Zoologischer Anzeiger 279 (2019) 38-51

Contents lists available at ScienceDirect

Zoologischer Anzeiger

journal homepage: www.elsevier.com/locate/jcz

Research paper

Two new species of land planarians (Platyhelminthes, Tricladida, Geoplanidae) from protected areas in the southern extreme of the Paranaense Rainforest, Argentina

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ARTICLE INFO

Article history: Received 21 November 2018 Received in revised form 9 January 2019 Accepted 10 January 2019 Available online 18 January 2019

Corresponding Editor: Sorensen

Keywords: Land flatworms Geoplaninae Obama Notogynaphallia Riverine forests Río de la Plata Basin

ABSTRACT

Here, we describe two new flatworm species of the genera Obama Carbayo et al., 2013 and Notogynaphallia Ogren & Kawakatsu, 1990 (Geoplaninae) found in riverine forests of central-eastern Argentina. Obama maculatentis sp. nov., which is about 50 mm in length, is characterized by black olive pigmentation on the dorsum, with two irregular rows of black spots, and light grey ventral surface. The eyes, with clear halos, extend to the dorsal surface. The foremost features of the copulatory apparatus include dorsal testes anterior to ovaries, extrabulbar prostatic vesicle with forked proximal portion, protrusible penis, male atrium with a large dorsal fold separating it from the female atrium, ovovitelline ducts ascending at the level of the gonopore, common ovovitelline duct dorsal to the distal portion of the female atrium, female canal ventro-anteriorly flexed, and female atrium with narrow lumen. Notogynaphallia fortuita sp. nov., which is about 30 mm in length, is characterized by beige ground pigmentation with tiny darker spots, cephalic region saffron yellow, and ventral surface light grey. The eyes, with small clear halos, extend to the dorsal surface. Regarding the internal anatomy, this new species shows parenchymatic musculature not organized in discrete layers, testes arising near the anterior end of the body, distal sperm ducts traversing the common muscle coat and joining in a short common sperm duct. The areas in which the land planarians were found represent the southernmost latitudes with representatives of the Interior Atlantic Forest (Paranaense Rainforest) biota, hence their biogeographical and conservation significance.

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1. Introduction

A significant proportion of Neotropical land planarians (Platyhelminthes, Geoplanidae, Geoplaninae) hitherto known have been described from the Brazilian Atlantic Forest. This also applies to species of the genera *Obama* Carbayo et al., 2013 and *Notogynaphallia* Ogren & Kawakatsu, 1990. The genus *Obama* comprises about 40 species, all of which have been recorded in Brazil (see Tyler et al. 2006–2016 and references herein). Only *Obama ladislavii* (Graff, 1899) and *Obama nungara* Carbayo et al., 2016 have also been found in Argentina (Lago-Barcia et al. 2015, 2018; Negrete & Brusa 2017). The latter has also been documented outside its

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native range in many countries of Europe, in which this species has been accidentally introduced (Justine et al. 2014; Lago-Barcia et al. 2015, 2018; Carbayo et al. 2016b). Like other geoplaninids, *Obama* species have been mostly recorded from the southern portion of the Atlantic Forest, where there are still many species to be described (Baptista & Leal-Zanchet 2010; Carbayo et al. 2002; Leal-Zanchet et al. 2011; Rossi et al. 2016). Regarding *Notogynaphallia*, a genus with eight species (plus four species *incertae sedis*), over 70% of the species have been described from Brazil. The remaining species are known from Peru, Colombia, Panama, Argentina, and Paraguay (Carbayo et al. 2013; Negrete et al. 2015).

In Argentina, although the state of knowledge on the diversity of land planarians is still in progress, significant advances have been made in the past few years, mainly in the Argentinean portion of the Atlantic Forest (Negrete et al. 2014; Negrete & Brusa 2017 and references therein). This ecoregion, which extends from the northeast to the southeast of Brazil, is one of the most diverse of





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South America and even in the world, but also one of the most threatened biotopes (Myers et al. 2000; Galindo-Leal & Câmara 2003). The inland portion of the Atlantic Forest is known as the Interior Atlantic Forest or Paranaense Rainforest, which covers the south-east of Brazil, the east of Paraguay and the north-east of Argentina. However, the riparian forests of the Río de la Plata Basin are 'green corridors' that extend the distribution of some of the floral and faunal elements of the Paranaense Rainforest toward southern latitudes, thus reaching the coast of the estuary of the Río de la Plata River in the central-east region of Argentina (Herrera & Torres Robles 2012). In the present contribution, we describe two new species of land planarians, one of the genus *Obama* and one *Notogynaphallia*, found in these riverine forests of the Río de la Plata River.

2. Material and methods

The specimens were manually collected between 2012 and 2018 from two protected natural areas, Reserva Natural Punta Lara (34°47′S, 57°59′W) and Monte Ribereño Isla Paulino, Isla Santiago $(34^{\circ}50'S, 57^{\circ}52'W)$, both located in the central-east region of Argentina (Buenos Aires province). These localities, situated on the coast of the Río de la Plata River, harbour different vegetation units, which include alluvial forests, grasslands, and riparian scrub. The prospected areas included native alluvial forests, locally named 'marginal forest', invaded by the exotic tree species Ligustrum lucidum (Dascanio et al., 1994). Land planarians were searched for beneath fallen logs and leaf litter during daylight, and by direct observation of the ground illuminated with a head lantern during the night. The external features of live animals were documented in situ. Once in the lab, they were photographed and then killed, using boiling water, fixed in 10% formaldehyde and preserved in 70% ethanol. Before fixation, the posterior end of the specimens was cut and stored in 100% ethanol. Fragments of the anterior, prepharyngeal, and pharyngeal regions as well as the copulatory apparatus were dehydrated in an ascending series of ethanol and subsequently embedded in Paraplast®. These fragments were then serially sectioned at intervals of $6-7 \,\mu\text{m}$ and stained with a modification of the Masson's trichrome method (Suvarna et al. 2013), which incorporates Ponceau Xylidine and Orange G in the solution of Acid fuchsin. The cutaneous musculature height to body height ratio (cutaneous muscular index, CMI) was measured according to Froehlich, C.G. (1955), from transverse sections of the prepharyngeal body region. The pigmentation of the specimens was estimated from the RAL colour standard chart (https://www.ralfarben.de). The line drawings with the reconstructions of the copulatory apparatus were made from photographs taken to the histological slides with a photographic camera adapted to the optical microscope. All figures are oriented with the anterior end of the body to the left.

The material examined was deposited at the Invertebrate Collection of Museo de La Plata (MLP), Argentina.

3. Results

Taxonomic part Family Geoplanidae Stimpson, 1857 Subfamily Geoplaninae Stimpson, 1857 Genus Obama Carbayo et al., 2013

3.1. Obama maculatentis sp. nov.

Material examined. Holotype: MLP He-7497, Reserva Natural Punta Lara, Ensenada, Buenos Aires province (Argentina) (34°47′32″S, 57°59′56″W), 15th November 2017, F. Brusa coll.; cephalic region: transverse sections on 13 slides; anterior region at the level of the ovaries: sagittal sections on 35 slides; prepharyngeal region: transverse sections on 6 slides; pharynx: sagittal sections on 24 slides; and copulatory apparatus: sagittal sections on 25 slides. Paratype: MLP He-7498, Reserva Natural Punta Lara, Ensenada, Buenos Aires province (Argentina), 18th November 2017, L. Negrete coll.; cephalic region: transverse sections on 10 slides; anterior region at the level of the ovaries: sagittal sections on 25 slides; pre-pharyngeal region: transverse sections on 8 slides; pharynx: sagittal sections on 22 slides; and copulatory apparatus: sagittal sections on 20 slides.

Additional material. MLP He-7499, Reserva Natural Punta Lara, Ensenada, Buenos Aires province (Argentina), 7th August 2018, L. Negrete coll. Preserved in 70% ethanol. MLP He-7500, Reserva Natural Punta Lara, Ensenada, Buenos Aires province (Argentina), 18th November 2017, L. Negrete coll. Specimen presumably immature, preserved in 70% ethanol.

Diagnosis. Species of *Obama* of 50 mm in length when fully extended. Dorsum black olive, with two irregular rows of black spots at the level of the testes. Male and female atria separated by a large dorsal fold. Ovaries drop-shaped, ovoid proximally and elongated and thin distally. Ovovitelline ducts arising from the first third of the dorsal side of the ovaries.

Type locality. Reserva Natural Punta Lara, Buenos Aires province, Argentina.

Habitat. The flatworms were found on the ground under fallen logs and crawling at night, in forests with native and exotic vegetation.

Etymology. The specific name, derived from Latin, is a combination of the words *macula* (spots) and *latentis* (hidden, camouflaged, occult), which refers to the pattern of spots scattered on the dorsum of the new species, which are difficult to see at first sight.

3.1.1. Description

External morphology. Body elongate, with parallel margins, narrowing progressively towards the anterior body region and more abruptly towards the posterior end (Fig. 1A). Anterior tip



Fig. 1. External aspect of *Obama maculatentis* sp. nov. **(A)** Dorsal view of the holotype (alive); inset showing it as seen with the naked eye. **(B)** Ventral view of the holotype (alive); arrowheads indicate position of the mouth and gonopore. **(C)** Dorsal view of the paratype (preserved), showing the eye pattern. Scale bar: 5 mm.

rounded; posterior end pointed (Fig. 1A, B). To the naked eve, the pigmentation of the dorsal surface is black-olive, with the cephalic region being paler (Fig. 1A, inset). Two irregular rows of black spots spread along the dorsum, scattering from the level in which the eyes spread dorsally, dissipating at the pre-pharyngeal level (Fig. 1A, C). This spotted pattern, discernible when live animals are photographed using flash, is also noticeable once the animals are preserved (Fig. 1C). Ventral surface light grev (Fig. 1B). Eves, with clear halos, are present along the entire body length. The eyes are monolobated and uniserial around the anterior tip and toward both margins of the body up to 4 mm from the anterior tip (Figs. 2A and 3). Between 4 and 8–9 mm from the anterior tip, the eyes continue monolobated and marginal in two or three irregular rows (Figs. 2B and 3). Thereafter, they spread onto the dorsal surface of the body, being mono- and trilobated (Figs. 1C and 2B). At a distance that corresponds to 30% and 40% of the body length (measured from the anterior margin), the eyes exhibit their maximum extension on the dorsum, occupying 30–35% of the body width on either side of the body, here being trilobated (Figs. 1C, 2C and 3). Eyes are numerous up to the pharyngeal region, being much less abundant and more isolated backwards, where they extend to the posterior-most body margin (Fig. 3). The largest specimen (holotype) reached 50 mm in length in maximum extension (Fig. 1A, B); after fixation, the body length ranged between 43 and 47 mm (Table 1). Mouth and gonopore located in the posterior third of the body at a distance of 65–66% and 81% from the anterior tip respectively (Table 1).

3.1.1.1. Internal morphology. Cephalic region. Sensory pits (30 µm deep) are present at the anterior tip and latero-ventral epidermis in a single irregular row (Fig. 4A). They extend up to 12–13% of the body length. Three types of secretory cells discharge through the dorsal epidermis, which is densely ciliated: rhabditogen cells with xanthophil secretion, abundant cells with fine granular erythrophil secretion and abundant cells with cyanophil secretion (Fig. 4A). The ventral epidermis, ciliated on the creeping sole, is provided with small rhabdites in the apex of epidermal cells, and receives abundant secretion (Fig. 4A). The glandular margin consists of abundant cells with fine granular cyanophil secretion (Fig. 4A). The glandular margin consists of abundant cells with fine granular cyanophil secretion, and scarce cyanophil granules (Fig. 4A). There are no musculo-glandular specializations.

Pre-pharyngeal region. Dorsal epidermis and ventral epidermis receiving the same secretory cells as the cephalic region of the body (Fig. 4B–D). The glandular margin is composed of abundant cells with fine granular erythrophil secretion, less abundant cells with fine granular xanthophil secretion, and scarce cyanophil granules (Fig. 4B, E). The creeping sole occupies the entire body width. The cutaneous musculature shows the typical arrangement of the



Fig. 3. Schematic drawing, in dorsal view, of the eye pattern of the holotype of *Obama maculatentis* sp. nov. The position of the mouth and gonopore is provided as reference. Scale bar: 5 mm.

Table 1

Measurements (mm) of the sectioned specimens of Obama maculatentis sp. nov. and thickness of the cutaneous musculature (CM) (μ m). All the measurements were obtained from fixed specimens.

	Holotype	Paratype
Body length	47.5	43.1
Maximum width	5.4	5.4
Height	1.5	1.5
DM	30.9	28.4
DM (%)	65%	66%
DG	38.4	34.8
DG (%)	81%	81%
DMG	7.5	6.4
CM dorsal		
circular	2.5	2.5
diagonal	5	5
longitudinal	25	30
CM ventral		
circular	2.5	2.5
diagonal	5	7.5
longitudinal	30	30

DG, distance of gonopore from anterior end; DM, distance of mouth from anterior end; DMG, distance between mouth and gonopore. Numbers in percentages represent position relative to the body length.

subfamily Geoplaninae, with nearly the same thickness both dorsally and ventrally (Table 1). Cutaneous muscular index (CMI): 5%. The parenchymatic musculature is not well organized and is composed of loose fibres. Below the cutaneous muscle layer there are isolated oblique muscle fibres, followed by supra-intestinal and sub-intestinal transverse fibres (Fig. 4B). Additionally, dorsoventral fibres are present among intestinal branches.

Pharyngeal region. Pharynx cylindrical, 3.2–3.6 mm in length (7–8% of the body length), with the dorsal insertion posteriorly displaced (~1 mm) from the ventral insertion (Fig. 5A). Mouth located in the second third of the pharyngeal pouch (5.4–5.6 mm in length).

The pharynx is covered by ciliated cuboidal epithelium, followed by a longitudinal, sub-epithelial muscle layer $(2.5-5 \,\mu m \, thick)$ and a subjacent circular layer $(5 \,\mu m \, thick)$ (Fig. 5B). The lining



Fig. 2. Details of the dorsal surface of the body of *Obama maculatentis* sp. nov. at different levels, showing the eye pattern (paratype). (A) Anterior end. (B) At 1/6th of the body length. (C) At 1/3rd of the body length.



Fig. 4. Transverse sections at the cephalic and pre-pharyngeal body regions of *Obama maculatentis* sp. nov. **(A)** Cephalic region (holotype). **(B)** Pre-pharyngeal region (paratype). **(C–E)** Details of transverse sections of the pre-pharyngeal region (paratype): (C) dorsal to the intestine and (D) ventral to the intestine, and (E) body margin. Scale bars: (A) 200 μm, (B) 500 μm, (C–E), 200 μm. Abbreviations: de, dorsal epidermis; dv, dorso-ventral parenchymatic muscles; e, eye; gm, glandular margin; i, intestine; lc, longitudinal cutaneous muscle layer; n, nerve plate; od, ovovitelline duct; rh, rhabditogen glands; sd, sperm duct; sp, sensory pit; t, testis; ve, ventral epidermis; vt, vitellaria.



Fig. 5. Sagittal sections of the pharynx of *Obama maculatentis* sp. nov. (paratype). **(A)** At the level of the mouth. **(B)** Detail at the level of the dorsal insertion of the pharynx. **(C)** Detail of communication of the pharyngeal lumen with the intestine. Scale bars: (A) 500 μm, (B) 100 μm, (C) 200 μm. Abbreviations: di, dorsal insertion of the pharynx; i, intestine; mo, mouth; oe, oesophagus; pg, pharyngeal glands; ph, pharyngeal lumen; pp, pharyngeal pouch; t, testis; vi, ventral insertion of the pharynx; vt, vitellaria.



Fig. 6. Schematic drawings from sagittal sections of the copulatory apparatus of *Obama maculatentis* sp. nov. (**A**) Holotype. (**B**) Paratype. Arrows indicate the paired portion of the prostatic vesicle. Scale bar: 1 mm. Abbreviations: co, common glandular ovovitelline duct; ej, ejaculatory duct; fa, female atrium; fc, female genital canal; go, gonopore; ma, male atrium; od, ovovitelline duct; p, penis papilla; pb, penis bulb; pv, prostatic vesicle; sd, sperm duct; sg, shell glands.

of the pharyngeal lumen comprises a ciliated columnar epithelium underlain by a thick layer of circular muscle fibres interspersed with some longitudinal fibres, being thicker ventrally than dorsally (150 and 75–100 μ m thick, respectively) (Fig. 5B, C). Two types of secretory cells discharge through the pharyngeal epithelium: abundant cells with xanthophil fine granular secretion and less abundant cells with cyanophil fine granules (Fig. 5B). Cell bodies of these pharyngeal glands are located in the surrounding parenchyma, anterior to the pharynx. The short oesophagus (~100 μ m in length) is lined by a ciliated columnar epithelium, underlain by the same muscle layer as the pharyngeal lumen (Fig. 5C).

Male reproductive system. Mature testes dorsal, arranged in two irregular rows on either side of the body, located below or among the supra-intestinal parenchymatic muscle fibres (Fig. 4B, C). The testes extend caudally from 7 to 8 mm behind the anterior tip to near the pharyngeal insertions and are anterior to the ovaries (Figs. 5A and 8F, Table 2). Sperm ducts, running dorsally and laterally to the ovovitelline ducts, located among the sub-intestinal parenchymatic muscle fibres (Fig. 4B, D). Laterally and posterior to the pharynx, the lumens of the sperm ducts are dilated and full of spermatozoa (Figs. 6A, B and 8A). Near the penis bulb, the sperm ducts gradually curve medially to open into the paired portion of the prostatic vesicle (Figs. 6A, B and 8A). The extrabulbar prostatic vesicle consists of a proximal, paired portion and a distal unpaired section, both being tubular-shaped (Fig. 6A, B). The paired portion consists of two short tubular 'arms' (~100 μ m in length), which converge in the median plane to communicate with the unpaired C-shaped portion (Figs. 6A, B. 7A, B and 8B). The latter traverses the penis bulb and continues as an ejaculatory duct (Fig. 6A, B). In the holotype, the ejaculatory duct is sinuous, obliquely traversing the penis papilla, and opening into



Fig. 7. Sagittal sections of the copulatory apparatus of *Obama maculatentis* sp. nov. **(A)** Holotype. **(B)** Paratype. Arrows indicate the cyanophil secretion of the male atrium. Arrowheads show the erythrophil secretion near the insertions of the penis papilla. Scale bar: 500 μm. Abbreviations: co, common glandular ovovitelline duct; ej, ejaculatory duct; fa, female atrium; fc, female genital canal; go, gonopore; ma, male atrium; p, penis papilla; pb, penis bulb; pv, prostatic vesicle; sg, shell glands; vt, vitellaria.



Fig. 8. Sagittal sections of the reproductive system of *Obama maculatentis* sp. nov. (holotype). (**A**) Distal portion of the sperm duct and proximal portion of the prostatic vesicle. (**B**) Unpaired portion of the prostatic vesicle. (**C**) Detail of the male copulatory system. (**D**) Male copulatory system at mid-sagittal section. (**E**) Male copulatory system at para-sagittal section. (**F**) Anterior-most testes and ovary. (**G**) Detail of the copulatory system, showing the transition between male and female atria. (**H**) Detail of the female reproductive system. (**I**) Detail of the female atria. (**H**) Detail of the female atria (**H**) Detail of the female atria. (**H**) Detail of the female atria (**H**) Detail of the female atria. (**H**) Detail of the female atria (**H**) Detail of the female atria (**H**) Detail of the female atria. (**H**) Detail of the female atria (**H**) Detail of the fe

the ventral-right side of the papilla, not at its apex (Figs. 6A and 8D). In the paratype, the ejaculatory duct traverses the papilla somewhat obliquely, following a smooth curve and ending at the tip of the papilla (Fig. 6B). The penis is the protrusible type, with the penis papilla being a massive expansion of the antero-dorsal wall of the male atrium, with its dorsal insertion posteriorly displaced, but not reaching the level of the gonopore (Figs. 6A, B and 7A, B). In the holotype, the male atrium, entirely occupied by the penis papilla, shows some folds in the proximal portion of its ventral wall, which are absent in the paratype (Figs. 6A, B and 7A, B). Dorsally to the gonopore, a large fold separates the male atrium from the female one (Figs. 6A, B and 7A, B).

Sperm ducts lined with ciliated, cuboidal epithelium (Fig. 4D) and surrounded by circular muscle fibres ($2.5 \,\mu$ m thick). Only distally, before opening in the prostatic vesicle, the epithelial lining of the sperm ducts becomes columnar (Fig. 8A). Prostatic vesicle lined with a columnar, ciliated epithelium, and traversed by abundant fine granular erythrophil secretion and scarce fine cyanophil granules (Fig. 8A, B). Muscle coat of the prostatic vesicle mainly consisting of circular fibres and some longitudinal and oblique intermingled fibres. This muscle coat is thicker on the unpaired portion ($40-50 \,\mu$ m thick) than on the forked portion ($15-20 \,\mu$ m thick) and the intrabulbar section ($\sim 20 \,\mu$ m thick). The ejaculatory duct is lined by a ciliated cuboidal epithelium that

Table 2

Measurements (mm) of the reproductive system of sectioned specimens of *Obama* maculatentis sp. nov.

	Holotype	Paratype
Anterior-most testes	8 (17%)	7 (16%)
Posterior-most testes	26.4 (56%)	24.6 (57%)
LMA	2.3	2.1
Location of ovaries	10.5 (22%)	9.2 (21%)
LCGD	0.25	0.25
LFC	0.25	0.25
LFA	1.2	1

LCGD, length of common glandular ovovitelline duct; LFA, length of female atrium; LFC, length of female canal; LMA, length of male atrium. Numbers in percentages represent position relative to the body length.

receives a fine granular erythrophil secretion, and is underlain by a thin circular muscle coat $(5-10 \,\mu\text{m}$ thick) (Fig. 8C). The penis papilla is lined with columnar epithelium (25 µm in height), with a subjacent muscle coat formed by circular fibres and some oblique intermingled ones, thicker at the level of the insertion of the penis $(20 \,\mu\text{m} \text{ thick})$ and thinner towards its apex $(10 \,\mu\text{m} \text{ thick})$ (Figs. 7A, B and 8C). The parenchyma of the penis papilla is traversed by variously oriented muscle fibres and by abundant glands discharging fine granular erythrophil secretion; the latter is discharged through its epithelium, accumulating at the apex of the cells (Fig. 8C). The cell necks of the erythrophil glands are more densely concentrated near the insertion of the papilla (Fig. 7A, B). Further, scarce cyanophil granules are discharged through the epithelium of the penis papilla. The cell bodies of these cyanophil penis glands are located outside the penis bulb, in the surrounding parenchyma. The male atrium is lined by a high columnar epithelium (30 µm in height dorsally, $15-25 \mu m$ in height ventrally), followed by a muscle coat with mainly circular fibres and some intermingled longitudinal ones (10–15 µm thick). The atrium receives a great amount of fine granular erythrophil secretion, and also abundant fine granular cyanophil secretion. The cell necks of the cyanophil glands accumulate in the ventral wall of the male atrium, discharging their secretion not only through the ventral wall of the atrium (Figs. 7A, B and 8C, D) but also through their latero-ventral walls (Fig. 8E).

Female reproductive system. Ovaries drop-shaped, ovoid proximally and elongated and thin distally (0.9 mm in length in longitudinal body axis in the holotype and 0.55 mm in length in the paratype) (Fig. 8F). They are located just behind the anterior-most testes, between the sub-intestinal parenchymatic muscle fibres and the nerve plate. The ovovitelline ducts arise from the first third of the dorsal side of the ovaries, containing spermatozoa in their anterior portions, both in the holotype and in the paratype (Fig. 8F). Posteriorly, the ovovitelline ducts run just above the nerve plate (Fig. 4B, D), receiving abundant secretion from the vitelline glands along their course. Vitellaria are located among the intestinal branches (Figs. 4B–D and 8F). Approximately at the level of the gonopore, the ovovitelline ducts progressively ascend and turn medial (Figs. 6A, B and 8E). Once above the female atrium, they join to form a short common glandular ovovitelline duct, which runs backwards to open into the female genital canal (Figs. 6A, B, 7A, B and 8H). This canal is ventro-anteriorly flexed and communicates with the female atrium (Figs. 6A, B, 7A, B and 8H). The female atrium, whose length is nearly half of that of the male atrium (Table 2), has a very narrow lumen $(50-100 \,\mu\text{m})$ as compared with the male atrium (Fig. 7A, B).

The ovovitelline ducts are lined with a ciliated, cuboidal epithelium and surrounded by circular muscle fibres (2.5 μ m thick) (Fig. 4D). The ascending portions of the ovovitelline ducts are lined with ciliated columnar epithelium and receive the secretion of the

shell glands (Fig. 8E). The lining epithelium of the common ovovitelline duct, ciliated and columnar, receives abundant erythrophil secretion from shell glands, whose cell bodies are located behind the female atrium (Figs. 7A, B and 8H). The common ovovitelline duct is enveloped by circular muscle fibres (10 µm thick). The female genital canal is lined with a tall non-ciliated stratified epithelium (55–65 µm in height), which receives a fine granular ervthrophil secretion (Fig. 8H), and is surrounded by circular muscle fibres (10–15 μ m thick). Only the ental portion of the female canal is lined with ciliated columnar epithelium. The female atrium is lined with a tall, non-ciliated stratified epithelium, with stratified appearance (100–160 µm in height) (Figs. 7A, B and 8G, I). The muscle coat of the female atrium consists of circular fibres interspersed with some oblique fibres (10–25 μ m thick). An abundant fine granular erythrophil secretion is discharged through the epithelium of the female atrium, which is therefore strongly erythrophil (Fig. 8G, I). The gonoduct is almost straight and lined with a ciliated columnar epithelium, and receives a fine granular erythrophil secretion, which concentrates in the apex of the epithelial cells as well as a much less abundant fine cyanophil secretion (Fig. 7A, B).

3.1.2. Discussion

The characteristics of the new species match the diagnosis of the genus *Obama*. This genus comprises Neotropical land planarians with the following features: body leaf-shaped, monolobated and trilobated eyes spreading onto the dorsum; pharynx cylindrical; prostatic vesicle extrabulbar and proximally forked; protrusible penis papilla, occupying the entire male atrium; common ovovitelline duct dorsal to the female atrium and communicating with the latter through a dorso-anteriorly flexed female canal (Carbayo et al. 2013).

Regarding its external appearance, mainly body shape, size, and dorsal colour pattern, Obama maculatentis sp. nov. shows certain similarities with Obama anthropophila Amaral et al., 2015 (in Alvarez-Presas et al. 2015), Obama decidualis Amaral & Leal-Zanchet, 2015 (in Alvarez-Presas et al. 2015), Obama nungara Carbayo et al., 2016, and Obama otavioi Carbayo, 2016 (in Carbayo et al. 2016a). The new species differs from O. otavioi because this species reaches a length of about 100 mm while its dorsum exhibits tiny blackish spots on a greenish or brownish beige background, which give it a dark aspect (Carbayo et al. 2016a). In contrast, in O. maculatentis sp. nov., the black olive pigmentation is almost homogeneous on the dorsum, excepting the cephalic region, where it is slightly paler. Otherwise, O. maculatentis sp. nov. might be confused at first sight with O. anthropophila or O. decidualis, because these species have a dark brown dorsum. Obama maculatentis sp. nov. might even be mistaken for specimens of O. nungara, at least with those with very dark, almost black, pigmentation on the dorsum (Alvarez-Presas et al. 2015; Carbayo et al. 2016b). However, none of these species have the pattern of blackish spots arranged in two irregular rows at the level of the testes as present in O. maculatentis sp. nov. This arrangement of specks has been observed only in specimens of O. nungara, which show a honey-yellow background, and in the green land planarian Obama ladislavii (Graff, 1899). It is worth mentioning that Obama maculatentis sp. nov. was found in sympatry with O. nungara in the prospected areas, and that in the field both were easily distinguishable from each other.

Details of its internal anatomy, however, amply differentiate the new species from *O. anthropophila*, *O. decidualis*, and *O. nungara*. *Obama marmorata* (Schultze & Müller, 1857), *O. otavioi*, and *Obama josefi* (Carbayo & Leal-Zanchet, 2001) will also be discussed because their internal morphology is rather similar to that of the new species. With respect to their copulatory apparatus, these six species are very similar and only histological details allow differentiation between them and the new species. Based on the general aspect of the penis papilla, we may arrange these Obama species into two groups. One group is formed by O. marmorata and O. otavioi, which have a massive penis papilla hanging from the 'roof' of the male atrium, with the dorsal insertion posteriorly displaced and located at the level of gonopore or even behind it. The other group comprises O. anthropophila, O. decidualis, O. josefi and *O. nungara*, in which the penis papilla is an oblique expansion of the male atrium, with its dorsal insertion posteriorly displaced but never reaching the level of the gonopore (Carbayo & Leal-Zanchet 2001; Álvarez-Presas et al. 2015; Carbayo et al. 2016a; 2016b; Lago-Barcia et al. 2018). In this regard, Obama maculatentis sp. nov. belongs to the second group. In the species of the first group, the length of the penis papilla corresponds to 75-80% of the total length of the copulatory apparatus (measured as the total length of the male atrium plus the female one) (Carbayo et al. 2016a; 2016b), whereas in the species of the second group, the penis papilla occupies about 45-50% of the total length of the copulatory apparatus (Álvarez-Presas et al. 2015; Carbayo & Leal-Zanchet 2001; Carbayo et al. 2016b; Lago-Barcia et al. 2018). In specimens of O. maculatentis sp. nov., the penis papilla ranges between 50 and 55% of the length of the copulatory apparatus, which implies that the relative size of the penis in the new species is similar to that of the second group.

Another remarkable feature in all these species of Obama is the presence of numerous glands producing a granular secretion that is concentrated in discrete bundles and is discharged through the ventral wall of the male atrium. This secretion has been described as having different affinities, namely, (a) cvanophilic, (b) ervthrophilic, and (c) mixed, with an erythrophilic 'central core' and a cyanophilic peripheral portion (Álvarez-Presas et al. 2015; Carbayo et al. 2016a; 2016b; Lago-Barcia et al. 2018). In O. nungara, this type of secretion also pierces the surface of the large fold that faces the penis and part of the left side of the male atrium, giving it the aspect of a secretory ring (Carbayo et al. 2016b; Lago-Barcia et al. 2018). In the other species, including O. maculatentis sp. nov., this secretion is not only discharged through the ventral wall of the atrium but also through the lateral walls. However, O. maculatentis sp. nov. differs from these species by showing a lobulated-like fold in the dorsal wall of the male atrium, leaving a narrow passage to the female atrium. Furthermore, O. nungara and O. otavioi are provided with an atrial fold that narrows the communication between both atria (Carbayo et al. 2016a; 2016b; Lago-Barcia et al. 2018). However, in these two species, the sheath-like fold arises from the ventral side of the atrium and extends to the dorsal wall, at the level of the gonopore, whereas in the new species, this expansion originates from the dorsal wall of the male atrium.

Regarding the female reproductive system, the new species and all six of the above-mentioned species of *Obama* are extremely similar, showing a female atrium with folded walls and narrow lumen, lined with a tall stratified columnar epithelium. Nevertheless, the ovaries of *O. maculatentis* sp. nov. are drop-shaped, whereas in the remaining species the ovaries are ovoid or ovalelongate in shape.

The morphological similarity of the six *Obama* species above discussed has been also revealed in several molecular analyses (Álvarez-Presas et al. 2015; Carbayo et al. 2016a; 2016b; Lago-Barcia et al. 2018). In this way, taking into account the anatomical similarity of *O. maculatentis* sp. nov. with these six species, their close evolutionary relationship is expected. This hypothesis may be corroborated by further research.

Family Geoplanidae Stimpson, 1857 Subfamily Geoplaninae Stimpson, 1857 Genus Notogynaphallia Ogren & Kawakatsu, 1990

3.2. Notogynaphallia fortuita sp. nov.

Material examined. Holotype: MLP He-6635–2, Isla Paulino, Berisso, Buenos Aires province (Argentina) (34°49′48′′S, 57°52′31′′W), 6th November 2012, L. Negrete coll.; cephalic region and anterior region at the level of the ovaries: transverse sections on 8 slides; rest of anterior region and pre-pharyngeal region: transverse sections on 14 slides; pharynx: sagittal sections on 8 slides; and copulatory apparatus: sagittal sections on 8 slides.

Diagnosis. Species of *Notogynaphallia* of about 30 mm in length when fully extended. Dorsum with beige ground pigmentation with tiny darker spots; cephalic region saffron-yellow; ventral surface light grey. Parenchymatic musculature not organized in discrete layers. Anterior-most testes very near the anterior end of the body. Distal portion of sperm ducts traversing the common muscle coat, curving antero-medially and, subsequently, joining to form a short common sperm duct.

Type locality. Isla Paulino, Buenos Aires province, Argentina.

Habitat. The specimen was found on the ground under a fallen log, in a forest with both native and introduced vegetation.

Etymology. The specific name derives from the Latin adjective *fortuitus* (which means casual, by chance), because only a single specimen was found after the area had been prospected on several occasions.

3.2.1. Description

External morphology. Body-shape between elongate and lanceolate, with nearly parallel margins, gradually tapering towards the anterior tip and more abruptly towards the posterior end, both extremities being blunt (Fig. 9). Dorsal surface with a beige ground pigmentation, with scattered tiny darker spots. This speckled pattern is less dense along a thin midline of the body, thus forming a pale stripe, poorly defined. Cephalic region saffron-yellow (Fig. 9); ventral surface is light grey. The monolobated eyes, with small clear halos, extend throughout the whole body length. They are uniserial around the anterior tip and the first two millimetres, extending onto the body margins (Fig. 10A). The eyes continue along the body margins, forming two or three irregular rows. At four millimetres from the anterior tip (20% of the body length), they spread onto the dorsal surface of the body (Fig. 10B). At the pharyngeal level, eyes are scarcer and become progressively less numerous towards the posterior end of the body, being restricted again on the body margins. The holotype reached ~30 mm in length in maximum extension (Fig. 9), and, after fixation, the body length was 19.4 mm and the maximum width 3.1 mm; body height 1.1 mm. Mouth and gonopore located at a distance of 48% and 70% from the anterior tip, respectively.

3.2.1.1. Internal morphology. Cephalic region. Sensory pits $(25 \,\mu\text{m} \text{deep})$ contour the anterior tip and extend posteriorly up to 1.8 mm (9% of the body length) from the anterior end of the body, being located in the latero-ventral epidermis in a single, irregular row



Fig. 9. External aspect, in dorsal view, of *Notogynaphallia fortuita* sp. nov. Scale bar: 5 mm.



Fig. 10. Details of the eye pattern at different levels of the body of Notogynaphallia fortuita sp. nov. (A) Anterior end. (B) At 1/5th of the body length.

(Fig. 11A, B). The dorsal epidermis, densely ciliated, receives secretion from three types of gland cells: (a) abundant rhabditogen cells (with xanthophil secretion), (b) scarce cells with fine granular erythrophil secretion, and (c) less abundant cells with cyanophil amorphous secretion (Fig. 11A, B). The ventral epidermis, ciliated on the creeping sole (~100% of the body width), is provided with small rhabdites in the apex of the epidermal cells, and also receives

abundant secretion from cells producing fine erythrophil granules and a less abundant amorphous cyanophil secretion. The glandular margin consists of abundant cells with fine granular erythrophil secretion, and scarce cells with an amorphous cyanophil secretion (Fig. 11B). There are no musculo-glandular specializations.

Pre-pharyngeal region. Dorsal epidermis and ventral epidermis receive the same secretions as in the cephalic region of the body



Fig. 11. Transverse sections at the cephalic and pre-pharyngeal body regions of *Notogynaphallia fortuita* sp. nov. **(A)** Cephalic region. **(B)** Anterior region at the level of the anteriormost testes. **(C)** Pre-pharyngeal region. **(D–F)** Details of transverse sections of the pre-pharyngeal region: (D) body margin, (E) dorsal to the intestine, and (F) ventral to the intestine. Scale bars: (A) 200 μm, (B, D–F) 100 μm, (C) 500 μm. Abbreviations: de, dorsal epidermis; e, eye; gm, glandular margin; i, intestine; lc, longitudinal cutaneous muscle layer; n, nerve plate; od, ovovitelline duct; rh, rhabditogen glands; sd, sperm duct; sp, sensory pit; t, testis; ve, ventral epidermis; vt, vitellaria.

(Fig. 11D–F). However, in the creeping sole, the amorphous cyanophil secretion is more abundant than in the cephalic region, while it is concentrated towards the body margins. The glandular margin, less defined than the cephalic region, shows some fine erythrophil granules and a scarce amorphous cyanophil secretion (Fig. 11C, D). The cutaneous musculature, with the typical Geoplaninae organization in three muscle layers, shows the same thickness dorsally and ventrally and is composed of the following layers: a circular sub-epithelial layer (2.5 µm thick), a diagonal intermediate layer (5 µm thick), and an inner longitudinal layer arranged into discrete bundles (20 µm thick). Cutaneous muscular index (CMI): 5%. Parenchymatic musculature composed of loose fibres without forming discrete layers (Fig. 11C). Beneath the cutaneous muscle layer, there are isolated decussate fibres, followed by supra-intestinal and sub-intestinal transverse fibres. There are also some dorso-ventral muscle fibres running between the intestinal branches.

Pharyngeal region. The cylindrical pharynx (2 mm in length, 10% of the body length) almost fully occupies the pharyngeal pouch (2.4 mm in length) (Fig. 12). Dorsal insertion of the pharynx slightly posteriorly displaced (350 µm) as compared with the ventral insertion, while the mouth is located in the second third of the pharyngeal pouch. The outer pharyngeal epithelium is cuboidal and ciliated and underlain by a thin longitudinal sub-epithelial muscle layer (5 μ m thick), followed by a circular muscle layer (10 μ m thick). Pharyngeal lumen lined by a ciliated and columnar epithelium, followed by a thick muscle layer consisting of circular fibres with some longitudinal interspersed fibres (50–75 μ m thick). Three types of secretory cells discharge through the pharyngeal epithelium: (a) abundant cells producing a xanthophil fine granular secretion, (b) less abundant cells with cyanophil fine granules, and (c) scarce cells with amorphous cyanophil secretion. The cell bodies of these pharyngeal glands are located in the surrounding parenchyma, anterior and laterally to the pharynx. The short oesophagus (~200 µm in length) is lined by a ciliated columnar epithelium and enveloped by the same muscle layers as the pharyngeal lumen.

Male reproductive system. Mature testes, arranged in three irregular rows on either side of the body, are located among the dorsal decussate parenchymatic muscle fibres, in close proximity of the longitudinal cutaneous muscle layer and between the intestinal branches (Fig. 11B, C, E). The anterior-most testes are located in the cephalic region (0.3 mm behind the anterior tip, i.e. at 1.5% of the body length), where they sit among the intestinal branches (Fig. 11B), and extend to near the root of the pharynx (43% of the



Fig. 12. Sagittal section of the pharynx of *Notogynaphallia fortuita* sp. nov. Scale bar: 200 µm. Abbreviations: di, dorsal insertion of the pharynx; i, intestine; mo, mouth; ph, pharynx; pp, pharyngeal pouch; vi, ventral insertion of the pharynx.

body length). The sperm ducts run dorso-medially to the ovovitelline ducts (Fig. 11F). At the level of the pharynx, the sperm ducts are dilated and form spermiducal vesicles that are full of spermatozoa (Fig. 14). At the level of the copulatory apparatus, the sperm ducts traverse the anterior. latero-ventral wall of the common muscle coat, subsequently curving medially and anteriorly. Then, the sperm ducts join to form a short common sperm duct (~100 um in length) just before the latter opens ventrally into the prostatic vesicle (Figs. 13 and 15A). The prostatic vesicle presents two intrabulbar portions: (1) a proximal, tubular, sinuous portion (Figs. 13 and 15A, B) and (2) a distal, ovoid portion with folded walls, which greatly restricts its lumen (Figs 13, 14, 15B, C and 16A–C). There is no ejaculatory duct, so that the distal portion of the prostatic vesicle communicates directly with the male atrium by means of small spaces in their folded walls (Figs 13, 14, 15B, C and 16A–C) that act to form an eversible penis. The lumen of the male atrium is narrow because its walls, mainly the ventral and lateral ones, are richly folded (Figs. 13 and 14). The male atrium (1.8 mm in length) is approximately two and a half times longer than the female atrium. The connection between the male and female atria is restricted by a large fold, dorsally to the gonopore, and a distal, ventral fold of the male atrium that extends backwards (Figs. 13 and 14).

The sperm ducts are lined with a ciliated, cuboidal epithelium. Muscle fibres enveloping the sperm ducts were not observed, excepting in the distal intrabulbar portions, where they are lined with a squamous epithelium and are surrounded by a longitudinal muscle layer (5 µm thick) (Fig. 15A). The epithelium of the short common sperm duct becomes columnar and ciliated (Fig. 15A). The two portions of the prostatic vesicle are lined with a columnar and ciliated epithelium, covered by longitudinal fibres, intermingled with obliquely running fibres $(5-10 \,\mu m \text{ thick})$. Two types of secretory cells discharge through both portions of the prostatic vesicle: (a) cells producing a fine granular xanthophil secretion, particularly abundant in the distal portion of the vesicle, and (b) scarce cells producing a fine granular erythrophil secretion (Figs. 14, 15A–C and 16A–C). The lining epithelium of the male atrium is columnar and non-ciliated (Fig. 15D) and is strongly erythrophil from the abundant fine granular secretion (Figs. 14 and 15C, D). The male atrium is covered by a muscle coat with circular and oblique fibres, thicker proximally $(25-30 \,\mu\text{m}$ thick) than distally $(10-15 \,\mu\text{m})$ thick). The common muscle coat consists of longitudinal, circular and intermingled obliquely oriented fibres (~20 µm thick).

Female reproductive system. The oval-elongated ovaries (0.5 mm in length and 0.2 mm in diameter) are located between the intestinal branches and the nerve plate (Fig. 17A). The ovovitelline ducts arise from the mid-dorsal side of the ovaries (Fig. 17A). The ovovitelline ducts run posteriad, dorsally to the nerve plate (Fig. 11C, F). Vitellaria are located among the intestinal branches (Figs. 11C and 17A, B). Behind the gonopore, the lateral ovovitelline ducts turn dorso-mediad (Fig. 17C) and join to form short common duct (0.1 mm in length) (Figs. 13 and 17E). This common duct is almost vertical and opens into the female genital canal (0.25 mm in length), which curves anteriad and then opens into the female atrium (Fig. 17E). The latter (0.7 mm in length) is provided with folded walls, thus leaving only a very narrow lumen (~100 μ m) (Figs. 13 and 17F).

The ovovitelline ducts are lined with a ciliated cuboidal epithelium, and surrounded by a circular muscle coat (5 μ m thick) (Figs. 11F and 17B). The ascending portions of the ovovitelline ducts are lined by a ciliated columnar epithelium (Fig. 17C), while the distal portions are devoid of cilia at the point where they start receiving the secretion from the shell glands (Fig. 17D). The common ovovitelline duct is lined with a columnar and non-ciliated epithelium that is pierced by the fine, abundant xanthophil



Fig. 13. Schematic drawing from sagittal sections of the copulatory apparatus of *Notogynaphallia fortuita* sp. nov. Scale bar: 500 μm. Abbreviations: cd, common sperm duct; cm, common muscle coat; co, common glandular ovovitelline duct; fa, female atrium; fc, female genital canal; go, gonopore; ma, male atrium; od, ovovitelline duct; pv1, proximal prostatic vesicle; pv2, distal prostatic vesicle; sd, sperm duct; sg, shell glands.



Fig. 14. Sagittal section of the copulatory apparatus of *Notogynaphallia fortuita* sp. nov. Scale bar: 500 µm. Abbreviations: cm, common muscle coat; co, common glandular ovovitelline duct; fa, female atrium; fc, female genital canal; go, gonopore; ma, male atrium; pv2, distal prostatic vesicle; sd, sperm duct; sg, shell glands.



Fig. 15. Sagittal sections of the male reproductive system of *Notogynaphallia fortuita* sp. nov. (**A**) Proximal portion of the prostatic vesicle. (**B**) Proximal and distal portions of the prostatic vesicle. (**C**) Detail of the communication of the distal prostatic vesicle with the male atrium. (**D**) Detail of the male atrium. Scale bars: (A, C, D) 50 μm, (B) 200 μm. Abbreviations: cd, common sperm duct; ma, male atrium; pv1, proximal prostatic vesicle; pv2, distal prostatic vesicle; sd, sperm duct.



Fig. 16. Series of sagittal sections of the copulatory apparatus of *Notogynaphallia fortuita* sp. nov., from the left (A) to the right side (C) of the body, showing the prostatic vesicle and the proximal region of the male atrium. Scale bar: 200 µm. Abbreviations: ma, male atrium; pv1, proximal prostatic vesicle; pv2, distal prostatic vesicle; sd, sperm duct.



Fig. 17. Transverse (A) and sagittal sections (B–F) of the female reproductive system of *Notogynaphallia fortuita* sp. nov. (A) Ovary. (B–D) Different sections of the ovovitelline ducts: (B) laterally to the pharynx, (C) ascending portion, laterally to the female atrium, and (D) distal portion, just before opening in the common ovovitelline duct. (E) Common ovovitelline duct, female genital canal, and opening into the female atrium. (F) Female atrium at the level of the gonopore. Scale bars: (A, C, D) 100 µm, (B, E) 50 µm, (F) 200 µm. Abbreviations: co, common glandular ovovitelline duct; cs, creeping sole; fa, female atrium; fc, female genital canal; go, gonopore; i, intestine; n, nerve plate; od, ovovitelline duct; ov, ovary; sg, shell glands; vt, vitellaria.

secretion of shell glands (Fig. 17E), and surrounded by a muscle coat consisting of circular fibres and some intermingled longitudinal ones (5 μ m thick). The female canal is lined by a non-ciliated columnar epithelium that receives some fine erythrophil granules, and is underlain by the same muscle coat as the common duct but slightly thicker (~10 μ m thick) (Fig. 17E). The female atrium is lined with a non-ciliated, stratified columnar epithelium (Fig. 17E, F), underlain by a musculature comprising circular fibres intermingled with some longitudinal ones (10–15 μ m thick). A finely erythrophil secretion is discharged through the epithelium of the female atrium (Fig. 17E, F). The gonoduct, vertically oriented, is lined with a columnar epithelium that is pierced by finely granular erythrophil secretion, as well as by a less abundant finely granular cyanophil secretion.

3.2.2. Discussion

The new species shows features that agree with the diagnosis of the genus *Notogynaphallia*, whose main characteristics are: body margins nearly parallel; eyes monolobated; pharynx cylindrical; intrabulbar prostatic vesicle broadly communicating with the male atrium; ejaculatory duct absent; male atrium with folded walls, functioning as an eversible penis; ascending distal portion of the ovovitelline ducts lateral to the posterior region of the female atrium; common ovovitelline duct located behind the female atrium; female genital canal dorso-anteriorly flexed (Ogren & Kawakatsu 1990; Carbayo et al. 2013).

Regarding the dorsal pigmentation, Notogynaphallia fortuita sp. nov., which presents a homogeneously pigmented dorsal surface, is easily distinguishable from species with a striped pattern, such as Notogynaphallia biseminalis (Riester, 1938), Notogynaphallia froehlichae Ogren & Kawakatsu, 1990, Notogynaphallia mourei (Froehlich, 1956), Notogynaphallia parca (Froehlich E.M., 1955), and Notogynaphallia sexstriata (Graff, 1899). Notogynaphallia fortuita sp. nov. also differs from Notogynaphallia plumbea (Froehlich, 1956) and Notogynaphallia nawei Negrete et al., 2015 because these last two species exhibit a homogeneous dark grey and black dorsum, respectively. The new species resembles Notogynaphallia modesta (Graff, 1899), which shows a lanceolate body with dark brown dorsum, paler in the sagittal region, thus forming a fine median stripe (Carbayo 2003). Regarding the species of Notogynaphallia incertae sedis (Carbayo et al. 2013), N. fortuita sp. nov. can also be distinguished from the striped species Notogynaphallia andina (Hyman, 1962), Notogynaphallia garua (du Bois-Reymond Marcus, 1951), and Notogynaphallia quinquestriata (Hyman, 1962), as well as from Notogynaphallia atra (Schultze & Müller, 1857), the latter having a homogeneous black dorsal colour.

Regarding its internal anatomy, the general configuration of the copulatory apparatus of *N. fortuita* sp. nov. resembles that of N. nawei and N. modesta, which are the only Notogynaphallia species described from outside of Brazil, being recorded in Argentina and Paraguay, respectively (Carbayo 2003; Negrete et al. 2015). The three species share the shape of the prostatic vesicle, being tubular and sinuous anteriorly, and posteriorly with an ample cavity with richly folded walls. However, the way in which the sperm ducts communicate with the tubular portion of the prostatic vesicle varies between these species. In N. fortuita sp. nov. and N. modesta, the sperm ducts unite to form a very short common sperm duct just before opening into the prostatic vesicle. However, in *N. modesta*, the sperm ducts join at the point where they penetrate the ventral side of the penis bulb (Carbayo 2003), whereas in *N. fortuita* sp. nov. the sperm ducts first penetrate the ventro-lateral wall of the common muscle coat and only thereafter join to form the common duct. Apart from the presence of this common sperm duct, the course of the sperm ducts in *N. nawei* and *N. fortuita* sp. nov. is very similar. In these two species, the morphology of the male atrium is also very similar, with its dorsal and ventral walls being richly folded, thus differing from that of *N. modesta*, in which the folds are almost restricted to the ventral wall (Carbayo 2003; Negrete et al. 2015). The female atrium of *N. fortuita* sp. nov. is an ample cavity, although its lateral walls are folded in such a way that the lumen actually is narrow. In contrast, the female atrium of N. modesta and N. nawei is not folded.

4. Final remarks

With respect to the biogeographic regionalisation of Argentina, the two new species herein described belong to the Pampean Province (Chacoan dominion). This biogeographic province covers central western Argentina (between 30° and 39°S), Uruguay, and the southern portion of the Brazilian state of Rio Grande do Sul (Morrone 2014). Nevertheless, the riparian forests of the Río de la Plata Basin, which is the second largest in South America after the Amazon Basin, function as corridors through which many faunal and floral components of the Paraná Province (= Interior Atlantic Forest) are able to extend into the Pampean Province. Thus, from a biogeographic point of view, the localities where O. maculatentis sp. nov. and *N. fortuita* sp. nov. were found are strategic because they represent the southernmost limit of this extended distribution of the Interior Atlantic Forest. These riparian forests are at serious risk because they are in close proximity of highly populated urban centres. They are also exposed to permanent discharge of industrial and urban sewage, which overflows to the forested areas during flooding of the rivers. Although this environment is highly sensitive to all of these anthropogenic disturbances, it still maintains a high biological diversity due to its protection status, which thus needs to be preserved.

Declarations of interest

None.

Funding

This study was partially financed by the Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET [grant number PIP 0635], the Agencia Nacional de Promoción Científica y Tecnológica, ANPCYT [grant number PICT 2016-3290], and Universidad Nacional de La Plata [grant numbers N886, PPID/N030], Argentina.

Acknowledgements

We thank the park managers of the natural reserve Reserva Natural Punta Lara (RNPL) for support during the fieldwork. We are also grateful to OPDS (Organismo Provincial para el Desarrollo Sostenible) of Buenos Aires province, Argentina, for permission to conduct sampling at RNPL. We thank the two anonymous reviewers whose comments and suggestions helped improve the early version of the manuscript.

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