

# New species of *Nanocladius* Kieffer, 1913 (Diptera: Chironomidae: Orthoclaadiinae) from Neotropical region

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**Abstract** – The genus *Nanocladius* Kieffer, 1913 (Diptera: Chironomidae: Orthoclaadiinae) has been frequently found in aquatic habitats of the Neotropical region. However, until now only one species was described living symphoretically on Ephemeroptera and no free-living species has been cited or formally described from the region. In the present study, two new species of *Nanocladius* are described and figured as male, pupa and larva: *N. communis* and *N. longispicula*. The specimens were collected from different streams in Brazil and are the first recorded species of the genus to the country.

**Key words:** Brazil / Chironomidae / *Nanocladius* / immature stages / taxonomy

## Introduction

Non-biting midges of the genus *Nanocladius* are minute dipterans with worldwide distribution. Sæther (1977) first revised the genus and defined two subgenus: *Nanocladius* str. usually with free-living larvae in lotic and lentic habitats (Cranston *et al.*, 1983), and *N. Plecopteracoluthus*, whose immatures are found living symphoretically on the larvae of Plecoptera, Megaloptera and Ephemeroptera (*e.g.*, Steffan, 1965; Epler, 1986; Hayashi, 1998). Prior to the present study *Nanocladius* comprised 34 species, of which only *N. (Plecopteracoluthus) bubrachiatatus* Epler occurs in the Neotropical region (Ashe and O'Connor, 2012). No species was formally described from the Australian region, although several morphotypes are known (*e.g.*, Cranston, 1996).

In Brazil, numerous ecological studies have recorded the larvae of *Nanocladius*, living symphoretically on other insects (Callisto and Goulart, 2000; Dorvillé *et al.*, 2000) or free-living on stones, litter, sediments and aquatic macrophytes in reservoirs and rivers (*e.g.*, Sanseverino and Nessimian, 1998, 2001; Callisto *et al.*, 2002; Henriques-Oliveira *et al.*, 2003; Suriano and Fonseca-Gessner, 2004; Janke and Trivinho-Strixino, 2007). In addition, several morphotypes were described (Ospina-Torres, 1992; Wiedenbrug, 2000). Although the larvae of *Nanocladius* are common in the benthos fauna, until the present study no species was described or cited from Brazil.

The Holarctic species of the genus were described and revised by several authors (*e.g.*, Sæther, 1977; Niitsuma, 1991; Harrison, 1994; Fu and Wang, 2009), but a comprehensive revision of the genus is still not available. In the present study, two new species of *Nanocladius* from Brazil are described and figured as adult, pupa and larva.

## Materials and Methods

Adults, pharate adults and pupal exuviae were collected with drift nets. Living larvae were collected with hand nets and isolated in small boxes (1 cm × 1 cm) half filled with stream water. Neither substratum nor food was given, except for some detritus carried over with the water. The boxes were checked twice a day for the emerged specimens. Larval skins, pupal exuviae and adults were preserved in 96% ethanol. The specimens examined were slide-mounted in Euparal.

Morphological terminology and abbreviations follow Sæther (1980). Data on larvae represent the 4th instar. Measurement methods followed Epler (1988). Mensural data are given as ranges, followed by the number of observed specimens in parenthesis if different from the number (*n*) stated at the beginning of the description. Seta counts are given as the ranges only. The holotypes and paratypes are deposited in the Museum of Zoology of the University of São Paulo (MZUSP), Brazil.

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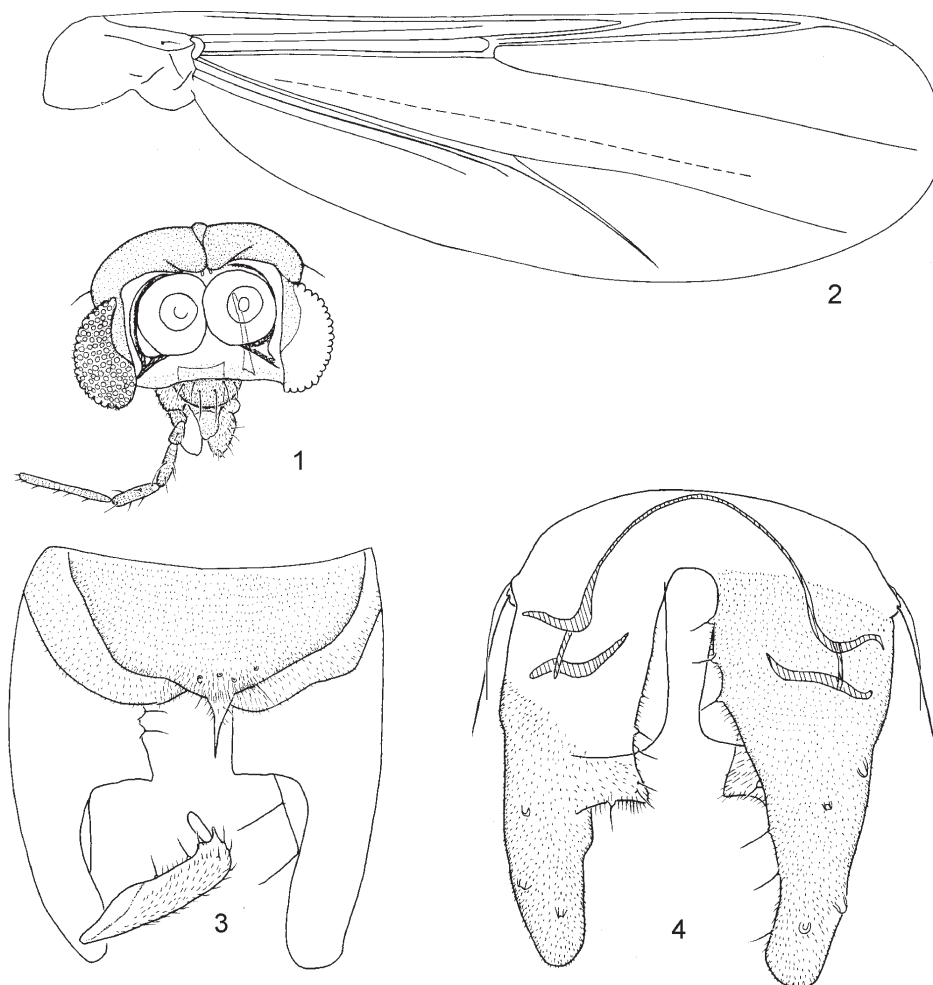


Fig. 1–4. *Nanocladius (N.) communis* sp. n., male. 1 head, 2 wing, 3 hypopygium (dorsal view), 4 hypopygium (ventral view).

## Taxonomy

*Nanocladius (N.) communis* sp. n. (Figs. 1–15)

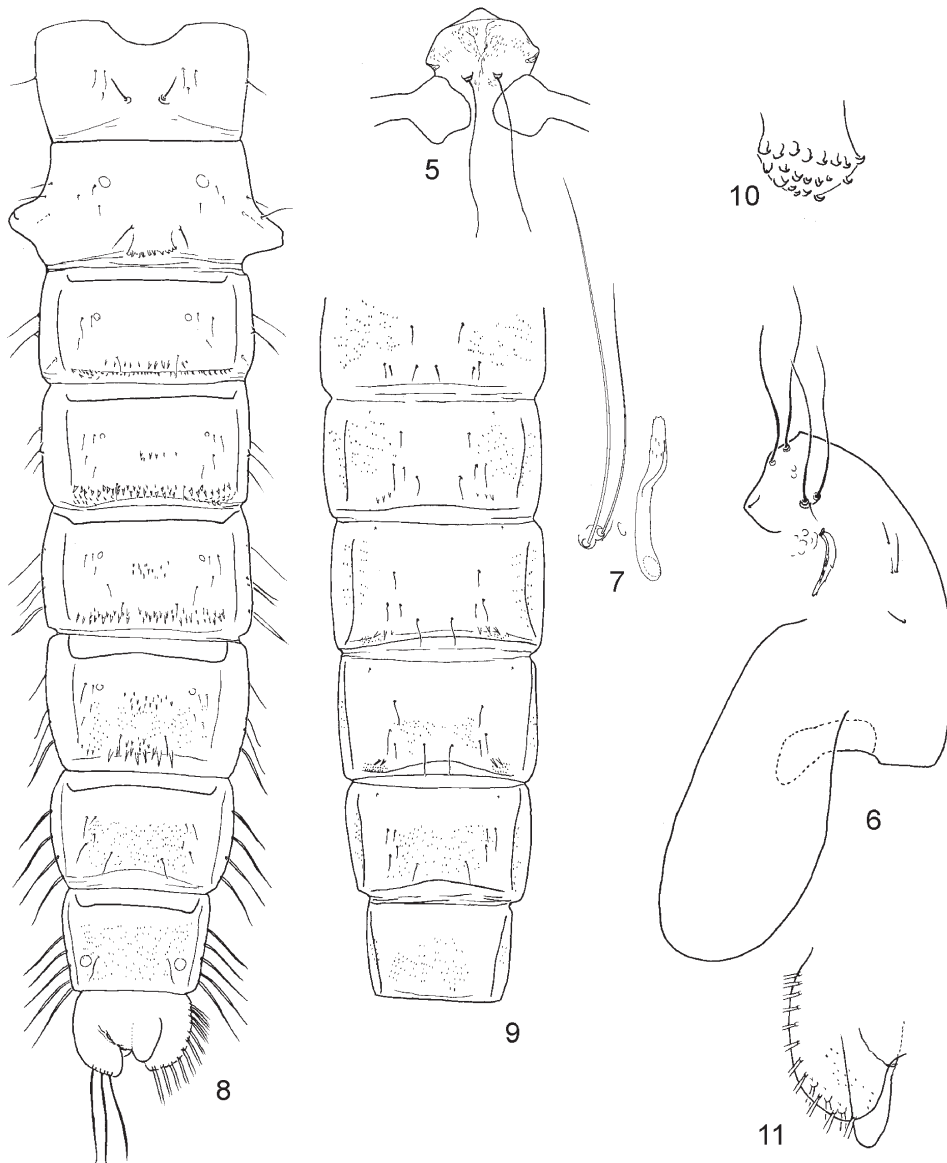
*Nanocladius (N.)* spec. 1 Wiedenbrug (2000)

**Type material.** Holotype male with pupal and larval exuviae, Brazil, São Paulo, São Carlos, Fazenda Canchin, 21°57.996'S, 47°50.604'W, 858 m a.s.l., 19.v.2011, S. Wiedenbrug. Paratypes: one male with pupal exuviae same data as holotype except for Ubatuba, stream near Ruínas da Lagoinha, 23°30.468'S, 45°11.923'W, 0 m a.s.l., xii.2000. Four pupal exuviae as previous except for Sertão da Quina, Cachoeira da Renata, bridge, 23°31.231'S, 45°14.625'W, 33 m a.s.l., 2.v.2007, two of them in the same slide as the paratypes of *N. (N.) longispicula* sp. n. One pharate male same data as before but 23°30.789'S, 45°14.442'W, 61 m a.s.l., 29.xii.2010.

**Additional material examined.** Five pupal exuviae, Brazil, Rio Grande do Sul, Taquara, Arroio do Mineiro, 15.xi.1994, S. Wiedenbrug. One pupal exuviae, same data as before but, São Francisco de Paula, Arroio dos Carros.

**Diagnostic characters.** The male of *N. N. communis* can be separated from other species of the genus by the following character combination: apical setae on antenna brown, straight and much shorter than apical flagellomere, AR 0.6, tergites with four to six setae uniserial distributed, phallapodeme without oral projections, inferior volsella triangular, anal point narrow, short, basally with microtrichia. The pupa can be distinguished due to the digitiform thoracic horn with spines, pedes spurii B conspicuous, hook row on protuberance, posterior lateral seta taeniate on segment VI and usually on V, tergites IV–VI with median spine-patches and posterior row of spines, on male pupa posterior ventral surface of anal lobe with shagreen points. The following characters are diagnostic for the larva: AR 1.14, ventromental plates very long rounded at caudal apex, with a ridge parallel to the anterior margin, premandible faint bifid, mentum with lateral teeth well defined, claws of anterior parapods serrated.

**Etymology.** Derived from the Latin *communis*, meaning common, and refers to a customary occurrence of this species in the benthic fauna of the sampled streams.



**Fig. 5–11.** *Nanocladius (N.) communis* sp. n., pupa. 5 frontal apotome, 6 thorax, 7 detail of thoracic horn and precorneals, 8 abdomen segments I–VIII (dorsal view) and anal lobe (left, dorsal view right, ventral view), 9 abdomen segments III–VIII (ventral view), 10 detail of hook row and 11 detail of anal lobe (ventral view, male).

## Description

*Adult male* ( $n = 2$  unless otherwise stated)

*Size.* Total length 1.10–1.18 mm. Wing length 0.89–0.90 mm. Total length/wing length 1.57–1.70. Wing length/length of profemur 2.31 (1).

*Coloration.* Head brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally dark brown; anteprenotum pale brown. Wing membrane transparent without spots and veins pale brown. Legs pale brown. Posterior margin of TIV–TV, anterior margin of TVI, TVIII and TIX brown, other tergites light brown. Gonocoxite and gonostylus light brown (1).

*Head* (Fig. 1). Eyes hairy. Temporal setae 1. Tentorium 120–125  $\mu\text{m}$  long, stipes not measurable. Small frontal tubercles present. Clypeus 38–40  $\mu\text{m}$  long, 68  $\mu\text{m}$  wide

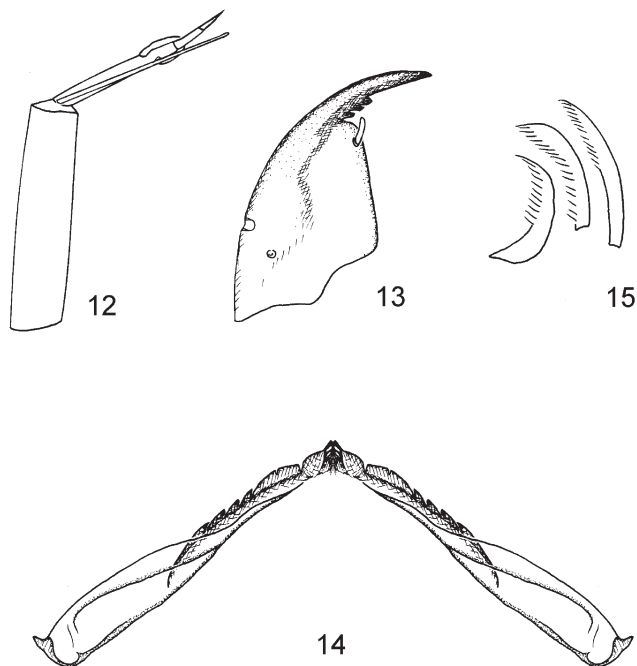
at largest part, bearing six to seven setae. Cibarial pump with anterior margin straight, 88–113  $\mu\text{m}$  long. Palpomere lengths 1–5 (in  $\mu\text{m}$ ): 23–25; 33; 53–58; 63–70; 120 (1). Antenna with 13 flagellomeres, flagellum 570–580  $\mu\text{m}$  long, apex slightly expanded, with short sensilla chaetica, setae on apex much shorter than last flagellomere, straight and light brown. Diameter of pedicel 80–100 (2)  $\mu\text{m}$ . AR 0.63–0.66.

*Thorax.* Anteprenotum with one lateral seta. Mid scutum with a scar, acrostichals not visible. Dorsocentrals 4–5, regularly uniserial. Prealars 1. Anapleural suture ratio 0.71. Scutellum with two setae. Anepisternals, preepisternals and postnotals absent.

*Wing* (Fig. 2). Width 0.31–0.32 mm. Costa 0.82–0.85 mm long, produced beyond  $R_{4+5}$ , ending very slightly beyond the tip of  $M_{3+4}$ .  $R_{2+3}$  not observed. VR 1.14–1.18.

**Table 1.** Lengths (in  $\mu\text{m}$ ) and proportions of leg segments in *Nanocladius communis* n. sp., adult male.

	Fe	Ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>
p <sub>1</sub>	300 (1)	380 (1)	250 (1)	210 (1)	140 (1)
p <sub>2</sub>	290–310	290–300	150 (1)	90 (1)	60 (1)
p <sub>3</sub>	310–340	390	200–210	120	80
	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV
p <sub>1</sub>	70 (1)	50 (1)	0.66 (1)	1.98 (1)	2.72 (1)
p <sub>2</sub>	20 (1)	20 (1)	0.52 (1)	3.95 (1)	4.00 (1)
p <sub>3</sub>	50	40	0.51–0.54	3.14–3.21	3.33–3.65

**Fig. 12–15.** *Nanocladius* (*N.*) *communis* sp. n., larva. 12 antenna, 13 mandible, 14 mentum and ventromental plates, and 15 claws of anterior parapods.

Brachiolum with one setae (1). Squama with one seta (1), veins bare.

**Legs.** Foreleg: width at apex of tibia 40  $\mu\text{m}$ , tibia with single, apical and pectinate spur 28 (1)  $\mu\text{m}$  long. Midleg: width at apex of tibia 30–40  $\mu\text{m}$ , tibial spur unmeasurable. Hindleg: width at apex of tibia 30–40  $\mu\text{m}$ , tibia with single, apical and pectinate spur 25–30  $\mu\text{m}$  long; comb with 10–11 setae. Legs with slender, hook-shaped claws. Pulvilli present. Lengths and proportion of leg segments as in Table 1.

**Abdomen.** Setae on tergites uniserial: Number of setae on tergite I–VIII: 4, 6, 6, 6, 5, 5, 4.

**Hypopygium** (Figs. 3 and 4). Tergite IX with four to five dorsal setae. Laterosternite with two setae. Anal point narrow and tapering toward the apex, with some microtrichia on anterior half. Sternapodeme narrow and convex anteriorly, without oral projections. Phallapodeme not observed. Superior volsella rounded, inferior volsella triangular. Gonocoxite stout, 88–115  $\mu\text{m}$  long, 33–40  $\mu\text{m}$  wide. GcR 2.69–2.88. Gonostylus 40–45  $\mu\text{m}$  long; megaseta enlarged, 8–10  $\mu\text{m}$  long. HR 1.95–2.88. HV 2.88–4.05.

**Pupa** ( $n = 6$  unless otherwise stated)

**Size.** Abdomen 0.85–0.98 (5) mm long

**Coloration.** Exuviae and thoracic horn light brown.

**Cephalothorax** (Figs. 5–7). Frontal apotome granulose, frontal setae 207  $\mu\text{m}$  long (1), on the small tubercles on the frontal apotome. Thorax smooth, slightly granulated on circular area ventral to the thoracic horn. Ocular field with two postorbital setae. Anteprenotum with two median setae and one lateral seta. Three precorneal setae, the longest 0.56 (1). Four dorsocentrals, Dc<sub>2</sub> and Dc<sub>3</sub> near each other. Prealar absent. Wing sheath smooth, 350–620  $\mu\text{m}$  long. Thoracic horn digitiform with scattered, pale spinules, 85–125  $\mu\text{m}$  long and 10  $\mu\text{m}$  (3) wide.

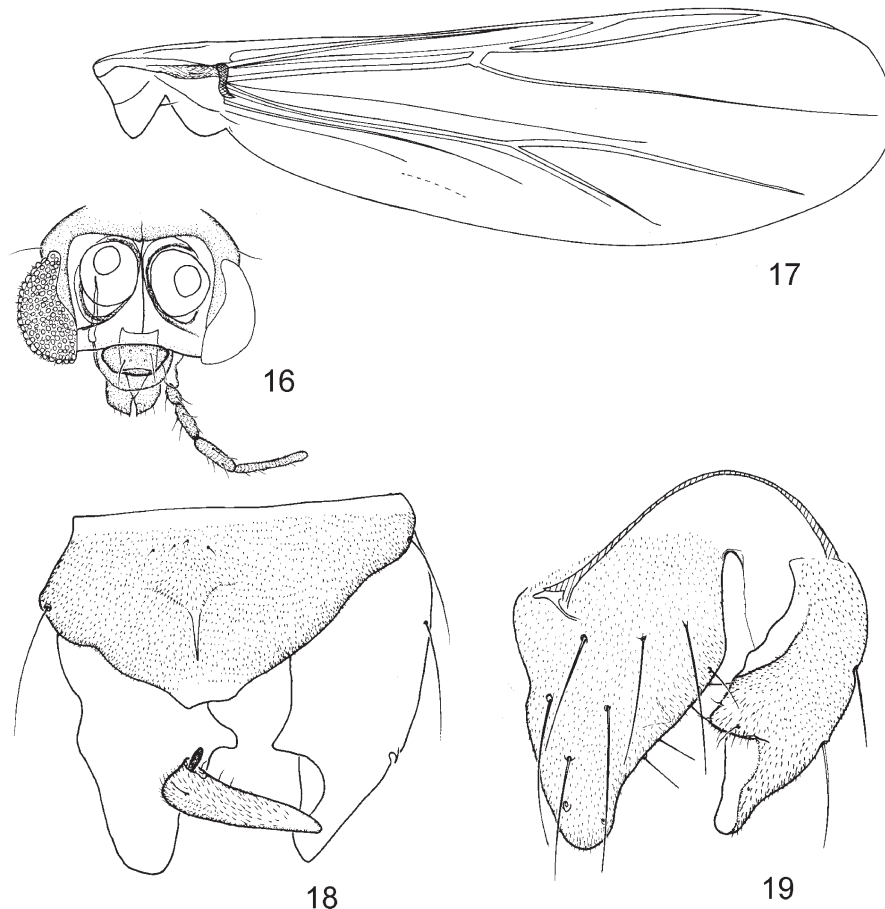
**Abdomen** (Figs. 8–11). Tergite and sternite I bare. TII with distinct median protuberance with up to three rows of small hooklets. TIII with a transverse row of spines on the posterior margin. TIV–VI posterior margin with about two irregular rows of spines, spines size increasing on posterior tergites. TIII–V with a few and sparse shagreen points (not drawn). TIV–VI with median patches of small spines. TVI–VIII with faint and sparse shagreen medially. Conjunctives on tergites III–IV, with transverse rows of small, curved hooklets, tergite IV with conjunctive hooklet rows usually medially interrupted. Tergite IX oral with a few points of very fine shagreen (not drawn). Pedes spurii B present on segment II. Sternites II–V with shagreen points distributed laterally, medially distributed on sternites VI–VIII, sternites IV–VI with a few posterolateral elongated spines. Abdominal chaetotaxy as in Figs. 8 and 9. Abdominal segment V with three non-taeniate lateral setae or with one additional posterior short taeniate lateral setae. Segment VI with one taeniate and three non-taeniate lateral setae, VII with four and VIII with five taeniae. Anal lobe 80–100 (5)  $\mu\text{m}$  long, fringe of 8–14 taeniae. Three pairs of strong anal macrosetae about 180  $\mu\text{m}$  (1) long. Anal lobe of male pupa with small points on posterior ventral surface.

**Larva** ( $n = 1$ )

**Coloration.** Head pale yellow, without maculation; postoccipital margin brown. Distal tooth of mandible and mentum brown. Posterior parapod claws all pale yellow.

**Head.** Length 300  $\mu\text{m}$ , 190  $\mu\text{m}$  wide. Surface smooth. All of the head capsule setae simple. Cephalic index 0.64.

**Antenna** (Fig. 12). Length 75  $\mu\text{m}$ , A<sub>1</sub> 40  $\mu\text{m}$  long, with ring organ placed 25  $\mu\text{m}$  from base, A<sub>2</sub> 30  $\mu\text{m}$  long. AR 1.14.



**Fig. 16–19.** *Nanocladius (N.) longispicula* sp. n., male. 16 head, 17 wing, 18 hypopygium (dorsal view) and 19 hypopygium (ventral view).

**Maxilla.** Maxillary palp elongated, with one a-seta. Chaetulae of palpiger and pecten galearis not observed.

**Labrum.** Labral sclerites not separate; with S1 and S2 simple setae. SI–SIII unobserved. Pecten epipharyngis with three simple, weakly pointed spines. Premandible faintly divided apically.

**Mandible** (Fig. 13). Length 68  $\mu\text{m}$ , apical tooth much longer than combined length of three inner teeth. Seta subdentalis pointed, no seta interna.

**Mentum** (Fig. 14). With one broad, partially double apical tooth and six pairs of distinct lateral teeth. Ventromental plate broad, long, tapering toward the mentum, with one long ridge parallel to the anterior margin and one or two small ridges perpendicular to it, on the lateral margin.

**Body.** Body segments with short simple setae. Parapods developed; anterior claws serrated (Fig. 15). Procercus and anal tubules not observed.

**Adult female.** Unknown

**Remarks.** In the key to *Nanocladius* by Sæther (1977), the following characters on male and pupa place *N. communis* in the subgenus *Nanocladius*: excavated genae, well-developed pulvilli and scutellum with two setae, pedes spurii B well developed, row of hooklets on protuberance, TIV–VI with median patches of spines

and TVII without caudal spines. The male of *N. (N.) pubescens* Makarchenko et Makarchenko, 2004 is similar to *N. N. communis* by having microtrichia on the anal point, inferior volsella triangular, sternapodeme rounded without oral projection and similar mensural features. However, *N. (N.) pubescens* can be distinguished by the gonostylus tapering toward the apex and the microtrichia present also on the distal half of the anal point. Immatures of *N. (N.) pubescens* are not known. According to Sæther (1977), the pupa of *N. (N.) communis* keys between *N. (N.) rectinervis* (Kieffer) and *N. (N.) spinipennis* Sæther. The taeniate posterior setae on segment V is mentioned by Sæther (1977) for *N. rectinervis* but the spines usually found on the ventral surface of the anal lobe seem to be characteristic to *N. N. communis*. Brundin (1966, p. 632) figured a pupa of *Nanocladius* from Patagonia, which is similar to *N. (N.) communis*; however, the thoracic horn of Brundin's morphotype is smooth and spinules on the anal lobe ventral surface are apparently absent.

***Nanocladius (N.) longispicula* sp. n.** (Figs. 16–35)

**Type material.** Holotype male with pupal exuviae, Brazil, São Paulo, Ubatuba, Sertão da Quina, near Cachoeira da Renata, 23°30.789'S, 45°14.442'W,

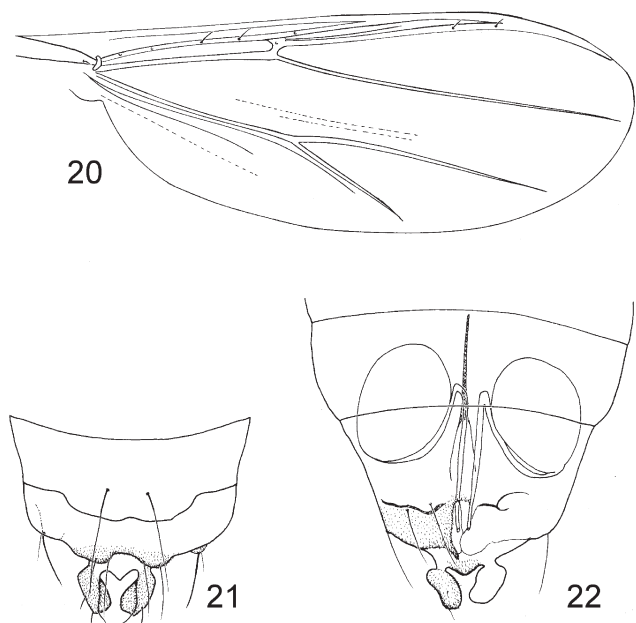


Fig. 20–22. *Nanocladius (N.) longispicula* sp. n., female. 20 wing, 21 genitalia (dorsal view) and 22 genitalia (ventral view).

61 m a.s.l., 23.iii.2008, S. Wiedenbrug. Paratypes: two female with pupal and larval exuviae same data as holotype except for São Carlos, Fazenda Canchin, 21°57.996'S, 47°50.604'W, 858 m a.s.l., 19.v.2011. Two pupal exuviae same data as holotype except for 2.v.2007 in the same slide with the paratypes of *N. (N.) communis* sp. n.

**Additional material examined:** One pharate male, with pupal and larval exuviae, Brazil, Mato Grosso do Sul, Bodoquena, Fazenda Califórnia, “2° riacho da trilha da Gruta”, 1.iv.2012, S. Wiedenbrug (SISBIOTA-Project). Two pupal exuviae, Rio Grande do Sul, Taquara, Arroio do Mineiro, 15.xi.1994, S. Wiedenbrug.

**Diagnostic characters.** The following characters are diagnostic for the male of *N. (N.) longispicula*: apical seta on antenna, brown, straight and shorter than last flagellomere, AR 0.34, tergites with four to six setae distributed uniserially, phallapodeme without oral projections, inferior volsella triangular, anal point narrow, short, without microtrichia. The pupa of *N. (N.) longispicula* can be separated from the pupae of other known species by having thoracic horn short, digitiform, with a few scale-shaped spines, pedes spuri B absent, hook row on protuberance, tergites III–VI with transverse row of long and strong spines on posterior margin, median patches of spines present only on tergite VI. The mentum with well-defined lateral teeth and the semi-ovoid shaped ridges on ventromental plate distinguish the larvae of *N. (N.) longispicula* from other species.

**Etymology.** Derived from the Latin *longis* meaning long and *spiculum* meaning sharp pointed, referring to the transverse row of long and strong spines on the pupal abdominal tergites.

## Description

*Adult male (n = 1)*

**Size.** Total length 1.10 mm. Wing length 0.81 mm. Total length/wing length 1.72. Wing length/length of profemur 2.61.

**Coloration.** Head brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally dark brown; anteprenotum pale brown. Wing membrane transparent without spots and veins pale brown. Legs pale brown. Abdomen brown. Hypopygium light brown.

**Head (Fig. 16).** Temporal setae 1. Tentorium 90 µm long, stipes not measurable. Frontal tubercles absent. Clypeus 63 µm long, 70 µm wide at largest part, bearing ten setae. Cibarial pump with anterior margin straight, 115 µm long. Palpomere lengths 1–5 (in µm): 18; 28; 50; 63; 95. Antenna with 13 flagellomeres, flagellum 470 µm long, apex slightly expanded, with short sensilla chaetica, diameter of pedicel 100 µm. AR 0.34.

**Thorax.** Anteprenotum with one lateral seta. Acrostichals 2, slightly scalpellate on mid scutum. Dorsocentrals 4, regularly uniserial; prealars 1. Anapleural suture ratio 0.79. Scutellum with two setae (1 per side). Anepisternals, preepisternals and postnotals absent.

**Wing (Fig. 17).** Width 0.28 mm. Costa 0.75 mm long, produced beyond  $R_{4+5}$ , ending very slightly beyond the tip of  $M_{3+4}$ .  $R_{2+3}$  along  $R_{4+5}$  (not drawn). VR 0.97. Brachiolum with one seta. Squama with one seta, veins bare.

**Legs.** Foreleg: width at apex of tibia 40 µm, tibia with single, apical and pectinate spur 18 µm long. Midleg: width at apex of tibia 40 µm, tibial spur unmeasurable. Hindleg: width at apex of tibia 40 µm, tibia with single, apical and pectinate spur 38 µm long; comb with 12 setae. Legs with slender, hook-shaped claws. Pulvilli present. Lengths and proportion of leg segments as in Table 2.

**Abdomen.** Number of setae on tergites III–VIII: 6, 6, 6, 5, 5, 4, tergites I and II not observed.

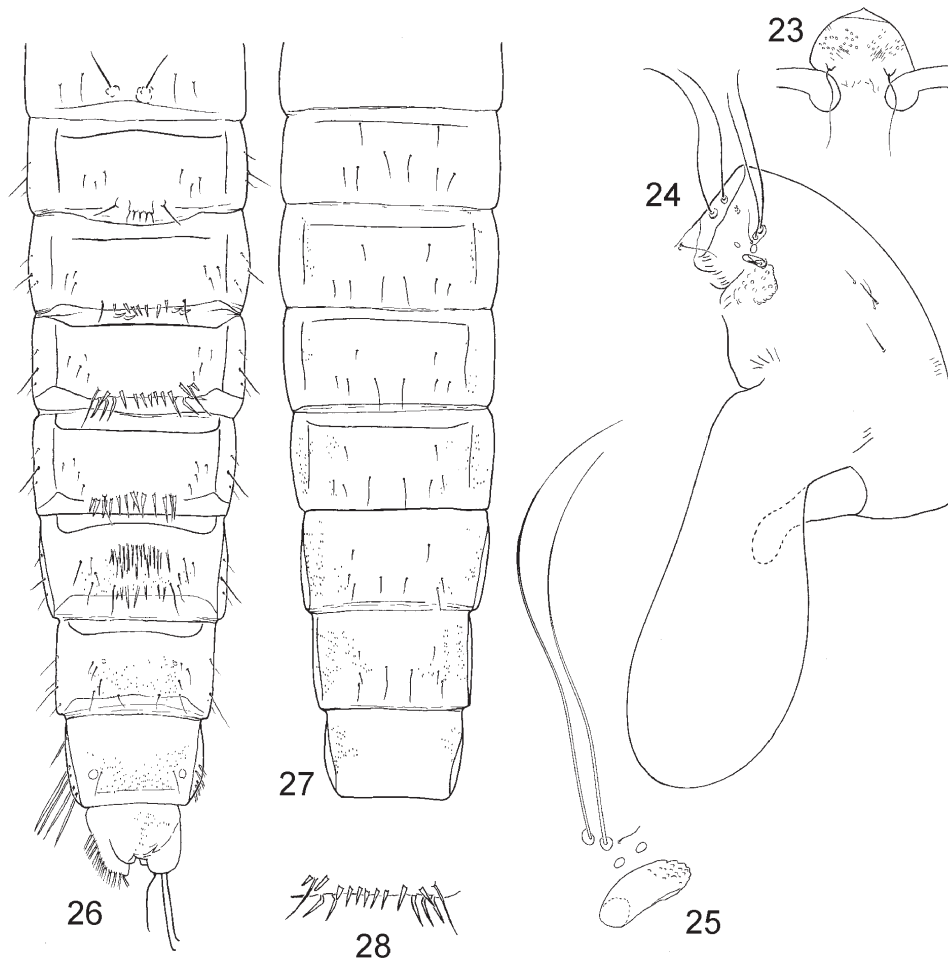
**Hypopygium (Figs. 18 and 19).** Tergite IX with four dorsal setae. Anal point narrow, short, bare and tapering toward the apex. Sternapodeme narrow and convex anteriorly, oral projections absent. Gonocoxite stout, 73 µm long, 43 µm wide. GcR 1.71. Gonostylus 38 µm long; megaseta enlarged, 13 µm long. HR 1.94. HV 3.71.

*Adult female (n = 2 unless otherwise stated)*

**Size.** Total length 0.71 (1) mm. Wing length 0.70–0.72 mm. Total length/wing length 1.02 (1). Wing length/length of profemur 1.00–1.40.

**Coloration.** Head, pedicel and antenna brown; maxillary palp pale brown. Thorax brown with mesonotum dorsally dark brown; anteprenotum pale brown. Wing membrane transparent without spots and veins pale brown. Legs pale brown. Abdomen wholly brown. Seminal capsules brown.

**Head.** Temporal setae 1. Tentorium and stipes unmeasured. Clypeus 63–65 µm long, 48–50 µm wide at largest part, bearing six setae. Cibarial pump with anterior margin straight, 78–80 µm long. Palpomere lengths



**Fig. 23–28.** *Nanocladius (N.) longispicula* sp. n., pupa. 23 frontal apotome, 24 thorax, 25 detail of thoracic horn and precorneals, 26 abdomen segments I–VIII (dorsal view) and anal lobe (left: ventral view, right: dorsal view), 27 abdomen segments I–VIII (ventral view) and 28 detail of postero-median margin of tergite III.

**Table 2.** Lengths (in  $\mu\text{m}$ ) and proportions of leg segments in *Nanocladius longispicula* n. sp., adult male.

	Fe	Ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>
p <sub>1</sub>	310	370	240	220	150
p <sub>2</sub>	290	290	150	90	50
p <sub>3</sub>	320	400	200	120	90
	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV
p <sub>1</sub>	90	50	0.65	1.81	2.83
p <sub>2</sub>	30	30	0.52	3.65	3.87
p <sub>3</sub>	50	40	0.50	3.07	3.60

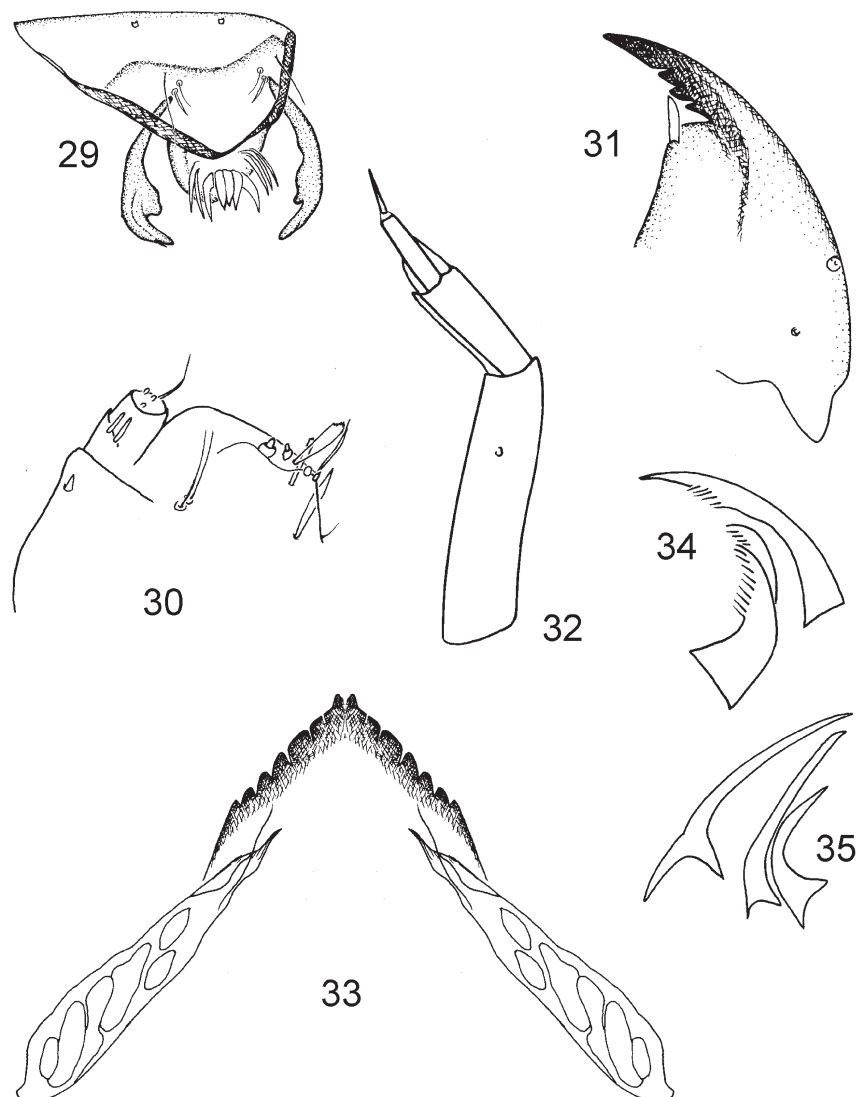
1–5 (in  $\mu\text{m}$ ): 15–20; 25; 43–50; 53–55; 90–93. Antenna with five flagellomeres, flagellum 188–215  $\mu\text{m}$  long, apex slightly expanded, with short sensilla chaetica, diameter of pedicel 48–53  $\mu\text{m}$ . AR 0.67–0.87.

**Thorax.** Antepronotum with one lateral seta. Acrostichals, two scalpellate setae on mid-scutum. Dorsocentrals 4, regularly uniserial; prealars 1. Anapleural suture ratio 0.75–0.83. Scutellum with two setae. Anepisternals, preepisternals and postnotals absent.

**Wing** (Fig. 20). Width 0.28–0.29 mm. Costa 0.65–0.70 mm long, produced beyond R<sub>4+5</sub>, ending very

beyond the tip of M<sub>3+4</sub>. R<sub>2+3</sub> present. VR 1.04–1.23. Brachiolum with one seta. R with 3–4 setae, R<sub>1</sub> with 1–2 and R<sub>4+5</sub> with 2–3. Squama not observed.

**Legs.** Foreleg: width at apex of tibia 40–50  $\mu\text{m}$ , tibia with single, apical and pectinate spur 18 (1)  $\mu\text{m}$  long. Midleg: width at apex of tibia 40  $\mu\text{m}$ , tibial spur 15–25  $\mu\text{m}$  long. Hindleg: width at apex of tibia 40  $\mu\text{m}$ , tibia with single, apical and pectinate spur 28–30  $\mu\text{m}$  long; comb with 12 setae. Legs with slender, hook-shaped claws. Pulvilli present. Lengths and proportion of leg segments as in Table 3.



**Fig. 29–35.** *Nanocladius (N.) longispicula* sp. n., larva. 29 labrum, 30 maxilla, 31 mandible, 32 antenna, 33 mentum and ventromental plates, 34 claws of anterior parapods and 35 claws of posterior parapods.

**Table 3.** Lengths (in  $\mu\text{m}$ ) and proportions of leg segments in *Nanocladius longispicula* n. sp., adult female.

	Fe	Ti	ta <sub>1</sub>	ta <sub>2</sub>	ta <sub>3</sub>
p <sub>1</sub>	230	270–300	190	120–130	90
p <sub>2</sub>	240	230–240	110	60	40–50
p <sub>3</sub>	220–250	280–290	130–140	70	50
	ta <sub>4</sub>	ta <sub>5</sub>	LR	BV	SV
p <sub>1</sub>	50	40	0.63–0.70	2.23–2.40	2.63–2.78
p <sub>2</sub>	20	20–40	0.46–0.52	3.53–3.70	3.93–4.45
p <sub>3</sub>	40	40–50	0.45–0.50	3.19–3.20	3.79–3.92

**Genitalia** (Figs. 21 and 22). Coxosternapodeme narrow, 85 (1)  $\mu\text{m}$  long. Cerci 25  $\mu\text{m}$  long and 13  $\mu\text{m}$  wide; with ten setae. Notum length 58 (1)  $\mu\text{m}$ . Seminal capsules large, spherical, with curved spermathecal ducts, length 33–65  $\mu\text{m}$ , maximum width 20–50  $\mu\text{m}$ . Length ratio SCa/No 1.13 (1). Tergite IX with two setigerous protrusions each with two setae. Gonocoxite IX, large with two setae.

*Pupa* ( $n = 5$  unless otherwise stated)

*Size.* Abdomen 0.81–0.94 mm long

*Coloration.* Exuviae and thoracic horn pale brown.

**Cephalothorax** (Figs. 23–25). Frontal apotome granu-lose, frontal setae on frontal apotome 80–112  $\mu\text{m}$  (2) long on the small tubercles. Thorax smooth, slightly granulated on the circular area ventral to thoracic horn. Ocular field



with two postorbital setae. Antepnotum with two long median setae and one lateral seta. Three precorneal setae, the longest about 260  $\mu\text{m}$  (1). Four dorsocentrals,  $\text{Dc}_2$  and  $\text{Dc}_3$  close together. Prealar absent. Wing sheath smooth, 450–610  $\mu\text{m}$  long. Thoracic horn digitiform, with few scale-shaped spines, 22–62 (3)  $\mu\text{m}$  long and 7–17  $\mu\text{m}$  wide. Thoracic horn ratio 2.50–3.58 (3).

**Abdomen** (Figs. 26–28). Tergites I–V and sternite I without shagreen. Tergites VI–IX with faint and sparse shagreen medially. TII with distinct protuberance with one row of small, curved spines. TIII–VI with transverse row of long and strong spines on posterior margin. Tergite VI with a median patch of long and fine spines. Tergite conjunctives III–IV with a few long and curved hooklets laterally distributed. Sternites II–VIII with weak shagreen, usually laterally distributed. Pedes spurii B absent on segment II. Abdominal chaetotaxy as in Figs. 26 and 27,  $\text{D}_5$  setae arising from a tubercle, smaller on the posterior segments. Abdominal segment VII with four taeniate setae, VIII with one anterior lateral taeniate seta and four posterior lateral taeniae. Anal lobe 80–100  $\mu\text{m}$  long, with a fringe of 10–13 taeniae. Three pairs of anal macrosetae.

*Larva* ( $n = 2$  unless otherwise stated)

**Coloration.** Head pale yellow, without maculation; postoccipital margin brown. Distal tooth of mandible and mentum brown. Posterior parapod claws all pale yellow.

**Head.** Length 240–260  $\mu\text{m}$ , 150–190  $\mu\text{m}$  wide. Surface smooth. All of the head capsule setae simple. Cephalic index 0.63–0.73.

**Antenna** (Fig. 32). Length 48–50  $\mu\text{m}$ ,  $\text{A}_1$  25–33  $\mu\text{m}$  long, with ring organ placed 20–23  $\mu\text{m}$  from base,  $\text{A}_2$  15–18  $\mu\text{m}$  long. AR 1.12–1.86.

**Maxilla** (Fig. 30). Maxillary palp elongated, with one a-seta. Bisensillum, seta maxillaris 1 and 2, antaxial setae, lacinial chaetae and sensillum basiconicum as figured.

**Labrum** (Fig. 29). S1 and S2 simple setae. SI–SIII unobserved. Pecten epipharyngis with three simple, weakly pointed spines. Premandible faintly divided apically.

**Mandible** (Fig. 31). Length 48–68  $\mu\text{m}$ , apical tooth as long as three inner teeth combined. Seta subdentalis pointed, no seta interna.

**Mentum** (Fig. 33). With one broad double apical tooth and six pairs of lateral teeth. Ventromental plate broad, long, tapering toward the mentum, with semi-ovoid ridges.

**Body.** Body segments with short simple setae. Parapods developed; longer anterior claws well serrated (Fig. 34), shorter basal claws simply hooked; posterior claws without serration (Fig. 35). Procercus and anal tubules not observed.

**Remarks.** *Nanocladius longispicula* belongs to the subgenus *Nanocladius* str. as defined by Sæther (1977) by having excavated genae, well developed pulvilli and scutellum with two setae. Although, the pupa has characters typical from species belonging to *Nanocladius* str. such as the hook row seated on protuberance, caudal

spines absent on TVII and TVI with median patches of spines, the absence of pedes spurii B is considered characteristic of the subgenus *N. plecopteracoluthus*. The fact that the larvae of the species here described were free-living corroborates with the position of the species *N. longispicula* in the subgenus *Nanocladius* str.

**Ecology and distribution.** Larvae of both species here described were collected from the surface of stones, in fast-flowing mountain and lowland streams, in the Atlantic Forest, São Paulo State. These species seem to have a wide distribution on streams of the mountains along the Brazilian coast since the pupal exuviae of both species were also collected in mountain streams in the same biome far south at Rio Grande do Sul State. In addition, larvae of *N. longispicula* were collected in small streams with stony bottom and coarse sand of a Semideciduous Forest in Mato Grosso do Sul, Brazil.

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