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RELATIONSHIPS OF BLACK-FLY SPECIES OF THE *SIMULIUM TUBEROSUM* SPECIES-GROUP (DIPTERA: SIMULIIDAE) IN PENINSULAR MALAYSIA, WITH KEYS TO TEN MALAYSIAN SPECIES

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ABSTRACT. — Two species of black flies, *Simulium* (*Simulium*) *jasmoni* Takaoka, Sofian-Azirun & Belabut, 2012 and *S.* (*S.*) *tiomanense* Takaoka, Sofian-Azirun & Belabut, 2012, described from Tioman Island, Pahang, Malaysia, are placed in two different lineages of the *tuberosum* species-group in the subgenus *Simulium* (*Simulium*) Latreille, and have close similarities to *S.* (*S.*) *tani* Takaoka & Davies, 1995 and *S.* (*S.*) *brevipar* Takaoka & Davies, 1995, respectively, which were the only members of the same species-group in the nearest mainland of Peninsular Malaysia. It is suggested that *S.* (*S.*) *jasmoni* and *S.* (*S.*) *tani* in one lineage, as well as *S.* (*S.*) *tiomanense* and *S.* (*S.*) *brevipar* in another, had a common vicariant origin and were isolated after sea levels rose. Comparisons of character states of several pupal morphological features among four species in one lineage and five species in another lineage show that both *S.* (*S.*) *jasmoni* and *S.* (*S.*) *tiomanense* on Tioman Island retain more putative ancestral characters than *S.* (*S.*) *tani* and *S.* (*S.*) *brevipar* and other species in the same lineages on the continent and its adjacent large islands. Keys to 10 Malaysian members of the *tuberosum* species-group are provided for females, males, pupae, and mature larvae.

KEY WORDS. — black fly, *Simulium*, Malaysia, relationship, identification key

INTRODUCTION

The *Simulium tuberosum* species-group of the subgenus *Simulium* Latreille of the genus *Simulium* Latreille (Diptera: Simuliidae), defined by Rubtsov (1956), is one of two among the 24 species-groups of the subgenus that have a wide geographical distribution from the Oriental Region through the Palearctic Region to the Nearctic Region (Takaoka, 1996, 2003). This species-group consists of 47 named species, of which 21 are recorded from the Oriental Region, 11 from the Palearctic Region, five from both the Oriental and Palearctic Regions, seven from the Nearctic Region, and three from both the Palearctic and Nearctic Regions (Adler & Crosskey, 2011). In Malaysia, where 69 species of black flies have been recorded (Adler & Crosskey, 2011; Takaoka et al., 2011a, 2011b), eight species were placed in the *tuberosum* species-group: *S.* (*S.*) *tani* Takaoka & Davies, 1995 complex and *S.* (*S.*) *brevipar* Takaoka & Davies, 1995 in Peninsular Malaysia (Takaoka & Davies, 1995); *S.* (*S.*) *aeneifacies* Edwards, 1933, *S.* (*S.*) *alberti* Takaoka, 2008, and *S.* (*S.*) *masilauense* Takaoka, 2008 in Sabah; *S.* (*S.*) *lunduense* Takaoka, 2008 in Sarawak; and *S.* (*S.*) *keningauense* Takaoka, 2008 and *S.* (*S.*) *sabahense* Smart & Clifford, 1969 in both Sabah and Sarawak (Edwards, 1933; Smart & Clifford, 1969; Takaoka, 2008).

Recently, two more new species of the *tuberosum* species-group were collected from Tioman Island, located in the southern section of the South China Sea, about 37 km east from the southeast coast of Peninsular Malaysia, and described as *S.* (*S.*) *jasmoni* Takaoka, Sofian-Azirun & Belabut, 2012 and *S.* (*S.*) *tiomanense* Takaoka, Sofian-Azirun & Belabut, 2012 (Takaoka et al., 2012). These two new species are placed in two different lineages of the *tuberosum* species-group, chromosomally shown by Tangkawanit et al. (2009), having close similarities to *S.* (*S.*) *tani* and *S.* (*S.*) *brevipar*, respectively, which were the only members of the same species-group in Peninsular Malaysia (Takaoka & Davies, 1995).

In this paper, the relationships of *S.* (*S.*) *jasmoni* and *S.* (*S.*) *tiomanense* on Tioman Island with *S.* (*S.*) *tani* and *S.* (*S.*) *brevipar* on the mainland of Peninsular Malaysia and other related species on the continent and adjacent large islands in two lineages are studied by comparing character states of several pupal morphological features.

The geographical distributions of the 10 species of the *tuberosum* species-group so far recorded from Malaysia are shown in Fig. 1, and keys to these Malaysian members are provided for females, males, pupae and mature larvae.

MATERIAL AND METHODS

For comparisons of morphological character states, following references of nine species in two different lineages of the *tuberosum* species-group were used: Takaoka & Davies (1995) for *S.* (*S.*) *tani* distributed in Peninsular Malaysia, Thailand, Sumatra, Vietnam, and China; Takaoka & Somboon (2008) for *S.* (*S.*) *rangjungense* Takaoka & Somboon, 2008 from Bhutan; Takaoka (2008) for *S.* (*S.*) *keningauense* from Sabah and Sarawak; Takaoka et al. (2012) for *S.* (*S.*) *jasmoni* from Tioman, in one lineage in which a minute subbasal projection on the female claw (Fig. 2E) is shared; Takaoka & Davies (1995) for *S.* (*S.*) *brevipar* distributed in Peninsular Malaysia, southern Thailand and Sumatra; Takaoka & Choochote (2005) for *S.* (*S.*) *yuphae* Takaoka & Choochote, 2005 from northern Thailand; Takaoka & Hadi (1991) for *S.* (*S.*) *sigiti*; Takaoka & Hadi, 1991 from Java; Chen et al. (2003) for *S.* (*S.*) *tianchi* Chen, Zhang & Yang, 2003 from Hainan; and Takaoka et al. (2012) for *S.* (*S.*) *tiomanense* from Tioman, in another lineage, which is characterised by the presence of pit-like organs on the pupal thoracic integument (Fig. 2K, L).

Morphological features examined for character states were all those of the pupa (character states assumed as plesiomorphic or apomorphic in parentheses): 1, the length of the mediodorsal trichomes on the thorax (long trichomes are plesiomorphic and short ones are apomorphic); 2, the shape of the tubercles on the frons and thorax (round tubercles are plesiomorphic and cone-shaped tubercles with pointed apices [Fig. 2N] are apomorphic) for one lineage; 3, the size of pit-like organs (small pit-like organs are plesiomorphic and large ones are apomorphic); and 4, the shape of tubercles on the frons and thorax (tubercles with smooth surface are plesiomorphic and tubercles with secondary projections [Fig. 2M] are apomorphic) for another lineage. Assumptions of the plesiomorphic or apomorphic character states are based on the fact that the more ancestral genus *Prosimulium* bears long mediodorsal trichomes, round tubercles, no pit-like organs, and tubercles with smooth surface, while certain species or subgenera of the most specialised genus *Simulium* have very short mediodorsal trichomes, cone-shaped tubercles with pointed apices, large pit-like organs, and tubercles with secondary projections, all of which have never been found in the genus *Prosimulium* (Takaoka, unpublished data). Terms for morphological features used here follow those of Takaoka (2003).

RESULTS AND DISCUSSION

The geographical distribution of the *tuberosum* species-group in the Oriental region covers most of the Southeast

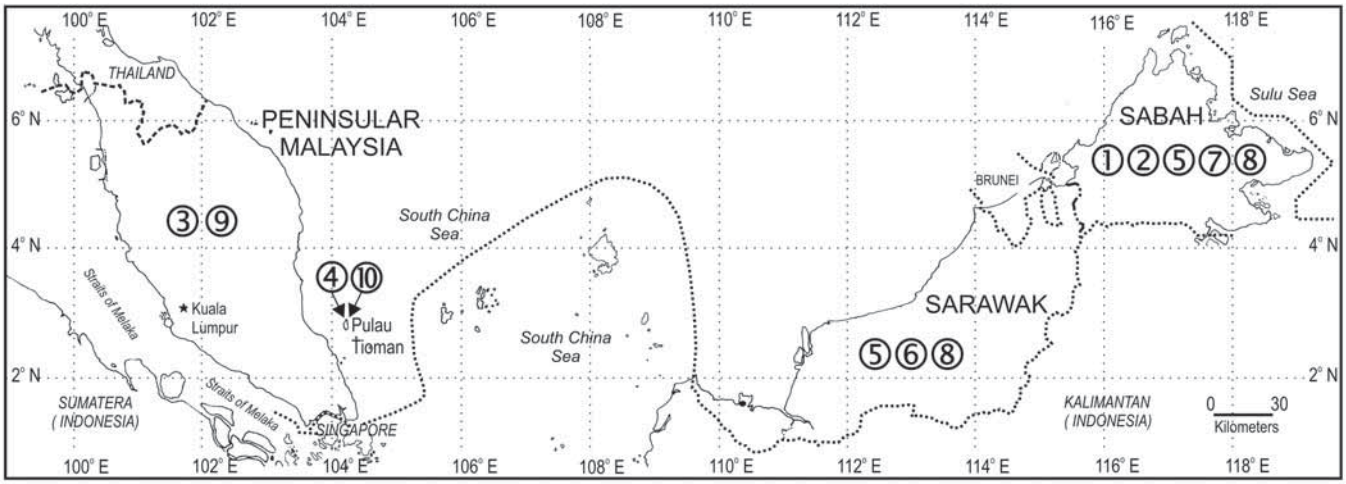

Fig. 1. The geographical distributions of 10 Malaysian species of the *Simulium tubesrosum* species-group. Numbers in circles correspond to the following species: 1, *Simulium aeneifacies*; 2, *S. alberti*; 3, *S. brevipar*; 4, *S. jasmoni*; 5, *S. keningauense*; 6, *S. lunduense*;7, *S. masilauense*; 8, *S. sabahense*; 9, *S. tani*;10, *S. tiomanense*. Dotted lines indicate international boundaries.

Asian areas of the Eurasian Continent, but its southward and eastward extensions are limited up to the so-called continental islands (e.g., Sumatra, Java, Borneo, Palawan, and Hainan), which were parts of the Sundaland formed by decreasing sea levels at the glacial epochs (Takaoka, 1996). It is not surprising that this species-group was also found on Tioman Island, one of the continental islands adjacent to the mainland of Peninsular Malaysia, characterised by mountainous terrain reaching 1,038 m in elevation, dominated by dipterocarp forests supporting a number of fast-flowing permanent streams suitable for habitats of the immature stages of black flies (Grismer, 2005). Given the dispersal capabilities of black flies over open waters (maximum flight range 116 km in still air; Hocking, 1953) and colonisation of near-mainland islands (within 100 km from the nearest source) by the resident species of the nearest mainland (Adler et al., 2005), the presence of the two new species of the *tuberosum* species-group on Tioman Island is unexpected because this island is only 37 km from the nearest mainland of Peninsular Malaysia.

The two new species, *S.* (*S.*) *jasmoni* and *S.* (*S.*) *tiomanense*, are of two different lineages, and have close similarities to *S.* (*S.*) *tani* and *S.* (*S.*) *brevipar*, respectively, which were the only members of the *tuberosum* species-group in the nearest mainland of Peninsular Malaysia (Takaoka & Davies, 1995). It is likely that *S.* (*S.*) *jasmoni* and *S.* (*S.*) *tani* in one lineage, as well as *S.* (*S.*) *tiomanense* and *S.* (*S.*) *brevipar* in another, had a common vicariant origin and were isolated after sea levels rose. This isolation, coupled with genetic drift and new selection pressures, attendant with living in new environments, likely promoted speciation in these isolated populations, as discussed for a significant portion of the flora and fauna endemic in the Tioman Archipelago (Sodhi et al., 1999).

In one lineage of the *tuberosum* species-group, in which a minute subbasal projection on the female claw (Fig. 2E) is shared, four species are involved: *S.* (*S.*) *tani* from Peninsular Malaysia, Thailand, Sumatra, Vietnam, and China; *S.* (*S.*) *rangjungense* from Bhutan; *S.* (*S.*) *keningauense* from Sabah and Sarawak; and *S.* (*S.*) *jasmoni* from Tioman Island. These four species are morphologically indistinguishable in the adults from one another but are distinguished in the pupa by the length of the mediodorsal trichomes on the thorax, the shape of the tubercles on the frons and thorax, and the divergence of the gill filaments. The mediodorsal trichomes are medium-long in *S.* (*S.*) *jasmoni* and *S.* (*S.*) *keningauense*, short in *S.* (*S.*) *tani* and very short in *S.* (*S.*) *rangjungense*, suggesting the direction of evolution in this order if the plesiomorphic state is assumed for the mediodorsal trichomes to be long. On the other hand, the tubercles on the frons and thorax are cone-shaped with pointed apices (Fig. 2N) in *S.* (*S.*) *keningauense* but rounded in the other species, suggesting that *S.* (*S.*) *jasmoni*, as well as *S.* (*S.*) *tani* and *S.* (*S.*) *rangjungense*, is more ancestral than *S.* (*S.*) *keningauense*. The pupal gill filaments are widely divergent in *S.* (*S.*) *jasmoni* but moderately so (e. g., Fig. 2K) in the three other species. However, it may be argued which character state of the gill filaments is plesiomorphic or apomorphic. Although only a few features were considered for their character states, *S.* (*S.*) *jasmoni* on Tioman Island appears to be the most ancestral among the four species in the same lineage.

Among species in the other lineage of the *tuberosum* species-group, five species are unique in having pit-like organs on the pupal thoracic integument (Fig. 2K, L): *S.* (*S.*) *brevipar* from Peninsular Malaysia, southern Thailand, and Sumatra; *S.* (*S.*) *yuphae* from northern Thailand; *S.* (*S.*) *sigiti* from Java; *S.* (*S.*) *tianchi* from Hainan; and *S.* (*S.*) *tiomanense* from Tioman. Among these, *S.* (*S.*) *tiomanense* is inferred to be the most ancestral since it has more plesiomorphic character states, such as small pit-like organs and smooth tubercles on the frons and thorax, than the four other species, all of which have medium-sized pit-like organs (Fig. 2K, L) and tubercles with secondary projections on their surface (Fig. 2M).

Evolutionary changes might have occurred less frequently in the populations of both *S.* (*S.*) *jasmoni* and *S.* (*S.*) *tiomanense* on Tioman Island, which retain a number of the putative ancestral characters, than in the populations of *S.* (*S.*) *tani* and *S.* (*S.*) *brevipar* on the mainland of Peninsular Malaysia and other related species in the same lineages on the continent and its adjacent large islands.

KEYS TO MALAYSIAN MEMBERS OF THE *SIMULIUM TUBEROSUM* SPECIES-GROUP

There are eight species-groups in the subgenus *Simulium* so far recorded from Malaysia: *argentipes* species-group (two species), *griseifrons* species-group (four species), *melanopus* species-group (seven species), *multistriatum* species-group (two species), *nobile* species-group (one species), *striatum* species-group (two species), *tuberosum* species-group (10 species), and *variegatum* species-group (one species) (Adler & Crosskey, 2011; Takaoka et al., 2012). The *tuberosum* species-group is distinguished from the other seven species-groups by the following diagnostic characteristics: in the female by a combination of the scutum with no longitudinal vittae and the claw simple or with a minute subbasal projection; in the male by the style with a short round basal protuberance bearing multiple cone-like spines; and in the pupa by the gill with six thread-like filaments coupled with a simple wall-pocket shaped cocoon (Fig. 2J), by which it is, though, not separable in the pupal stage from the *variegatum* species-group (Takaoka & Davies, 1995, 1996). Few larval characteristics are available to separate the *tuberosum* species-group from other species-groups (Takaoka & Davies, 1995, 1996). Most morphological features used in the following keys are illustrated in Fig. 2A–S.

Females*

1. Claw with a minute subbasal projection (Fig. 2E) 2
– Claw simple, without any subbasal projection (Fig. 2F) 4
2. Sensory vesicle medium-long (0.42–0.48 times as long as 3rd maxillary palpal segment) (Fig. 2B) .... *S. keningauense* Takaoka
– Sensory vesicle elongate (over 0.5 times as long as 3rd maxillary palpal segment) (Fig. 2C) 3

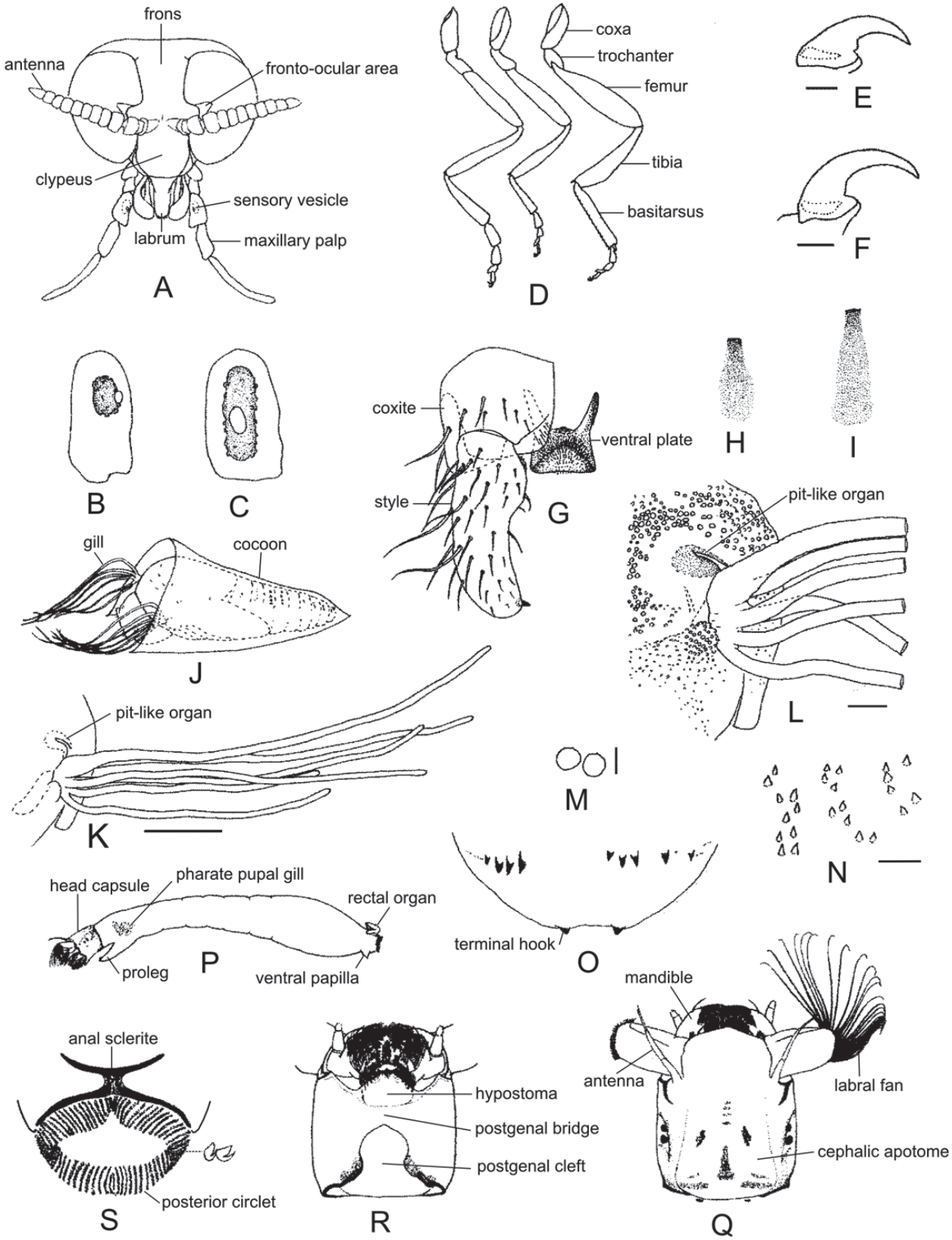

Fig. 2. Morphological features of the genus *Simulium*. A, female head (front view); B, C, 3rd segments of female maxillary palps showing medium-sized and elongate sensory vesicles, respectively (B, *S. brevipar*; C, *S. tani*); D, female leg (fore, mid and hind legs from left); E, female claw with minute round subbasal tooth (*S. keningauense*); F, female claw without subbasal tooth (*S. alberti*); G, male genitalia (ventral view, left coxite and style and parameres omitted, *S. brevipar*); H, median sclerite with apical 1/2 nearly parallel sided (*S. brevipar*); I, median sclerite gradually widened toward apex (*S. tani*); J, pupa and cocoon; K, right pupal gill and pit-like organ (outer view, *S. brevipar*); L, enlargement of basal portion of pupal gill, pit-like organ and tubercles on thoracic integument (*S. brevipar*); M, relatively large tubercles with minute secondary projections on thoracic integument (*S. brevipar*); N, cone-shaped tubercles with pointed apices on dorsomedial surface of thorax (*S. keningauense*); O, 9th abdominal segment of pupa (dorsal view, *S. tani*); P, whole body of mature larva (lateral view); Q, R, head capsules of larva (Q, dorsal view; R, ventral view);S, posterior tip of larval abdomen (dorsal view). Scale bars = 0.2 mm for K; 0.05 mm for L; 0.04 mm for N; 0.01 mm for E, F, M.

3. Minute subbasal projection of claw nodule-like, rounded (Fig. 2E) ..... *S. tani* Takaoka & Davies
– Minute subbasal projection of claw pointed apically .... *S. jasmoni* Takaoka, Sofian-Azirun & Belabut
4. Sensory vesicle elongate (0.59 times as long as 3rd maxillary palpal segment) 5
– Sensory vesicle medium-long (0.31–0.49 times as long as 3rd maxillary palpal segment) 6
5. Height of frons against its narrowest width 1.23 .... *S. alberti* Takaoka
– Height of frons against its narrowest width 1.39 .... *S. masilauense* Takaoka
6. Fore basitarsus moderately dilated, 5.22–5.65 times as long as its greatest width ..... *S. aeneifacies* Edwards
– Fore basitarsus greatly dilated, 4.30–4.91 times as long as its greatest width 7
7. Height of frons against its narrowest width 1.35 .... *S. brevipar* Takaoka & Davies
– Height of frons against its narrowest width 1.04–1.26 8
8. Hind basitarsus 0.64 times as wide as hind femur .... *S. sabahense* Smart & Clifford
– Hind basitarsus 0.57 times as wide as hind femur .... *S. lunduense* Takaoka

* *Simulium tiomanense* is not included because its female remains unknown.

Males

1. Abdominal segments 2, 6 and 7 each with pair of pruinose spots. Median sclerite broad, nearly parallel-sided at least on apical 1/2 (Fig. 2H) 2
– Abdominal segments 2, 5, 6 and 7 each with pair of pruinose spots. Median sclerite gradually widened from base toward apex (Fig. 2I) 3
2. Head holoptic ..... *S. brevipar* Takaoka & Davies
– Head dichoptic .... *S. tiomanense* Takaoka, Sofian-Azirun & Belabut
3. Hind basitarsus as wide as or little wider than hind femur .. 4
– Hind basitarsus narrower than hind femur 7
4. Enlarged upper-eye facets in 12–14 vertical columns and 13–15 horizontal rows ..... *S. sabahense* Smart & Clifford
– Enlarged upper-eye facets in 16–20 vertical columns and 17–22 horizontal rows 5
5. Style in ventrolateral view narrowed from basal 1/3 to apical 1/3, then slightly widened toward apex .... *S. alberti* Takaoka
– Style in ventrolateral view nearly parallel-sided or narrowed from middle toward apex 6
6. Style in ventrolateral view abruptly narrowed near middle, then slightly narrowed toward apex, with apical portion 0.53 times as wide as greatest width at base ..... *S. aeneifacies* Edwards
– Style in ventrolateral view moderately narrowed just before middle, then nearly parallel-sided toward apex, with apical portion 0.71 times as wide as greatest width at base .... *S. masilauense* Takaoka
7. Hind basitarsus nearly parallel-sided, 4.50–4.62 times as long as its greatest width ..... *S. lunduense* Takaoka
– Hind basitarsus spindle- or wedge-shaped, 3.50–3.89 times as long as its greatest width 8
8. Fore basitarsus 6.43 times as long as its greatest width .... *S. jasmoni* Takaoka, Sofian-Azirun & Belabut
– Fore basitarsus 5.52–5.70 times as long as its greatest width 9
9. Hind basitarsus 3.50 times as long as its greatest width .... *S. tani* Takaoka & Davies
– Hind basitarsus 3.81–3.89 times as long as its greatest width. ..... *S. keningauense* Takaoka

Pupae

1. Pit-like organ adjacent to base of gill present (Fig. 2K, L). Terminal hooks absent 2
– Pit-like organ adjacent to base of gill absent. Terminal hooks present (Fig. 2O) 3
2. Tubercles on frons with minute secondary projections (Fig. 2M) ..... *S. brevipar* Takaoka & Davies
– Tubercles on frons smooth, without secondary projection .... *S. tiomanense* Takaoka, Sofian-Azirun & Belabut
3. Basal portions of dorsal and ventral filaments moderately divergent at angle of 90 degrees or little more when viewed laterally ..... *S. jasmoni* Takaoka, Sofian-Azirun & Belabut
– Basal portions of dorsal and ventral filaments somewhat divergent at angle of 40–80 degrees (e.g., Fig. 2K) 4
4. Most of tubercles on thoracic integument cone-shaped with pointed apex (Fig. 2N) 5
– Most of tubercles on thoracic integument rounded 6
5. Six gill filaments arranged as 1+(1+2)+2 filaments from dorsal to ventral ..... *S. keningauense* Takaoka
– Six gill filaments arranged as 2+2+2 filaments from dorsal to ventral ..... *S. lunduense* Takaoka
6. Stalks of dorsal, middle and ventral paired filaments medium-long to long ..... *S. alberti* Takaoka
– Stalks of dorsal, middle and ventral paired filaments very short to short 7
7. Mediodorsal trichome as long as or longer than posterolateral trichome 8
– Mediodorsal trichome shorter than posterolateral trichome 9
8. Longest dorsal filament 1.8–2.1 mm long and shortest ventral filament 1.3–1.5 mm long ..... *S. masilauense* Takaoka
– Longest dorsal filament 1.4–1.7 mm long and shortest ventral filament 0.7–1.0 mm long ..... *S. sabahense* Smart & Clifford
9. Two anterolateral trichomes short, subequal in length to each other ..... S. *tani* Takaoka & Davies
– Two anterolateral trichomes different in length from each other (1 short and 1 medium-long) ..... *S. aeneifacies* Edwards

Mature Larvae

1. Postgenal cleft triangular, gradually narrowed from base to apex 2
– Postgenal cleft nearly parallel-sided, or somewhat widened from base to middle, arrow-head shaped, or mitre-shaped (Fig. 2R) 3
2. Posterior circlet with 74 rows of up to 12 hooklets per row .. .. *S. brevipar* Takaoka & Davies
– Posterior circlet with 84 rows of up to 14 hooklets per row .. .. *S. tiomanense* Takaoka, Sofian-Azirun & Belabut
3. Lateral surface of head capsule light to medium brown around eye-spot region 4
– Lateral surface of head capsule whitish-yellow to yellow around eye-spot region 6
4. Labral fan with 34–36 main rays..... *S. masilauense* Takaoka
– Labral fan with 38–44 main rays 5
5. Rectal organ with 15–18 secondary lobules per lobe .... *S. alberti* Takaoka
– Rectal organ with 10–12 secondary lobules per lobe .... *S. aeneifacies* Edwards
6. Postgenal cleft arrow-head shaped, nearly parallel-sided or slightly widened from base to middle 7
– Postgenal cleft mitre-shaped, somewhat to moderately widened from base to middle 8
7. Postgenal cleft relatively wide, widest in middle 0.91 times length of postgenal cleft .... *S. jasmoni* Takaoka, Sofian-Azirun & Belabut
– Postgenal cleft moderately wide, widest in middle 0.74 times length of postgenal cleft ..... *S. tani* Takaoka & Davies

8. Labral fan with 34 main rays ..... *S. keningauense* Takaoka
– Labral fan with 36–42 main rays 9
9. Posterior circlet with up to 13 hooklets per row .... *S. sabahense* Smart & Clifford
– Posterior circlet with up to 16 hooklets per row .... *S. lunduense* Takaoka

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LITERATURE CITED

Adler, P. H. & R. W. Crosskey, 2011. *World Blackflies (Diptera: Simuliidae): A Comprehensive Revision of the Taxonomic and Geographical Inventory [2011]*. http://entweb.clemson.edu/biomia/pdfs/blackflyinventory.pdf .117 pp. (Accessed 16 Aug.2011).

Adler, P. H., D. J. Giberson & L. A. Purcell, 2005. Insular black flies (Diptera: Simuliidae) of North America: Tests of colonization hypotheses. *Journal of Biogeography*, **32**: 211–220.

Chen, H. B., C. L. Zhang & M. Yang, 2003. Descriptions of two new species of the subgenus *Nevermannia* Enderlein and subgenus *Simulium* Latreille from Hainan Island, China (Diptera, Simuliidae). *Acta Zootaxonomica Sinica*, **28**: 745–750.

Edwards, F. W., 1933. Diptera Nematocera from Mount Kinabalu. *Journal of Federated Malay States Museum*, **17**: 223–296.

Grismer, L. L., 2005. *Amphibians and Reptiles of the Tioman Archipelago, Malaysia*. Forest Department Peninsular Malaysia, Kuala Lumpur, Malaysia. 215 pp.

Hocking, B., 1953. On the intrinsic range and speed of flight of insects. *Transactions of the Royal Entomological Society of London*, **104**: 223–345.

Rubtsov, I. A., 1956. *Blackflies (Simuliidae). Fauna of the USSR. 2nd Edition, n. s. No. 61, Diptera 6 (6)*. Acad. Nauk SSSR, Moscow and Leningrad. 859 [+1] pp. [Translated from Russian by Amerind Publ. Co. Pot. Ltd., Deli, India, 1989].

Smart, J. & E. A. Clifford, 1969. Simuliidae (Diptera) of Sabah (British North Borneo). *Zoological Journal of Linnean Society*, **48**: 9–47.

Sodhi, N. S., H. S. Yong & P. K. Ng (eds.), 1999. The biodiversity of the Pulau Tioman, Peninsular Malaysia. *Raffles Bulletin of Zoology, Supplement*, **6**: 1–288.

Takaoka, H., 1996. The geographical distribution of the genus *Simulium* Latreille in the Oriental and Australasian Regions. *Japanese Journal of Tropical Medicine and Hygiene*, **24**: 113–124.

Takaoka, H., 2003. *The Black Flies (Diptera: Simuliidae) of Sulawesi, Maluku and Irian Jaya.* Kyushu University Press, Fukuoka, Japan. xxii +581 pp.

Takaoka, H., 2008. Taxonomic revision of *tuberosum* species-group of *Simulium* (*Simulium*) in Sabah and Sarawak, Malaysia (Diptera: Simuliidae). *Medical Entomology and Zoology*, **59**: 55–80.

Takaoka, H. & W. Choochote, 2005. Two new species of black flies (Diptera: Simuliidae) from northern Thailand. *Medical Entomology and Zoology*, **56**: 319–334.

Takaoka, H. & D. M. Davies, 1995. *The Black Flies (Diptera: Simuliidae) of West Malaysia*. Kyushu University Press, Fukuoka, Japan. viii +175 pp.

Takaoka, H. & D. M. Davies, 1996. The black flies (Diptera: Simuliidae) of Java, Indonesia. B*ishop Museum Bulletin in Entomology*, **6**: 1–81.

Takaoka, H. & U. K. Hadi, 1991. Two new blackfly species of *Simulium* (*Simulium*) from Java, Indonesia (Diptera: Simuliidae). *Japanese Journal of Tropical Medicine and Hygiene*, **19**: 357–370.

Takaoka, H., M. Sofian-Azirun & D. M. Belabut, 2012. Descriptions of two new species of *Simulium* (*Simulium*) (Diptera: Simuliidae) from Tioman Island, Peninsular Malaysia. *Raffles Bulletin of Zoology*, **60**: 399–409.

Takaoka, H., M. Sofian-Azirun & R. Hashim, 2011a. *Simulium* (*Gomphostilbia*) *sofiani*, a new species of black fly (Diptera: Simuliidae) from Peninsular Malaysia. *Tropical Biomedicine*, **28**: 389–399.

Takaoka, H., M. Sofian-Azirun, R. Hashim & Z. Ya'cob, 2011b. Two new species of *Simulium* (*Gomphostilbia*) (Diptera: Simuliidae) from Peninsular Malaysia, with keys to Peninsular Malaysian members of the *Simulium ceylonicum* species-group. *ZooKeys*, **118**: 53–74.

Takaoka, H. & P. Somboon, 2008. Eleven new species and one new record of black flies (Diptera: Simuliidae) from Bhutan. *Medical Entomology and Zoology*, **59**: 213–262.

Tangkawanit, U., C. Kuvangkadilok, V. Baimai & P. H. Adler, 2009. Cytosystematics of the *Simulium tuberosum* group (Diptera; Simuliidae) in Thailand. *Zoological Journal of the Linnean Society*, **155**: 289–315.