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Increasing diversity of land planarians (Platyhelminthes: Geoplanidae) in the Interior Atlantic Forest with the description of two new species and new records from Argentina

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Abstract

Here, we describe two new species of land planarians of the genera *Pasipha* and *Imbira* from the Atlantic Forest of Argentina: *Pasipha quirogai* **sp. nov.** and *Imbira negrita* **sp. nov.** Their external features and internal anatomy distinguish them from their congeners. *Pasipha quirogai* **sp. nov.** is about 40mm long, characterized by brown beige dorsal pigmentation with a golden yellow median band and thin graphite black para-median stripes, cylindrical pharynx, extrabulbar prostatic vesicle with paired proximal portion, male atrium with small folds in its proximal part, and female canal opening into the dorso-caudal wall of the female atrium. *Imbira negrita* **sp. nov.** is about 110mm long, with jet black dorsal pigmentation, pharynx collar-type, short female canal opening in the postero-dorsal portion of the female atrium, and epithelial lining of female atrium with stratified appearance. We also provide exhaustive descriptions of *Geoplana quagga*, *Obama ladislavii*, and *Paraba multicolor*, all recorded for the first time in Argentina, by analysing anatomical features of taxonomic relevance. In addition, we improve the description of *G. quagga*, mainly regarding the musculature, secretory cells, and the copulatory apparatus.

Key words: Tricladida, Land flatworms, Geoplaninae, *Imbira*, *Pasipha*, *Geoplana*, *Obama*, *Paraba*, Neotropical Region, north-eastern Argentina

Introduction

The subfamily Geoplaninae Stimpson, 1857 (Platyhelminthes, Continenticola, Geoplanidae) is distributed along the Neotropical Region, except for a few species accidentally introduced in other continents (Arndt 1934; Lago-Barcia *et al.* 2015; Carbayo *et al.* 2016). In South America, the highest richness of land planarians is found in the Atlantic Forest. This ecoregion, which includes the Atlantic coast of Brazil, east of Paraguay and north-east of Argentina, is one of the biodiversity hotspots of the world (Myers *et al.* 2000). However, it is also one of the most endangered biomes, being now confined to less than 13% of its original extent (Ribeiro *et al.* 2011). In recent years, we started to evaluate the diversity of land planarians with the main long-term goal of approaching to the knowledge achieved in the southernmost portion of the Brazilian Atlantic Forest, which began almost 20 years ago (Leal-Zanchet & Carbayo 2000). This diversity was totally unknown in natural reserves from the Interior Atlantic Forest in Argentina until recently (Negrete 2013; Negrete & Brusa 2012, 2016a, b; Negrete *et al.* 2014a, b). Thus, in this paper, we describe two new land planarian species of the genera *Imbira* Carbayo *et. al.*, 2013 and *Pasipha* Ogren & Kawakatsu, 1990 and report the first records outside Brazil for *Geoplana quagga* Marcus, 1951 *incertae sedis*, *Obama ladislavii* (Graff, 1899) and *Paraba multicolor* (Graff, 1899).

Many species of land planarians are usually described based on few specimens or even only one. This is mainly due to the difficulty of finding more individuals because they belong to the cryptozoic soil fauna, spending most of the day hidden in soil shelters. There is also an ecological reason because many species show a very low abundance and the probability to find more individuals is therefore low (Carbayo *et al.* 2002; Fick *et al.* 2006; Baptista & Leal-Zanchet 2010; Negrete *et al.* 2014a). In such cases, the intraspecific variation is incompletely

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known and the co-specificity of new specimens from different localities can be questioned (Leal-Zanchet & Matos 2011). In view of this, we also analysed anatomical features of taxonomic relevance of *G. quagga*, *O. ladislavii*, and *P. multicolor* from Argentina and compared them with their original descriptions and recent literature in order to provide new information about these species.

Materials and methods

All the material studied was collected from surveys conducted in the Interior Atlantic Forest, north-eastern Argentina (Misiones province) (Fig. 1). Specimens of *Imbira negrita* **sp. nov.** were collected in Moconá Provincial Park, whereas the specimen of *Pasipha quirogai* **sp. nov.** was collected in Osununú Private Reserve. Specimens of both species were collected during nocturnal sampling, when they were seen creeping on the ground in trails of well-preserved native forests. *Geoplana quagga* and *Paraba multicolor* were collected in anthropized areas of San Antonio town. *Obama ladislavii* was found in Salto Encantado Provincial Park and in a private reserve (Centro de Investigación Antonia Ramos, CIAR), both located in native forests. All flatworms were collected by hand and photographed *in vivo*. Then, they were placed onto Petri dishes, one at a time, and boiling water was poured over them when they were fully extended. The specimens were histologically processed and their external and internal anatomy analysed following the methodology described in Negrete *et al.* (2014a). The histological slides (6–8µm thick) were stained using the Masson's trichrome method (Suvarna *et al.* 2013).

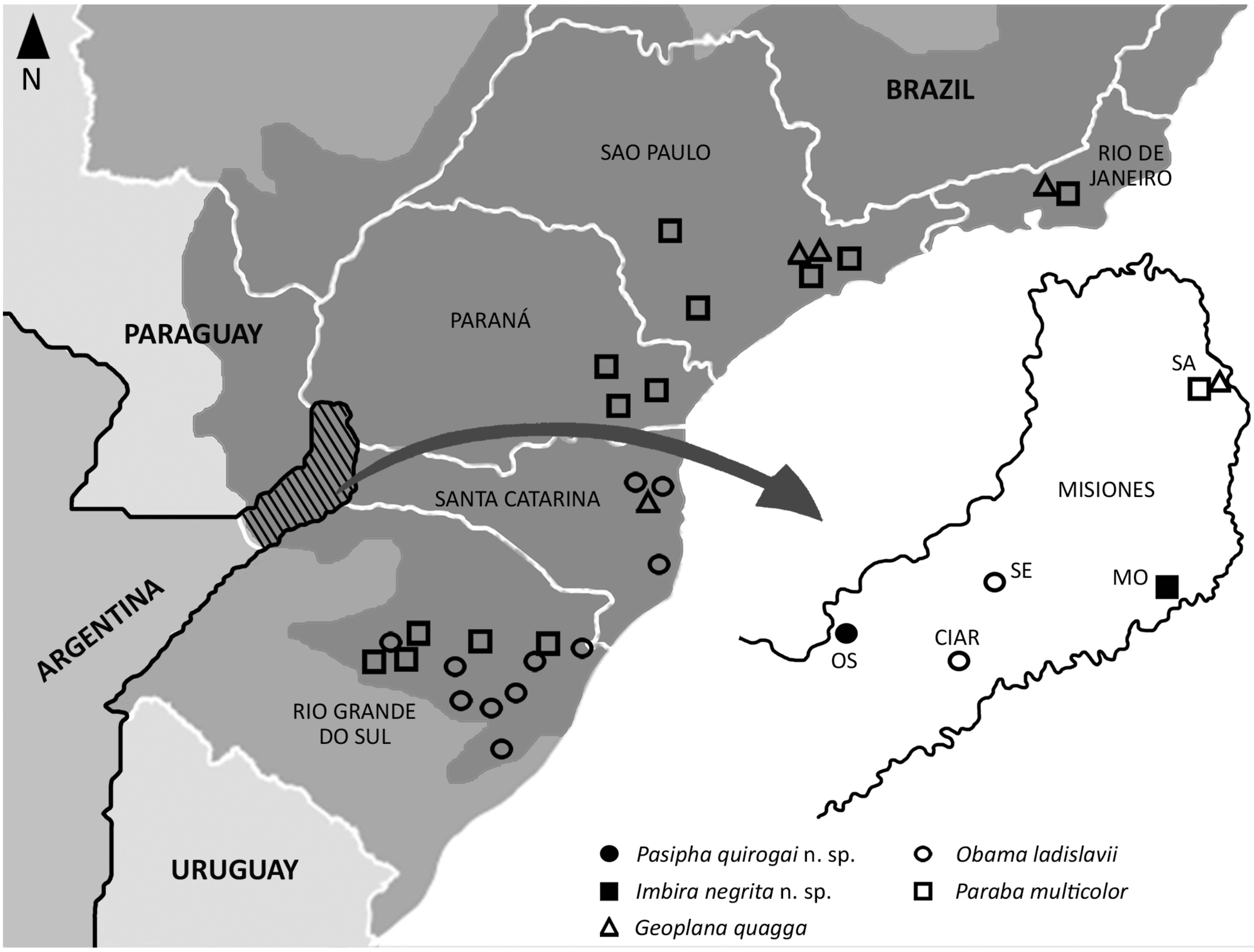

FIGURE 1. Map with the known distribution of *Geoplana quagga*, *Obama ladislavii*, and *Paraba multicolor* throughout Brazil (original extension of the Atlantic Forest in dark grey). In detail, the Argentine portion of the Atlantic Forest (Misiones province) with the new records of these species and localities of the new species here described. (CIAR) Centro de Investigaciones Antonia Ramos, (MO) Moconá Provincial Park, (OS) Osununú Private Reserve, (SA) San Antonio, (SE) Salto Encantado Provincial Park.

The pigment of land planarians was estimated as matches to the RAL colour chart (http://www.ralcolor.com/).

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

Abbreviations. (cm) cutaneous musculature, (co) common muscle coat, (cod) common glandular ovovitelline duct, (cs) creeping sole, (de) dorsal epidermis, (di) dorsal insertion of pharynx, (dp) dorsal parenchymal muscle layer, (dvp) dorso-ventral parenchymal muscle layer, (e) eyes, (ej) ejaculatory duct, (fa) female atrium, (fc) female genital canal, (gm) glandular margin, (go) gonopore, (i) intestine, (lc) longitudinal cutaneous muscle layer, (ma) male atrium, (mo) mouth, (n) nervous plate, (od) ovovitelline duct, (oe) oesophagus, (ov) ovary, (p) eversible penis, (pb) penis bulb, (ph) pharynx, (phl) pharyngeal lumen, (php) pharyngeal pouch, (pp) penis papilla, (pv) prostatic vesicle, (rb) rhabditogen cells, (sbp) sub-intestinal parenchymal muscle layer, (sd) sperm duct, (sg) shell glands, (sp) sensory pits, (spp) supra-intestinal parenchymal muscle layer, (t) testes, (ve) ventral epidermis, (vi) ventral insertion of pharynx, (vit) vitellaria.

Results

Taxonomic account

Genus *Pasipha* Ogren & Kawakatsu, 1990

Pasipha quirogai sp. nov.

(Figs. 2–3)

Type material. Holotype. MLP–He 7018, Osununú Private Reserve, 28 February 2015; cephalic region: transversal sections on 17 slides; anterior region at the level of the ovaries: sagittal sections on 8 slides; pre-pharyngeal region: transverse sections on 7 slides; pharynx and copulatory apparatus: sagittal sections on 18 slides.

Diagnosis. Elongated body with parallel margins; ~40mm in length in maximum extension; dorsal surface brown beige with median band golden yellow; thin brown beige line along the median band and thin graphite black para-median stripes; ventral surface light grey; pharynx cylindrical; extrabulbar prostatic vesicle piriform, with folded walls, and tubular paired proximal portion; male atrium richly folded, with small folds in its most proximal part; female genital canal opening into the dorso-caudal wall of the female atrium.

Type locality. Osununú Private Reserve (27°16’46”S, 55°34’43”W), near San Ignacio, Misiones province, Argentina (Fig. 1).

Etymology. The name of this species is in honour to Horacio Quiroga (1878–1937), an Uruguayan writer who lived in San Ignacio town, in the vicinity of the type locality of the new species. He wrote wonderful stories in which, with the rainforest as setting, he used the supernatural to show the struggle of men and animals to survive.

Description. *External morphology.* Body elongate, with parallel margins, and flattened in cross section (Fig. 2A, B). The anterior tip is blunt, and the posterior tip ends sharply pointed. The dorsal surface is brown, with a median band light brown to yellowish, which is approximately 1/4 of body width, although it is a bit wider at the level of the pharynx and copulatory apparatus (Fig. 2A). The median band has a narrow darker brown longitudinal line. Thin black para-median stripes extend along the whole body, except near the cephalic apex (Fig. 2A). The ventral surface is light grey. After fixation, the dorsal pigment was conserved almost as in live, although a bit paler (Fig. 2A). The holotype was ~40mm long when crawling and 35.5mm after fixation. Its maximum width was 2.3mm, and its maximum height was 1mm. The mouth and gonopore were located at 24.7mm (70% of body length) and 29.6mm (83% of body length) of the anterior tip, respectively. The eyes, with clear halos, extend along the whole body (Fig. 2A), and are uniserial around the anterior tip and in the first millimetre of the body. Then, they sparse bi- and triserially, and, at 5mm from the anterior tip, they spread on the dorsal surface, reaching 6–8 rows in their maximum extension, which is at 12–15mm from the anterior tip. Towards the posterior body region, the eyes remain dorsal but scarcer and at the level of the pharynx they are less numerous (3–4 rows on each side of the body). At the level of the copulatory apparatus, they are more isolated, reaching the posterior end.

Internal morphology. Sensory pits (25µm deep), as simple invaginations of ventral epidermis, contour the anterior tip and extend along the body margins in a single row until 4mm from the anterior end (Fig. 2B). Four different types of secretory cells discharge through the dorsal epidermis (25µm high) at the pre-pharyngeal region: rhabditogen cells with xanthophil rhammites, which expand along the whole dorsal epidermis, abundant cells with fine granular erythrophil secretion, and less numerous cells with fine granular cyanophil secretion and erythrophil amorphous secretion (Fig. 2B–D). Glandular margin with abundant fine granular erythrophil and cyanophil secretions, and erythrophil and cyanophil amorphous secretions in less quantity (Fig. 2B, C). Ventral epidermis (15µm high) ciliated on the creeping sole, which occupies 95% of the body width. The same types of secretory

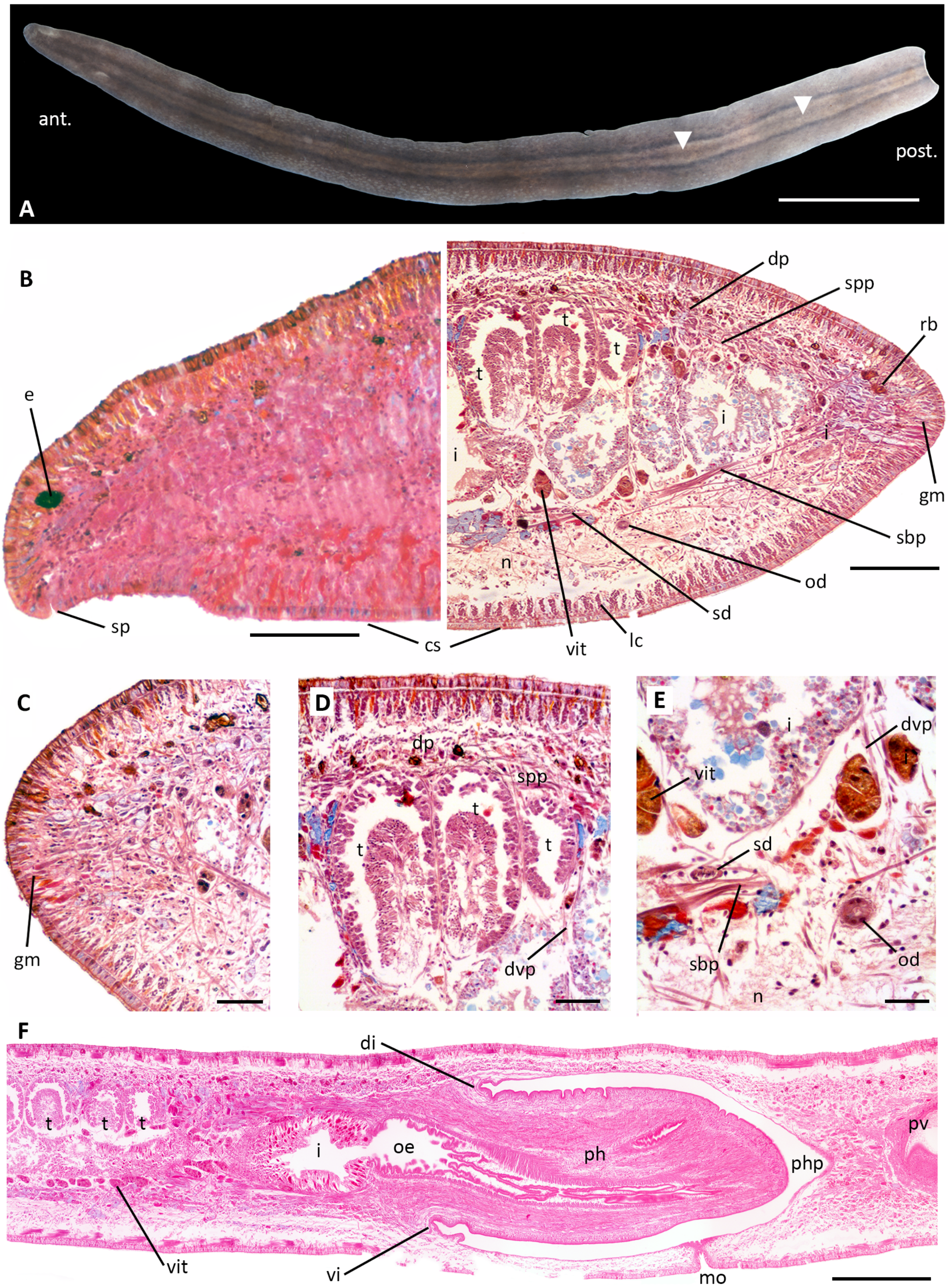

FIGURE 2. *Pasipha quirogai* **sp. nov. (A)** Photograph of preserved specimen (holotype), in dorsal view (arrowheads indicate the position of the mouth and gonopore, respectively). **(B)** Half of a transverse section at the cephalic region (on the left) and at the pre-pharyngeal region (on the right). **(C–E)** Transverse sections of the pre-pharyngeal region with details: glandular margin (C), dorsal to intestine (D), ventral to intestine (E). **(F)** Sagittal section of the pharynx. Scale bars: A: 5mm, B: 200µm, C: 50µm, D, E: 100µm, F: 500µm.

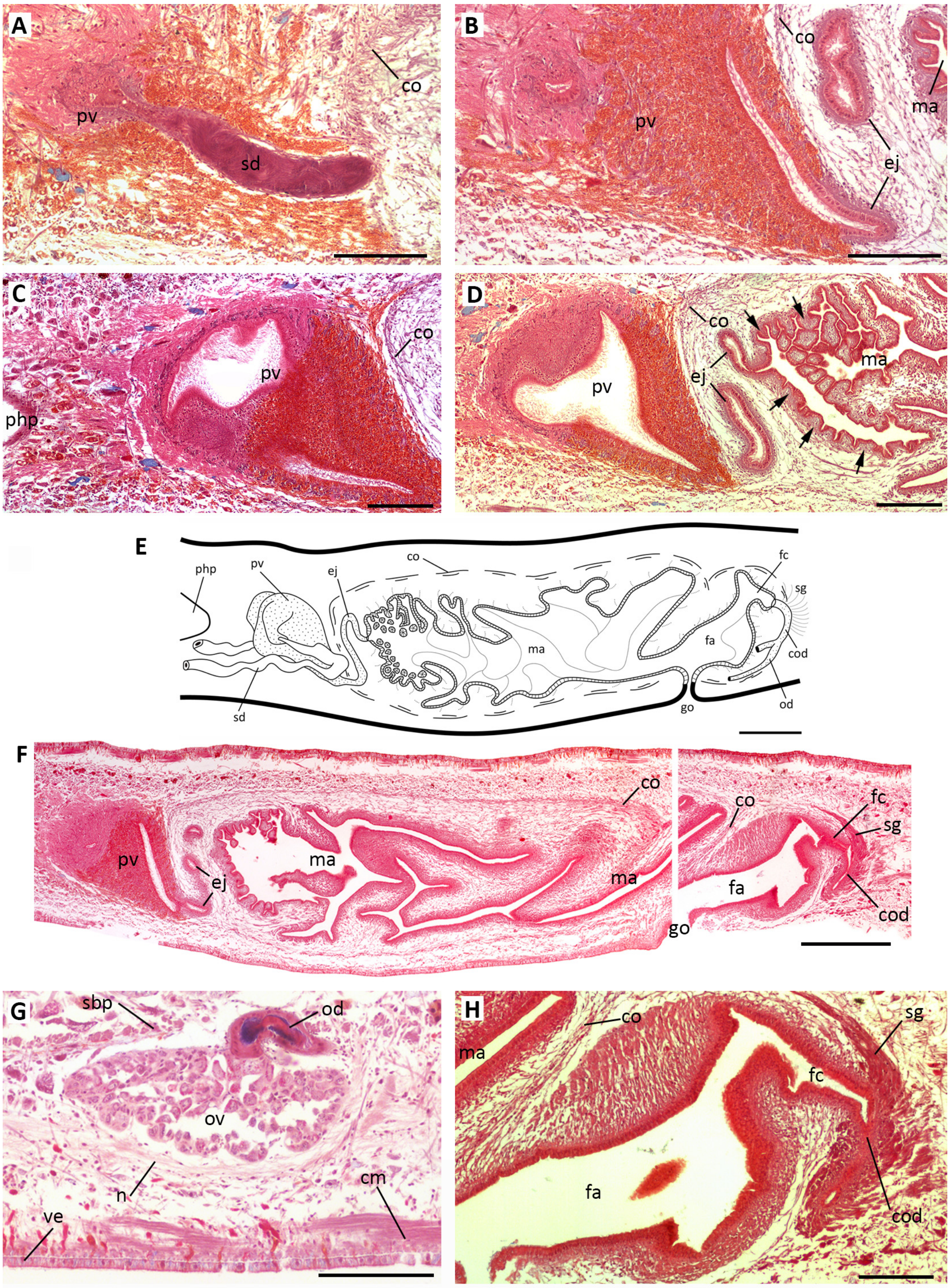

FIGURE 3. *Pasipha quirogai* **sp. nov. (A–D)** Sagittal sections at the level of the prostatic vesicle (small folds of the male atrium indicated by arrows). **(E)** Schematic reconstruction of the copulatory apparatus in sagittal view. **(F–H)** Sagittal sections of the reproductive system: copulatory apparatus (F), ovary (G), detail of the female atrium (H). Scale bars: A–D, G, H: 200µm, E, F: 500µm.

cells discharge through the ventral epidermis as in the dorsal surface, although there are small dermal rhabdites (Fig. 2B, C). Fine granular cyanophil and erythrophil amorphous secretions are more abundant than in the dorsal epidermis, and fine granular erythrophil secretion is scarcer. The dorsal and ventral epidermis of the cephalic region receives the same types of secretory cells as in the pre-pharyngeal region, except for the dorsal epidermis, which receives abundant fine granular xanthophil secretion instead of erythrophil secretion (Fig. 3A). Also, erythrophil amorphous secretion is highly abundant in the creeping sole (70% of body width) (Fig. 2B).

Cutaneous musculature with a subepidermal circular layer (2.5µm thick), an intermediate diagonal layer (10µm thick), and an internal longitudinal layer (50µm thick dorsally, 60µm thick ventrally). The thickness of the cutaneous musculature represents 13% of the body height (CMI). Parenchymal musculature thinner than the cutaneous one (9% of body height), constituted by a dorsal decussate layer (50µm thick) followed by a transverse supra-intestinal (15µm thick) and a sub-intestinal (25µm thick) muscle layer. In addition, dorso-ventral fibres accommodate among intestinal branches (Fig. 2B, D, E).

Pharynx cylindrical, 2mm in length (6% of body length), occupying most of the pharyngeal pouch (2.3mm in length) (Fig. 2F). Pharynx lined by ciliated cuboidal epithelium, followed by a longitudinal subepithelial muscle layer (5µm thick) and a subjacent circular layer (10µm thick). Pharyngeal lumen lined by ciliated columnar epithelium, followed by a circular subepithelial muscle layer (30µm thick) and a subjacent longitudinal layer (10µm thick). Two types of secretory cells, the cell bodies of which are located in the surrounding parenchyma anterior to the pharynx, discharge through the pharyngeal epithelium: abundant cells with fine granular erythrophil secretion and less abundant fine granular cyanophil secretion (Fig. 2F). Short oesophagus (350µm in length) lined by ciliated columnar epithelium, followed by a subepithelial circular layer (25µm thick) and a subjacent longitudinal layer (10µm thick). The oesophagus:pharynx ratio is 17%.

Dorsal mature testes ovoid in shape, arranged in three irregular rows on each side of the body and located immediately below the supra-intestinal parenchymal muscle layer (Fig. 2B, D). Testes extending from behind the ovaries to the proximity of the pharyngeal root (24% and 65% of body length from the anterior end, respectively), occupying up to 35% of the body height at the pre-pharyngeal region. Sperm ducts medial and slightly dorsal to the ovovitelline ducts, located among fibres of the sub-intestinal parenchymal muscle layer (Fig. 2B, E). At the level of the pharynx, sperm ducts are dilated, with their lumen full of spermatozoa, constituting spermiducal vesicles. Sperm ducts extend backwards, laterally to the prostatic vesicle, until the common muscle coat, and then bend antero-dorsally, and to the sagittal plane, and open into the forked parts of the prostatic vesicle (Fig. 3A, E). Extrabulbar prostatic vesicle, almost piriform in shape, located very close (~300µm) to the pharyngeal pouch (Figs. 2F, 3E). The lumen of the prostatic vesicle is highly irregular due to its folded walls, especially the distal portion, which has a narrow lumen (Fig. 3A–D, F). The proximal portion of the prostatic vesicle has tubular forked parts, and it is also distinguished from the distal unpaired portion by the type of glandular secretions that each receives (see below). The ejaculatory duct (~700µm long) runs sinuously within the common muscle coat and opens into the bottom of the male atrium (Fig. 3D–F). The male atrium (2.5mm long), three times larger than the female atrium, is highly folded with its lumen very narrow (Fig. 3E, F). The most proximal region is distinguished from the rest by having numerous small folds (Fig. 3D–F).

Sperm ducts lined by ciliated cuboidal epithelium, without underlying musculature at pre-pharyngeal level, while spermiducal vesicles exhibit squamous epithelium with a thin subepithelial longitudinal muscle layer (5µm thick). The lining epithelium of the prostatic vesicle is columnar and ciliated, followed by musculature composed of circular fibres intermingled with some longitudinal ones (25–35µm thick). The epithelium of the proximal portion, which involves the forked tubular parts, receives abundant fine granular erythrophil secretion, whereas the distal portion is traversed by abundant coarse granular xanthophil secretion (Fig. 3A–D, F). The cell bodies of these glands are located in the surrounding parenchyma anteriorly to the prostatic vesicle and laterally to the distal part of the pharyngeal pouch. The ejaculatory duct is lined by ciliated columnar epithelium, with subjacent muscle coat composed of circular and oblique fibres (10–15µm thick). The most proximal part of the male atrium, which exhibits small folds, is lined by columnar and ciliated epithelium. The rest of the atrial epithelium is columnar and non-ciliated. The muscularis of the male atrium is composed mainly of circular fibres with some longitudinal and oblique fibres (20–40µm thick). The whole atrial epithelium only receives abundant fine granular erythrophil secretion. Common muscle coat with longitudinal fibres (10–15µm thick).

Ovaries (500µm long and 200µm diameter) ovoid in shape, located between the sub-intestinal parenchymal muscle layer and the nervous plate (Fig. 3G), at 8mm from the anterior end (22% of body length). Ovovitelline ducts arising in the mid-dorsal third of the ovaries (Fig. 3G). Backwards, they are situated below the sub-intestinal parenchymal muscle layer (Fig. 2B, E). Behind the gonopore, the ovovitelline ducts run ventro-laterally to the female atrium, contouring the common muscle coat, and posteriorly ascend slightly and join in a common glandular ovovitelline duct (~300µm long). Common duct almost vertical and distally curved to open into female atrium through a short female canal (150µm long). Female canal opening into the dorso-caudal wall of the female atrium (Fig. 3E, H). The female atrium (800µm long) is an oblique cavity poorly folded and almost tubular in shape, partially separated from the male atrium by an oblique fold which hangs from the roof between both atria at the level of the gonopore (Fig. 3E, F, H). Vitelline follicles well developed, located among intestinal branches (Fig. 2B, E).

Ovovitelline ducts lined by ciliated cuboidal epithelium, followed by a thin circular muscle layer (5µm thick). Lining epithelium of common glandular ovovitelline duct columnar and ciliated, receiving abundant erythrophil amorphous secretion from shell glands (Fig. 3F, H); common duct coated by a muscle layer composed of circular fibres intermingled with some oblique fibres (20–25µm thick). Female canal lined by a ciliated columnar epithelium followed by a muscle layer (20µm thick) with the same arrangement as in the common ovovitelline duct. The female canal receives abundant fine granular erythrophil secretion and erythrophil amorphous secretion in less quantity (Fig. 3H). Lining epithelium of female atrium columnar and non-ciliated, filled with abundant fine granular erythrophil secretion and scarce erythrophil amorphous secretion. Cell bodies of these glands located in the surrounding parenchyma. Muscularis of the female atrium much thicker than that of the male atrium (~200µm thick), being composed of circular fibres with some interspersed longitudinal fibres, although thinner near the gonopore (25–50µm thick). Common muscle coat not well organized as in the male atrium, consisting of loose longitudinal fibres (5–10µm thick).

Parasitism. Nematode larvae were found in the anterior body region (inside the nervous plate and parenchyma very close to the ovaries) and inside the pharynx.

Comparative discussion. The new species here described exhibits the combination of diagnostic characters of the genus *Pasipha* Ogren & Kawakatsu, 1990, namely: slender body with parallel margins; extrabulbar prostatic vesicle histologically differentiated according to the secretions it receives and commonly with a bifurcated proximal portion; ejaculatory duct opening directly into a long and richly folded male atrium; ovovitelline ducts ventral to the female atrium and joining each other behind it; female genital canal ventrally flexed (Ogren & Kawakatsu 1990; Carbayo *et al.* 2013). Besides, species of *Pasipha* tend to exhibit a thick cutaneous musculature in relation to body height, varying approximately between 10% and 20% (values of CMI). The internal anatomy of the different species of *Pasipha*, mainly concerning the copulatory apparatus, is extremely similar, making difficult any comparison among them. So, they are usually distinguished by differences in the external appearance and histological details of the internal anatomy.

In comparison with the other species of the genus, *Pasipha quirogai* **sp. nov.** externally resembles *Pasipha mbya* Negrete & Brusa, 2016, species described for Argentina (Negrete & Brusa 2016a). Both species exhibit a median yellowish band with black para-median stripes on the dorsum. However, in *P. mbya*, whose ground colour is dark grey, the median band is mottled with black spots, while in *P. quirogai*, whose ground colour is brown, there is a thin brownish line along the median band. Regarding the copulatory apparatus, they are also similar but *P. quirogai* **sp. nov.** can be differentiated from *P. mbya* in some details. The prostatic vesicle of both species is globose with folded walls, with the proximal portion bifurcated and receiving fine granular erythrophil secretion. Nevertheless, in *P. mbya*, the distal part of the prostatic vesicle is pierced by coarse erythrophil granules, whereas, in *P. quirogai* **sp. nov.**, it receives coarse xanthophil granules. We also observed differences in the secretions that pierce the epithelium of the male atrium. In *P. quirogai* **sp. nov.**, it receives only fine granular erythrophil secretion, whereas, in *P. mbya*, it is also pierced by amorphous erythrophil secretion and cyanophil granules (Negrete & Brusa 2016a). Regarding the female system, *P. quirogai* **sp. nov.** is distinguished from *P. mbya* because the female atrium of *P. quirogai* **sp. nov.** is lined by a columnar epithelium, whereas that of *P. mbya* is tall columnar of pseudostratified aspect (Negrete & Brusa 2016a).

Regarding the colour pattern, *Pasipha quirogai* **sp. nov.** also shares some similarity with the Brazilian species *Pasipha penhana* (Riester, 1938), *Pasipha tapetilla* (Marcus, 1951), *Pasipha backesi* Leal-Zanchet *et al.*, 2012, and *Pasipha mesoxantha* Amaral & Leal-Zanchet, 2016, all of which exhibit a yellowish median band along the dorsum. *Pasipha penhana* and *P. tapetilla* differ from the new species because they exhibit lateral black stripes on a greyish background (Riester 1938; Marcus 1951), absent in *P. quirogai* **sp. nov.** The dorsal colour pattern of *P. backesi*, unlike that of *P. quirogai* **sp. nov.**, also shows lateral stripes of dark brown pigment (Leal-Zanchet *et al.* 2012). *Pasipha mesoxantha* lacks para-median stripes, whereas, in *P. quirogai* **sp. nov.**, they are present.

Regarding the copulatory apparatus, the new species can be differentiated from the above-mentioned Brazilian species due to some anatomical and histological details. In *P. penhana* and *P. tapetilla*, the prostatic vesicle is tubular and unforked, while in *P. quirogai* **sp. nov.**, it is globose with the proximal portion forked, as *P. backesi* and *P. mesoxantha*. The epithelial lining of the male atrium of *P. backesi* and *P. mesoxantha* receives different secretions along its length, whereas that of *P. quirogai* **sp. nov.** receives only erythrophil granules. In addition, the female atrium of *P. backesi* and *P. mesoxantha* is oval-elongate with highly folded walls (Amaral & Leal-Zanchet 2016; Leal-Zanchet *et al.* 2012), contrasting with the poorly folded atrium of *P. quirogai* **sp. nov.**, which is almost tubular in shape. Also, the position of the common glandular ovovitelline duct differs between these species because in *P. backesi* and *P. mesoxantha* it is slightly ascendant and runs posteriorly, while in *P. quirogai* **sp. nov.** it is almost vertical and distally anteriorly flexed before opening into the female genital canal.

Genus *Imbira* Carbayo *et al.*, 2013

Imbira negrita sp. nov.

(Figs. 4–6, Tables 1, 2)

Type material. Holotype. MLP–He 7415, Moconá Provincial Park, 6 November 2015; cephalic region: transversal sections on 21 slides; anterior region at the level of the ovaries: sagittal sections on 46 slides; pre-pharyngeal region: transverse sections on 6 slides; pharyngeal region: sagittal sections on 30 slides; post-pharyngeal region: sagittal sections on 17 slides; copulatory apparatus: sagittal sections on 25 slides. *Paratype 1*, MLP–He 7416-1, Moconá Provincial Park, 6 November 2015; cephalic region: transversal sections on 17 slides; anterior region at the level of the ovaries: sagittal sections on 24 slides; pre-pharyngeal region: transverse sections on 6 slides; pharyngeal region: sagittal sections on 13 slides; copulatory apparatus: sagittal sections on 12 slides. *Paratype 2*, MLP–He 7416-2, Moconá Provincial Park, 6 November 2015; cephalic region: transversal sections on 15 slides; anterior region at the level of the ovaries: sagittal sections on 21 slides; pre-pharyngeal region: transverse sections on 6 slides; pharynx and copulatory apparatus: sagittal sections on 16 slides. *Paratype 3*, MLP–He 7417, Moconá Provincial Park, 8 October 2016; preserved in 70% ethanol.

Diagnosis. Elongated body with parallel margins; 50–110mm in length in maximum extension; dorsal surface jet black and ventral surface ivory; eyes marginal along the body; short female canal opening in the postero-dorsal portion of the female atrium; epithelial lining of female atrium with stratified appearance.

Type locality. Moconá Provincial Park (27°08'56"S, 53°53'54"W), Misiones province, Argentina (Fig. 1).

Etymology. The species name alludes to the black pigmentation of the dorsum (black=negra/o in Spanish), since in Argentina and other south American Spanish-speaking countries, *negrita* is a colloquial way to call someone or something black or very obscure.

Description. *External morphology.* Body elongate, with parallel margins, and flattened in cross section (Fig. 4A–C). Anterior tip blunt, and posterior body region gradually sharpening, with pointed end (Fig. 4A). Dorsal surface jet black and ventral surface ivory, only whitish around the mouth and gonopore (Fig. 4A). After fixation, dorsal ground colour black grey and ventral surface oyster white. Eyes, with small clear halos, exclusively marginal, being uniserial throughout the body, except in the middle third, where they are irregularly bi-serial. When crawling, the maximum length of the largest specimen was 110mm, whereas that after fixation was 90.5mm. The maximum length of the smallest specimen was 36.4mm after fixation (Table 1). Maximum width ranged between 2.9mm and 5.3mm, whereas maximum height ranged between 1 and 1.6mm. The mouth and the gonopore are located at 71–81% (mean: 76%) and 88–93% (mean: 90%) from the anterior tip, respectively (Table 1).

Internal morphology. Sensory pits (30–50μm deep), as simple invaginations of ventral epidermis, contour the anterior tip and extend along the body margins in a single irregular row until ~5mm from the anterior end (Fig. 4B). Sensory pits at intervals of 30–40μm near anterior tip, posteriorly becoming more gradually spaced. At the pre-pharyngeal region, the ventral epidermis (25–40μm high), which is ciliated on the creeping sole (~100% of body width at the pre-pharyngeal region), receives fine granular cyanophil secretion (more abundant in the ventro-lateral margins of the body), fine and coarse granular erythrophil secretions, and coarse granular xanthophil secretion. Cell bodies of these secretions located in the subjacent parenchyma, below cutaneous musculature (Fig. 4C, E). Additionally, small rhabdites are highly abundant. The same types of secretory cells discharge through the dorsal epidermis (20–25μm high), with rhammites covering the whole dorsal epidermis (Fig. 4C, D). There is no glandular margin. The dorsal and ventral epidermis of the cephalic region receives the same types of secretory cells as the pre-pharyngeal region, except xanthophil secretion. The creeping sole of the cephalic region occupies ~85% of the body width.

TABLE 1. Measurements (mm) from fixed specimens of *Imbira negrita* **sp. nov.** CS: width of creeping sole; DG: distance from gonopore to anterior end; DM: distance from mouth to anterior end. The numbers given in parentheses represent the position relative to body length (%). Thickness (µm) of cutaneous (CM) and parenchymal (PM) musculatures at the pre-pharyngeal region. CMI (cutaneous muscular index): ratio of height of cutaneous musculature to body height. PMI (parenchymal muscular index): ratio of height of parenchymal musculature to body height. Both indices measured at pre-pharyngeal region. (-) not measured.

	Holotype	Paratype 1	Paratype 2	Paratype 3
Length	90.5	55.7	36.4	80
Width	5.3	3.6	2.9	5
Height	1.6	1.3	1	-
DM	64 (71%)	45.1 (81%)	27.8 (76%)	62 (77%)
DG	80 (88%)	52.1 (93%)	31.9 (88%)	72 (90%)
CS	100%	100%	90%	-
CM dorsal	62.5	65	62.5	-
CM ventral	75	75	57.5	-
CMI	9%	11%	12%	-
PM	90	95	50	-
PMI	6%	7%	5%	-

Cutaneous musculature composed of a subepidermal circular layer (2.5–5µm thick), an intermediate diagonal layer (5–15µm thick), and an inner longitudinal layer (50–60µm thick) (Fig. 4D, E). The thickness of the cutaneous musculature occupies 9–12% of the body height (Table 1). Parenchymal musculature constituted by a dorsal, not well organized, layer with oblique and some longitudinal fibres, followed by a transverse supra-intestinal and a sub-intestinal muscle layer (Fig. 4C–E). Longitudinal fibres (20–30µm thick) accommodate immediately above the sub-intestinal layer (Fig. 4E). Also, dorso-ventral fibres are arranged among intestinal branches (Fig. 4D). The parenchymal musculature occupies 5–7% of the body height (Table 1). In the cephalic region, and even at the level of the ovaries, parenchymal muscles exhibit the same arrangement as the pre-pharyngeal region, but longitudinal fibres are more abundant. They form discrete bundles (30–45µm thick) above the sub-intestinal muscle layer and below it mainly towards the body margins (Figs. 4B, 6F, G). Numerous parenchymal muscle fibres variously oriented traverse the nervous plate along the cephalic region (Fig. 4B).

The pharynx is collar-type, 2.6–5.1mm in length (6–7% of body length), occupying most of the pharyngeal pouch (2.3–6.5mm in length) (Fig. 5A). Dorsal insertion is 1.4–3.5mm posteriorly displaced (Fig. 5A). The pharynx is lined by ciliated cuboidal epithelium, followed by a longitudinal muscle layer (2.5–5µm thick) and a subjacent circular muscle layer (5–10µm thick). Pharyngeal lumen lined with ciliated columnar epithelium, followed by a circular subepithelial muscle layer (50–75µm thick) and a subjacent longitudinal muscle layer (5–10µm thick). Two types of secretory cells, whose cell bodies are located in the surrounding parenchyma anterior and lateral to the pharynx, discharge through the pharyngeal epithelium: highly abundant cells with fine granular erythrophil secretion and abundant fine granular cyanophil secretion (Fig. 5A). Oesophagus (0.4–1mm in length) lined with ciliated columnar epithelium, followed by a subepithelial circular muscle layer (100–130µm thick) and a thin subjacent longitudinal muscle layer (5–15µm thick). The oesophagus:pharynx ratio is 15–21%.

The dorsal mature testes are ovoid in shape, arranged in one or two irregular rows on each side of the body. They are located below the supra-intestinal parenchymal muscle layer (Fig. 4C, D), extending from behind the ovaries to near the pharynx (17–33% and 67–76% of body length from the anterior tip, respectively) (Table 2). They occupy 18–20% of the body height at the pre-pharyngeal region. Sperm ducts, dorsally and laterally displaced from the ovovitelline ducts, located among fibres of the sub-intestinal parenchymal muscle layer (Fig. 4C, E). Laterally and behind the pharynx, sperm ducts are slightly dilated and full of spermatozoa. Their distal courses turn to the sagittal plane to open ventro-laterally into the forked portion of the extrabulbar prostatic vesicle (Figs. 5B–D, 6A). These long tubular paired portions (1–2.2mm long) run laterally sinuous and continue as an unpaired intrabulbar portion whose course is also tortuous (0.4–0.7mm long) (Figs. 5B–D, 6B–E). Very close before opening into the male atrium, the prostatic vesicle connects with the ejaculatory duct, which is a short canal with a narrow lumen (150–300μm long) (Figs. 5B–D, 6C, E). Male atrium (0.7–2.5mm long), with highly folded walls, leaving a reduced lumen inside the cavity (Figs. 5B–D, 6B–E). The folded walls of its proximal part, where the ejaculatory duct opens, conform a kind of papilla: the eversible penis (Figs. 5B–D, 6B–E). Only the male atrium of the holotype exhibits dorsal folds posteriorly to the eversible penis (Figs. 5B, 6B, C).

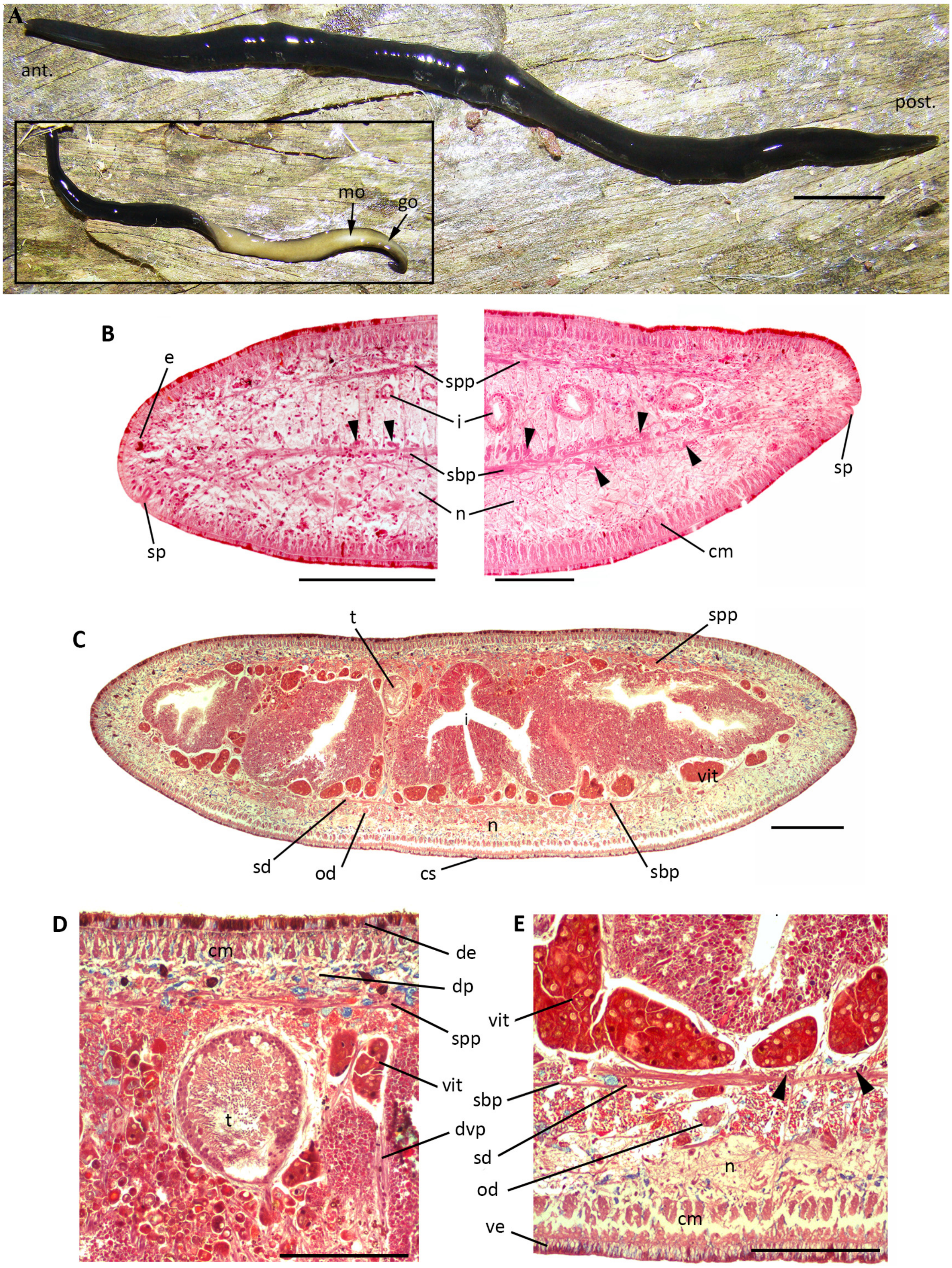

FIGURE 4. *Imbira negrita* **sp. nov. (A)** Photograph of a live specimen (holotype), in dorsal view. In the inset, the same specimen showing part of the ventral surface. **(B)** Half of a transverse section at the cephalic region (on the left) (paratype 1) and near the ovaries (on the right) (holotype). Arrowheads indicate longitudinal parenchymal muscle fibres. **C** Transverse section of the pre-pharyngeal region. **(D, E)** Transverse sections of the pre-pharyngeal region with details: dorsal to intestine (D), ventral to intestine (E). Scale bars: A: 10mm, B, D, E: 250μm, C: 500μm.

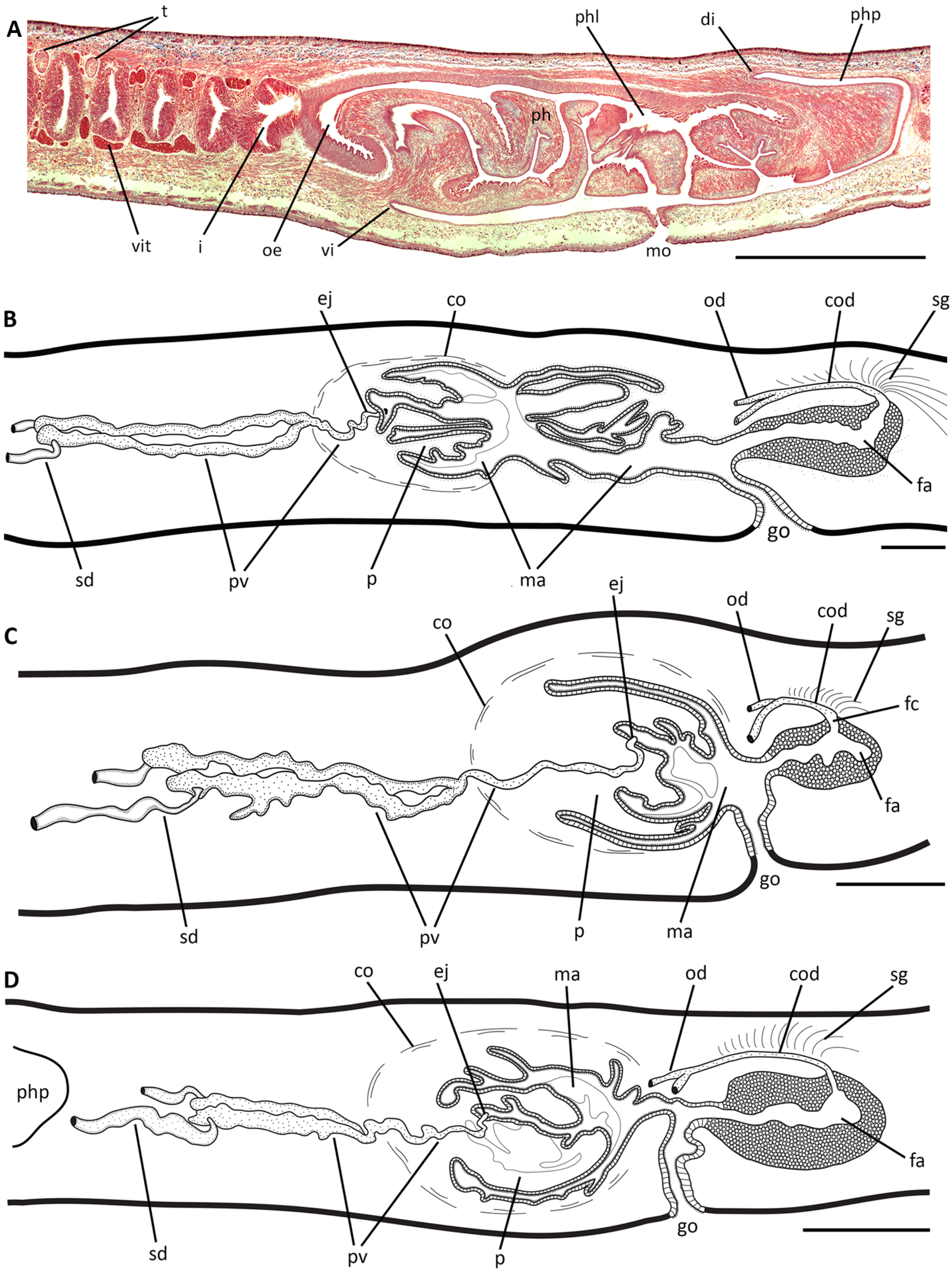

FIGURE 5. *Imbira negrita* **sp. nov.** (holotype). **(A)** Sagittal section of the pharynx. **(B–D)** Schematic reconstructions of the copulatory apparatus in sagittal view: holotype (B), paratype 1 (C), paratype 2 (D). Scale bars: A: 1mm, B–D: 500µm.

Sperm ducts lined with ciliated cuboidal epithelium, without underlying musculature at pre-pharyngeal level. Epithelial lining of the prostatic vesicle, both paired and unpaired portions, columnar and ciliated, filled with abundant erythrophil fine granular secretion. Cell bodies of prostatic glands located in the surrounding parenchyma between the pharyngeal pouch and the prostatic vesicle (Fig. 6A). Musculature of the prostatic vesicle mainly composed of longitudinal fibres with some interspersed oblique fibres (10–15µm thick). Ejaculatory duct lined with cuboidal to columnar ciliated epithelium, which is pierced by scarce erythrophil fine granules, surrounded by a thin longitudinal muscle layer (2.5µm thick). Epithelial lining of the male atrium columnar and densely ciliated, strongly erythrophil, receiving abundant fine granular secretion (Fig. 6B–E). Its muscularis, composed of a subepithelial circular layer and a subjacent longitudinal layer, is thicker dorsally (20–30µm thick and 10–25µm thick, respectively) than ventrally (5–10µm thick and 5–10µm thick, respectively). Common muscle coat formed by loose longitudinal fibres (10–15µm thick). Male atrium connected with the female atrium by a narrow lumen (Figs. 5B–D, 6C–E).

TABLE 2. Measurements (mm) of reproductive organs of *Imbira negrita* **sp. nov.** DPVP, distance between prostatic vesicle and pharyngeal pouch; LCOD, length of common glandular ovovitelline duct; LFA, length of female atrium; LFC, length of female canal; LMA, length of male atrium; LPP, length of penis papilla; LPV, length of prostatic vesicle. The numbers given in parentheses represent the position relative to body length (%).

	Holotype	Paratype 1	Paratype 2
Anteriormost testes	15.2 (17%)	9.9 (18%)	12 (33%)
Posteriormost testes	60.4 (67%)	42.5 (76%)	26.3 (72%)
LPV (extrabulbar portion)	2.2	1	1
LPV (intrabulbar portion)	0.7	0.7	0.4
DPVP	0.5	1.7	0.7
LMA	2.5	0.8	0.75
Location of ovaries	12.7 (14%)	9.4 (17%)	10.8 (30%)
LCOD	0.8	0.25	0.55
LFC	0.05	0.05	0.05
LFA	1.6	0.5	0.75

Ovaries (150–220µm long and 130–210µm diameter) almost spherical in shape, located immediately beneath the intestine branches and among muscle fibres of the sub-intestinal parenchymal layer (Fig. 6F, G). They are at 9.5–12.7mm from the anterior end (14–30% of body length) (Table 2). Ovovitelline ducts emerge from the latero-ventral face of ovaries (Fig. 6F). In the pre-pharyngeal region, they are located between the sub-intestinal muscle fibres and nervous plate (Fig. 4C, E). Before the gonopore, ovovitelline ducts ascend in their course to the median plane of the body. At the level of the gonopore, they join each other in a long common glandular ovovitelline duct (250–800µm long). This duct runs dorsally to the female atrium with a convex course and curves distally to open into the postero-dorsal region of the female atrium through a very short female canal (~50µm in length) (Figs. 5B–D, 6H). Female atrium (0.5–1.6mm in length) without folded walls, but with a narrow lumen because it is lined with a tall epithelium (see below) (Figs. 5B–D, 6C–E, H). Vitellaria, well developed (except paratype 1), located among intestinal branches (Figs. 4C–E, 6F, G).

Lining epithelium of the ovovitelline ducts ciliated and cuboidal, wrapped by a thin circular muscle layer (5µm thick). Common glandular ovovitelline duct lined with cuboidal to columnar ciliated epithelium, followed by a muscle layer with circular fibres and some oblique and longitudinal fibres (10–15µm thick). Similarly to the ascending portions of the ovovitelline ducts, it receives erythrophil secretion from shell glands (Figs. 5B–D, 6C–E, H). Female canal lined with ciliated and columnar epithelium, pierced by fine erythrophil granules, followed by circular muscle fibres (5–10µm thick). Lining epithelium of female atrium with stratified appearance, except near the gonopore, where it is columnar (Figs. 5B–D, 6C–E, H). Abundant fine granular erythrophil secretion discharges into the atrial epithelium. Muscularis of female atrium composed of circular, oblique and longitudinal fibres (25–35µm thick).

Comparative discussion. The new species here described fits well with the diagnosis of *Imbira* Carbayo *et al.*, 2013. This genus, which includes only two species, both recorded in the Brazilian Atlantic Forest, characterizes geoplaninid flatworms with elongated (around 90 to 200mm in length, in maximum extension), slender and flattened body, and with parallel margins; eyes marginal along the body; and presence of longitudinal parenchymal muscle fibres. In addition, the reproductive system shows a tubular prostatic vesicle with paired proximal portions; male atrium folded with eversible penis; ovovitelline ducts ascending laterally to gonopore and joining each other dorsally to female atrium; short female canal dorso-anteriorly flexed; and lining epithelium of female atrium with multi-layered aspect (Marcus 1951; Leal-Zanchet & Carbayo 2001; Carbayo *et al.* 2013).

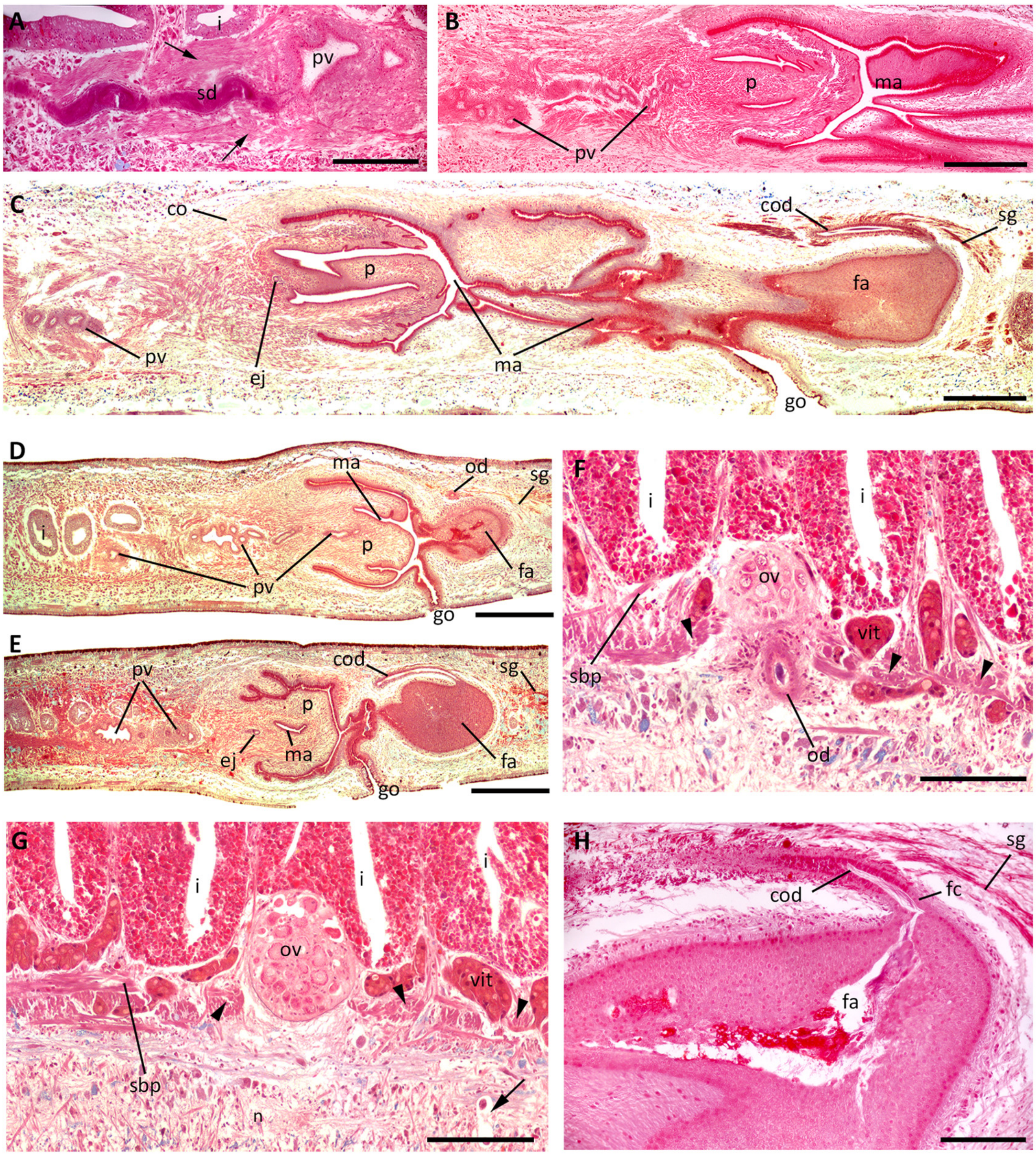

FIGURE 6. *Imbira negrita* **sp. nov.** Sagittal sections of the reproductive system. **(A)** Detail of the proximal portion of the prostatic vesicle (arrows indicate prostatic secretion) (holotype). **(B)** Detail of the male reproductive system (holotype). **(C)** Copulatory apparatus of the holotype. **(D)** Copulatory apparatus of paratype 1. **(E)** Copulatory apparatus of paratype 2. **(F)** Latero-external portion of the ovary (holotype). **(G)** Middle portion of the ovary (arrow indicates nematode larva) (holotype). **(H)** Detail of the female atrium (holotype). In F and G, arrowheads indicate longitudinal parenchymal muscle fibres in discrete bundles. Scale bars: A: 250µm, B–E: 500µm, F, G: 200µm, H: 100µm.

Externally, *Imbira negrita* **sp. nov.**, whose dorsal pigmentation is homogeneous black, is easily distinguished from the other species of the genus: in *Imbira marcusi* Carbayo *et al.*, 2013 (see *Geoplana goetschi* after Marcus (1951)), the dorsal ground colour is dark green olive, sometimes brownish or black, with a sub-marginal yellowish stripe contouring the body (Marcus 1951; Álvarez-Presas *et al.* 2011), whereas in *Imbira guaiana* (Leal-Zanchet & Carbayo, 2001), the dorsal surface is greyish-olive (Leal-Zanchet & Carbayo 2001). Regarding the body shape, the new species resembles *I. guaiana* because both species look like a ribbon, whereas in *I. marcusi*, which is the widest species of the genus, the body is almost lanceolate (Marcus 1951, photos in http://planarias.each.usp.br). The pharynx of *I. negrita* **sp. nov.** is similar to that of *I. guaiana*, which is collar-type (Leal-Zanchet & Carbayo 2001). In the new species, it is between bell-shaped and collar-type, although in the largest sectioned specimen the pharynx is collar-type. Although Marcus (1951) stated that the pharynx in *I. marcusi* is cylindrical, with dorsal insertion at the level of the mouth (Marcus 1951, p. 191), according to Álvarez-Presas *et al.* (2011), who reviewed

Marcus material, the pharynx is bell-shaped. The copulatory apparatus of the three species is quite similar, differing in some details. The prostatic vesicle of *I. negrita* **sp. nov.** and *I. guaiana* exhibits two portions. Proximally, it consists of two long tubular extrabulbar branches which run backward parallel to each other, and distally they unite into an unpaired and tubular intrabulbar portion. This unpaired portion runs sinuously and opens into a short ejaculatory duct whose lumen is narrow. In *I. marcusi*, the proximal part is also bifurcated and extrabulbar, but it opens directly into the ejaculatory duct, which runs along the penis bulb and traverses the eversible penis (Marcus 1951; Álvarez-Presas *et al.* 2011). It would be interesting to corroborate whether this long part identified by Marcus (1951) and Álvarez-Presas *et al.* (2011) as the ejaculatory duct is in fact the intrabulbar part of the prostatic vesicle. Similarly to that observed in *I. negrita* **sp. nov.**, the eversible penis and the male atrium of *I. marcusi* and *I. guaiana* are lined by ciliated epithelium.

Regarding the female reproductive system, the new species can be distinguished from *I. marcusi* and *I. guaiana* because, in these species, the ovovitelline ducts emerge from the dorsal region of the ovaries (Marcus 1951; Leal-Zanchet & Carbayo 2001), whereas in *I. negrita*, they emerge from the latero-ventral face of the ovaries. In the three species, the ovovitelline ducts join dorsally, at the level of the gonopore or slightly behind it, with some intraspecific variation, as observed both in *I. negrita* **sp. nov.** and the Brazilian species. The same applies to the point where the female genital canal (which is extremely short in all species) opens into the female atrium. In the largest specimen of *I. negrita* **sp. nov.**, the female canal opens into the most distal part of the dorsal wall of the female atrium, whereas in the smaller specimens, the female canal opens into the distal third of the female atrium but not terminally. According to the schematic drawings of specimens studied by Marcus (1951), something similar appears to occur in *I. marcusi*. However, Marcus reconstructed the copulatory apparatus of an animal after copulation, therefore mature, with the female canal opening in the middle third of the female atrium.

Genus *Geoplana* Stimpson, 1857

Geoplana quagga Marcus, 1951 *incertae sedis*

(Figs. 7–8, Tables 3, 4)

Material examined. MLP–He 7412, San Antonio town, 8 May 2008; cephalic and anterior region at the level of the ovaries: sagittal sections on 37 slides; pre-pharyngeal region: transverse sections on 11 slides; pharynx and copulatory apparatus: sagittal sections on 31 slides. MLP–He 7411-1, San Antonio town, 7 May 2008; cephalic and anterior region at the level of the ovaries: sagittal sections on 13 slides; pharynx and copulatory apparatus: sagittal sections on 15 slides. MLP–He 7411-2, San Antonio town, 7 May 2008; preserved in ethanol.

Locality. San Antonio town (26°03'17.25"S, 53°43'50.29"W), Misiones province, Argentina (Fig. 1).

Description. *External morphology.* Body elongate, with parallel margins. When crawling, the first quarter of the body is elongated and much thinner than the rest of the body, with the anterior tip blunt and the posterior region ending abruptly (Fig. 7A, B). The dorsal surface is black grey with a pure white median stripe flanked by jet black para-median stripes formed by very dense pigment on each side of the median stripe (Fig. 7A, C). Along the first quarter of the body, the median band is very thin and traversed by narrow pure white irregular stripes (Fig. 7A, C). The ventral surface and body margins are light grey. The eyes, with small clear halos, are exclusively marginal. They are uniserial throughout the body, except in the middle third, where they are irregularly bi-serial (Fig. 7B, C). When crawling, maximum length was ~30mm, whereas after fixation was 20.5–22.6mm. Maximum width was 3–3.4mm. The mouth and gonopore distances from the anterior tip are 65–74% and 78–83% respectively relative to body length.

Internal morphology. Rhammites cover the entire dorsal epidermis (20µm high), which receives fine granular erythrophil and cyanophil secretions. The ventral epidermis (25µm high), which is ciliated on the creeping sole (~95% of body width), receives the same types of secretions, being cyanophil secretions more abundant than erythrophil ones. Small rhabdites are present along the creeping sole. Glandular margin with coarse granular erythrophil secretion and abundant rhammites. Sensory pits (50µm deep), as simple invaginations of ventral epidermis, contour the cephalic region.

Cutaneous musculature composed of a subepidermal circular layer (2.5–5µm thick), an intermediate diagonal layer (5–10µm thick), and an inner longitudinal layer (25–50µm thick). The cutaneous musculature occupies 5–9%

of the body height (CMI). Parenchymal musculature constituted by a dorsal layer, with decussate fibres, followed by a transverse supra-intestinal and a sub-intestinal muscle layer. Also, scarce dorso-ventral fibres intermingle among intestinal branches. The parenchymal musculature occupies 7–12% of the body height.

The cylindrical pharynx, 1.3–1.5mm in length (6–7% of body length), occupies most of the pharyngeal pouch (1.6–1.7mm in length), with the mouth located at the distal third of the latter (Fig. 7D). Pharynx lined by ciliated cuboidal epithelium, followed by a longitudinal subepithelial muscle layer (5µm thick) and a subjacent circular layer (5–10µm thick). Pharyngeal lumen lined by ciliated columnar epithelium, followed by a single muscle layer composed mainly of circular fibres with some longitudinal fibres intermingled (40–70µm thick). Pharyngeal stroma pierced by highly abundant fine granular erythrophil and cyanophil secretions which open in the pharyngeal tip (Fig. 7D). A short oesophagus (~100µm in length) connects the pharyngeal lumen with the intestine.

TABLE 3. Measurements (mm) from fixed specimens of *Geoplana quagga* and *Paraba multicolor*. CS: width of creeping sole; DG: distance from gonopore to anterior end; DM: distance from mouth to anterior end. The numbers given in parentheses represent the position relative to body length (%). Thickness (µm) of cutaneous (CM) and parenchymal (PM) musculatures at the pre-pharyngeal region. CMI (cutaneous muscular index): ratio of height of cutaneous musculature to body height. PMI (parenchymal muscular index): ratio of height of parenchymal musculature to body height. Both indices measured at the pre-pharyngeal region. (-) not measured.

	G. quagga MLP–He 7412	*G. quagga* MLP–He 7411-1	*P. multicolor* MLP–He 6471-3	*P. multicolor* MLP–He 6471-2
Length	22.6	20.5	42.5	35.3
Width	3.4	3	5.6	4.6
Height	1.4	1.3	2	1.5
DM	14.6 (65%)	15.2 (74%)	28.7 (67%)	22.2 (63%)
DG	17.6 (78%)	17.1 (83%)	34.7 (82%)	26.3 (74%)
CS	95%	-	100%	100%
CM dorsal	65	32.5	32.5	27.5
CM ventral	65	32.5	52.5	72.5
CMI	9%	5%	4%	7%
PM	175	87.5	250	190
PMI	12%	7%	12%	13%

TABLE 4. Measurements (mm) from fixed specimens of *Geoplana quagga* and *Paraba multicolor*. DPVP, distance between prostatic vesicle and pharyngeal pouch; LCOD, length of common glandular ovovitelline duct; LFA, length of female atrium; LFC, length of female canal; LMA, length of male atrium; LPP, length of penis papilla; LPV, length of prostatic vesicle. The numbers given in parentheses represent the position relative to body length (%).
(-) not measured. (*) distal portion of prostatic vesicle. (+) eversible penis.

	G. quagga MLP–He 7412	*G. quagga* MLP–He 7411-1	*P. multicolor* MLP–He 6471-3	*P. multicolor* MLP–He 6471-2
Anteriormost testes	4.2 (19%)	5.1 (25%)	7.6 (18%)	5.5 (16%)
Posteriormost testes	12.4 (55%)	13.2 (64%)	25.4 (60%)	18.5 (52%)
LPV	0.85	0.75	1.1(*)	0.7(*)
DPVP	0.9	0.1	2.1	1.6
LPP	0.55(+)	0.45(+)	0.8	0.9
LMA	0.65	0.5	1.5	1.5
Location of ovaries	3.4 (15%)	5 (24%)	7 (16%)	5 (14%)
LCOD	0.1	0.1	0.07	0.07
LFC	0.25	0.3	-	-
LFA	0.5	0.45	1	0.8

FIGURE 7. *Geoplana quagga*. **(A)** Dorsal view of live specimen (MLP-He 7411-2). **(B)** Detail of the cephalic region of preserved specimen (MLP-He 7412). **(C)** Dorsal view of preserved specimen (MLP-He 7411-1). **(D)** Sagittal section of the pharynx (MLP-He 7411-1). **(E, F)** Sagittal sections of the anterior region (MLP-He 7412): at the level of testes (E), at the level of the ovaries (F). **(G)** Body region immediately posterior to the pharynx, with nematode larva (MLP-He 7412). Scale bars: A, C: 10mm, B: 1mm, D, G: 500µm, E, F: 200µm.

Dorsal testes located below the supra-intestinal parenchymal muscle layer (Fig. 7E), constituting two irregular rows on each side of the body. They are posterior to the ovaries, extending until the proximity of the pharyngeal root (19–25% and 55–64% of body length from the anterior end, respectively). At pre-pharyngeal level, sperm ducts are dorsal and laterally displaced to the ovovitelline ducts, located among fibres of the sub-intestinal parenchymal muscle layer. Behind the pharynx, they are expanded and full of spermatozoa, forming spermiducal vesicles. Their distal portions bend medially to open laterally into the extrabulbar prostatic vesicle, which is C-shaped in sagittal view (Fig. 8A–C). Then, the prostatic vesicle penetrates the antero-dorsal face of the penis bulb and communicates with the ejaculatory duct, which runs obliquely to reach the centre of the bulb (Fig. 8A, B). The ejaculatory duct opens in a sinuous duct of the male atrium, which is formed by their folded walls (Fig. 8A, B, D). These folds of the atrium project backward forming a kind of papilla (eversible penis) (450–550µm long) (Fig. 8A, B). The male atrium (500–600µm long) houses this papilla in its entire cavity, which is well separated from the female atrium by a dorsal fold located at the level of the gonopore (Fig. 8A, B). Sperm ducts lined by ciliated cuboidal epithelium, without underlying musculature at the pre-pharyngeal level. The extrabulbar prostatic vesicle is lined with a ciliated, columnar epithelium, pierced by erythrophil fine granules very densely arranged and intensely stained (Fig. 8B, C), and surrounded by a thick muscle layer mainly composed of circular fibres with interspersed oblique and longitudinal fibres (65–80µm thick). The ejaculatory duct, whose lumen is more dilated than the prostatic vesicle, is also lined with a ciliated, columnar epithelium, which receives fine granular erythrophil secretion, in less quantity compared with the prostatic vesicle (Fig. 8B–D). The ejaculatory duct is wrapped by a muscle layer arranged as the prostatic vesicle but thinner (25–30µm thick). The cell bodies of the glands which pierce the ejaculatory duct are in the surrounding parenchyma, outside the penis bulb. The male atrium is lined by columnar epithelium, followed by a circular and a longitudinal muscle layer, being thicker dorsally (25–30µm thick) than ventrally (10µm thick). The atrial epithelium is filled with scarce fine granular erythrophil secretion discharged by cell glands which lie below its muscularis. The lining epithelium of the male atrial folds is columnar, non-ciliated and strongly stained because it is crossed by abundant erythrophil fine granules (Fig. 8B, D, E), whose cell bodies lie immediately below the circular muscle layer (5–10µm thick) and a subjacent longitudinal layer (25–30µm thick). Also, abundant cell necks with fine erythrophil granules traverse the atrial folds and open in the ectal region of the papilla (Fig. 8B, E). The cell bodies of these glands are located outside the bulb (Fig. 8A, B).

Ovaries proximally ovoid and distally elongate, drop-shaped, ventral to the intestine, located just above the nervous plate (Fig. 7F). They appear at 3.4–5mm from the anterior tip (15–24% of body length). Ovovitelline ducts originate dorsally from the ovoid part of the ovaries and run backwards. Numerous vitelline follicles, which discharge throughout the entire length of the ovovitelline ducts, accommodate among intestine branches (Fig. 7E–G). At the level of the gonopore, the ovovitelline ducts gradually ascend and turn towards the sagittal body plane and join in a short common glandular ovovitelline duct (~100µm long) located above the posterior region of the female atrium (Fig. 8A, B). The female genital canal (250–300µm in length), projecting dorso-anteriorly, connects the common ovovitelline duct with the female atrium, whose proximal part is funnel-shaped (Fig. 8A). The rest of the atrium is a poorly folded ample cavity (450–500µm long) (Fig. 8A, B). The ovovitelline ducts are lined with cuboidal ciliated epithelium surrounded by a thin longitudinal muscle layer (2.5µm thick). Their distal ascending portions are pierced by amorphous cyanophil secretion from the shell glands, which also discharge into the common ovovitelline duct. This common duct is lined with columnar ciliated epithelium continuous with the female canal, but in the latter is non-ciliated. Both the common duct and female canal are mainly wrapped by circular muscle fibres with some oblique ones (10–15µm thick). The epithelium of the female atrium is pseudostratified, with nuclei at different heights, pierced by abundant erythrophil fine granules (Fig. 8B). Its muscularis is composed of circular fibres with some interspersed longitudinal and oblique fibres, thicker dorsally (120–150µm thick) than ventrally (40–75µm thick). The gonopore canal is lined with a columnar ciliated epithelium, filled with erythrophil fine granules densely grouped apically (Fig. 8B).

Parasitism. Nematode larvae were found in the specimen MLP-He 7412 inside the parenchyma, near the copulatory apparatus (Fig. 7G).

Remarks and comparative discussion. The anatomical features of specimens from San Antonio (Argentina) are coincident with the original description of *Geoplana quagga* provided by Marcus (1951) from material collected in São Paulo (Brazil). This species is easily distinguished from other geoplaninids due to its characteristic colour pattern, consisting of white transversal stripes irregularly arranged on both sides of an also white median band, and a black background. These transversal stripes occupy the anterior region, approximately one-fifth of the body length in live specimens. The eyes pattern of Argentinean specimens also agrees with that of the Brazilian specimens, being mostly uniserial along the body and marginally arranged (Marcus 1951). Besides, the body size and relative position of the mouth and gonopore regarding body length are quite similar between Argentinean and Brazilian flatworms.

Geoplana quagga is now considered as a species *incertae sedis* because the diagnosis of the genus *Geoplana* was emended (Carbayo *et al.* 2013). Some features of *G. quagga*, mainly regarding the reproductive system, are incongruent with the re-definition of *Geoplana*, namely: prostatic vesicle intrabulbar, penis papilla protrusible extending even along the female atrium, male atrium not folded, ascending portion of the ovovitelline ducts lateral to female atrium (Carbayo *et al.* 2013). In *G. quagga*, the reproductive system exhibit, among other features, extrabulbar prostatic vesicle, eversible penis (no permanent penis papilla) formed by folds of the male atrium, and ovovitelline ducts ascending at the level of gonopore. Although *G. quagga* does not belong to the genus *Geoplana*, we decided to maintain it in this genus until new evidence supports their inclusion in an appropriate one.

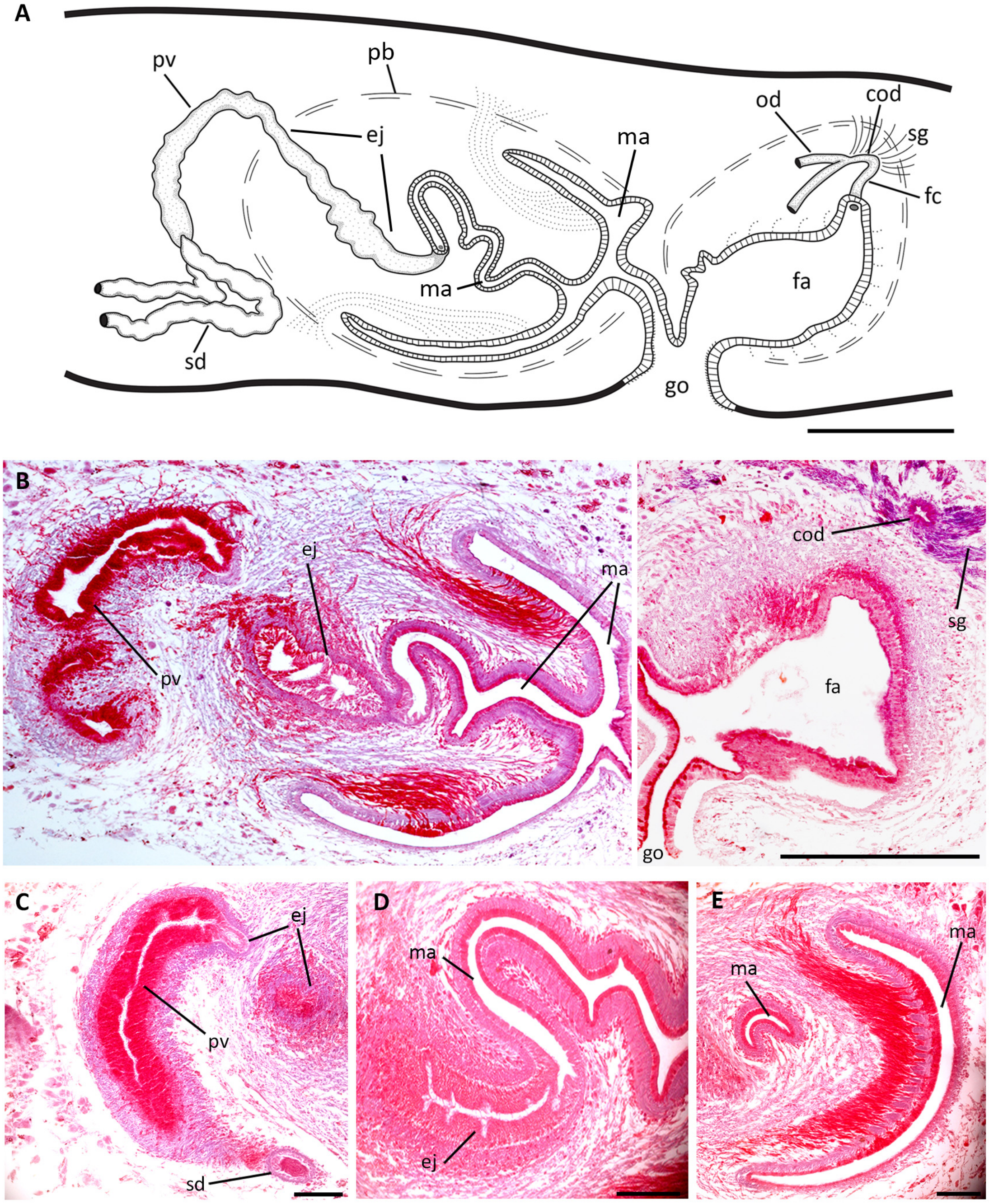

FIGURE 8. *Geoplana quagga*. **(A)** Schematic reconstruction of the copulatory apparatus, in sagittal view (MLP-He 7412). **(B–E)** Sagittal sections of the reproductive system: copulatory apparatus (MLP-He 7412) (B), detail of the extrabulbar prostatic vesicle (MLP-He 7411-1) (C), detail of the ejaculatory duct and male atrium (MLP-He 7411-1) (D), detail of male atrium (MLP-He 7411-1) (E). Scale bars: A, B: 500µm, C–E: 100µm.

Although the description of the reproductive system and the reconstruction of the copulatory apparatus of *G. quagga* sketched by Marcus (1951) fit well with our specimens, we found some small differences from the histological point of view. In this regard, Marcus pointed out the presence of a long ejaculatory duct which starts outside the penis bulb with a narrow lumen, and follows inside the bulb proximally as a dilated canal (obliquely oriented) and distally with a narrow lumen and highly sinuous, before opening on the tip of the penis papilla. Based on our specimens, we have interpreted that the ejaculatory duct corresponds only with the dilated, obliquely oriented, part mentioned by Marcus. Besides, the distal sinuous and narrow portion corresponds with the narrow lumen left by the folds of the male atrium, which take the form of a papilla (eversible penis).

Regarding the female reproductive system, according to Marcus (1951), the female atrium is an ample cavity with folded walls, but in our specimens, it is poorly folded.

The finding of *Geoplana quagga* in Argentina (Misiones province) is the first report of this species outside Brazil, thus expanding its distribution range. Previous reports place this species in the states of São Paulo, Rio de Janeiro and Santa Catarina (Marcus 1951; Froehlich 1956a, 1959) (Fig. 1). However, because the Argentinean specimens were found in proximity to the frontier with the state of Paraná (Brazil), the presence of *G. quagga* in this state is highly expected. Froehlich (1955a) catalogued *G. quagga* as a 'man-follower species' because it has always been found in anthropized areas, both in cities (gardens, backyards, vacant lots) and peri-urban areas (near houses) (Marcus 1951; Froehlich 1956a, 1959). Similarly, the specimens from San Antonio were collected under fallen logs in a man-disturbed riparian native forest, near human settlements.

Although Marcus (1951) did not give an indication or explanation concerning the origin of the species name, we believe that it could have derived from an extinct subspecies of plains zebra, *Equus quagga quagga*, an equid that lived in South Africa until the 19th century. This equid was distinguished from other zebras by its limited pattern of white stripes mainly on the anterior part of the body (Skinner & Chimimba 2005). This striped pattern may have reminded Marcus (1951) of this extinct animal, thereby giving the species name to this land planarian.

Genus *Paraba* Carbayo *et al.*, 2013

Paraba multicolor (Graff, 1899)

(Figs. 9–11, Tables 3, 4)

Material examined. MLP–He 6471-3, San Antonio town, 6 November 2010; cephalic and anterior region at the level of the ovaries: sagittal sections on 52 slides; pre-pharyngeal region: transverse sections on 5 slides; pharynx and copulatory apparatus: sagittal sections on 52 slides. MLP–He 6471-2, San Antonio town, 6 November 2010; cephalic and anterior region at the level of the ovaries: sagittal sections on 60 slides; pre-pharyngeal region: transverse sections on 10 slides; pharynx and copulatory apparatus: sagittal sections on 40 slides. MLP–He 6471-1, San Antonio town, 6 November 2010; preserved in ethanol.

Locality. San Antonio town (26°03'26.63"S, 53°44'07.91"W), Misiones province, Argentina (Fig. 1).

Description. *External morphology.* Body lanceolate, with the anterior region gradually narrowing, and the posterior body region ending abruptly (Fig. 9A, B). Dorsum graphite black with a wide yellow orange median band (~30% of body width) along the entire body except a small portion in the cephalic region (Fig. 9A, B). A thin red orange stripe runs throughout the median band, which is a bit thicker at the level of the pharynx and copulatory apparatus (Fig. 9A, B). The pigment bordering the median band is more concentrated, forming jet black para-median stripes, darker than the background colour. After fixation, the dorsal pigment becomes slightly faded and the para-median stripes as well as the red orange median stripe are better discernible (Fig. 9B). The ventral surface is cream. The eyes, with clear halos, are uniserial around the anterior tip and up to 2mm on the body margins; posteriorly, they remain marginal and extend in two and three irregular rows for 4–6mm. Then, they spread dorsally and pluriserially, forming 10–12 irregular rows for 10–12mm, being posteriorly more isolated and less numerous. At the pre-pharyngeal level, they form 6–8 rows of eyes, and posteriorly remain dorsal, reaching the posterior end (Fig. 9A, B). When crawling, maximum length was ~50mm. After fixation, maximum length was 35.3–42.5mm and maximum width was 4.6–5.6mm. The position of the mouth and gonopore relative to body length varies from 63% to 67% and from 74% to 82% respectively.

Internal morphology. The dorsal epidermis (30–35µm high), with abundant rhabdites, receives abundant fine granular xanthophil secretion, and scarce fine granular erythrophil and cyanophil secretions (Fig. 9C–E). The ventral epidermis (35–40µm high), ciliated on the creeping sole (~100% of body width), is filled with small rhabdites which occupy the apex of epidermal cells, and abundant fine granular cyanophil secretion. The glandular margin contains abundant coarse xanthophil granules and scarce fine cyanophil granules (Fig. 9C, D). The sensory pits are simple invaginations (30–50µm deep) distributed in a single row along the cephalic region and extending up to ~500µm from the anterior tip. The cutaneous musculature, organized with the three typical layers of geoplaninids, is almost twice thicker ventrally than dorsally. Its thickness relative to body height at the pre-pharyngeal region is 4–7% (Table 3). The parenchymal musculature is organized with three layers: a dorsal layer with decussate diagonal fibres, a supra-intestinal layer with transverse fibres, and a sub-intestinal layer with transverse fibres. Its thickness relative to body height at the pre-pharyngeal region is 12–13% (Table 3).

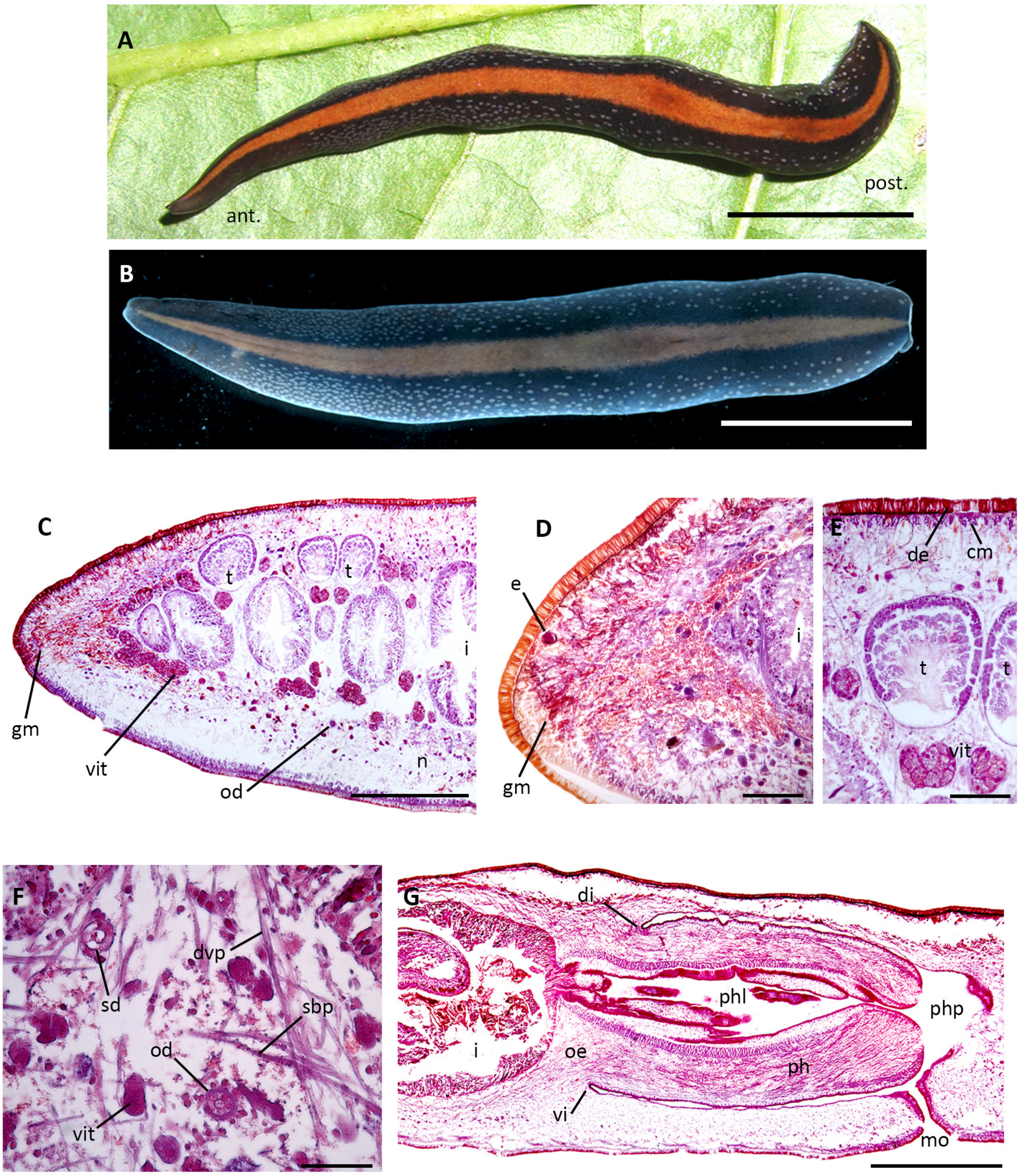

FIGURE 9. *Paraba multicolor*. **(A)** Live specimen in dorsal view (MLP-He 6471-2). **(B)** Preserved specimen in dorsal view (MLP-He 6471-3). **(C–F)** Transverse sections of the pre-pharyngeal region with details (MLP-He 6471-3): pre-pharyngeal region (C), detail of glandular margin (D), detail of testes (E), detail of sperm duct and ovovitelline duct (F). **(G)** Sagittal section of the pharynx (MLP-He 6471-2). Scale bars: A, B: 10mm, C, G: 1mm, D, E: 100µm, F: 50µm.

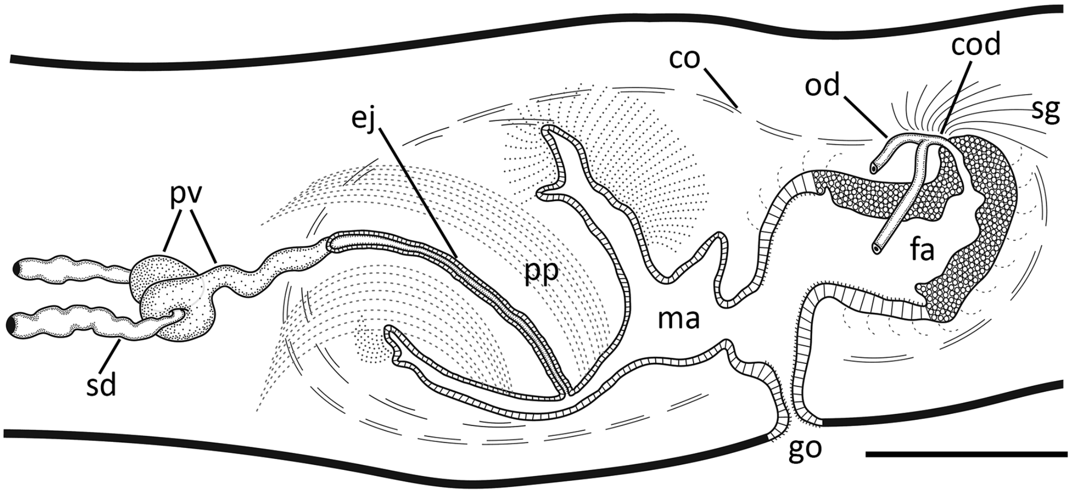

FIGURE 10. *Paraba multicolor.* Schematic reconstruction of the copulatory apparatus, in sagittal view (MLP-He 6471-3). Scale bar: 1mm.

Pharynx cylindrical (2.3–2.6mm long, 6% of body length), with the dorsal insertion posteriorly displaced (300–350µm), and mouth located in the distal third of the pharyngeal pouch (3–3.8mm long) (Fig. 9G). Pharyngeal stroma traversed by abundant fine granular erythrophil and cyanophil secretions, whose cell bodies are placed in the surrounding parenchyma anterior to the pharynx and pierce the pharyngeal tip. Pharynx lined by ciliated cuboidal epithelium, followed by a thin layer of longitudinal muscle fibres (2.5µm thick) and a subjacent circular muscle layer (5–10µm thick). The epithelium of the pharyngeal lumen is columnar and ciliated, surrounded by a thick muscle layer consisting of circular fibres with some longitudinal fibres interspersed (35–60µm thick). An oesophagus is present (22–24% of pharyngeal length).

Dorsal testes, rounded, located between the supra-intestinal parenchymal muscle layer and intestinal branches (Fig. 9C, E). They form three irregular rows on each side of the body and extend behind the ovaries to near the pharyngeal root (16–18% and 52–60% of body length from the anterior end, respectively). At pre-pharyngeal level, sperm ducts, which are located among fibres of the sub-intestinal parenchymal muscle layer, are dorsal and medially displaced to the ovovitelline ducts (Fig. 9F). Behind the pharynx, they are expanded and full of spermatozoa, forming spermiducal vesicles, and open laterally into the paired portions of the prostatic vesicle (Figs. 10, 11B). The extrabulbar prostatic vesicle is composed of proximal globose paired portions and a distal unpaired portion, which is tubular (Figs. 10, 11A, C, D). The latter (0.7–1.1mm long) runs sinuously, traverses the penis bulb and, after a short intrabulbar tract, communicates with the ejaculatory duct, which is slightly ventrally displaced inside the penis papilla (Figs. 10, 11D). The slightly assymetrical penis papilla (0.8–0.9mm long) occupies most of the male atrium (1.5mm long). Folds of the dorsal wall of the male atrium separate this from the female atrium (Figs. 10, 11A). Sperm ducts are lined with cuboidal epithelium, while distally (spermiducal vesicles) they are lined with columnar epithelium (Fig. 11B). The two portions of the prostatic vesicle are lined with non-ciliated columnar epithelium that receives apically cyanophil secretion as well as abundant fine granular erythrophil secretion, which is more abundant in the paired portions (Fig. 11A–D). The cell bodies of these erythrophil glands are located in the surrounding parenchyma, anterior to the copulatory apparatus. The musculature of the prostatic vesicle, composed of a circular layer with some interspersed longitudinal fibres, is thicker in the paired portions (20–25µm thick) than in the unpaired portion (5–10µm thick). The epithelial lining of the ejaculatory duct is cuboidal and ciliated, followed by a thin circular muscle layer (2.5µm thick). The penis papilla is lined by a cuboidal epithelium, pierced by highly abundant fine granular erythrophil secretion (Fig. 11A, D). Also, amorphous erythrophil secretion and cyanophil granules discharge mainly at the level of penis insertions (Fig. 11A, D). The musculature of the penis is composed of circular fibres (5–10µm thick). The male atrium is lined with a columnar epithelium filled with erythrophil and cyanophil granules. The muscularis of the male atrium is composed of a circular layer with interspersed longitudinal fibres (5–15µm thick).

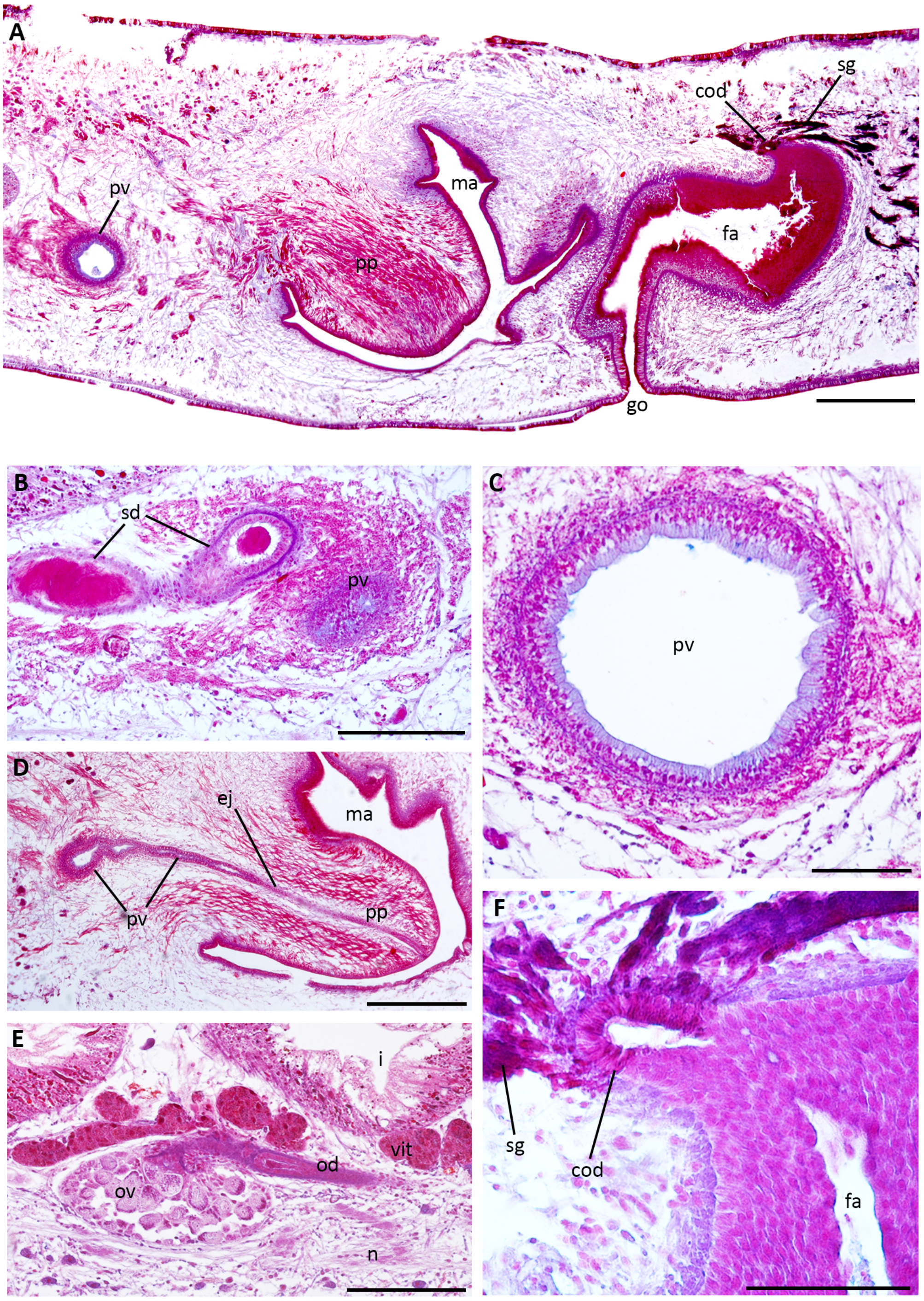

FIGURE 11. *Paraba multicolor.* **(A–F)** Sagittal sections of the reproductive system: copulatory apparatus (MLP-He 6471-3) (A), opening of the sperm duct into the prostatic vesicle (MLP-He 6471-3) (B), proximal portion of the prostatic vesicle (MLP-He 6471-3) (C), detail of the male system (MLP-He 6471-2) (D), ovary (MLP-He 6471-3) (E), detail of the female atrium (MLP-He 6471-2) (F). Scale bars: A, D: 500µm; B, E: 200µm; C, F: 100µm.

The ovaries, ovoid in shape, are located between the sub-intestinal parenchymal muscle layer and the nervous plate (Fig. 11E). The ovovitelline ducts arise from the mid-dorsal third of the ovaries (Fig. 11E). Vitelline follicles, well developed, are located among intestine branches and discharge along the ovovitelline ducts (Figs. 9C, E, F, 11E). At the level of the gonopore, the ovovitelline ducts ascend and run to the middle plane, joining each other in a short common duct (~100µm long) located above the posterior region of the female atrium (Figs. 10, 11A, F). The common ovovitelline duct is horizontal and, distally, it is ventrally flexed to open into the female atrium, which is almost tubular in shape (Figs. 10, 11A, F). Histologically, the female atrium can be differentiated in two portions (see below). The ental part of the proximal portion is vertical, with a narrow lumen, and continues almost horizontally and more spacious, while the distal portion curves ventrally before opening in the gonopore (Figs. 10, 11A). The ovovitelline ducts are lined with cuboidal ciliated epithelium followed by a circular muscle layer (2.5µm thick). Their distal ascending portions receive abundant secretion from shell glands as the common ovovitelline duct (Fig. 11A, F). The latter is lined by ciliated columnar epithelium surrounded by circular muscle fibres (10–15µm thick). The epithelial lining of the proximal region of the female is columnar and non-ciliated with stratified appearance (180–250µm high), while the distal portion is lined with columnar ciliated epithelium (60–100µm high) (Figs. 10, 11A, F). The female atrium receives abundant fine granular erythrophil secretion, more abundant in the proximal portion, and less abundant fine cyanophil granules. Its muscularis is composed of a circular subepithelial layer (15–25µm thick) and a subjacent longitudinal layer (10–15µm thick). A common muscle coat, composed of longitudinal and oblique fibres, well organized around the male atrium (50–70µm thick), is less conspicuous around the female atrium.

Remarks and comparative discussion. *Paraba multicolor* (Graff, 1899) has been reported in some localities of Brazil (states of Paraná, Rio de Janeiro, Rio Grande do Sul, and São Paulo) (Graff 1899; Marcus 1951; Froehlich 1955b, 1956a, 1956b, Leal-Zanchet & Matos 2011) (Fig. 1), and in Germany (Hamburg) as an introduced species (Arndt 1934). The external appearance of *P. multicolor* exhibits high variability since the dorsal ground colour can be dark brown, dark grey or black, with a median band pigmented from pale yellowish, yellow, ochre, orange to reddish, flanked by white or black para-median stripes (Graff 1899; Marcus 1951; Froehlich 1956a; Leal-Zanchet & Matos 2011). Also, in some specimens, the occurrence of a narrow stripe of a rust-red pigment along the median band can be observed (Leal-Zanchet & Matos 2011). The specimens from Argentina fit with the description of the holotype from São Paulo, with a yellow orange median band ('fulvo-ferrugineus' according to Graff) with a narrow red orange median stripe, although the para-median stripes in the Argentinean specimens are black and those in the specimen studied by Graff (1899) are whitish. Flatworms from Rio Grande do Sul also agree in general with Argentinean specimens, but do not exhibit the rust-red pigment along the median band (Leal-Zanchet & Matos 2011). Regarding body size, specimens from Argentina (45–50mm long in maximum extension) are intermediate between those from Rio Grande do Sul (~35mm long) (Leal-Zanchet & Matos 2011) and those from São Paulo (70mm long) (Marcus 1951). Once fixed, the relative distance of the mouth and gonopore in relation to the anterior end is similar to that observed in specimens from all localities from Brazil. Although Graff (1899) described only the external aspect of *P. multicolor*, Marcus (1951) and Leal-Zanchet & Matos (2011) provided more comprehensive descriptions of both the external and internal anatomy of this species. Froehlich (1955b) also included some notes regarding internal features of the musculature and glandular secretions of the pre-pharyngeal region. The presence of a glandular margin, with abundant coarse granular xanthophil secretion, and the thickness of the cutaneous musculature relative to body height at the pre-pharyngeal region (~6% on average) are congruent with specimens from Rio Grande do Sul and Rio de Janeiro (Froehlich 1955b; Leal-Zanchet & Matos 2011). Also, the cylindrical pharynx with the dorsal insertion a bit posteriorly displaced, short oesophagus and mouth opening in the distal part of the pharyngeal pouch agree with descriptions given by Marcus (1951) and Leal-Zanchet & Matos (2011). Regarding both the general morphology and histology of the copulatory apparatus, the Argentinean specimens are similar to those from Rio Grande do Sul and São Paulo (Marcus 1951; Leal-Zanchet & Matos 2011). Leal-Zanchet & Matos (2011) re-analysed material studied by Marcus (1951) and interpreted that the female canal considered by Marcus corresponds to the most proximal portion of the female atrium, whose lumen is narrow. We agree with this opinion, based on observations of the Argentinean specimens. Also, Leal-Zanchet & Matos (2011) referred to a vagina (*i.e.* female canal) that connects the short common ovovitelline duct with the ental portion of the female atrium. However, histologically, we did not distinguish a female canal in specimens from Argentina.

Like *Geoplana quagga* and other geoplaninids, *Paraba multicolor* is common in anthropized areas (backyards, vacant lots) (Froehlich 1955a, 1956a, 1958), although it is not restricted to these disturbed environments since it has been also found in native forests (Leal-Zanchet & Matos 2011). Likewise, specimens from San Antonio (Argentina) were found below flower plots in a backyard, probably being an accidental introduction through the trade of ornamental plants.

Genus *Obama* Carbayo *et al.*, 2013

Obama ladislavii (Graff, 1899)
(Fig. 12)

Material examined. MLP–He 7017, Salto Encantado Provincial Park, 25 February 2015; pre-pharyngeal region: transverse sections on 6 slides; pharynx: sagittal sections on 20 slides; copulatory apparatus: sagittal sections on 22 slides. MLP–He 7413, CIAR (27°26’40”S, 54°56’24”W), 11 November 2015; preserved in ethanol. MLP–He 7414, CIAR (27°26’40”S, 54°56’24”W), 13 October 2016; preserved in ethanol.

Localities. Salto Encantado Provincial Park (27°03’31”S, 54°49’44”W), and Centro de Investigación Antonia Ramos (CIAR) (27°26’40”S, 54°56’24”W), Misiones province, Argentina (Fig. 1).

Description. *External morphology.* Body lanceolate, with the anterior body region gradually narrowing to the anterior tip, and posterior region ending abruptly (Fig. 12A, B). Dorsal surface yellow green, with black spots splattering the dorsum in two irregular rows at the level of the testes (Fig. 12A). Ventral surface golden yellow with margins yellow green (Fig. 12B). Once fixed, specimens become immediately beige. Eyes are uniserial and marginal around the anterior tip and up to 4mm from the tip. Then, they continue along the body margins in two or three irregular rows between 4 and 12mm from the anterior tip, and posteriorly become dorsal, reaching the posterior end. The maximum extension of the eyes on the dorsum is between 14 and 28mm from the anterior tip (28–57% relative to body length). When crawling, maximum length varied from 45 to 60mm. After fixation, the length of the specimen studied was 49mm, and maximum width was 6.5mm. The position of the mouth and gonopore relative to body length was 63% and 77% respectively.

Internal morphology. Dorsal epidermis (50µm high) with three types of glandular secretion: abundant rhabdites and fine granular erythrophil secretion, and scarce fine granular cyanophil secretion. Ventral epidermis (40µm high), ciliated on the creeping sole (~100% of body width), with numerous small rhabdites occupying the apex of cells, and receiving fine granular erythrophil secretion and abundant cyanophil granules. Glandular margin absent. Cutaneous musculature, arranged with the three typical layers of Geoplaninae, a bit thicker ventrally (55µm thick) than dorsally (40µm thick). The thickness of the cutaneous musculature relative to body height at the pre-pharyngeal region is 5%. Parenchymal musculature organized in three layers, with loose fibres: a dorsal decussate layer with oblique fibres, a supra-intestinal transverse layer and a sub-intestinal transverse layer. The thickness of the parenchymal musculature relative to body height at the pre-pharyngeal region is ~3%.

Pharynx cylindrical (3.2mm in length, 6% of body length), with dorsal insertion slightly displaced backwards (450µm). Mouth located at the level of the pharyngeal apex, in the middle third of the pharyngeal pouch (5.5mm long). Abundant erythrophil and xanthophil fine granules and less abundant cyanophil fine granules traverse the pharyngeal stroma and pierce the pharyngeal tip. Pharynx lined by ciliated cuboidal epithelium, followed by a subepithelial longitudinal muscle layer (5µm thick) and a subjacent circular layer (20µm thick). Pharyngeal lumen lined by ciliated columnar epithelium, with a subjacent muscle coat composed of circular fibres with longitudinal interspersed fibres (75–100µm thick). A short oesophagus is present (380µm long).

Dorsal testes, located below the supra-intestinal parenchymal muscle layer, forming two irregular rows on each side of the body. At pre-pharyngeal level, sperm ducts located among sub-intestinal muscle fibres and dorso-medial to the ovovitelline ducts. Behind the pharynx, sperm ducts are dilated and full of spermatozoa, forming spermiducal vesicles (Fig. 12C–E). Their distal portions ascend and curve to the sagittal plane and open into also ascending paired portions of the prostatic vesicle (Fig. 12C–E). Paired tubular portions, obliquely oriented, join in an unpaired portion which runs to the dorsum, contouring the common muscle coat (Fig. 12C, D). The prostatic vesicle penetrates the antero-dorsal face of the penis bulb and, after a short intrabulbar tract, continues as ejaculatory duct inside the penis papilla (Fig. 12C, D). The ejaculatory duct traverses the penis papilla, running proximally sinuous and distally almost straight, opening near the tip of the penis, a bit ventrally displaced (Fig. 12C, D). The penis papilla projects obliquely from the dorsal wall of the male atrium, with its dorsal insertion posteriorly displaced (Fig. 12C, D). The male atrium (1.6mm long), without folded walls, is almost totally occupied by the penis papilla (1.4mm long) and broadly communicated with the female atrium (Fig. 12C). Distal portions of sperm ducts lined by a ciliated squamous epithelium, with a thin longitudinal muscle layer (2.5µm thick). Prostatic vesicle lined with a ciliated, columnar epithelium, pierced by fine erythrophil granules highly abundant in the unpaired portion (Fig. 12F). Musculature of prostatic vesicle composed of circular and some longitudinal fibres (15–25µm thick). The ejaculatory duct is lined by a ciliated columnar epithelium, filled with erythrophil fine granules, followed by a thin layer of circular fibres (2.5–5µm thick). The penis papilla is lined with non-ciliated columnar epithelium, followed by a circular muscle layer with some interspersed longitudinal fibres (10–15µm thick). Stroma of penis papilla with abundant fine granular erythrophil secretion and scarce cyanophil granules (Fig. 12D). Dorsal wall of the male atrium lined with columnar epithelium (up to 60µm high), and ventral wall lined with cuboidal epithelium (Fig. 12D), both pierced by erythrophil granules. Muscularis of male atrium with circular fibres with some interspersed longitudinal fibres (5–10µm thick). Abundant cyanophil fine granules extend from the dorsal to the antero-ventral side of the male atrium, spreading from the left wall of the atrium (Fig. 12C, D, G). Cyanophil granules also spread onto the dorsal part of the penis papilla (Fig. 12D).

Vitelline follicles mature but scarce, located in the surrounding parenchyma among intestine branches. Ovovitelline ducts located immediately below the sub-intestinal parenchymal muscle layer. At the level of the gonopore, the ovovitelline ducts ascend to above the female atrium joining each other (Fig. 12C, H). The common glandular ovovitelline duct (200µm long) runs backwards and ventrally flexed (Fig. 12C, D) to communicate with the female canal (180µm long), which runs downwards and anteriorly (Fig. 12C, D). The female canal opens into the female atrium (600µm long), which is funnel-shaped in sagittal section (Fig. 12C). The ovovitelline ducts are lined with cuboidal ciliated epithelium with a subjacent thin circular muscle layer (2.5µm thick). Distal ascending portions of ovovitelline ducts receive erythrophil secretion from shell glands (Fig. 12C, H). Common glandular ovovitelline duct lined with ciliated columnar epithelium, pierced by shell glands (Fig. 12C, D, H), followed by a subjacent muscle layer consisting of circular and longitudinal fibres (10–15µm thick). Epithelial lining of the female canal, columnar and non-ciliated, with abundant fine granular erythrophil secretion, wrapped by longitudinal and circular muscle fibres (10µm thick). Female atrium lined with non-ciliated columnar epithelium of stratified appearance (60–75µm high) (Fig. 12D), which receives erythrophil granules and scarce xanthophil amorphous secretion. Muscularis with the same arrangement and thickness of the female genital canal. Gonopore canal lined with ciliated columnar epithelium, apically erythrophil, with abundant cyanophil granules.

Remarks and comparative discussion. *Obama ladislavii* (Graff, 1899) is one of the land planarian species most easily distinguished from others, due to the striking bright green pigmentation of its dorsum. The external aspect of specimens from Argentina (Misiones) matches well with that of Brazilians specimens, which have been reported in many localities of the southern region of this country (Graff 1899; Froehlich 1959; Álvarez-Presas *et al.* 2015). Our specimens also exhibit black spots, which spread on the dorsum at the level of the testes, as observed in specimens from Santa Catarina and Rio Grande do Sul (Froehlich 1959; Álvarez-Presas *et al.* 2015). According with the original description, maximum extension in living specimens ranges from 46 to 100mm (Graff 1899). The specimens studied by us (~50mm in maximum extension, on average) are similar to those studied by Froehlich (1959) from Rio Grande do Sul (the type locality, among others) and Santa Catarina. Álvarez-Presas *et al.* (2015), who re-described *O. ladislavii* based on material from new localities and specimens previously studied by Graff (1899) and material collected by Froehlich (1959), found specimens with ~70–80mm in maximum extension. The relative position of the mouth and gonopore in relation to body length (63% and 77% respectively, in the specimen sectioned) is similar to that of the specimens analysed by Froehlich (1959) (average: 61% and 76%) and Álvarez-Presas *et al.* (2015) (average: 64% and 83%). Regarding the internal anatomy, the thickness of the cutaneous musculature relative to the body height at the pre-pharyngeal region of the Argentinean specimen is concordant with that of specimens studied by Álvarez-Presas *et al.* (2015). However, these authors observed a glandular margin at the pre-pharyngeal region, not observed in the sectioned specimen from Argentina, although this could be due to variations in the fixation and staining protocols. The cylindrical pharynx presents insertions almost at the same level in sagittal view, with its apical portion located at the level of the mouth, as illustrated by Froehlich (1959) and Álvarez-Presas *et al.* (2015). Similarly to specimens from the type locality (Taquara, Rio Grande do Sul), the pharynx is pierced by erythrophil, xanthophil and cyanophil secretions, with the mouth in the middle of the pharyngeal pouch (Álvarez-Presas *et al.* 2015). The body region at both the level of the ovaries and the level of the anteriormost testes could not be compared because the specimen could not be sectioned. In general, the aspect of the copulatory apparatus agrees with descriptions provided by Froehlich (1959) and Álvarez-Presas *et al.* (2015) but, as pointed out by the latter authors, we also found differences with the schematic reconstruction illustrated by Graff (1899), namely: intrabulbar prostatic vesicle without paired portions, and ovovitelline ducts ascending behind the female atrium. Froehlich (1959) pointed out the presence of a dorsal fold of the male atrium separating it partially from the female atrium, absent in the specimen from Argentina and those studied by Álvarez-Presas *et* *al.* (2015), in which both atria are broadly communicated. It is also noteworthy that both atria, particularly the female one, exhibit tall columnar epithelium (~100–200µm high) (Froehlich 1959; Álvarez-Presas *et al.* 2015), although in the Argentinean specimen its maximum height (75µm) was lower than that in the Brazilian ones, similarly to the male atrium. This is probably because the specimen studied was not fully mature, evidenced by the scarcity of vitelline follicles.

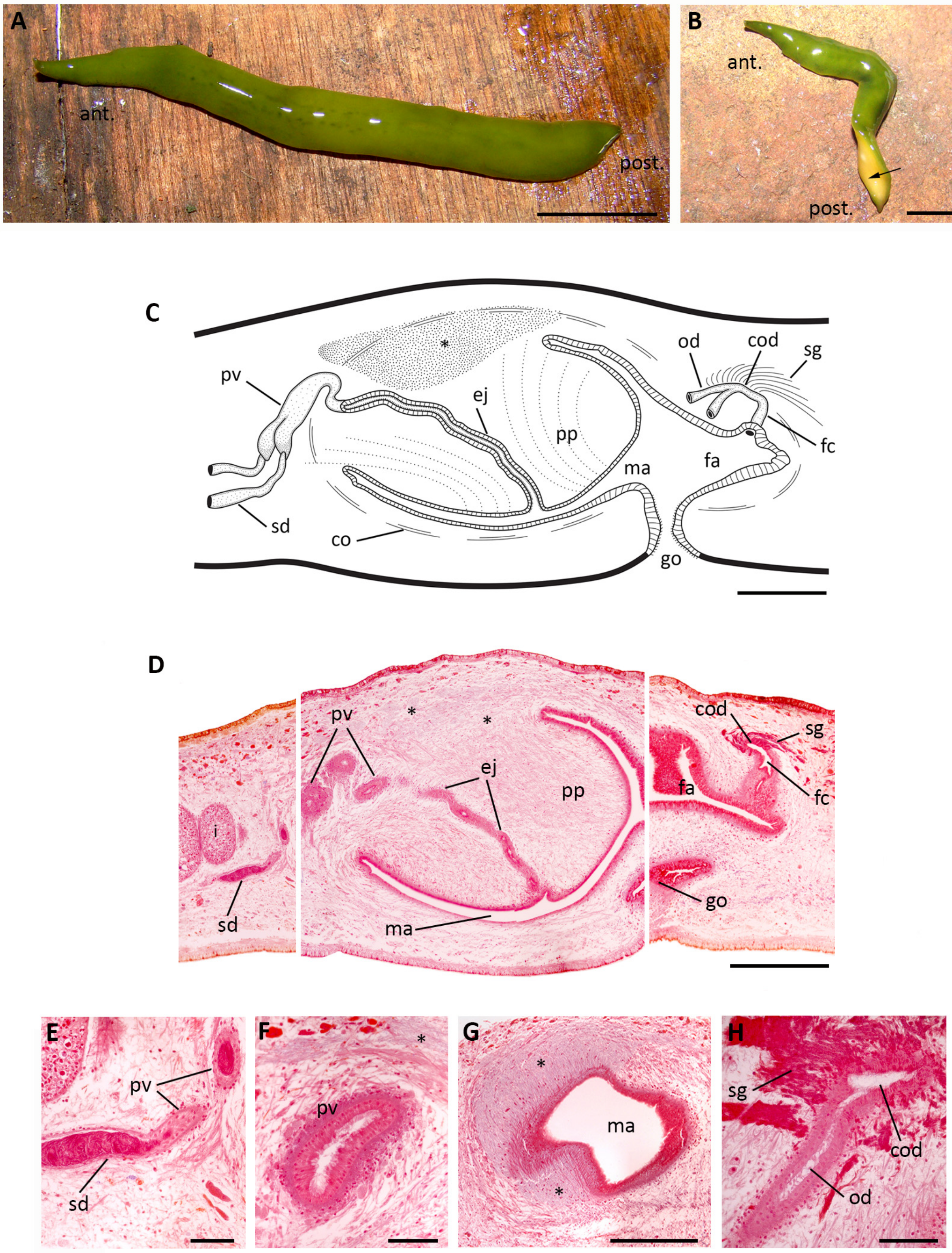

FIGURE 12. *Obama ladislavii*. **(A–B)** Photographs of live specimens in dorsal view: MLP-He 7413 (A), MLP-He 7414 (B) (arrow indicates the position of the gonopore). **(C)** Schematic reconstruction of the copulatory apparatus, in sagittal view (MLP-He 7017). Asterisk indicates the position of cyanophil granular secretion in the middle sagittal plane. **(D–H)** Sagittal sections of the reproductive system (MLP-He 7017) (asterisk indicates the position of cyanophil granular secretion): copulatory apparatus (D), detail of the proximal portion of the prostatic vesicle (E), detail of the distal portion of the prostatic vesicle (F), para-sagittal section at the level of the male atrium (G), detail of the ascending section of one of the ovovitelline ducts (H). Scale bars: A, B: 10mm, C, D, G: 500µm; E, F, H: 100µm.

Final remarks

The new species here described were found in natural reserves which conserve remnants of the Interior Atlantic Forest, in north-eastern Argentina (Misiones province). *Pasipha quirogai* **sp. nov.** was found in the southernmost portion of the Atlantic Forest, characterized by grasslands in basalt hills which extend along the Paraná River coast in this part of Misiones. This vegetation is mixed with woodlands which take the form of islets and galleries along watercourses (Giraudo *et al.* 2003). Although this ecosystem, which limits with the Chaco biogeographic province, exhibits a singular flora with numerous endemism, it is in danger because of the reduced area conserved and the absence of green corridors. By contrast, the Moconá Provincial Park, where *Imbira negrita* **sp. nov.** was found, conserves large extensions of native forests and, together with other reserves, is part of a great green corridor. Although this ecosystem is under legal protection, it is suffering the impact of human activities such as deforestation and furtive hunting, which puts biodiversity at risk. Thus, it is critical to better know the land planarian diversity in these highly susceptible ecosystems, especially since these flatworms are very sensitive to changes in their habitats, reason why they can be used in studies of the quality of the environment (Carbayo *et al.* 2002; Negrete *et al.* 2014a).

We also considered relevant to describe as completely as possible the species *Geoplana quagga*, *Obama ladislavii*, and *Paraba multicolor*, which represent the first records in Argentina. We analysed anatomical features of taxonomic relevance to provide new information and to consider the intraspecific variation in these species. We focused on the description of *G. quagga* because it was described long ago and because some features, mainly regarding the internal anatomy, which are now taxonomically informative, were not assessed by Marcus (1951). *Obama ladislavii* and *P. multicolor*, which were described at the end of the nineteenth century, have been recently redescribed (Leal-Zanchet & Matos 2011; Álvarez-Presas *et al.* 2015).

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