RESEARCH ARTICLE



# Three new species of Cratera Carbayo et al., 2013 from Araucaria forests with a key to species of the genus (Platyhelminthes, Continenticola)

Ilana Rossi<sup>1</sup>, Ana Leal-Zanchet<sup>1</sup>

Instituto de Pesquisas de Planárias and Programa de Pós-Graduação em Biologia, Universidade do Vale do Rio dos Sinos, 93022-750 São Leopoldo, Rio Grande do Sul, Brazil

Corresponding author: Ana Leal-Zanchet (zanchet@unisinos.br)

Academic editor: D. Gibson	Received 11 November 2016	Accepted 7 December 2016	Published 5 January 2017
	http://zoobank.org/F0716052-7604	-4828-A7D8-D788D5A241CF	

**Citation:** Rossi I, Leal-Zanchet A (2017) Three new species of *Cratera* Carbayo et al., 2013 from *Araucaria* forests with a key to species of the genus (Platyhelminthes, Continenticola). ZooKeys 643: 1–32. https://doi.org/10.3897/ zooKeys.643.11093

## Abstract

Areas of *Araucaria* moist forest have been considered to constitute hotspots of land flatworm diversity, harbouring a high number of undescribed species. Herein we describe three new species of land flatworms of *Cratera* Carbayo et al., 2013 occurring in such type of forest in south Brazil. The three species are differentiated from their congeners mainly by their colour pattern, anatomy of the pharynx and prostatic vesicle, and details of the penis papilla and male atrium. An identification key to species of the genus in the Neotropical region is provided.

#### Keywords

Atlantic Forest, Geoplaninae, land flatworms, Neotropical region, taxonomy, Tricladida

## Introduction

The subfamily Geoplaninae, which has a Neotropical distribution, shows high diversity in Brazilian tropical forests (Winsor et al. 1998, Sluys 1999, Álvarez-Prezas et al. 2014). Among the phytophysiognomies which constitute the Brazilian Atlantic Forest, the mixed ombrophilous forest (*Araucaria* moist forest) has been considered to constitute

hotspots of land flatworm diversity, harbouring many yet undescribed species (Leal-Zanchet and Baptista 2009, Leal-Zanchet et al. 2011). Most flatworm species described from the *Araucaria* moist forest occur in areas from its southern portion (Lemos and Leal-Zanchet 2008, Amaral et al. 2012, Leal-Zanchet et al. 2012, Lemos et al. 2014, Rossi et al. 2014, 2015).

The subfamily Geoplaninae is currently composed of 24 genera (Sluys et al. 2009, Carbayo et al. 2013), six of them recently proposed based on a combination of morphological and molecular analyses to encompass some of the species that belonged to the genus *Geoplana* Stimpson, 1857. Among them, the genus *Cratera* Carbayo, Álvarez-Presas, Olivares, Marques, Froehlich and Riutort, 2013 was proposed for five species occurring in areas of the Brazilian Atlantic Forest. Recently, another five species were described (Rossi et al. 2014, 2015; Carbayo and Almeida 2015; Negrete and Brusa 2016). Herein three new species are described, occurring in areas covered by *Araucaria* moist forest in south Brazil and a taxonomic key provided for species of *Cratera*.

#### Materials and methods

Land planarians were collected in two protected areas located in the Iguassu River Drainage Basin, namely the Três Barras National Forest (26°09.27'–26°16.9'S; 50°16.0'–50°21.22'W), in Três Barras, state of Santa Catarina, and a private reserve named Araucaria Natural Heritage Private Reserve (26°20.35'–26°26.13'S; 51°19.49'– 51°25.29'W), in General Carneiro, state of Paraná, both in south Brazil. They were collected during the day by direct sampling in leaf litter, under and inside fallen logs and under stones or during the night, when they are more active, by visual search.

Colour pattern and body shape and dimensions of live specimens were recorded. Specimens were then killed with boiling water and fixed in neutral formalin 10% and subsequently maintained in 70% ethyl alcohol. Methods described by Rossi et al. (2015) were used for histological processing of material and analysis of external and internal characters. The material was sectioned at intervals of 6  $\mu$ m and stained with Masson's trichrome method or haematoxylin and eosin (Romeis 1989).

Type-material is deposited in the Museu de Zoologia da Universidade do Vale do Rio dos Sinos, São Leopoldo, state of Rio Grande do Sul, Brazil (**MZU**), and the Helminthological Collection of Museu de Zoologia da Universidade de São Paulo, state of São Paulo, Brazil (**MZUSP**).

#### Abbreviations used in the figures

dorsal band

db

cg	cyanophil glands		
cmc	common muscle coat	de	dorsal epidermis
cov	common glandular ovovitelline duct	df	dorsal flecks

- di
- di dorsal insertion

dm	dorsal cutaneous musculature	om	outer musculature
dsm	dorsal subcutaneous mesenchymatic	ov	ovovitelline ducts
	musculature	р	penis papilla
e	eyes	рр	pharyngeal pouch
eg	erythrophil glands	ps	paramarginal stripe
ej	ejaculatory duct	pv	prostatic vesicle
es	oesophagus	rg	rhabditogen glands
fa	female atrium	sg	shell glands
fc	female canal	sbm	sub-intestinal transverse mesen-
gm	glandular margin		chymatic musculature
go	gonopore	sd	sperm ducts
ĥ	halos	sp	sensory pit
i	intestine	spm	supra-intestinal transverse mesen-
im	internal musculature		chymatic musculature
lu	pharyngeal lumen	SV	spermiducal vesicle
m	mouth	t	testes
ma	male atrium	v	vitelline follicles
mas	marginal stripe	ve	ventral epidermis
mes	median stripe	vi	ventral insertion
n	nerve cord	vm	ventral cutaneous musculature
0	ovary	xg	xanthophil glands

## Taxonomy

Family Geoplanidae Stimpson, 1857 Subfamily Geoplaninae Stimpson, 1857 *Cratera* Carbayo et al., 2013

#### Cratera cryptolineata sp. n.

http://zoobank.org/0A70BDC4-AA06-46E6-A8E3-3F31686E6513

**Material examined.** Holotype: MZUSP PL.1690: *leg.* I. Rossi, 3 June 2015, Três Barras (Três Barras National Forest), state of Santa Catarina, Brazil – anterior tip: transverse sections on 12 slides; anterior region at the level of the ovaries: sagit-tal sections on 25 slides; pre-pharyngeal region: transverse sections on 16 slides; pharynx: sagittal sections on 30 slides; copulatory apparatus: sagittal sections on 25 slides.

Other specimens: all specimens sampled in the same locality as the holotype. MZU PL.00217: *leg.* J. A. L. Braccini, 29 July 2015 – anterior tip: transverse sections on 15 slides; anterior region at the level of the ovaries: sagittal sections on 16 slides; pre-pharyngeal region: transverse sections on eight slides; pharynx and copulatory apparatus: sagittal sections on 19 slides. MZU PL.00218: *leg.* J. A. L. Braccini, 27 July 2015 – pre-pharyngeal region: transverse sections on 14 slides; pharynx and copulatory



#### 10 mm



apparatus: sagittal sections on 20 slides. MZU PL.00219: leg. J. A. L. Braccini, 2 June 2015 – copulatory apparatus: horizontal sections on 10 slides.

Diagnosis. Species of Cratera with dark-brown dorsal colour, thin median stripe and greyish margins; eyes dorsal with clear halos; pharynx cylindrical; prostatic vesicle almost horizontal; penis papilla conical and symmetrical occupying distal portion of female atrium.

Description. External features. Body elongate with parallel margins and dorsal surface slightly convex; anterior tip rounded and posterior tip pointed (Fig. 1). When creeping, maximum length 52mm. After fixation, maximum length 40mm (Table 1). Mouth and gonopore located at posterior fourth of body in average (Table 1).

Live specimens with dorsal surface homogeneous dark-brown (Fig. 1). Under stereomicroscope, greyish ground colour visible on anterior tip, on body margins, as well as on thin median stripe occurring along body except for cephalic region. Ventral surface light brown. After fixation, dorsal pigmentation becomes light brown with darker body margins, constituting marginal stripes; thin median stripe remains greyish (Figs 2, 4–5). Ventral surface becomes light grey.

Eyes monolobate, initially uniserial, surround anterior tip (Figs 3-4). After first millimetre of body, eyes become larger and spread onto dorsal surface, occupying maximum width of about one-third of body width on either side of body. Eyes remain dorsal and relatively numerous towards posterior tip (Fig. 3). Inconspicuous clear halos may occur around dorsal eyes (Figs 4–5). Diameter of pigment cups 20–30 µm.

Sensory organs, epidermis and body musculature. Sensory pits (Figs 6-7), as simple invaginations (30-40 µm deep), contour anterior tip and occur ventromarginally in irregular, single row in anterior 1/6th of body. Creeping sole occupies the whole body width in pre-pharyngeal region (Fig. 11).

Three types of glands discharge through whole epidermis of pre-pharyngeal region: rhabditogen glands with xanthophil secretion (ventrally with smaller rhabdites)

Measurement	Holotype MZUSP PL.1690	Specimen MZU PL.00217	Specimen MZU PL.00218	Specimen MZU PL.00219
Maximum length in extension	50	43	52	45
Maximum width in extension	3	2	2	3
Length at rest	25	10	30	20
Width at rest	6	5	4	7
Length*	40	35	33	34
Width*	4	3	3.5	3.5
DM*	29 (72%)	27 (77%)	25 (76%)	27 (79%)
DG*	37 (92%)	32.5 (93%)	30 (91%)	31 (91%)
DMG*	8	5.5	5	4
DPVP*	4	2.6	2.7	-
Ovaries	9 (22%)	8 (23%)	-	-
Anteriormost testes	9.5 (24%)	10 (29%)	-	_
Posteriormost testes	25.5 (64%)	23.5 (67%)	22 (67%)	-
Length of prostatic vesicle	0.4	0.3	0.3	0.35
Length of penis papilla	0.8	0.6	0.6	0.35
Length of male atrium	0.7	0.5	0.5	_
Length of female atrium	0.5	0.3	0.3	_

**Table 1.** Measurements, in mm, of specimens of *Cratera cryptolineata* sp. n. Abbreviations: – not measured; \* after fixation; DG distance of gonopore from anterior end; DM distance of mouth from anterior end; DMG distance between mouth and gonopore; DPVP distance between prostatic vesicle and pharyngeal pouch. The numbers given in parentheses represent the position relative to body length.

and cyanophil glands with amorphous secretion, besides scarce xanthophil glands with fine granular secretion (Figs 10–11). Glandular margin (Figs 8–9) visible after first millimetre of body. At least four types of glands constitute glandular margin: numerous erythrophil glands with fine granules and xanthophil glands with coarse granules; scarcer cyanophil glands with fine granules and rhabditogen glands with xanthophil rhabdites. Glands discharging through anterior tip of body similar to those of prepharyngeal region (Figs 6–7).

*Cutaneous musculature* with usual three layers (circular, oblique and longitudinal layers); longitudinal layer with thick bundles (Figs 8–11, Table 2), becoming progressively lower towards body margins. Thickness of cutaneous musculature between two and five times that of epidermis (Table 2). Ventral musculature with similar thickness or slightly thicker than dorsal musculature at sagittal plane in pre-pharyngeal region (Table 2). In relation to body height, cutaneous musculature thinner in pre-pharyngeal region than in cephalic region (Table 2); thickness gradually diminishes towards anterior tip (Fig. 6).

*Mesenchymal musculature* (Figs 6, 8, 10–11) well developed, mainly composed of three layers: (1) dorsal subcutaneous, located close to cutaneous musculature, with decussate fibres (3–9 fibres thick), (2) supra-intestinal transverse (5–10 fibres thick) and (3) sub-intestinal transverse (6–15 fibres thick). Mesenchymal musculature less developed in anterior region (Fig. 6) than in pre-pharyngeal region.



**Figures 2–5.** *Cratera cryptolineata* sp. n., dorsal view, **2** holotype, pattern of pigmentation **3** specimen MZU PL.00217, eye pattern **4–5** holotype, anterior extremity (**4**) and median third of body (**5**).

Table	e <b>2</b> .	Body	height	and	cutaneous	muscu	lature	in the	media	in region	of a	transversal	sect	ion	of the
pre-pł	hary	ngeal	(PP) an	d cej	phalic (CE	) regior	ns, in	micror	netres,	and ratio	of t	he thickne	ss of	cuta	neous
muscu	ılatu	re to	the heig	ht of	f the body	( <i>mc:h</i> i1	ndex)	of spec	imens	of Crater	a cry	<i>otolineata</i> s	p. n.		

Measurement	Holotyp PL	e MZUSP .1690	Specime PL.00	n MZU 0217	Specimen MZU PL.00218	
	PP	CE	PP	CE	РР	
Dorsal cutaneous musculature	50	47	43	51	45	
Ventral cutaneous musculature	58	55	41	50	53	
Dorsal epidermis	9	9	12	9	12	
Ventral epidermis	25	12	19	12	19	
Body height	1252	794	1054	818	955	
<i>Mc:h</i> (%)	9	13	8	12	10	



**Figures 6–13.** *Cratera cryptolineata* sp. n., holotype, **6–7** anterior region, transverse section **8–11** prepharyngeal region, transverse sections: body margin (9), dorsal portion (10), ventral portion (11) 12 pharynx, sagittal section 13 ovary, sagittal section.



**Figures 14–15.** *Cratera cryptolineata* sp. n., **14** holotype, sagittal composite reconstruction of copulatory apparatus **15** specimen MZU PL.00219, horizontal composite reconstruction of copulatory apparatus.

**Digestive system.** *Pharynx* cylindrical, nearly 5% of body length, occupies 81% of pharyngeal pouch. Pharyngeal dorsal insertion posteriorly shifted next to end of anterior third of pharyngeal pouch. Mouth slightly posterior to dorsal insertion (Fig. 12). Oesophagus short, with folded walls. Oesophagus: pharynx ratio 5%–9%.

Pharynx and pharyngeal lumen lined by ciliated, cuboidal epithelium, becoming squamous towards pharyngeal tip, with insunk nuclei. Pharyngeal glands constituted by four gland types: erythrophil glands of two types (with coarse and fine granular secretion); xanthophil glands with fine granular secretion and cyanophil glands with amorphous secretion. Outer pharyngeal musculature (4–8  $\mu$ m thick) comprised of subepithelial layer of longitudinal fibres followed by layer of circular fibres. Inner pharyngeal musculature (30–40  $\mu$ m thick) composed of thick subepithelial layer with circular fibres, followed by thin layer of longitudinal fibres. Both muscle layers become thinner towards pharyngeal tip. Oesophagus lined by ciliated, cuboidal to columnar epithelium with some insunk nuclei; Musculature of oesophagus (60–100  $\mu$ m thick) composed of thick subepithelial layer with longitudinal fibres.

**Reproductive organs.** *Testes* in one irregular row on either side of body, located beneath dorsal transverse mesenchymal muscles, between intestinal branches (Figs 8, 10), begin slightly posteriorly to ovaries, in anterior fourth of body, and extend to near root of the pharynx (Table 1). Sperm ducts medial to ovovitelline ducts, under or among fibres of sub-intestinal transverse mesenchymal musculature, in pre-pharyngeal region (Fig. 11). They form spermiducal vesicles posteriorly to pharynx. Distally, spermiducal vesicles enter laterally into proximal portion of prostatic vesicle (Figs 14–15, 17). Extrabulbar prostatic vesicle, unpaired, located near common muscle coat, with proximal portion ample and distal portion tubular and sinuous. Proximal portion laterally expanded and T-shaped (Figs 15, 17), almost horizontal, but located closer to ventral epidermis than to dorsal epidermis (Figs 14, 16). Ejaculatory duct almost straight, opening through expansion at tip of penis papilla (Figs 14, 18). Male atrium without folds. Penis papilla conical and symmetrical, projecting into distal portion of female atrium (Figs 14–18, Table 1).

Sperm ducts lined with ciliated, cuboidal epithelium and coated with thin muscularis (approximately 2  $\mu$ m thick) constituted of interwoven circular and longitudinal fibres. Prostatic vesicle lined with ciliated, tall columnar epithelium. Muscularis of prostatic vesicle (8–20  $\mu$ m thick) comprises longitudinal and circular intermingled fibres. Ejaculatory duct lined with ciliated, columnar epithelium, with irregular height at expanded portion (Fig. 18). Muscle coat of ejaculatory duct thin (about 4  $\mu$ m), mainly constituted of circular fibres. Numerous erythrophil glands with fine granular secretion as well as glands with amorphous cyanophil secretion open into both prostatic vesicle and ejaculatory duct. Penis papilla and male atrium columnar (nearly 40  $\mu$ m thick) lined with non-ciliated, columnar epithelium. Xanthophil and erythrophil glands with fine granular secretion, besides glands with amorphous cyanophil secretion open through penis papilla and male atrium. Openings of cyanophil glands more numerous into male atrium and concentrated at dorso-lateral wall (Fig. 16). Muscula-



**Figures 16–17.** *Cratera cryptolineata* sp. n., **16** holotype, copulatory apparatus, sagittal section **17** specimen MZU PL.00219, copulatory apparatus, horizontal section.



Figures 18–19. *Cratera cryptolineata* sp. n., holotype, sagittal sections, 18 penis papilla 19 female organs. Arrows indicate lacunae.

ris of penis papilla (10–20  $\mu$ m thick) and male atrium (6–10  $\mu$ m thick) composed of subepithelial circular layer, followed by longitudinal layer.

Vitelline follicles (Figs 8, 10–11, 13) situated between intestinal branches. Ovaries ovoid (approximately 200  $\mu$ m in diameter), dorsal to ventral nerve plate, in anterior fourth of body (Fig. 13, Table 1). Ovovitelline ducts emerge dorsally from median third of ovaries (Fig. 13) and run posteriorly immediately above nerve plate. Ascending portion of ovovitelline ducts located lateral to female atrium. Common glandular ovovitelline duct short, located dorsally to posterior third of female atrium (Figs 14–16, 19). Female genital duct dorso-anteriorly curved. Female atrium funnel-shaped without folds (Figs 16, 19), shorter than male atrium (Table 1).

Ovovitelline ducts and common ovovitelline duct lined with ciliated, columnar epithelium and covered with 5-µm-thick layer of intermingled circular and longitudinal muscle fibres. Numerous shell glands with erythrophil secretion empty into common glandular ovovitelline duct as well as into distal half of ascending portion of ovovitelline ducts (Figs 14–16, 19). Epithelial lining of female genital duct and atrium with irregular height (40–90 µm thick), stratified appearance; epithelial cells with some lacunae containing secretion (Fig. 19). Abundant cyanophil glands with amorphous secretion and erythrophil glands with fine granular secretion, as well as few xanthophil glands with fine granular secretion open into female duct and atrium. Muscularis of female duct and atrium (10–20 µm thick) composed of interwoven longitudinal and circular fibres. Specimens MZU PL.00218, MZU PL.00217 and MZU PL.00219 not fully mature, with poorly developed vitelline follicles, but showing shell glands opening into ovovitelline ducts and common glandular oviduct.

Male and female *atria* with ample communication, without separating folds (Figs 14–17). Common muscle coat thin along both male and female atria, thicker dorsally than ventrally, composed of circular, longitudinal and oblique fibres. Gonoduct vertical, lined with ciliated, columnar epithelium. Numerous cyanophil glands with amorphous

secretion and rhabditogen glands with xanthophil secretion, as well as scarce erythrophil glands with fine granular secretion empty into gonoduct. Muscularis of gonoduct comprised of subepithelial layer of circular fibres, followed by longitudinal layer.

**Etymology.** The specific name is a composite of the Greek adjective *kryptós* (hidden) and the Latin noun *linea* (stripe), referring to the thin median stripe, visible only under the stereomicroscope.

**Distribution.** Known only from the type locality.

#### *Cratera nigrimarginata* sp. n. http://zoobank.org/2EA2144E-2F1B-4752-9CBB-2AD34CA51633

**Material examined.** Holotype: MZUSP PL.1691: *leg.* I. Rossi, 18 July 2015, General Carneiro (Araucaria Natural Heritage Private Reserve), state of Paraná, Brazil – anterior region in three fragments on 114 slides; pre-pharyngeal region: transverse sections on 10 slides; pharynx: sagittal sections on 19 slides; copulatory apparatus: sagittal sections on 17 slides.

Other specimens: all specimens sampled in the same locality as the holotype. MZU PL.00220: *leg.* I. Rossi, 6 February 2015 – anterior tip: transverse sections on 24 slides; anterior region at the level of the ovaries: sagittal sections on 78 slides; pre-pharyngeal region: transverse sections on 16 slides; pharynx: sagittal sections on 40 slides; copulatory apparatus: sagittal sections on 33 slides. MZU PL.00221: *leg.* J. L. A. Braccini, 4 June 2015 – copulatory apparatus: horizontal sections on 20 slides.

**Diagnosis.** Species of *Cratera* with light-brownish dorsal colour bordered by dark margins; eyes dorsal with clear halos and bilobed appearance; pharynx cylindrical; prostatic vesicle with unbranched and dilated proximal portion; tip of penis papilla with infolds projecting into ejaculatory duct; cyanophil glands pierce male atrium evenly distributed.

**Description. External features.** *Body* elongate, flat and with parallel margins; anterior tip rounded and posterior tip pointed (Figs 20–22). When creeping, maximum length 57mm. After fixation, maximum length 47mm. Mouth and gonopore located at posterior fourth of body (Table 3).

Live animals with dorsal surface light-brownish, constituting broad band, bordered by greyish or black margins; cephalic region greyish (Figs 20–22). Ventral surface pale yellow (Fig. 21). Under stereomicroscope, dorsal band bordered by thin black paramarginal stripes. After fixation, besides dorsal band and paramarginal stripes, dorsal surface may contain inconspicuous median stripe (Fig. 23); ventral surface becomes whitish with greyish margins and anterior tip. In preserved specimens, dorsal band with maximum width of about two thirds of body width. Paramarginal stripes, with nearly 1/12<sup>th</sup> of body width, begin behind the cephalic region (approximately anterior 1/9<sup>th</sup> of body) and converge towards posterior tip (Figs 23, 25).

*Eyes*, initially uniserial and monolobate, surround anterior tip (Figs 24–25). After second millimetre of body, eyes become larger and with bilobated appearance (Fig. 27), spreading onto dorsal surface and occupying almost all body width in anterior



Figures 20–22. *Cratera nigrimarginata* sp. n., habitus, dorsal view, 20 holotype, anterior region 21 specimen MZU PL.00220 with part of ventral surface visible 22 specimen MZU PL.00221.

**Table 3.** Measurements, in mm, of specimens of *Cratera nigrimarginata* sp. n. Abbreviations: – not measured; \* after fixation; DG distance of gonopore from anterior end; DM distance of mouth from anterior end; DMG distance between mouth and gonopore; DPVP distance between prostatic vesicle and pharyngeal pouch. The numbers given in parentheses represent the position relative to body length.

Measurement	Holotype MZUSP PL.1691	Specimen MZU PL.00220	Specimen MZU PL.00221
Maximum length in extension	55	57	55
Maximum width in extension	4	4	4
Length at rest	30	46	35
Width at rest	6	5	5
Length*	46	47	45
Width*	5	5.5	4.5
DM*	35.5 (77%)	37 (79%)	37 (82%)
DG*	43.5 (94%)	42 (89%)	41 (91%)
DMG*	8	5	4
DPVP*	0.7	0.6	0.8
Ovaries	10 (22%)	11.5 (24%)	_
Anteriormost testes	13 (28%)	15.5 (33%)	_
Posteriormost testes	30.5 (66%)	33.5 (71%)	-
Length of prostatic vesicle	0.8	0.8	0.7
Length of penis papilla	1.2	1.3	1
Length of male atrium	0.9	0.9	0.9
Length of female atrium	1.2	1.6	1.2

third of body (Fig. 24). Some eyes surrounded by inconspicuous small clear halos over paramarginal stripes (Figs 25–26). Eyes less numerous towards posterior tip. Diameter of pigment cups 15–40  $\mu$ m.



Figures 23–26. *Cratera nigrimarginata* sp. n., dorsal view, 23 holotype, pattern of pigmentation 24–26 specimen MZU PL.00220, eye pattern (24), anterior extremity (25) and median third of body (26).

Sensory organs, epidermis and body musculature. Sensory pits (Fig. 28), as simple invaginations (30–40  $\mu$ m deep), contour anterior tip and occur ventromarginally in irregular, single row in anterior 1/8th of body. Creeping sole occupies 90% of body width in pre-pharyngeal region.

Three types of glands discharge through whole epidermis of pre-pharyngeal region: rhabditogen cells with xanthophil secretion (ventrally with smaller rhabdites), cyanophil glands with amorphous secretion and xanthophil glands with fine granular secretion (Figs 31–32). Few erythrophil glands with fine granular secretion open through ventral epidermis. Glandular margin conspicuous (Figs 29–30), after first millimetre of body. At least five types of glands constitute glandular margin: xanthophil glands with coarse granules of two types (heavily and slightly stained), cyanophil glands of two types (coarse granular and amorphous secretion) and erythrophil glands with coarse granules. Glands discharging through anterior tip of body similar to those of pre-pharyngeal region (Fig. 28).

*Cutaneous musculature* with usual three layers (circular, oblique and longitudinal layers); longitudinal layer with thick bundles (Figs 29–32, Table 4). Thickness of cu-



**Figures 27–34.** *Cratera nigrimarginata* sp. n., holotype, **27** dorsal eye, horizontal section **28** anterior region of body, transverse section **29–32** pre-pharyngeal region, transverse sections, body margin (**30**), dorsal portion (**31**), ventral portion (**32**) **33** pharynx, sagittal section **34** ovary, sagittal section.

Mannant	Holotype MZ	USP PL.1691	Specimen MZU PL.00220		
Measurement	PP	CE	РР	CE	
Dorsal cutaneous musculature	71	37	72	35	
Ventral cutaneous musculature	85	85	70	77	
Dorsal epidermis	15	15	19	15	
Ventral epidermis	22	15	22	15	
Body height	1277	484	1104	484	
<i>Mc:h</i> (%)	12	25	13	23	

**Table 4.** Body height and cutaneous musculature in the median region of a transverse section of the pre-pharyngeal (PP) and cephalic (CE) regions, in micrometres, and ratio of the thickness of cutaneous musculature to the height of the body (mc:h index) of specimens of *Cratera nigrimarginata* sp. n.

taneous musculature between two and four times that of epidermis (Table 4). Ventral musculature with similar thickness or slightly thicker than dorsal musculature at sagittal plane in pre-pharyngeal region (Table 4). Musculature becoming progressively lower towards body margins. In relation to body height, cutaneous musculature thinner in pre-pharyngeal region than in cephalic region, especially ventral musculature (Table 4, Fig. 28); thickness gradually diminishes towards anterior tip.

*Mesenchymal musculature* (Figs 28–29, 31–32) well developed, mainly composed of three layers: (1) dorsal subcutaneous, located close to cutaneous musculature, with decussate fibres (6–10 fibres thick), (2) supra-intestinal transverse (8–14 fibres thick) and (3) sub-intestinal transverse (10–18 fibres thick). Mesenchymal musculature more developed in cephalic region (Fig. 28) than in pre-pharyngeal region, especially dorsal subcutaneous musculature (12–20 fibres thick).

**Digestive system.** *Pharynx* cylindrical, approximately 6% of body length, occupies 70% of pharyngeal pouch. Pharyngeal dorsal insertion slightly shifted posteriorly (Fig. 33); mouth in median third of pharyngeal pouch. Oesophagus short with folded wall. Oesophagus: pharynx ratio 4%–12%.

Pharynx and pharyngeal lumen lined by ciliated, cuboidal epithelium with insunk nuclei. Pharyngeal glands constituted by four secretory cell types: abundant erythrophil glands with fine granular secretion, xanthophil glands with coarse granular secretion, as well as two types of cyanophil glands (with amorphous and fine granular secretions). Outer pharyngeal musculature (10–30  $\mu$ m thick) comprised of thin subepithelial layer of circular fibres, followed by thin layer of longitudinal fibres. Inner pharyngeal musculature (70–90  $\mu$ m thick) comprises thick subepithelial layer of circular fibres, followed by thinner layer of longitudinal fibres. Outer and inner muscle layers gradually become thinner towards pharyngeal tip. Oesophagus lined by ciliated, cuboidal to columnar epithelium with insunk nuclei. Musculature of oesophagus (70–120  $\mu$ m thick) composed of thick layer with circular fibres, followed by layer of longitudinal fibres.

**Reproductive organs.** *Testes* in one irregular row on either side of body, located beneath dorsal transverse mesenchymal muscles (Figs 29, 31), begin slightly posteriorly to ovaries, in anterior third of body, and extend to near root of pharynx (Table 3).

Sperm ducts medial to ovovitelline ducts, among fibres of sub-intestinal transverse mesenchymal musculature, forming spermiducal vesicles laterally to pharynx. Distally, spermiducal vesicles penetrate into lateral wall of proximal portion of prostatic vesicle (Figs 35–36, 38). Prostatic vesicle extrabulbar, unpaired, consisting of two portions: proximal portion short and dilated and distal portion tubular and sinuous. Proximal portion displaced ventrally in relation to distal portion and located closer to ventral epidermis than to dorsal epidermis (Figs 35, 37). Prostatic vesicle of specimen MZU PL.00220 showing larger lumen, filled with secretions. Ejaculatory duct almost straight, expanding at tip of penis papilla. Male atrium without folds. Penis papilla conical and symmetrical (Figs 35–38, Table 3). Tip of penis papilla occupying distal part of female atrium; with infolds projecting into ejaculatory duct (Fig. 40).

Lining epithelium of *sperm ducts* cuboidal and ciliated; thin muscularis (about 5  $\mu$ m thick) constituted of interwoven circular and longitudinal fibres. Prostatic vesicle lined with ciliated, columnar epithelium. Muscularis of prostatic vesicle (20–40  $\mu$ m thick) comprises mainly circular fibres mixed with longitudinal and oblique fibres (Fig. 39). Ejaculatory duct lined with ciliated, tall columnar epithelium (Fig. 40). Muscle coat of ejaculatory duct (5–10  $\mu$ m) constituted of interwoven circular and longitudinal fibres. Erythrophil glands with fine granular secretion as well as cyanophil glands with amorphous secretion open into both prostatic vesicle and ejaculatory duct (Fig. 39). Penis papilla and male atrium lined with non-ciliated, columnar epithelium. Numerous cyanophil glands with amorphous secretion and few erythrophil glands with fine granular secretion open evenly distributed through penis papilla and male atrium. Muscularis of penis papilla (10–20  $\mu$ m thick) and male atrium (6–10  $\mu$ m thick) comprised of subepithelial layer of circular fibres, followed by layer of longitudinal fibres.

Vitelline follicles (Figs 29, 31, 34) situated between intestinal branches. Ovaries ovoid (approximately 200  $\mu$ m in diameter) located dorsal to ventral nerve plate, in anterior fourth of body (Fig. 34, Table 3). Ovovitelline ducts emerge laterally from posterior half of ovaries and run posteriorly immediately above nerve plate. Ascending portion of ovovitelline ducts located lateral to female atrium. Common glandular ovovitelline duct short, located dorsally to posterior third of female atrium. Female genital duct dorso-anteriorly curved (Figs 35–37, 41). Female atrium oval-elongate with folded walls (Figs 35, 37), longer than male atrium (Table 3).

Ovovitelline ducts and common ovovitelline duct lined with ciliated, cuboidal to columnar epithelium and covered with intermingled circular and longitudinal muscle fibres (3–10  $\mu$ m). Abundant shell glands with erythrophil secretion, besides cyanophil glands, empty into common glandular ovovitelline duct as well as into distal third of ascending portion of ovovitelline ducts (Figs 35–38, 41). Epithelial lining of female genital duct and atrium tall columnar, showing irregular height and sometimes stratified appearance (50–300  $\mu$ m thick), ciliated in female duct. Epithelial cells with some lacunae containing cyanophil secretion (Figs 37, 41). Abundant cyanophil glands with amorphous secretion and less numerous erythrophil glands with fine granules open into female duct and atrium. Muscularis (10–20  $\mu$ m thick) of female genital duct



1 mm

**Figures 35–36.** *Cratera nigrimarginata* sp. n., **35** holotype, sagittal composite reconstruction of copulatory apparatus **36** specimen MZU PL.00221, horizontal composite reconstruction of copulatory apparatus.

and atrium composed of interwoven circular and longitudinal fibres. Specimen MZU PL.00220 shows poorly developed vitelline follicles, but copulatory organs, including shell glands, fully developed.



Figures 37–38. *Cratera nigrimarginata* sp. n., 37 holotype, copulatory apparatus, sagittal section 38 specimen MZU PL.00221, copulatory apparatus, horizontal section.

Male and female *atria* broadly communicated each other, without separating folds (Figs 35–38). Common muscle coat thin along both male and female atria, thicker dorsally than ventrally, composed of circular, longitudinal and oblique fibres. Gonod-uct vertical, lined with ciliated columnar epithelium. Numerous cyanophil glands with amorphous secretion and rhabditogen glands open into gonoduct. Muscularis of gonoduct comprised of thin subepithelial layer of circular fibres, followed by thin layer of longitudinal fibres.

**Etymology.** The specific name is a composite of the Latin adjective *niger* (black) and the Latin noun *margo* (margin), referring to the colour pattern with dark margins.

**Distribution.** Known only from its type locality.



Figures 39–41. *Cratera nigrimarginata* sp. n., holotype, sagittal sections, **39** prostatic vesicle **40** penis papilla **41** female organs. Arrows indicate lacunae.

## *Cratera aureomaculata* sp. n. http://zoobank.org/E4F5F32E-D05F-49C7-92A6-670A85E1D052

**Material examined.** Holotype: MZUSP PL.1692: *leg.* J. L. A. Braccini, 3 June 2015, Três Barras (Três Barras National Forest), state of Santa Catarina, Brazil – anterior tip: transverse sections on 19 slides; anterior region at the level of the ovaries: sagittal sections on 56 slides; pre-pharyngeal region: transverse sections on 14 slides; pharynx: sagittal sections on 35 slides; copulatory apparatus: sagittal sections on 31 slides.

**Diagnosis.** Species of *Cratera* with dorsal ground colour yellowish covered by brownish pigmentation in cephalic region and blackish pigmentation constituting irregular flecks over rest of dorsum; eyes dorsal with clear halos; pharynx cylindrical; prostatic vesicle unpaired with proximal portion displaced ventrally, laterally expanded and T-shaped; penis papilla conical and symmetrical with ventral insertion posteriorly displaced.

**Description. External features.** *Body* elongate with parallel margins and dorsal surface slightly convex; anterior tip rounded and posterior tip obtuse (Fig. 42). When creeping, maximum length 55mm. After fixation, maximum length 46mm. Mouth and gonopore located at posterior fourth of body (Table 5).

Live specimens with dorsal ground colour yellowish, covered by brownish pigmentation in cephalic region. Behind cephalic region, blackish pigmentation constitutes irregular flecks over dorsal surface, larger laterally and more concentrated towards posterior tip (Figs 42–43). Ventral surface light grey with yellowish margins; cephalic region (nearly anterior 1/8th of body length) brownish with darker margins.

*Eyes* monolobate, initially uniserial, surround anterior tip (Figs 44–45). After first millimetre of body, eyes become larger and spread onto dorsal surface, occupying maximum width of approximately one-third of body width on either side of body. Eyes remain dorsal, but less numerous towards posterior tip (Figs 44, 46). Some eyes over dorsal flecks surrounded by inconspicuous clear halos (Figs 45–46). Diameter of pigment cups  $20-40 \mu m$ .



10 mm

Figure 42. Cratera aureomaculata sp. n., holotype, habitus, dorsal view.

**Table 5.** Measurements, in mm, of the holotype of *Cratera aureomaculata* sp. n. Abbreviations: \* after fixation; DG distance of gonopore from anterior end; DM distance of mouth from anterior end; DMG distance between mouth and gonopore; DPVP distance between prostatic vesicle and pharyngeal pouch. The numbers given in parentheses represent the position relative to body length.

Measurement	Holotype MZUSP PL.1692
Maximum length in extension	55
Maximum width in extension	4
Length at rest	30
Width at rest	6
Length*	46
Width*	5
DM*	35.5 (77%)
DG*	43.5 (95%)
DMG*	8
DPVP*	4
Ovaries	12.5 (27%)
Anteriormost testes	15.5 (34%)
Posteriormost testes	30 (65%)
Length of prostatic vesicle	0.3
Length of penis papilla	0.7
Length of male atrium	0.8
Length of female atrium	0.6

Sensory organs, epidermis and body musculature. Sensory pits (Figs 47–48), as simple invaginations (30–60  $\mu$ m deep), contour anterior tip and occur ventromarginally in irregular, single row in anterior third of body. Creeping sole occupies whole body width in pre-pharyngeal region (Fig. 52).

Three types of *glands* discharge through whole epidermis of pre-pharyngeal region: abundant rhabditogen cells with xanthophil secretion (rhammites), cyanophil glands with amorphous secretion and xanthophil glands with fine granular secretion (Figs 51–52). Glandular margin (Figs 49–50) visible after anterior 1/16th of body. At least four types of glands constitute glandular margin: xanthophil and erythrophil glands, both

42



**Figures 43–46.** *Cratera aureomaculata* sp. n., holotype, dorsal view, **43** pattern of pigmentation **44** eye pattern **45–46** anterior extremity (**45**) and median third of body (**46**).

with coarse granular secretions, besides few xanthophil and cyanophil glands with fine granules. Glands discharging through anterior tip of body similar to those of pre-pharyngeal region (Figs 47–48).

*Cutaneous musculature* with usual three layers (circular, oblique and longitudinal layers); longitudinal layer with thick bundles (Figs 49–52, Table 6). Thickness of cutaneous musculature between four and five times that of epidermis (Table 6). Ventral musculature thicker than dorsal at sagittal plane in pre-pharyngeal region (Table 6). Musculature becoming progressively lower towards body margins. In relation to body height, cutaneous musculature slightly thinner in pre-pharyngeal region than in cephalic region (Figs 47–48), especially ventral musculature (Table 6); thickness gradually diminishes towards anterior tip.

*Mesenchymal musculature* (Figs 49, 51–52) well developed, mainly composed of three layers: (1) dorsal subcutaneous, located close to cutaneous musculature, with decussate fibres variously oriented (3–5 fibres thick), (2) supra-intestinal transverse (8–14 fibres thick) and (3) sub-intestinal transverse (8–18 fibres thick). Mesenchymal musculature less developed in cephalic region (Fig. 47) than in pre-pharyngeal region.



Figures 47–54. *Cratera aureomaculata* sp. n., holotype, 47–48 anterior region, transverse section
49–52 pre-pharyngeal region, transverse sections: body margin (50), dorsal portion (51), ventral portion (52)
53 pharynx, sagittal section 54 ovary, sagittal section.

M	Holotype MZUSP PL.1692				
Measurement	РР	CE			
Dorsal cutaneous musculature	57	42			
Ventral cutaneous musculature	79	50			
Dorsal epidermis	15	9			
Ventral epidermis	22	12			
Body height	1240	719			
Mc:h (%)	11	13			

**Table 6.** Body height and cutaneous musculature in the median region of a transversal section of the pre-pharyngeal (PP) and cephalic (CE) regions, in micrometres, and ratio of the thickness of cutaneous musculature to the height of the body (mc:h index) of the holotype of *Cratera aureomaculata* sp. n.

**Digestive system.** *Pharynx* cylindrical, approximately 4% of body length, occupies 90% of pharyngeal pouch. Pharyngeal dorsal insertion slightly shifted posteriorly. Mouth in median third of pharyngeal pouch (Fig. 53). Oesophagus short with folded wall. Oesophagus: pharynx ratio 24%.

Pharynx and pharyngeal lumen lined by ciliated, cuboidal epithelium with insunk nuclei. Pharyngeal glands constituted by four secretory cell types: numerous erythrophil and xanthophil glands, both with fine granular secretion and cyanophil glands with amorphous secretion, as well as less numerous xanthophil glands with coarse granular secretion. Outer pharyngeal musculature (6–12  $\mu$ m thick) comprised of thin subepithelial layer of longitudinal muscles, followed by layer of circular fibres. Inner pharyngeal musculature (60–110  $\mu$ m thick) comprises thick subepithelial layer of circular fibres, followed by layer of longitudinal fibres. Outer and inner muscle layers gradually become thinner towards pharyngeal tip. Oesophagus lined by ciliated, cuboidal to columnar epithelium with insunk nuclei. Musculature of oesophagus (30–50  $\mu$ m thick) composed of thick layer with circular fibres, followed by layer of longitudinal fibres.

**Reproductive organs.** *Testes* in one irregular row in either side of body, located beneath dorsal transverse mesenchymal muscles (Figs 49, 51), begin slightly behind anterior third of body and extend to near root of pharynx (Table 5). Sperm ducts medial to ovovitelline ducts, among fibres of sub-intestinal transverse mesenchymal musculature, form spermiducal vesicles posteriorly to pharynx. Distally, spermiducal vesicles bend to enter laterally into proximal expanded portion of prostatic vesicle (Fig. 55). Prostatic vesicle extrabulbar, unpaired, located near common muscle coat, with ample proximal portion and tubular distal portion. Proximal portion laterally expanded and T-shaped, displaced ventrally in relation to distal portion and located closer to ventral epidermis than to dorsal epidermis (Figs 55–57). Ejaculatory duct with slightly sinuous proximal portion and expanded distal portion (Figs 57, 58). Male atrium without folds. Penis papilla conical and symmetrical with ventral insertion posteriorly displaced (Figs 55–56, 58, Table 5).

Sperm ducts lined with ciliated, cuboidal epithelium and coated with thin muscularis (about 3  $\mu$ m thick) constituted of interwoven circular and longitudinal fibres.



**Figure 55.** *Cratera aureomaculata* sp. n., holotype, sagittal composite reconstruction of copulatory apparatus.

Prostatic vesicle lined with ciliated, tall columnar epithelium (Fig. 57). Muscularis of prostatic vesicle (8–20  $\mu$ m thick) comprises interwoven circular, longitudinal and oblique fibres. Ejaculatory duct lined with ciliated, columnar epithelium (Fig. 58). Muscle coat of ejaculatory duct thin (about 6  $\mu$ m), mainly constituted of circular fibres. Numerous glands with fine granular, mixed secretion (cyanophil external part and erythrophil internal core) empty into both prostatic vesicle and ejaculatory duct; erythrophil glands with fine granules open into ejaculatory duct. Penis papilla and male atrium lined with non-ciliated, columnar or pseudostratified epithelium (approximately 40  $\mu$ m thick). Erythrophil glands with fine granules, as well as cyanophil glands with amorphous secretion open through penis papilla and male atrium, besides xanthophil glands through penis papilla (Figs 56–58); cyanophil glands concentrate their numerous openings at dorso-lateral wall of male atrium (Figs 55–57). Muscularis of penis papilla (nearly 10  $\mu$ m thick) and male atrium (5–10  $\mu$ m thick) composed of subepithelial circular layer, followed by longitudinal layer.

Vitelline follicles (Figs 49, 51, 54) situated between intestinal branches. Ovaries oval-elongate (Fig. 54), two times longer than wide (approximately 200  $\mu$ m in diameter), located dorsal to ventral nerve plate, in anterior third of body (Table 5).



Figures 56–59. *Cratera aureomaculata* sp. n., holotype, sagittal sections, 56 copulatory apparatus 57 prostatic vesicle 58 penis papilla 59 female organs. Arrows indicate lacunae.

Ovovitelline ducts emerge dorsally from median third of ovaries and run posteriorly immediately above nerve plate. Ascending portion of ovovitelline ducts located at level of gonopore. Common glandular ovovitelline duct short, located dorsally to median third of female atrium. Female genital duct dorso-anteriorly curved (Figs 55, 59). Female atrium funnel-shaped. Length of female atrium about half that of male atrium (Figs 55–56, 59, Table 5).

*Ovovitelline ducts* and common ovovitelline duct lined with ciliated, cuboidal to columnar epithelium and covered with intermingled circular and longitudinal muscle

fibres (approximately 5  $\mu$ m thick). Abundant shell glands with erythrophil secretion, besides cyanophil glands, empty into common glandular ovovitelline duct as well as into distal third of ascending portion of ovovitelline ducts (Figs 55–56, 59). Epithelial lining of female genital duct and atrium with irregular height and stratified appearance (30–120  $\mu$ m thick); epithelial cells with some lacunae (Figs 56, 59). Abundant cyanophil glands with amorphous secretion and erythrophil glands with fine granules empty into female duct and atrium. Muscularis of female duct and atrium (8–20  $\mu$ m thick) composed of interwoven circular and longitudinal fibres.

Male and female *atria* with ample communication, without separating folds (Figs 55–56). Common muscle coat thin along both male and female atria, thicker dorsally than ventrally, composed of circular, longitudinal and oblique fibres. Gonoduct anteriorly inclined, lined with ciliated columnar epithelium. Numerous cyanophil glands with amorphous secretion, besides rhabditogen glands, open into gonoduct. Muscularis of gonoduct comprised of subepithelial layer of circular fibres, followed by longitudinal layer.

**Etymology.** The specific name is a composite of the Latin adjective *aureus* (golden) and the Latin noun *macula* (spot), referring to the colour pattern with yellowish ground colour covered by black irregular flecks.

Distribution. Known only from its type locality.

## Notes on ecology and distribution

*Cratera cryptolineata* and *Cratera aureomaculata* are sympatric in its type-locality, the Três Barras National Forest, in areas of *Araucaria* moist forest. They were recorded during night samplings in areas characterized by the dominance of *Bromelia antiachanta* Bentol. in the understorey (Fig. 60). *Cratera cryptolineata* showed high abundance in such areas, whereas *Cratera aureomaculata* was represented by a single specimen.



**Figures 60–61.** Sampling sites in type localities, **60** Três Barras National Forest, state of Santa Catarina, Brazil **61** Araucaria Natural Heritage Private Reserve, state of Paraná, Brazil.

*Cratera nigrimarginata* occurred only in its type-locality, the Araucaria Natural Heritage Private Reserve, in a site of *Araucaria* moist forest showing an initial stage of regeneration with poorly developed understorey (Fig. 61). The species showed low abundance during both day and night samplings.

#### Discussion

The three new species herein described can be easily assigned to the genus *Cratera* Carbayo et al., 2013, by presenting its diagnostic features, such as ejaculatory duct forming a distal cavity in the penis papilla, position of the ovovitelline ducts by approaching the female atrium and funnel-shaped female atrium.

By showing dorsal eyes and a cylindrical pharynx, the three species herein described share superficial similarities with four other species of *Cratera*: *C. joia* (Froehlich, 1956), *C. anamariae* Carbayo, 2015, *C. ochra* Rossi et al., 2015 and *C. viridimaculata* Negrete and Brusa, 2016 (Froehlich 1956, Carbayo and Almeida 2015, Rossi et al. 2015, Negrete and Brusa 2016), to which they are comparatively discussed.

#### Cratera cryptolineata sp. n.

Regarding the colour pattern, by having an almost homogeneous, dark brown dorsal surface with a thin median stripe, *Cratera cryptolineata* can be differentiated from *C. anamariae* and *C. ochra*, which show a yellowish ground colour with black pigmentation forming stripes or bands (Carbayo and Almeida 2015, Rossi et al. 2015), and from *C. viridimaculata*, with dorsal surface stippled with dark grey fine spots on a light olive green background. The colour pattern of *C. cryptolineata* is similar to that of *C. joia*, but the latter has a broader median stripe and conspicuous clear halos surrounding eyes, whereas in *C. cryptolineata* clear halos are inconspicuous (Froehlich 1956).

With respect to the copulatory apparatus, *C. cryptolineata*, showing a penis papilla tip slightly posterior to the gonoduct, can be differentiated from *C. joia*, in which the penis papilla is longer, occupying half of the female atrium length. In addition, *C. cryptolineata* differs from *C. joia*, *C. anamariae* and *C. viridimaculata* by having a prostatic vesicle unforked and an almost horizontal orientation, whereas in these three species it is curved ventrally, besides being forked in *C. anamariae* (Froehlich 1956, Carbayo and Almeida 2015, Negrete and Brusa 2016). By showing the penis papilla with both insertions at the same transversal level, *C. cryptolineata* can be distinguished from *C. ochra* and *C. viridimaculata*, which show the penis papilla with the ventral insertion posteriorly displaced (Rossi et al. 2015, Negrete and Brusa 2016). The anatomy of the female atrium of *C. cryptolineata*, ample and without folds, also differs from that of *C. anamariae*, which has lateral folds (Carbayo and Almeida 2015).

By showing a light-brownish dorsal colour bordered by dark marginal stripes, *Cratera nigrimarginata* can be easily differentiated from *C. anamariae*, which has two broad lateral stripes, *C. ochra*, with dispersed pigmentation forming two broad bands, and *C. viridimaculata*, which show dispersed pigmentation without forming bands (Carbayo and Almeida 2015, Rossi et al. 2015, Negrete and Brusa 2016). *Cratera nigrimarginata* can also be differentiated from *C. joia* and *C. cryptolineata*, both with a light median stripe and the rest of the dorsum strongly pigmented (Froehlich 1956). In addition, *C. nigrimarginata* differs from their congeners by having dorsal eyes with a bilobated appearance, whereas other species show typical monolobated eyes along the body.

Regarding the copulatory apparatus, *C. nigrimarginata* shows an unbranched prostatic vesicle with dilated proximal portion, being differentiated from *C. cryptolineata*, *C. ochra* and *C. joia* with a prostatic vesicle showing proximal diverticula. In addition, it differs from *C. anamariae* and *C. viridimaculata*, which show a prostatic vesicle with forked proximal portions, C-shaped in *C. viridimaculata*. By having openings of cyanophil glands evenly distributed into the male atrium, *C. nigrimarginata* also differs from these species, in which the openings of cyanophil glands concentrate dorsolaterally into the male atrium.

#### Cratera aureomaculata sp. n.

*Cratera aureomaculata* shows a distinctive colour pattern, showing a blackish pigmentation constituting irregular flecks over the yellowish dorsal ground colour and a brownish pigmentation in the cephalic region. Thus, it differs from stripped species, such as *C. nigrimarginata* and *C. anamariae*, as well as from species showing a strongly pigmented dorsal surface with a light median stripe, such as *C. joia* and *C. cryptolineata* (E.M. Froehlich 1955, Froehlich 1956, Carbayo and Almeida 2015). It can also be distinguished from *C. ochra*, which shows dispersed pigmentation forming two broad bands, and from *C. viridimaculata* with dark grey body margins and cephalic region (Rossi et al. 2015, Negrete and Brusa 2016).

With respect to the copulatory apparatus, *C. aureomaculata* shows a prostatic vesicle with proximal portion laterally expanded and T-shaped, differing from *C. nigrimarginata*, which has a prostatic vesicle with dilated proximal portion, as well as from *C. anamariae* and *C. viridimaculata* which show a prostatic vesicle with forked proximal portions, C-shaped in the latter. By showing the penis papilla with the ventral insertion posteriorly displaced and the proximal portion of the prostatic vesicle ventrally displaced, *C. aureomaculata* differs from *C. cryptolineata* with both insertions at the same transversal level and prostatic vesicle almost horizontal. *C. aureomaculata* shows the penis papilla tip anterior to the gonoduct and a common ovovitelline duct dorsal to the female atrium, being differentiated from *C. joia*, in which the penis papilla is longer, occupying half of the female atrium length, and a common ovovitelline duct is absent. *C. aureomaculata* 

can be distinguished from *C. ochra* by the position of the proximal portion of the prostatic vesicle, which is more ventrally located in relation to the rest of the vesicle in *C. aureomaculata* than in *C. ochra*, in which the prostatic vesicle is almost horizontal.

# Key to the species of the genus Cratera in the Neotropical region

1	Colour pattern with stripes or bands2
_	Colour pattern without stripes or bands8
2	Eyes spreading over the dorsal surface
_	Eyes exclusively on the margins or lateral parts of the body9
3	Pharynx cylindrical
_	Pharynx bell-form
4	Prostatic vesicle with proximal portion laterally expanded and T-shaped5
_	Prostatic vesicle with another form
5	Dark-brown dorsal colour, with a thin median stripe and greyish margins <i>C. cryptolineata</i> sp. n.
-	Yellow-ochre dorsal colour with dispersed greyish or greyish-brown pigmen- tation constituting two broad dorsal bands <i>C. ochra</i> Rossi et al., 2015
6	Unbranched prostatic vesicle with dilated proximal portion
_	Prostatic vesicle with forked proximal portion or with proximal diverticula 7
7	Dark-grevish dorsal colour with rusty median stripe, anterior tip and mar-
/	gins
_	Yellow dorsal colour with two paramedian black stripes
	<i>C. anamariae</i> Carbavo, 2015
8	Prostatic vesicle tubular and C-shaped with forked proximal portion
-	Prostatic vesicle with proximal portion laterally expanded and T-shaped
9	Short and wide penis papilla with a large intra-penial cavity
-	<i>C. cuarassu</i> Carbavo & Almeida, 2015
_	Conical and symmetrical penis papilla without intra-penial cavity
10	Pharvnx bell-form
_	Pharynx cylindrical
11	Orange ground colour with a light median stripe and greenish pigmentation
	on the anterior tip
_	Colour pattern with four thin, black stripes, besides orange marginal bands
	and thin median stripe
12	Black dorsal surface with a thin and light median stripe
_	Yellowish ground colour with brownish pigmentation forming bands

We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (Nr. 306853/2015-9 and CNPq/PELD Site 9, Nr. 403817/2012-9) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for research grants and fellowships in support of this study. We are very grateful to Prof. Dr. C.A. Sanquetta and his research group in the Universidade Federal do Paraná, especially Dr. A.L. Rodrigues, for their help in the planning and organization of the field trips, as well as for the opportunity to take part in the research project supported by CNPq/PELD Site 9. We acknowledge M.Sc. J.A.L. Braccini for his help in sampling flatworms and the laboratory technicians R. Canello and L. Guterres for their help in section preparation. We also thank M.Sc. E. Benya for an English review of the text, M.Sc. P. Boll for suggestions regarding species names and Dr. H. Jones (Natural History Museum, London, United Kingdom) and Dr. F. Carbayo (Universidade de São Paulo, São Paulo, Brazil) for comments and suggestions on an early draft of the manuscript.

## References

- Álvarez-Presas M, Sánchez-Gracia A, Carbayo F, Rozas J, Riutort M (2014) Insights into the origin and distribution of biodiversity in the Brazilian Atlantic forest hotspot: A statistical phylogeographic study using a low-dispersal organism. Heredity 112(6): 656–665. https:// doi.org/10.1038/hdy.2014.3
- Amaral SV, Oliveira SM, Leal-Zanchet AM (2012) Three new species of land flatworms and comments on a complex of species in a genus *Geoplana* Stimpson (Platyhelminthes: Continenticola). Zootaxa 3338: 1–32.
- Carbayo F, Almeida AL (2015) Anatomical deviation of male organs of land planarians from Rio de Janeiro, Brazil, with description of two new species of *Cratera* (Platyhelminthes, Tricladida). Zootaxa 3931: 27–40. https://doi.org/10.11646/zootaxa.3931.1.2
- Carbayo F, Álvarez-Presas M, Olivares CT, Marques FPL, Froehlich EM, Riutort M (2013) Molecular phylogeny of Geoplaninae (Platyhelminthes) challenges current classification: proposal of taxonomic actions. Zoologica Scripta 42(5): 508–528. https://doi. org/10.1111/zsc.12019
- Froehlich CG (1956) Planárias terrestres do Paraná. Dusenia 7(4): 173–191.
- Froehlich EM (1955) Sôbre espécies brasileiras do gênero *Geoplana*. Boletim da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo, Ser. Zoologia 19: 289–369.
- Leal-Zanchet AM, Baptista VA (2009) Planárias terrestres (Platyhelminthes, Tricladida) em remanescentes de Floresta com Araucária. In: Fonseca CR, Souza AF, Leal-Zanchet AM, Dutra T, Backes A, Ganade G (Eds) Floresta com Araucária: Ecologia, Conservação e Desenvolvimento Sustentável. Holos, Ribeirão Preto, 199–207.
- Leal-Zanchet AM, Baptista VA, Campos LM, Raffo JF (2011) Spatial and temporal patterns of land flatworm assemblages in Brazilian Araucaria forests. Invertebrate Biology 130: 25–33. https://doi.org/10.1111/j.1744-7410.2010.00215.x

- Leal-Zanchet AM, Rossi I, Seitenfus ALR, Alvarenga J (2012) Two new species of land flatworms and comments on the genus *Pasipha* Ogren & Kawakatsu, 1990 (Platyhelmintes: Continenticola). Zootaxa 3583: 1–21.
- Lemos SV, Cauduro GP, Valiati VH, Leal-Zanchet AM (2014) Phylogenetic relationships within the flatworm genus *Choeradoplana* Graff (Platyhelminthes: Tricladida) inferred from molecular data with the description of two new sympatric species from *Araucaria* moist forests. Invertebrate Systematics 28: 605–627. http://dx.doi.org/10.1071/IS14003
- Lemos SV, Leal-Zanchet AM (2008) Two new species of *Notogynaphallia* Ogren & Kawakatsu (Platyhelminthes: Tricladida: Terricola) from Southern Brazil. Zootaxa 1907: 28–46.
- Negrete L, Brusa F (2016) First report of the genus *Cratera* (Platyhelminthes, Geoplanidae) in Argentina, with description of a new species and comments on the species of the genus. ZooKeys 610: 1–12. https://doi.org/10.3897/zookeys.610.9465
- Romeis B (1989) Mikroskopische Technik. Urban und Schwarzenberg, München, 697 pp.
- Rossi I, Amaral SV, Ribeiro GG, Cauduro GP, Fick I, Valiati VH, Leal-Zanchet AM (2015) Two new Geoplaninae species (Platyhelminthes: Continenticola) from Southern Brazil based on an integrative taxonomic approach. Journal of Natural History 50: 1–29. https:// doi.org/10.1080/00222933.2015.1084057
- Rossi I, Fontoura M, Amaral S, Leal-Zanchet AM (2014) A new species of land flatworm (Platyhelminthes: Continenticola) from areas of Araucaria Forest in southern Brazil. Zootaxa 3794(4): 514–524. https://doi.org/10.11646/zootaxa.3794.4.2
- Sluys R (1999) Global diversity of land planarians (Platyhelminthes, Tricladida, Terricola): a new indicator-taxon in biodiversity and conservation studies. Biodiversity and Conservation 8(12): 1663–1681. https://doi.org/10.1023/A:1008994925673
- Sluys R, Kawakatsu M, Riutort M, Baguña J (2009) A new higher classification of planarian flatworms (Platyhelmintes, Tricladida). Journal of Natural History 43(29/30): 11763– 1777. https://doi.org/10.1080/00222930902741669
- Winsor L, Johns PM, Yeates GW (1998) Introduction, and ecological and systematic background, to the Terricola (Tricladida). Pedobiologia 42: 389–404.