

Description of male, pupa and larva of *Simulium* (*Asiosimulium*) *wanchaii* (Diptera: Simuliidae) from Thailand, with keys to identify four species of the subgenus *Asiosimulium*

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Abstract. The male, pupa and mature larva of *Simulium* (*Asiosimulium*) *wanchaii* Takaoka & Choochote, one of the four species of the small Oriental black fly subgenus *Asiosimulium*, are described for the first time based on samples collected from Thailand. The male *S. (A.) wanchaii* is characterized based on the enlarged hind basitarsus and the ventral plate which is much wider than long. The pupa and larva are characterized by the gill with 19 filaments and the deep postgenal cleft, respectively. Keys are provided to identify all the four species of the subgenus *Asiosimulium* for females, males, pupae and mature larvae.

INTRODUCTION

Simulium (*Asiosimulium*) Takaoka & Choochote, one of two small black fly subgenera, endemic in the Oriental Region, is represented by four species, i.e., *S. (A.) furvum* Takaoka & Srisuka, *S. (A.) oblongum* Takaoka & Choochote and *S. (A.) wanchaii* Takaoka & Choochote, all described from Thailand (Takaoka & Choochote, 2005, 2006; Takaoka *et al.*, 2013), and *S. (A.) suchitrae* Takaoka from Nepal (Takaoka & Shrestha, 2010). The male, pupa and larva of *S. (A.) wanchaii* and the male of *S. (A.) suchitrae* are unknown.

The biting habit and medical and veterinary importance of the subgenus *Asiosimulium* remains to be investigated, though females of *S. (A.) wanchaii* were reported to be caught on a human attractant in Doi Pui National Park, Chiang Mai, Thailand (Takaoka & Choochote, 2006).

In a recent survey of larvae and pupae of black flies in Maewa National Park, Lampang

Province, Thailand, we collected pupae of the subgenus *Asiosimulium*, of which the gill is arborescent, consisting of 19 thread-like filaments, a number of filaments differing from those of three known species. The adult females reared from these pupae morphologically agreed with the original description of the female of *S. (A.) wanchaii* (Takaoka & Choochote, 2006).

We describe the male, pupa and mature larva of *S. (A.) wanchaii* for the first time based on reared males, pupae and mature larvae. Keys to identify all the four species of the subgenus *Asiosimulium* are given for females, males, pupae and mature larvae.

The methods of collection, description and illustration, and terms for morphological features used here follow those of Takaoka (2003). The specimens used are deposited at the Entomology Section, Queen Sirikit Botanic Garden, Chiang Mai, Thailand.

Male. Body length 2.6–2.9 mm. **Head.** Nearly as wide as thorax. Holoptic. Upper eye medium brown, consisting of large facets in

18 or 19 vertical columns and 19 or 20 horizontal rows. Clypeus brownish-black, moderately covered with dark brown long hairs interspersed with yellow fine hairs. Antenna composed of scape, pedicel and nine flagellomeres, dark brown except scape, pedicel and base of first flagellomere yellow; first flagellomere somewhat elongate, 1.67 times as long as second one. Maxillary palp composed of five segments, greyish-brown except third segment dark brown; proportional lengths of third, fourth, and fifth segments 1.0:1.1:1.7; third segment (Fig. 1A) of moderate size; sensory vesicle (Fig. 1A) ellipsoidal, 0.22 times as long as third segment, with large opening apically. **Thorax.** Scutum medium to dark brown except anterolateral calli ochreous, thinly gray pruinose, faintly with two dark narrow submedian longitudinal vittae, slightly shiny when illuminated at certain angles, and densely covered with golden-yellow short hairs and with several light brown upright long hairs on prescutellar area. Scutellum dark brown, with light brown upright long hairs and golden-yellow short hairs. Postnotum medium brown, slightly shiny when illuminated at certain angles, and bare. Pleural membrane bare. Katepisternum longer than deep, dark brown, gray pruinose, shiny when illuminated at certain angles and bare. **Legs.** Entirely medium to dark brown except base of posterior surface of mid trochanter, posterior surface of hind trochanter, and extreme bases of all tibiae yellow. Fore basitarsus 7.5 times as long as its greatest width. Hind basitarsus (Fig. 1B) enlarged, spindle-shaped, 4.05 times as long as its greatest width, 0.94 and 0.88 times as wide as greatest widths of hind tibia and femur, respectively; calcipala well developed, slightly shorter than its width at base, and 0.25 times as wide as greatest width of basitarsus; pedisulcus weakly developed. **Wing.** Length 2.1–2.2 mm. Costa with dark short spines as well as dark hairs. Subcosta bare. Hair tuft on base of radial vein dark. Basal portion of radius fully haired. R1 with dark spinules and hairs. R2 with dark hairs only. Basal cell and basal median cell absent. **Abdomen.** Basal scale brownish-black, with

fringe of light brown long hairs. Dorsal surface of abdomen dark brown to brownish-black, moderately covered with dark hairs; tergite 2 with pair of dorsolateral shiny patches when illuminated at certain angles; other tergites without such shiny patches; ventral surface of abdominal segments 3–8 somewhat shiny when illuminated at certain angles. Abdominal segment 9 in ventral view (Fig. 1C) with sternite in form of transverse thick bar. **Genitalia.** Coxite in ventral view (Fig. 1C) rectangular, 2.2 times as long as its greatest width. Style in ventral view (Fig. 1C) short, 0.66 times as long as coxite, gently tapered and with apical spine (right style in one male with two apical spines); style in ventrolateral view (Fig. 1D) wide basally, 0.55 times as wide as long, tapered toward apex; style in medial view (Fig. 1E) gently curved inward and tapered toward apex; style in caudal view (Fig. 1F) twisted inward and tapered toward pointed apex. Ventral plate in ventral view (Fig. 1C) with body wide, much wider than long, somewhat rounded laterally, with anterior margin slightly produced medially, with posterior margin slightly rounded posteriorly (or nearly straight and slightly concave medially when ventral plate somewhat tilted), with median portion produced ventrally, and densely covered with setae except both lateral portions bare; basal arms short, directed anteriorly; ventral plate in lateral view (Fig. 1G) moderately produced ventrally; ventral plate in caudal view (Fig. 1H) inverted-V shaped, produced ventrally, and covered with setae on median portion of posterior surface. Median sclerite (Fig. 2G, I) thin, plate-like, with several sutures on apical portion. Paramere (Fig. 1J) of moderate size, well sclerotized, without hook. Aedeagal membrane (Fig. 1J) with minute setae; dorsal plate not developed. Ventral surface of tenth abdominal segment with four or five hairs laterally near cercus on each side. Cercus (Fig. 1K, L) in form of narrow lobe, covered with five to seven hairs.

Pupa. Body length 3.0–3.5 mm. **Head.** Integument dark yellow, moderately covered with round tubercles; frons with two unbranched long trichomes (Fig. 2A) on each side; face with one unbranched long trichome

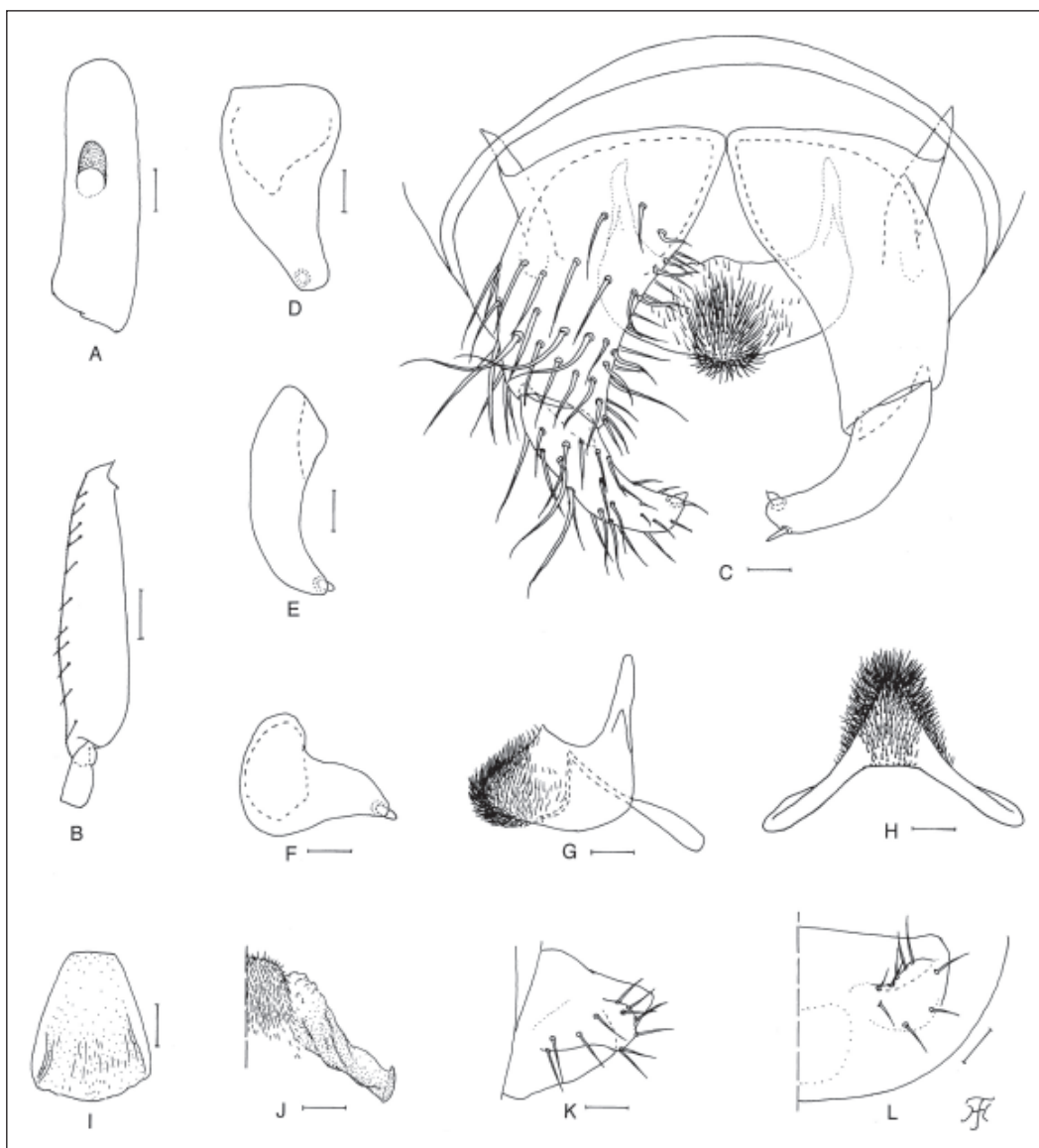


Figure 1. Male of *S. (A.) wanchaii*. A, third segment of maxillary palp with sensory vesicle bearing a large opening (front view; left side); B, basitarsus and second tarsomere of hind leg (left side; outer view); C, sternite of abdominal segment 9, coxites, styles and ventral plate (ventral view); D–F, right styles (D, ventrolateral view; E, medial view; F, caudal view); G, ventral plate with median sclerite (lateral view); H, ventral plate (caudal view); I, median sclerite (caudal view); J, paramere and aedeagal membrane (left half; caudal view). (J); K and L, tenth abdominal segments and cerci (K, right side; lateral view; L, left side; caudal view). Scale bars = 0.1 mm for B; 0.02 mm for A and C–L.

(Fig. 2B) on each side; antennal sheath without any projection or tubercles. **Thorax.** Integument dark yellow, moderately covered with tubercles; thorax on each side with two long mediodorsal trichomes (Fig. 2C), two anterolateral trichomes (one long, one

medium-long) (Fig. 2D), one medium-long mediolateral trichome (Fig. 2E) and three ventrolateral trichomes (one medium-long, two short) (Fig. 2F); all trichomes unbranched. Gill (Fig. 2G) of arborescent type, composed of 19 short to medium-long

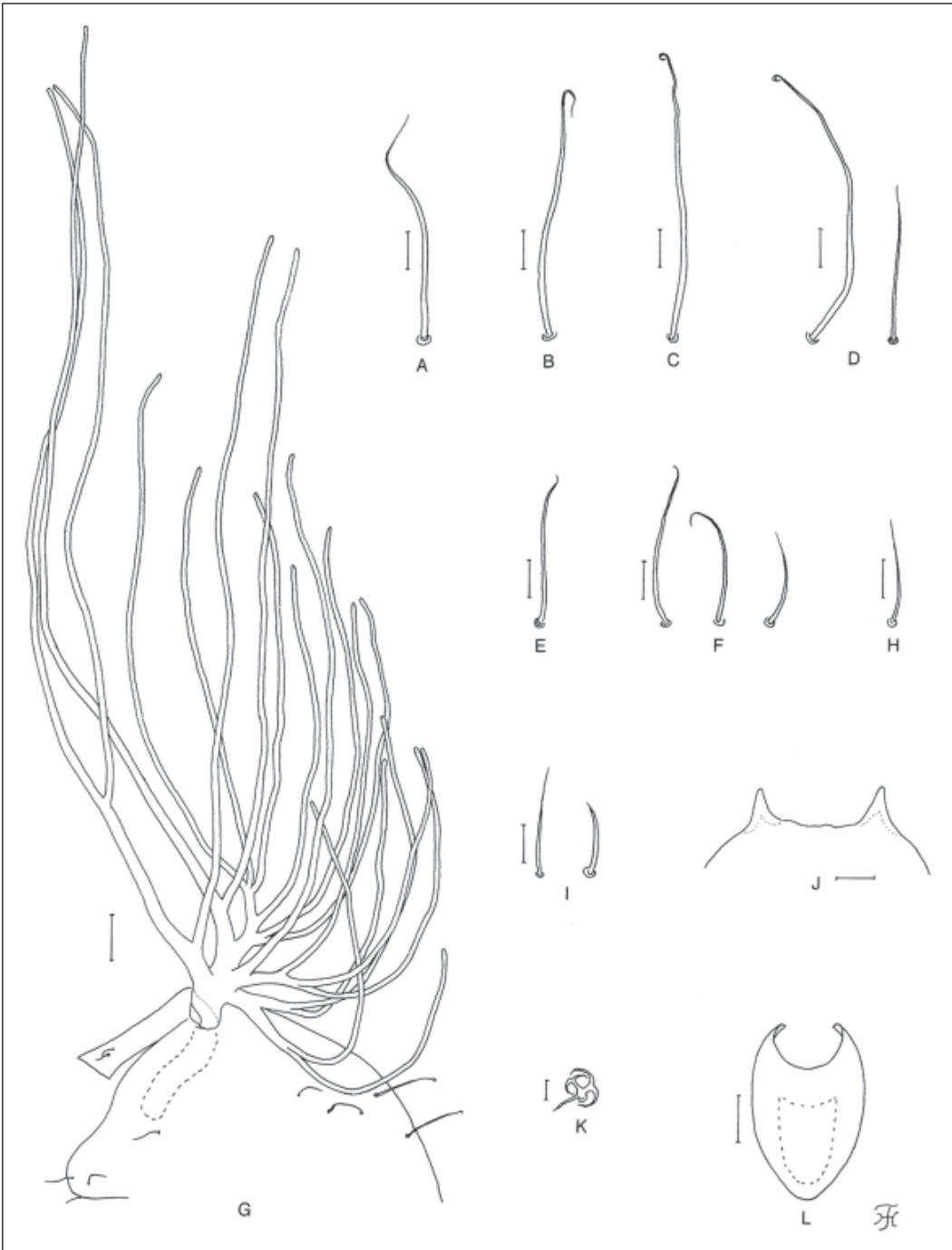


Figure 2. Pupa of *S. (A.) wanchaii*. A, frontal trichome; B, facial trichome, C–F, thoracic trichomes (C, mediodorsal; D, anterolateral; E, mediolateral; F, ventrolateral); G, gill filaments (left side; outer view); H, hair-like slender seta on dorsal surface of abdominal segment 1; I, hair-like slender seta and spinous seta on dorsal surface of abdominal segment 2; J, terminal hooks (caudal view); K, grapnel-shaped hooklet; L, cocoon (dorsal view). Scale bars = 1.0 mm for L; 0.1 mm for G; 0.02 mm for A, B, C–F and H–J; 0.01 mm for K.

slender thread-like filaments arranged from dorsal to ventral as four groups, i.e., dorsal, middle outer, middle inner, and ventral groups; dorsal group composed of four filaments always arranged as 2+2 filaments, middle outer group composed of four filaments arranged as (2+1)+1 filaments (or 2+2 filaments in left gill of one pupa, or 2+1 filaments lacking one filament in right gill of another pupa making a total number of filaments 18), middle inner group composed of six filaments in dorsal subgroup and two paired filaments in ventral subgroup, six filaments in dorsal subgroup variously arranged, e.g., (2+1)+3 filaments, or (1+2)+(1+2) filaments, or [(1+1)+1]+(1+2) filaments, or (1+2+1)+2 filaments, 2+4 filaments, or 4+2 filaments, or 3+(1+2) filaments, or 2+2 filaments in right gill of one pupa lacking two filaments making a total number of filaments 17 in right gill of one pupa), ventral group with three filaments always arranged as 1+2 filaments; all filaments light to medium brown, variable in length ranging from 0.8 mm to 2.6 mm, with three filaments of ventral group and two filaments of ventral subgroup of middle inner group relatively longer than other filaments, and with annular ridges and furrows and densely covered with minute tubercles; basal fenestra at base of gill of normal size. **Abdomen.** Dorsally, all segments unpigmented except segments 1, 2 and 9 yellowish; segment 1 without tubercles, with one short slender seta (Fig. 2H) on each side; segment 2, without tubercles, and with one short slender seta, five short spinous setae (Fig. 2I) on each side; segments 3 and 4 each with four stout hooks and one or two short spinous setae on each side; segments 6–9 each with comb-like groups of minute spines, and without spine-combs on each side; segment 9 with pair of small cone-shaped terminal hooks (Fig. 2J). Laterally, segments 2–4 each with three short spinous setae on each side; segment 9 with two grapnel-shaped hooklets (Fig. 2K) on each side in male pupae. Ventrally, all segments unpigmented except segment 9 yellowish; segment 4 with pair of unbranched dark hooklets and few short setae on each side; segments 5–7 each with pair of

unbranched stout dark hooks and few short setae on each side; segments 4–8 each with comb-like groups of minute spines on each side. **Cocoon** (Fig. 2L). Wall-pocket shaped, moderately woven, with anterodorsal margin somewhat thickly woven, and slightly extended ventrolaterally: individual threads invisible; 4.0–5.0 mm long by 2.0–3.4 mm wide.

Mature larva. Body length 5.0–6.2 mm. Body grayish. Cephalic apotome whitish on anterior half, yellowish on posterior half except narrow area along posterior margin dark brown; head spots distinct, medium to dark brown except anterior spot of posterolateral spots on each side light brown; posterior spot of posterolateral spots on each side merged in medium brown background and posterior spot of mediolongitudinal spots also connected posteriorly to medium brown area along posterior margin. Lateral surface of head capsule yellow except area above eye-spot region light brown, with light to medium brown spots, i.e., two large spots and two small ones in front of posterior margin and two isolated small spots below eye-spot region. Ventral surface of head capsule (Fig. 3C) yellow except median portions on each side of postgenal cleft and posterior portions along posterior margin light to medium brown, with elongate spot on each side of postgenal cleft merged into dark background. Cervical sclerites composed of two yellow small elliptical pieces, not fused to occiput, widely separated medially from each other. Antenna consisting of three segments and apical sensillum, nearly as long as stem of labral fan; proportional lengths of first, second, and third segments 1.00:1.15–1.19:0.96–1.00. Labral fan with 38–40 main rays. Mandible (Fig. 3A) with mandibular serration consisting of two teeth (one large and one small); large tooth at right angle to mandible on apical side; comb-teeth composed of three teeth decreasing in size from first to third; one or two supernumerary serrations present. Hypostoma (Fig. 3B) with nine apical teeth in row; median tooth most prominent, followed by corner teeth; lateral serrations weakly developed anteriorly; seven to nine hypostomal bristles per side,

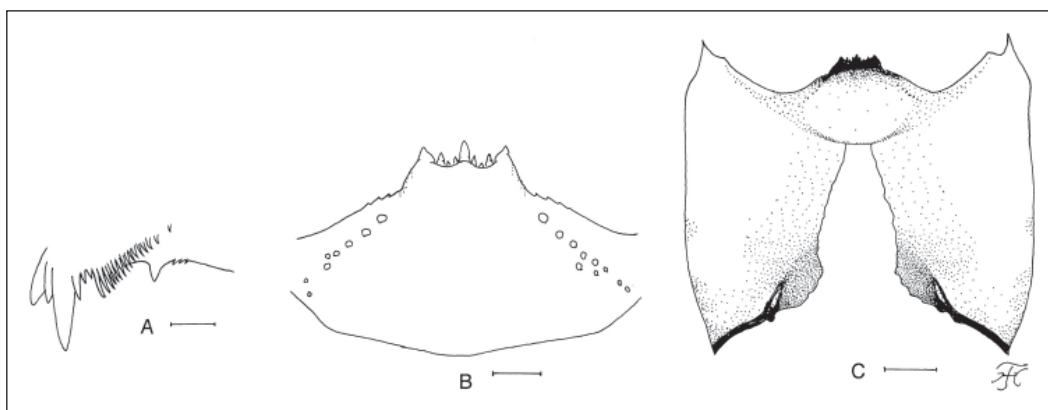


Figure 3. Mature larva of *S. (A.) wanchaii*. A, apical portion of mandible; B, hypostoma; C, head capsule showing postgenal cleft (ventral view). Scale bars = 0.1 mm for C; 0.04 mm for B; 0.02 mm for A.

lying slightly divergent posteriorly from lateral margin. Postgenal cleft (Fig. 3C) deep, reaching posterior margin of hypostoma; subesophageal ganglion unpigmented. Thoracic and abdominal cuticle bare except both sides of anal sclerite moderately covered with simple colorless setae. Rectal scales weakly developed, unpigmented. Rectal organ compound (number of secondary lobules per lobe was uncountable because all rectal organs were detracted). Anal sclerite X-shaped, with anterior arms nearly as long as posterior ones; anterior arms broadened, and space between arms widely sclerotized basally; six sensilla just posterior to basal juncture; accessory sclerite absent. Last abdominal segment somewhat expanded ventrolaterally forming bulge, ventral tip of which is visible as small ventral papilla when viewed from side. Posterior circlet with 80 or 81 rows of up to 14–16 hooklets per row.

Materials examined. Five females, five males (all adults reared from pupae and preserved with associated pupal exuviae and cocoons in 80% ethanol) and three mature larvae (QSBG-2012-249), THAILAND: Maewa National Park, Thoen District, Lampang Province, 23-IX-2012, Wichai Srisuka.

Distribution. Thailand.

Biology. The biting habit of females of *S. (A.) wanchaii* is unknown. The pupae and larvae of this species were collected from

grasses trailing in the water of a small stream slowly flowing on rocks (water temperature 27.6 °C, exposed to sun, altitude 468 m) (17°37'19.7" N, 99°18'49.0" E). Associated species was *S. (A.) furvum*.

Remarks. The male of *S. (A.) wanchaii* is distinguished from that of *S. (A.) oblongum* by the sensory vesicle with a large opening (Fig. 1A) and the enlarged spindle-shaped hind basitarsus (Fig. 1B), the ventral plate much wider than long (Fig. 1C), and from that of *S. (A.) furvum* by the number of upper-eye facets in 18 or 19 vertical columns and 19 or 20 horizontal rows, and the ventral plate with arms not diverged (Fig. 1C).

The pupa of *S. (A.) wanchaii* is readily distinguished from the three other related species by the number of gill filaments, i.e., 19 filaments in *S. (A.) wanchaii* versus 31–33 in *S. (A.) oblongum*, 28 in *S. (A.) suchitrae* and 22 in *S. (A.) furvum*. The pupa of *S. (A.) wanchaii* is also distinguished from that of *S. (A.) suchitrae* by the frons and thorax covered with tubercles and the absence of the bulbous basal fenestra at the base of the gill.

The larva of *S. (A.) wanchaii* is similar to those of *S. (A.) oblongum* and *S. (A.) suchitrae* but is distinguished from *S. (A.) furvum* by lacking a pigmented subesophageal ganglion.

Keys to identify four species of the subgenus *Asiosimulium*

Females

1. Cercus much elongated, about twice as long as its basal width.... *S. (A.) oblongum*
Cercus normal or somewhat elongated, less than 1.3 times as long as its basal width..... 2
2. Spermatheca globular.... *S. (A.) wanchaii*
Spermatheca pear-shaped..... 3
3. Genital fork with stout projection directed anteriorly *S. (A.) furvum*
Genital fork without projection directed anteriorly..... *S. (A.) suchitrae*

Males*

1. Hind basitarsus slightly widened, 0.75 times as wide as hind tibia.....
S. (A.) oblongum
Hind basitarsus much widened, 0.94–0.96 times as wide as hind tibia..... 2
2. Upper-eye facets in 16 vertical columns and 17 horizontal rows; ventral plate with arms widely diverged..... *S. (A.) furvum*
Upper-eye facets in 18 or 19 vertical columns and 19 or 20 horizontal rows; ventral plate with arms not diverged.....
S. (A.) wanchaii

*The male of *S. (A.) suchitrae* is unknown.

Pupae

1. Gill with enlarged basal fenestra; head and thoracic integument bare.....
S. (A.) suchitrae
Gill without enlarged basal fenestra; head and thoracic integument with tubercles 2
2. Gill with 31–33 filaments.....
S. (A.) oblongum
Gill with 19 or 22 filaments..... 3
3. Gill with 19 filaments..... *S. (A.) wanchaii*
Gill with 22 filaments..... *S. (A.) furvum*

Larvae

1. Postgenal cleft with pigmented subesophageal ganglion..... *S. (A.) furvum*
Postgenal cleft without pigmented subesophageal ganglion..... 2

2. Labral fan with 30 main rays.....
..... *S. (A.) suchitrae*
Labral fan with 38-45 main rays..... 3
3. Labral fan with 38-40 main rays; mandible with supernumerary serrations.....
..... *S. (A.) wanchaii*
Labral fan with 43-45 main rays; mandible without supernumerary serrations.....
..... *S. (A.) oblongum*

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REFERENCES

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

Takaoka, H. & Choochote, W. (2005). A new subgenus and a new species of *Simulium* s. l. (Diptera: Simuliidae) from Thailand. *Medical Entomology and Zoology* **56**: 33-41.

Takaoka, H. & Choochote, W. (2006). A new species of the subgenus *Simulium* (*Asiosimulium*) (Diptera: Simuliidae) from Thailand. *Medical Entomology and Zoology* **57**: 45-48.

Takaoka, H. & Shrestha, S. (2010). New species of black flies (Diptera: Simuliidae) from Nepal. *Zootaxa* **2731**: 1-62.

Takaoka, H., Srisuka, W., Saeung, A. & Choochote, W. (2013). *Simulium* (*Asiosimulium*) *furvum*, a new species of black fly (Diptera: Simuliidae) from Thailand. *Journal of Medical Entomology* **50**: 493-500.