iwatense* from Hokkaido, Japan was not determined because of a small amount of template DNA for sequencing.

DISCUSSIONS

The abnormal form of the male was molecularly identified as *S. (S.) iwatense*, the only species in the *S. (S.) ornatum* species-group known from Japan (Shiraki, 1935; Takaoka et al., 2021), although certain morphological characteristics (apart from unusual appendages on the sternite 8) were different from those of normal males of *S. (S.) iwatense* as noted above. Of these characteristics, the number of upper-eye (large) facets is one of the key characteristics used for species identification in most species-groups of black flies. In addition, the cercus, which is longer than its basal width (when viewed laterally), is very rare in both males and females of black flies. Considering the high genetic similarity, we interpret that this male is an abnormal form of *S. (S.) iwatense*, and all its differing characteristics (including the reduced number of upper-eye facets and elongate cercus) have yielded, accompanying the major abnormality occurring on its sternite 8.

Non-sex mosaic morphological abnormalities were rarely recorded in adult black flies (e.g., Takaoka, 2003). The abnormality occurring on abdominal segment 8 of the male in the present case probably is recorded for the first time.

The reason and mechanism of this abnormality are unknown, although it may have taken place during a process of morphogenesis at the preimaginal stage probably due to an error of expression of gene(s) relating to male genital organs or that of homeotic gene(s) determining the location of male genital organs, or both.

The male genitalia of *Drosophila* are formed by two parts, one derived from a male genital primordium corresponding to segment 9 and the other from an anal primordium corresponding to segments 10 and 11 (Sánchez & Guerrero, 2001).

If the male genitalia of black flies are similarly formed, the male genital primordium in this abnormal male might have been wrongly induced on segment 8 in addition to segment 9.

Another possibility of the abnormality in this male is parasitism by mermithid nematodes. However, this is excluded because neither the presence of a mermithid nor the presence of an exit hole for a mermithid in the fly's abdomen was observed.

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. This study was funded by the Ministry of Education, Malaysia, under the Higher Institution Centre of Excellence (HiCoE) niche area vector and vector-borne diseases (Project no. MO002-2019).

Conflict of Interests

The author declares that they have no conflict of interests.

REFERENCES

- Adler, P.H., Currie, D.C. & Wood, D.M. (2004). The Black Flies (Simuliidae) of North America. Ithaca: Cornell University Press, xv + pp. 941.
- Otsuka, Y., Takaoka, H., Aoki, C. & Choochote, W. (2003). Phylogenetic analysis of the subgenus *Himalayum* within the genus *Simulium* s.l. (Diptera: Simuliidae) using mitochondrial 16S rRNA gene sequences. *Medical Entomology and Zoology* **54**: 113-120. <https://doi.org/10.7601/mez.54.113>
- Sánchez, L. & Guerrero, I. (2001). The development of the *Drosophila* genital disc. *BioEssays*, **23**: 698-707. <https://doi.org/10.1002/bies.1099>
- Shiraki, T. (1935). Simuliidae of the Japanese Empire. *Memoirs of the Faculty of Science and Agriculture Taihoku Imperial University* **16**: 1-90.
- 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 asakoeae* species-group. *Acta Tropica* **197**: 105043. <https://doi.org/10.1016/j.actatropica.2019.105043>
- Takaoka, H. (2003). The Black Flies (Diptera: Simuliidae) of Sulawesi, Maluku and Irian Jaya. Fukuoka: Kyushu University Press, xxii + 581 pp.
- Takaoka, H., Aoki, C. & Hayakawa, H. (1992). Natural infections of blackflies with larvae of zoonotic *Onchocerca* spp. in northeast Japan. *Japanese Journal of Tropical Medicine and Hygiene* **20**: 1-9. <https://doi.org/10.2149/tmh1973.20.1>
- Takaoka, H., Iwasa, M., Otsuka, Y. & Fukuda, M. (2021). Two new species and a newly recorded species of black flies (Diptera: Simuliidae) from Hokkaido, Japan, with the description of the male of *Simulium* (*Simulium*) *iwatense* (Shiraki). *Medical Entomology and Zoology* **72**: (in press).