Journal of Natural History
Vol. 46, Nos. 19–20, May 2012, 1153–1177

Three new Brazilian species of the land planarian *Choeradoplana* (Platyhelminthes: Tricladida: Geoplaninae), and an emendation of the genus

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(*Received 21 December 2011; final version received 11 January 2012; printed 5 April 2012*)

Three new Brazilian species of the neotropical land planarian genus *Choeradoplana* (Platyhelminthes: Tricladida: Continenticola: Geoplaninae) are described, making a total of nine species within the genus. All the new species share unique derived characters typical of the genus. Two of the new species exhibit important features representing morphological variations that were previously unknown for the genus: the dorsal cutaneous longitudinal muscle layer, as well as the ventral one, partially sunken into the parenchyma in one species, and the common ovovitelline duct approaching the copulatory apparatus ventrally in the other. As a consequence of these morphological variations, an emendation of the genus is proposed.

Keywords: Flatworms; Turbellaria; Continenticola; neotropical; taxonomy; Brazil

Introduction

The subfamily Geoplaninae is restricted to the Neotropical region, including, with only a few exceptions, all the native species of the region. Most of the species are described from Brazil, although we are still far from attaining a comprehensive estimate of Brazil's terrestrial flatworm richness (Carbayo and Froehlich 2008).

Choeradoplana Graff, 1896 is one of the 17 geoplaninid genera presently recognized. The genus was established by Graff (1896) and thereafter reviewed by Froehlich (1955), who restudied in detail *Choeradoplana iheringi* Graff, 1899 and emended some important points of Graff's description, such as the true nature (i.e. cutaneous sunken fibres) of the supposedly parenchymal longitudinal muscle fibres. He indicated *C. iheringi* as the type species, and diagnosed the genus "as *Geoplana*, but with two glandular cushions at anterior end. Part of the ventral sub-epidermal longitudinal muscle bundles internal to sub-muscular nerve plexus. These bundles originate the retractor of the glandulo-muscular organ (glandular cushions)".

In their index, Ogren and Kawakatsu (1990) transcribed Froehlich's redefiniton of the genus adding to it, as a note: "female canal enters genital antrum dorsally". They also commented on the presence/absence of the penial papilla in the different species.

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ISSN 0022-2933 print/ISSN 1464-5262 online

http://dx.doi.org/10.1080/00222933.2012.657699
http://www.tandfonline.com

Later Carbayo and Leal-Zanchet (2003) added to Froehlich's diagnosis some other important morphological aspects that Froehlich had noticed but did not include in the diagnosis. So, the present diagnosis is as follows: Geoplanidae of elongated body; cephalic region with a glandulo-muscular organ composed of two cushions; eyes and sensory pits absent in the anterior apex; creeping sole broad, more than a third of the body width; strong cutaneous longitudinal musculature, ventrally with a portion internal to the subcutaneous nerve plexus all throughout the body and, in cephalic region, entirely sunk into the parenchyma constituting the glandulo-muscular retractor organ; some dorsal longitudinal cutaneous muscle fibres oriented dorso-ventrally in the cephalic region; true parenchymal longitudinal musculature, weak or absent; bodies of rhabditogen cells located between the retractor muscle and the ventral epidermis of the cephalic region; dorsal testes; sensory papillae absent; copulatory apparatus without adenodactyls.

As a contribution to the knowledge of the turbellarian fauna inhabiting the Brazilian Atlantic Rainforest, three new species of the genus *Choeradoplana* are herein described.

Materials and methods

The specimens studied were collected manually, mainly during the night, in Atlantic Rainforest areas in the State of São Paulo, Brazil, during 2008–2009. The specimens were photographed, and then killed with boiling water. A small piece of tissue was immediately cut off, measured and preserved in absolute ethanol for DNA extraction; it is standard practice in our laboratory to measure unidentified specimens or those belonging to species that are poorly known. The worms were fixed with 10% formalin and subsequently stored in 80% ethanol. The anterior region of the body (from extremity up to the pre-pharyngeal region) was cut into a number of pieces: cephalic region, anterior region 1, anterior region 2, etc., besides pieces with the pharynx and the copulatory apparatus. The pieces were embedded in Paraplast, sectioned at 7-μm thickness, and stained with Mallory/Cason trichrome stain (Romeis 1989). Drawings were prepared using a camera lucida.

The following abbreviations are used throughout the text and figures: a, anterior end of the body; cm, common muscular coat; CMI, cutaneous musculature thickness relative to body height at the pre-pharyngeal region; co, common glandular ovovitelline duct; cr, creeping sole; dd, dorso-diagonal parenchymal muscles; de, dorsal epidermis; dv, dorso-ventral muscles; e, eye; fa, female genital atrium; g, gonopore; i, intestine; ls, normal longitudinal cutaneous muscles; m, muscles; ma, male genital atrium; mo, mouth; MZUSP, Museu de Zoologia da Universidade de São Paulo; ov, ovovitelline duct; ph, pharyngeal pocket; pv, prostatic vesicle; sb, subintestinal transverse muscles; sc, subcutaneous nerve net; sd, sperm duct; sg, shell glands; sk, sunken longitudinal cutaneous muscles; sp, supraintestinal transverse muscles; t, testis; v, vagina; vi, vitellaria; vn, ventral nerve plate.

Systematic account

Order **TRICLADIDA** Lang, 1884
Suborder **CONTINENTICOLA** Carranza et al., 1998
Family **GEOPLANIDAE** Stimpson, 1857

Subfamily **GEOPLANINAE** Stimpson, 1857
Choeradoplana von Graff, 1896
Choeradoplana bocaina sp. nov.
(Figures 1–8; Tables 1, 2)

Type material

Holotype. MZUSP PL 997: Parque Nacional da Serra da Bocaina, São José do Barreiro/SP, Brazil, *c*.22°45′08″ S, 44°37′19″ W, F. Carbayo et al. col., 8 September 2008. Cephalic region: sagittal sections on 10 slides; pre-pharyngeal region: transverse sections on three slides; pharynx: sagittal sections on 13 slides; copulatory apparatus: sagittal sections on six slides.

Paratypes. MZUSP PL 998: ibid., 22°45′08″ S, 44°37′19″ W, F. Carbayo et al. col., 7 September 2008. Anterior region 2: horizontal sections on seven slides; anterior region 3: transverse sections on four slides; pharynx plus copulatory apparatus: sagittal sections on 14 slides; MZUSP PL 999: ibid., 22°45′08″ S, 44°37’19″ W, F. Carbayo et al., col., 10 February 2008. Cephalic region: transverse sections on five slides; pharynx and copulatory apparatus: sagittal sections on five slides.

Type locality

Parque Nacional da Serra da Bocaina, São José do Barreiro/SP, Brazil, covered with primary Atlantic Rainforest.

Etymology

The specific epithet refers to the Tupi-Guarani (Indigenous Brazilian tribe) name of the National Park where the type material was collected.

Diagnosis

Choeradoplana species with small dark pigmented spots dispersed on the dorsum; pharynx bell-shaped; extra-bulbar portion of prostatic vesicle proximally two-forked, dish-shaped distally; posterior section of the female atrium narrow.

Description

The largest live specimen (paratype MZUSP PL 998) measures up to 48 mm in length and 4 mm in width (see Table 1 for size of fixed specimens). The body is slender (Figure 1). Dorsum highly convex in cross-section; body margins rounded; ventral side slightly convex. The cephalic region, which bears the glandular cushions typical of the genus, is kept rolled up in the living worm when creeping and resting. The posterior end is pointed. The creeping sole is as wide as 74% of body width at the pre-pharyngeal region. Mouth 19.5 mm from anterior end, gonopore at 23 mm (holotype).

Ground colour of dorsum is yellowish-brown; ventral side pale brown in the cephalic region, cream in rest of the body. Dorsum of holotype and of paratype MZUSP PL 999 with dark brownish spots of different size, more densely concentrated paramedianly, so delineating an irregular longitudinal stripe on each side. In the

Table 1. *Choeradoplana bocaina* sp. nov. – body measurements of fixed specimens (in mm and % of body measurements).

Specimen	Holotype	Paratype MZUSP PL 999	Paratype MZUSP PL 998
Length (mm)	36	24	32
Width (mm)	3	2	3
Mouth to anterior tip	19.5	14.5	18.0
Relative M : L	54.2%	60.4%	53.1%
Gonopore to anterior tip	23	19	23
Relative G : L	63.9%	79.2%	72.0%
Creeping sole: width	73.8%	?	73.6%

G, gonopore; L, length; M, mouth; W, width.

Figure 1. *Choeradoplana bocaina* sp. nov. Holotype creeping in dorsal and lateral (inset) view. About 36 mm in length.

paratype MZUSP PL 998 the dorsal region is almost completely covered by a dense web of brown pigment. There is medianly a darker longitudinal stripe of brownish pigment.

Eyes formed by one pigmented cup, 40–45 μm in diameter. No clear halos around them. Eyes absent in the first 3.3 mm of body (paratype MZUSP PL 999). Posteriorly they are irregularly arranged in a marginal row of three or four eyes throughout body length. At pre-pharyngeal region the eyes spread onto the back in a band 12% of body

width. Sensory pits distributed ventro-laterally in a uniserial row from 1.8 mm behind the anterior end to near ovaries (paratype MZUSP PL 999).

Epidermis ciliated only in creeping sole. Contrary to what occurs along body, in cephalic region the bodies of the rhabditogen cells that discharge through the glandular cushions are located between retractor muscle and ventral epidermis.

Ventral nerve plate 70 μm in thickness; cerebral ganglia not visible in available horizontal sections.

The cutaneous musculature comprises the three typical layers of Geoplaninae (Table 2): a subepithelial circular layer followed by two diagonal with decussate fibres, and then a strongly developed longitudinal layer with fibres arranged in bundles (Figures 2, 3). As characteristic of the genus, some of the muscle fibres of ventral

Table 2. *Choeradoplana bocaina* sp. nov. – thickness (in μm) of cutaneous musculature in pre-pharyngeal region.

Specimen	Holotype	Paratype MZUSP PL 998
Dorsal circular	5 (1–4)	5 (1–3)
Dorsal diagonal	22.5 (2–5)	15 (2–5)
Dorsal longitudinal	122.5 (50–60)	92.5 (28–73)
Dorsal total	150.0	112.5
Sunken ventral longitudinal	42.5 (18–22)	32.5 (23–41)
Normal ventral longitudinal	70.0 (15–25)	42.5 (5–16)
Ventral diagonal	20.0 (2–3)	7.5 (2–5)
Ventral circular	5 (1–3)	5 (1–4)
Ventral total	137.5	87.5
CMI	28.7%	20.0%

Lowest and highest number of muscle fibres per bundle are given in parentheses.
CMI, cutaneous musculature thickness relative to body height at the pre-pharyngeal region.

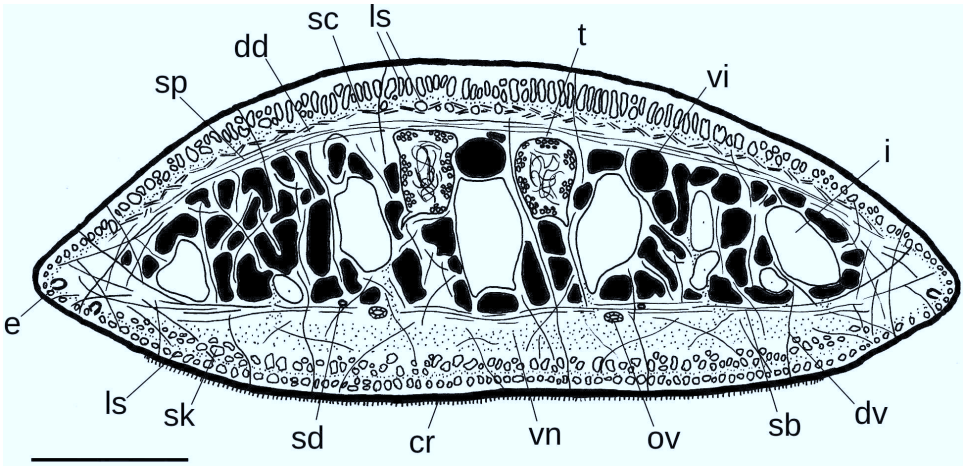

Figure 2. *Choeradoplana bocaina* sp. nov. Holotype. Diagrammatic reconstruction of a transverse section of pre-pharyngeal region. Scale bar 0.5 mm.

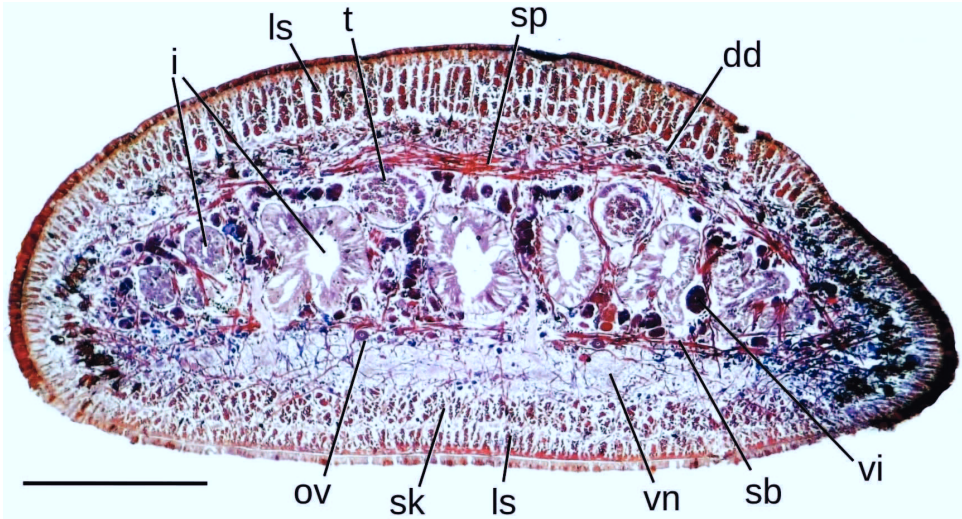

Figure 3. *Choeradoplana bocaina* sp. nov. Paratype MZUSP PL 998. Micrograph of a transverse section of the pre-pharyngeal region. Scale bar 0.5 mm.

longitudinal layer are sunk into parenchyma. Medianly, in paratype MZUSP PL 998 a few dorsal longitudinal fibres are also sunk into parenchyma and intermingled with those of parenchymal dorsal layer of diagonal decussate fibres (Figure 2). CMI, 20.0–28.7%. In cephalic region, ventral longitudinal layer is completely sunk into parenchyma, constituting cephalic retractor muscle, as described for *C. iheringi* and three other species by Froehlich (1955).

The three usual parenchymal muscle layers are present throughout the body: a well-developed dorsal layer of diagonal decussate fibres (38 μm thick, holotype), a transverse supraintestinal layer (30–35 μm), a transverse subintestinal layer (20–25 μm). In cephalic region there is an additional layer of transverse fibres, between ventral nerve plate and retractor muscle: the subneural muscle layer.

Mouth lying in middle of pharyngeal pouch (Figure 4). Pharynx bell-shaped, having dorsal insertion approximately at mouth level. Epithelium lining pharyngeal pocket squamous, non-ciliated, surrounded by a thin circular subepithelial layer. Outer and inner pharyngeal epithelia flat, ciliated. Outer one underlain by a one-fibre thick layer of longitudinal muscle fibres followed by a layer of circular fibres (10 μm); inner epithelium underlain by a layer of circular fibres (40 μm) with interspersed longitudinal fibres.

Testes located under supraintestinal transverse muscle layer, partially placed between the intestinal diverticula (Figure 2). They extend from level of ovaries to root of pharynx (paratype MZUSP PL 998). Sperm ducts run immediately above subintestinal muscle layer, slightly dorsal and lateral to ovovitelline ducts. Near prostatic vesicle, they curve dorso-anteriorly to communicate with the two vertical, dilated branches of extra-bulbar portion of vesicle (Figures 5–8). The latter is dish-shaped, diagonally inclined, and receives the branches through its anterior ventral face. It continues inside penial bulb as an ample, almost horizontal, tubular portion. Its opening into male atrium is narrow in holotype and wide in paratype MZUSP PL 998. Male atrium has

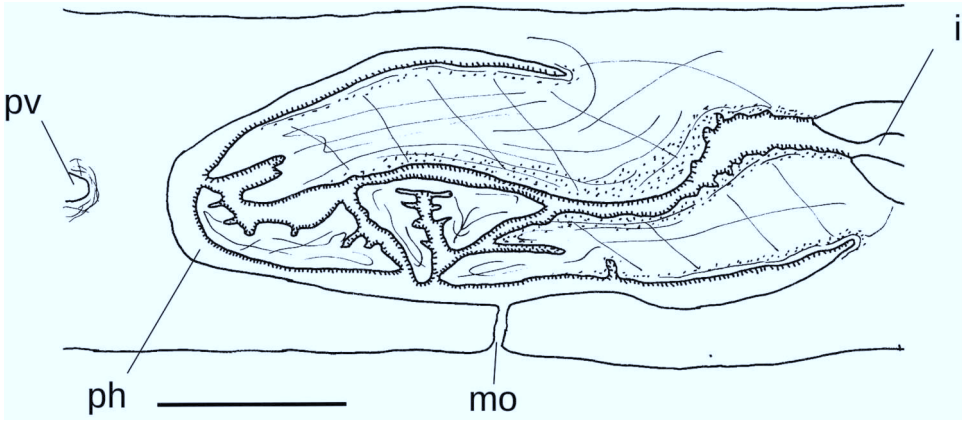

Figure 4. *Choeradoplana bocaina* sp. nov. Paratype MZUSP PL 999. Diagrammatic representation of the pharynx from sagittal sections. Scale bar 0.5 mm.

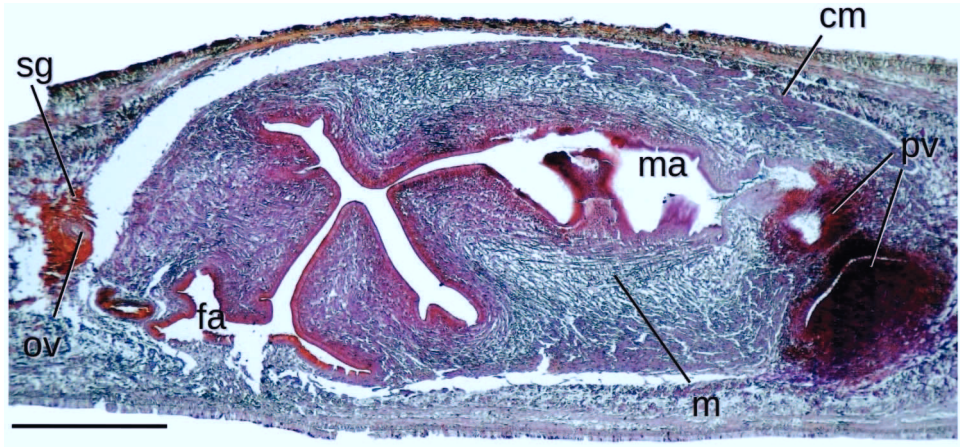

Figure 5. *Choeradoplana bocaina* sp. nov. Holotype. Micrograph of a sagittal section of the copulatory apparatus. Scale bar 0.5 mm.

some small folds entally, and a large ventral fold ectally. There is no ejaculatory duct as a differentiated portion.

Whole prostatic vesicle lined with columnar, ciliated epithelium. Extrabulbar portion receives abundant granulous erythrophil secretion. Additionally, receives gross granular xanthophil secretion around openings of branches. Intrabulbar portion pierced by gland cells, each containing a different type of secretion. They are distributed into rings, from proximal to distal end of vesicle, as follows: gross xanthophil granules, fine erythrophil granules mixed with fine xanthophil ones, and very fine erythrophil granules. Extrabulbar portion surrounded by interwoven muscle fibres, and intrabulbar portion by a muscular layer of circular fibres interspersed with longitudinal ones. The male atrium lined with cuboidal to columnar epithelium, non-ciliated, apically xanthophil, and crossed by cells containing erythrophil granular secretions. Male

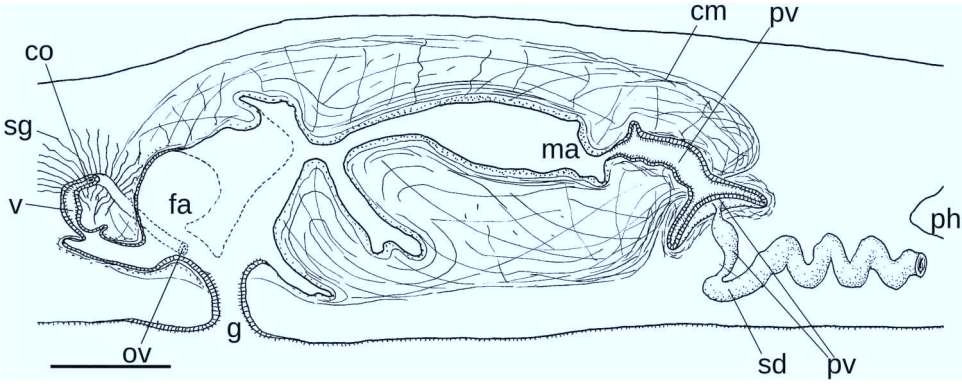

Figure 6. *Choeradoplana bocaina* sp. nov. Holotype. Diagrammatic reconstruction of copulatory apparatus from sagittal sections. Only one-side half represented. Scale bar: 0.5 mm.

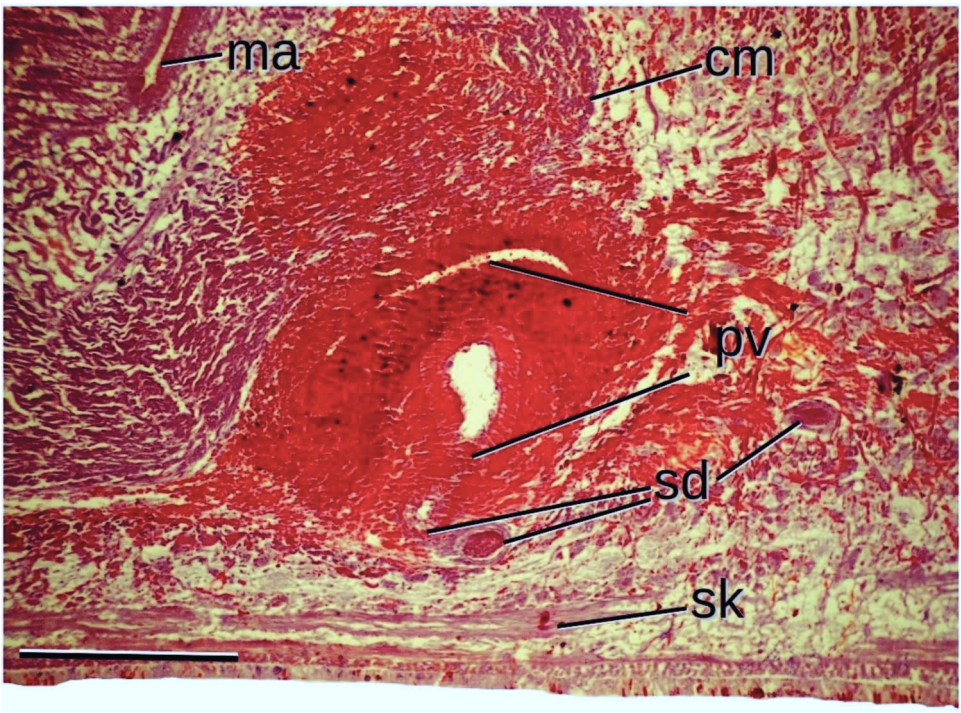

Figure 7. *Choeradoplana bocaina* sp. nov. Holotype. Micrograph of a para-sagittal section of the paired portion of the prostatic vesicle. Scale bar 0.25 mm.

atrium is clothed with a dense layer of circular muscle fibres (25 μm thick) followed by one of longitudinal fibres (50–80 μm).

Ovaries rounded, *c*.300 μm in diameter, placed 11 mm from anterior end, above the ventral nerve plate (paratype MZUSP PL 998). Ovovitelline ducts emerge from dorso-external aspect. Laterally to female atrium, they rise posteriorly and are medianly

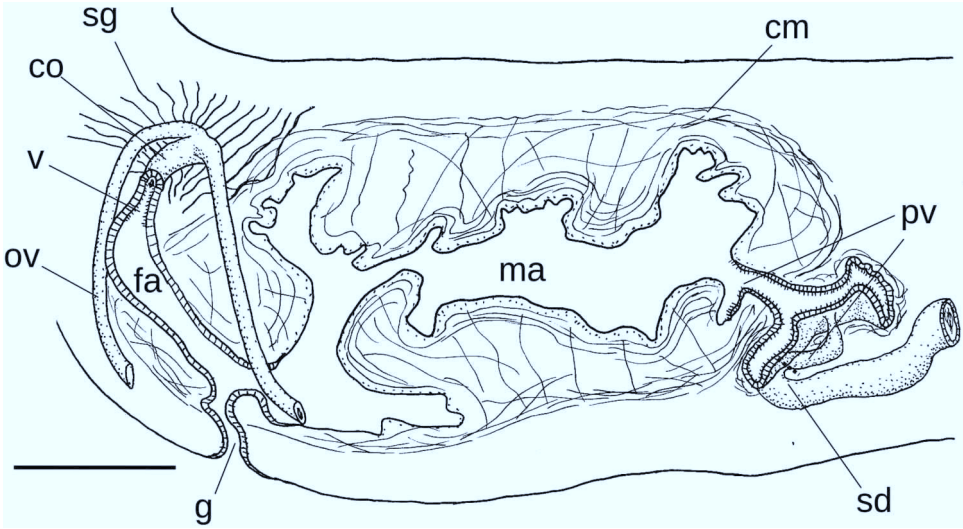

Figure 8. *Choeradoplana bocaina* sp. nov. Paratype MZUSP PL 998. Diagrammatic reconstruction of the copulatory apparatus from sagittal sections. The fixation caused distortion in the female organs. Only one-side half of the prostatic vesicle represented. Scale bar 0.5 mm.

inclined, then unite dorsally, in the sagittal plane, to the common glandular ovovitelline duct (Figures 5, 6, 8). They receive shell glands into distal portion. Common glandular ovovitelline duct is short, and directed downwards; continuous with vagina, an upward diverticle of female atrium. In the holotype female atrium is dilated anteriorly and abruptly narrowed posteriorly; in paratype MZUSP PL 998, whole atrium is a narrow cavity. Female atrium is as long as one-third to half of male atrium. At level of gonopore, an irregular dorsal fold separates male and female atria.

Vagina is lined with a columnar to cuboidal ciliated epithelium, crossed by scarce gland cells containing granular erythrophil secretion. Coated with thin layer of circular muscle fibres. Female atrium lined with columnar, non-ciliated epithelium, apically xanthophil, and penetrated by scarce cells containing granular erythrophil secretion. It is surrounded by a thin longitudinal muscle layer followed by a circular one (12 μm). Common muscular coat formed, especially in anterior portion, by a very dense layer, 120 μm thick, of decussate fibres.

Discussion

The genus *Choeradoplana* currently contains six species: *C. ehrenreichi* von Graff, 1899, *C. iheringi*, *C. langi* von Graff, 1899, *C. bilix* Marcus, 1951, *C. catua* Froehlich, 1955 and *C. marthae* Froehlich, 1955. Regarding the colour pattern, all of them, except for *Choeradoplana iheringi*, differ from *C. bocaina* in that they have sharp dark longitudinal lines or bands on a clear ground, instead of the dark speckles of variable size and density, as in *C. bocaina.* In *C. iheringi*, the back is homogeneously mottled with minute brown spots.

Regarding the internal morphology, *C. bocaina* does not have any peculiar outstanding characteristics. However, morphological details, or the combination of

features of the copulatory complex as well as those of the pharynx, do not allow its identification with any of the previously described species. *Choeradoplana catua*, a species with dark lines along the body, is the most similar as regards the general anatomy, but its prostatic vesicle is completely intrabulbar, contrasting with that of *C. bocaina*, which is partially extrabulbar. In addition, in *C. catua*, the dorsal insertion of the pharynx and mouth are at the same transverse level, whereas in *C. bocaina*, dorsal insertion is anterior to the mouth.

Choeradoplana banga sp. nov.
(Figures 9–15; Tables 3, 4)

Type material

Holotype. MZUSP PL 1000: Parque Estadual da Serra da Cantareira, São Paulo/SP, Brazil, *c*.23°25′45″ S, 46°37′57″ W, F. Carbayo et al. col., 30 January 2008. Cephalic region: sagittal sections on six slides; anterior region 2: sagittal sections on 12 slides; anterior region 3: horizontal sections on five slides; pre-pharyngeal region: transverse sections on five slides; pharynx and copulatory apparatus: sagittal sections on 15 slides.

Paratypes. MZUSP PL 1001: ibid., 23°25′44.9" S, 46°37′57″ W, F. Carbayo et al., col., 14 December 2008. Pharynx: sagittal sections on 21 slides; copulatory apparatus: sagittal sections on 17 slides; MZUSP PL 1002. ibid., 23°25′44.9" S, 46°37′57″ W, F. Carbayo et al., col., 14 December 2008. Cephalic region: sagital sections on six slides; anterior region 2: sagittal sections on eight slides; pre-pharyngeal region: transverse sections on three slides; pharynx: sagittal sections on 10 slides; copulatory apparatus: sagittal sections on 18 slides.

Type locality

Parque Estadual da Serra da Cantareira, São Paulo/SP, Brazil, covered with secondary Atlantic Rainforest.

Etymology

In Tupi-Guarani language *banga* means *bent* or *reverse*; it alludes to the ventral, behind the atrium, approach of the common glandular ovovitelline duct, in contrast to what has been described for the genus.

Diagnosis

Choeradoplana with pale ochre dorsum, with numerous brown-to-black irregular small spots arranged in a network design; pharynx bell-shaped; prostatic vesicle intrabulbar; narrow female atrium; common glandular ovovitelline duct approaching the vagina behind the atrium, from the ventral side; a massive sphincter ventral to male and female atria, adjacent to the gonopore canal.

Description

Paratype MZUSP PL 1002, the largest specimen when alive, measured up to 45 mm in length and 2 mm in width. After fixation paratype measured 44 mm in length and

Table 3. *Choeradoplana banga* sp. nov. – body measurements of fixed specimens (in mm and % of body measurements).

Specimen	Holotype	Paratype MZUSP PL 1001	Paratype MZUSP PL 1002
Length	36	?	44
Width	3	3.5	3
Mouth to anterior tip	20	?	28
Relative M : L	57.1 %	?	63.6 %
Gonopore to anterior tip	28.5	?	33
Relative G : L	81.5 %	?	75.0 %
Creeping sole : width	81.2 %		78.6 %

G, gonopore; L, length; M, mouth; W, width.

3 mm in width (Table 3). Body elongated, with anterior end slightly dilated, provided with the two glandular cushions ventrally separated by a longitudinal groove, diagnostic of the genus (Figure 9). As characteristic for the genus, live worms held the anterior extremity dorso-posteriorly rolled. Posterior end pointed. Dorsum highly convex in cross-section; body margins rounded; ventral side slightly convex. Dorsal background colour pale ochre, with numerous small irregular dark brown spots, most confluent, creating a network design. Body margins slightly ferruginous; ventral side pale yellowish. Mouth 20 mm and gonopore at 28.5 mm from anterior end (holotype).

Eyes formed by one pigmented cup *c*.55 μm in diameter, absent in the first 0.25 mm. From this point on, distributed marginally in an irregular row of two or three eyes, up to posterior end of body. Sensory pits 35 μm deep, located ventro-marginally, from 2 mm to 8.2 mm behind anterior end (paratype MZUSP PL 1001).

Epidermis ciliated only over creeping sole. Rhabditogen cells open onto whole cephalic surface, more densely through epithelium of glandular cushions. Cellular bodies of cephalic cushion erythrophil glands located between longitudinal cutaneous muscle layer and epithelium.

At anterior end, 2.2 mm behind apex, ventral nerve plate (100 μm thick) gives rise to an ill-defined cerebral ganglion (approximately 200 μm thick and 2 mm long) (holotype).

Cutaneous musculature comprising three typical layers of Geoplaninae species, namely, a subepithelial more external layer of circular fibres, and then a double layer of diagonal decussated fibres, and then one of longitudinal fibres, arranged into dense bundles (Figure 10; Table 4). Ventrally, layer of longitudinal fibres partially sunk into the parenchyma and with its fibres seemingly not joined into bundles. CMI 29.5%. In cephalic region sunken cutaneous longitudinal muscle fibres gather medianly forming retractor, unroller of whole extremity, as described by Froehlich (1955) for *C. iheringi*.

Three parenchymal muscle layers: a dorsal weak layer of diagonal, decussated fibres (30–35 μm thick, holotype), located immediately under the dorsal subcutaneous nerve net, and two transverse layers, one supraintestinal (80–105 μm thick) and other subintestinal (75 μm thick) (Figure 10). In cephalic region there is an additional layer (20 μm thick) of transverse muscle fibres, located under ventral nerve plate.

Figure 9. *Choeradoplana banga* sp. nov. Holotype initiating movement. About 30 mm in length.

Mouth in middle of pharyngeal pouch (Figure 11), 20 mm from anterior body end (holotype). Pharyngeal pouch lined with non-ciliated, cubic-to-flat epithelium, underlain by a one-fibre thick layer of circular muscle fibres. Pharynx bell-shaped, tending to collar-shape. Outer pharyngeal epithelium flat, ciliated, underlain by one-fibre thick longitudinal muscle layer, then by a layer of circular fibres (15 μm thick) with interspersed longitudinal fibres. Inner pharyngeal epithelium cuboidal, ciliated, underlain by a circular muscle layer (50 μm thick) with interspersed longitudinal fibres.

Testes located between supraintestinal muscle layer and intestine. Distributed on each side of the body in a single-to-triple row from ovarian level (6.6 mm from anterior end) to dorsal pharyngeal insertion, at 23 mm from anterior end (holotype). Anteriormost testes 2.5 mm behind cerebral ganglia. Sperm ducts running immediately above subintestinal muscle layer, dorsal to ovovitelline ducts. Close to copulatory apparatus they curve anterodorsally and medianly, before penetrating the penial bulb to open at each side of proximal tubular portion of prostatic vesicle (Figures 12–15). Distally, vesicle widens acquiring an irregular shape and very folded walls. It communicates amply with male cavity, without an ejaculatory duct. Entally,

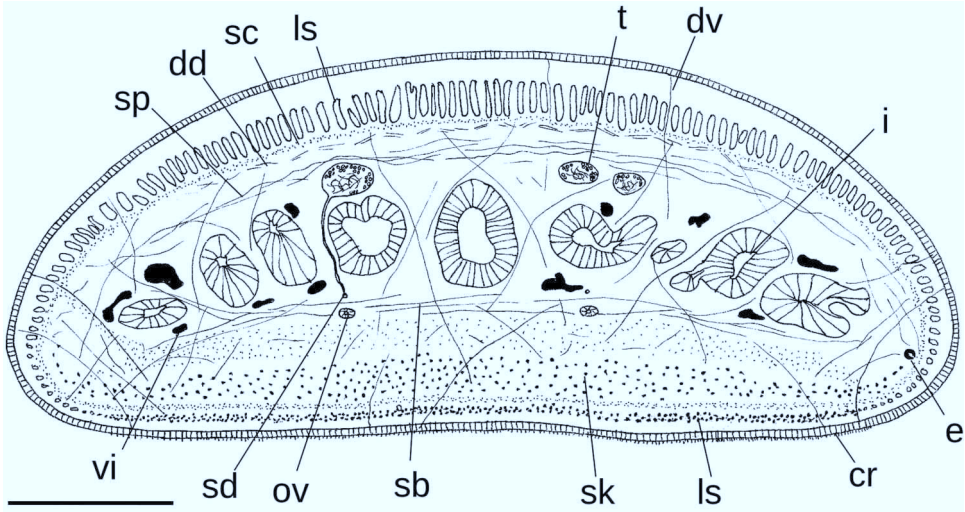

Figure 10. *Choeradoplana banga* sp. nov. Holotype. Diagrammatic transverse section of pre-pharyngeal region. Scale bar 0.5 mm.

male atrium is spacious with several small folds, especially from dorsal wall; ectally, mainly because of a bulky fold from the ventral wall, it becomes a long narrow canal.

Epithelium of tubular portion of prostatic vesicle columnar, ciliated, crossed by cell glands with fine erythrophil granules; dilated portion lined with columnar, irregularly ciliated epithelium. Pierced by cell glands containing gross erythrophil granules, and underlain by a 15-μm-thick circular muscle layer. In holotype, tubular portion of prostatic vesicle contains sperm.

Male atrium clothed proximally with a columnar epithelium that becomes cubic distally; irregularly ciliated all along. It is crossed by secretory cells of two types,

Table 4. *Choeradoplana banga* sp. nov. – thickness (in μm) of cutaneous muscle layers in the pre-pharyngeal region.

Specimen	Holotype
Dorsal circular	10 (1–2)
Dorsal diagonal	40 (1–4)
Dorsal longitudinal	110 (50–75)
Dorsal total	160
Sunken ventral longitudinal	110 (1–8)
Normal ventral longitudinal	25 (1–3)
Ventral diagonal	15 (1–3)
Ventral circular	5 (1–2)
Ventral total	150
CMI	29.5%

Lowest and highest number of muscle fibres per bundle are given in parenthesis.
CMI, cutaneous musculature thickness relative to body height at the pre-pharyngeal region.

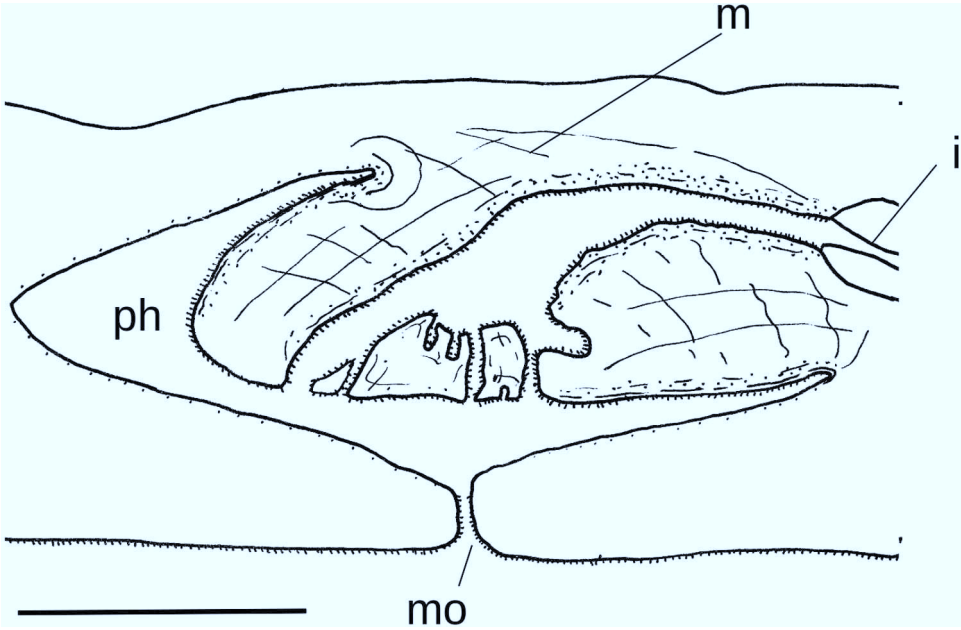

Figure 11. *Choeradoplana banga* sp. nov. Holotype. Diagrammatic represention of the pharynx from sagittal sections. Scale bar 1 mm.

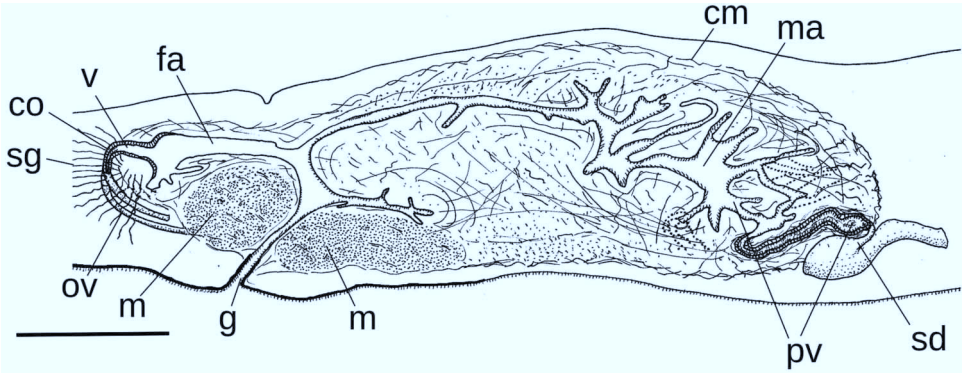

Figure 12. *Choeradoplana banga* sp. nov. Holotype. Diagrammatic representation of the copulatory apparatus from sagittal sections. Only one-side half of the prostatic vesicle represented. Scale bar 1 mm.

containing erythrophil and cyanophil granular secretions, respectively. Its muscularis is an 8-μm-thick layer of circular fibres. Between common muscular coat and muscularis is a dense mass of muscle fibres without clear orientation. In paratype MZUSP PL 1001, male atrial cavity is partially filled by an ejaculate with sperm and erythrophil secretion.

Ovaries globular, approximately 320 μm in diameter, located 2.5 mm behind cerebral ganglia, immediately above ventral nerve plate (holotype). Ovovitelline ducts arise

Figure 13. *Choeradoplana banga* sp. nov. Holotype. Micrograph of a sagittal section of the massive sphincter ventral to the male and female atria. Scale bar 0.5 mm.

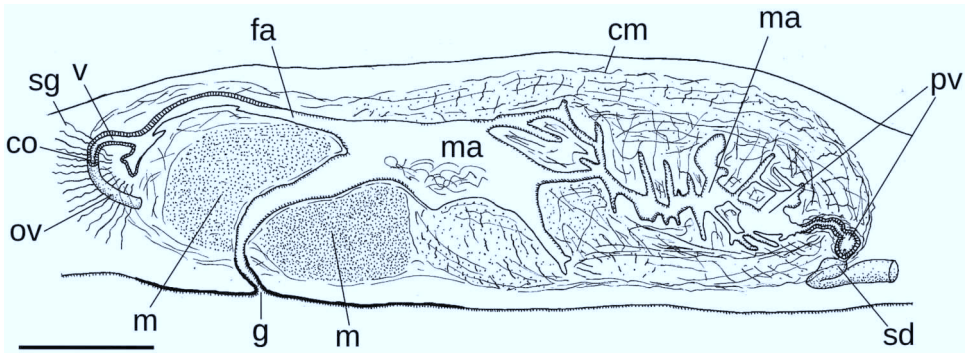

Figure 14. *Choeradoplana banga* sp. nov. Paratype MZUSP PL 1001. Diagrammatic representation of the copulatory apparatus from sagittal sections. Only one-side half represented. Scale bar 1 mm.

from external side of ovaries, and run backwards between muscle fibres of subintestinal transverse muscle layer. They proceed inclined medianly and slightly to the dorsum passing female atrium. Behind female atrium at half the height of dorsum, they curve upwards, and unite with short common glandular ovovitelline duct. The latter joins the vagina, a posteriorly directed tubular diverticle of female atrium. Female atrium is a narrow cavity, as long as one-quarter of male atrium.

Female atrium and vagina, both lined with columnar non-ciliated epithelium, crossed by two types of glands, respectively, erythrophil and cyanophil. Female atrium with 6-μm-thick coat of interwoven muscle fibres; vagina with thin coat of circular fibres.

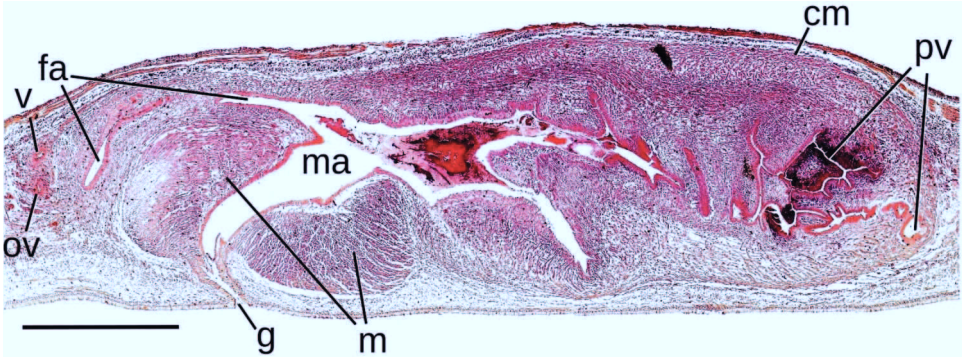

Figure 15. *Choeradoplana banga* sp. nov. Paratype MZUSP PL 1001. Micrograph of a sagittal section of the copulatory apparatus. Scale bar 1 mm.

Ventrally, in the wall of male and female atrium adjacent to gonopore canal, is an extraordinarily massive muscular sphincter, 500 μm thick, composed of circular fibres interspersed with very scarce longitudinal fibres. Gonopore canal lined with columnar, ciliated epithelium underlain by 20-μm-thick circular musculature.

Common muscular coat consists of a dense layer, 80–120 μm thick, of interwoven fibres embracing the whole copulatory complex.

Discussion

Externally, *C. banga* is hardly distinguishable from *C. iheringi*, to such an extent that, convinced that we were dealing with a *C. iheringi* specimen, we removed and degraded a piece of the paratype MZUSP PL1001 for DNA extraction without having previously measured the worm's length. Specimens of *C. iheringi* have been collected in Teresópolis, RJ (Riester 1938), Ribeirão Pires, SP (Marcus 1951) and Salesópolis, SP (Leal-Zanchet and Souza 2003). Ribeirão Pires is the closest to the type locality of *C. banga*, approximately 40 km away.

Regarding the anatomy, the copulatory complex of *C. banga* possesses two important peculiarities. One is the female genital canal (vagina plus common glandular ovovitelline duct) flexing downwards to receive both ovovitelline ducts that finish their ascending path behind the copulatory complex, before attaining the dorsum. This is a unique arrangement of the female genital ducts (female genital canal plus ovovitelline ducts) previously not described in *Choeradoplana.* All the previously described species, *C. iheringi* included, as remarked by Ogren and Kawakatsu (1990), have the female genital canal dorsally or dorso-anteriorly flexed, and the ovovitelline ducts ascending, up to the dorsum, laterally to the female atrium, and approaching anteriorly to the female canal. The other outstanding peculiarity is the presence of the massive sphincter ventrally to male and female atria, a feature that is not only unique for a *Choeradoplana* species, but also unique among known Geoplaninae species. The two peculiarities of the genital anatomy reinforces Froehlich's (1955) statement that the copulatory apparatus of *Choeradoplana* is of a simple type, but shows high variation in the details.

Choeradoplana gladismariae sp. nov.
(Figures 16–22; Tables 5, 6)

Type material

Holotype. MZUSP PL 1003: Parque Estadual Intervales, Ribeirão Grande/SP, Brazil, 24°16′35″ S, 48°24′56″ W, F. Carbayo et al., col., 12 December 2008. Cephalic region: transverse sections on four slides; anterior region 2 (ovaries): sagittal sections on five slides; anterior region 3: horizontal sections on four slides; pre-pharyngeal region: transverse sections on three slides. Pharynx and copulatory apparatus: sagittal sections on 10 slides.

Paratype. MZUSP PL 1004: ibid., 24°16′35″ S, 48°24′56″ W, F. Carbayo et al. col., 7 July 2009. Cephalic region: horizontal sections on two slides; anterior region 2: sagittal sections on three slides; pre-pharyngeal region: transverse sections on one slide. Pharynx and copulatory apparatus: sagittal sections on four slides.

Type locality

Parque Estadual Intervales, Ribeirão Grande/SP, Brazil, covered with primary Atlantic Rainforest.

Etymology

Specific name from Gladis Maria Schmidt, Carbayo's wife, to whom this species is dedicated.

Diagnosis

Choeradoplana species with a thin dark-brown mid-stripe and two para-median bands of brown spots on a pale yellowish dorsum; both dorsally and ventrally, the cutaneous muscle layer of longitudinal muscle is partially sunken into the parenchyma; prostatic vesicle wall bellows-like and pleated.

Description

Body relatively wide, with convex dorsum, rounded margins and slightly convex ventral side. Anterior end ventrally provided with pair of glandular cushions separated by longitudinal groove, and kept up and rolled backwards by the live worm. Posterior end pointed. Largest specimen (holotype) measured, when alive, up to 32 mm in length and 2 mm in width; after fixation, with cephalic region unrolled, 35 mm (Table 5). Mouth 18.5 mm from anterior end, and gonopore 22.7 mm (holotype).

Dorsally, rolled anterior end excepted, ground colour yellowish with thin longitudinal dark brown mid-stripe (1/25 of body width) and two para-median bands (1/4 of body width) of brown specks, disposed as to create a marmorean aspect (Figure 16). In the rolled cephalic extremity, median line ends before the apex, and the bands are reduced to externalmost bold limits; the exposed ventral surface is greyish, especially on margins of median groove. In posterior extremity, median stripe and abruptly narrowed bands merge. Ventral side is whitish medianly, and faintly yellow marginally.

Table 5. *Choeradoplana gladismariae* sp. nov. – body measurements of fixed specimens (in mm and % of body measurements).

Specimen	Holotype	Paratype MZUSP PL 1004
Length	35	25
Width	5	2
Mouth to anterior tip	18.5	13.50
Relative M : L	52.8%	54.0 %
Gonopore to anterior tip	22.7	21.0
Relative G : L	64.8%	84.0 %
Creeping sole : width	84.6%	78.2 %

G, gonopore; L, length; M, mouth; W, width.

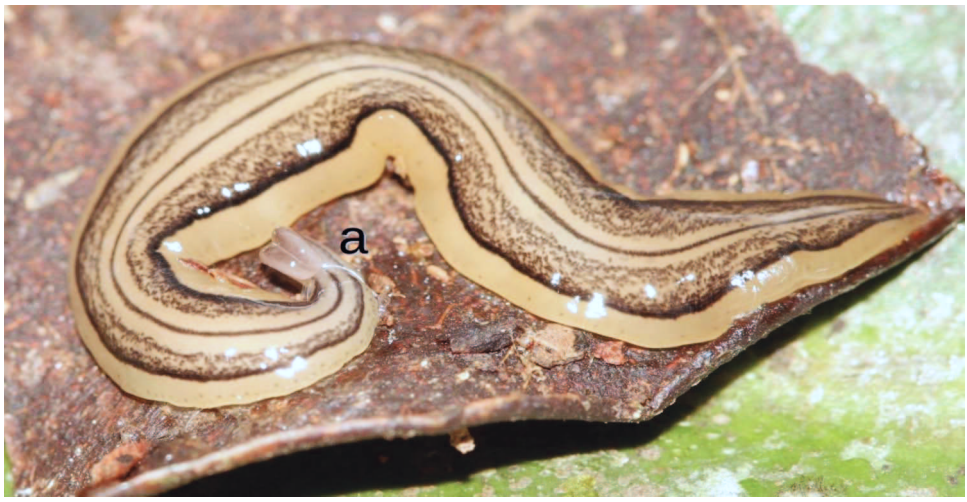

Figure 16. *Choeradoplana gladismariae* sp. nov. Holotype resting. About 30 mm in length.

Eyes comprise one pigmented cup, 50–70 μm in diameter, roughly arranged as two or three marginal rows up to posterior end. Absent in very anterior tip. Without clear halos. Sensory pits, 20–30 μm deep, as a uniserial ventrolateral row, from little behind the anterior end through 11 mm (holotype).

Epidermis ciliated just over creeping sole. In cephalic end, rhabditogen cells open onto entire surface, but more densely through epithelium of glandular cushions. Their cellular bodies lay in parenchyma between subcutaneous nerve plexus and epithelium.

Cerebral ganglia 3 mm behind apex. Although not clearly delimited from ventral nerve plate, 80 μm thick; it is approximately 5 mm long and 130 μm thick (holotype).

Three usual geoplaninid cutaneous muscle layers present: one circular, two diagonal with decussate fibres, one longitudinal with fibres arranged into bundles (Table 6). Dorsally, as well as ventrally, body margins excluded; longitudinal layer partially sunk into parenchyma. Dorsal sunken fibres gathered into well-delimited, compact bundles, 107 μm thick; ventral ones much less numerous, gathered into a layer (45 μm

Table 6. *Choeradoplana gladismariae* sp. nov. – thickness (in μm) of cutaneous muscle layers in the pre-pharyngeal region.

Specimen	Holotype	Paratype MZUSP PL 1004
Dorsal circular	2.5 (1–2)	2.5 (1–2)
Dorsal diagonal	15 (1–3)	5 (1–2)
Dorsal normal longitudinal	45 (32–50)	15 (3–8)
Dorsal sunken longitudinal	107 (45–130)	28 (20–35)
Dorsal total	169.5	92.5
Ventral sunken longitudinal	67 (4–30)	30 (2–6)
Ventral normal longitudinal	20 (4–18)	10 (1–4)
Ventral diagonal	5 (1–3)	5 (1–2)
Ventral circular	2.5 (1–2)	2.5 (1–2)
Ventral total	94.5	47.5
CMI	22.0 %	21.2 %

Lowest and highest number of muscle fibres per bundle are given in parenthesis.
CMI, cutaneous musculature thickness relative to body height at the pre-pharyngeal region.

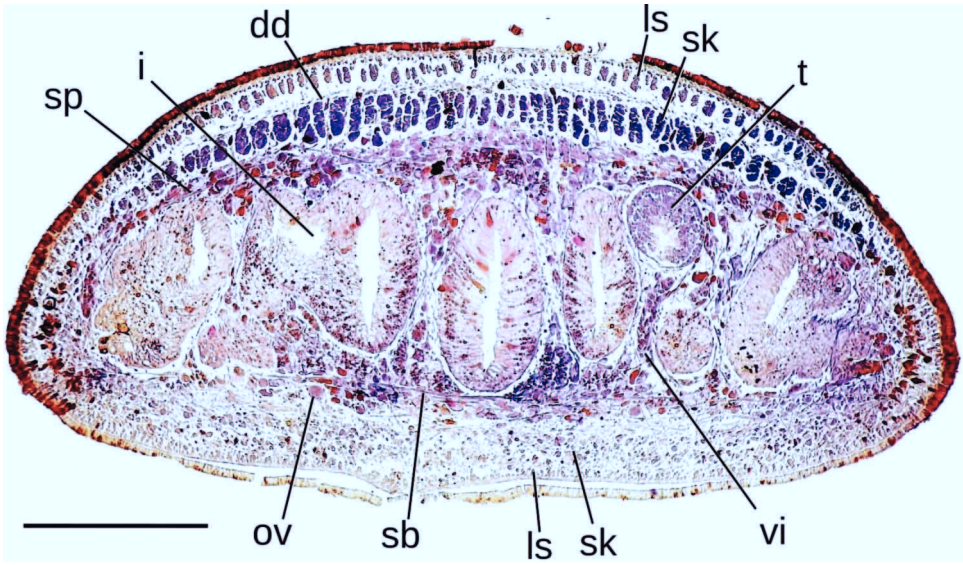

Figure 17. *Choeradoplana gladismariae* sp. nov. Holotype. Transverse section of pre-pharyngeal region. Scale bar 0.5 mm.

thick) with loose, smaller, ill-delimited bundles (Figures 17–19). In the cephalic region dorsal longitudinal sunken fibres are less apparent; direction of fibres in histological sections is not clearly discernible. CMI, 21.2–22.0%. Otherwise, arrangement of cutaneous muscle fibres in cephalic region as in *C. iheringi* (see Froehlich 1955; Carbayo and Leal-Zanchet 2003).

Parenchymal musculature: besides dorsoventral muscles, layer of dorsodiagonal fibres (20 μm thick, holotype) (followed by the sunken layer of longitudinal muscles mentioned above), the transverse supraintestinal (60 μm thick) and subintestinal

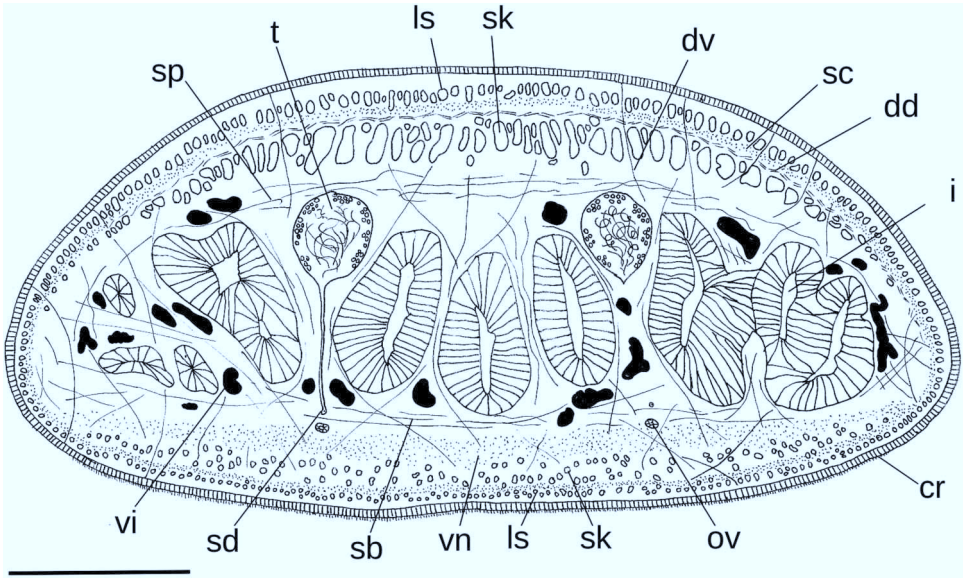

Figure 18. *Choeradoplana gladismariae* sp. nov. Holotype. Diagrammatic representation of a transverse section of pre-pharyngeal region. Scale bar 0.5 mm.

(48 μm thick) fibres (Figures 17, 18), and, in cephalic region, a transverse subneural muscle layer (25 μm thick), between ventral nerve plate and retractor muscle. Arrangement of parenchymal muscle fibres in cephalic region as in type species (Froehlich 1955; Carbayo and Leal-Zanchet 2003).

Mouth located in middle of pharyngeal pouch. Pharynx bell-shaped, with folded free margin (Figure 20). Dorsal insertion approximately at the same transverse level as mouth. Lining epithelium of pharyngeal pouch flat, non-ciliated, underlain by one-fibre-thick layer of circular muscle fibres. Outer pharyngeal epithelium flat ciliated, with sunken nuclei. Underlain by a 5-μm-thick layer of longitudinal muscle fibres, followed by one with circular fibres (6 μm thick). Inner pharyngeal epithelium cubic-to-flat, ciliated, followed by layer of circular fibres (45 μm thick) with interspersed longitudinal ones.

Testes located between supraintestinal parenchymal muscle layer and intestine, extending as a single-to-triple row from 1 mm anterior to each ovary (3.5 mm behind cerebral ganglia) up to level with dorsal pharyngeal insertion (holotype). Sperm ducts run immediately above subintestinal muscle layer, just dorsally to ovovitelline ducts. Near the copulatory complex, sperm ducts communicate with the paired extrabulbar, branches of prostatic vesicle. Prostatic branches penetrate into penial bulb, unite as a short tube that opens into the final, dilated portion of prostatic vesicle (Figures 21, 22). This portion has a complex structure, with pleated lateral walls, the whole organ reminiscent of a bellows. On each side of the vesicle, the pleats, disposed in a dorsoventral series, are oriented as to converge ectally, where they end leaving the ectal, final, portion of vesicular cavity free of pleats. Medianly the cavity, ample in sagittal plane but very narrow transversally, separates two lateral sets of pleats. Both sides of vesicle, between each pair of pleats in dorsoventral series, a bag, open to the median cavity, is formed. Ectally, vesicular cavity continues through male atrium. Lumen of atrium is narrow and its wall folded.

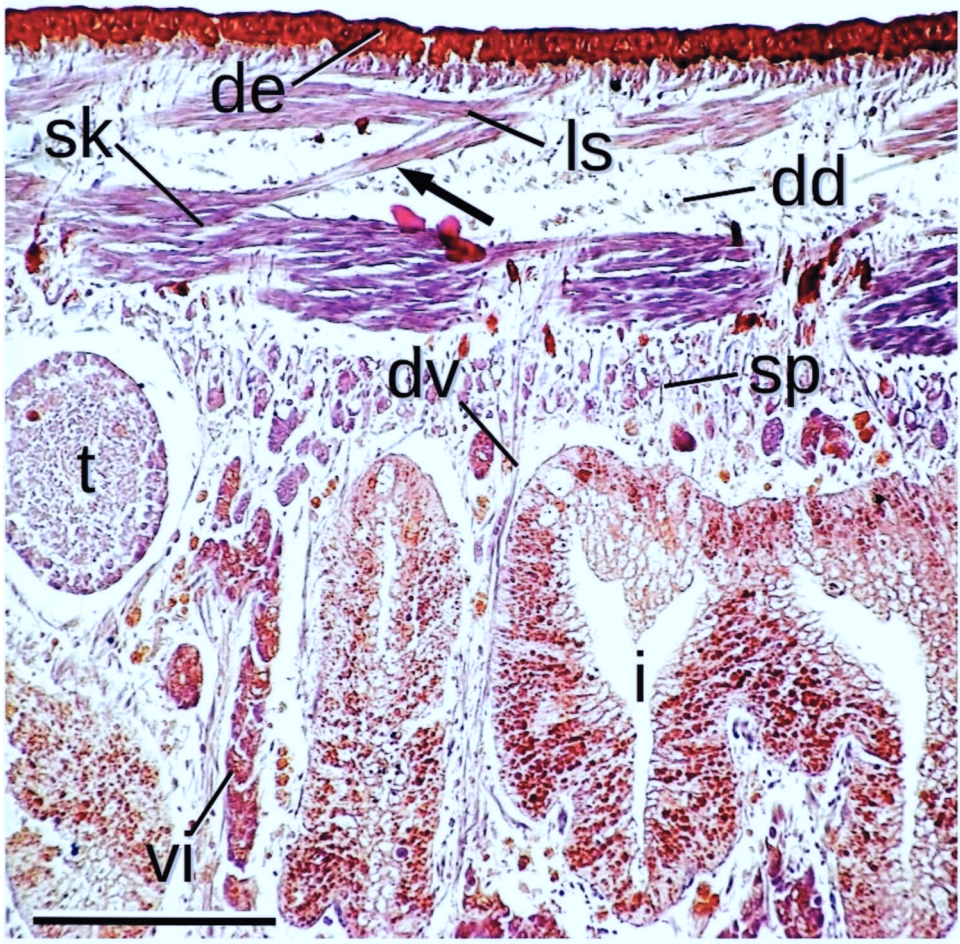

Figure 19. *Choeradoplana gladismariae* sp. nov. Holotype. Micrograph of a sagittal section of the anterior region of the body showing the most anterior testis. Arrow indicates longitudinal muscle fibres crossing subcutaneous nerve net sunk into the parenchyma. Scale bar 0.2 mm.

Branches and tubular portion of prostatic vesicle are clothed with a columnar ciliated epithelium, which is crossed by secretory cells containing fine erythrophil granules. In dilated portion, pleats are mostly lined with non-ciliated cubic epithelium; in rest of cavity wall it is ciliated. Regarding their secretions, pleats are clearly differentiated into two regions: a basal region, the most extensive, and a distal one. The former receives two types of glands, respectively: one, very abundant, with erythrophil granules, and the other, very scarce, with xanthophil granules; both types have their cellular bodies in parenchyma, anterior to copulatory complex. Distal region is very richly pierced by secretory cells containing fine highly erythrophil granules, whose cellular bodies lay below epithelium. A 12-μm-thick layer of circular muscle fibre underlies epithelium of pleats. Involving the whole dilated portion, a mass of glandular tissue is fully saturated with coarse granules of an erythrophil secretion. There are some interspersed muscle fibres. Male atrium lined with cubic-to-columnar epithelium, entally ciliated where glands discharge their cyanophil granular secretion. Ectally epithelium

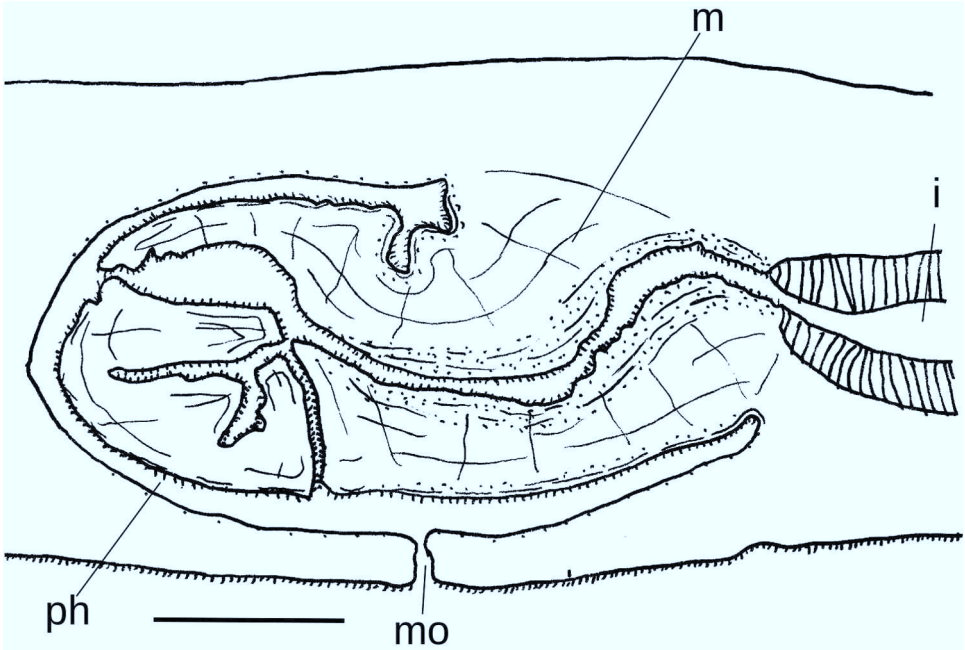

Figure 20. *Choeradoplana gladismariae* sp. nov. Holotype. Diagrammatic representatation of the pharynx from sagittal sections in lateral view. Scale bar 0.5 mm.

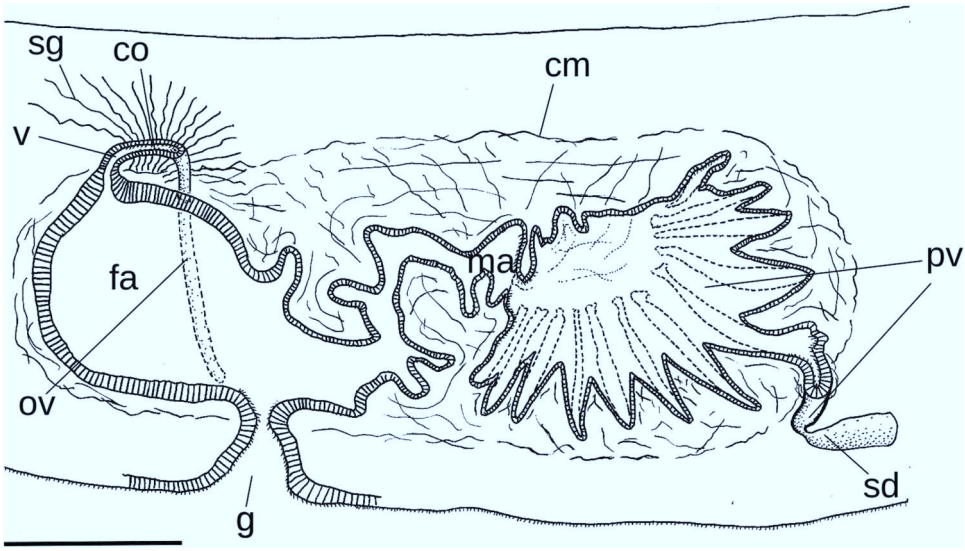

Figure 21. *Choeradoplana gladismariae* sp. nov. Holotype. Diagrammatic reconstruction of the copulatory apparatus from sagittal sections. Only one-side half represented. Scale bar 0.5 mm.

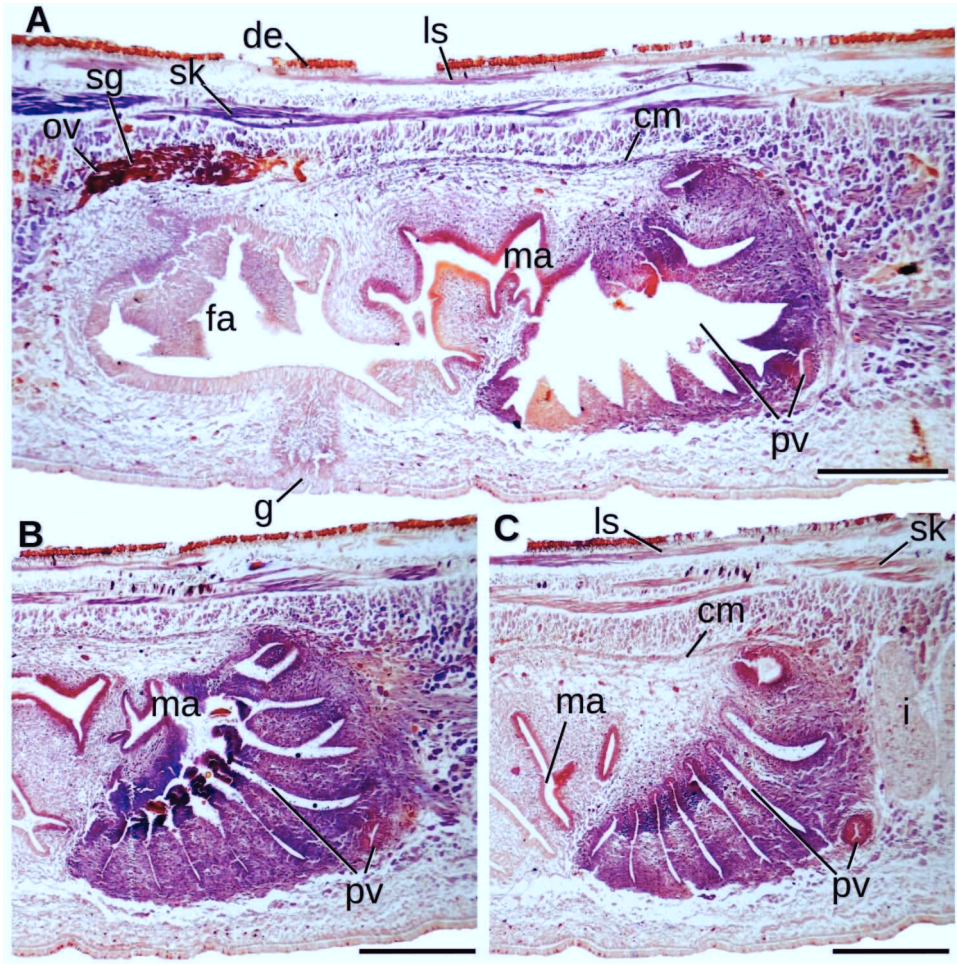

Figure 22. *Choeradoplana gladismariae* sp. nov. Holotype. Micrographs of (A) the mid-sagittal section the copulatory apparatus; (B) a para-sagittal section of the prostatic vesicle; (C) a lateral sagittal section of the prostatic vesicle. Scale bars 0.5 mm.

receives an erythrophil granular secretion. Male atrium coated with a layer of circular muscle fibres, 8 μm thick.

Ovaries roughly ellipsoid, 250 μm in diameter. Located immediately above ventral nerve plate, at 11 mm from anterior tip, and 4.5 mm behind cerebral ganglia (holotype). Ovovitelline ducts arise from external side of ovaries, and run backwards above ventral nerve plate. Behind gonopore they ascend laterally to female atrium, posteriorly and medianly inclined, then unite dorsally to atrium, and continue as common glandular ovovitelline duct. The latter runs backwards to communicate with vagina (Figures 21, 22). Short vagina arises dorsally from female atrium, and proceeds curved forward, then receives common glandular ovovitelline duct. Shell glands also open into distal portions of paired ovovitelline ducts. Female atrium is an ample cavity dorsoventrally dilated and laterally narrowed. It is two-thirds the length of male atrium.

Vagina and female atrium lined with columnar, non-ciliated epithelium, which receives two types of glands, with erythrophil and fine cyanophil secretions, respectively. It is underlain by a 6–10-μm-thick circular muscle layer.

Common muscular coat of the copulatory complex composed of a weak layer, 80–120 μm thick, of intermingled fibres.

Discussion

Among striped *Choeradoplana* species, only *C. gladismariae* and *C. ehrenreichi* have an odd number of three distinct longitudinal dark stripes on a clear ground. They differ, however, in that *C. ehrenreichi* has three equally wide stripes, whereas in *C. gladismariae* the lateral stripes are much wider than the median one. They are bands rather than stripes.

Regarding the internal morphology, *C. gladismariae* has an outstanding characteristic, unique for the genus, that is, the strong dorsal layer of insunk cutaneous longitudinal fibres. In the *Choeradoplana* species described up to now, a similar muscle arrangement occurs only ventrally, as is stated in Froehlich's diagnosis of the genus (Froehlich 1955). Besides *Choeradoplana*, three other Geoplaninae genera, the three monotypic, have been described with the cutaneous longitudinal musculature partially sunken into the parenchyma, namely *Gusana*, E.M. Froehlich, 1978 *Liana* E.M. Froehlich, 1978 and *Supramontana* Carbayo and Zanchet, 2003. Only *Gusana cruciata*, the Chilean unique species of the genus, has the cutaneous longitudinal muscles partially sunken both dorsally and ventrally, like *C. gladismariae*. However, in *G. cruciata*, dorsal sunken muscle fibres do not gather into bundles but occur loosely in the parenchyma between the subcutaneous nerve plexus and the ventral nerve plate (E.M. Froehlich 1978). *Choeradoplana* and *Gusana*, as well as *Liana* and *Supramontana*, are distinct genera through the morphology of their cephalic extremities.

Choeradoplana **emended diagnosis**

As a result of the present study the diagnosis of *Choeradoplana* must be emended on some points, as follows: Geoplaninae of elongated subcylindrical body. Cephalic region with two glandular cushions, ventrally separated by a longitudinal groove; kept rolled up and backwards in live worms. Eyes and sensory pits absent in the apex. Broad creeping sole, more than one-third of body width. Strong cutaneous longitudinal muscles partially sunken into the parenchyma, exclusively ventrally or, more rarely, ventrally and dorsally too. Anteriorly all sunken ventral longitudinal fibres concentrated medianly, constituting the retractor unroller of the cephalic extremity. Common glandular ovovitelline duct approaching vagina dorsally from anterior direction, more rarely approaching behind the female atrium from the ventral direction.

Acknowledgements

We are grateful to Gladis Maria Schmidt for helping with diurnal and nocturnal samplings throughout the years; to Júlio Pedroni for the pictures of the living specimens of *C. bocaina* and *C. banga*; to the Instituto Florestal (Secretaria do Meio Ambiente do Estado de São Paulo, Brazil) and Instituto Chico Mendes de Conservação da Biodiversidade (Brazil) for

licensing the fieldwork; to two anonymous reviewers for their valuable comments and suggestions. AcademicEnglishSolutions.com revised the English. F.C. has financial support from FAPESP (Brazil) and Fundación BBVA (Spain).

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