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Two new species of *Notogynaphallia* Ogren & Kawakatsu (Platyhelminthes: Tricladida: Terricola) from Southern Brazil

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Abstract

Two new species of *Notogynaphallia* Ogren & Kawakatsu, 1990, from South Brazil, are described. *Notogynaphallia pseudoceciliae* sp. nov. and *Notogynaphallia arturi* sp. nov., present elongate bodies with parallel margins and the dorsum with five dark longitudinal stripes on a yellowish background. Additionally, *N. arturi* has two large lateral bands of brown pigment between the paramedian and lateral stripes. Both species, which are appended to a complex of striped species previously described for the genus *Notogynaphallia*, show a unique combination of characters of external and internal morphology.

Key words: Geoplaninae, striped species, taxonomy, morphology

Introduction

Land planarians are represented in South America mainly by the subfamily Geoplaninae, a relatively new group which has successfully spread throughout this region (Froehlich 1967; Winsor *et al.* 1998). Approximately 260 species have already been described in 16 genera in this subfamily (Ogren *et al.* 1997; Seitenfus & Leal-Zanchet 2004), some of these species having been included in a collective group for species inquirendae and nomina dubia (Ogren & Kawakatsu 1990). The highest species richness of land planarians world-wide is registered in the southern hemisphere (Winsor *et al.* 1998), in areas which were originally covered by the south-eastern Brazilian Atlantic Rain Forest (Sluys 1998; 1999; Fick *et al.* 2006), where taxonomical studies were concentrated from the 19th to the middle of the 20th centuries. Recent studies have indicated that the southern portions of this biome also shelter a high number of species (Carbayo *et al.* 2002; Fick *et al.* 2006). For the type-locality of the species herein described, the National Forest of São Francisco de Paula, located in South Brazil, forty species of land planarians have been registered (Leal-Zanchet & Carbayo 2000), six of them belonging to the genus *Notogynaphallia*. Three of these species were recently described (Froehlich & Leal-Zanchet 2003; Leal-Zanchet & Froehlich 2006). These form a species complex which is characterized by an elongate body with parallel margins, and the dorsum with five or seven dark longitudinal stripes on a yellowish background (Froehlich & Leal-Zanchet 2001; Leal-Zanchet & Froehlich 2006). Two additional species, herein described, are added to this complex.

Material and methods

Specimens were collected by direct sampling in soil litter, under and inside fallen logs and branches, and under rocks in the National Forest of São Francisco de Paula (29°23'-29°27'S, 50°23'-50°25W), São Fran-

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cisco de Paula, state of Rio Grande do Sul, Brazil.

For histological processing of material and analysis of external and internal characters, the methods described by Froehlich and Leal-Zanchet (2003) were used. The material was sectioned at 5-7µm.

Type-material was deposited in the following reference collections: Museu de Zoologia da Universidade do Vale do Rio dos Sinos (MZU), São Leopoldo, Rio Grande do Sul, Brazil, and the Helminthological Collection of Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo, São Paulo State, Brazil.

Taxonomic part

Family Geoplanidae Stimpson, 1857

Subfamily Geoplaninae Stimpson, 1857

Notogynaphallia Ogren et Kawakatsu, 1990

Notogynaphallia pseudoceciliae sp. nov.

Notogynaphallia sp. 6 Leal-Zanchet & Carbayo, 2000
Notogynaphallia sp. 4 Carbayo, Leal-Zanchet & Vieira, 2001
Notogynaphallia sp. 3 Carbayo, Leal-Zanchet & Vieira, 2002

Etymology: The specific name refers to external similarity with *Notogynaphallia ceciliae* Froehlich & Leal-Zanchet, 2003.

Type-material: Holotype: MZUSP PL.301: T. Fleck, leg. 25. IX. 98 – pre-pharyngeal region: transversal sections on 6 slides; pharynx: sagittal sections on 9 slides; copulatory apparatus: sagittal sections on 12 slides. Paratypes: MZU PL.00070: F. Carbayo, leg. 16. XII. 98 - pharynx: sagittal sections on 3 slides; body region between pharynx and copulatory apparatus: sagittal sections on 14 slides; copulatory apparatus: sagittal sections on 18 slides; MZU PL.00071: M. Cardoso, leg. 16. XII. 98 – anterior region in three fragments: transversal sections on 8 slides; horizontal sections on 4 slides and sagittal sections on 15 slides; pre-pharyngeal region: transversal sections on 12 slides; pharynx: sagittal sections on 10 slides; copulatory apparatus in two fragments: sagittal sections on 25 slides; MZU PL.00072: I. Fick, leg. 08. VII. 99 – anterior region at the level of the ovaries: sagittal sections on 15 slides; pre-pharyngeal region: transversal sections on 5 slides; pharynx: sagittal sections on 11 slides; copulatory apparatus: sagittal sections on 12 slides.

Type-locality: São Francisco de Paula, state of Rio Grande do Sul (RS), Brazil.

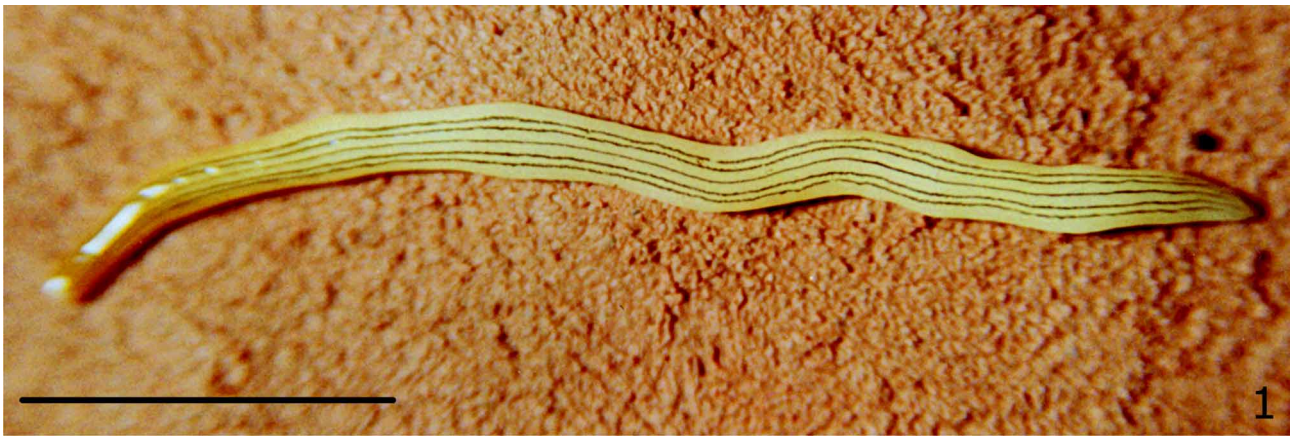
Distribution: São Francisco de Paula, Rio Grande do Sul, Brazil.

Diagnosis: Dorsum light-yellow with five dark longitudinal stripes, thin but distinct; wide marginal zone, free of stripes; eyes dorsal, without clear halos; typical glandular margin absent; mc:h, 18-21%; pharynx cylindrical with dorsal insertion posteriorly displaced, folded margins; esophagus absent; foremost testes posterior to ovaries, most posterior ones near root of pharynx; efferent ducts open laterally into distal portion of tubular branches of prostatic vesicle; extrabulbar prostatic vesicle, with two long tubular branches exceeding the posterior end of pharyngeal pouch; male atrium long and highly folded, with histologically differentiated proximal and distal portions; ejaculatory duct opening through a small projection into the proximal portion of male atrium; oviducts emerging dorsally from median third of ovaries, and ascending posteriorly to gonopore; common glandular oviduct dorsal to female atrium; vagina directed dorsally and forwards; female atrium oval-elongate, almost as long as the male atrium, presenting epithelium with lacunose multilayered aspect.

Description

External morphology: Body elongate with parallel margins (Fig. 1), anterior end obtuse and posterior pointed. When creeping, maximal length may reach 67 mm (Table 1). Mouth distance from anterior tip varies

from 64% to 77% relatively to body length, gonopore from 79% to 88% (Table 1). Dorsal and ventral ground colour yellowish. Dorsum with five dark brown longitudinal stripes, one median, two paramedian, and two lateral (Figs. 1, 3A-B). In paratype MZU PL.00071, median stripe begins, discontinuously, at 2 mm from anterior tip (ca. 3% of body length), slightly behind the beginning of paramedian stripes (1 mm from anterior tip or 2% of body length) and ahead of lateral stripes (ca. 3 mm from anterior tip or 5% of body length) (Fig. 3A). Near posterior end, on each side of body, paramedian and lateral stripes converge; paramedian stripes become discontinuous at approximately 6 mm from posterior tip, whereas lateral ones almost reach this tip (Fig. 3B). Median stripe ends at approximately 2 mm from posterior tip (96% of body length). At median third of body of paratype MZU PL.00071, lateral stripes located at 0.7 mm from the body margins (one-fifth of body width), so that a wide marginal zone remains free of stripes. Median stripe is the thinnest (approx. 0.07 mm or 2% of body width), followed by paramedian (approx. 0.11 mm or 3% of body width) and lateral (approx. 0.14 mm or 4% of body width) stripes.

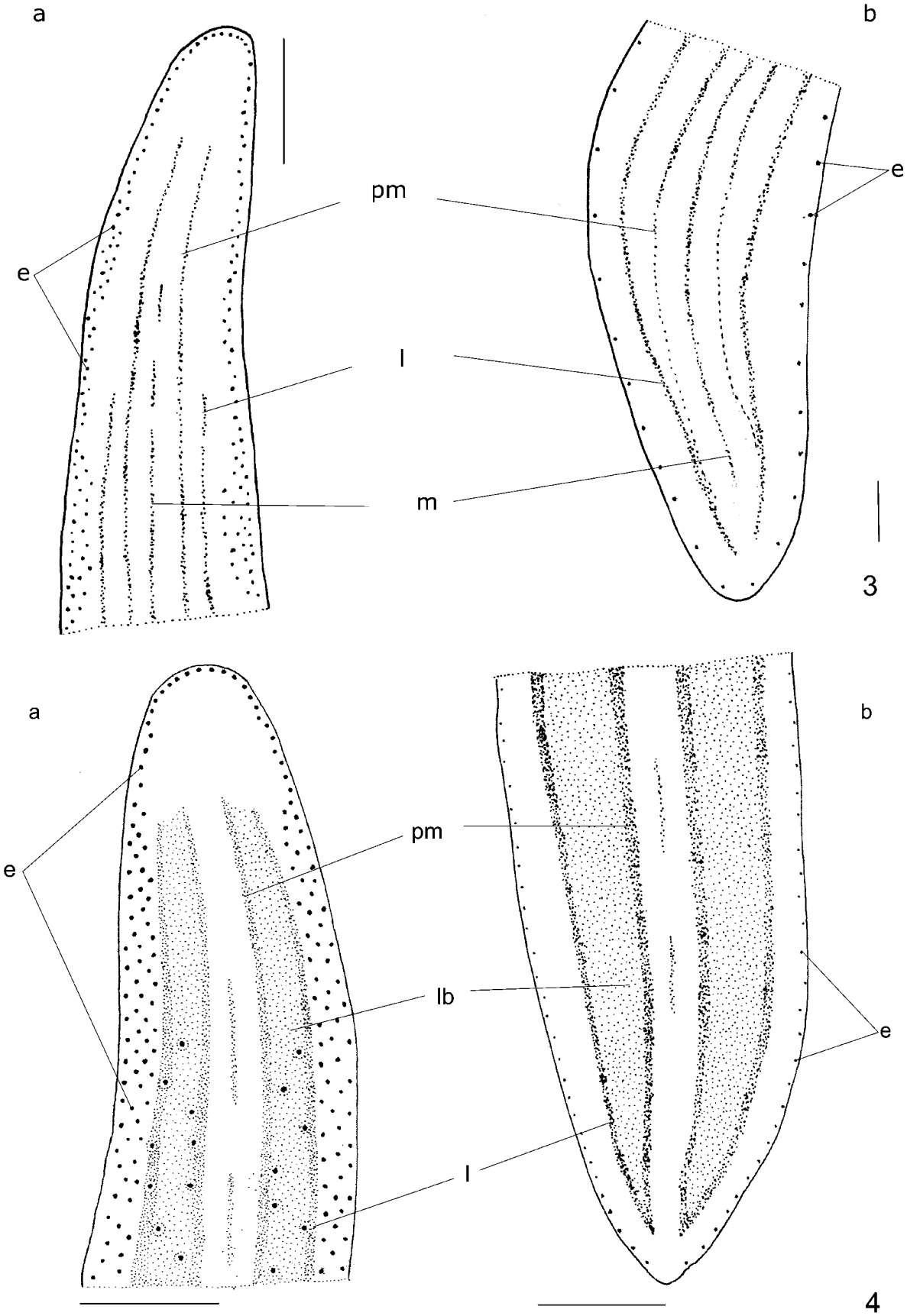


FIGURES 1–2. Photographs of live specimens, dorsal view: *N. pseudoceciliae* sp. nov. (holotype) (1); *N. arturi* sp. nov. (paratype MZU PL.00076) (2). Scale bar: 10 mm.

Eyes, initially marginal and uniserial, surround the anterior tip. In paratype MZU PL.00071, eyes become pluriserial after the second millimeter (approx. 4% of body length). Between approximately 4 mm and 9 mm (ca. 7% and 14% of body length) from the tip they become dorsal, forming two to three lateral irregular series (Fig. 3A). Sparser backwards, become limited to body margins, occurring up to near the posterior tip (Fig. 3B). No clear halos.

Internal morphology

Epidermis and musculature at pre-pharyngeal region: Creeping sole, 64% and 58% of body width, respectively, in holotype and paratype MZU PL.00072 (Table 1).



FIGURES 3–4. Detail of colour pattern of anterior (a) and posterior (b) body regions of preserved specimens of *N. pseudoceciliae* sp. nov. (paratype MZU PL.00071) (3) and *N. arturi* sp. nov. (paratype MZU PL.00080) (4). (e) eyes, (l) lateral stripe, (lb) lateral band, (m) median stripe, (pm) paramedian stripe. Scale bar: 1 mm.

Three types of secretory cells discharge through dorsal epidermis and body margins: (1) abundant rhabditogen cells with xanthophil secretion (2) cells with coarse erythrophil secretion; (3) few cells with cyanophil amorphous secretion. The erythrophil cells and a fourth type of cell, with fine granulous xanthophil secretion, are more abundant towards body margins, but do not form a typical glandular border (Fig. 5). Creeping sole receives necks of few rhabditogen cells, cells with fine granulous erythrophil secretion, and numerous cells with amorphous cyanophil secretion.

Cutaneous musculature with the usual three layers, longitudinal layer with thick bundles, being approximately four times higher than the other two. At the sagittal plane, ventral musculature higher than dorsal (Table 2), the latter becoming higher paramedianly. Towards body margins cutaneous musculature progressively lower (Fig. 5). Mc:h 18% to 21% (Table 2).

Mesenchymatic musculature composed of four layers: dorsal subcutaneous with oblique fibers variously oriented (ca. 3 fibers thick); supra-intestinal transversal (approx. 4-6 fibers thick); sub-intestinal transversal (4-5 fibers thick); and subneural transversal (ca. 4-5 fibers thick) (Fig. 5). In addition, scattered ventral subcutaneous oblique fibers as well as dorsoventral ones are present. If existent, longitudinal fibers are indiscernible, few and very scattered.

TABLE 1. Measurements, in mm, of type-specimens of *N. pseudoceciliae* sp. nov. –: not measured; *: After fixation; ** Specimens with damaged anterior tip (lost or regenerating); DG: distance of gonopore from anterior end; DM: distance of mouth from anterior end; DMG: distance between mouth and gonopore; DPVP: distance between prostatic vesicle and pharyngeal pouch. The numbers given in parentheses represent the position relative to body length.

	Holotype MZUSP PL.301**	Paratype MZU PL.00070**	Paratype MZU PL.00071	Paratype MZU PL.00072
Maximum length in extension	40	35	67	45
Maximum width in extension	2	2.5	3.5	2.5
Length at rest	22	22	45	27
Width at rest	3.5	3	4.5	3.5
Length*	38	21	57	47
Width*	2.5	2.5	3.5	3
DM*	–	–	44 (77)	30 (64)
DG*	–	–	50 (88)	37 (79)
DMG*	8.5	–	6	7
DPVP*	0	0	0	–
Creeping sole %	64	–	–	58
Ovaries	11 (23)	–	–	–
Anteriormost testes	13 (28)	–	–	–
Posteriormost testes	25 (66)	–	–	30 (64)
Prostatic vesicle	3.9	5.4	2.6	–
Male atrium	1.6	1.9	1.1	–
Female atrium	1.5	1.5	0.8	–

Pharynx (Fig. 7): Pharynx of cylindrical type with dorsal insertion posteriorly displaced, but still in anterior third of pharyngeal pouch, and with folded margins. Mouth in median third of pharyngeal pouch. No esophagus. Pharyngeal glands with cell bodies located in mesenchyme, mainly anterior and posteriorly as well as lateral to pharyngeal pouch. Three secretory cell types: cells with coarse granulous xanthophil secretion, cells with fine granulous erythrophil secretion, and cells with cyanophil amorphous secretion. Outer musculature of pharynx (ca. 18µm thick) constituted of thin longitudinal subepithelial layer, followed by a thicker circular one, mixed internally with few longitudinal fibers. Towards pharyngeal tip, circular layer

becomes as thin as longitudinal one. Inner pharyngeal musculature (ca. 15µm thick) composed of thick circular subepithelial layer, mixed mainly externally with some longitudinal fibers. Inner musculature gradually thins down outwards, and, though mainly dorsally, also inwards.

TABLE 2. Cutaneous musculature, in µm, in the median region of a transversal section of the pre-pharyngeal region, and ratio of the height of cutaneous musculature to the height of the body (mc:h index) of specimens of *N. pseudoceciliae* and *N. arturi*.

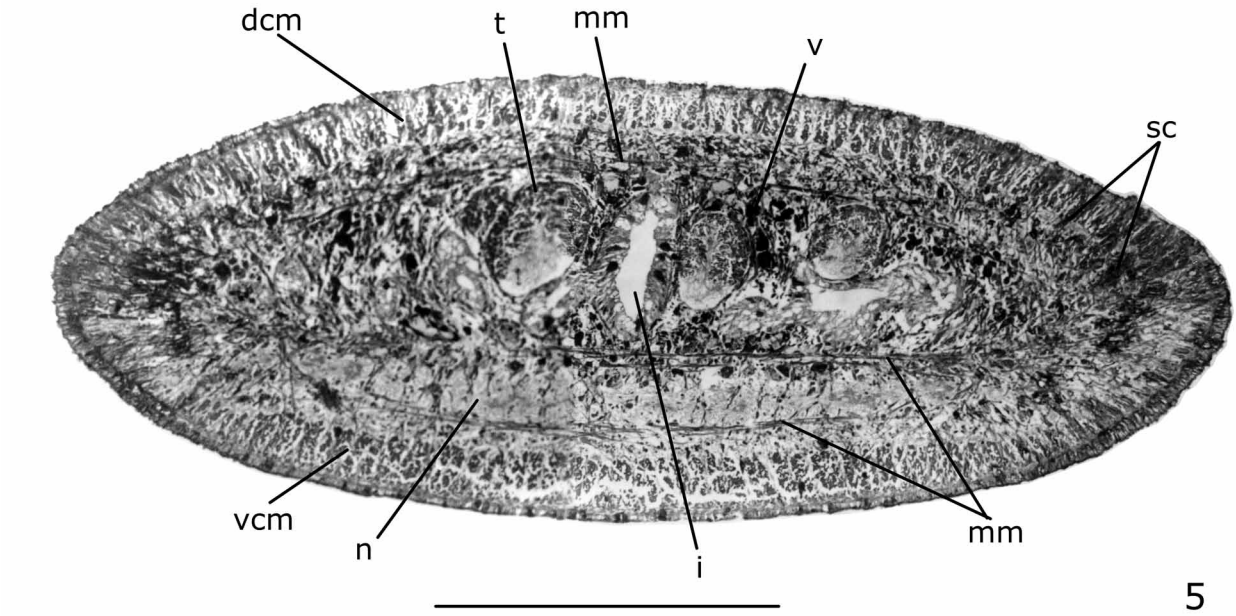
	N. pseudoceciliae		*N. arturi*			
Specimens	Holotype MZUSP PL.301	Paratype MZU PL.00072	Holotype MZUSP PL.302	Paratype MZU PL.00073	Paratype MZU PL.00075	Paratype MZU PL.00079
Dorsal musculature	70	66	71	112	62	62
Ventral musculature	86	75	63	108	74	56
Mc:h	21%	18%	12%	20%	17%	14%

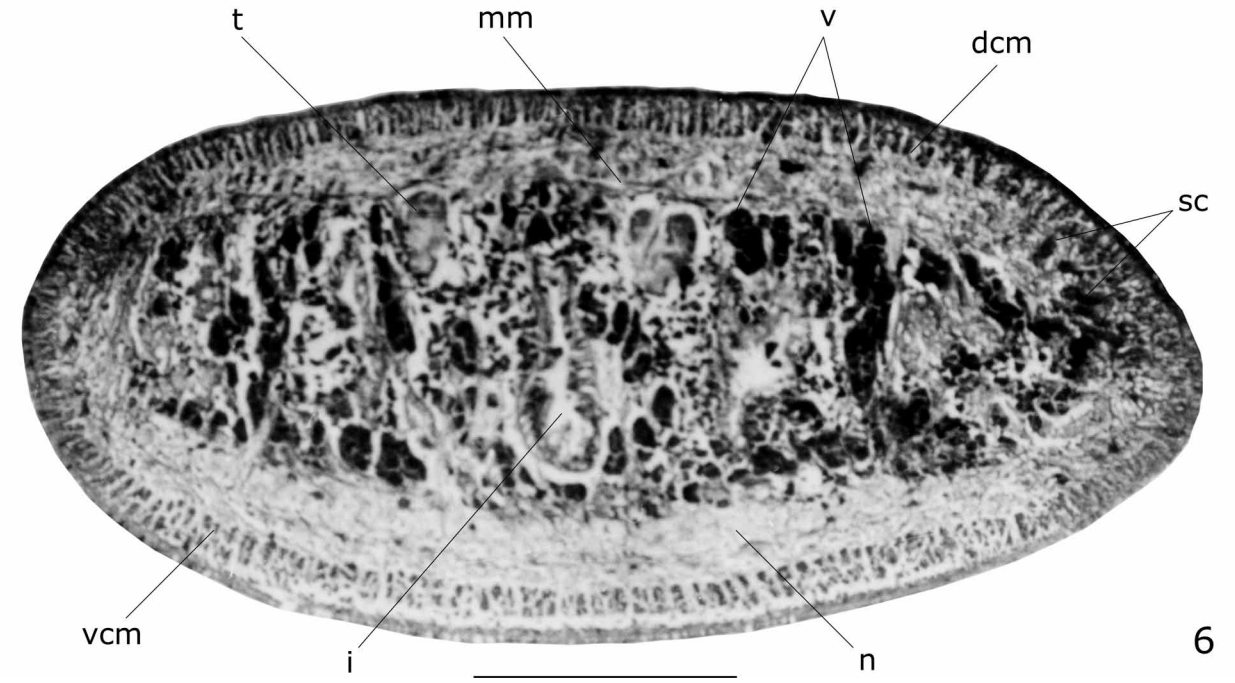
Reproductive apparatus: Foremost testes posterior to ovaries; most posterior ones, near root of pharynx, slightly posteriorly to or at same transversal level as ventral insertion of pharynx (Table 1). Testes in two irregular rows beneath the dorsal transversal mesenchymatic muscles dorsally and intersticially to the intestinal branches on each side of the body (Fig. 5). Efferent ducts run dorsally to oviducts, sometimes laterally displaced. Behind pharynx, the right and left efferent ducts form false seminal vesicles, and enter prostatic vesicle laterally in distal region of forked portions, respectively, an approximate distance of 1.3 mm and 1.5 mm from penial bulb (Fig. 9A). Prostatic vesicle (Table 1, Figs. 10, 13, 14), tubular and forked. The two long tubular extrabulbar branches extend anteriorly near to or a little beyond posterior end of pharyngeal pouch. Ca. 1 mm from bulbar muscular coat, paired branches of prostatic vesicle unite, forming a single canal which enters the bulbar muscular coat and forms a sinuous and ample ejaculatory duct. The latter opens, through a small projection, into proximal portion of male atrium (Figs. 9B, 11). Male atrium oval-elongate (Table 1, Figs. 9B, 11, 14) with numerous folds, irregular in shape, which are higher ectally.

Lining epithelium of efferent ducts cuboidal ciliated; thin muscularis (ca. 3µm thick) mainly constituted of circular fibers. Prostatic vesicle lined with columnar to pseudostratified ciliated epithelium, receiving abundant erythrophil granulous secretion from secretory cells with bodies lying in mesenchyme, mainly around vesicle. Muscularis of vesicle (10-15µm thick) constituted of interwoven longitudinal and circular fibers. Ejaculatory duct lined with columnar ciliated epithelium, without openings from secretory cells, being coated with weakly developed muscularis (ca. 3-5µm thick) constituted of mixed longitudinal and circular fibers.

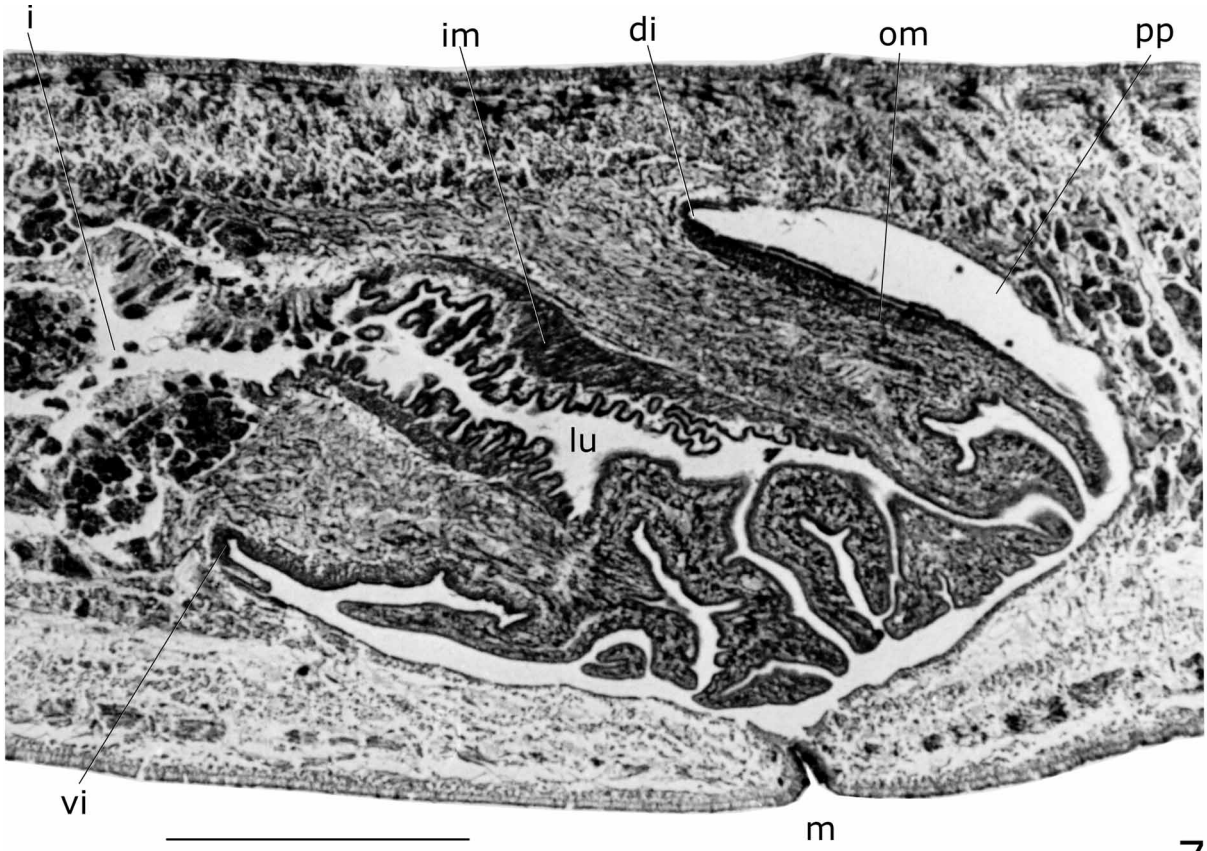
Epithelial lining of male atrium, columnar non-ciliated. Four types of secretory cells, with cell bodies external to common muscle coat, empty through the epithelium: cells with fine granulous slightly erythrophil secretion; cells with granulous heavily stained erythrophil secretion; cells with coarse granulous xanthophil secretion; and cells with cyanophil amorphous secretion. Openings from xanthophil and heavily stained erythrophil cells are more numerous into the ectal portion of male atrium; those from slightly erythrophil cells into the ental portion of male atrium. Muscularis weakly developed (3-7µm) throughout male atrium, but thicker (approx. 40µm) in the most ectal folds; mainly composed of circular layer with some mixed longitudinal fibers.

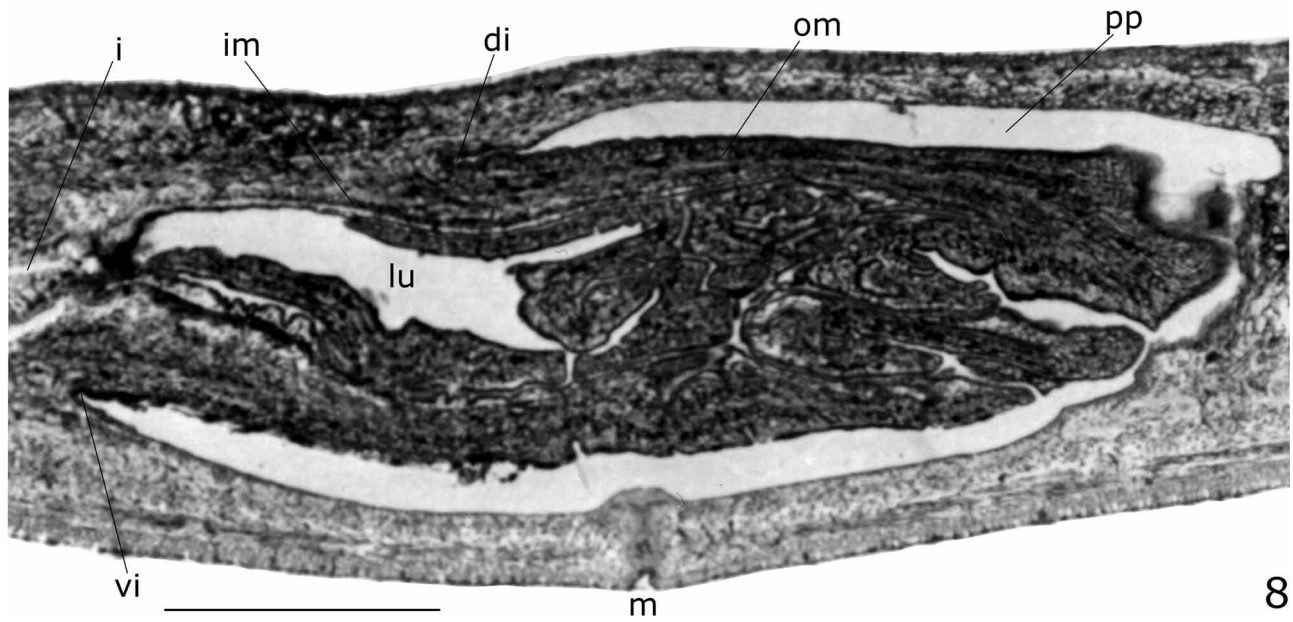
Ovaries oval-elongate in shape, measuring 0.3 mm anterior-posteriorly and 0.05 mm dorso-ventrally in paratype MZU PL.00072. Oviducts emerging dorsally from median third of ovaries, lead backwards immediately dorsal to nerve plate. Behind gonopore, oviducts ascend posterior and medially inclined, to unite dorsally to the female atrium, thus forming the common glandular oviduct (Figs. 9B, 15). The latter, a long canal, dorsal to female atrium, that leads backward to communicate with vagina. Vagina as a terminal dorsally and forwardly directed diverticulum of female atrium. Female atrium, oval-elongate in shape and with a narrowed lumen, almost as long as male one (Table 1, Figs. 9B, 12, 15).





FIGURES 5–6. Pre-pharyngeal region of *N. pseudoceciliae* sp. nov. (paratype MZU PL.00071) (5) and *N. arturi* sp. nov. (holotype) (6) in transverse section. (dcm) dorsal cutaneous musculature, (i) intestine, (mm) mesenchymatic muscles, (n) nerve plate, (sc) secretory cells, (v) vitellaria, (vcm) ventral cutaneous musculature, (t) testes. Scale bar: 500 µm.





FIGURES 7–8. Pharynx of *N. pseudoceciliae* sp. nov. (holotype) (7) and *N. arturi* sp. nov. (holotype) (8). Sagittal section. (di) dorsal insertion, (i) intestine, (im) internal musculature, (lu) pharyngeal lumen, (m) mouth, (om) outer musculature, (pp) pharyngeal pouch, (vi) ventral insertion. Scale bar: 500 µm.

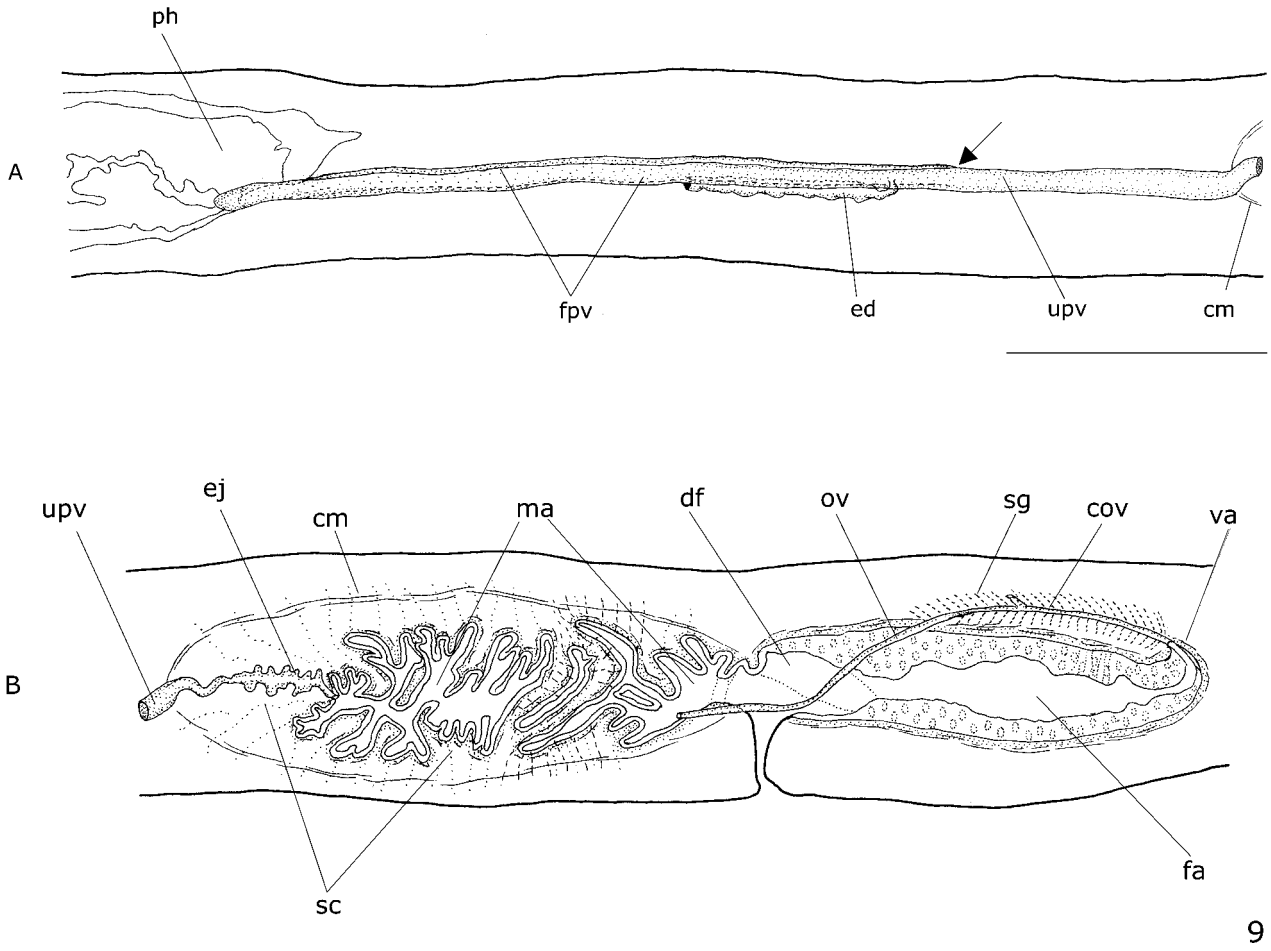


FIGURE 9. Diagrammatic composite reconstruction of copulatory apparatus of *N. pseudoceciliae* sp. nov. from sagittal sections (holotype): prostatic vesicle (A) and male and female atria (B). (cm) common muscle coat, (cov) common glandular oviduct, (df) dorsal fold, (ed) efferent duct, (ej) ejaculatory duct, (fa) female atrium, (fpv) forked portions of prostatic vesicle, (go) gonopore, (m) mouth, (ma) male atrium, (ov) oviducts, (ph) pharynx, (sc) secretory cells, (sg) shell glands, (upv) unpaired portion of prostatic vesicle, (va) vagina. Arrow indicates joining position of paired branches of prostatic vesicle. Scale bar: 1 mm.

Paired oviducts lined with cuboidal ciliated epithelium which becomes, near copulatory apparatus, columnar ciliated, as is that of common glandular oviduct. Paired oviducts as well as common glandular oviduct coated with thin circular muscle layer with some interposed longitudinal fibers. Abundant shell glands with xanthophil secretion empty into distal third of ascending portion of paired oviducts, besides into common glandular oviduct (Figs. 9B, 15).

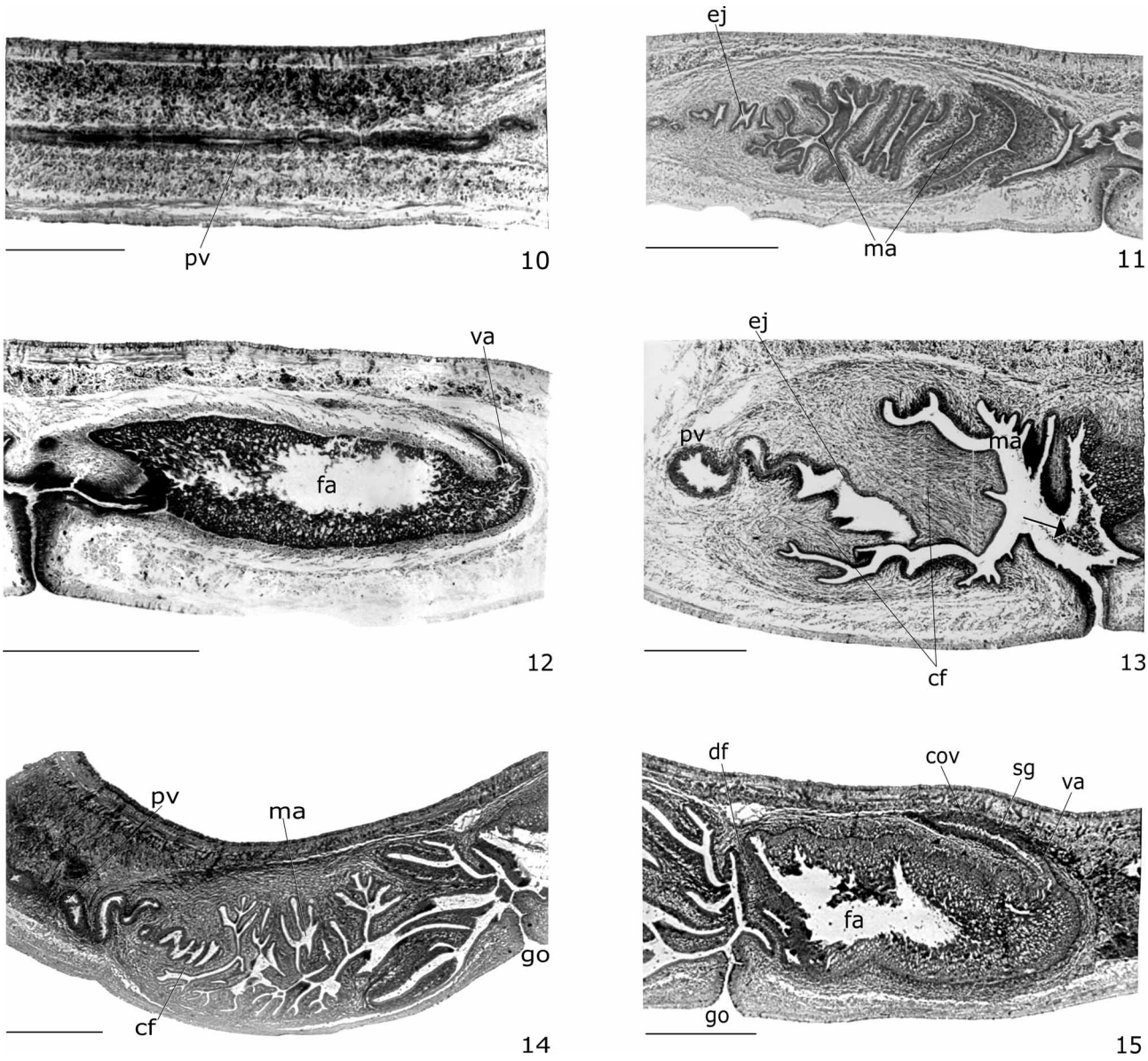
Lining of female atrium very thick with multilayered aspect (Figs. 9B, 12, 15). It presents many lacunae containing cyanophil secretion and others with either xanthophil or erythrophil secretion. Near to gonopore, the thick epithelial lining is substituted by a columnar epithelium. Vagina lined ectally with a thinner epithelium than that of female atrium, showing multilayered aspect, and entally with a columnar epithelium (Fig. 12). Erythrophil cells with granulous secretion and cells with cyanophil amorphous secretion discharge into vagina and female atrium. Cell bodies of cyanophil glands are external to the muscle common coat and those of erythrophil cells are internal to the muscle common coat. Muscularis (15-17µm) mainly constituted of circular fibers mixed with a number of longitudinal fibers.

Straight gonopore canal. Dorsal fold, approximately at same transversal level as gonopore canal, leads to venter, inclining posteriorly, and fuses with ventral wall of female atrium (Figs. 9B, 15).

Common muscle coat with circular, longitudinal and oblique fibers, thicker around ental portion as well as

along dorsal wall of male than around female atrium. Between atrial muscularis and common muscle coat, a stroma with muscle fibers variously oriented.

Remarks: Vitellaria are inconspicuous in the holotype and paratype MZU PL.00072, although well developed in paratypes MZU PL.00070 and MZU PL.00071. Paratype MZU PL.00072, probably the younger worm, and paratype MZU PL.00071, highly contracted, reveal a circular fold in the male atrium, similar to a temporary penis papilla, this being traversed by the ejaculatory duct (Fig. 13). In both the holotype and paratype MZU PL.00071, there is abundant holocrine secretion, produced by cells of the multilayered-like lining, in the lumen of female atrium. Secretory cells of the copulatory apparatus have been described based on paratype MZU PL.00070.



FIGURES 10–15. Copulatory apparatus of *N. pseudoceciliae* sp. nov. in sagittal sections: prostatic vesicle (10), male atrium (11), and female copulatory organs (12) of the holotype; male copulatory organs of the paratype MZU PL.00071 (13); unpaired portion of prostatic vesicle and male atrium (14), and female copulatory organs (15) of the paratype MZU PL.00070. (cf) circular fold, (cov) common glandular oviduct, (df) dorsal fold, (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ma) male atrium, (pv) prostatic vesicle, (sg) shell glands, (va) vagina. Arrow: holocrine secretion. Scale bar: 500 μm.

Notogynaphallia arturi sp. nov.

Notogynaphallia sp. 2 Leal-Zanchet & Carbayo, 2000
Notogynaphallia sp. 1 Carbayo, Leal-Zanchet & Vieira, 2001
Notogynaphallia sp. 1 Carbayo, Leal-Zanchet & Vieira, 2002

Etymology: the specific epithet is in homage to Artur Soligo, manager of the National Forest of São Francisco de Paula (FLONA/SFP).

Type-material: Holotype: MZUSP PL.302: F. Carbayo, leg. 14.I.98 – Anterior region at the level of the ovaries: sagittal sections on 17 slides; pre-pharyngeal region: transversal sections on 15 slides; pharynx: sagittal sections on 20 slides; copulatory apparatus: sagittal sections on 19 slides.Paratypes: MZU PL.00073: F. Carbayo, leg. 19.X.96 – Pre-pharyngeal region: transversal sections on 11 slides; pharynx: sagittal sections on 10 slides; copulatory apparatus: sagittal sections on 37 slides; MZU PL.00074: F. Carbayo, 20.V.98 – preserved in ethanol 70%; MZU PL.00075: F. Carbayo, leg. 25.IX. 98 – Anterior region in two fragments: sagittal sections on 21 slides; pre-pharyngeal region: transversal sections on 5 slides; pharynx: sagittal sections on 15 slides; copulatory apparatus: sagittal sections on 9 slides; MZU PL.00076: F. Carbayo, 29.IV. 99 – preserved in clove oil; MZU PL.00077: F. Carbayo, 26.VII.99 – copulatory apparatus: sagittal sections on 10 slides; MZU PL.00078: F. Carbayo, leg. 12.VIII.99 – copulatory apparatus: sagittal sections on 8 slides; MZU PL.00079: F. Carbayo, leg. 14.IX. 99 – pre-pharyngeal region: transversal sections on 7 slides; pharynx: sagittal sections on 12 slides; copulatory apparatus: sagittal sections on 16 slides; MZU PL.00080: M. Fontoura, leg. 23.V.2003 – Anterior tip: transversal sections on 7 slides; posterior tip: transversal sections on 7 slides.

Type-locality: São Francisco de Paula, state of Rio Grande do Sul, Brazil.

Distribution: São Francisco de Paula, Rio Grande do Sul, Brazil.

Diagnosis: Dorsum yellowish to light-brown with two dark brown lateral bands, wherein at the external and internal limits the very dense pigment creates, at each side of the dorsum, a lateral and a paramedian stripe; median stripe discontinued, sometimes indistinct; eyes dorsal, with inconspicuous clear halos; glandular margin absent; mc:h, 12-20%; esophagus absent; pharynx bell-shaped; foremost testes approximately level with ovaries, most posterior ones approximately level with ventral insertion of pharynx; efferent ducts open into anterior portion of prostatic vesicle; extrabulbar prostatic vesicle, oval-elongate, without bifurcation; male atrium long and highly folded, with histologically differentiated proximal and distal portions; oviducts emerging dorsally from median third of ovaries, and ascending behind gonopore; common glandular oviduct dorsal to female atrium; vagina curved dorso-anteriorly; female atrium oval-elongate and folded; male atrium approx. twice as long as female atrium.

Description

External morphology: Body elongate with parallel margins (Fig. 2), anterior end obtuse, posterior end pointed. When crawling, maximal length reaches 38 mm (Table 3). Mouth distance from anterior tip varies from 50% to 72% relatively to body length, gonopore from 71% to 81% (Table 3). Dorsal ground colour yellowish to light-brown; ventral one pale yellow or brownish with denser pigmentation at the margins. Live specimens with anterior tip brown, reddish or orange dorsally and ventrally. There are two dark-brown lateral bands overlaying the dorsal ground colour, whereby at the external and internal limits the very dense pigment creates, on each side of the dorsum, a paramedian and a lateral stripe (Figs. 2, 4A-B). In addition, there is a discontinuous, sometimes indistinct, dark median stripe. Bands and stripes begin at ca. 1 mm from anterior tip (ca. 4% of body length of paratype MZU PL.00080) (Fig. 4A). Near posterior end, on each side of body, paramedian and lateral stripes converge forming an elongated V (Fig. 4B). At median third of body of paratype MZU PL.00080, lateral bands 0.5 mm wide (25% of body width). Paramedian and lateral stripes, respectively, ca. 0.1 mm wide (5% of body width) at median third of body of paratype MZU PL.00080.

Eyes, initially marginal and uniserial, contour anterior tip. In paratype MZU PL.00080, become pluriserial approximately 1 mm behind anterior tip (4% of body length). Approximately 2 mm behind anterior tip (ca.

9% of body length), they spread close to the sagittal plane, here being surrounded by inconspicuous clear halos (Fig. 4A). After 4 mm behind anterior tip (ca. 17% of body length), become lateral and sparse (Fig 4B). After fixation halos are indiscernible.

TABLE 3. Measurements, in mm, of type–specimens of *N. arturi* sp. nov. –: not measured; *: After fixation; ** Specimens with damaged anterior tip; DG: distance of gonopore from anterior end; DM: distance of mouth from anterior end; DMG: distance between mouth and gonopore; DPVP: distance between prostatic vesicle and pharyngeal pouch. The numbers given in parentheses represent the position relative to body length.

	Holotype MZUSP PL.302	Paratype MZU PL.00073	Paratype MZU PL.00074	Paratype MZU PL.00075	Paratype MZU PL.00076	Paratype MZU PL.00077**	Paratype MZU PL.00078**	Paratype MZU PL.00079	Paratype MZU PL.00080
Maximum length in extension	38	–	23	34	19	35	25	32	33
Maximum width in extension	2.5	–	1.5	1.5	1.5	2.5	1.5	2	1.5
Length at rest	23	–	16	21	14	21	15	23	17
Width at rest	3	–	2	3	2.5	3	2.5	3	2.5
Length*	23	20	10	27	9	14.5	10.5	21	23
Width*	3	2	2	2	2	2	2	3	2
DM*	13 (56)	13 (65)	5 (50)	16 (59)	6.5 (72)	–	–	11 (52)	14 (61)
DG*	17 (74)	15 (75)	–	22 (81)	–	–	–	15 (71)	–
DMG*	4	2	–	6	–	5	2.5	4	–
DPVP*	0.1	0	–	0.2	–	–	–	0.3	–
Creeping sole %	95	–	–	–	–	–	–	70	–
Ovaries	4 (17)	–	–	–	–	–	–	6 (29)	–
Anteriormost testes	4 (17)	–	–	–	–	–	–	6 (29)	–
Posteriormost testes	12 (52)	–	–	–	–	–	–	11 (52)	–
Prostatic vesicle	0.7	0.7	–	0.7	–	–	–	0.5	–
Male atrium	1.5	1.5	–	1.4	–	–	–	1.4	–
Female atrium	0.9	0.8	–	0.8	–	–	–	0.9	–

Internal morphology

Epidermis and musculature at pre-pharyngeal region: Width of creeping sole, measured in paratype MZU PL.00079 and holotype, represented, respectively, 70% and 95% of body width (Table 3).

Four types of secretory cells open through dorsal epidermis and body margins: (1) rhabdithogen cells with xanthophil secretion; (2) cells with coarse granulous erythrophil secretion; (3) cells with fine granulous erythrophil secretion; and (4) cells with amorphous cyanophil secretion. There is no glandular margin (Fig. 6). Creeping sole receives abundant cells with amorphous cyanophil secretion, and a small amount of cells with coarse erythrophil secretion, besides few rhabdithogen cells.

Cutaneous musculature with constitution similar to that described for *N. pseudoceciliae*. Longitudinal layer approximately three to five times higher than both the circular and oblique together. Cutaneous musculature higher paramedianly than medianly. Towards body margins progressively lower (Fig. 6). Dorsal cutaneous musculature higher than ventral. Mc:h 12% to 20% (Table 2).

Mesenchymatic musculature mainly composed of three layers: supra-intestinal transversal (approx. 11-16 fibers thick); sub-intestinal transversal (approx. 4-8 fibers thick); and subneural transversal (ca. 6-10 fibers thick). Dorsal subcutaneous layer with oblique fibers inconspicuous. Scattered ventral subcutaneous oblique fibers, besides some dorsoventral fibers. If existent, longitudinal fibers are indiscernible.

Pharynx (Fig. 8): Pharynx bell-shaped, highly folded. Mouth in median third of pharyngeal pouch,

slightly posterior to dorsal insertion. No esophagus. Pharyngeal glands, with cell bodies in mesenchyme, mainly anteriorly to pharynx, of three types: cells with coarse granulous erythrophil secretion; cells with granulous slightly erythrophil secretion; and cells with amorphous cyanophil secretion. Outer and inner pharyngeal musculatures similar to those of *N. pseudoceciliae*, but more developed, each measuring ca. 40-50µm thick.

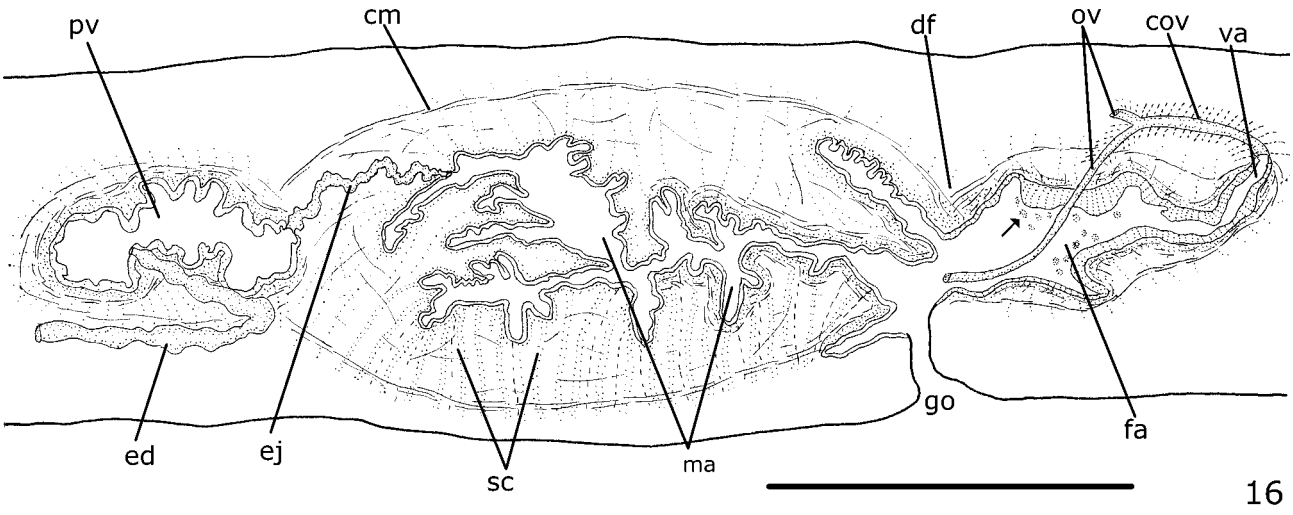


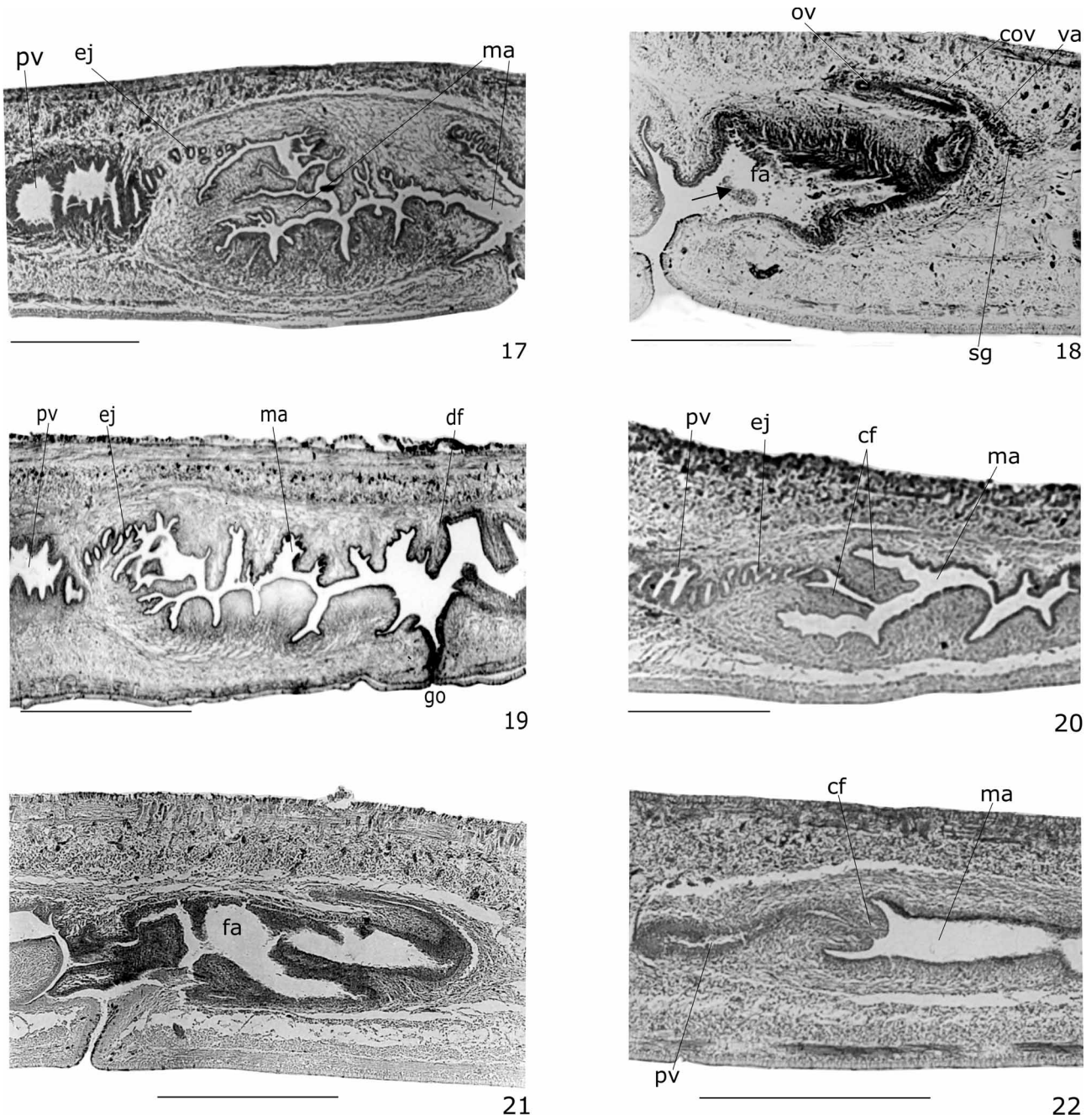
FIGURE 16. Diagrammatic composite reconstruction of copulatory apparatus of *N. arturi* sp. nov. from sagittal sections (holotype). (cm) common muscle coat, (cov) common glandular oviduct, (df) dorsal fold, (ed) efferent duct, (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ma) male atrium, (ov) oviducts, (pv) prostatic vesicle, (sc) secretory cells, (va) vagina. Arrow: holocrine secretion. Scale bar: 1 mm.

Reproductive apparatus: Testes begin at approximately the same level as ovaries and extend almost to the same level as the ventral insertion of pharynx (Table 3). Pre-pharyngeally, efferent ducts dorsal to oviducts, sometimes medianly displaced. Lateral to posterior third of pharyngeal pouch, efferent ducts become false seminal vesicles, which run until near to posterior end of prostatic vesicle, to then turn anteriorly, opening laterally into anterior third of prostatic vesicle (Fig. 16). Extrabulbar prostatic vesicle spacious, oval-elongate with a folded internal wall (Table 3, Figs. 16, 17, 19, 20). Entering penis bulb, vesicle narrows, constituting a sinuous ejaculatory duct, which opens, dorsally dislocated, into male atrium (Figs. 16, 17, 19). Male atrium oval-elongate (Table 3, Figs. 16, 17, 19, 20) with abundant folds which are higher and more irregular in shape dorsally than ventrally.

Lining epithelium of efferent ducts cuboidal ciliated, underlain by a thin muscularis composed of mainly circular fibers. Prostatic vesicle lined with pseudostratified columnar epithelium, ciliated, traversed by two gland types with granulous secretion and cell bodies in surrounding mesenchyme: abundant cells with erythrophil secretion and fewer cells with xanthophil secretion. Muscularis of vesicle (ca. 20-35µm thick) composed of interwoven circular and longitudinal fibers. Lining epithelium of ejaculatory duct columnar, ciliated, receiving openings of cyanophil cells with cell body in the mesenchyme, external to common muscle coat. Ejaculatory duct muscularis thin (ca. 5µm thick) with intermixed circular and longitudinal fibers.

Male atrium lined with cuboidal to columnar non-ciliated, epithelial cells with xanthophil irregular apical surface. Three types of secretory cells pierce the epithelium: numerous cells with granulous xanthophil secretion, cells with granulous erythrophil secretion, and cells with amorphous cyanophil secretion. The three types of cells with bodies external to common muscle coat in surrounding mesenchyme. Openings from secretory cells more numerous distally and in the ventral male atrial wall. Muscularis thin (10-15µm), mainly consti-

tuted of circular fibers with few intermixed longitudinal fibers, becoming thinner in proximal atrial region, towards ejaculatory duct opening, as well as in dorsal wall of male atrium.



FIGURES 17–22. Copulatory apparatus of *N. arturi* sp. nov. in sagittal sections: male (17) and female (18) copulatory organs of the holotype; male copulatory organs of the paratype MZU PL.00073 (19); male (20) and female (21) copulatory organs of the paratype MZU PL.00079; copulatory organs of the paratype MZU PL.00078 (22). (cov) common glandular oviduct, (cf) circular fold, (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ma) male atrium, (ov) oviducts, (pv) prostatic vesicle, (sg) shell glands, (va) vagina. Arrow: holocrine secretion. Scale bar: 500 μm.

Ovaries oval-elongate, measuring ca. 0.3 mm anterior-posteriorly and 0.1 mm dorso-ventrally. Oviducts arise from dorsal side of median third of ovaries and run posteriorly, immediately above nerve plate. Behind gonopore, oviducts proceed dorsomediad, to join above female atrium, here forming common glandular oviduct which continues backwards, to communicate with vagina (Fig. 16). This is a dorso-anteriad curved diverticulum of female atrium. Female atrium oval-elongate, the length of which corresponds to half that of the

male atrium, with ample cavity and folded walls (Table 3, Figs. 16, 18, 21).

Paired oviducts as well as common glandular oviduct lined with cuboidal to columnar ciliated epithelium, and coated with mixed circular and longitudinal fibers. Shell glands with xanthophil secretion opening into distal quarter of ascending portion of paired oviducts, and also into common glandular oviduct (Figs. 16, 18).

Epithelium lining vagina and female atrium tall pseudostratified columnar epithelium, irregular in height, ciliated in the ental portion of vagina, and with irregular and xanthophil apical surface in the atrium. Glands with either cyanophil amorphous or granulous erythrophil secretions, both with cell bodies external to common muscle coat, besides glands with granulous xanthophil secretion with cell bodies internal to common muscle coat, all discharge into whole epithelium. Muscularis of vagina and female atrium, thicker in the female atrium (approx. 32 - 64 µm), consisting of interwoven longitudinal, circular, and oblique fibers.

Straight gonopore canal. Dorsal fold, posterior to the level of gonopore canal, leads to the venter (Figs. 16, 19), inclining anteriorly, to finally fuses with ventral wall of female atrium.

Common muscle coat (approx. 12 - 30 µm thick) constituted of longitudinal with several mixed circular and oblique fibers. Between muscularis and common muscle coat, a stroma with muscle fibers variously oriented.

Remarks: The pharynx and copulatory apparatus are extremely contracted in the holotype. In paratypes MZU PL.00075 and MZU PL.00079, the dorsal insertion of the pharynx is located at the end of the anterior third of the pharyngeal pouch. There are well-developed yolk glands in the holotype; these in an earlier developmental stage in paratype MZU PL.00073, and inconspicuous in paratypes MZU PL.00075 and MZU PL.00079. Paratype MZU PL.00079 shows ovaries in an early stage of maturation with small ovocytes. Paratypes MZU PL.00077 and MZU PL.00078 each reveal a copulatory apparatus in an earlier stage of development (Fig. 22), with incompletely formed gonopore canal, vagina, and common glandular oviduct. Paratypes MZU PL.00075, MZU PL.00077, MZU PL.00078, and MZU PL.00079 present a circular ental fold in the male atrium, with the ejaculatory duct opening into the bottom (Figs. 20, 22). The very contracted copulatory apparatus of paratype MZU PL.00079 shows higher folds in the ventral wall of male atrium than in the dorsal wall. In the holotype (Figs. 16, 18), as well as in the paratypes MZU PL.00073 and MZU PL.00079, there is an abundant holocrine secretion in the lumen of the female atrium. The holotype oviposited between a half and 2 hours before fixation. Secretory cells of the copulatory apparatus were described based on the holotype and paratype MZU PL.00079.

Discussion

The genus *Notogynaphallia* was erected by Ogren and Kawakatsu (1990) for all Geoplaninae species without penial papilla, the male atrium with folded walls, and the female canal entering dorsally into the atrium. Froehlich and Leal-Zanchet (2003), analyzing mainly the reproductive organs of the species belonging to *Notogynaphallia*, have pointed the heterogeneity of this taxon. Later, Carbayo (2006) described the presence of a retractor muscle in two species of the genus, pointing to the absence of it in the type-species of the genus.

Froehlich and Leal-Zanchet (2003) differentiated two groups of species in this genus, based on characters of external and internal morphology. The species of group 1 present a diversified body-shape and colour-pattern, differing from the species of group 2, besides possessing a compact genital apparatus with an intrabulbar prostatic vesicle and a small female atrium. Group 2 is mainly comprised of slender worms with longitudinal stripes on the dorsum and a long genital apparatus with an extrabulbar prostatic vesicle. Thus, the two species herein described, *Notogynaphallia pseudoceciliae* and *Notogynaphallia arturi*, are included in group 2.

There are, however, other species with a striped dorsum which are known exclusively through their external morphology: *Notogynaphallia modesta* (Graff), *Notogynaphallia quinquestriata* (Hyman), and *Pseudogeoplana bohlsi* (Graff). These three species will be considered in the present discussion.

External morphology

Regarding external aspects, *N. pseudoceciliae* can be included in the complex of species proposed by Leal-Zanchet and Froehlich (2001), with specimens characterized by an elongate body with parallel margins and a dorsum with five or seven dark longitudinal stripes on a yellowish-background. The species which constitute this complex are: *N. abundans* (Graff), *N. caissara* (Froehlich), *N. ceciliae* Froehlich & Leal-Zanchet, *N. ernesti* Leal-Zanchet & Froehlich, and *N. graffi* Leal-Zanchet & Froehlich. These species, besides *N. guaiana* Leal-Zanchet & Carbayo, *N. muelleri* (Diesing), and *N. fita* (Froehlich), constitute group 2 of Froehlich and Leal-Zanchet (2003).

As already commented by Froehlich and Leal-Zanchet (2006), when comparing the species of the complex, these can be separated from each other by details such as the relative width of the different stripes, arrangement of stripes relative to body width and origin of stripes near the extremities of the body, especially the caudal end. Regarding relative width of the different stripes, *N. pseudoceciliae* shows, as does *N. ceciliae*, all stripes of a similar width. When specimens of both species were collected in the same type-locality, they were first confused, one with the other. Later, they could be separated by analyzing the arrangement of the stripes when related to body width, as the stripes of *N. ceciliae* run closer to the median line of the body than in *N. pseudoceciliae*. Concerning origin of stripes near the anterior tip in relation to the body length, stripes of *N. pseudoceciliae*, especially the paramedian stripes, begin closer to the anterior tip than those of *N. ceciliae*. Regarding stripe position near to the caudal end of the body, *N. pseudoceciliae* differs from *N. ceciliae* and other species of the complex, except *N. graffi*, in that the paramedian and lateral stripes converge on both sides of the body, prior to the posterior end.

Characteristics of external morphology easily separate *N. arturi* as well as *N. guaiana*, *N. muelleri* and *N. fita* (Froehlich & Leal-Zanchet 2003; Leal-Zanchet & Froehlich 2006), from the other species of group 2. *Notogynaphallia arturi* presents a distinctive colour pattern, due to the presence of two wide lateral bands on the dorsum.

Eye distribution throughout body length is similar, in *N. pseudoceciliae* and *N. arturi*, to most species of group 2, which, except for *N. fita* and *N. guaiana*, present eyes spreading dorsally in at least some portion of the body between the anterior tip and the pharynx (Froehlich & Leal-Zanchet 2003).

On comparing *N. pseudoceciliae* and *N. arturi* with the other above-cited species presenting a striped dorsum, i.e. *N. modesta*, *N. quinquestriata* and *P. bohlsi*, we can distinguish two patterns*: N. pseudoceciliae* and *N. quinquestriata*, which are five-striped, and *N. modesta, P. bohlsi* and *N. arturi* with two lateral bands limited by lateral and medial darker stripes.

As already stated by Froehlich and Leal-Zanchet (2003), when describing *N. ceciliae*, the five-striped *N. quinquestriata* presents marginal instead of lateral stripes, these surrounding the whole body including the anterior and posterior tips. In addition these marginal stripes are wider and darker than the median and paramedian ones. These characteristics as well as the occurrence of exclusively marginal eyes, are, in our opinion, enough to distinguish *N. pseudoceciliae* and *N. quinquestriata*. Furthermore, the distribution of *N. quinquestriata* in Barro Colorado Island, Panama, is an indication of the impossibility of conspecificity in *N. pseudoceciliae* and *N. quinquestriata*, due to the high degree of endemism generally presented by land planarians, as mentioned by Froehlich and Leal-Zanchet (2003), for *N. ceciliae*, another species of the complex.

For the description of *Geoplana bohlsi* from Asunción, Paraguay, Graff (1899, p. 333) studied two fragments of a specimen without either of the two extremities, the stage of development being unknown. The dorsum presented a dark median line and two grey bands, one on each side and on a yellowish-background. Both margins of grey bands, the external and internal, the latter twice as wide as the former, were darker and, as seen in figure 17 (Taf. VII), rather clearly defined. Graff (1899) commented that if the grey between them had been fainter, one would have been able to consider the worm as being five-striped. As already commented by Leal-Zanchet and Froehlich (2006), Marcus (1951) suggested that *G. bohlsi* was probably a synonym of the species renamed as *N. graffi*. Leal-Zanchet and Froehlich (2006) corroborated the assignment of *G. bohlsi* by

Ogren and Kawakatsu (1990) to the collective group *Pseudogeoplana*. *N. arturi* and *P. bohlsi* displayed a very similar pattern. Graff (1899) considered it a very peculiar pattern, in such a way that he stated it would not be difficult to recognize *P. bohlsi* on finding new material. However, our studies with communities of land planarians in South Brazil, indicate that this pattern is not rare; we encountered undescribed species belonging to different genera with a very similar colour pattern (A.M. Leal-Zanchet, personal observation). In addition, the provenance of *P. bohlsi* does not seem to coincide with the distribution of *N. arturi*.

Graff (1899) described *N. modesta*, also from Asunción, Paraguay, based on several specimens which displayed a dark yellow ground colour mixed with brown pigment. They possess two lateral and two paramedian dark stripes, the former being more often darker and wider than the latter. Graff commented that when the lateral and paramedian stripes are of similar width, there is a darker lateral band between the two. However, the eyes distribution pattern in *N. modesta*, seeming to be exclusively marginal, serves to distinguish this species from *N. arturi*. As suggested by Froehlich and Leal-Zanchet (2003) for *N. quinquestriata*, the allocation of *N. modesta* to *Notogynaphallia* has to be taken as a tentative measure, although it would be better to include this among the species gathered by Ogren and Kawakatsu (1990) into the collective group *Pseudogeoplana*.

Internal anatomy

As regards morphology of the pharynx, *N. pseudoceciliae* presents, as is the case of both *N. caissara* and *N. graffi*, a cylindrical type of pharynx. *N. guaiana* stands apart from the other species of group 2 by having a collar-shaped pharynx.

Concerning morphology of the male organs, Leal-Zanchet and Froehlich (2006) assembled the seven species with a striped dorsum of group 2, as follows: on one side, *N. graffi*, *N. caissara* and *N. fita*, which present a very long prostatic vesicle with a comparatively narrow cavity and efferent ducts entering laterally into the prostatic vesicle, and on the other, *N. ernesti* and *N. muelleri*, with a voluminous and compact prostatic vesicle without branches or diverticula, much shorter than in the former species, with also efferent ducts opening into the ental portion of the prostatic vesicle, either terminally or subterminally. In addition, Leal-Zanchet and Froehlich (2006) separate *N. abundans* and *N. ceciliae* from the other species, seeing that they combine characters similar to those of both groups. As regards general aspects of the male organs, *N. pseudoceciliae* is similar to *N. graffi*, *N. caissara* and *N. fita*, being distinguished from the latter by the long bifurcated portions of its prostatic vesicle, as well as by its longer male atrium and slighter projection of the ental wall. The long bifurcated portions of the prostatic vesicle of *N. pseudoceciliae* also discriminate it from *N abundans* and *N. ceciliae*, which have an unpaired prostatic vesicle or only a bifurcation of the proximal portion of this organ. On considering the above-mentioned characteristics, *N. arturi* can be assembled with *N. ernesti* and *N. muelleri*, these differing, one from the other, by details of the morphology of the prostatic vesicle and the relative length of both the male and female atria. Nevertheless, *N. muelleri* stands apart from the other two species by presenting an S-shaped vesicle with the ental portion dorsally directed at the point where the efferent ducts open. Furthermore, in this species the length of the male atrium is seven times that of the female one, whereas in *N.ernesti* and *N. arturi* the length of the male atrium is, respectively four and two times that of the female one.

The morphology of the female atrium, with a lining composed of tall epithelium of a multilayered aspect, differentiates *N. pseudoceciliae* from most other species of *Notogynaphallia*, with the exception of *N. guaiana*. Both these species share a prostatic vesicle with long forked portions, although the relative length of the vesicle, varying from 5% to 26% of body-length, is much greater in *N. pseudoceciliae* than in *N. guaiana* (ca. 1% of body-length). In addition, the efferent ducts open into the proximal portions of the prostatic vesicle in *N. guaiana*, and laterally into the ectal third in *N. pseudoceciliae*.

As regards the female atrium, *N. mueleri*, *N. ernesti* and *N. arturi* also present similar general characteristics, differing in certain details such as the length of the female atrium in relation to body-length. The relative length of the female atrium is larger in *N. arturi* (between 3% and 4% of body-length) than in *N. ernesti*

(between 0.7% and l.6% of body-length). In a specimen of *N. muelleri* drawn by Froehlich (1959: Fig. 16), the length of the female atrium corresponds to ca. 0.6% of body-length.

In conclusion, the combination of characters of the external and internal morphology of *N. pseudoceciliae* and *N. arturi*, distinguish them from the other known species of *Notogynaphallia*, as well as from the species discussed above, which are known exclusively from their external morphology.

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