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Female black flies of *Simulium* (Diptera: Simuliidae) collected on humans in Tam Dao National Park, Vietnam: Description of a new species and notes on four species newly recorded from Vietnam

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Abstract. A total of 29 female black flies were captured by a hand net as they swarmed around humans in Tam Dao National Park, Vinh Phuc Province, Vietnam. They included one species of the subgenus *Gomphostilbia* (*Simulium* (*Gomphostilbia*) *asakoae* Takaoka & Davies) and five species of the subgenus *Simulium*, of which one species is described as *Simulium* (*Simulium*) *vietnamense* sp. nov. and the other four species (*S.* (*S.*) *chungi* Takaoka & Huang, *S.* (*S.*) *grossifilum* Takaoka & Davies, *S.* (*S.*) *maenoi* Takaoka & Choochote, and *S.* (*S.*) *rufibasis* Brunetti) are newly recorded from Vietnam.

INTRODUCTION

Black flies are well known as biting dipteran insects of medical and veterinary importance. In Southeast Asian countries, collections of adult black flies on humans have been scarcely carried out except in northern parts of Thailand, where adult females of 22 species were captured on humans (Choochote *et al.*, 2005) and, among them, three human-biting species (*Simulium* (*Gomphostilbia*) *asakoae* Takaoka & Davies, *Simulium* (*Simulium*) *nigrogilvum* Summers and *Simulium* (*Simulium*) *nodosum* Puri) were implicated as vectors of filarial species (Fukuda *et al.*, 2003; Takaoka *et al.*, 2003; Ishii *et al.*, 2008).

In Vietnam, only 16 species of black flies were known, including *S.* (*G.*) *asakoae*, *S.* (*S.*) *nigrogilvum* and *S.* (*S.*) *nodosum* (Adler & Crosskey, 2014; Crosskey & Howard, 1997; Pham, 1998, 1999). Biting habits of these three and the other species in Vietnam remain to be studied.

On November 9, 2013, we collected 29 adult female black flies as they flew around humans in the afternoon (1300–1500 hour) at a road traversing a mountain slope (about 500 m inside the gate, altitude 997 m, N21°28'104", E105°38'687") in Tam Dao National Park, Vinh Phuc Province, about 90 km north of Hanoi, in Vietnam. The weather was clear, with no wind, and the air temperature was 23–24°C. Landing and biting of female black flies on humans were not confirmed. All female black flies were preserved in vials with 80% ethanol. They were identified using keys and original descriptions of species from Thailand (Takaoka & Suzuki, 1984; Takaoka & Choochote, 2004), Peninsular Malaysia (Takaoka & Davies, 1995) and China (Chen & An, 2003) and other individual relevant papers on black flies from Taiwan (Takaoka

& Huang, 2006; Huang & Takaoka, 2009) and Thailand (Takaoka & Choochote, 2002, 2005). As a result, female black flies were identified as one species of the subgenus *Gomphostilbia* and five species of the subgenus *Simulium*, including one new species.

We describe this new species as *Simulium* (*Simulium*) *vietnamense* sp. nov., and record the other four known species, i.e., *S.* (*S.*) *chungi* Takaoka & Huang, *S.* (*S.*) *grossifilum* Takaoka & Davies, *S.* (*S.*) *maenoi* Takaoka & Choochote, and *S.* (*S.*) *rufibasis* Brunetti, for the first time from Vietnam.

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

The holotype and paratype of the new species are deposited in the Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.

1. *Simulium* (*Gomphostilbia*) *asakoae* Takaoka & Davies, 1995

Simulium (*Gomphostilbia*) *asakoae* Takaoka & Davies, 1995: 55–60 (female, male, pupa and larva).

Specimens examined. Two females.

Distribution. Peninsular Malaysia, Thailand, Hong Kong, Vietnam.

Remarks. Two females were assigned to the *Simulium asakoae* species-group of the subgenus *Gomphostilbia* defined by Takaoka (2012) by having the yellow hair tuft on the stem vein, hind tibiae yellowish on the basal 2/3 and genital fork without a distinct projection directed posteromedially. According to keys for 21 species of the group (Takaoka *et al.*, 2014), two females were identified as *S.* (*G.*) *asakoae*.

As mentioned above, *S.* (*G.*) *asakoae* was reported as a human biter (Choochote *et al.*, 2005) and a vector of unknown filarial species in Thailand (Fukuda *et al.*, 2003; Ishii *et al.*, 2008).

2. *Simulium* (*Simulium*) *vietnamense* Takaoka, Sofian-Azirun & Chen, sp. nov.

Female. Body length 2.6–3.2 mm. ***Head***. Slightly narrower than thorax. Frons black, shiny, with bluish reflection when illuminated at certain angles, with several dark stout hairs along lateral margins; frontal ratio 1.23–1.35:1.00:0.95–1.00; frons:head ratio 1.00:3.41–3.63. Fronto-ocular area well developed, short, directed laterally, and pointed apically. Clypeus brownish-black, shiny, white pruinose when illuminated at certain angles, moderately covered with dark-brown medium-long hairs (though mediolongitudinal portion of upper 2/3 widely bare) interspersed with several dark-brown longer curved hairs on each side of lower portion. Labrum 0.64–0.68 times length of clypeus. Antenna composed of scape, pedicel and nine flagellomeres; first flagellomere 1.30 times length of second one; scape and pedicel yellow, fifth to seventh flagellomeres dark-brown to brownish-black, and first to fourth flagellomeres dark yellow to medium brown (gradually darkened apically) when viewed from above but yellow to dark yellow when viewed ventrally; first to sixth flagellomeres each with pit-like depression with many sensilla on inner and outer sides. Maxillary palp with five segments, light brown except first and second segments yellow; proportional lengths of third, fourth, and fifth segments 1.00:1.11–1.21:2.62–2.72; third segment (Fig. 1A) of normal size, with medium-sized ellipsoidal sensory vesicle (0.33–0.42 times length of third segment) having medium-sized opening. Maxillary lacinia with 13–15 inner and 13 or 14 outer teeth. Mandible with 24–26 inner and 12–14 outer teeth. Cibarium (Fig. 1B) with 54–57 minute processes near posterodorsal margin. ***Thorax***. Scutum black except anterolateral calli dark brown, shiny, with bluish reflection when illuminated at certain angles, grayish pruinose when illuminated at certain angles, moderately covered with brassy recumbent short hairs interspersed with dark-brown short hairs on anterior portion near anterior margin, and several dark-brown long upright hairs on prescutellar area. Scutellum dark brown, covered with dark-brown upright long hairs as well as brassy short hairs. Postnotum brownish-black, bare. Pleural membrane bare. Katepisternum brownish-black, longer than deep, bare, and shiny when illuminated at certain angles. ***Legs***. Foreleg: coxa whitish-yellow; trochanter yellow; femur yellow to

dark yellow; tibia whitish except apical 1/4 brownish-black; tarsus black, with moderate dorsal hair crest; basitarsus greatly dilated, 5.53–5.65 times as long as its greatest width. Midleg: coxa dark brown; trochanter and femur yellow; tibia yellow though posterior surface widely whitish; tarsus dark brown except basal 2/3 of basitarsus yellow. Hind leg: coxa dark brown; trochanter yellow; femur yellow except apical cap medium brown; tibia (Fig. 1C) in outer view yellow on little less than basal 2/5, light to dark brown on apical 3/5 (though whitish narrowly along posterior margin except apical 1/4); tibia in posterior view widely whitish and shiny on basal 3/4 when illuminated at certain angles; tarsus brownish-black except little less than basal 3/5 of basitarsus, and basal 1/2 or little less of second tarsomere yellowish-white; basitarsus (Fig. 1D) nearly parallel-sided, 5.50–5.81 times as long as wide, and 0.81 and 0.67–0.70 times as wide as greatest widths of hind tibia and femur, respectively; calcipala (Fig. 1D) moderately developed, nearly as long as wide, and 0.40–0.43 times as wide as greatest width of basitarsus; pedisulcus (Fig. 1D) well developed. Tarsal claw simple, without basal or subbasal tooth. ***Wing***. Length 2.3 mm. Costa with dark spinules and hairs; subcosta haired except near apex bare; basal section of radial vein haired on apical 1/2–3/4; R_1 with dark-brown spinules and hairs; R_2 with dark-brown hairs; hair tuft on stem vein dark brown; basal cell absent. ***Abdomen***. Basal scale medium brown, with fringe of dark hairs. Dorsal surface of abdomen medium-brown to brownish-black except little less than basal 1/2 of second segment whitish, with light to dark-brown short hairs; tergite 2 shiny and white pruinose when illuminated at certain angles and tergites 6–9 shiny. Ventral surface of segment 7 with pair of weakly sclerotized submedian sternal plates. ***Genitalia***. Sternite 8 (Fig. 1E) with 13–19 dark-brown medium-long to long stout hairs and few yellow short hairs on each lateral surface. Ovipositor valves (Fig. 1E) wide, somewhat thick except narrow areas along inner and posterior margins thin and transparent, somewhat rounded ventrally, densely covered with microsetae (except narrow transparent portions along inner and posterior margins bare) interspersed with 19–24 yellow short hairs; inner margins slightly sinuous, somewhat separated from each other. Genital fork (Fig. 1F, G) of inverted-Y form, with narrow well sclerotized stem; arms of moderate width, each with heavily sclerotized lateral portion, of which anterior corner directed anterodorsally. Paraproct in ventral view (Fig. 1H) rounded, subequal in length to greatest width, strongly pigmented on anterior surface, with 30–32 dark-brown short to medium-long hairs (except few hairs on ventral surface yellow) on lateral and ventral surfaces, and with 8 or 9 short sensilla on anteromedial surface; paraproct in lateral view (Fig. 1I) 0.95 times as long as wide, and somewhat protruding ventrally beyond ventral margin of cercus. Cercus in lateral view (Fig. 1I) short, 0.53 times as long as wide, with numerous medium-long hairs, and rounded posteriorly. Spermatheca (Fig. 1J) nearly ovoid, 1.22–1.27 times as long as greatest width, well sclerotized except small portion of junction with duct unsclerotized, with faintly defined reticulate surface patterns only near junction; internal setae present; accessory ducts subequal in thickness to each other, and slightly thicker than major duct.

Male, **pupa** and **mature larva**. Unknown.

Type specimens. Holotype female, collected by a hand net around a human on the road in the Tam Dao National Park, Vinh Phuc Province, Vietnam, 9-XI-2013, by C.D. Chen & K.W. Lau. Paratype: one female, same data and date as those of the holotype.

Etymology. The species name *vietnamense* refers to the name of the country.

Remarks. The female of this new species is characterized by the haired basal portion of the radial vein, unpatterned scutum and simple claws. Among the species of the subgenus *Simulium* in the Oriental Region, nine of 47 species of the *griseifrons* species-group share a combination of these female characteristics: *S.* (*S.*) *ephippioidum*, Chen & Wen, *S.* (*S.*) *fuzhouense* Zhang & Wang, *S.* (*S.*) *ledongense* Yang & Chen, *S.* (*S.*) *mediaxisus* An, Guo & Xu, *S.* (*S.*) *qini* Cao, Wang & Chen, *S.* (*S.*) *shangchuanense* An & Hao, *S.* (*S.*) *spoonatum* An & Yan, *S.* (*S.*)

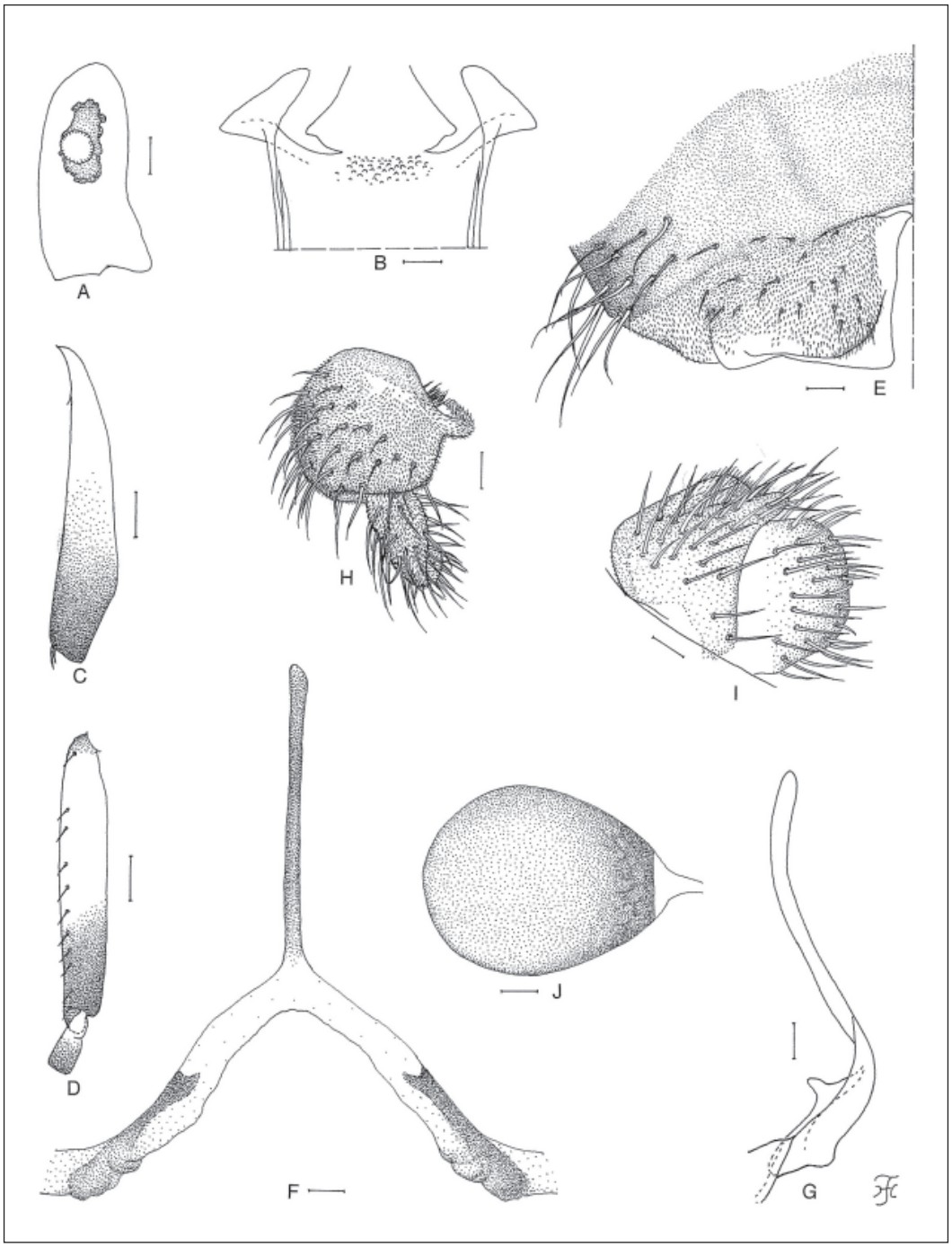

Figure 1. Female of *Simulium* (*Simulium*) *vietnamense* sp. nov. A, third segment of right maxillary palp with sensory vesicle (front view); B, cibarium; C, tibia of left hind leg (outer view); D, basitarsus with calcipala and second tarsomere with pedisulcus of left hind leg (outer view); E, sternite 8 and ovipositor valve (only right half shown; ventral view); F & G, genital forks (F, ventral view; G, lateral view); H & I, right paraprocts and cerci (H, ventral view; I, lateral view); J, spermatheca. Scale bars. 0.1 mm for C & D; 0.02 mm for A, B & E–J.

tenuatum Chen, all from China (Chen & An, 2003), and *S. yongi* from Peninsular Malaysia and Thailand (Takaoka & Davies, 1997). All the Chinese species except *S.* (*S.*) *fuzhouense* and *S.* (*S.*) *qini* differ from *S.* (*S.*) *vietnamense* sp. nov. by the hind tibia, which is yellowish on the basal 2/3 and dark on the rest, and the different shape of the ovipositor valve (Chen & An, 2003). *Simulium* (*S.*) *fuzhouense*, of which the legs were not described, is distinguished from this new species by the smaller number of short hairs on the ovipositor valve (8 in *S.* (*S.*) *fuzhouense* versus 19–24 in *S.* (*S.*) *vietnamense* sp. nov.) and the globular spermatheca (Chen & An, 2003). *Simulium* (*S.*) *qini*, of which the legs were also not described, has a triangular ovipositor valve (Chen & An, 2003), and *S.* (*S.*) *yongi* has much darker legs (Takaoka & Davies, 1997).

This new species appears to be related to *S.* (*S.*) *rudnicki* Takaoka & Davies described from Malaysia and *S.* (*S.*) *ufengense* Takaoka from Taiwan, by having a similar ovipositor valve, unpatterned scutum, simple claws and similar color pattern of the legs, but is distinguished from the latter two species by having the haired basal portion of the radial vein and the number of minute processes on the cibarium (54–57 in this new species versus 74 in *S.* (*S.*) *rudnicki* and 44–48 in *S.* (*S.*) *ufengense*) (Takaoka & Davies, 1995; Huang & Takaoka, 2009). The female of *S.* (*S.*) *ufengense* differs from this new species by the relative width of the hind basitarsus against the hind tibia (0.67 in *S.* (*S.*) *ufengense* versus 0.81 in *S.* (*S.*) *vietnamense* sp. nov.)

3. *Simulium* (*Simulium*) *chungi* Takaoka & Huang, 2006

Simulium (*Simulium*) *chungi* Takaoka & Huang, 2006: 219–227 (female, male, pupa and larva).

Specimens examined. 14 females.

Distribution. Taiwan and Vietnam (**New record**).

Remarks. This species is assigned to the *Simulium griseifrons* species-group and its female is characterized by the striped scutum, haired basal portion of the radial vein, simple claws, and ovipositor valve having a narrow apical tip (Takaoka & Huang, 2006). Eleven of the 14 females agreed morphologically with the original description of the female of *S.* (*S.*) *chungi* but three females have the darker hind tibia than that of *S.* (*S.*) *chungi*, which is yellowish-white on the basal 1/2 and dark brown to brownish-black on the apical 1/2 (cf., yellowish-white on the basal 2/3 and brownish-black on the apical 1/3 in *S.* (*S.*) *chungi*). It remains to be studied whether the difference in the color of legs is due to intraspecific or interspecific variation.

4. *Simulium* (*Simulium*) *grossifilum* Takaoka & Davies, 1995

Simulium (*Simulium*) *grossifilum* Takaoka & Davies, 1995: 105–115 (female, male, pupa and larva).

Specimens examined. Seven females.

Distribution. Peninsular Malaysia, Thailand and Vietnam (**New record**).

Remarks. This species was originally described from Peninsular Malaysia and is assigned to the *griseifrons* species-group (Takaoka & Davies, 1995). The female is characterized by the striped scutum, haired basal portion of the radial vein, simple claws, and flap-like ovipositor valve having an elongated projection on the dorsal surface (Takaoka & Davies, 1995). All seven females agreed morphologically with the original description of the female of *S.* (*S.*) *grossifilum*. The female of *S.* (*S.*) *grossifilum* is similar to that of *S.* (*S.*) *phayaoense* Takaoka & Choochote described from Thailand (Takaoka & Choochote, 2005) but is distinguished by the smaller size of the sensory vesicle against the third maxillary palpal segment (0.34 in *S.* (*S.*) *grossifilum* versus 0.41 in *S.* (*S.*) *phayaoense*).

5. *Simulium* (*Simulium*) *maenoi* Takaoka & Choochote, 2002

Simulium (*Simulium*) *maenoi* Takaoka & Choochote, 2002: 115–120 (female, male, pupa and larva).

Specimens examined. Three females.

Distribution. Thailand and Vietnam (**New record**).

Remarks. This species was described from Thailand and was assigned to the

griseifrons species-group (Takaoka & Choochote, 2002). The female is characterized by the striped scutum, bare basal portion of the radial vein, simple claws and triangular ovipositor valves (Takaoka & Choochote, 2002).

6. *Simulium* (*Simulium*) *rufibasis* Brunetti, 1911

Simulium rufibasis Brunetti: 1911: 282–288 (female).

Simulium (*Simulium*) *rufibasis* Brunetti: Puri, 1932: 899–903 (female, male and pupa).

Specimen examined. One female.

Distribution. India, China, Japan, Korea, Myanmar, Nepal, Pakistan, Taiwan, Thailand and Vietnam (**New record**).

Remarks. This species was originally described from India (Brunetti 1911) and is assigned to the *Simulium tuberosum* species-group (Adler & Crosskey, 2014). The female is characterized by the unpatterned scutum and submedian paired clusters of long hairs on the ventral surface of the seventh abdominal segment (Puri, 1932).

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