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‘Endless forms most beautiful’: taxonomic revision of the planarian *Geoplana vaginuloides* (Darwin, 1844) and discovery of numerous congeners (Platyhelminthes: Tricladida)

ANA LAURA ALMEIDA[1], FERNANDO P. L. MARQUES[2] AND FERNANDO CARBAYO[1*]

[1]*Laboratório de Ecologia e Evolução, Escola de Artes, Ciências e Humanidades (EACH), Universidade de São Paulo (USP), Av. Arlindo Bettio, 1000, São Paulo, SP, 03828-000, Brazil*
[2]*Departamento de Zoologia/Instituto de Biociências, Universidade de São Paulo (USP), Rua do Matão, Travessa 14, Cidade Universitária, São Paulo, SP, 05508-900, Brazil*

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In 1832, Darwin discovered two ‘elegantly coloured true Planaria’ in Rio de Janeiro, one of which he later formally named as *Planaria vaginuloides* (Darwin, 1844). Currently named *Geoplana vaginuloides* (Darwin, 1844), it is the type species of the genus and of the subfamily Geoplaninae. Many chromatic patterns of the body were recorded for the species, but its polyphyletic status was recently pointed out. Herein we revise the three-species-rich genus by combining morphological and molecular data within an integrative taxonomic framework. Our results support the monophyly of the genus, but also reveal that there are at least four nominal species recorded in the literature under the name of *G. vaginuloides*. This number increases to ten if the diagnostic features used by former authors are applied, i.e. the eye-catching dorsal colour pattern and the typical, extraordinarily long penis papilla. Our results also indicate that *Geoplana chita* Froehlich, 1956 is a complex of species and that *Geoplana pulchella* Schultze & Müller, 1857 is monophyletic. We describe herein 13 species of the genus. Surprisingly, the number of species of *Geoplana* was overshadowed by their beauty.

ADDITIONAL KEYWORDS: Atlantic forest – Continenticola – DNA – genes – Geoplaninae – histology – microcomputed tomography – morphology – phylogeny – systematics.

INTRODUCTION

Land planarians are chiefly pantropical in distribution, and the Neotropical region houses ~260 species, all considered as members of Geoplaninae Stimpson, 1857. Throughout its distribution, the Atlantic forest is credited to be one of the areas with the highest species densities worldwide (Sluys, 1998, 2016). It was in this region that a few specimens of land planarians caught the attention of Charles Darwin in the 19th century. Enchanted by the tropical forest near Rio de Janeiro, southeastern Brazil, Darwin confessed that ‘nothing has so much interested me as finding two species of elegantly coloured true Planaria inhabiting the dewy forest!’ (1832, letter to H. S. Henslow). Land planarians were not phytovorous, as Darwin thought (1832, p. 48, in Keynes, 2000); they are predators of soil invertebrates (Froehlich, 1966). Back in England, Darwin described a few new species of land flatworms from around the world, including the colourful *Planaria vaginuloides* Darwin, 1844 (currently in *Geoplana* Schultze & Müller, 1857), which he had found in the surroundings of Rio de Janeiro.

Darwin lived in the pre-histological era; therefore, the formal description of the species was based merely on the external aspect. With respect to its chromatic pattern, he described *G. vaginuloides* as having “Sides and foot coloured dirty ‘orpiment orange’; above, with two stripes on each side of pale ‘primrose-yellow’, edged externally with black; on centre of the back a stripe of glossy black; these stripes become narrow towards both extremities”. The information provided in the

*Corresponding author. E-mail: baz@usp.br
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original description is all we have available to diagnose *G. vaginuloides*, because the type material seems to be lost (H. D. Jones, personal communication).

Riester (1938) and Froehlich (1956a) examined additional worms from Teresópolis [State of Rio de Janeiro (RJ)], Marcus (1951, 1952) from São Paulo [State of São Paulo (SP)], and Froehlich (1958) from Itanhaém (SP). Additionally, Froehlich (1955a) examined one specimen from an unknown locality (see Supporting Information, Table S1). Based on these studies, up to seven chromatic body patterns are currently known for this species (Darwin, 1844; Riester, 1938; Marcus, 1952; Froehlich, 1958). Riester (1938) and Marcus (1951) examined the internal organs from histological slides, and Froehlich (1958) summarily studied these organs in one cleared specimen.

Apart from the records of *G. vaginuloides* mentioned above, Prudhoe (1949) reported this species from Trinidad and Tobago. However, Marcus (1951, 1952) doubted the conspecificity of Prudhoe's specimens owing to the very short copulatory apparatus relative to its body length (see also Froehlich, 1967; Carbayo & Leal-Zanchet, 2001). Kawakatsu *et al.* (2002) considered the specimens from Trinidad and Tobago to represent a new species of *Geoplana*, viz., *Geoplana prudhoei* Kawakatsu, Ogren, Froehlich & Sasaki, 2002.

No more taxonomic works have been published on *G. vaginuloides*, with the exception of a molecular phylogenetic study of the subfamily Geoplaninae, in which the polyphyletic status of *G. vaginuloides* (represented by two individuals) was pointed out in a recent paper (Carbayo *et al.*, 2013). In that paper, the diagnosis of *Geoplana* was also emended, and 100 species of the genus were transferred to other genera or were considered *incertae sedis*. Currently, there are only three species in the genus, namely, *G. vaginuloides*, *Geoplana pulchella* Schultze & Müller, 1857 and *Geoplana chita* Froehlich, 1956, all from Brazil. The only taxonomic study of *G. chita* is the original description, which included details of its internal organs (Froehlich, 1956b). The original description of *G. pulchella*, from Blumenau [State of Santa Catarina (SC), Brazil], is based on its external aspect (Schultze & Müller, 1857). An additional specimen from Brusque (state of SC, Brazil) was examined by du Bois-Reymond Marcus (1951). However, this specimen was considered not to be conspecific and is currently placed in *Obama* Carbayo *et al.*, 2013. Froehlich (1955b) reported an immature specimen of *G. pulchella* from the type locality.

The polyphyletic status of *G. vaginuloides* is problematic owing to the fact that it is the type species of the genus and of the subfamily (Froehlich, 1955a). Consequently, the nomenclatural stability of the genus and subfamily is dependent on the unequivocal circumscription of this taxon. This work is aimed at an integrative taxonomic revision of the genus. We analysed the morphology of the material originally examined by Riester (1938), Marcus (1951, 1952) and Froehlich (1956a), and additional specimens collected by or donated to the late Professor Eudóxia Maria Froehlich (University of São Paulo). In addition, we have included specimens collected by ourselves or donated to us, from which we obtained mitochondrial and nuclear gene fragments to infer their phylogenetic relationships. Finally, we have also revised the taxonomy of the two other nominal species of the genus (see Carbayo *et al.*, 2013), i.e. *G. chita* and *G. pulchella* Schultze & Müller, 1857, by examining the available type specimens and additional individuals collected by us and from Eudóxia's collection, and we describe, for the first time, the internal morphology of *G. pulchella*.

MATERIAL AND METHODS

Fieldwork

We actively collected planarians in areas covered with Atlantic Forest in the States of Rio de Janeiro, São Paulo, Paraná and Santa Catarina, Brazil (Fig. 1). After collection, they were photographed and their external aspect was recorded before they were killed in boiling water. Before fixing them for histological studies, we removed a small piece of the worm and preserved it in absolute ethanol and stored this material in a freezer. Specimens from E. M. Froehlich's collection were probably fixed with Susa. Specimens collected in 2005 or later were fixed in 100% ethanol or in 10% formalin buffered solution.

Histology

We cut the specimens into several pieces, dehydrated these in a graded ethanol series, cleared them in clove oil, and infiltrated and embedded them in Paraplast tissue embedding medium. We then sectioned these tissue blocks at 3–7 μm intervals using a retracting rotary microtome, affixed the sections with albumin and glycerol (1:1) onto glass slides placed on a hotplate, and stained them following Cason (1950). Finally, sections were dehydrated in a graded ethanol series, cleared in xylene and mounted in synthetic balsam (Merck and Synth). We examined the slides with an optical compound microscope and made drawings of the pharynges and the copulatory apparatuses with the aid of a camera lucida attached to the microscope. Photomicrographs were taken with a DP72 digital camera attached to the microscope, and images were edited in GIMP (GNU Image Manipulation Program 2.8.16; The GIMP team www.gimp.org, 1995–2016). Colour descriptions of the body of live or fixed specimens follow online RAL palette colours (© RAL gemeinnützige GmbH, available at

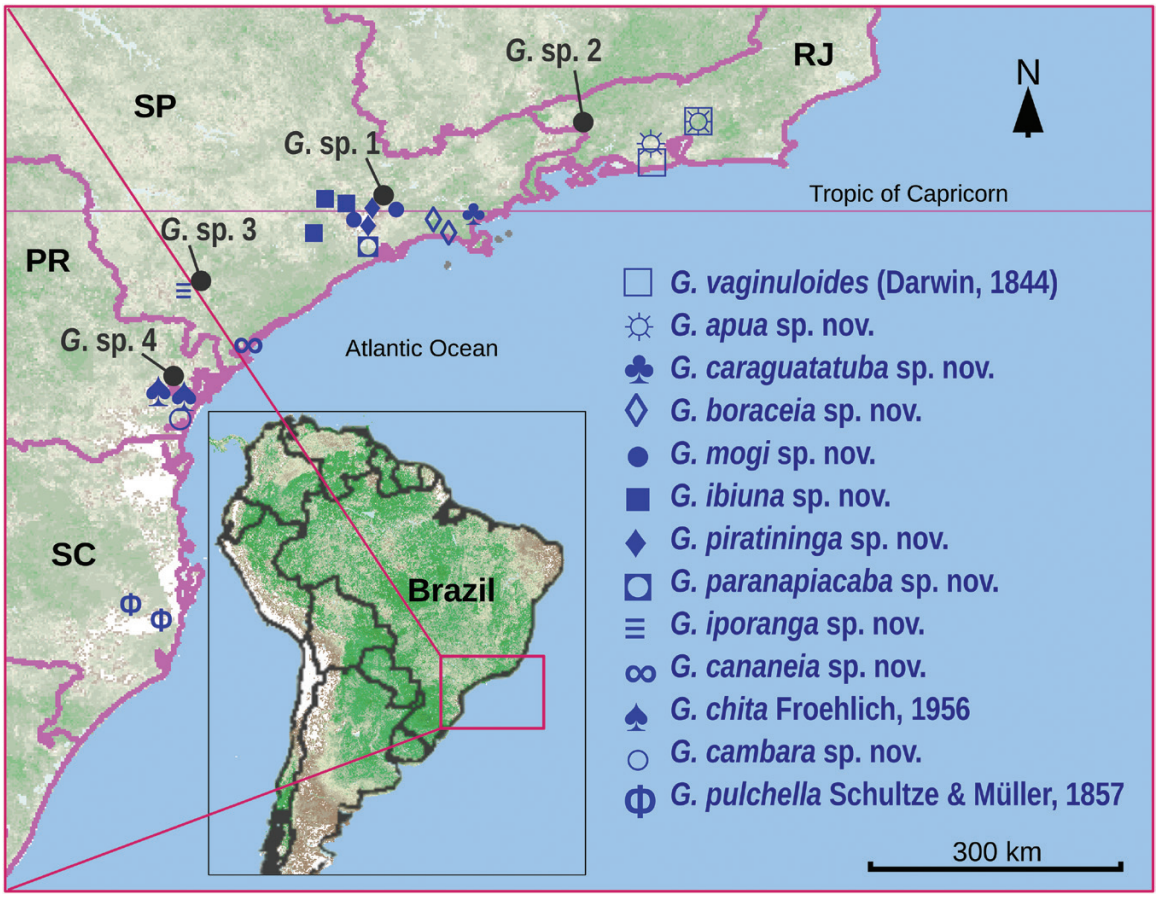

Figure 1. Distribution map of the species of *Geoplana* addressed in this paper. Abbreviations for the Brazilian States are as follows: PR, Paraná; RJ, Rio de Janeiro; RS, Rio Grande do Sul; SC, Santa Catarina; SP, São Paulo.

https://www.ral-farben.de/uebersicht-ral-classic-farben.html?&L=1) through comparison with digital images of the specimens on a computer screen. The thickness of cutaneous musculature is expressed as a percentage of body height in the prepharyngeal region (CMI). The figures of dorsal, sagittal and horizontal views are oriented so that the anterior end of the body is to the left.

The material we collected or that was donated to us was deposited at the Museu de Zoologia da Universidade de São Paulo (MZUSP). We also examined material deposited in the Senckenberg Museum Frankfurt (SMF) and in Professor Eudóxia Maria Froehlich's Collection (EMF, provisionally under the care of F.C.; see details in the respective section 'Material examined' for each species).

Microcomputed tomography

Virtual two-dimensional sections were made of specimens MZUSP PL 2080 and MZUSP PL 2095. The specimens were submerged in 100% ethanol containing 1% iodine in absolute ethanol for approximately for 4 weeks. One day before scanning, the specimens were rinsed in absolute ethanol for 5 min and, subsequently, the region of the copulatory apparatus was scanned in an X-ray micro-computed tomography (μCT) SkyScan1272 scanner or Skyscan 1272 (Bruker MicroCT, Kontich, Belgium). The operational settings for scanning the specimen MZUSP PL 2080 were as follows: 21 kV, source current of 175 μA, image pixel size of 3.249953 μm, no filter applied, depth of 16 bits, exposure of 1458 ms, rotation step of 0.080°, and frame averaging of 2. The operational settings for scanning the specimen MZUSP PL 2095 were as follows: 73 kV, source current of 104 μA, image pixel size of 5.69 μm, no filter applied, depth of 16 bits, exposure of 1000 ms, rotation step of 0.15°, and frame averaging of 12. Reconstructions were carried out as bmp formatted images using the software Nrecon version 1.6.6.0 (Bruker MicroCT) with the following settings: smoothing = 0, ring artefact correction = 15, beam hardening correction = 63% (specimen MZUSP PL 2080) and 0% (MZUSP PL 2095), and HU calibration = off. Sets

of virtual sections were reconstructed with the software package DataViewer (http://www.skyscan.be/products/downloads.htm), and a selection of sets with the original grey scales were saved. The contrast of grey-scale virtual sections was enhanced with GIMP (GNU Image Manipulation Program 2.8.10). After the virtual sections had been acquired, we rinsed the specimens in 80% ethanol and, subsequently, dehydrated and treated them, following the histological procedure described in the previous subsection.

Material examined

Specimens identified and described are listed below in the 'Material examined' section for the respective species. We made an effort to find and identify all other specimens of the genus recorded in the literature. Those specimens whose identification could not be ascertained are listed in the Supporting Information (Table S1). Those specimens are immature, the histological preparations of their copulatory apparatus are of limited quality, or they have been lost. In the Supporting Information (Table S1) there are also recently collected representatives of undescribed species of *Geoplana* (named *Geoplana* sp. 1–4 in the molecular analyses) that are outside the scope of the present work.

Acquisition of molecular data

We selected 42 specimens of putative *Geoplana* members for molecular studies. Genomic DNA extractions were performed with the Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA) following Álvarez-Presas *et al.* (2011). Polymerase chain reaction (PCR) was used to amplify partial sequences of the mitochondrial region of Cytochrome Oxidase I (*COI*) and the nuclear region of the Elongation Factor-1-alpha promoter (*EF-1-α*). Amplifications were performed in a 20 µL volume containing 1 µL of DNA, 4 µL of 5× Phusion Buffer GC (Thermo Scientific) 1.5 mM Tris–HCl (pH 8.4), 50 mM KCl, 1 µL of l M dNTPs, 1.0–3.0 mM $MgCl_2$, 1 µL of l M of each primer, and 0.2 µL of Phusion High-Fidelity DNA Polymerase (Thermo Scientific). General PCR conditions included initial denaturation for 3 min at 98 °C, 35 cycles of denaturation for 10 s at 98 °C, annealing for 30 s at specific temperatures (see below), extension for 1 min at 72 °C, and a final extension for 5 min at 72 °C. Amplifications and sequencing were performed with following primer sets: *COI* with 'FlatwormCOI-F' (5′-GCAGTTTTTGGTTTTTTGGACATCC-3′) and 'FlatwormCOI-R' (5′-GAGCAACAACATAATAAGTATCATG-3′) (Sunnucks *et al.*, 2006) at 45 °C; BarT 5′-ATGACDGCSCATGGTTTA-3′, BarS 5′-GTTATGCCTGTAATGATTG-3′ (Álvarez-Presas et al., 2011) and COIR 5′-CCWGTYARMCCHCCWAYAGTAAAA-3′ (Lázaro *et al.*, 2009) at 45 °C; and *EF-1-α* with EFplatF 5′-GATTGCYCCWGGYCATCG-3′ and EFplatR 5′-GCRATWGAYTCGTGRTGC-3′ (Carbayo *et al.*, 2013) at 45–48 °C. The PCR products were purified using an Agencourt AMPure XP DNA Purification and Cleanup kit (Beckman Coulter Inc.). Products were subsequently cycle sequenced directly from both forward and reverse directions using ABI Big-Dye Sequence Terminator (v. 3.1), cleaned with ethanol precipitation, and sequenced on an ABI Prism Genetic Analyzer (3131XL) automated sequencer (Applied Biosystems/ThermoFisher).

Sequence reads were assembled using the Consed/Phred/Phrap package (Ewing & Green 1998; Ewing *et al.*, 1998; Gordon, Abajian & Green, 1998; Gordon, Desmarais & Green, 2001). Before submitting the data to phylogenetic analysis, we aligned the sequences with MAFFT (Katoh *et al.*, 2002) using the G-INS-i iterative refinement method with 1000 cycles, and visualized and edited the sequences in BioEdit (Hall, 1999). Aligned sequences of *COI* and *EF-1-α* were checked for stop codons using the DNA-to-protein translation online resource by Bikandi *et al.* (2004).

Phylogenetic inference

We considered two datasets: the first one, hereafter referred as all-terminals, included all relevant terminals for which we had sequences of *COI* and/or *EF-1-α*, which included terminals with missing fragments; the second, hereafter referred as selected-terminals, included a subset of the all-terminals dataset, which included terminals for which both fragments (i.e. *COI* and *EF-1-α*) were available. These two datasets allowed us to evaluate clade stability owing to missing data, which was relevant to us because for some putative species we had only one of the fragments. We proceeded with phylogenetic inferences by using two optimality criteria: maximum parsimony (MP) and maximum likelihood (ML). Phylogenetic analyses under parsimony were carried out with TNT (Goloboff, Farris & Nixon, 2008) using its New Technology searches (Goloboff, 1999; Nixon, 1999) with the following search parameters: rep 100, ratchet 20, fuse 20 and hold 10. For ML analyses, model selection was performed in jModelTest (version 2.1.6; Guindon *et al.*, 2003; Darriba *et al.*, 2012), considering 88 candidate models ranked by corrected Akaike information criterion (AICc) scores, and tree searches were performed in GARLI (version 2.0; Zwickl, 2006), considering both single and partition models for each genomic region for 1000 independent search replicates. Following Carbayo *et al.* (2013), we considered five representatives of Rhynchodeminae as outgroups: *Arthurdendyus triangulatus* (Dendy, 1895); *Artioposthia testacea* Hutton, 1880; *Australoplana* sp.;

Dolichoplana striata Moseley, 1877; and *Endeavouria septemlineata* (Hyman, 1939) (see Table 1).

For both datasets and optimality criteria, the genomic regions were concatenated and analysed simultaneously. We also analysed individual partitions (i.e. *COI* and *EF-1-α*) to evaluate the information content of each fragment. However, our working topology was based on the simultaneous analyses of both regions. Direct measures of nodal support (*sensu* Grant & Kluge, 2008a) were accessed in reference to optimality criteria.

For MP analyses, nodal support for selected nodes was evaluated using Goodman–Bremer values (GBS; Goodman *et al.*, 1982; Bremer, 1988; Bremer, 1994; see Grant & Kluge, 2008a), calculated with a modified version of the script BREMER.RUN distributed with TNT. This script considered 1000 replicates with ten repetitions of ratchet and drift (Nixon, 1999; Goloboff *et al.*, 2008) in constrained searches and the remaining default parameters. For ML analyses, log-likelihood difference support (LLD; *sensu* Lee & Hugall, 2003; see Meireles *et al.*, 1999; Grant & Kluge, 2008b) was calculated for selected nodes using constrained negative searches in GARLI under the same configuration settings as the initial tree search. Finally, putative transformations for selected branches were compiled using the consensus tree obtained by TNT with YBYRÁ (Machado, 2015).

Species recognition

Species hypothesis was based on congruence of molecular clades and cohesive morphology. Initially, we recognized all specimens sharing the same set of external and internal traits as morphospecies, as generally used for the taxonomy of the geoplaninids. These morphospecies or putative species were subsequently tested against molecular phylogenies. If morphological and molecular results were congruent with each other for certain morphospecies, we considered the species status as corroborated by molecular and morphological data. By doing so, we are mirroring the phylogenetic concept of species, meaning 'diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent' (Cracraft, 1983). If the molecular data did not contradict the species hypothesis (usually owing to lack of resolution) but we had morphological evidence support our species hypotheses, we erected the species based on morphological attributes alone.

Abbreviations used in figures

cg, cyanophil gland cells; co, common glandular ovovitelline duct; e, eye; ej, ejaculatory duct; ep, esophagus; fa, female atrium; fd, female genital duct; g, gonopore; i, intestine; lc, longitudinal cutaneous muscles; lp, longitudinal parenchymatic muscles; m, muscle; ma, male atrium; mo, mouth; ms, thin muscle fibre; o, ovary; ov, ovovitelline duct; pb, penis bulb; ph, pharyngeal pouch; pp, penis papilla; px, pharynx; sb, subintestinal transverse muscles; sd, sperm duct; se, necks of secretory cells; sg, shell glands; sp, supraintestinal transverse muscles; t, testis; vi, vitellaria; vn, ventral nerve plate.

MOLECULAR RESULTS

We compiled nucleotide sequences of 154 terminals, including five outgroup taxa members of Rhynchodeminae and Geoplaninae individuals (Table 1). These sequences or gene fragments were either obtained from GenBank or they were generated by us. *Geoplana* was represented by 42 individuals, within which we generated new nucleotide sequences for 37. The remaining ones (five) were obtained from GenBank.

The all-terminals dataset consisted of 146 sequences of *COI* (36 of *Geoplana*) with 676 bp, and 121 sequences of *EF-1-α* (28 of *Geoplana*) with 548 bp. For this dataset, all individuals assigned to *Geoplana* were represented by both gene fragments, except for *G. chita* and *G. mogi* sp. nov. (see below), which were represented by sequences of *EF-1-α* only, and *G. apua* sp. nov., represented by *COI* only. The selected-terminals dataset, for which both gene fragments were available, included a total of 106 individuals of Geoplaninae and five outgroup taxa, within which there were 27 individuals of *Geoplana*.

The parsimony analysis for the all-terminals dataset resulted in 27 equally parsimonious topologies (MPTs) with 5469 steps (Figs 2, 3; see also Supporting Information, Figs S1, S2). The parsimony analysis of the selected-terminals dataset resulted in ten MPTs with 4980 steps. The consensus tree of the selected-terminals dataset (Supporting Information, Fig. S1) recovered similar clades for *Geoplana*, thus suggesting that missing data did not affect the evidential support for putative species within the genus.

Maximum likelihood analyses considered the TPM1uf+I+Γ model for *COI*, SYM+I+Γ for *EF-1-α*, and GTR+I+Γ for the concatenated dataset. Analyses including all terminals assuming a single substitution model for all partitions resulted in a topology with a score of log-likelihood (lnL) –25150.054229, whereas the score for partition models (assuming different substitution models for each fragment) was lnL –24525.1728. The latter partition model was favoured by the AICc (50119.8095 vs. 51476.3247). Analyses including selected terminals assuming a single model for all partitions resulted in a topology with a score of lnL –23020.1415, whereas the score assuming different substitution models for each fragment was lnL –22437.9183. As before, our analyses favoured the the

Table 1. Individuals used in the study, museum code used in the figures and text, collecting locality data, identification base and GenBank accession numbers for all sequences

Species	Field number	Specimen's accession number (MZUSP PL)	Collecting locality	Collecting date	Latitude	Longitude	Identification	*COI*	EF-1-alpha
Rhynchodeminae									
Arthurdendyus triangulatus (Dendy, 1895)							GenBank	DQ666027	KC614559
Artioposthia testacea Hutton, 1880							GenBank	AF178305	KC614560
Australoplana sp.							GenBank	DQ666028	KC614558
Dolichoplana striata Moseley, 1877	F0379	1005	Igrejinha, RS	10 July 2004	–29.57200	–50.77800	GenBank	KC608226	KC614465
Endeavouria septemlineata (Hyman, 1939)	F0988	657	Parque Ecológico do Tietê, São Paulo, SP	30 March 2006	–23.48570	–46.50959	GenBank	KC608233	KC614471
	F0192	1184	Maquiné, RS	10 February 2004	–29.67774	–50.20640	GenBank	KC608222	KC614461
Geoplaninae									
Cephaloflexa araucariana Carbayo & Leal-Zanchet, 2003	F3387	1073	Floresta Nacional de São Francisco de Paula, RS	21 January 2009	–29.42736	–50.39838	GenBank	KC608316	KC614536
	F3426	1076	Floresta Nacional de São Francisco de Paula, RS	23 January 2009	–29.42739	–50.39072	GenBank	KC608319	KC614539
Cephaloflexa bergi (Graff, 1899)	F1034	303	São Sebastião, SP	7 September 2006	–23.75203	–45.631027	GenBank	KC608238	KC614472
	F1038	305	São Sebastião, SP	7 September 2006	–23.75203	–45.631027	GenBank	KC608240	KC614474
Cephaloflexa sp. 1	F2103	1211	Parque Nacional Serra da Bocaina, SP	10 February 2008	–23.65388	–45.88910	GenBank	KC608272	–
	F2108	1019	Parque Nacional Serra da Bocaina, SP	10 February 2008	–23.65388	–45.88910	GenBank	KC608274	KC614501

Table 1. *Continued*

Species	Field number	Specimen's accession number (MZUSP PL)	Collecting locality	Collecting date	Latitude	Longitude	Identification	*COI*	EF-1-alpha
Rhynchodeminae									
Choeradoplana banga Carbayo & E. M. Froehlich, 2012	F2023	1000	Parque Estadual Serra da Cantareira, SP	30 January 2008	–23.42916	–46.63250	GenBank	KC608267	–
	F3011	1002	Parque Estadual Serra da Cantareira, SP	14 December 2008	–23.42914	–46.63250	GenBank	KC608301	KC614523
Choeradoplana bocaina Carbayo & E. M. Froehlich, 2012	F2104	999	Parque Nacional Serra da Bocaina, SP	10 February 2008	–22.75222	–44.62194	GenBank	KC608273	KC614500
	F2803	998	Parque Nacional Serra da Bocaina, SP	7 September 2008	–22.75222	–44.62194	GenBank	KC608283	KC614509
	F2822	997	Parque Nacional Serra da Bocaina, SP	8 September 2008	–22.75000	–44.61666	GenBank	KC608288	–
Choeradoplana gladismariae Carbayo & E. M. Froehlich, 2012	F3092	1003	Parque Estadual Intervales, SP	12 December 2008	–24.27638	–48.41556	GenBank	KC608306	KC614528
	F3802	1004	Parque Estadual Intervales, SP	7 July 2009	–24.27638	–48.41556	GenBank	KC608326	–
Choeradoplana iheringi Graff, 1899	F3449	533	Floresta Nacional de São Francisco de Paula, RS	2 April 2012	–29.42367	–50.38711	GenBank	KC608320	KC614540
Choeradoplana pucupucu Carbayo *et al.*, 2017	F2840	540	Parque Nacional Serra da Bocaina, SP	September 2008	–22.74277	–44.61639	GenBank	KC608293	KC614517
Cratera crioula (E. M. Froehlich, 1955)	F3708	1078	Parque Estadual Serra da Cantareira, SP	19 April 2009	–23.43084	–46.63413	GenBank	KC608323	KC614543

Table 1. *Continued*

Species	Field number	Specimen's accession number (MZUSP PL)	Collecting locality	Collecting date	Latitude	Longitude	Identification	*COI*	EF-1-alpha
Rhynchodeminae									
	F3715	1079	Parque Estadual Serra da Cantareira, SP	19 April 2009	–23.43084	–46.63413	GenBank	KC608324	KC614544
Cratera pseudovaginuloides (Riester, 1938)	F1244	670	Parque Nacional da Serra dos Órgãos, RJ	16 December 2007	–22.45528	–42.99750	GenBank	KC608251	KC614482
	F1245	671	Parque Nacional da Serra dos Órgãos, RJ	16 December 2007	–22.45528	–42.99750	GenBank	KC608252	KC614483
Cratera tamoia (E. M. Froehlich, 1955)	F1139	665	Parque Nacional da Serra dos Órgãos, RJ	15 January 2007	–22.45528	–42.99750	GenBank	KC608246	KC614478
	F1336	672	Parque Nacional da Serra dos Órgãos, RJ	18 December 2007	–22.49870	–42.99620	GenBank	KC608254	KC614484
Geobia subterranea (Schultze & Müller, 1857)	F0358	650	Colégio Cristo Rei, São Leopoldo, RS	30 May 2004	–29.78828	–51.15084	GenBank	KC608225	KC614464
	F1355	673	Parque Nacional da Serra dos Órgãos, RJ	18 December 2007	–22.49870	–42.99620	GenBank	KC608255	KC614485
Geoplana apua Almeida & Carbayo **sp. nov.**	F5236	2072	Nova Iguaçú, RJ	4–6 April 2012			Holotype	MG653226*	–
Geoplana boraceia Almeida & Carbayo **sp. nov.**	F4112	2070	Estação Biológica de Boraceia, SP	21 December 2009	–23.65389	–45.88917	Holotype	–	MG653220*
	F4358	1086	São Sebastião, SP	26 February 2010	–23.75203	–45.631027	Paratype	KC608329	KC614547
	F4389	2123	São Sebastião, SP	26 February 2010	–23.746	–45.632	Paratype	MG653225*	–

Table 1. *Continued*

Species	Field number	Specimen's accession number (MZUSP PL)	Collecting locality	Collecting date	Latitude	Longitude	Identification	*COI*	EF-1-alpha
Rhynchodeminae									
	F4409	1087	São Sebastião, SP	27 February 2010	–23.75203	–45.631027	External morphology similar to type material	KC608330	KC614548
	F6026	2095	São Sebastião, SP	11 January 2014	–23.74439	–45.63715	Paratype	MG653227*	MG653194*
	F6055	2125	São Sebastião, SP	11 January 2014			Paratype	MG653237*	MG653205*
	F6068	2124	São Sebastião, SP	12 January 2014			Paratype	MG653244*	–
	F6357	2073	São Sebastião, SP	27 September 2014	–23.74685	–45.63213	Paratype	MG653238*	MG653206*
	F6575	2077	São Sebastião, SP	13 February 2015	–23.74685	–45.63213	Paratype	MG653239*	MG653208*
	F6578	2078	São Sebastião, SP	13 February 2015	–23.74685	–45.63213	Paratype	MG653240*	MG653209*
Geoplana cambara Almeida & Carbayo **sp. nov.**	F1614	1009	Parque Nacional Saint Hilaire / Lange, PR	10 January 2008	–25.76437	–48.62266	GenBank (named *G. vaginuloides* in GenBank)	KC608262	KC614492
	F1635	2063	Parque Nacional Saint Hilaire/ Lange, PR	11 January 2008			Paratype	MG653229*	MG653198*
Geoplana cananeia Almeida & Carbayo **sp. nov.**	F4096	2067	Parque Estadual da Ilha do Cardoso/ Cananeia, SP	18 October 2009	–25.091235	–47.928957	Holotype	MG653223*	MG653196*
	F4097	2068	Parque Estadual da Ilha do Cardoso/ Cananeia, SP	18 October 2009	–25.091235	–47.928957	Paratype	MG653233*	MG653201*

Table 1. *Continued*

Species	Field number	Specimen's accession number (MZUSP PL)	Collecting locality	Collecting date	Latitude	Longitude	Identification	*COI*	EF-1-alpha
Rhynchodeminae									
	F4098	2069	Parque Estadual da Ilha do Cardoso/ Cananeia, SP	18 October 2009	–25.091235	–47.928957	Paratype	MG653224*	–
Geoplana caraguatatuba Almeida & Carbayo **sp. nov.**	F6727	2080	Parque Estadual Serra do Mar/ Caraguatatuba, SP	14 May 2016	–23.594012	–45.429672	Paratype	MG653247*	–
	F6976	2126	Parque Estadual Serra do Mar/ Caraguatatuba, SP	14 January 2016	–23.593880	–45.429746	Paratype	MG653251*	–
Geoplana chita Froehlich, 1956	F1710	715	Morretes, PR	13 January 2008	–25.469604	–48.813739	External and internal morphology similar to that of type material	–	MG653218*
	F1730	716	Morretes, PR-25.469604	13 January 2008	–25.469604	–48.813739	External and internal morphology similar to that of type material	–	MG653219*
Geoplana ibiuna Almeida & Carbayo **sp. nov.**	F3562	2066	Parque Estadual do Jurupará/ Ibiúna, SP	February 2009			Holotype	MG653232*	–
	F6907	2081	Santana de Parnaíba, SP		–23.449229	–47.012175	Paratype	MG653248*	MG653214*
	F6908	2082	Santana de Parnaíba, SP		–23.449229	–47.012175	Paratype	MG653249*	MG653215*
	F6909	2083	Santana de Parnaíba, SP		–23.449229	–47.012175	Paratype	MG653250*	–

Table 1. *Continued*

Species	Field number	Specimen's accession number (MZUSP PL)	Collecting locality	Collecting date	Latitude	Longitude	Identification	*COI*	EF-1-alpha
Rhynchodeminae									
Geoplana iporanga Almeida & Carbayo **sp. nov.**	F3166	2065	Parque Estadual de Intervales, SP	13 December 2008	–24.269387	–48.405467	Holotype	MG653231*	MG653200*
	F3132	2064	Parque Estadual de Intervales/ Ribeirão Grande, SP	13 December 2008	–24.269387	–48.405467	Paratype	–	MG653221*
Geoplana mogi Almeida & Carbayo **sp. nov.**	F1022	2061	Mogi das Cruzes, SP	27 May 2006	–23.451536	–46.227993	Paratype	–	MG653217*
Geoplana paranapiacaba Almeida & Carbayo **sp. nov.**	F6660	2079	Cidade Turística de Paranapiacaba, SP	5 June 2015	–23.77746	–46.30145	Holotype	MG653246*	MG653213*
Geoplana piratininga Almeida & Carbayo **sp. nov.**	F1163	666	Parque Jardim Previdência/SP	17 June 2007	–23.579662	–46.727503	Paratype	KC608247	KC614479
	F0509	2060	Parque Ecológico do Tietê/SP	27 May 2005	–23.490918	–46.516424	Paratype	MG653222*	MG653195*
Geoplana pulchella Schultze & Müller, 1857	F3249	1068	Parque Estadual da Serra do Tabuleiro, SC	14 January 2009	–27.98139	–48.74806	External aspect similar to that of original description	KC608310	KC614532
	F6528	2075	RPPN Vale das Pedras, SC	16 January 2015	–27.61695	–049.34778	External aspect similar to that of original description	MG653245*	MG653212*

Table 1. *Continued*

Species	Field number	Specimen's accession number (MZUSP PL)	Collecting locality	Collecting date	Latitude	Longitude	Identification	*COI*	EF-1-alpha
Rhynchodeminae									
	F6544	2075	RPPN Vale das Pedras, SC	18 January 2015	–27.61696	–049.34813	External aspect similar to that of original description	–	MG653207*
Geoplana quagga Marcus, 1951	F1003	1188	Parque Ecológico do Tietê, São Paulo, SP	30 March 2006	–23.48570	–46.50959	Genbank	KC608236	–
Geoplana sp. 1	F2934	2127	Parque Estadual da Cantareira, SP	26 October 2008			Molecular and morphological support	MG653241*	–
	F2943	2128	Parque Estadual da Cantareira, SP	26 October 2008			Molecular and morphological support	MG653242*	MG653210*
	F2944	2129	Parque Estadual da Cantareira, SP	26 October 2008			Molecular and morphological support	MG653243*	MG653211*
Geoplana sp. 2	F5145	2130	Parque Nacional de Itatiaia, RJ	4 April 2012			Molecular and morphological support	MG653234*	MG653202*
	F5163	2131	Parque Nacional de Itatiaia, RJ	4 April 2012			Molecular and morphological support	MG653235*	MG653203*
	F5204	2132	Parque Nacional de Itatiaia, RJ	6 April 2012			Molecular and morphological support	MG653236*	MG653204*
Geoplana sp. 3	F2638	2133	Parque Estadual de Intervales, SP	24 December 2008	–24.269387	–48.405467	Molecular support	MG653230*	MG653199*

Table 1. *Continued*

Species	Field number	Specimen's accession number (MZUSP PL)	Collecting locality	Collecting date	Latitude	Longitude	Identification	*COI*	EF-1-alpha
Rhynchodeminae									
Geoplana sp. 4	F1707	2134	Morretes, PR	13 January 2008	–25.469604	–48.813739	Molecular support	MG653252*	MG653216*
Geoplana vaginuloides (Darwin, 1844)	F6387	2074	Parque Estadual da Pedra Branca, RJ	13 December 2014	–22.93707	–43.46098	Neotype	MG653228*	MG653197*
Geoplana goetschi Riester, 1938, *incertae sedis*	F2870	412	Parque Estadual Serra da Cantareira, SP	26 October 2008	–23.46047	–46.63672	GenBank	KC608294	–
Geoplana hina Marcus, 1951, (*incertae sedis*)	F1613	1008	Parque Nacional Saint Hilaire/ Lange, PR	10 January 2008	–25.76437	–48.62266	GenBank	KC608261	KC614491
Geoplana phocaica Marcus, 1951 (*incertae sedis*)	F2827	457	Parque Nacional Serra da Bocaina, SP	September 2008	–22.73389	–44.61639	GenBank	KC608289	KC614514
Geoplana phocaica Marcus, 1951 (*incertae sedis*)	F2830	460	Parque Nacional Serra da Bocaina, SP	September 2008	–22.73389	–44.61639	GenBank	KC608290	KC614515
Geoplaninae 1	F2033	1193	Parque Nacional Serra da Bocaina, SP	7 February 2008	–22.75000	–44.61666	GenBank	KC608269	–
Geoplaninae 2	F2807	1050	Parque Nacional Serra da Bocaina, SP	7 September 2008	–22.75222	–44.62194	Named as *Geoplana* sp. 1 in GenBank	KC608284	KC614510
	F2821	1052	Parque Nacional Serra da Bocaina, SP	8 September 2008	–22.75000	–44.61666	Named as *Geoplana* sp. 1 in GenBank	KC608287	KC614513
Geoplaninae 3	F2783	1048	Parque Estadual Intervales, SP	29 July 2008	–24.27333	–48.41694	Named as *Geoplana* sp. 2 in GenBank	KC608281	KC614508

Table 1. *Continued*

Species	Field number	Specimen's accession number (MZUSP PL)	Collecting locality	Collecting date	Latitude	Longitude	Identification	*COI*	EF-1-alpha
Rhynchodeminae									
Geoplaninae 4	F2796	1194	Parque Nacional Serra da Bocaina, SP	7 September 2008	–22.73389	–44.61639	Named as *Geoplana* sp. 3 in GenBank	KC608282	–
Geoplaninae 5	F2031	1014	Parque Nacional Serra da Bocaina, SP	7 February 2008	–22.73333	–44.60000	Named as *Geoplana* sp. 4 in GenBank	KC608268	KC614497
	F2809	1051	Parque Nacional Serra da Bocaina, SP	8 September 2008	–22.73389	–44.61639	Named as *Geoplana* sp. 4 in GenBank	KC608285	KC614511
Geoplaninae 6	F2189	348	Parque Estadual do Desengano, RJ	18 March 2008	–21.87667	–41.91972	Named as *Geoplana* sp. 5 in GenBank	KC608278	KC614505
Geoplaninae 7	F3191	1066	Parque Estadual da Serra do Tabuleiro, SC	9 January 2009	–27.95989	–48.75761	Named as *Geoplana* sp. 6 in GenBank	KC608308	KC614530
	F3247	1067	Parque Estadual da Serra do Tabuleiro, SC	13 January 2009	–27.98139	–48.74806	Named as *Geoplana* sp. 6 in GenBank	KC608309	KC614531
Gusana sp. 1	F4421	1088	Parque Hualpén (Chile)	7 July 2010	–36.79611	–73.15244	GenBank	KC608331	KC614549
	F4428	1089	Parque Hualpén (Chile)	7 July 2010	–36.79611	–73.15244	GenBank	KC608332	–
Imbira guaiana (Leal-Zanchet & Carbayo, 2001)	F0432	653	Floresta Nacional de São Francisco de Paula, RS	1 August 2004	–29.41666	–50.38333	GenBank	KC608229	KC614467
	F3361	1071	Floresta Nacional de São Francisco de Paula, RS	20 January 2009	–29.42367	–50.38711	GenBank	KC608314	–

Table 1. *Continued*

Species	Field number	Specimen's accession number (MZUSP PL)	Collecting locality	Collecting date	Latitude	Longitude	Identification	*COI*	EF-1-alpha
Rhynchodeminae									
Imbira marcusi Carbayo *et al.*, 2013	F1041	1190	São Sebastião, SP	8 September 2006	–23.75203	–45.631027	GenBank	KC608241	–
	F2833	404	Parque Nacional Serra da Bocaina, SP	September 2008	–22.75222	–44.62194	GenBank	KC608291	KC614516
Issoca jandaia Froehlich, 1955	F2043	1015	Parque Nacional Serra da Bocaina, SP	9 February 2007	–22.78222	–44.60583	GenBank	KC608270	KC614498
Issoca rezendei (Schirch, 1929)	F1182	667	Parque da Previdência, São Paulo, SP	9 August 2007	–23.58080	–46.72709	GenBank	KC608248	KC614480
	F1679	1010	Parque Nacional Saint Hilaire/ Lange, PR	13 January 2008	–25.46972	–48.81222	GenBank	KC608263	KC614493
Issoca sp. 1	F2158	1020	Parque Estadual do Desengano, RJ	16 March 2008	–21.87694	–41.92583	GenBank	KC608275	KC614502
	F2507	1045	Reserva Biológica Augusto Ruschi, ES	24 May 2008	–19.88740	–40.54319	GenBank	KC608279	KC614506
	F4085	1085	Parque Estadual do Desengano, RJ	13 August 2009	–21.87555	–41.91361	GenBank	KC608328	KC614546
Luteostriata abundans (Graff, 1899)		no voucher	São Leopoldo, RS	1998	–29.78828	–51.15084	GenBank	KC631621	KC614556
	F0205	646	Parobé, RS	27 March 2004	–29.62940	–50.83110	GenBank	KC608223	KC614462
	F0238	648	Parobé, RS	27 March 2004	–29.62940	–50.83110	GenBank	KC608224	KC614463
Luteostriata cecilliae (Froehlich & Leal-Zanchet, 2003)	F3451	1077	Floresta Nacional de São Francisco de Paula, RS	25 January 2009	–29.42367	–50.38711	GenBank	KC608321	KC614541

Table 1. *Continued*

Species	Field number	Specimen's accession number (MZUSP PL)	Collecting locality	Collecting date	Latitude	Longitude	Identification	*COI*	EF-1-alpha
Rhynchodeminae									
Luteostriata ernesti (Leal-Zanchet & Froehlich, 2006)		no voucher	São Paulo, SP	2005	–23.49080	–46.52090	GenBank	–	KC614555
	F3358	1070	Floresta Nacional de São Francisco de Paula, RS	25 January 2009	–29.42367	–50.38711	GenBank	KC608313	KC614534
Luteostriata graffi (Leal-Zanchet & Froehlich, 2006)	F0384	652	Taquara, RS	28 January 2010	–29.660524	–50.746782	GenBank	KC608227	KC614466
	F0440	654	Morro das Aranhas, Florianópolis, SC	9 September 2004	–27.468485	–48.377724	GenBank	KC608230	KC614468
	F3381	1072	Floresta Nacional de São Francisco de Paula, RS	21 January 2009	–29.42963	–50.39780	GenBank	KC608315	KC614535
Luteostriata muellerleri (Diesing, 1861)		No voucher					GenBank	–	KC614554
	F3268	1199	Parque Estadual da Serra do Tabuleiro, SC	15 January 2009	–27.84278	–48.92583	GenBank	KC608311	–
Luteostriata sp.	F1192	668	Parque da Previdência, São Paulo, SP	9 August 2007	–23.58080	–46.72709	GenBank	KC608249	KC614481
Luteostriata sp. 1	F1036	659	São Sebastião, SP	7 September 2006	–23.75203	–45.631027	GenBank	KC608239	KC614473
	F1078	663	Estação Biológica de Boraceia, Salesópolis, SP, Brazil	2 February 2006	–23.65388	–45.88910	GenBank	KC608243	KC614476
Luteostriata sp. 2	F0441	655	Santinhos, Florianópolis, SC	9 September 2004	–27.46370	–48.38100	GenBank	KC608231	KC614469

Table 1. *Continued*

Species	Field number	Specimen's accession number (MZUSP PL)	Collecting locality	Collecting date	Latitude	Longitude	Identification	*COI*	EF-1-alpha
Rhynchodeminae									
Matuxia matuta (E. M. Froehlich, 1955)	F2184	1021	Parque Estadual do Desengano, RJ	17 March 2008	–27.95989	–48.75761	GenBank	KC608276	KC614503
	F2187	1022	Parque Estadual do Desengano, RJ	17 March 2008	–21.87138	–41.91472	GenBank	KC608277	KC614504
Matuxia tuxaua E. M. Froehlich, 1955	F1275	1192	Parque Nacional da Serra dos Órgãos, RJ	16 December 2007	–22.45528	–42.99750	GenBank	KC608253	–
	F3059	1058	Parque Estadual Serra da Cantareira, SP	14 December 2008	–23.42914	–46.63250	GenBank	KC608302	KC614525
Notogynaphallia albonigra (Riester, 1938)	F4081	1083	Parque Estadual do Desengano, RJ	13 August 2009	–21.87555	–41.91361	GenBank	KC608327	KC614545
Notogynaphallia parca (E. M. Froehlich, 1955)	F2972	1196	Parque Estadual Serra da Cantareira, SP	26 October 2008	–23.42914	–46.63250	GenBank	KC608298	–
Notogynaphallia sexstriata (Graff, 1899)	F0792	656	Cidade Universitária, USP, São Paulo, SP	19 December 2005	–23.56671	–46.73017	GenBank	KC608232	KC614470
	F1413	680	Parque Nacional da Serra de Itajaí, SC	4 January 2008	–27.04836	–49.09200	GenBank	KC608257	KC614487
Obama anthropophila Amaral, Leal-Zanchet & Carbayo, 2015	F1399	1007	Parque Nacional da Serra do Itajaí, Blumenau, SC	4 January 2008	–27.05306	–49.08528	GenBank	KC608256	KC614486
	F1504	698	Parque Nacional da Serra do Itajaí, Blumenau, SC	6 January 2008	–27.05306	–49.08528	GenBank	KC608259	KC614489

Table 1. *Continued*

Species	Field number	Specimen's accession number (MZUSP PL)	Collecting locality	Collecting date	Latitude	Longitude	Identification	*COI*	EF-1-alpha
Rhynchodeminae									
	F1566	707	Parque Nacional da Serra do Itajaí, Blumenau, SC	7 January 2008	–27.05306	–49.08528	GenBank	KC608260	KC614490
Obama burmeisteri (Schultze & Müller, 1857)	F0989	658	Parque Ecológico do Tietê, São Paulo, SP	30 March 2006	–23.48570	–46.50959	GenBank	KC608234	–
	F2886	733	Parque Estadual Serra da Cantareira, SP	26 October 2008	–23.46047	–46.63672	GenBank	KC608297	KC614520
Obama carinata (Riester, 1938)	F3084	1062	Parque Estadual Intervales, SP	12 December 2008	–24.27638	–48.41556	GenBank	KC608304	KC614527
	F3093	1064	Parque Estadual Intervales, SP	12 December 2008	–24.27638	–48.41556	GenBank	KC608307	KC614529
Obama josefi (Carbayo & Leal-Zanchet, 2001)	F3389	1074	Floresta Nacional de São Francisco de Paula, RS	21 January 2009	–29.42736	–50.39838	GenBank	KC608317	KC614537
	F3403	1075	Floresta Nacional de São Francisco de Paula, RS	21 January 2009	–29.42736	–50.39838	GenBank	KC608318	KC614538
Obama ladislavii (Graff, 1899)	F1418	681	Parque Nacional da Serra de Itajaí, SC	5 January 2008	–27.04836	–49.09200	GenBank	KC608258	KC614488
Paraba franciscana (Leal-Zanchet & Carbayo, 2001)	F3357	1069	Floresta Nacional de São Francisco de Paula, RS	2 November 2006	–29.42894	–50.39200	GenBank	KC608312	KC614533
	F5485	935	Floresta Nacional de São Francisco de Paula, RS	12 August 1999	–29.36666	–50.38333	GenBank	KC608335	KC614552
Paraba multicolor (Graff, 1899)	F1736	594	Parque Nacional Saint Hilaire/ Lange, PR	14 January 2008	–25.46972	–48.81222	GenBank	KC608265	KC614495

Table 1. *Continued*

Species	Field number	Specimen's accession number (MZUSP PL)	Collecting locality	Collecting date	Latitude	Longitude	Identification	*COI*	EF-1-alpha
Rhynchodeminae									
	F2997	1055	Parque Estadual Serra da Cantareira, SP	14 December 2008	–23.42914	–46.63250	GenBank	KC608299	KC614521
Paraba rubidolineata (Baptista & Leal-Zanchet, 2005)	F5482	936	Floresta Nacional de São Francisco de Paula, RS	26 August 1999	–29.36666	–50.38333	GenBank	KC608333	KC614550
Pasipha chimbeva (E. M. Froehlich, 1955)	F2813	403	Parque Nacional Serra da Bocaina, SP	8 September 2008	–22.73389	–44.61639	GenBank	KC608286	KC614512
	F3010	476	Parque Estadual Serra da Cantareira, SP	14 December 2008	–23.42914	–46.63250	GenBank	KC608300	KC614522
Pasipha pasipha (Marcus, 1951)	F1841	1012	Parque Estadual Alberto Löfgren, São Paulo, SP	26 January 2008	–23.45925	–46.63730	GenBank	KC608266	KC614496
	F2871	1053	Parque Estadual Serra da Cantareira, SP	26 October 2008	–23.46047	–46.63672	GenBank	KC608295	KC614518
Pasipha pinima (E. M. Froehlich, 1955)	F1714	1011	Parque Nacional Saint Hilaire/ Lange, PR	13 January 2008	–25.46972	–48.81222	GenBank	KC608264	KC614494
	F2585	717	Parque Nacional da Serra dos Órgãos, RJ	10 July 2008	–22.45528	–42.99750	GenBank	KC608280	KC614507
Pasipha rosea (E. M. Froehlich, 1955)	F1061	660	Parque Estoril, São Bernardo do Campo, SP	22 October 2006	–23.77368	–46.51469	GenBank	KC608242	KC614475
Pasipha sp. 2	F1138	664	Parque Nacional da Serra dos Órgãos, RJ	14 January 2007	–22.38455	–42.97681	GenBank	KC608245	KC614477
Pasipha tapetilla (Marcus, 1951)	F2878	732	Parque Estadual Serra da Cantareira, SP	26 October 2008	–23.46047	–46.63672	GenBank	KC608296	KC614519

Table 1. *Continued*

Species	Field number	Specimen's accession number (MZUSP PL)	Collecting locality	Collecting date	Latitude	Longitude	Identification	*COI*	EF-1-alpha
Rhynchodeminae									
	F5631B	938	São Paulo, SP	May 2007	–29.36666	–50.38333	GenBank	KC608336	KC614553
Polycladus sp. 1	F0397	1186	Valdívia (Chile)	2004	–39.81000	–73.23000	GenBank	KC608228	–
Supramontana irritata Carbayo & Leal-Zanchet, 2003	F3452	772	Floresta Nacional de São Francisco de Paula, RS	25 January 2009	–29.42064	–50.39394	GenBank	KC608322	KC614542
	F5483	937	Floresta Nacional de São Francisco de Paula, RS	9 February 2006	–29.36666	–50.38333	GenBank	KC608334	KC614551
Xerapoa hystrix Froehlich, 1955	F3057	1057	Parque Estadual Serra da Cantareira, SP	12 December 2008	–24.27638	–48.41556	GenBank	–	KC614524
Xerapoa pseudo-rhynchodemus (Riester, 1938)	F0992	1187	Parque Ecológico do Tietê, São Paulo, SP	30 March 2006	–23.48570	–46.50959	GenBank	KC608235	–
	F1013	1189	Cidade Universitária, USP, São Paulo, SP	17 March 2006	–23.56671	–46.73017	GenBank	KC608237	–
Xerapoa trina (Marcus, 1951)	F1134	1191	Cascata Imbuí, Teresópolis, RJ	14 January 2007	–22.38455	–42.97681	GenBank	KC608244	–
	F1239	669	Parque Nacional da Serra dos Órgãos, RJ	15 December 2007	–22.45528	–42.99750	GenBank	KC608250	–

*All sequences obtained for this study. All specimens were collected in Brazil, unless specified otherwise. Abbreviations for the Brazilian States are as follows: PR, Paraná; RJ, Rio de Janeiro; RS, Rio Grande do Sul; SC, Santa Catarina; SP, São Paulo.

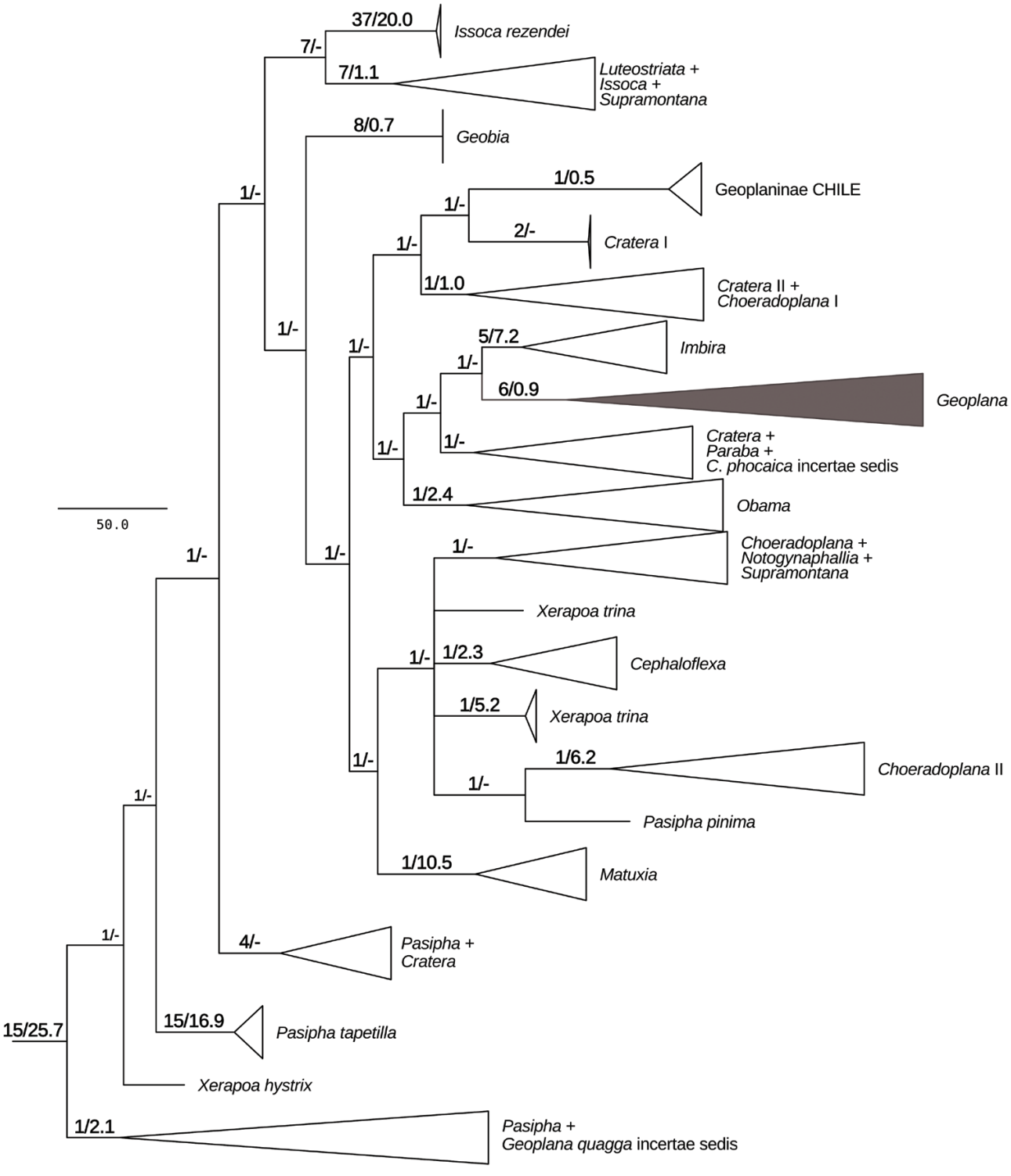

Figure 2. Summary of sister-group relationships within Geoplaninae based on the simultaneous analysis of *COI* and *EF-1-α* partial sequences for all-terminals dataset using parsimony as the optimality criteria with 5469 steps in length. Support values for selected nodes are above branches [Goodman–Bremer (GBS)/log-likelihood difference (LLD)]. Absence of values for LLD indicated that the node was not recovered by the maximum likelihood (ML) analyses.

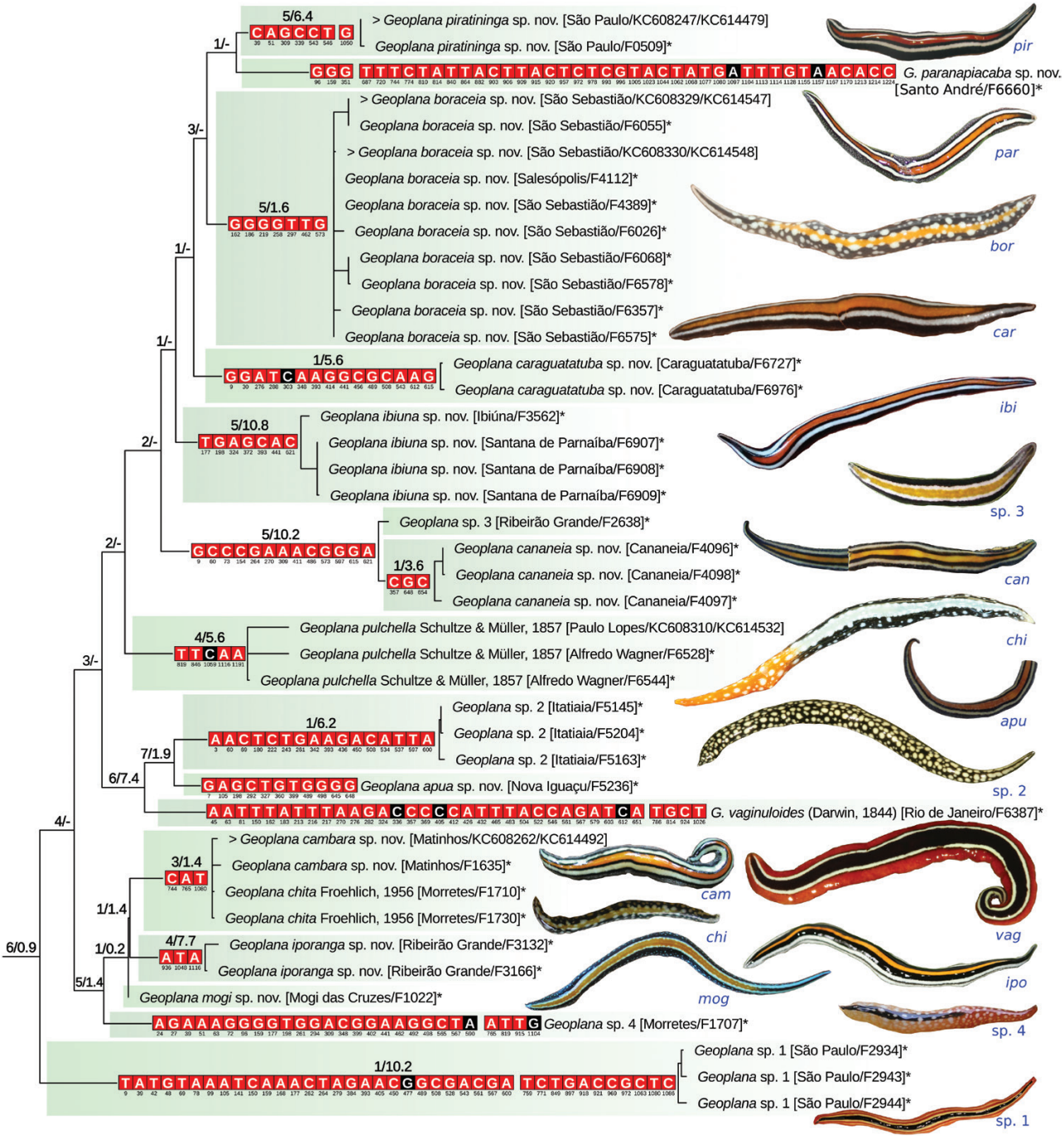

Figure 3. Phylogenetic hypotheses for species of *Geoplana* based on the simultaneous analysis of *COI* and *EF-1-α* partial sequences for all-terminals dataset using parsimony as the optimality criteria. Bases on branches represent unambiguous unique synapomorphies (black) and homoplastic synapomorphies (red) for the respective clades (numbers below bases indicate position of the transformations). Nucleotide positions 1–676 refer to *COI*, and 677–1225 refer to *EF-1-α*. Support values for selected nodes are above branches [Goodman–Bremer (GBS)/log-likelihood difference (LLD)]. Absence of values for LLD indicated that the node was not recovered by the maximum likelihood (ML) analyses. *Sequences were obtained for this study. >Species name updated with respect to GenBank. Pictures of specimens are at different scales.

latter (AICc of 45633.2577 vs. 46779.9146). The results of ML analyses are partly congruent with the phylogenetic pattern recovered by the MP analyses (Supporting Information, Figs S2, S3). In general, internal nodes are sensitive to optimality criteria (i.e. MP vs. ML); however, both recovered the clades we recognize as putative species of *Geoplana*.

Phyletic status of Geoplaninae and its main taxonomic groups

All phylogenetic analyses revealed that Geoplaninae and five of its genera, namely *Cephaloflexa* Carbayo & Leal-Zanchet, 2003, *Geoplana* Schultze & Müller, 1857, *Imbira* Carbayo *et al.*, 2013, *Obama* Carbayo *et al.*, 2013 and *Matuxia* Carbayo *et al.*, 2013, are monophyletic, regardless of the dataset and optimality criterion applied. Two suprageneric assemblages, one constituted by *Issoca* Froehlich, 1955a + *Luteostriata* Carbayo, 2010 + *Supramontana* Carbayo & Leal-Zanchet, 2003 (from Brazil), another by *Gusana* Froehlich, 1978 + *Polycladus* Blanchard, 1845 (from Chile), were also recovered in all trees. *Choeradoplana* Graff, 1896, *Issoca*, *Luteostriata*, *Paraba* Carbayo *et al.*, 2013, *Pasipha* Ogren & Kawakatsu, 1990 and *Supramontana* always resulted in polyphyletic assemblages. Sister-group relationships are unstable for these assemblages and for each of these genera (e.g. compare Fig. 2 with Supporting Information, Fig. S3).

Species of *Geoplana*: congruence between molecular and morphological data

Most species possess sets of molecular homoplastic synapomorphies, and a few species, namely *Geoplana paranapiacaba*, *Geoplana caraguatatuba*, *G. pulchella*, *G. vaginuloides*, *Geoplana* sp. 1 and *Geoplana* sp. 4, additionally exhibit between one and three unambiguous unique synapomorphies (Fig. 3). That is to say that at least these six species can be diagnosed on a molecular basis. However, we found it premature to include those sets as part of the formal diagnosis of putative species given the nature of most transformations.

Congruence between molecular and morphological data of members of *Geoplana* was retrieved in most analyses. The instances in which phylogenetic analyses were not congruent with our initial concept of morphospecies involved lack of phylogenetic resolution. The phylogenetic analysis of *COI* (Fig. S5) recovered clades with congruent specimens that share cohesive morphological attributes. However, there were no sequences of this marker for *G. chita* (see below). Analyses including only *EF-1-α* recovered most species, but the reciprocal monophyly of *Geoplana boraceia*, *Geoplana cambara*, *Geoplana cananeia*, *G. chita* and *Geoplana ibiuna* was not observed because their representatives nested in a polytomy (Supporting Information, Fig. S6). The simultaneous phylogenetic analyses of *COI* and *EF-1-α*, independently of taxonomic representation (i.e. all-terminals or selected-terminals datasets) and optimality criteria (i.e. MP and ML) recovered the monophyly of most putative species of *Geoplana* (see Fig. 3; Supporting Information Figs S1–S6). There was a single exception to this general pattern for *G. cambara* and *G. chita*, for which the resolution among haplotypes remained unresolved (Fig. 3), most probably attributable to the lack of *COI* sequences for *G. chita*.

Four lineages, namely *Geoplana* sp. 1–4, which are not described here, were also retrieved as distinct monophylic groups; two of these lineages are represented by immature individuals (Supporting Information, Table S1).

MORPHOLOGICAL RESULTS

Geoplanidae Stimpson, 1857

Geoplaninae Stimpson, 1857

Geoplana Stimpson, 1857

Geoplana vaginuloides (Darwin, 1844)

Figs 4 and 5

Planaria vaginuloides Darwin, 1844, p. 244.

Geoplana vaginuloides: Riester, 1938, p. 72–75 [part]; Froehlich, 1956a, p. 314–315 [part]; Marcus, 1951, p. 54–56 (misidentification), 1952: p. 76–77 (misidentification).

Material examined

Neotype MZUSP PL 2074 (field number F6387): Parque Estadual da Pedra Branca, Rio de Janeiro/RJ, Brazil (22°56′13.5″S, 043°27′39.5″W). F. Carbayo *et al.*, collected (coll.), 13 December 2014. Cephalic region: horizontal sections on two slides; ovarian region: horizontal sections on eight slides; piece behind ovarian region: horizontal sections on ten slides; pre-pharyngeal region: transverse sections on ten slides; pharynx: sagittal sections on five slides; copulatory apparatus: sagittal sections on ten slides.

SMF N574: Barreira, Teresópolis, Rio de Janeiro/RJ, Brazil. E. Bresslau coll., 1914. Cephalic region preserved in balsam on one slide; pre-pharyngeal region: transverse sections on one slide; pharynx: sagittal sections on two slides; copulatory apparatus: sagittal sections on three slides; tail preserved in balsam on one slide.

SMF N628: Barreira, Teresópolis, Rio de Janeiro/RJ, Brazil. E. Bresslau, *Coll.* 1914. Cephalic region and tail preserved in balsam on one slide; pre-pharyngeal

region: transverse sections on one slide; pharynx and copulatory apparatus: sagittal sections on three slides.

Note

We could not confirm conspecificity of some specimens studied by Riester (1938) and specimens examined by Froehlich (1955a, 1956a, 1958) (see Supporting Information, Table S1).

Distribution

Areas covered with Atlantic forest in the municipalities of Rio de Janeiro and Teresópolis, state of Rio de Janeiro.

Diagnosis

Dorsal colour pattern constituted by a median black band, bounded on either side by a fine white-yellow stripe, externally to which is a very thin black line, and this is externally bordered by a marginal reddish-iron band. Inner pharynx musculature with two layers. Anteroventral region of penis bulb more developed. Penis papilla with dorsal insertion strongly displaced anteriorly to level the ventral insertion.

Type locality

Rio de Janeiro, Brazil.

External aspect

Live animals ~70 mm long and 4 mm wide. Elongated body, with margins approximately parallel; anterior end rounded, posterior pointed. Dorsum convex, ventral side slightly convex. Dorsal colour constituted by a median black band (one-third of body width), bounded on either side by a fine white-yellow stripe (two-ninths), externally to which is a very thin black line (one-ninth), and this is bordered externally by a marginal reddish-iron band (one-third) (Fig. 4A). Ventral body surface grey with dark margins at cephalic end, and saffron yellow on the remaining surface. After fixation, body colour faded slightly. Two types of eyes: (1) a conical type (zuckerhutförmig, sugarloaf shaped), 90 µm high and 40 µm in width; and (2) a cup-shaped type, 40 µm in diameter, with the former contouring the anterior end. Cup-shaped-type eyes spread as a band, one-third of body width on either side. In the posterior half of the body, the eyes even reach to the median black band. Sensory pits simple invaginations, 20 µm deep, located ventromarginally in a single row from the very anterior body tip up to at least the first one-quarter of body length. Relative position mouth-to-body length, 48%. Relative position gonopore-to-body length, 61%.

Internal morphology

Creeping sole 97% of body width. Very abundant rhabditogen cells piercing dorsal and marginal epidermis. Glandular margin absent. The cutaneous musculature comprises three layers, namely a subepithelial circular layer, followed by a double diagonal layer with decussate fibres, and then a well-developed longitudinal layer. Muscle fibres of the longitudinal, innermost layer (30 µm thick) arranged into bundles with 15–35 fibres each. CMI, 8%. Three parenchymal muscle layers present, all weakly developed: dorsal layer of decussate diagonal fibres, supraintestinal layer of transverse and longitudinal fibres, and subintestinal layer with transverse and longitudinal fibres (Fig. 4B). Ventral nerve plate present.

Mouth situated at a distance from the root of the pharynx equivalent to 67% of pharyngeal pocket length. Pharynx cylindrical (Fig. 4C). Oesophagus-to-pharynx ratio, 12%. Outer pharyngeal musculature consisting of a subepithelial layer (6 µm) of longitudinal muscle, followed by a layer (30 µm) of circular fibres. Inner pharynx musculature consisting of a subepithelial layer of circular fibres (40 µm), followed by a layer of of longitudinal fibres (10 µm).

Testes dorsally located between supraintestinal parenchymal muscle layer and intestine; anteriormost testes at a distance equivalent to 17% of body length; posterior follicles near to the pharynx root. Penis bulb oval, relatively strongly developed, more developed ventro-anteriorly; it extends from 0.5 mm anterior to penis papilla to being level with the gonopore region (specimen MZUSP PL 2074). Penis bulb consists of tightly packed muscle fibres variously orientated anteriorly, and longitudinally and obliquely orientated posteriorly; in this posterior region, fibres on the right side run obliquely downwards to embrace the ventro-anterior portion of the female atrium and, subsequently, presumably anchor on ventral epidermis. Sperm ducts are dorso-internal to the ovovitelline ducts. Lateral to penis insertion, sperm ducts bend medially and then ventrolaterally to penetrate the bulb. The ducts subsequently run anteriad and join to continue posteriorly and sinuously as the ejaculatory duct (Fig. 4D). Ejaculatory duct traverses central region of penis papilla to open at its tip. Sperm ducts lined with a cuboidal, ciliated epithelium and surrounded by a 5-µm-thick layer of circular muscles. Wall of ejaculatory duct stained reddish, consisting of a cuboidal, ciliated epithelium, surrounded by circular muscles, which constitute a muscular cylinder with a diameter equivalent to three times the diameter of the duct (Fig. 5A).

Penis papilla very long, 5 mm, with dorsal and ventral insertions at the same horizontal plane (Fig. 4D). This papilla is cylindrical along most of its length; total length equal to 17 times its diameter, entirely occupying both male and female atria; dorsal insertion strongly displaced anteriorly and very ventrally, so that it projects from the anteroventral portion of the male atrium. Subepithelial musculature consisting of a dense, 42-µm-thick layer of circular muscle,

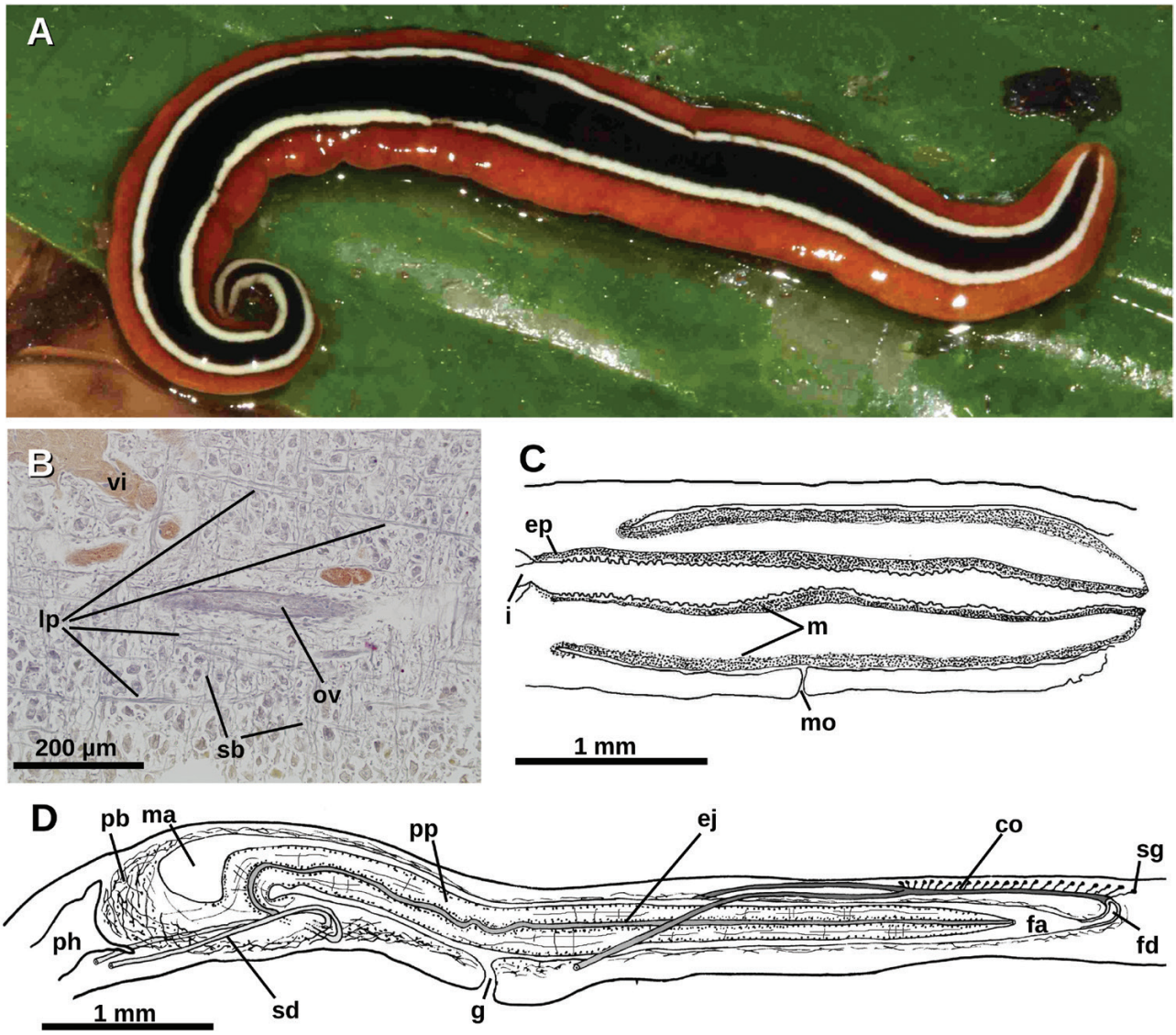

Figure 4. *Geoplana vaginuloides* (Darwin, 1844). Specimen MZUSP PL 2074. A, dorsal view of live specimen, ~60 mm in length. B, photomicrograph of longitudinal and transverse muscles of the subintestinal parenchymatic layer in a horizontal section. C, diagrammatic representation of the pharynx from sagittal sections. D, diagrammatic representation of the copulatory apparatus from sagittal sections.

tending to decussate distally, followed by a single layer of longitudinal muscle. Epithelium of papilla pierced by reddish secretions. Poor condition of sections does not display further histological details. Male atrium long and ample, not folded.

Ovaries oval shaped, 250 µm in maximal length, and situated at a distance from anterior end equivalent to one-fifth of body length. Ovovitelline ducts emerge from an unknown region of ovaries, lateral to female atrium. Then, they curve medially and join to form the common glandular ovovitelline duct, dorsally to the posterior third of this atrium (Fig. 4D). Common glandular ovovitelline duct as long as one-third of female atrium length and communicates with the female genital duct; this duct is a projection of the posterior region of the female atrium and is dorso-anteriorly orientated. The female genital duct is lined with an epithelium provided with intraepithelial gaps (Fig. 5B). Poor condition of sections does not display further histological details.

Female atrium without folds, two to three times as long as male atrium, lined with a tall epithelium, which posteriorly presents intraepithelial gaps. Atrial epithelium surrounded by a thin layer of longitudinal muscle fibres, followed by a layer of muscles with criss-cross arrangement; thickness of both layers equal to 25 µm, distally doubling in thickness.

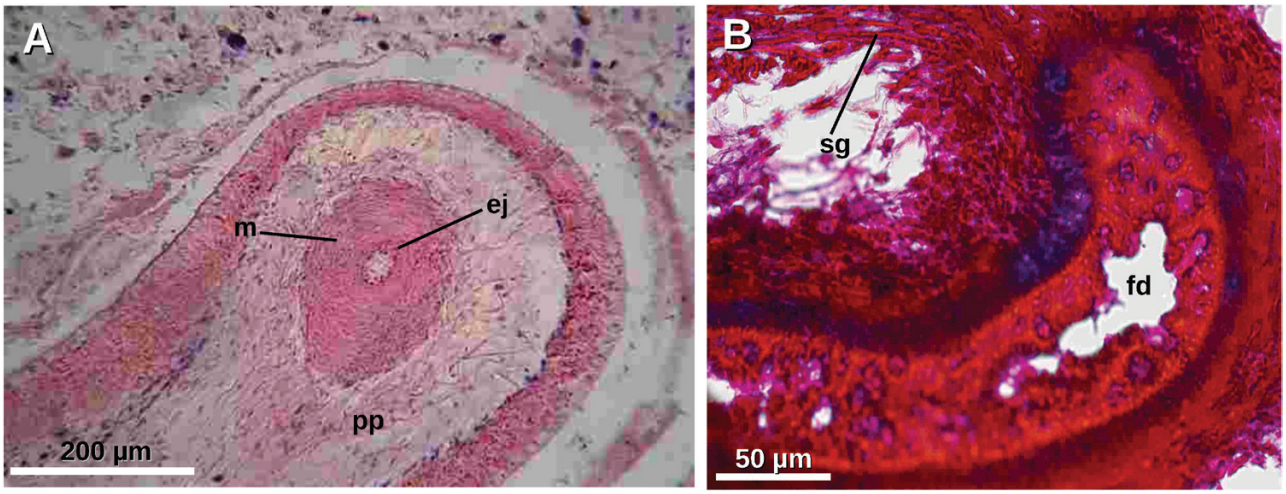

Figure 5. *Geoplana vaginuloides* (Darwin, 1844). A, photomicrograph of part of the penis papilla of specimen N574 on a sagittal section, showing the ejaculatory duct and its surrounding muscle. B, photomicrograph of the female genital duct of specimen MZUSP PL 2074 on a sagittal section.

Remarks

The original description of the dorsal chromatic pattern of this species may be ambiguous ("Sides and foot coloured dirty 'orpiment orange'; above with two stripes on each side of pale 'primrose-yellow', edged externally with black; on centre of the back a stripe of glossy black"; Darwin, 1844, p. 244). From Darwin's description, Marcus deduced that 'an additional black lateral stripe should exist on each side, albeit only the external stripe was mentioned' (Marcus, 1952 [original in Portuguese]). No specimen matching Marcus' description was ever found, but this ambiguity is removed in Darwin's field notes. In an entry on 17 June, 1832, he states: "Colours: back with glossy black stripe; on each side of this a primrose white one edged externally with black; these stripes reach to extremities, & become uniformly narrower. sides & foot dirty 'orpiment orange'" (in Keynes, 2000, p. 47). We found this precise colour pattern displayed by the specimen MZUSP PL 2074 from the type locality. Hence, it is most probable that this specimen and that found by Darwin are conspecific. A conclusive comparison is not possible because there is no record of Darwin's specimen in the Natural History Museum, London, where the material collected by Darwin is deposited (H. Jones, personal communication).

Neotype designation satisfies provisions of the ICZN code (Art. 75.3.1.–7.) as follows: (1) the neotype is designated with the express purpose of clarifying the taxonomic status or the type locality of the name *Geoplana vaginuloides*; (2) the characters differentiating *G. vaginuloides* from any other taxa are stated in the diagnosis above; (3) these data and the description of the species are sufficient to ensure recognition of the specimen designated; (4) after Dr Hugh Jones [from Natural History Museum London (NHM)], the name-bearing type specimen is lost or even has never been deposited in the NHM; (5) the 'Remarks' section shows that neotype is consistent with the former name-bearing type from the original description and from Darwin's field notes (in Keynes, 2000); (6) the neotype came from the original type locality; and (7) the neotype is the property of the Museu de Zoologia da Universidde de São Paulo (MZUSP), Brazil.

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FIGS 6 AND 7

Geoplana vaginuloides: Riester, 1938, p. 72–75 [part]: Froehlich, 1956a, p. 314–315 [part].

Material examined

Holotype MZUSP PL 2072 (field number F5236): Nova Iguaçú/RJ, Brazil. Cristiano Sampaio *et al.*, coll., 4–6 April 2012. Cephalic region: horizontal sections on eight slides; ovarian region: sagittal sections on five slides; pre-pharyngeal region: transverse sections on ten slides; pharynx: sagittal sections on six slides; copulatory apparatus: sagittal sections on 11 slides.
Paratype MZUSP PL 2090 (field number F7001): Teresópolis, Rio de Janeiro/RJ, Brazil. E. M. Froehlich coll., 20 July 1952. Copulatory apparatus: sagittal sections on 18 slides.

Paratype SMF N460: Barreira, Teresópolis, Rio de Janeiro/RJ, Brazil. Ernest Bresslau coll., 25 February 1914. Cephalic region and tail preserved in balsam on one slide; pre-pharyngeal region: transverse sections on one slide; pharynx and copulatory apparatus: sagittal sections on two slides.

Distribution
Areas covered with Atlantic forest in the municipalities of Nova Iguaçú and Teresópolis, State of Rio de Janeiro, Brazil.

Diagnosis
Dorsal colour pattern constituted by a median luminous orange band, bounded on either side by a black stripe, externally to which is a greenish-yellow stripe, and this again is bordered externally by a marginal black band. Inner pharynx musculature with two layers. Anterodorsal region of penis bulb more developed. Penis papilla with dorsal insertion strongly displaced anteriorly and very ventrally, as long as 18 times its diameter.

Etymology
The specific epithet is a Tupi (indigenous Brazilian tribe) word that means mound (Tibiriçá, 1984), referring to the hilly terrain where the species was collected.

External aspect
Live animals 45 mm long when creeping. Body margins parallel; anterior end rounded, posterior end pointed. In preserved specimens, dorsum convex, ventral side slightly convex. Dorsal colour pattern of the preserved specimen F5236 constituted by a median luminous orange band (one-third of body width), bounded on either side by a black stripe (1/12th), externally to which is a greenish-yellow stripe (1/12th), and this again is bordered externally by a marginal black band (one-sixth) (Fig. 6A). Ventral body surface pale orange. In specimen F7001 (preserved for > 60 years), black became brownish, remaining colours became cream. Two types of eyes: a conical type, 80 µm high and 30 µm wide; and a cup-shaped type, 50 µm in diameter. The former type of eye contours the anterior portion of the body. Four millimetres behind anterior end, cup-shaped-type eyes spread dorsally onto the yellowish stripes until rear end of body, where the eyes are sparsely distributed. Sensory pits simple invaginations, 25 µm deep, located ventromarginally in a single row and extending from the very anterior end up to at least the first one-fifth of body length. Relative position mouth-to-body length, 55%. Relative position gonopore-to-body length, 67%.

Internal morphology
Creeping sole 77% of body width. Glandular margin absent. Cutaneous musculature same as in the type species of the genus in terms of organization. CMI, 7%. Muscle fibres of the longitudinal layer (15–20 µm thick) arranged into bundles with ten to 30 fibres each. Parenchymal musculature same as in the type species of the genus in terms of organization and relative development (Fig. 6B). Ventral nerve plate present.

Mouth situated at a distance from root of the pharynx equivalent to 54% of pharyngeal pocket length. Pharynx cylindrical (Fig. 6E). Oesophagus-to-pharynx ratio, 18%. Pharyngeal musculature (Fig. 6D) same as in the type species of the genus in terms of organization and development, with the exception of the inner longitudinal layer, which reaches 35 µm in thickness.

Testes dorsally located between supraintestinal parenchymal muscle layer and intestine; anteriormost testes at a distance from anterior end equivalent to 13% of body length; posteriormost near the pharynx root. Penis bulb oval shaped, relatively strongly developed, consisting of tightly packed muscle fibres variously orientated. The bulb is more developed dorso-anteriorly, and extends from 0.5 mm anterior to penis papilla up to the gonopore region (specimen MZUSP PL 2072). Diagonal fibres surrounding distal section of penis bulb run obliquely downwards on the right side of the body to embrace ventrally the anterior portion of the female atrium. Sperm ducts run laterally to the ovovitelline ducts. Laterally to ventral insertion of penis papilla, sperm ducts bend anteriorly and medially, then penetrate penis bulb ventrolaterally, and maintain this course until they join each other. From this point onwards, the ejaculatory duct runs posteriorly, centrally traversing the penis papilla to open at its tip. Sperm present in extrabulbar portion of sperm ducts; in one specimen, sperm was also present at the anterior section of the intrabulbar lumen.

Sperm ducts lined with a cuboidal, ciliated epithelium and surrounded by a 5- to 8-µm-thick layer of circular muscles. Proximal half of ejaculatory duct lined with cuboidal, ciliated epithelium and pierced by gland cells producing weakly erythrophil, fine granules; distal half lined with squamous, non-ciliated epithelium pierced by gland cells producing erythrophil, coarse granules. Anterior half of this duct surrounded by circular muscles, which constitutes a muscular cylinder in the distal portion with a diameter equivalent to five times the diameter of this duct.

Penis papilla thin, somewhat conical, because it narrows very gradually, and very long; total length equal to 18 times its diameter, occupying almost entirely both male and female atria; dorsal insertion strongly displaced anteriorly and very ventrally, so that it projects from anteroventral portion of male atrium. Squamous lining epithelium of the penis papilla pierced by numerous gland cells producing xanthophyl and erythrophil fine granules, and underlain by a dense 85-µm-thick layer of circular muscle, distally tending to decussate, followed by a single layer of longitudinal muscle (Fig. 6F).

Male atrium not folded (Fig. 7A, B), lined with a low, non-ciliated epithelium, and underlain by a 5-µm-thick layer of circular muscles, followed by an equally thin layer of longitudinal fibres. A set of muscle fibres is also present as longitudinal fibres extending around the gonopore canal laterally to the male atrium, and being intermingled with those of the penis bulb.

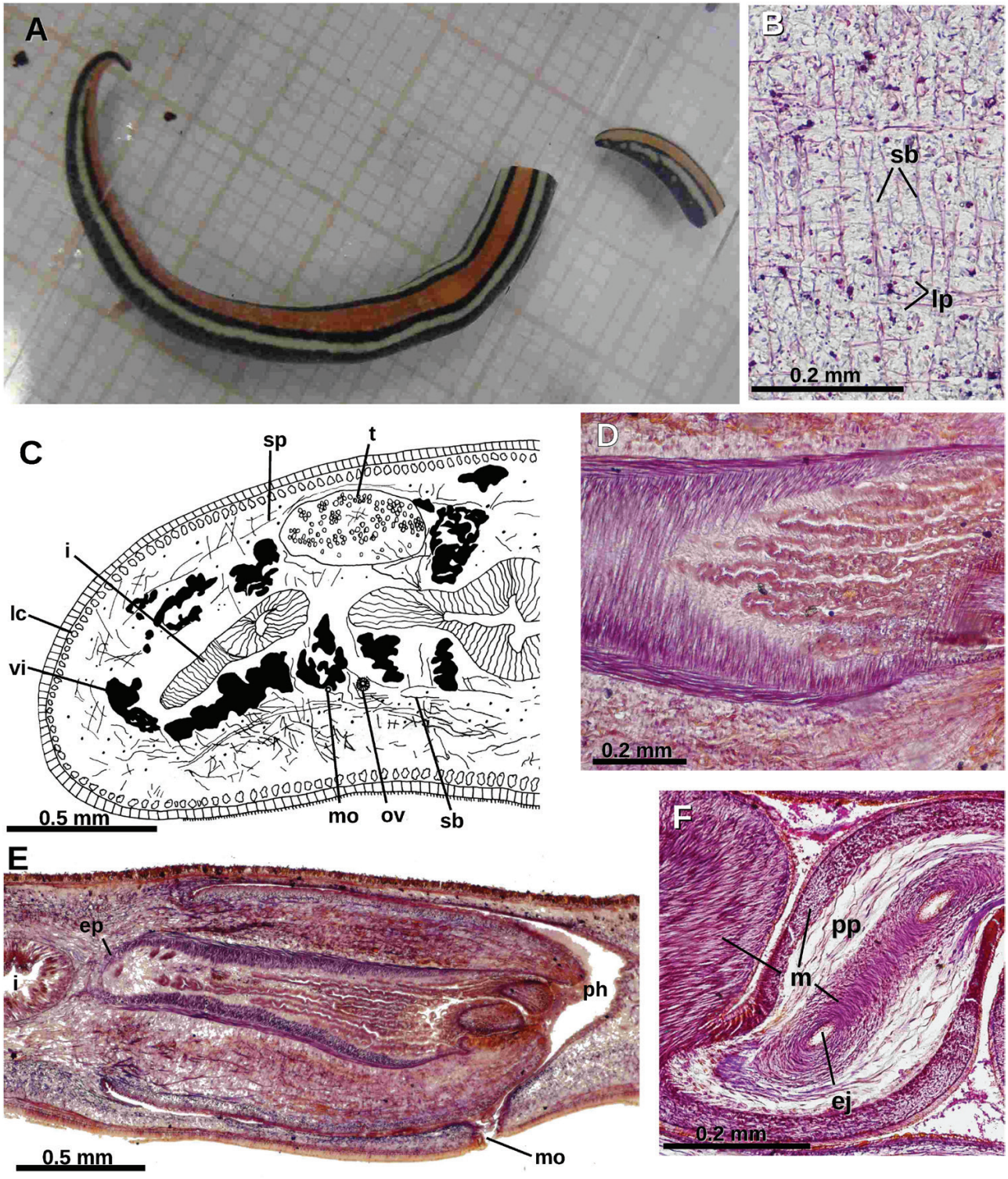

Figure 6. ***Geoplana apua*** **sp. nov.** A, dorsal view of the MZUSP PL 2072 preserved specimen on millimetre graph paper after cutting off a piece of the body. B, photomicrograph of longitudinal and transverse muscles of the subintestinal parenchymatic layer in a horizontal section of specimen MZUSP PL 2072. C, diagramatic reconstruction of half of a transverse section of the pre-pharyngeal region of the holotype; dots represent longitudinal parenchymatic muscle fibres. D, photomicrograph of the two muscle layers of the internal musculature of the pharynx in sagittal section. E, photomicrograph of a sagittal section of the pharynx of specimen MZUSP PL 2072. F, photomicrograph of the ejaculatory duct and its surrounding muscle of specimen MZUSP PL 2072 in sagittal section.

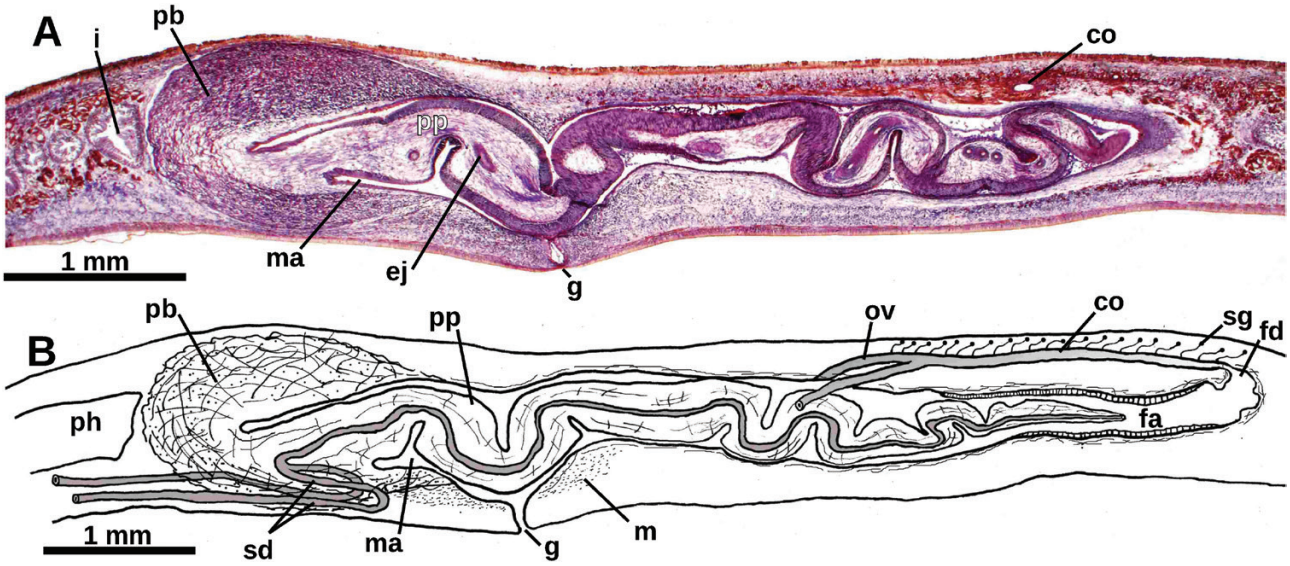

Figure 7. ***Geoplana apua*** **sp. nov.** A, photomicrograph of a sagittal section of the copulatory apparatus of specimen MZUSP PL 2072. B, diagrammatic representation of the copulatory apparatus of specimen MZUSP PL 2072 from sagittal sections.

Ovaries oval shaped, 300 µm in maximal diameter, and situated at a distance from anterior end equivalent to 22.5% of body length. Ovovitelline ducts emerge from the dorsolateral aspect of ovaries. The oviducts run laterally to the female atrium, curve medially and then join to form the common glandular ovovitelline duct, dorsally to this atrium. Common glandular ovovitelline duct as long as 40% of female atrium length, communicating with female genital duct (Fig. 7B); this duct is a projection of the posterior region of the female atrium, bent dorsally and lined with a squamous, non-ciliated epithelium. A cyanophil fibrillar mass, 5–10 µm thick, is immediately present underneath this epithelium of the female atrium, with very thin circular filaments (< 1 µm) resembling muscle fibres in stain affinity and orientation, followed by a layer of longitudinal muscle fibres.

Female atrium without folds, as long as three times that of male atrium, and lined with a cuboidal-to-columnar epithelium, which is pierced by glands producing xanthophil granules and is underlain by a thin layer of muscle fibres in a criss-crossed arrangement, followed by a loose layer (25 µm) of longitudinal fibres.

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FIGS 8 AND 9

Geoplana vaginuloides: Marcus, 1951, p. 54–56.

Material examined

Holotype MZUSP PL 2089 (field number F7000): Parque do Estado, São Paulo/SP, Brazil. O. Françoso and E. M. Froehlich, coll., 29 May 1985. Cephalic region: sagittal sections on ten slides; ovarian region: horizontal sections on 12 slides; pre-pharyngeal region: transverse sections on ten slides; pharynx: sagittal sections on 20 slides; copulatory apparatus: sagittal sections on 35 slides.

Paratype Marcus' specimen (1951) (from EMF collection): Eldorado neighborhood, West zone of the city of São Paulo/SP, Brazil. E. Marcus and E. M. Froehlich, coll., 1 November 1947. Copulatory apparatus: sagittal sections on five slides.

Paratype MZUSP PL 2061 (field number F1022): Mogi das Cruzes/SP, Brazil (23°27′05.5″S, 046°13′40.8″W). F. Carbayo, coll., 27 May 2006. Cephalic region: transverse sections on 13 slides; ovarian region: horizontal sections on seven slides; pre-pharyngeal region: transverse sections on five slides; pharynx: sagittal sections on eight slides; copulatory apparatus: sagittal sections on five slides.

Distribution

Areas covered with secondary Atlantic forest in the municipalities of São Paulo and Mogi das Cruzes, state of São Paulo, Brazil.

Etymology

The specific epithet refers to the Tupi-Guarani (indigenous Brazilian tribe) name of the municipality where the species was collected.

Diagnosis

Dorsal colour pattern constituted by a median orange-ochre band, bounded on either side by a thin whitish stripe, and in turn this is bordered externally by a thin

black line, externally to which is a grey band with dense white mottling, externally to which is a thin marginal black line. Longitudinal parenchymal muscle fibres disposed around intestine as a loose muscular tube. Diameter of muscular cylinder surrounding ejaculatory duct equivalent to 1.3 times the diameter of this duct.

External aspect

Preserved animals 50 mm long and 4 mm wide. Body margins parallel; anterior end rounded, posterior pointed. Dorsum strongly convex, ventral side flat. On histological slides, body 1.25 mm in height. In live animals, dorsal colour pattern constituted by a median orange-ochre band (one-third of body width) that passes into the black of the cephalic region (one-fifth of body width), bounded on either side by a thin whitish stripe (one-ninth), and in turn this is bordered externally by a thin black line, externally to which is a grey band (the two latter totalling two-ninths) with dense white mottling, externally to which is a thin marginal black line (Fig. 8A). This marginal band was discovered in a detailed examination of the preserved specimen; this band remained hidden in dorsal view owing to the strong convexity of the dorsum. Ventral body surface whitish, with blackish margins. In preserved specimen F1022, whitish, blackish and greyish colours lost brightness; orange-ochre faded. In specimen F7000, median band was ochre-brown bordered on either side by cream lines, in turn this was bordered by a brown green line, externally to which was a beige grey band. Two types of eyes: a conical type, 90 µm high and 40 µm in width; and a cup-shaped type, 40 µm in diameter. The former type contours the anterior tip of the body. Behind the first millimetre, cup-type eyes spread progressively onto the dorsum, reaching the whole dorsum from the pharynx region until posterior end. Sensory pits simple invaginations, 55 µm deep, located ventromarginally in a single row, at least along first third of body from very anterior end. Relative position mouth-to-body length, 55%. Relative position gonopore-to-body length, 71%.

Internal morphology

Creeping sole 78% of body width. Glandular margin absent. Three typical geoplaninid cutaneous muscle layers present, being as thick as 9.5% of body height. Muscle fibres of the longitudinal, innermost layer (40 µm thick) arranged into bundles with 18–20 fibres each. Three parenchymal muscle layers present: dorsal layer of decussate diagonal fibres, supraintestinal layer of transverse fibres, and subintestinal layer with transverse fibres. Longitudinal fibres around intestine present, constituting a loose tube with fibres mixing with transverse ones. Ventral nerve plate present.

Mouth situated at a distance from root of the pharynx equivalent to 44–46% of pharyngeal pocket length. Pharynx cylindrical, with dorsal insertion slightly

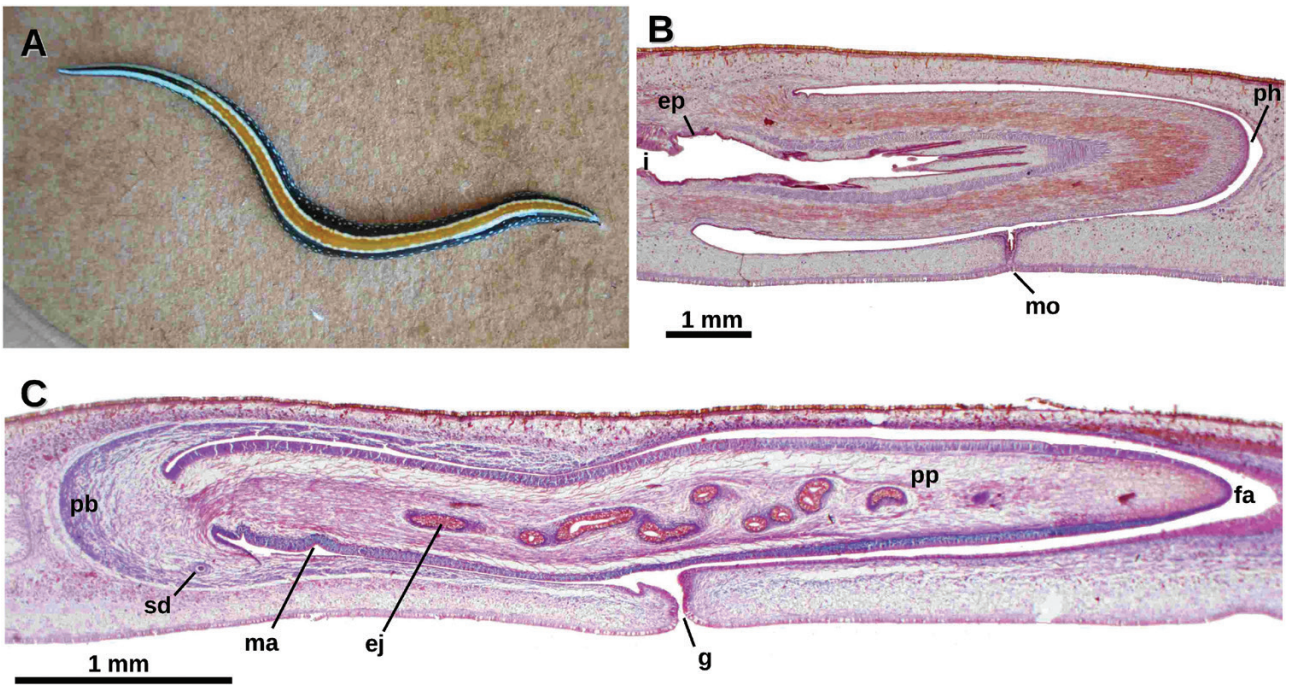

Figure 8. ***Geoplana mogi*** **sp. nov.** A, dorsal view of the live specimen MZUSP PL 2061. B, photomicrograph of a sagittal section of the pharynx of specimen MZUSP PL 2089. C, photomicrograph of a sagittal section of the copulatory apparatus of specimen MZUSP PL 2089.

posterior to ventral one (Fig. 8B). Oesophagus-to-pharynx ratio, 15%. Outer pharyngeal musculature consisting of a subepithelial layer (5 µm) of longitudinal muscle, followed by a layer (70 µm) of circular fibres, the innermost of which are intermingled with longitudinal muscle fibres. Inner pharynx musculature consisting of a thin subepithelial layer of longitudinal muscle, followed by a layer (70 µm) of circular fibres and an innermost layer (30 µm) of longitudinal fibres.

Testes dorsally located between supraintestinal parenchymal muscle layer and intestine; anteriormost testes at a distance from anterior end equivalent to 22% of length of body; posteriormost follicles near pharynx root. Penis bulb elongated, extending posteriorly from 0.5 mm (specimen MZUSP PL 2061) anterior to penis papilla to the level of the gonopore. The bulb consists of tightly packed muscle fibres, variously orientated. Diagonal fibres on the right side of the bulb run obliquely downwards to embrace ventrally the anterior portion of female atrium. These fibres apparently cross the nerve plate to anchor on the ventral epidermis. Sperm ducts run dorsally to the ovovitelline ducts. Laterally to penis insertion, sperm ducts bend medially and then penetrate the penis bulb ventrolaterally. Subsequently, the ducts recurve and separately open into the sinuous ejaculatory duct (Figs 8C, 9A). The latter traverses the central region of the penis papilla to open at its tip. Sperm present in sperm ducts, also along its course within penis bulb. Sperm ducts lined with a cuboidal, ciliated epithelium and surrounded by a 20-µm-thick layer of circular muscles. Ejaculatory duct lined with columnar, ciliated epithelium; epithelium of extrapenial portion pierced by glands producing weakly erythrophil, fine granules, and being surrounded by 60-µm-thick layer of circular muscle fibres. Epithelium of intrapenial portion of ejaculatory duct richly pierced by glands producing erythrophil granules and surrounded by a 20-µm-thick layer of circular muscles, which constitutes a muscular cylinder possessing a diameter equivalent to 1.3 times the diameter of this duct (Fig. 9B).

Penis papilla long and cylindrical along most of its length, with dorsal insertion slightly anterior to the ventral. Total length of papilla equal to ten times its greatest diameter, and occupying male atrium and almost entire female atrium; lined with a cuboidal, non-ciliated epithelium, pierced by two types of gland cells, producing coarse erythrophil granules and fine cyanophil granules, respectively. Erythrophil glands pierce anterior and distal third of penis; necks of cyanophil glands pierce dorsal epithelium of second third, and sometimes also the ventral one, and pack space immediately beneath epithelium so that subepithelial muscle fibres are apart from it (Fig. 9C). Subepithelial musculature of penis papilla consisting of dense 80- to

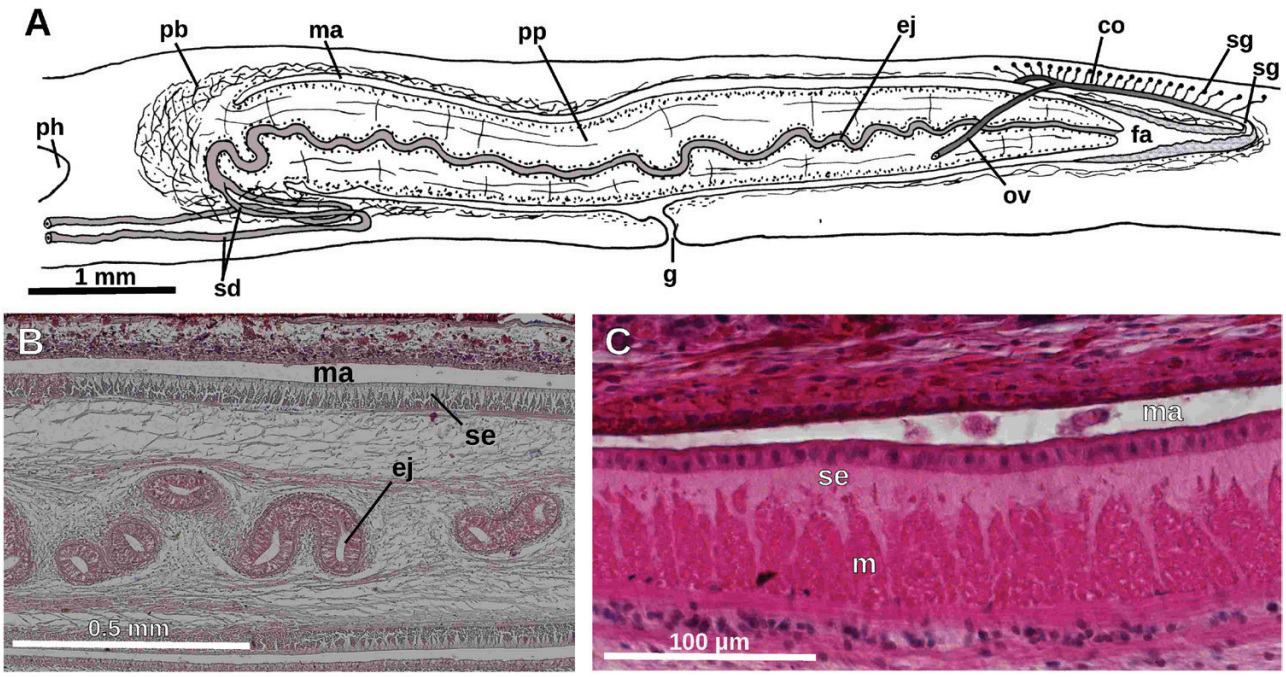

Figure 9. ***Geoplana mogi*** **sp. nov.** A, diagrammatic representation of the copulatory apparatus of the specimen MZUSP PL 2089 from sagittal sections. B, photomicrograph of the ejaculatory duct and its surrounding musculature of specimen MZUSP PL 2089 in sagittal section. C, photomicrograph of dorsal epithelium of penis papilla and erythrophil glands located immediately beneath epithelium of the unlabelled specimen studied by Marcus (1951) in sagittal section.

90-µm-thick layer of circular muscle, the fibres of which are decussate distally, followed by a 10- to 20-µm thick layer of longitudinal muscle.

Male atrium not folded, lined with a low, non-ciliated epithelium, pierced by some glands secreting amorphous substance, and underlain by 7-µm-thick layer of circular muscles, followed by a nearly inconspicuous layer of longitudinal fibres.

Ovaries elongated, 1.1 mm in maximal anteroposterior diameter, 0.2 mm in dorsoventral width, and situated at a distance from anterior end equivalent to 22% of body length. Ovovitelline ducts emerge from dorsolateral aspect of ovaries laterally to female atrium, curve medially and join common glandular ovovitelline duct dorsally to this atrium. Common glandular ovovitelline duct as long as a third of female atrium length; it communicates with female genital duct, which is a dorso-anteriorly orientated projection of the posterior region of the female atrium (Fig. 9A) and is lined with a 25-µm-tall cuboidal, ciliated epithelium.

Female atrium without folds, posterior portion funnel shaped; as long as 1.5 times that of male atrium; anteriorly lined with a cuboidal epithelium; posteriorly lined with a tall epithelium with stratified appearance and intraepithelial holes. Epithelium pierced by two types of glands, producing erythrophil granules and, less abundantly, xanthophil. Epithelium underlain by 5- to 45-µm-thick layer of circular-to-decussate muscle fibres, followed by some longitudinal fibres, and then a layer (50–150 µm thick) of thicker longitudinal fibres.

GEOPLANA PIRATININGA ALMEIDA & CARBAYO SP. NOV.

FIGS 10 AND 11

Geoplana vaginuloides: Marcus, 1952, p. 76–77.

Material examined

Holotype MZUSP PL 2071 (field number F5134): Parque Jardim Previdência, São Paulo/SP, Brazil (23°34′48.7″S, 046°43′39.3″W). F. Carbayo *et al.*, coll., 5 February 2006. Cephalic region: horizontal sections on four slides; ovarian region: horizontal sections on two slides; region behind ovaries: horizontal sections on ten slides; pharynx: sagittal sections on six slides; copulatory apparatus: sagittal sections on eight slides.

Paratype MZUSP PL 2060 (field number F0509): Parque Ecológico do Tietê, São Paulo/SP, Brazil (23°29′27.1″S, 046°31′01.6″W). F. Carbayo, coll., 27 May 2005. Pharynx and post-pharynx region: sagittal sections on six slides (incompletely mature).

Paratype MZUSP PL 666 (field number F1163): Parque Jardim Previdência, São Paulo/SP, Brazil (23°34′46.8″S, 046°43′39.0″W). O. M. Junior, coll., 17 June 2007. Pharynx: sagittal sections on 13 slides; copulatory apparatus: sagittal sections on seven slides (incompletely mature).

Distribution

Areas covered with secondary Atlantic forest and garden in the municipalities of São Paulo and Mongaguá, state of São Paulo, Brazil.

Etymology

The specific epithet is a Tupi (indigenous Brazilian tribe) word formerly referring to the city of São Paulo, where the species was collected.

Diagnosis

Dorsal colour pattern constituted by a median traffic red band, bounded on either side by a black stripe, externally to which is a white stripe, and in turn this is externally bordered by a black band, externally to which is a thin marginal white line. Dorsal and ventral insertions of penis papilla at the same transverse plane. Wall of female atrium not folded. Female-to-male atrial length ratio, 2.0.

External aspect

Live animals 25 mm long and 4 mm wide. Body relatively short, with margins nearly parallel; anterior end rounded, posterior pointed. Dorsum strongly convex, ventral side slightly convex. Dorsal body surface constituted by a median traffic red band (1/3.5th of body width), passing into black part of cephalic region (one-sixth of body length) and of posterior tip, bounded on either side by a black stripe, externally to which is a white stripe (one-seventh), and in turn this is bordered externally by a black band (one-seventh), externally to which is a thin marginal white line (Fig. 10A). Ventral side whitish. After fixation, dorsal black colour faded, red passed into yellow-orange and white into cream. Two types of eyes: a conical type, contouring only anterior end; and a cup-shaped type, spreading dorsally onto the black bands. Sensory pits simple invaginations, 25 µm deep, located ventro-marginally in a single row from the very anterior end up to at least first one-fifth of body length. Relative position mouth-to-body length, 42.5%. Relative position gonopore-to-body length, 64%.

Internal morphology

Creeping sole ~85% of body width, as measured on horizontal or sagittal sections. Glandular margin absent. Three typical geoplaninid cutaneous muscle layers present, being as thick as 5% of body height. Muscle fibres of the longitudinal, innermost layer (25 µm thick) arranged into bundles. Three parenchymal muscle layers present, all weakly developed: dorsal layer

of decussate diagonal fibres, supraintestinal layer of transverse and longitudinal fibres, and subintestinal layer with transverse fibres. Additionally, longitudinal fibres surround intestine as a loose tube, the ventral fibres of which are mixed up with those of the subintestinal layer. Ventral nerve plate present.

Mouth situated at a distance from root of the pharynx equivalent to 50–65% of pharyngeal pocket length. Pharynx cylindrical, with dorsal insertion slightly posterior to ventral one (Fig. 10B). Oesophagus-to-pharynx ratio, 12%. Outer pharyngeal musculature consisting of a subepithelial layer (5 µm) of longitudinal muscle, followed by a layer (45 µm) of circular fibres, the innermost of which are intermingled with longitudinal ones. Inner pharynx musculature consisting of a thin subepithelial layer of longitudinal muscle, followed by a layer (40 µm) of circular fibres and an innermost layer (10 µm) of longitudinal fibres (Fig. 10C).

Testes dorsally located between supraintestinal parenchymal muscle layer and intestine; anteriormost testes at a distance from anterior end equivalent to 25.5% of body length; posteriormost follicles a little anterior to pharynx root. Penis bulb elongated, somewhat oval, extending from 0.3 mm anterior to penis papilla; from thereon the bulb extends ventrally, whereas dorsally it extends posteriorly for only short distance. The bulb consists of variously orientated packed muscle fibres. Diagonal fibres on the right side of the bulb run obliquely downwards to embrace ventrally the anterior portion of the female atrium. These fibres apparently mix with the subintestinal parenchymal musculature, but their anchor point could not be

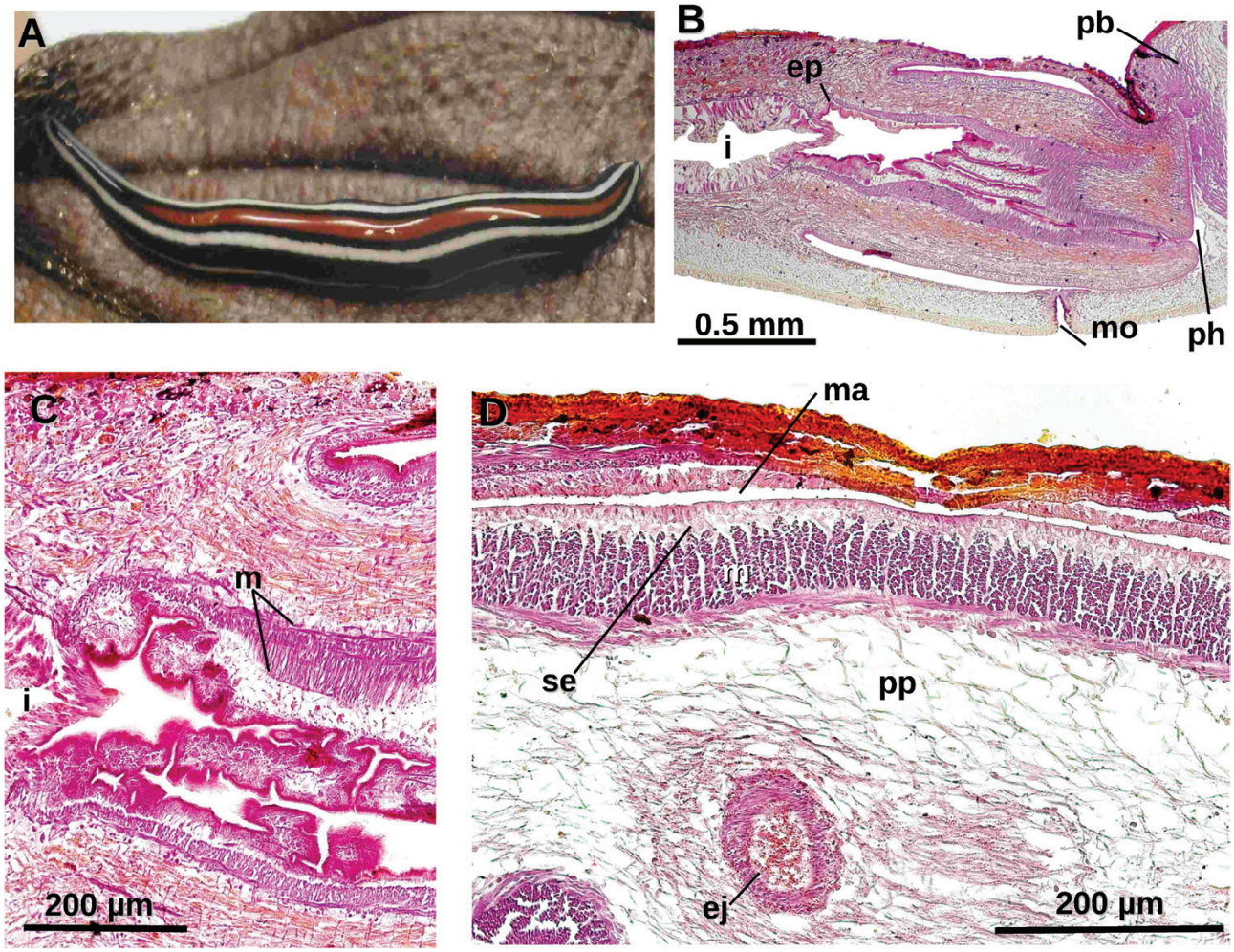

Figure 10. ***Geoplana piratininga*** **sp. nov.** A, dorsal view of the live specimen MZUSP PL 2060; anterior to the left, ~32 mm in length. B, photomicrograph of a sagittal section of the pharynx of specimen MZUSP PL 2071. C, photomicrograph of the three muscle layers of the internal musculature of the pharynx in sagittal section of specimen MZUSP PL 2071. D, photomicrograph of dorsal epithelium of penis papilla and erythrophil glands located immediately beneath the epithelium of specimen MZUP PL 2071 in sagittal section.

discerned. Sperm ducts run dorsally to the ovovitelline ducts. Sperm ducts bend medially behind insertions of penis papilla and penetrate penis bulb ventrolaterally. The ducts subsequently recurve to join and thus form the ejaculatory duct, which sinuously posteriad, then traverses the penis papilla to open at its tip. Distal portion of sperm ducts containing sperm, along their course both outside and inside penis bulb.

Sperm ducts lined with a cuboidal, ciliated epithelium and surrounded by a 6-µm-thick layer of circular muscles. Extrapapillar portion and anterior portion of intrapapillar section of ejaculatory duct lined with columnar ciliated epithelium, pierced by glands producing erythrophil fine granules. Remaining section of the ejaculatory duct also columnar and ciliated, pierced by numerous gland cells producing erythrophil granules. Entire ejaculatory duct surrounded by a layer of circular muscle, 35 µm thick around anterior portion, otherwise 10 µm thick (Fig. 10D). Diameter of muscular cylinder surrounding ejaculatory duct is equivalent of 3.5 times the diameter of this duct.

Penis papilla is long, horizontal and cylindrical along most of its length, with dorsal and ventral insertions at the same transverse plane (Fig. 11A, B). Total length of papilla equal to five times its greatest diameter, and completely filling both male and female atrium; papilla lined with a cuboidal epithelium, pierced by gland cells producing coarse erythrophil granules; glands producing cyanophil granules packed in space immediately beneath epithelium of the dorsal second third of penis papilla, so that subepithelial muscle fibres are separated from it (Fig. 10D). Penial subepithelial musculature consisting of dense 90-µm-thick layer of circular muscle, followed by a layer (25 µm thick) of longitudinal muscle.

Male atrium ample, not folded, lined with a low, non-ciliated epithelium, pierced by glands secreting erythrophil amorphous substance and underlain by a 15-µm-thick layer of circular muscles, followed by an inconspicuous layer of longitudinal fibres.

Ovaries incompletely developed, 500 µm in length along anteroposterior body axis, located above ventral nerve plate and situated at a distance from anterior end equivalent to ~20.5% of body length. Laterally to the female atrium, the oviducts curve medially and join to form a common glandular ovovitelline duct. This duct is as long as one-third of the female atrium length and communicates with the female genital duct, the latter being a funnel-shaped, anterodorsally orientated projection of the posterior region of the female atrium.

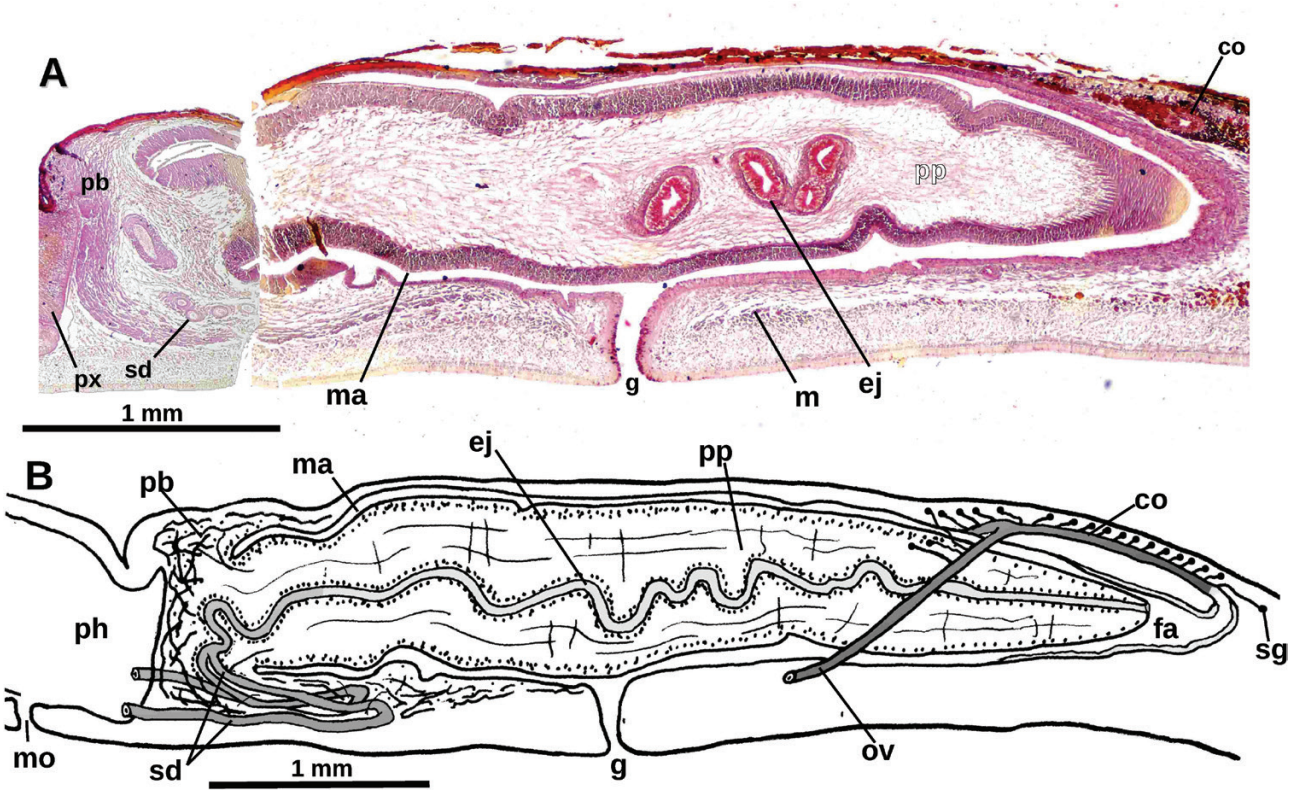

Figure 11. ***Geoplana piratininga*** **sp. nov.** A, photomicrograph of a sagittal section of the copulatory apparatus of specimen MZUSP PL 2071. B, diagrammatic representation of the copulatory apparatus of specimen MZUSP PL 2071 from sagittal sections.

Female atrium funnel shaped and without folds, as long as two times the male atrium length (Fig. 11B); in its anterior portion it is lined with cuboidal epithelium, posteriorly with a 40-µm-high epithelium with stratified aspect; pierced by two types of glands producing cyanophil and xanthophil granules, respectively. Epithelium underlain by a 5- to 40-µm-thick layer of circular-to-decussate muscle fibres, followed by a 25-µm-thick layer of longitudinal fibres. Additionally, 4-µm-thick longitudinal fibres around these muscles constitute a kind of muscle coat.

GEOPLANA PARANAPIACABA ALMEIDA & CARBAYO SP. NOV.

FIGS 12 AND 13

Material examined

Holotype MZUSP PL 2079 (field number F6660): Cidade Turística de Paranapiacaba, Santo André/SP, Brazil (23°46′38.9″S, 046°18′05.2″W). F. Carbayo *et al*., coll., 5 June 2015. Cephalic region: horizontal sections on four slides; ovarian region: horizontal sections on nine slides; region behind ovaries: horizontal sections on 14 slides; pre-pharyngeal region: transverse sections on 16 slides; pharynx: sagittal sections on 17 slides; copulatory apparatus: sagittal sections on 20 slides.

Paratype MZUSP PL 2087 (field number F6998): Cidade Turística de Paranapiacaba, Santo André/SP, Brazil. E. M. Froehlich *et al*., coll., 13 May 1990. Copulatory apparatus: sagittal sections on 39 slides.

Distribution

Only known from the type locality.

Etymology

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

Diagnosis

Dorsal colour pattern constituted by a median stripe of orange-ochre, bounded on either side by a black line, externally to which is a broader white line, and in turn this is externally bordered by a thin black line, externally to which is a grey band with dense white mottling, bordered by a thin marginal black line. Diameter of muscular cylinder round ejaculatory duct equivalent to 0.5 times the diameter of this duct. Female-to-male atrial length ratio, 3.5–4.

External aspect

Preserved animals up to 50 mm long, and 4 mm wide. Body margins nearly parallel; anterior end rounded, posterior end pointed. Dorsum strongly convex, ventral side slightly convex. Dorsal body surface constituted by a median stripe of orange-ochre (one-quarter of body width) that passes into the black of cephalic region (one-fifth of body length), bounded on either side by a black line (1/16th) posteriorly merging with each other, externally to which is a broader white line (one-eighth), and this is externally bordered by a black stripe (1/16th), externally to which is a grey stripe (one-eighth) with dense white mottling, then bordered by a marginal thin black line (Fig. 12A). Ventral body surface is whitish with blackish margins. In preserved specimen F6660, dorsal colour slightly faded. In preserved specimen F6998, stripes were varied from faded black grey to pearl orange. Two types of eyes: a conical type contouring only anterior end; and a cup-shaped type, spreading dorsally onto the black stripes. Sensory pits simple invaginations, 40 µm deep, located ventromarginally in a single row from the very anterior tip to at least the end of the anterior fifth of the body. Relative position mouth-to-body length, 61%. Relative position gonopore-to-body length, 74%.

Internal morphology

Creeping sole 78% of body width. Glandular margin absent. Three typical geoplaninid cutaneous muscle layers present, being as thick as 8% of body height at pre-pharyngeal region. Longitudinal muscle fibres of the innermost layer (35–50 µm thick) arranged into bundles with 17–30 fibres each. Three parenchymal muscle layers present: a dorsal layer of decussate diagonal fibres, a loose supraintestinal layer of transverse and longitudinal fibres, and a loose subintestinal layer with traverse and longitudinal fibres (Fig. 12B, C). Ventral nerve plate present.

Mouth situated at a distance from root of the pharynx equivalent to 66% of pharyngeal pocket length. Pharynx cylindrical (Fig. 12D). Oesophagus-to-pharynx ratio, 26%. Outer pharyngeal musculature consisting of a subepithelial layer (5 µm) of longitudinal muscle, followed by a layer (70 µm) of intermingled circular and longitudinal muscle fibres. Inner pharynx musculature consisting of a thin subepithelial layer of longitudinal muscle, followed by a layer (90 µm) of circular fibres and an innermost layer (15 µm) of longitudinal fibres.

Testes dorsally located between supraintestinal parenchymal muscle layer and intestine; anteriormost testes (at a distance from anterior end equal to 22% of body length) behind ovarian region, posteriormost near root of pharynx. Penis bulb elongated, strongly developed, consisting of tightly packed muscle fibres variously orientated in its anterior section, while posteriorly the fibres are orientated diagonally, extending from 1 mm (holotype) anterior to penis papilla to the level of the gonopore, even more posteriorly in the dorsal body region. Diagonal fibres on the right side of the bulb run obliquely downwards to

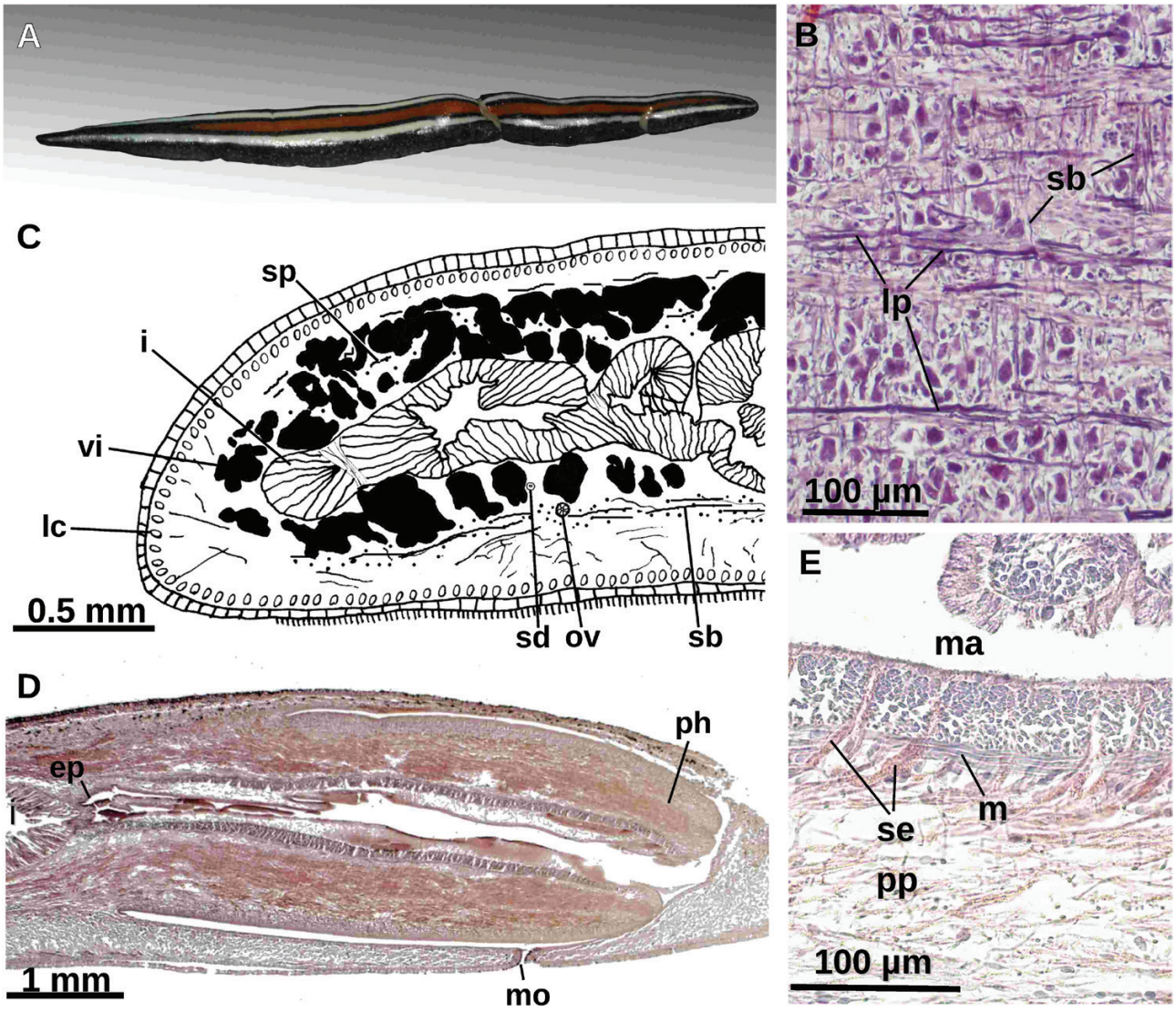

Figure 12. ***Geoplana paranapiacaba*** Almeida & Carbayo **sp. nov.** A, dorsal view of the 50-mm-long specimen MZUSP PL 2079, immediately after having been killed with boiling water. It was accidentally cut into two pieces at the moment of collection. B, photomicrograph of longitudinal and transverse muscles of the subintestinal parenchymatic layer in horizontal section of specimen MZUSP PL 2079. C, diagramatic reconstruction of half of a transverse section of the pre-pharyngeal region of the holotype; dots represent longitudinal parenchymatic muscle fibres. D, photomicrograph of a sagittal section of the pharynx of specimen MZUSP PL 2079. E, photomicrograph of dorsal epithelium of penis papilla and erythrophil glands located immediately beneath the epithelium of specimen MZUP PL 2079 in sagittal section.

embrace ventrally the anterior portion of the female atrium; further course of these fibres could not be determined owing to tissue damage. Sperm ducts run dorsolaterally to the ovovitelline ducts. Laterally to penis bulb, sperm ducts bend anteriorly and medially, and then ventrolaterally penetrate the bulb. The ducts subsequently recurve and join to form an unpaired, sinuous sperm duct directed posteriorly (Fig. 13A, B). The unpaired portion continues as an ejaculatory duct inside the penis papilla, which traverses centrally to open at its tip. Sperm present in sperm duct, both along its course outside and in part of anteriorly directed portion inside penis bulb. Epithelium of sperm ducts and unpaired sperm duct pierced by glands producing minute weakly cyanophil granules; surrounded by a 7-µm-thick layer of circular muscles. Ejaculatory duct lined by 15 µm, ciliated epithelium pierced by abundant glands producing erythrophil granules; surrounded by a layer of circular muscle fibres constituting a muscular cylinder with a diameter equivalent to 0.5 times the diameter of this duct.

Penis papilla long, with dorsal and ventral insertions at the same transverse plane; it is horizontal and cylindrical, with a length (4 mm) equal to ten times its diameter, even occupying more than half of the female atrium. Papilla covered with a cuboidal, non-ciliated epithelium, pierced by gland cells especially distally, producing erythrophil granules (Figs 12E, 13D). Subepithelial penis musculature consisting of a dense 25- to 105-µm-thick layer of circular muscle, followed by a thin layer (12 µm) of longitudinal muscle.

Male atrium not folded (Fig. 13A, B), lined with a low, non-ciliated epithelium, pierced by glands producing an amorphous erythrophil substance and underlain by a 15-µm-thick layer of circular muscles, followed by a thin layer of longitudinal fibres.

Ovaries oval shaped, 550 µm in maximal anteroposterior diameter and 250 µm in dorsoventral diameter, and situated at a distance from anterior end equivalent to 16% of body length. Ovovitelline ducts emerge from dorsolateral aspect of ovaries. The oviducts run laterally to the female atrium, then curve medially and join to form a common glandular ovovitelline duct, dorsally to this atrium. Common glandular ovovitelline duct as long as half of the female atrium length, communicates with female genital duct; the latter being a 40-µm-long projection of the dorsoposterior region of the female atrium (Fig. 13A, B, D).

Female atrium long, without folds, as long as 3.5–4 times the male atrium length (Fig. 13A, B, D) and lined with a tall epithelium (70 µm). Posterior section of female

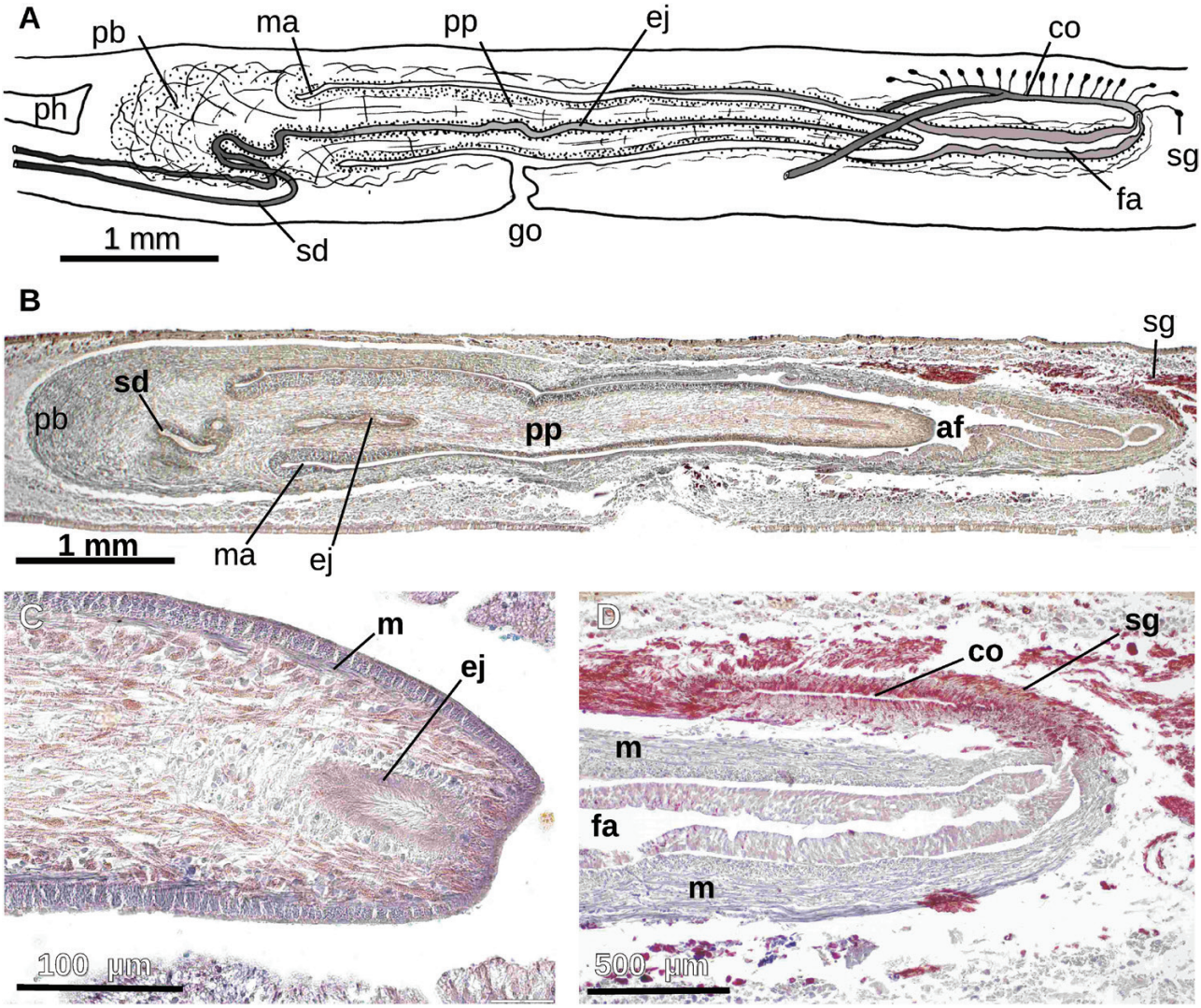

Figure 13. ***Geoplana paranapiacaba*** Almeida & Carbayo **sp. nov.** A, diagrammatic representation of the copulatory apparatus of specimen MZUSP PL 2079 from sagittal sections. B, photomicrograph of a sagittal section of the copulatory apparatus of specimen MZUSP PL 2079. C, photomicrograph of a sagittal section of the penis papilla tip of specimen MZUSP PL 2079. D, photomicrograph of posterior section of female atrium of specimen MZUSP PL 2079.

atrium lined with an epithelium with stratified aspect and some internal gaps; surrounded by a thin layer of longitudinal muscle fibres, followed by 70-µm-thick layer of circular fibres, and a third layer (115 µm) of fibres in criss-cross arrangement. No evident distinction between atrial fibres and those of common muscle coat.

GEOPLANA CARAGUATATUBA ALMEIDA & CARBAYO SP. NOV.

FIGS 14 AND 15

Material examined

All specimens were collected in Parque Estadual Serra do Mar, Caraguatatuba/SP, Brazil.

Holotype MZUSP PL 2091 (field number F7004; 23°35′38.0″S, 045°25′47.1″W): F. Carbayo *et al.*, coll., 14 May 14 2016. Ovarian region: horizontal sections on eight slides; pre-pharyngeal region: transverse sections on seven slides; pharynx and copulatory apparatus: sagittal sections on 34 slides.

Paratype MZUSP PL 2080 (field number F6727; 23°35′38.4″S, 045°25′46.8″W): F. Carbayo *et al.*, coll., 2 July 2015. Before sectioning, copulatory apparatus studied on images of orthogonal virtual sections obtained by µCT. Ovarian region: horizontal sections on eight slides; pre-pharyngeal region: transverse sections on nine slides; pharynx and copulatory apparatus: sagittal sections on eight slides.

Paraype MZUSP PL 2126 (field number F6976; 23°35′38.0″S, 045°25′47.1″W): F. Carbayo *et al.*, coll., 1 January 2016. In absolute ethanol.

Distribution

Only known from borders of Atlantic forest in the municipality of Caraguatatuba, state of São Paulo, Brazil.

Etymology

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

Diagnosis

Dorsal colour pattern constituted by a median orange-ochre band, bounded on either side by a black stripe, externally to which is a white stripe, and this in turn is externally bordered by a black stripe that merges into a grey band with white mottling, which fades gradually towards its outer margin, while this margin is bordered by a black stripe. Penis bulb extending anteriorly 0.8 mm from penis papilla. Wall of female atrium not folded.

External aspect

Live animals 60 mm long and 4 mm wide when extended. Body margins nearly parallel; anterior end rounded, posterior pointed. Dorsum convex, ventral side slightly convex. Dorsal body surface constituted by a median orange-ochre band (one-quarter of body width) that grades into the black of the cephalic region (one-ninth of body length) and of posterior end, bounded on either side by a black line (one-tenth) that join each other posteriorly, external to which is a white stripe (one-ninth), which join each other at the posterior end, and in turn this is externally bordered by a black stripe (one-ninth) that merges into a grey band (one-ninth) with white mottling and which gradually fades towards its outer margin. This margin is bordered by a black line (Fig. 14A). Ventral body surface whitish with black margins. In preserved specimens, dorsal black colour became dark greyish, and grey became cream; whitish remained unchanged. Two types of eyes: a conical type contouring only the anterior end; and a cup-shaped type, spreading dorsally onto the surface external to the white stripes. Sensory pits simple invaginations, 50 µm deep, located ventromarginally in a single row from the very anterior end up to the end of first one-third of body. Relative position mouth-to-body length, 55%. Relative position gonopore-to-body length, 69%.

Internal morphology

Creeping sole 80% of body width. Glandular margin absent. Three typical geoplaninid cutaneous muscle layers present, being as thick as 6% of body height. Muscle fibres of the longitudinal innermost layer (15 µm thick) arranged into bundles with 20–30 fibres each. Three parenchymal muscle layers present: dorsal layer of decussate diagonal fibres, supraintestinal layer of transverse and longitudinal fibres, and subintestinal layer with transverse and longitudinal fibres. Longitudinal fibres rarely lateral to intestine. Ventral nerve plate present.

Mouth situated at a distance from root of the pharynx equivalent to 34–60% of pharyngeal pocket length. Pharynx cylindrical, with dorsal insertion posteriorly placed (Fig. 14B). Oesophagus-to-pharynx ratio, 14%. Outer pharyngeal musculature consisting of a subepithelial layer (5 µm) of longitudinal muscle, followed by a layer (55 µm) of circular fibres, intermingled with longitudinal ones at its innermost portion. Inner pharynx musculature consisting of a thin subepithelial layer of longitudinal muscle, followed by a layer (35 µm) of circular fibres and an innermost layer (10 µm) of longitudinal fibres (Fig. 14C).

Testes dorsally located between supraintestinal parenchymal muscle layer and intestine; anteriormost testes (at a distance from anterior end equal to 25% of body length) behind ovarian region, posteriormost near root of pharynx. Penis bulb elongated, extending from 0.8 mm anterior to penis papilla to the level of the gonopore region; strongly developed, consisting

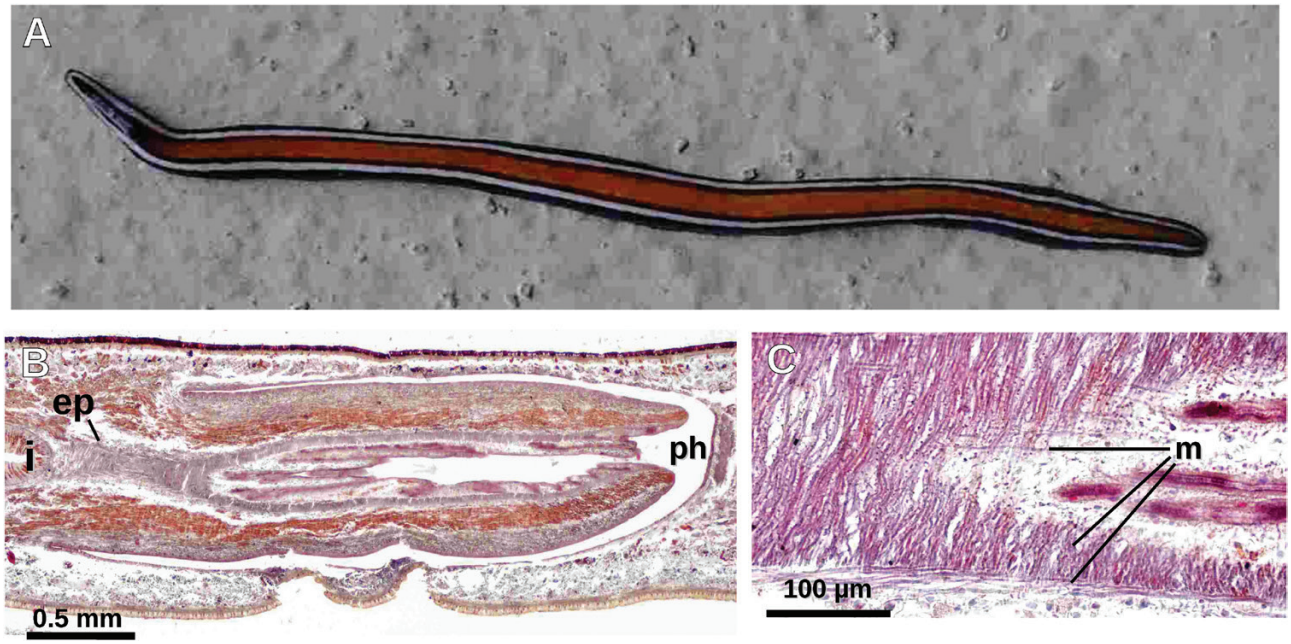

Figure 14. ***Geoplana caraguatatuba*** Almeida & Carbayo **sp. nov.** A, dorsal view of the living specimen MZUSP PL 2080, ~42 mm in length. B, photomicrograph of a sagittal section of the pharynx of specimen MZUSP PL 2080. C, photomicrograph of the three muscle layers of the internal musculature pharynx in sagittal section of specimen MZUSP PL 2080.

of tightly packed muscle fibres variously orientated, somewhat more developed dorsally than ventrally. Bulb fibres on the right side run obliquely downwards to embrace ventrally the anterior portion of the female atrium, subsequently anchoring presumably on ventral epidermis. Sperm ducts run dorsolaterally or dorsally to the ovovitelline ducts. Laterally to penis bulb, sperm ducts bend anteriorly and medially, and then ventrolaterally penetrate the bulb. The ducts subsequently join to form the ejaculatory duct, with initial section outside penis papilla, then crossing it centrally to open at its tip (Fig. 15A). Sperm ducts lined with a cuboidal, ciliated epithelium and surrounded by a 5- to 25-µm-thick layer of circular muscles. Sperm contained in distal portion of sperm ducts, both along their course outside and inside penis bulb. Ejaculatory duct lined with tall, ciliated epithelium; pierced by numerous gland cells producing erythrophil granules, except in its first millimetre. Ejaculatory duct surrounded by a 25-µm-thick layer of circular muscle fibres constituting a muscular cylinder with a diameter equivalent to 1.7 times the diameter of this duct.

Penis papilla 5 mm long, horizontal and cylindrical along most of its length, with dorsal and ventral insertions at the same transverse plane (Fig. 15A–C). Total length of papilla equal to seven times its diameter. Papilla covered with a cuboidal epithelium pierced by gland cells producing erythrophil granules; dorsal epithelium of second third of papilla pierced by glands producing cyanophil granules; space immediately beneath this epithelium packed by necks of these cells (Fig. 15D, E). Subepithelial musculature consisting of dense layer of circular muscle, 130 µm thick dorsally, 60 µm thick ventrally, followed by a 20-µm-thick layer of longitudinal muscle.

Male atrium ample, not folded, lined with a low, non-ciliated epithelium and underlain by an 8-µm-thick layer of circular muscles, followed by an 8-µm-thick layer of longitudinal fibres. Additional longitudinal muscle fibres around atrium dorsal to gonopore canal.

Ovaries are oval shaped, 450 µm in length in longitudinal axis, and situated at a distance from anterior end equivalent to one-quarter of body length. Ovovitelline ducts emerge from dorsolateral aspect of ovaries. Ovaries run laterally to posterior portion of female atrium, then curve medially to communicate with common glandular ovovitelline duct. The latter duct is as long as one-seventh of female atrium length and opens into the female genital duct, a projection of the dorsoposterior region of the female atrium.

Female atrium without folds, cylindrical along most of its course, funnel shaped posteriorly; as long as 1.5 times that of male atrium (Fig. 15A); funnel-shaped portion lined with tall (20–25 µm) epithelium with stratified aspect, being pierced by two types of glands, producing erythrophil and xanthophil amorphous substance, respectively; cylindrical portion lined with cuboidal epithelium. Female atrium surrounded

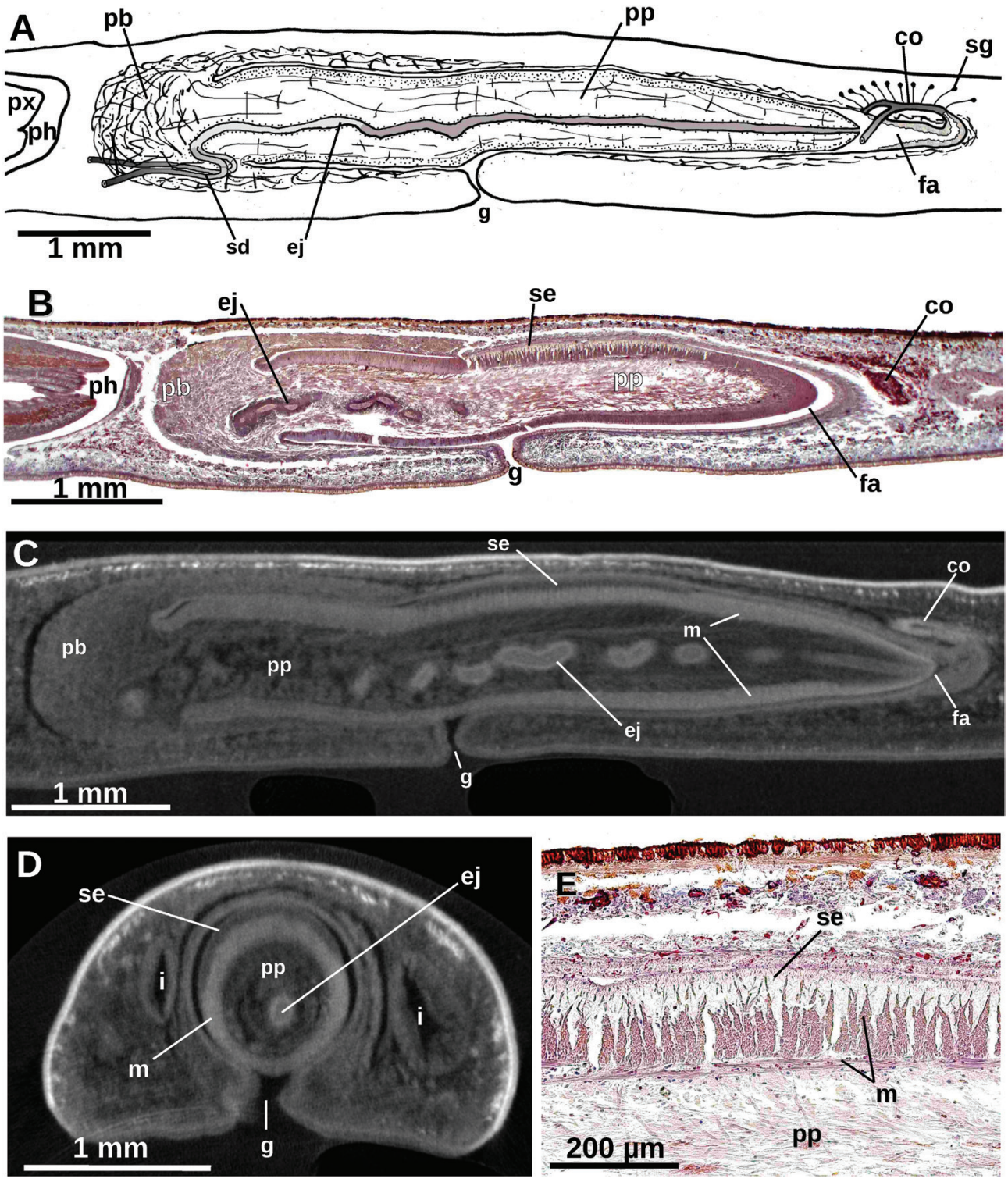

Figure 15. ***Geoplana caraguatatuba*** Almeida & Carbayo **sp. nov.** Specimen MZUSP PL 2080. A, diagrammatic representation of the copulatory apparatus from sagittal sections. B, photomicrograph of a sagittal section of the copulatory apparatus. C, virtual sagittal section of the copulatory apparatus derived from the micro-computed tomography dataset. D, virtual transverse section derived from the micro-computed tomography dataset of the body at the level of the gonopore. E, photomicrograph of dorsal epithelium of penis papilla and cyanophil glands located immediately beneath epithelium in sagittal section.

by 10- to 35-μm-thick layer of circular-to-decussate muscle fibres, followed by a 10- to 65-μm-thick layer of longitudinal fibres.

GEOPLANA IBIUNA ALMEIDA & CARBAYO SP. NOV.

FIG. 16

Material examined

Holotype MZUSP PL 2066 (field number F3562): Parque Estadual do Jurupará, Ibiúna/SP, Brazil. F. Carbayo, coll., February 2009. Cephalic region: horizontal sections on 14 slides; ovarian region: horizontal sections on 12 slides; pre-pharyngeal region: transverse sections on ten slides; pharynx and copulatory apparatus: sagittal sections on 33 slides.
Paratype MZUSP PL 2081 (field number F6907): Santana de Parnaíba/SP, Brazil (23°26′57.2″S, 047°00′43.8″W). F. Carbayo coll., 15 December 2015. Cephalic region: horizontal sections on 12 slides; ovarian region: horizontal sections on six slides; region behind ovaries: horizontal sections on six slides; pre-pharyngeal region: transverse sections on 19 slides; pharynx and copulatory apparatus: sagittal sections on 21 slides.

Paratype MZUSP PL 2082 (field number F6908): Santana de Parnaíba/SP, Brazil (23°26′57.2″S, 047°00′43.8″W). F. Carbayo coll., 12 December 2015. Pharynx and copulatory apparatus: sagittal sections on 11 slides.

Paratype MZUSP PL 2083 (field number F6909): Santana de Parnaíba/SP, Brazil (23°26′57.2″S, 047°00′43.8″W). F. Carbayo coll., 12 December 2015. Pharynx and copulatory apparatus (underdeveloped): sagittal sections on 17 slides.

Paratype MZUSP PL 2088 (field number F6999): Pico do Jaraguá, São Paulo/SP, Brazil (23°27′28.7″S, 046°45′49.9″W). E. M. Froehlich *coll.* 15 August 1951. Ovarian region: horizontal sections on 18 slides; pre-pharyngeal region: transverse sections on 12 slides; copulatory apparatus: sagittal sections on 43 slides.

Distribution

Secondary Atlantic forest in the municipalities of Ibiúna and Santana de Parnaíba, state of São Paulo, Brazil.

Etymology

The specific epithet refers to the Tupi-Guarani (indigenous Brazilian tribe) name of one of the municipalities where the type material was collected.

Diagnosis

Dorsal colour pattern constituted by a median orange-ochre stripe, bounded on either side by a black stripe, externally to which is a white stripe, and this in turn is externally bordered by a black band, externally to which is a thin marginal white line. Dorsal insertion of penis papilla strongly displaced anteriorly and very ventrally. Posterior wall of female atrium richly folded. Female-to-male atrial length ratio, 1.0.

External aspect

Live animals 40–50 mm long and 3 mm wide. Body margins nearly parallel; anterior end rounded, posterior pointed. Dorsum convex, ventral side slightly convex. Dorsal body surface constituted by a median orange-ochre band, varying from one-quarter to one-seventh of body width, that grades into the black of the cephalic region (one-ninth of body length) and posterior end, bounded on either side by a black stripe (one-fifth), which merge posteriorly, externally to which is a white stripe (one-quarter), and this is bordered externally by a black band (one-third), externally to which is a thin marginal white line (Fig. 16A). Ventral surface of the body whitish. In preserved specimens, the dorsal colour remained unchanged. Two types of eyes: a conical type, 100 μm high and 35 μm in width; and a cup-shaped type, 40 μm in diameter. The former contour the anterior end of the body. Cup-shaped-type eyes spread onto the dorsum, reaching whitish stripes all along the body. Sensory pits located ventromarginally in a single row, from the very anterior end up to the end of first quarter of body. Relative position mouth-to-body length, 50–58%. Relative position gonopore-to-body length, 64–71%.

Internal morphology

Creeping sole 82% of body width. Glandular margin absent. Three typical geoplaninid cutaneous muscle layers present, being as thick as 6–8% of body height. Muscle fibres of the longitudinal, innermost layer (30 μm thick) arranged into bundles with 15–25 fibres each. Three parenchymal muscle layers present: dorsal layer of decussate diagonal fibres, supraintestinal layer of transverse and longitudinal fibres, and subintestinal layer with transverse and longitudinal fibres. Longitudinal fibres around intestine constituting a loose tube whose fibres mix up with transverse ones. Ventral nerve plate present.

Mouth situated at a distance from root of the pharynx equivalent to 42–59% of pharyngeal pocket length. Pharynx cylindrical (Fig. 16B). Oesophagus-to-pharynx ratio, 17%. Outer pharyngeal musculature consisting of a subepithelial layer (8 μm) of longitudinal muscle, followed by a layer (85 μm) of intermingled circular and longitudinal muscle fibres. Inner pharynx musculature consisting of a thin (5 μm) subepithelial layer of longitudinal muscle, followed by a layer (75 μm) of circular fibres and an innermost layer (12 μm) of longitudinal fibres (Fig. 16C).

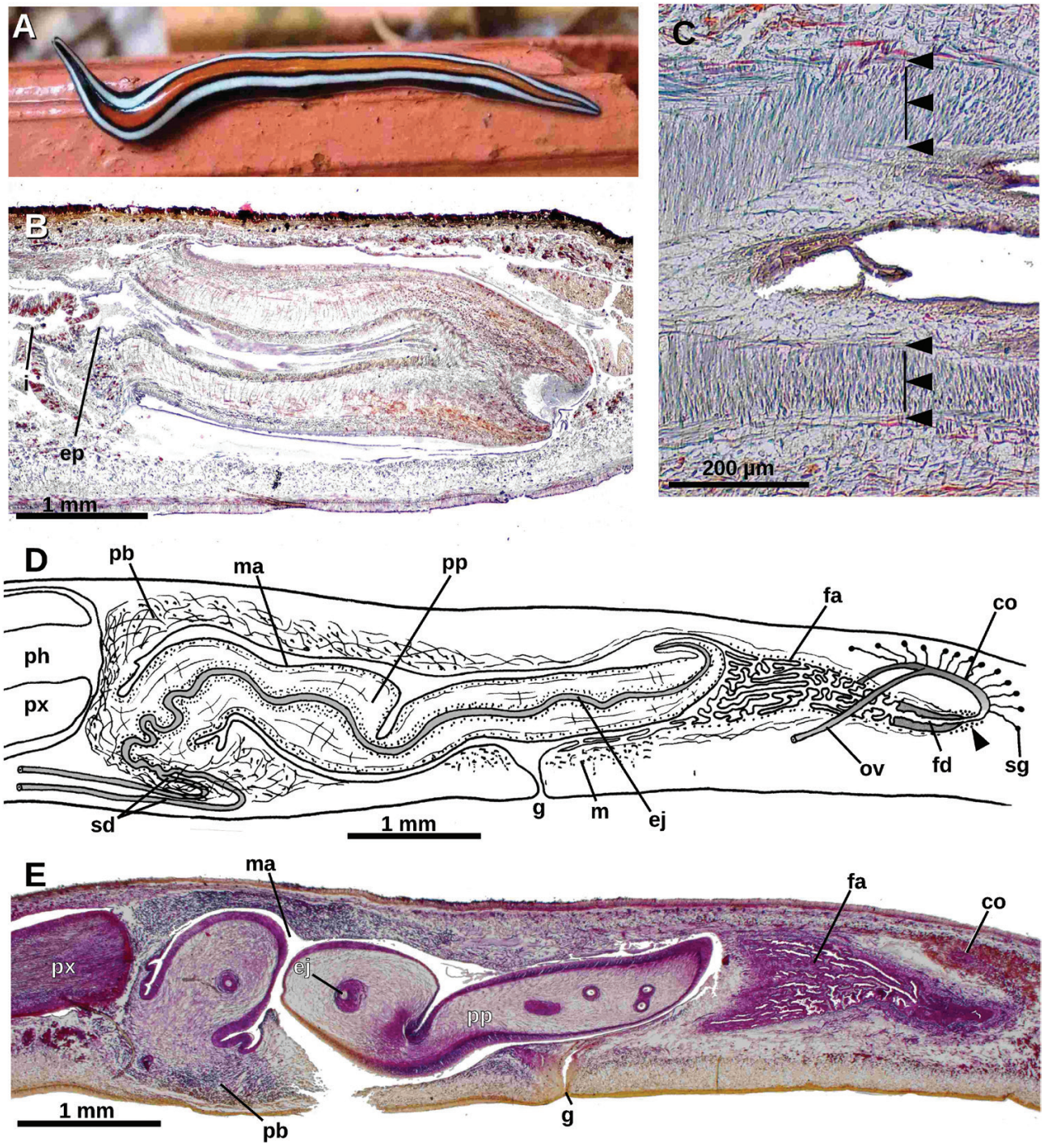

Figure 16. ***Geoplana ibiuna*** Almeida & Carbayo **sp. nov.** A, dorsal view of the living specimen MZUSP PL 2081, 50 mm in length. B, photomicrograph of a sagittal section of the pharynx of specimen MZUSP PL 2081. C, photomicrograph of a sagittal section of the inner pharyngeal epithelium showing its three muscle layers (each indicated with an arrowhead) of specimen MZUSP PL 2066. D, diagrammatic representation of the copulatory apparatus of specimen MZUSP PL 2066 from sagittal sections. Arrowhead points to the sphincter embracing posterior portion of female atrium. E, photomicrograph of a sagittal section of the copulatory apparatus of specimen MZUSP PL 2066.

Testes dorsally located between supraintestinal parenchymal muscle layer and intestine; anteriormost testes behind ovaries, at a distance roughly equivalent to 10% of body length; posteriormost follicles slightly anterior to root of pharynx. Penis bulb elongated, extending anteriad ~0.3 mm anterior to penis papilla; in the dorsal portion of the body, the penis bulb extends posteriorly to the level of the gonopore; in the ventral body region, the penis bulb extends posteriorly only to about halfway between the root of the penis papilla and the gonopore. The bulb is strongly developed, consisting of tightly packed muscle fibres variously orientated. Penis bulb muscle more densely packed dorsally than ventrally (Fig. 16D, E). Bulb fibres on the right side run obliquely downwards to embrace ventrally the anterior portion of the female atrium, subsequently anchoring presumably on ventral epidermis. Sperm ducts run dorsolaterally to the ovovitelline ducts. Posteriorly to level of insertion of penis papilla, sperm ducts bend anteriorly and medially and then penetrate penis bulb ventrolaterally. The ducts subsequently recurve and join to form the ejaculatory duct. Extrapapillar portion of this duct considerably sinuous; traversing penis papilla centrally to open at its tip. Sperm present in distal portion of sperm duct, along its course both outside and inside penis bulb. Sperm ducts lined with a cuboidal, ciliated epithelium and surrounded by a 20-µm-thick layer of circular muscles. Anterior portion of ejaculatory duct lined with squamous-to-cuboidal, ciliated epithelium pierced by numerous gland cells producing erythrophil granules and surrounded by a coat of circular muscle fibres constituting a muscular cylinder with a diameter equivalent to three times the diameter of this duct.

Penis papilla long, horizontal and progressively thinner towards its tip; this tip very thin. Dorsal insertion strongly displaced anteriorly and very ventrally, so that it projects from anteroventral portion of male atrium. Penis papilla completely fills male atrium and a variable part of the female atrium, probably owing to fixation artefacts. Total length of papilla equal to six to seven times its greatest diameter. Penis papilla covered with a cuboidal epithelium, pierced by gland cells producing fine erythrophil granules. Necks of these glands fill a moderate space immediately beneath epithelium of second third of penis papilla. Subepithelial musculature consisting of dense 45-µm-thick layer of circular muscle, followed by a 30-µm-thick layer of longitudinal muscle.

Male atrium ample, not folded (Fig. 16D, E), lined with a low epithelium and underlain by a 5-µm-thick layer of decussate muscles. Circular muscle fibres surround both innermost portion of gonopore canal and nearby atrium.

Ovaries oval shaped, 400 µm in length in longitudinal body axis and situated at a distance from anterior end equivalent to one-fifth of body length. Ovovitelline ducts emerge from dorsolateral aspect of ovaries. The oviducts run laterally to posterior portion of female atrium, then curve medially and join to form common glandular ovovitelline duct. This duct is as long as one-sixth of female atrium length and communicates with female genital duct; the latter is a very narrow projection of the posterior region of female atrium, lined with a 6-µm-tall ciliated epithelium and surrounded by a small but distinct sphincter made up of circular muscle fibres.

Female atrium narrow and long, somewhat funnel shaped; as long as male atrium (Fig. 16D, E). Atrial wall very richly folded, either along anterior half or along almost all posterior half, thus narrowing its lumen. Lining epithelium mostly cuboidal, 12 µm high, posteriormost portion tall, 40 µm and with stratified aspect. Two types of gland cells pierce epithelium, producing cyanophil granules and erythrophil substance, respectively. Female atrium surrounded by a 5-µm-thick layer of longitudinal muscle fibres, which do not occur on the posterior portion, followed by a 40-µm-thick layer of decussate fibres. A loose muscle coat of thick (10 µm) longitudinal fibres envelopes this atrium.

GEOPLANA CANANEIA ALMEIDA & CARBAYO SP. NOV.

FIG. 17

Material examined

All specimens were collected in Parque Estadual da Ilha do Cardoso, Cananeia/SP, Brazil (25°05′28.4″S, 047°55′44.2″W). J. Pedroni, coll., 18 October 2009.

Holotype MZUSP PL 2067 (field number F4096): Cephalic region: horizontal sections on five slides; ovarian region: horizontal sections on 12 slides; prepharyngeal region: transverse section on 12 slides; pharynx: sagittal sections on 17 slides; copulatory apparatus: sagittal sections on 45 slides.

Paratype MZUSP PL 2068 (field number F4097): pharynx: transverse sections on six slides; undeveloped copulatory apparatus: sagittal sections on three slides.

Paratype MZUSP PL 2069 (field number F4098): pharynx: sagittal sections on eight slides; copulatory apparatus: sagittal sections on 13 slides.

Distribution

Area covered with Atlantic forest in Cardoso Island, municipality of Cananeia, state of São Paulo.

Etymology

The specific epithet refers to the Tupi-Guarani (indigenous Brazilian tribe) name of the municipality where the species was collected.

Diagnosis

Dorsal colour pattern constituted by a median yellow orange band, bounded on either side by a black stripe, externally to which is a white stripe, and this in turn is externally bordered by a black stripe, which merges into a grey band with white mottling, which gradually fades towards its outer margin, while this margin is bordered by a thin black stripe. Penis bulb extending anteriorly 1 mm from penis papilla. Stroma of the penis papilla with necks of glands gathered in bundles resembling an orange in cross-section. Wall of posterior half of female atrium richly folded.

External aspect

Adults up to 66 mm long and 5 mm wide. Body margins nearly parallel; anterior end rounded, posterior pointed. Dorsum strongly convex, ventral side flat. Dorsal body surface constituted by a median yellow-orange band (approximately one-quarter of body width) that grades into the black of cephalic region (1/13th of body length), bounded on either side by a black stripe (one-sixth) which join posteriorly, externally to wh ich is a white stripe (one-seventh), and this in turn is bordered externally by a black stripe, which merges into a grey band with white mottling (one-sixth), which gradually fades towards its outer margin, while this margin is bordered by a thin black stripe (Fig. 17A). Ventral surface is whitish with black margins. The dorsal colours of preserved specimens remained unchanged. Two types of eyes: a conical type, in a single row around anterior tip; and a cup-shaped type, spreading onto dorsum, reaching whitish stripes except at the anterior extremity of the body. Sensory pits simple invaginations, 20 µm deep, located ventromarginally in a single row, from the very anterior end up to at least first one-sixth of body length. Relative position mouth-to-body length, 55%. Relative position gonopore-to-body length, 70%.

Internal morphology

Creeping sole 84% of body width. Glandular margin absent. Three typical geoplaninid cutaneous muscle layers present, being as thick as 5–6% of body height. Muscle fibres of the longitudinal, innermost layer (25 µm thick) arranged into bundles with 25–33 fibres each. Three parenchymal muscle layers present: dorsal layer of decussate diagonal fibres, supraintestinal layer of transverse and longitudinal fibres, and subintestinal layer with transverse and longitudinal fibres. Longitudinal fibres around intestine are present, constituting a loose tube whose fibres mix with transverse ones. Ventral nerve plate present.

Mouth situated at a distance from root of the pharynx equivalent to 29% of pharyngeal pocket length. Pharynx cylindrical, with dorsal insertion slightly posterior to ventral one (Fig. 17B). Oesophagus-to-pharynx ratio, 12%. Outer pharyngeal musculature consisting of a subepithelial layer (5 µm) of longitudinal muscle, followed by a layer (50 µm) of circular muscle fibres with longitudinal ones in its innermost region. Inner pharynx musculature consisting of a thin subepithelial layer of longitudinal muscle, followed by a layer (50 µm) of circular fibres and an innermost layer (15 µm) of longitudinal fibres.

Testes dorsally located between supraintestinal parenchymal muscle layer and intestine; anteriormost testes slightly behind ovarian region (equivalent to 20% of body length), posteriormost follicles slightly anterior to the root of the pharynx. Penis bulb elongated, extending from 1 mm anterior to penis papilla to the level of the gonopore region; well developed, consisting of tightly packed muscle fibres variously orientated. Diagonal fibres on the right side of the bulb run obliquely downwards to embrace ventrally the anterior portion of the female atrium. These fibres apparently mix with the subintestinal parenchymal musculature. Sperm ducts run dorsolaterally to the ovovitelline ducts. Laterally to ventral insertion of penis papilla, sperm ducts bend anteriorly and medially and then ventrolaterally to penetrate the bulb. The ducts subsequently recurve, then join to form the ejaculatory duct. This wide canal (Fig. 17C) initially curves dorsoposteriorly, then enters penis papilla and traverses it sinuously to open at its distal tip. Sperm present in distal portion of sperm ducts, along their course both outside and inside penis bulb. Ejaculatory duct lined with columnar, ciliated epithelium, 25–40 µm high in anterior portion, 40–50 µm high inside the papilla, and surrounded by a 40-µm-thick layer of circular muscle fibres, which constitutes a muscular cylinder with a diamter equivalent to 1.5 times the diameter of this duct. Extrapapillar and anterior portion of ejaculatory duct pierced by numerous gland cells producing fine erythrophil granules; from that portion until nearly the tip of papilla the duct is pierced by cells producing coarse erythrophil granules.

Penis papilla long, horizontal and cylindrical, with dorsal insertion slightly anterior to the ventral part; tip blunt. The papilla occupies the male atrium and anterior two-fifths of the female atrium. Total length of papilla equal to six times its greatest diameter. It is covered with a squamous epithelium, which is pierced by two types of very abundant gland cells, producing fine erythrophil and cyanophil granules, respectively, with the former being more abundant. Necks of these two types of glands progressively gather in larger and less numerous bundles; in the distal portion of the papilla, erythrophil necks are gathered in nine to 14 bundles, which are separated from each other by cyanophil glands, thus giving rise to a picture resembling an orange in cross-section

(Fig. 17D, E). Subepithelial musculature consisting of dense 50-µm-thick layer of circular muscle, slightly decussate, followed by a 20-µm-thick layer of longitudinal muscle.

Male atrium ample, not folded (Fig. 17E), lined with a squamous epithelium pierced by glands secreting an amorphous erythrophil substance, and underlain by a 5-µm-thick layer of circular muscles, followed by a thin layer of longitudinal fibres. Circular muscle fibres surround both innermost portions of gonopore canal and nearby atrium.

Ovaries oval shaped, 500 µm in length in the longitudinal axis, and situated at a distance from anterior end equivalent to one-fifth of body length. Ovovitelline ducts emerge from dorsolateral wall of ovaries. Laterally to hind region of female atrium, the ducts curve medially and join to form common glandular ovovitelline duct. This duct is as long as one-sixth of female atrium length; it first bends posteroventrally, then curves anteriorly to open into female genital duct; the latter is a projection of the posterior region of the female atrium and lined with a 12-µm-high columnar, ciliated epithelium.

Female atrium long, twice as long as male atrium (Fig. 17E); anterior half spacious and lined with cuboidal epithelium; hind half has richly folded wall projected into its own lumen consisting of 25-µm-high, stratified-like epithelium. Two types of gland pierce female epithelium, producing erythrophil and cyanophil granules, respectively, the second being more abundant in posterior half.

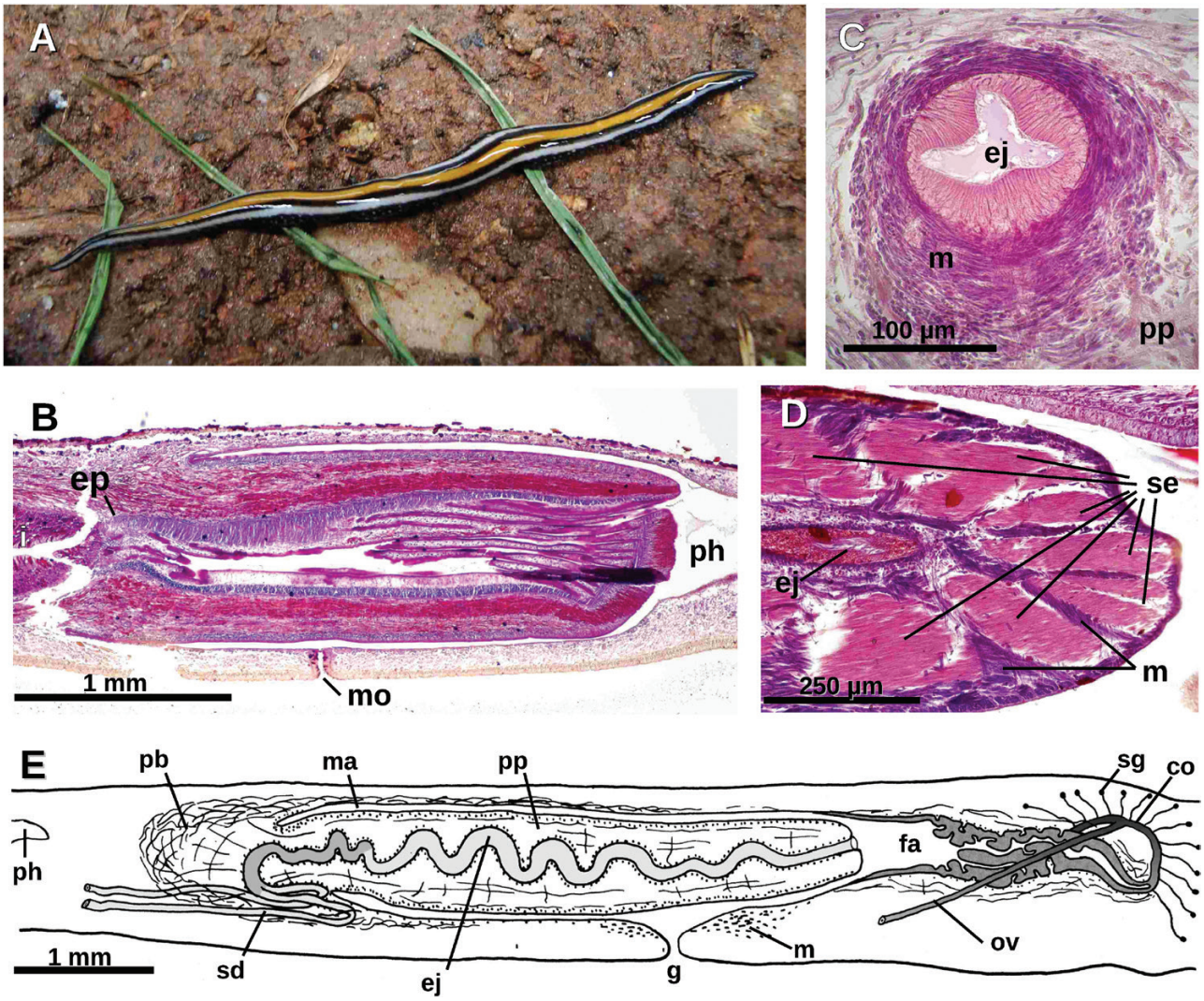

Figure 17. ***Geoplana cananeia*** Almeida & Carbayo **sp. nov.** Specimen MZUSP PL 2067. A, dorsal view of the living specimen, ~65 mm in length. B, photomicrograph of a sagittal section of the pharynx. C, photomicrograph of a transverse section of ejaculatory duct. D, photomicrograph of an oblique section of the distal portion of the penis papilla; note the gland necks gathered into dense bundles. E, diagrammatic representation of the copulatory apparatus from sagittal sections.

Atrium surrounded by 25- to 40-µm-thick subepithelial layer of circular muscle fibres, followed by a layer of longitudinal fibres, partly interwoven with the circular ones.

GEOPLANA CAMBARA ALMEIDA & CARBAYO SP. NOV.

FIGS 18 AND 19

Material examined

All specimens were collected in Parque Nacional de Saint-Hilaire/Lange, Matinhos/PR, Brazil.

Holotype MZUSP PL 2062 (field number F1611): F. Carbayo *et al*., coll., 10 January 2008. Cephalic region: transverse sections on 21 slides; ovarian region: horizontal sections on 13 slides; pharynx: transverse sections on 18 slides; copulatory apparatus: sagittal sections on 15 slides.

Paratype MZUSP PL 2063 (field number F1635) (immature): F. Carbayo *et al*., coll., 11 January 2008. Cephalic region: horizontal sections on nine slides; pharynx: sagittal sections on five slides; portion behind pharynx: sagittal sections on four slides.

Paratype MZUSP PL 1009 (field number F1614): F. Carbayo *et al*., coll., 1 January 2008 (25°45′51.7″S, 048°37′21.6″W). Preserved in absolute ethanol.

Distribution

Areas covered with Atlantic forest in Parque Nacional de Saint-Hilaire/Lange, Matinhos, state of Paraná, Brazil.

Etymology

The specific epithet refers to the Tupi-Guarani (indigenous Brazilian tribe) name of the river flowing nearby the sampling site.

Diagnosis

Dorsal colour pattern constituted by a median orange stripe, bounded on either side by a thin black stripe, externally to which is a broader white stripe, and this in turn is externally bordered by a black line, externally to which is a grey band with dense white mottling, externally to which is a marginal black line. Longitudinal parenchymal muscle fibres disposed around intestine as a loose muscular tube. Diameter of muscular cylinder surrounding ejaculatory duct equivalent to 12 times the diameter of this duct.

External aspect

Live animals 43 mm long and 3.5 mm wide. Body elongated, margins nearly parallel; anterior end rounded, posterior pointed. Dorsum strongly convex, ventral side flat. Dorsal colour constituted by a median orange band, bounded on either side by a thin black stripe (one-third of body width) that grades into the black of the cephalic region (1/12th of body length), externally to which is a broader white stripe (one-sixth), and this in turn is externally bordered by a black line, externally to which is an area (one-sixth) coloured with a grey band with dense white mottling, externally to which is a marginal black line (Fig. 18A). Ventral body surface cream, with black margins. The dorsal colours of preserved specimens remained unchanged. Two types of eyes: a conical type, around anterior tip; and a cup-shaped type, which are located from a short distance behind anterior tip to posterior end, extending dorsally onto whitish stripes. Sensory pits simple invaginations, located ventromarginally in a single row from the very anterior end up to at least a distance equivalent to 13% of body length. Relative position mouth-to-body length, 50%. Relative position gonopore-to-body length, 64%.

Internal morphology

Creeping sole 80% of body width. Glandular margin absent. Three typical geoplaninid cutaneous muscle layers present, being as thick as 6% of body height. Muscle fibres of the longitudinal, innermost layer (6–12 µm thick) arranged into bundles with seven to 20 fibres each. Three parenchymal muscle layers present: dorsal layer of decussate diagonal fibres, supraintestinal layer of transverse and longitudinal fibres, and subintestinal layer with transverse and longitudinal fibres. Longitudinal fibres around intestine present, constituting a loose tube of fibres mixed up with transverse ones. Ventral nerve plate present.

Mouth situated at a distance from root of the pharynx equivalent to 47% of pharyngeal pocket length. Pharynx cylindrical (Fig. 18B). Oesophagus-to-pharynx ratio, 17%. Outer pharyngeal musculature consisting of a subepithelial layer (5 µm) of longitudinal muscle, followed by a layer (65 µm) of intermingled circular and longitudinal muscle fibres. Inner pharynx musculature (Fig. 18C) consisting of a thin subepithelial layer of longitudinal muscle, followed by a layer (45 µm) of circular fibres and an innermost layer (10 µm) of longitudinal fibres.

Testes dorsally located between supraintestinal parenchymal muscle layer and intestine; anteriormost testes slightly anterior to ovarian region, posteriormost follicles slightly anterior to root of pharynx. Penis bulb elongated, extending from 1.2 mm anterior to penis papilla to behind level of gonopore; very strongly developed dorsally to male atrium. It consists of tightly packed muscle fibres variously orientated anteriorly. Diagonal fibres on the right side of the bulb run obliquely downwards to embrace ventrally the anterior portion of the female atrium. Those fibres seem to cross the nerve plate and to

anchor on ventral epidermis. Sperm ducts run dorsolaterally to the ovovitelline ducts. Laterally to ventral insertion of penis papilla, sperm ducts bend anteriorly and medially and then ventrolaterally to penetrate the bulb. The ducts subsequently join near root of penis papilla to form the ejaculatory duct. This duct traverses the central region of penis papilla to open at its tip (Fig. 18D, E). Sperm present in distal portion of sperm ducts, along their course both outside and inside penis bulb. Sperm ducts lined with a cuboidal, ciliated epithelium, pierced by glands producing fine weakly erythrophil granules; sperm ducts and anterior portion of ejaculatory duct surrounded by a circular muscle. Distal portion of ejaculatory duct surrounded by a layer of circular-to-decussate muscles. Thickness of muscles surrounding the ejaculatory duct increases from 50 µm proximally to a maximum of 150 µm in mid-papilla, and constituting a muscular cylinder with a diameter equivalent to 12 times the diameter of this duct.

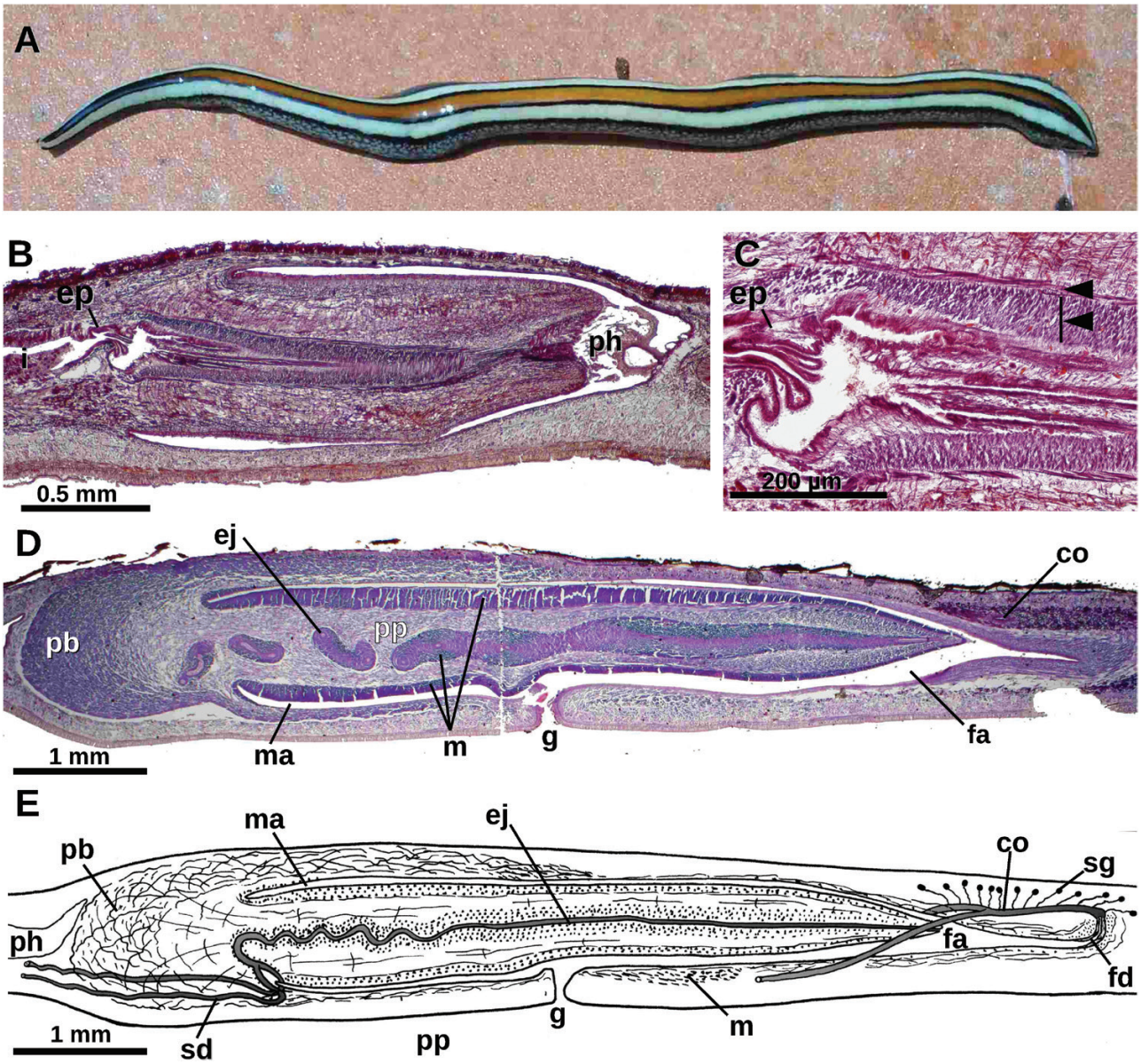

Figure 18. ***Geoplana cambara*** Almeida & Carbayo **sp. nov.** A, dorsal view of the living specimen MZUSP PL 2062, ~43 mm in length. B, photomicrograph of a sagittal section of the pharynx of specimen MZUSP PL 2063. C, photomicrograph of a sagittal section of the inner pharyngeal epithelium showing its two muscle layers (each indicated with an arrowhead) of specimen MZUSP PL 2063. D, photomicrograph of a sagittal section of the copulatory apparatus of specimen MZUSP PL 2062. D, diagrammatic representation of the copulatory apparatus of specimen MZUSP PL 2062.

Penis papilla long, horizontal and cylindrical, with dorsal and ventral insertions at the same transverse plane; tip pointed (Fig. 18D, E). It occupies the entire male atrium and most of the female atrium. Total length of papilla equal to seven times its greatest diameter. Papilla covered with a cuboidal epithelium, pierced by two types of gland cells, producing erythrophil and cyanophil granules, respectively. Space immediately beneath mid-dorsal epithelium of papilla packed by necks of latter type of gland cells (Fig. 19A, B). Subepithelial musculature consisting of dense layer of circular muscle, tending to decussate distally, 185 µm thick dorsally, 145 µm ventrally, followed by a 15-µm-thick layer of longitudinal muscle. Distally, abundant decussate fibres in stroma. Male atrium ample, not folded, lined with a squamous epithelium, and underlain by a 5-µm-thick layer of circular muscles, followed by an inconspicuous layer of longitudinal fibres.

Ovaries oval shaped, 425 µm in diameter in longitudinal axis, and situated at a distance from anterior end equivalent to 17% of body length. Ovovitelline ducts emerge from dorsolateral wall of ovaries. Laterally to female atrium, the oviducts curve medially and join to form common glandular ovovitelline duct. Common glandular ovovitelline duct as long as a quarter of female atrium length, after a knee-shaped bend communicating with a 30-µm-long female genital duct lined with cuboidal, ciliated epithelium, the latter opening into the female atrium.

Female atrium long, not folded (Figs 18D, 19C, D) and as long as two times the male atrium. Posterior, curved section lined with 20-µm-tall stratified-like, non-ciliated epithelium and surrounded by a 30-µm-thick mass of very thin, seemingly circular muscle fibres, mixed with apparently minute erythrophil granules (Fig. 19D). Anterior section of female atrium lined with cuboidal epithelium and surrounded by a

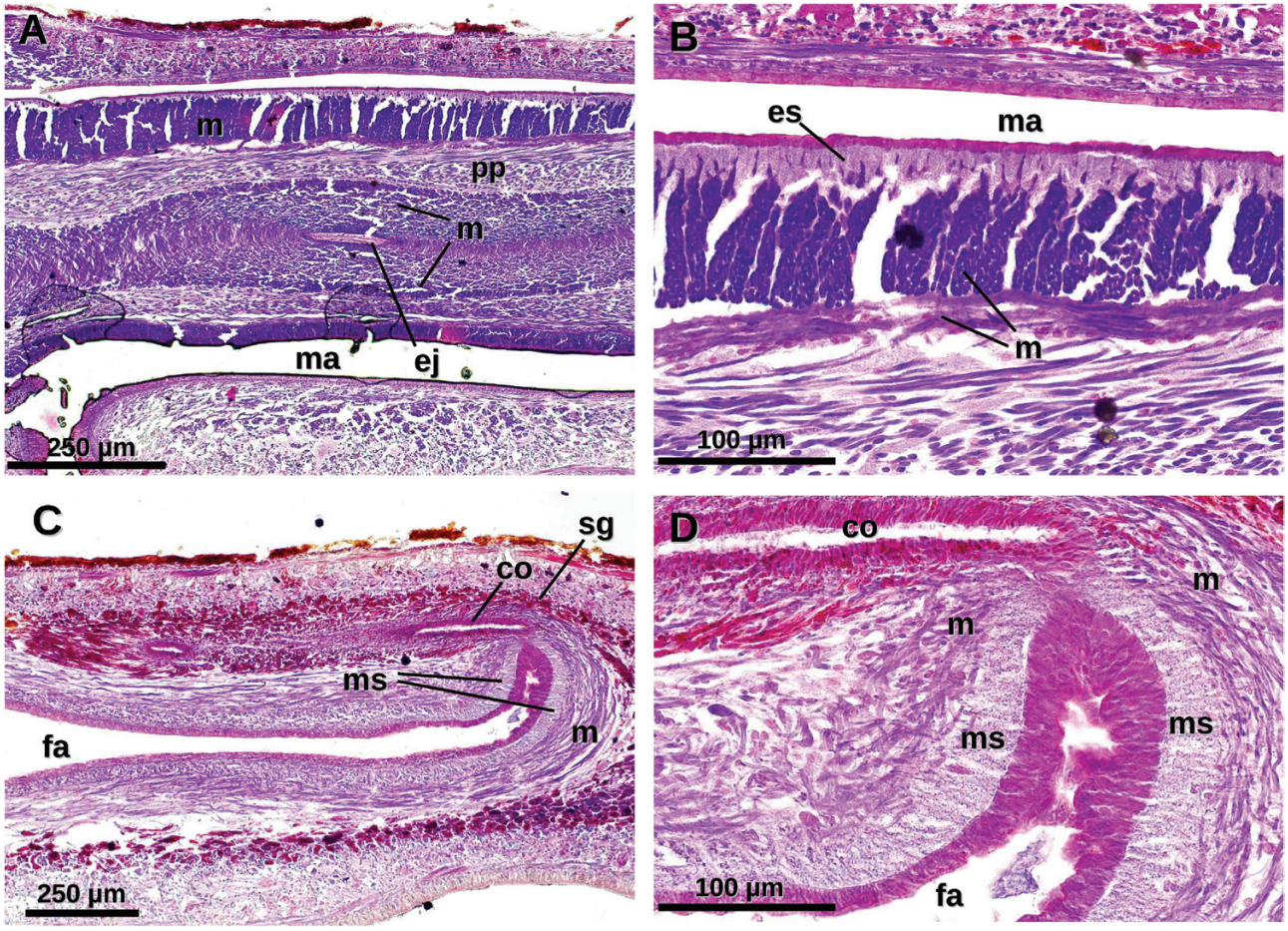

Figure 19. ***Geoplana cambara*** Almeida & Carbayo **sp. nov.** Photomicrographs of sagittal sections of specimen MZUSP PL 2062. A, mid-portion of penis papilla, with the ejaculatory duct and its surrounding muscle. B, dorsal epithelium of penis papilla and cyanophil glands located immediately beneath epithelium. C, female atrium. D, female genital duct; note layer of very thin muscle fibres surrounding the duct.

10- to 40-μm-thick layer of circular and longitudinal muscle fibres. A 10- to 110-μm-thick layer of longitudinal muscles embraces entire female atrium.

GEOPLANA IPORANGA ALMEIDA & CARBAYO SP. NOV.

FIGS 20 AND 21

Material examined

All specimens were collected in Parque Estadual de Intervales, Ribeirão Grande/SP, Brazil (24°16′09.8″S, 048°24′19.7″W). F. Carbayo *et al.*, coll., 13 December 2008.

Holotype MZUSP PL 2065 (field number F3166): Ovarian region: sagittal sections on 17 slides; prepharyngeal region: transverse sections on seven slides; pharynx: sagittal sections on 11 slides; male genital atrium: sagittal sections on 21 slides; female genital atrium: sagittal sections on nine slides.

Paratype MZUSP PL 2064 (field number F3132) (immature): Cephalic region: horizontal sections on three slides; pharynx and copulatory apparatus: sagittal sections on 12 slides.

Distribution

Areas covered with Atlantic forest in Parque Estadual de Intervales, Ribeirão Grande, state of São Paulo, Brazil.

Etymology

The specific epithet is the name of another locality within the conservation area from where the species was collected.

Diagnosis

Dorsal colour pattern constituted by a median melon yellow band, bounded on either side by a black band, externally to which is a white band divided longitudinally in half by a black line. Penis bulb extending anteriorly 2 mm from penis papilla. Sperm ducts join inside penis papilla. Diameter of muscular cylinder around ejaculatory duct equivalent to ten times the diameter of this duct.

External aspect

Live animals ~50 mm long and 4 mm wide. Body margins nearly parallel; anterior end rounded, posterior pointed. Dorsum and ventral side slightly convex. Dorsal body surface constituted by a median melon yellow band (one-fifth of body width) that grades into black of cephalic region (one-tenth of body length), bounded on either side by a black band (one-quarter), externally to which is a white band (one-fifth) that is longitudinally divided into half by a black line fading towards rear end (Fig. 20A). Ventral surface white, with blackish margins. The dorsal colours of preserved specimens became slightly paler. Two types of eyes: a conical type, in a single row around anterior tip; and a cup-shaped type, spreading onto dorsum and reaching the black bands from immediately behind anterior end to the rear end. Sensory pits simple invaginations, 45 μm deep, located ventromarginally in a single row, from the very anterior end up to at least first one-quarter of body length. Relative position mouth-to-body length, 52%. Relative position gonopore-to-body length, 67%.

Internal morphology

Creeping sole 81% of body width. Glandular margin absent. Three typical geoplaninid cutaneous muscle layers present, being as thick as 5% of body height. Muscle fibres of the longitudinal, innermost layer (25–30 μm thick) arranged into bundles with 20–40 fibres each. Three parenchymal muscle layers present: dorsal layer of decussate diagonal fibres, supraintestinal layer of transverse and longitudinal fibres, and subintestinal layer with transverse and longitudinal fibres. Longitudinal fibres around intestine constituting a loose tube whose fibres mix with transverse ones. Ventral nerve plate present.

Mouth situated at a distance from root of the pharynx equivalent to 37–39% of pharyngeal pocket length. Pharynx cylindrical, dorsal insertion slightly posterior. Oesophagus-to-pharynx ratio, 12% (Fig. 20B). Outer pharyngeal musculature consisting of a subepithelial layer (7 μm) of longitudinal muscle, followed by a layer (55 μm) of intermingled circular and longitudinal muscle fibres. Inner pharynx musculature consisting of a thin subepithelial layer of longitudinal muscle, followed by a layer (110 μm) of circular fibres and an innermost layer (40 μm) of longitudinal fibres.

Testes located dorsally between supraintestinal parenchymal muscle layer and intestine; anteriormost testes at a distance from anterior end equivalent to 22% of body length; posteriormost follicles slightly anterior to root of pharynx. Penis bulb elongated, extending from 2 mm anterior to penis papilla to the level of the gonopore region; very strongly developed, mainly dorsally to male atrium. Penis bulb consists of tightly packed muscle fibres; fibres variously orientated proximally, and diagonally orientated distally. Distal fibres on the right side run obliquely downwards to embrace ventrally the anterior portion of the female atrium; these fibres seem to join fibres of the subintestinal parenchymal muscle layer, thus forming bundles; their anchor points could not be discerned.

Sperm ducts run dorsally to the ovovitelline ducts. Shortly before attaining the level of penial insertion, sperm ducts bend medially and ventrolaterally penetrate the bulb. The ducts subsequently run posteriorly, penetrate penis papilla and join to form the ejaculatory duct; this traverses the central region of penis papilla

to open, presumably, at its tip, but this tip is missing. Sperm present in distal portion of sperm ducts, along their course both outside and inside penis bulb and penis papilla. Sperm ducts lined with a cuboidal, ciliated epithelium and surrounded by a 10-µm-thick layer of circular muscles. Ejaculatory duct lined with cuboidal, ciliated epithelium containing fine cyanophil granules, and surrounded by layer of circular muscles, tending to decussate towards tip of penis; layer with a maximal thickness of 140 µm on mid papilla, and constituting a muscular cylinder with a diameter equivalent to ten times the diameter of this duct (Fig. 20C).

Penis papilla horizontal and cylindrical along most of its length, with dorsal and ventral insertions at the same transverse plane (Fig. 21A, B). Papilla occupies entire male atrium and most of female atrium. Total length of papilla equal to six times its greatest diameter. Penis papilla covered with a low epithelium pierced by two types of gland cells, producing erythrophil and cyanophil granules, respectively. A moderate space, 10 µm thick, immediately beneath mid-dorsal epithelium of papilla packed by necks of latter gland type (Fig. 20C). Subepithelial musculature consisting of dense layer of circular muscle, tending to decussate distally, 200 µm thick dorsally, 100 µm thick ventrally, followed by a 10-µm-thick layer of longitudinal muscle. Stroma rich in diagonal fibres, especially distally. Male atrium ample, not folded, lined with a cuboidal epithelium, underlain by a 12-µm-thick layer of circular muscles, followed by a thin layer of longitudinal fibres.

Ovaries oval shaped, ~200 µm in anteroposterior diameter, situated at a distance from anterior end equivalent to one-quarter of body length. Laterally to female atrium, the oviducts curve medially and join to form the common glandular ovovitelline duct. Common glandular ovovitelline duct as long as one-third of female atrium length, and communicating with posterior portion of female atrium, the latter being 200 µm long and curved towards dorsal body surface.

Female atrium without folds, as long as three times that of male atrium (Fig. 21B). The very short posterior portion (200 µm in length) of the female atrium is lined with a 25-µm-high stratified-like, non-ciliated epithelium, which is surrounded by a 40-µm-thick layer of very thin muscle fibres. Otherwise, the female atrium is lined with cuboidal epithelium and pierced by gland cells producing cyanophil granules. Epithelium of female atrium and that of female genital duct are

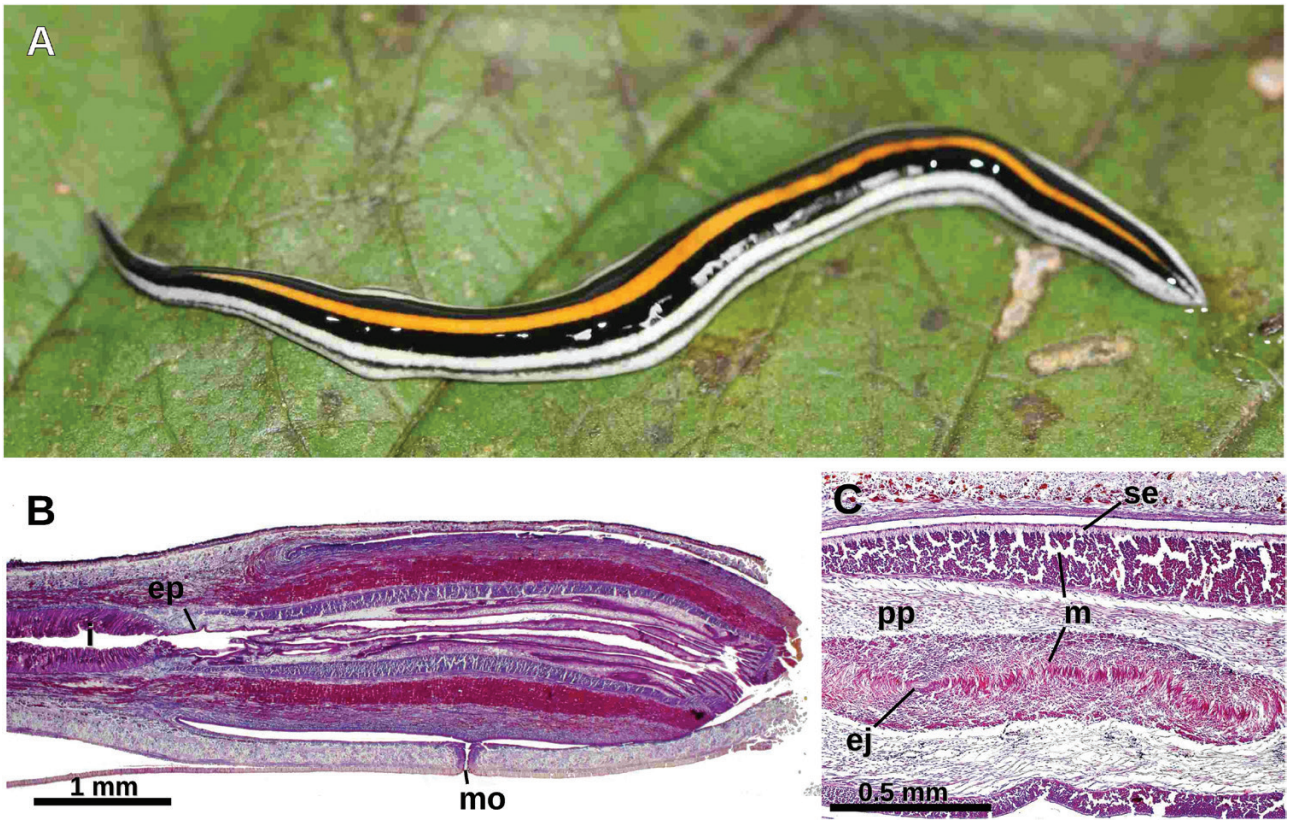

Figure 20. ***Geoplana iporanga*** Almeida & Carbayo **sp. nov.** Specimen MZUSP PL 2065. A, dorsal view of the live specimen, ~53 mm in length. B, photomicrograph of a sagittal section of the pharynx. C, mid-portion of penis papilla, with the ejaculatory duct and its surrounding muscle.

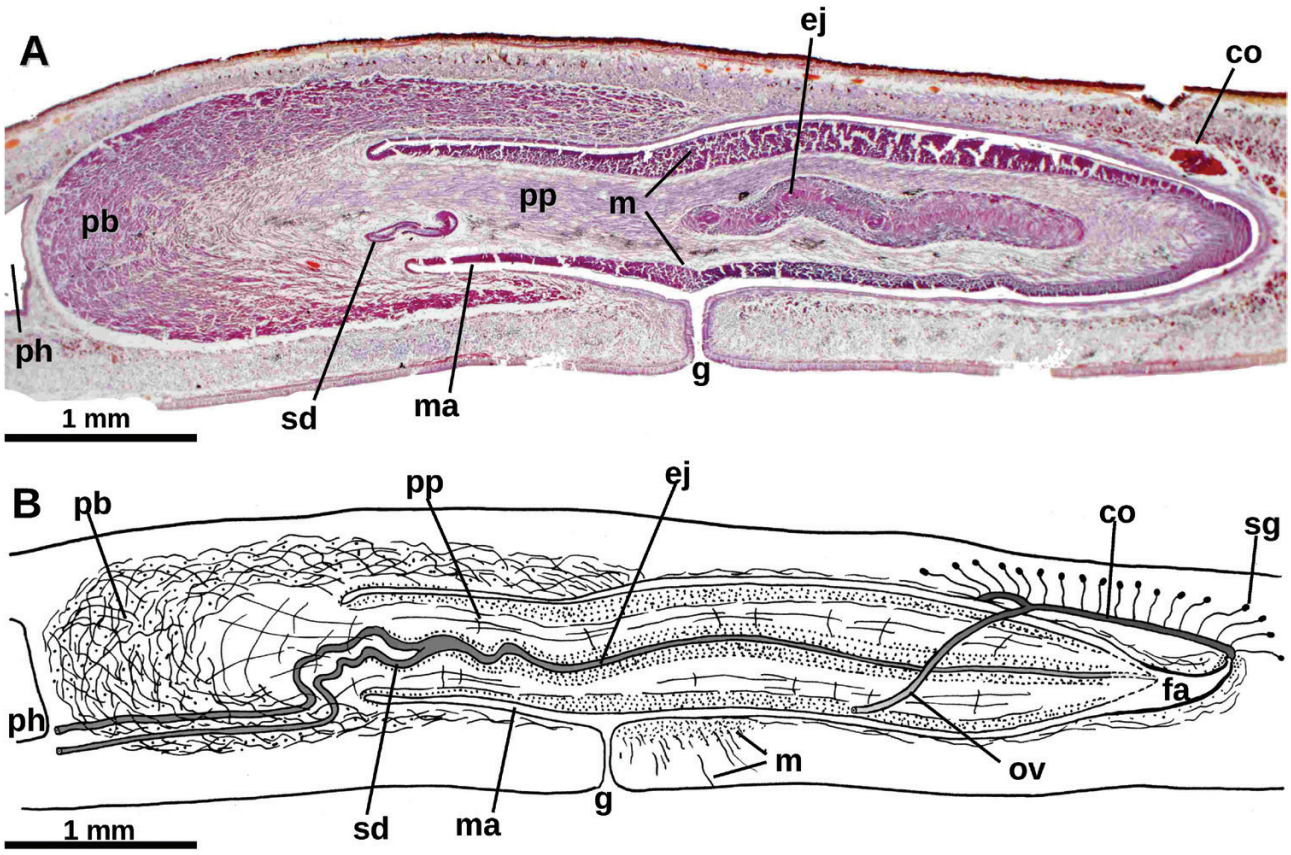

Figure 21. ***Geoplana iporanga*** Almeida & Carbayo **sp. nov.** Sagittal views of copulatory apparatus of specimen MZUSP PL 2065. A, microphotograph. B, diagrammatic representation.

surrounded by 5- to 20-µm-thick layer of circular-to-decussate muscle fibres, followed by a 5- to 55-µm-thick layer of longitudinal fibres.

GEOPLANA PULCHELLA SCHULTZE & MÜLLER, 1857

FIGS 22 AND 23

Geoplana pulchella Schultze & Müller, 1857: p. 25; Froehlich, 1955b, p. 189–190; du Bois-Reymond Marcus, 1951, p. 234–235 (misidentification).

Material examined

MZUSP PL 1068 (field number F3249): Parque Estadual da Serra do Tabuleiro, Paulo Lopes/SC, Brazil (27°58′53.0″S, 048°44′53.0″W). F. Carbayo, *et al*., coll., 14 January 2009. Cephalic region: transverse sections on 11 slides; ovarian region: horizontal sections on six slides; region behind ovaries: sagittal section on nine slides; pre-pharyngeal region: sagittal sections on ten slides; pharynx: sagittal sections on 11 slides; copulatory apparatus: sagittal sections on 11 slides.

MZUSP PL 2075 (field number F6528): RPPN Vale das Pedras, Alfredo Wagner/SC, Brazil (27°37′01.0″S, 049°20′52.0″W). A. L. Almeida *et al*., coll., 16 January 2015. Cephalic region: horizontal sections on four slides; ovarian region: horizontal sections on five slides; pre-pharyngeal region: transverse sections on four slides; pharynx: sagittal sections on four slides; copulatory apparatus: sagittal sections on nine slides.

MZUSP PL 2076 (field number F6544): RPPN Vale das Pedras, Alfredo Wagner/SC, Brazil (27°37′01.1″S, 049°20′53.3″W). A. L. Almeida *et al*., coll., 18 January 2015. Cephalic region: horizontal sections on five slides; region behind ovaries: horizontal sections on four slides; pre-pharyngeal region: transverse sections on seven slides; pharynx: sagittal sections on five slides; copulatory apparatus: sagittal sections on seven slides.

Note

We could not confirm identification of a specimen studied by Froehlich (1955b); see Supporting Information (Table S1).

Distribution

Areas covered with Atlantic forest in municipalities of Blumenau, Paulo Lopes, Alfredo Wagner, Eastern part of Santa Catarina state, South Brazil.

Diagnosis

Anterior third of dorsal body pure orange; remaining dorsal side constituted by a median light grey band, bordered on either side by a black band; entire dorsum sprinkled with conspicuous whitish specks. Penis bulb extending anteriorly 1 mm from penis papilla. Papilla as long as seven to nine times its diameter and with a very thin tip. A conspicuous mass of very thin circular muscle fibres around posterior section of female atrium.

External aspect

Live animal 30 mm long and 3 mm wide when extended. Body elongated, margins nearly parallel; anterior end rounded, posterior pointed. Dorsum strongly convex, ventral side slightly convex. Anterior third of dorsal body surface pure orange; remaining two-thirds with a median light grey wide band (one-third), bordered on either side by a black band (one-third), with the exception of the very anterior end; entire dorsum sprinkled with conspicuous whitish specks (Fig. 22A). Anterior third of ventral body surface traffic white; rest of ventral surface squirrel grey. The dorsal colours of preserved specimens became slightly paler. Two types of eyes: a conical type, 80 µm high and 35 µm wide; and a cup-shaped type, 35 µm in diameter. The former contour the anterior third of the body. Cup-shaped type eyes in lateral bands, approximately one-third of body width. Sensory pits simple invaginations, 30 µm deep, located ventromarginally in a single row, from the very

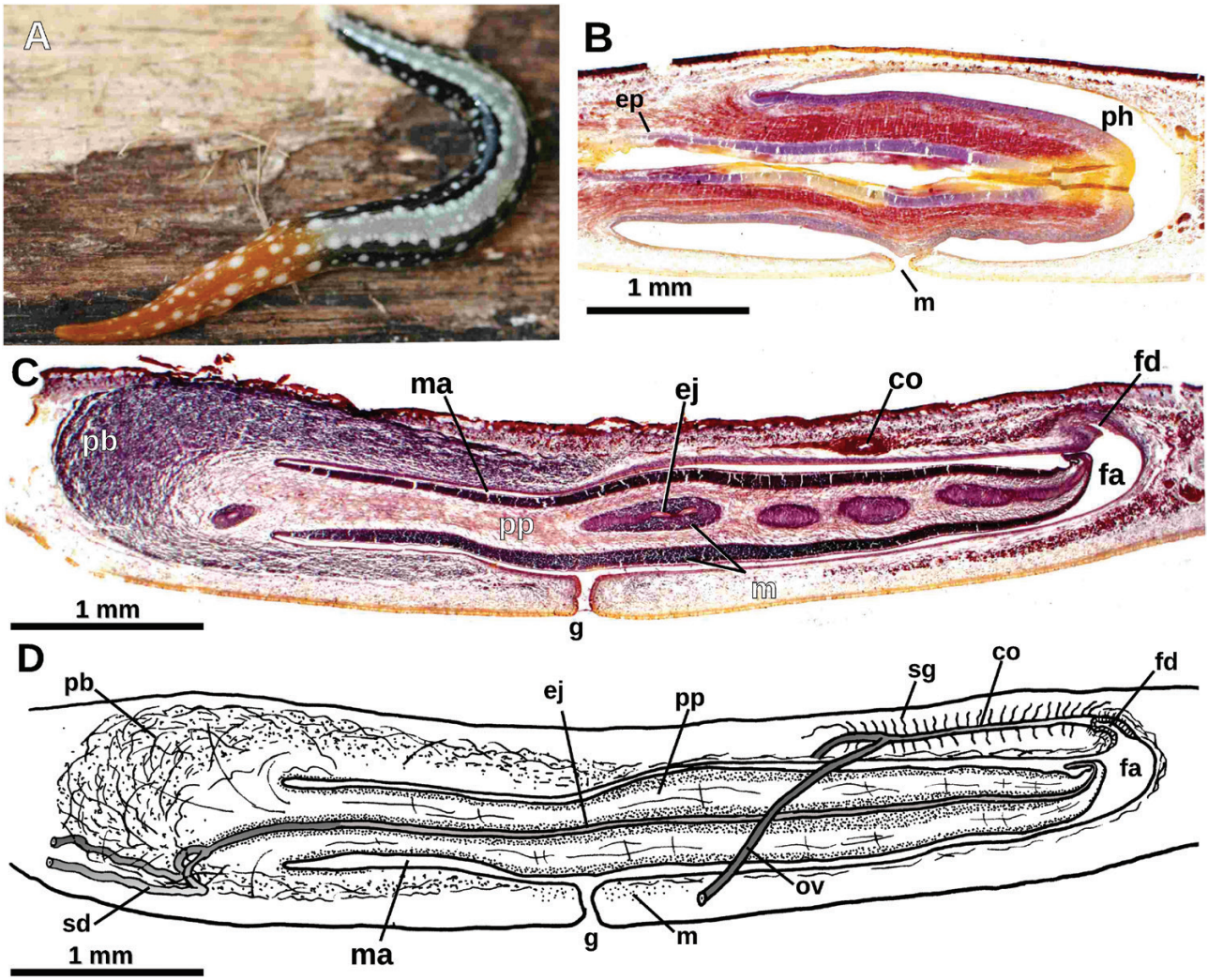

Figure 22. *Geoplana pulchella* Schultze & Müller, 1857. A, dorsal view of the living specimen MZUSP PL 1068, 38 mm in length. B, photomicrograph of a sagittal section of the pharynx of specimen MZUSP PL 1068. C, photomicrograph of a sagittal section of the copulatory apparatus of specimen MZUSP PL 2076. D, diagrammatic representation of the copulatory apparatus from sagittal sections of specimen MZUSP PL 2076.

anterior end up to the pre-pharyngeal region. Relative position mouth-to-body length, 53%. Relative position gonopore-to-body length, 67%.

Internal morphology

Creeping sole 85% of body width. Glandular margin absent. Three typical geoplaninid cutaneous muscle layers present, being as thick as 5% of body height. Muscle fibres of the longitudinal, innermost layer (20 µm thick) arranged into bundles with 17–21 fibres each. Three parenchymal muscle layers present: dorsal layer of decussate diagonal fibres, supraintestinal layer of transverse and longitudinal fibres, and a subintestinal layer with transverse and longitudinal fibres. Ventral nerve plate present.

Mouth situated at a distance from root of the pharynx equivalent to 51% of pharyngeal pocket length. Pharynx cylindrical. Oesophagus-to-pharynx ratio, 22% (Fig. 22B). Outer pharyngeal musculature consisting of a subepithelial layer (5 µm) of longitudinal muscle, followed by a layer (50 µm) of intermingled circular and longitudinal muscle fibres. Inner pharynx musculature consisting of a thin subepithelial layer of longitudinal muscle, followed by a layer (65 µm) of circular fibres and an innermost layer (20 µm) of longitudinal fibres.

Testes dorsally located between supraintestinal parenchymal muscle layer and intestine; anteriormost testes at a distance from anterior end equivalent to 27% of body length; posteriormost follicles a little anterior to the root of the pharynx. Penis bulb elongated, extending from 1 mm anterior to penis papilla to level of gonopore region (Fig. 22C, D); strongly developed, consisting of tightly packed muscle fibres variously orientated proximally, whereas diagonally orientated distally. Penis bulb more developed dorsally to male atrium than ventrally. Oblique muscular ring embraces dorsodistal portion of male atrium and anteroventral portion of female atrium; some fibres detach from the ring and continue downwards to ventral epidermis behind gonopore region (Fig. 23A). Sperm ducts run dorsolaterally to the ovovitelline ducts. Laterally to penis bulb, sperm ducts bend anteriorly and medially and then ventrolaterally penetrate the bulb. The ducts subsequently join to form an unpaired duct that continues as the ejaculatory duct near the root of the penis papilla, and traverses its central region to open at its tip. Sperm present in distal portion of sperm ducts, along their course both outside and inside penis bulb. Sperm ducts lined with a cuboidal, ciliated epithelium and surrounded by a 7- to 10-µm-thick layer of circular muscles. Ejaculatory duct lined with tall, ciliated epithelium, pierced by conspicuous and numerous gland cells producing erythrophil granules. It is surrounded by a layer of circular muscles, tending to decussate towards penis tip; the layer has maximal thickness of 75 µm in the posterior half of the papilla, constituting a muscular cylinder with a diameter equivalent to four times the diameter of this duct.

Penis papilla long, horizontal and cylindrical along most of its length, with dorsal and ventral insertions at the same transverse plane; the very tip of this papilla is thin and long. This papilla occupies the entire male atrium and most of the female atrium. Total length of papilla equal to seven to nine times its greatest diameter. The papilla is covered with a cuboidal, non-ciliated epithelium, pierced by gland cells producing erythrophil

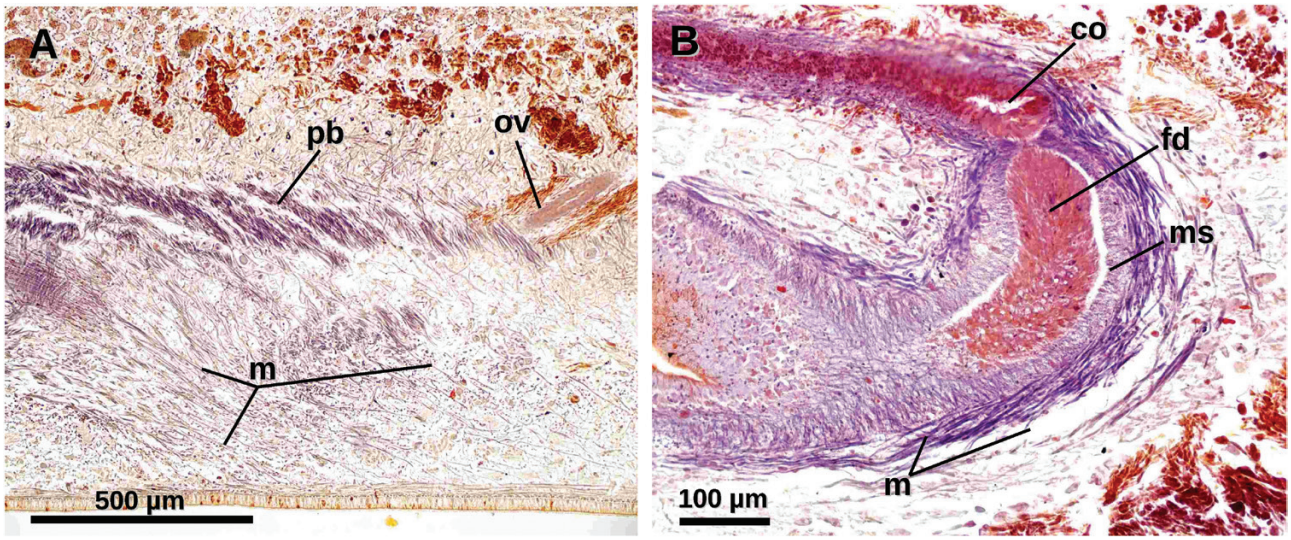

Figure 23. *Geoplana pulchella* Schultze & Müller, 1857. Photomicrographs of sagittal sections of specimen MZUSP PL 1068. A, fibres seemingly derived from penis bulb running downwards to ventral epidermis behind gonopore region. B, female genital duct and surrounding layer of very thin muscle fibres.

granules; glands progressively more densely distributed towards the distal portion of the penis papilla. Subepithelial musculature consisting of dense 90-µm-thick layer of circular muscle, tending to decussate distally, followed by a single layer of longitudinal muscle. Stroma of papilla traversed by numerous diagonal muscle fibres, especially distally. Male atrium ample, not folded; lined with a low, non-ciliated epithelium, and underlain by a 5-µm-thick layer of circular muscles, followed by an inconspicuous layer of longitudinal fibres.

Ovaries oval shaped, 260 µm in diameter in anterior–posterior axis, and situated at a distance from anterior end equivalent to one-quarter of body length. Ovovitelline ducts emerge from dorsolateral wall of ovaries. Laterally to female atrium, the oviducts curve medially and join to form common glandular ovovitelline duct. Common glandular ovovitelline duct as long as half of female atrium length, and communicating with female genital duct; this duct is a projection of the dorsoposterior region of thefemale atrium and is lined with a 25-µm-tall stratified-like, ciliated epithelium, with an erythrophil-free surface. A cyanophil fibrillar mass 10–20 µm thick is present immediately underneath this epithelium, with very thin circular filaments (< 1 µm); it might be of glandular nature, albeit their circular arrangement resembles that of muscle fibres (Fig. 23B).

Female atrium without folds (Fig. 22C, D), twice as long as the male atrium, and lined with a cuboidal epithelium. This epithelium and that of female genital duct surrounded by 5- to 20-µm-thick layer of circular-to-decussate muscle fibres, followed by a 5- to 55-µm-thick layer of longitudinal fibres.

GEOPLANA CHITA FROEHLICH, 1956

FIG. 24

Geoplana chita – Froehlich, 1956b [in part].

Material examined

Original type material (from EMF collection): MZUSP PL 2092 (field number Specimen A): Curitiba/PR, Brazil. Froehlich, 21 November 1952. Copulatory apparatus: sagittal sections on two slides.

MZUSP PL 2093 (field number Specimen B): Curitiba/PR, Brazil. E. M. Froehlich *coll.*, 21 November 1952. Copulatory apparatus: sagittal sections on four slides. Specimen A or B: pre-pharyngeal region: transverse sections on two slides; pharynx: sagittal sections on one slide.

Other specimens: MZUSP PL 2084 (field number F6993): Morretes/PR, Brazil. E. M. Froehlich *et al.*, coll., 17 June 1953. Pharynx and copulatory apparatus: sagittal sections on nine slides.

Additional material: MZUSP PL 2086 (field number F6995): Morretes/PR, Brazil. Magno C. Segalla, coll., 1 November 1987. Pharynx and copulatory apparatus: sagittal sections on 37 slides.

MZUSP PL 715 (field number F1710) (imature): Morretes/PR, Brazil (25°28′10.6″S, 048°48′49.5″W). F. Carbayo *et al.*, coll., 13 January 2008. Pre-pharyngeal region: transverse sections on three slides; pharynx: sagittal sections on five slides; region behind pharynx: sagittal sections on four slides.

MZUSP PL 716 (field number F1730) (incompletely mature): Morretes/PR, Brazil (25°28′10.6″S, 048°48′49.5″W). F. Carbayo *et al.*, coll., 13 January 2008. Cephalic region: horizontal sections on four slides; pharynx and copulatory apparatus: sagittal sections on six slides.

Note

An unlabelled specimen from the original type material is not conspecific (see Discussion below and Supporting information, Table S1).

Distribution

Areas covered with Atlantic forest in the municipalities of Curitiba and Morretes, State of Paraná, South Brazil.

Diagnosis

Dorsal colour pattern constituted by a median mustard-coloured band, bounded on either side by a black band; entire dorsum sprinkled with conspicuous whitish specks. Penis bulb extending anteriorly 1.5 mm from penis papilla. Diameter of muscle muscular cylinder around ejaculatory duct equivalent to ten times the diameter of this duct. Female-to-male atrial length ratio, 2.

External aspect

Preserved adults 58 mm long and 4 mm wide. Body margins nearly parallel; anterior end rounded, posterior pointed. Dorsum strongly convex, ventral side flat. Dorsal body surface of live specimens constituted by a median mustard-coloured band (one-third of body width) that grades into the black of cephalic region (one-sixth of body length), bounded on either side by a black band (one-third); entire dorsum sprinkled with conspicuous whitish specks (Fig. 24A, B). Ventral surface whitish with blackish margins. The dorsal colours of preserved specimens collected in 2008 became slightly paler; the dorsal side of one specimen collected in 1952 consisted of a beige red median band bordered by greyish lateral bands. Two types of eyes: a conical type, in a single row around anterior tip; and a cup-shaped type, spreading onto dorsum along lateral bands being one-third of body width. Relative position

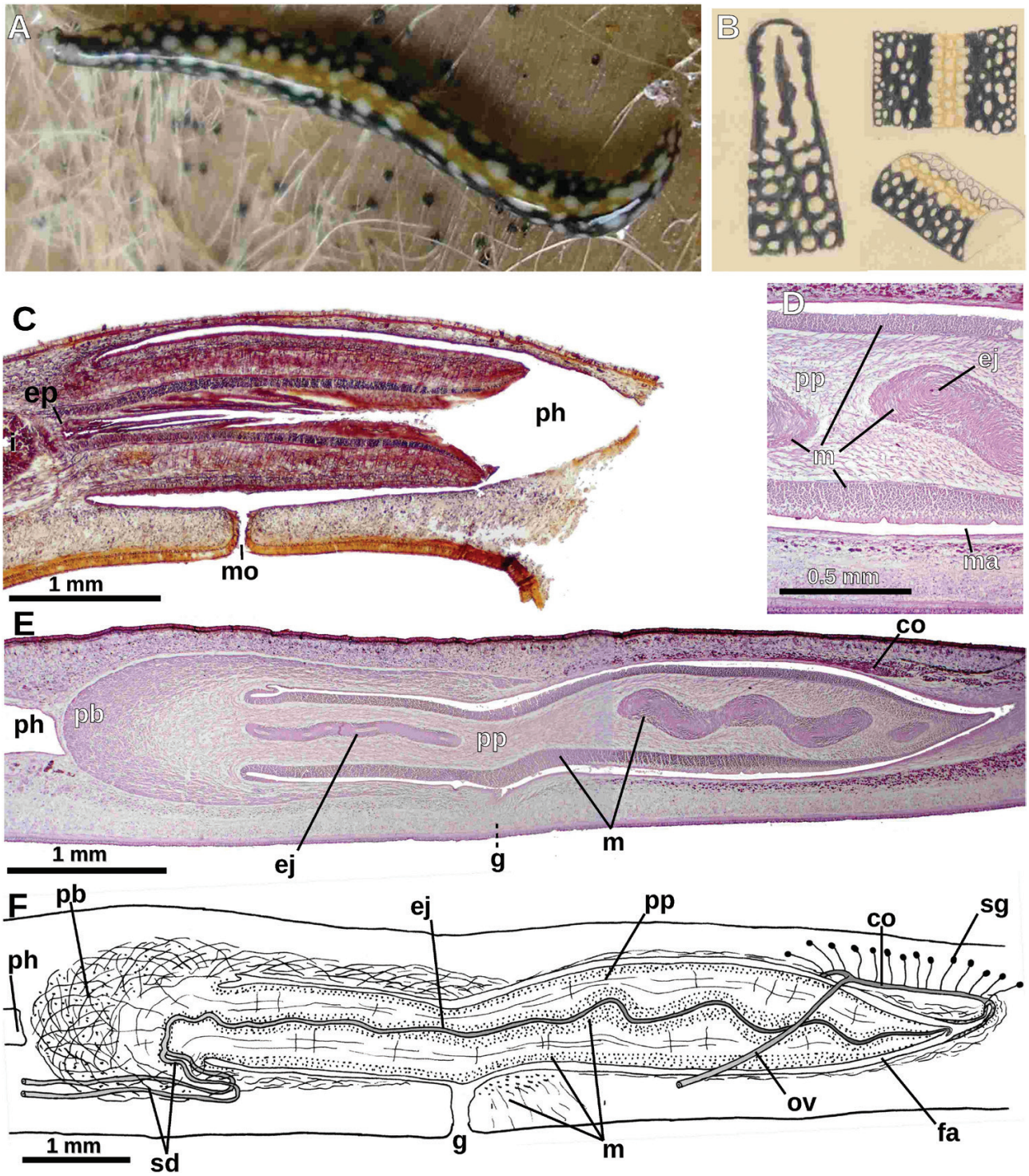

Figure 24. *Geoplana chita* Froehlich, 1956. A, dorsal view of the live specimen MZUSP PL 716, underdeveloped. B, drawings of dorsum of anterior end (left) and mid-region in dorsal view (top, right) and in three-dimensional section (bottom, right) of the body of a type specimen from Paraná; drawings by E. M. Froehlich; scale bar not available. C, photomicrograph of a sagittal section of the pharynx of specimen MZUSP PL 715. D, photomicrograph of a sagittal section of the copulatory apparatus of specimen MZUSP PL 2086. E, photomicrograph of part of the penis papilla of specimen MZUSP PL 2086 in sagittal section, showing the ejaculatory duct and its surrounding muscle. F, diagrammatic representation of the copulatory apparatus from sagittal sections of specimen MZUSP PL 2086.

mouth-to-body length, 65%. Relative position gonopore-to-body length, 76%.

Internal morphology

Creeping sole 86% of body width. Glandular margin absent. Three typical geoplaninid cutaneous muscle layers present, being as thick as 6% of body height. Muscle fibres of the longitudinal, innermost layer (10–20 µm thick) arranged into bundles with seven to 30 fibres each. Three parenchymal muscle layers present: dorsal layer of decussate diagonal fibres, supraintestinal layer of transverse fibres, and subintestinal layer with transverse fibres. Longitudinal fibres around intestine present, constituting a loose tube whose fibres mix with transverse ones. Ventral nerve plate present.

Mouth situated at a distance from root of the pharynx equivalent to 30–45% of pharyngeal pocket length. Pharynx cylindrical; dorsal insertion posteriorly placed. Oesophagus-to-pharynx ratio, 14% (Fig. 24C). Outer pharyngeal musculature consisting of a thin subepithelial layer of longitudinal muscle, followed by a layer (35 µm) of intermingled circular and longitudinal muscle fibres. Inner pharynx musculature consisting of a thin subepithelial layer of longitudinal muscle, followed by a layer (38 µm) of circular fibres and an innermost layer (10 µm) of longitudinal fibres.

Testes dorsally located between supraintestinal parenchymal muscle layer and intestine; anteriormost testes very slightly anterior to ovarian region, posteriormost testes near root of pharynx. Penis bulb elongated, extending from 1.5 mm anterior to penis papilla to level of gonopore region; very strongly developed, consisting of tightly packed muscle fibres variously orientated proximally, whereas diagonally orientated distally. Distal fibres on the right side run obliquely downwards to embrace ventrally the anterior portion of the female atrium and subsequently anchor on ventral epidermis. Penis bulb more developed dorsally to male atrium than ventrally. Sperm ducts run dorsally, or slightly lateral to the ovovitelline ducts. Sperm ducts lined with a cuboidal, ciliated epithelium and surrounded by a 10- to 15-µm-thick layer of circular muscles. Ejaculatory duct lined with cuboidal, ciliated epithelium, pierced by gland cells apparently producing erythrophil granules; surrounded by a layer of circular muscles with maximal thickness of 140 µm on the posterior half, and constituting a muscular cylinder with a diameter equivalent to ten times the diameter of this duct (Fig. 24D–F).

Penis papilla long, horizontal and cylindrical along most of its length, with dorsal and ventral insertions at the same transverse plane (Fig. 24D, F); posterior half of this papilla dilated, tapering to form a very narrow tip. Papilla occupies entire male and female atrium. Total length of papilla equal to seven times its greatest diameter. Papilla covered with a cuboidal, non-ciliated epithelium, pierced by gland cells producing erythrophil granules. A moderate space of 10 µm immediately beneath epithelium of mid-papilla fully packed by necks of gland cells producing cyanophil granules. Subepithelial musculature consisting of a dense layer of circular muscle, tending to decussate distally, 80 µm thick dorsally and 160 µm thick ventrally, followed by a 10-µm-thick layer of longitudinal muscle. Male atrium ample, not folded; lined with a low, non-ciliated epithelium, and underlain by a 10-µm-thick layer of circular muscles followed by a 10-µm-thick layer of longitudinal fibres.

Ovaries oval shaped, 500 µm in diameter in anteroposterior axis, and situated at a distance from anterior end equivalent to one-quarter of body length. Ovovitelline ducts emerge from dorsolateral wall of ovaries. Laterally to female atrium, the oviducts curve medially and join to form the common glandular ovovitelline duct. Common glandular ovovitelline duct as long as approximately one-third of the female atrium length, and communicating with posterior portion of female atrium, the latter being 300 µm long, and bent posterodorsally.

Female atrium without folds, and being twice the length of the male atrium (Fig. 24F). Posterior section with 200 µm in length of female atrium lined with a 20-µm-high, stratified-like, non-ciliated epithelium, which is surrounded by a 30-µm-thick layer of very thin muscle fibres. Remaining epithelium of female atrium lined with cuboidal epithelium, pierced by gland cells producing erythrophil granules. This epithelium and that of female genital duct surrounded by 5- to 40-µm-thick layer of circular-to-decussate muscle fibres, followed by a 40- to 150-µm-thick layer of longitudinal fibres.

GEOPLANA BORACEIA ALMEIDA & CARBAYO SP. NOV.

FIGS 25 AND 26

Material examined

Holotype MZUSP PL 2070 (field number F4112): Estação Biológica de Boraceia, Salesópolis/SP, Brazil (23°39′14.0″S, 045°53′21.0″W). F. Carbayo *et al*., coll., 21 December 2009. Cephalic region: horizontal sections on 11 slides; ovarian region: horizontal sections on four slides; pre-pharyngeal region: transverse sections on eight slides; pharynx: sagittal sections on six slides; copulatory apparatus: sagittal sections on 12 slides.

Paratype MZUSP PL 2077 (field number F6575): São Sebastião/SP, Brazil (23°44′48.7″S, 045°37′55.7″W). A. L. Almeida *et al*., coll., 13 February 2015. Ovarian region: horizontal sections on seven slides; pre-pharyngeal region: transverse sections on ten slides; pharynx and copulatory apparatus: sagittal sections on 15 slides.

Paratype MZUSP PL 2078 (field number F6578): São Sebastião/SP, Brazil (23°44′48.7″S, 045°37′55.7″W). A. L. Almeida *et al*., coll., 13 February 2015. Ovarian region: horizontal sections on seven slides; pre-pharyngeal region: transverse sections on ten slides; pharynx and copulatory apparatus: sagittal sections on nine slides.

Paratype MZUSP PL 2073 (field number F6357): São Sebastião/SP, Brazil (23°44′48.7″S, 045°37′55.7″W). A. L. Almeida *et al*., coll., 29 April 2015. Copulatory apparatus: sagittal sections on 11 slides.

Paratype MZUSP PL 2123 (field number F4389): São Sebastião/SP, Brazil (23°44′45.6″S, 045°37′55.2″W). F. Carbayo *et al*., coll., 26 February 2010. Entire specimen in 80% ethanol.

Paratype MZUSP PL 2095 (field number F6026): São Sebastião/SP, Brazil (23°44′39.8″S, 045°38′13.7″W). Carbayo *et al*., coll., 11 January 2014. Preserved in 80% ethanol.

Paratype MZUSP PL 2085 (field number F6994): Estação Biológica de Boraceia, Salesópolis/SP, Brazil. O. Froehlich *et al*., coll., 14 September 1986. Pharynx and copulatory apparatus: sagittal sections on 48 slides.

Paratype MZUSP PL 2124 (field number F6068): São Sebastião/SP, Brazil (23°44′48.7″S, 045°37′55.7″W). Carbayo *et al*., coll., 12 January 2014. Preserved in 100% ethanol.

Paratype MZUSP PL 2125 (field number F6055): São Sebastião/SP, Brazil (23°44′24″S 45°37′48″W). Carbayo *et al*., coll., 11 January 2014. Preserved in 80% ethanol.

Paratype MZUSP PL 1086 (field number F4358): São Sebastião/SP, Brazil (23°45′07.3″S, 045°37′51.7″W). Carbayo *et al*., coll., 26 February 2010. Pre-pharyngeal region: transverse sections on five slides; pharynx: sagittal sections on 12 slides; copulatory apparatus: sagittal sections on 12 slides.

Distribution

Areas covered with Atlantic forest in the municipalities of Salesópolis and São Sebastião, state of São Paulo, Brazil.

Diagnosis

Dorsal colour pattern constituted by a median mustard-coloured band, bounded on either side by a black band; entire dorsum sprinkled with conspicuous whitish specks. Penis bulb extending anteriorly 0.8 mm from penis papilla. Diameter of muscular cylinder around ejaculatory duct equivalent to 1.5 times the diameter of this duct. Female-to-male atrial length ratio, ~1.0.

Etymology

The specific epithet refers to the name of the biological station where the type material was collected.

External aspect

Preserved adults 50 mm long and 4 mm wide. Body elongated, margins nearly parallel; anterior end rounded, posterior pointed. Dorsum strongly convex, ventral side flat. Dorsal body surface constituted by a median mustard-coloured band (one-third of body width) that grades into the black of cephalic region (one-sixth of body length), bounded on either side by a black band (one-third); entire dorsum sprinkled with conspicuous whitish specks (Fig. 25A). Ventral surface is grey whitish, with blackish margins. In fixed specimens, dorsal colours faded, mostly the median band. Two types of eyes: a conical type, in a single row around anterior tip; and a cup-shaped type, spreading onto dorsum along lateral bands with one-third of body width. Sensory pits simple invaginations, 40 µm deep, located ventromarginally in a single row, from the very anterior end up to at least the first one-eighth of body length. Relative position mouth-to-body length, 57%. Relative position gonopore-to-body length, 70%.

Internal morphology

Creeping sole 85% of body width. Glandular margin absent. Three typical geoplaninid cutaneous muscle layers present, as thick as 9% of body height. Muscle fibres of the longitudinal, innermost layer (20–25 µm thick) arranged into bundles with nine to 30 fibres each. Three parenchymal muscle layers present: dorsal layer of decussate diagonal fibres, supraintestinal layer of transverse and longitudinal fibres, and subintestinal layer with transverse and longitudinal fibres. Longitudinal fibres around intestine present, constituting a loose tube whose fibres mix with transverse ones. Ventral nerve plate present.

Mouth situated at a distance from root of the pharynx equivalent to 34–49% of pharyngeal pocket length. Pharynx cylindrical, dorsal insertion slightly posterior. Oesophagus-to-pharynx ratio, 10% (Fig. 25B). Outer pharyngeal musculature consisting of a subepithelial layer (2.5 µm) of longitudinal muscle, followed by a layer (35 µm) of circular fibres, the innermost of which are intermingled with longitudinal ones. Inner pharynx musculature consisting of a thin subepithelial layer of longitudinal muscle, followed by a layer (50 µm) of circular fibres and an innermost layer (15 µm) of longitudinal fibres.

Testes dorsally located between supraintestinal parenchymal muscle layer and intestine; anteriormost testes at a distance from anterior end equivalent to 22% of body length; posteriormost testes slightly

anterior to root of pharynx. Penis bulb elongated, extending from 0.8 mm anterior to penis papilla to the level of gonopore region; well developed, dorsally more than ventrally. It consists of tightly packed muscle fibres variously orientated proximally. Part of the muscle fibres from the right dorsal portion of penis bulb detach and, subsequently, run obliquely downwards to embrace ventro-anterior portion of the female atrium (Fig. 25D, E). Further course of these fibres could not be determined. A lower number of muscle fibres from the dorsoposterior left portion of penis bulb run anteroventrally towards ventral

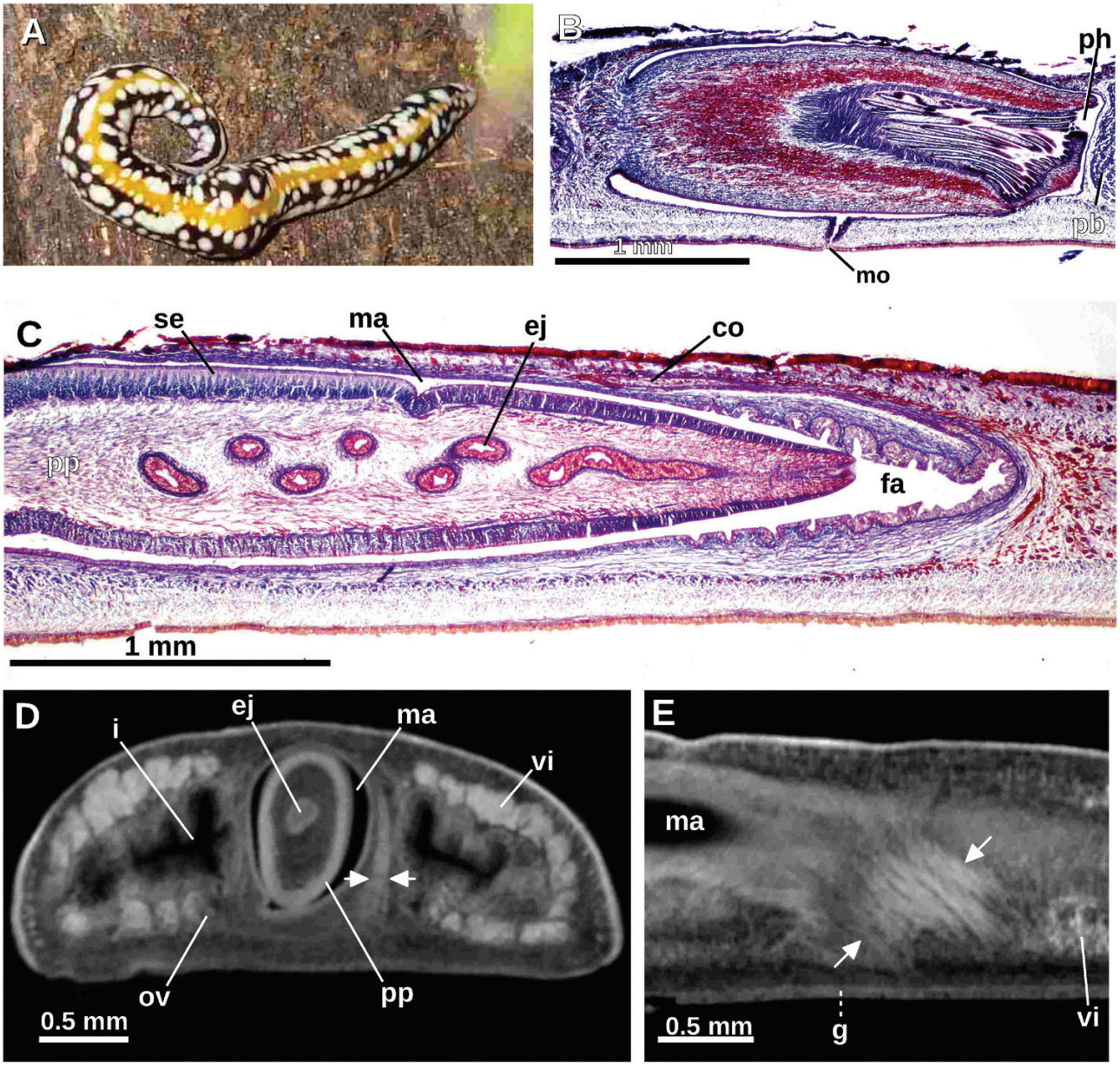

Figure 25. ***Geoplana boraceia*** Almeida & Carbayo **sp. nov.** A, dorsal view of the living specimen MZUSP PL 1086 (field number F4358), ~40 mm in length. B, photomicrograph of a sagittal section of the pharynx of specimen MZUSP PL 2077. C, photomicrograph of a sagittal section of penis papilla and female atrium of specimen MZUSP PL 2070. D, micro-computed tomography-based image of a transverse section of the penis papilla of specimen MZUSP PL 2095. Arrows point to muscle detached from the penis bulb running towards the ventral epidermis. Right body side to the right. E, micro-computed tomography-based image of a parasagittal section of the right side of penis bulb of specimen MZUSP PL 2095. Arrows point to muscles detached from the penis bulb running towards the ventral epidermis.

epidermis; further course of these fibres could not be determined.

Sperm ducts run dorsolaterally or dorso-internally to the ovovitelline ducts. Laterally to level of the insertion of penis papilla, the sperm ducts bend medially and then ventrolaterally to penetrate the bulb. The ducts subsequently recurve and join to continue posteriorly as an unpaired, curved duct; the unpaired portion continues as the ejaculatory duct. The latter follows a sinuous course to open at the tip of the penis papilla (Figs 25C, D, 26A, B). Sperm present in distal portion of sperm ducts, along both their course outside and inside penis bulb. Sperm ducts lined with a cuboidal-to-columnar, ciliated epithelium and surrounded by a 12-µm-thick layer of circular muscles. Ejaculatory duct lined with tall, ciliated epithelium; extrapapillar section pierced by gland cells producing fine, weakly cyanophil granules, but this cell type may extend along intrapapillar section until the level of the gonopore; intrapapillar section pierced by conspicuous and numerous gland cells producing erythrophil-to-xanthophil granules; surrounded by a 10-µm-thick layer of circular muscles, and constituting a muscular cylinder with a diameter equivalent of 1.5 times the diameter of this duct.

Penis papilla long, horizontal and cylindrical along most of its length; distally conical. Dorsal and ventral insertions of this papilla approximately at the same transverse plane. This papilla occupies the entire male atrium and the first half of the female atrium. Total length of papilla equal to six to seven times its greatest diameter. Penis papilla covered with a squamous-to-cuboidal epithelium, pierced by two types of gland cells, producing strongly erythrophil granules and fine cyanophil granules, respectively; former glands pierce entire epithelium except for mid-dorsal; this area pierced by cyanophil glands, the necks of which pack the space immediately beneath this epithelium (Fig. 25C). Subepithelial musculature consisting of a dense layer of circular muscle, decussates distally, 110 µm thick dorsally, 55 µm ventrally, followed by a 15-µm-thick layer of longitudinal muscle. Male atrium ample, not folded, and lined with a low, non-ciliated epithelium, pierced by gland cells producing erythrophil granules and underlain by a 10-µm-thick layer

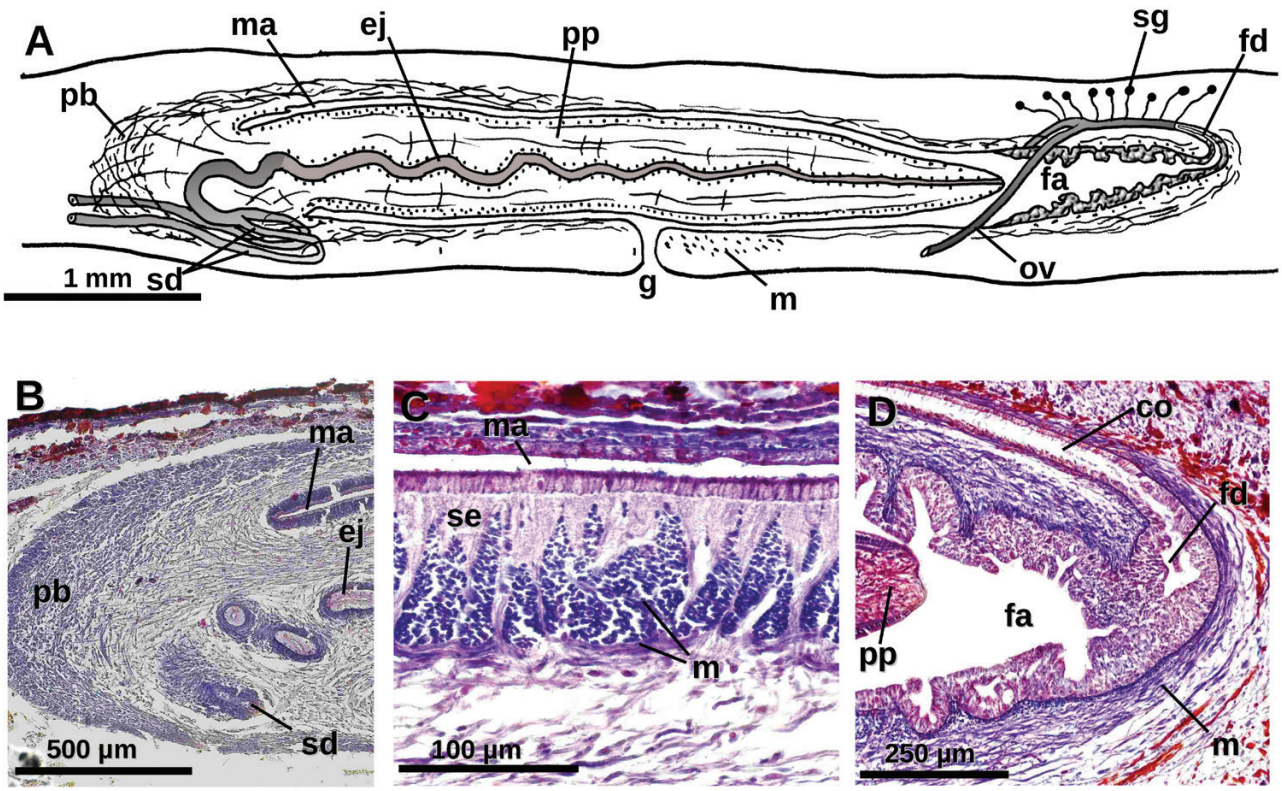

Figure 26. ***Geoplana boraceia*** Almeida & Carbayo **sp. nov.** Sagittal views. A, diagrammatic representation of the copulatory apparatus of specimen MZUSP PL 2070. B, photomicrograph of a sagittal section of the penis bulb of specimen MZUSP PL 2070. C, photomicrograph of dorsal epithelium of penis papilla and cyanophil glands located immediately beneath epithelium of specimen MZUSP PL 2077. D, photomicrograph of posterior portion of female atrium and female genital duct of specimen MZUSP PL 2070.

of circular muscles, followed by a 10-µm-thick layer of longitudinal fibres.

Ovaries oval shaped, 500 µm in diameter in anterior–posterior axis, and situated at a distance from anterior end equivalent to one-fifth of body length. Ovovitelline ducts emerge from dorsolateral wall of ovaries. Laterally to female atrium, the oviducts curve medially and join to form the common glandular ovovitelline duct. This duct is as long as 20–30% of female atrium length and communicates with female genital duct; the latter is a canalicular projection of the posterior region of the female atrium, and is lined with a 25-µm-tall stratified-like, ciliated epithelium.

Female atrium cylindrical, becoming conical posteriorly, sometimes here with small folds (Figs 25C, 26A, D); a little longer than the male atrium. Conical portion lined with a stratified-like epithelium; cylindrical portion lined with cuboidal epithelium; both epithelia pierced by two types of gland cells, producing amorphous substance; surrounded by 5- to 20-µm-thick layer of circular-to-decussate muscle fibres, followed by a layer of longitudinal fibres, 125 µm thick in posterior portion, 20 µm in anterior portion.

DISCUSSION

Phyletic status of the genera

The monophyly of Geoplaninae was addressed by Carbayo *et al.* (2013). In that paper, earlier presumed sister-group relationships were demonstrated to be unstable, but more recently resolved interrelationships resulted in stable groups. The classification of geoplaninids proposed by them was based on morphological homogeneity of species within every clade. As a result, the authors erected new genera and emended the diagnoses of some other genera, including *Geoplana*. Contrary to their results, we found no support for the monophyly of *Choeradoplana*, *Issoca*, *Luteostriata*, *Paraba*, *Pasipha* or *Supramontana*. This disagreement might be attributable to the different markers used; we considered only *COI* and *EF-1-α* partial sequences, whereas Carbayo *et al.* (2013) also included the nuclear fragments 18S and 28S rDNA.

Nonetheless, our results agree with Carbayo *et al.* (2013) in recovering the monophyly of the subfamily Geoplaninae, as well as the genera *Cephaloflexa*, *Geoplana*, *Imbira*, *Obama* and *Matuxia*. Moreover, regardless of the explored molecular dataset and applied optimality criteria, the 42 individuals of *Geoplana*, representing 17 species, were always recovered as a monophyletic group with high support values (GBS, LLD). This independent molecular test of monophyly corroborates the validity of the genus as diagnosed on a morphological basis.

Deconstructing the species-complex *Geoplana* cf. *vaginuloides*

Coincidence of the similar dorsal colour pattern and the extraordinarily long penis papilla most probably misled former researchers (Riester, 1938; Marcus 1951, 1952; Froehlich, 1956a, 1958) so as to confound species-specific diagnostic traits with intraspecific morphological variation. Indeed, it was only through independent evidence from molecular phylogenies that we were able to reconsider the taxonomic value of some morphological attributes and, ultimately, to propose species hypotheses. Thus, different from the conspecificity reported by Riester (1938), Marcus (1951, 1952) and Froehlich (1956a, 1958), *G. vaginuloides*, *G. apua*, *G. mogi* and *G. piratininga* should each be considered as an independent lineages. Moreover, if we apply the morphological criteria used by Riester (1938), Marcus (1951, 1952) and Froehlich (1956a) to delimit the species of *Geoplana*, the following additional species would have been considered as cryptic: *G. cananeia* Almeida & Carbayo sp. nov., *G. paranapiacaba*, *G. caraguatatuba*, *G. cambara*, *G. ibiuna* and *G. iporanga*.

It is true that four species pairs, namely *G. cambara*–*G. paranapiacaba*, *G. caraguatatuba*–*G. cananeia*, *G. ibiuna*–*G. piratininga* and *G. chita*–*G. boraceia*, have nearly identical colour patterns (see below). Regarding the internal morphology, two species pairs are indistinguishable from each other, namely *G. mogi*–*G. boraceia* and *G. cambara*–*G. chita*. However, all 13 species of the genus present a unique combination of external and internal features.

Moreover, two of the species pairs, namely *G. cambara*–*G. paranapiacaba* and *G. caraguatatuba*–*G. cananeia*, can also be distinguished from each other by unambiguous unique molecular synapomorphies. These synapomorphies are present only in *G. paranapiacaba* and *G. caraguatatuba*. Indeed, *G. paranapiacaba* can be diagnosed by two nucleotide positions of *EF-1-α* that comprise unique unequivocal synapomorphies, whereas *G. caraguatatuba* can be identified by one nucleotide position of *COI*, which constitutes a unique unequivocal synapomorphy. It is not usual to identify a species on the basis of nucleotide positions (Trevisan, Primon & Marques, 2017), but this approach can be a decisive taxonomic tool for specimens lacking a copulatory apparatus or from which there is only a small tissue fragment of the body available.

Morphological distinction of the species of *Geoplana*

A total of five species are distinguished from the remaining congeners in the chromatic pattern of the dorsum, namely *G. vaginuloides* (median black band,

white-yellow para-median stripes externally bordered by black lines, and lateral reddish-iron bands), *G. apua* (median luminous orange band, bounded on either side by a black stripe, externally to which is a greenish-yellow stripe, and this again is bordered externally by a marginal black band), *G. mogi* (median orange-ochre band, bounded on either side by a thin whitish stripe, and in turn this is bordered externally by a thin black line, externally to which is a grey band with dense white mottling, externally to which is a thin marginal black line), *G. iporanga* (median melon yellow band, bounded on either side by a black band, externally to which is a white band divided longitudinally in half by a black line), and *G. pulchella* (anterior third of dorsal body pure orange; remaining dorsal side constituted by a median light grey band, bordered on either side by a black band; entire dorsum sprinkled with conspicuous whitish specks).

There are six pairs of species that are indistinguishable by means of only either the external aspect (*G. piratininga–G. ibiuna*, *G. chita–G. boraceia*, *G. caraguatatuba–G. cananeia* and *G. cambara–G. paranapiacaba*) or the internal organs (*G. cambara–G. chita* and *G. mogi–G. boraceia*). Their profound similarity means that we rely on a combination of both external and internal atributes to recognize every species.

The dorsum of *G. piratininga* and *G. ibiuna* is very alike (median traffic red or orange-ochre band, bounded on either side by a black stripe, externally to which is a white stripe, and in turn this is externally bordered by a black band, externally to which is a thin marginal white line). However, their copulatory apparatus differs in that in *G. piratininga* the penis papilla is as long as five times its diameter, and the female-to-male atrial length ratio is 2.0, whereas in *G. ibiuna* the penis papilla is as long as seven times its diameter, and the female-to-male atrial length ratio is 1.0.

The dorsum of *G. chita* and *G. boraceia* is also indistinguishable from each other (median mustard-coloured band, bounded on either side by a black band; entire dorsum sprinkled with conspicuous whitish specks). The internal most conspicuous difference between them is that the diameter of the muscular cylinder around the ejaculatory duct is equivalent to ten times the diameter of this duct in *G. chita*, whereas in *G. boraceia* it is equivalent to 1.5 times.

Geoplana caraguatatuba and *G. cananeia* present a dorsal colour pattern also very alike (median orange-ochre or yellow-orange band, bounded on either side by a black stripe, externally to which is a white stripe, and this in turn is externally bordered by a black stripe that merges into a grey band with white mottling, which fades gradually towards its outer margin, whereas this margin is bordered by a black stripe). However, the penis papilla of *G. caraguatatuba* is pointed, the female atrium presents smooth walls, and female-to-male atrial length ratio is 2.0, whereas in *G. cananeia* the penis papilla is blunt, the posterior half of the female atrium has a richly folded wall and the female-to-male atrial length ratio is 1.0. Additionally, in the latter species the stroma of the penis papilla presents necks of glands gathered in gross bundles.

The pair of species *G. cambara–G. paranapiacaba* are externally very alike (median stripe of orange or orange-ochre, bounded on either side by a black line, externally to which is a broader white line, and in turn this is externally bordered by a thin black line, externally to which is a grey band with dense white mottling, bordered by a thin marginal black line). Internally, they can be distinguished in that the longitudinal parenchymal muscle fibres are disposed around intestine as a loose muscular cylinder in *G. cambara*, whereas in *G. paranapiacaba* the muscle fibres are positioned only dorsally and ventrally to the intestine. Furthermore, in *G. cambara* the diameter of the muscular cylinder around the ejaculatory duct is equivalent to 12 times the diameter of this duct, whereas in *G. paranapiacaba* it is equivalent to 2.5 times.

Finally, the species of each of two pairs of species (*G. cambara–G. chita* and *G. mogi–G. boraceia*) cannot be differentiate from each other with regard to the internal organs, especially the copulatory apparatus. However, the chromatic pattern of *G. cambara* is constituted by a median orange stripe plus five pairs of lines (lateralmost lines are difficult to discern) either black, white or grey, whereas the dorsum of *G. chita* displays a median mustard-coloured band, bounded on either side by a black band, with entire dorsum sprinkled with conspicuous whitish specks.

The internal organs of *G. mogi* and *G. boraceia* are also very similar to each other, but the dorsum in *G. mogi* is constituted by a median orange-ochre band plus four pairs of whitish, black or grey lines, whereas in *G. boraceia* the median mustard-coloured band is bounded on either side by a black band; with the entire dorsum sprinkled with conspicuous whitish specks.

A COMPLEX OF SPECIES UNDER THE CONCEPT OF *G. CHITA*

Our morphological data support the notion that *G. chita* was originally described on the basis of material of two species, one from Curitiba and another from Eldorado. Ultimately, there are three species with a general appearance like that of *G. chita*. One of these species is *G. chita*, represented by the specimens listed above in the 'Material examined' section. This material includes two specimens of the original type material from Curitiba (see also Supporting Information, Table S1), plus additional individuals more recently collected in Curitiba and Morretes. A second species is represented by only one individual from Eldorado,

which also belongs to the original type material of *G. chita*. The remains of this single individual (see Supporting Information, Table S1) are the pharynx and the incompletely sectioned copulatory apparatus. The internal morphology of this specimen, for which, unfortunately, there was no tissue available for molecular studies, suggests that it should be considered a new species of *Geoplana*. The description of this taxon is beyond the scope of the present study.

A third cryptic species is *G. boraceia*, represented by individuals from Salesópolis and São Sebastião. This species also displays the same external aspect as *G. chita*. Indeed, the paratype MZUSP PL 2085, from Eudóxia's collection, was identified by her as *G. chita* (E. M. Froehlich, unpublished observation). However, its internal organs and molecular data exclude *G. boraceia* being from conspecific with *G. chita* (see diagnoses and diagnostic key below).

Two species, *G. chita* and *G. cambara* (Fig. 3), nested in a polytomy. Hence, nothing can be inferred about the phyletic status of these putative species. This result is most likely to be an artefact given that the former species is represented only by the *EF-1-α* sequence, and that the dorsal colour pattern of these two species is different from each other.

Reassessment of the diagnosis of *Geoplana*

As a result of our better comprehension of the morphological features of this group, the current diagnosis of the genus *Geoplana* (see Carbayo *et al.*, 2013) is here emended as follows.

Geoplaninae with medium-sized body, 25–100 mm in length; slender body, with nearly parallel margins; dorsum strongly convex; eyes monolobulated, cone shaped in the anterior region of the body; parenchymal longitudinal muscle fibres present and organized in a muscular cylinder around the intestine, or in two layers, one supra-, another subintestinal, each intermingled with parenchymal transverse fibres; pharynx cylindrical; prostatic vesicle absent; part of penis bulb musculature detached and running obliquely towards ventral epidermis, behind gonopore; thick, well-delimited male genital muscle coat; penis papilla protrusible, cylindrical, very long, extending into female genital atrium; muscle fibres of penis papilla and ejaculatory duct densely packed in a thick layer; male atrium not folded; ascending portion of the ovovitelline ducts lateral to female atrium, joining each other above it; female genital duct dorso-anteriorly flexed, arising from the posterior region of the female atrium; female atrium long, generally not folded.

To the best of our knowledge, the peculiar arrangement of penis bulb fibres is unique among land planarians. This arrangement consists of a detachment of part of its fibres from the right body side, thereupon running backwards towards the ventral epidermis behind the gonopore. There exists some variation between the species of *Geoplana*. In *G. pulchella*, these fibres detach from both sides of the body to embrace the female atrium ventrally. In *G. boraceia*, additional muscle fibres from the left body side detach and follow downwards and anteriorly towards the ventral epidermis from the distal portion of the penis bulb. Furthermore, we did not observe this muscular arrangement in *G. ibiuna*. This peculiar course of these muscles was best traced in virtual sections derived from μCT datasets. Application of this non-destructive technique (Ziegler *et al.*, 2010) before the histological sectioning could have provided details that are hard to observe in traditional histological sections.

Reassessment of the diagnosis of Geoplaninae

One of the few diagnostic features of the subfamily is the absence of parenchymal longitudinal muscles, as proposed by Ogren & Kawakatsu (1990). Parenchymal longitudinal muscles are present in Rhynchodeminae (Geoplanidae). In fact, it has only been recently that this parenchymal longitudinal musculature was discovered in geoplaninids, i.e. *Imbira* Carbayo *et al.*, 2013. The diagnosis of Geoplaninae is here emended as follows.

Broad ciliated creeping sole covering most of ventral surface (or sparse body cilia as in the genus *Geobia*); mouth immediately behind midbody (in *Polycladus*, mouth is in posterior quarter); dorsal testes; well-developed subepithelial or cutaneous longitudinal musculature arranged in bundles; additional parenchymal muscle layers may be present and organized in longitudinal fibres, either dorsally and ventrally to the intestine, or as a tube around it.

Final remarks

We have discovered a number of *Geoplana* species in the Atlantic forest that were formerly obscured by their beauty. After having uncovered the morphological features differentiating all these species from each other, they might more appropriately be called pseudocryptic species, in the sense of Sáez & Lozano (2005). As new specimens are examined from museum collections or from new samplings, more new species of *Geoplana* may well be discovered. Thus, what initially would seem to be a species-poor genus with only three species, may in fact include no less than 17 species, 13 of which were (re)described herein.

On the basis of samplings from Argentinean (Negrete, 2013) and Brazilian forests (our samplings), more species of the *Geoplana* will be unveiled. The unexpected proliferation of species from the pretty *G. vaginuloides* resembles the last words of Darwin's own book *On the Origin of Species*, 'Endless forms most beautiful'.

IDENTIFICATION KEY TO SPECIES OF *GEOPLANA*

1. Dorsal median black band.....*Geoplana vaginuloides*
   Dorsal median band with another colour.....2
2. Dorsal median grey band.....*Geoplana pulchella*
   Dorsal median yellow-orange or reddish band.....3
3. Dorsal median band bounded on either side by a thin whitish stripe.....*Geoplana mogi*
   Dorsal median band bounded on either side by a black stripe.....4
4. Additional pair of fine paramedian greenish-yellow stripes.....*Geoplana apua*
   Additional pair of fine paramedian whitish stripes.....5
5. Dorsum sprinkled with whitish specks.....6
   Dorsum not sprinkled with whitish specks.....8
6. White mottling only on the lateral-most stripes.....7
   Entire dorsum sprinkled with conspicuous whitish specks.....9
7. Outermost zone fades from black to grey towards its external margin.....10
   Outermost zone divided into an internal black line and a grey band.....11
8. Outermost zone white, also continuous with white body margins.....*Geoplana iporanga*
   Outermost zone black, bounded by white body margins.....12
9. Diameter of muscular cylinder around ejaculatory duct equivalent to 1.5 times the diameter of this duct .....*Geoplana boraceia*
   Diameter of muscular cylinder around ejaculatory duct equivalent to ten times the diameter of this duct .....*Geoplana chita*
10. Penis papilla cylindrical and blunt, with stroma with necks of glands characteristically gathered in bundles that resemble an orange in cross-section.....*Geoplana cananeia*
    Penis papilla pointed.....*Geoplana caraguatatuba*
11. Longitudinal parenchymal muscle fibres disposed around intestine as a loose muscular cylinder .....*Geoplana cambara*
    Supraintestinal and subintestinal parenchymal muscle layers constituted of mixed transverse and longitudinal fibres.....*Geoplana paranapiacaba*
12. Penis papilla with dorsal insertion strongly displaced anteriorly and very ventrally.....*Geoplana ibiuna*
    Dorsal and ventral insertions of penis papilla at the same transverse plane.....*Geoplana piratininga*

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Phylogenetic hypothesis for 106 terminals of Geoplanidae based on the simultaneous analysis of *COI* and *EF-1-α* partial sequences using parsimony as the optimality criterion, including only terminals for which both regions were present (selected-terminals dataset).

Figure S2. Phylogenetic hypothesis for 144 terminals of Geoplanidae based on the simultaneous analysis of *COI* and *EF-1-α* partial sequences using parsimony as the optimality criteria, including terminals with missing data (all-terminals dataset).

Figure S3. Phylogenetic hypothesis for 106 terminals of Geoplanidae based on the simultaneous analysis of *COI* and *EF-1-α* partial sequences using maximum likelihood as the optimality criterion, including only terminals for which both regions were present (selected-terminals dataset).

Figure S4. Phylogenetic hypothesis for 144 terminals of Geoplanidae based on the simultaneous analysis of *COI* and *EF-1-α* partial sequences using maximum likelihood as the optimality criterion, including terminals with missing data (all-terminals dataset).

Figure S5. Phylogenetic hypothesis for 137 terminals of Geoplanidae based on analysis of *COI* sequences using maximum likelihood as the optimality criterion.

Figure S6. Phylogenetic hypothesis for 123 terminals of Geoplanidae based on analysis of *EF-1-α* sequences using maximum likelihood as the optimality criterion.

Table S1. Representatives of different species of *Geoplana* that could not be identified or probably represent undescribed species.