



Article

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Two new species of land flatworms and comments on the genus *Pasipha* Ogren & Kawakatsu, 1990 (Platyhelminthes: Continenticola)

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Abstract

Two new species of Geoplaninae, *Pasipha backesi* Leal-Zanchet, Rossi & Seitenfus, **sp. nov.** and *P. brevilineata* Leal-Zanchet, Rossi & Alvarenga, **sp. nov.**, from southern Brazil are described herein. The two new sympatric species can be distinguished from each other by differences in colour pattern and the copulatory apparatus, especially regarding the prostatic vesicle. They show similar characteristics regarding the relative position of the ovaries and anteriormost testes in relation to body length, the position where ovovitelline ducts emerge from the ovaries, pharyngeal morphology and general morphology of the copulatory apparatus. Comments on the most important morphological characters of the 22 species originally assigned to the genus *Pasipha* are presented in order to highlight the heterogeneity of the genus as well as facilitate the comparative discussion of the new species. In addition, based on the current diagnosis of the genus, we propose the placement of *G. cafusa* into *Pasipha*.

Key words: Geoplaninae, taxonomy, land planarians, triclad

Introduction

The subfamily Geoplaninae currently encompasses 17 genera, besides the collective group *Pseudogeoplana* Ogren & Kawakatsu, 1990 (Ogren & Kawakatsu, 1990, Grau *et al.*, 2012). Many of these genera are based solely on an analysis of the literature, considering the informal groups outlined by E.M. Froehlich (1955) and Froehlich (1967) (Ogren & Kawakatsu, 1990, Grau *et al.*, 2012). This is the case, for example, of the genera split from *Geoplana*, which include *Amaga*, *Gigantea*, *Notogynaphallia* and *Pasipha*. The three former taxa have been discussed in recent papers (Froehlich & Leal-Zanchet, 2003, Leal-Zanchet & Froehlich, 2006, Carbayo, 2008, 2010, Grau *et al.*, 2012), whereas the situation of the genus *Pasipha* has yet to be adequately addressed.

The genus *Pasipha* was created for the Geoplaninae species that have an elongate body, a folded male atrium, a female canal approaching from the ventral side, and lack a (permanent) penis papilla. The absence of unique diagnostic features results in a heterogeneous group of 22 species. The known distribution of the genus includes localities in Brazil, Chile, Peru and Panama.

In our evaluation of the land planarian communities from areas of *Araucaria* Forest of three conservation units in Southern Brazil, we recorded the occurrence of five species that were tentatively assigned to the genus *Pasipha* (Leal-Zanchet *et al.*, 2011). Here we describe two of these species and comment on other species included in the genus in order to highlight its heterogeneity and facilitate comparative discussion of the species.

Material and Methods

Specimens of *P. backesi* Leal-Zanchet, Rossi & Seitenfus, **sp. nov.** and *P. brevilineata* Leal-Zanchet, Rossi & Alvarenga, **sp. nov.** were collected from São Francisco de Paula (National Forest of São Francisco de Paula) (29°23'–29°27'S; 50°23'–50°25'W), located in the state of Rio Grande do Sul, Brazil. Sampling took place in areas of Mixed Ombrophilous Forest, as well as in areas with *Araucaria* plantations, and in the neighborhood of man-made buildings in this National Forest.

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

The ratio of the height of the cutaneous musculature to the height of the body (mc:h index in Froehlich, 1955) was determined in the median region of a transverse section of the pre-pharyngeal region. Mesenchymatic muscle fibers were counted in transverse sections of the same region. Colour descriptors, based on the uptake of dyes of particular colours, were used for classifying secretions with trichrome methods: erythrophil, xanthophil and cyanophil. The term cyanophil is also applied to secretions that have an affinity for the green dye of Goldner's Masson.

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

Taxonomic Part

Family Geoplanidae Stimpson, 1857

Subfamily Geoplaninae Stimpson, 1857

Genus *Pasipha* Ogren & Kawakatsu, 1990

***Pasipha backesi* Leal-Zanchet, Rossi & Seitenfus, sp. nov.**

Pasipha sp. 1: Leal-Zanchet & Carbayo, 2000

Pasipha sp. 1: Leal-Zanchet & Baptista, 2009

Pasipha sp. 2: Leal-Zanchet *et al.*, 2009

Pasipha sp. 1: Leal-Zanchet *et al.*, 2011

Etymology: the specific name is in honor of Prof. Dr. Albano Backes and his worthy contribution to the knowledge of the ecology of the *Araucaria* Forest.

Type-material.

Holotype: MZUSP PL.1201: *leg.* M. Cardoso, 31. July 1998, São Francisco de Paula, RS, Brazil—anterior tip: transverse sections on 12 slides; anterior region at the level of the ovaries: sagittal sections on 15 slides; pre-pharyngeal region: transverse sections on 10 slides; pharynx: sagittal sections on 15 slides; copulatory apparatus: sagittal sections on 19 slides;

Paratypes: MZU PL.127: *leg.* F. Carbayo, 19. October 1996, São Francisco de Paula, RS, Brazil—pre-pharyngeal region: transverse sections on 8 slides; pharynx and copulatory apparatus: sagittal sections on 39 slides; MZU PL.128: *leg.* R. Murowaniecki, 10. July 1998, São Francisco de Paula, RS, Brazil—anterior region at the level of the ovaries: horizontal sections on 7 slides; pre-pharyngeal region: transverse sections on 5 slides; pharynx: sagittal sections on 9 slides; copulatory apparatus: sagittal sections on 14 slides; MZU PL.129: *leg.* L.B. Matos, 05. October 2003, São Francisco de Paula, RS, Brazil—anterior tip: transverse sections on 19 slides; pre-pharyngeal region: transverse sections on 6 slides; pharynx: sagittal sections on 11 slides; copulatory apparatus: horizontal sections on 8 slides.

Diagnosis:

Dorsum brown with yellowish median stripe, bordered by dark-brown to black paramedian stripes; eyes dorsal, with clear halos; glandular border with three cell types; mc:h, 18%–19%; pharynx cylindrical; esophagus conspicuous; esophagus: pharynx ratio, 17%–20%; anteriormost testes posterior to ovaries; most posterior testes near root of pharynx; sperm ducts opening dorsally displaced into lateral walls of proximal region of vesicle; prostatic vesicle globose, extrabulbar, consisting of two distinct regions: a forked ental region and a large unforked ectal region; ejaculatory duct sinuous; male atrium long, highly folded, with two main regions; ovovitelline ducts emerging dorsally, but laterally displaced, from posterior half of ovaries, and uniting below female atrium; common glandular ovovitelline duct posteriorly directed, slightly ascendent; female canal almost vertical; female atrium oval-elongate, highly folded; length of male atrium, about two times that of female atrium; gonopore canal

slightly posteriorly inclined; male and female atria with independent musculatures; no folds separating male and female atria.

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

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

TABLE I. Measurements, in mm, of specimens of *Pasipha backesi* Leal-Zanchet, Rossi & Seitenfus, **sp. nov.** –: not measured; *: After fixation; 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.1201	Paratype MZU PL.127	Paratype MZU PL.128	Paratype MZU PL.129
Maximum length in extension	33	-	49	28
Maximum width in extension	1.5	-	2.0	1.5
Length at rest	-	28	23	13
Width at rest	-	5.0	4.0	2.0
Length*	38	28	26	18
Width*	3.0	2.0	2.5	2.0
DM*	27 (71)	13 (46)	18 (69)	13 (72)
DG*	31 (82)	20 (71)	22 (85)	14.5 (80)
DMG*	4	7	4	3
DPVP*	0	-	0	-
Creeping sole %	95	-	98	-
Ovaries	4.5 (12)	-	6 (23)	-
Anteriormost testes	5.7 (15)	-	7 (27)	-
Posteriormost testes	26 (68)	-	17 (65)	-
Prostatic vesicle	0.5	-	0.3	-
Male atrium	1.9	-	1.5	-
Female atrium	1.1	-	0.4	-

Description

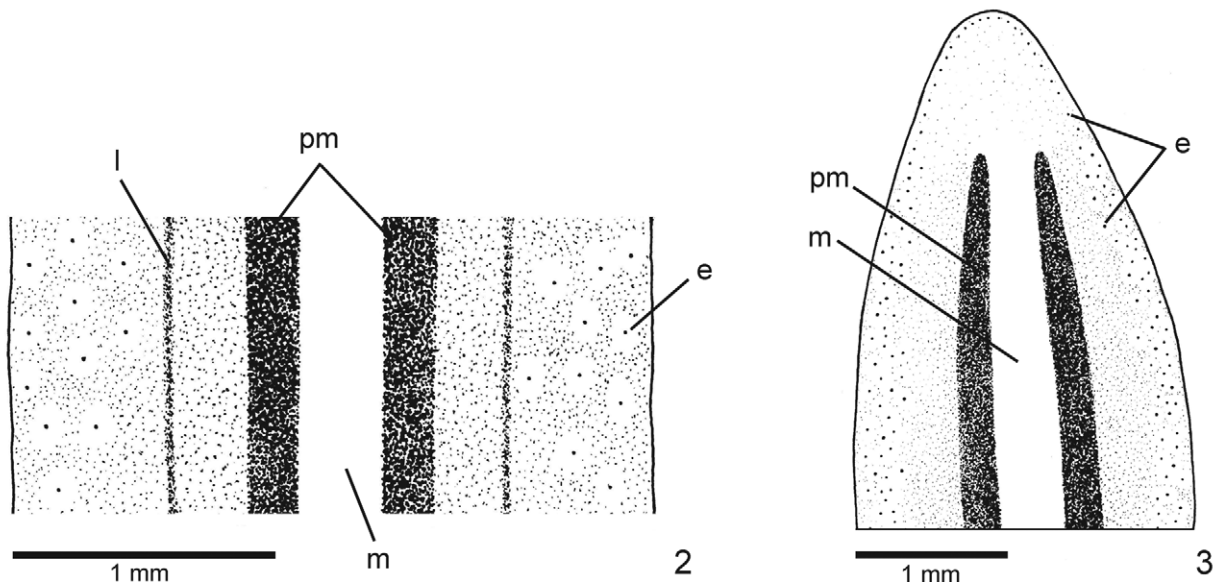
External morphology

Body elongate with parallel margins. When creeping, body becomes very slender and sub-cylindrical in cross-section; maximal length reaches 33 mm in the holotype (Table 1). Mouth distance from anterior tip, 71%; gonopore distance from anterior tip, 82% relative to body length in the holotype (Table 1). Alive, dorsum brown with yellowish median stripe, bordered by dark-brown to black paramedian stripes (Fig. 1). Under the stereomicroscope, pale-brown dorsal ground-colour visible on anterior tip and body margins. Except for the anterior tip, body margins and the yellowish median stripe, dorsal ground colour overlain by dense dark-brown pigmentation. It concentrates on bordering the median stripe, forming two black paramedian stripes (Fig. 2). This pigmentation also concentrates more laterally, constituting two dark-brown lateral stripes. Ventral surface greyish pale yellow with dark grey anterior tip. After fixation, anterior tip pale yellow (first 1 mm in both the holotype and paratype MZU PL.128, corresponding to 3% and 4% of body length, respectively) with overlaying pigmentation being absent. Median and paramedian stripes begin approximately at 1 mm from anterior tip in both the holotype (Fig. 3) and paratype MZU PL.128. Both stripes end close to posterior tip where median stripe gradually becomes thinner and paramedian stripes converge. Lateral stripes less conspicuous after fixation, especially on anterior and posterior thirds of the body. Median stripe with variable width; maximum width about 0.4 mm or 0.8 mm, corresponding to 15% or 36% of body width in the holotype and paratype MZU PL.128, respectively.



FIGURE 1. *Pasipha backesi* Leal-Zanchet, Rossi & Seitenfus, **sp. nov.**: photograph of a live specimen (paratype MZU PL.127) in dorsal view.

Eyes, initially uniserial, surround anterior tip. After the first millimeter, eyes are larger and become pluriserial. They extend over the dorsal surface, being surrounded by clear halos. Three to five eyes occur on the same transversal level, occupying about 0.3 mm on each side of the dorsal surface (11% of body width) (Figs. 2–3). Eyes become less numerous towards posterior tip, but are still dorsal and surrounded by clear halos next to this tip.



FIGURES 2–3. *Pasipha backesi* Leal-Zanchet, Rossi & Seitenfus, **sp. nov.**, holotype: (2) colour pattern of the median third of the body of the live specimen in dorsal view; (3) detail of eyes pattern in the anterior body third of the preserved specimen in dorsal view. (e) eyes, (l) lateral stripes, (m) median stripe; (pm) paramedian stripes.

Internal morphology

Anterior region

Sensory pits, as simple invaginations, about 20 μm to 25 μm deep. They contour anterior tip, occurring initially at intervals of about 18 μm . The sensory pits become gradually sparser posteriorly, until they disappear approximately 4 mm from anterior tip (about 10% of body length). Eyes, approximately 15 μm to 35 μm in diameter, contour the anterior tip in a single row; then they run along both sides of the body. Cutaneous

musculature with different arrangement to that of pre-pharyngeal region (see next section). The ventral longitudinal cutaneous muscles form a thick median bundle on the first millimeter of the body. This median bundle is four times thicker than laterally and about two times thicker than the dorsal cutaneous musculature at the same transversal level (Figs. 4–5). The ventral cutaneous longitudinal muscles gradually become more developed laterally, reaching the same thickness as medianly about 1 mm from anterior tip. Mesenchymal musculature poorly developed on anterior tip, comprising fibers in various directions; it becomes similar to that of the pre-pharyngeal region approximately 1 mm from anterior tip. Openings of rhabditogen cells are absent and four types of secretory cells open throughout entire body margin on anterior tip: abundant cells with granular erythrophil secretion of two types (densely arranged heavily stained granules and sparse, weakly stained granules), two types of cyanophil cells (one with fine granular secretion and the other with amorphous secretion). Openings of glands producing coarse granular xanthophil secretion appear on body margins about 0.3 mm posterior to the anterior tip. Erythrophil cells, xanthophil cells, cyanophil cells with amorphous secretion and rhabditogen cells open through the dorsal surface, three of them also through ventral surface (erythrophil, cyanophil and rhabditogen cells), approximately 1.6 mm after the tip. Four types of cells become more numerous on body margins, thus forming the glandular margin, viz. cells with densely arranged heavily stained erythrophil secretion, cells with sparse weakly stained erythrophil granules, xanthophil cells and cyanophil cells.

Epidermis and musculature at pre-pharyngeal region

Width of creeping sole, 95% of body width in the holotype (Table 1). Three types of secretory cells discharge through dorsal epidermis and body margins: (1) numerous cells with coarse granular xanthophil secretion; (2) rhabditogen cells with xanthophil secretion; (3) cells with amorphous cyanophil secretion. Creeping sole receives abundant amorphous cyanophil secretion, coarse granular xanthophil secretion, and scarce xanthophil secretion from rhabditogen cells. Glandular margin (Fig. 6) receiving three types of secretion: coarse, densely arranged erythrophil granules, sometimes coalescing to an amorphous mass; coarse granular xanthophil secretion; rod-shaped cyanophil secretion.

Cutaneous musculature with the usual three layers (circular, oblique and longitudinal layers), longitudinal layer with thick bundles (Table 2). At the sagittal plane, ventral musculature higher than dorsal. Musculature higher medially, becoming progressively lower towards body margins. Mc:h 18% in the holotype (Table 2).

TABLE II. Body height and cutaneous musculature in the median region of a transversal section of the pre-pharyngeal region, in micrometers, and ratio of the height of cutaneous musculature to the height of the body (mc:h index) of specimens of *Pasipha backesi* Leal-Zanchet, Rossi & Seitenfus, **sp. nov.**

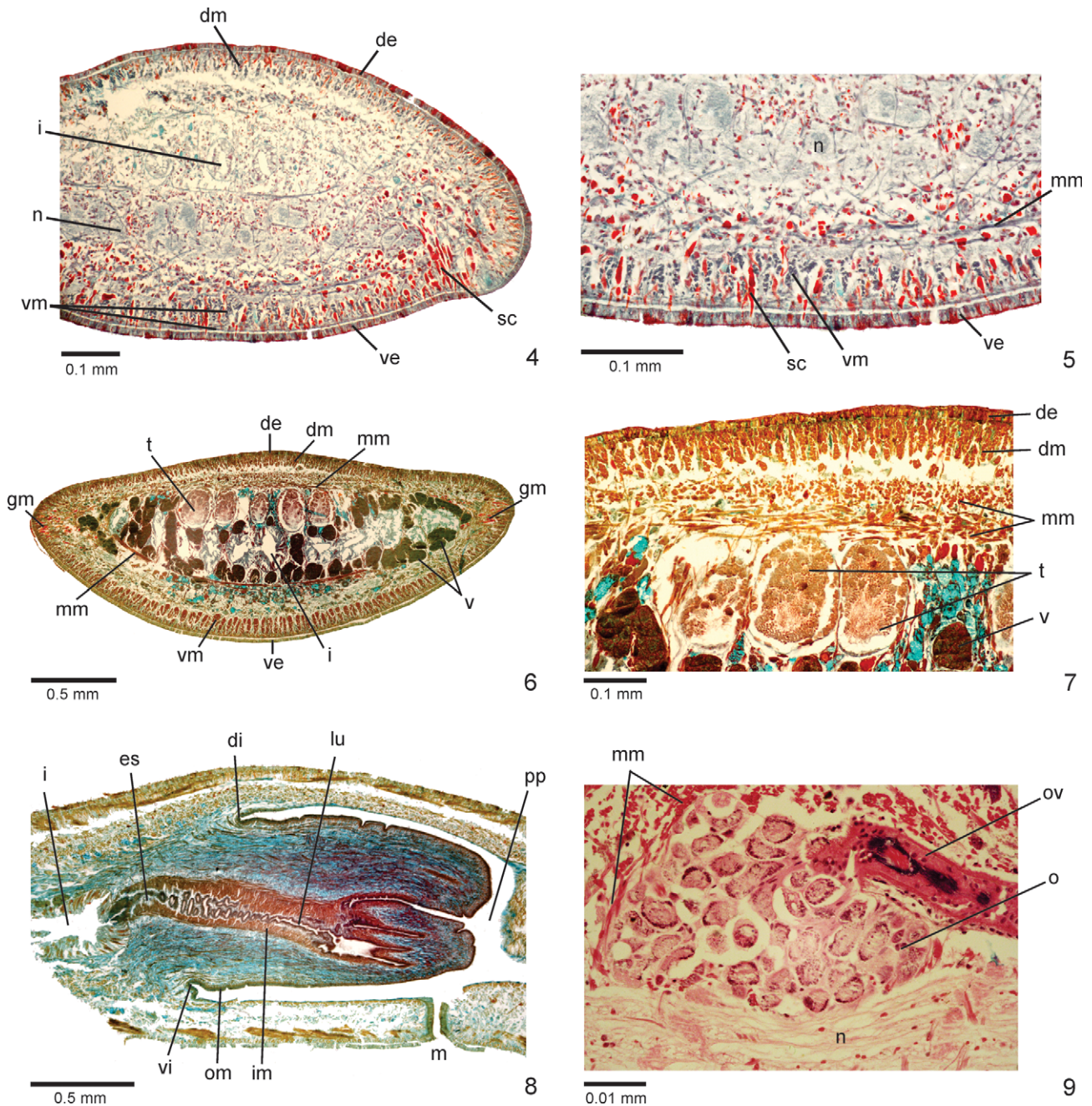
Specimens	Holotype MZUSP PL.1201	Paratype MZU PL.128	Paratype MZU PL.129
Dorsal musculature	78	72	33
Ventral musculature	124	68	78
Body height	1125	750	625
Mc:h	18%	19%	18%

Mesenchymal musculature (Figs. 6–7) well developed, mainly composed of three layers: (1) dorsal subcutaneous, located mainly close to the cutaneous musculature, with oblique fibers variously oriented (about 3–10 fibers thick), some of them very much inclined; (2) supra-intestinal transverse (about 2–8 fibers thick); (3) sub-intestinal transverse (approximately 2–8 fibers thick). In addition, there are scattered transverse subneural fibers, ventral subcutaneous oblique fibers, as well as dorsoventral ones.

Pharynx

Pharynx cylindrical with dorsal insertion slightly posteriorly displaced (Fig. 8). Mouth at end of median third of pharyngeal pouch. Esophagus conspicuous; it is lined by ciliated cuboidal epithelium with insunk nuclei. The esophagus is coated with a thick circular subepithelial muscle layer, followed by longitudinal fibers. Esophagus: pharynx ratio, 20% in the holotype. Pharynx and pharyngeal lumen lined by ciliated cuboidal epithelium with

insunk nuclei. Pharyngeal glands of three types: abundant cells with strong erythrophil, fine granular secretion; cells with amorphous cyanophil secretion; and cells with densely arranged, fine granular, weakly erythrophil secretion. Cell bodies of pharyngeal glands located in the mesenchyme, anterior, lateral and posteriorly to pharynx. Pharyngeal outer musculature (about 21 μm 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 (about 56 μm thick) comprises a thick circular subepithelial layer, followed by longitudinal fibers. Inner musculature gradually becomes thinner towards pharyngeal tip.



FIGURES 4–9. *Pasipha backesi* Leal-Zanchet, Rossi & Seitenfus, **sp. nov.**, holotype: (4) anterior region of the body in transverse section; (5) detail of ventral surface of the anterior region of the body in transverse section; (6) pre-pharyngeal region in transverse section; (7) detail of the dorsal surface of the pre-pharyngeal region in transverse section; (8) pharynx in sagittal section; (9) ovary in sagittal section. (de) dorsal epidermis, (di) dorsal insertion, (dm) dorsal cutaneous musculature, (es) esophagus, (gm) glandular margin, (i) intestine, (im) internal musculature, (lu) pharyngeal lumen, (m) mouth, (mm) mesenchymal muscles, (n) nerve plate, (o) ovary, (om) outer musculature, (ov) ovovitelline duct, (pp) pharyngeal pouch, (sc) secretory cells, (t) testes, (v) vitellaria, (ve) ventral epidermis, (vi) ventral insertion, (vm) ventral cutaneous musculature.

Reproductive organs

Testes in three irregular rows dorsal or dorso-interstitial to the intestinal branches on each side of the body (Figs. 6–7). They begin slightly posterior to the ovaries and extend near to the root of the pharynx (Table 1). Sperm ducts medial to ovovitelline ducts in pre-pharyngeal region. They form spermiducal vesicles slightly anterior to pharynx. Spermiducal vesicles extend laterally to the penis bulb, turn anteriorly, ascend and enter laterally into proximal region of the prostatic vesicle. Extrabulbar prostatic vesicle, adjacent to the pharyngeal pouch. Prostatic vesicle globose, with narrow lumen due to its folded wall, presenting two distinct regions, a large ectal region and a short, forked ental region (Figs. 10–12). Ejaculatory duct sinuous, arising ventrally displaced from the ectal region of the prostatic vesicle; it penetrates the muscular coat of the penis bulb and ascends to open into the lower part of the male atrium (Fig. 10). Male atrium long (Table 1, Figs. 10–12), with two anatomically and histologically differentiated main regions. The first region corresponds to the most ental part of the atrium (about 1/5 of male atrium length); it is obliquely disposed and laterally expanded, presenting numerous small folds. The second region shows abundant, tall folds that greatly narrow the whole cavity.

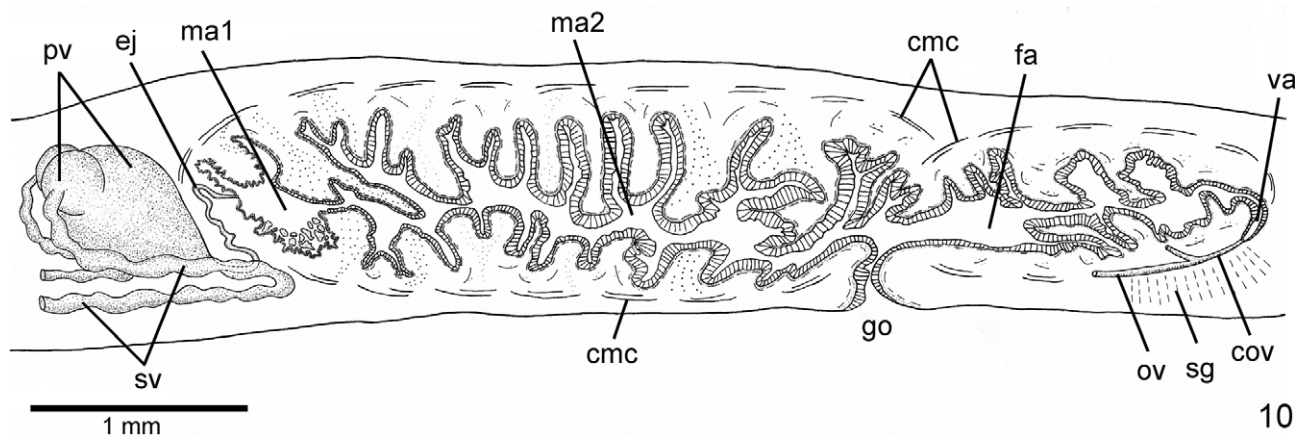


FIGURE 10. *Pasipha backesi* Leal-Zanchet, Rossi & Seitenfus, **sp. nov.**, holotype: sagittal composite reconstruction of the copulatory apparatus. (cmc) common muscle coat, (cov) common glandular ovovitelline duct, (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ma1) ental region of male atrium, (ma2) ectal region of male atrium, (ov) ovovitelline ducts, (pv) prostatic vesicle, (sg) shell glands, (sv) spermiducal vesicle, (va) vagina.

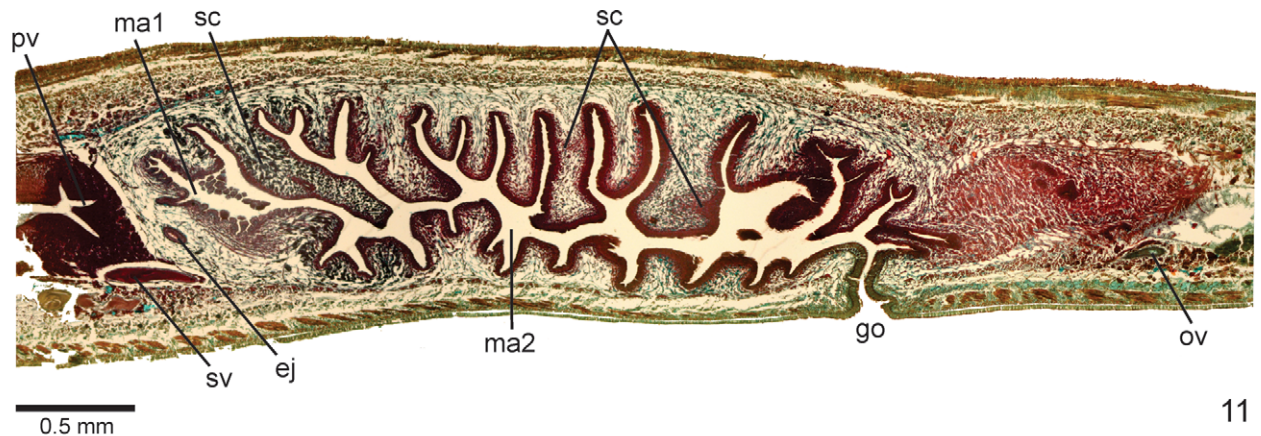
Lining epithelium of sperm ducts cuboidal and ciliated; thin muscularis (4 μm) mainly constituted of circular fibers. Prostatic vesicle lined by ciliated columnar epithelium, traversed by numerous glands with erythrophil secretion arising from cell bodies in the surrounding mesenchyme. Muscularis of prostatic vesicle (about 50 μm –110 μm thick) comprises interwoven longitudinal, oblique and circular fibers. The ental region of prostatic vesicle receives fine granular and relatively weakly stained erythrophil secretion, whereas the ectal region receives a coarse, heavily stained erythrophil secretion. Ejaculatory duct lined by ciliated columnar or pseudo-stratified epithelium, receiving few openings from secretory cells with weakly cyanophil, amorphous secretion. These glands show subepithelial cell bodies. Muscle coat of ejaculatory duct thin (5 μm thick), mainly constituted of circular fibers.

Region 1 of male atrium lined by a columnar to cuboidal, ciliated epithelium, irregular in height; region 2 by a non-ciliated columnar epithelium, which gradually increases in height distally. The epithelial lining of distal half of the male atrium shows an irregular height and contains an irregular xanthophil apical surface. Region 1 with scattered openings from secretory cells producing an amorphous, cyanophil secretion, as well as cells showing weakly erythrophil fine granular secretion. Both glands with subepithelial cell bodies.

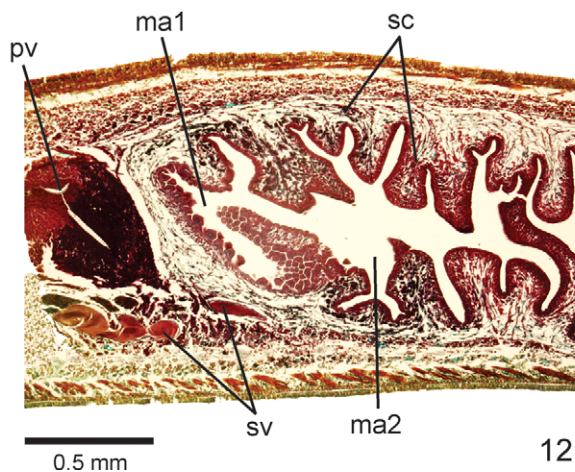
Region 2 of male atrium receives secretions of at least six types: (I) coarse granular erythrophil secretion; (II) fine granular, weakly stained, erythrophil secretion; (III) coarse granular xanthophil secretion; (IV) coarse granular, heavily stained cyanophil secretion; (V) amorphous, heavily stained cyanophil secretion. Cell type I is the most numerous one in the proximal third of region 1; types I and II are more abundant in the median third; and types III and IV are the most characteristic and numerous cells in the distal third of region 1. Cell type V occurs in the ental and median thirds. Cell types I, III, and IV have their cell bodies external to the common muscle coat; cell types II and V have their cell bodies located internally to the common muscle coat.

Muscularis of region 1 (about 5 μm thick) comprises circular fibers mixed with few longitudinal fibers. Muscularis of region 2 with similar thickness throughout the entire region (25 μm –30 μm) and constituted of circular subepithelial and subjacent longitudinal fibers, partially interwoven.

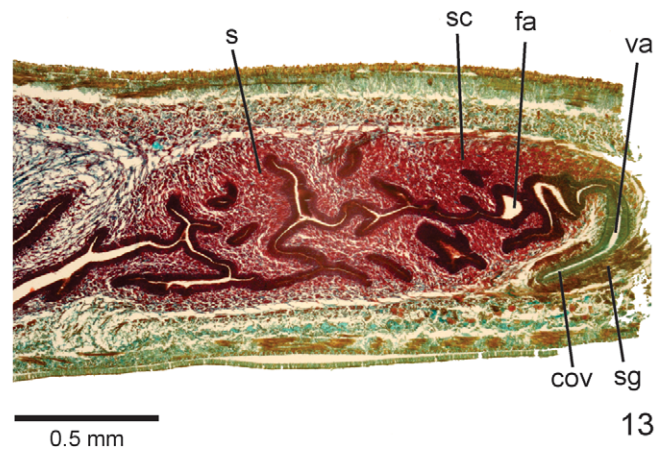
Ovaries ovoid, measuring about 0.25 mm anterior-posteriorly, 0.2 mm dorso-ventrally in the holotype. Ovitelline ducts emerge dorsally from the posterior half of ovaries (Fig. 9), slightly laterally displaced, and run posteriorly immediately above the nerve plate. Below proximal third of female atrium, the ovitelline ducts unite to form a common glandular ovitelline duct (Figs. 10, 13). This slightly ascendent duct runs posteriorly, contouring the common muscle coat and, subsequently, opens into the almost vertical female canal (vagina). The latter enters the common muscle coat to open into the end of the female atrium (postflex condition with ventral approach) (Figs. 10, 13). Female atrium oval-elongate with highly folded walls. Length of female atrium about half of male atrial length (Table 1).



11



12



13

FIGURES 11–13. *Pasipha backesi* Leal-Zanchet, Rossi & Seitenfus, **sp. nov.**, holotype: (11) copulatory apparatus in sagittal section; (12) detail of prostatic vesicle and ental half of male atrium in sagittal section; (13) female atrium in sagittal section. (cov) common glandular ovovitelline duct, (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ma1) ental region of male atrium, (ma2) ectal region of male atrium, (ov) ovovitelline duct, (pv) prostatic vesicle, (s) stroma, (sc) secretory cells, (sg) shell glands, (sv) spermiducal vesicle, (va) vagina.

Paired ovovitelline ducts as well as common ovovitelline duct lined by cuboidal to columnar ciliated epithelium and covered with intermingled circular and longitudinal muscle fibers. Abundant shell glands empty into common ovovitelline duct as well as in the distal sections of the ovovitelline ducts.

Female atrium lined by a tall-columnar epithelium, with irregular height and xanthophil apical surface. Four types of secretory cells open through the epithelium of the female atrium: (1) abundant cells with coarse granular erythrophil secretion; (2) cells with coarse granular xanthophil secretion; (3) cells with amorphous cyanophil secretion; (4) cells with fine granular, chromophobic secretion. Xanthophil cells show cell bodies external to the common muscle coat. The other glands have their cell bodies located internally to the common muscle coat. Muscularis of female atrium thin (about 5 μm –10 μm), comprising interwoven circular and longitudinal fibers. This muscularis is poorly defined against the atrial stroma.

Female canal lined by tall-columnar to pseudo-stratified epithelium, being ciliated in the proximal part of this canal. Lining of the female canal is traversed by coarse granular xanthophil secretion as well as amorphous cyanophil secretion. Both secretions are produced by cells with cell bodies in the surrounding mesenchyme. Glands with fine granular, chromophobic secretion and subepithelial cell bodies also open into the female canal. Muscularis of female canal (about 10 μm –12 μm) composed of circular subepithelial fibers, partially mixed with some longitudinal fibers.

Gonopore canal slightly posteriorly inclined. Male and female atria with independent muscular coats and without separating folds, but female atrium and canal laterally displaced in relation to the gonopore canal (Figs. 10–11). Epithelial lining of gonopore canal columnar ciliated, receiving the openings of cyanophil cells producing an amorphous secretion. Sparse glands containing coarse granular erythrophil secretion also open into the gonopore canal. Muscularis of gonopore canal comprises subepithelial circular fibers and subjacent longitudinal fibers.

Common muscle coat with longitudinal, oblique and circular fibers, slightly thicker around male atrium (about 8–10 fibers thick) than around female atrium (about 6–8 fibers thick). Between atrial muscularis and common muscle coat, a stroma with many, variously orientated well developed muscle fibers (Fig. 13), sometimes forming bundles (*Eigenmusculatur* of Graff, 1899). The common muscle coat is poorly defined from this stroma around female atrium. Mesenchymal muscle fibers of the transverse layers located adjacent to the common muscle coat.

Vitellaria, mainly situated between intestinal branches, open into the ovovitelline ducts.

Remarks: Vitellaria well developed in the holotype and in paratypes MZU PL.127 and MZU PL.128 and inconspicuous in paratype MZU PL.129. The latter was immature, thus presenting small testes and no copulatory organs. Paratype MZU PL.127 was in an initial stage of decomposition when was fixed; its internal morphology shows artifacts. The dorsal and ventral cutaneous musculatures of paratype MZU PL.128 have almost the same height in contrast to the cutaneous musculature of the holotype and paratype MZU PL.129.

***Pasipha brevilineata* Leal-Zanchet, Rossi & Alvarenga, sp. nov.**

Pasipha sp. 3: Leal-Zanchet & Carbayo, 2000

Pasipha sp. 3: Leal-Zanchet & Baptista, 2009

Pasipha sp. 2: Leal-Zanchet *et al.*, 2009

Pasipha sp. 3: Leal-Zanchet *et al.*, 2011

Etymology: the specific name refers to the thin (=brevi) median stripe (=lineata) over the dorsum.

Type-material.

Holotype: MZUSP PL.1202: *coll.* A. M. Leal-Zanchet, 08. September 2000, São Francisco de Paula, RS, Brazil—anterior tip in two fragments: transverse sections on 20 slides; anterior region at the level of the ovaries: sagittal sections on 10 slides; pre-pharyngeal region: transverse sections on 6 slides; pharynx: sagittal sections on 10 slides; copulatory apparatus: sagittal sections on 19 slides;

Paratypes: MZU PL.130: *leg.* F. Carbayo, 14. January 1998, São Francisco de Paula, RS, Brazil—pre-pharyngeal region: transverse sections on 10 slides; pharynx and copulatory apparatus: sagittal sections on 13 slides; MZU PL.131: *leg.* M. Cardoso, 04. May 1998, São Francisco de Paula, RS, Brazil—anterior tip: horizontal sections on 5 slides; pre-pharyngeal region: transverse sections on 11 slides; pharynx: sagittal sections on 16 slides; copulatory apparatus: sagittal sections on 15 slides; MZU PL.132: *leg.* L. S. Teixeira, 08. June 1998, São Francisco de Paula, RS, Brazil—anterior region at the level of the ovaries in two fragments: horizontal sections on 15 slides; pre-pharyngeal region: transverse sections on 4 slides; pharynx: sagittal sections on 7 slides; copulatory apparatus: sagittal sections on 11 slides; MZU PL.133: *coll.* A. M. Leal-Zanchet, 14. January 1999, São Francisco de Paula, RS, Brazil—preserved in ethanol 70°; MZU PL.134: *leg.* M. Cardoso, 23. March 1999, São Francisco de Paula, RS, Brazil—preserved in clove oil; MZU PL.135: *leg.* V. Baptista, 27. May 2004, São Francisco de Paula, RS, Brazil—anterior tip: transverse sections on 20 slides; pre-pharyngeal region: transverse sections on 10 slides; pharynx and copulatory apparatus: sagittal sections on 17 slides; MZU PL.136: *leg.* J. Alvarenga, 05. August 2005, São Francisco de Paula, RS, Brazil—copulatory apparatus: horizontal sections on 13 slides; MZU PL.137: *leg.* L.B. Matos, 05. August 2005, São Francisco de Paula, RS, Brazil—copulatory apparatus: sagittal sections on 10 slides.

Diagnosis:

Dorsum black or dark-brown; median stripe pale yellow to grayish, thin and sometimes interrupted, becoming whitish and wider next to posterior tip; eyes dorsal with clear halos; glandular border with three cell types; mc:h, 17%-19%; pharynx cylindrical; esophagus conspicuous; esophagus: pharynx ratio, 27%-37%; anteriormost testes posterior to ovaries; most posterior testes near root of pharynx; sperm ducts opening into end of proximal region of vesicle; prostatic vesicle, mainly globose, extrabulbar with two distinct regions: a forked, laterally expanded and ventrally disposed ental region and a large unforked ectal region; ejaculatory duct sinuous; male atrium long, intensely folded, with two main regions; ovovitelline ducts emerging dorsally from end of posterior half of ovaries, uniting ventrally to the female atrium; common glandular ovovitelline duct almost vertical, slightly anteriorly arched; female canal posteriorly directed and slightly recurved; female atrium oval-elongate, highly folded; length of male atrium, approximately two times that of female atrium; gonopore canal slightly posteriorly inclined; male and female atria with independent musculatures; dorsal elongated fold separating male and female atria.

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

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

TABLE III. Measurements, in mm, of specimens of *Pasipha brevilineata* Leal-Zanchet, Rossi & Alvarenga, **sp. nov.** -: not measured; --: gonopore absent; *: After fixation; 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.1202	Paratype MZU PL.130	Paratype MZU PL.131	Paratype MZU PL.132	Paratype MZU PL.133	Paratype MZU PL.134	Paratype MZU PL.135	Paratype MZU PL.136	Paratype MZU PL.137
Maximum length in extension	55	33	39	50	43	24	36	37	35
Maximum width in extension	2.0	1.5	1.5	2.5	2.0	1.0	2.0	2.0	2.0
Length at rest	-	-	17	20	23	12	30	10	15
Width at rest	-	-	3.0	4.0	3.0	2.5	3.0	3.0	2.0
Length*	29	18	21	31	27	15	24	25	25
Width*	3.0	2.0	2.0	3.5	2.0	1.5	2.0	2.5	2.5
DM*	20 (69)	13 (72)	15 (71)	21.5 (69)	19 (70)	10 (67)	15.5 (65)	17 (68)	18 (72)
DG*	24 (83)	--	18 (86)	25.5 (82)	22 (81)	12 (80)	19 (79)	21 (84)	21 (84)
DMG*	4.0	--	3.0	4.0	3.0	2.0	3.5	4.0	3.0
DPVP*	0	-	-	0	-	-	0	-	0
Creeping sole %	99	-	-	98	-	-	99	-	-
Ovaries	6.5 (22)	-	-	6.6 (21)	-	-	3.8 (16)	-	-
Anteriormost testes	8.3 (29)	-	-	7.8 (25)	-	-	-	-	-
Posteriormost testes	19 (65)	-	-	20 (64)	-	-	14 (58)	-	-
Prostatic vesicle	0.6	-	-	0.6	-	-	0.5	-	0.3
Male atrium	2.5	-	-	2.5	-	-	2.4	-	1.9
Female atrium	1.0	-	-	1.4	-	-	0.9	-	0.8

Description**External morphology**

Body elongate with parallel margins. When crawling, maximal length reaches 55 mm in the holotype (Table 3). Mouth distance from anterior tip, 69%; gonopore distance from anterior tip, 83% relative to body length in the

holotype (Table 3). Alive, dorsum black or dark-brown with median stripe pale yellow to greyish (ground-colour). The latter is thin and sometimes interrupted, becoming whitish and wider next to posterior tip (Fig. 14). Under the stereomicroscope, dorsal ground-colour visible on anterior tip and body margins as well as forming the thin median stripe. The ground-colour also appears on irregularly distributed spots which are more numerous next to posterior tip, sometimes coalescing with median stripe. Dorsal ground colour overlain by fine and dense black or dark-brown pigmentation. Median stripe begins close to anterior tip, being interrupted or inconspicuous next to the pharynx. After that, the median stripe is still visible until the posterior tip (Fig. 15). Ventral surface greyish with fine pigmentation and dark grey anterior tip. In preserved specimens dorsal colour fades, especially in the anterior tip due to show looser pigmentation than the rest of the dorsum (Fig. 15).



FIGURE 14. *Pasipha brevilineata* Leal-Zanchet, Rossi & Alvarenga, **sp. nov.**: photograph of a live specimen (paratype MZU PL.133) in dorsal view.

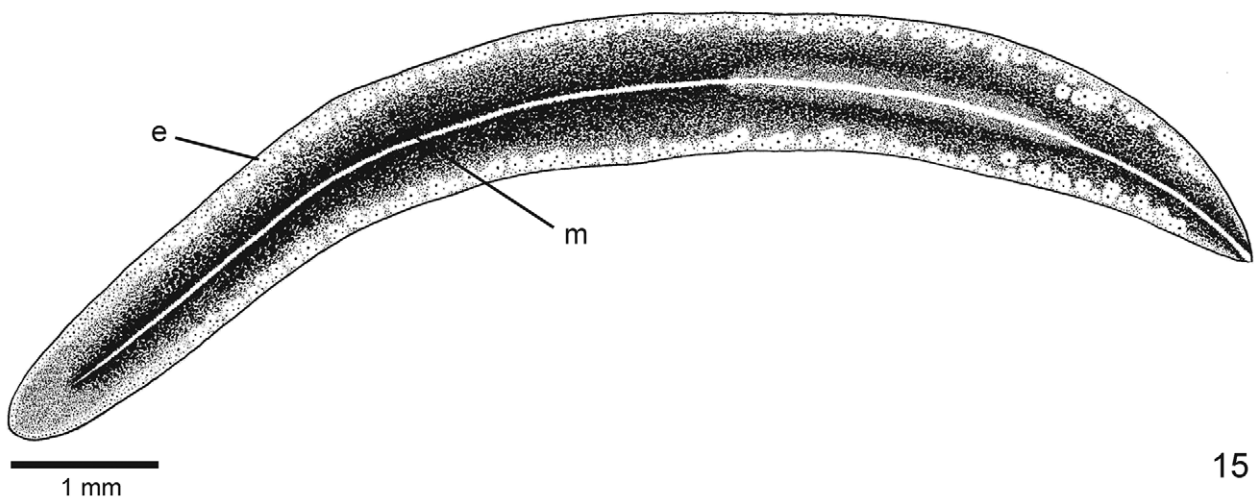
Eyes uniserially surround anterior tip (Fig. 16). After the tip, they become pluriserial and extend over the dorsal surface, being surrounded by clear halos. Three to four eyes occur on the same transversal level, occupying about one-fourth of body width on each side of the dorsal surface. They also occur next to the posterior end (Figs. 15, 17).

Internal morphology

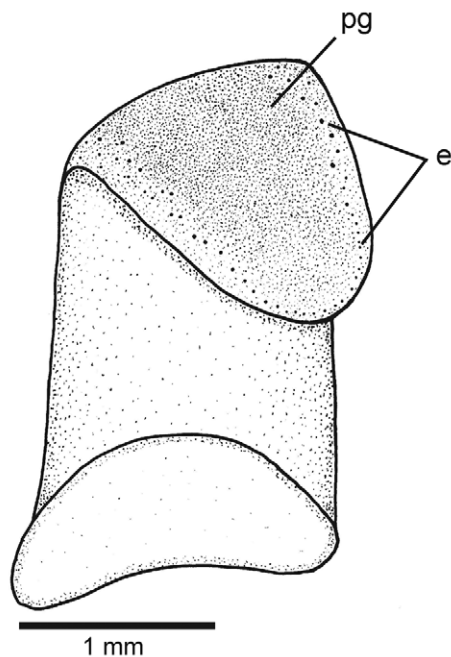
Anterior region

Sensory pits, as simple invaginations (Fig. 18), about 25 μm to 30 μm deep. They occur in the first 5 mm of the body (17% of body length). Eyes (about 20 μm to 35 μm in diameter) contour the anterior tip in a single row, then run along both sides of the body. Cutaneous musculature with different arrangement to that of pre-pharyngeal

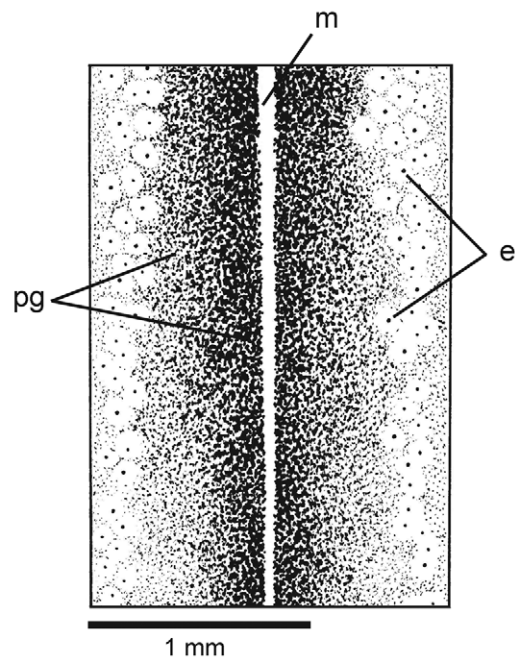
region (see next section). The ventral longitudinal cutaneous muscles form a thick median bundle on first 1.5 mm of body length (Fig. 18); it is about two times thicker than laterally as well as than the dorsal cutaneous musculature at the same transversal level. The ventral longitudinal cutaneous muscles gradually become more developed laterally, reaching the same thickness as medianly about 1.5 mm from anterior tip. Mesenchymal musculature poorly developed on anterior tip and with fibers in various directions. A transverse sub-intestinal mesenchymal muscle layer appears approximately 0.4 mm from anterior tip; oblique, very inclined muscle fibers mixed with some traverse fibers aggregate ventrally. Rhabditogen cells absent on first 0.5 mm of body length. Four types of secretory cells open throughout entire body surface on the anterior tip: weakly stained cyanophil cells with amorphous secretion; heavily stained cyanophil cells with densely arranged, granular secretion; numerous cells with granular xanthophil secretion; and abundant cells with densely arranged, granular erythrophil secretion. Openings of cyanophil cells more numerous on dorsal surface and body margins; openings of erythrophil cells more abundant on ventral surface, being absent on sensory margin. Glands with densely arranged cyanophil secretion joined in bundles throughout dorsal surface on the anterior tip. Erythrophil cells become more numerous on body margins, forming the glandular margin, approximately 1.5 mm from anterior tip. In addition, xanthophil cells gradually become relatively scarcer and rhabditogen cells more numerous on dorsal surface.



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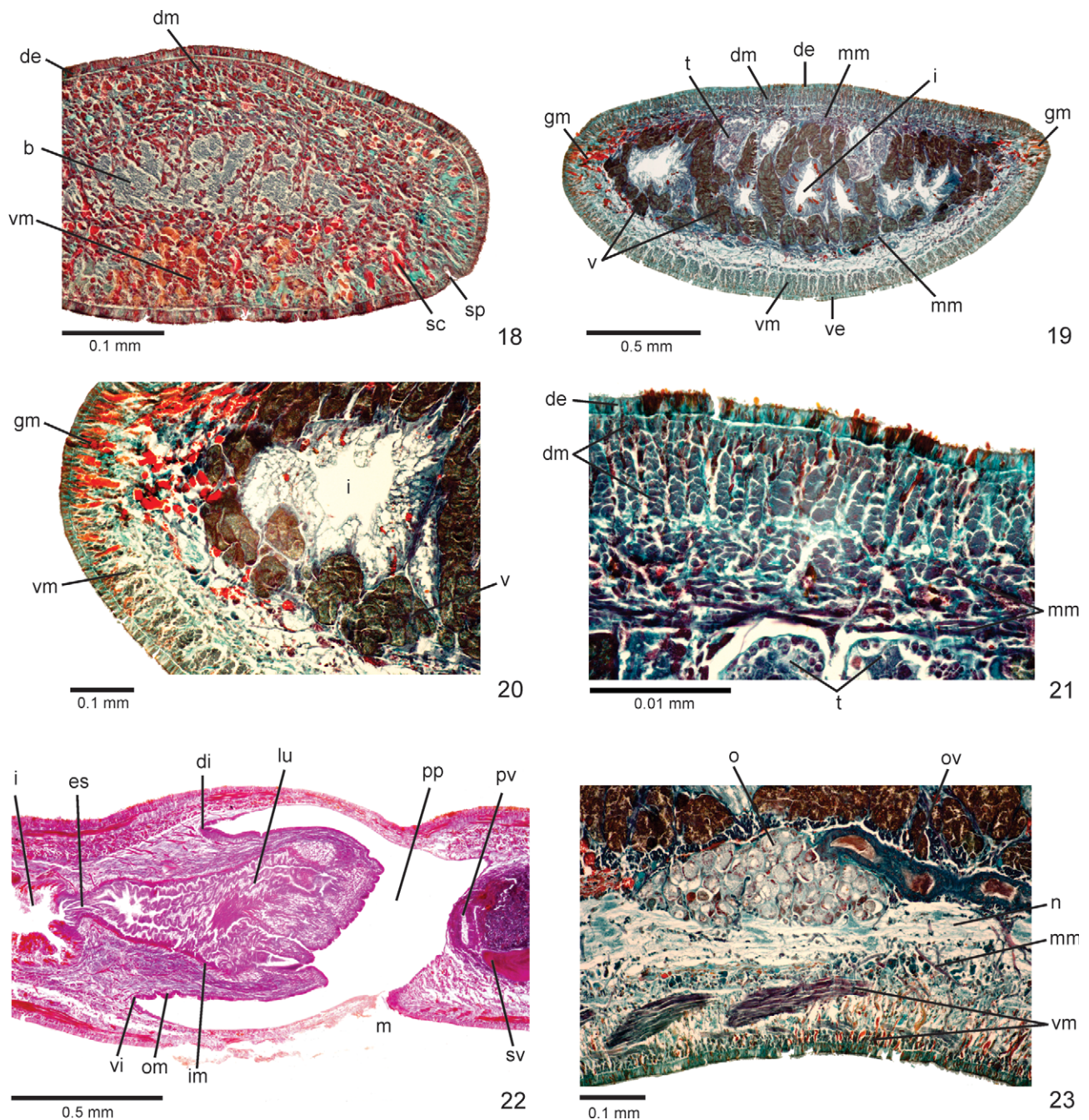


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FIGURES 15–17. *Pasipha brevilineata* Leal-Zanchet, Rossi & Alvarenga, **sp. nov.**: (15) colour pattern of a preserved specimen (paratype MZU PL.136) in dorsal view; (16) detail of eyes pattern of the anterior tip of a preserved specimen (holotype) in dorsal view; (17) detail of colour pattern of the median body third of the body of a preserved specimen (holotype) in dorsal view. (e) eyes, (m) median stripe, (pg) pigmentation.



FIGURES 18–23. *Pasipha brevilineata* Leal-Zanchet, Rossi & Alvarenga, **sp. nov.**, holotype: (18) anterior region of the body in transverse section; (19) pre-pharyngeal region in transverse section; (20) detail of the body margin in the pre-pharyngeal region in transverse section; (21) detail of the dorsal surface of the pre-pharyngeal region in transverse section; (22) pharynx in sagittal section; (23) ovary in sagittal section. (b) brain, (de) dorsal epidermis, (di) dorsal insertion, (dm) dorsal cutaneous musculature, (es) esophagus, (gm) glandular margin, (i) intestine, (im) internal musculature, (lu) pharyngeal lumen, (m) mouth, (mm) mesenchymal muscles, (n) nerve plate, (o) ovary, (ov) ovovitelline duct, (om) outer musculature, (pp) pharyngeal pouch, (pv) prostatic vesicle, (sc) secretory cells, (sv) spermiducal vesicle, (sp) sensory pit, (t) testes, (v) vitellaria, (ve) ventral epidermis, (vm) ventral cutaneous musculature, (vi) ventral insertion.

Epidermis and musculature at pre-pharyngeal region

Creeping sole broad, occupying 99% of body width in the holotype (Table 3). Four types of glands open through dorsal epidermis and body margins: (1) abundant cells producing rod-shaped granular, erythrophil secretion; (2) rhabditogen cells showing xanthophil secretion; (3) cells producing coarse, densely arranged, erythrophil granules, sometimes coalescing to an amorphous masse; (4) cells with amorphous cyanophil secretion. Creeping sole receives abundant amorphous cyanophil secretion; coarse granular, xanthophil secretion; two types of erythrophil secretion, one fine granular and the other with rod-shaped granules. In addition, a small quantity of rhabditogen

cells with xanthophil secretion open through the creeping sole. Glandular margin (Figs. 19–20) comprises four types of secretion: coarse, densely arranged erythrophil granules, sometimes coalescing to an amorphous masse; rod-shaped, erythrophil granules; scarce coarse xanthophil granules; amorphous cyanophil secretion.

Cutaneous musculature tripartite (circular, oblique and longitudinal muscle layers), longitudinal layer with thick bundles (Table 4). At the sagittal plane, ventral musculature higher than dorsal. Musculature higher medially, becoming progressively lower towards body margins. Mc:h 19% in the holotype (Table 4).

TABLE IV. Body height and cutaneous musculature in the median region of a transversal section of the pre-pharyngeal region, in micrometers, and ratio of the height of cutaneous musculature to the height of the body (mc:h index) of specimens of *Pasipha brevilineata* Leal-Zanchet, Rossi & Alvarenga, **sp. nov.**

Specimens	Holotype MZUSP PL.1202	Paratype MZU PL.132	Paratype MZU PL.135
Dorsal musculature	75	60	80
Ventral musculature	98	80	87
Body height	925	800	887
Mc:h	19%	17%	19%

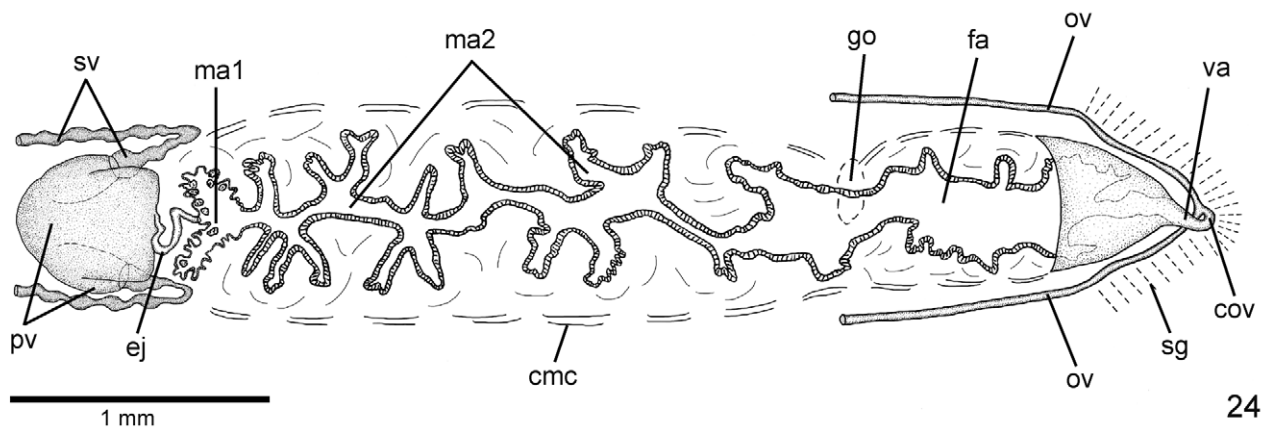
Mesenchymal musculature well developed (Fig. 21), comprised mainly of three layers: (1) dorsal subcutaneous, located mainly close to the cutaneous musculature, with oblique fibers variously oriented (about 8–10 fibers thick), some of them very much inclined; (2) supra-intestinal transverse; (3) sub-intestinal transverse (each about 10–14 fibers thick). In addition, there are scattered transverse subneural fibers, dorsal and ventral oblique fibers, as well as dorsoventral ones.

Pharynx

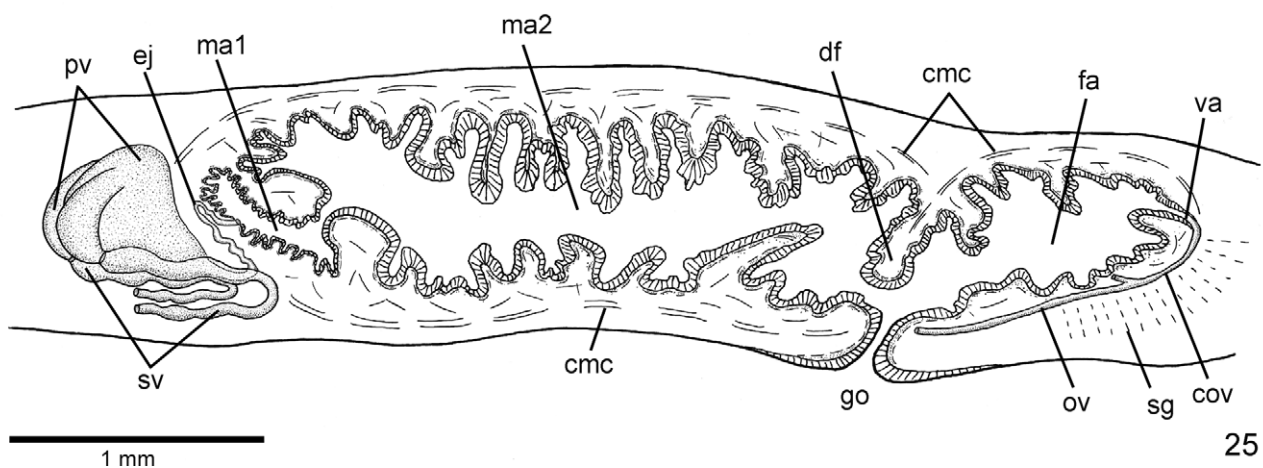
Pharynx cylindrical with dorsal insertion slightly posteriorly displaced (Fig. 22). Mouth located at end of median third or in posterior third of pharyngeal pouch. Esophagus conspicuous (Fig. 22), being lined by cuboidal to columnar ciliated epithelium with insunk nuclei. It is coated with a thick circular subepithelial muscle layer, followed by longitudinal fibers. Esophagus: pharynx ratio, 27% in the holotype. Pharynx and pharyngeal lumen lined by ciliated cuboidal epithelium with insunk nuclei. Pharyngeal glands constituted by three secretory cell types: numerous secretory cells with fine granular erythrophil secretion; cells with amorphous cyanophil secretion; and cells with densely arranged, fine granular xanthophil secretion. Cell bodies of pharyngeal glands located in the mesenchyme, anterior, lateral and posteriorly to pharynx. Pharyngeal outer musculature (10 μm –17 μm thick) comprised of thin subepithelial layer of longitudinal muscles, followed by a thicker circular layer, mixed internally with few longitudinal fibers. Circular layer becomes as thin as longitudinal one towards pharyngeal tip. Inner pharyngeal musculature (about 38 μm thick) comprises a thick circular subepithelial layer, followed by longitudinal fibers. Inner musculature gradually becomes thinner towards pharyngeal tip.

Reproductive organs

Testes in two to three irregular rows dorsal or dorso-interstitial to the intestinal branches (Figs. 19, 21), on each side of the body. They extend from slightly posterior of the ovaries to just anterior of the pharynx (Table 3). Pre-pharyngeally, sperm ducts dorsal to ovovitelline ducts, medially displaced. Sperm ducts begin to form spermiducal vesicles laterally to the pharynx. Spermiducal vesicles extend laterally to the penis bulb, turn anteriorly and enter ventrally into the forked portions of the prostatic vesicle (Figs. 25, 26). Extrabulbar prostatic vesicle, adjacent to the pharyngeal pouch. Prostatic vesicle comprises two parts: a forked, ventrally directed ental portion, tubular in shape, and an ectal portion with narrow lumen due to its folded wall, globose in shape (Figs. 22, 24–27). Ejaculatory duct sinuous, arising ventrally displaced from the ectal portion of the prostatic vesicle (Fig. 27). This duct penetrates the muscular coat of the penis bulb and ascends to open into the lower part of the male atrium. Male atrium long (Table 3, Figs. 24, 25), with two anatomically and histologically differentiated main regions. The most ental part or region 1 corresponds to about 1/5 of male atrium length; it is obliquely disposed and laterally expanded, presenting abundant small folds (Figs. 26, 27). The ectal part or region 2 shows numerous, tall folds that greatly narrow the whole cavity (Fig. 26–28).



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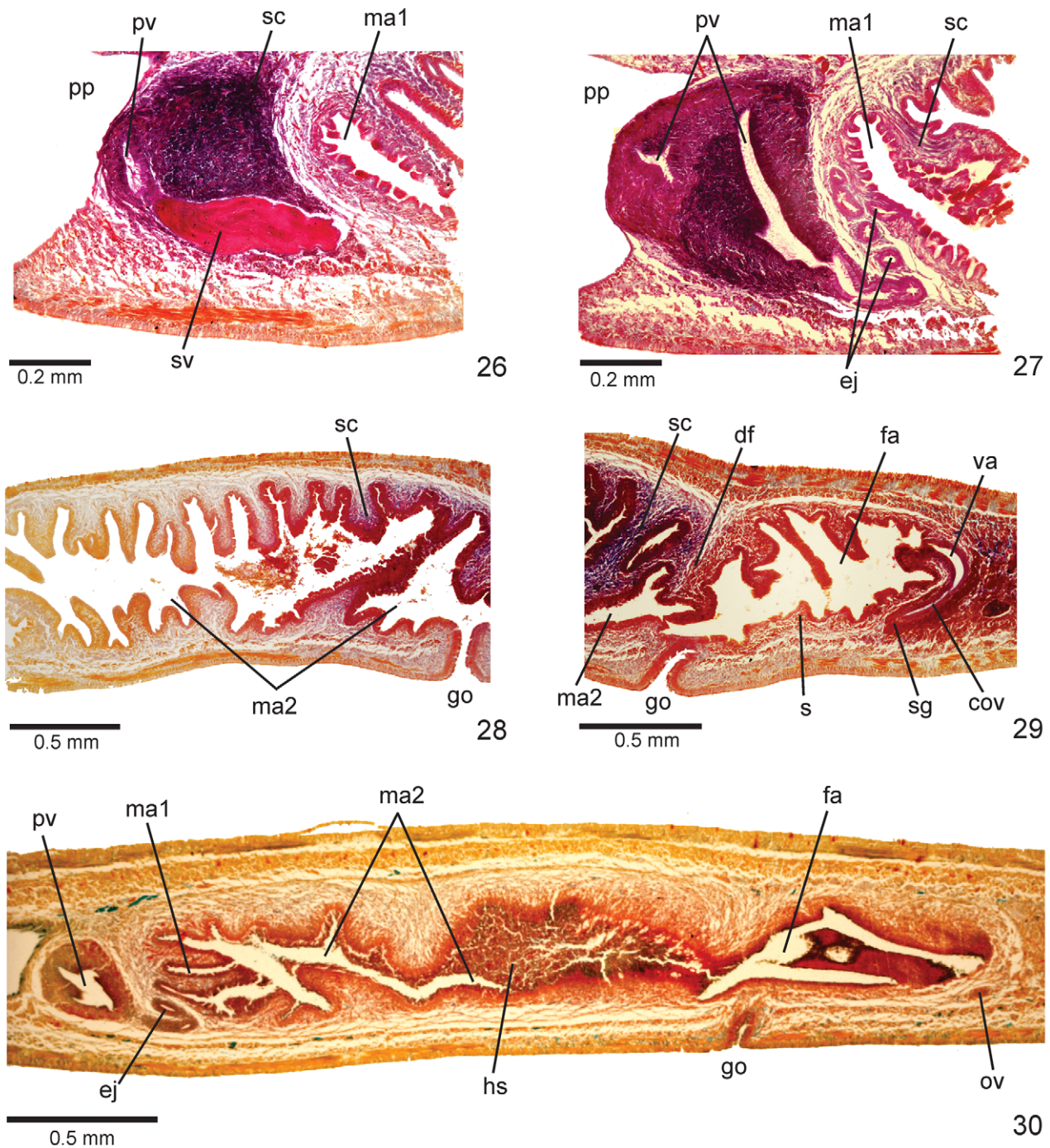
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FIGURES 24–25. *Pasipha brevilineata* Leal-Zanchet, Rossi & Alvarenga, **sp. nov.**: (24) diagrammatic horizontal composite reconstruction of the copulatory apparatus of paratype MZU PL.136; (25) sagittal composite reconstruction of the copulatory apparatus of the holotype. (cmc) common muscle coat, (cov) common glandular ovovitelline duct, (df) dorsal fold separating male and female atria, (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ma1) ental region of male atrium, (ma2) ectal region of male atrium, (ov) ovovitelline ducts, (pv) prostatic vesicle, (sg) shell glands, (sv) spermiducal vesicle, (va) vagina.

Sperm ducts lined by ciliated cuboidal epithelium; they are coated with thin muscularis (3 μm) comprised mainly of circular fibers. Prostatic vesicle lined by columnar ciliated epithelium traversed by abundant glands. These glands comprise two types, one containing fine granular erythrophil secretion and the other producing coarse granular erythrophil secretion. They open into the ental and ectal portions of the prostatic vesicle, respectively. Both cell types have their cell bodies in the surrounding mesenchyme. Muscularis of vesicle (about 45 μm –110 μm thick) comprised of interwoven longitudinal and circular fibers. Ejaculatory duct lined by ciliated columnar epithelium, gradually diminishing its height towards male atrium. This duct receives scarce weakly cyanophil, amorphous secretion. Glands producing this secretion have subepithelial cell bodies. Muscularis of ejaculatory duct thin (5 μm –12 μm thick), mainly comprised of circular fibers.

Male atrium with ental part lined by a columnar to cuboidal, ciliated epithelium, irregular in height; ectal part with columnar, non-ciliated cells, which gradually increase in height distally. The epithelial cells of the ectal part show xanthophil irregular apical secretion, partially broken and discharged into the male atrium cavity. Ental part of male atrium with numerous openings from secretory cells with amorphous, slightly cyanophil secretion. These glands have subepithelial bodies. Ectal part of male atrium receives abundant secretion of at least five types: (I) coarse granular erythrophil secretion; (II) fine granular xanthophil secretion; (III) fine granular, ovoid, erythrophil secretion; (IV) coarse granular heavily stained cyanophil secretion; (V) weakly cyanophil amorphous secretion. Cell type I is the most numerous one in proximal third of the ectal portion of male atrium; cell types II and III are more abundant in the median third; and cell type IV is the most characteristic and numerous one in the distal third of the ectal portion of male atrium. Cell type V opens along the entire length of male atrium. The cell bodies of the five types of glands are located internally to the common muscle coat.

Muscularis with similar thickness throughout the ectal portion of male atrium (12 μm –18 μm), but thinner in the ental portion (12 μm). It is comprised of a circular subepithelial layer and subjacent longitudinal fibres, partially interwoven.



FIGURES 26–30. *Pasipha brevilineata* Leal-Zanchet, Rossi & Alvarenga, **sp. nov.**: (26) opening of spermiducal duct into one of the paired portions of prostatic vesicle of the holotype in sagittal section; (27) detail of prostatic vesicle and ejaculatory duct of the holotype in sagittal section; (28) male atrium of the holotype in sagittal section; (29) female atrium of the holotype in sagittal section; (30) copulatory apparatus of paratype MZU PL.137. (cov) common glandular ovovitelline duct, (df) dorsal fold, (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (hs) holocrine secretion, (ma1) ental region of male atrium, (ma2) ectal region of male atrium, (ov) oviduct, (pp) pharyngeal pouch, (pv) prostatic vesicle, (s) stroma, (sc) secretory cells, (sg) shell glands, (sv) spermiducal vesicle, (va) vagina.

Ovaries oval-elongate, measuring about 0.4 mm anterior-posteriorly, 0.2 mm dorso-ventrally in the holotype. Ovovitelline ducts emerge dorsally from the posterior half of ovaries (Fig. 23), then recurve immediately dorsal to

the nerve plate. Ventrally to female atrium, the ovovitelline ducts unite to form a common glandular ovovitelline duct. The latter is an almost slightly ascendent duct that contours the common muscle coat (Figs. 25, 29). The common glandular ovovitelline duct penetrates this coat to open dorsally, through the female canal (vagina), into the end of the female atrium (postflex condition with ventral approach). The female canal is posteriorly directed and slightly recurved. Female atrium oval-elongate in shape, relatively long, and highly folded (Figs. 24, 25, 28). Length of female atrium about half of male atrial length (Table 3).

Paired ovovitelline ducts as well as common glandular ovovitelline duct lined by cuboidal to columnar ciliated epithelium; muscularis comprised of interwoven circular and longitudinal fibers. Numerous shell glands open into common glandular ovovitelline duct as well as in the distal portions of the paired ovovitelline ducts.

Female atrium lined by a tall-columnar epithelium, with irregular height and xanthophil apical secretion. This epithelium receives three types of secretions: (1) abundant coarse granular xanthophil secretion; (2) amorphous cyanophil secretion; (3) sometimes ovoid, granular erythrophil secretion. The three types of glands have their cell bodies located internally to the common muscle coat. Muscularis of female atrium thin (about 5 μm); it is comprised mainly of circular fibers with some interposed longitudinal fibers. This muscularis is poorly defined against the atrial stroma.

Female canal lined by tall-columnar to pseudo-stratified epithelium, with xanthophil apical secretion in the distal part of the canal. Its lining is ciliated in the proximal part of the female canal. Three types of secretions are discharged into the lumen of the female canal: coarse granular xanthophil secretion, amorphous cyanophil secretion, and granular erythrophil secretion. The latter is produced by glands with subepithelial cell bodies; the other two gland types have their cell bodies between fibers of the atrial stroma. Muscularis of female canal (about 8 μm –10 μm) comprised of circular subepithelial fibers partially interwoven with some longitudinal ones.

Gonopore canal slightly posteriorly inclined. Male and female atria with independent muscular coats. Dorsal elongated fold, arising from distal wall of the female atrium, leads anteriorly, and fuses with ventral wall of the male atrium (Figs. 25, 29). Gonopore canal lined by ciliated columnar epithelium, receiving the openings of numerous glands containing amorphous cyanophil secretion and abundant rhabditogen cells. Scattered erythrophil glands also discharge their granular secretion into the lumen of the gonopore canal. Muscularis of gonopore canal comprised of circular subepithelial fibers and subjacent longitudinal fibers.

Common muscle coat thin, with longitudinal, oblique and circular fibers, slightly thicker around male atrium (approximately 6–8 fibers thick) than around female atrium (about 4–6 fibers thick). This coat is separated from the atrial muscularis by a well developed stroma with many, variously oriented muscle fibers, sometimes forming bundles. The common muscle coat is poorly defined from this stroma around female atrium. Mesenchymal muscle fibers of the transverse layers located adjacent to the common muscle coat.

Vitellaria, situated mainly between intestinal branches, open into the ovovitelline ducts.

Remarks: Vitellaria well developed in the holotype and paratype MZU PL.132, and inconspicuous in paratypes MZU PL.130, MZU PL.131, MZU PL.135, and MZU PL.137. The latter had copulatory organs in an advanced stage of formation in relation to other paratypes in maturation. It showed a conical projection of the ental wall of the male atrium containing the histologically and anatomically differentiated ental male atrial region. This region forms a large and folded cavity with the ejaculatory duct opening into the bottom of this cavity. The ectal portion of the male atrium is filled with holocrine secretion (Fig. 30).

Discussion

Historical background. In her study of Brazilian species of *Geoplana* sensu Stimpson, 1857, E.M. Froehlich (1955) outlined an informal “group A” for specimens with parallel body margins, elongated and narrow body when crawling and with flattened and shortened body when resting. These specimens ranged from 48 mm to 130 mm in length, and had small eyes all over the dorsal surface. The following species were included in this group: *Geoplana pasipha* Marcus, 1951, *G. penhana* Riester, 1938, *G. pinima* E.M. Froehlich, 1955, *G. plana* Schirch, 1929, *G. rosea* E.M. Froehlich, 1955, *G. velina* Froehlich, 1955 and *G. velutina* Riester, 1938, as well as *G. astraera* Marcus, 1951 and *G. chimbeva* E.M. Froehlich, 1955. While studying Neotropical land planarians, C.G. Froehlich (1967) added to this group *G. caeruleonigra* Riester, 1938, *G. cafusa* Froehlich, 1956, *G. chilensis* Graff, 1899, *G. hauseri* Froehlich, 1959, *G. oliverioi* Froehlich, 1955, and *G. splendida* Graff, 1899. He commented also that *G. aphalla* Hyman, 1941 might be assigned to it. Ogren & Kawakatsu (1990) proposed the genus *Pasipha* for all of these

species, with exception of *G. cafusa*, and included another six species in the genus, viz. *G. biseminalis* Riester, 1938, *G. diminutiva* Hyman, 1955, *G. ercilla* Froehlich, 1978, *G. pulchella* Schultze & Müller, 1857, *G. tapetilla* Marcus, 1951, *G. trina* Marcus, 1951, and *G. weyrauchi* Du Bois-Reymond Marcus, 1951. Ogren & Kawakatsu (1990) maintained *G. cafusa* in the genus *Geoplana*, although it was initially included in the same group as *G. pasipha* by Froehlich (1967). However, this species shows all of the diagnostic characters proposed for the genus *Pasipha*, such as an elongate body, a folded male atrium, absence of a (permanent) penis papilla, and a female canal approaching from the ventral side, except that the latter is only slightly ventrally inclined, which is also the case in other species of the genus. Thus, we also propose to place *G. cafusa* in the genus *Pasipha*.

External morphology in species of the genus *Pasipha*. Regarding body length, eight species of *Pasipha*, (*P. aphalla*, *P. astraea*, *P. cafusa*, *P. diminutiva*, *P. ercilla*, *P. pulchella*, *P. splendida*, *P. tapetilla*, and *P. trina*), have a small body (up to 30 mm), usually between 15 mm and 30 mm, with the exception of *P. aphalla*, which may be 5 mm long. Three species (*P. oliverioi*, *P. velina*, and *P. weyrauchi*) have an intermediate body length (between 30 mm and 50 mm). The remaining species, including the type-species *P. pasipha*, have a large body that reaches over 50 mm in length. There is no data about the body length of *P. biseminalis*, which is only known from a small post-pharyngeal fragment.

As commented by E. M Froehlich (1955), for the species of group “A”, most *Pasipha* species have eyes spread over the dorsum and usually surrounded by clear halos. Four species (*P. chilensis*, *P. chimbeva*, *P. oliverioi*, and *P. trina*) have eyes exclusively on the body margins. For two species, *P. biseminalis* and *P. diminutiva*, the eye distribution is not clearly known.

In regards to the dorsal colour pattern, some species, such as *P. aphalla*, *P. astraea*, *P. caeruleonigra*, *P. chimbeva*, *P. diminutiva*, and *P. pinima*, have a homogeneous pattern. Most species, however, possess a striped pattern with at least a median stripe (*P. cafusa*, *P. chilensis*, *P. hauseri*, *P. pasipha*, *P. plana*, *P. rosea*, *P. splendida*, and *P. velina*) or various stripes over the dorsum (*P. oliverioi*, *P. penhana*, *P. tapetilla*, *P. trina*, and *P. weyrauchi*). Three species, e.g. *P. ercilla*, *P. pulchella*, and *P. velutina*, have a marbled dorsal surface. There was no data about colour pattern for *P. biseminalis*.

Internal morphology in species of the genus *Pasipha*. The cutaneous and mesenchymal musculatures in the anterior or pre-pharyngeal body regions, which are diagnostic characters at the genus level, are almost unknown in species of *Pasipha*. According to Grau (2010), *P. pasipha* shows a tripartite cutaneous musculature with longitudinal muscles in bundles and an anterior body region with the same arrangement as in the pre-pharyngeal region. The author indicated that the mesenchymal musculature is composed of three main layers: a dorsal diagonal with decussate fibers, a supra-intestinal with transverse decussate fibers, and a sub-intestinal transverse. The mc:h index of *P. pasipha* is about 16% (Françoso Jr., 1992). Besides *P. pasipha*, there is information about the cutaneous and mesenchymal musculatures of *P. chilensis* and *P. ercilla*. According to E.M. Froehlich (1978, p. 29), the cutaneous musculature of *P. ercilla*, especially the longitudinal layer, is strong and the mc:h index varies from 22% to 23%. In contrast, the cutaneous musculature of *P. chilensis* is weak and the mc:h index is 2% (E.M. Froehlich, 1978, p. 27). The mesenchymal musculature was described as thick for *P. ercilla*, especially the sub-intestinal transverse layer. According to E.M Froehlich, the mesenchymal musculature of *P. chilensis* is weak, with some supra-intestinal transverse fibers. The cutaneous and mesenchymal musculatures of other species of *Pasipha* are unknown.

In relation to the pharyngeal anatomy, the pharynx is cylindrical in eleven species of the genus (*P. chilensis*, *P. chimbeva*, *P. ercilla*, *P. oliverioi*, *P. pasipha*, *P. pinima*, *P. pulchella*, *P. tapetilla*, *P. trina*, *P. velina*, and *P. weyrauchi*). According to Riester (1938, p. 20), the pharynx seems to be cylindrical in *P. caeruleonigra*. The pharynx of *P. astraea*, *P. cafusa*, *P. diminutiva*, and *P. splendida* is bell-shaped, whereas in *P. hauseri*, *P. penhana*, *P. plana*, *P. rosea*, and *P. velutina*, the pharynx is collar-shaped. The pharyngeal anatomy of *P. aphalla* and *P. biseminalis* is unknown.

Regarding the male copulatory organs, most species currently included in the genus *Pasipha* have a large, extra-bulbar prostatic vesicle that receives the sperm ducts at its ental portion. Its ectal portion communicates with the male atrium by means of a sinuous ejaculatory duct that opens into the lower part of the atrium.

The prostatic vesicle is usually unforked, with the exception of *P. chilensis* and *P. hauseri*. Regarding its shape, in some species, such as *P. astraea*, *P. oliverioi*, *P. penhana*, *P. plana*, *P. rosea*, and *P. tapetilla*, the vesicle has the shape of an inverted U with two histologically distinct portions. In three species, *P. chimbeva*, *P. oliverioi* and *P. pasipha*, the ental portion of the vesicle is ovoid, whereas the ectal portion is tubular. In *P. pinima* and *P. velina*, it

is oval-elongate. In *P. cafusa*, *P. chilensis* and *P. weirauchy*, the vesicle is tubular. In contrast to most species of the genus, the prostatic vesicle is exclusively intrabulbar in *P. hauseri*, while in *P. biseminalis* it is short, ovoid and close to the common muscle coat. In *P. aphalla*, *P. ercilla*, *P. penhana*, and *P. trina*, there is no prostatic vesicle according to the original description of these species. However, for *P. penhana*, Riester (1938, p. 15) described a glandular ejaculatory duct that may actually be the prostatic vesicle. There was no data about the prostatic vesicle of *P. caeruleonigra*.

The sperm ducts open terminally (*P. biseminalis*, *P. chilensis*, *P. hauseri*, and *P. splendida*), ventrally (*P. plana* and *P. velina*) or laterally (*P. cafusa*, *P. chimbeva*, *P. pasipha*, *P. rosea*, *P. tapetilla*, and *P. velutina*) into the ental portion of the prostatic vesicle. In *P. oliverioi*, they open through the medial wall of the prostatic vesicle. In *P. pinima*, the opening of the sperm ducts is posteriorly displaced. In *P. penhana* and *P. weyrauchi*, the sperm ducts unite and form a seminal duct, which opens terminally into the prostatic vesicle.

The male atrium is usually richly folded and very long, at least twice as long as the female atrium. *Pasipha pasipha*, *P. biseminalis*, and *P. oliverioi* show a proximal differentiated region in the male atrium. This region contains very small and numerous folds with the ejaculatory duct opening into its lower part. This proximal region of the male atrium may actually function as a penis papilla. One of the species, *P. tapetilla*, shows a barrel-shaped papilla in the ental part of the male atrium that may not be a permanent organ, but may be formed by this differentiated ental part of the male atrium. In some species, such as *P. biseminalis*, *P. penhana*, and *P. weyrauchi*, the male atrium is relatively short.

In relation to the female organs, the ovovitelline ducts run laterally to the female atrium to unite and form the common glandular ovovitelline duct, located posteriorly or ventrally to the female atrium. Thus, the common glandular ovovitelline duct usually ascends to open into the ental part of the female atrium by means of a female canal (vagina) located behind this atrium. In only three species of the genus the common glandular ovovitelline duct does not ascend, but is instead horizontal and located behind the female canal (*P. biseminalis*, *P. trina*, and *P. weyrauchi*). The female canal might be slightly curved to the ventral surface, as illustrated by Marcus (1951) for *P. pasipha* (figs. 239–241), with the same occurring in *P. astraea*, *P. chimbeva*, and *P. splendida*. In many species of the genus the female canal is horizontal or almost horizontal, such as in *P. biseminalis*, *P. cafusa*, *P. caeruleonigra*, *P. hauseri*, *P. penhana*, *P. pinima*, *P. plana*, *P. tapetilla*, *P. trina*, *P. velutina*, and *P. weyrauchi*. Usually the female canal is tubular, but in *P. caeruleonigra* it is globose. In *P. chilensis*, *P. oliverioi*, and *P. velina* there is no female canal, so that the common glandular ovovitelline duct opens directly into the female atrium. In *P. diminutiva* and *P. ercilla* the common glandular ovovitelline duct and the female canal are unknown, while the description is not clear regarding this region in *P. aphalla*. No female canal was mentioned in the description of *P. rosea* (E.M. Froehlich, 1955, p. 304-305), but figure 67, which illustrates the copulatory apparatus of a mature worm, indicates a ventrally curved canal without any openings of shell glands.

The female atrium in species of *Pasipha* is usually long, ample and folded, but in six species it is relatively short, viz. *P. aphalla*, *P. astraea*, *P. cafusa*, *P. chilensis*, *P. tapetilla*, and *P. trina*. *Pasipha velina* has atria with a small quantity of folds and a more narrowed ectal portion of the male atrium. *Pasipha ercilla* presents very long, almost contiguous atria, with complex folds, presenting numerous short, narrow and blindly ending canals. Four species have an epithelium with stratified appearance in the female atrium, viz. *P. caeruleonigra*, *P. chimbeva*, *P. pinima*, and *P. splendida*.

Most species currently included in the genus *Pasipha* have atria that are separated from each other by a dorsal fold, such as in *P. cafusa*, *P. chimbeva*, *P. caeruleonigra*, *P. oliverioi*, *P. penhana*, *P. pinima*, *P. velutina*, and *P. weyrauchi*, or a sphincter in the ectal portion of the male atrium, as is the case of *P. plana* and *P. rosea*. In some species the male and female atria also show independent musculature, as for example in *P. cafusa*, *P. oliverioi*, and *P. splendida*. In the type-species *P. pasipha* no separation between male and female atria was indicated by Marcus (1951).

In four species (*P. aphalla*, *P. caeruleonigra*, *P. ercilla*, and *P. diminutiva*), some features of the copulatory apparatus could not be verified. In *P. aphalla* the reconstruction of the female part of the copulatory apparatus is incomplete. The holotype of *P. caeruleonigra* has a copulatory apparatus that is incompletely sectioned and lacks the ental part of the male atrium and the prostatic vesicle. The type-specimens of *P. diminutiva* and *P. ercilla* were incompletely mature. In addition, the copulatory apparatus of *P. pulchella* is unknown. This species, originally described on external morphology alone, was redescribed by Froehlich (1955a), based on an immature specimen.

Affinities of the new species in this paper. Mature specimens of *P. backesi* Leal-Zanchet, Rossi & Seitenfus

sp. nov. and *P. brevilineata* Leal-Zanchet, Rossi & Alvarenga **sp. nov.**, which are intermediate in body length, particularly differ from the smaller species of *Pasipha*: *P. aphalla*, *P. astraea*, *P. diminutiva*, *P. trina*, *P. caeruleonigra*, and *P. ercilla*. *Pasipha backesi* and *P. brevilineata* can also be distinguished from larger species, such as *P. chimbeva*, *P. pinima* and *P. velutina*, by their intermediate body length. The dorsal eyes of *Pasipha backesi* and *P. brevilineata* differ from the exclusively marginal eyes of *P. biseminalis*, *P. chilensis*, *P. chimbeva*, *P. oliverioi*, and *P. trina*. Regarding colour pattern, *Pasipha backesi* and *P. brevilineata* clearly differ from each other. *Pasipha backesi*, with lateral bands and median and paramedian stripes, differs from *P. brevilineata*, which has only a median, inconspicuous or sometimes interrupted median stripe. Regarding colour pattern, both species also differ from other species described for the genus. An exception is *Pasipha brevilineata*, which shows a colour pattern that is similar to *P. cafusa* and *P. velina*.

Regarding pharyngeal anatomy, *P. backesi* and *P. brevilineata*, which have a cylindrical pharynx, can be distinguished from *P. astraea*, *P. cafusa*, *P. diminutiva*, *P. hauseri*, *P. penhana*, *P. plana*, *P. rosea*, *P. splendida*, and *P. velutina*, which show a bell-shaped or collar-shaped pharynx.

Pasipha backesi and *P. brevilineata* differ from the remaining species, *P. pasipha*, *P. tapetilla*, *P. velina*, and *P. weyrauchi*, by details of the copulatory apparatus. The globular prostatic vesicle of *P. backesi* and *P. brevilineata* differs from the tube-shaped vesicles in *P. pasipha* and *P. weyrauchi*. In addition, both the common glandular ovovitelline duct and the female canal are horizontal in *P. weyrauchi*, whereas these ducts have an ascendant position in *P. backesi* and *P. brevilineata*. Both species differ from *P. tapetilla* in the shape of the prostatic vesicle, with two tubular, vertically disposed portions, and the short female atrium in the latter. *Pasipha backesi* and *P. brevilineata* can be distinguished from *P. velina* by the unfolded, relatively shorter male and female atria and by the absence of a female canal in the latter. In addition, the opening of the sperm ducts into the prostatic vesicle is sub-terminal, dorsally displaced, into expanded lateral portions of the vesicle in *P. backesi* and terminal, into the expansions of the forked portions of the vesicle in *P. brevilineata*, whereas sub-terminal and ventral in *P. velina*. The shape of the ental portions of the prostatic vesicle and the opening of the sperm ducts are important features to distinguish *P. backesi* and *P. brevilineata* from each other. In *P. backesi* the ental paired portions of the prostatic vesicle are spheroid and laterally disposed. In *P. brevilineata* the ental portions of the prostatic vesicle are tubular and ventrally disposed. In *P. backesi* the sperm ducts run dorsally and open into the lateral expansions of the prostatic vesicle, whereas they remain ventral and open into the ental portions of the prostatic vesicle in *P. brevilineata*. In addition, there are no folds separating male and female atria in *P. backesi*, whereas a dorsal, elongated fold separates the atria in *P. brevilineata*.

The two new sympatric species can be distinguished from each other especially by their colour pattern and the details of the copulatory apparatus. Both species show similar characteristics regarding relative position of ovaries and anteriormost testes in relation to body length, the position where ovovitelline ducts emerge from the ovaries, pharyngeal morphology as well as the general morphology of the copulatory apparatus. Both new species show a strong mesenchymal musculature and a higher development of the ventral cutaneous musculature close to the anterior tip in relation to the pre-pharyngeal region. The arrangement of the cutaneous musculature, both in the anterior region and in the pre-pharyngeal region, and the arrangement of the mesenchymal musculature provide important characters for defining genera (Froehlich, 1955b, Carbayo & Leal-Zanchet, 2003). However, these characteristics are not well known for various Geoplaninae species, especially those that were included in genera split off from *Geoplana*. Regarding these species, the body musculature should be analyzed both in the anterior tip as well as in the pre-pharyngeal region, in order to delimit natural groups. In light of the above discussion, the genus *Pasipha* is a heterogeneous assemblage of species. Besides a re-description of the type-species *P. pasipha*, a comparative analysis of the body musculature and the morphology of the copulatory apparatus of the species included in this genus is necessary.

Acknowledgements

We thank the Brazilian Research Council (CNPq) for grants and fellowships supporting this study. Acknowledgements are due to Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA), Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) and the staff of the National Forest of São Francisco de Paula for supporting fieldwork activities. We are grateful to the laboratory technicians

Aline Centa and Letícia Guterres for section preparation and to all those who collaborated with specimen collecting. Thanks are also due to Teresinha H. Oliveira for the pictures of live specimens of *P. backesi* and *P. brevilineata*. Dr. Hugh Jones and two anonymous reviewers are acknowledged for their constructive comments.

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