



<http://dx.doi.org/10.11646/zootaxa.3931.1.2>

<http://zoobank.org/urn:lsid:zoobank.org:pub:3371D5F0-26A4-400A-ABD7-3CD9B4B9403E>

## Anatomical deviation of male organs of land planarians from Rio de Janeiro, Brazil, with description of two new species of *Cratera* (Platyhelminthes, Tricladida)

FERNANDO CARBAYO<sup>1,2</sup> & ANA LAURA ALMEIDA<sup>1,3</sup>

<sup>1</sup>Laboratório de Ecologia e Evolução, Escola de Artes, Ciências e Humanidades, Universidade de São Paulo – USP, Av. Arlindo Bettio, 1000, CEP 03828-000, São Paulo, SP, Brazil. E-mail: baz@usp.br

<sup>2</sup>Programa de Pós-Graduação em Zoologia, Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, Trav. 14, 321, Cidade Universitária, CEP 05508-900, São Paulo, SP, Brazil.

<sup>3</sup>Programa de Pós-Graduação em Sistemática, Taxonomia Animal e Biodiversidade. Museu de Zoologia da Universidade de São Paulo, Avenida Nazaré, 481, CEP 04263-000, Ipiranga São Paulo, SP, Brazil. E-mail: ana\_laura\_xp@hotmail.com

### Abstract

Two new land planarian species, collected in the State of Rio de Janeiro, Brazil, are described. Their external aspect is similar to that of *Imbira marcusii* Carbayo *et al.*, 2013 and *Pseudogeoplana theresopolitana* (Schirch, 1929), respectively. The analysis of the internal organs, however, revealed they belong to the genus *Cratera*. The male copulatory organs of one species is very different from any other geoplaninid, for the penis papilla holds a large, distal cavity receiving the ejaculatory duct and, furthermore, the papilla projects vertically downwards from the roof of the male atrium. Thus we consider it as a new species, *Cratera cuarassu* **sp. nov.** The second species differs from its congeners in that the dorsal insertion of the penis papilla is anterior to the ventral one, and in that the female atrium is narrowed in the anterior portion. The species was found in the type locality of *Pseudogeoplana theresopolitana* (Schirch, 1929) and compares well with it in the external features. However, since its internal organs are unknown and the type material of the species is seemingly lost, we describe it as *Cratera anamariae* Carbayo, **sp. nov.**

**Key words:** Anatomy, Continenticola, flatworms, morphology, taxonomy

### Introduction

The native Neotropical land planarians (Platyhelminthes, Tricladida, Geoplanidae) are nearly all Geoplaninae. In a recent article, a part of the Geoplaninae in-groups was revised and the species-rich genus *Geoplana* Stimpson, 1857, the type-genus of Geoplaninae, was split into six genera: *Geoplana* Stimpson, 1957; *Barreirana* Ogren & Kawakatsu, 1990 (formerly a subgenus); *Cratera* Carbayo *et al.*, 2013; *Matuxia* Carbayo *et al.*, 2013; *Obama* Carbayo *et al.*, 2013; and *Paraba* Carbayo *et al.*, 2013 (Carbayo *et al.*, 2013). Thus, the genus *Geoplana* went from including over 100 species to only three (plus 47 species considered *incertae sedis*).

Currently the genus *Cratera* includes five species transferred from *Geoplana* when the genus was proposed, plus a new one recently described (Rossi *et al.*, 2014). The main diagnostic feature of the genus is a cavity continued from the ejaculatory duct located at the tip of the protrusible penis papilla. Different from the eversible penis type, which lacks a permanent penis papilla (it is "mainly formed from the ejaculatory duct which is sheathed in the everted male atrium" (Winsor, 1998)), the protrusible penis is characterized by "a conspicuous, permanent papilla and muscular bulb which, through contraction of circular muscles with consequent narrowing of the bulb and extension of longitudinal muscles, is protruded through the gonopore" as defined by Winsor (1998). The six known species of the genus possess this type of penis papilla, in a similar horizontal position.

In samplings in the Brazilian Atlantic forest in the State of Rio de Janeiro we collected two species displaying the color patterns of *Imbira marcusii* Carbayo *et al.*, 2013, and *Pseudogeoplana theresopolitana* (Schirch, 1929), respectively. However, both species present the main diagnostic feature of *Cratera*, but the former deviates from its

congeners in having a penis papilla projecting vertically downwards from the roof of the male atrium. In the following we provide a description of both species and a discussion of their identity.

## Material and methods

We conducted the samplings in areas covered with Atlantic Forest in the State of Rio de Janeiro, Brazil. We actively collected the animals during the day (under rocks and rotting logs) and during the night (in open places such as trails) between December 2007 and December 2010. We then photographed them and took notes on their external aspect, killed the specimens in boiling water and, before fixing them in 10% formalin solution for histological studies, removed a small piece of the worm and preserved it in absolute ethanol. The specimens, cut into a variable number of pieces containing the anterior region, the pre-pharyngeal region, the pharynx, and the copulatory apparatus, were dehydrated in a graded ethanol series, cleared in xylene or clove oil, and infiltrated and embedded in Paraplast® Tissue Embedding Medium. We sectioned these tissue blocks at 7 µm intervals using a retracting rotary microtome, affixed with albumin-glycerol (1:1) on glass slides placed on a slide warmer, stained them with the method Mallory-Heidenhain modified by Cason (1950), dehydrated in a graded ethanol series, cleared in xylene, and mounted them in Entellan® mounting medium. After some months, the stain of some sections faded. For these sections we removed the cover slide, and soaked the sections in the fixative Heidenhain's "Susa" (in Kiernan, 1990) for 20 hours. Subsequently the sections were restained following Mallory's method after Krichesky (1931; apud Humason, 1967). We analysed the slides with an optical microscope, and made drawings of the pharynx and the copulatory apparatuses with a camera lucida attached to the microscope. The colors of the body of living and fixed specimens were named after the palette RAL colours (RAL gemeinnützige GmbH, available at <https://www.ral-farben.de/uebersicht-ral-classic-farben.html?&L=1>) by comparison with digital pictures of the specimens on a computer screen. The material was deposited in the Museu de Zoologia da Universidade de São Paulo (MZUSP).

### List of abbreviations in figures

- (a) anterior end of the body
- (c) circular cutaneous muscles
- (cg) cyanophilic gland cells
- (co) common glandular ovovitelline duct
- (d) diagonal cutaneous muscles
- (dc) distal intra-penial cavity
- (de) dorsal epidermis
- (dv) dorso-ventral muscles
- (e) eye
- (ej) ejaculatory duct
- (fa) female atrium
- (fd) female genital duct
- (g) gonopore
- (gm) glandular margin
- (i) intestine
- (l) longitudinal cutaneous muscles
- (m) muscles
- (ma) male genital atrium
- (mc) muscular coat
- (mo) mouth
- (o) ovary
- (ov) ovovitelline duct
- (pc) proximal intra-penial cavity
- (pp) penis papilla

(pv) prostatic vesicle  
(sb) suprainestinal transverse muscles  
(sd) sperm duct  
(sg) shell glands  
(sp) suprainestinal transverse muscles  
(t) testis  
(ve) ventral epidermis  
(vi) vitellaria  
(vn) ventral nerve plate

## **Taxonomy**

### **Systematic account**

#### **Order Tricladida Lang, 1884**

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

#### **Geoplanidae Stimpson, 1857**

#### **Geoplaninae Stimpson, 1857**

#### ***Cratera* Carbayo, Álvarez-Presas, Olivares, Marques, Froehlich & Riutort, 2013**

#### ***Cratera cuarassu* sp. nov.**

**Etymology.** The name *cuarassu* is a free composition of the Tupi (indigenous Brazilian language) words *cuara* (meaning *hole, cave*) and *assu* (meaning *large* (Tibiriçá, 1984)). It refers to the large intra-penial cavity.

**Type material.** All specimens were collected in areas covered with Atlantic forest in the Parque Estadual do Desengano, Santa Maria Madalena, State of Rio de Janeiro, Brazil, by Júlio Pedroni *et al.* (see Acknowledgements). Holotype PL 348 (field number, F2189): (S 2152'36", W 4155'11"). 18/Mar/2008. Anterior end: sagittal sections on 5 slides; portion containing ovaries: sagittal sections on 64 slides; body region posterior to ovaries: horizontal sections on 27 slides; pre-pharyngeal region: transverse sections on 20 slides; pharynx: sagittal sections on 40 slides; copulatory apparatus: sagittal sections on 76 slides. Paratype PL 349 (field number, F2191): (S 2152'17", W 4154'53"). 17/Mar/2008. Portion containing ovaries: horizontal sections on 31 slides; body region posterior to ovaries: sagittal sections on 57 slides; pre-pharyngeal region: transverse sections on 17 slides; copulatory apparatus: sagittal sections on 70 slides. Paratype PL 350 (field number, F2192): (S 2152'36", W 4155'11"). 18/Mar/2008. Anterior end: horizontal sections on 3 slides; pre-pharyngeal region: transverse sections on 12 slides; pharynx and copulatory apparatus: sagittal sections on 82 slides. Paratype PL 351 (field number, F2193): (S 2152'36", W 4155'11"). 18/Mar/2008. Pre-pharyngeal region: transverse sections on 32 slides; copulatory apparatus: sagittal sections on 45 slides. Paratype PL 809 (field number, F4074): 13/Aug/2009. Incompletely mature. Pharynx and copulatory apparatus: sagittal sections on 6 slides. Paratype PL 805 (field number, F4006): Preserved in 80% ethanol.

**Type-locality.** Parque Estadual do Desengano, municipality Santa Maria Madalena/RJ, Brazil.

**Synonymy.** *Geoplana* sp. 5 in Carbayo *et al.* (2013).

*Cratera* sp. 5 in Carbayo *et al.* (2013).

**Diagnosis.** The species is distinguished from its congeners by its black blue dorsum with submarginal zinc yellow stripe. The peculiar shape of the male organs, i.e., a very short and wide penis papilla projecting vertically downwards from the roof of the male atrium, and possessing a large intra-penial cavity, readily distinguishes this from any other species of Geoplaninae.

**External aspect.** Only the paratype PL 805 was measured in life, reaching 11 cm in length and 0.6 cm in width at maximum extension (Fig. 1). After fixation adults measured 7 to 11 centimeters in length. Body margins parallel, anterior end rounded, posterior one pointed. Dorsum convex-flat, ventral side flat, 1 to 1.9 mm in height.

Dorsal color black blue with a submarginal zinc yellow stripe on each side of the body. The stripes have 1/9th of body width, running from nearly the anterior tip of the body to the posterior end. Ventral side light ivory, becoming brown beige in the anterior end (Fig. 2). After fixation, colors of dorsal and ventral side faded and a faint graphite black dark midline of the dorsum became visible (Fig. 3).

In the six, fixed mature specimens, the mouth lies at a distance from anterior tip equal to 69–76% (mean 70%) of body length; the gonopore, 78–86% (mean 82%). The eyes are of the pigmented-cup type, 35–40  $\mu\text{m}$  in diameter, surrounded by clean halos, and arranged marginally from the very anterior tip of the body to the posterior end. They are distributed in an irregular row consisting of one to three eyes in the black-blue colored body margin.



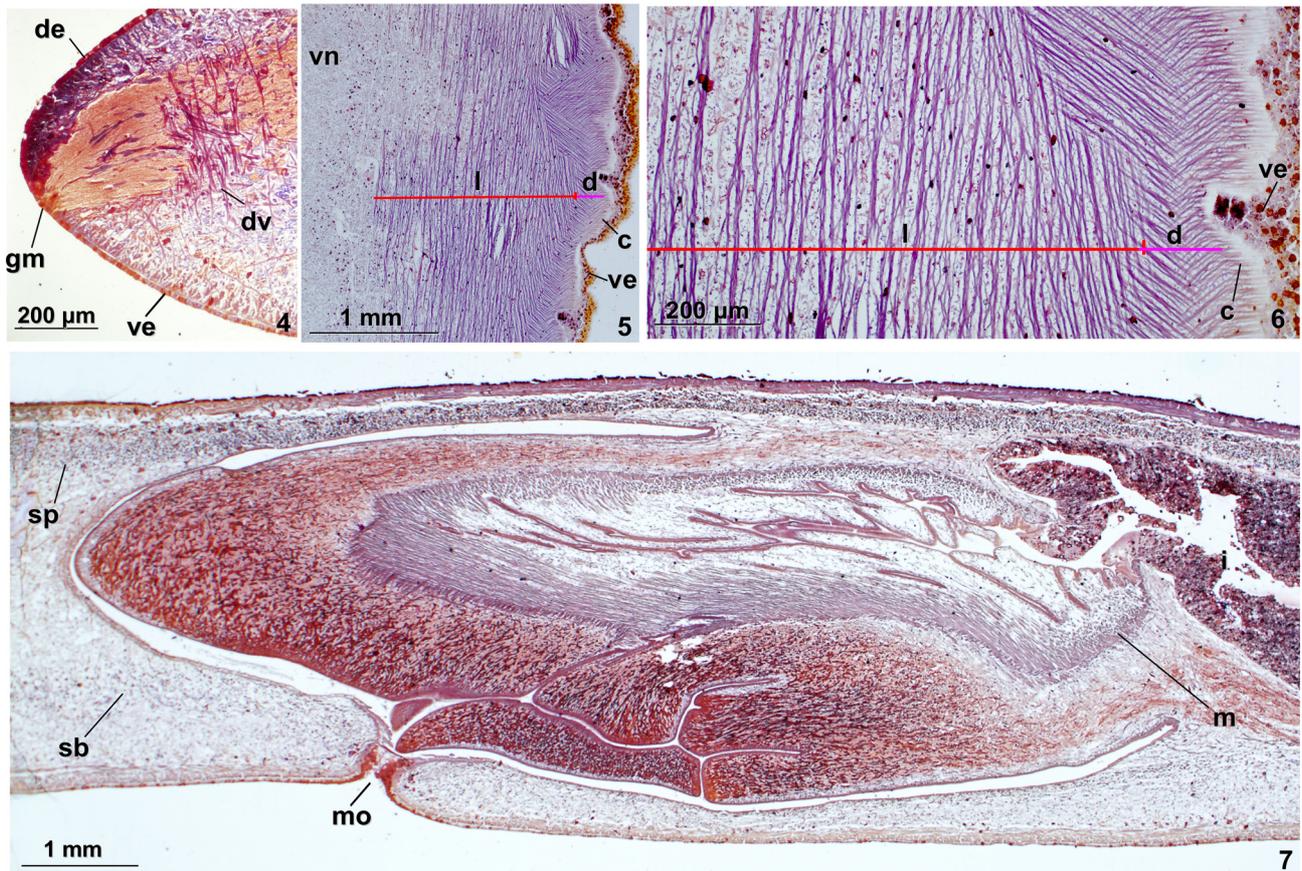
**FIGURES 1–3.** *Cratera cuarassu* sp. nov. (1) living paratype PL 805 creeping in dorsal view. About 60 mm in length. (2) living paratype PL 805 in ventral view. (3) fixed holotype on a millimetered paper after cutting off a piece of the central body.

**Internal morphology.** The sensory pits are simple invaginations 35  $\mu\text{m}$  deep, located ventro-marginally in a single row in the anterior third of the body. The width of the creeping sole is 90% of body width at the pre-pharyngeal region.

The conspicuous glandular margin (Fig. 4) is constituted by very abundant gland cells containing xanthophilic granules and scarce gland cells containing cyanophilic granules. The cutaneous musculature comprises the three typical layers of Geoplaninae in the same way throughout the body, i.e., a subepithelial circular layer followed by two diagonal layers with decussate fibers, and then a longitudinal layer with fibers arranged in bundles of 43–73 fibers each. The longitudinal fibers lying immediately under the decussate fibers do not run in the anteroposterior axis of the body, but slightly obliquely to the body margins (Figs. 5–6). The thickness of cutaneous muscle ranges from 6% to 14% (mean 10%) to body height in the pre-pharyngeal region. The three common geoplaninid parenchymal muscle layers, all well developed, are present throughout the body: a dorsal layer of diagonal decussate fibers, a transverse supraintestinal layer, and a transverse subintestinal one (Fig. 7).

The mouth lies in the middle of the pharyngeal pouch. The bell-shaped pharynx (Fig. 7) occupies about 2/3 of the pharyngeal pouch. An esophagus is absent. The pouch is lined by a flat ciliated epithelium, surrounded by a thin muscle layer of longitudinal muscle. Three types of glands (erythrophilic, xanthophilic and scarce cyanophilic) secrete through the distal pharyngeal epithelium.

The outer and inner pharyngeal epithelia are flat, ciliated. The outer epithelium is underlain by a layer of longitudinal muscle (15  $\mu\text{m}$ ) followed by a layer of circular muscle (45  $\mu\text{m}$ ); the inner epithelium is underlain by a layer of circular muscle (60  $\mu\text{m}$ ) followed by a layer of longitudinal muscle (10  $\mu\text{m}$ ).

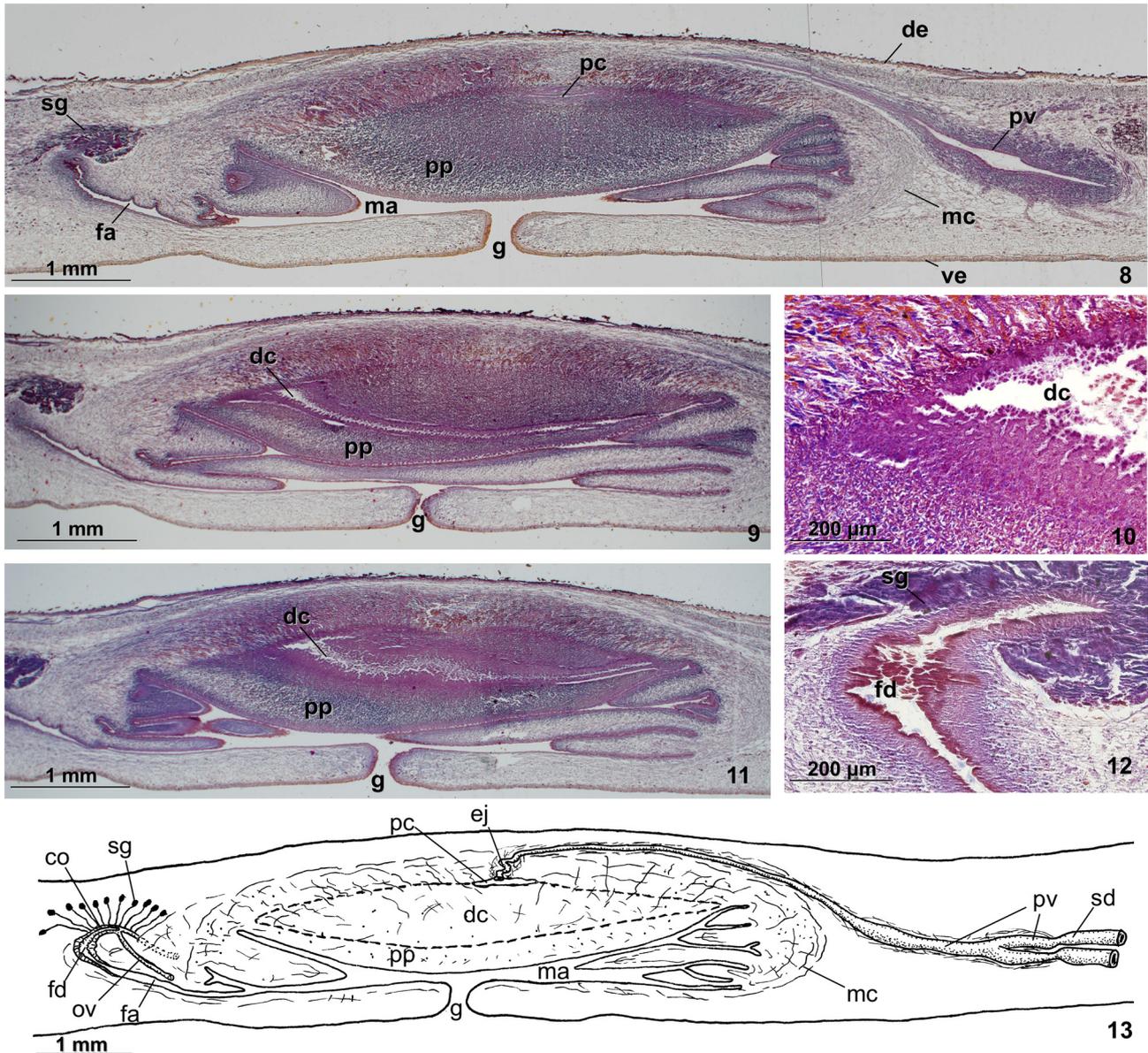


**FIGURES 4–7.** *Cratera cuarassu* sp. nov. Micrographs of the holotype. (4) micrograph of the glandular margin of holotype in a transverse section of the pre-pharyngeal region. (5) micrograph of the ventral cutaneous musculature tangentially cut in a horizontal section. Red line indicates longitudinal fibers; the pinkish line indicates decussate fibers. Anterior to the bottom. (6) micrograph of a detail of ventral cutaneous musculature tangentially cut in a horizontal section. Red line indicates longitudinal fibers; the pinkish line delimits the decussate fibers. Anterior to the bottom. (7) micrograph of a sagittal section of the pharynx of the holotype. Anterior to the right.

The testes are dorsal, located between the suprainestinal parenchymal muscle layer and the intestine, extending posteriad to ovaries (9 mm in the holotype, 8% of body length) to nearly level with dorsal pharyngeal insertion. The sperm ducts run immediately above the subintestinal muscle layer. Each sperm duct communicates with the anterior tip of the respective branch of the very long, canalicular prostatic vesicle (Figs. 8, 13–15). This vesicle runs posteriorly, gradually inclined dorsad, and subsequently penetrates the dorso-anterior aspect of the common muscle coat and continues posteriorly to nearly level with the gonopore. The epithelium of the prostatic vesicle is lined with a ciliated, columnar to cuboidal epithelium, pierced by numerous openings of gland cells containing erythrophilic granules. It is underlain by a layer of nearly longitudinal muscles. The ejaculatory duct is a sinuous, descending canal, lined with a cuboidal epithelium, opening into a small 700-µm-long proximal cavity at the base of a large, distal intra-penial cavity (Figs. 9–11, 13–15). This distal cavity is laterally compressed, and 4-mm long. The penis is of the protrusible type. The proximal cavity is lined with a non-ciliated epithelium underlain by a layer of circular muscles. It is pierced by scarce gland cells producing xanthophilic granules. The distal cavity is lined with a ciliated, apically erythrophilic epithelium, the free surface of which is sinuous. This epithelium is pierced by openings of numerous gland cells containing xanthophilic granules. Additionally, two types of gland cells discharge their secretion into the cavity, those producing pale-cyanophilic granules pierce the intermediate portion of the epithelium; those producing cyanophilic large granules pierce the distal portion of the cavity. The epithelium of this large cavity is surrounded by a subepithelial layer of circular muscle, followed by a layer of longitudinal muscle.

The penis papilla is short and much widened along the longitudinal axis of the body. It projects vertically downwards from the roof of the male atrium, even in incompletely mature specimens (Fig. 17), slightly inclined to

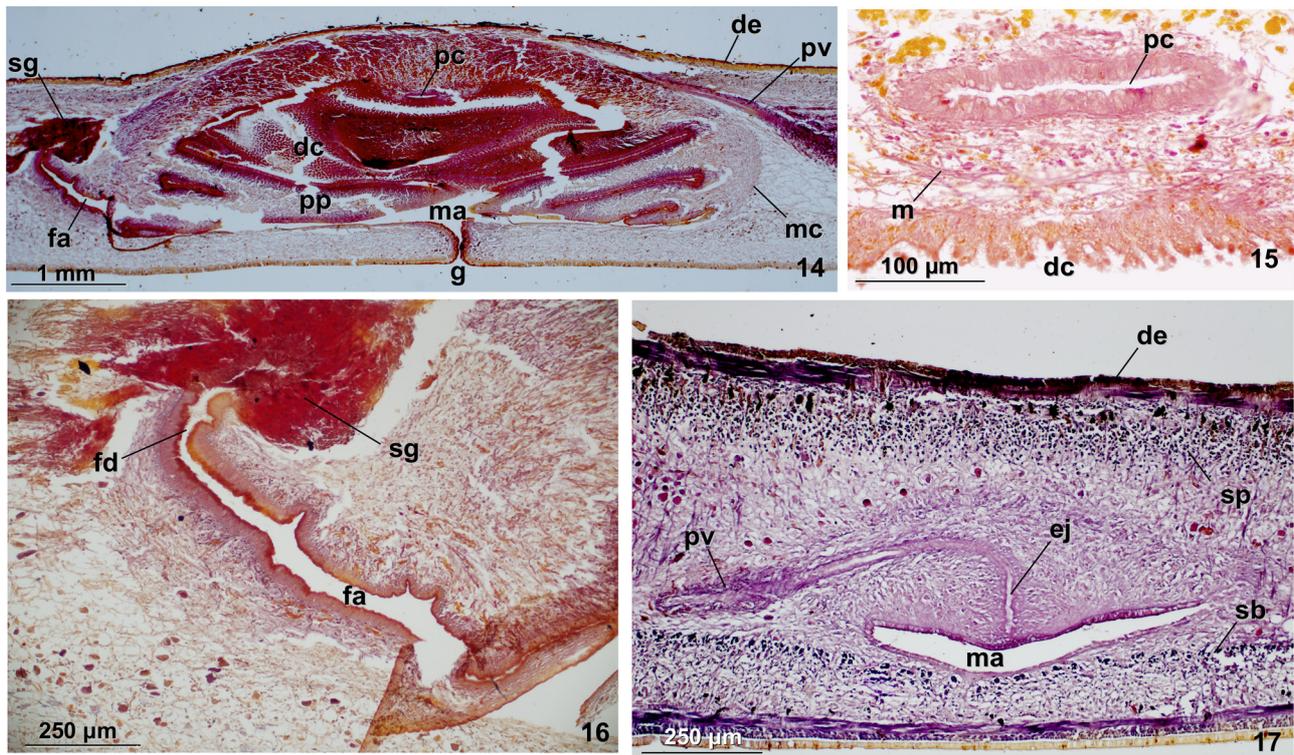
the right. The penis papilla is covered with a nucleated, ciliated epithelium transversed by gland cells containing xanthophilic granules. It is underlain with a subepithelial layer of circular muscles, followed by a layer of longitudinal muscles. The penis bulb is well muscularized, comprising decussated fibers. The penis papilla fills almost all the male atrium, this having two conspicuous, horizontal folds that encircle the papilla. Anteriorly, these folds may be divided into 3–5 minor folds. The folds are covered with a ciliated, apically erythrophilic epithelium, through which sparsely scattered xanthophilic glands discharge, and is underlain by a subepithelial thin layer of circular muscles, followed by a layer of longitudinal muscles. The gonopore lies in the middle of the male atrium.



**FIGURES 8–13.** *Cratera cuarassu* sp. nov. Holotype. Anterior to the right. (8–9) micrographs of a sagittal section of the copulatory apparatus of the holotype. (10) micrograph of a detail of the large cavity within the penis papilla of the holotype. (11) micrograph of a parasagittal section of the copulatory apparatus of the holotype. (12) micrograph of the genital female canal and posterior aspect of the female atrium of the holotype in a sagittal section. (13) diagrammatic reconstruction of copulatory apparatus of the holotype from sagittal sections.

The ovaries are ellipsoid, ~300 µm in diameter. They are located immediately above the ventral nerve plate, at a distance from the anterior tip equal to 21% of body length. The ovovitelline ducts arise from the dorso-external side of the ovaries, and run backwards above the ventral nerve plate, externally to the sperm ducts. The ovovitelline ducts pass posteriorly and in the proflex condition with dorsal approach, unite with the female genital duct. The ovovitelline ducts join each other to form the common glandular ovovitelline duct. This duct opens into the genital

female canal, a C-shaped duct in lateral view. It is slightly dilated in the intermediate portion; the distal portion runs forwards ventrally to open into the posterior region of the female atrium (Figs. 12–14, 16). The female atrium is a dorso-ventrally compressed, narrow cavity. The length of the female atrium: male atrium ratio is 1:6.



**FIGURES 14–17.** *Cratera cuarassu* sp. nov. Micrographs. (14) common glandular duct and female atrium of the paratype F2192 in a sagittal section. Anterior to the right. (15) sagittal section of the paratype F2192 showing the small cavity into which opens the ejaculatory duct. (16) sagittal section of the paratype F2192 showing the genital female canal and posterior aspect of the female atrium. Anterior to the right. (17) sagittal section of the copulatory apparatus of the paratype PL 809, incompletely developed. Anterior to the left.

Ovovitelline ducts and common glandular ovovitelline duct are lined with a columnar, ciliated epithelium. The female genital canal is lined with an apically erythrophilic epithelium, pierced by gland cells containing cyanophilic fine granules. It is ciliated only in the portion close to the common glandular ovovitelline duct. The epithelial cells lining the dilated portion are taller and have the free surface sinuous. The female atrium is lined with a flat, non-ciliated erythrophilic epithelium, pierced with scarce openings of glands secreting fine cyanophilic granules, and surrounded by a thin layer of circular muscle.

**Remarks.** Among the geoplaninids, the external aspect of living specimens of *Cratera cuarassu* sp. nov. can be confounded with *Imbira marcusii*. The latter species is widely distributed across the Atlantic forest of Southeast and South Brazil, but *Cratera cuarassu* sp. nov. and *Imbira marcusii* are not sympatric. *Imbira marcusii* displays several patterns in the color of the dorsum which differentiate it from that of the new species, and even in the most similar combination of color and pattern its lateral yellow stripes are half or less in width than those of *Cratera cuarassu* sp. nov.

Among the current 23 genera of Geoplaninae, the new species matches only the diagnostic features of *Cratera*, excepting in one feature. The cavity into which the ejaculatory duct opens does not occupy only the apical portion of the penis papilla but extends inwards to the base of the papilla, resulting in a large intra-penial cavity.

This large intra-penial cavity and the remarkably vertical projection from the roof of the male atrium of the papilla are characters that distinguish the new species from the others of the genus, even from any other species of Geoplaninae. Our decision about placing the species in *Cratera* is supported by molecular data shown in Carbayo *et al.* (2013); the new species forms part of a clade constituted exclusively by species of *Cratera*.

The presumed torsion of the penis papilla in *C. cuarassu* results in a morphological deviation from its congeners, and thus it resembles geoplaninids of other genera, viz., *Issoca rezendei* (Schirch, 1929), *Gigantea*

*gouvernoni* Jones & Sterrer, 2005, and the following Peruvian species of *Geoplana* listed as *incertae sedis*: *Geoplana cantuta* du Bois-Reymond Marcus, 1951, *G. chalona* du Bois-Reymond Marcus, 1951, *G. gabriellae* du Bois-Reymond Marcus, 1951, *G. lambaya* du Bois-Reymond Marcus, 1958, *G. quichua* du Bois-Reymond Marcus, 1951, *G. shapra* du Bois-Reymond Marcus, 1957 (see Carbayo *et al.*, 2013).



**FIGURES 18–21.** *Cratera anamariae* sp. nov. Living specimens. (18) holotype, about 30 mm in length. (19) partial view of the ventral side of the holotype. (20) dorsal view of paratype PL 1566, about 35 mm in length. (21) dorsal view of paratype PL 1568, about 35 mm in length.

***Cratera anamariae* Carbayo, sp. nov.**

**Etymology.** The specific name pays homage to Ana Maria Leal-Zanchet (Universidade do Vale do Rio dos Sinos, São Leopoldo, RS, Brazil), for her contribution to the knowledge of the triclad.

**Type material.** All specimens collected in Parque Nacional da Serra dos Órgãos, municipality of Teresópolis/RJ, Brazil by F. Carbayo *et al.* Holotype PL 1567 (field number, F2557): (S 22°27'19", W 42°59'51"). 8/Jul/2008. Anterior end: sagittal sections on 12 slides; portion containing testes: horizontal sections on 10 slides; pre-pharyngeal region: transverse sections of 7 slides; pharynx and copulatory apparatus: sagittal sections on 23 slides. Paratype PL 1566 (field number, F1337): (S 22°27'19", W 42°59'51"). 19/Dez/2007. Anterior end: sagittal sections on 16 slides; portion containing the ovaries: horizontal sections on 10 slides; pre-pharyngeal region: transverse sections of 2 slides; pharynx and copulatory apparatus: sagittal sections on 35 slides. Paratype PL 1571 (field number, F2605): (S 22°27'19", W 42°59'51"). 13/Jul/2008. Anterior end: horizontal sections on 5 slides; pharynx and copulatory apparatus: sagittal sections on 40 slides. Paratype PL 1568 (field number, F2559): (S 22°27'19", W 42°59'51"). 8/Jul/2008. Copulatory apparatus: sagittal sections on 26 slides. Paratype PL 1570 (field number, F2595): (S 22°27'19", W 42°59'51"). 8/Jul/2008. Copulatory apparatus: sagittal sections on 13 slides. Paratype PL 1572 (field number, F4190): (S 22°27'23.1", W 42°59'42.5"). 6/Jan/2010. Pharynx and copulatory apparatus: sagittal sections on 11 slides. Paratype PL 1569 (field number, F2594): (S 22°27'19", W 42°59'51"). 10/Jul/2008. Anterior end: sagittal sections on 32 slides; pre-pharyngeal region: transverse sections of 6 slides; pharynx and copulatory apparatus: sagittal sections on 28 slides.

**Type-locality.** Parque Nacional da Serra dos Órgãos, Teresópolis, State of Rio de Janeiro, Brazil.

**Diagnosis.** The species is distinguished from its congeners by the following combination of characters: yellow dorsum with two, sometimes four longitudinal dark stripes; cylindrical pharynx, with the dorsal and ventral insertion in the same transverse plane; and an anteriorly-narrowed female atrium.

**External aspect.** At rest the adults are up to 4 cm in length and 1 cm in width. The body is lanceolate, with both ends pointed. The body is about 1 mm in height. When creeping its movement is smooth. The dorsum is convex, the ventral side flat. The ground color is luminous yellow or sulfur yellow, with two paramedian black stripes with 1/9th to 1/5th of the body width (Figs. 18–23). The stripes are thinner towards the ends of the body, joining each other anteriorly. The dorsum between these stripes may be cream colored or light pink. The margins of the anteriormost 5 mm are black. Two submarginal, black brown stripes may also be present throughout the body. The ventral side is cream in color, becoming dark along the margins of the anteriormost 5 mm. In the six fixed, mature specimens, the mouth lies at a distance from anterior tip equal to 70–80% (mean 77%) of body length; the gonopore, 84–94% (mean 89%).

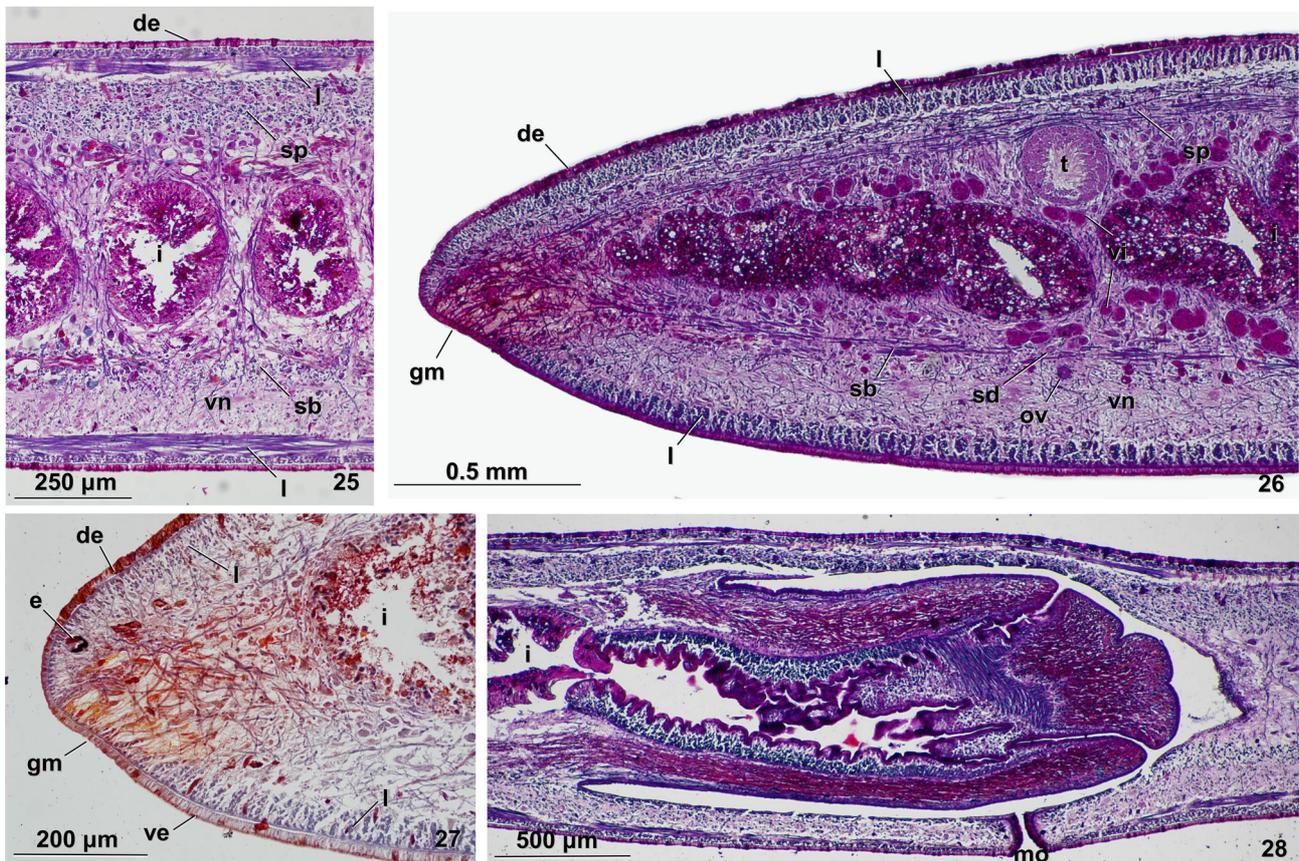
The eyes are of the pigmented-cup type, 35  $\mu$ m in diameter. They are distributed from the very anterior tip back to the very posterior end. From the very anterior end, they spread over the dorsum on a band with one third of the body width, i.e., they lie beneath the lateral dark stripes (Fig. 24). Those under pigmented stripes are surrounded by clear halos in the fixed specimens.

The sensory pits are distributed ventro-marginally in a single row from the very anterior tip of the body posteriorly to an extension of 29% of body length. The creeping sole is as wide as 90% of body width at the pre-pharyngeal region.

The epidermis is ciliated only in the creeping sole. In the pre-pharyngeal region the dorsal epidermis is pierced by rhabditogen cells and cells containing erythrophilic granules. The ventral epidermis is crossed by cells containing erythrophilic granules, scarce rhabditogen cells and scarce cyanophilic glands. There is a conspicuous glandular margin constituted of gland cells of three types, one containing erythrophilic granules, other xanthophilic granules, and a third one, scarce, producing amorphous erythrophilic secretion.



**FIGURES 22–24.** *Cratera anamariae* sp. nov. (22) living paratype PL 1570, about 30 mm in length. (23) wounded paratype PL 1572, as found in nature. (24) anterior end of paratype PL 1570 in ethanol.



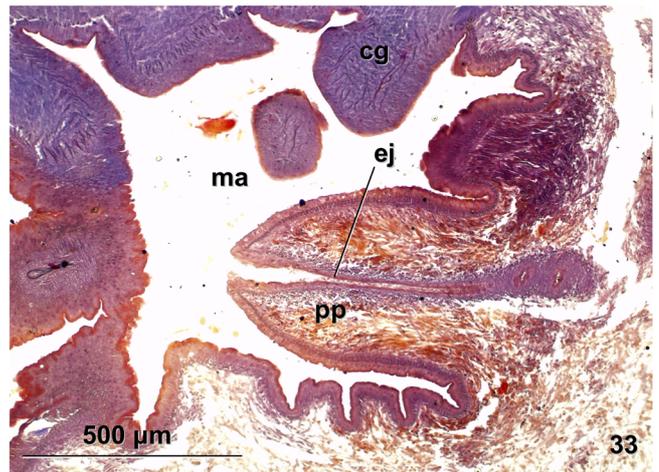
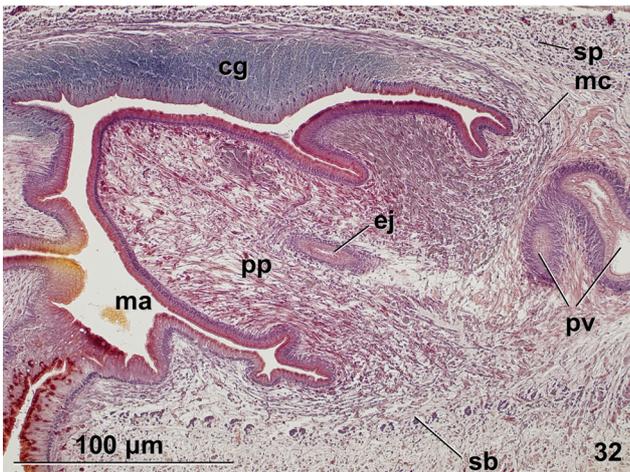
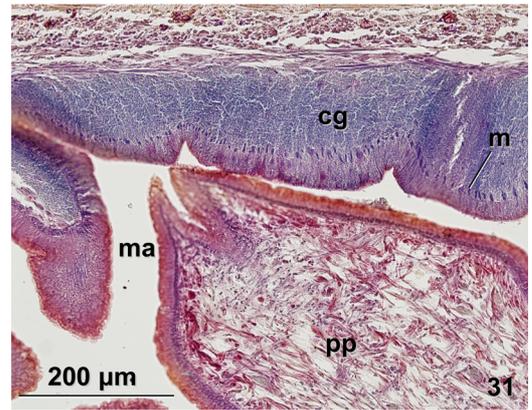
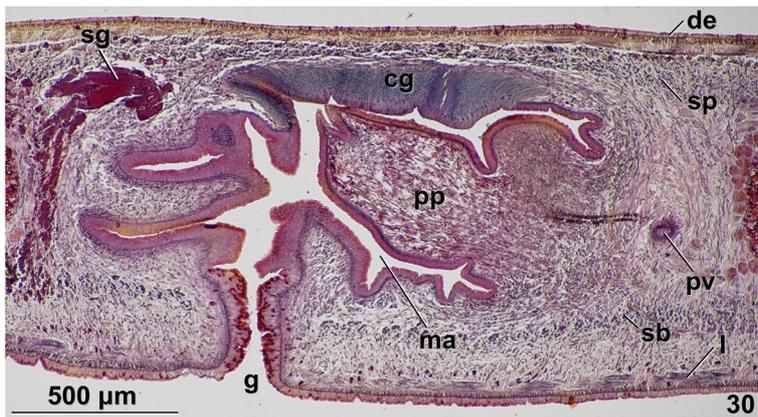
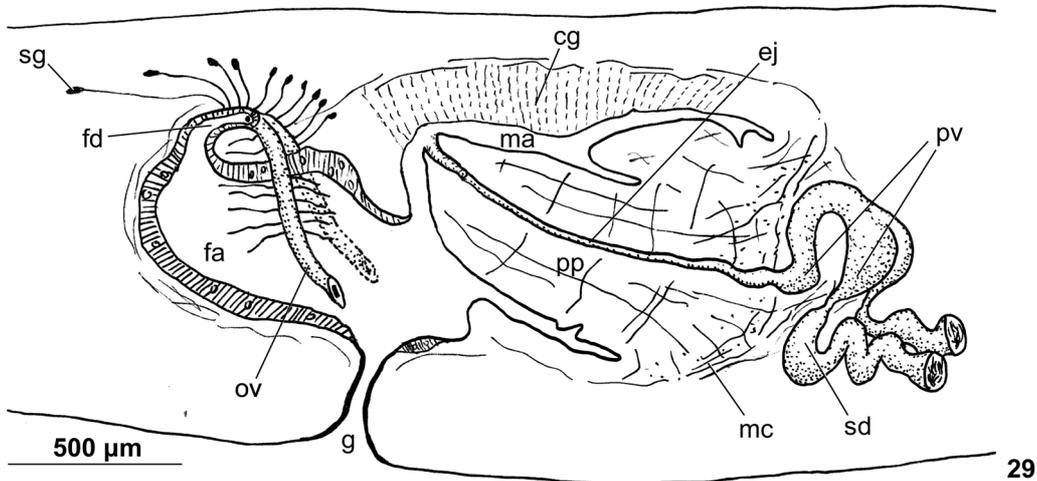
**FIGURES 25–28.** *Cratera anamariae* sp. nov. Micrographs. (25) sagittal section a region anterior to the pharynx of paratype PL 1572. (26) general view of a transverse section of the pre-pharyngeal region of the holotype. (27) glandular margin of the paratype PL 1569 in a transverse section. (28) sagittal section of the pharynx paratype PL 1572. Anterior to the left.

**Internal morphology.** The cutaneous musculature comprises the three typical layers of Geoplaninae, viz., a circular layer followed by two diagonal layers with decussate fibers, and then a longitudinal layer with fibers arranged in bundles (dorsally, 15–30 fibers each; ventrally, 25–30 fibers; (Figs. 25–27). The thickness of the cutaneous muscle is 11% of body height at the pre-pharyngeal region. The three usual parenchymal muscle layers are present throughout the body: a dorsal layer of diagonal decussate fibers (15  $\mu\text{m}$  thick in the pre-pharyngeal region), a transverse suprainestinal layer (50  $\mu\text{m}$ ), and a transverse subintestinal one (40  $\mu\text{m}$ ), the latter with its fibers more densely distributed than those of the suprainestinal layer (Figs. 25–26).

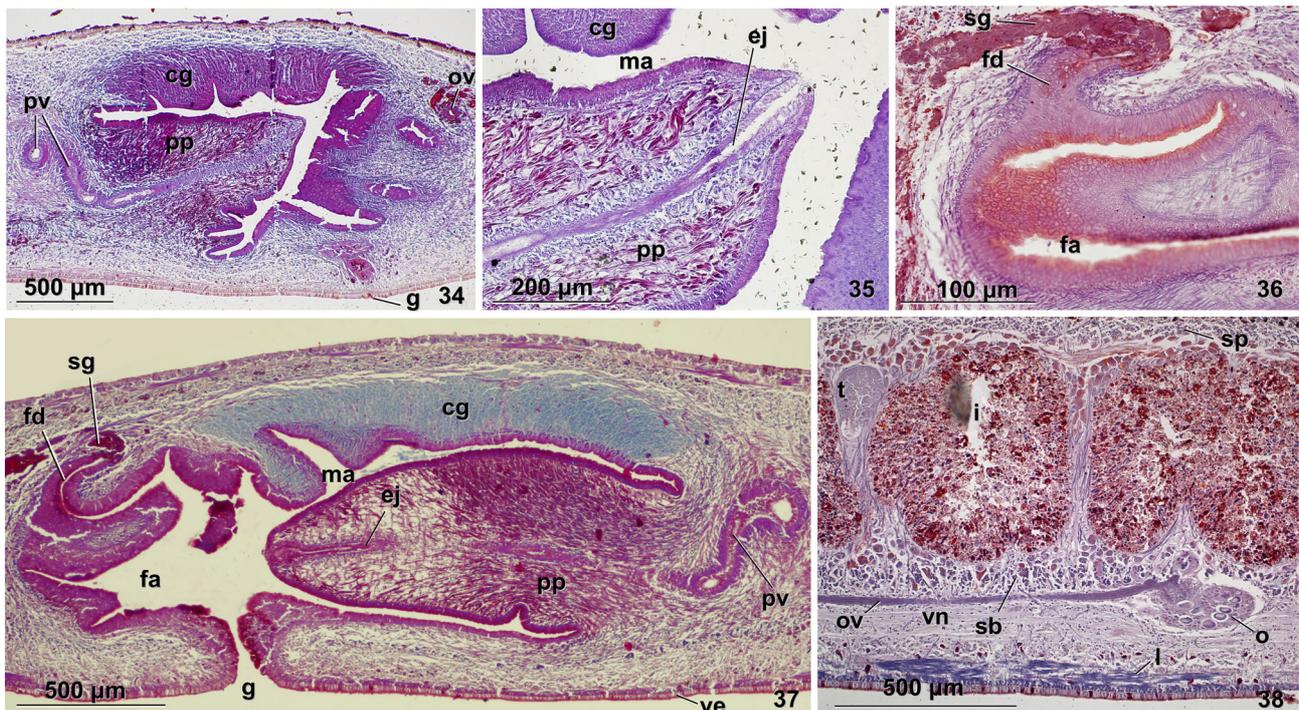
Mouth situated nearly in the middle of the pharyngeal pouch (Fig. 28). The pharynx is cylindrical. The pharyngeal pocket is lined with a non-ciliated epithelium. Three types of gland cells secrete through the distal pharyngeal epithelium, each producing either erythrophilic granules, or cyanophilic granules, or xanthophilic granules; the former is the most abundant. The outer and inner pharyngeal epithelia are ciliated. The outer one is underlain by a 5- $\mu\text{m}$ -thick layer of longitudinal muscle fibers followed by a layer of circular fibers (5  $\mu\text{m}$ ). The inner epithelium is underlain by a layer of circular fibers (70  $\mu\text{m}$ ) followed by a layer (10  $\mu\text{m}$ ) of longitudinal fibers.

The testes are dorsal and located under the suprainestinal transverse muscle layer, partially interstitial to intestinal diverticula (Figs. 26). They extend from 0.5 mm behind the ovaries to the level of the esophagus. The sperm ducts run between the fibers of the subintestinal muscle layer, dorsad to the ovovitelline ducts (Fig. 26). Their distal portion is curved anteriorly to the sagittal plane to communicate with the paired branches of the prostatic vesicle. The branches run dorso-anteriorly. Subsequently, they continue as an unpaired portion running ventrally and posteriad to penetrate the penis bulb. The prostatic vesicle is clothed with a columnar, ciliated epithelium. It is wrapped by a 20- $\mu\text{m}$ -thick layer of circular muscle. Gland cells containing fine erythrophilic granules discharge into the vesicle. The penis is of the protrusible type. The ejaculatory duct penetrates the penis papilla medially (Figs. 29–35, 37). Near the tip of the papilla, the duct is widened so as to form a kind of cavity.

The ejaculatory duct is lined with a cuboidal ciliated epithelium, grading in height from 10  $\mu\text{m}$ , to 20  $\mu\text{m}$  in the widened portion. The conical penis papilla points dorso-posteriorly, and is clothed with a 10–15  $\mu\text{m}$  high, non-ciliated epithelium. It is pierced by very numerous thick-necked gland cells (4  $\mu\text{m}$  in diameter), producing erythrophilic granules. Gland cells producing reddish-purple secretion discharge through the epithelium of the proximal third of the papilla. The papilla epithelium is underlain by a layer of circular muscle (12  $\mu\text{m}$  thick), followed by a layer of longitudinal muscles (5  $\mu\text{m}$ ).



**FIGURES 29–33.** *Cratera anamariae* sp. nov. Sagittal views. Anterior to the right. (29) diagrammatic reconstruction of the copulatory apparatus of the holotype. (30) micrograph of the copulatory apparatus of the holotype. (31) micrograph of the tip of the penis papilla of the holotype. (32) micrograph of the penis papilla of the holotype. (33) micrograph of the penis papilla of paratype PL 1568.



**FIGURES 34–38.** *Cratera anamariae* sp. nov. Sagittal views. (34) micrograph of the penis papilla of paratype PL 1566. Anterior to the left. (35) micrograph of the penis papilla of paratype PL 1566. Anterior to the left. (36) micrograph of the female genital canal and atrium of the holotype. (37) micrograph of the copulatory apparatus of paratype PL 1571. Anterior to the right. (38) micrograph of the ovary and anteriormost testis of paratype PL 1569. Anterior to the right.

The male and female atria are embedded in a muscularis comprising inner circular muscles (male atrium 5  $\mu$ m thick, female atrium 10  $\mu$ m thick) external to which is a thinner layer of longitudinal muscles (male atrium 2  $\mu$ m thick, female atrium 20  $\mu$ m thick). The atria are delineated anatomically by folding of their communal wall resulting in an inter-atrial aperture (Fig. 29), the opening of which is about half their separate heights.

Histologically the male atrium is characterized by a non-ciliated cuboidal-to-columnar epithelium. Very abundant cyanophilic secretion is discharged through the dorsal covering epithelium, whereas through the ventral one sparse erythrophilic secretion is discharged.

The ovaries are ovoid, above the ventral nerve plate (Fig. 38) and at a distance from the anterior end equal to 24% of body length. The ovovitelline ducts emerge from the dorso-external aspect of each ovary, pass posteriorly and in the proflex condition with dorsal approach, open into the female genital duct (Figs. 29, 36–37). The female atrium is ample in lateral view, but is occluded by lateral folds continued from the folding of the common atrial wall. The distal portion of ovovitelline ducts receives shell glands; the common glandular ovovitelline duct receiving these glands is markedly reduced. The female atrium is lined with a non-ciliated columnar epithelium up to 100  $\mu$ m in height, with a multilayered aspect, displaying vacuolation and penetrated by gland cells secreting erythrophilic granules. The length of the female atrium to male atrium ratio is about 1:1.

**Remarks.** Currently, the genus *Cratera* contains six species, namely *C. crioula* (E. M. Froehlich, 1955), *C. joia* (Froehlich, 1956), *C. pseudovaginuloides* (Riester, 1938), *C. steffeni* Rossi *et al.*, 2014, *C. tamoia* (E. M. Froehlich, 1955), and *C. yara* (E. M. Froehlich, 1955), besides *C. cuarassu* sp. nov. Regarding the color pattern of the dorsum, the species clearly differs from *C. crioula*, *C. joia*, *C. tamoia* and *C. yara* in that in these species the dorsum is dark excepting a narrow, pale midline. It also differs from *Cratera pseudovaginuloides* and *C. steffeni*, in that in these species the longitudinal stripes run on an orange ground color. Furthermore, in *C. pseudovaginuloides* the stripes are restricted to the median region, whereas in *C. steffeni* they are thin and nearly marginal. The new species also differs from *Cratera cuarassu* sp. nov. in that the ground color of the latter is only visible as a pair of submarginal light stripes on a predominant dark color.

Regarding the internal morphology, the new species differs from all other species of *Cratera*, namely, the dorsal insertion of the penis papilla is anterior to the ventral one, so that the organ is dorso-posteriad oriented, and the anterior portion of the female atrium is narrowed.

There are still 59 nominal species of Geoplaninae (Tyler *et al.*, 2006–2013), the internal anatomy of which remains unknown. These species are placed under the collective genus *Pseudogeoplana* Ogren & Kawakatsu, 1990. The genus was proposed for geoplaninid *species inquirendae* and *nomina dubia* (Ogren & Kawakatsu, 1990). Six species within this group show a body color pattern consisting of one or two pairs of dark stripes on a yellowish ground, namely *P. brittlebanki* (Von Graff, 1897), *P. nobilis* (Von Graff, 1899), *P. oerstedii* (Von Graff, 1899), *P. perspicillata* (Von Graff, 1899), *P. rostrata* (Von Graff, 1899) and *P. theresopolitana* (Schirch, 1929).

*Pseudogeoplana brittlebanki*, from Tigre (near Buenos Aires, Argentina), and *P. nobilis*, from nearby Corral (Chile), differ from the new species in that their paramedian black stripes are wider, 24% and 27% of the body width, respectively. Furthermore, in *P. nobilis* these stripes are more closely placed each other, the posterior end is red-brownish, and the ventral side presents a color pattern similar to that of the dorsum. *Pseudogeoplana oerstedii*, from Palermo (Buenos Aires, Argentina) differs from the new species in that its ventral body margins are brownish. *Pseudogeoplana perspicillata* and *P. rostrata*, both from Blumenau (Santa Catarina, Brazil), differ from the new species in that their lateralmost stripes are marginal and wider, and the ventral side displays a grayish lateral bands on each side of the body. Additionally, the ground color of the dorsum in *P. perspicillata* is dark brown-reddish, whereas in *P. rostrata* the pair of dark bands fades before reaching the anterior end of the body.

Unlike the original descriptions of species of *Pseudogeoplana* discussed earlier, *P. theresopolitana* was only very briefly described (Schirch, 1929). Besides, it was described from a single specimen, the anterior end of which was lacking. The original description reads "*Geoplana theresopolitana* nov. spec. 30 mm in length, 4 mm in width. The cephalic end of the specimen here described is lacking. The dorsum is shiny yellow and with two black stripes with the same width. The eyes are arranged in a row, becoming scarce posteriorly. The ventral side is light, but with the help of a lens fine yellowish spots become visible. Species close to *G. rostrata*, even possibly identical. One specimen from Teresópolis" (original description is in Portuguese; Schirch, 1929). Schirch did not illustrate his species, but Graff did for *P. rostrata*. The dorsum of the latter is very similar to that of specimens of *C. anamariae* Carbayo, **sp. nov.** having a two-stripe color pattern. Thus, *C. anamariae* Carbayo, **sp. nov.** matches well the features of *P. theresopolitana* except for the yellowish spots on the ventral side, that are absent in the new species, and the distribution of the eyes. In regard to the latter, we are of the opinion that distribution of the eyes might not have been precisely outlined. Schirch's description subtly implied that the eyes are marginal ("arranged into a row"), but he also suggests conspecificity with *P. rostrata* (Von Graff, 1899), whose eyes are dorsal, as in the new species.

While the features of *P. theresopolitana* do not exclude conspecificity with *C. anamariae* **sp. nov.**, they do not confirm it, either. Further investigation of Schirch's species is impracticable as the type-specimen was not found in the Museu Nacional do Rio de Janeiro (MNRJ, Rio de Janeiro, Brazil), where he was employed. It is apparently lost (Guilherme Muricy, pers. comm.). Therefore, we here describe these specimens as a new species.

## Acknowledgements

Instituto Chico Mendes de Conservação da Biodiversidade (Brazil) and Instituto Estadual do Ambiente (RJ) licensed the field work; Júlio Pedroni, Cláudia Olivares, Welton Araújo, Marília da Costa, Débora Redivo, Alceli Rodrigues and Leonardo Nunes (EACH, USP) helped collection of the specimens; Adriano Maximiano, Diego Santos and Ana Cristina Vasconcellos (EACH, USP) made the histological preparations; Dr. Guilherme Muricy (MNRJ) provided information on type of *P. theresopolitana*. Dr. Leigh Winsor is specially thanked for his critical reading of a previous version of the manuscript. Dr. Hugh Jones and Dr. Ana Maria Leal-Zanchet are fully acknowledged for their constructive comments. São Paulo Research Foundation (FAPESP, process no. 2014/13661–8) and Fundación BBVA provided financial support to FC.

## References

- Bois-Reymond Marcus, E. du (1951) On South American geoplanids. *Boletim da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo, Série Zoologia*, 16, 217–255.  
<http://dx.doi.org/10.11606/issn.2318-5988>
- Bois-Reymond Marcus, E. du (1957) On Turbellaria. *Academia Brasileira de Ciências*, 29 (1), 153–159.

- Carbayo, F., Álvarez-Presas, M., Olivares, C.T., Marques, F.P.L. Froehlich, E.M. & Riutort, M. (2013) Molecular phylogeny of Geoplaninae (Platyhelminthes) challenges current classification: Proposal of taxonomic actions. *Zoologica Scripta*, 42, 508–828.  
<http://dx.doi.org/10.1111/zsc.12019>
- Cason, J.E. (1950) A rapid one-step Mallory-Heidenhain stain for connective tissue. *Stain Technology*, 25 (4), 225–226.  
<http://dx.doi.org/10.3109/10520295009110996>
- Froehlich, C.G. (1956) Planárias terrestres do Paraná. *Dusenía*, 7 (4), 173–196.
- Froehlich, E.M. (1955) Sobre espécies brasileiras do gênero *Geoplana*. *Boletim da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo, Série Zoologia*, 19, 289–369.  
<http://dx.doi.org/10.11606/issn.2318-5988.v2i0p3-10>
- Humason, G.L. (1967) *Animal tissue techniques*. W. H. Freeman and Company, San Francisco, 569 pp.
- Jones, H.D. & Sterrer, W. (2005) Terrestrial planarians (Platyhelminthes, with three new species) and nemertines of Bermuda. *Zootaxa*, 1001, 31–58.
- Kiernan, J.A. (1990) *Histological and histochemical methods: theory and practice*. 2<sup>nd</sup> Edition. Pergamon Press, Oxford, 433 pp.
- Marcus, E. (1951) Turbellaria brasileiros (9). *Boletim da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo, Série Zoologia*, 16, 5–215.  
<http://dx.doi.org/10.11606/issn.2318-5988>
- Ogren, R.E. & Kawakatsu, M. (1990) Index to the species of the family Geoplanidae (Turbellaria, Tricladida, Terricola) Part I: Geoplaninae. *Bulletin of the Fuji Women's College, Serie II*, 28, 79–166.
- Riester, A. (1938) Beiträge zur Geoplaniden-Fauna Brasiliens. *Abhandlungen der Senckenbergischen naturforschenden Gesellschaft*, 441, 1–88.
- Rossi, I., Fontoura, M., Amaral, S. & Leal-Zanchet, A.M. (2014) A new species of land flatworm (Platyhelminthes: Continenticola) from areas of Araucaria Forest in southern Brazil. *Zootaxa*, 3794 (4), 514–524.  
<http://dx.doi.org/10.11646/zootaxa.3794.4.2>
- Schirch, P. (1929) Sobre as planarias terrestres do Brasil. *Boletim do Museu Nacional do Rio de Janeiro*, 5, 27–38.
- Tibiriçá, L.C. (1984) *Dicionário Tupi-Português*. Traço ed., São Paulo, 200 pp.
- Tyler, S., Schilling, S., Hooge, M. & Bush, L.F. (comp.) (2006–2013) Turbellarian taxonomic database. Version 1.7 Available from: <http://turbellaria.umaine.edu/turbella.php> (accessed 1 October 2014)
- Von Graff, L. (1899) *Monographie der Turbellarien: II. Tricladida Terricola*. Engelmann, Leipzig, 575 pp.
- Winsor, L. (1998) Aspects of taxonomy and functional histology in terrestrial flatworms (Tricladida, Terricola). *Pedobiologia*, 42, 412–432.