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Geoplana multicolor Graff (Platyhelminthes: Tricladida): new records and taxonomic re-interpretation of morphological characters

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Abstract

Many species of land flatworms have been described based on specimens, or even on a single specimen, from a sole locality. Thus, populational or individual variations are poorly known. The external morphology of *Geoplana multicolor* Graff, 1899 was first described based on a single specimen from the vicinities of the city of São Paulo, South Brazil. Marcus (1951) analysed specimens from São Paulo with a similar external aspect and considered them as conspecific with *G. multicolor*, describing their internal anatomy. In the present work, we amplify the known distribution of the species, by registering its occurrence in four localities of southern Brazil (state of Rio Grande do Sul), and comparatively analyse this relatively ample material, which includes different developmental phases. The description of histological aspects, especially those regarding musculature and secretory cells of the copulatory organs, has been enhanced and amplified after the present analysis. Thus, the description of *G. multicolor* is here complemented, with an improvement on the interpretation of important taxonomical characters. In addition, the paper reports, for the first time, the occurrence of an ejaculate attached to the atrial wall of a species of Geoplaninae which presents a penis papilla, and briefly discusses its significance.

Key words: Geoplaninae, taxonomy, developmental stages, spermatophore

Introduction

Many species of land flatworms have been described based on a few specimens, or even a single specimen, from a sole locality. Thus, populational variation is poorly known. Later the species may be found in other localities usually by other researchers, being re-described, after facing the difficulties of being convinced of the co-specificity of the new material. Researchers must also face morphological differences between specimens due to different maturation stages and/or physiological phases. Such difficulties are increased by the fact that flatworms are soft-bodied animals which may contract during fixation.

The external morphology of *Geoplana multicolor* was first described by Graff (1899) based on a single specimen collected in the neighborhood of the city of São Paulo, South Brazil. Marcus (1951) analysed specimens from São Paulo with a similar external aspect and considered them as conspecific with *G. multicolor*, describing their internal anatomy. Later, Froehlich (1955) described aspects of the musculature and epidermal glands of *G. multicolor* sensu Marcus, followed (Froehlich, 1956a, b, 1957) by an analysis of specimens of *G. multicolor* from the states of Rio de Janeiro and Paraná. He considered their external and internal morphology to be, in general, coincident with Marcus' description (1951).

In the present work, we study several specimens from four localities of southern Brazil (state of Rio Grande do Sul) and re-analyse the specimens studied by Marcus (1951), from the state of São Paulo, in order to re-define characters of taxonomical significance.

Material and methods

Specimens were collected by direct sampling in soil-litter, under and inside fallen logs and branches, and under rocks in the following localities of the state of Rio Grande do Sul, Brazil: the town of Caxias do Sul (29°10'S;

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51°10'W); on a trail and under a fallen trunk near an area of Mixed Ombrophilous Forest, in the National Forest of São Francisco de Paula (29°23' – 29°27'S; 50°23' – 50°25W), São Francisco de Paula; in areas of Caducifolious Forest at two sites in the municipality of Santa Maria, namely the "Campo de Instrução do Exército – CISM" (29º43' – 29º44'S; 53º42' – 53º44'W), in the district of São Valentin, and Três Barras (29º41'S; 53º44'W), a district of Arroio Grande, located 20 km to the northeast of the seat of the municipality. One specimen (MZU PL.00089) was collected on a rainy day on one of the inner walls of a house in the municipality of Santa Maria. The external morphology of live worms was examined while alive. Afterwards these were killed in boiling water and fixed with neutral formaldehyde.

Two additional specimens were collected in pitfall traps with drift-fences. These consisted of 100-liter containers placed on the ground with the tops level with the surface of the soil (Castro & Leal-Zanchet, 2005), in areas of man-disturbed native grassland at the following localities: Ibarama (29º25'S; 53º08'W); and Santa Maria, in the district of Pains, located ca. 10 km from the municipality seat (29º44'S; 53º44'W). These specimens were directly fixed in formaldehyde.

Methods described by Froehlich and Leal-Zanchet (2003) were used for both the histological processing of material and the analysis of external and internal characters. The material was sectioned at 5–7µm thick. In addition, Marcus' original slides of *G. multicolor* deposited in the Collection of Eudóxia Maria Froehlich (EMF Collection), in the University of São Paulo, were examined.

The specimens were deposited in the Museu de Zoologia da Universidade do Vale do Rio dos Sinos (MZU), São Leopoldo, Rio Grande do Sul, Brazil.

Taxonomic part

Family Geoplanidae Stimpson, 1857

Subfamily Geoplaninae Stimpson, 1857

Geoplana Stimpson, 1857

Geoplana multicolor Graff, 1899

Geoplana multicolor Graff, 1899 (Tafel VII, Figs. 12–14: external aspect).
Geoplana multicolor: Marcus, 1951
Geoplana multicolor: Froehlich, 1955
Geoplana multicolor: Froehlich, 1956a
Geoplana multicolor: Froehlich, 1956b
Geoplana multicolor: Froehlich, 1957
Geoplana sp. 7: Leal-Zanchet & Carbayo, 2000
Geoplana sp. 3: Castro & Leal-Zanchet, 2005

Material examined. EMF Nr. 1–4: São Paulo, SP, Brazil – Four specimens collected and studied for the first time by E. Marcus. EMF 1: Pharynx and copulatory apparatus: sagittal sections on 4 slides/ Figs. 176 and 177 (Marcus, 1951: p. 187); EMF 2: copulatory apparatus: sagittal sections on 4 slides; EMF 3: copulatory apparatus: sagittal sections on 2 slides; EMF 4: pharynx and copulatory apparatus: sagittal sections on 5 slides; MZU PL.00081: São Francisco de Paula, RS, Brazil – R. Murowaniecki, leg. 04.V.1998 – Pre-pharyngeal region: transverse sections on 4 slides; pharynx: sagittal sections on 12 slides; copulatory apparatus: sagittal sections on 19 slides; MZU PL.00082: São Francisco de Paula, RS, Brazil – A. Leal-Zanchet, coll. 04.V.1998 – preserved in ethanol 70%; MZU PL.00083: São Francisco de Paula, RS, Brazil – G. Fiorentin, leg. 30.V.1998 – Anterior region in three fragments: horizontal sections on 6 and 9 slides, respectively, and transverse sections on 19 slides; pre-pharyngeal region: transverse sections on 7 slides; pharynx: sagittal sections on 12 slides; copulatory apparatus: sagittal sections on 10 slides; MZU PL.00084: Santa Maria (CISM), RS, Brazil – C. Cristofoli, leg. 19.XI.2000 – Pre-pharyngeal region: transverse sections on 6 slides; pharynx: sagittal sections on 17 slides; copulatory apparatus: sagittal sections on 16 slides; MZU PL.00085: Santa Maria (CISM), RS, Brazil – A. Seitenfus, leg. 13.I.2001 – preserved in ethanol 70%; MZU PL.00086: Santa Maria (Três Barras), RS, Brazil – M. Fontoura, leg. 09.VI.2001 – preserved

in ethanol 70%; MZU PL.00087: Ibarama, RS, Brazil – T. Santos, leg. 21.I.2001 – Pre-pharyngeal region: sagittal sections on 32 slides; pharynx: sagittal sections on 22 slides; copulatory apparatus: sagittal sections on 25 slides; MZU PL.00088: Santa Maria (CISM), RS, Brazil – V. Baptista, leg. 29.IX.2001 – Anterior region in two fragments: sagittal sections on 25 slides; pre-pharyngeal region: transverse sections on 8 slides; pharynx: sagittal sections on 13 slides; copulatory apparatus: sagittal sections on 22 slides; MZU PL.00089: Santa Maria (Camobi), RS, Brazil – S. Cechin, leg. 29.IX.2001 – Pre-pharyngeal region: transverse sections on 5 slides; pharynx: sagittal sections on 9 slides; copulatory apparatus: sagittal sections on 9 slides; MZU PL.00090: Santa Maria (CISM), RS, Brazil – V. Dias, leg. 24.I.2002 – Pre-pharyngeal region: transverse sections on 5 slides; pharynx: sagittal sections on 11 slides; copulatory apparatus: sagittal sections on 11 slides; MZU PL.00091: Santa Maria (CISM), RS, Brazil – R. Castro, leg. 24.I.2002 – Anterior region at the level of the ovaries: horizontal sections on 36 slides; pre-pharyngeal region: transverse sections on 29 slides; pharynx: sagittal sections on 47 slides; copulatory apparatus: horizontal sections on 67 slides; MZU PL.00092: Santa Maria (CISM), RS, Brazil – M. Fontoura, leg. 24.I.2002 – preserved in ethanol 70%; MZU PL.00093: Santa Maria (Pains), RS, Brazil – T. Santos, leg. 18.X.2001 – Pre-pharyngeal region: transverse sections on 5 slides; pharynx: sagittal sections on 17 slides; posterior region: sagittal sections on 11 slides; MZU PL.00094: Santa Maria (Três Barras), RS, Brazil – R. Castro, leg. 11.VII.2002 – preserved in ethanol 70%; MZU PL.00095: Caxias do Sul, RS, Brazil – R. Fleck, leg. 17.IX.2002 – Pre-pharyngeal region: transverse sections on 15 slides; pharynx: sagittal sections on 23 slides; copulatory apparatus: sagittal sections on 19 slides; MZU PL.00096: Santa Maria (Três Barras), RS, Brazil – R. Castro, leg. 15.XII.2002 – preserved in ethanol 70%; MZU PL.00097: Santa Maria (Três Barras), RS, Brazil – L. Matos, leg. 15.XII. 2002 – preserved in ethanol 70%; MZU PL.00098: Santa Maria (Três Barras), RS, Brazil – L. Matos, leg. 15.XII.2002 – Pre-pharyngeal region: transverse sections on 1 slide; pharynx: sagittal sections on 4 slides; copulatory apparatus: sagittal sections on 3 slides; MZU PL.00099: Santa Maria (Três Barras), RS, Brazil – V. Baptista, leg. 15.XII.2002 – preserved in ethanol 70%; MZU PL.00100: Santa Maria (Três Barras), RS, Brazil – R. Castro, leg. 23.VIII.2002 – Copulatory apparatus: sagittal sections on 30 slides.

Type locality. São Paulo state, Brazil.

Distribution. Rio de Janeiro (Teresópolis), São Paulo (Alto da Serra, Avaré, Mogi das Cruzes, São Paulo), Paraná (Curitiba, Guapiara, Ponta Grossa, Lapa), Rio Grande do Sul (São Francisco de Paula, Caxias do Sul, Ibarama, Santa Maria) – Brazil

Diagnosis. Dorsum brown with light median stripe, which may contain a median concentration of rust red or brownish pigment inside it, bordered by black paramedian stripes; eyes dorsal, with clear halos; conspicuous glandular margin; mc:h, 3–9%; pharynx cylindrical; esophagus: pharynx ratio, 13%–30%; foremost testes approximately level with ovaries, most posterior ones near root of pharynx; efferent ducts open laterally into anterior end of prostatic vesicle; prostatic vesicle consisting of two portions, a forked, extrabulbar, proximal portion with ample lumen and an unpaired, much narrower, distal portion which enters the bulbar muscular coat; male atrium oval-elongate, with almost the entire cavity occupied by the penis papilla; ejaculatory duct opens ventrally dislocated, near the tip of the truncate, asymmetrical papilla; oviducts emerging, dorsally and laterally dislocated, from median third of ovaries, and ascending behind gonopore; common glandular oviduct short; vagina as a dorso-anteriorly curved ental portion of female atrium, sometimes indiscernible; female atrium oval-elongate, obliquely disposed, with a narrowed lumen, presenting two distinct regions, the ental one lined by an epithelium with multilayered aspect; male atrium at least as long as female atrium; asymmetric gonopore canal; ample dorsal fold separating male and female atria which open to gonopore canal in different parasagittal planes.

Description. External morphology.

Body broad and flat, anterior end as well as posterior pointed. When crawling, maximal length reaches 55mm (Table 1). Mouth distance from anterior tip varies from 52% to 76%; gonopore between 62% and 83%, relative to body length (Table 1). Dorsal ground-colour brown; the venter is pale yellow or greyish with denser pigmentation at the margins. Dorsally, there is a broad, orange or reddish median stripe, whereby at the external limits a very dense pigment creates a black paramedian stripe on each side of the dorsum. Lateral to these, there is dark-brown pigmentation overlaying the dorsal ground colour (Figs. 1–3). Specimens from São Paulo may contain a median concentration of rust red or brownish pigment in the middle of the broad median stripe.

In specimen MZU PL.00092, from Santa Maria (Fig. 2), median and paramedian stripes begin at approximately 1 mm from anterior tip (ca. 3% of body length) and extend up to 0.7 mm from posterior one (ca. 98% ofbody length). At median third of body, median and paramedian stripes, respectively, 1.2 mm and 0.5 mm wide (24% and 10% of body width, respectively). After fixation, the median stripe becomes pale yellow.

TABLE 1. Measurements, in mm, of specimens of *Geoplana multicolor* Graff, 1899. -: not measured; --: gonopore absent; *: After fixation; ** Specimens with damaged anterior tip or without posterior third of body (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.

	MZU PL.0 0081	MZU PL.0 0082	MZU PL.0 0083	MZU PL.0 0084	MZU PL.0 0085 **	MZU PL.0 0086 **	MZU PL.0 0087	MZU PL.0 0088	MZU PL.0 0089	MZU PL.0 0090	MZU PL.0 0091	MZU PL.0 0092	MZU PL.0 0093	MZU PL.0 0094	MZU PL.0 0095	MZU PL.0 0096 **	MZU PL.0 0097	MZU PL.0 0098	MZU PL.0 0099	MZU PL.0 0100
Maximum length in extension	-	35	38	35	45	18	-	35	28	35	55	45	-	17	32	-	40	23	-	-
Maximum width in extension	-	3	3	2.5	4	2	-	2	3	3	3	3	-	1	3	-	3	2	-	-
Length at rest	-	-	-	25	35	15	-	17	12	-	-	30	-	8	25	19	20	11	-	-
Width at rest	-	-	-	3	5	3.5	-	4	5	-	-	5	-	4	4	4	4	3	-	-
Length*	22	-	24	22	27	10	27	28	23	25	41	29	23	11	27	15	29	17	15	27
Width*	4	-	4.5	5	4.5	4	4	4	3.5	4	7	5	3	2	4.5	3	4	2	2.5	3.5
DM*	14.5 (66)	-	15 (62)	14 (64)	16 (59)	8 (--)	17 (63)	18.5 (66)	17 (74)	16 (64)	25 (61)	18 (62)	16.5 (72)	7.5 (68)	15 (55)	13 (--)	15 (52)	13 (76)	9.5 (63)	18 (67)
DG*	17.5 (79)	-	18 (75)	--	20 (74)	--	20 (74)	22 (79)	--	19.5 (78)	30 (73)	21.5 (74)	19 (83)	--	20 (74)	-	18 (62)	--	--	22 (81)
DMG*	3	-	3	--	4	--	3	3.5	--	3.5	5	3.5	2.5	--	5	-	3	--	--	4
DPVP*	-	-	0.6	-	-	-	-	1.5	-	-	2.1	-	-	-	0.7	-	-	-	-	-
Creeping sole %	-	-	96	-	-	-	-	98	-	-	98	-	-	-	-	-	-	-	-	-
Ovaries	-	-	4.7 (20)	-	-	-	-	4.5 (18)	-	-	6.5 (16)	-	-	-	-	-	-	-	-	-
Anteriormost testes	-	-	5 (21)	-	-	-	-	4.7 (19)	-	-	6.8 (17)	-	-	-	-	-	-	-	-	-
Posteriormost testes	-	-	13.5 (56)	-	-	-	-	16 (63)	-	-	22 (54)	-	-	-	-	-	-	-	-	-
Prostatic vesicle (forked portions)	-	-	0.2	-	-	-	-	0.3	-	-	0.4	-	-	-	0.2	-	-	-	-	0.3
Prostatic vesicle (unforked portion)	-	-	0.4	-	-	-	-	0.6	-	-	1.0	-	-	-	0.4	-	-	-	-	0.4
Penis papilla	-	-	0.8	-	-	-	-	0.6	-	-	0.5	-	-	-	1.0	-	-	-	-	0.9
Male atrium	-	-	0.8	-	-	-	-	1.3	-	-	1.0	-	-	-	1.2	-	-	-	-	1.6
Female atrium	-	-	0.6	-	-	-	-	0.7	-	-	1.0	-	-	-	0.8	-	-	-	-	0.8
Vagina	-	-	0.06	-	-	-	-	0.3	-	-	0.1	-	-	-	0.08	-	-	-	-	0.2
Common oviduct	-	-	0.06	-	-	-	-	0.1	-	-	0.1	-	-	-	0.06	-	-	-	-	0.12

Eyes uniserially surround anterior tip, becoming pluriserial immediately after. In specimen MZU PL.00092, between 2 mm and 4 mm (approx. 12% and 45% of body length) behind anterior end, these form three series extending near to body margins. Between approx. 5 mm and 15 mm from anterior tip, dorsal eyes surrounded by clear halos become abundant. They occur up to paramedian stripes, sometimes invading these. Towards posterior end, they become less numerous (Figs. 2–3).

FIGURE 1. Photograph of a live specimen of *G. multicolor* Graff, 1899, in dorsal view, from São Francisco de Paula.

Internal morphology.

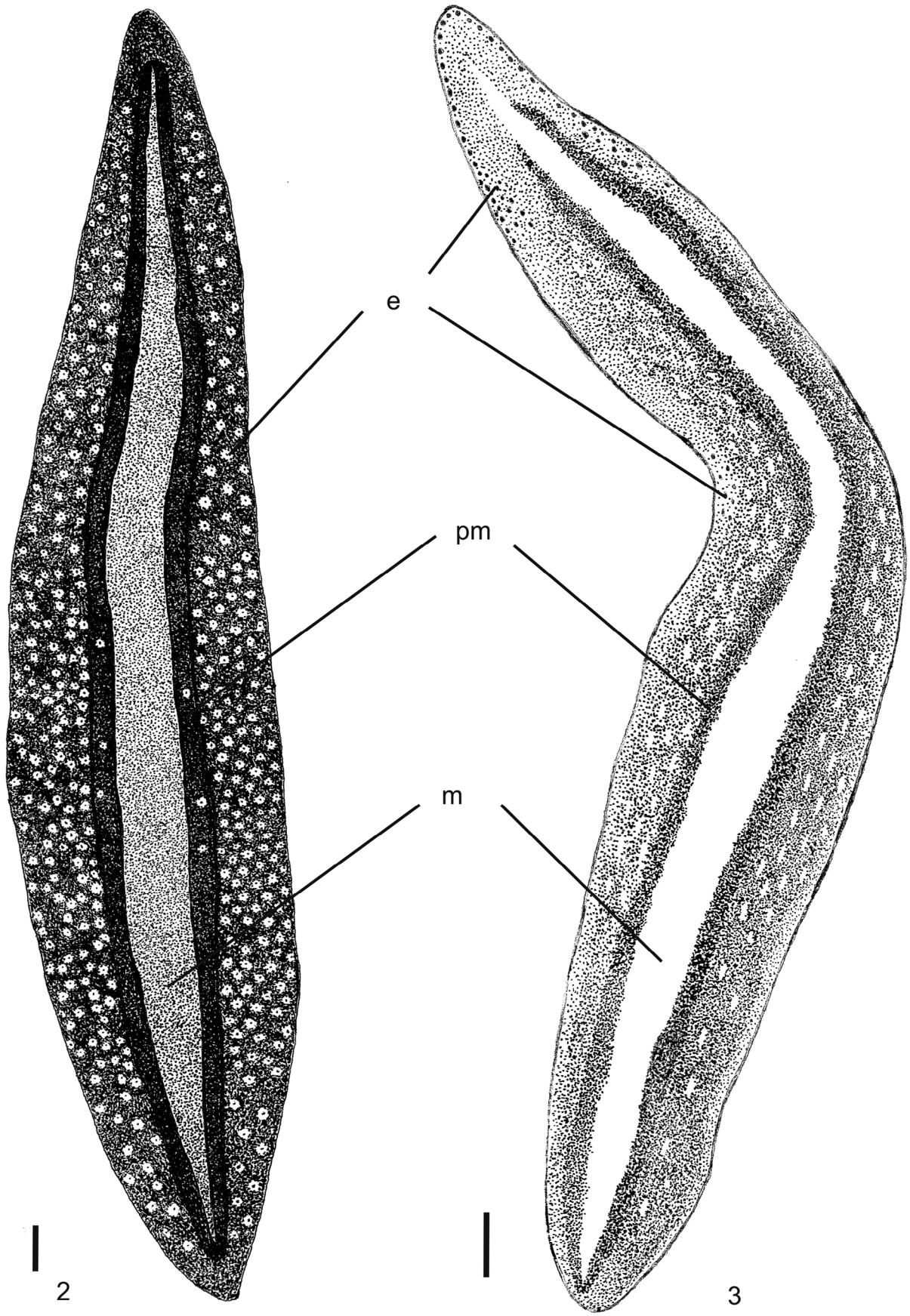
Epidermis and musculature at pre-pharyngeal region (Fig. 4): Creeping sole broad (Table 1). Three types of secretory cells open through dorsal epidermis and body margins: (1) rhabditogen cells with xanthophil secretion; (2) cells with coarse xanthophil secretion, more numerous towards body margins; (3) cells with xanthophil amorphous secretion and subepithelial bodies; and (4) cells less frequent with cyanophil amorphous secretion. Creeping sole receives abundant cells with cyanophil amorphous secretion and less numerous secretory cells of two types: rhabditogen cells and cells with coarse xanthophil secretion. Glandular margin constituted by numerous cells with coarse xanthophil secretion; cells with xanthophil amorphous secretion and subepithelial bodies; and some rhabditogen cells.

Cutaneous musculature with the usual three layers, longitudinal layer with small bundles, being approximately three times higher than the other two. At the sagittal plane, ventrally and dorsally with similar thickness. Towards body margins cutaneous musculature progressively lower. Mc:h 3% to 9% (Table 2).

TABLE 2. Cutaneous musculature in the median region of a transverse 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 *Geoplana multicolor* Graff, 1899. * Specimen collected in a pitfall trap and directly fixed in formaldehyde.

	MZU PL.00081	MZU PL.00083	MZU PL.00088	MZU PL.00091	MZU PL.00093*	MZU PL.00095
Dorsal musculature	23	32	15	28	48	35
Ventral musculature	27	37	12	22	34	43
Mc:h	6%	7%	3%	3%	9%	7%

Mesenchymatic musculature mainly composed of transversal, oblique, and dorsoventral fibres, the former ones may constitute a supra-intestinal transversal and a sub-intestinal transversal layer, each of them approx. 3–4 fibers thick. If existent, longitudinal fibers are indiscernible.



FIGURES 2–3. Detail of colour pattern of preserved specimens of *G. multicolor* Graff, 1899 from Southern Brazil: (2) specimen MZU PL.00092, from Santa Maria; (3) specimen MZU PL.00095, from Caxias do Sul. (e) eyes, (m) median stripe, (pm) paramedian stripes. Scale bar: 1mm.

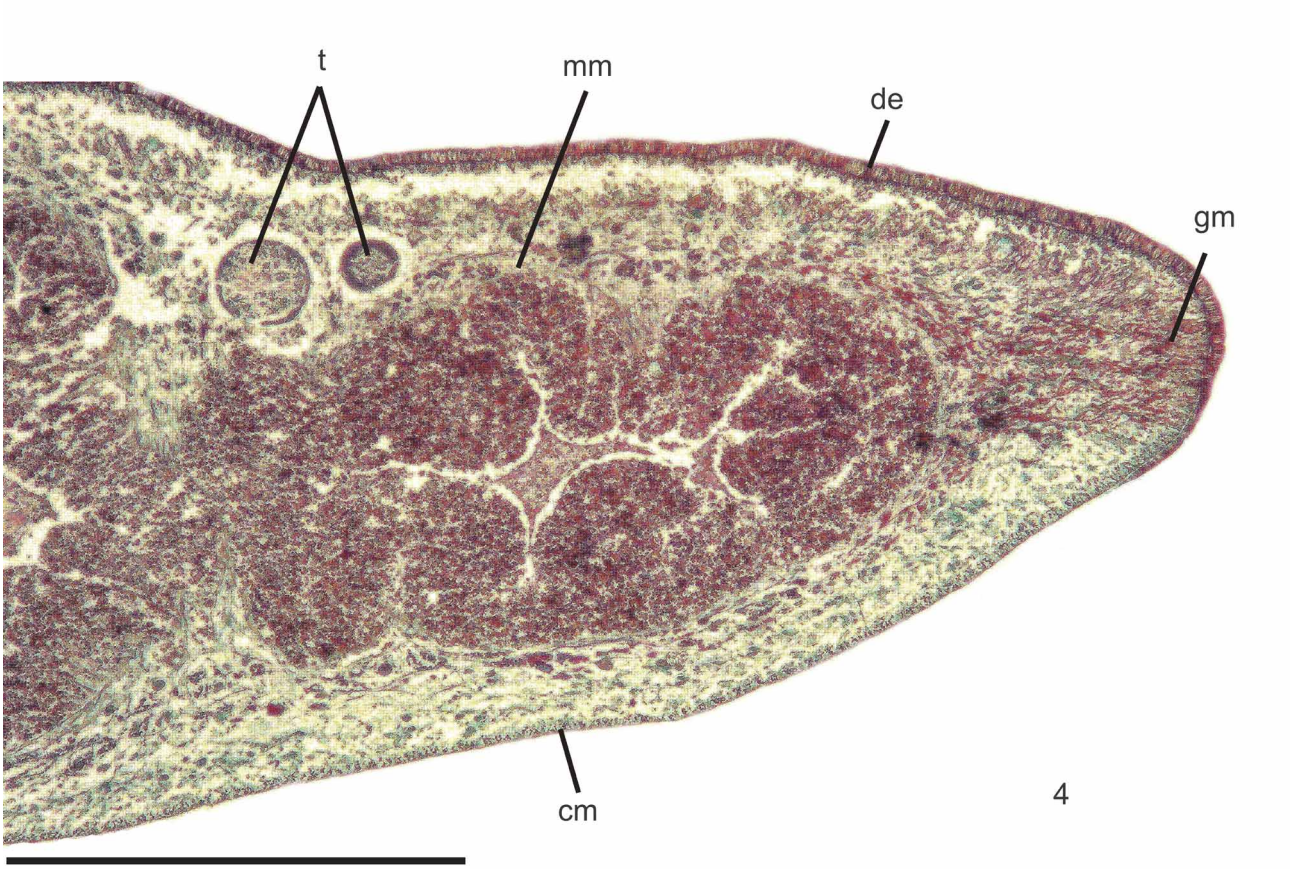


FIGURE 4. Detail of the pre-pharyngeal region in transverse section of specimen MZU PL.00091 of *G. multicolor* Graff, 1899. (cm) cutaneous musculature, (de) dorsal epidermis, (gm) glandular margin, (i) intestine, (mm) mesenchymatical muscles, (t) testes. Scale bar: 1mm.

Pharynx (Fig. 5): Pharynx of cylindrical type with dorsal and ventral insertions almost at the same transversal level. Mouth near the end of pharyngeal pouch. Short esophagus lined with columnar ciliated epithelium presenting some insunk nuclei and coated with interwoven circular and longitudinal fibers. Pharynx and pharyngeal lumen lined with columnar ciliated epithelium showing some insunk nuclei, those of the pharyngeal lumen located among fibers of inner pharyngeal musculature. Pharyngeal glands with cell bodies located in mesenchyme, mainly anteriorly to pharyngeal pouch. Four secretory cell types: (1) cells with densely arranged, xanthophil granulous secretion; (2) cells with strongly erythrophil granulous secretion; (3) cells with cyanophil amorphous secretion; and (4) cells with strongly cyanophil, fine granulous secretion. Outer musculature of pharynx (ca. 19 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 (ca. 20 µm thick) composed of thick circular subepithelial layer, mixed with some longitudinal fibers. Inner musculature gradually thins down towards pharyngeal tip. Esophagus:pharynx ratio from 13% to 34%.

Reproductive apparatus: Testes begin at approximately the same level as ovaries and extend near to root of the pharynx (Table 1), constituting two irregular rows dorsal to the intestinal branches on each side of the body (Fig. 4). Pre-pharyngeally, efferent ducts dorsal to oviducts, sometimes laterally displaced. Behind and/or lateral to pharynx these form false seminal vesicles, opening laterally into anterior third of prostatic vesicle (Figs. 6, 7, 9, 12).

Prostatic vesicle comprises two portions, a forked, extrabulbar, proximal portion with ample lumen, and an unpaired much narrower distal portion which proceeds posteriorly and enters the bulbar muscular coat, continuing into the ejaculatory duct. The latter traverses the truncate asymmetrical penis papilla to open, ventrally displaced, near its tip. Oval-elongate male atrium with almost the entire cavity or at least the ental half occupied by the penis papilla. Male atrium may contain folds in its ectal portion (Tables 1, 3, Figs. 6–11, 13).

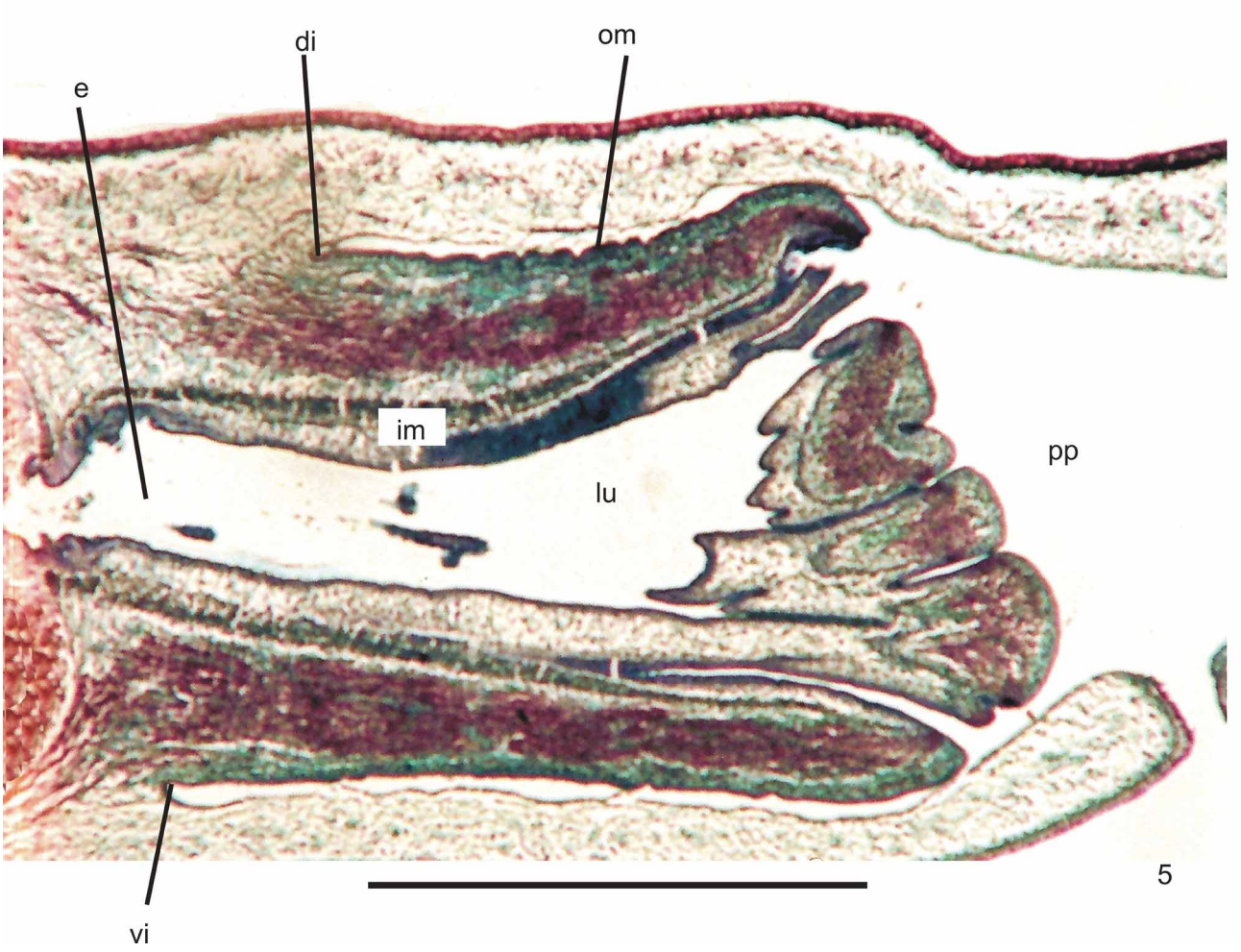
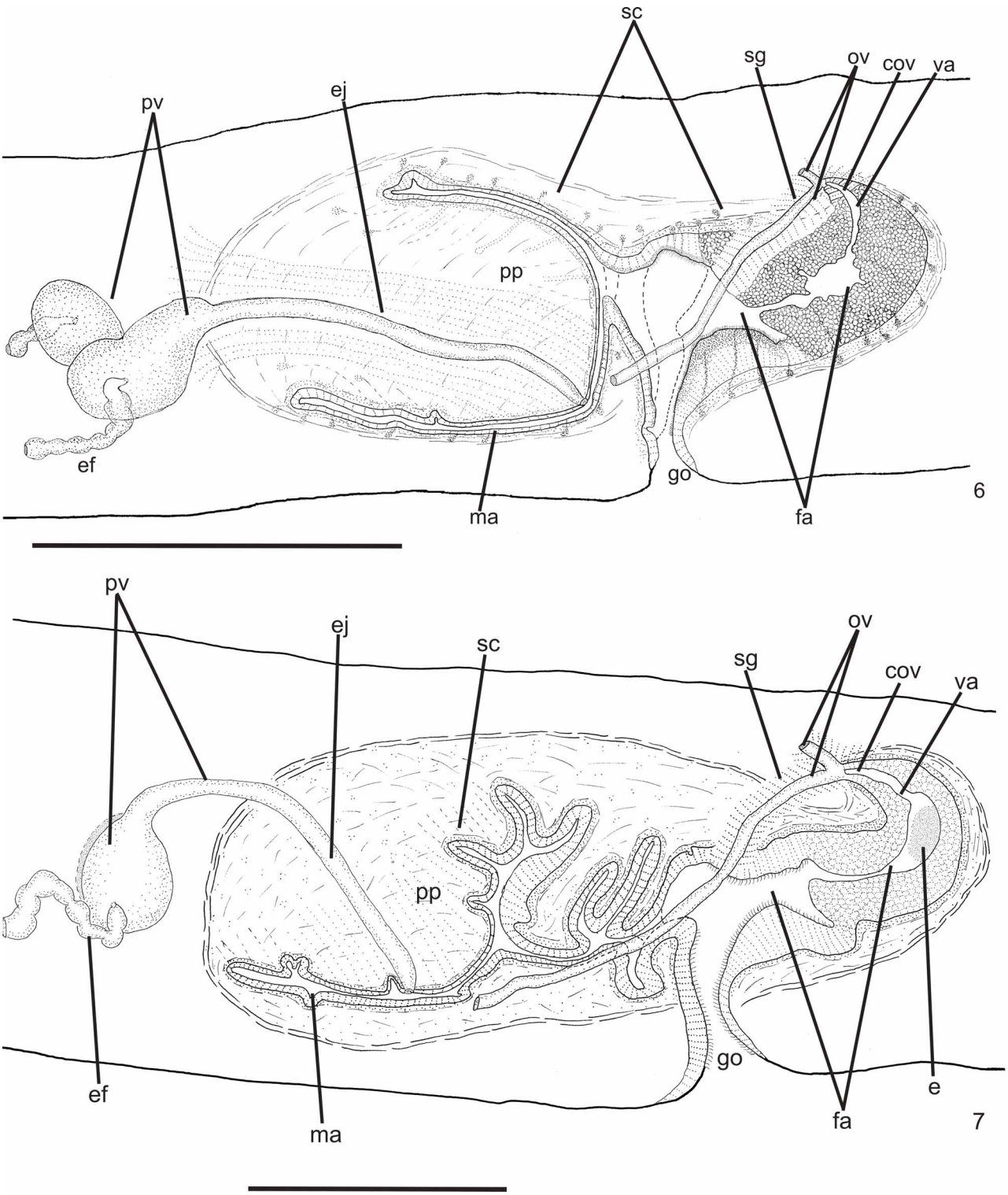


FIGURE 5. Pharynx of specimen MZU PL.00091 of *G. multicolor* Graff, 1899. Sagittal section. (di) dorsal insertion, (e) esophagus, (im) internal musculature, (lu) pharyngeal lumen, (om) outer musculature, (pp) pharyngeal pouch, (vi) ventral insertion. Scale bar: 1mm.

Lining epithelium of efferent ducts cuboidal ciliated; thin muscularis (ca. 2–4µm thick) mainly of circular fibers. Prostatic vesicle lined with irregular columnar to pseudostratified non-ciliated epithelium, gradually diminishing its height towards ejaculatory duct. Erythrophil cells with granular secretion and cyanophil cells with amorphous secretion, both with bodies lying in mesenchyme, mainly around vesicle, show numerous openings into the proximal portion and scarce openings into the distal one. Muscularis of vesicle (35–60 µm and 10–25 µm thick, respectively, in the proximal and distal portions) constituted of interwoven circular, oblique, and longitudinal fibers.

Ejaculatory duct lined with columnar ciliated epithelium, receiving numerous openings from secretory cells with amorphous, cyanophil secretion and subepithelial bodies. It is coated with muscularis (ca. 10–15 µm thick) of circular fibers interposed with some longitudinal ones. Penis papilla lined with columnar, non-ciliated epithelium, changing from cuboidal to squamous towards its tip, presenting insunk nuclei, mainly next to the tip. Three types of secretory cells run longitudinally in the papilla, with numerous openings through its lining epithelium: (1) cells with fine densely arranged granular slightly erythrophil secretion opening mainly at the tip of the papilla; (2) cells with granular heavily stained erythrophil secretion opening mainly laterally next to the papilla root; and (3) cells with cyanophil amorphous secretion and openings more numerous laterally next to the papilla root. A fourth type of cell, with xanthophil amorphous secretion and openings mainly at the tip of the papilla, occurs scarcely. Erythrophil cells present cell bodies external to common muscle coat; cyanophil and xanthophil cells, intrabulbar or intrapapillar cells bodies. Muscularis (5–23µm) mainly composed of circular layer with some mixed longitudinal fibers; thinner towards the tip of the papilla. Longitudinal, radial and oblique muscle fibers cross the papilla.

Epithelial lining of male atrium, columnar non-ciliated, becoming ciliated in the near of gonopore. Epithelial cells with xanthophil or erythrophil apical secretion, higher distally. Two types of secretory cells, with cell bodies internal to common muscle coat, empty through the epithelium: abundant cells with cyanophil amorphous secretion and cells with fine granulous erythrophil secretion. Muscularis weakly developed (8µm) throughout male atrium, constituted of circular subepithelial fibers and longitudinal subjacent ones.



FIGURES 6–7. Diagrammatic sagittal composite reconstructions of the copulatory apparatus of *G. multicolor*: (6) from São Francisco de Paula (MZU PL.00083); (7) from Santa Maria (MZU PL.00100). (cov) common glandular oviduct, (e) ejaculate, (ef) efferent duct, (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ma) male atrium, (ov) oviducts, (pp) penis papilla, (pv) prostatic vesicle, (sc) secretory cells, (sg) shell glands, (va) vagina. Scale bar: 1mm.

Ovaries pear-shaped. Oviducts emerge dorsally and laterally dislocated from median third of ovaries, then lead backwards immediately dorsal to nerve plate. Behind the gonopore, oviducts ascend posteriorly and medially inclined, to unite dorsally to the female atrium, thus forming the common glandular oviduct. A short common glandular oviduct opens into ental portion of female atrium. Female atrium obliquely disposed and oval-elongate in shape, and with an irregular lumen, narrower in the ental half. Length of female atrium equal to that of male atrium or approx. half male atrium length. Ental portion presents a short, dorso-anteriorly curved diverticulum (vagina) (Tables 1, 3, Figs. 6–10, 14–17).

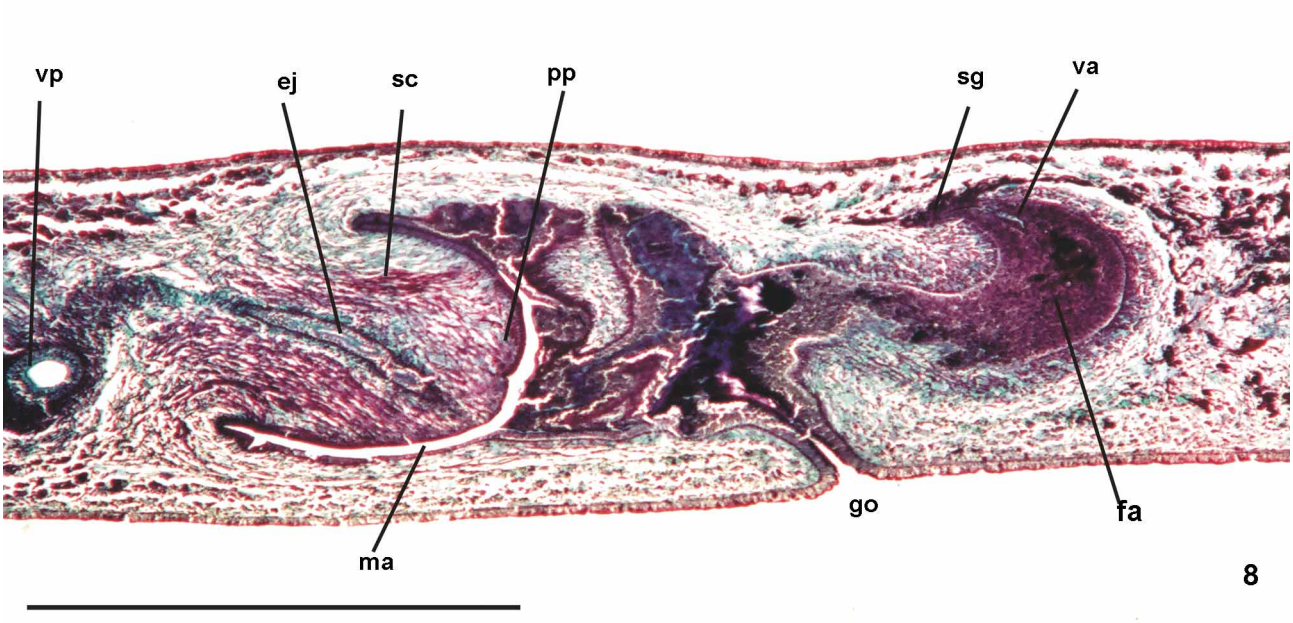


FIGURE 8. Copulatory apparatus of *G. multicolor* Graff, 1899 from Santa Maria (MZU PL.00088) in sagittal section. (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ma) male atrium, (pp) penis papilla, (pv) prostatic vesicle, (sc) secretory cells, (sg) shell glands, (va) vagina. Arrow indicates holocrine secretion. Scale bar: 1mm.

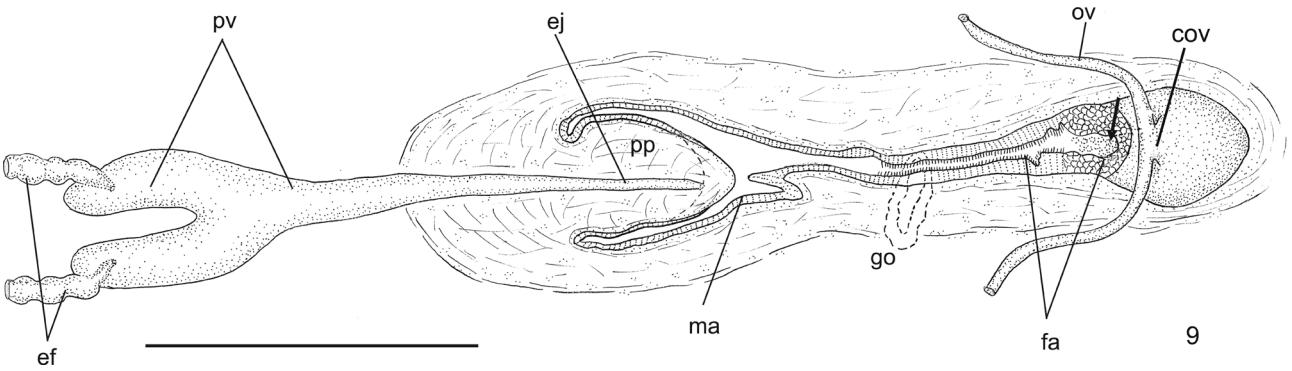


FIGURE 9. Diagrammatic horizontal composite reconstruction of the copulatory apparatus of *G. multicolor*, from Santa Maria (MZU PL.00091). (cov) common glandular oviduct, (ef) efferent duct, (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ma) male atrium, (ov) oviducts, (pp) penis papilla, (pv) prostatic vesicle. Arrow indicates holocrine secretion. Scale bar: 1mm.

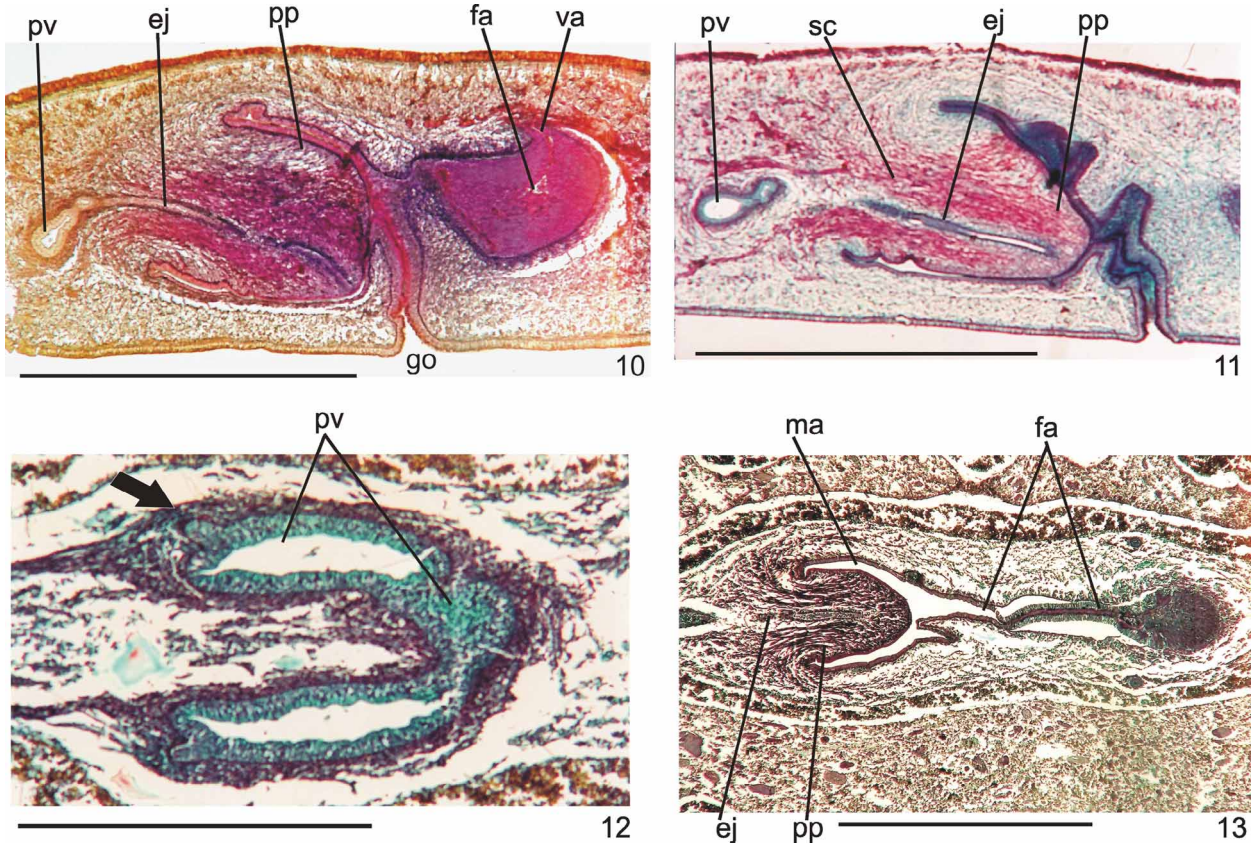
Lining epithelium of paired oviducts cuboidal to columnar ciliated, of common oviduct columnar ciliated; muscle coat of paired and common oviducts mainly with circular fibers and some interposed longitudinal fibers. Shell glands with xanthophil or erythrophil secretion opening into distal ascending portion of paired oviducts, and also into common glandular oviduct.

Lining of vagina and ental portion of female atrium very thick with multilayered aspect; more irregular in height, changing to columnar ciliated, in the ectal portion of female atrium (Figs. 6–8, 14–16). Epithelial cells of the proximal portion non-ciliated, presenting erythrophil cytoplasm; those of the distal portion with xanthophil or erythrophil apical secretion. Numerous secretory cells with cyanophil amorphous secretion and less frequent cells

with granulous erythrophil or xanthophil secretion discharge into female atrium. Cell bodies of both glands are mainly internal to the common muscle coat, those of cyanophil cells may occur externally to this coat. Muscularis of female atrium constituted of interwoven circular and longitudinal fibers, thicker (20–40µm) than in male atrium. Muscularis of vagina mainly of circular fibers with some interposed longitudinal fibers (16–30µm).

TABLE 3. Measurements, in mm, of copulatory organs of specimens of *Geoplana multicolor* Graff, 1899 studied by Marcus (1951).

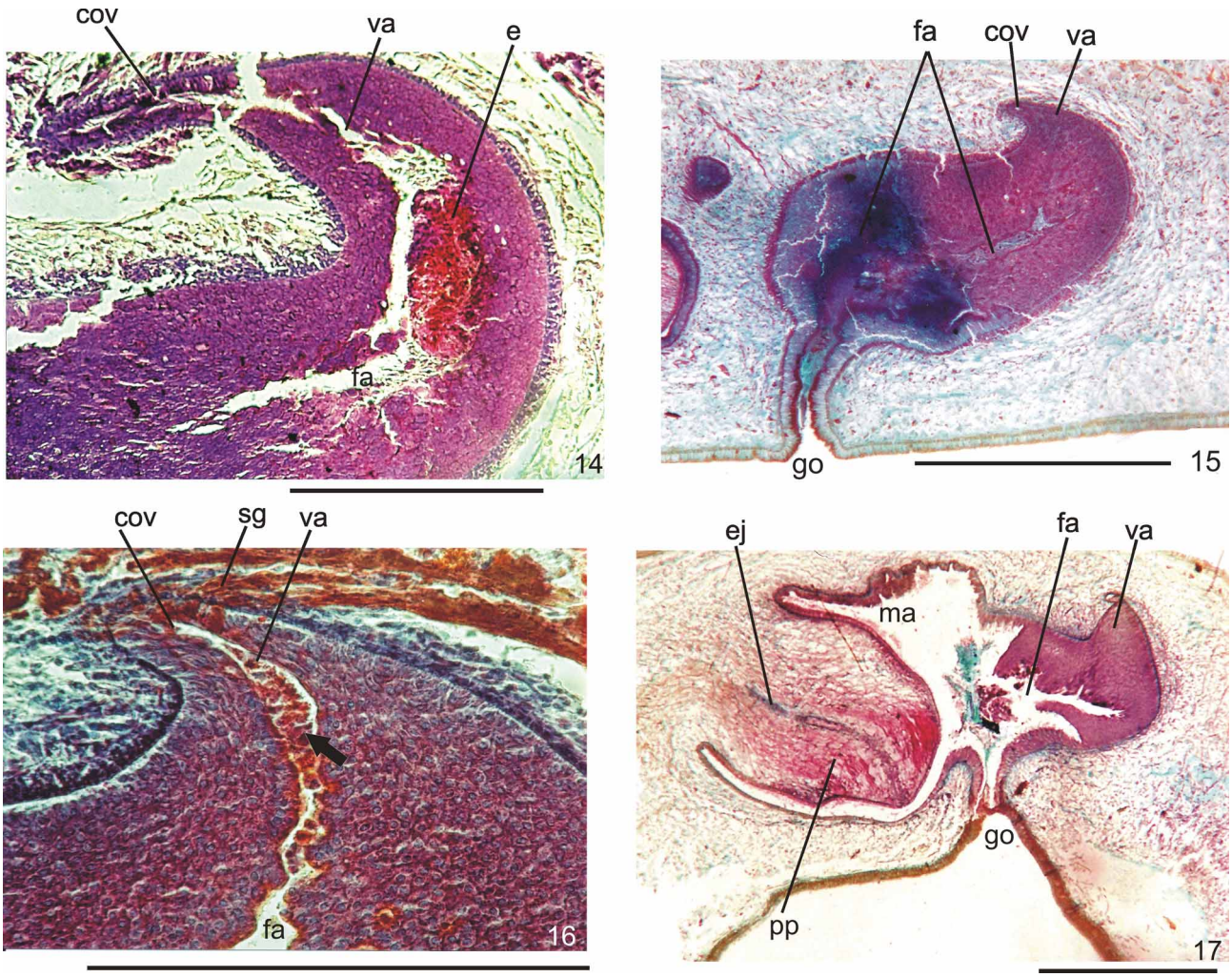
	EMF 1	EMF 2	EMF 3	EMF 4
Prostatic vesicle (forked portions)	0.3/0.4	0.3/0.4	0.3/0.4	0.3/0.4
Prostatic vesicle (unforked portion)	0.4	0.4	0.4	0.3
Penis papilla	0.7	0.9	1.0	0.7
Male atrium	0.9	1.3	1.1	0.8
Female atrium	0.8	0.7	0.9	0.7
Vagina	0.06	0.06	0.1	0.08
Common oviduct	0.04	0.08	0.08	0.06



FIGURES 10–13. Copulatory apparatus of *G. multicolor* Graff, 1899: (10) from São Francisco de Paula (MZU PL.00083) in sagittal section; (11) from Caxias do Sul (MZU PL.00095) in sagittal section; (12) prostatic vesicle and (13) male and female atria of specimen MZU PL.00091 from Santa Maria in horizontal view. (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ma) male atrium, (pp) penis papilla, (pv) prostatic vesicle, (sc) secretory cells, (va) vagina. Arrows indicate openings of the efferent ducts into the prostatic vesicle. Scale bar: 1mm (10, 11, 13); 500µm (12).

Gonopore canal approximately vertical in sagittal plane, asymmetric, inclining forwards or backwards to communicate with male or female atria which open to the canal in different parasagittal planes. A dorsal fold approximately at the level of gonopore canal leads to venter and fuses with ventral wall (Figs. 6–8, 10, 11). Gonopore canal lined with columnar ciliated epithelium with numerous openings of cells with cyanophil amorphous secretion, rhabditogen cells, and cells with granulous erythrophil secretion. Muscularis of circular fibers with some interposed longitudinal fibers.

Common muscle coat thin with circular, longitudinal and oblique fibers, inconspicuous around female atrium. Between the atrial muscularis and common muscle coat, a poorly developed stroma is present with muscle fibers variously oriented.



FIGURES 14–17. Copulatory apparatus of *G. multicolor* Graff, 1899: (14) detail of female organs of specimen MZU PL.00100 from Santa Maria in sagittal section, (15) female organs of specimen MZU PL.00095 from Caxias do Sul in sagittal view; (16) female organs of specimen MZU PL.00083 from São Francisco de Paula in sagittal section; (17) copulatory apparatus of specimen MZU PL.00087 from Ibarama in sagittal section. (cov) common glandular oviduct, (e) ejaculate, (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ma) male atrium, (pp) penis papilla, (sg) shell glands, (va) vagina. Arrow indicates holocrine secretion. Scale bar: 250µm (14, 16), 500µm (15, 17).

Remarks. Vitellaria were inconspicuous in specimens MZU PL.00090, MZU PL.00091, and MZU PL.00095. They were in an early stage of maturation in specimens MZU PL.00083 and MZU PL.00087, although well developed in specimens MZU PL.00081, MZU PL.00088 and MZU PL.00100. In specimens MZU PL.00081, MZU PL.00083, MZU PL.00088, MZU PL.00091 and MZU PL.00100 there was an abundant holocrine secretion produced by cells of a multilayered-like lining, in the lumen of the female atrium (Figs. 8–10, 14, 16). Specimens MZU PL.00088, MZU PL.00087 and MZU PL.00100 show signs of recent copulation. Specimen MZU PL.00088 had a great amount of erythrophil amorphous secretion, cyanophil amorphous secretion and sperm in the female cavity (Fig. 8), as well as sperm in the common glandular duct. Specimen MZU PL.00100 presented a more asymmetrical penis papilla, a highly folded male atrium and an ejaculate constituted of a very condensed mass of erythrophil secretion associated with sperm and cyanophil secretion fixed in the ental portion of the female atrium near the vagina (Fig. 14). The specimens MZU PL.00088 and MZU PL.00100, from Santa Maria, and MZU PL.00095, from Caxias do Sul, presented a longer male atrium than the specimens from other localities in Southern Brazil and those from São Paulo (Tables 1, 3). The very contracted and curled specimen MZU PL.00087 from Ibarama,

directly fixed after falling into a pitfall, showed anatomical and histological characters similar to the specimens from other localities in Southern Brazil and those from São Paulo. It differed especially due to the ample communication between the male and female atria in the sagittal plane which seemed to be caused by its curled condition (Fig. 17). This specimen was in an early phase of maturation as the development of vitellaria and secretory cells denotes. Specimens MZU PL.00084, MZU PL.00089, MZU PL.00093 and MZU PL.00098 were juveniles. Specimens MZU PL.00093 and MZU PL.00098 showed only a cell-agglomerate encircling a tubular or ovoid organ lined by a columnar epithelium which is interpreted as the initial formation of the copulatory organs. In specimens MZU PL.00084 and MZU PL.00089, the copulatory organs were in an early stage, being lined by a columnar epithelium of variable thickness, becoming pluristratified in the female atrium of specimen MZU PL.00084. There were neither a penis papilla nor a separation between atria in both specimens, and the openings of the female and male ducts into the copulatory organs were indiscernible.

Discussion

In general, specimens of *G. multicolor* from Rio Grande do Sul (RS) are smaller than those from São Paulo (SP). Marcus (1951) commented on specimens reaching a length of 70 mm and a width of 7 mm. Nevertheless, the author also remarked that most of the analysed worms were much smaller. Mature specimens from RS present a length of at least 35 mm and at the maximum 55 mm, and a width of 3 mm. In all specimens from this region, the dorsum presents a light coloured median stripe, which may be orange or reddish, without the occurrence of a median concentration of rust-red or brownish pigment therein. This pattern has also been observed by Froehlich (1956a, 1957) in specimens from Rio de Janeiro and Paraná states as well as by Marcus (1951) in some specimens from São Paulo. Marcus (1951) illustrated the pattern in figure 291, thereby pointing to the similarity to *Geoplana metzi* Graff (1899). The body form, the relative position of the mouth and gonopore, the pattern of distribution of the eyes and the ventral colour of the specimens herein studied are concordant with Marcus' description (1951). The specimens from RS, in general, also agree with the description of the holotype by Graff (1899) with the exception of the absence of the median concentration of rust-red pigment in the light median stripe.

Concerning the secretory cells opening through the dorsal and ventral epidermis and the constitution of the mesenchymatic musculature, the specimens analysed herein are similar to those from SP described by Froehlich (1955). Regarding the thickness of the cutaneous musculature, some specimens from Santa Maria present certain divergent characteristics in relation to those from São Francisco de Paula, Caxias and specimens studied by Froehlich (1955), namely, the dorsal cutaneous musculature being slightly thicker than the ventral cutaneous and the mc:h index smaller. These small variations were interpreted as due to populational differences.

The cylindrical pharynx presents dorsal and ventral insertions almost at the same transversal level or, a slightly posterior displacement of the dorsal insertion, as illustrated by Marcus (1951, Fig. 176). All specimens from São Paulo sectioned by Marcus, and at present in the EMF collection, reveal a short esophagus, similar to the specimens from the state of Rio Grande do Sul (RS). This was also illustrated by Marcus (1951).

The copulatory apparatus of specimens from RS was similar to that of the worms analysed by Marcus (1951), though we present an improved interpretation of the female canal and common glandular oviduct. That portion of the copulatory apparatus considered by Marcus as the female canal is the narrow lumen of the female atrium and not a proximal diverticulum of the female atrium. A dorso-anteriorly curved diverticulum is observed in specimens of *G. multicolor* from Santa Maria, Ibarama and Caxias do Sul, but its occurrence might be difficult to note in specimens from São Francisco de Paula and São Paulo. The extension of shell gland openings differs from Marcus's description which seems to be based on a not fully mature specimen (EMF 1) which was illustrated in Fig. 177 (Marcus, 1951). Three further specimens from São Paulo in the EMF collection (EMF 2 to 4) show numerous shell gland openings into the short common glandular oviduct.

Regarding male copulatory organs, the specimens from RS show a more asymmetrical penial papilla than those from São Paulo in the EMF collection. The male atrium is also longer and with a pleated distal portion in the specimens from Santa Maria when compared to those from São Francisco de Paula and most specimens from São Paulo of the EMF collection. Folds in the ectal portion of the male atrium may be absent or less developed in adult specimens which are not fully mature. A histological detail observed in all specimens from both RS and São Paulo is the occurrence of dense cilia and an accumulation of apical erythrophyl or xanthophil secretion in the lining epithelium of the distal portions of the male and female atria. As to other histological aspects, especially those regarding mus-

culature and secretory cells of the copulatory organs, their description was further enhanced and amplified after analyzing the specimens from RS.

Concerning the development of the copulatory organs in *G. multicolor*, Marcus (1951) mentioned a 14 mm long, almost completely mature worm, as presenting the lining of the female atrium with a multilayered aspect. Froehlich (1957) also described an incipiently mature, 15 mm long worm from Paraná state, as presenting the female atrium filled with a mass of cells. In the case of four juvenile specimens from Santa Maria, with lengths ranging between 17 mm and 23 mm after fixation, copulatory organs in an initial developmental phase could be distinguished in only two, whereas, there was a tubular or ovoid organ without differentiated regions in the others. The lining of the female atrium was beginning to show a multilayered aspect in only one of the juvenile specimens.

Indication of recent copulation was observed in three specimens of *G. multicolor* from RS. One of them presented an ejaculate attached to the ental atrial wall, near the vagina, which may be from a partner during copulation. This was the first observation of an ejaculate attached to the atrial wall of a species of Geoplaninae with penis papilla. In five other species of Geoplaninae, in which an ejaculate was described before, a penis papilla is absent (Souza & Leal-Zanchet, 2004). According to Souza & Leal-Zanchet, some ejaculates may perhaps act as spermatophores, playing a role in sperm transfer during copulation in species without a penis papilla. The ejaculate observed in *G. multicolor* is different from that observed in other Geoplaninae, because erythrophil secretions packed sperm in an inner core. It might be more similar to the ejaculate observed by Winsor (1991, 1998) in the rhynchodeminid *Pimea monticola*. Possibly the occurrence of spermatophores may be widespread in land flatworms and their role in sperm transfer needs to be more intensely assessed.

Geoplana multicolor is similar to 14 other species of *Geoplana* by presenting a female atrium with a very narrow lumen due to the occurrence of an epithelial lining with a multilayered aspect (Baptista & Leal-Zanchet, 2005). Among these, *G. multicolor*, *G. caapora*, *G. prudhoei*, and *G. incognita* show similar orientation of the main axis of the female atrium which is obliquely disposed with a tendency to verticality. In other species of *Geoplana*, the orientation of this axis is horizontal, with the exception of *G. preta* and *G. saima*, where it is vertical (Baptista & Leal-Zanchet, 2005). A distinctive feature *G. multicolor* is the occurrence of two distinct histological regions in the female atrium herein described. Concerning the relative extension of the vagina and the common glandular oviduct, *G. multicolor* is similar to *G. crawfordi*, *G. crioula*, *G. gaucha*, *G. rubidolineata* and *G. saima*, in presenting a very short common glandular oviduct, although the occurrence of an ental, dorso-anteriorly curved diverticulum, discernible in most analysed specimens of *G. multicolor*, distinguishes this species from the others. *Geoplana multicolor* and *G. gaucha* also share asymmetry of the gonopore canal (Froehlich, 1959).

In relation to characters of the male copulatory organs, and on considering the 14 above-mentioned species, *G. multicolor* shares with *G. caapora*, *G. carrierei*, and *G. gaucha* the occurrence of an extra-bulbar prostatic vesicle with a forked proximal portion. *Geoplana multicolor* differs from *G. carrierei* and *G. gaucha* by having a shorter prostatic vesicle, and from *G. gaucha* and *G. caapora* by the proximal position of the opening of the efferent ducts into the prostatic vesicle.

In conclusion, some specimens of *G. multicolor*, including those from Southern Brazil described herein, express external similarity to those of *G. metzi* Graff (1899), although the internal morphology of both species is distinct. The combination of characters of external and internal morphology also allows for distinguishing *G. multicolor* from other species of *Geoplana* which possess a female atrium lined by a tall epithelium with multilayered aspect.

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