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On a collection of enchytraeids (Oligochaeta) from first order streams in São Paulo State, Brazil

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Abstract. A collection of 21 ethanol-preserved specimens of enchytraeids collected in first order streams in São Paulo State, Brazil, was whole-mounted on slides and investigated under light microscope. Eight species taxa were distinguished, belonging to *Achaeta*, *Guaranidrilus*, and *Marionina*. Five species could be named, one of them tentatively: *Achaeta neotropica* Černosvitov, 1937, *Achaeta singularis* Schmelz, 2008, *Guaranidrilus oiepe* Righi, 1974, *Marionina argentea* (Michaelsen, 1889) s.l., *Marionina* cf. *seminuda* Xie & Rota, 2001. Three further unnamed species taxa of *Guaranidrilus* were distinguished; two of them may be new species. The presence of sexually mature specimens of *A. singularis* allowed an emendation of the original description which was based on juvenile specimens. The rod-shaped crystals in the coelom of *A. singularis* are similar to raphides in plants and sponges, and may consist of calcium oxalate. The material is deposited in the Zoological Museum of the University of São Paulo, Brazil.

Keywords. Annelida, Clitellata, freshwater biodiversity, South America, raphides.

INTRODUCTION

Enchytraeids are frequently found in samples from South American freshwater sediments (Brinkhurst & Marchese 1989, Gorni & Alves 2008). In small streams with oxygen-rich water up to 30–50% of the oligochaete fauna may consist of enchytraeids (Rodrigues *et al.* 2015, 2016). However, species are rarely identified because of the lack of identification guides and taxonomic expertise in South America (Brown *et al.* 2013). Furthermore, enchytraeids are best identified *in vivo*, whereas the other aquatic oligochaetes are identified on fixed material. As a result, while 171 species and subspecies of other microdrile oligochaete families have been counted in South American freshwaters (Christoffersen 2007), not more than 7 species of Enchytraeidae have been recorded from freshwater sediments (Table 1).

Here we report on a small collection of ethanol-preserved enchytraeids sampled in first-order forest streams of the State of São Paulo, southeastern Brazil. Sexually adult specimens that could be identified to species are described and illustrated. The investigation profited from previous work on terrestrial enchytraeids in the neighbouring Paraná State in the framework of the SOLOBIOMA project (Römbke *et al.* 2007, Schmelz *et al.* 2008, 2009, 2011). It turned out that almost half of all specimens belonged to a species discovered during that project: *Achaeta singularis* Schmelz, 2008 (Schmelz *et al.* 2008). It had been described based on juvenile specimens, and the presence of adults in this collection allowed to complement the original description with details of the reproductive organs. This species is peculiar by rod-shaped crystals in the coelom. They are re-described and their possible function is discussed. Furthermore, a single adult specimen of *Marionina argentea* sensu lato is described. The remaining specimens in the collection are characterized briefly.

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Table 1. List of enchytraeid species identified from freshwater sediment samples in South America, based on Christoffersen (2009) and original literature.

Species	Reference	Habitat, comments
Buchholzia appendiculata (Buchholz)	Martínez-Ansemil & Giani 1986	small stream in Bolivia
Enchytraeus buchholzi Vejdovský	Christoffersen 2009	Argentina, Peru, Bolivia, Venezuela. Species complex.
Enchytraeus gordioides Černosvitov	Černosvitov 1941	sediment of a small stream in Loreto, Argentina
Fridericia bisetosa (Levinsen)	Botea 1987	Venezuela, stream and phreatic sediment. Records doubtful.
Hemienchytraeus loksai Dózsa-Farkas	Černosvitov 1939	Peru, Lake Lagunilla, as *H. stephensoni* (see Schmelz & Collado 2007)
Henlea perpusilla Friend	Martínez-Ansemil & Giani 1986	small stream in Bolivia
Globulodrilus riparius (Bretscher)	Martínez-Ansemil & Giani 1986	small stream in Bolivia, as *Marionina riparia*
Lumbricillus lineatus (Müller)	Černosvitov 1939	shore of Lake Titicaca, as *Pachydrilus lineatus*

MATERIAL AND METHODS

The collection contained 21 ethanol-preserved specimens of enchytraeids in 18 vials, collected from river sediments in the State of São Paulo, Brazil. Most of the material (17 specimens) was collected in first order streams in the "Parque Estadual Campos do Jordão", São Paulo State, Brazil, in different sampling campaigns of aquatic oligochaetes, from 2006 to 2008, by Ana Emiliar Siegloch. Methods and some results are available in Gorni & Alves (2008). The remaining four specimens are from other sites in São Paulo State. Sampling took place within the framework of the project BIOTA/FAPESP, "Research Program on Biodiversity Characterization, Conservation, Restoration and Sustainable Use" (www.biota.org.br). Geographical coordinates were not available for all sampling localities.

The specimens were passed through an ethanol-xylene dehydration series and whole-mounted in Canada balsam between two coverslips to allow investigation from both sides. The coverslip preparation was framed with an aluminium slide, the so-called Cobb slide used in nematology (Neuhaus *et al.* 2017). Microscopical investigation was carried out at x40 to x400 magnification. For the redescription of *A. singularis*, the type series was consulted as well. In the descriptions, taxonomically important details of the morphology that could not be observed due to the mode of fixation are indicated by "not distinguished". All material is in the possession of the Zoological Museum of the University of São Paulo, Brazil (MZUSP, #1232 – #1249).

RESULTS

Identification

Eight species taxa of three genera were distinguished: two species of *Achaeta*, three species of *Guaranidrilus*, and two species of *Marionina*. Five species could be named, one of them tentatively: *Achaeta neotropica* Černosvitov, 1937, *Achaeta singularis* Schmelz, 2008, *Guaranidrilus oiepe* Righi, 1974, *Marionina argentea* (Michaelsen, 1889) s.l., and *Marionina* cf. *seminuda* Xie & Rota, 2001. In the other cases, only the genus or the family could be assigned.

Comments on species and specimens

Achaeta singularis Schmelz, 2008

(Figures 1–2)

Achaeta singularis Schmelz. Schmelz, Collado & Römbke 2008: 26–28, Fig. 6, Table 4.

Material examined. Ten specimens, 4 adult, 3 subadult, 3 juvenile, São Paulo State, Parque Estadual Campos do Jordão, collected by Ana Emiliar Siegloch. MZUSP #1239, 1 adult specimen, Parque Estadual Campos do Jordão - Campo do Meio, 22°41'56.6"S, 45°29'16.8"W. 21-08-2006. MZUSP #1240, 1 subadult specimen, Parque Estadual Campos do Jordão – Camhambora, 22°42'29.3"S, 45°30'6.7"W, 07-10-2007. MZUSP #1242, 1 adult specimen, Parque Estadual Campos do Jordão – Mimosinha, 22° 41'29.3"S, 45°27'36"W, 08-10-2007. MZUSP #1243, 1 subadult and 1 juvenile specimen, Parque Estadual Campos do Jordão - Serrote, 22°39'39"S, 45°26'58.4"W, 20-08-2006. MZUSP #1244, 1 subadult specimen, Parque Estadual Campos do Jordão – Casguilho, 22°40'20.6"S, 45°27'53"W, 07-10-2007. MZUSP #1245, 1 adult specimen, record data as in #1240. MZUSP #1247, 1 juvenile specimen, record data as in #1239. MZUSP #1248, 1 juvenile specimen, record data as in #1242. MZUSP #1249, 1 adult specimen, record data as in #1240.

Further material for comparison, not included in this description: type series, 7 specimens (MZUSP # 1370: holotype, MZUSP #1371: paratypes).

Description. (The description is based on adult and subadult specimens unless stated otherwise.) Worms stout with tapering body ends. Largest adult specimen 8 mm long, diameter 0.45 mm at V, 0.6 mm at XI, 0.48 mm at XX. Remaining adult and subadult specimens 5–6 mm long and up to 0.4–0.55 mm wide. Juveniles 3.5–4 mm long, 0.3–0.4 mm wide. Segment numbers 25–30 in adult and subadult specimens, juveniles with 18–22 segments. Paired prostomial ganglia and inner prostomial papillae present. Body wall very thin, usually 6 µm thick, 8–10 µ in some regions of the largest adults, longitudinal muscle layer ca. 2.5–3 µm thick, not thicker than outer layer of epidermis and ring muscles. Pygidium flattened dorso-ventrally, rectal muscles inserting laterally, no ventral nerve cord. Epidermal gland cells not seen, pyriform glands absent. Brain incised anteriorly and posteriorly. Ventral nerve cord ganglionated from II on, ganglia of II–IV not fused, ganglia ovoid, rounded, separate from each other by elongate connectives free of perikarya. Towards growth zone in caudal segments, connectives with a narrow line of ventral perikarya, and ganglia increasingly fused into a medullar strand with segmental bulges (the ganglion precursors). Pharyngeal glands all united dorsally and with ventral lobes, dorsal connection usually wide, in VI sometimes narrow. Oesophageal appendages in V, dorso-laterally of oesophagus, paired, outline irregular. Chloragocytes as a thin, continuous layer from VI on, absent in X. Intestinal diverticula absent, gut widening more or less gradually in preclitellar segments, pars tumida inconspicuous, circumferal. Dorsal blood vessel from VII in juveniles and adults. Preclitellar nephridia at 6/7 and 7/8, *ca*. 4x as long as wide, c. 220 µm long and 60 µm wide in largest adult, compact and without constriction at septum. Anteseptale short, truncate, postseptale slightly tapering towards ectal pore; terminal vesicle present; epidermis thickened around ectal pore. Postclitellar nephridia from 11/12, of similar shape and size as in preclitellar region, those of hindmost 5–7 segments increasingly reduced in size towards pygidium. Nephridia unpaired or absent in several segments of the mid-body region. Coelomocytes three types: (1) flat, filled with pale vesicles, length *ca.* 25–40 µm; (2) flat, pale, matrix hyaline, cell perimeter brimmed, *ca*. 25 µm; (3) completely filled with packages of crystalline, needle-like bodies; needles pointed at both ends, length of packages c. 25 µm, thickness varying but never exceeding length.

Clitellum girdle-shaped, cells in *ca*. 24 separate or dense rows. Hyalocytes dorsally and laterally, alternating with granulocytes. Hyalocyte diameter *ca*. 15–25 µm, granulocyte diameter *ca*. 10–15 µm, height max. 20–30 µm; cells thickened around male pores. Rows of border cells (*i.e.* the 3–4 most anterior and most posterior rows, devoid of hyalocytes) interrupted mid-ventrally. Transverse row at the level of male pores wider than adjacent rows, situated in the middle of clitellum (ca. 12th row). Testis in IX, ovary in X in all specimens. Gonads unpaired. In adults large

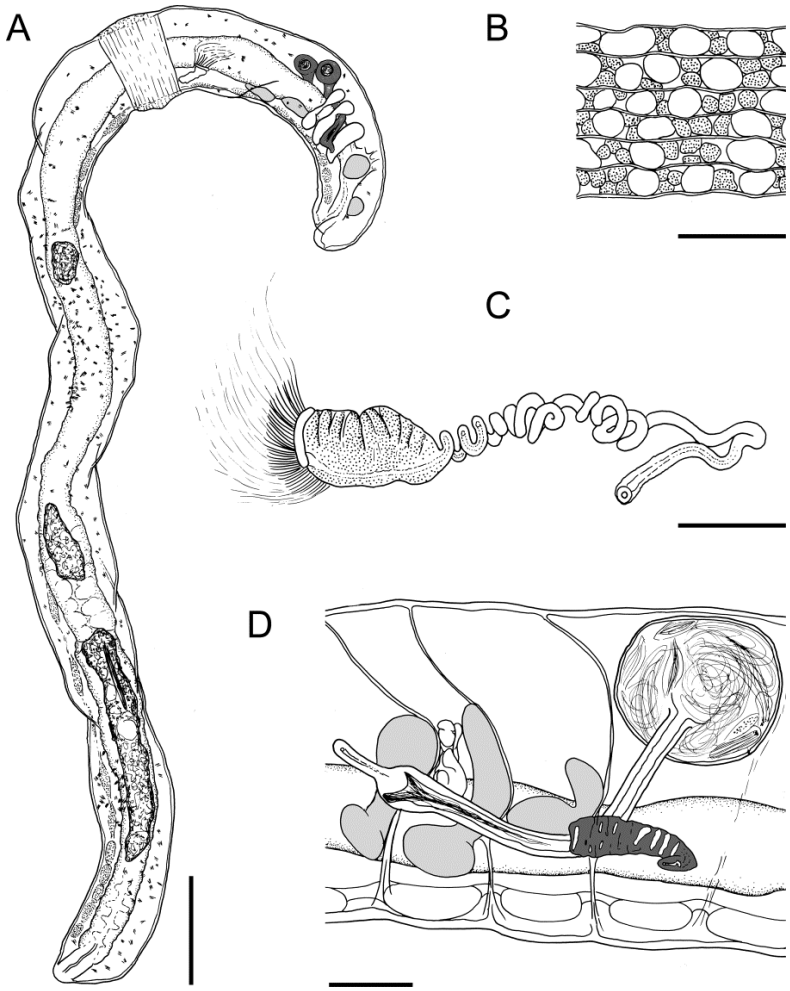

Figure 1. *Achaeta singularis* Schmelz, 2008. A. MZUSP #1243, adult specimen. Oocyte, postclitellar nephridia, and dorsal blood vessel not shown. Dark-grey: spermathecae. Light-grey, from right to left: brain, pharyngeal pad, preclitellar nephridia. Coelom with raphides, intestine with three pellets of ingested organic and inorganic matter (sand grains up to 100 µm diameter). Circumferal midgut pars tumida between 2nd and 3rd pellet. Perkarya of last five segments non-ganglionic. B. MZUSP #1243, detail of clitellum dorsolaterally, head region to the top. The second row from the top is the one that encloses the male pores. C. MZUSP #1243, male efferent apparatus. D. MZUSP #1245, segments IV–VII, lateral view, with pharyngeal glands (light-grey), oesophageal appendage in V, nephridium 6/7 (dark-grey), ventral nerve cord, and spermatheca.
2nd preclitellar nephridium at 7/8 not shown (specimen damaged here).

masses of developing and mature sperm present dorsally in IX. One specimen with a paired bulge anteriad of septum 8/9 dorsally, bulge unpaired in another specimen (seminal vesicle?). Spermatozoa about 140 µm long, heads *ca*. 40 µm long. Sperm funnels small, length less than half of body diameter, about twice as long as wide (*e.g*., 130 µm long, 70 µm wide); funnel body cylindrical, tapering towards vas deferens; collar apparently of varying width: as wide as funnel body, or up to twice as wide (*ca*. 100 µm), judging from masses of sperm attached in parallel. Vasa deferentia tightly and irregularly coiled, of equal diameter throughout (*ca*. 7 µm), barely distinguishable near male pore; coil extending posteriad without loops. Male pores ventrally on body surface, widely separate, without surrounding glands, inconspicuous. Pores in the middle of clitellum. No further accessory glands seen. Spermathecae with short ectal duct and large ampulla; ampulla subdivided

into ectal part, connecting tube and ental reservoir. Ectal pores lateral; ectal glands absent; ectal duct short, in V, length *ca*. 60 µm, diameter ca. 18 µm, connection with ampulla thickened (ental bulb). Ampullar ectal part in V, dilated (diameter 50–60 µm) and with distinct lumen, sperm arranged here in parallel with nuclei oriented ectad. Ampullar ectal part gradually narrowing into connecting tube with distinct walls and lumen, diameter *ca*. 25–30 µm; connecting tube widening more or less abruptly in VII into thin-walled, spherical or egg-shaped ental reservoir, length ca. 140 µm, diameter 100–120 µm.

Remarks. Comparison with the original description. This redescription of *Achaeta singularis* enhances the original description, which was based on juvenile material, confirms most of the characters as originally described, and clarifies several doubtful points. In the following we list the peculiar and species-distinguishing characters of *A singularis* as given in Schmelz *et al.* (2008: 27) and compare them with our material: (1) Short brain with deep anterior and posterior concavities. Confirmed. (2) Ganglia of segments II–IV not fused into a suboesophageal ganglion. Confirmed. (3) Anterior ganglia rounded like ovoid bodies, not subdivided into an anterior and a posterior lobe. Confirmed. (4) Posterior ganglia fused into a common medullar strand. Confirmed, but less pronounced in subadult and adult specimens, where ganglia can always be distinguished even though they are in close contact with each other (Fig. 1A). (5) Presumably small body size and low segment number. Not confirmed. Adult specimens have the size and segment number in the medium range for species of *Achaeta* (comp. Schmelz & Collado 2010). The juvenile specimens of the type series measure 2 mm in length and 0.12–0.15 in body diameter, segment number 14–24. (6) Pyriform glands absent. Confirmed. (7) Oesophageal appendages present in V. Confirmed. (8) All pharyngeal glands united dorsally. Confirmed. (9) Secondary ventral pharyngeal gland lobes absent. Confirmed. (10) Nephridia at 6/7 and 7/8. Confirmed. (11) Dorsal blood vessel originating in preclitellar region. The origin was observed always in VII in juvenile and adult specimens alike, and serves hence as a taxonomic character in all age groups. The origin may vary in other species: In the European *A. camerani*, juveniles have the origin in VII and adults in VIII (Schmelz & Collado 2010). (12) Spermathecal ectal pores in lateral position. Confirmed. Furthermore the girdle-shape of the clitellum and the wide spacing of the male pores is confirmed. The vas deferens is coiled and not straight (as seen in one paratype specimen). The originally described prostomial ganglia and prostomial papilla could not be investigated due to the poor mode of fixation.

The details of the reproductive system added here support the hypothesis in Schmelz *et al.* (2008) that *A. singularis* belongs to an ancient lineage within *Achaeta*. Girdle-shape of the clitellum, the uniform distribution of hylaocytes, and the absence of glands near the orifice of the spermatheca and of the male efferent duct are most likely plesiomorphies in the genus. Contrasting characters in other species of *Achaeta* (and hypothesized apomorphies) are: Clitellum interrupted dorsally and ventrally, hyalocytes concentrated into dorso-lateral longitudinal rows, spermathecal ectal duct swollen into a glandular mass, glands around male pore (see, *e.g.*, Schmelz & Collado 2010). The hypothesis was originally based on the absence of a suboesophageal ganglion (see above, point 2).

In the following we comment on two further peculiarities: the forward shift of the gonadal region by two segments, and the presence of rod-shaped crystals in the coelom. Both characters, while present in only some specimens of the type series, are present in all specimens of the new collection.

Displacement forwards of the gonadal region. The gonadal region is displaced two segments forwards in all specimens of the present collection, with testis in IX instead of XI, and ovary, male pores and clitellum in X instead of XII. In the type series this shift ahead is present only in one paratype specimen (Schmelz *et al.* 2008); the other six type specimens have the gonadal region in the usual position (XI–XII). A displacement

forwards of the gonadal region by one segment occurs in several species of enchytraeids, especially in *Achaeta*, where hitherto seven species are known to share this character. A shift ahead by *more* than one segment, however, is – with one exception – only found in taxa with the faculty to reproduce asexually by fragmentation (in the genera *Cognettia/Chamaedrilus*, *Buchholzia* and *Enchytraeus*). The only exception is *E. gordioides* Černosvitov, 1942 (Table 1), but this species may be fragmenting as well, considering its similarity with the fragmenting *E. bigeminus* Nielsen & Christensen, 1963 and *E. dudichi* Dózsa-Farkas, 1995. Fragmentation, if not directly observed, can be inferred from incomplete front or rear ends in many specimens. It is unknown in *Achaeta*, and the material of *Achaeta singularis* gives no indication of fragmentation either: all specimens are complete anteriorly and posteriorly. This seems to indicate a new type of variation in the family: a shift of the gonadal region ahead by more than one segment in a species with exclusively sexual reproduction.

Only some of the specimens have a forward shift of the gonadal region (the holotype is not among them), and up to now there are only two morphs, one with male pores in X and one with male pores in XII. The resulting possibility that *A. singularis* presents, in fact, two different species should be tested with DNA sequencing methods.

In this context we have to notify an error in a figure within the original description of *Achaeta singularis* (Schmelz *et al.* 2008). In Figure 6A, testis and ovary are shown in X and XI, respectively, counting the ganglia. Reinvestigation of the paratype specimen from which the drawing was made (MZUSP #1371) revealed that, in fact, they are situated in XI and XII, respectively, in accordance with the text of the original description.

Raphides in the coelom of *A. singularis*. Bundles of microscopic, needle-like crystals are present in the coelom of all specimens of the São Paulo State material (Fig. 2), and also in some of the type specimens (Schmelz *et al.* 2008), the holotype included (Schmelz, pers. obs.). These crystals are named here "raphides" (Greek raphidos = needle), in accordance with the use of this term in the biological literature. Raphides occur in sponges, fungi, and plants, and they consist mostly of calcium oxalate (plants, fungi) (Arnott 1995, Horner & Wagner 1995) or aragonite (sponges) (Hooper & Van Soest 2006). In *A. singularis* each bundle of raphides is about 8 µm wide and 4 µm thick. They are inclusions of coelomocytes, because most bundles are surrounded by a membrane, which also encloses a nucleus, closely attached to the needles either laterally or apically, at one side of the needle tips (comp Fig. 2C, bottom). The crystals do no disappear after clearing with xylene. As to their chemical composition, it may be calcium oxalate. Raphides are common in plants, and most raphides in plants consist of calcium oxalate. Shape and size of raphides in *A. singularis* are similar to the crystals in plants (comp. Coté 2009, Saadi & Modal 2011). Their function is usually considered to be a repellent against predation. We hypothesized a similar function in *A. singularis* (Schmelz *et al.* 2008), but this would imply that possible predators are able to distinguish between different species of enchytraeids. Further possible functions are related to calcium metabolism. Calcium is rare in tropical soils, and oxalate, abundantly produced by fungi, binds calcium. A capacity to ingest and to metabolize oxalate to bind calcium has been suggested for a Nearctic earthworm (*Arctiostrotus* sp.) (Spiers *et al.* 1986), and the North American earthworm *Diplocardia mississippiensis* Smith may selectively feed on fungi to derive calcium via the fungal oxalates (Lachnicht & Hendrix 2001). A similar function as a calcium-binding device may be assumed for the oxalate crystals in *A. singularis*, which may result from fungal grazing as well. On the other hand, the target may not be calcium but oxalate. In the strongly acid soil or freshwater sediment, a surplus of oxalate may be assimilated, increasing acidity in the coelomic fluid. Low pH is a problem for soil biota in the tropics (Lavelle *et al.* 1995). The binding of oxalate by calcium in the coelomocytes of *A. singularis* may therefore be a mechanism to regulate the pH in the body fluid, similar to one of the functions of the so-called calciferous glands in earthworms.

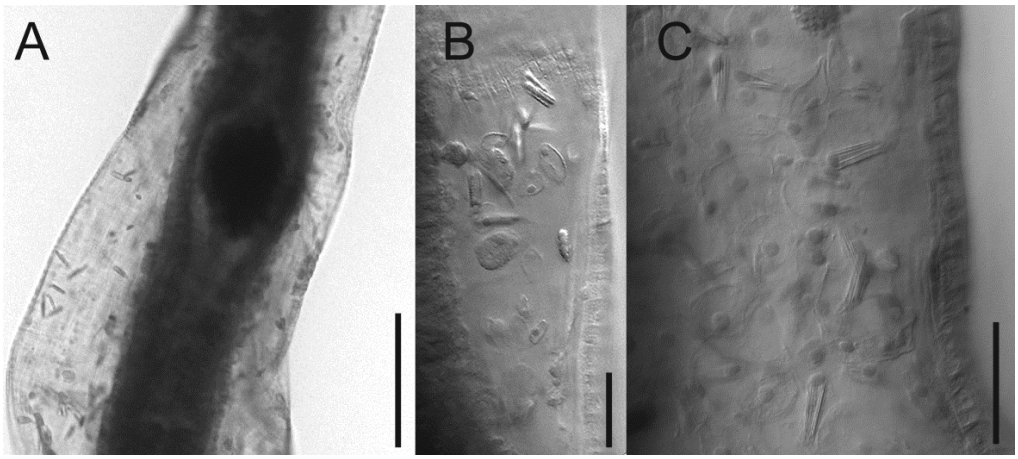

Figure 2. *Achaeta singularis* Schmelz, 2008. MZUSP #1243, micrographs of coelomocytes with and without raphides, at different magnifications. Scale bars = 200 μm in A, 50 μm in B, C.

Raphides are also present in sponges (Hooper & Van Soest 2006), here as aragonite crystals, and with a different shape. To our knowledge, raphides are unknown in other animal taxa. With more material sampled in the future, the chemical nature of raphides in *A. singularis* may be determined with certainty.

Raphides were present in only three specimens (out of seven) of the type series and therefore excluded from the list of species-distinguishing characters (Schmelz *et al.* 2008). However, their presence in all specimens of our collection here suggests that raphides should be included as a taxonomic character for *A. singularis*. All specimens of the type series are small juveniles, and it may take some time during growth and maturation to metabolize or to store calcium oxalate in the coelomocytes. We have observed a similar case in some European specimens of *Marionina argentea* (Michaelsen) sensu lato, a complex of semi-aquatic species (Rota 2013), characterized by strongly refractile bodies in the coelomocytes. Presence of these bodies is so constant that it is a diagnostic character of the species complex, but some very small specimens are without these bodies (Schmelz, pers. obs.). It seems that, as in *A. singularis*, these bodies are metabolites that are not yet present immediately after hatching of the worms from the cocoon. The chemical composition of the refractile bodies in *Marionina argentea* is unknown; but they are not calcium crystals, because they disappear during clearing with xylene.

Achaeta neotropica Černosvitov, 1937

Achaeta neotropica Černosvitov, 1937b: 154–157, Figs 16–26.

Achaeta neotropica Cern.—Righi 1974: 127–129, Figs 1–6; Christoffersen 1979: 153–154, Figs 1–10; Righi 1981: 427. Schmelz *et al.* 2008: 14–21, Fig. 4.

?*Achaeta becki* Schmelz & Collado, 2005: 49–57, Figs 1–2, Table 1.

Material examined. MZUSP #1235, 1 juvenile specimen, Parque Estadual Campos do Jordão - Serrote, 22°39'39"S, 45°26'58.4"W, 20-08-2006, col.: Ana Emilia Siegloch.

Description. Length 3 mm, chaetae and pyriform glands absent, oesophageal appendages not seen, cuticle thick, anterior nephridia at 6/7 and 8/9, absent at 7/8, with terminal vesicle.

A. neotropica is possibly a complex of species (Schmelz *et al.* 2008). The specimen was identified mainly on account of the peculiar distribution of preclitellar nephridia.

***Marionina argentea* (Michaelsen, 1889) sensu lato**

(Figure 3, Table 3)

Enchytraeus argenteus Michaelsen, 1889: 15–16, Fig. 6.
Pachydrilus (*Marionina*) *argenteus* (Michaelsen). Černosvitov 1937a: 293.
Marionina argentea (Michaelsen). Nielsen & Christensen 1959: 113–114, Figs 138–141. Chalupský 1992: 145, Fig. 15. Rota 1995: 220. Schmelz & Collado 2010: 100, Fig. 39A.
Marionina argentea (Michaelsen) sensu lato. Rota 2013: 136.

Material examined. MZUSP #1233, 1 adult specimen on a slide with 2 specimens (the other one being an unidentified juvenile specimen of enchytraeids, possibly *Hemienchytraeus* sp., see below). Ubatuba, Rio da Pipoca, 23º24'1.7"S, 45º6'47.9"W, 05-10-2007, col.: Ana Emilia Siegloch.

Description. Body length 1 mm, diameter c. 0.07 mm (0.07 mm in V, 0.09 mm in XI, 0.06 mm in XX). 20 segments. Body wall thin, mostly *ca*. 3 µm thick. Prostomium and first two segments strongly contracted, with deep external transverse furrows (fixation artefact). Prostomial epithelium with frontal recess, location of head pore unknown. Chaetae two per bundle, absent laterally at II, straight with ental hook, ectally slightly bent, length *ca*. 22 µm in anterior segments, 25 µm posteriorly. Pharyngeal glands dorsal in IV and V, elongate and ventral in VI. Brain shape unknown due to lateral position of worm, ventral nerve cord medullar, wide and lobed in II–IV. Shape of nephridia not distinguished; lobed tissue seen in nephridial positions in VIII and IX. Coelomocytes present, shape and size unknown. Dorsal blood vessel origin in XI or 1/2 XII, anterior bifurcation not visible. Gonadal region shifted one segment forward: Testes in X, male gland in XI, clitellum in XI–1/2 XII. Clitellum saddle-shaped, ventral border roughly at level of male glands; cells in *ca*. 14 dense, transverse rows, granulocytes *ca*. 8 µm wide, hyalocytes *ca*. 12 µm wide, roughly alternating with tendency to form longitudinal rows. Ventral borders of clitellum formed by one longitudinal row of hyalocytes on each side; each line interrupted by one granulocyte dorsally of male gland (Fig. 3). Seminal vesicle absent, developing sperm present dorsally in X. Sperm funnel and vas deferens not distinguished. Male glandular bulb spherical, not larger than clitellar gland cells, diameter 8–10 µm. Spermatheca small, consisting of short ectal duct and globular ampulla, the latter smaller than pharyngeal gland lobes, connected with oesophagus; further details (*e.g.* glands) not distinguished.

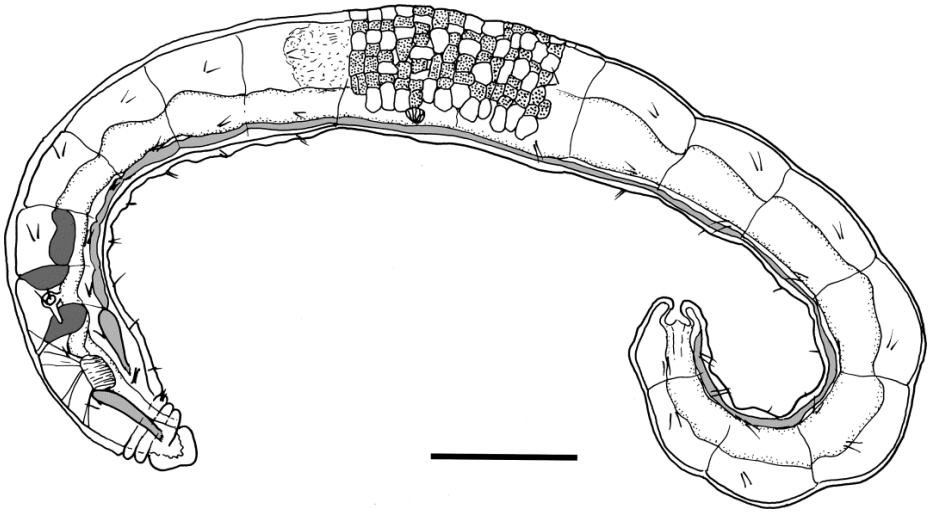

Figure 3. *Marionina argentea* (Michaelsen, 1889) *sensu lato*. Light-grey: brain and ventral nerve cord. Dark-grey: pharyngeal glands.

Remarks. Although several details were not distinguishable – brain shape, texture of coelomocytes, spermathecal glands, nephridia, sperm funnels – this specimen is safely assigned to *Marionina argentea* (Michaelsen, 1889) due to the peculiar chaetal pattern, together with small body size, low segment number, medullar ventral nerve cord, shape of the pharyngeal glands, small size of the reproductive organs, and low amounts of developing sperm. *Marionina argentea* (Michaelsen, 1889) sensu auctorum was recently split into four species by Rota (2013) and a fifth species was added in Martin *et al.* (2015) (Table 3). Among these five species under the umbrella of *M. argentea* sensu lato, the single specimen described here is most similar to *M. deminuta* Rota, 2013 (Table 3) by the small body size, the forward shift of the gonadal region by one segment, the small size of the male gland and the almost identical pattern of clitellar gland cells (compare Fig. 3 with Fig. 3B,C in Rota 2013), here especially the ventro-lateral row of hyalocytes, interrupted by one granulocyte at the level of the male gland. Hence, this specimen belongs either to *M. deminuta* or to a new species. This is the first record of *Marionina argentea* sensu lato from South America.

Marionina cf. *seminuda* Xie & Rota, 2001

Material examined. MZUSP #1237, 1 juvenile specimen, Parque Estadual Campos do Jordão - Celestina, 19-08-2006, col.: Ana Emilia Siegloch.

Description. Body length 2 mm, diameter 0.09 (V), 0.075 (XII), 0.08 (XX). 22 segments. Chaetae straight ectally, ental hook almost absent, 2 per bundle, laterals absent, ventrals from III (= absent in II). Cuticle conspicuous, 1.5 µm thick. Brain apparently indented posteriorly. Ventral nerve cord medullar. Pharyngeal glands in IV, V with ventral lobes and unpaired dorsal lobe, in VI elongate, separate ventral lobes. No oesophageal appendages or intestinal diverticula seen. Preclitellar nephridia at 7/8, 9/10. Dorsal blood vessel from XII. Coelomocytes floppy, length 10–20 µm, with pale blurred vesicles, coagulated masses of cells in anterior segments, but most of coelom empty. Precursors of testis and ovary in XI and XII, respectively.

The peculiar distribution pattern of the chaetae is known only from *M. seminuda* Xie & Rota, 2001, a terrestrial species described from China. The other details of this juvenile specimens also agree with the description of *M. seminuda*, which hence may be a peregrine species. Sexually mature material is necessary to confirm the identification.

Guaranidrilus oiepe Righi, 1974

Guaranidrilus oiepe Righi, 1974: 140–141, Figs 32–40. Righi 1981: 427–428. Coates & Diaz 1988: 779–780, Fig. 2.

Material examined. MZUSP #1232, 1 adult specimen, Picinguaba - Riacho Sabesp, Sao Paulo State, Brazil, 04-10-2007, col.: Ana Emilia Siegloch.

Description. Body length 4 mm, diameter 0.14 mm (V), 0.16 mm (XII, XX), 29 segments. Two chaetae per bundle absent at XII, ventral chaetae ca. 32 : 3 µm, caudal chaetae *ca*. 56 : 5 µm; *i.e.*, caudal chaetae almost twice as large as anterior chaetae. Epidermal gland cells present, pattern not distinguished. Body wall thin (*e.g.*, 8 µm dorsally in XVI), cuticle 1.5–2 µm thick. Secondary lobes of pharyngeal glands in VI. Oesophageal appendages absent. Intestinal diverticula in VII, directed anteriad only, no posterior projection. Dorsal blood vessel from XI or XII. Preclitellar nephridia 2 pairs, at 8/9 and 9/10. Coelomocytes sparse, with very fine, regular, blurred vesicles.

Clitellum saddle-shaped, well-developed, cell height dorso-laterally 16 µm, on dorsal half hyalocytes and granulocytes, ventro-laterally only granulocytes, *ca*. 33 dense rows; hyalocytes contagious. Seminal vesicle absent. Sperm length *ca*. 50 µm, heads *ca*. 20 µm. Sperm funnel small, cylindrical, 30 µm long, 20 µm thick, collar high, not wider than funnel body. Vas deferens in tight isodiametric coils. Male pores on body surface, no male glands distinguished. Spermatheca small, elongate, bent in V, extending into VI. No accessory glands seen.

This specimen agrees with the description of *G. oiepe* Righi, 1974, except for the body size (length 5.7–8.5 mm, diameter ca. 0.25 at XII, *ca*. 0.2 in rest of body, 32–34 segments). However, a subsequent record of the species (Righi 1981) gives dimensions that agree better with our specimen (length 3.5–4.1 mm, diameter *ca*. 0.14 mm at XII, *ca*. 0.13 mm in rest of body, 31–32 segments). Number of preclitellar nephridia is unknown in *G. oiepe*, only the presence of the 1st nephridium at 8/9 is originally described. Apart from *G. oiepe*, only *G. hoeferi* Schmelz *et al.* 2011 has preclitellar nephridia in 8/9 and 9/10, in other species the 1st nephridium is placed more anteriorly. *G. hoeferi* differs by huge spermathecae and seminal vesicle, among other characters. *G. oiepe* was originally found in decaying wood inside humus-rich soil in Minas Gerais, and there are two more records from Brazil, from the States Rio de Janeiro (Righi 1975) and Mato Grosso (Righi 1981); this is the first record from São Paulo State.

Guaranidrilus sp. 1

Material examined. MZUSP #1234, 1 subadult specimen, Núcleo Santa Virgínia (São Luis do Paraitinga, SP), Ponte Três Paus, 31-10-2008, col. Ana Emilia Siegloch.

Description. Length 3 mm, 26 segments. Two chaetae per bundle, 60 µm long in caudal segments, 26 µm anteriorly. No epidermal gland cells seen. Cuticle 1.5–2 µm thick. Brain incised posteriorly. Pharyngeal glands all united dorsally. Presence or absence of oesophageal appendages not ascertained. Intestinal diverticula in VII, filling out segment. Dorsal blood vessel from IX or further back. Preclitellar nephridia at 6/7–8/9. Coelomocytes 12–18 µm long, filled with brown granules, cells very conspicuous against rest of body. Clitellum girdle-shaped, in c. 35 rows. Compact mass of cysts in XI dorsally. Spermatozoa short: *ca*. 40 µm long, heads *ca*. 10 µm, or longer. Sperm funnels small, 32 µm : 16 µm, collar as wide as funnel body. Vasa deferentia in tight isodiametric coils, male pores on body surface, no "penial bulb", clitellum thickened around male pores. Spermathecae stump-like and very short, *ca*. 30 µm long, diameter 10 µm, possibly extending into VI.

Remarks. This specimen probably belongs to a new species. It is conspicuous by the strong-brown coelomocytes and the girdle-shaped clitellum. The only species of *Guaranidrilus* currently known with a girdle-shaped clitellum is *G. cingulatus* Schmelz *et al.*, 2011, a species with pale coelomocytes.

Guaranidrilus sp. 2

Material examined. MZUSP #1236, 1 juvenile specimen, Parque Estadual Campos do Jordão - Campo do Meio, 22°41’56.6”S, 45°29’16.8”W, 27-04-2006. MZUSP #1241, 1 juvenile specimen, Ubatuba - Rio do Veio, 23°21'35"S, 44°47'0,4"W, 03-10-2007. Col. Ana Emilia Siegloch.

Description. Length 3 mm. Chaetae *ca*. 50 µm in caudal segments, 25 in anterior segments (MZUSP #1236), or 26 µm anteriorly, 36 µm posteriorly (MZUSP #1241). Cuticle thin (< 1 µm). Secondary pharyngeal gland lobes in V and VI. Oesophageal appendages paired anteriorly in VI, small. Intestinal diverticula in VII. Preclitellar nephridia 3 pairs, from 6/7 to 8/9. Coelomocytes small, ca. 10 µm long.

Remark. The two juvenile specimens of *Guaranidrilus* sp. 2 differ in the length of the chaetae and may belong to different species.

Guaranidrilus sp. 3

Material examined. MZUSP #1246, one adult specimen. Parque Estadual Campos do Jordão - Serrote, 22°39'39"S, 45°26'58.4"W, 15-02-2007, col. Ana Emilia Siegloch.

Description. Length 7 mm, diameter 0.2–0.25 mm. 36 segments. Head pore on prostomium. Chaetae 2 throughout, anteriorly ca. 40–45 µm long, in caudal segments 60–65 µm. Epidermal gland cells at least 6 rows dorsally in anterior segments; shape not distinguished. Body wall *ca*.

15–18 µm thick dorsally, cuticle *ca*. 2.5 µm thick, longitudinal muscle layer 8–10 µm. Septa not conspicuously thickened. Pharyngeal glands with secondary ventral lobes in VI (possibly also in V, small). Oesophageal appendages absent. Intestinal diverticula extending over 3 segments, VII–IX, with anterior and posterior projections. Dorsal blood vessel from XI. Preclitellar nephridia 4 pairs, 6/7–9/10. Coelomocytes concentrated at body ends, length *ca*. 30–50 µm, 1.5–2x as long as wide, with very fine blurred vesicles.

Clitellum saddle-shaped, *ca*. 33 rows, mid-ventral interruption narrower than distance of male pores. Maximum cell height 18 µm dorsally and 14 µm ventrally around male pores. Seminal vesicle absent, few cysts dorsally in XI. Spermatozoa present on sperm funnel, length *ca*. 85 µm, heads 32 µm. Sperm funnel pear-shaped, *ca*. 60 µm long, *ca*. 1.5x as long as wide, tapering at both ends, the tapering being more pronounced ectally, towards vas deferens; collar narrower than funnel body. Vas deferens wound in tight isodiametric coils. Male pores on body surface, male glands small or absent, no "penial bulb". Spermatheca small, without sperm, extending into anterior region of VI only, length *ca*. 170 µm, ectal duct 16 µm wide, ental reservoir 24 µm wide, transition from ectal duct to ampulla inconspicuous. No accessory glands seen. Two mature eggs.

Remarks. The specimen agrees with most details of the original description of *Guaranidrilus glandulosus* Černosvitov, 1937 (Černosvitov 1937b), notably the large intestinal diverticula, so far unique in the genus (in other species the diverticula extend over 1 or 2 segments). However, *G. glandulosus* has large male glands ("bulbes péniales") around the male pores, so this specimen most probably belongs to a new species. Furthermore, *G. glandulosus* is incompletely known and in need of revision, because one important character is controversial: Oesophageal appendages are present in VI according to the original description ("peptonéphridies"), but they appear to be absent in the type material (Coates & Diaz 1988).

Enchytraeidae gen. sp.

Material examined. MZUSP #1233, one juvenile specimen, Ubatuba – Rio da Pipoca, 23°24'1.7"S, 45°6'27.9"W, 05-10-2007. MZUSP #1238, two juvenile specimens, Pindamonhangaba - Cachoeira Nilceia, 22°45'25.6"S, 45°28' 38.2"W, 28-09-2007. Col. Ana Emilia Siegloch.

Remarks. The three juvenile specimens have two strongly sigmoid chaetae per bundle and a medullar nerve cord. They may belong to *Hemienchytraeus*, but genus-diagnostic characters (*e.g.* oesophageal appendages) were not distinguished.

DISCUSSION

Achaeta singularis, *Guaranidrilus oiepe* and *Marionina argentea* sensu lato are new records for the State of São Paulo, and *M. argentea* is also new for South America. The latter, very common in Europe, is a complex of semi-aquatic species, often found in moist soil, at river banks, but also in river sediments (Rota 2013). As was mentioned above, among the five species currently distinguished within *M. argentea* sensu lato, the specimens resemble most *M. deminuta* Rota, 2013.

Better knowledge of freshwater enchytraeids in South America faces three challenges: First, the species diversity of enchytraeids in South America is still largely unexplored (Schmelz *et al.* 2013) and any sampling may result in collecting undescribed species. Second, taxonomic work with enchytraeids requires the investigation of living specimens. Third, identification of the aquatic species requires also knowledge of the terrestrial species, because many species commonly considered as terrestrial may survive or even reproduce in aquatic habitats, provided that no oxygen-deficiency occurs. In Europe, enchytraeids are species-rich and abundant in the ecotone between soil and freshwater (*e.g.*, Erséus *et al.* 2005, Plum & Filser 2005, Beylich & Graefe 2007). It will be highly interesting to sampling this ecotone in tropical and subtropical Brazil and to compare the fauna with the aquatic and the

terrestrial one. For example, *A. singularis* was originally found on "terra firme" but it makes up almost half of the specimens from the freshwater samples. A sampling campaign along the moisture gradient from soil to freshwater should reveal the true moisture preferences of this species that was considered to be a remnant of the old autochthonous forest (Schmelz *et al.* 2008).

Another point of interest will be the percentage of native *vs*. peregrine species in the aquatic enchytraeid assemblage. Among all taxa identified here, *M. argentea* and *M.* cf. *seminuda* may be considered as peregrine species, all others are likely native species.

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Table 2. List of enchytraeid specimens, with MZUSP accession numbers, sampling sites, and identification results.

MZUSP accession numbers	Sampling locality	Identification result
MZUSP 1232	Picinguaba - Riacho Sabesp	*Guaranidrilus oiepe* Righi, 1974
MZUSP 1233a,b	Ubatuba – Rio da Pipoca	a: *Marionina argentea* s.l. (Michaelsen, 1889) b: Enchytraeidae gen. sp.
MZUSP 1234	Núcleo Santa Virgínia (São Luis do Paraitinga, SP) Ponte Tręs Paus	*Guaranidrilus* sp. 1
MZUSP 1235	Parque Estadual Campos do Jordão - Serrote	*Achaeta neotropica* Černosvitov, 1937
MZUSP 1236	Parque Estadual Campos do Jordão - Campo do Meio	*Guaranidrilus* sp. 2
MZUSP 1237	Parque Estadual Campos do Jordão - Celestina	*Marionina* cf. *seminuda* Xie & Rota, 2001
MZUSP 1238 a,b	Pindamonhangaba - Cachoeira Nilceia	a, b: Enchytraeidae gen. sp.
MZUSP 1239	Parque Estadual Campos do Jordão - Campo do Meio	*Achaeta singularis* Schmelz, 2008
MZUSP 1240	Parque Estadual Campos do Jordão - Camhambora	*Achaeta singularis*
MZUSP 1241	Ubatuba - Rio do Veio	*Guaranidrilus* sp. 2
MZUSP 1242	Parque Estadual Campos do Jordão - Mimosinha	*Achaeta singularis*
MZUSP 1243a,b	Parque Estadual Campos do Jordão - Serrote	*Achaeta singularis*
MZUSP 1244	Parque Estadual Campos do Jordão - Casguilho	*Achaeta singularis*
MZUSP 1245	Parque Estadual Campos do Jordão - Camhambora	*Achaeta singularis*
MZUSP 1246	Parque Estadual Campos do Jordão - Serrote	*Guaranidrilus* sp. 3
MZUSP 1247	Parque Estadual Campos do Jordão - Campo do Meio	*Achaeta singularis*
MZUSP 1248	Parque Estadual Campos do Jordão - Mimosinha	*Achaeta singularis*
MZUSP 1249	Parque Estadual Campos do Jordão - Camhambora	*Achaeta singularis*

Table 3. Comparison of specimen MZUSP #1233, identified as *Marionina argentea* s.l., with known species of the *Marionina argentea* (Michaelsen, 1889) species complex. Literature data from Rota (2013) and Martin *et al.* (2015).

	Marionina argentea (Michaelsen, 1889) s. l. MZUSP #1233	*Marionina argentea* (Michaelsen, 1889) s.s. (Rota 2013)	*Marionina mendax* Rota, 2013	*Marionina mimula* Rota, 2013	*Marionina deminuta* Rota, 2013	*Marionina sambugarae* Schmelz, 2015
Body length	1 mm	1.8–2.4 mm	1.5–2.4 mm	1.5–1.7 mm	0.9–1.4 mm	1.5 mm
Chaetae, length	22–25 µm	16–32 µm	19–42 µm	18–38 µm	16–29 µm	28–38 µm
Pharyngeal glands in VI: dorsal lobe	absent	present	absent	present	present	present
Gonadal region	X–XII	XI–XIII	XI–XIII	XI–XIII	X–XII or XI–XIII	XI–XIII
Clitellum dorsally	present	present	present	present	present	absent
Clitellum, ventral margin	hyalocytes	granulocytes	granulocytes	unknown	hyalocytes	granulocytes
Male glandular bulb length	10 µm	29–37 µm	16–27 µm	32–48 µm	17–19 µm	20–32 µm

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