



A new Peruvian land planarian species of the genus *Gigantea* (Platyhelminthes, Tricladida, Geoplanidae)

LISANDRO H. L. NEGRETE¹, FRANCISCO BRUSA^{1,3}, FERNANDO CARBAYO²

¹CONICET. División Zoología Invertebrados, Facultad de Ciencias Naturales y Museo, Paseo del Bosque s/n. 1900. La Plata, Argentina. E-mails: lnegrete@fcnym.unlp.edu.ar; fbrusa@fcnym.unlp.edu.ar

²Escola de Artes, Ciências e Humanidades, Universidade de São Paulo. São Paulo, Brazil. E-mail: baz@usp.br

³Corresponding author

Abstract

A new species of Geoplaninae land planarian from south Peru, *Gigantea urubambensis* **sp. nov.**, is described. The most outstanding features of the new species are the very dorsal location of the testes, between the dorsal diagonal and the supra-intestinal parenchymatic muscle layers; a penis papilla projecting from the roof of the male atrium and acentrally traversed by the ejaculatory duct; and the absence of glandular ridges on the penis papilla.

Key words: Flatworm, terrestrial fauna, Continenticola, Neotropical Region, Peru, very dorsal testes

Introduction

In South America, Peru has the second highest number of land planarian species, with 33 species. Twenty-nine of these species belong to the Geoplaninae (two species of *Amaga* Ogren & Kawakatsu, 1990, twenty of *Geoplana* Schultze & Müller, 1857, two of *Gigantea* Ogren & Kawakatsu, 1990, one of *Notogynaphallia* Ogren & Kawakatsu, 1990, two of *Pasipha* Ogren & Kawakatsu, 1990, and two of *Pseudogeoplana* Ogren & Kawakatsu, 1990), two species to the Microplaninae (*Incapora* du Bois-Reymond Marcus, 1953 and *Microplana* Vejdovsky, 1889), one species to the Rhynchodeminae (*Dolichoplana* Moseley, 1877), in addition to the cosmopolitan species *Bipalium kewense* Moseley, 1878 (Bipaliinae). Most of these records date back to the mid-twentieth century (Meixner 1906; Beauchamp 1939; du Bois-Reymond Marcus 1951, 1953, 1957, 1958; Hyman 1955), and no new species of Peruvian terrestrial planarians have been described since then.

Gigantea comprises twelve species represented in Colombia, Costa Rica, Panama, Trinidad & Tobago, and Peru. The two known Peruvian species of the genus, *Gigantea idaia* (du Bois-Reymond Marcus, 1951) and *G. unicolor* (Hyman, 1955), were described from the proximities of Cusco at 4000 masl and northwest of Peru at 2100 masl, respectively.

In the present paper we describe a new land planarian species of the genus *Gigantea* from the Urubamba river basin, Peru.

Material and methods

The single specimen was collected in July 2007, near Camisea (11° 51' S; 72° 56' W) at 400 masl, department of Cusco, in the Lower Urubamba Region (LUR), east of the Andes Mountains, southern Peru. The area derives its name from the Lower Urubamba River, which forms the primary drainage basin in the region (Alonso *et al.* 2001). The LUR comprises approximately 600 km² of tropical rainforest that enclose some

areas impacted from exploitation of natural gas.

The worm was collected by hand, photographed *in vivo* and its external morphology and colour pattern described. Body size was measured both in the live animal and after fixation. The worm was fixed with 10% formaldehyde and thereafter preserved in 70% ethanol. For histological studies, fragments of the anterior region, pre-pharyngeal region, pharynx, and copulatory apparatus were cut and dehydrated in ascending ethanol series, and subsequently embedded in Paraplast. Sagittal, horizontal and transverse serial sections (6–10 µm thick) of different body regions were stained with haematoxylin-eosin and Cason trichrome stain (Romeis, 1989).

The ratio of height of cutaneous musculature to height of body (cutaneous muscular index, CMI or mc:h) was calculated after Froehlich (1955). To calculate the thickness of the epithelium and the cutaneous and parenchymatic muscular layers, four cross-sections on each of the four slides of the pre-pharyngeal region were measured and the mean for each measurement was calculated. The same procedure was followed for sagittal sections of the pharyngeal musculature and copulatory apparatus.

The holotype is deposited in the Invertebrate Collection at Museo de La Plata (MLP), Argentina.

Abbreviations used in figures

cm, common muscle coat; cml, cutaneous muscle layers; co, common glandular ovovitelline duct; cs, creeping sole; di, dorsal insertion of the pharynx; dp, dorsal parenchymatic muscle layer; dvp, dorso-ventral parenchymatic muscle fibers; e, eyes; ed, ejaculatory duct; ef, efferent duct; ep, epithelium; fa, female genital atrium; fp, folds of the pharynx; g, gonopore; i, intestine; lb, lateral bands; m, muscles; ma, male genital atrium; md, median band; mo, mouth; od, ovovitelline duct; ov, ovary; ph, pharyngeal pouch; pm, paramedian stripes; pp, penis papilla; pv, prostatic vesicle; sbp, sub-intestinal parenchymatic muscle layer; sg, shell glands; spp, supra-intestinal parenchymatic muscle layer; t, testes; v, vagina; vi, ventral insertion of the pharynx; vit, vitellaria.

Systematic account

Order Tricladida Lang, 1884

Suborder Continenticola Carranza, Littlewood, Clough, Ruiz-Trillo, Baguña & Riutort, 1998

Superfamily Geoplanoidea Stimpson, 1857

Family Geoplanidae Stimpson, 1857

Subfamily Geoplaninae Stimpson, 1857

Genus *Gigantea* Ogren & Kawakatsu, 1990

Gigantea urubambensis sp. nov.

Material. Holotype, MLP 6083. Anterior region: transverse sections on 49 slides; horizontal sections on 18 slides; sagittal sections on 72 slides. Pre-pharyngeal region: transversal sections on 45 slides. Pharynx: sagittal sections on 81 slides. Copulatory apparatus: sagittal sections on 99 slides. Rest of body in 70%–100% ethanol.

Diagnosis. Body large (length: 160 mm, maximum width: 13 mm, maximum height: 5.5 mm), elongated and lanceolated; dorsally with two thin paramedian reddish stripes along a median yellowish orange band, black lateral bands; body margins whitish; eyes initially marginal and uniserial encircling the anterior tip, backwards becoming dorsal and pluriserial with clear halos along the black lateral bands and yellowish

orange median band; dorsal epithelium ciliated in the cephalic region; glandular margin absent; CMI, 5.0–6.9%; pharynx collar-type; esophagus absent; testes very dorsal, between dorsal diagonal and supra-intestinal parenchymatic muscle layers; prostatic vesicle absent; penis papilla large, ventrally projected from the roof of the male atrium, acentrally traversed by the ejaculatory duct; glandular ridges on penis papilla absent; ovovitelline ducts ascend posterior to the female atrium; common glandular ovovitelline duct absent; female atrium wide.

Type locality. Tropical rainforest near Camisea (11° 51' S; 72° 56' W) in the Lower Urubamba Region, in Cusco, Peru.

Etymology. The specific name refers to the Lower Urubamba River Valley of southern Peru, where the specimen was collected.

Description. External features. The live specimen was 160 mm long and 13 mm in maximum width at pharynx level. The body is elongated and lanceolated, with a blunt rear end. After fixation, the worm was 120 mm long and 12 mm in maximum width (10% width:length ratio). Maximum height was 5.5 mm, 63 mm from the anterior end (53.0% of body length). Mouth and gonopore are located at 70 mm (58.3%) and 90 mm (75.0%), respectively, from the anterior end.

The dorsal side shows two thin paramedian reddish stripes along a median yellowish orange band (41% of body width); two black lateral bands (42% of body width). Further markings consist of two marginal whitish stripes (17% of body width) (Fig. 1). The ventral side is light whitish gray.



FIGURE 1. *Gigantea urubambensis* sp. nov. Holotype. Photograph of live specimen in dorsal view.

Eyes initially marginal and uniserial, encircling the anterior tip. At 9 mm from the anterior tip (8% of body length) they become dorsal and pluriserial with clear halos along the black lateral bands and the margins of the median yellowish orange band (Fig. 2A). Posterior to 48 mm from the anterior tip (40% of body length), the eyes become scattered along the black lateral bands to reach the posterior body region (Fig. 2B).

Epidermis and secretions. The ventral epithelium is ciliated across the entire width of the body, thus forming a broad creeping sole. The dorsal epithelium is only ciliated in the anterior region. At the level of the pre-pharyngeal region, the epithelium is 41 μ m high on the dorsal side, 36 μ m ventrally and 38 μ m at the body margins. Erythrophilic granular glands open through the entire epithelium but a glandular margin is absent. Abundant rhabditogenic cells traverse the entire epithelium, being much more abundant on the dorsal side than on the ventral. In the cephalic region, cyanophilic granular secretions traverse the latero-ventral epithelium.

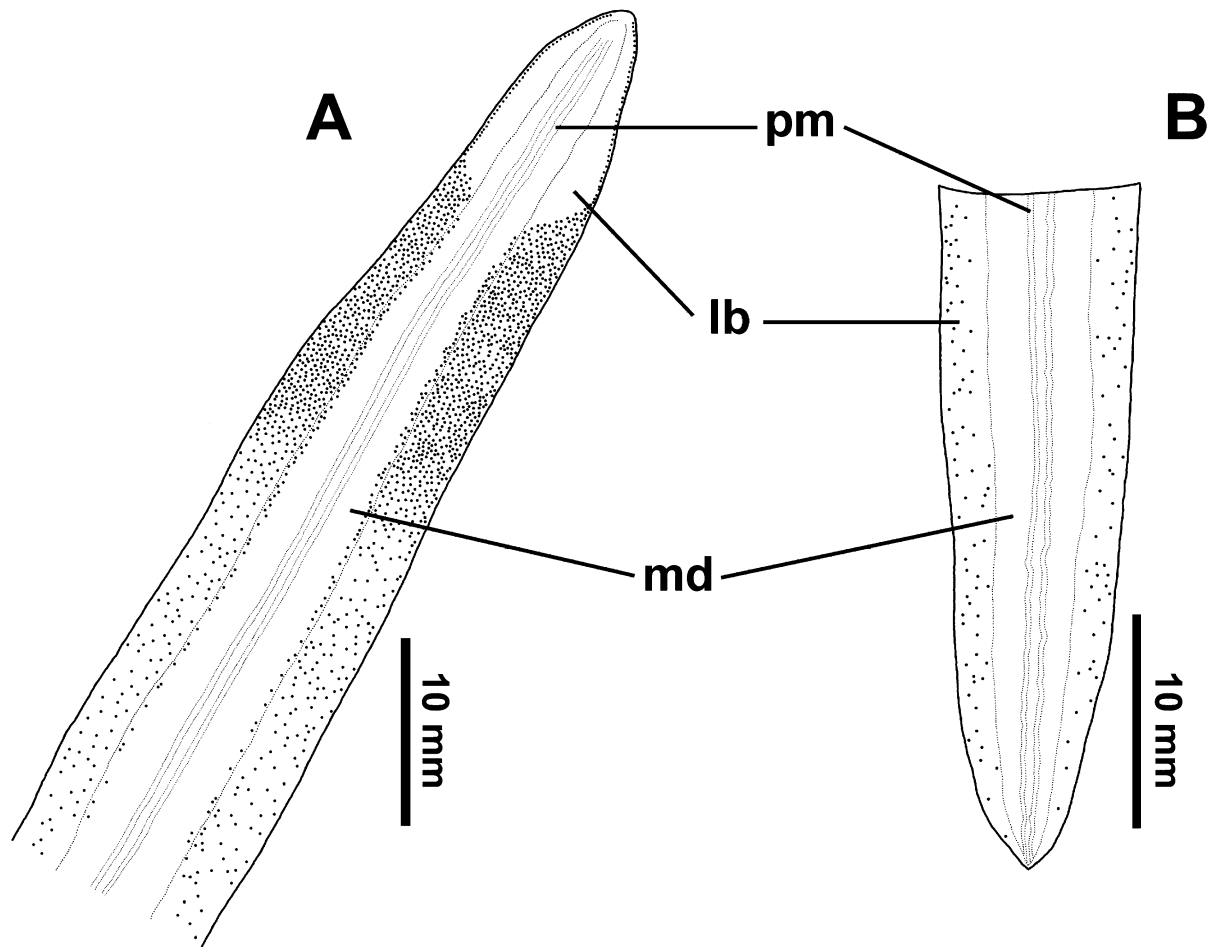


FIGURE 2. *Gigantea urubambensis* sp. nov. Holotype. Schematic drawing of the eyes of anterior (A) and posterior (B) body regions in dorsal view from preserved specimen (the marginal bands were omitted).

Cutaneous musculature. The cutaneous musculature is formed by the three layers that are typical of the Geoplaninae: an external circular layer, a double diagonal layer with decussated bundles, and a longitudinal layer also organized in bundles (Figs. 3A, 4A). In the pre-pharyngeal region, the circular layer is thicker dorsally than ventrally, being 34 μm and 15 μm respectively. The diagonal musculature is equally thick at the dorsal and ventral regions, ranging from 19 to 34 μm . The longitudinal muscular layer is 53 to 97 μm thick, equally developed dorsally and ventrally. CMI ranges between 5.0% and 6.9%. The cutaneous muscle layers grow thinner towards the sides of the body, with the longitudinal muscle being most conspicuous. In the cephalic region the layers also grow thinner with no changes in orientation of the muscle fibers, but the anterior most 0.5 mm was missing.

Parenchymal musculature. In the pre-pharyngeal region, the parenchymal muscle fibers are arranged in various directions, with the dorso-ventral ones being most abundant. Other parenchymal fibers are arranged in three layers that together correspond to 10% of body height: (1) a dorsal diagonal layer with decussated fibers (22.5 μm thick) located under the dorsal submuscular peripheral nerve net; (2) a supra-intestinal transverse layer (125 μm thick); (3) a sub-intestinal transverse layer (100 μm thick) (Fig. 3A). The dorsal diagonal layer is formed by bundles of 4–7 fibers each. The other two layers do not present bundles. Numerous oblique fibers run between the intestinal branches, from dorsal to ventral epidermis extending to the body margins. Muscular fibers become scarce towards the anterior end of the body and disappear near the tip, whose first 0.5 mm was missing.

Digestive system. The mouth is located in the middle of the pharyngeal pouch. The pharynx, collar type, is 7.6 mm long (~6% of body length) (Fig. 3B) and strongly folded. An esophagus is absent. The lining

epithelium of the pharyngeal pouch is flat, not ciliated, and columnar around the mouth; it is surrounded by sub-epithelial muscle layer, one-fiber thick, which is longitudinal at the pharyngeal region, and decussated posteriorly to it.

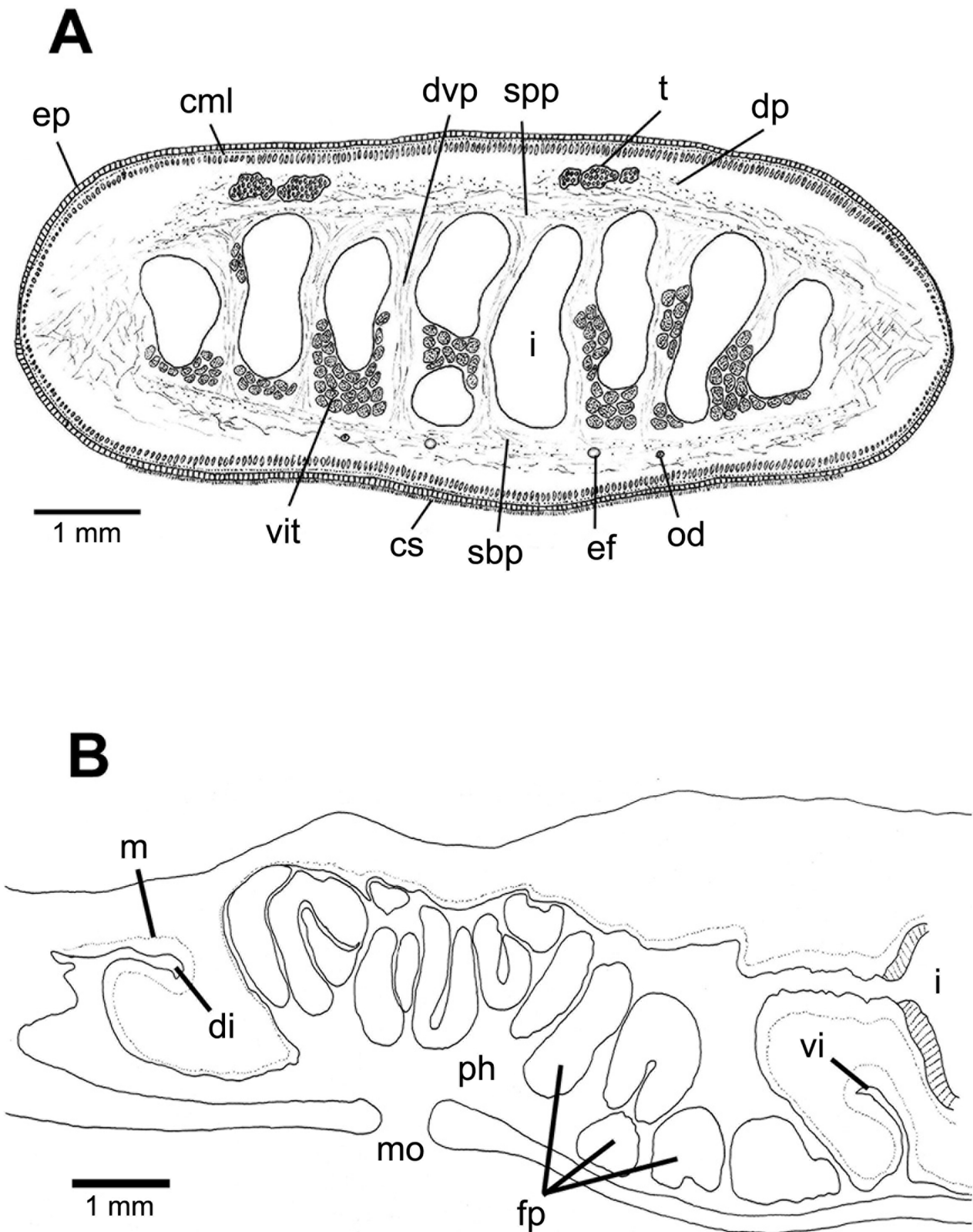


FIGURE 3. *Gigantea urubambensis* **sp. nov.** Holotype. A. Diagrammatic transverse section of pre-pharyngeal region. B. Sagittal reconstruction of the pharynx; anterior end to the right.

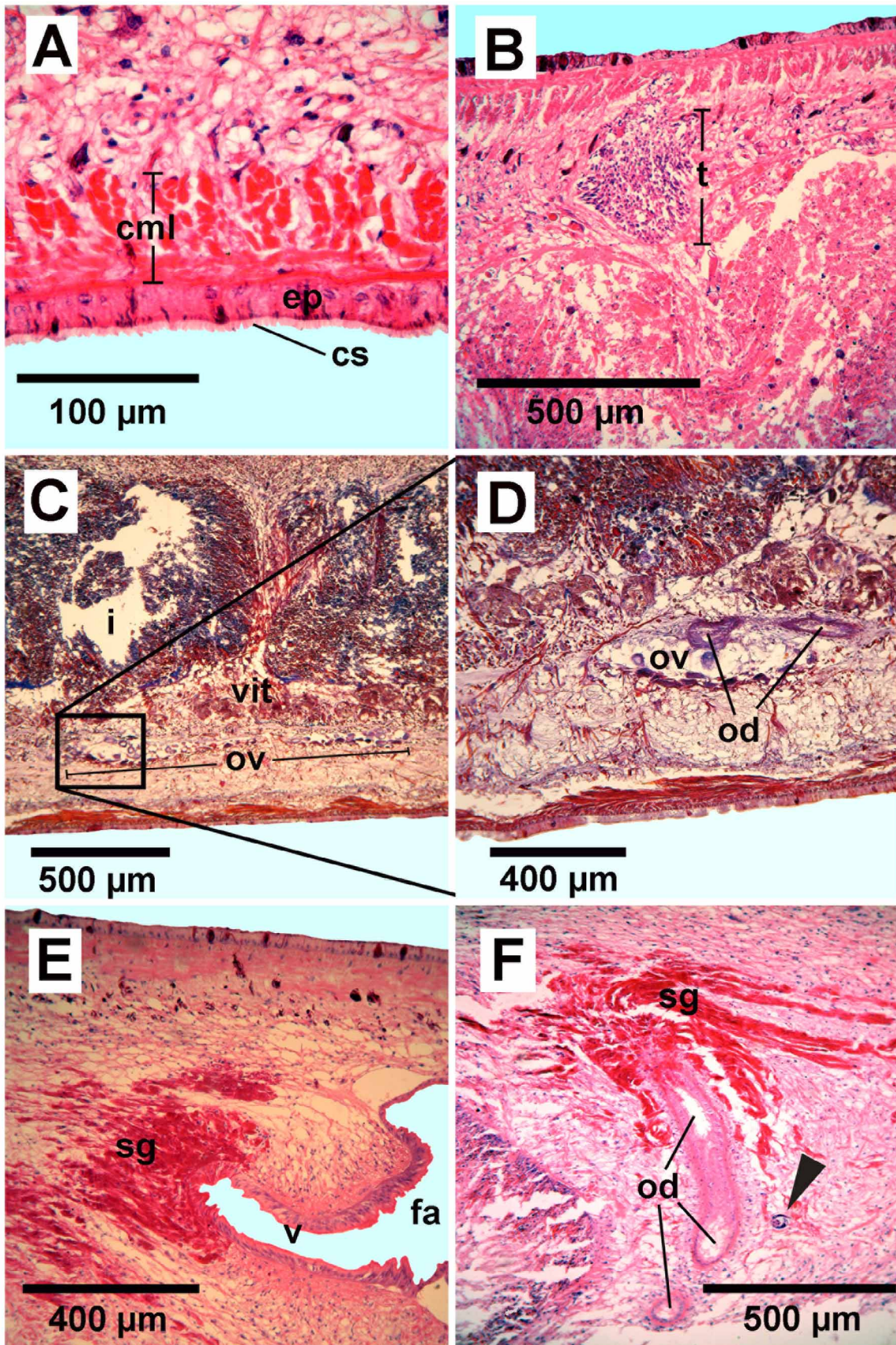


FIGURE 4. *Gigantea urubambensis* sp. nov. Holotype. A. Ventral median part of transversal section through pre-pharyngeal region. B. Dorsal median part of transversal section through pre-pharyngeal region. C-D. Sagittal section through anterior region. D. Detailed view of communication between ovary and ovovitelline duct. E-F. Sagittal section through female reproductive system. Arrowhead marks nematode larva.

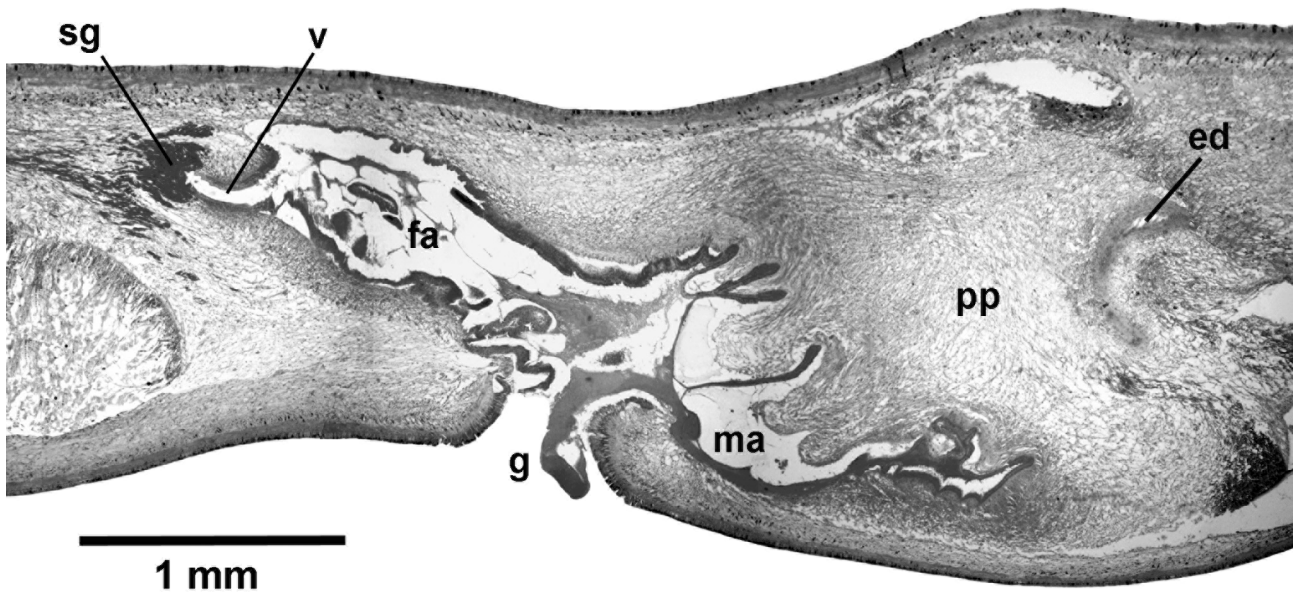


FIGURE 5. *Gigantea urubambensis* sp. nov. Holotype. Sagittal section through the copulatory apparatus; anterior end to the right.

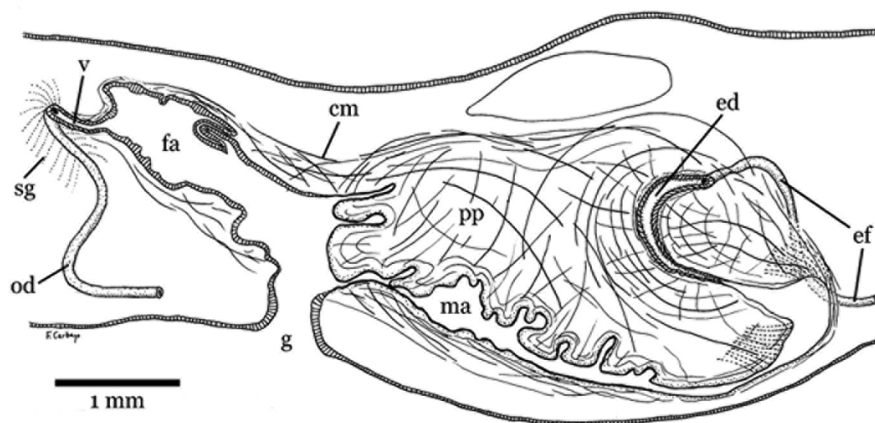


FIGURE 6. *Gigantea urubambensis* sp. nov. Holotype. Sagittal reconstruction of the copulatory apparatus; anterior end to the right. Only left efferent and left ovovitelline ducts are shown.

The outer pharyngeal epithelium is squamous, with the nuclei sunken below its muscularis, and columnar with basal nuclei at the pharyngeal insertion. It is traversed by two types of granular secretions, erythrophilic and cyanophilic. The outer musculature comprises a longitudinal layer (10 μm thick) followed by a circular muscle (19–24 μm thick). The epithelium of the pharyngeal lumen is squamous, with the nuclei sunken into the parenchyma; a circular muscular layer (190–385 μm thick) lies underneath, with some interspersed longitudinal fibers.

Male reproductive system. The testes are rounded and located dorsally between the dorsal diagonal and the supra-intestinal parenchymatic muscle layers, occupying about one-sixth of the dorso-ventral height in the pre-pharyngeal region (Figs. 3A, 4B). The follicles are arranged in 1–5 rows on either side of the body. The posterior-most testes are lateral to the pharynx. The anterior-most and the posterior-most testes are located at 10.0% and 59.6%, respectively, from the anterior tip.

The efferent ducts run ventrally to the parenchymal subintestinal muscular layer, above the ovovitelline

ducts and slightly internal to them (Fig. 3A). Their epithelium is cuboidal and ciliated, and the distal lumen contains spermatozoa. They are surrounded by a decussated 13 μm thick muscle layer. In the vicinity of the proximal region of the male atrium, the efferent ducts turn dorsally, then curve to the sagittal plane, penetrate horizontally the common muscle coat, communicate with each other, and open into the ejaculatory duct (Fig. 6). The latter is a slightly dilated canal, C-shaped in lateral view, with its the distal section oriented forward (Figs. 5, 6). It traverses the penis papilla acentrally and opens near the ventral insertion of the latter. The columnar and ciliated epithelium of the ejaculatory duct is 18 μm high. The apical portion of its cells is inclined towards the opening of the duct. Scarce cells with an erythrophilic granular secretion open onto the lumen. The ejaculatory duct is surrounded by a one-fiber thick circular muscle layer, followed by a 10 μm -thick layer of longitudinal fibers.

The penis papilla, large and distally folded, occupies the entire male atrium and projects ventrally from its roof. The epithelium of the penis papilla is columnar with basal nuclei. If cilia are present, then they are inconspicuous due to secretion in male atrium (Fig. 5). Around the opening of the ejaculatory duct the epithelium of the penis papilla is squamous, non ciliated, traversed by numerous cells with an erythrophilic granular secretion. The muscularis of the penis papilla comprises a sub-epithelial circular layer, 25 μm thick, followed by a longitudinal layer, 70 μm thick. The parenchyma of the penis papilla possesses abundant muscle fibers arranged in several directions.

The male atrium is a long cavity completely occupied by the penis papilla. It is twice as long as the female atrium. Its epithelium is squamous, apically erythrophilic. The presence of secretion in the atrial lumen prevented us from discerning whether this epithelium is ciliated or not (Fig. 5). The epithelium is underlain by a circular muscle layer, 12–17 μm thick, followed by a longitudinal layer, 63–70 μm thick. Numerous cells with erythrophilic granular secretion, underneath the longitudinal muscle layer, open together in the transition area between male and female atrium.

Female reproductive system. The ventral, elongated ovaries (1.2 mm x 0.15 mm) are located between the sub-intestinal muscle layer and ventral nerve plate (Fig. 4C, D), at a distance equal to 8% of body length from the anterior end of the body. The vitellaria are well developed, distributed among and beneath the intestinal branches (Figs. 3A, 4C).

The ovovitelline ducts arise from the antero-dorsal part of the ovaries (Fig. 4C, D). The ducts run within the parenchymatic sub-intestinal muscle layer. They run posterior to the gonopore and to the female atrium, and then turn dorsally to join directly with the vagina (Fig. 6). A common glandular ovovitelline duct is absent. The vagina, formed by an almost horizontal and elongated diverticulum of the female atrium, opens in the proximal region of the latter. The female atrium is an oblique and wide cavity, 1.3 mm long occupying at least the dorsal half of the body height (Figs. 5, 6), presenting some folds that project into its lumen.

The ovovitelline ducts have a ciliated epithelium and the distal third of the ascendant portion receives the shell glands (Fig. 4F). The vagina has a non-ciliated, apically erythrophilic, pseudo-stratified epithelium, with the distal portion of its cells inclined towards the ovovitelline ducts (Fig. 4E). This epithelium overlies a decussated muscular layer, 8 μm thick. The lining epithelium of the female atrium is pseudo-stratified, non-ciliated, apically erythrophilic. Beneath the epithelium there is a thin decussated muscle layer, followed by a circular layer and another with intermingled longitudinal and circular muscle fibers. The proximal ventral epithelium of the female atrium is traversed by cells with an erythrophilic granular secretion, and their bodies are beneath the intermingled muscle fibers. The gonopore canal is straight and lined with a columnar, ciliated epithelium.

The common muscle coat is formed by a thick layer of longitudinal and oblique fibers, more developed in the male organ than the female.

Parasitism. Nematode larvae were found in the parenchyma, both near the ejaculatory duct and below the vagina (Fig. 4F).

Discussion

In the present paper we followed the classification of triclad flatworms as recently published by Sluys *et al.* (2009). The new species fits well into the subfamily Geoplaninae since the following diagnostic features of the subfamily are present: broad ciliated creeping sole; mouth immediately behind mid-body; subcutaneous longitudinal musculature well developed and arranged in bundles; longitudinal parenchymal muscle absent; testes dorsal. This subfamily comprises 16 genera, including the collective genus *Pseudogeoplana* for insufficiently known species, especially regarding their copulatory apparatus (Ogren & Kawakatsu 1990; Sluys *et al.* 2009).

The external morphology of *Gigantea urubambensis* **sp. nov.** resembles some *Pseudogeoplana* species. Fifteen of the 55 species of *Pseudogeoplana* are distinguished from the new species because they lack bands or stripes. Most of the 40 remaining species show a dorsal color pattern ranging from a brown to a gray ground colour with yellow or black bands, or vice versa, to a light brown or yellowish ground colour with bands or stripes from light brown to black. This is very different from the striking pattern of bands observed in *G. urubambensis* **sp. nov.** Only two species have a colour pattern resembling that of *G. urubambensis* **sp. nov.**, viz. *Pseudogeoplana lumbricoides* (Schirch, 1929) and *Pseudogeoplana bonita* (Schirch, 1929). In *P. lumbricoides* the dorsal surface is reddish-orange, forming a wide median band, the latter with attenuated pigmentation in its central portion, and black-blue lateral stripes. In *G. urubambensis* **sp. nov.**, however, the median band is yellow-orange with two paramedian reddish stripes, and the black lateral bands are much wider than in *P. lumbricoides*. *Pseudogeoplana bonita* has two yellow-orange bands, similar to those of *G. urubambensis* **sp. nov.**, but they are separated by a wide greenish median stripe.

Regarding the other Geoplaninae genera, the body shape and the size of the new species preclude its inclusion in the genera *Cephaloflexa* Carbayo & Leal-Zanchet, 2003, *Choeradoplana* Graff, 1896, *Geobia* Diesing, 1862, *Issoca* Froehlich, 1955, *Gusana* Froehlich, 1978, *Enterosyringa* Ogren & Kawakatsu, 1990, *Xerapoa* Froehlich, 1955, and *Polycladus* Blanchard, 1845. Six of the remaining geoplaninid genera are also excluded due to differences in important anatomical structures: either the penis papilla is small or absent (*Pasipha*, *Notogynaphallia*, *Amaga*, *Liana* Froehlich, 1978), or the female genital canal curves anterodorsally from the female atrium (*Geoplana*, *Notogynaphallia*, *Amaga*, and *Supramontana* Carbayo & Leal-Zanchet, 2003). Therefore, the only remaining genus is *Gigantea*, to which the generic key of Ogren & Kawakatsu (1990) also leads. This genus typically possesses male accessory genital organs (glandular ridges according to the diagnosis of the genus) in the penis papilla, which are absent in the new species, as well as in some *Gigantea* species (see below).

It has been suggested that the genus is heterogeneous due to variation in the male accessory genital organs (Carbayo 2008). When Carbayo (2008) proposed a new species for the genus, he divided it into two informal groups. The first group includes *Gigantea* species in which the penis papilla has a horizontal orientation, while the ejaculatory duct traverses the papilla horizontally. The second group includes species with penis papilla that obliquely emerges from the dorsal wall of the male atrium, while the ejaculatory duct runs postero-ventrally through the papilla. Two species of the genus, *G. idaia* (du Bois-Reymond Marcus, 1951) and *G. sandersoni* (Prudhoe, 1949), were left ungrouped because they do not clearly fit into any of the groups.

The new species fits into this second group, which comprises *G. bistrinata* (Hyman, 1962), *G. cameliae* (Fuhrmann, 1914), *G. gouvernoni* Jones & Sterrer, 2005, and *G. maupoi* Carbayo, 2008. Additionally, the new species is compared to *G. idaia* and *G. sandersoni*.

Gigantea urubambensis **sp. nov.** differs from *G. bistrinata*, *G. idaia*, *G. maupoi* and *G. sandersoni* in that the male accessory genital organs (glandular ridges) are absent in the new species. *G. cameliae* and *G. gouvernoni* also lack these organs, but differing from the new species, the ejaculatory duct of *G. cameliae* centrally traverses a cylindrical and horizontally placed penis papilla, while the ejaculatory duct of *G. gouvernoni* is much longer than that of the new species. It remains unclear as to why *G. cameliae* was transferred from *Geoplana* to *Gigantea* by Ogren and Kawakatsu (1990) in view of the fact that the position of its female genital canal is unknown.

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