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## ***Limnophyes guarani* sp. n., a new hygropetric Orthocladiinae from southern Brazil (Diptera: Chironomidae)**

LUIZ CARLOS PINHO<sup>1,3</sup> & TROND ANDERSEN<sup>2</sup>

<sup>1</sup>*Universidade Federal de Santa Catarina, Centro de Ciências Biológicas, Depto. de Ecologia e Zoologia, CEP 88040-901, Florianópolis - SC, Brazil*

<sup>2</sup>*Department of Natural History, University Museum of Bergen, University of Bergen, P.O. Box 7800, NO-5020 Bergen, Norway*

<sup>3</sup>*Corresponding author. E-mail: luiz.pinho@ufsc.br*

### **Abstract**

A new species of *Limnophyes* Eaton collected in the Corvo Branco Mountains in Santa Catarina State is described and figured, based on male and female adults, larva and pupa. The species groups with *L. griseata* (Edwards) and *L. bidumus* Sæther as the adults have a pronounced humeral pit with lanceolate setae and an additional group of lanceolate setae just posterior to antepronotum. The adults are, however, distinctly smaller, have a lower AR and have more lanceolate setae in the group just posterior to antepronotum than the two other species. The pupa can easily be separated from the pupa of *L. bidumus* as the anal macroseta is longer than the anal lobe. The larva lives hygropetric on vertical rock surfaces. A key to male adults of Neotropical *Limnophyes* is given.

**Key words:** taxonomy, Neotropical Region, immatures, key

### **Introduction**

The genus *Limnophyes* Eaton, 1875, with about 90 species, has a worldwide distribution. The Holarctic and Afrotropical species were reviewed by Sæther (1990a), the Neotropical species by Sæther (1990b). Eight species of *Limnophyes* are listed for the Neotropical region (Ashe & O'Connor 2012), four of which were originally described by Edwards (1931) and redescribed by Sæther (1990b). Of the remaining four species, two were described by Sublette and Sasa (1994) from Guatemala; the third is an uncertain record of the cosmopolitan *L. minimus* (Meigen, 1818) from Juan Fernández Island in Chile, and the last is *L. gercinoi* (Oliveira, Messias & Santos, 1995), described as *Corytibacladius* by Oliveira *et al.* (1995) and redescribed by Mendes *et al.* (2007).

Below we describe a new *Limnophyes* species from the Santa Catarina State in southern Brazil. The larva was collected in the Corvo Branco Mountains, where it lives hygropetric on vertical rock surfaces.

### **Methods and terminology**

Larvae were collected on rock surfaces with a thin layer of running water and reared in laboratory in small petri dishes with wet moss. The specimens were then preserved in alcohol and later mounted in Euparal following the procedure outlined by Sæther (1969). The general morphology follows Sæther (1980).

The holotype and paratype of the new species will be deposited in the Museu de Zoologia da Universidade de São Paulo (MZUSP).

### **Key to adult males of Neotropical *Limnophyes* Eaton**

1. Lanceolate setae clustered in dorsal humeral pit and/or with lanceolate setae clustered just behind middle of antepronotum,

	sometimes in a deep pit .....	2
1'.	Lanceolate setae not clustered in dorsal humeral pit or behind middle of antepronotum, sometimes with scattered lanceolate setae in humeral area .....	4
2.	Dorsal humeral pit without lanceolate setae, but with single lanceolate seta in humeral area; with cluster of about 14 lanceolate setae in deep pit behind middle of antepronotum. Antepronotal lobe with about 17 lateral setae (Sæther 1990b, fig. 1). Argentina .....	<i>L. collaris</i> (Edwards)
2'.	Dorsal humeral pit with cluster of 15–20 lanceolate setae; area behind middle of antepronotum with lanceolate setae, but never in deep pit. Antepronotal lobe with 3–7 lateral setae .....	3
3.	Wing length 0.97 mm. Antepronotal lobe with dorsal projection, with 7 strong dorsal setae (Fig. 1). Brazil .....	<i>L. guarani</i> sp. n.
3'.	Wing length 1.54 mm. Antepronotal lobe without dorsal projection, with 3 weak dorsal setae (Sæther 1990b, fig. 5). Argentina .....	<i>L. griseata</i> (Edwards)
4.	Humeral setae lanceolate .....	5
4'.	Humeral setae not lanceolate .....	7
5.	Antepronotal lobe with strong dorsal projection. Epimeron II with 3–4 setae. Squama with 0–2 setae (Mendes <i>et al.</i> 2007, figs 2–4). Brazil .....	<i>L. gercinoi</i> (Oliveira, Messias & Santos)
5'.	Antepronotal lobe not to moderately projected. Epimeron II with or without setae. Squama with 4–7 setae .....	6
6.	Antenna with 13 flagellomeres. Epimeron II without setae. Gonostylus with low, rounded, medially placed crista dorsalis (Sæther, 1990b, fig. 3). Argentina .....	<i>L. subnudicolis</i> (Edwards)
6'.	Antenna with 12 flagellomeres. Epimeron II with 5–7 setae. Crista dorsalis very weak (Sublette & Sasa 1994, figs 83–86). Guatemala .....	<i>L. mariae</i> Sublette & Sasa
7.	Front tibial spur reduced, 19 µm long. Posterior anespisternum II and epimeron II without setae. Acrostichals absent (Sæther 1990b, fig. 4). Argentina, Chile .....	<i>L. brachyarthra</i> (Edwards)
7'.	Front tibial spur normal, 30–52 µm long. Posterior anespisternum II and epimeron II with or without setae. Acrostichals present or absent .....	8
8.	Virga present, with 2 or 3 spines. Anal point proper absent (large elevation of tergite IX referred to as “anal point” by Sæther (1990a) present) (Sæther 1990a, fig. 23; Sæther 1975, figs 2–3, as <i>L. hudsoni</i> ). Holarctic, Afrotropical and ?Neotropical (uncertain record from Chile) .....	<i>L. minimus</i> (Meigen)
8'.	Virga absent. Anal point small and triangular (Sublette & Sasa 1994, figs 79–82). Guatemala .....	<i>L. guatemalensis</i> Sublette & Sasa

### *Limnophyes guarani* sp. n.

(Figures 1–18)

**Type material.** Holotype: male adult with pupal exuviae: Brazil, Santa Catarina State, Grão-Pará, Serra do Corvo Branco, 28°03'21" S 49°22'00" W, 1241m a.s.l., 07.x.2014, seepage, LC Pinho & A Pitaluga leg. (MZUSP). Paratypes: 1 female adult with larval and pupal exuvia, as holotype (MZUSP).

**Etymology.** The specific epithet refers to the Guarani Aquifer, that seeps at the road cut where the larvae were collected.

**Diagnostic characters.** The adults group with *L. griseata* (Edwards, 1931) and *L. bidumus* Sæther, 1990 in having a pronounced humeral pit with lanceolate setae and an additional group of lanceolate setae just posterior to antepronotum. They are, however, smaller with a wing length of 0.97 mm in the male and 0.85 mm in the female, compared to 1.54 mm in male *L. griseata*, on average 1.61 mm in male *L. bidumus* and 1.25 mm in female *L. bidumus*; the female of *L. griseata* is not known. The new species also has a lower AR, 0.24 in the male and 0.36 in the female, compared to 0.41 in male *L. griseata*, on average 0.60 in male *L. bidumus* and 0.45 in female *L. bidumus*. Further, the new species has a distinctly higher number of lanceolate setae in the group directly behind antepronotum, 17 in the male and 15 in the female, compared to 6 in male *L. griseata*, 2–6 in male *L. bidumus* and 3 in female *L. bidumus*. Further, *L. griseata* and *L. bidumus* have at most two short, dorsal antepronotals, while there are 7 and 10 comparatively long, dorsal setae in male and female *L. guarani* sp. n., respectively. It shares a dorsal projection of the antepronotal lobe with long, numerous dorsal setae with *L. gercinoi*, but this projection is much more pronounced in *L. gercinoi*.

The pupa of the new species can easily be separated from the pupa of *L. bidumus* as the anal macroseta is longer than the anal lobe, while in *L. bidumus* these setae are only about 0.6 times as long as the anal lobe. Further, L1–L5 on segment VIII increase in size for *L. guarani* sp. n., while in *L. bidumus* they are alternate in size; the pupa of *L. griseata* is not known. The only pupae with anal macrosetae longer than anal lobe, like *L. guarani*, are *L. asquamatus* Andersen, 1937, *L. spinigus* Sæther, 1990 and maybe *L. gurgicola* (Edwards, 1929).

The larvae of neither *L. griseata* nor *L. bidumus* are known. The larva of *L. guarani* sp. n. can be distinguished from other described *Limnophyes* larvae by its smaller size, with a head capsule length of 0.22 mm, while the lengths in the remaining described species range between 0.24 and 0.33 mm. Well developed supraanal macrosetae 235 µm long, procerus 25 µm high, AR 2.00, and antennal blade distinctly longer than flagellum will also help to characterize the larva of *L. guarani* sp. n.

**Description. Male** (n = 1). Total length 2.00 mm. Wing length 0.97 mm. Total length/wing length 2.06. Wing length/length of profemur 2.46.

*Colouration:* Blackish brown; wings light brown; legs uniformly dark brown except for trochanter and the very base of femora pale.

*Head.* Antenna with 12 flagellomeres, AR 0.24. Ultimate flagellomere 93 µm long. Temporal setae 4, including 1 inner vertical, 1 outer vertical and 2 postorbitals. Clypeus with 13 setae. Tentorium 100 µm long, 17 µm wide. Stipes 87 µm long, 32 µm wide. Palp segment lengths (in µm): 22, 25, 57, 65, 105. Third palpomere with 1 sensillum clavatum in apical third; 10 µm long.

*Thorax* (Figure 1). Antepronotal lobes slightly projected dorsally. Antepronotals consisting of 3 lateral and 7 dorsal setae. Acrostichals very small and difficult to observe, apparently 4 in mid scutum. Dorsocentrals consisting of 17 lanceolate close to antepronotum, 15 lanceolate in dorsal humeral pit, 18 lanceolate prescutellar setae and 13 simple setae; prealars 7, extending anteriorly; supraalar absent; preepisternum with 4 setae; posterior anepisternum II with 2 setae; epimeron II with 6 setae. Scutellum with 7 setae.

*Wing* (Figure 2). VR 1.42. Costal extension 47 µm long. R with 4 setae, brachiolum with 1 seta, other veins bare. Squama with 2 setae.

*Legs.* Spur of fore tibia 37 µm long, spurs of mid tibia 17 µm and 20 µm long, spurs of hind tibia 40 µm and 17 µm long. Width at apex of fore tibia 27 µm, of mid tibia 27 µm, of hind tibia 35 µm. Comb with 13 setae, longest 37 µm, shortest 15 µm. Tarsomere 4 shorter than 5. Lengths and proportions of legs as in Table 1.

**TABLE 1.** Lengths (in µm) and proportions of legs of *Limnophyes guarani* sp. n., male (n = 1).

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV	BR
p <sub>1</sub>	394	463	246	148	108	59	64	0.53	2.91	3.48	2.1
p <sub>2</sub>	414	424	187	99	69	39	49	0.44	3.47	4.48	2.5
p <sub>3</sub>	414	463	266	128	118	49	59	0.57	3.29	3.30	2.1

*Hypopygium* (Figures 3 and 4). Anal point low, broad based, bluntly triangular, covered with microtrichia and with about 12 marginal setae; laterosternite IX with 2 setae. Phallapodeme 67 µm long; transverse sternapodeme 62 µm long. Virga consisting of two spines, 27 µm long. Gonocoxite 110 µm long; inferior volsella of *minimus*-group type. Gonostylus 72 µm long; crista dorsalis absent; megaseta 15 µm long. HR 1.53; HV 1.81.

**Female** (n = 1). Total length 1.74 mm. Wing length 0.85 mm. Total length/wing length 2.05. Wing length/length of profemur 2.47.

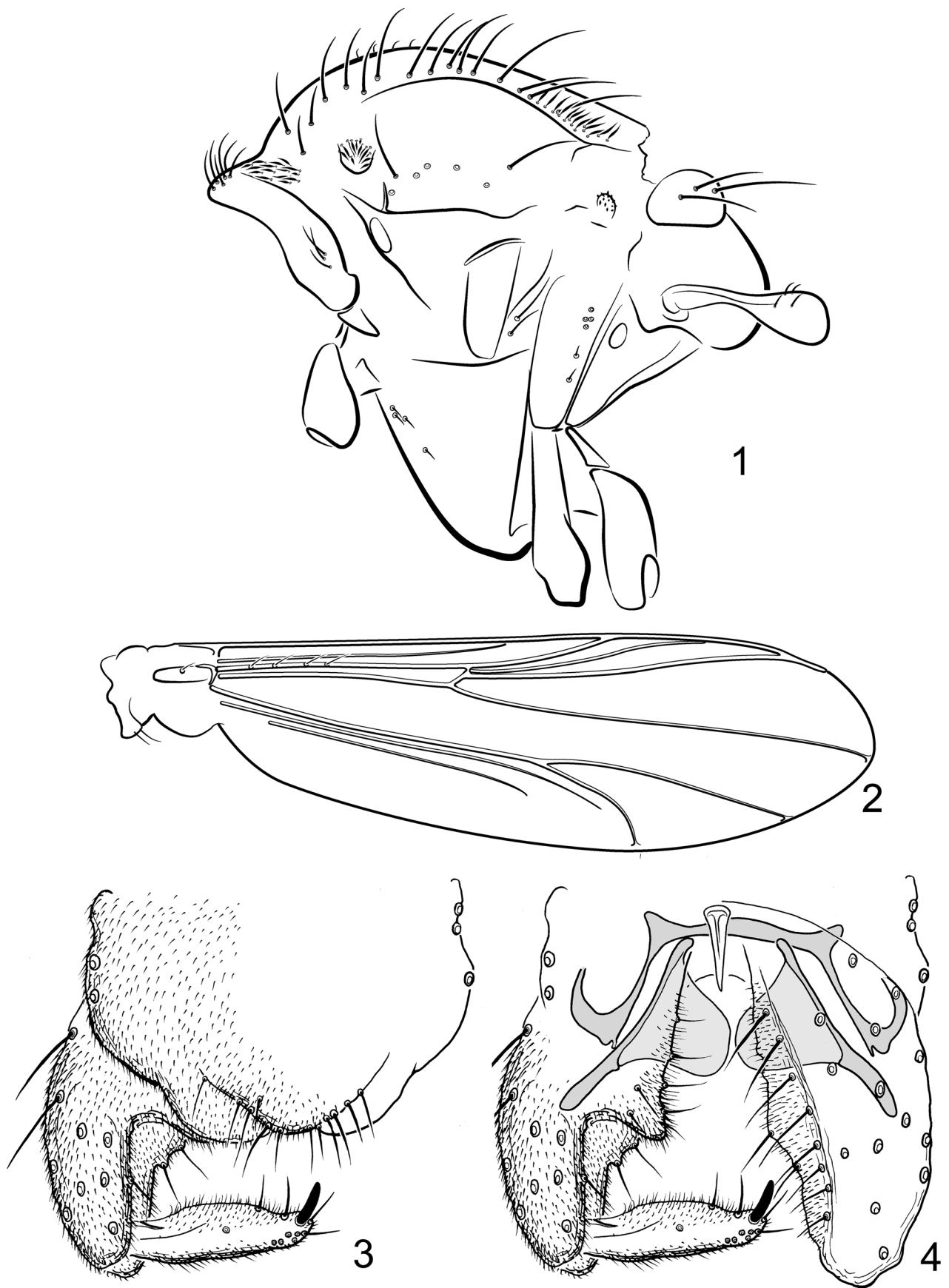
*Colouration:* As in male.

*Head.* AR 0.36. Flagellomere length/width (in µm): 37/22, 37/22, 42/20, 42/20, 57/25. Temporal setae 4, including 1 inner vertical, 1 outer vertical, and 2 postorbitals. Clypeus with 14 setae. Tentorium 75 µm long, 10 µm wide. Stipes 75 µm long, 30 µm wide. Palp segment lengths (in µm): 20, 22, 60, 62, 62. Third palpal segment with 1 sensillum clavatum in apical third, 12 µm long.

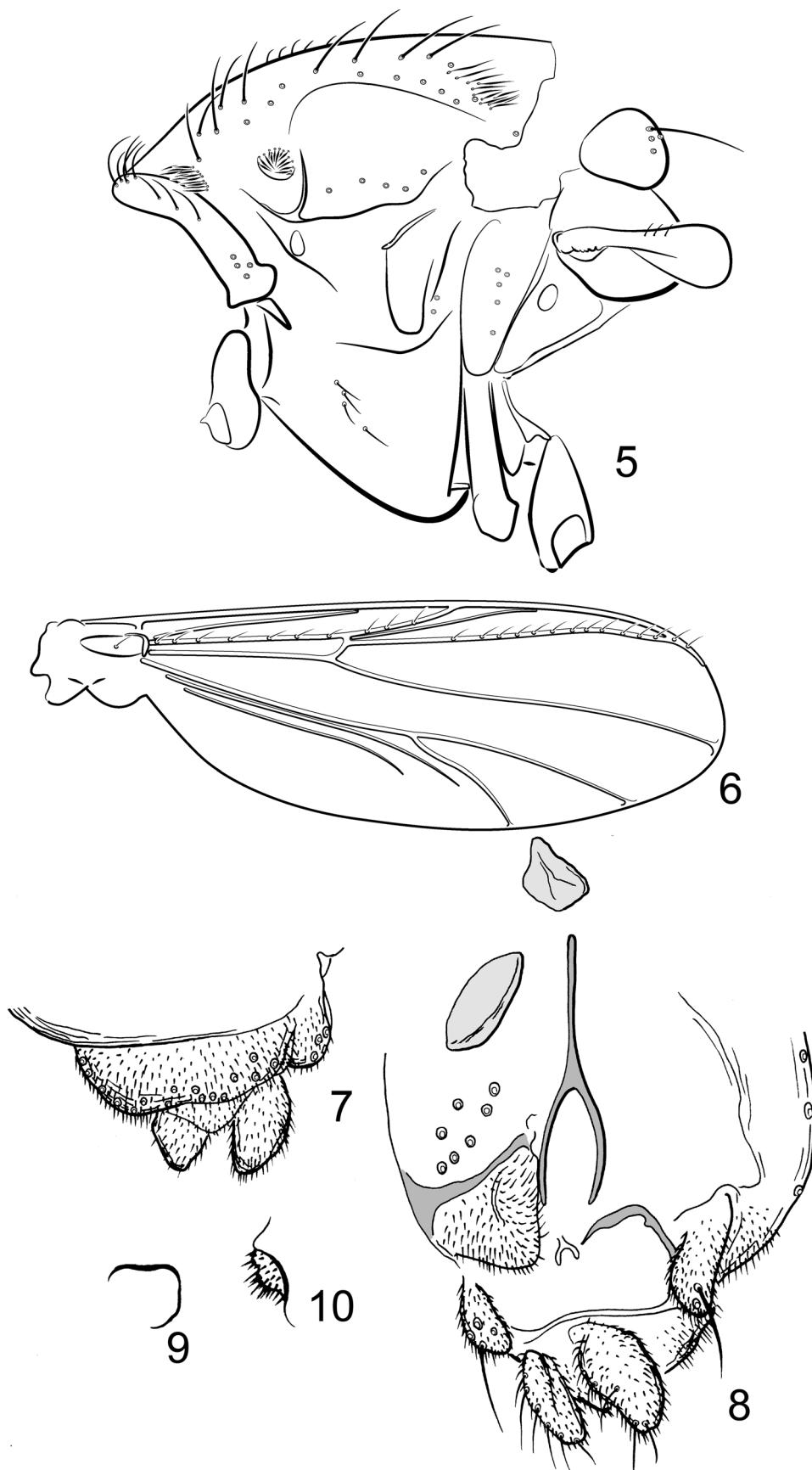
*Thorax* (Figure 5). Antepronotal lobes slightly projected dorsally. Antepronotals consisting of 4 lateral, 3 median, and 10 dorsal setae. Acrostichals 5 in mid scutum. Dorsocentrals consisting of 15 lanceolate close to antepronotum, 14 lanceolate in dorsal humeral pit, 12 lanceolate prescutellar setae and 19 simple setae; prealars 6, extended anteriorly; supraalar 1; preepisternum with 4 setae; posterior anepisternum II with 2 setae; epimeron II with 5 setae. Scutellum with 6 setae.

*Wing* (Figure 6). VR 1.36. Costal extension 80 µm long, with 2 non marginal setae. R with 7 setae, R<sub>1</sub> with 3 setae, R<sub>4+5</sub> with 12 setae, brachiolum with 1 seta, remaining veins bare. Squama bare.

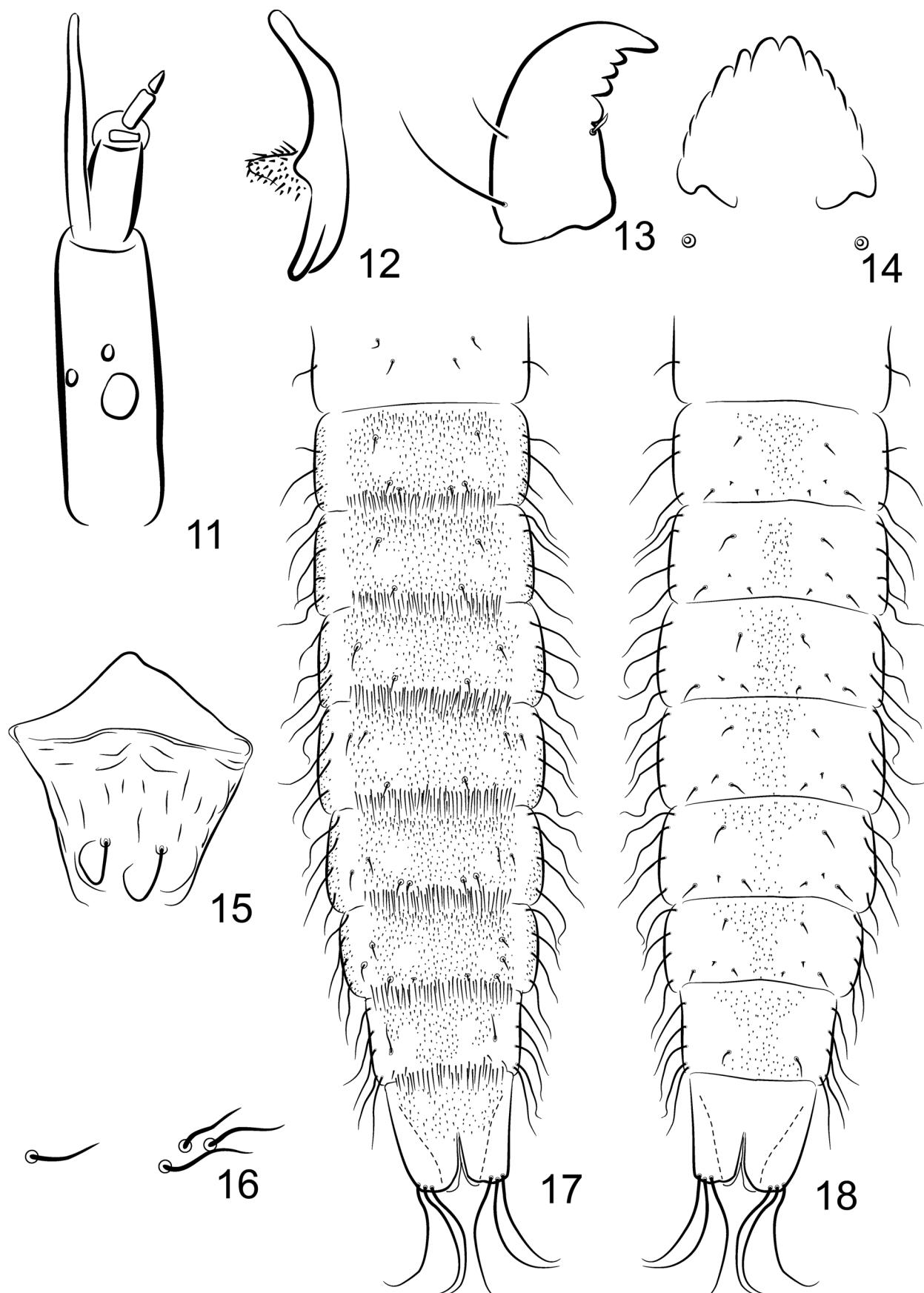
*Legs.* Spur of fore tibia 25 µm long, spurs of mid tibia 17 µm and 15 µm long, spurs of hind tibia 27 µm and 15 µm long. Width at apex of fore tibia 27 µm, of mid tibia 30 µm, of hind tibia 35 µm. Comb with 11 setae, longest 30 µm, shortest 22 µm. Tarsomere 4 shorter than 5. Lengths and proportions of legs as in Table 2.



**FIGURES 1–4.** *Limnophyes guarani* sp. n., male adult. 1, Thorax. 2, Wing. 3, Hypopygium, dorsal view. 4, Hypopygium with anal point and tergite IX removed, dorsal aspect to the left and ventral aspect to the right.



**FIGURES 5–10.** *Limnophyes guarani* sp. n., female adult. 5, Thorax. 6, Wing. 7, Genitalia, dorsal aspect. 8, Genitalia, ventral aspect. 9, Apodeme lobe. 10, Dorsomesal lobe.



**FIGURES 11–18.** *Limnophyes guarani* sp. n., larva (11–14) and pupa (15–18). 11, Antenna. 12, Premandible. 13, Mandible. 14, Mentum. 15, Frontal apotome. 16, Dorsocentrals. 17, Abdomen, dorsal view. 18, Abdomen, ventral view.

**TABLE 2.** Lengths (in  $\mu\text{m}$ ) and proportions of legs of *Limnophyes guarani* sp. n., female ( $n = 1$ ).

	fe	ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV	BR
p <sub>1</sub>	343	392	201	123	83	44	54	0.51	3.08	3.66	1.2
p <sub>2</sub>	353	358	162	74	54	34	49	0.45	4.14	4.39	1.6
p <sub>3</sub>	387	407	221	98	103	39	49	0.54	3.51	3.59	2.5

*Genitalia* (Figures 7–10). Gonocoxite IX 60  $\mu\text{m}$  long, with 3 strong and 2 weak setae. Tergite IX undivided, with 20 setae. Cercus 55  $\mu\text{m}$  long. Seminal capsules 50  $\mu\text{m}$  long, neck not observed. Notum 72  $\mu\text{m}$  long.

**Pupa** ( $n = 1$ –2). Total length 2.07–2.22 mm. Exuviae transparent.

*Cephalothorax*. Frontal setae (Fig. 15) 67–70  $\mu\text{m}$  long. Longest median antepronotal 68–92  $\mu\text{m}$  long. Distance between Dc<sub>1</sub> and Dc<sub>2</sub> 7–8  $\mu\text{m}$  (Fig. 16), between Dc<sub>2</sub> and Dc<sub>3</sub> 7–8  $\mu\text{m}$ , between Dc<sub>3</sub> and Dc<sub>4</sub> 55–60  $\mu\text{m}$ .

*Abdomen* (Figures 17–18). Tergite I bare, T II–VIII with coarse and extensive shagreen, T IX with relative coarse anterior shagreen. Sternite I bare; S II–VIII with fine, sparse median spinules; S IX bare. Number of caudal spines on T II–VIII as: 88–120, 99–103, 109–112, 91–103, 68–86, 61–72, 52–54. Maximum length of caudal spines 47–60  $\mu\text{m}$  long, slightly shorter on T II–III. Conjunctives II/III–VI/VII with 4–6 rows of spinules. Lengths (in  $\mu\text{m}$ ) of L<sub>1</sub> to L<sub>5</sub> on segment VIII as: 95–105, 100–112, 110–120, 125 (1), 132–149, increasing in size. L<sub>4</sub> absent in female. Anal lobe 132–154  $\mu\text{m}$  long; anal macrosetae 187–207  $\mu\text{m}$  long. Genital sac of male reaching apex of anal lobe; 40  $\mu\text{m}$  short of apex of lobe in female.

**Larva** ( $n = 1$ ). Total length 2.05 mm. Head capsule 0.22 mm long.

*Head*. Antenna as in Figure 11. Length of antennal segments (in  $\mu\text{m}$ ): 40, 10, 2, 6, 2. AR = 2.00. Basal antennal segment 13  $\mu\text{m}$  wide; distance from base to ring organ 15  $\mu\text{m}$ , to basal mark of seta 17  $\mu\text{m}$ , to distal mark 20  $\mu\text{m}$ . Blade 27  $\mu\text{m}$  long; accessory blade and apical style of second segment not distinguishable; Lauterborn organ 6  $\mu\text{m}$  long. S1 with branches reduced; median chaetulae laterales apparently smooth. Premandible (Fig. 12) 48  $\mu\text{m}$  long, with 2 teeth; premandibular brush present. Mandible (Fig. 13) blackish in apical half, 62  $\mu\text{m}$  long, with four inner teeth; seta subdentalis indistinguishable. Seta interna 6  $\mu\text{m}$  long. Mentum (Fig. 14) apparently with 12 teeth. Postmentum 132  $\mu\text{m}$  long.

*Abdomen*. Procerus 25  $\mu\text{m}$  high, 20  $\mu\text{m}$  wide, with 6 anal setae, 270  $\mu\text{m}$  long. Supraanal seta 235  $\mu\text{m}$  long. Longest body seta 55  $\mu\text{m}$  long.

**Distribution and bionomics.** The larvae were collected in the Corvo Branco Mountains in southern Brazil, where they were found in the thin water film running down the vertical rock surface in a road cut. Larvae of *Podonomus* sp. and an undescribed species of Thaumaleidae were also found at the same site.

## Discussion

According to Sæther (1990a) *Limnophyes* species are often difficult to distinguish from each other due to extensive intraspecific variation. The number of lanceolate humerals and prescutellars can be highly variable in the same species, but is still of great importance in species delimitation, as for *L. guarani*. The number of lanceolate humerals close to antepronotum found in the male of *L. guarani* sp. n. (17) differs quite strongly from the number found in *L. griseata* (6) and in *L. bidumus* (2–6). Even larger variation is found in the same species for lanceolate prescutellars and humerals restricted to the pit area, however these characters in addition to the diagnostic characters for the immatures strengthen the differentiation between the species.

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