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Larval Description of *Simulium* (*Notolepria*) *cuasiexiguum* and *Simulium* (*Chirostilbia*) *obesum* and New Records of Black Fly Species (Diptera: Simuliidae) in the States of São Paulo and Minas Gerais, Brazil

MATEUS PEPINELLI[1], NEUSA HAMADA[2] AND SUSANA TRIVINHO-STRIXINO[1]

[1]*Lab. Entomologia Aquática, Depto. Hidrobiologia, Univ. Federal de São Carlos, Rod. Washington Luís, km 235 13565-905, C. postal 676, São Carlos, SP*
[2]*Coordenação de Pesquisas em Entomologia, Instituto Nacional de Pesquisas da Amazônia, C. postal 478, 69011-970 Manaus, AM*

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Descrição das Larvas de *Simulium* (*Notolepria*) *cuasiexiguum* e *Simulium* (*Chirostilbia*) *obesum* e Novos Registros de Espécies de Borrachudos (Diptera: Simuliidae) para os Estados de São Paulo e Minas Gerais

RESUMO - Neste manuscrito são descritos os estágios larvais de *Simulium cuasiexiguum* Shelley, Luna Dias, Maia-Herzog & Lowry e *Simulium obesum* Vulcano. São fornecidos ainda novos registros para quatro espécies de borrachudos para o estado de São Paulo (*Simulium cuasiexiguum, Simulium metallicum s.l.* Bellardi, *Simulium stellatum* Gil Azevedo, Figueró & Maia-Herzog e *Simulium oyapockense s.l.* Floch & Abonnenc) e de três para Minas Gerais (*Simulium duodenicornium* Pepinelli, Hamada & Trivinho-Strixino, *Simulium obesum* e *Lutzsimulium pernigrum* Lutz).

PALAVRAS-CHAVE: Inseto aquático, simulídeo, distribuição geográfica, taxonomia

ABSTRACT - We describe the larval stage of *Simulium cuasiexiguum* Shelley, Luna Dias, Maia-Herzog & Lowry and *Simulium obesum* Vulcano and provide new distribution records of four black fly species in the State of São Paulo (*Simulium cuasiexiguum, Simulium metallicum s.l.* Bellardi, *Simulium stellatum* Gil Azevedo, Figueró & Maia-Herzog and *Simulium oyapockense s.l.* Floch & Abonnenc) and three species in the State of Minas Gerais (*Simulium duodenicornium* Pepinelli, Hamada & Trivinho-Strixino, *Simulium obesum* and *Lutzsimulium pernigrum* Lutz).

KEY WORDS: Aquatic insect, geographical distribuition, taxonomy

Knowledge about all life stages of an organism is important to clarify taxonomic problems, particularly in black flies where there are many species complexes (Hamada *et al*. 2003). Pupae and adults of black fly species in Brazil are relatively well known, especially the females, because some species in this family have medical and veterinary importance. However, knowledge about the larval stages usually is neglected, mainly due to the difficulty of distinguishing closely related species in this family, at this life stage.

In this paper we describe the larvae (last-instar) of two species, one belonging to the subgenus *Notolepria* (*S. cuasiexiguum* Shelley, Luna Dias, Maia-Herzog & Lowry) and the other to the subgenus *Chirostilbia* (*S. obesum* Vulcano).

The subgenus *Notolepria* is composed of nine species, only four of which occur in Brazil: *S. cuasiexiguum*, *S. exiguum* Roubaud, *S. incertum* Lutz and *S. paraguayense* Schrottky (Crosskey & Howard 2004). The subgenus *Chirostilbia* Enderlein is composed of 15 species found only in South America, 14 of which occur in Brazil (Crosskey & Howard 2004, Hamada & Pepinelli 2004, Hamada *et al*. 2006).

During ecological surveys carried out between 2002 and 2005, in the states of São Paulo and Minas Gerais (on the border with São Paulo) four black fly species were recorded for the first time in the first state and three, in the second.

One of the newly found black fly species in the state of São Paulo is *Simulium metallicum s.l.* Bellardi; this species is known from Central America and northern South America (e.g. Conn *et al*. 1989, Arteaga & Muñhoz de Hoyos 1999, Hamada & Fouque 2001, Hamada & Grillet 2001). There are at least 12 recognized cytotypes in this nominal species (Conn *et al*. 1989, Arteaga & Muñhoz de Hoyos 1999) and, in some regions, such as Mexico, Guatemala and northern Venezuela, it is incriminated as a vector of *Onchocerca volvulus* Leuckart (Dalmat 1955, Collins 1979, Grillet *et al*. 1995). Since in this paper we report the southern most geographical distribution for this species, we present a short morphological description of the larvae, pupae and adults

collected. Crosskey & Howard (1997, 2004) listed this species in the subgenus *Simulium*; however, Coscarón *et al*. (1999), in a phylogenetic study, suggested that the subgenus *Aspathia* Enderlein should be revalidated and all the Neotropical species that were formerly placed in the subgenus *Simulium* should be included in this subgenus. Recently, Adler *et al*. (2004) corroborated the Coscarón *et al*. (1999) conclusion.

Materials and Methods

The material examined was collected between 2002 and 2005 at different localities in the states of São Paulo and Minas Gerais (see Examined Material).

The techniques for collection and rearing of specimens are those detailed in Hamada & Pepinelli (2004) and Pepinelli *et al*. (2005). Images illustrating the morphology were obtained directly from specimens using either a Sony or a Nikon digital camera attached to either a dissecting or to a compound microscope. Some of the specimens in the study are deposited in the Invertebrate Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, AM, Brazil, and some specimens are deposited in the collection of the Laboratório de Entomologia Aquática da Universidade Federal de São Carlos (UFSCar), SP, Brazil.

Description

Simulium (*Notolepria*) *cuasiexiguum* Shelley, Luna Dias, Maia-Herzog & Lowry, 2001, Figs. 1–17. (Figs. 1–7)

Larva (last instar) (Figs. 1–7)**.** Length: 3.2–3.9 mm (mean = 3.5 mm, SD = 0.2, n = 5); head capsule lateral length: 0.30–0.33 mm (mean = 0.32 mm, SD = 0.05, n = 5); dorsal width: 0.58–0.61 mm (mean = 0.59 mm, SD = 0.07, n = 5). Body color varying from pale gray to dark gray, with intersegmental bands well defined (in Carnoy's solution). Head capsule (in dorsal view) pale, without pattern (Fig. 1). Postgenal cleft rounded, widest at middle (Figs. 2, 3), postgenal bridge 0.68 times as long as hypostoma (Fig. 3). Subesophageal ganglion pigmented (Fig. 2). Antenna as long as labral fan stalk; proportions of articles, proximal to distal, excluding apical sensillum, 1:1.1–1.2:1–0.9. Hypostoma (Fig. 4) with median tooth shorter than lateral teeth; two paralateral teeth; 4–5 lateral serrations and four setae (per side). Labral fan with 26–27 primary rays. Lateral mandibular process not seen. Pupal gill histoblast dark brown, dissected, with six filaments. Ventral posterior tubercle absent. Anterodorsal arms of anal sclerite shorter in length than posteroventral arms (Fig. 6). Posterior proleg bearing 70–72 rows (mean = 71, n = 3) with 11–13 hooks (mean = 12, n = 6). Anal papillae with three branches, each with 9–11 finger-shaped lobes (Fig. 5).

Diagnosis. The larva of *S. cuasiexiguum* is very similar to that of *S. exiguum*. Differences include the number of labral fan primary rays, Rondônia´s (Brazil) *S. exiguum* population has 41–42 rays, while Shelley *et al*. (1997) reported 30–43 rays. *S. cuasiexiguum* larvae examined had 26–27 labral fan primary rays. Both species can be distinguished by the number of gill filaments in the histoblast; after dissection, *S. cuasiexiguum* has six filaments (Fig. 7) and *S. exiguum* has eight (Fig. 8).

Simulim (*Chirostilbia*) *obesum* Vulcano, 1959, Figs. 1–44. (Figs. 9–16)

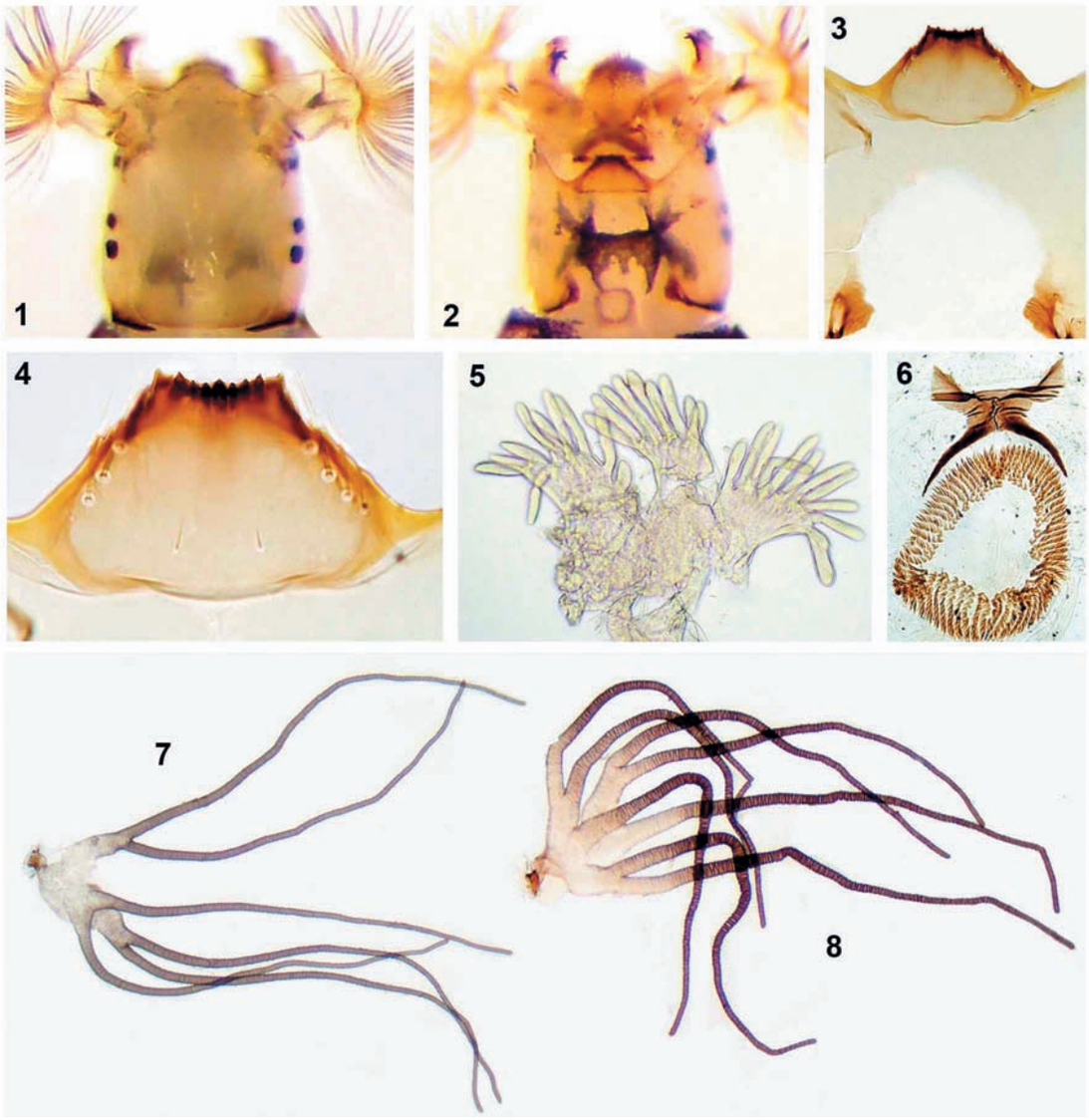
Larva (last instar) (Figs. 9–16)**.** Length: 9.3–10.6 mm (mean = 10 mm, SD = 0.5, n = 5); head capsule lateral length: 0.65–0.7 mm (mean = 0.67 mm, SD = 0.02, n = 5); dorsal width: 0.92–1.0 mm (mean = 0.97 mm, SD = 0.05, n = 5). General color dark brown (in Carnoy's solution). Head capsule (in dorsal view) with positive spots (Figs. 9, 11). Cervical sclerites small, elliptical, free in membrane, each with thin, elongated sclerite on its anterior region (Figs. 9, 11). Postgenal cleft shallow, with apex triangular or rounded, widest at its base (Figs. 10, 12); postgenal bridge 1.75 times as long as hypostoma (Fig. 12). Subesophageal ganglion not pigmented but area of postgenal cleft covered with dark membrane (Fig. 10). Antenna (Fig. 13) as long as labral fan stalk; proportions of articles, proximal to distal, excluding apical sensillum, 1:1.35–1.42:1.2–1.25. Hypostoma (Fig. 14) with median tooth larger than lateral teeth; two paralateral teeth; 4–5 lateral serrations and 9–11 setae (per side). Labral fan with 38–42 primary rays. One thick lateral mandibular process. Pupal gill histoblast dark brown with more than 100 filaments. Body covered with simple setae. Ventral posterior tubercle absent. Anterodorsal arms of anal sclerite shorter in length than posteroventral arms (Fig. 15). Posterior proleg bearing 149–151 rows (mean = 150, n = 3) with 18–22 hooks (mean = 20, n = 6). Anal papillae with three branches, each with approximately 20 finger-shaped lobes.

Diagnosis. The last-instar larvae of *S. obesum* can be distinguished from the known last-instar larvae of the other species in the subgenus *Chirostilbia* mainly by the shallow postgenal cleft (Figs. 10, 12), large postgenal bridge (1.75 times as long as hypostoma) and gill histoblast with, after dissection, more than 100 filaments (Fig. 16).

New Records of Black Fly Species in the States of São Paulo and Minas Gerais

Four species were collected for the first time in the state of São Paulo: *S. cuasiexiguum, S. metallicum s.l.*, *S. stellatum* Gil Azevedo, Figueró & Maia-Herzog and *S. oyapockense s.l.* Floch & Abonnenc. Three species were collected for the first time in the state of Minas Gerais: *S. duodenicornium* Pepinelli, Hamada & Trivinho-Strixino, *S. obesum* and *Lutzsimulium pernigrum* Lutz.

S. cuasiexiguum and *S. oyapockense s.l.* were collected in the Aguapeí River, which belongs to the Paraná River hydrological basin. The larvae and pupae of these species were collected from submerged deciduous leaves and roots

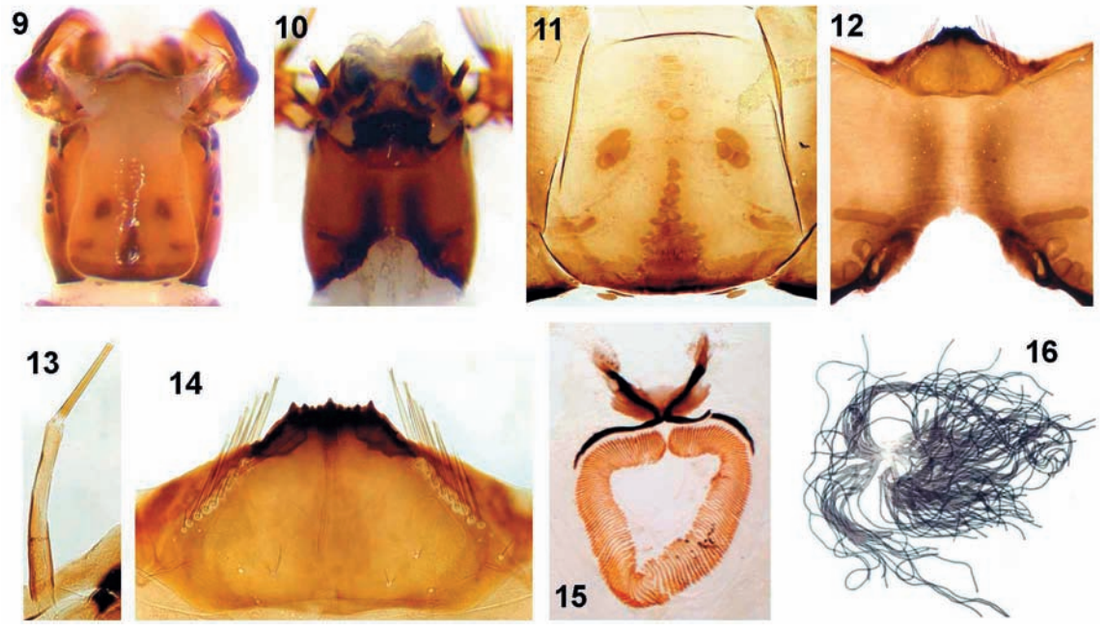


Figs. 1–8. *S. cuasiexiguum* larvae. Fig. 1: head capsule, dorsal view. Fig. 2: head capsule, ventral view. Fig. 3: postgenal cleft, ventral view. Fig. 4: hipostoma. Fig. 5: anal papillae. Fig. 6: anal sclerite. Fig. 7: dissected gill histoblast. Fig. 8: *S. exiguum* dissected gill histoblast.

and leaves of the streamside vegetation. *S. cuasiexiguum* was known from northern Goiás and Mato Grosso states, collected from small to medium-sized (10–100 m), fast-flowing rivers (Shelley *et al*. 2001). *S oyapockense s.l.* is a species complex (Shelley *et al*. 1997) with a wide geographical distribution, known from Venezuela, Guyana, Colombia, Ecuador, Brazil, French Guiana and Argentina (Crosskey & Howard 2004). This species has medical importance because it is one of the vectors of *O. volvulus* in the Amazonian onchocerciasis focus in Brazil and Venezuela (Shelley *et al*. 1997).

S. stellatum was collected in a small headwater stream, in a section of the stream with reduced flow, attached to leaves. This species was known only from its type locality, a stream in Itatiaia National Park, in Rio de Janeiro state.

S. metallicum s. l. was collected in two small headwater streams, one a tributary of the Rio Capivara in Botucatu municipality and one a tributary of the Rio Santa Maria in Brotas municipality, São Paulo state. Larvae and pupae were collected from deciduous and submerged leaves of the streamside vegetation. The only previous record of this species in Brazil was in Roraima state (Hamada & Grillet 2001). Some morphological structures are presented in Figs. 17–29 and are described here. Female scutum black, with anterior illumination, 1+1 median and 1+1 sublateral, silver whitish pruinose vittae extending from anterior to posterior margin of thorax (Fig. 17). With posterior illumination, the color of this pattern changes, i.e., areas that were silver



Figs. 9–16. *S. obesum* larvae. Fig. 9: head capsule, dorsal view. Fig. 10: head capsule, ventral view. Fig. 11: head capsule, positive-spot pattern. Fig. 12: postgenal cleft, ventral view. Fig. 13: antenna. Fig. 14: hypostoma. Fig. 15: anal sclerite. Fig. 16: dissected gill histoblast.

whitish turn black (Fig. 18). The female abdominal pattern, in lateral view, is shown in Fig. 21. The hypogynial valves (= gonapophyses) are shorter than the eighth sternite (Fig. 24). Cercus and anal lobe as in Fig. 23. Male scutum black; with anterior illumination with 1+1 submedian, silver pruinose cunae on anterior half (Fig. 19). With posterior illumination, thorax black and margin with silver pruinosity (Fig. 20). Male abdominal pattern, in lateral view is shown in Fig. 22. Genitalia black; gonocoxite subrectangular, gonostyle elongated, with single terminal spine (Fig. 25). Ventral plate and median sclerite as in Fig. 26. Larval head with positive pattern (Fig. 27), although some larvae showed no head pattern. Postgenal cleft rounded, postgenal bridge twice as long as hypostoma (Fig. 28). Pupal cocoon, flattened, rounded in dorsal view, with six gills (Fig. 29). Pupae of *S. metallicum s.l.* collected in the state of Roraima (Hamada & Grillet 2001) have gills shorter and distributed in a more open arrangement than the pupae collected in the state of São Paulo, whereas the larvae, females and males are similar.

S. duodenicornium was known only from its type locality, Joanópolis municipality, Cachoeira dos Pretos, in São Paulo state (Pepinelli *et al*. 2005). In Minas Gerais this species was collected in the Rio Lourenço Velho, Cachoeira Pilões, Itajubá municipality, using Podostemacea leaves and rock as substrate. Both rivers where this species was collected belong to the Rio Tietê hydrological basin.

S. obesum was collected in areas with fast-flowing waters in streams in Campos do Jordão and Santo Antonio do Pinhal municipalities, in São Paulo state and Camanducaia municipality, in Minas Gerais state, using bedrock as substrate. This species was collected previously in São Paulo, Rio de Janeiro, and Santa Catarina states (Vulcano 1959, Coscarón 1991).

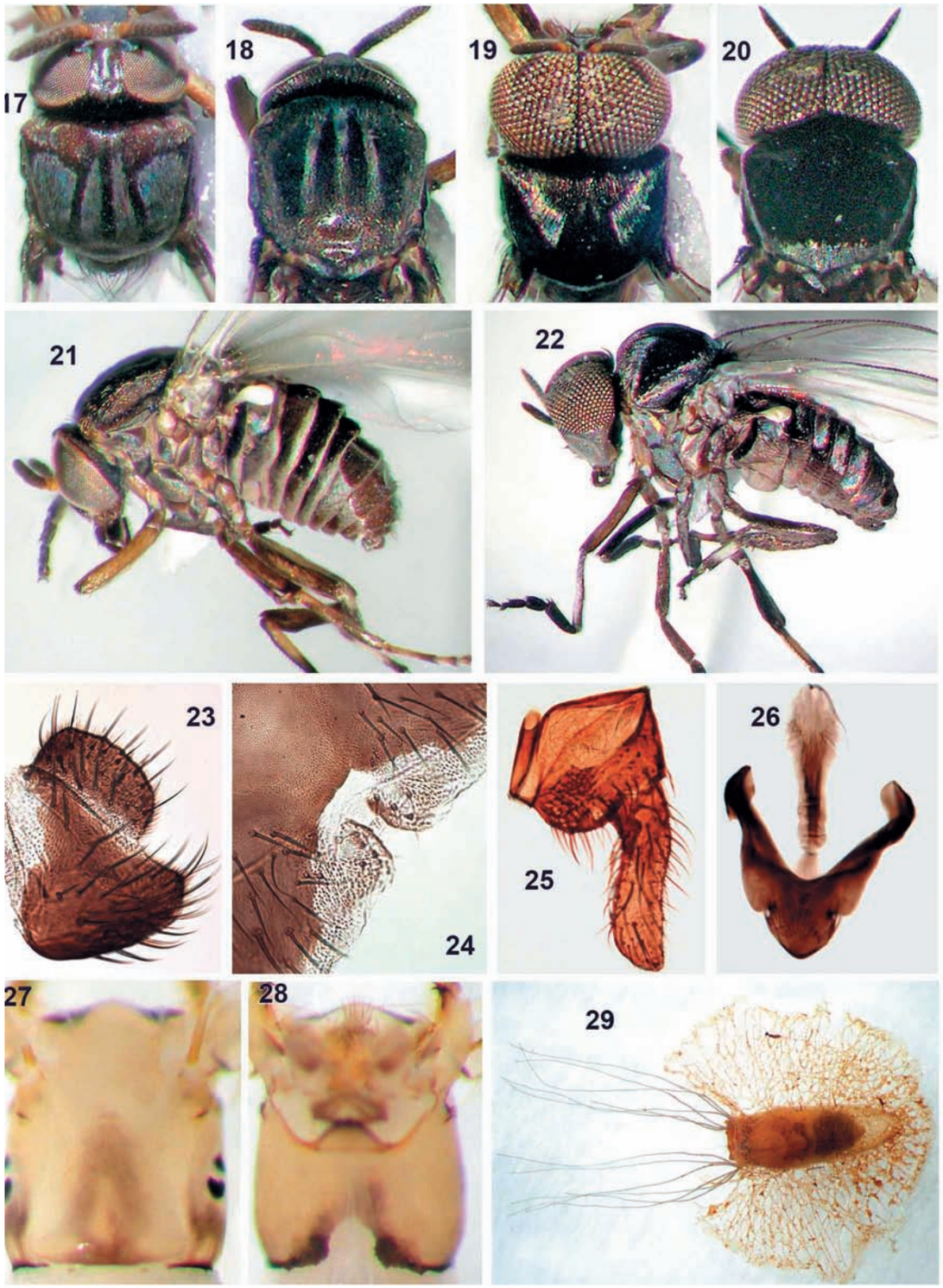
L. pernigrum was collected in a stream in Sariqui farm, Marmelópolis municipality in the state of Minas Gerais, attached to deciduous and submerged leaves of the streamside vegetation. This species was known from the states of São Paulo, Rio de Janeiro and Rio Grande do Sul (Crosskey & Howard 2004).

With these new black fly species records the number of species in Minas Gerais increases from 32 (Crosskey & Howard 2004, Gil-Azevedo *et al*. 2005) to 35 and in São Paulo from 51 (Crosskey & Howard 2004, Pepinelli *et al*. 2003, Hamada & Pepinelli 2004, Pepinelli *et al*. 2005) to 55.

Of the four species recently discovered in São Paulo, *S. metallicum s.l.* represents an unexpected finding, because it was known only from Central America and northern South America, representing a large increase in its geographical distribution.

Examined Material

Simulium cuasiexiguum. Brazil, São Paulo State, Lucélia municipality, Rio Aguapeí (Salto Botelho) (21°27'S; 50°55'W), 28.xii.2002, Hamada N, five last-instar larvae, on slide mounts (INPA); 03.iv.2004, Pepinelli M, Shimbori EM, 10 last-instar larvae, in ethanol (UFSCar).
Simulium duodenicornium. Brazil, Minas Gerais state, Itajubá municipality, Rio Lourenço Velho, Cachoeira Pilões (22°22'S; 45°18'W), 10.ix.2005, Pepinelli M, Hamada N, five last-instar larvae and five pupae in ethanol (INPA).
Simulium exiguum s.l.. Brazil, Rondônia State, Ji-Paraná



Figs. 17–29. *S. metallicum s.l.* Fig. 17: female scutum, dorsal view (anterior illumination). Fig. 18: female scutum, dorsal view (posterior illumination). Fig. 19: male scutum, dorsal view (anterior illumination). Fig. 20: male scutum, dorsal view (posterior illumination). Fig. 21: female, lateral view. Fig. 22: male, lateral view. Fig. 23: cercus and anal lobe, lateral view. Fig. 24: hypogynial lobes, ventral view. Fig. 25: gonocoxite and gonostylus. Fig. 26: ventral plate and median sclerite. Fig. 27: larva head, in situ, dorsal view. Fig. 28: larva head, in situ, ventral view. Fig. 29: pupa, dorsal view.

municipality, Rio Urupá (10º07'S; 61º54'W), 02.viii.2002, Hamada N, three last-instar larvae, in slide mount (INPA). *Simulium metallicum s.l.*. Brazil, São Paulo state, Botucatu municipality, Fazenda Indiana, small stream (22º54'S; 48º23'W), 11.v.2004, Hamada N, Pepinelli M, 10 last-instar larvae in ethanol, three pupae in ethanol, one female pinned with pupal exuviae, one male pinned with pupal exuviae, one male on slide mount, one pharate female on slide mount (INPA). Brotas municipality, small tributary of the right margin of Córrego Santa Maria, above the waterfall (22º18'S, 48º12'W), 08.v.2004, Hamada N Pepinelli M, thee last-instar larvae in ethanol (INPA).
Simulium obesum. Brazil, São Paulo state, Campos do Jordão municipality, Parque Estadual Campos do Jordão, Córrego Galharada (22º41'S; 45º27'W), 18.v.2004, Pepinelli M Hamada N, 10 last-instar larvae in ethanol; four last-instar larvae on slide mounts (UFSCar), 10 last-instar larvae in ethanol (INPA). Santo Antonio do Pinhal municipality, Cachoeira do Lefreve (22º49'S; 45º37'W), 17.v.2004, Pepinelli M, Hamada N, 10 last-instar larvae in ethanol and five last-instar larvae, on slide mounts (UFSCar), 10 last-instar larvae in Carnoy solution (INPA). Minas Gerais state, Camanducaia municipality, Monte Verde district, Mina d'água waterfall (22º52'S, 46º02'W), 22.v.2004, Pepinelli M, Hamada N, 10 last-instar larvae in ethanol (INPA).
Simulium oyapockense s.l.. Brazil, São Paulo state, Lucélia municipality, Rio Aguapeí, Salto Botelho (21º27'S; 50º55'W), 28.xii.2002, Hamada N, three last-instar larvae in ethanol, two last-instar larvae, on slide mounts (INPA).
Simulium stellatum. Brazil, São Paulo state, São José do Barreiro municipality, Bairro da Onça, small stream near the Escola Bairro da Onça (22º50'S; 44º33'W), 16.ix.2005, Hamada N, Pepinelli M, 1 pupa in ethanol (INPA).
Lutzsimulium pernigrum, Brazil, Minas Gerais state, Marmelópolis municipality, stream at Fazenda Sariqui (22º30'S; 45º09'W), 11.ix.2005, Hamada N, Pepinelli M, two last-instar larvae in ethanol (INPA).

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