Zootaxa 4137 (2): 187–210
http://www.mapress.com/j/zt/
Copyright © 2016 Magnolia Press

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://doi.org/10.11646/zootaxa.4137.2.2
http://zoobank.org/urn:lsid:zoobank.org:pub:94661073-A6EB-4134-B709-BF4ED7AF50EA

Land flatworms of the genus *Pasipha* (Platyhelminthes, Geoplanidae) in Argentina, with description of three new species

LISANDRO NEGRETE[1, 2] & FRANCISCO BRUSA[1, 2, 3]
[1]*División Zoología Invertebrados, Museo de La Plata. Facultad de Ciencias Naturales y Museo. Universidad Nacional de La Plata. Boulevard 120 & 61, B1900FWA, La Plata, Argentina*
[2]*CONICET – Consejo Nacional de Investigaciones Científicas y Técnicas*
[3]*Corresponding author. E-mail: fbrusa@fcnym.unlp.edu.ar*

Abstract

The genus *Pasipha* Ogren & Kawakatsu, 1990 currently includes 22 species, most of them recorded in the Brazilian Atlantic Forest. Recently, *Pasipha hauseri* was documented in north-eastern Argentina, thus extending its distribution range. This paper reports new records of the genus *Pasipha* in the Interior Atlantic Forest ecoregion in Argentina, with the description of three new species: *Pasipha atla* **sp. nov.**, *Pasipha johnsoni* **sp. nov.**, and *Pasipha mbya* **sp. nov.** These new species exhibit similarities in internal anatomy, such as pharynx cylindrical, extrabulbar prostatic vesicle with folded walls and proximally forked, male atrium highly folded, being at least two times longer than the female atrium and with small folds in their proximal portion. However, they can be distinguished from each other mainly by the secretion types discharged into the prostatic vesicle as well as into the male and female atria. In addition, they have different colour patterns on the dorsal surface.

Key words: land planarians, Geoplaninae, Interior Atlantic Forest ecoregion, north-eastern Argentina

Introduction

The highest species richness in the world for land flatworms is found in the Atlantic Forest ecoregion (Fick *et al.* 2006), which is a mosaic of ecosystems extending along the Atlantic coast of Brazil and inland in this country, eastern Paraguay and north-eastern Argentina, where it is known as Interior Atlantic Forest. About 180 species of land flatworms have been described in Brazil (Carbayo *et al.* 2009), but there is comparatively very little information about them in Paraguay and Argentina. Accordingly, we recently began to study the diversity of land flatworms of the Interior Atlantic Forest in north-eastern Argentina (Negrete 2013; Negrete & Brusa 2012; Negrete *et al.* 2014a, b), and have recorded five morphospecies that were allocated to the genus *Pasipha* plus the species *Pasipha hauseri* (Froehlich, 1959) (Negrete *et al.* 2014a), the latter so far recorded only in Brazil.

The genus *Pasipha* Ogren & Kawakatsu, 1990 is one of 24 currently recognized genera of land flatworms (Platyhelminthes, Geoplanidae) of the subfamily Geoplaninae (Tyler *et al.* 2006–2015), the distribution of which covers the Neotropical Region. It includes 22 species of land flatworms, most of them described for Brazil. They show slender, elongate body, folded male atrium without a permanent penis papilla, extrabulbar prostatic vesicle, and female canal approaching from the ventral side before entering the female atrium (Carbayo *et al.* 2013; Leal-Zanchet *et al.* 2012).

This paper describes three of the previously recognized morphospecies by Negrete *et al.* (2014a) in the Interior Atlantic Forest in north-eastern Argentina, contributing to the knowledge of this genus, particularly in Argentina.

Materials and methods

Sampling was conducted at three localities: Urugua-í Wildlife Reserve (25° 59’ S, 54° 05’ W), San Antonio Strict

Accepted by W. Sterrer: 25 May 2016; published: 11 Jul. 2016

Nature Reserve (26° 03’ S, 53° 46’ W) and Iguazú National Park (25° 39’ S, 54° 27’ W), located in the south of the Interior Atlantic Forest ecoregion, north-eastern Argentina (Misiones Province). These are mixed rainforests dominated by *laurel* and *gautambu* forests with abundant tree ferns in the understory (Giraudo *et al.* 2003).

Land flatworms were collected manually during the day from beneath fallen logs, leaf litter and stones, and during the night through direct observation of soil on forest trails by the light of a headlamp. The external morphology of specimens was recorded, after which they were killed with boiling water, fixed in 10% formaldehyde and preserved in 70% ethanol. Body fragments of the collected specimens were dehydrated in an ascending series of ethanol and embedded in Paraplast®. Sagittal and transverse serial sections (6–8 µm thick) of the anterior body region, transverse sections of the pre-pharyngeal region (6–8 µm thick) and sagittal serial sections of the pharynx and the copulatory apparatus (6–8 µm thick) were performed with a microtome and stained with Masson’s trichrome method (Subarna *et al.* 2013). The ratio of the height of cutaneous musculature to the height of the body (cutaneous muscular index, CMI) and the ratio of the height of parenchymatic musculature to the height of the body (parenchymatic muscular index, PMI) were calculated at the pre-pharyngeal level following Froehlich (1955a) and Winsor (1983), respectively. The types and additional specimens studied were deposited in the Invertebrate Collection at Museo de La Plata (MLP), Argentina.

Results

Taxonomy

Order Tricladida Lang, 1884

Suborder Continenticola Carranza *et al.*, 1998

Family Geoplanidae Stimpson, 1857

Subfamily Geoplaninae Stimpson, 1857

Genus *Pasipha* Ogren & Kawakatsu, 1990

Pasipha atla sp. nov.

(Figs. 1–4)

Pasipha sp. 2: Negrete *et al.* 2014a

Type material. Holotype: MLP-He 6173. San Antonio Strict Nature Reserve, Misiones Province, Argentina, 29.X.2008, L. Negrete, coll.; cephalic region: transverse sections on 11 slides (7 µm); anterior region at level of ovaries: sagittal sections on 26 slides (7 µm); pre-pharyngeal region: transverse sections on 6 slides (6 µm); pharynx and copulatory apparatus: sagittal sections on 31 slides (7 µm).

Paratypes: MLP-He 6461–1. Urugua-í Wildlife Reserve, Misiones Province, Argentina, 22.VIII.2009, L. Negrete, coll.; cephalic region and anterior region at level of ovaries: transverse sections on 35 slides (8 µm); pre-pharyngeal region: transverse sections on 4 slides (8 µm); pharynx and copulatory apparatus: sagittal sections on 35 slides (8 µm). MLP-He 6461–2. Urugua-í Wildlife Reserve, Misiones Province, Argentina, 22.VIII.2009, L. Negrete, coll.; cephalic region: sagittal sections on 20 slides (8 µm); anterior region at level of ovaries: sagittal sections on 32 slides (8 µm); pre-pharyngeal region: transverse sections on 5 slides (8 µm); pharynx and copulatory apparatus: sagittal sections on 26 slides (8 µm).

Diagnosis. Dorsum dark brown with a whitish median stripe only present on the cephalic and posterior body regions. Eyes dorsal with clear halos. Pharynx cylindrical. Prostatic vesicle extrabulbar, tubular, with narrow lumen, and proximally forked. Male atrium twice the length of the female atrium, highly folded. Proximal part of male atrium with small folds. Ovaries anterior to the anteriormost testes. Distal sections of ovovitelline ducts ventral to the female atrium, joining behind it. Common ovovitelline duct vertical and posterior to the female atrium, and female genital canal antero-dorsally flexed. Female atrium with folded walls.

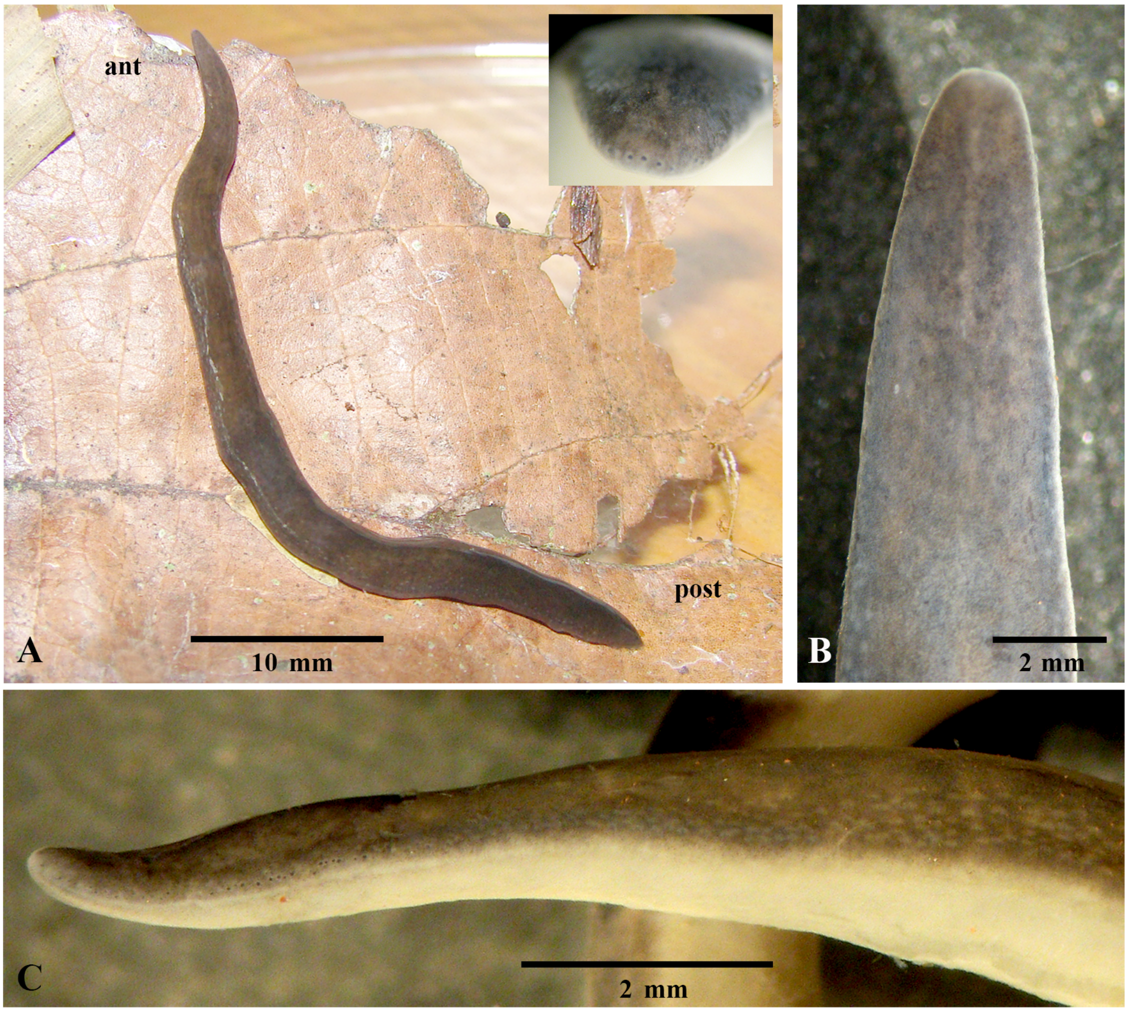

FIGURE 1. *Pasipha atla* **sp. nov. (A)** Photograph of a live specimen (MLP-He 6173), with detail of the cephalic region. Dorsal (MLP-He 6173) **(B)** and lateral (MLP-He 6461–1) **(C)** view of anterior body region of preserved specimens.

Type locality. San Antonio Strict Nature Reserve (26° 03' S, 53° 46' W), Misiones Province, Argentina.

Etymology. The scientific name is a combination of the initials of Urugua-í Wildlife Reserve park managers Ariel Tombo and Laura Aréjola, in gratefulness of their valuable assistance during fieldwork.

External morphology. The body is elongate with parallel margins. In living specimens, the anterior region gradually narrows, with blunt ending, and the posterior region is sharply pointed (Fig. 1A, B). The dorsal colour pattern is homogeneous dark brown (Fig. 1A). A thin whitish median stripe is distinguished in the cephalic region, extending to about 5 mm from the anterior tip (Fig. 1B), and close to the posterior tip (only visible under stereomicroscope). The ventral surface is greyish. After fixation, the dorsal colour pattern is light brown (Fig. 1B, C). The eyes, with clear halos, surround the anterior tip and extend uniserially on both body margins along 3–5 mm from the anterior tip, continuing bi- and tri-serially for 2–4 mm (Fig. 1A, C). Then the eyes extend pluriserially over the dorsal surface. At the pharyngeal level they become restrict to the body margins and less numerous, and extend to the posterior end. Length of the fixed specimens ranged from 35 to 48 mm, maximum width was 2.4–5.4 mm, and maximum height 1.5–2.2 mm. The mouth was located at a distance of 66–78% from the anterior tip, and the gonopore at 77–88% (Table 1).

Internal morphology. *Epidermis, secretions and musculature in the cephalic region.* Dorsal epidermis (20–25 µm µmhigh) receives abundant rhabditogen and erythrophil fine granular secretions, and erythrophil and cyanophil amorphous secretions in less quantity. Ventral epidermis (30 µm high), ciliated in the creeping sole (~90% of body width), receives the same type of secretions. Sensory pits (20–25 µm deep), as simple invaginations, contour the anterior tip and spread on both body margins in a single row up to ~7 mm from the anterior tip. The cutaneous musculature has the same arrangement (see below) and similar thickness (10% to 15% of body height) as in the pre-pharyngeal region. The parenchymatic musculature is composed of the same layers as the pre-pharyngeal region, ranging from 5% to 10% of body height (Fig. 2A). There is no musculo-glandular specialization in the cephalic region.

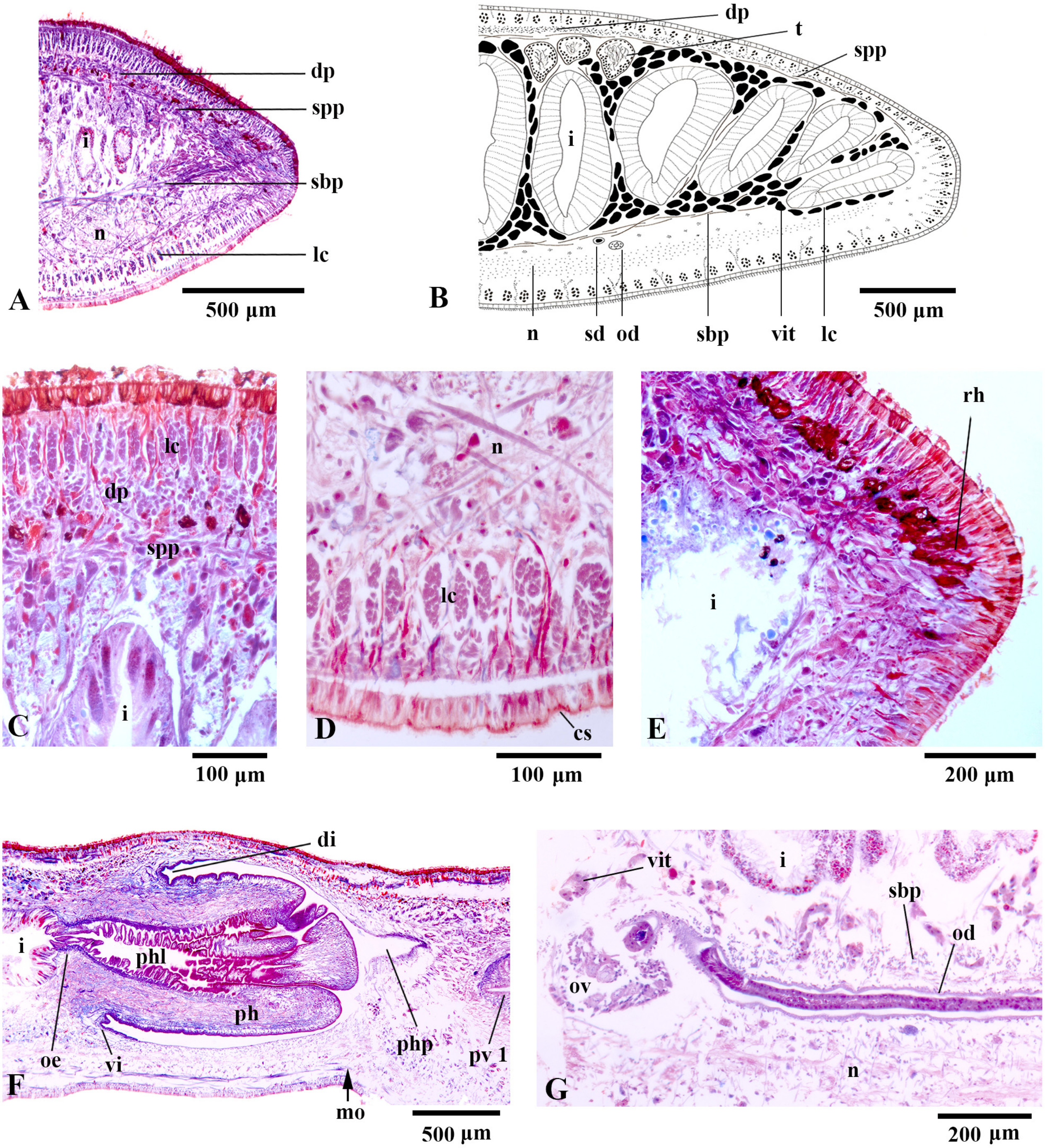

FIGURE 2. *Pasipha atla* **sp. nov.** (MLP-He 6173). **(A)** Half of a transverse section of the cephalic region. **(B)** Schematic drawing of half of a transverse section of the pre-pharyngeal region. **(C–D)** Detail of the dorsal **(C)** and ventral **(D)** portion of a transverse section in pre-pharyngeal region. **(E)** Detail of the marginal portion of a transverse section in pre-pharyngeal region. **(F)** Sagittal section of the pharynx. **(G)** Sagittal section of the anterior region. (di) dorsal insertion; (dp) dorsal parenchymatic muscle layer; (i) intestine; (lc) longitudinal cutaneous muscle layer; (mo) mouth; (n) nervous plate; (od) ovovitelline duct; (oe) oesophagus; (ov) ovary; (ph) pharynx; (phl) pharyngeal lumen; (php) pharyngeal pouch; (pv1) proximal prostatic vesicle; (rh) rhabditogen glands; (sbp) sub-intestinal parenchymatic muscle layer; (sd) sperm duct; (spp) supra-intestinal parenchymatic muscle layer; (t) testes; (vi) ventral insertion; (vit) vitellaria.

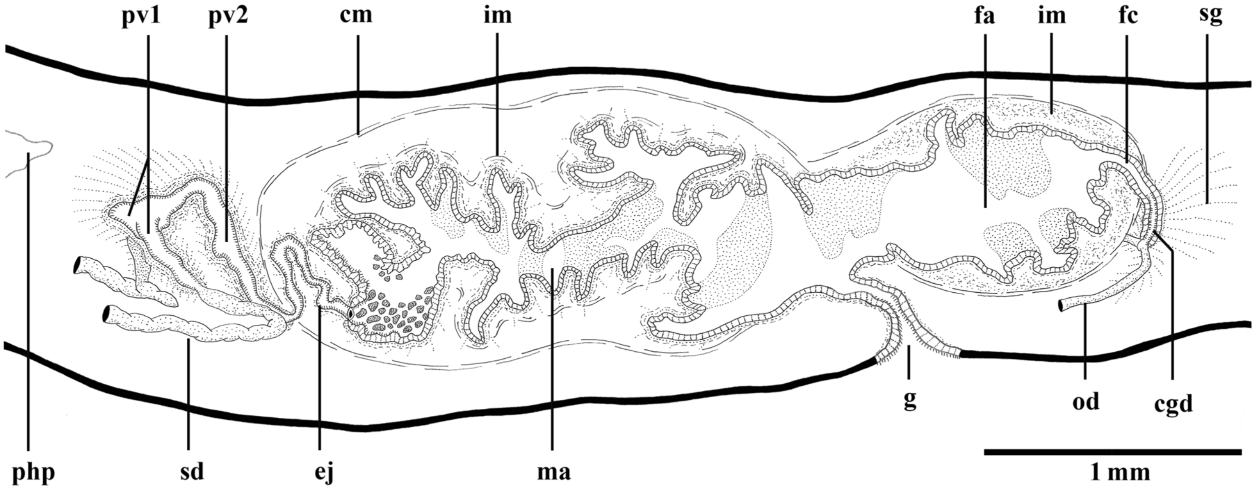

FIGURE 3. *Pasipha atla* **sp. nov.** (MLP-He 6173). Sagittal reconstruction of the copulatory apparatus. (cgd) common glandular ovovitelline duct; (cm) common muscle coat; (ej) ejaculatory duct; (fa) female atrium; (fc) female genital canal; (im) intermediate muscle fibres; (g) gonopore; (ma) male atrium; (od) ovovitelline duct; (php) pharyngeal pouch; (pv1) proximal prostatic vesicle; (pv2) distal prostatic vesicle; (sd) sperm duct; (sg) shell glands.

TABLE 1. Measurements (mm) of fixed specimens of *Pasipha atla* **sp. nov.** (DG) distance of gonopore from anterior end, (DM) distance of mouth from anterior end, (DMG) distance between mouth and gonopore. The numbers in parentheses represent the position relative to body length (%).

	MLP-He 6173	MLP-He 6461–1	MLP-He 6461–2
Length	35.3	42	35
Maximum width	2.6	2.4	5.4
Maximum height	1.5	1.6	2.2
DM	27.5 (78%)	32 (76%)	23 (66%)
DG	30 (85%)	37 (88%)	27 (77%)
DMG	2.5	5	4
Creeping sole (%)	90%	90%	90%

Epidermis, secretions and musculature in the pre-pharyngeal region. Abundant rhabditogen cells with erythrophil secretion, numerous cells with fine granular and amorphous erythrophil secretions, and fine granular cyanophil secretion in less quantity discharge through dorsal epidermis (25–30 µm high) and body margins (Fig. 2C, E). Ventral epidermis (30 µm high), ciliated in the creeping sole (85–90% of body width), presents abundant small dermal rhabdites densely arranged at the apex of cells. Also, abundant amorphous cyanophil and fine granular erythrophil secretions, and less numerous amorphous erythrophil secretion are present in ventral epidermis (Fig. 2D). There is no glandular margin (Fig. 2B, E).

The cutaneous musculature consists of the three typical layers of Geoplaninae, a thin circular subepidermal layer, with the same thickness dorsally and ventrally, followed by an intermediate layer with diagonal fibres, and an internal thicker longitudinal layer arranged in bundles, which is thicker ventrally than dorsally (Table 2). CMI ranges from 9% to 15%. Parenchymatic musculature is composed of three layers: a dorsal layer with decussate fibres (situated below the longitudinal cutaneous layer), which is the thickest, a supra-intestinal and a sub-intestinal transverse layer (Fig. 2B, C). Dorso-ventral fibres are arranged among intestinal branches. PMI ranges from 4% to 7% (Table 2).

Digestive system. The pharynx (1.2–1.6 mm in length, about 3–4% of body length) is cylindrical, with the dorsal insertion slightly posteriorly displaced (100–350 µm) (Fig. 2F). The mouth is located in the posterior third of the pharyngeal pouch (Fig. 2F). The epithelium lining of the outer surface of the pharynx is cuboidal and densely ciliated, and the outer pharyngeal musculature is arranged in two layers: a thin longitudinal subepithelial layer (5–7.5 µm thick) followed by an inner circular layer (10–25 µm thick). The epithelium lining the pharyngeal lumen is columnar and ciliated, and the inner pharyngeal musculature consists of a circular subepithelial layer (10–50 µm thick) followed by a subjacent longitudinal layer (15–50 µm thick). Secretory cells, the cell bodies of which are located anterior and lateral to the pharynx, discharge abundant erythrophil and cyanophil fine granular secretion in the pharyngeal epithelium (Fig. 2F).

TABLE 2. Thickness of cutaneous (CM) and parenchymatic (PM) musculatures (µm), and CMI and PMI indices at pre-pharyngeal region of *Pasipha atla* **sp. nov.**

	MLP-He 6173	MLP-He 6461–1	MLP-He 6461–2
CM dorsal			
circular	2.5	5	5
diagonal	10	12.5	17.5
longitudinal	60	75	62.5
CM ventral			
circular	2.5	5	5
diagonal	15	25	32.5
longitudinal	85	125	80
CMI (%)	12%	15%	9%
PM dorsal	30	40	50
PM supra-intestinal	25	35	25
PM sub-intestinal	25	30	25
PMI (%)	5%	7%	4%

The oesophagus (100–600 µm in length) (Fig. 2F) is lined by a columnar epithelium followed by a subjacent circular muscle layer (15–25 µm thick) and a longitudinal layer (10–15 µm thick). The oesophagus: pharynx ratio ranges from 19% to 32%.

Male reproductive system. The testes, ovoid in shape, are arranged in two to four irregular rows on each side of the body, being dorsal to the intestinal branches and located below the supra-intestinal parenchymatic muscle layer (Fig. 2B). Testes occupy 11–13% of the body height. They appear behind the ovaries and extend to the pre-pharyngeal region, being located at a distance between 21–27% and 61–75% of the body length from the anterior end (Table 3). The sperm ducts are located among fibres of the sub-intestinal parenchymatic muscle layer or just below them, being slightly dorsal and medial to ovovitelline ducts in the pre-pharyngeal region (Fig. 2B). Behind the pharynx, sperm ducts are tortuous and distally dilated (Fig. 3) with lumen full of spermatozoa. In the vicinity of the common muscle coat, sperm ducts bend toward the dorsum and forward, and open into the proximal region of the extrabulbar prostatic vesicle (Figs. 3, 4B). The prostatic vesicle, located just behind the pharynx (Figs. 2F, 3), is tubular with a narrow irregular lumen and presents two anatomically and histologically distinguishable regions (see below) (Figs. 3, 4A–C). The proximal region is proximally forked, communicating with sperm ducts (Figs. 3, 4A–C). The distal region is ventro-posteriorly curved and opens into the ejaculatory duct exactly at the boundary of the common muscle coat (Figs. 3, 4A, D). The ejaculatory duct is sinuous and opens into the bottom of the male atrium, sometimes slightly displaced ventrally (Figs. 3, 4D). The walls of the most proximal part of the male atrium present numerous small folds (Figs. 3, 4A, D, E) while the rest of the atrium is a cavity with large folds. Thus, its lumen is narrow (Figs. 3, 4A). The male atrium is approximately twice the length of the female atrium (Table 3).

Sperm ducts are lined by a ciliated cuboidal epithelium surrounded by a circular muscle layer (5 µm thick). The prostatic vesicle is lined with ciliated columnar epithelium, traversed in its proximal region by abundant fine granular pale cyanophil secretion, and abundant coarse granular erythrophil secretion in its distal region (Fig. 4A–D). The glandular cell bodies are located in the surrounding parenchyma laterally and anteriorly to the prostatic vesicle. The prostatic musculature of both regions comprises intermingled longitudinal and circular fibres (25–55 µm thick). The ejaculatory duct is lined by a ciliated columnar epithelium, which receives scarce fine granular cyanophil secretion, followed by a subjacent muscular layer with circular fibres intermingled with some longitudinal fibres (10–20 µm thick).

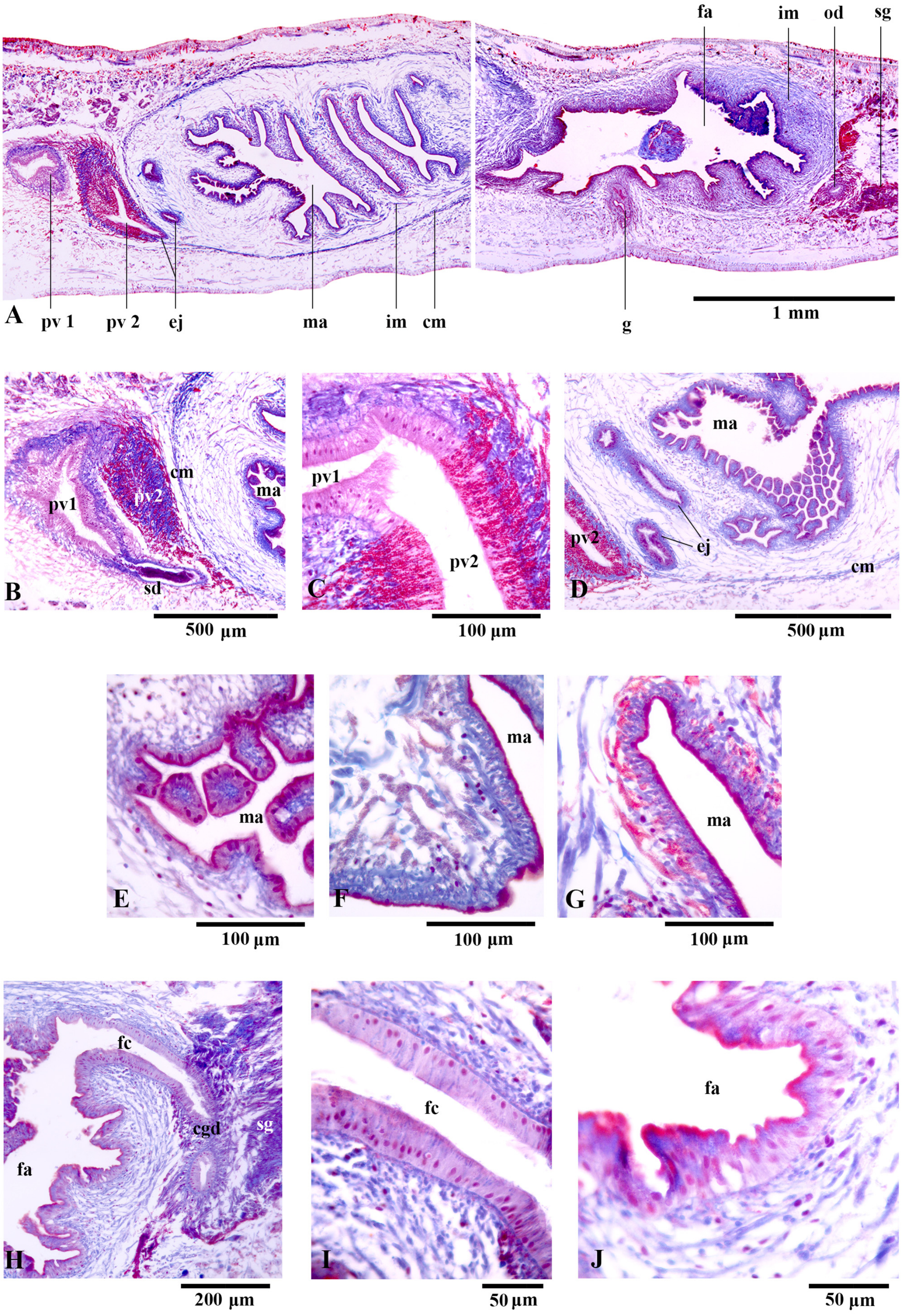

FIGURE 4. *Pasipha atla* **sp. nov.** (MLP-He 6173). **(A)** Sagittal section of the copulatory apparatus. **(B–G)** Details of the male reproductive system, in sagittal view. **(H–J)** Details of the female reproductive system, in sagittal view. (cgd) common glandular ovovitelline duct; (cm) common muscle coat; (ej) ejaculatory duct; (fa) female atrium; (g) gonopore; (im) intermediate muscle fibres; (ma) male atrium; (od) ovovitelline duct; (pv1) proximal prostatic vesicle; (pv2) distal prostatic vesicle; (sd) sperm duct; (sg) shell glands.

TABLE 3. Measurements (mm) of reproductive organs of *Pasipha atla* **sp. nov.** LCGD, length of common glandular ovovitelline duct; LFA, length of female atrium; LFC, length of female canal; LMA, length of male atrium; LPV, length of prostatic vesicle. The numbers in parentheses represent the position relative to body length (%).

	MLP-He 6173	MLP-He 6461–1	MLP-He 6461–2
Anteriormost testes	7.5 (21%)	12 (29%)	9.4 (27%)
Posteriormost testes	24.7 (70%)	31.5 (75%)	21.4 (61%)
LPV (proximal)	0.6	0.85	0.95
LPV (distal)	0.6	0.9	1.2
LMA	3	3.25	2.5
Location of ovaries	4.5 (13%)	9.2 (22%)	7.6 (22%)
LCGD	0.35	0.6	0.6
LFC	0.35	0.37	0.4
LFA	1.2	2	1.1

The lining epithelium of the small folds of the most proximal part of the male atrium is columnar and ciliated, with the apex of the cells filled with strongly stained fine granular erythrophil secretion (Fig. 4E). The epithelium of the rest of the male atrium is cuboidal and non-ciliated except in the distal portion, which is columnar. The male atrium receives abundant fine granular cyanophil secretion along its entire length, and in its middle third also receives abundant fine granular erythrophil secretion (Fig. 4F, G). Cell bodies of both types of glands are located below the epithelial lining of the atrium. The muscularis consists of a subepithelial circular layer (10–25 µm thick) followed by a longitudinal layer (5–10 µm thick). Longitudinal fibres, forming a not well organized coat (40–100 µm thick) (named *eigenmusculatur* by Graff (1899); here named intermediate muscle fibres), are located between the muscularis and the common muscle coat (Figs. 3, 4A). The common muscle coat consists of longitudinal and less abundant circular and oblique fibres, thicker dorsally (20–50 µm thick) than ventrally (10–20 µm thick).

Female reproductive system. The ovaries (300–500 µm in length, 200–400 µm high), ovoid in shape, are situated at a distance of 13–22% of the body length from the anterior end (Fig. 2G, Table 3). They are located between the sub-intestinal parenchymatic muscle layer and the nervous plate (Fig. 2G). The ovovitelline ducts emerge from the medial dorsal side of the ovaries, and run posteriorly, being located just above the nervous plate in the pre-pharyngeal region (Fig. 2B, G). Their distal sections are located ventrally to the female atrium. Just behind this atrium, they contour the common muscle coat and run to the sagittal plane in a slight ascending course. The ovovitelline ducts join behind the atrium to form a common glandular ovovitelline duct (Figs. 3, 4H). It is almost vertically oriented and distally curves forward, passing through the common muscle coat, and opens into the female genital canal (Figs. 3, 4H). The latter continues ascending and opens into the bottom of the female atrium, dorsally displaced (Fig. 3, 4H). The female atrium presents folded walls, although it is not as richly folded as the male atrium (Figs. 3, 4A). At the level of the gonopore there is a dorsal fold that separates the atria (Fig. 3).

The ovovitelline ducts are lined with a ciliated cuboidal epithelium, followed by a circular muscle layer (5 µm thick). Both the distal portions of the ovovitelline ducts and the common glandular ovovitelline duct receive abundant secretion from the shell glands (Figs. 3, 4A, H). The lining epithelium of the latter, which also receives scarce fine granular cyanophil secretion, is columnar and ciliated followed by a circular muscle layer (10 µm thick). The female canal is lined by a non-ciliated columnar epithelium, which receives abundant fine granular cyanophil secretion (Fig. 5I). The muscularis of the female canal consists of circular and longitudinal intermingled fibres (10–20 µm thick). Like the female canal, the lining epithelium of the female atrium is columnar and non-ciliated, but in the latter, the apical part of the epithelium is filled with abundant fine granular erythrophil and cyanophil secretions (Fig. 4J). In the distal portion of the female atrium erythrophil secretion is more abundant. Cell bodies of these glands are subepithelial, located in the surrounding parenchyma within the common muscle coat. The muscularis of the female atrium consists of a thin layer of intermingled circular and longitudinal fibres (5–10 µm thick). There is a thick muscle layer with intermingled circular and some longitudinal and oblique fibres (35–100 µm thick) (here named intermediate muscle fibres) located between the atrial muscularis and the common muscle coat (Figs. 3, 4A). The common muscle coat consists of longitudinal fibres, thicker dorsally (35–50 µm thick) than ventrally (15–25 µm thick). Vitellaria are well developed in all specimens. At the pre-pharyngeal level, vitelline follicles are arranged dorsally and ventrally to the intestinal branches and among them (Fig. 2B).

Parasitism. Nematode larvae were found in all study specimens, located in the parenchyma among intestinal branches and vitellaria of the anterior body region.

Pasipha johnsoni **sp. nov.**

(Figs. 5–9)

Pasipha sp. 3: Negrete *et al.* 2014a

Type material. Holotype. MLP-He 6489. Urugua-í Wildlife Reserve, Misiones Province, Argentina, 31.X.2010, L. Negrete, coll.; cephalic region and anterior region at level of ovaries: sagittal sections on 14 slides (8 µm); pre-pharyngeal region: transverse sections on 5 slides (8 µm); pharynx and copulatory apparatus: sagittal sections on 15 slides (8 µm).

Additional material examined. MLP 6488. Urugua-í Wildlife Reserve, Misiones Province, Argentina, 21.VIII.2009, L. Negrete, coll.; cephalic region and anterior region at level of ovaries: transversal sections on 33 slides (8 µm); pharynx: sagittal sections on 36 slides (6 µm) (immature specimen).

Diagnosis. Dorsum dark grey with two black lateral stripes. Eyes dorsal with clear halos. Pharynx cylindrical. Prostatic vesicle extrabulbar, globose with folded walls, and proximally forked. Male atrium richly folded, three times longer than the female atrium. Ovaries anterior to the anteriormost testes. Distal sections of ovovitelline ducts ventral to the female atrium, joining behind it. Common ovovitelline duct vertical and located behind the female atrium. Female genital canal almost horizontal. Female atrium without folded walls.

Type locality. Urugua-í Wildlife Reserve (25° 59’ S, 54° 05’ W), Misiones Province, Argentina.

Etymology. The specific name is dedicated to Andrés Johnson (1956-2009), eminent naturalist who made a significant contribution to the knowledge of Atlantic Forest biodiversity in Argentina.

External morphology. The body is elongate, with parallel margins, and ovoid in cross section (Figs. 5, 7B). The anterior tip is rounded and the posterior tip ends in a sharp point (Fig. 5A, B). The colour pattern of the dorsal surface is dark grey with two black lateral stripes, visible at naked eye (each ~1/10 of body width). They extend along the whole body except in the cephalic region, where pigment is light grey (Fig. 5A, B). Towards the body margins, pigment becomes gradually lighter. Ventral surface is light grey. After fixation, the dorsal ground colour becomes paler, but lateral stripes are evenly visible. The eyes, with clear halos, surround the cephalic region uniserially and spread towards body margins in a single row up to 4–6 mm from the anterior tip. Then the eyes spread progressively dorsally in two and three rows (Fig. 6). At the level of pharynx, they are again marginal, uniserial and less numerous, reaching the posterior end of body.

The largest specimen was 40 mm long *in vivo* (Fig. 5A). After fixation, body length ranged from 29 mm to 37 mm, maximum width was 1.7–1.9 mm, and maximum height 1–1.3 mm. The mouth and gonopore were located at a distance of 67% and 81% from the anterior tip, respectively.

Internal morphology. *Epidermis, secretions and musculature in the cephalic region.* Dorsal epidermis (15 µm height) receives abundant rhabditogen secretion, with rhabdites covering the entire dorsal surface, as well as abundant fine granular erythrophil secretion and less numerous amorphous erythrophil secretions discharge both in dorsal and ventral epidermis. Scarce fine granular cyanophil secretion open through body margins. Ventral epidermis (20 µm height) is ciliated on the creeping sole (90% of body width). Sensory pits (25 µm deep), as simple invaginations, surround the anterior tip and continue toward body margins in a single row up to ~2 mm from the anterior tip (Fig. 7A). The cutaneous musculature, organized as in the pre-pharyngeal region (see below), is somewhat thicker than at the pre-pharyngeal level (13% of the body height). The thickness of the parenchymatic musculature ranges from 7% to 13%. There is no musculo-glandular specialization in the cephalic region.

Epidermis, secretions and musculature in the pre-pharyngeal region. Abundant rhabditogen cells with erythrophil secretion, numerous cells with fine granular erythrophil secretion and less abundant cells with amorphous erythrophil and cyanophil secretions open through dorsal epidermis (25–30 µm height) and body margins (Fig. 7B, C). Ventral epidermis (25 µm height), ciliated in the creeping sole (90% of body width), presents abundant small dermal rhabdites, and receives fine granular erythrophil secretion in less quantity than the dorsal epidermis. Cell bodies of the erythrophil glands are located below the cutaneous musculature. There is no glandular margin (Fig. 7B).

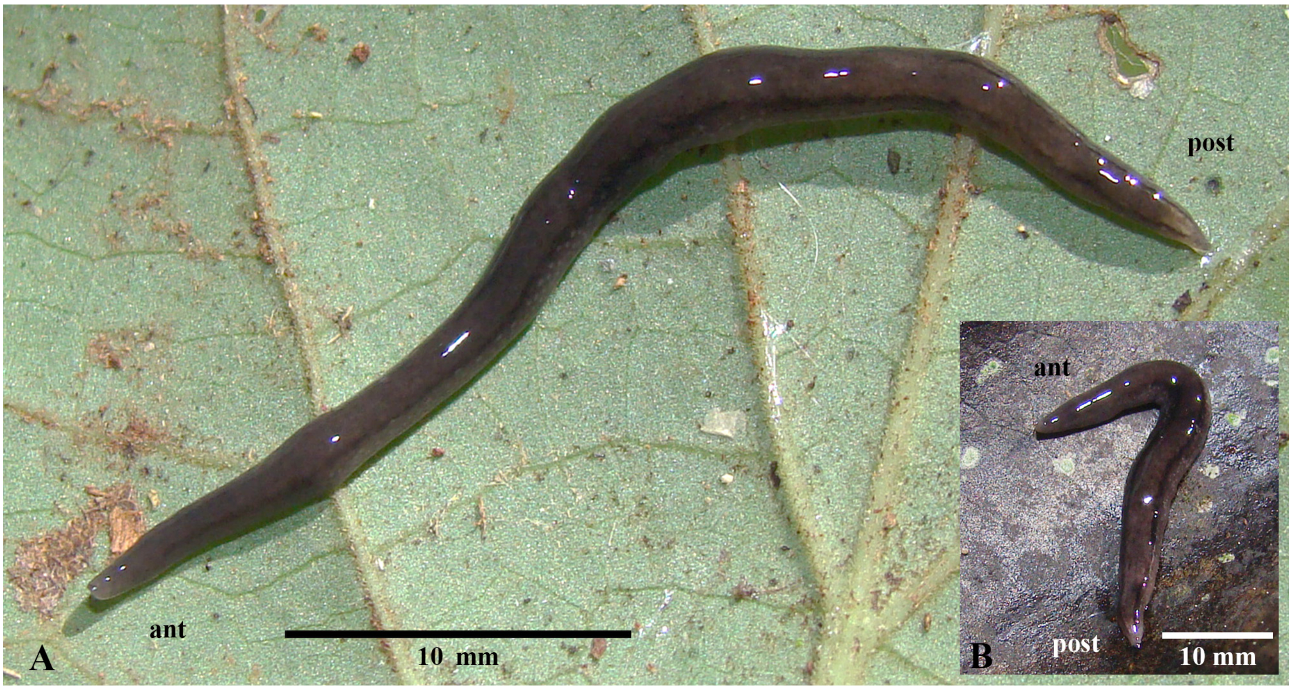

FIGURE 5. *Pasipha johnsoni* **sp. nov.** Photographs of live specimens, in dorsal view. **(A)** Holotype (MLP-He 6489). **(B)** Immature specimen (MLP-He 6488).

FIGURE 6. *Pasipha johnsoni* **sp. nov.** (MLP-He 6489). Schematic drawing of the colour pattern and eyes at the anterior body region, in dorso-lateral view.

The cutaneous musculature consists of three typical layers of Geoplaninae, a thin subepidermal circular layer (2.5 µm thick), an intermediate diagonal layer (5–12.5 µm thick), and an internal thicker longitudinal layer organized in bundles (40–45 µm thick dorsally, 50–70 µm thick ventrally). CMI ranges from 9% to 11%. The parenchymatic musculature consists of a dorsal decussate layer (30–40 µm thick), a supra-intestinal layer and a sub-intestinal transverse layer (25–40 µm thick each) (Fig. 7C, D). Dorso-ventral fibres are located among intestinal branches (Fig. 7C). PMI ranges from 7% to 10%.

Digestive system. The pharynx (1.2–1.3 mm in length, about 3–4% of body length) is cylindrical, with the dorsal insertion slightly posteriorly displaced, with mouth located in the posterior third of the pharyngeal pouch (1.5–1.7 mm in length) (Fig. 7E). The epithelial lining of the outer surface of the pharynx is cuboidal and ciliated, followed by a thin longitudinal subepithelial muscle layer (2.5–5 µm thick) and a subjacent circular layer (10 µm thick). The epithelium of the pharyngeal lumen is columnar and ciliated. The inner pharyngeal musculature consists of a circular subepithelial layer (20–40 µm thick) and a subjacent longitudinal layer (10–25 µm thick). Abundant cells with fine erythrophil and cyanophil granules discharge their secretions onto the pharyngeal

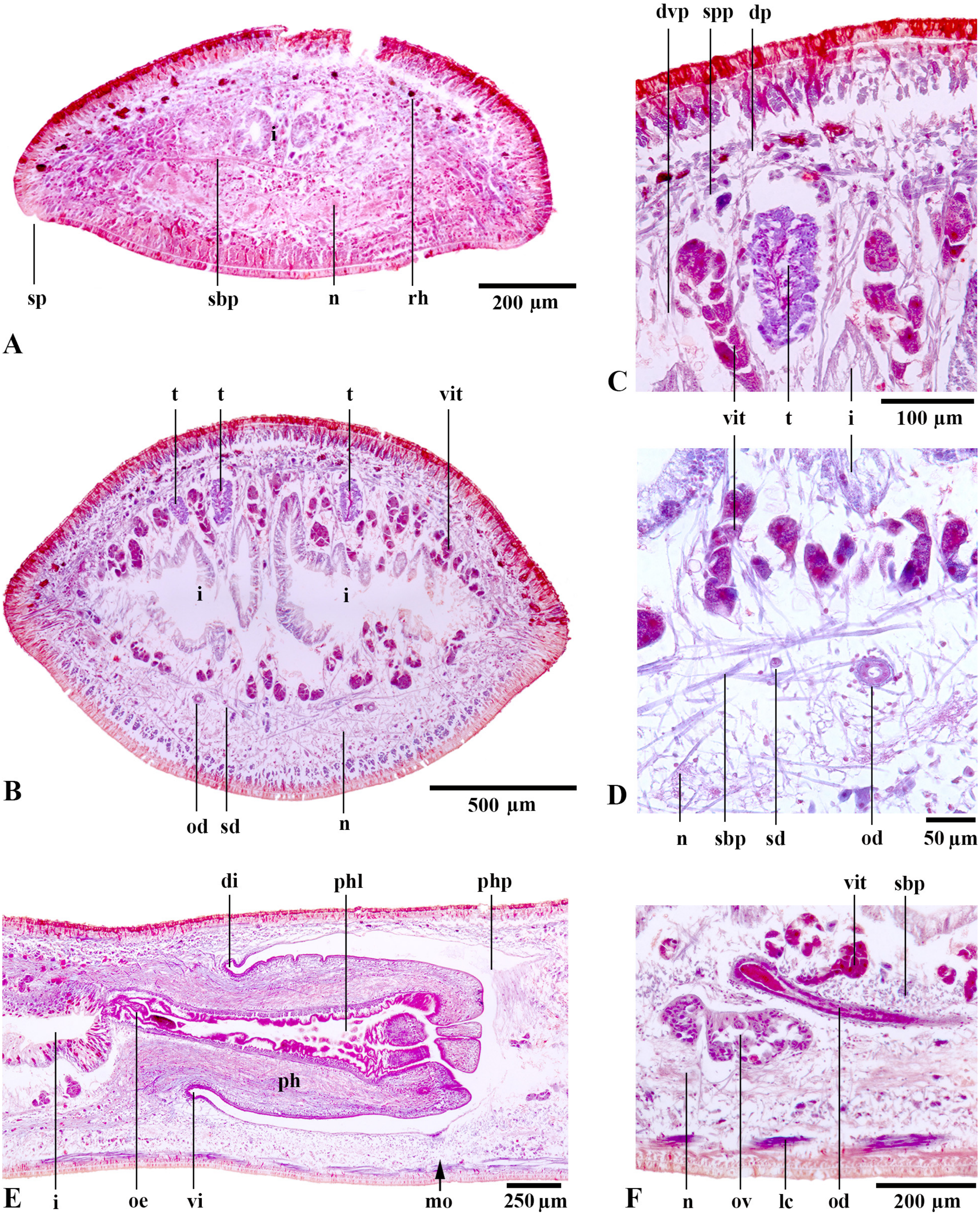

FIGURE 7. *Pasipha johnsoni* **sp. nov.** (MLP-He 6489). **(A)** Transverse section of the cephalic region. **(B)** Transverse section of the pre-pharyngeal region. **(C–D)** Detail of the dorsal **(C)** and ventral **(D)** portion of a transverse section in pre-pharyngeal region. **(E)** Sagittal section of the pharynx. **(F)** Sagittal section of the anterior region. (di) dorsal insertion; (dp) dorsal parenchymatic muscle layer; (dvp) dorso-ventral parenchymatic musculature; (i) intestine; (lc) longitudinal cutaneous muscle layer; (mo) mouth; (n) nervous plate; (od) ovovitelline duct; (oe) oesophagus; (ov) ovary; (ph) pharynx; (phl) pharyngeal lumen; (php) pharyngeal pouch; (rh) rhabditogen glands; (sbp) sub-intestinal parenchymatic muscle layer; (sd) sperm duct; (sp) sensory pit; (spp) supra-intestinal parenchymatic muscle layer; (t) testes; (vi) ventral insertion; (vit) vitellaria.

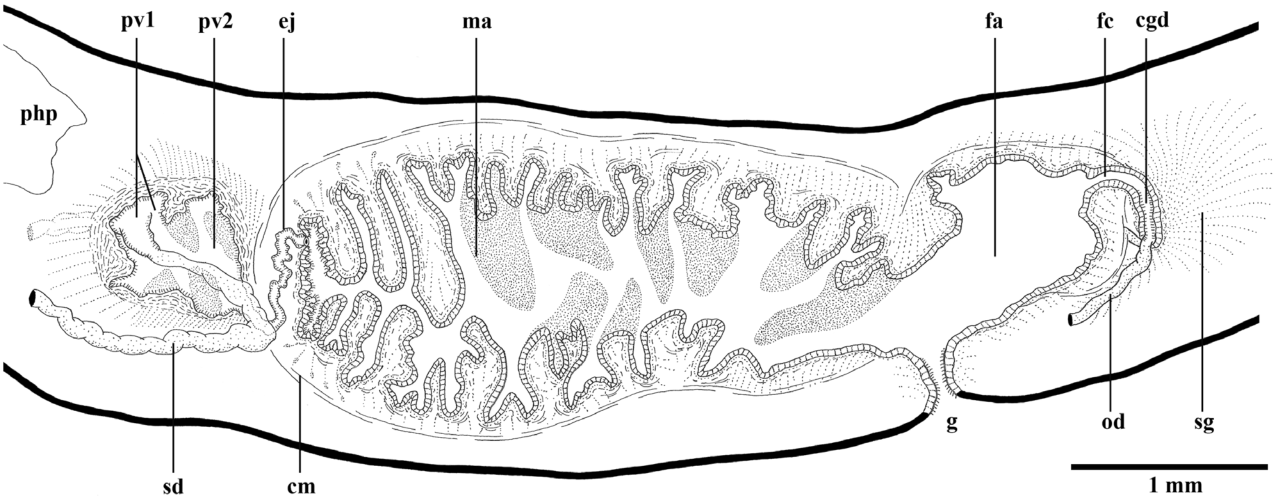

FIGURE 8. *Pasipha johnsoni* **sp. nov.** (MLP-He 6489). Sagittal reconstruction of the copulatory apparatus. (cgd) common glandular ovovitelline duct; (cm) common muscle coat; (ej) ejaculatory duct; (fa) female atrium; (fc) female genital canal; (g) gonopore; (ma) male atrium; (od) ovovitelline duct; (php) pharyngeal pouch; (pv1) proximal prostatic vesicle; (pv2) distal prostatic vesicle; (sd) sperm duct; (sg) shell glands.

epithelium. Cell bodies of these glands are located anterior to the pharynx and extend their necks towards the pharyngeal epithelium. The oesophagus (400–500 µm in length) is lined by a columnar epithelium, followed by a subepithelial circular muscular layer (15–20 µm thick) and a subjacent longitudinal one (10 µm thick). The oesophagus: pharynx ratio is 31–38%.

Male reproductive system. The testes, ovoid in shape, are located just below the supra-intestinal parenchymatic muscular layer, forming two irregular rows on each side of body (Fig. 7B, C). They are pre-pharyngeal and extend from behind the ovaries to the vicinity of the pharyngeal root, being located at a distance between 25% and 63% of the body length from anterior end. Testes occupy 18% of the body height. The sperm ducts are medial to ovovitelline ducts in the pre-pharyngeal region, being located between fibres of the sub-intestinal parenchymatic muscular layer (Fig. 7B, D). Posterior to the pharynx they are dilated and their lumen is full of spermatozoa, and in the vicinity to the common muscular coat they bend antero-dorsally and to the sagittal plane and open into the forked portions of the prostatic vesicle (Figs. 8, 9B). The extrabulbar prostatic vesicle, located just behind the pharynx (Fig. 9A), is globose and consists of two distinctive regions. Both exhibit folded walls with narrow lumen but differ histologically, in the type of glandular secretions that they receive (see below) and anatomically, since the proximal region is proximally forked (Figs. 8, 9A–C). The distal portion of the prostatic vesicle gradually diminishes in diameter and opens into the ejaculatory duct, which has a small portion external to the common muscle coat (Figs. 8, 9C). Once within the common muscle coat, the ejaculatory duct has an ascending sinuous course and opens into the bottom of the male atrium, dorsally displaced (Figs. 8, 9A). The male atrium (2.6 mm in length) is a richly folded cavity with a narrow lumen; its length is three times larger than that of the female atrium (Figs. 8, 9A). The most proximal region presents numerous small folds while the rest of the atrium exhibits larger folds (Figs. 8, 9A).

The lining epithelium of the sperm ducts is cuboidal and ciliated, and no underlying musculature is observed at pre-pharyngeal level, while posterior to the pharynx they exhibit squamous epithelium surrounded by a thin circular muscle layer (5 µm thick). The prostatic vesicle, lined by a ciliated columnar epithelium, is wrapped by a muscle coat with longitudinal and some circular fibres (40–60 µm thick), which is thinner in the proximal region (15–20 µm thick). The epithelium of proximal region of the prostatic vesicle is traversed by abundant fine granular pale erythrophil secretion while its distal part receives abundant coarse granular erythrophil secretion, strongly stained (Fig. 9A–C). Cell bodies of both glandular secretions are located in the surrounding parenchyma laterally and anteriorly to the prostatic vesicle. The ejaculatory duct is lined by a ciliated columnar epithelium followed by a circular muscle layer (5–10 µm thick) and a thin subjacent longitudinal (2.5 µm thick). The male atrium is lined in its most proximal region, in which there are small folds, by a columnar and ciliated epithelium (Fig. 9D). The rest of the male atrium is lined by a non-ciliated, cuboidal to columnar epithelium. The male atrial epithelium can be

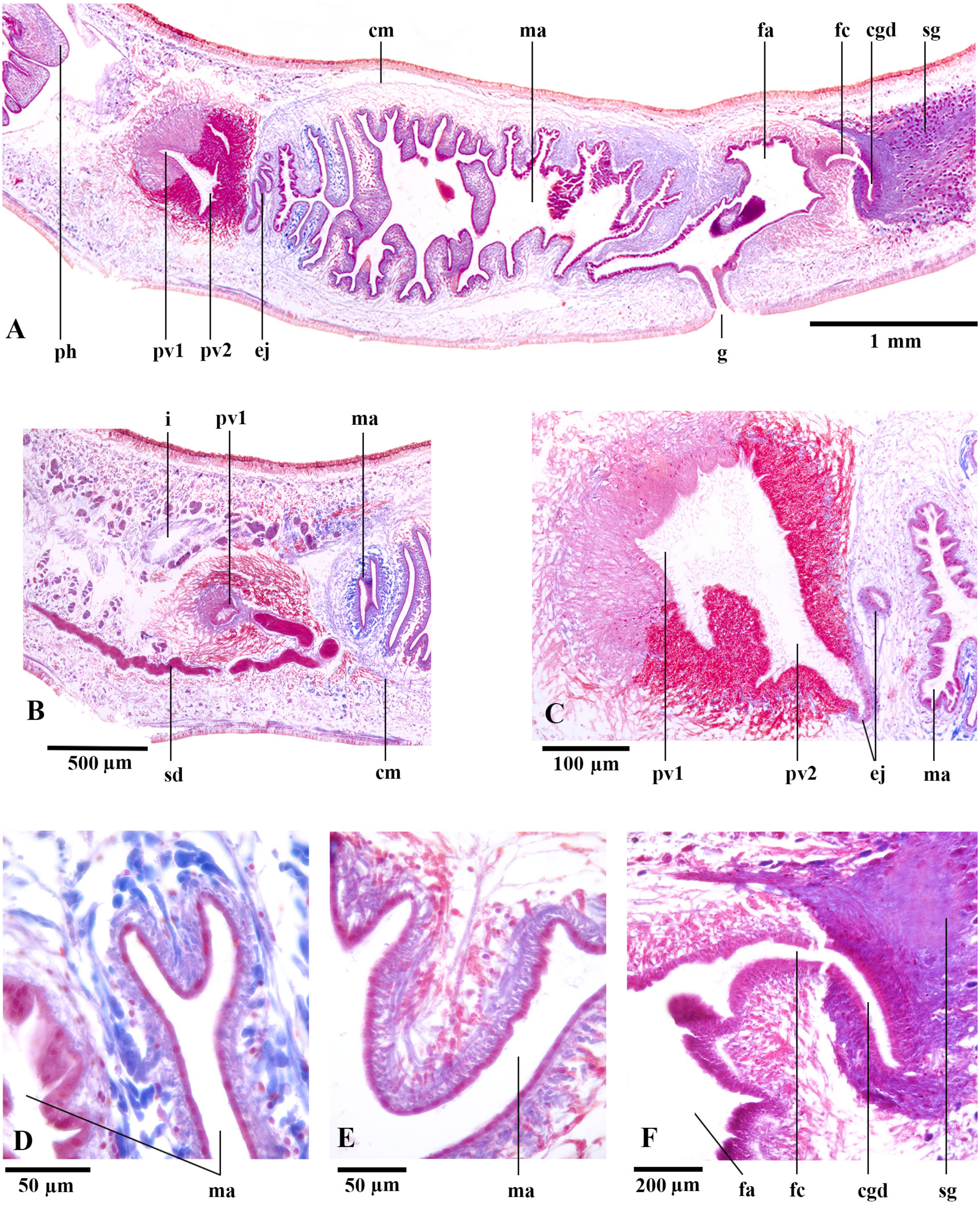

FIGURE 9. ***Pasipha johnsoni*** **sp. nov.** (MLP-He 6489). **(A)** Sagittal section of the copulatory apparatus. **(B–E)** Details of the male reproductive system, in sagittal view. **(F)** Detail of the female reproductive system, in sagittal view. (cgd) common glandular ovovitelline duct; (cm) common muscle coat; (ej) ejaculatory duct; (fa) female atrium; (fc) female genital canal; (g) gonopore; (i) intestine; (ma) male atrium; (ph) pharynx; (pv1) proximal prostatic vesicle; (pv2) distal prostatic vesicle; (sd) sperm duct; (sg) shell glands.

differentiated into three regions according to the glandular secretions that discharge into it. The proximal region is traversed by amorphous cyanophil secretion; the median region receives abundant fine granular erythrophil secretion, and the distal region receives abundant fine granular cyanophil secretion (Fig. 9A, D, E). The muscularis of the male atrium consists of a subepithelial circular layer (5 µm thick) followed by a longitudinal layer (10 µm thick). The common muscle coat consists of longitudinal fibres (15–30 µm thick) that bend and enter into the atrial folds, but without forming a coherent layer (Fig. 8).

Female reproductive system. The ovaries (300 µm in length and 200 µm high, in the holotype), ovoid in shape, are located ventrally to the intestine, between the sub-intestinal parenchymatic muscle layer and the nervous plate (Fig. 7F). They are situated at 7.7 mm from the anterior end (21% of body length). The ovovitelline ducts arise from the dorsal side of the ovaries, and backwards they are located below the sub-intestinal parenchymatic muscle layer at pre-pharyngeal level (Fig. 7D). Behind the gonopore they still run below the copulatory apparatus and progressively bend to the sagittal plane. In their distal course the ovovitelline ducts contour the common muscle coat and join in a common glandular ovovitelline duct (250 µm length) which runs vertically behind the female atrium (Figs. 8, 9A, F). The female genital canal is an almost horizontal duct (240 µm length) that connects the common glandular duct to the female atrium (Figs. 8, 9F). The female atrium (850 µm length) is a poorly folded cavity, although there is large fold shared by both atria which hangs from their dorsal walls (Figs. 8, 9A).

The ovovitelline ducts are lined with a cuboidal and ciliated epithelium, followed by a circular muscle layer (5 µm thick). Their distal ascending portions receive secretion from the shell glands. The lining epithelium of the common glandular ovovitelline duct is columnar and ciliated, traversed by abundant secretion from shell glands, followed by a subjacent muscle layer consisting of circular and oblique interwoven fibres (15 µm thick) (Fig. 9A, F). The epithelium of the female canal is columnar, followed by a thin muscle layer of circular fibres (5 µm thick). The female atrium is lined by a columnar epithelium and its muscularis consists of a circular layer (5 µm thick) followed by a loosely organized longitudinal layer (10 µm thick). The female canal and atrium receive abundant fine granular erythrophil secretion (Fig. 9A, F), and the epithelium of the distal portion of the female atrium is traversed by abundant fine granular cyanophil secretion (Fig. 9A). Cell bodies of these glands are located in the surrounding parenchyma outside the common muscle coat. This coat consists of longitudinal fibres (10–15 µm thick). The gonopore canal is lined by a columnar and ciliated epithelium, receiving openings of scarce cyanophil glands with fine granular secretion. Vitellaria are developed only in the mature specimen (holotype), with follicles located dorsal and ventral to the intestinal branches (Fig. 7B–D, F).

Parasitism. In the holotype, nematode larvae were found in the parenchyma of the anterior body region and in the pharyngeal region.

Pasipha mbya **sp. nov.**

(Figs. 10–14)

Pasipha sp. 1: Negrete *et al.* 2014a

Type material. Holotype. MLP-He 6476. Urugua-í Wildlife Reserve, Misiones Province, Argentina, 21.VIII.2009, L. Negrete, coll.; cephalic region and anterior region at level of ovaries: transverse sections on 42 slides (8 µm); pre-pharyngeal region: transverse sections on 12 slides (8 µm); pharynx and copulatory apparatus: sagittal sections on 30 slides (8 µm).

Paratype. MLP-He 6978. Iguazú National Park, Misiones Province, Argentina, 13.IV.2013, L. Negrete coll.; cephalic region: transverse sections on 36 slides (6–8 µm); anterior region at level of ovaries: sagittal sections on 28 slides (6–8 µm); pre-pharyngeal region: transverse sections on 5 slides (6 µm); pharynx and copulatory apparatus: sagittal sections on 35 slides (7 µm).

Diagnosis. Dorsum dark grey with a yellow median longitudinal band, and thin black para-median stripes. Eyes dorsal with clear halos. Glandular margin present. Pharynx cylindrical. Prostatic vesicle extrabulbar, globose, with folded walls, and proximally forked. Male atrium richly folded, with small folds in its proximal part, 2.5–4 times longer than the female atrium. Ovaries anterior to the anteriormost testes. Ovovitelline ducts ventral to the female atrium, joining behind it. Common glandular ovovitelline duct vertical, and female genital canal almost horizontal. Female atrium without folded walls.

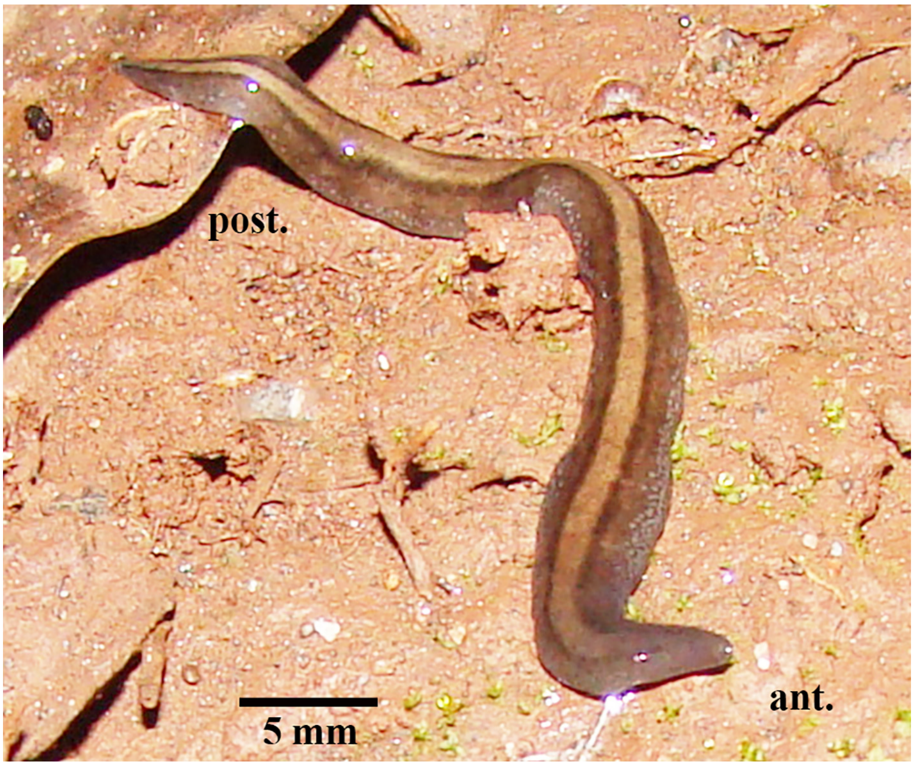

FIGURE 10. *Pasipha mbya* **sp. nov.** (MLP-He 6476). Photograph of a live specimen, in dorsal view.

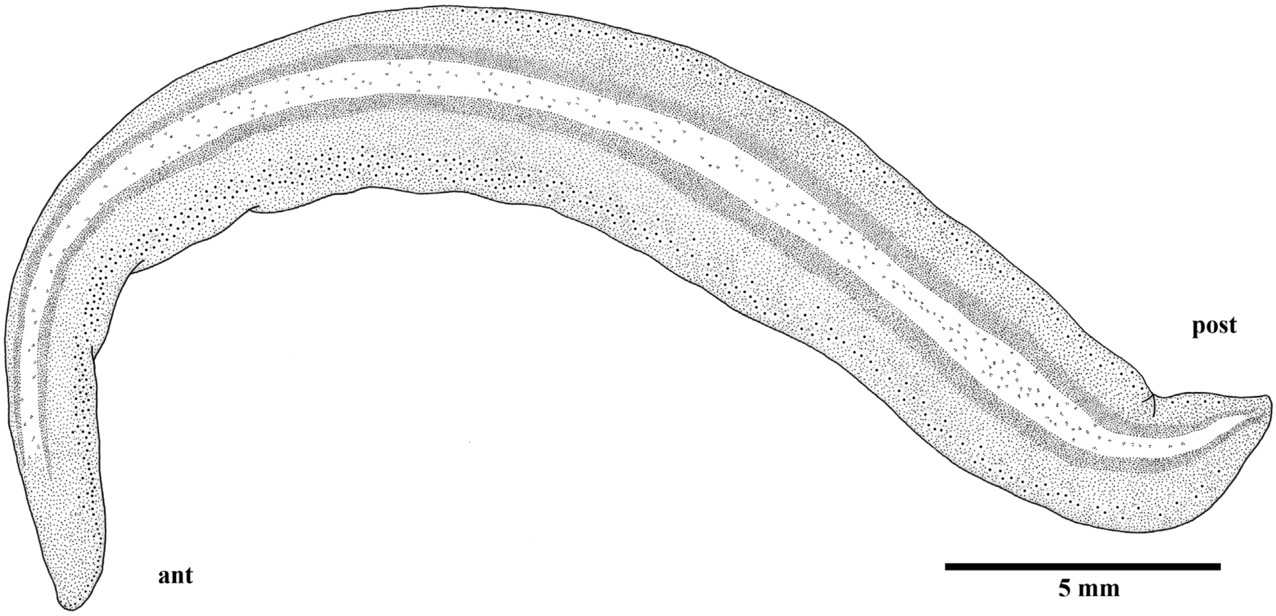

FIGURE 11. *Pasipha mbya* **sp. nov.** (MLP-He 6476).Schematic drawing of the colour pattern and eyes, in dorso-lateral view.

Etymology. The specific name is devoted to the Mbyá indigenous community, member of the Guaraní Tribe, native people of the rainforests of Misiones Province, Argentina.

Type locality. Urugua-í Wildlife Reserve (25° 59' S, 54° 05' W), Misiones Province, Argentina.

External morphology. The body is elongate, with parallel margins. The anterior tip is slightly rounded and the posterior end is sharply pointed. The dorsal surface is dark grey with a longitudinal yellow median band which arises a few millimetres from the tip and extends along the body (Fig. 10). The yellow band, the width of which is approximately one quarter of the body width; it is slightly mottled with black spots, mostly concentrated at the level of pharynx and copulatory apparatus. Thin black para-median stripes border the median band (Figs. 10, 11).

After fixation, the ground colour becomes paler, and para-median stripes are no longer distinguishable. The ventral surface is light grey. The eyes are initially distributed in a single row around the anterior tip. Posteriorly, the eyes are located uniserially on the body margins along 2 mm and biserially for 2 mm. Then they spread dorsally, forming 4–5 irregular rows on each side of the body. The dorsal eyes are surrounded by clear halos. At the level of the pharynx, the eyes are less numerous and at the level of the copulatory apparatus they are again uniserial and scattered on the body margins, reaching the posterior end (Fig. 11). The holotype was 40 mm long and ~2.5 mm wide when crawling. After fixation, body length of study specimens ranged from 28 mm (holotype) to 46.2 mm, maximum width was 3–3.2 mm, and maximum height 1.3–1.5 mm. The mouth and the gonopore were located at a distance of 70–72% and 83–84% from the anterior tip, respectively (Table 4).

TABLE 4. Measurements (mm) from fixed specimens of *Pasipha mbya* **sp. nov.** (DG) distance of gonopore from anterior end, (DM) distance of mouth from anterior end, (DMG) distance between mouth and gonopore. The numbers in parentheses represent the position relative to body length (%).

	MLP-He 6476	MLP-He 6978
Length	28	46.2
Maximum width	3	3.2
Maximum height	1.3	1.5
DM	20.2 (72%)	32.4 (70%)
DG	23.6 (84%)	38.4 (83%)
DMG	3.4	6
Creeping sole (%)	90%	90%

Internal morphology. *Epidermis, secretions and musculature in the cephalic region.* Dorsal epidermis (20–25 µm height) receives abundant rhabditogen secretion and amorphous erythrophil secretion. Scarce fine granular erythrophil and cyanophil secretions discharge through dorsal epidermis, although they are more abundant on the body margins (Fig. 12A). Ventral epidermis (25–30 µm height) presents small dermal rhabdites and receives abundant fine granular erythrophil secretion, and scarce amorphous erythrophil and fine granular cyanophil secretions. A single row of sensory pits (20–25 µm deep) surrounds the cephalic region and they spread toward body margins up to 1.5 mm from the anterior tip.

The cutaneous musculature, composed of the same layers as the pre-pharyngeal region (see below), is slightly thicker than at the pre-pharyngeal level (14–16% of the body height). The thickness of the parenchymatic musculature is also larger than in the pre-pharyngeal region (9–14% of the body height). In the anterior body region, there is a subneural muscular transverse layer (15–25 µm thick) situated below the nervous plate, which arises very close to the anterior tip and extends for ~2 mm (Fig. 12A). There is no musculo-glandular specialization in the cephalic region.

Epidermis, secretions and musculature in the pre-pharyngeal region. Dorsal epidermis (20–35 µm high) and body margins contain abundant rhabdites (Fig. 12B, C). Rhabditogenic glands are situated in the parenchyma below the cutaneous musculature. There are glands with amorphous erythrophil and scarce cyanophil secretions which discharge through the dorsal epidermis. Ventrally, the epidermis (25–35 µm high) is ciliated, forming a wide creeping sole (~90% of body width). In the ventral epidermis, amorphous cyanophil glandular secretion is more abundant that on the dorsal surface and there is scarce amorphous erythropil secretion, with small dermal rhabdites occupying the apex of the epidermal cells. The glandular margin consists of abundant fine granular erythrophil and cyanophil secretions (Fig. 12D).

The cutaneous musculature consists of an external subepithelial circular layer, followed by a diagonal layer, and an internal longitudinal layer arranged in bundles, which is thicker ventrally than dorsally (Table 5). CMI ranges from 13% to 14%. Parenchymatic musculature is arranged in three layers: a dorsal layer with decussate fibres (35 µm thick), located below the cutaneous dorsal longitudinal bundles, a supra-intestinal layer and a sub-intestinal transverse layer (7.5–10 µm thick and 25–35 µm thick, respectively). PMI ranges from 6% to 9% (Table 5). Dorso-ventral fibres are intermingled among the intestinal branches.

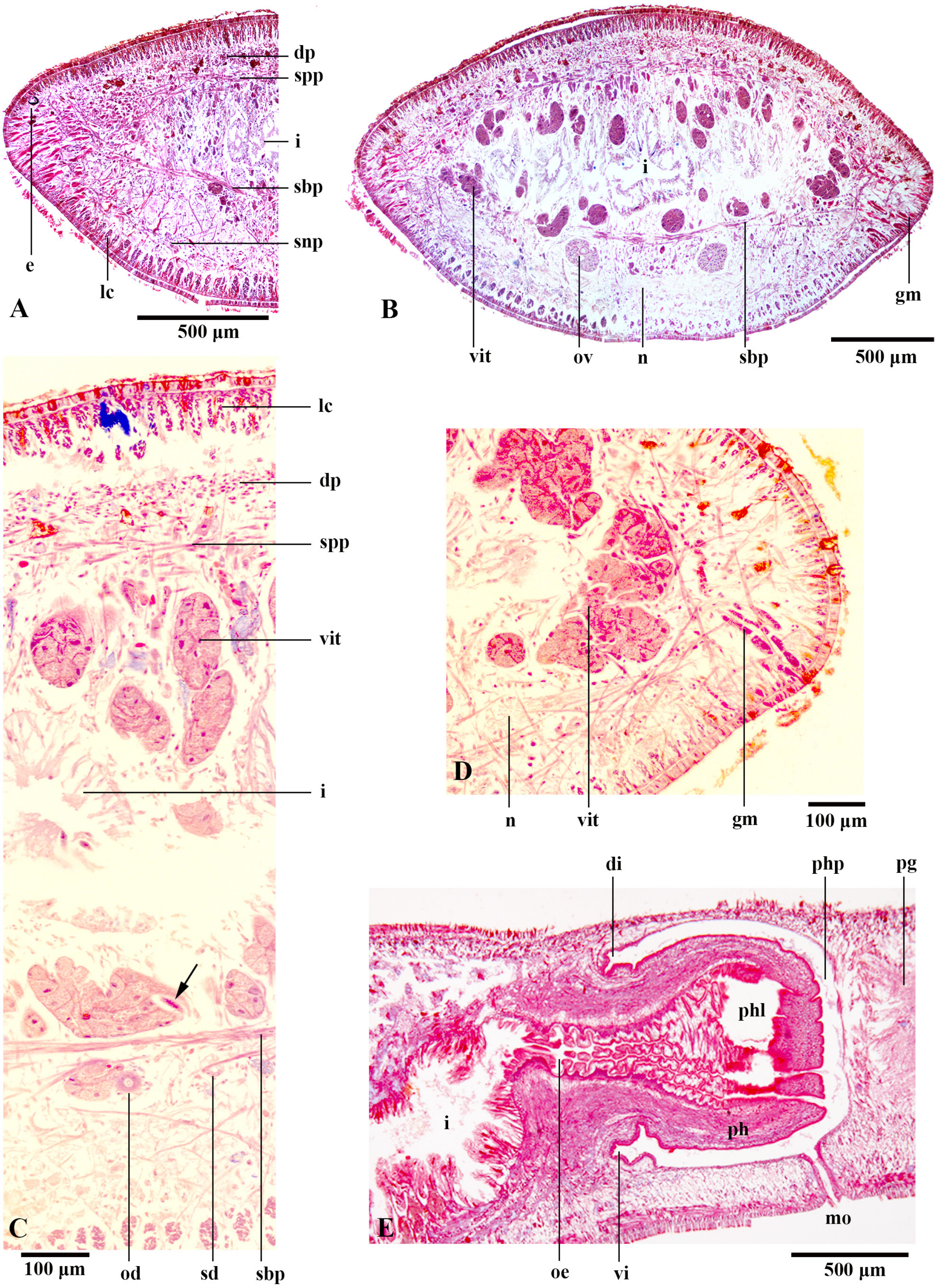

FIGURE 12. *Pasipha mbya* **sp. nov. (A)** Half of a transverse section of the cephalic region (MLP-He 6978). **(B)** Transverse section of the anterior region (MLP-He 6978). **(C–D)** Details of transverse sections at pre-pharyngeal level (MLP-He 6978). **(E)** Sagittal section of the pharynx (MLP-He 6476). (di) dorsal insertion; (dp) dorsal parenchymatic muscle layer; (e) eye; (gm) glandular margin; (i) intestine; (lc) longitudinal cutaneous muscle layer; (mo) mouth; (n) nervous plate; (od) ovovitelline duct; (oe) oesophagus; (ov) ovary; (ph) pharynx; (phl) pharyngeal lumen; (php) pharyngeal pouch; (pg) prostatic glands; (sbp) sub-intestinal parenchymatic muscle layer; (sd) sperm duct; (snp) sub-neural parenchymatic muscle layer; (spp) supra-intestinal parenchymatic muscle layer; (vi) ventral insertion; (vit) vitellaria. Arrow indicates nematode larva.

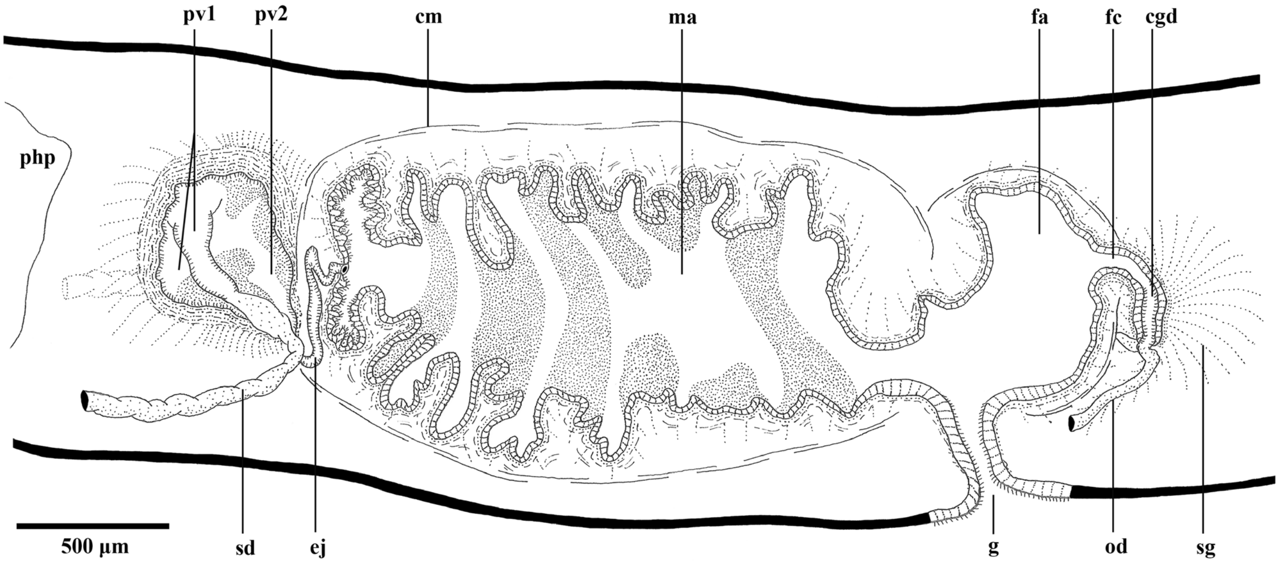

FIGURE 13. *Pasipha mbya* **sp. nov.** (MLP-He 6476). Sagittal reconstruction of the copulatory apparatus. (cgd) common glandular ovovitelline duct; (cm) common muscle coat; (ej) ejaculatory duct; (fa) female atrium; (fc) female genital canal; (g) gonopore; (ma) male atrium; (php) pharyngeal pouch; (od) ovovitelline duct; (pv1) proximal prostatic vesicle; (pv2) distal prostatic vesicle; (sg) shell glands; (sd) sperm duct.

TABLE 5. Thickness of cutaneous (CM) and parenchymatic (PM) musculatures (µm), and CMI and PMI indices at pre-pharyngeal region of *Pasipha mbya* **sp. nov.**

	MLP-He 6476	MLP-He 6978
CM dorsal		
circular	5	2.5
diagonal	12.5	10
longitudinal	45	75
CM ventral		
circular	5	5
diagonal	12.5	10
longitudinal	82.5	100
CMI (%)	13%	14%
PM dorsal	35	60
PM supra-intestinal	10	35
PM sub-intestinal	35	35
PMI (%)	6%	9%

Digestive system. The pharynx (0.95–1.9 mm in length, about 3–4% of body length) is cylindrical, with the mouth opening into the distal portion of the pharyngeal pouch (1–2.5 mm in length) (Fig. 12E). The epithelial lining of the outer surface of the pharynx is cuboidal and ciliated, followed by a longitudinal subepithelial muscle layer (5–10 µm thick) and a subjacent circular layer (20–25 µm thick). The epithelium of the pharyngeal lumen is columnar and ciliated. The inner pharyngeal musculature consists of a circular layer (15–35 µm thick) followed by a longitudinal layer (10–15 µm thick). Fine granular erythrophil and cyanophil secretions from cell glands located in the surrounding parenchyma before the pharynx discharge through the pharyngeal epithelium. Amorphous erythrophil and cyanophil secretions are present in less quantity, the cell bodies of which are subepithelial. Oesophagus (350–400 µm in length) is present (Fig. 12E). The oesophagus: pharynx ratio ranges from 21% to 37%.

Male reproductive system. The oval testes are arranged in two or three irregular rows on each side of the body.

They are located dorsally to the intestine, beneath the supra-intestinal parenchymatic muscle layer. The ratio of the height of the testes to the height of body ranges from 16% to 26%. The testes are located at a distance between 24% and 63–66% (Table 6) of the body length from the anterior end. The sperm ducts are located beneath the sub-intestinal parenchymatic muscle layer, and medial to the ovovitelline ducts in the pre-pharyngeal region (Fig. 12C). Behind the pharynx, the sperm ducts follow their lateral course, ventrally to the prostatic vesicle, reaching the vicinity of the common muscle coat of the male atrium. Then they bend antero-dorsally and run to the sagittal plane, opening into the bifurcated portions of the proximal prostatic vesicle (Fig. 13). At this point, the sperm ducts are widened and their lumen full of spermatozoa. The extrabulbar prostatic vesicle, located immediately behind the pharynx, is globose with irregular lumen (Figs. 13, 14A, B). It consists of two morphologically and histologically distinct regions (see below), namely: a proximal region, which is proximally forked, and a distal region, which communicates with the ejaculatory duct exactly at the boundary of the common muscle coat (Figs. 13, 14A, B). The ejaculatory duct has an ascending course and is sinuous distally, opening into the bottom of the male atrium (Figs. 13, 14C). The richly folded male atrium is 2.5 to 4 times longer than the female atrium. Their proximal part is characterized by small folds, while the rest of the atrial cavity exhibits larger folds (Figs. 13, 14A, B).

TABLE 6. Measurements (mm) of reproductive organs of *Pasipha mbya* **sp. nov.** LCGD, length of common glandular ovovitelline duct; LFA, length of female atrium; LFC, length of female canal; LMA, length of male atrium; LPV, length of prostatic vesicle. The numbers in parentheses represent the position relative to body length (%).

	MLP-He 6476	MLP-He 6978
Anteriormost testes	6.7 (24%)	11 (24%)
Posteriormost testes	18.6 (66%)	29 (63%)
LMA	1.6	4
Location of ovaries	6.2 (22%)	10 (22%)
LCGD	0.2	0.35
LFC	0.2	0.35
LFA	0.4	1.6

The prostatic vesicle is lined with a ciliated columnar epithelium. The proximal part is provided with abundant fine granular erythrophil secretion, whereas the distal part receives abundant coarse granular erythrophil secretion (Fig. 14A, B). Cell bodies of glands opening into both regions are located in the surrounding parenchyma (Fig. 12E). The thick muscle coat which envelops the prostatic vesicle consists of circular, longitudinal and oblique intermingled fibres (70–90 µm thick). The ejaculatory duct presents a ciliated columnar epithelium, which receives in its distal course erythrophil amorphous secretion (Fig. 14C). The lining epithelium of the most proximal region of the male atrium is columnar and ciliated, and receives fine granular erythrophil and cyanophil secretions. The remaining walls of the male atrium are lined by a non-ciliated, cuboidal to columnar epithelium. Excepting the most proximal portion, the remaining proximal half of the male atrium receives only abundant fine granular erythrophil secretion. The epithelium of the distal half is provided with abundant fine granular and amorphous erythrophil secretions, and fine granular cyanophil secretion which is more abundant at the level of the dorsal fold. The muscularis of the male atrium consists of a subepithelial circular layer (5–10 µm thick) followed by a longitudinal layer (15–30 µm thick). The common muscle coat consists dorsally of intermingled longitudinal and oblique fibres (40–60 µm thick), and ventrally of longitudinal fibres (25 µm thick). The male atrium is separated from the female atrium by a dorsal fold, so that the lumen between both atria is narrow (Figs. 13, 14A).

Female reproductive system. The ovaries (250–300 µm in length and 250 µm high, in the holotype) are located at a distance of 22% of the body length from the anterior end (Table 6). They are located between the sub-intestinal parenchymatic muscle layer and the nervous plate (Fig. 12B). The ovovitelline ducts emerge from the postero-dorsal region of the ovaries, with spermatozoa in their lumen. Behind the gonopore, the ovovitelline ducts ascend slightly, run to the sagittal plane and open into a vertical common glandular ovovitelline duct located behind the female atrium (Figs. 13, 14A, D). The female genital canal runs forward almost horizontally to open into the female atrium, with poorly folded walls (Figs. 13, 14A, D).

The epithelium of the ovovitelline ducts is ciliated and cuboidal, surrounded by a thin longitudinal muscle layer (5 µm thick). The common glandular ovovitelline duct is lined by a ciliated columnar epithelium followed by a subjacent circular muscle layer (10–15 µm thick). Both the distal portions of the ovovitelline ducts and the common glandular ovovitelline duct receive abundant secretion from shell glands (Figs. 13, 14D). The female genital canal is lined by a columnar and non-ciliated epithelium, and its musculature consists of circular (10 µm thick) and longitudinal (10 µm thick) layers. The lining epithelium of the female canal is filled with fine granular erythrophil secretion and scarce fine granular cyanophil secretion. The female atrium is lined by a non-ciliated pseudostratified columnar epithelium, and its muscularis consists of circular and longitudinal intermingled fibres (10–15 µm thick). The atrial epithelium receives abundant openings from glands with fine granular cyanophil secretion and less abundant glands with fine granular erythrophil secretion. The common muscular coat consists dorsally of longitudinal and some oblique fibres (50 µm thick), and ventrally of only longitudinal fibres (30 µm thick). The gonopore is an almost straight canal, and its ciliated columnar epithelium receives openings of scarce glands containing fine granular cyanophil secretion. Vitellaria are well developed in both specimens studied, both dorsal and ventral to the intestine branches and between them (Fig. 12B–D).

Parasitism. Nematode larvae were found in the parenchyma of the anterior and pre- pharyngeal region, and within vitelline follicles (Fig. 12C).

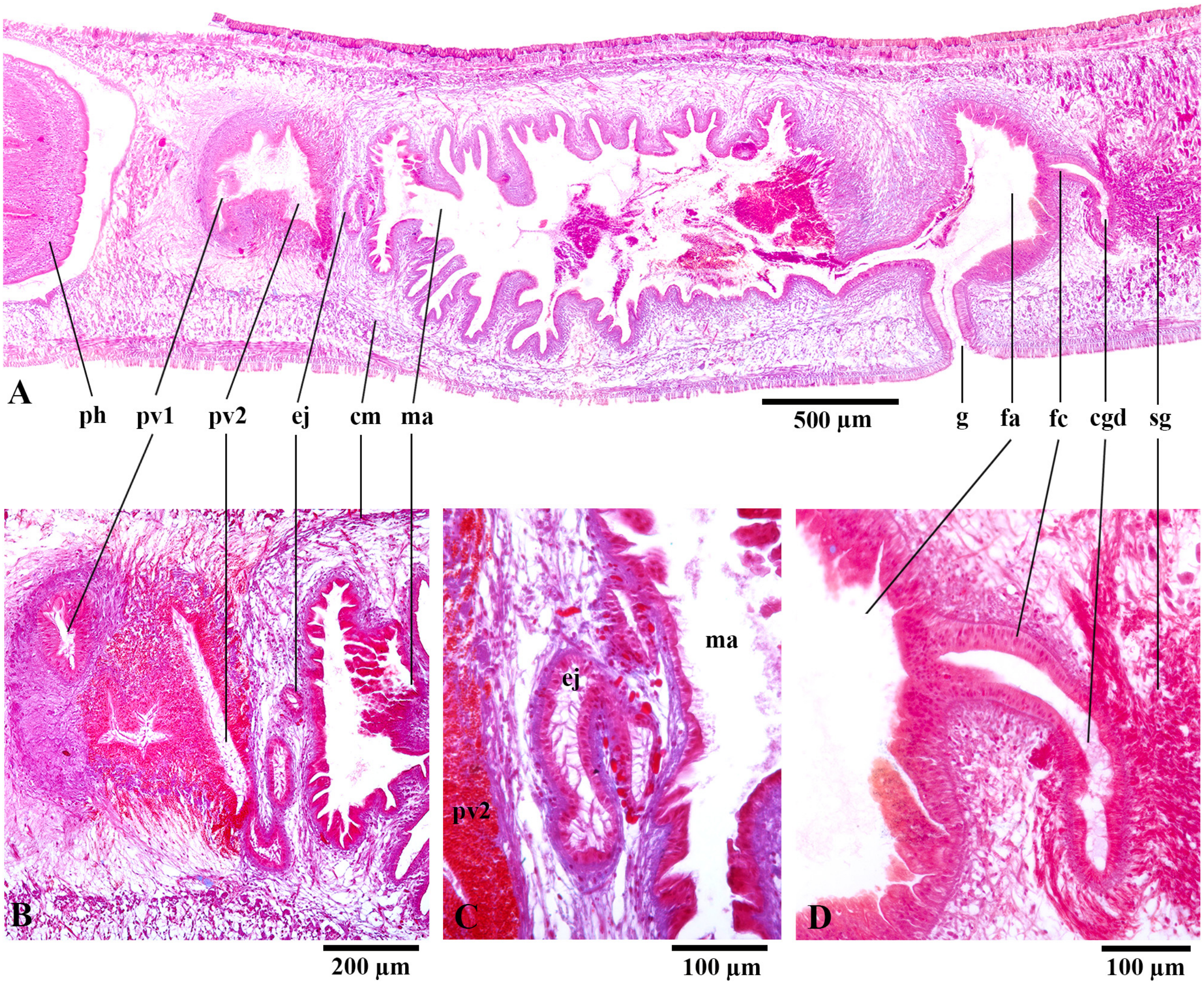

FIGURE 14. *Pasipha mbya* **sp. nov.** (MLP-He 6476). **(A)** Sagittal section of the copulatory apparatus. **(B–C)** Details of the male reproductive system, in sagittal view. **(D)** Detail of the female reproductive system, in sagittal view. (cgd) common glandular ovovitelline duct; (cm) common muscle coat; (ej) ejaculatory duct; (fa) female atrium; (fc) female genital canal; (g) gonopore; (ma) male atrium; (ph) pharynx; (pv1) proximal prostatic vesicle; (pv2) distal prostatic vesicle; (sg) shell glands.

Discussion

The genus *Pasipha* was formally proposed by Ogren & Kawakatsu (1990) to split some species of *Geoplana* Stimpson, 1857 on the basis of previous works by EM Froehlich (1955) and CG Froehlich (1967). Ogren & Kawakatsu (1990) grouped species "characterized by an elongate body, absence of a penis papilla, folded male atrium, and female genital canal arriving from the ventral side" (Froehlich 1967). Since then, this combination of characters has been approximately sufficient to identify the genus. However, Carbayo *et al.* (2013) established an amended diagnosis of the genus, adding new features regarding both the external and internal anatomy, which have restricted its definition. Also, Carbayo *et al.* (2013) considered six species as *incertae sedis*, owing to the scarcity of morphological data or features incongruent with the diagnosis.

The three new species described herein are consistent with the current diagnosis of the genus *Pasipha*. Externally, they exhibit a slender flattened body, especially when crawling, with parallel margins. Regarding the reproductive system, the male atrium lacks a permanent penis papilla, although the eversion of their richly folded walls may act as a temporary penis (Winsor 1998). In addition, the male atrium is separated from the female atrium by a dorsal fold, the prostatic vesicle is extrabulbar and differentiated into regions by the secretions that it receives, and the ovovitelline ducts which run below the female atrium join each other behind it (Carbayo *et al.* 2013).

Many *Pasipha* species exhibit homogeneous internal anatomy, mainly with respect to the copulatory apparatus, sometimes making it difficult to compare species. Therefore, the combination of the external appearance and details of the internal anatomy (such as the occurrence of glandular margin, the shape of the prostatic vesicle and the type of secretions it receives, the position of the openings of the sperm ducts into the prostatic vesicle, the epithelial lining of the male and female atria and the secretions which traverse them, the morphology and position of the common ovovittelline duct and female canal) is very useful for purposes of comparison. For greater clarity, the new species described herein are discussed separately.

***Pasipha atla* sp. nov.** According to the pattern of pigmentation of the dorsal surface, *Pasipha atla* **sp. nov.** resembles the type species *Pasipha pasipha* (Marcus, 1951), *Pasipha hauseri* (Froehlich, 1959), *Pasipha brevilineata* Leal-Zanchet *et al.*, 2012, all of them described from Brazil, and two species considered by Carbayo *et al.* (2013) as *incertae sedis* namely, *Pasipha aphalla* (Hyman, 1941) and *Pasipha ercilla* (Froehlich, 1978), from Panama and Chile, respectively. *Pasipha pasipha* has a brown pigmentation similarly to *Pasipha atla* **sp. nov.**, but differs from the new species in which the thin median stripe running along the entire body, and in the cephalic region there is a thin transversal stripe (Marcus 1951), which is absent in *P. atla* **sp. nov.** With regard to the copulatory apparatus, even though the prostatic vesicle is similar in both species, being tubular and proximally forked, in *P. pasipha* it is highly sinuous, mainly in the distal part, opening into the male atrium through the ejaculatory duct which runs mostly outside the common muscle coat (Marcus 1951), while in *P. atla* **sp. nov.** the ejaculatory duct is surrounded by the muscle fibres of the common muscle coat. *Pasipha hauseri* presents a greyish-brown pigment on the dorsal surface with a lighter stripe that is continuous along the body, unlike the new species (Froehlich 1959). Internally, one remarkable difference between both species is the pharynx, which is collar type in *P. hauseri* but cylindrical in *P. atla* **sp. nov.** *Pasipha brevilineata* has a dark-brown pigmentation with a thin median stripe which is sometimes interrupted, becoming conspicuous in the anterior and posterior body regions (Leal-Zanchet *et al.* 2012). Those specimens in which the median stripe is incompletely disposed along the dorsum could be appear similar to *P. atla* **sp. nov.** However, *P. brevilineata* differs from the new species in regards to the internal anatomy. In the Brazilian species was described a glandular margin, composed by four types of secretion, absent in *P. atla* **sp. nov.** Also, the cutaneous musculature of *P. brevilineata* is somewhat thicker (CMI: 17–19%) than in *P. atla* **sp. nov.** (CMI: 9–15%). In both species, the extrabulbar prostatic vesicle comprises two parts, being forked its proximal portion and tubular in shape. However, in *P. brevilineata*, the distal portion is globose in shape, meanwhile in *P. atla* **sp. nov.** is tubular. Besides, *P. brevilineata* can be distinguished from the new species considering the type of secretions which traverse the prostatic epithelium, particularly in the proximal forked portion. In *P. brevilineata*, the epithelial lining is traversed by fine granular erythrophil secretion (Leal-Zanchet *et al.* 2012), but in *P. atla* **sp. nov.** it receives fine granular cyanophil secretion. In relation to the female reproductive system, the common ovovitelline duct in *P. brevilineata* is a slightly ascendent canal that contours the common muscle coat and runs below the distal part of the female atrium. By contrast, the common ovovitelline duct in *P. atla* **sp. nov.** is vertical and posterior to the female atrium.

With respect to the *incertae sedis* species, *P. aphalla* has uniform dark brown colour. Unfortunately, the internal anatomy is not sufficiently known and in her reconstruction of the copulatory apparatus, Hyman (1941) did not mention the presence of a prostatic vesicle connecting the sperm ducts to the male atrium, so it may have been an incompletely mature specimen, since it was 5 mm in length. *Pasipha ercilla* exhibits some similarity with *P. atla* **sp. nov.**, whose dorsal surface is marbled as a result of the combination of dark brown and black pigmentation, but differs from the new species in a thin median longitudinal stripe running along the dorsum (Froehlich 1978). The specimen on which the original description is based lacks a fully developed copulatory apparatus, making any comparison difficult. The incompleteness development of the copulatory apparatus is evident due to the absence of an extrabulbar prostatic vesicle, the ventral wall of the male atrium is smooth, and the whole atrial cavity is lined with a flat epithelium (Froehlich 1978). Additionally, there is no separation between male and female atria.

Pasipha johnsoni. Among species of the genus, *Pasipha johnsoni* **sp. nov.** can be distinguished externally from others by its distinctive colour pattern, with two thin black lateral stripes on a dark grey ground. Regarding internal anatomy, the new species shares affinities with *Pasipha chimbeva* (Froehlich, 1955), described for Brazil, and *Pasipha mbya* **sp. nov.** Like *P. johnsoni* **sp. nov.**, these species present a globose prostatic vesicle with folded walls, located just behind the cylindrical pharynx, female atrium without folded walls and much shorter than the male atrium, a dorsal fold separating the two atrial cavities, and the common glandular ovovitelline duct and female genital canal almost vertically and horizontally oriented, respectively. However, *P. johnsoni* **sp. nov.** can be distinguished from *P. chimbeva* because in the latter species the prostatic vesicle only receives a single type of erythrophil secretion and its proximal portion is not forked (EM Froehlich 1955). Another distinguishing feature between *P. johnsoni* **sp. nov.** and this Brazilian species is the ejaculatory duct, which has both an extrabulbar and intrabulbar course in *P. chimbeva* (EM Froehlich 1955), whereas in the new species it is only intrabulbar. The copulatory apparatus of *P. johnsoni* **sp. nov.** is highly similar to *P. mbya* **sp. nov.**, although the male atrium of *P. johnsoni* **sp. nov.** is characterized by having three regions according to the different secretions that discharge into the atrial epithelium, which is not observed in *P. mbya* **sp. nov.**

Pasipha mbya. The colour pattern of *Pasipha mbya* **sp. nov.** resembles that of *Pasipha backesi* Leal-Zanchet *et al.*, 2012, *Pasipha penhana* (Riester, 1938) and *Pasipha tapetilla* (Marcus, 1951), all of them described from Brazil. These species have a yellowish median band with black para-median stripes on the dorsum. However, *P. backesi* and *P. penhana* also have thin dark lateral stripes, while in *P. tapetilla,* lateral and marginal black stripes are also arranged on the dorsal surface (Riester 1938; Marcus 1951; Leal-Zanchet *et al.* 2012). The pattern of eye distribution differs between both Brazilian flatworms and the new species. In the former, the eyes are spread pluriserially very close to the anterior tip on the dorsum, but in *P. mbya* **sp. nov.**, they become gradually dorsal and pluriserial.

With regard to internal morphology *P. mbya* **sp. nov.** can be distinguished from *P. penhana* because in the latter species the pharynx is collar-shaped, and the prostatic vesicle, named "seminal duct and glandular ejaculatory duct" by Riester (1938), is tubular. In *P. tapetilla*, whose pharynx is cylindrical as in *P. mbya* **sp. nov.**, the prostatic vesicle is also tubular. The reproductive system of *P. mbya* **sp. nov.**, mainly the male reproductive system, is similar to *P. backesi,* since both species have an extrabulbar prostatic vesicle, globose in shape, with folded walls and narrow lumen, forked in its proximal part. Also, in both the new species and *P. backesi* the prostatic vesicle is characterized by receiving the similar types of secretions, so that the vesicle can be differentiated into two portions (Leal-Zanchet *et al.* 2012). Similarly to *P. backesi*, in the new species, the ejaculatory duct ascends sinuously, within the common muscle coat, and opens almost into the bottom of the male atrium which exhibits numerous small folds in its proximal part. However, *Pasipha mbya* **sp. nov.** can be distinguished from *P. backesi* because in the latter species, the common glandular ovovitelline duct is almost horizontal and ventrally located to the female atrium, and the female genital canal has an almost vertical position. In *Pasipha mbya* **sp. nov.**, the relation is reverse, with the common ovovitelline duct running vertically and posterior to the female atrium, and the female canal turning almost horizontally before opening into the female atrium.

The three new species of *Pasipha* can be distinguished from each other mainly by their colour pattern of the dorsal surface and some details of the internal anatomy, mainly with respect to the copulatory apparatus. All species herein described exhibit similar characteristics regarding the thickness of the cutaneous (CMI) and parenchymatic (PMI) musculatures, pharyngeal morphology, relative position of ovaries regarding the anteriormost testes, and relative position of testes in relation to body length. However, they show dissimilarities regarding the presence/absence of glandular margin, the position where ovovitelline ducts emerge from the ovaries, the morphology and secretions of the prostatic vesicle, and the type of secretions which discharge along the male and female atria.

Acknowledgements

The authors thank Fundación Vida Silvestre Argentina and Ministerio de Ecología y Recursos Naturales Renovables de Misiones (Misiones, Argentina) for permission to conduct surveys at Urugua-í Wildlife Reserve (UWR). We are grateful to Administración de Parques Nacionales for permission to conduct sampling at Iguazú National Park (INP). We are thankful to Instituto Nacional de Tecnología Agropecuaria (INTA) for permission to perform surveys at San Antonio Strict Nature Reserve. We also thank UWR park rangers Ariel Tombo, Laura Aréjola and Marcelo Rivero for their support during the fieldwork, and Marcelo Kostlin for his cooperation in the survey at INP. This work was partially financed by CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas) PIP 112–201201–00635 CO, Agencia Nacional de Promoción Científica y Tecnológica (FONCyT) PICT 2014-0768, and Universidad Nacional de La Plata (UNLP) projects PPID/N013 and 11/N728. We would like to thank Dr. Ana M. Leal-Zanchet and Dr. Wolfgang Sterrer for their constructive comments on an early version of the manuscript.

References

Carbayo, F., Froehlich, E.M., Leal-Zanchet, A.M. & Amato, S.B. (2009) Turbelários (Platyhelminthes). *In:* Moreira da Rocha, R. & Pereira Boeger, W.A. (Eds.), *Estado da arte e perspectivas para a Zoologia no Brasil.* Editora da Universidade Federal do Paraná, Paraná, pp. 49–64.

Carbayo, F., Álvarez-Presas, M., Olivares, M.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, 508–528.
http://dx.doi.org/10.1111/zsc.12019

Fick, I.A., Leal-Zanchet, A.M. & Vieira, E.M. (2006) Community structure of land flatworms (Platyhelminthes, Terricola): comparisons between Araucaria and Atlantic forest in Southern Brazil. *Invertebrate Biology*, 125, 306–313.
http://dx.doi.org/10.1111/j.1744-7410.2006.00062.x

Froehlich, C.G. (1955a) Sôbre morfologia e taxonomia das Geoplanidae. *Boletins da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo, Série Zoologia*, 19, 195–279.

Froehlich, C.G. (1955b) Notas sobre Geoplanas brasileiras (Turbellaria; Tricladida). *Papéis Avulsos do Departamento de Zoologia*, 12, 189–198.

Froehlich, C.G. (1956) Tricladida Terricola das regiões de Teresópolis e Ubatuba. *Papéis Avulsos do Departamento de Zoologia*, 12, 313–343.

Froehlich, C.G. (1959) On Geoplanids from Brazil. *Boletins da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo, SérieZoologia*, 22, 201–265.

Froehlich, C.G. (1967) A contribution to the zoogeography of Neotropical land planarians. *Acta Zoologica Lilloana*, 23, 153–162.

Froehlich, E.M. (1955) Sôbre espécies brasileiras do gênero *Geoplana*. *Boletins da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo, Série Zoologia*, 19, 289–369.

Froehlich, E.M. (1978) On a collection of chilean landplanarians. *Boletim de Zoologia da Universidade de São Paulo*, 3, 7–80.

Froehlich, E.M. & Froehlich, C.G. (1972) Land Planarians from the Amazonian Region. *Papéis Avulsos de Zoologia*, 26, 29–45.

Giraudo, A.R., Povedano, H., Belgrano, M.J., 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. (Eds.), *The Atlantic Forest of South America: biodiversity status, threats, and outlook*. Island Press, Washington DC, pp. 160–180.

Graff, L. (1899) *Monographie der Turbellarien: II. Tricladida Terricola (Landplanarien).*Verlag von Wilhelm Engelmann, Leipzig, 574 pp.

Hyman, L.H. (1941) Terrestrial flatworms from the Canal Zone, Panamá. *American Museum Novitates*, 1105, 1–11.

Leal-Zanchet, A.M., Rossi, I., Seitenfus, A.L.R. & Alvarenga, J. (2012) Two new species of land flatworms and comments on the genus *Pasipha* Ogren & Kawakatsu, 1990 (Platyhelminthes: Continenticola). *Zootaxa*, 3583, 1–21.

Marcus, E. (1951) Turbellaria brasileiros (9). *Boletins da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo, Série Zoologia*, 16, 5–215.

Negrete, L. (2013) *Diversidad de planarias terrestres (Platyhelminthes, Tricladida, Geoplanidae) en el Bosque Paranaense Argentino*. Doctoral Thesis. Universidad Nacional de La Plata, La Plata, 309 pp.

Negrete, L. & Brusa, F. (2012) *Choeradoplana crassiphalla* sp. nov. (Platyhelminthes: Tricladida: Geoplanidae): a new species of land planarian from the Atlantic Forest of Argentina. *Studies on Neotropical Fauna and Environment*, 47, 227–237.
http://dx.doi.org/10.1080/01650521.2012.735071

Negrete, L., Colpo, K.D. & Brusa, F. (2014a) Land planarian assemblages in protected areas of the Interior Atlantic Forest:

implications for conservation. *PLoS ONE*, 9, e90513.
http://dx.doi.org/10.1371/journal.pone.0090513

Negrete, L., Leal-Zanchet, A.M. & Brusa, F. (2014b) A new species of *Supramontana* Carbayo & Leal-Zanchet (Platyhelminthes, Continenticola, Geoplanidae) from the Interior Atlantic Forest. *Zootaxa*, 3753 (2), 177–186.
http://dx.doi.org/10.11646/zootaxa.3753.2.7

Ogren, R.E. & Kawakatsu, M. (1990) Index to the species of the family Geoplanidae (Turbellaria, Tricladida, Terricola) Part I: Geoplaninae. *The Bulletin of Fuji Women's College*, 28, 79–166.

Riester, A. (1938) Beiträge zur Geoplaniden-Fauna Brasiliens. *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft*, 441, 1–88.

Tyler, S., Schilling, S., Hooge, M. & Bush, L.F. (Comp.) (2006–2015) Turbellarian taxonomic database. Version 1.8. Available from: http://turbellaria.umaine.edu/turbella.php (accessed 12 November 2015)

Subarna, S.K., Layton, C. & Bancroft, J.D. (2013) *Bancroft's theory and practice of histological techniques*. Churchill Livingstone, Elsevier, London, 637 pp.

Winsor, L. (1983) A revision of the cosmopolitan land planarian *Bipalium kewense* Moseley, 1878 (Turbellaria: Tricladida: Terricola). *Zoological Journal of the Linnean Society*, 79, 61–100.
http://dx.doi.org/10.1111/j.1096-3642.1983.tb01161.x

Winsor, L. (1998) Aspects of taxonomy and functional histology in terrestrial flatworms (Tricladida: Terricola). *Pedobiologia*, 42, 412–432.