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Taxonomy of the *Simulium perflavum* species-group
(Diptera: Simuliidae),
with description of a new species from Brazil

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Abstract: The larva, pupa, male, and female of *Simulium trombetense* n. sp. are described and illustrated. This species was collected in the Brazilian Amazon region in the states of Amapá, Amazonas, Pará, and Roraima near the edges of the crystalline basement-rock formation of the Pre-Cambrian Guiana Shield. Keys for larvae, pupae, males, and females of species in the *Simulium perflavum* Species-Group are presented, as well as new observations on adult, pupal, and larval characters. Evidence is given to support the species status of *S. maroniense* Floch and Abonnenc, previously considered synonymous with *S. rorotaense* Floch and Abonnenc.

Introduction

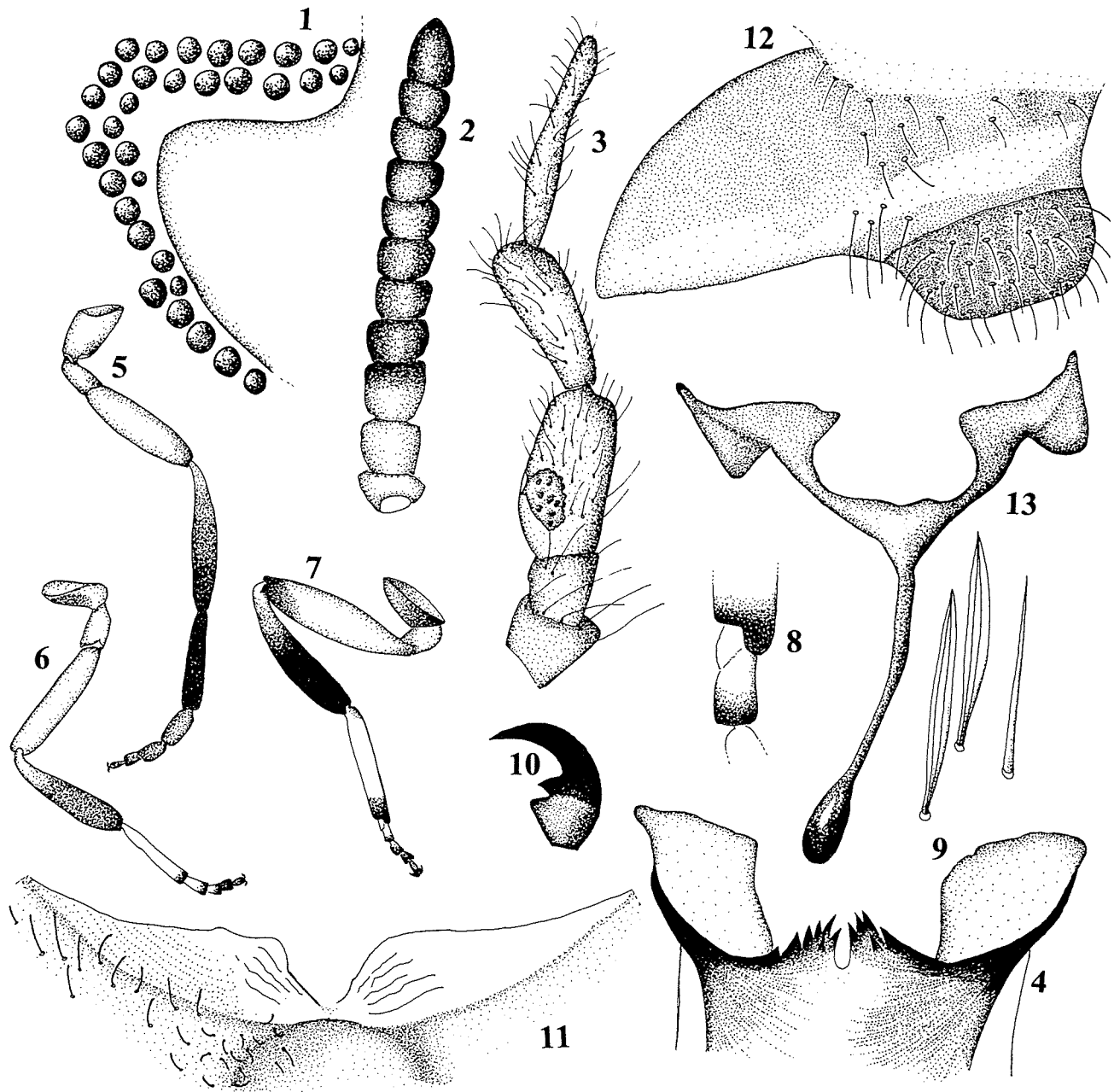
The subgeneric classification of South American black flies is controversial, particularly with regard to the subgenera *Ectemnaspis* and *Psilopelmia* (Coscarón, 1990; Crosskey and Howard, 1997), which are treated by some workers as full genera (Py-Daniel and Sampaio, 1994). Nonetheless, division of these taxa into species-groups shows general agreement. For example, Coscarón (1984) and Crosskey and Howard (1997) recognized the *Simulium perflavum* Species-Group, which is common in Brazil and Venezuela. Miranda Esquivel and Muñoz de Hoyos (1995) suggested that a number of species-groups, including the *S. perflavum* Species-Group, should be elevated to subgenus. However, their only representative for this species-group was *Simulium perflavum* Roubaud, the most autapomorphic member of the group.

The members of the *S. perflavum* Species-Group are *S. kabanayense* Ramírez Perez and Vulcano; *S. perflavum*; *S. rorotaense* Floch and Abonnenc; and *S. suarezi* Ramírez Perez, Rassi and Ramírez (Crosskey and Howard, 1997). We

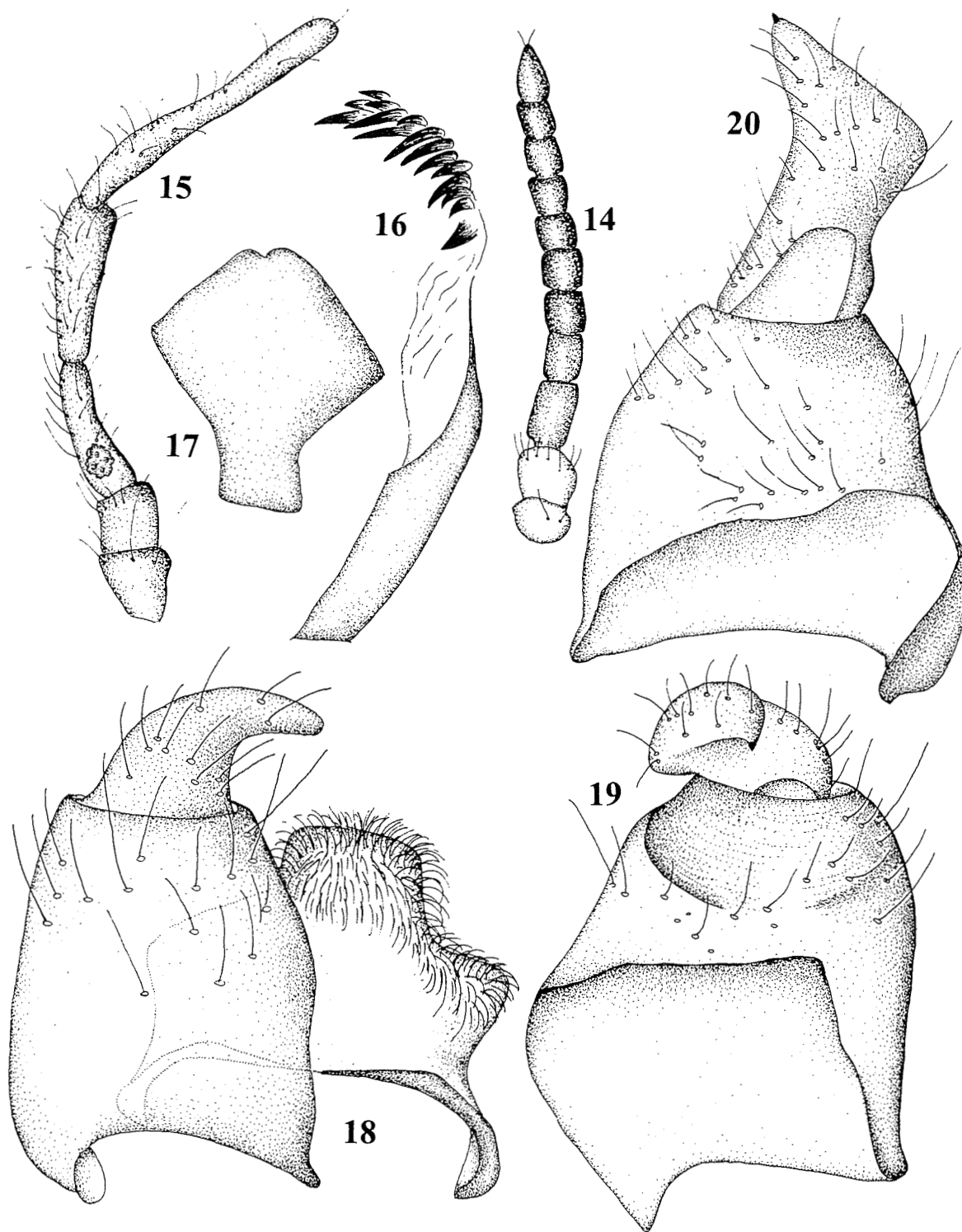
describe a new species in this group, based on larval, pupal, and adult characters, and provide evidence that *Simulium maroniense* Floch and Abonnenc is a good species. Keys are presented to distinguish members of the *S. perflavum* Species-Group, and comments are provided on adult-leg setae, larval antennae, and pupal cephalic trichomes. In the keys, we also include *S. ignacioi* Ramírez Perez and Vulcano, previously considered a synonym of *S. rorotaense* (Shelley *et al.*, 1984, 1997). However, cytological information indicates that it is a good species (N. Hamada, P.H. Adler and M.E. Grillet, unpublished data).

Materials and Methods

Larvae and pupae were fixed in 75% ethanol or Carnoy's solution (1 part glacial acetic acid: 3 parts absolute ethanol). Some pupae were reared to adults. When necessary, structures were clarified in hot 95% lactic acid; clarified genitalia were preserved in glycerine. Euparal[®] was used as the mounting medium for permanent slides. Nomenclature follows that of Adler (1990), Coscarón (1987), and Py-Daniel (1981).



Figures 1-13. *Simulium trombetense* n. sp., female. (1) Fronto-ocular triangle (frontal view). (2) Antenna (dorsal view). (3) Maxillary palpus (lateral view). (4) Cibarium. (5) Fore leg (lateral view). (6) Middle leg (lateral view). (7) Hind leg (lateral view). (8) Calcipala and pedisulcus (lateral view). (9) Scale-like and filiform setae. (10) Tarsal claw (lateral view). (11) Hypogynial valves (ventral view). (12) Anal lobe and cercus (lateral view). (13) Genital fork (ventral view).



Figures 14-20. *Simulium trombetense* n. sp., male. (14). Antenna (dorsal view). (15) Maxillary palpus (ventrolateral view). (16) Paramere (ventral view). (17) Median sclerite. Gonocoxite and gonostylus: (18) ventral view, with ventral plate; (19) dorsal view; (20) dorsal view, flattened between slide and coverslip.

Larvae, pupae, and adults of *S. maroniense*, *S. perflavum*, and *S. rorotaense* were collected in the states of Amazonas and Roraima (Brazil); those of *S. ignacioi*, *S. kabanayense*, and *S. suarezi* were collected in the state of Bolivar (Venezuela). Characters used to distinguish males of *S. ignacioi*, *S. kabanayense*, and *S. suarezi* are from the original descriptions (Ramírez Perez and Vulcano, 1973; Ramírez Perez et al., 1977) and a subgeneric revision by Coscarón (1984, 1990).

The holotype and paratypes are deposited in the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Amazonas, Brazil. Other paratypes are deposited in the Clemson University Arthropod Collection (CUAC), Clemson, SC, U.S.A.; the Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo, Brazil; the National Museum of Natural History (NMNH), Washington, D.C., U.S.A., and the Natural History Museum (NHM), London, England.

***Simulium trombetense* Hamada, Py-Daniel, and Adler, New Species**

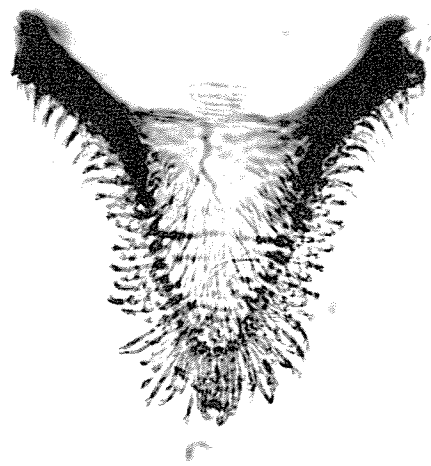
Figures 1-38

[Note: The second author (Py-Daniel) of the species name believes the species should be placed in genus *Ectemnaspis*].

Simulium "X" Hamada, Adler, and McCreddie, 1996.

Simulium "X" Hamada, 1997.

Female (in 80% ethanol): General body color orange; length: body 2.3-2.6 mm ($n = 4$); thorax 1.1-1.2 mm ($n = 6$). Wing: length 2.2 mm ($n = 5$); width 1.1-1.2 mm ($n = 5$). Frons, clypeus, and occiput with silvery blue pruinosity; frons longer than wide; fronto-ocular triangle small (Fig. 1). Antenna (Fig. 2) 0.54-0.59 mm long; pedicel and scape brownish yellow; flagellum brown, with silver pubescence. Palpus yellowish brown; sensory vesicle small, round, with short neck (Fig. 3); palpomere V 1.7-1.8 times as long as III and 1.7 times as long as IV. Mandible with 9-10 external serrations and 24-26 internal teeth; lacinia with 24-25 retrorse teeth. Cibarium (Fig. 4) with 2 anterior submedian elevations, each with group of strong teeth. Pharynx with cluster of sharp teeth proximally. Scutum orange, with brown and golden setae; lateral margin with silver band. Anepisternum brownish orange anteriorly; other regions and katepisternum brown, with silver pruinosity.



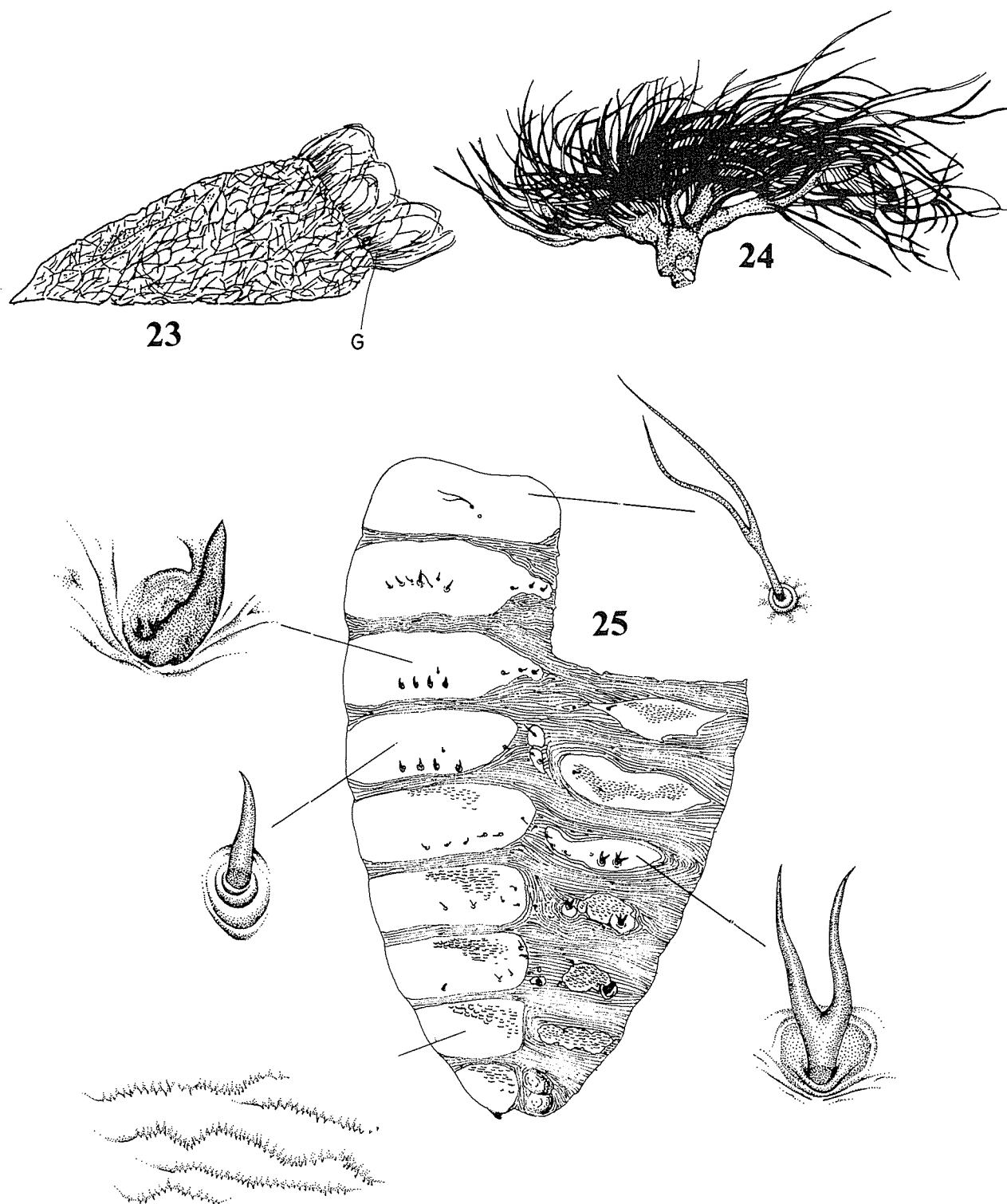
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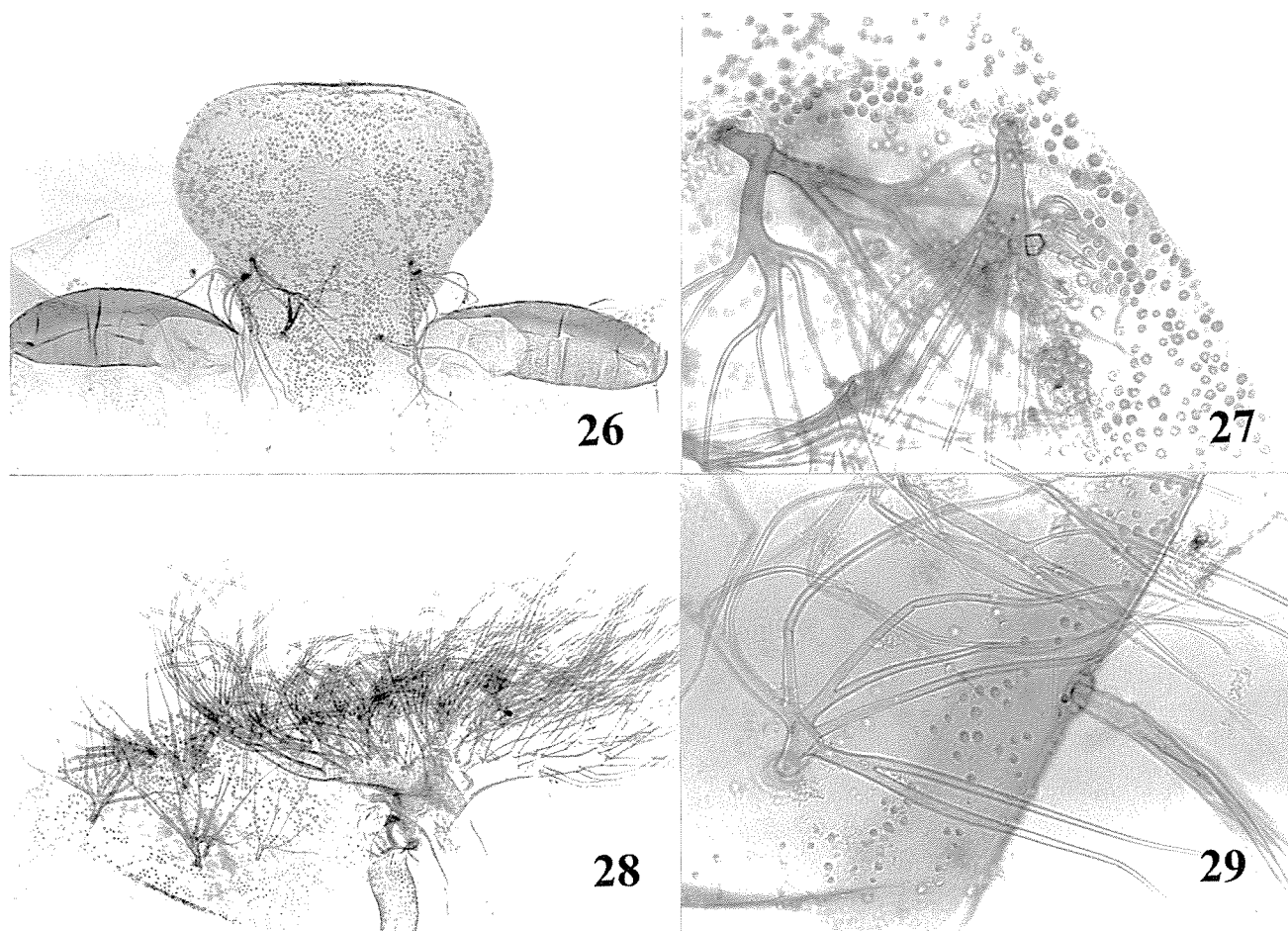
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Figures 21, 22. *Simulium trombetense* n. sp., male.
Ventral plate: (21) terminal view; (22) ventral view.

Scutellum orange, with long brown and golden setae; postnotum orange to brownish orange, with silver pruinosity. Wing veins yellowish brown; base of R and Sc with setae. Halter with basal region tan and terminal region yellow to whitish yellow. Fore leg (Fig. 5) from coxa to femur without dark patches, and with tibia and tarsomeres dark brown to black; middle leg (Fig. 6) with coxa, tibia, and tarsomeres bearing dark patches, and other segments without patches; hind leg (Fig. 7) with all segments bearing dark patches; calcipala and pedisulcus (Fig. 8) well developed. Femora and tibiae with filiform and scale-like setae (Fig. 9);



Figures 23-25. *Simulium trombetense* n. sp., pupa. (23) Cocoon (lateral view), G = gill filaments. (24) Gill (lateral view). (25) Abdomen: dorsal view (left side), ventral view (right side).

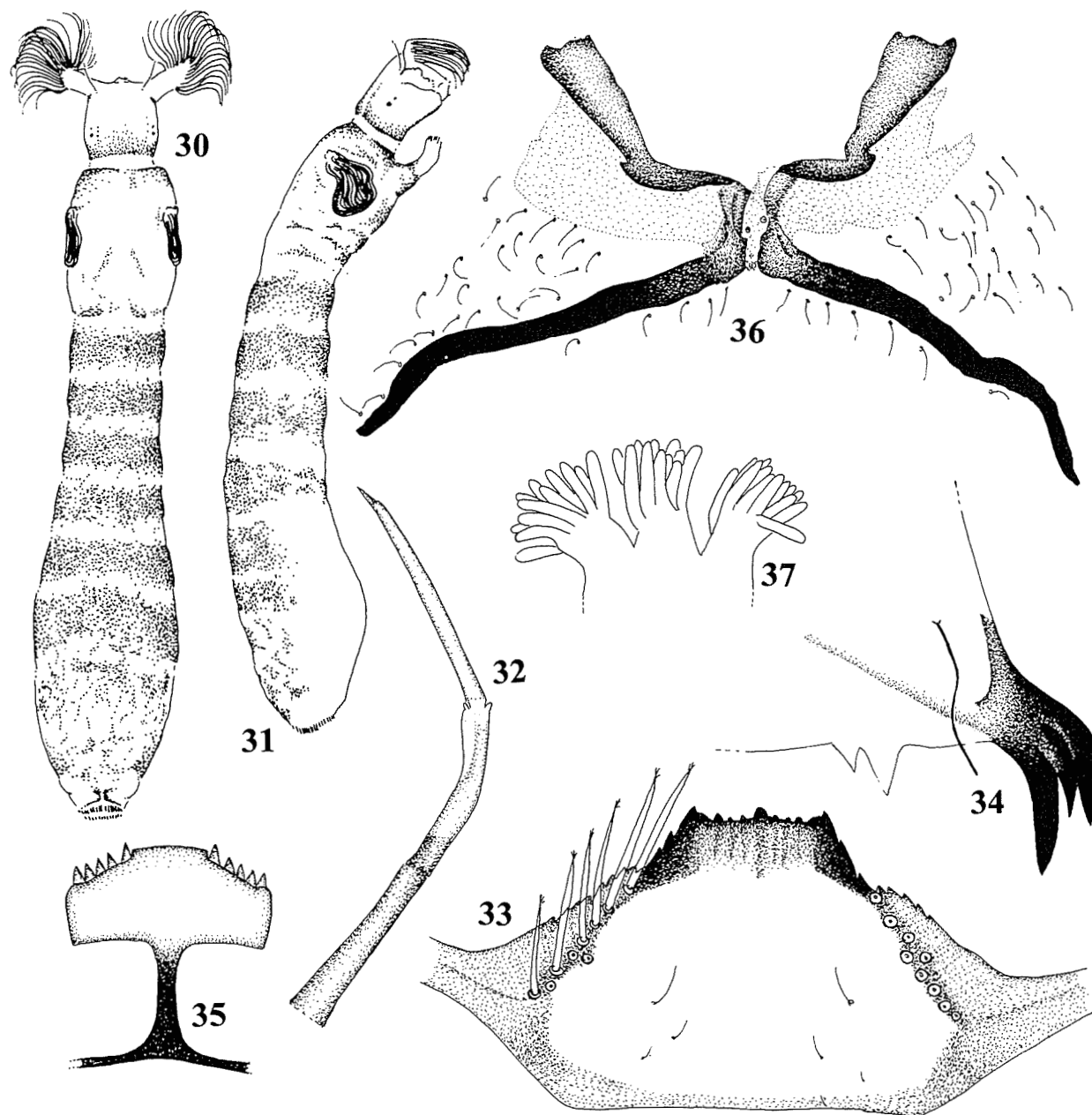


Figures 26-29. *Simulium trombetense* n. sp., pupa. (26) Cephalic plate and antennal sheaths (dorsal view). Thoracic trichomes: (27) from Presidente Figueiredo (Amazonas) and (29) from Trombetas River (Pará). (28) Thoracic cuticle and gill (lateral view).

tarsal claws each with 1 subbasal tooth (Fig. 10). Abdominal tergites I and II with predominant orange patches; other tergites with some orange spots; membranous areas greenish to gray. Basal fringe of long, thin, brown setae with golden reflections. Tergite II with silver pruinosity; tergites VI-VIII with varnish-like appearance. In lateral view, cercus subrectangular and anal lobe elongate, subtriangular (Fig. 12). Hypogynial lobes (Fig. 11) subtriangular. Genital fork (Fig. 13) with stem moderately long and slender, expanded anteriorly; lateral arms forming subrectangular space in region of bifurcation. Spermatheca subspherical with cuticular microspines; attachment area of spermathecal duct membranous.

Male (in 80% ethanol): General body color orange; length: body 2.7-4.6 mm ($n = 5$); thorax 0.9-

1.1 mm ($n = 5$). Wing: length 2.0-2.1 mm ($n = 4$); width 1.0-1.1 mm ($n = 4$). Frons, clypeus, and occiput with silvery blue pruinosity. Antenna (Fig. 14) 0.6 mm long; pedicel and scape brownish orange; flagellum brown, with silver pruinosity. Palpus (Fig. 15) brownish orange; palpomere V about 1.9-2.3 times as long as palpomere III and 1.8-2.0 times as long as palpomere IV. Sensory vesicle small, subspherical. Scutum orange, with brown and golden setae. Scutellum orange, with long, golden and brown setae. Postnotum orange to brownish orange, with silver pruinosity. Anepisternum with anterior region brownish orange; other regions and katepisternum brown. Wing veins brownish yellow; setae and spines brown; Sc without setae; base of R with setae. Halter and legs with color pattern and scales like those of female. All abdominal tergites brown, with



Figures 30-37. *Simulium trombetense* n. sp., larva. Habitus: (30) dorsal view, (31) lateral view. (32) Antenna (lateral view). (33) Hypostoma (ventral view). (34) Apex of mandible with lateral mandibular process (lateral view). (35) Labral sclerite (frontal view). (36) Anal sclerite (dorsal view). (37) Anal papillae (dorsal view).

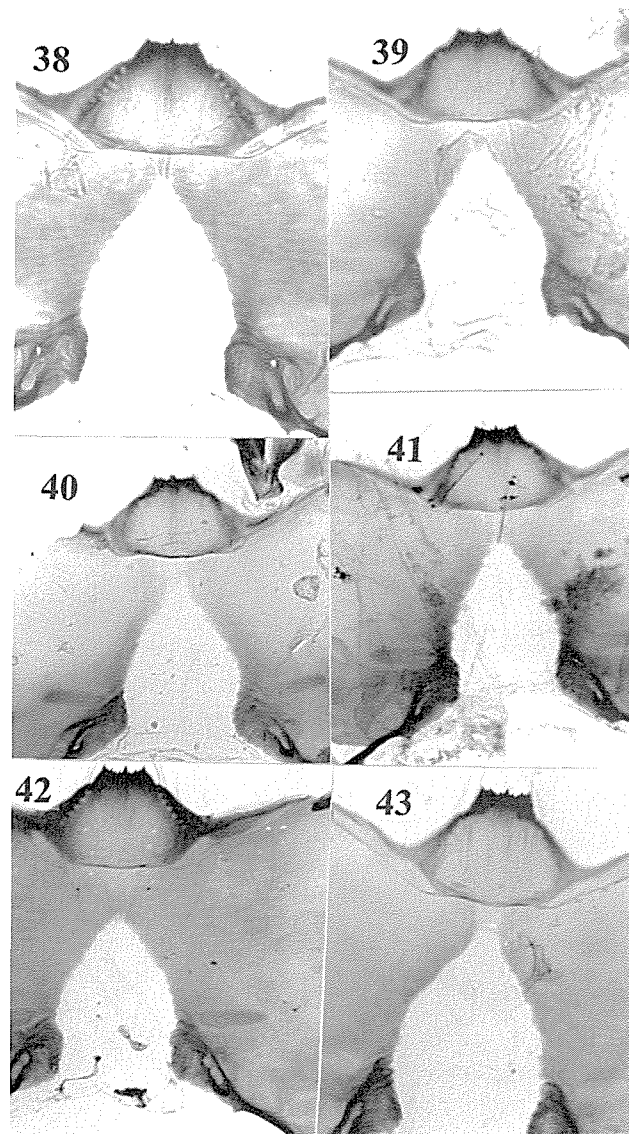
orange patches, sometimes with orange midline. Basal fringe of thin, long, light brown setae with golden highlights. Tergite II with silver pruinosity; tergites V-VIII with lateral silver pruinosity. Paramere as in Fig. 16. Median sclerite as in Fig.

17. Gonocoxite and gonostylus brown, with silver pubescence; gonocoxite about as long as wide (Figs. 18, 19, 20). Gonostylus bearing one apical tooth; gonostylus flattened between coverslip and slide: 0.7 times as long as gonocoxite, longer than

wide (Fig. 20); *in situ*, distal 1/3 curved (Figs. 18, 19). Ventral plate, in ventral view subrectangular (Fig. 22), with lip perpendicular to body and best viewed by directing posterior margin of plate ventrally (Fig. 18); in terminal view subtriangular (Fig. 21).

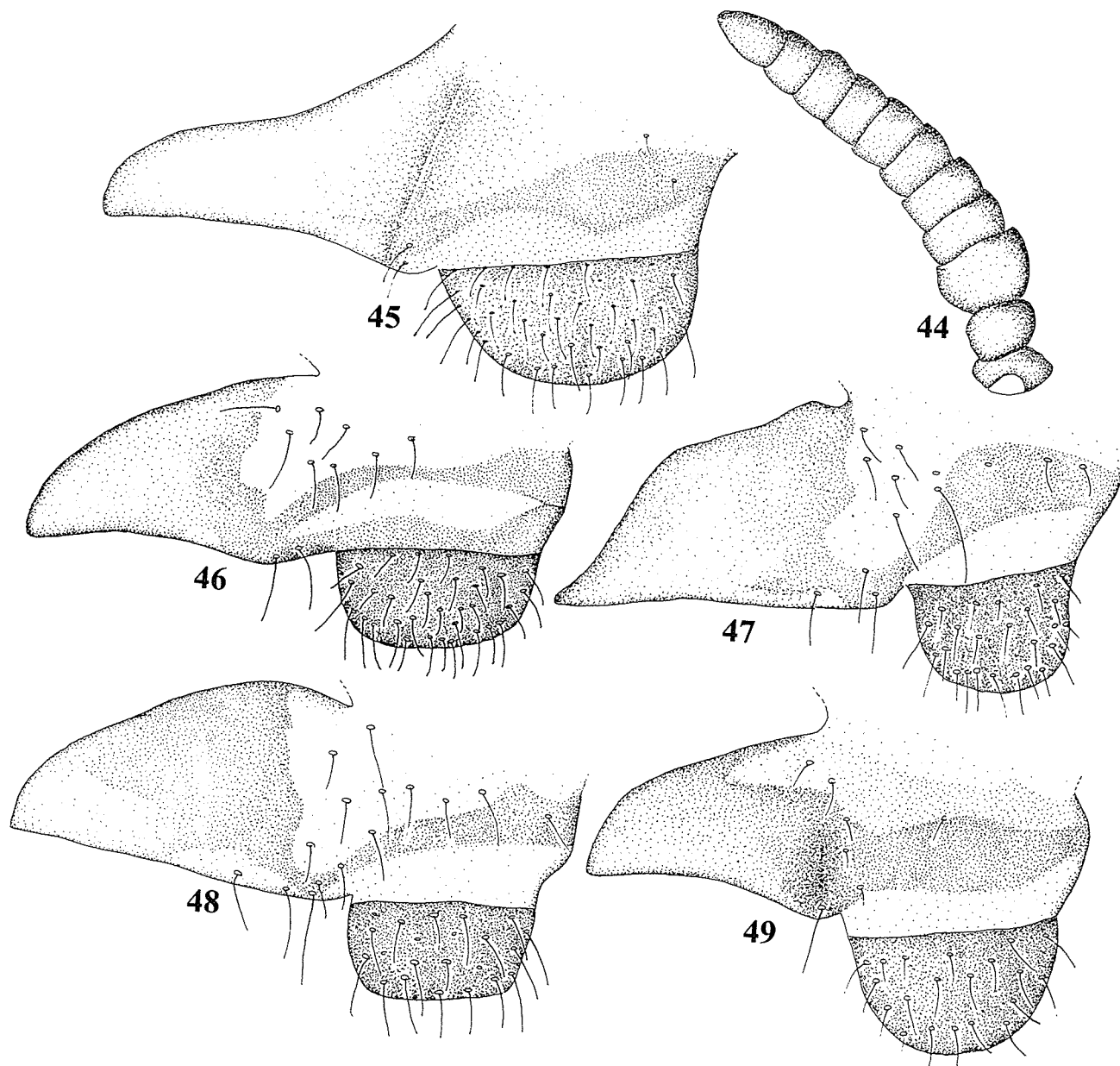
Pupa (in 80% ethanol): length 2.4-2.5 mm ($n = 3$); pupae from Pará (PA) state larger, length 3.2-3.4 mm ($n = 4$). Cocoon (Fig. 23) dark brown, almost boot-shaped, parchment-like, with open loops anteriorly; individual threads distinct; length along dorsal surface 3.4-3.7 mm (PA = 3.6-4.1 mm); ventral surface 3.2-3.5 mm (PA = 4.0-4.3 mm); longest length = 4.4-4.9 mm (PA = 4.9-5.3 mm). Head projected downward, with small dome-shaped tubercles (Fig. 26); 2 pairs of frontal trichomes, each with 6-8 branches, and 1 facial pair with 6-9 branches. Thorax with small, dome-shaped and some pointed tubercles (Fig. 28); 6 pairs of dorsal thoracic trichomes, each with 3-15 branches (Figs. 27 and 29); 2-4 branched trichomes on each lateral region. Frontoclypeus and thoracic region with tubercles variable in density; specimens from Presidente Figueiredo County with very dense tubercles (Fig. 27); specimens from Trombetas River with tubercles less dense (Fig. 29). Gill with 7 thick trunks bearing 160-250 thin, short filaments (Figs. 23, 24, 28). Abdominal chaetotaxy as in Fig. 25 (1 side only): tergite I with 1 pair of setae (1 dark, long and bifid; the other pale, thin, posterior); tergite II with 3 hooks directed anteriorly, some thin setae intercalated, and 3 small setae laterally; tergites III and IV each with 4 anteriorly directed hooks posteriorly, and 1 small seta anterior to second outermost hook; tergites V to IX each with group of fine, posteriorly directed comb-like spines along anterior margin; tergite IX with 1 short, slightly curved, dorsally directed terminal spine. Pleural membrane of segments II to VI each usually with 1-3 minute setae per side. Sternites III, IV and V with 2 plates divided by membranous region, the first 2 sternites each with group of fine, posteriorly directed comb-like spines anteriorly; sternite V with 2 bifid or trifid hooks and sublateral setae. Sternites VI and VII each with 2 plates divided by membranous region, the smaller plate bearing 1 hook, the larger plate with group of fine, posteriorly directed comb-like spines and 1 bifid-quadrifid hook medially.

Larva (mature, in Carnoy's solution): Length 5.9-6.7 mm ($n = 15$); larvae from state of Pará larger, length 7.2-8.2 mm ($n = 8$). Larval habitus as



Figures 38-43. Larval head capsule (ventral view). (38) *Simulium trombetense* n.sp. (39) *S. suarezi*. (40) *S. rorotaense*. (41) *S. maroniense*. (42) *S. kabanayense*. (43) *S. perflavum*.

in Figs. 30, 31; general coloration light to dark green. Head palest anterodorsally, with brown anteromedian and anterolateral spots and with brown spot along posterior margin; line over eye spots translucent, leading into brown area posteriorly; lateral length 0.4-0.5 mm; dorsal width 0.5-0.7 mm; setae of head capsule simple. Cervical sclerites small, elliptical, free in membrane. Postgenal cleft (Fig. 38) about 1.5 times as long as wide, extended about 3/4 distance



Figures 44-49 Female. (44) Antenna, *Simulium perflavum* (dorsal view). Anal lobe and cercus (lateral view): (45) *S. perflavum*, (46) *S. rorotaense*, (47) *S. maroniense*, (48) *S. suarezi*, (49) *S. kabanayense*.

to hypostomal groove, widest at midpoint, tapered apically. Postgenal bridge 0.2-0.3 times as long as hypostoma length. Antenna (Fig. 32) subequal in length to labral fan stalk; medial article translucent, distal article longer than medial and proximal articles; proportions of articles (proximal to distal, excluding apical sensillum) 1.0:0.9-1.0:1.1-1.3. Labral fan with 45-50 primary rays. Hypostoma (Fig. 33) with median tooth as large as

lateral teeth; sublateral teeth small; lateral margin of hypostoma with 2 paralateral teeth and 5-6 lateral serrations per side; 6-9 lateral setae per side, and 2-6 on central disk. Subesophageal ganglion not pigmented. Mandibular teeth with 1 apical, 2 external, 3 subapical (third smaller than second and both smaller than first), and 10-12 internal teeth; mandibular sensillum single. Lateral mandibular process (Fig. 34) thin,

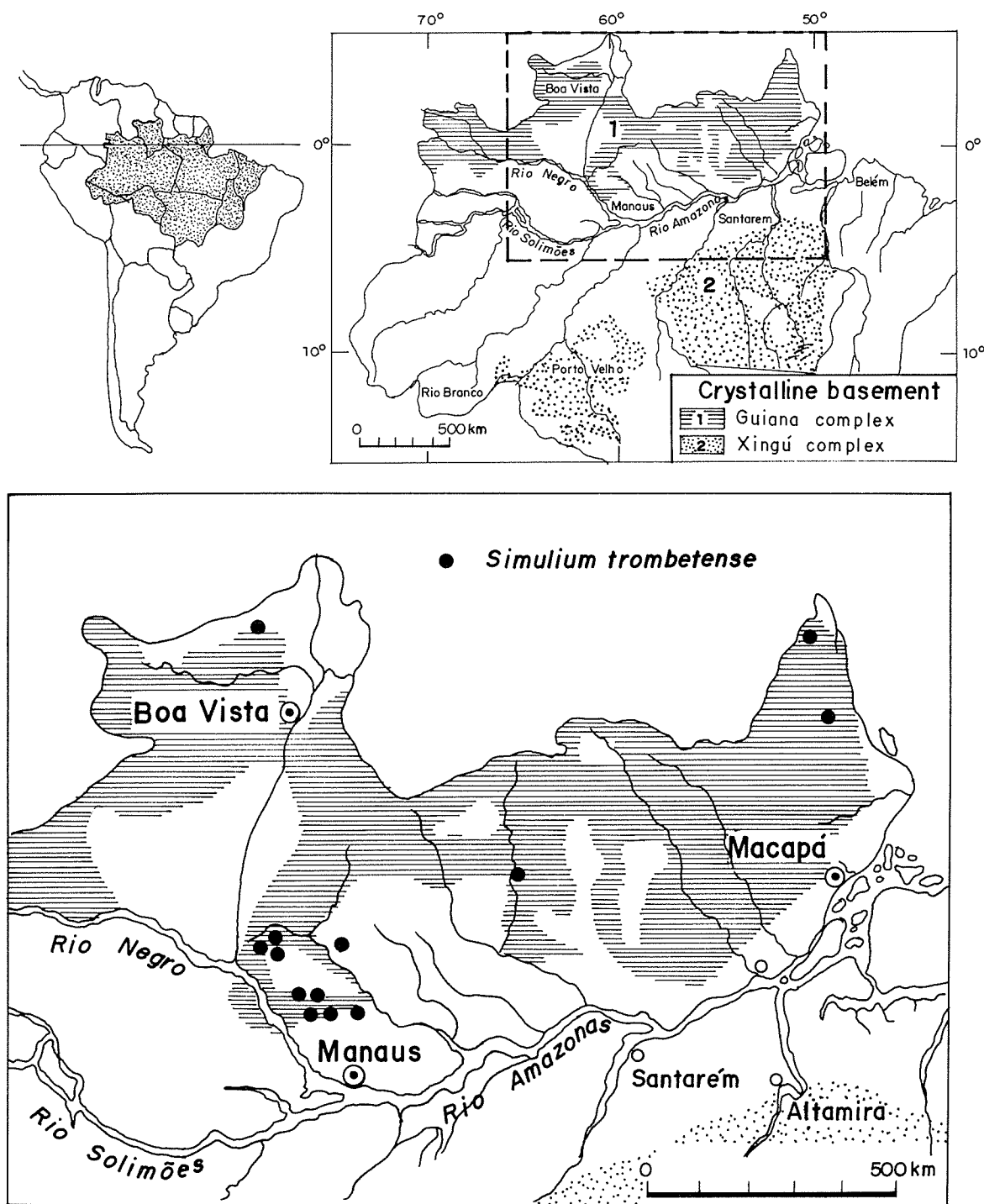
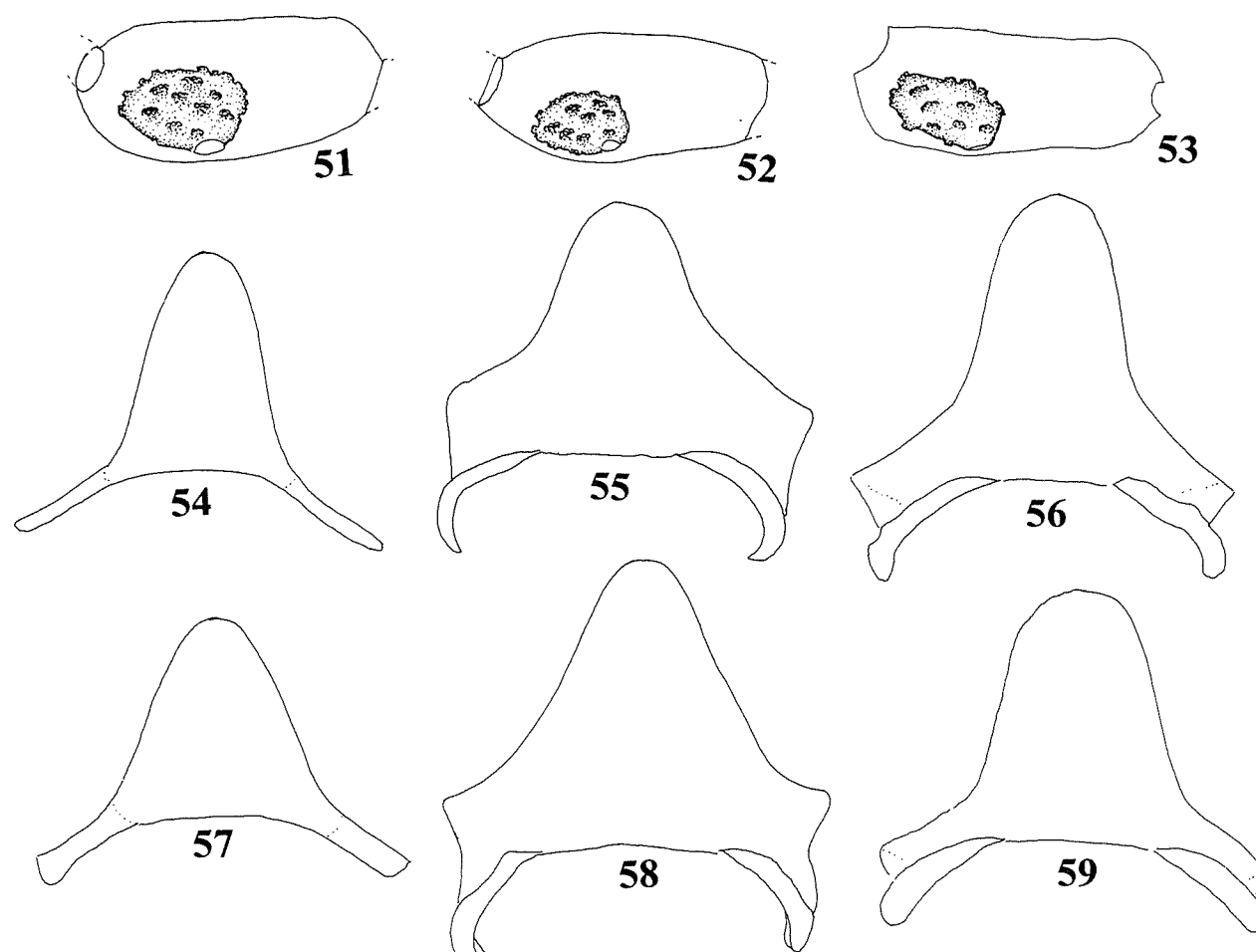


Figure 50. Collection sites of *Simulium trombetense* n.sp. (Diptera: Simuliidae) in relation to the crystalline basement-rock formation of the Guiana Shield, Brazil (map modified from Santos, 1981).

filament-like, longer than inferior margin of mandible. Labral sclerite as in Fig. 35. Body with simple setae; intersegmental bands unpigmented, distinct. Gill histoblast *in situ* (Fig. 31) longer than

wide, reniform; when dissected (Fig. 24) with 160-250 filaments. Anterodorsal arms of anal sclerite (Fig. 36) thinner and shorter in length than posteroventral arms, and associated with elongate,

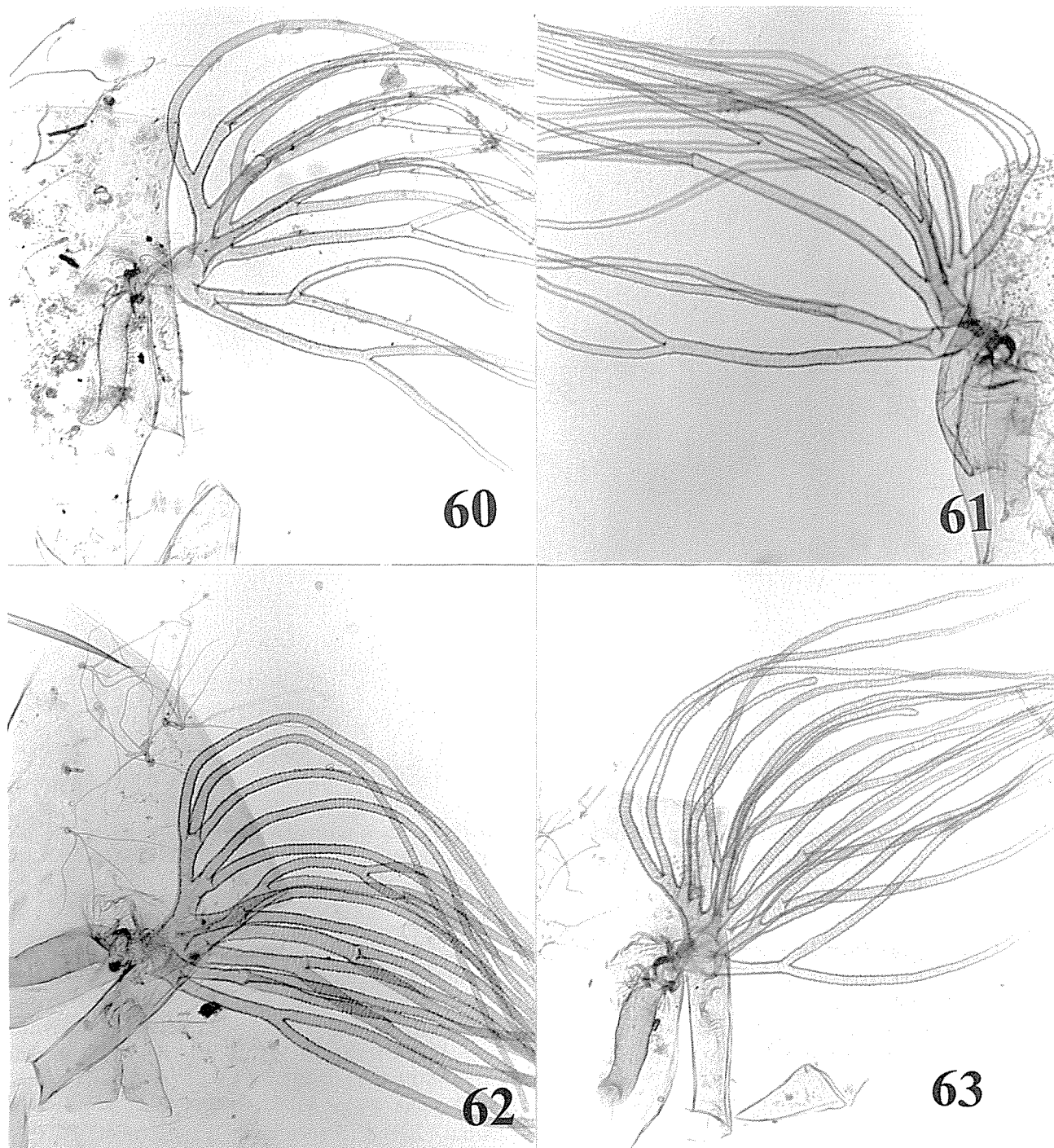


Figures 51-59. Palpal sensory vesicle of female (ventrolateral view): (51, 52) *Simulium maroniense*, (53) *S. rorotaense*. Male ventral plate: *S. maroniense* (54) terminal and (55, 56) ventral views; *S. rorotaense* (57) terminal and (58, 59) ventral views.

thin, simple setae. Posterior proleg bearing 14-16 hooks in 89-113 rows. Anal papillae (Fig. 37) composed of 3 lobes, each with 11-13 lobules.

Types. Holotype: G (pinned) with pupal exuviae (in glycerin microvial), Igarapé da Onça, Highway (HW) AM201 km 20, Presidente Figueiredo County, Amazonas (02°02'S 59°50'W) 22/VI/1996, coll. N. Hamada and F.F. Xavier Filho (INPA). Paratypes: State of Amazonas: same locality and collectors as holotype, 22/VI/1996 (5GG, 5EE-INPA; 5GG, 5EE- MZUSP), 28/III/96 (4 pupae-INPA). Tributary of Igarapé Lajes, BR174 km 113, Presidente Figueiredo (01°59'S 60°01'W), 19/VII/95, coll. N. Hamada and F.F. Xavier Filho (2GG, 3EE- CUAC; 1G, 1E-NMNH; 1G, 1E-NHM).

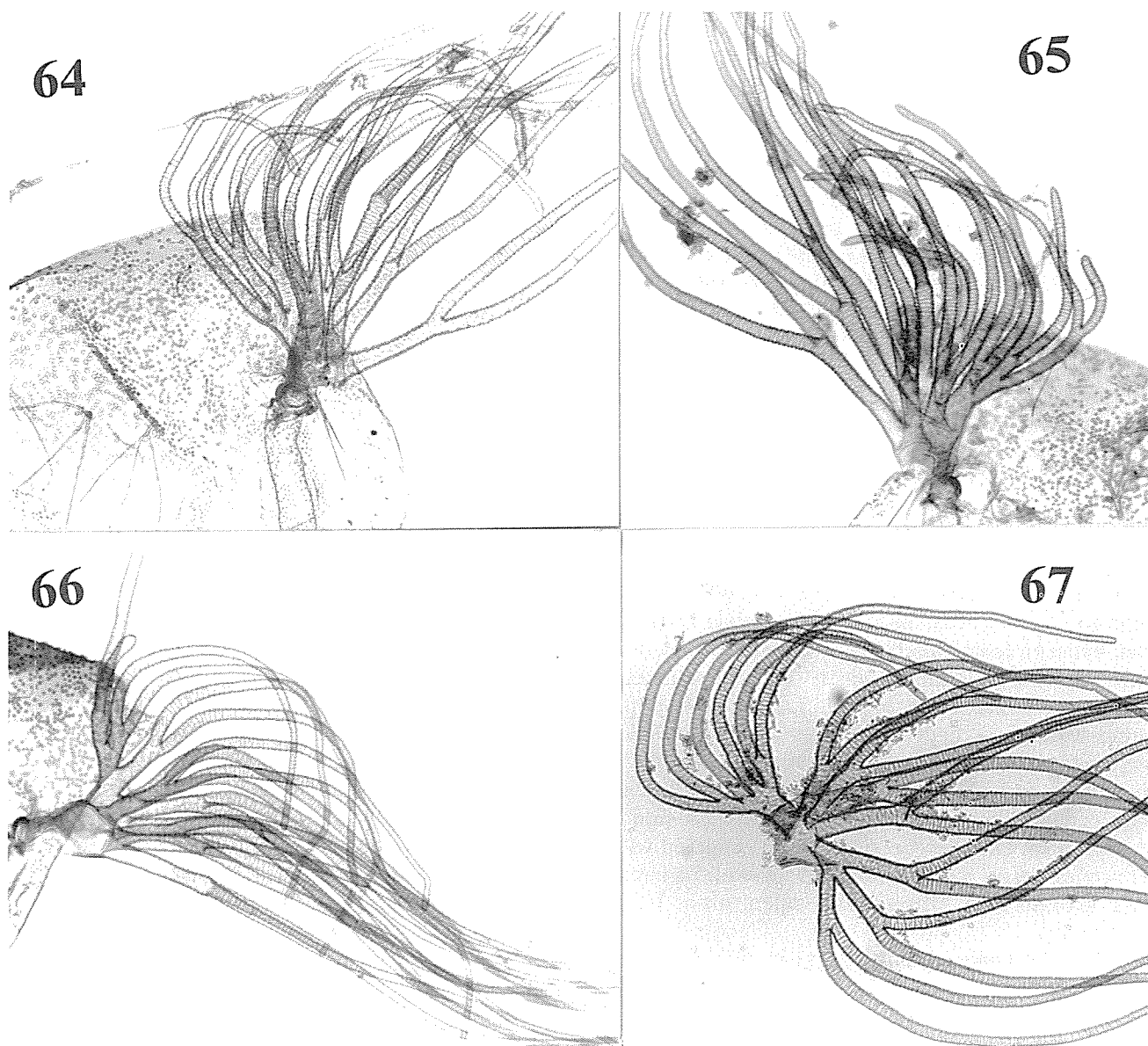
Cachoeira Santuário, HW AM210 km 12, Presidente Figueiredo (02°03'S 59°55'W), 18/VII/95, coll. N. Hamada and F.F. Xavier Filho (3 larvae-INPA). Abandoned pisciculture tanks, Urubuí River, HW BR174 km 112, Presidente Figueiredo (2°01'S 60°01'W), 18/VII/95, coll. N. Hamada and F.F. Xavier Filho (1 larva-INPA). Tributary of Urubuí River, Presidente Figueiredo, 02°01'S 60°01'W, 17.VII.95, coll. N. Hamada and F.F. Xavier Filho (10 larvae, 6 pupae-INPA), 26/III/96, coll. N.Hamada and F.F. Xavier Filho (4 larvae-INPA). Tributary of Urubuí River, bridge on way to Cachoeira Iracema, HW BR174 km 113, Presidente Figueiredo, 01°59'S 60°03'W, 26/III/96, coll. N. Hamada and F.F.Xavier Filho (2 pupae-INPA).



Figures 60-63. *Simulium rorotaense* pupal gill, showing variation (lateral view).

Cachoeira do Boto, unpaved road to Cachoeira Morena, Balbina Dam, Presidente Figueiredo, 02°05'S 59°20'W, coll. N. Hamada and F.F. Xavier Filho (4 larvae-INPA). State of Amapá: HW BR 156, near Carnot village, enroute to town of

Oiapoque, 02°40'N 51°21'W, 26/VII/96, coll. N. Hamada and L.S. Aquino (1 pupa-INPA). Oiapoque County, near airport, 03°52'N 51°47'W, 27/VII/96, coll. N. Hamada and L.S. Aquino (3 larvae, 5 pupae-INPA). State of Pará: tributary from left



Figures 64-67. *Simulium maroniense* pupal gill, showing variation (lateral view, except Fig. 67 which is mounted to show rosette-type arrangement of filaments).

bank of Trombetas River, between mouth of Rio Turuna and Cachoeira Fumaça, ca. 0°03'S 52°00'W, 14/X/85, coll. equipe Comander (10 larvae, 14 pupae- INPA). State of Roraima: small stream near Igarapé Sorocaima, Taurepang Indian village, 04°25'N 61°09'W, 05/VI/96, coll. N. Hamada and F. Xavier Filho (1 pupa-INPA).

Etymology. This species is named for the Trombetas River, in the state of Pará where this species was first collected.

Diagnosis. The last-instar larva of *S. trombetense* is distinguished from larvae of other

species in the *S. perflavum* Species-Group by the shape of the histoblast (Fig. 31). Younger larvae are difficult to separate from those of *S. ignacioi*, *S. maroniense*, *S. rorotaense*, and *S. suarezi* because the postgenal cleft is of similar shape (Figs. 38-41) and the head-capsule pattern and body color are similar. Larvae of the new species can be distinguished from those of *S. perflavum* and *S. kabanayense* by the shape of the postgenal cleft (Figs. 42-43), body color and body shape. The pupa of *S. trombetense* can be distinguished by the number of gill filaments (160-250 filaments); all

other species in the group have 8-23 gill filaments. Adults of *S. perflavum* can be distinguished from those of the new species by the pattern of the middle legs (without dark patches), by the female antenna which has enlarged basal flagellomeres (Fig. 44), and by the shape of the anal lobe (Fig. 45). Adults of other species in this group are similar, but *S. trombetense* females have, in lateral view (Fig. 12), a small, light area on the anterior margin of the anal lobe, whereas females of *S. rorotaense*, *S. maroniense*, and *S. suarezi* have this light area expanded more than 50% of the distance toward the base of the cercus (Figs. 46-48); *S. kabanayense* (Fig. 49) has, in lateral view, a slight concavity on the free posterior margin of the anal lobe. Males of the new species can be distinguished from those of *S. ignacioi*, *S. kabanayense*, *S. maroniense*, *S. rorotaense*, and *S. suarezi* by the small orange patches on all tergites.

Biology. Larvae of *S. trombetense* were collected in forested areas (Terra Firme and Campina) from streams and rivers with bedrock, fast current (some sections with waterfalls), and high discharge (mean width = 9.0 m; mean discharge = 2.2 m³ s⁻¹; *n* = 12). Larval and pupal substrates were bedrock, fallen leaves, and live vegetation. Water temperature was between 24°C and 25°C and pH was between 4.2 and 6.3. This species usually was found with *S. rorotaense* and *S. maroniense*. On one occasion it was collected with *S. perflavum* in a small forested stream; however, this stream ran through an open area 1 km upstream.

Female feeding habits are unknown; we never collected this species biting humans at the collection sites. Oviposition was observed once and is of the "egg dabbling" type (*sensu* Crosskey, 1990), which is similar to that of *S. rorotaense* (Gorayeb, 1981) and *S. perflavum* (Hamada, 1997).

The general distribution of *S. trombetense* is associated with the edges of the crystalline basement-rock formation of the Guiana Shield (Fig. 50). This is the oldest rock formation of this Pre-Cambrian shield (Santos, 1981). *Simulium trombetense* has a patchy distribution, even in Presidente Figueiredo County where collecting effort was greatest. In Presidente Figueiredo County, for example, this species was collected in one stream (Igarapé Lajes) but not in others (Igarapé dos Veados at Cachoeira Santa Cláudia and Cachoeira Orquídea). From a human perspective, these streams are very similar, located about 10 km apart in forested areas, with

low pH, low conductivity, high velocity, high discharge, and rapids and waterfalls.

New information on adult, pupal, and larval characters

Coscarón (1987, 1990) stated that legs of the adults of species in the subgenus *Ectemnaspis* have only filiform setae; however, all species in the *S. perflavum* Species-Group have scale-like setae distributed with filiform ones on their femora and tibiae.

Coscarón's (1987) key to *Simulium* larvae stated that species in the *S. perflavum* Species-Group have antennae shorter than the labral fan stalk and that the medial article is longer than the distal one. Larvae of most species in the group were unknown prior to the present study; therefore, the only species that Coscarón (1987) examined was *S. perflavum*, which has the characteristics he reported. However, all other species in the group have larval antennae as long as the labral-fan stalk and have a medial article subequal to or shorter than the distal one.

Ramírez Pérez *et al.* (1977) reported three cephalic trichomes per side on pupae of *S. suarezi*; however, the specimens that we collected with M.E. Grillet, J.W. McCreadie, and C. Delgado in the Grand Savanna region, Bolívar state, Venezuela, have four cephalic trichomes per side (three frontal and one facial).

Species status of *Simulium maroniense*

Simulium maroniense was considered by Shelley *et al.* (1984, 1997) to be synonymous with *S. rorotaense* based on the original description and their examination of two syntypes each of *S. rorotaense* (1G, 1E) and *S. maroniense* (1G, 1E) housed in the Pasteur Institute, Paris. Only the female of *S. rorotaense* had its complete pupal pelt; the syntypes of *S. maroniense* had only the abdominal portions of their pupal pelts. Shelley *et al.* (1984) stated that the differences in the form of the anal lobes and hypogynial lobes used to distinguish these species in the original description were artifacts and that differences in pigmentation, tubercle size and density, and trichome and gill branching of the pupa represented intraspecific variation.

Coscarón (1987) listed *S. maroniense* as a synonym of *S. rorotaense*, but in his revision of the subgenus, Coscarón (1990) decided that both were

good species, stating that the female of *S. rorotaense* has a larger palpal sensory vesicle and that the pupa has dorsal gill filaments as long as the ventral ones, a frontoclypeus and thorax with smaller and fewer tubercles, and smaller trichomes (with 2-3 branches). Py-Daniel and Sampaio (1995) did not include *S. maroniense* in their Brazilian black fly list, suggesting that they did not recognize it as a valid species.

We collected both *S. maroniense* and *S. rorotaense* in the states of Amapá, Amazonas, Roraima and Pará. The female cibaria of both species are similar, as they are in all species that belong to this species-group, and the palpal sensory vesicle of the female of *S. maroniense* (Figs. 51-52) can be smaller or as large as that of *S. rorotaense* (Fig. 53); the genitalia, especially of the female, are very similar between the two species. In ventral and apical views, the male ventral plate of both species has slight differences in shape (Figs. 54-59); however, more observations are necessary to confirm shape as a diagnostic character.

Floch and Abonnenc (1946) distinguished *S. maroniense* and *S. rorotaense* in the pupal stage. *Simulium rorotaense* is polymorphic for tubercle density (almost none to very dense, Figs. 60-63, 68, 70) and trichome branching (2-9), depending on geographical region. In *S. maroniense*, these characters are more consistent throughout its range; it has slightly larger tubercles that are present in high density (Figs. 64-66, 69, 71) and trichomes with 2-11 branches. Pigmentation of the head and thorax is darker in *S. maroniense* than in *S. rorotaense*. However, the character that best distinguishes these species is the thickness and length of the gill filaments, together with their arrangement. *Simulium maroniense* has thicker, more darkly pigmented gills, with the dorsal filaments shorter than the ventral ones and branching near the base (Figs. 64-67). The 18-21 gill filaments, in anterior view, present an open rosette at the base (Fig. 67); this rosette can be very open, as in *S. suarezi* (Fig. 74), or more closed, but is always present. *Simulium rorotaense* has 17-23 thinner, more lightly pigmented filaments, varying in size and branching pattern, and the filaments are projected forward, not forming an open rosette at the base (viewed anteriorly) (Figs. 60-63). In the larval stage, both species are similar, but the gill characteristics can be used to distinguish mature larvae by removing the histoblast. Cytotaxonomic

studies of their polytene chromosomes (Hamada, 1997) also indicate that these two species are distinct.

Keys for females, males, pupae, and larvae of the *Simulium perflavum* Species-Group

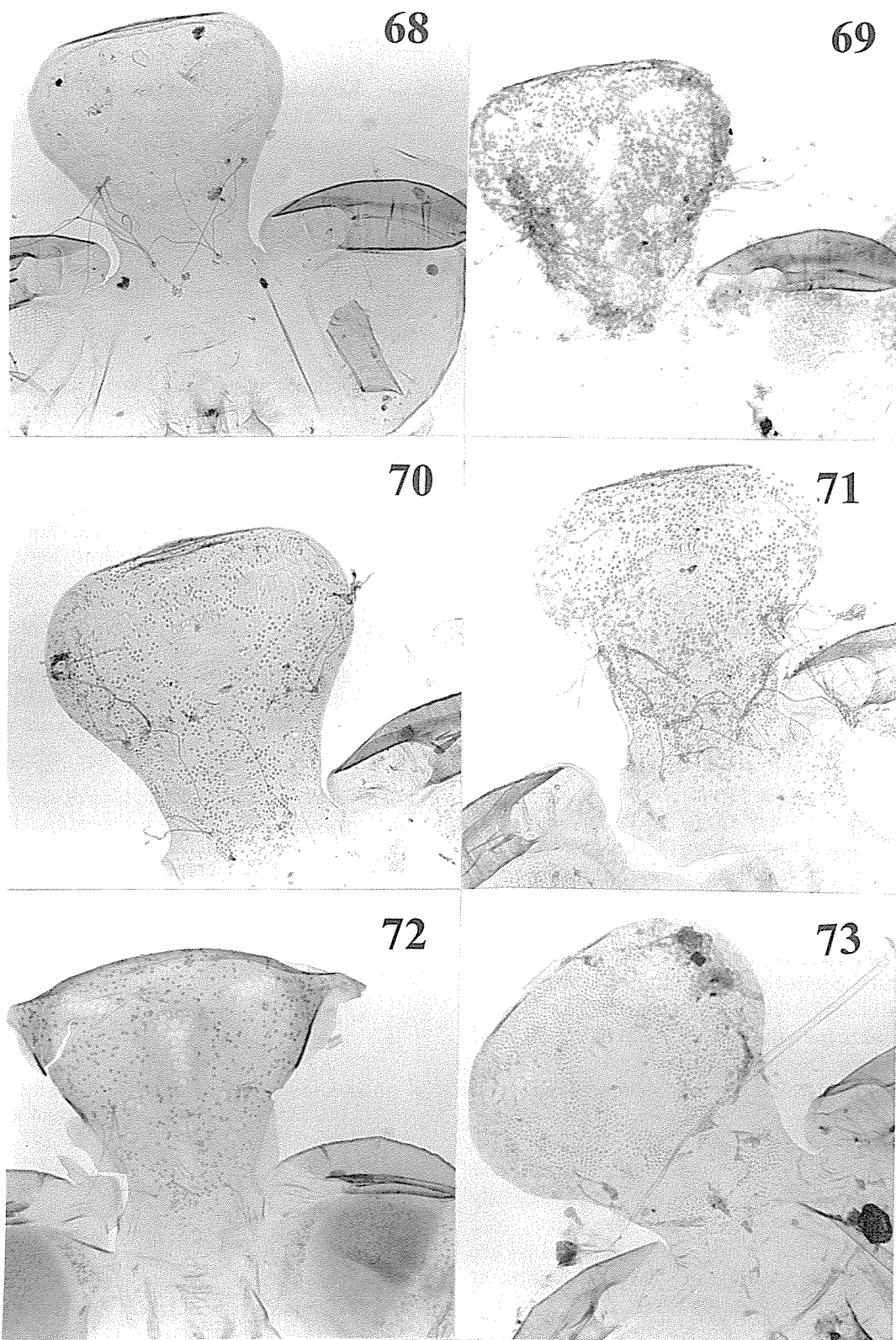
(*Simulium ignacioi* was not included in this key because of a lack of material and because the original description does not contain adequate information).

Females

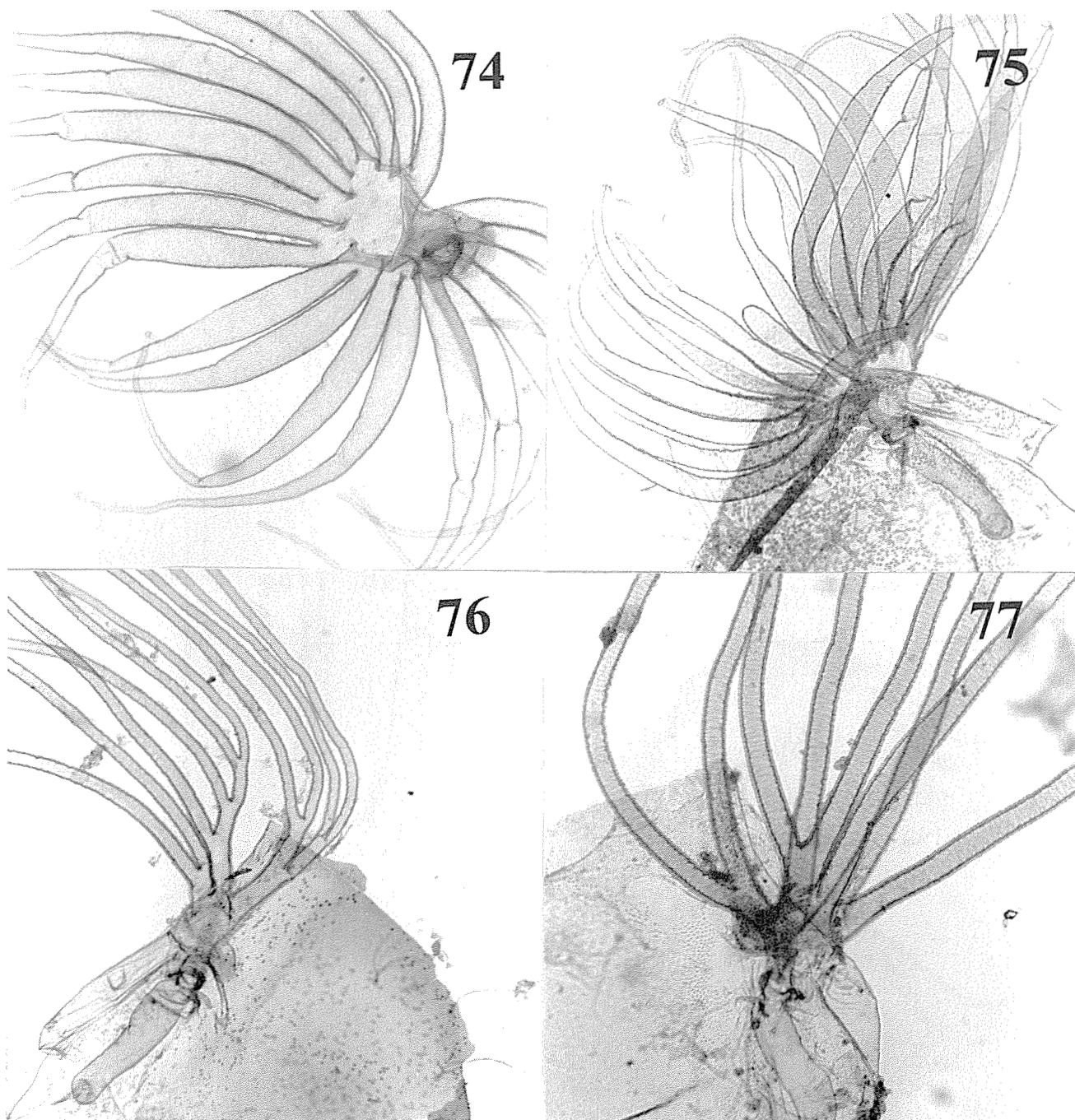
1. Middle leg without dark areas on femur and tibia; antenna broadest at basal flagellomere, without dark areas (Fig. 44); projection of anal lobe, in lateral view, with anterior margin slightly concave (Fig. 45) *S. perflavum*
- Middle leg with dark patches; antenna not broadest at basal flagellomere, with dark areas; projection of anal lobe, in lateral view, with anterior margin convex 2
- 2(1). Projection of anal lobe (beyond cercus), in lateral view, 1.9 times as long as basal width (Figs. 46-48) *S. maroniense*, *S. rorotaense*, *S. suarezi*
- Projection of anal lobe (beyond cercus), in lateral view, 1.2-1.3 times as long as basal width (Figs. 12, 49) 3
- 3(2). Projection of anal lobe, in lateral view, with posterior margin slightly concave (Fig. 49) *S. kabanayense*
- Projection of anal lobe, in lateral view, with posterior margin slightly convex (Fig. 12) *S. trombetense*, new species

Males

1. Middle leg and antenna without dark regions; ventral plate (ventral view) almost twice as long as wide *S. perflavum*
- Middle leg and antenna with dark regions; ventral plate (ventral view) less than twice as long as wide 2
- 2(1). All abdominal tergites with yellowish orange patches *S. trombetense*, new species
- Not all abdominal tergites with yellowish orange patches 3
- 3(2). Abdominal segment I yellowish brown; segment II dark brown to black *S. kabanayense*
- Abdominal segments I-II yellowish brown *S. ignacioi*, *rorotaense*, *S. maroniense*, *S. suarezi*



Figures 68-73. Pupal cephalic region (dorsal view). (68, 70) *Simulium rorotaense*. (69, 71) *S. maroniense*. (72) *S. kabanayense*. (73) *S. perflavum*.

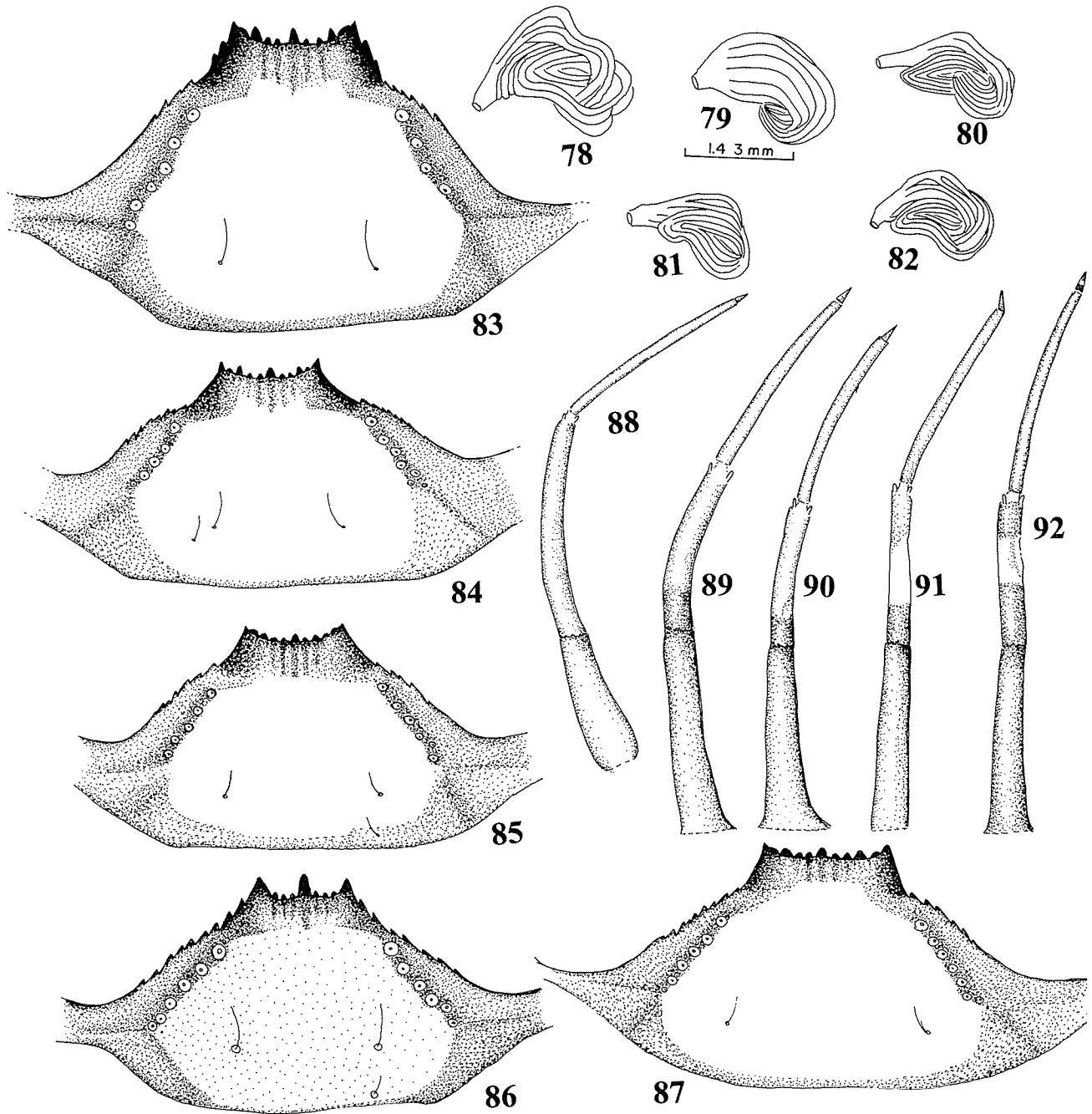


Figures 74-77. Pupal gill (lateral view, except Fig. 74 which is mounted to show rosette-type arrangement of filaments). (74, 75) *Simulium suarezi*. (76) *S. kabanayense*. (77) *S. perflavum*.

Pupae

1. Gill with 12 or fewer filaments 2
- Gill with 14 or more filaments 3
- 2(1). Gill with 8 filaments (Fig. 77); ocular region without dark patch (Fig. 73) *S. perflavum*

- Gill with 10-12 filaments (Fig. 76); ocular region with dark patch (Fig. 72) *S. kabanayense*
- 3(1). Gill with 160-250 filaments (Fig. 28) *S. trombetense*, new species
- Gill with 14-23 filaments 4



Figures 78-92. Larva. Gill histoblast (lateral view): (78) *Simulium perflavum*, (79) *S. suarezi*, (80) *S. kabanayense*, (81) *S. rorotaense*, (82) *S. maroniense*. Hypostoma (ventral view): (83) *S. perflavum*, (84) *S. suarezi*, (85) *S. maroniense*, (86) *S. kabanayense*, (87) *S. rorotaense*. Antenna (lateral view): (88) *S. perflavum*, (89) *S. rorotaense*, (90) *S. maroniense*, (91) *S. suarezi*, (92) *S. kabanayense*.

4(3). Gill with 20 filaments, branching near a single, enlarged central base; filaments thicker proximally than distally (Figs. 74-75), without furrows; four pairs of cephalic trichomes *S. suarezi*

— Gill with 14-23 filaments, not branching near a single, enlarged central base; filaments subequal in thickness proximally and distally, with furrows; three pairs of cephalic trichomes 5

- 5(4). Gill with thin, lightly pigmented filaments, varying in size and branching pattern (Figs 60-63); in anterior view, filaments projected forward, not forming rosette; tubercles of cephalic and thoracic region nearly absent to dense (Figs. 68, 70) *S. ignacioi*, *S. rorotaense*
- Gill with thick, darkly pigmented filaments; dorsal filaments shorter than ventral ones, branching near base (Figs. 64-66); in anterior view, filaments forming open rosette basally (Fig. 67); cephalic tubercles slightly larger and in greater density (Figs. 69, 71) *S. maroniense*

Last-Instar Larvae
(in Carnoy's Solution)

1. Histoblast in situ large, with filaments or at least base of filaments thick (Figs. 31, 78, 79) 2
- Histoblast in situ small, with filaments not thickened (Figs. 80-82) 4
- 2(1). Body pale green to green; dissected histoblast with 8 filaments; median tooth of hypostoma not covered by anterior hypostomal margin (Fig. 83); antenna with medial article longer than distal one (Fig. 88) *S. perflavum*
- Body green to dark green; dissected histoblast with more than 8 filaments; median tooth of hypostoma almost covered by anterior hypostomal margin (Figs. 33, 84); antenna with medial article not longer than distal one (Figs. 32, 91) 3
- 3(2). Histoblast in situ with at least 5 thick, long branches (Fig. 79) (20 total filaments when dissected, Fig. 74); posterior proleg with 98-101 rows of 16 hooks; labral fan with about 40 primary rays .. *S. suarezi*
- Histoblast in situ with thick branches giving rise to many thin filaments (Fig. 31) (160-250 total filaments when dissected, Fig. 24); posterior proleg with 89-113 rows of 13-16 hooks; labral fan with 45-50 primary rays *S. trombetense*, new species
- 4(1). Abdomen brown or dark green, with dark, irregular dorsal spots and with ventral tubercles; antenna lacking pigmentation on middle 1/3 of medial article; apical sensillum bicolored (Fig. 92); hypostoma with median tooth projected beyond anterior margin (Fig. 86); labral fan with 44-49 primary rays; posterior proleg with 83-85 rows of 12-13 hooks *S. kabanayense*
- Abdomen yellowish brown or green to dark green, without dark, irregular dorsal spots and without ventral tubercles; antenna with medial article pigmented only on proximal 1/3; apical sensillum not bicolored (Figs. 89, 90); hypostoma with median tooth partially covered by anterior

margin (Figs. 85, 87); labral fan with 41-45 primary rays; posterior proleg with 87-104 rows of 13-15 hooks 5

- 5(4). Dissected histoblast with thick, dark filaments branching near base, and with dorsal filaments shorter than ventral ones; in anterior view, filaments forming open rosette basally (Fig. 67) *S. maroniense*
- Dissected histoblast with thin, lightly pigmented filaments branching in irregular pattern; in anterior view, filaments projected forward, not forming open rosette basally *S. ignacioi*, *S. rorotaense*

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