

Choeradoplana crassiphalla sp. nov. (Platyhelminthes: Tricladida: Geoplanidae): a new species of land planarian from the Atlantic Forest of Argentina

Lisandro Negrete^{a,b} & Francisco Brusa^{a,b*}

^aDivisión Zoología Invertebrados, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, La Plata, Argentina; ^bCONICET

(Received 25 January 2012; accepted 26 September 2012)

A new species of land planarian from northeastern Argentina, *Choeradoplana crassiphalla* sp. nov., is described. This is the first report of *Choeradoplana* in the Atlantic Forest of Argentina and the second species of the genus for this country. *Choeradoplana* includes nine species of Neotropical land planarians. The new species is light brown with a thin clearer median stripe. The pharynx is bell-shaped. The copulatory apparatus presents a short and very high and thick penis papilla. *Choeradoplana crassiphalla* is compared with other species of the genus, especially with *Ch. marthae*, due to the similarity of the copulatory apparatus.

Keywords: terrestrial flatworm; Geoplaninae; Choeradoplana species; northeastern Argentina

Introduction

The genus Choeradoplana includes nine species of Neotropical land planarians. Eight of the nine known species have been described for Brazil (von Graff 1899; Marcus 1951; Froehlich 1955; Carbayo & Froehlich 2012), whereas Ch. langi (von Graff, 1894) has been described for Paraguay, and recorded in Argentina (Buenos Aires province) and Brazil (Du Bois-Reymond Marcus 1951; Ogren & Kawakatsu 1990). These species are characterized by their small size (20-50 mm length) and the anterior tip dilated and rolled towards the back when the flatworms crawl above the soil. The anterior tip possesses two glandular cushions, separated by a median groove. The glandular cushions are the glandulo-muscular organ, named "Drüsenpolster" by von Graff (1899). Also, the genus is characterized by possessing part of the cutaneous longitudinal muscle layer partially sunk into the parenchyma. The cephalic retractor muscle (derived from that layer) has a triangular shape in cross section (Carbayo & Leal-Zanchet 2003).

The land planarian fauna from Argentina is poorly known. Most of the descriptions and records date back to the nineteenth century (von Graff 1894, 1897, 1899). With the recent record of a non-native species, *Caenoplana coerulea* Moseley, 1877 (Negrete et al. 2011), 14 species of land planarians (Geoplanidae) are known for Argentina. Three subfamilies are represented: 10 species of Geoplaninae, three species of Rhynchodeminae and one species of Bipaliinae.

There are no records of land planarian species in the Argentinean portion of the Atlantic Forest. Only *Bipalium kewense* Moseley, 1878 was found in the vicinity of native forests (Negrete et al. 2011). The Atlantic Forest of South America is one of the world's 25 recognized biodiversity hotspots, with more than 60% of all terrestrial species on earth (Galindo-Leal & Câmara 2003). The Atlantic Forest is a complex of ecoregions that span the Atlantic coast of Brazil, extending westward into eastern Paraguay and northeastern Argentina (Misiones province) (Di Bitetti et al. 2003).

The aim of this paper is to describe a new species of land planarian of the genus *Choeradoplana* from the Atlantic Forest of Argentina.

Material and methods

The land flatworms were collected between 2009 and 2010 in the Reserva de Vida Silvestre Urugua-í (RVSU) (25°59′ S, 54°05′ W) and Campo Anexo INTA in San Antonio County (SA) (26°02′ S, 53°47′ W), Misiones province, Argentina. The two sampling locations belong to the Atlantic Forest ecosystem. The first locality is within the Upper Paraná Atlantic Forest ecoregion, characterized by a semideciduous seasonal forest, with trees ranging in height from 20 to 50 m, with strata of smaller trees and a dense undergrowth of bamboo (Giraudo et al. 2003). The second one belongs to the *Araucaria* moist forest ecoregion, with

the predominance of the Paraná pine *Araucaria angustifolia* and tree ferns.

The geoplanids were manually collected during the day beneath fallen logs, leaf litter and stones, and during the night, when planarians are active. The animals were photographed in vivo and their external morphology and color pattern were recorded. The specimens were killed with boiling water, fixed in 10% formaldehyde and conserved in 70% ethanol. Body fragments were dehydrated in an ascending series of ethanol and embedded in Paraplast[®]. Sagittal and transverse serial sections (8 µm thick) of the anterior body region, transverse sections of the pre-pharyngeal region (8 µm thick) and sagittal serial sections of the pharynx and the copulatory apparatus (8 µm thick) were stained with Masson's trichrome method and hematoxylin-eosin method (Romeis 1989). The ratio of the height of cutaneous musculature to the height of the body (cutaneous muscular index, CMI) was calculated according to Froehlich (1955). The copulatory apparatus of the specimens was reconstructed for descriptive and diagnostic purposes.

The holotype, paratypes and additional specimens were deposited in the Invertebrate Collection at Museo de La Plata (MLP), Argentina.

Results

Family **Geoplanidae** Stimpson, 1857 Subfamily **Geoplaninae** Stimpson, 1857 Genus *Choeradoplana* von Graff, 1896 *Choeradoplana crassiphalla* sp. nov. (Figures 1–7)

Type series

Holotype: MLP 6561. Argentina, Misiones, RVSU, 23 May 2009, L. Negrete, coll.; anterior region: transverse sections on five slides; pre-pharyngeal region: transverse sections on five slides; pharynx and copulatory apparatus: sagittal sections on 11 slides. Paratypes: MLP 6225. Argentina, Misiones, SA, 16 May 2009, L. Negrete, coll.; anterior region: sagittal sections on 23 slides; pre-pharyngeal region: transverse sections on 14 slides; pharynx and copulatory apparatus: sagittal sections on 15 slides. MLP 6226-3. Argentina, Misiones, RVSU, 22 August 2009, L. Negrete, coll.; anterior region 1: transverse sections on 12 slides; anterior region 2: sagittal sections on eight slides; pre-pharyngeal region: transverse sections on five slides; pharynx and copulatory apparatus: sagittal sections on 12 slides. MLP 6481. Argentina, Misiones, SA, 5 November 2010, L. Negrete, coll.; anterior region 1: transverse sections on 15 slides; anterior region 2: sagittal sections on 14 slides; prepharyngeal region: transverse sections on four slides; pharynx and copulatory apparatus: sagittal sections on 19 slides. MLP 6562. Argentina, Misiones, RVSU, 21 August 2009, L. Negrete, coll.; anterior region: transverse sections on eight slides; pre-pharyngeal region: transverse sections on three slides; pharynx and copulatory apparatus: sagittal sections on nine slides.

Additional material examined

MLP 6563. Argentina, Misiones, RVSU, 22 August 2009, L. Negrete, coll.; preserved in 70% ethanol. MLP 6564. Argentina, Misiones, SA, 18 May 2009, L. Negrete, coll.; preserved in 70% ethanol.

Diagnosis

The new species can be distinguished from the other species in the genus by the following particular combination of features: light brown colored dorsal surface with a thin clear median longitudinal stripe, and yellowish brown body margin; intra-bulbar prostatic vesicle consisting of a large and highly folded chamber; the short, high and thick penis papilla – ventrally to the prostatic vesicle – hanging from the roof of the male atrium; female atrium 3–5 times shorter than male atrium.

Etymology

The specific name is derived from the Latin: *crassus* (thick) and *phallus* (penis), due to the features of the penis papilla.

Type locality

Reserva de Vida Silvestre Urugua-í, northern Misiones province, Argentina.

Description

External morphology

Dorsum brown, ranging from light to darker pigmentation. Very thin median longitudinal stripe, lighter than ground color, being 5% with respect to body width (Figure 1A, B). Median stripe starting posteriorly of light gray anterior tip, continuing throughout the body and disappearing just before the posterior end. Body margins yellowish brown, ventral surface pale yellowish brown to whitish. Some specimens with posterior end black. Anterior tip blunt, ventrally with striations separated by a median groove. Posterior end narrowing gradually.

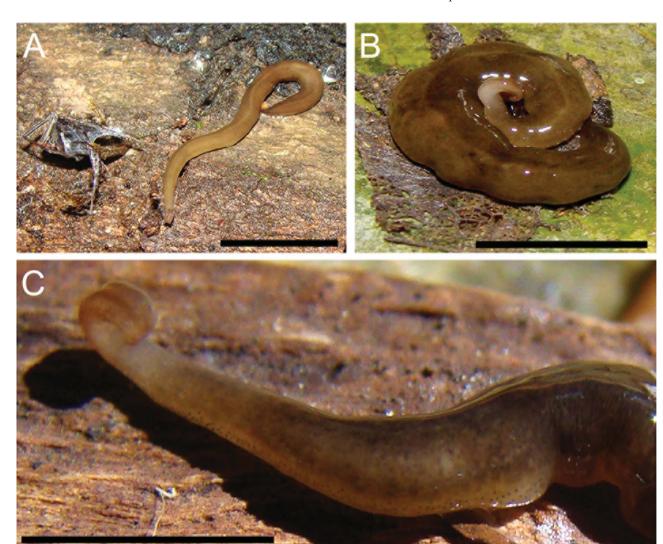


Figure 1. Photographs of living specimens of *Choeradoplana crassiphalla* sp. nov. (A) Holotype, with harvestman trapped in mucus. Scale bar: 10 mm. (B) Paratype MLP 6226-3, at rest. Scale bar: 10 mm. C. Detail of the anterior end of another specimen. Scale bar: 5 mm.

On both sides eyes beginning 1–2 mm behind anterior end, being absent at the tip. Eyes initially uniserial and marginal, then bi- or triserial and marginal, very close to each other for 4–5 mm (Figure 1C). Thereafter, eyes bi- or uniserial, some scattered, less numerous at mouth level, reaching posterior end of the body.

After fixation, body length between 22 and 43 mm, maximum width 1.5–3.5 mm, maximum height 0.9–1.9 mm. Mouth located at a distance of 55.5–62.5% from anterior tip; gonopore at 66.3–73.3%, respectively (Table 1).

Internal morphology

Cephalic region with musculo-glandular organ, consisting of very abundant erythrophil and rhabditogen

secretion, and a retractor muscle. Secretions forming two glandular cushions, separated by a median groove, on both sides of retractor muscle (Figure 2A). Fibers of retractor muscle concentrated in median ventral region, beneath nervous plate. Retractor muscle triangle-shaped in cross section (Figure 2A). Thin layer of transversal muscles (5 μ m thick) present between nervous plate and retractor muscle, thus forming a sub-neural layer. In anterior region, supraintestinal fibers well developed forming an intermingled muscle (Figure 2A). Fibers of cutaneous longitudinal muscle layer partially sunk into the parenchyma.

Sensory pits as simple invaginations (30–40 μ m deep) present in ventral body margins (Figure 2B). They appear posteriorly from anterior tip, forming a sensory border until 1–1.5 mm from anterior tip.

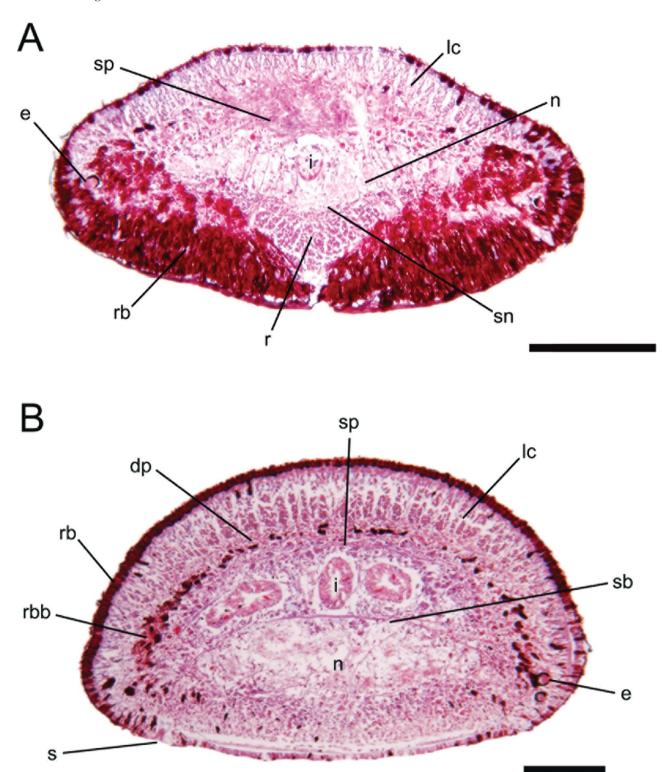


Figure 2. Transverse sections of the cephalic region of the body of *Choeradoplana crassiphalla* sp. nov. (paratype MLP 6481). (A) Close to the anterior tip. (B) Immediately behind the glandular cushions. Abbreviations: dp: dorsal parenchymatic muscle layer; e: eye; i: intestine; lc: longitudinal cutaneous muscle layer; n: nervous plate; r: retractor muscle; rb: rhabdites; rbb: rhabditogen cellular bodies; s: sensory pit; sb: sub-intestinal transverse parenchymatic muscle layer; sn: sub-neural transverse parenchymatic muscle layer; sp: supra-intestinal transverse parenchymatic muscle layer. Scale bars: 250 µm.

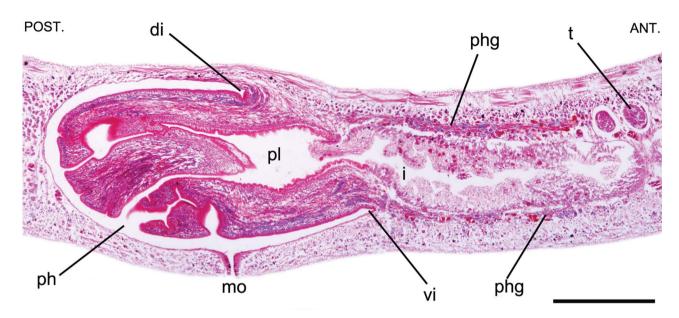


Figure 3. Sagittal section of the pharynx of *Choeradoplana crassiphalla* sp. nov. (holotype). Abbreviations: di: dorsal insertion of the pharynx; i: intestine; mo: mouth; phg: pharyngeal glands; ph: pharyngeal pouch; pl: pharyngeal lumen; t: testes; vi: ventral insertion of the pharynx. Scale bar: 500 μm.

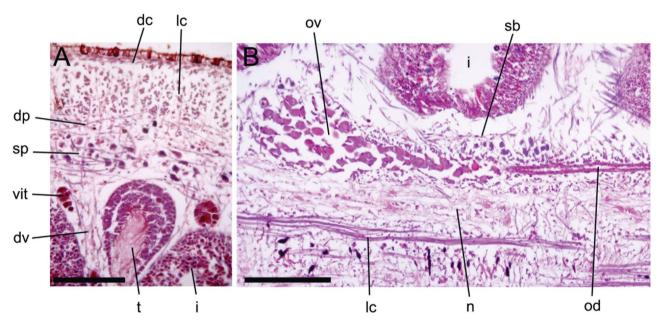


Figure 4. (A) Detail of a sagittal section of the anterior region of the body at the level of the ovaries of *Choeradoplana* crassiphalla sp. nov. (paratype MLP 6481). (B) Detail of a sagittal section of the pre-pharyngeal region of *Choeradoplana* crassiphalla sp. nov. (paratype MLP 6481). Abbreviations: dc: diagonal cutaneous muscle layer; dp: dorsal parenchymatic muscle layer; dv: dorsoventral parenchymatic muscles; i: intestine; lc: longitudinal cutaneous muscle layer; n: nervous plate; od: ovovitelline duct; ov: ovary; sb: sub-intestinal transverse parenchymatic muscle layer; t: testes; vit: vitellaria. Scale bars: 100 μm (A), 200 μm (B).

Ventral epidermis ciliated on creeping sole, which is approximately 70–85% of body width at prepharyngeal region. Ventral epidermis 20–35 μ m high; dorsal epidermis 15–20 μ m high. Rhabdites very abundant over entire dorsal epidermis and body

margins (Figure 2B). In ventral epidermis secretion is less abundant, occupying the apex of the cells. Rhabditogen cellular bodies located beneath cutaneous longitudinal muscle layer, or among fibers of dorsal parenchymatic muscle layer. Scarce cyanophil

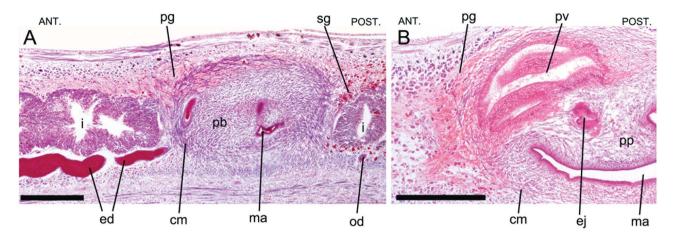


Figure 5. (A) Sagittal section of the copulatory apparatus of *Choeradoplana crassiphalla* sp. nov. (paratype MLP 6226-3). (B) Detail of a sagittal section of *Choeradoplana crassiphalla* sp. nov. (holotype), showing the prostatic vesicle. Abbreviations: cm: common muscle coat; ed: efferent duct; ej: ejaculatory duct; i: intestine; ma: male atrium; od: ovovitelline duct; pb: penis bulb; pg: prostatic glands; pp: penis papilla; pv: prostatic vesicle; sg: shell glands. Scale bars: 500 μm.

secretion discharging through epidermis. Glandular body margin absent.

Cutaneous musculature composed of an external layer of circular muscles, a diagonal layer, and an internal longitudinal layer formed by bundles (Figure 2B, Table 2). The latter most strongly developed and ventrally subdivided into two layers. Innermost layer sunk into parenchyma, located between cutaneous nerve net and nervous plate. The CMI ranging from 16.3% to 28.3% (Table 2).

Parenchymatic musculature arranged in three layers with similar thickness: a dorsal layer with diagonal fibers, a supra-intestinal and a sub-intestinal transverse layer (Figure 2B). Also, dorsoventral fibers intermingled among the intestinal branches. The thickness of the parenchymatic musculature represents 34.2–43% of the cutaneous musculature (Table 2).

Pharynx bell-shaped. Mouth located in the middle of pharyngeal pouch (Figure 3). Pharynx 1.27–1.7 mm long. Pharyngeal epithelium cuboidal and ciliated. Inner musculature of pharynx well developed, being composed of a circular subepithelial layer (15–30 μm thick), under which there is a longitudinal somewhat thicker layer (15–50 μm thick). External musculature of pharynx less developed than internal, being arranged in circular (25–30 μm thick) and longitudinal (5–20 μm thick) fibers, respectively. Numerous cell necks of erythrophil secretory cells and, less abundant, cyanophil cell necks, whose cellular bodies are found in the parenchyma of the pre-pharyngeal region, traversing the pharynx to open through the pharyngeal epithelium (Figure 3).

Testes of all specimens mature, with spermatozoa in their lumen. Testes spherical or oval, dorsal, located

beneath supra-intestinal parenchymatic muscle layer (Figure 4A). Testes arranged in two or three rows on each side of body. Foremost testes anterior of ovaries, spreading until close to pharynx (Figure 3, Table 3). Testes height between 10.5% and 25% of body height.

Efferent ducts at pre-pharyngeal level, running above and among fibers of sub-intestinal parenchymatic muscle layer, located slightly dorsal to ovovitelline ducts. Efferent ducts lined with a ciliated squamous epithelium. Distally, behind pharynx, efferent ducts widened like false seminal vesicles, their lumen full of spermatozoa (Figure 5A), and their epithelium cuboidal. Distal tracts of efferent ducts entering penis bulb, bending almost 90° towards dorsum and connecting laterally with first third of prostatic vesicle (Figures 5A, 7). Prostatic vesicle located inside the common muscle coat, therefore being intra-bulbar (Figures 5B, 6, 7), dorsal of male atrium, oval shaped and its walls folded (Figures 5B, 6B, 7). Its length variable (Table 3). Prostatic vesicle lined with ciliated cylindrical epithelium, provided with abundant xanthophil granulous secretion. Secretion highly abundant, being discharged from secretory cells whose cellular bodies are located both anterior and posterior to prostatic vesicle, and both inside and outside of common muscle coat (Figures 6, 7). Prostatic vesicle joining ejaculatory duct, which runs almost concentrically along the penis papilla. Ejaculatory duct with cuboidal epithelium and receiving scarce cyanophil and xanthophil secretions (Figure 7).

Penis papilla short, very high and thick (Figures 6, 7, Table 3), hanging from the roof of the male atrium, occupying most of its cavity. Penis

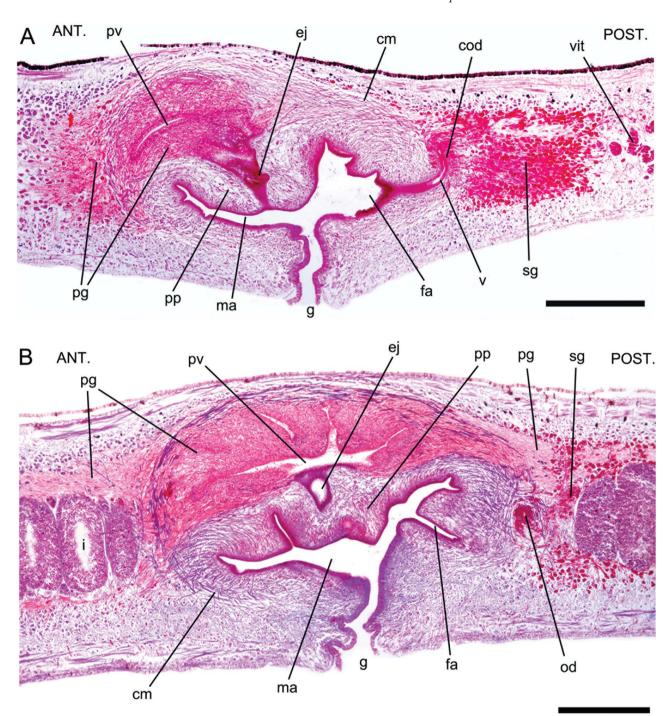
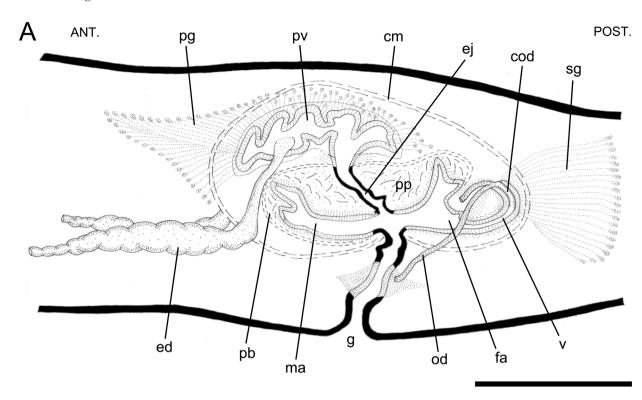


Figure 6. Sagittal section of the copulatory apparatus of (A) holotype and (B) paratype MLP 6226-3 of *Choeradoplana crassiphalla* sp. nov. Abbreviations: cm: common muscle coat; cod: common ovovitelline glandular duct; ej: ejaculatory duct; fa: female atrium; g: gonopore; i: intestine; ma: male atrium; od: ovovitelline duct; pg: prostatic glands; pp: penis papilla; pv: prostatic vesicle; sg: shell glands; v: vagina; vit: vitellaria. Scale bars: 500 μm.

papilla dorsoventrally to slightly obliquely oriented (Figures 6, 7), with cuboidal epithelium receiving abundant erythrophil and scarce cyanophil secretions. Its stroma possessing abundant intermingled muscle fibers. Male atrium 3–5 times longer than

female (Figures 6, 7, Table 3). Its epithelium ranging between cuboidal and cylindrical, apically erythrophil. Muscularis of male atrium composed of a subepithelial longitudinal muscle layer (5–12 μ m thick) followed by a circular one (12–17 μ m thick).



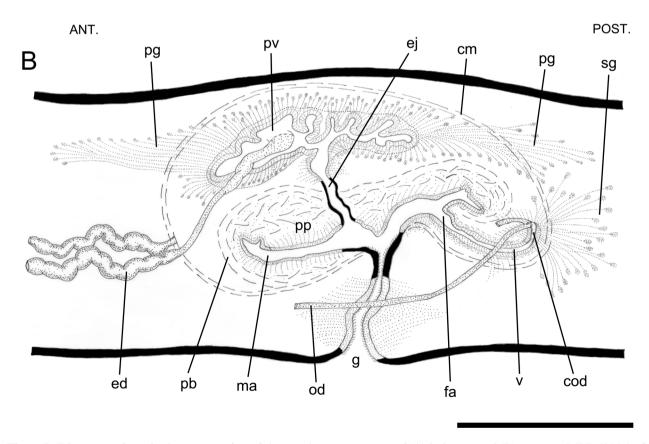


Figure 7. Diagrammatic sagittal reconstruction of the copulatory apparatus of (A) holotype and (B) paratype MLP 6226-3 of *Choeradoplana crassiphalla* sp. nov. Abbreviations: cm: common muscle coat; cod: common ovovitelline glandular duct; ed: efferent duct; ej: ejaculatory duct; fa: female atrium; g: gonopore; ma: male atrium; od: ovovitelline duct; pb: penis bulb; pg: prostatic glands; pp: penis papilla; pv: prostatic vesicle; sg: shell glands; v: vagina. Scale bars: 1 mm.

Table 1. Measurements (mm) of the specimens of *Choeradoplana crassiphalla* sp. nov. All the measurements were obtained from fixed specimens.

	Holotype MLP 6561	Paratype MLP 6226-3	Paratype MLP 6562	Paratype MLP 6481	Paratype MLP 6225
Length	22	43	24	43	38
Maximum width	1.5	3.5	1.8	2.15	2.2
Height	0.95	1.4	0.9	1.4	1.93
DM	12.9	26.5	15	26	21.1
DM (%)	58.6	61.6	62.5	60.5	55.5
DG	15.7	31.5	17	31	25.2
DG (%)	71.4	73.3	70.8	72.1	66.3
DMG	2.8	5	2	5	4.1
CS (%)	75	71.3	70	76	76.4

Note: Abbreviations: DG: distance of gonopore from anterior end; DG (%): DG/body length; DM: distance of mouth from anterior end; DM (%): DM/body length. DMG: distance between mouth and gonopore; CS: creeping sole width.

Table 2. Thickness of cutaneous (CM) and parenchymatic (PM) musculature (µm) and CMI in the pre-pharyngeal region of specimens of *Choeradoplana crassiphalla* sp. nov.

	Holotype MLP 6561	Paratype MLP 6226-3	Paratype MLP 6562	Paratype MLP 6481	Paratype MLP 6225
CM dorsal circular	5	2.5	2.5	5	5
CM dorsal diagonal	20	12.5	15	40	40
CM dorsal longitudinal	100	95	87.5	145	120
CM dorsal total	125	110	105	190	165
CM ventral circular	5	5	5	5	5
CM ventral diagonal	15	12.5	20	25	20
CM ventral longitudinal	100	175	125	175	125
CM ventral total	120	192.5	150	205	150
CMI (%)	25.8	21.6	28.3	18.4	16.3
PM dorsal	25	50	35	50	50
PM supraintestinal	37.5	50	25	60	50
PM subintestinal	25	30	30	25	35
PM:CM total (%)	35.7	43	35.3	34.2	42.9

Table 3. Measurements (mm) of the reproductive system of specimens of Choeradoplana crassiphalla sp. nov.

	Holotype MLP 6561	Paratype MLP 6226-3	Paratype MLP 6562	Paratype MLP 6481	Paratype MLP 6225
Anteriormost testes	4.8 (21.8%)	_	_	4 (9.3%)	8.25 (21.7%)
Posteriormost testes	11.8 (53.6%)	25 (58.1%)	13.9 (57.9%)	23.5 (54.6%)	19.6 (51.6%)
T:BH	23%	18.7%	25%	10.5%	15.5%
$LPV \times HPV$	0.65×0.21	1.25×0.5	0.65×0.3	0.62×0.28	0.8×0.35
$LPP \times WPP \times HPP$	$0.3 \times 0.7 \times 1$	$0.3 \times 0.92 \times 1.3$	$0.25 \times 0.7 \times 0.6$	$0.15 \times 0.6 \times 0.85$	$0.3 \times 0.42 \times 1.1$
LMA	0.85	1.32	0.55	0.88	1.2
Location of ovaries	4.9 (22.3%)	_	_	4.1 (9.5%)	8.5 (22.3%)
LGCD	0.09	0.08	0.15	0.08	0.12
LV	0.13	0.38	0.28	0.13	0.32
LFA	0.3	0.25	0.2	0.3	0.25
LFA:LMA	35.3%	18.9%	36.4%	34.1%	20.8%

Note: Abbreviations: LFA: length of female atrium; LGCD: length of common glandular duct; LMA: length of male atrium; LPP: length of penis papilla; WPP: width of penis papilla; HPP: height of penis papilla; LPV: length of prostatic vesicle; HPV: height of prostatic vesicle; LV: length of vagina; T: testes height; BH: body height.

Erythrophil secretion discharging through epithelium of male atrium.

Ovaries located ventrally, between sub-intestinal parenchymatic muscle layer and nervous plate

(Figure 4B). Ovaries situated at a distance of $\sim 20\%$ body length from anterior end. In one young specimen (without shell glands) ovaries situated at a distance of 9.5% of body length (Table 3). Ovaries with

globose anterior portion and long, narrow posterior one (Figure 4B). Ovovitelline ducts emerging laterally and externally from first portion of ovaries. In several specimens, proximal part of ovovitelline ducts with spermatozoa. In the pre-pharyngeal region, ovovitelline ducts immediately beneath sub-intestinal parenchymatic muscle layer. Their epithelium cuboidal and ciliated. Ovovitelline ducts running backwards, reaching copulatory apparatus, and then taking an ascending direction immediately behind gonopore, bending toward sagittal plane and opening into common glandular ovovitelline duct (Figure 7). Distal ascending portions of ovovitelline ducts and of the common glandular duct receiving abundant secretion from shell glands (Figure 7). Short common glandular duct lined with ciliated cuboidal high epithelium. This is dorsoventral and slightly curved forward, and in some specimens is C-shaped. Common glandular duct opening into a long vagina (Figures 6A, 7, Table 3). Epithelium of vagina ranging from non-ciliated cuboidal high to cylindrical. Muscularis of vagina composed of a layer of circular and longitudinal intermingled fibers (10 µm thick). Vagina curving ventrally and opening onto female atrium (Figures 6A, 7). Female atrium very short (Figures 6, 7, Table 3). Ratio between length of female and male atrium between 18.9% and 36.4% (Table 3). Female atrium lined by cuboidal epithelium. Its muscularis formed by thin circular layer (2.5 μ m thick) and a longitudinal one (7–12 μ m thick). The latter adjacent to common muscle coat of variable thickness (62–187 µm thick), which is composed of longitudinal and oblique fibers. Scarce erythrophil and cyanophil secretions open through the epithelium of the vagina and the female atrium (Figure 7B).

Vitellaria well developed and abundant. In the prepharyngeal region, vitellaria extending both dorsally and ventrally of intestine and between its branches (Figure 4A). In the copulatory apparatus region, vitellaria adjacent to ovovitelline ducts and common glandular duct (Figure 6A).

Gonopore canal high, its epithelium cylindrical and ciliated, receiving abundant cyanophil secretion and scarce erythrophil granulous secretion.

Discussion

The features of the external and internal morphology of the new species fit into *Choeradoplana*. The reproductive system of this genus is highly heterogeneous, basically in regards to the copulatory apparatus. Except for *Ch. ehrenreichi* von Graff, 1899, for which internal morphology has not yet been described, in *Ch. banga* Carbayo & Froehlich, 2012, *Ch. bocaina*

Carbayo & Froehlich, 2012, *Ch. catua* Froehlich, 1955, *Ch. gladismariae* Carbayo & Froehlich, 2012, *Ch. iheringi* von Graff, 1899, and *Ch. langi*, the male reproductive system has a folded male atrium. However, in *Ch. catua* and *Ch. langi* the atrium could act as a temporary penis papilla. The species that have a folded male atrium have a prostatic vesicle different from the new species. This prostatic vesicle is anterior to the copulatory apparatus; in the new species it is dorsal to the copulatory apparatus.

In *Ch. bilix* Marcus, 1951 and *Ch. marthae* Froehlich, 1955, there is a penis papilla like in *Ch. crassiphalla* sp. nov. The new species can be externally distinguished from *Ch. bilix* due to the latter having a yellow ground color at the dorsum with two lateral grayish brown bands. Regarding the internal morphology, the pharynx of *Ch. bilix* is between bell-shaped and the collar type, and the prostatic vesicle is extra-bulbar and anterior to the penis papilla.

Choeradoplana crassiphalla sp. nov. resembles Ch. marthae, mainly in features of the copulatory apparatus, while the color pattern of Ch. marthae presents two dark brown to black bands and a thin median light yellow band. Also, the pharynx is cylindrical. Regarding the copulatory apparatus, the course of the ovovitelline ducts in Ch. marthae is very similar to the new species, ascending posteriorly to the gonopore and joining dorsally a short common glandular duct. The latter is approximately at the same horizontal level as the prostatic vesicle, whereas in Ch. crassiphalla sp. nov. it is below the prostatic vesicle. Both in Ch. crassiphalla sp. nov. and Ch. marthae the distal portions of efferent ducts go through the bulbar musculature and ascend vertically to join the prostatic vesicle laterally. Nevertheless, Ch. marthae has an almost spherical prostatic vesicle, smaller than in Ch. crassiphalla sp. nov., and this is anterior to the penis papilla. In addition, Ch. crassiphalla sp. nov. can be distinguished from Ch. marthae because this species has a small and cylindrical penis papilla (Froehlich 1955), which is slightly oblique and directed towards the posterior end of the body.

Clearly, there is dissimilarity among the copulatory apparatus of the species of the genus *Choeradoplana* as some species have a permanent penis papilla while the others do not. Therefore, phylogenetic approaches are needed to provide a better idea about the relationships of the *Choeradoplana* species.

Acknowledgments

The authors are grateful to Fundación Vida Silvestre Argentina and Ministerio de Ecología, Recursos Naturales Renovables y

Turismo, Misiones for permission to conduct sampling at Reserva de Vida Silvestre Urugua-í, and especially to the park managers Ariel Tombo and Laura Aréjola for support during the fieldwork. We are thankful to the Administración de Parques Nacionales and INTA San Antonio for permission to conduct sampling at the Campo Anexo, in San Antonio County. We wish to acknowledge Eudoxia M. Froehlich and Fernando Carbayo for sending us photos of the slides of the holotype of *Choeradoplana marthae*. The authors acknowledge the anonymous reviewer for their useful suggestions. This work was financed by CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), Agencia Nacional de Promoción Científica y Tecnológica (FONCyT) PICT 2007-01287, and UNLP (Universidad Nacional de La Plata) 11/N600.

References

- Carbayo F, Froehlich EM. 2012. Three new Brazilian species of the land planarian *Choeradoplana* (Platyhelminthes: Tricladida: Geoplaninae), and an emendation of the genus. J Nat Hist. 46(19–20):1153–1177.
- Carbayo F, Leal-Zanchet AM. 2003. Two new genera of geoplaninid land planarians (Platyhelminthes: Tricladida: Terricola) of Brazil in the light of cephalic specializations. Inv Syst. 17(3):449–468.
- Di Bitetti MS, Placci G, Dietz LA. 2003. Una Visión de Biodiversidad para la Ecorregión del Bosque Atlántico del Alto Paraná: Diseño de un Paisaje para la Conservación de la Biodiversidad y prioridades para las acciones de conservación. Washington (DC): World Wildlife Foundation. 154 p.
- Du Bois-Reymond Marcus E. 1951. On South American Geoplanids. Bol Fac Fil Ciênc Letr, Sér Zool. 16:217–255.

- Froehlich CG. 1955. Sôbre morfologia e taxonomia da: Geoplanidae. Bol Fac Fil Ciênc Letr, Sér Zool. 19:195–279.
- Galindo-Leal C, Câmara G, editors. 2003. Atlantic forest hotspot status: an overview. In: The Atlantic Forest of South America: biodiversity status, threats, and outlook. Washington (DC): Island Press. p. 3–11.
- Giraudo AR, Povedano H, Belgrano MJ, Krauczuk E, Pardiñas U, Miquelarena A, Ligier D, Baldo D, Castelino M. 2003.
 Biodiversity status of the interior Atlantic forest of Argentina. In: Galindo-Leal C, Câmara G, editors. The Atlantic Forest of South America: biodiversity status, threats, and outlook. Washington (DC): Island Press. p. 160–180.
- Marcus E. 1951. Turbellaria brasileiros (9). Bol Fac Fil Ciênc Letr, Sér Zool. 16:5–215.
- Negrete LHL, Brusa F, Winsor L. 2011. The blue land planarian *Caenoplana coerulea*, an invader in Argentina. Rev Mex Biod. 82:287–291.
- Ogren RE, Kawakatsu M. 1990. Index to the species of the family Geoplanidae (Turbellaria, Tricladida, Terricola) Part I: Geoplaninae. Bull Fuji Women's Coll. 28(2):79–166.
- Romeis B. 1989. Mikroskopische Technik. München (Germany): Urban und Schwarzenberg. 697 p.
- von Graff L. 1894. Landplanarien. Viaggio del dott. Alfredo Borelli nella Republica Argentina e Paraguay, V. Boll Mus Zool Anat Comp Torino. 9(182):1–4.
- von Graff L. 1897. Neue Landplanarien. Viaggio del Dott. A. Borelli nel Chaco Boliviano e nella Republica Argentina, IX. Boll Mus Zool Anat Comp Torino. 12(296):1–3.
- von Graff L. 1899. Monographie der Turbellarien: II. Tricladida Terricola (Landplanarien). Leipzig (Germany): Verlag von Wilhelm Engelmann. 574 p.