

A new black fly species of subgenus *Gomphostilbia* Enderlein, 1921 (Diptera, Simuliidae) from Eastern Ghats, India

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ABSTRACT: *Simulium* (*Gomphostilbia*) *alagarensis* **sp. nov.**, was described based on adults (female and male), pupae, and larvae. This new species is placed in the *S. batoense* species group of the subgenus *Gomphostilbia* Enderlein by having an antenna with nine flagellomeres, pleural membrane bare, female subcostal bare, pupal gill composed of 8 slender threat-like filaments in the pupa, hypostome bristles per side lying parallel to lateral margin in the larvae. It is morphologically similar to *S. (G.) kumbakkaraiensis* recently described in Tamil Nadu, south India (Anbalagan *et al.*, 2019).

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KEY WORDS: Lotic dipterans, Karanthamalai, *S. batoense*

INTRODUCTION

Black flies (Family: Simuliidae) are significant both medically and veterinary-wise. Black flies spread many diseases, but the most common is onchocerciasis, or river blindness, by the parasitic worm *Onchocerca volvulus* (WHO, 2022; Gyasi *et al.*, 2023). Blindness, skin sores, and excruciating itching can all result from this illness. Black flies can cause distress to livestock through their persistent biting, leading to reduced feeding and weight gain, lower milk production, and overall stress. Leucocytozoonosis is a disease caused by protozoan parasites of the genus *Leucocytozoon* (Piratae *et al.*, 2021). These parasites infect birds and are transmitted by black flies (Simuliidae) and

biting midges (Ceratopogonidae). Globally, 2415 black fly species were recorded (Adler, 2025), and 87 species (74 named and 13 unnamed) were found in India (Vijayan and Anbalagan, 2023). Among these 87 species belong to six subgenera in the genus *Simulium* Latreille (Adler, 2024): *Eusimulium* (one species named and one unnamed), *Gomphostilbia* (20 named and 2 unnamed species), *Montisimulium* (four named and one unnamed species), *Nevermannia* (eight named and two unnamed species), *Simulium* (40 named and seven unnamed species) and *Wilhelmia* (one species). Twenty-two species in *Gomphostilbia* in India were recorded under three species groups: *S. (G.) batoense* (13 species), *S. (G.) ceylonicum* (one species), *S. (G.) darjeelingensis* (one species), *S. (G.) epistum*

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(three species), *S. (G.) gombakense* (two species) and *S. (G.) gomphostilbia* (two unplaced species). These species were assigned to the *S. batoense* species group of the subgenus *Gomphostilbia*. This study explores black flies in the Alagar hills of Eastern Ghats, south India. Among these specimens, a novel species of the genus *Gomphostilbia* Enderlein was found. Therefore, a description of this new species was provided.

MATERIALS AND METHODS

Larvae and pupae were collected manually with a fine brush and forceps from stream substrates (leaf litter, boulders, pebbles, and submerged human waste materials like polythene, snack covers, and cloths). Sterilized water was used for rearing to prevent introducing fungal spores into the system. Collected larvae and those not used for rearing pupae were preserved separately in the field in 70 per cent ethanol. The pupae were separated and placed on wet filter paper in a small plastic container for rearing. They were kept in an incubator adjusted to be similar to their habitat (21–27°C) for one or two days until adult emergence. Maintained regular inspection and adequate ventilation in the rearing area to prevent stagnant air and replaced rearing substrates to prevent the accumulation of organic matter that can promote fungal growth. Employed disinfectants, including 70 per cent ethanol, to sterilize all containers, instruments, and surfaces beforehand. This practice is effective in reducing microbial load and preventing fungal outbreaks (Hunter *et al.*, 1994; Jonusaite and Buda, 2002; Anbalagan *et al.*, 2011; Vijayan *et al.*, 2017; Vasiliev and Aibulatov, 2023). Morphological characteristics of larvae, pupae, and adults were examined under a compound and stereo microscope. Dissected parts (heads, thoraxes, abdomens, legs, and wings) were cleared with lactic acid (85%) and transferred to glycerin for mounting. Permanent slides were made using a small drop of Canada balsam and were kept for two weeks without disturbing them to become dry. Clear nail polish was applied to the edges of the dried slides to be sealed. The mounting slides were examined under a Leica® DM300 LED compound microscope. Morphology characters were

measured using a micrometer. Morphology characters were manually illustrated using a camera lucida attached to a Leica® MZ16 stereo microscope. Description, illustrations, and terms for morphological features were according to Takaoka (2003), Adler *et al.* (2004), Vijayan *et al.* (2019) and Anbalagan *et al.* (2020a). Dichotomous keys (Takaoka, 2012; Takaoka *et al.*, 2019; Pramual *et al.*, 2022) were used for the identification of specimens. Emerging specimens were stored in ethanol at “20°C, and all type specimens were deposited at the Department of Animal Health and Management, Science Campus, Alagappa University, Karaikudi, Tamil Nadu, India (DAHMA).

RESULTS

Simulium (Gomphostilbia) alagarensis Vijayan & Anbalagan sp. nov. (Figs. 1–3)

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Diagnosis. Female adult: the only species of the *S. batoense* species group with antenna nine flagellomeres, pleural membrane bare, subcosta bare, and tibia light to dark brown with basal 1/4 white, hind basitarsus narrow and nearly parallel-sided, 6.1 times as long as wide, and 0.58 and 0.63 times as wide as greatest width of tibia and femur.

Female (n = 1). Body length 2.5–2.6 mm. **Head.** Nearly as wide as the thorax. Frons grey, shiny, moderately covered with yellowish-white scale-like recumbent short hairs; frontal ratio 1.9:1.0:2.9; head ratio 1.0:5.9. Fronto-ocular area well developed, narrow, directed dorsolaterally. Clypeus brownish-black, densely covered with yellowish-white recumbent hairs interspersed with a few to several dark longer hairs on each side. Labrum 0.7 times as long as clypeus. Antenna composed of scape, pedicel, and nine flagellomeres, grey to white except scape, pedicel, and base of a first flagellomere dark yellow, proportional length of first and second flagellomeres 1.0:0.7. Maxillary palp composed of five palpomeres, dark brown, proportional length of third, fourth, and fifth palpomeres 1.0:1.1:2.3; third palpomere of moderate

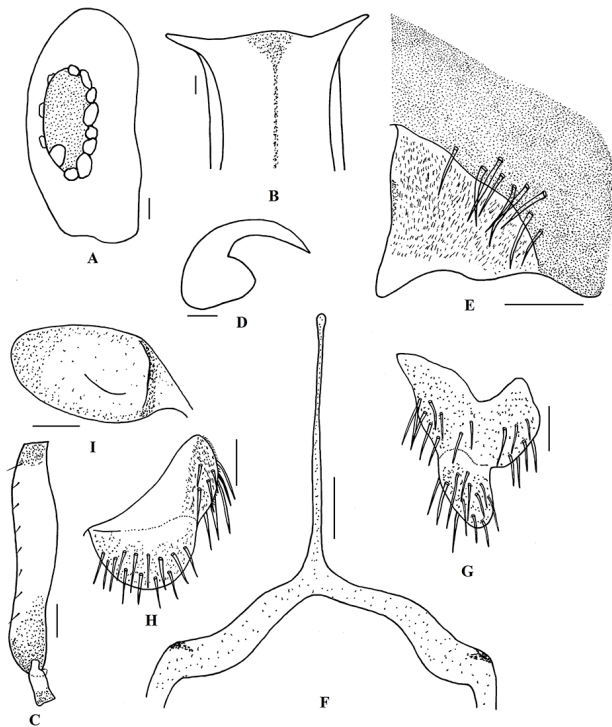


Fig. 1 Female of *Simulium* (*Gomphostilbia*) *alagarensis* sp. nov. A, 3rd segment of right maxillary palp showing sensory vesicle (frontal view); B, cibarium (frontal view); C, basitarsus and 2nd tarsomere of left hind leg showing calcipala and pedisulcus (frontal view); D, tarsal claw; E, 8th sternite, ovipositor valve (ventral view); F, genital fork (ventral view); G & H, right paraprocts and cercus (G, ventral view; H, lateral view); I, spermatheca (lateral view). Scale bars. 0.01mm for B; 0.02mm for A, E and F; 0.05mm for G–I; 0.1mm for C

size (Fig. 1A); sensory vesicle ellipsoidal, long, 0.6 times length of third palpomere and with a medium-sized opening. Maxillary lacinia with 11 inner and 15 outer teeth. Mandible with 35 inner and 11 outer teeth. Cibarium (Fig. 1B) medially forming sclerotized plate folded forward from the posterior margin, with a strongly sclerotized medial longitudinal ridge with a well-sclerotized cup-like apex. **Thorax.** Scutum dark brown except anterolateral calli dark ochreous, with 3 brownish-black longitudinal vittae (1 narrow median and 2 lateral), median vitta united anteriorly to anterior calli, lateral vittae united posteriorly to prescutellar area; scutum shiny when illuminated at certain angles, moderately covered with yellow scale-like recumbent short hairs interspersed with dark brown

long upright hairs on prescutellar area. The scutellum is shiny, with dark brown short hairs. Postnotum brownish-yellow and bare. Pleural membrane bare. Katepisternum brownish-black to black, longer than deep, shiny when illuminated at certain angles, moderately covered with fine short hairs. **Legs.** Foreleg: coxa yellow; trochanter yellow except apical portion somewhat darkened; femur light brown; tibia light brown; tarsus brownish black, with moderate dorsal hair crest; basitarsus slightly dilated, 5.3 times as long as its greatest width. Midleg: coxa yellowish brown except posterior surface dark brown; trochanter yellow to light brown; femur and tibia brown; tarsus brownish-black except basal 1/2 of basitarsus dark yellow. Hind leg: coxa yellowish brown; trochanter yellow; femur medium brown with base yellow and apical cap dark brown; tibia light to dark brown with basal 1/4 white, covered with brownish fine hairs on outer and posterior surfaces; tarsus brownish-black except basal 2/3 of basitarsus (though base light brown); hind basitarsus (Fig. 1C) narrow, nearly parallel-sided, 6.1 times as long as wide, and 0.58 and 0.63 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 1C) slightly shorter than width at base, and 0.57 times as wide as greatest width of basitarsus. Pedisulcus (Fig. 1C) well defined. Claw (Fig. 1D) with large basal tooth 0.4 times as long as claw. **Wing.** Length 1.9 mm. Costa with dark brown spinules and light brown hairs. Hair tuft on a base of radial vein dark brown. The basal portion of the radius was fully haired. Basal cells absent. Halter. Clear white except for the margin and basal stem darkened. **Abdomen.** Basal scale dark yellow, with a fringe of yellowish-white hairs. Dorsal surface of the abdomen is medium to dark brown except for segment 2 light brown though middle portion of the tergal plate is ochreous, and moderately covered with dark short to long hairs; tergites of segments 2 and 6–9 is shiny when illuminated at certain angles. The ventral surface of segments 2–4 is creamy, and those of other segments are light to dark brown; sternal plate on segment 7 is undeveloped. **Genitalia.** Sternite 8 (Fig. 1E) is well sclerotized and bare medially, covered with 7–9 long hairs on each side. Ovipositor valves are tongue-like, thin, membranous, and moderately covered with

microsetae; inner margins are convex, somewhat sclerotized, and slightly separated from each other. Genital fork (Fig. 1F) of usual inverted-Y forms; stem slender and well sclerotized; arms of moderate width, lateral plate of each arm with thin lobe directed posteromedially and small stout projection directed anterodorsally. Paraproct in ventral view concave anterolaterally, with 3 sensilla on an anteromedial surface; paraproct in lateral view (Fig. 1G) much produced ventrally, 0.54 times as long as wide, with 3–5 medium-long too long hairs on ventral and lateral surfaces. Cercus in lateral view (Fig. 1H) is short, rounded posteriorly, 0.55 times as long as wide. Spermatheca (Fig. 1I) ellipsoidal, 1.8 times as long as its greatest width, well sclerotized except duct and a small area near the juncture with duct unsclerotized, and with a hexagonal pattern on the surface; internal setae absent; both accessory ducts slender, subequal in diameter to major one.

Male ($n = 1$). Body length 3.2 mm. **Head**, somewhat wider than thorax. Upper eye yellowish brown, consisting of 13 vertical columns and 13 horizontal rows of large facets. Face brownish black, grayish-white pruinose. Clypeus brownish black, whitish pruinose, densely covered with golden-yellow scale-like medium-long hairs (directed upward and lateral) interspersed with several dark brown simple longer hairs. Antenna 1st flagellomere elongates, 1.7 times as long as 2nd one. Maxillary palp light to medium brown, proportional lengths of 3rd, 4th, and 5th segments 1.0:1.1:2.1; 3rd segment (Fig. 2A) widened apically; sensory vesicle (Fig. 2A) ellipsoidal, small (0.25 times as long as 3rd segment), and with medium-sized opening. **Thorax**. Scutum is slightly darker than female and short hairs on the scutum is golden yellow. **Legs**. Foreleg: coxa yellow; trochanter yellow with some portions light brown; femur light brown except apical cap brown; tibia brown with median 2/3 light brown and covered with dark brown hairs; tarsus brown to dark brown; basitarsus moderately dilated 6.6 times as long as its greatest width. Midleg: coxa yellowish brown; trochanter yellow to brown; femur yellow except apical 1/4 brown; tibia medium brown to dark brown; tarsus dark brown to brownish-black except the anterior



Fig. 2 Male of *Simulium* (*Gomphostilbia*) *alagarensis* sp. nov. A, 3rd segment of left maxillary palp showing small sensory vesicle (frontal view); B, basitarsus and 2nd tarsomere of left hind leg showing calcipala and pedisulcus (frontal view); C, coxites, styles, ventral plate and median sclerite (ventral view); D, left style (frontal view); E, ventral plate and median sclerite (lateral view); F, ventral plate (end view); G, left paramere and aedeagal membrane (hind view); H, 10th abdominal segment and cercus (right side and lateral view). Scale bars. 0.02mm for A, G and H; 0.05mm for C–F; 0.1mm for B

surface of little less than basal 1/2 of basitarsus dark yellow to light brown. Hindleg: coxa dark yellow to brown; trochanter yellow; femur light brown except apical 1/2 dark brown; tibia brown except basal and apical portion dark brown; tarsus medium to dark brown except basal 1/2 (or little less) of basitarsus whitish-yellow and little less than basal 1/3 of 2nd tarsomere white; basitarsus (Fig. 2B) slender, spindle-shaped, 4.7 times as long as wide, and 0.6 and 0.7 times as wide as greatest width of tibia and femur, respectively; calcipala (Fig. 2B) nearly as long as wide, and 0.39 times as wide as greatest width of basitarsus. Pedisulcus (Fig. 2B) well defined. **Wing**. Length 1.9 mm. Costa with dark brown spinules as well as dark brown hairs

except the basal portion with a patch of yellowish hairs. Subcosta bare. Hair tuft on stem vein dark brown. Basal portion of radius fully haired; R^1 with dark spinules and hairs and R^2 with hairs only. Basal cells absent. Halter. Yellowish brown except outer surface ochreous, basal stem darkened, and apex white. **Abdomen.** Basal scale dark brown, with a fringe of light to medium brown hairs. Dorsal surface of abdomen is dark brown except for segment 2 light brown (though posterior 1/4 of dorsal surface brown), covered with dark brown short to long hairs; segments 2, 5–7 with shiny dorsolateral or lateral patches; ventral surface of segment 2 yellow, those of segments 3 and 4 yellow except sternites medium brown, and those of other segments medium to dark brown. **Terminalia.** Coxite in ventral view (Fig. 2C) is nearly rectangular, 1.7 times as long as its greatest width. Gonostyle in ventral view slightly bent inward, slightly tapered from base to apex, sharpened apically and with apical spine; style in medial view (Fig. 2D) longer than coxite (0.71 times as long as coxite), gently bent inward, nearly parallel-sided, with apical spine; style in ventrolateral view very slightly tapered toward apical 3/4, with rounded apex. Ventral plate in ventral view (Fig. 2C) with body transverse, 0.81 times as long as wide, tapered posteriorly, with anterior margin produced antero-medially, and posterior margin concave medially, densely covered with microsetae on ventral surface; basal arms of long, directed forward, then convergent apically; ventral plate in lateral view (Fig. 2E) moderately produced ventrally; ventral plate in end view (Fig. 2F) triangular dorsally, without microsetae on posterior surface. Median sclerite is thin, plate-like, wide. Paramere (Fig. 2G) of moderate size, with 3 distinct long and stout hooks and several smaller ones. Aedeagal membrane is moderately setose and slightly sclerotized at the base but the dorsal plate is not well defined. Ventral surface of abdominal segment 10 without distinct hairs near posterior margin. Cercus in lateral view (Fig. 2H) is small, and rounded, with 8–10 hairs.

Pupa (n = 4). Body length 3.1–3.2 mm. **Head.** Integument dark yellow, moderately covered with small round tubercles; antennal sheath with protuberance; face with pair of simple very long

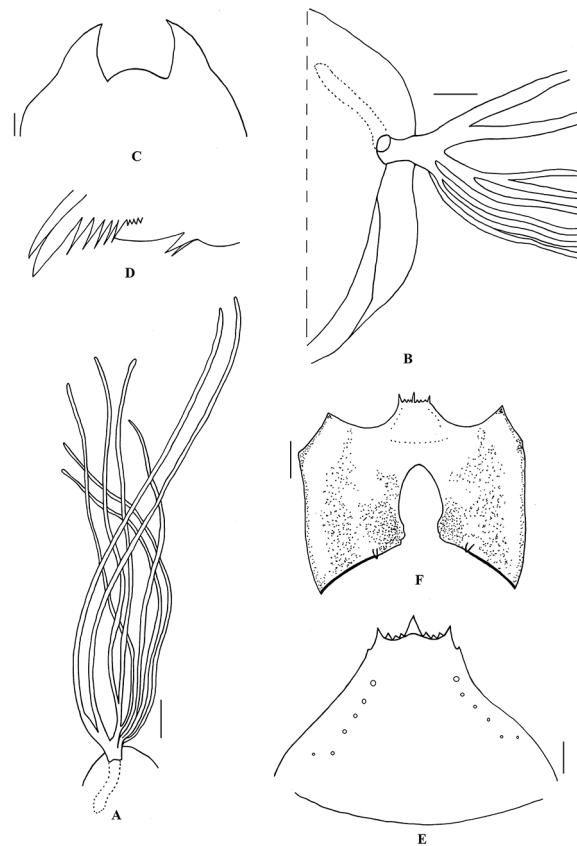


Fig. 3 Pupa and larva of *Simulium* (*Gomphostilbia*) *alagarensis* sp. nov. A–C, pupa; D–F, larva. A, left gill filaments (lateral view); B, basal portion gill (outer view); C, terminal hooks (hind view); D, right mandible (lateral view); E, hypostoma (ventral view); F, head capsule showing postgenal cleft and hypostoma (ventral view). Scale bars. 0.01mm for D; 0.02mm for E; 0.05mm for C; 0.1mm for A, B and F

trichomes with coiled apices, and frons with 3 pairs of simple very long trichomes with coiled apices; 2 frontal trichomes on each side arising close together, subequal in length to one another and slightly longer than facial one. **Thorax.** Integument yellow, covered with round tubercles, with 3 simples very long dorsomedial trichomes with coiled apices, 1 simple very long anterolateral trichome with coiled apices, 1 simple very long mediolateral trichome with uncoiled apex, and 3 simples ventrolateral trichome with uncoiled apices (1 long and 2 short) on each side. Gill (Fig. 3A) composed of 8 slender thread-like filaments, arranged as $[(1 + 2) + (1 + 2)] + 2$ filaments from dorsal to ventral, with medium-long common basal stalk having somewhat

swollen transparent organ ventrally at base (Fig. 3B); common basal stalk 0.5 times length of interspiracular trunk; both primary and secondary stalks of dorsal triplet short, primary stalk of middle triplet medium-long, but secondary stalk short; length of primary and secondary stalks of middle triplet combined slightly longer than stalk of ventral pair; stalk of ventral pair short, 0.9–1.0 times length of common basal stalk and 0.55–0.6 times length of interspiracular trunk; stalk of ventral pair 1.5–1.52 times as thick as primary stalk of middle triplet, and 1.7–1.8 times as thick as primary stalk of dorsal triplet; primary stalk of dorsal triplet lying against stalk of ventral pair at angle of 40–60 degrees or little more when viewed laterally; all filaments yellowish brown, gradually tapered toward apex; entire length of filaments (measured from base of gill to tips of filaments) based on one pupa as follows: 1.5–1.6 mm for dorsal triplet, 2.0–2.1 mm for middle triplet and 2.7–2.8 mm for ventral paired filaments. Abdomen. Segment 9 with a pair of triangular flat terminal hooks, of which the outer margin is slightly longer than the inner margin and not crenulated (Fig. 3C). **Cocoon** ($n = 8$). 3.3–3.5 mm long by 3.0–3.1 mm wide; wall pocket-shaped, thinly and moderately woven, widely extended ventrolaterally; anterior margin somewhat thickly woven, with the dorsal portion not or slightly produced anteriorly when viewed dorsally; posterior 1/2 with floor roughly or moderately woven.

Mature larva ($n = 8$). Body length 5.8–5.9 mm. Body creamy to colour markings as follows: thoracic segment 1 encircled with an ochreous broad transverse band (though disconnected ventrally), proleg greyish, thoracic segments 2 and 3 yellowish-grey dorsally and each with distinct ochreous wide areas ventrally, abdominal segments 1–4 each encircled with yellowish brown broadband, abdominal segments 5–8 almost entirely covered by yellowish brown transverse broadband on dorsal and dorsolateral surfaces; abdominal segments 5 and 6 each with W-shaped broad transverse greyish-brown band on dorsolateral surfaces of posterior 1/2 of each segment; abdominal segment 7 and 8 with a transverse yellowish-brown band on ventral surface; Cephalic apotome yellowish brown, and sparsely covered with simple minute setae;

head spots indistinct. Lateral surface of the head capsule is yellowish brown except the eye-spot region yellow, and very sparsely covered with simple minute setae; spots indistinct. Ventral surface of the head capsule is yellowish brown except a somewhat darkened area near the posterior margin on each side of postgenal cleft, and very sparsely covered with simple minute setae. Antenna composed of 3 segments and apical sensillum, 1.4 times longer than the stem of labral fan; proportional lengths of 1st, 2nd, and 3rd segments 1.00:1.2:1.4. Labral fan with 26–28 main rays. Mandible (Fig. 3D) with 3 comb-teeth decreasing in length from 1st to 3rd; mandibular serrations composed of 2 teeth (1 medium-sized and 1 small); major tooth at an acute angle against mandible on the apical side; supernumerary serrations absent. Hypostoma (Fig. 3E) with a row of 9 apical teeth; median and each corner tooth prominent (though median tooth slightly longer than corner teeth) and much longer than 3 intermediate teeth on each side; lateral margin smooth; 5 hypostomal bristles per side lying parallel to the lateral margin. Postgenal cleft (Fig. 3F) is arrowhead-shaped, 2.7 times as long as postgenal bridge. Cervical sclerite is composed of 2 very pale small pieces, not fused to occiput, moderately separated medially from each other. Anal sclerite of usual X-form, with anterior arms a little shorter (0.71 times as long as posterior arms) than posterior ones, broadly sclerotized at base; accessory sclerite absent. Posterior circlet with 68–70 rows of hooklets with up to 13–14 hooklets per row.

Type series. Holotype (in alcohol): Female (with associated pupal exuviae and cocoon, in 80% ethanol), collected from a small stream (width 1–1.5 m, depth 9 cm, water temperature 23.5°C, more than partially shaded, elevation 363 m, 10°17'23.51" N; 78°14'44.38" E), slow running in a natural forest, Ayyan odai, Malaiyur, Karanthamalai, Dindigul district, Tamil Nadu, India, 16-XII-2021, by S. Vijayan & S. Anbalagan, Paratypes: (in alcohol) One female, one male, eight pupae, thirteen mature larvae and twenty-one immature larvae, same data as those of the holotype.

Biological notes. Able to collect the larvae and pupae of this newly discovered species off the leaves. Associated simuliids were *S. (S.) striatum*,

and *S. (G.) sp. n1*.

Distribution. Ayyan odai, Ayyan aruvi, Uluppakkudi Falls, Nuburagangai, Periyathoppu stream, Sithan aruvi, and Mavoor stream.

Etymology. The species name *alagarensis* refers to the name of Alagar Hill, where this new species was collected.

Remarks. The following morphological characteristics of *Simulium (G.) alagarensis* sp. nov. place it in the subgenus *Gomphostilbia* in *Simulium*: haired katepisternum in the adults, abdominal segment 9 with pair of triangular flat terminal hooks in the pupa and smooth lateral margins of hypostoma in the larva. In addition, this new species is assigned to the *Simulium batoense* species group of the subgenus *Gomphostilbia* by having a bare pleural membrane, dark brown hair tuft on the base of the radius, dark tibiae of the female and male, slender male hind basitarsus, eight-gill filaments, and grapnel-like hooklets in the pupa (Takaoka, 2012).

Simulium (G.) alagarensis sp. nov., is morphologically similar to *S. (G.) kumbakkaraiensis* recently described from Tamil Nadu, South India (Anbalagan *et al.*, 2019) in sharing the following characteristics: labrum 0.7 times as long as clypeus, scutum with three brownish-black longitudinal vittae and tarsal claw with large basal tooth in the female, body length of male 3.2 mm, the respiratory gill with 8 slender thread-like filaments in the pupa and wall pocket-shaped cocoon and antenna proportional length of 1st, 2nd and 3rd segments 1.0: 1.2: 1.2 in the larva.

However, this new species is distinguished from *S. (G.) kumbakkaraiensis* by the following characters (those of *S. (G.) kumbakkaraiensis* in parentheses): in the female by the hind basitarsus 6.1 times as long as wide (5.3 times as long as wide) and sternite 8 covered with 9 long hairs on each side (10–13 long hairs on each side), in the male by the antenna 1st flagellomere elongates, 1.3 times as long as 2nd one (antenna 1st flagellomere elongates, 1.7 times as long as 2nd one) and fore basitarsus 6.6 times as long as its greatest width

(6.0 times as long as its greatest width), in the pupa by the common basal stalk 0.41 times length of interspiracular trunk (1.0 times length of interspiracular trunk) and in the larva by the postgenal cleft 2.7 as long as postgenal bridge (1.5 as long as postgenal bridge).

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