Simulium (Psilopelmia) virescens, a new black-fly species (Diptera: Simuliidae) from the southwestern region of the state of Bahia, Brazil

Neusa Hamada¹, Nayra Gomes da Silva¹, Eleny da Silva Pereira²

¹Coordenação de Biodiversidade, Instituto Nacional de Pesquisas da Amazônia, Manaus, AM, Brasil
²Curso de Pós-graduação em Biologia Animal, Universidade de Brasília, Brasília, DF, Brasil

The last-instar larva, pupa, male and female of Simulium virescens sp. nov. are described and illustrated. This species has a peculiar larva, which has an elongated head capsule and light-green colour. The first thoracic segment has tubercle on its dorsal region and the third thoracic segment has one pair of tubercles; the first to the fourth abdominal segments have one pair of tubercles on each segment. Until now this new species had only been collected at the type locality, which is on the middle stretch of the Correntina River in the southwestern portion of the state of Bahia, Brazil. Females were voraciously biting humans during the field work. This new species represents the second species of Simulium (Psilopelmia) in Brazil and the first registered outside of the Brazilian Amazon Region.

Key words: aquatic insects - neotropical region - agricultural region - São Francisco River basin

The subgenus Simulium (Psilopelmia) has a mostly neotropical distribution, although some species occur in the Nearctic region (Coscarón et al. 1996). The number of species included in this subgenus differs depending on the author [see Coscarón and Coscarón-Arias (2007) and Adler and Crosskey (2011)]. This difference occurs due to disagreement over which species belong to S. (Psilopelmia) and which to Simulium (Ectemnaspis), with several species groups (sensu Coscarón 1984) of the latter subgenus being included in S. (Psilopelmia) by Crosskey (1988) and Adler and Crosskey (2011).

We are following the classification scheme of Coscarón and Coscarón-Arias (2007) until a complete revision of both subgenera is done. In Brazil, only one species in this subgenus has been previously reported: Simulium iracouboense Floch and Abonnenc, 1946, which is widely distributed in the Amazon Region (Adler & Crosskey 2011). During an aquatic insect survey in the southwestern region of the state of Bahia (BA), Brazil, we collected specimens of an undescribed black-fly species with characteristics that allow us to include it in S. (Psilopelmia). In the present paper we describe the last-instar larva, pupa, female and male of Simulium virescens sp. nov. and present some information on the bionomics of the new species.

MATERIALS AND METHODS

The new species was collected in the Correntina River, located in municipality of Correntina, BA. This river belongs to the sub-hydrographic basin of the Corrente River, a tributary on the left bank of the middle stretch of the São Francisco River in the western portion of BA. This area is covered mainly by cerrado (central Brazilian savanna) vegetation and the regional topography is dominated by a plateau located at 700-900 m above sea level (asl). Annual precipitation in this area averages 1.100 mm, with a rainy season occurring between October-April, when 94% of the total precipitation falls (Andrade et al. 2002).

The techniques for collection and rearing of specimens are those detailed in Pepinelli et al. (2005). Measurements of specimens and the terminology used in the species description are detailed in Adler et al. (2004). The description of the adult thoracic pattern was based on specimens recovered from alcohol using the technique of Sabrosky (1966). Images illustrating the morphology were obtained directly from specimens using a Leica M165C stereomicroscope in conjunction with the Leica auto montage program and using an Olympus BX51 compound microscope in conjunction with an Olympus digital acquisition system (DP 72 model using the Cell D program). The tridimensional images were obtained by joining the photographs obtained with the equipment cited above, using the Combine ZM program (2007) (public domain software by A Hadley, release date 1st December 2007). Type specimens are deposited in the Invertebrate Collection of National Institute of Amazonian Research (INPA), Manaus, state of Amazonas, Brazil and Museum of Zoology, University of São Paulo (MZUSP), São Paulo, state of São Paulo, Brazil.

S. (Psilopelmia) virescens

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(Figs 1-13)

Diagnosis - Female: cibarium with an elaborated cibarial armature composed of sharp spines that also cover part of the sclerotized cornuae; cibarium central region sclerotized with small, blunt, serrations; scutum brown covered with pale golden greenish setae; with...
anterior illumination, antepronotal lobe yellowish with silver pruinosity, with posterior illumination, with 1 + 1 short, silver spots; postpronotal lobe and paratergite yellowish with silver pruinosity in lateral view; basal region of R and Sc without setae; claws with basai tooth; anepisternal membrane and abdomen light yellowish green; dorsal, median region of the third-fifth abdominal segments with dark brown, subquadrature spots; anal lobe short. Male: scutum brown covered with golden setae; with anterior illumination, antepronotal lobe yellowish with silver pruinosity; postpronotal lobe and paratergite yellowish with silver pruinosity in lateral view; ventral plate as wide as high; gonostylius shorter than gonocoxite with one subterminal spine and four-six thick spines distributed along the external margin. Pupa: with six gill filaments shorter than its cocoon; cephalic plate and thorax with few tubercles; cephalic plate with 2 + 2 short, unbranched fronton trichomes and 1 + 1 longer, unbranched or bifid dorsal trichomes; thorax with bifid trichomes. Larva light green in colour; first thoracic segment, dorsally, with one pointed tubercle; third thoracic segment, dorsally, with one pair of pointed tubercles; first-fourth abdominal segments, dorsally, each with one pair of pointed tubercles; without ventral tubercles; ventral, distal region of the abdomen with a flat, straight ending.

Female (reared from pupa) (Figs 1-3) - General scutal colour dark brown, anepisternal membrane and abdomen light green (Fig. 1A-D). Body length 2.18-2.33 mm [mean = 2.24 mm, standard deviation (SD) = 0.07, n = 3]; thorax lateral length 0.71-0.74 mm (mean = 0.72 mm, SD = 0.01, n = 3); thorax dorsal length 0.64-0.79 mm (mean = 0.71 mm, SD = 0.07, n = 3). Wing length 1.99-2.15 mm (mean = 2 mm, SD = 0.06, n = 5); wing width 0.88-1.05 mm (mean = 0.97 mm, SD = 0.06, n = 5). Fronto-ocular triangle small, wider than high (Fig. 2A). Frons covered with silver pruinosity, clypeus and occiput light gray, with silver pruinosity. Antenna, 0.42-0.49 mm in length (mean = 0.44 mm, SD = 0.02, n = 6); scape, pedicel and base of first flagellomere light brown, remaining flagellomere dark brown (Fig. 2C). Maxillary palpus brown; sensory vesicle elongated occupying more than 1/2 of the length of palpomere III; palpomere V slightly longer than the lengths of palpomeres III and IV together (Fig. 2D). Mandible with 7-9 external serrations (mean = 7.2, SD = 1.3, n = 6) and 25-29 internal teeth (mean = 26.8, SD = 1.4, n = 6). Lacinia with 21-27 retorse teeth (mean = 24.2, SD = 1.3, n = 5). Cibarium (Fig. 2B) with an elaborated cibarial armature composed of sharp spines that also cover part of the sclerotized cornuae; cibarium central region sclerotized with small, blunt serrations. Thorax (Fig. 1A, B, D) with scutum brown covered with pale golden greenish setae; with anterior illumination (Fig. 1A), 1 + 1 faintly silver spots on anterior region and, usually, with a large, irregular gray area in the central median region, antepronotal lobe yellowish with silver pruinosity; with posterior or illumination (Fig. 1B), 1 + 1 silver, subtriangular spot on anterior region; postpronotal lobe and paratergite yellowish with silver pruinosity, in lateral view. Scutellum brown with long dark brown and pale golden greenish setae. Postnotum dark brown, with silver pruinosity. Anepisternal membrane light green and katepisternum brown, covered with silver pruinosity. Costa of wing with spines and setae (Fig. 2E). Basal section of R and Sc bare (Fig. 2E). Coxa, femur and tibiae of all legs bearing filiform and flat scale-like setae (Fig. 2K, L). Foreleg with coxa, trochanter and femur light brown, tibiae with 1/3 of distal region dark brown, basitarsus and tarsomeres I-IV dark brown (Fig. 2G). Middle leg with basal region of coxa, light brown, trochanter, femur and tibia yellowish-brown, distal region of basitarsus brown, tarsomeres I-IV brown (Fig. 2H). Hind leg with coxa and trochanter yellowish-brown, 1/3 of distal region of femur light brown, remaining areas yellowish-brown, distal 2/3 of tibia brown, apical region of basitarsus brown, tarsomeres I-IV brown (Fig. 2I). Calcipala not reaching pedipulus. Claws curved, with basal tooth (Fig. 2J). Abdominal tergite II with 1 + 1 lateral silver spot; tergites III-V with median, sub-rectangular dark, brown spot, each with lateral, elongated brown spot (Fig. 1C); tergite plates VI-VIII, light yellow, with varnish-like appearance (Fig. 1C). Basal fringe with whitish setae. Sternites light yellowish green, genitalia brown. Eighth sternite weakly sclerotized with evenly distributed setae; hypognyal valves (= gonapophyses) subtriangular to rounded, slightly divergent, membranous (only internal margin

Fig. 1: Simulium virescens sp. nov. (Diptera: Simuliidae) female. A: head and thorax, dorsal view, anterior illumination; B: head and thorax, dorsal view, posterior illumination; C: thorax and abdomen, dorsal view: D: lateral habitus (Bar = 0.5 mm).
slightly sclerotized) and with few microtrichia (Fig. 3D). Cercus rounded, covered with small hairs and long dark setae; anal lobe short, subtriangular (Fig. 3B). Genital fork with anteriorly directed apodeme, well-developed lateral plate (Fig. 3C). Spermatheca globular, with internal spicules (Fig. 3A) arranged on transversal line; spermathecal duct and duct insertion area unpigmented and not sclerotized.

**Male (reared from pupa) (Figs 4-6)** - General scutal colour brown, anepisternal membrane light green, abdomen light greenish brown (Fig. 4A-D). Body length 2-2.50 mm (mean = 2.20 mm, SD = 0.2, n = 3); thorax lateral length 0.68-0.75 mm (mean = 0.71 mm, SD = 0.03, n = 3); thorax dorsal length 0.55-0.68 mm (mean = 0.6 mm, SD = 0.07, n = 3). Wing length 1.57-1.73 mm (mean = 1.6 mm, SD = 0.07, n = 4); wing width 0.74-0.83 mm (mean = 0.8 mm, SD = 0.01, n = 4). Antennae (Fig. 5A) 0.42-0.46 mm in length (mean = 0.44 mm, SD = 0.01, n = 5); scape, pedicel and base of first flagellomere light brown; remaining flagellomere dark brown. Palpus dark brown, sensory vesicle occupying less than 1/3 of palpomere III; palpomere V slightly longer than the length of palpomeres III and IV together (Fig. 5B). Scutum brown, covered with golden greenish setae (Fig. 4A, B); with anterior illumination, antepronotal lobe yellowish with silver pruinosity; postpronotal lobe and paratergite yellowish with silver pruinosity in lateral view. Scutellum dark brown with long dark brown and golden setae. Postnotum dark brown with silver pruinosity. Anepisternum and katepisternum covered with silver pruinosity. Costa of wing with spines and setae (Fig. 5C). Subcosta usually bare, basal section of R bare (Fig. 5C). Leg col-

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![Fig. 2: Simulium virescens sp. nov. (Diptera: Simuliidae) female. A: fronto-ocular triangle; B: cibarium; C: antenna; D: maxillary palp; E: base of wing; F: basitarsus and tarsus, showing the calcipala and pedisulcus; G: fore leg; H: middle leg; I: hind leg; J: tarsal claw from the middle leg; K, L: scale-like setae on the legs.](image_url)
oration same as in the female (Fig. 5D-F). Claws with basal tooth. Calcipala not reaching pedisulcus (Fig. 5G). Basal fringe with long, thin, black and golden hairs. Abdominal tergites greenish, median region of tergites III-V with sub-rectangular, dark brown spots (Fig. 4C); tergites III-VIII with posterior margin dark. Abdominal segments II, VI-VIII with lateral, silver bands. Genitalia dark brown; gonocoxite and gonostylus covered with small hairs and long setae; gonocoxite sub-rectangular (Fig. 6A, B); gonostylus sub-triangular (Fig. 6A-C), as long as wide, with one subterminal spinule and four-six thick spines, distributed along the external margin in dorsal view (Fig. 6E). Ventral plate as long as wide, covered by setae, as in Fig. 6D. Median sclerite elongated, as in Fig. 6E. Paramere with developed and sclerotized basal process and numerous parameral spines (Fig. 6F); aedeagal membrane with small setae.

**Pupae (Figs 7-10)** - Cocoon length dorsally 2.5-2.9 mm (mean = 2.7 mm, SD = 0.2, n = 5), ventrally 3.1-3.3 mm (mean = 3.2 mm, SD = 0.06, n = 5); pupa lateral length 2.2-2.6 mm (mean 2.43 mm, SD = 0.1, n = 5). Cocoon slipper-shaped, tightly woven, whitish brown (Fig. 7A, B). Cephalic plate with 2 + 2 short, unbranched frontal trichomes and 1 + 1 longer, unbranched or bifid dorsal trichomes; mostly without tubercles; rounded tubercles present on the external area to the dorsal trichomes (Fig. 8B). Thorax with 5 + 5 pairs of short, simple or bifid trichomes near margin of dorsal cleft (Fig. 8C); tubercles mostly pointed, sparsely distributed over surface and more concentrated near the wing sheath. Gill length 0.9-1.4 mm (mean = 1.16 mm, SD = 0.2, n = 4), pale brownish. Gill with six filaments, main trunk short, giving rise to three short sets of primary branches, each consisting of two secondary branches (Fig. 8D). All gill filaments approximately the same length, with rounded ends. Abdominal tergites as in Fig. 9A-D. Sternites III with 2 + 2 spine comb patch in the median-lateral region (Fig. 9A). Sternite IV with 1 + 1 bifid hook and 1 + 1 spine comb patch in the median-latero region. Sternite V with 2 + 2 laterally distributed, bifid hooks and 1 + 1 spine-comb patch on the posterior region of these hooks at the median-latero region (Fig. 9A, B). Sternites VI and VII with 2 + 2 simple hooks, distributed evenly along the width of the segment; 1 + 1 spine comb patch distributed on the median-lateral region, between the two lateral combs (Fig. 9C, D). Sternites VIII and IX with spine comb distributed on the anterior region without hooks. Abdominal tergites as in Fig. 10. Teruite I with 1 + 1 filiform setae. Terague II with 6 + 6 filiform setae (Fig. 10A). Terugites III-V with 4 + 3-4 submedian simple hooks (Fig. 10B-D). Terugites VI-VII with small patch of comb-like spines on the latero-anterior region (Fig. 10E). Terugite IX with spine combs on the anterior margin and 1 + 1 small hooks.

**Larva (last-instar) (Figs 11-13)** - Body length: 4.9-5.3 mm (n = 5); head capsule lateral length: 0.48-0.53 mm (mean = 0.5 mm, SD = 0.05, n = 5); dorsal width of head capsule: 0.29-0.32 mm (mean = 0.31 mm, SD = 0.01, n = 5). General body colour light-green (in Carnoy’s solution) (Fig. 11A). Frontoclypeal apotome with faintly pattern (Fig. 12A), cuticle with abundant and stout setae.
Cervical sclerites small, rounded to elliptical, free in the membrane (Fig. 12A, F). Postgenal cleft rounded anteriorly (Fig. 12B, E). Postgenal bridge as long as hypostoma (Fig. 12E). Hypostoma with strongly pigmented anterior margin and nine apical teeth (Fig. 12D). Median tooth simple, either similar in length (Fig. 12D) or longer than the 3 + 3 sublateral teeth (Fig. 12E), 1 + 1 lateral tooth wider than median and sublateral teeth, but similar in height, 1-2 + 1-2 paralateral teeth and approximately 6-10 + 6-10 small lateral serrations; hypostoma with 1 + 1 line of 6-8 lateral setae and 1 + 1 short setae parallel to lateral margin and 3-4 + 3-4 setae in posterior region of hypostoma near hypostomal groove (Fig. 12D). Subesophageal ganglion slightly pigmented (Fig. 12B). Antenna shorter than labral fan stalk; base of proximal article whitish and median to distal region of the medial article whitish, distal article dark brown (Fig. 12H); article proportions (proximal: medial: distal) 1: 1.2-1.75: 1-1.2 (n = 6). Mandible with three apical teeth, 10-12 internal teeth, 1-2 large mandibular sub-serration and one or two lateral mandibular processes (n = 6), longer than mandible margin. Labral fan with 40-46 rays (mean = 41.1, SD = 5.15, n = 6). Thorax, with first segment, dorsally, bearing one pointed tubercle, third thoracic segment, dorsally, bearing one pair of pointed tubercles (Fig. 11A, B). Gill histoblast, dissected, with six filaments. Abdomen, first to fourth abdominal segments, dorsally, each bearing one pair of pointed tubercles (Fig. 11A, B). Abdominal segment IX without ventral tubercles (Fig. 11A). Anterodorsal arms of anal sclerite shorter in length than posteroverentral arms (Fig. 12J). Posterior proleg bearing 98-105 rows (mean = 101.5, SD = 2.5, n = 4), each with 14-23 hooks (n = 4). Rectal papillae (Fig. 12I) with three branches, each with approximately
12-15 finger-shaped lobes (n = 5). Body cuticle covered with wide scale-like setae (Fig. 13A-D), varying in shape according with body distribution. Some of the scale-like setae with fringed margin (Fig. 13A) distributed on the posterior region of the body.

Type data and depository - Holotype: Brazil, BA, Correntina. Vila Treviso, BA-349, ± km-92. Rio Correntina (S13°31'08.6” W45°21'02.4”). Entrance ± 12 km from Posto Cacheoeira. 20.VI.2009. Cols Hamada N, Silva NG, Pereira ES & Silva JO Fêmea (F) (INPA). Paratypes: Brazil, BA, Correntina. Vila Treviso, BA-349, ± km-92. Rio Correntina (S13°31'08.6” W45°21'02.4”). Entrance ± 12 km from Posto Cacheoeira. 20.VI.2009. Cols Hamada N, Silva NG, Pereira ES & Silva JO.4 F mounted on a pinned triangle with pupal exuviae preserved in glycerin (INPA). 13 F preserved in 80% ethanol: 10F (INPA) and 3F (MZUSP). One male (M) dissected, mounted in slide (INPA). Two pupae (P) dissected, mounted on slide (INPA). Six last-instar larvae (L) dissected, mounted in slide (INPA). Ten larvae preserved in 80% ethanol: 5 L (INPA) e 5 L (MZUSP). Five P preserved in 80% ethanol: two P (INPA) and three P (MZUSP). 19.VI.2009. Cols Hamada N, Silva NG, Pereira ES & Silva JO. Eight F mounted on a pinned triangle (biting humans during field work): five F (INPA) and three F (MZUSP). Five F dissected, mounted on a slide (INPA). Eight M mounted on a pinned triangle with pupal exuviae preserved in glycerin (INPA). 31.X.2008. Cols Hamada N & Kikuchi RM. Eight M mounted on a pinned triangle with pupal exuviae preserved in glycerin: five M (INPA) and three M (MUZSP). Twelve F mounted on a pinned triangle (biting humans during field work): 10 F (INPA) and two F (MZUSP). Six F dissected, mounted on a slide (INPA). Five M dissected, mounted on a slide (INPA).

Distribution - S. virescens sp. nov. has only been collected in the Correntina River, in the western region of BA, which is in the São Francisco River hydrographic basin.

Bionomics - Larvae and pupae were collected mostly over grass leaves, located under the water and on the banks of the Correntina River (Fig. 14A-C). During the field work, females were observed in clouds, voraciously biting humans, especially in the afternoon. The study site is located in the cerrado biome at an altitude of 700 m asl and is exposed to high levels of solar radiation. This river has width of 20 m, bottom composed of rocks and sand and some stretches with rifles and waterfalls. At the time of the collection the water in the Correntina River was very clear with a temperature of approximately 25°C, electrical conductivity below 10 µS/cm and pH of 6.5. We sampled black flies in several streams near the headwaters of the Correntina River and S. virescens sp. nov. was not found at these sites. Also, we failed to collect this new species when we sampled at a location only 100 km downstream from the type locality. These facts suggest that the distribution of S. virescens n. sp is limited to the median stretch of the Correntina River; however, additional field work should be done on the other rivers in the region, including in the nearby state of Minas Gerais.

The streams and rivers located in the southwestern region of Bahia have unique fauna. Simulium cerradense Coscarón, Cerqueira, Sato and La Salvia, 1992 is currently only known from this region (Coscarón et al. 1992, Hamada et al. 2004). Recently, a new genus and species of dragonfly (Orionothemis felixorioni Fleck, Hamada and Carvalho, 2009) collected in this region was described (Fleck et al. 2009). The high diversity and unique fauna observed in the study area indicate that preservation actions should be undertaken very soon, as this region is unprotected by any conservation units.

Etymology - The species name derives from the Latin word virescens meaning “greenish” in reference to the green colour of the larvae and of the female abdomen.

Fig. 7: Simulium virescens sp. nov. (Diptera: Simuliidae) pupae. A: pupal habitus, lateral view; B: pupal habitus, dorsal view (Bar = 1.0 mm).

Fig. 8: Simulium virescens sp. nov. (Diptera: Simuliidae) pupae. A: partial view of cephalic plate, showing the frontal and dorsal trichomes; B: partial view of cephalic plate, showing the frontal trichomes; C: pupal gill filaments (Bar = 0.05 mm); D: thoracic trichomes (Bar = 0.2 mm).
DISCUSSION

*S. virescens* sp. nov. is placed in the subgenus *S. (Psilopelmia)* following some of the characters given in Coscarón et al. (1996). These authors stated that *S. (Psilopelmia)* can be divided into two groups, one that represents the Nearctic fauna and the other the Neotropical areas of Mexico, Central and South America. Later on, Coscarón and Coscarón-Arias (2007) included the Blancasi species group in *S. (Psilopelmia)* and stated that this subgenus is composed of two species groups (Escomeli and Blancasi).

According with Coscarón et al. (1996), the Nearctic species in *S. (Psilopelmia)* can be morphologically characterized by having females with cibarium with strong, small spines, abdomen with three longitudinal bands formed by the dark spots on the median region and anal lobe without incision between the anterior projection and the basal lobe. The males of these Nearctic species can be characterized by having 1 + 1 silver, anterior bands that can reach the median region of the scutum, gonostylus...
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relatively short, slight concave distally. In the immature stages, pupae of the Nearctic species can be characterized by the presence of eight gill filaments, shorter than the cocoon and larvae by having cephalic apotome without defined ornamentation (except *Simulium mediiovittatum* Knab, 1915). Although *S. virescens* sp. nov. has Neotropical distribution, it has several of the characteristics of the species that Coscarón et al. (1996) reported for the species that occur in the Nearctic region, as can be observed in the new species diagnosis and description presented above.

The only other *S. (Psilopelmia)* species, *sensu* Coscarón and Coscarón-Arias (2007) that occurs in Brazil is *S. iracouboense*. Both species can be distinguished by their scutal coloration, which is yellowish orange in *S. iracouboense*, with paired, silver stripes in the female and paired subtriangular, anterior, silver spots in the male. The morphology of the genitalia of the two species also differs. The two species also can be easily distinguished at the pupal and larval stages: the *S. iracouboense* pupa has eight gill filaments while *S. virescens* sp. nov. has six.

The other *S. (Psilopelmia)* Neotropical species with six gill filaments are *S. dandrettai* Vargas, Martínez Palacios and Díaz Nájera, 1946, *Simulium zempoalense* Vargas, Martínez Palacios and Díaz Nájera, 1946 and *Simulium trivittatum* Malloch, 1914 (Coscarón & Coscarón-Arias 2007), but the new species can be easily distinguished from these by the adults and by the larval morphology. The larvae of *S. virescens* sp. nov. have a unique morphology, with dorsal tubercles on some thoracic and abdominal segments and an elongated head capsule.
In this study we report for the first time in Brazil the presence of *S. (Psilopelmia)* outside of the Amazonian Region.

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