

A species complex in the genus *Notogynaphallia* Ogren and Kawakatsu (Platyhelminthes : Tricladida : Terri-cola) with a taxonomic revision of homonyms of *Geoplana marginata* Schultze & Müller and a reinterpretation of *Notogynaphallia caissara* (Froehlich) anatomy

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ABSTRACT. Three species of Geoplaninae from Southern Brazil with elongated body, parallel margins and yellowish dorsum with five to seven dark longitudinal stripes are studied. The three species, besides *Notogynaphallia ceciliae*, constitute a complex presenting a long prostatic vesicle, a folded and usually very long male atrium, a female atrium ending in a dorsally or dorso-anteriorly directed diverticulum (vagina), and a long common glandular oviduct approaching dorso-anteriorly. Besides details of the external morphology, the species are mainly differentiated by anatomical characters, such as thickness of the cutaneous musculature (mc : index), position of the ovary as related to first testes, posterior limit of the serial testes, rising of the oviducts as related to the gonopore, site of the efferent duct entrance into the prostatic vesicle, morphology of the prostatic vesicle, and morphology of the male and female atria. Two of the five-striped species were identified, one as *Geoplana marginata* sensu Graff, and the other as *G. marginata* sensu Marcus, confirming that they are different species. Both are re-defined and re-named, respectively as *N. graffi* sp. nov. and *N. ernesti* sp. nov. The third, seven-striped, species was identified as *N. abundans* and its anatomy is described for the first time.

KEY WORDS : land planarians, Geoplaninae, morphology, taxonomy

INTRODUCTION

LEAL-ZANCHET & FROEHLICH (2001) proposed a complex of four species within the genus *Notogynaphallia* Ogren & Kawakatsu, 1990, all of them characterized by an elongate body with parallel margins, and dorsum with five or seven dark longitudinal stripes on yellowish background. In addition, comments were made on the present taxonomic status of *Geoplana marginata* Schultze & Müller, 1857 and of the four species misidentified and described by several subsequent authors as this same species.

One of the four species of the complex, *Notogynaphallia ceciliae*, was described by FROEHLICH & LEAL-ZANCHET (2003). The remaining three species of the complex, the three among those previously misidentified as *G. marginata*, are described now.

MATERIAL AND METHODS

Material of *N. ernesti* sp. nov. (= *G. marginata* sensu Marcus) comprised specimens from Jundiá (23°10'60''S, 46°52'W), Valinhos (22°56'60''S, 47°1'W) and the Botanical Garden of São Paulo (23°31'60''S, 46°37'W), state of São Paulo; from Curitiba (25°25'S, 49°15'W),

state of Paraná; and from the National Forest of São Francisco de Paula (29°23'-29°27'S, 50°23'-50°25'W), São Francisco de Paula, state of Rio Grande do Sul. Specimens from São Paulo and Paraná belong to the Land Planarian Scientific Collection of E. M. Froehlich (EMF coll.). Studied specimens of *N. graffi* sp. nov. (= *G. marginata* sensu Graff) were from São Francisco de Paula (National Forest of São Francisco de Paula), Salvador do Sul (29°26'60''S, 51°31'W) and Três Coroas (29°31'60''S, 50°47'60''W), state of Rio Grande do Sul. Studied specimens of *Notogynaphallia abundans* (GRAFF, 1899) were from Campo Bom (29°40'60''S, 51°2'60''W), Glorinha (29°52'S, 50°47'60''W), Novo Hamburgo (29°40'60''S, 51°7'60''W), Salvador do Sul (29°26'60''S, 51°31'W), Poço das Antas (29°26'60''S, 51°40'W), São Leopoldo (29°46'S, 51°8'60''W) and Tupandi (29°28'S, 51°25'W), state of Rio Grande do Sul. In addition, Marcus' original slides of *N. ernesti* as well as C. G. Froehlich's slides of *N. abundans* were examined. Besides, slides of the pharynx and copulatory apparatus of three other species of the same intragenetic group, as delimited in FROEHLICH & LEAL-ZANCHET (2003), *N. caissara* (E.M. Froehlich, 1955), *N. muelleri* (Diesing, 1861) and *N. fita* (Froehlich, 1959), were studied for comparison. The material of the three species belongs to EMF coll.

For analysis of external and internal characters as well as processing of the newly collected material, methods described in FROEHLICH & LEAL-ZANCHET (2003) were used. The material was sectioned at 6-10µm. Worms from EMF coll. were treated in the same way; they had been previously fixed in Formalin/Alcohol/Acetic Acid (FAA) or 4% formalin (ROMEIS, 1989) and maintained in 70% ethanol.

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 transversal section of the pre-pharyngeal region. Mesenchymatic muscle fibers were counted in transversal 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 (red-loving), xanthophil (orange-loving) and cyanophil (dark blue-loving). The term cyanophil also applies to secretions which have an affinity for the green dye of Goldner's Masson.

Specimens and type-material have been deposited in the following reference collections : Museu de Zoologia da Universidade do Vale do Rio dos Sinos (MZU), São Leopoldo, Rio Grande do Sul, Brazil, the Helminthological Collection of Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo, São Paulo State, Brazil, as well as in the Land Planarian Scientific Collection of E.M. Froehlich (EMF), Department of Zoology, Universidade de São Paulo, São Paulo, São Paulo State, Brazil.

TAXONOMIC PART

Family Geoplanidae Stimpson, 1857

Subfamily Geoplaninae Stimpson, 1857

Notogynaphallia Ogren & Kawakatsu, 1990

Notogynaphallia ernesti sp. nov.

Geoplana marginata : Marcus, 1951

Geoplana abundans : Almeida, Yamada & E.M. Froehlich, 1988 (misidentification)

Geoplana marginata (auctorum) : Almeida, Yamada & E.M. Froehlich, 1991

Notogynaphallia marginata (in part) : comb. nov. Ogren, Kawakatsu & Froehlich, 1992

Notogynaphallia sp. 5 : Leal-Zanchet & Carbayo, 2000

Notogynaphallia marginata sensu Marcus, 1951 : Leal-Zanchet & Froehlich, 2001; Carbayo, Leal-Zanchet & Vieira, 2001

Geoplana marginata sensu Marcus, 1951 : Carbayo, Leal-Zanchet & Vieira, 2002

Nec *Geoplana marginata* Schultze & Müller, 1857

Nec *Geoplana marginata* : Graff, 1899

Nec *Geoplana marginata* : Riester, 1938

Etymology

The specific name is homage to Ernst Gotthelf Marcus and the importance of his, in several aspects, pioneer work regarding Brazilian turbellarians.

Type material

Holotype : EMF Nr. 4 : Cidade Jardim, São Paulo/ SP. Collected (03.V.47) and studied for the first time by E. Marcus. Copulatory apparatus : sagittal sections on 4 slides/ Fig. 153 (Marcus, 1951 : p. 181); pharynx : sagittal sections on one slide/ Fig. 152 (Marcus, 1951 : p. 181); and pre-pharyngeal region : transversal sections on one slide (Marcus, 1951 : p. 181).

Paratypes : EMF Nr. 5 : Cidade Jardim, São Paulo/ SP. Collected (03.V.47) and studied for the first time by E. Marcus. Copulatory apparatus : sagittal sections on 4 slides/ Fig. 154 (Marcus, 1951 : p. 181); EMF Nr. 595a : O. Froehlich & M. Schweiger, leg. 22.VII.85, Valinhos/ SP – preserved in ethanol 70°; EMF Nr. 595b : O. Froehlich & M. Schweiger, leg. 22.VII.85, Valinhos/ SP, anterior region at level of ovaries : sagittal sections on 7 slides; pre-pharyngeal region : transversal sections on 4 slides; pharynx : sagittal sections on 4 slides; copulatory apparatus : sagittal sections on 10 slides; EMF Nr. 677 : O. Françoso Júnior & M. Ramos, leg. 09.I.87, Botanical Garden, São Paulo/ SP, pre-pharyngeal region : transversal sections on 4 slides; pharynx : sagittal sections on 9 slides; copulatory apparatus : sagittal sections on 10 slides; EMF Nr. 761a : O. Françoso Júnior, leg. 09.XI.87, Parque do Museu de História Natural, Curitiba/ PR – preserved in ethanol 70°; EMF Nr. 761b : O. Françoso Júnior, leg. 09.XI.87, Parque do Museu de História Natural, Curitiba/ PR, pre-pharyngeal region : transversal sections on 3 slides; pharynx : sagittal sections on 6 slides; copulatory apparatus : sagittal sections on 8 slides; EMF Nr. 927 : C. F. Rocha, leg. 22.VI.96, Serra do Japi, Jundiá/ SP, pre-pharyngeal region : transversal sections on 5 slides; pharynx : sagittal sections on 16 slides; copulatory apparatus : sagittal sections on 7 slides; MZUSP PL.173 : F. Carbayo, leg. 25.IX.98, São Francisco de Paula/ RS, anterior region at level of ovaries : sagittal sections on 11 slides; pre-pharyngeal region : transversal sections on 5 slides; pharynx : sagittal sections on 7 slides; copulatory apparatus : sagittal sections on 11 slides; MZUSP PL.174 : R.A. Castro, leg. 05.XII.2000, São Francisco de Paula/ RS - preserved in ethanol 70°; MZU PL.00046 : F. Carbayo, leg. 25.IX.98, São Francisco de Paula/ RS – preserved in ethanol 70°; MZU PL.00047 : F. Carbayo, leg. 25.IX.98, São Francisco de Paula/ RS, pre-pharyngeal region : transversal sections on 8 slides; pharynx in two fragments : sagittal sections on 13 slides; copulatory apparatus in two fragments : sagittal sections on 18 slides; MZU PL.00048 : F. Carbayo, leg. 23.X.98, São Francisco de Paula/ RS, region anterior to ovaries : sagittal sections on 12 slides; anterior region at level of ovaries : sagittal sections on 17 slides; pre-pharyngeal region in two fragments : transversal sections on 17 slides; pharynx : sagittal sections on 13 slides; copulatory apparatus : sagittal sections on 20 slides; MZU PL.00049 : F. Carbayo, leg. 13.V.99, São Francisco de Paula/ RS, copulatory apparatus : horizontal sections on 8 slides; MZU PL.00050 : F. Carbayo, leg. 14.IX.99 – pre-pharyngeal region : transversal sections on 6 slides; pharynx : sagittal sections on 7 slides; copulatory apparatus : sagittal sections on 9 slides.

Type-locality

Cidade Jardim, São Paulo, state of São Paulo (SP), Brazil.

Distribution

São Paulo (Ribeirão Pires, São Paulo, Jundiaí, Valinhos), Paraná (Curitiba), Rio Grande do Sul (São Francisco de Paula) - Brazil.

Diagnosis

Dorsum yellowish with five brownish to black longitudinal stripes; median stripe thin and discontinued; paramedian and lateral stripes distinct, continued, of variable width; eyes dorsal, without clear halos except those in lateral stripes; glandular margin mainly with abundant xanthophil cells; mc :h, 15-20%; pharynx bell-shaped with folded margins; most anterior testes level with ovaries, most posterior ones anterior or lateral to pharynx; efferent ducts opening into anterior third of prostatic vesicle;

extrabulbar prostatic vesicle, oval to oval-elongate, unforked; male atrium, long, highly folded, with many secretory cells, less frequent proximally and distally; oviducts emerging from dorsal side of median third of ovaries and rising anteriorly to gonopore; common glandular oviduct dorsal to female atrium; female atrium relatively short, with some folds, continuing dorsally by slightly anteriorly bent vagina; length of male atrium, 4.0 to 5.0 times that of female one.

External morphology

Body elongate with parallel margins. Anterior end obtuse, posterior pointed. When creeping maximal length 70mm (Table 1). Mouth and gonopore distance from anterior tip vary a great deal in specimens of different provenances (Table 1), in part due to different maturation stages of the worms. Back pale yellow, ventral side cream. Live specimens from São Francisco de Paula, with anterior tip orange dorsally and ventrally. Dorsum with five longitudinal stripes, one median, two paramedian and two lateral ones (Figs 1, 4-6), former brownish, others black. In preserved specimens groundcolour fades, but stripes maintain colour.

TABLE 1

Measurements, in mm, of type-specimens of *N. ernesti* sp. nov. - : not measured; * : After fixation; ** Specimens with damaged anterior tip;

DG : distance of gonopore from anterior end; DM : distance of mouth from anterior end;

DMG : distance between mouth and gonopore; DPVP : distance between prostatic vesicle and pharyngeal pouch.

The numbers given in parentheses represent the position relative to body length.

	paratype EMF 595a	paratype EMF 595b	paratype EMF 677	paratype EMF 761a**	paratype EMF 761b**	paratype EMF 927	paratype MZU/SP PL.173	paratype MZU/SP PL.174	paratype MZU PL.00046	paratype MZU PL.00047	paratype MZU PL.00048	paratype MZU PL.00049	paratype MZU PL.00050
Maximum length in extension	-	-	-	-	-	70	35	60	30	42	57	49	35
Maximum width in extension	-	-	-	-	-	2.0	2.5	3.0	2.0	2.5	2.5	2.5	2.5
Length at rest	-	-	-	-	-	-	22	-	16	20	22	35	15
Maximum width at rest	-	-	-	-	-	-	4	-	3.5	3	4.5	3.0	4.0
Length*	21	35	38	28	27	59	29	42	21.5	33	37	31	30
Width*	2.0	2.5	2.5	2.5	1.5	2.5	2.5	3.0	2.0	2.0	3.5	2.5	2.5
DM*	6	20.5	22	12	10	35.5	18	29	11	21.5	23	18.5	16
	(29%)	(58%)	(58%)	(43%)	(37%)	(60%)	(62%)	(69%)	(51%)	(65%)	(77%)	(60%)	(53%)
DG*	10	27.5	28	18	17	45.5	22	32	15	24	28	23.5	22
	(47%)	(79%)	(74%)	(64%)	(63%)	(77%)	(76%)	(76%)	(70%)	(73%)	(76%)	(76%)	(73%)
DMG*	4.0	7.5	6.0	6.0	7.0	10	4.0	3.0	4.0	2.5	5.0	5.0	6.0
DPVP*	-	1.1	3.8	-	0.2	2.7	0.4	-	-	0.35	0.45	-	0.8
Creeping sole	-	81%	86%	-	82%	-	-	-	-	79%	79%	-	-
Ovaries	-	9.0	9.0	-	-	-	7.0	-	-	-	10	-	-
	-	(27%)	(24%)	-	-	-	(23%)	-	-	-	(27%)	-	-
Anteriormost testes	-	9.5	9.0	-	-	-	7.0	-	-	-	10	-	-
	-	(27%)	(24%)	-	-	-	(23%)	-	-	-	(27%)	-	-
Posteriormost testes	-	19.5	21	-	-	35	17	-	-	-	23	-	-
	-	(56%)	(55%)	-	-	(59%)	(59%)	-	-	-	(61.5%)	-	-
Prostatic vesicle	-	1.5	0.3	-	0.7	0.55	0.6	-	-	0.35	0.35	-	0.7
Male atrium	-	1.9	1.3	-	3.5	2.3	1.5	-	-	2.2	2.0	-	1.7
Female atrium	-	0.5	0.6	-	0.7	0.4	0.3	-	-	0.6	0.4	-	0.4



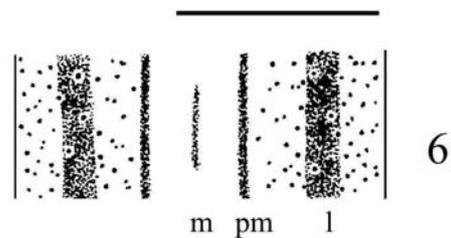
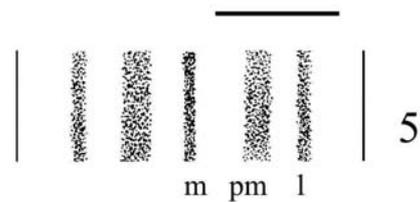
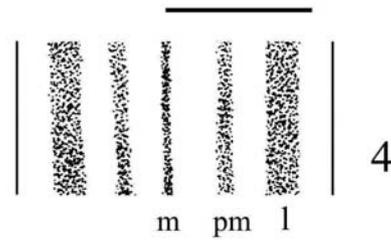
Figs 1-3. – Photographs of live specimens, dorsal view : (1) *N. ernesti* sp. nov., from São Francisco de Paula; (2) *N. graffi* sp. nov., from São Francisco de Paula; (3) *N. abundans* (Graff, 1899), from Poço das Antas.

In specimens from São Paulo state (Valinhos, Botanical Garden and Jundiaí), the median, paramedian and lateral stripes begin between ca. 0.5mm and 2mm from the anterior end (2% to 4% of body length). However, in paratype from Jundiaí, paramedian stripes begin very close to anterior tip (less than 0.5mm or 0.8% of body length). Median and paramedian stripes extend up to 0.5mm, 1.5mm or 2mm from posterior end (96% to 98% of body length); lateral stripes converge towards this tip. Lateral stripes are the widest (approx. 0.3mm or 11% of body width), then paramedian (approx. 0.1mm or 5% of body width) and median (approx. 0.05mm or 2% of body width) ones (Fig. 4).

In paratype EMF 761a, from Curitiba, with damaged anterior tip, median stripe becomes discontinued a little before ending at 2mm from posterior tip (93% of body length), paramedian stripes extend up to 1mm from posterior end (96% of body length); lateral stripes converge and extend up to this tip. Paramedian stripes the widest (approx. 0.2mm or 8% of body width), followed by lateral (approx. 0.09mm or 4% of body width) and median (approx. 0.05mm or 2% body width) ones (Fig. 5).

In paratype MZU PL.00046, from São Francisco de Paula, median stripe begins at ca. 4mm from anterior end (ca. 19% of body length) and extends up to ca. 2.5mm from posterior one (88% of body length). Paramedian and

lateral stripes begin at approx. 1.7mm (ca. 8% of body length), former ends ca. 1.0mm from posterior tip (ca. 95% of body length), later converges towards it. Lateral stripes the widest (approx. 0.16mm or 8% of body width), followed by paramedian (approx. 0.05mm or 2.5% of body width) and then median (approx. 0.03mm or 1.5% of body width) stripes (Fig. 6). Latter is discontinuous.



Figs 4-6. – Detail of colour pattern of preserved specimens of *N. ernesti* sp. nov. : (4) paratype EMF 677, from Botanical Garden, São Paulo; (5) paratype EMF 761b, from Curitiba; (6) paratype MZU PL.00046, from São Francisco de Paula. (l) lateral stripe, (m) median stripe, (pm) paramedian stripe. Scale bar : 1mm.

Eyes, initially uniserial, contour anterior tip. In paratype from Jundiaí, become pluriserial approx. 1.5mm behind anterior tip (2% of body length). Between 5mm and 12mm (ca. 8% and 20% of body length) from the tip they are more abundant, and spread up to near paramedian stripes. After 15mm (25% of body length) behind the tip they become sparser. In paratype MZU PL.00046, from São Francisco de Paula, eyes become pluriserial after approx. 0.65mm from anterior end (3% of body length). More numerous between second and sixth millimeter (ca. 9% and 28% of body length) spread up to

near paramedian stripes. Sparser backwards, become exclusively marginal from ninth millimeter (ca. 42% of body length) on. Those in lateral stripes are surrounded by clear halos (Fig. 6).

Epidermis and musculature at pre-pharyngeal region

Width of creeping sole, measured in five specimens, varied from 79% to 86% of body width (Table 1).

Four types of secretory cells discharge through dorsal epidermis and body margins: (1) cells with xanthophil secretion of coarse very dense granulation; (2) rhabdithogen cells; (3) cells with fine, weakly erythrophil secretion; (4) cells with amorphous cyanophil secretion. First two cell types are very numerous; xanthophil, even more abundant at body margins, creates a kind of glandular border. Creeping sole receives abundant cells with amorphous cyanophil secretion, cells with erythrophil granular secretion, and small quantity of rhabdithogen cells.

Cutaneous musculature with the usual three layers, longitudinal layer with thick bundles (Table 4). Musculature higher paramedianly, especially on ventral side where may be 30µm higher than medianly. Towards body margins progressively lower. Mc:h 15% to 20% (Table 4). Well developed mesenchymatic musculature composed of four layers: dorsal subcutaneous with oblique fibers variously oriented (ca. 3-5 fibers thick); supra-intestinal transversal (approx. 5 fibers thick); sub-intestinal transversal (6-8 fibers thick); and subneural transversal (ca. 3 fibers thick). In addition, scattered ventral subcutaneous oblique fibers as well as dorsoventral ones are present. Longitudinal fibers are indiscernible, if existent, few and very scattered.

Pharynx

Pharynx bell-shaped with folded margins (Fig. 7). Mouth at end of anterior third or at median one of pharyngeal pouch, slightly anterior to or on same transversal level of dorsal insertion. Proximal part of pharyngeal lumen communicating directly with intestine, so esophagus is absent. Pharyngeal glands, with cell bodies in mesenchyme, mainly anterior and lateral to pharynx, of four types: two types of secretory cells with erythrophil secretion, one with strongly stained, irregular granules, mostly fine, other with weakly stained, fine granules; cells with amorphous cyanophil secretion; and cells with dense, granulous xanthophil secretion.

Outer musculature of pharynx constituted of thin longitudinal subepithelial layer (ca. 3µm thick), followed by circular one (ca. 25µm thick), mixed internally with few longitudinal fibers. Towards pharyngeal tip, circular layer becomes as thin as longitudinal one. Inner pharyngeal musculature composed of thick circular subepithelial layer (ca. 29µm thick), followed by some longitudinal fibers. Inner musculature gradually thins outwards, and, mainly dorsally, also inwards.

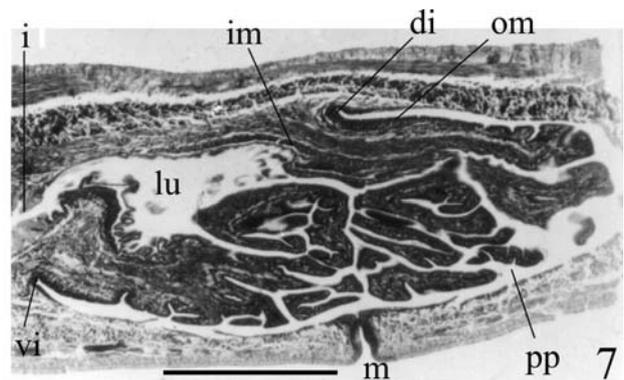


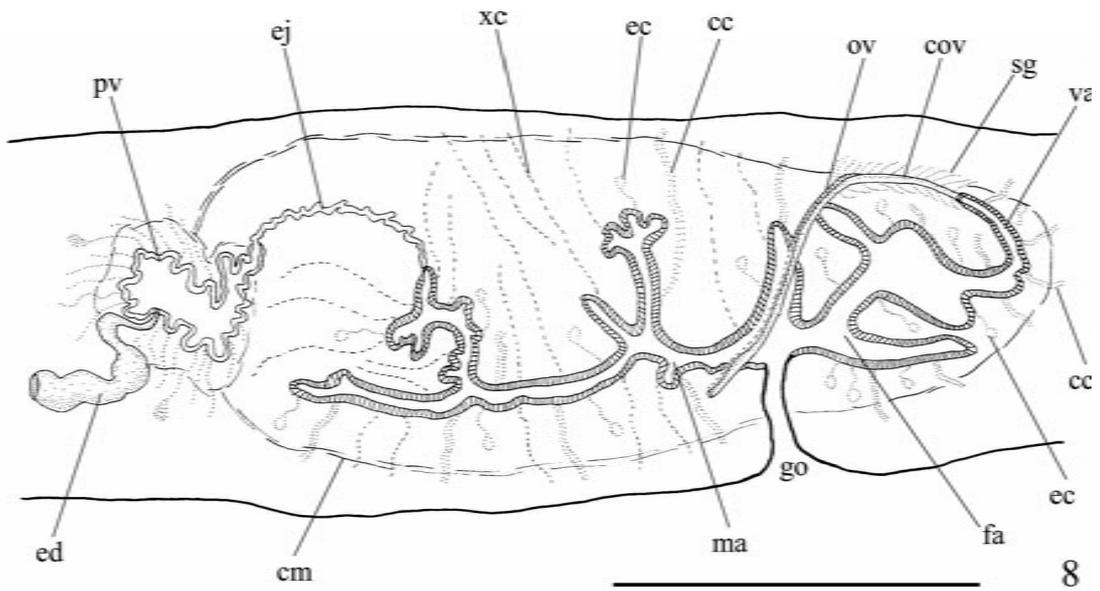
Fig. 7. – Pharynx of *N. ernesti* sp. nov. (paratype MZUSP PL.173) from São Francisco de Paula. Sagittal section. (di) dorsal insertion, (i) intestine, (im) internal musculature, (lu) pharyngeal lumen, (m) mouth, (om) outer musculature, (pp) pharyngeal pouch, (vi) ventral insertion. Scale bar: 500µm.

Reproductive organs

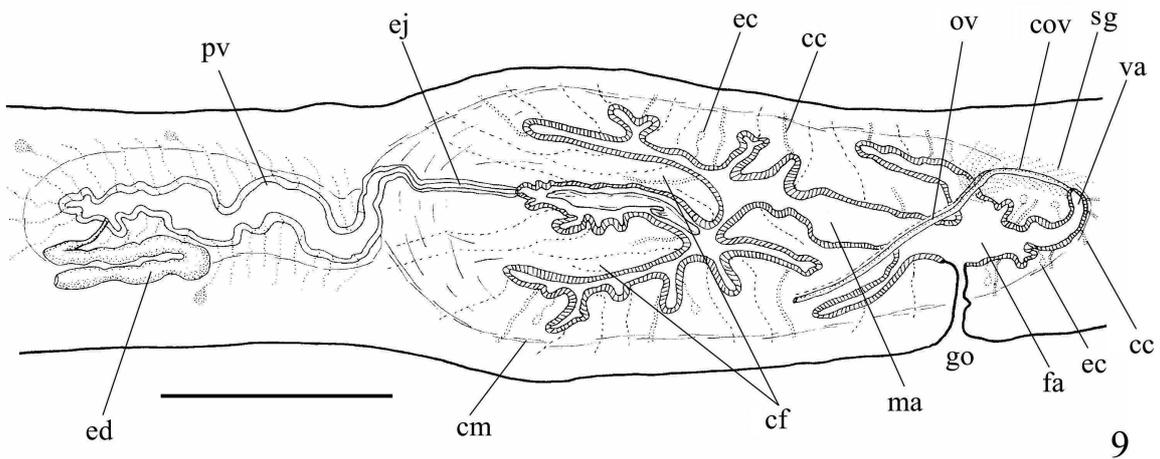
Most anterior testes approximately level with ovaries; most posterior ones anterior or lateral to pharynx, up to mouth level (Table 1). Efferent ducts, dorsolateral to oviducts in pre-pharyngeal region, run backwards, form false seminal vesicles behind pharynx, and, laterally to prostatic vesicle, turn anteriorly, ascend and enter into the ventral (Figs 8-10) or dorsal (Fig. 11) wall of vesicle near its anterior third. They are lined with ciliated cuboidal epithelium.

Extrabulbar prostatic vesicle spacious, oval to oval-elongate (Figs 8-13); more globose in paratype from Botanical Garden (Fig. 8), more elongate in that from Valinhos (Fig. 9). With no diverticula or branches; and although with a folded internal wall, the thick muscularis gives it a rather smooth external surface. Length of vesicle and distance between vesicle and pharyngeal pouch considerably variable (Table 1). Lining epithelium columnar, ciliated, of irregular height, traversed by abundant glands with coarse xanthophil secretion and cell bodies in surrounding mesenchyme. Muscularis (ca. 23µm thick) composed of interwoven longitudinal and circular fibers. Entering penis bulb, vesicle narrows, constituting a nearly rectilinear to mostly sinuous ejaculatory duct, which opens into bottom of male atrium. This opening dorsally dislocated in the worm from Botanical Garden (Fig. 8). Epithelial lining of ejaculatory duct with cuboidal to columnar ciliated cells, irregular in height, and with few openings of cyanophil secretory cells. Muscle coat thin (7µm thick) with intermixed longitudinal and circular fibers.

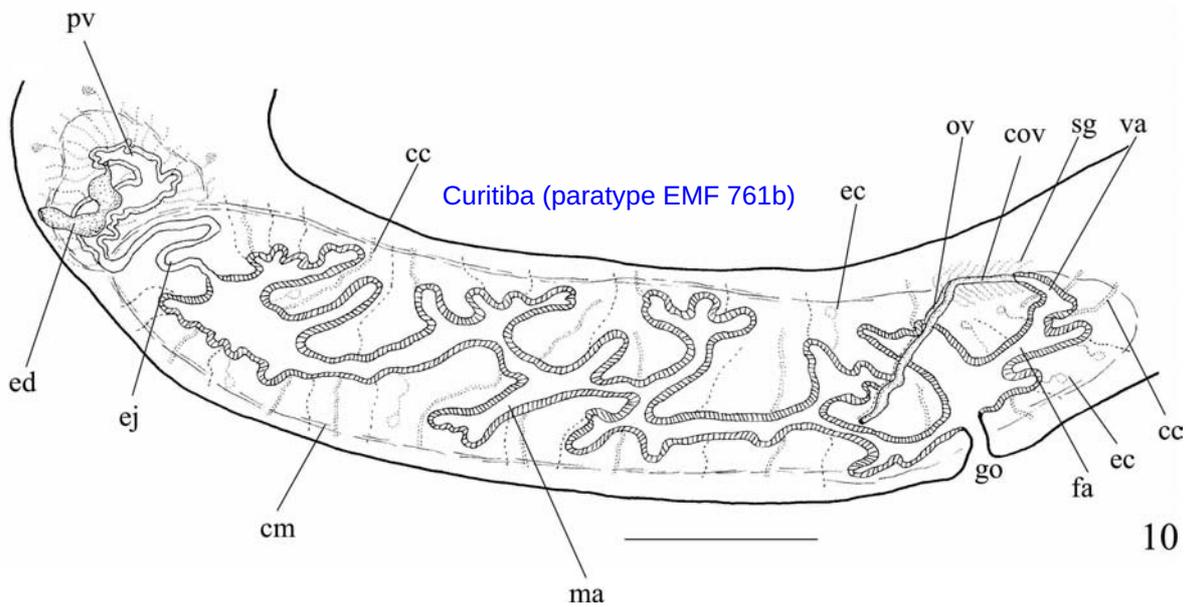
Male atrium long (Table 1), with numerous high folds that greatly restricts the whole cavity. However, in some specimens, folds near to gonopore, mainly those of ventral wall, lower, leaves atrial cavity more spacious. Folds vary considerably, regarding size, form and localization in atrial wall, in different specimens (Figs 8-12). In specimen from Valinhos, similarly to that described by MARCUS (1951) for one of his specimens (Fig. 154, p. 181), there is a circular fold, delimiting a long canal in bottom of which lies the opening of ejaculatory duct, bulging from the atrial wall. This set occupies the ental half of male cavity (Fig. 9).



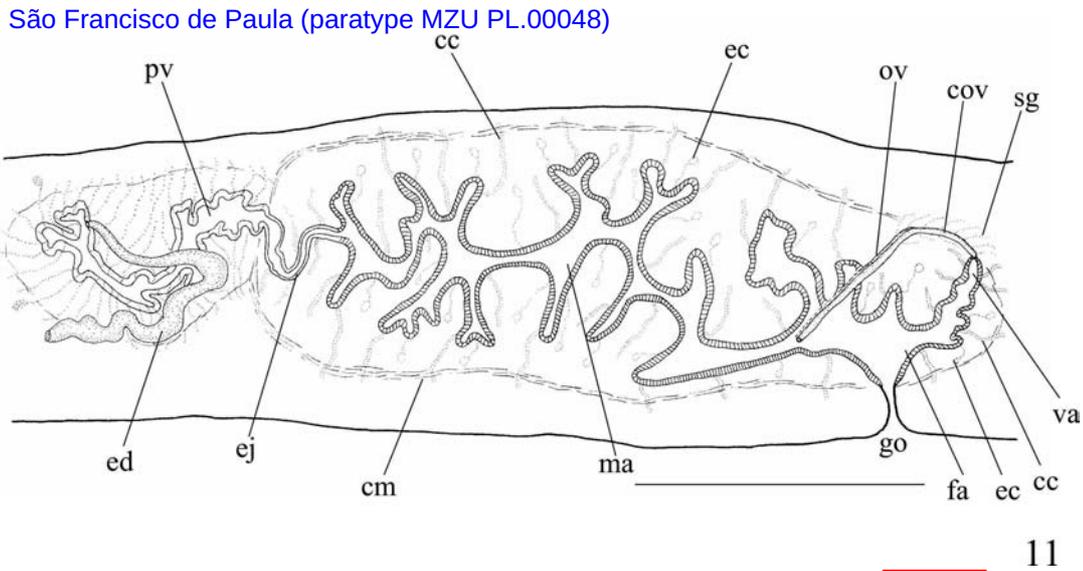
Botanical Garden, São Paulo (paratype EMF 677)



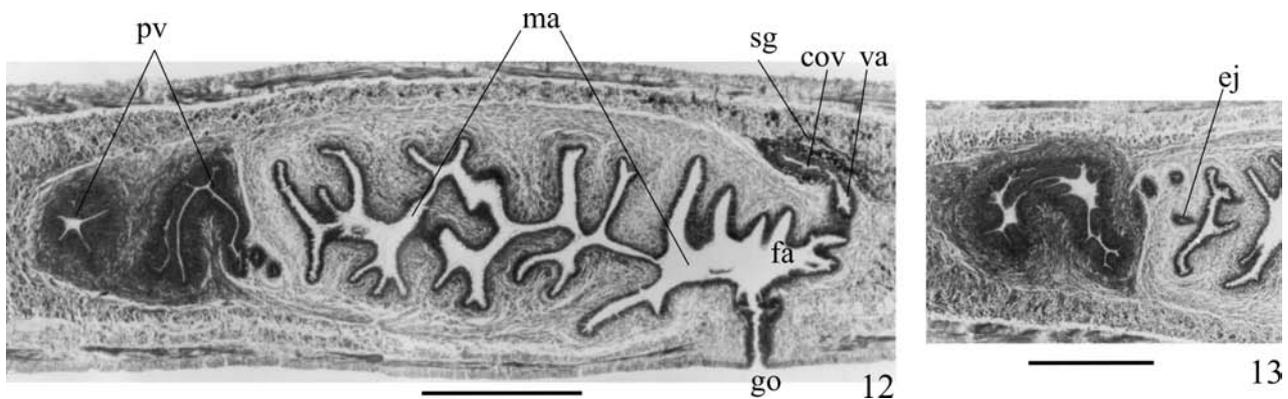
Valinhos/ SP (paratype EMF 595b)



Curitiba (paratype EMF 761b)



Figs 8-11. – Diagrammatic sagittal composite reconstruction of the copulatory apparatus of *N. ernesti* sp. nov. : (8) from Botanical Garden, São Paulo (paratype EMF 677); (9) from Valinhos/ SP (paratype EMF 595b); (10) from Curitiba (paratype EMF 761b); (11) from São Francisco de Paula (paratype MZU PL.00048). (cc) cyanophil secretory cells, (cf) circular fold, (cm) common muscle coat, (cov) common glandular oviduct, (ec) erythrophil secretory cells, (ed) efferent duct, (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ma) male atrium, (ov) oviducts, (pv) prostatic vesicle, (sg) shell glands, (va) vagina, (xc) xanthophil secretory cells. Scale bar : 1mm.



Figs 12-13. – Copulatory apparatus of *N. ernesti* sp. nov. from São Francisco de Paula (paratype MZUSP PL.173) in sagittal section : (12) overall view; (13) detail of proximal portion of male atrium. (cov) common glandular oviduct, (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ma) male atrium, (pv) prostatic vesicle, (sg) shell glands, (va) vagina. Scale bar : 500µm.

Male atrium epithelium with columnar non-ciliated cells of irregular height, and xanthophil irregular apical surface, partially broken and discharged into male atrium cavity. Three types of abundant secretory cells pearce the epithelium : cells with xanthophil granulous secretion and cells with fine granulous erythrophil secretion, both with bodies internal to common muscle coat; third type cells with fine granulous cyanophil secretion and bodies external to common muscle coat in surrounding mesenchyme. Secretory cells less numerous in the proximal atrial portion, where ejaculatory duct opens, and in the most distal one. Muscularis thick (61-68µm) constituted of circular subepithelial and subjacent longitudinal fibers, partially interwoven. In proximal atrial region, towards ejaculatory duct opening, muscularis becomes thinner, and crossed by

some fibers of stroma between muscularis and common muscle coat.

Oviducts emerging dorsally from median third of ovaries. Anterior to gonopore, oviducts ascend posterior and medially inclined, dorsally to the female atrium unite and form common glandular oviduct (Figs 8-12). The latter, a long canal slightly inclined to dorsum that leads backward to communicate with vagina. Paired oviducts as well as common oviduct lined with columnar ciliated epithelium, and coated with thin layer mainly of circular muscles. Abundant shell glands empty into common oviduct besides distally in paired oviducts.

Vagina, curved dorsoanteriorly, emerges from posterior extremity of female atrium (Figs 8-12). Female atrium short (Table 1) with some folds (Figs 8-12), and length

equal to one-quarter or one-fifth part of male atrium length in specimens from São Francisco de Paula, Valinhos and Curitiba, to half male atrium length in specimen from Botanical Garden.

Vagina and atrium lined with columnar epithelium, distally ciliated in the vagina, and with irregular height and xanthophil surface. Glands with cyanophil amorphous secretion with bodies external to common muscle coat and erythrophil glands with granulous secretion, short necks, and subepithelial bodies, discharge into the whole epithelial surface. Muscularis, weakly developed when compared with male atrium muscularis (15-25µm thick in female atrium), composed of circular fibers mixed with some longitudinal ones.

Gonopore canal vertical or slightly inclined backwards (Figs 8-12).

Common muscle coat with longitudinal, oblique and circular fibers, thicker around male (ca. 32µm thick) than around female (ca. 17µm thick) atrium. Between atrial muscularis and common muscle coat, a stroma with many, variously orientated muscle fibers (Eigenmusculatur of GRAFF, 1899), is well developed.

Notogynaphallia graffi sp. nov.

Geoplana marginata: Graff, 1899 (Tafel V, Fig. 27-29: external aspect; Textfig. 36: copulatory apparatus). Localization of original material unknown

Notogynaphallia marginata (in part): comb. nov. Ogren, Kawakatsu & E.M. Froehlich, 1992

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

Notogynaphallia marginata sensu Graff, 1899: Leal-Zanchet & Froehlich, 2001; Carbayo, Leal-Zanchet & Vieira, 2001

Geoplana marginata sensu Graff, 1899: Carbayo, Leal-Zanchet & Vieira, 2002

Nec *Geoplana marginata* Schultze & Müller, 1857

Nec *Geoplana marginata*: Riester, 1938

Nec *Geoplana marginata*: Marcus, 1951

Etymology

The specific epithet is homage to Ludwig von Graff who first described the species, and his Herculean work regarding turbellarians, and land planarians in particular.

Type material

Holotype: MZUSP PL. 176: F. Carbayo, leg. 25.IX.98, São Francisco de Paula/RS, region anterior to ovaries: sagittal sections on 4 slides; anterior region at the level of the ovaries: sagittal sections on 12 slides; pre-pharyngeal region: transversal sections on 5 slides; pharynx: sagittal sections on 10 slides; copulatory apparatus: sagittal sections on 11 slides.

Paratypes: MZUSP PL.175: F. Carbayo, leg. 10.VII.98, São Francisco de Paula/RS – preserved in ethanol 70°; MZUSP PL.177: F. Carbayo, leg. 14.X.99, São Francisco de Paula/RS, copulatory apparatus: horizontal sections on 12 slides; MZU PL.00051: F. Carbayo, leg. 26.X.97, Três Coroas/RS, copulatory apparatus in two fragments: sagittal sections on 15 slides; MZU PL.00052: M. Cardoso, leg. 11.XII.97, São Francisco de

Paula/RS, anterior region at the level of the ovaries: sagittal sections on 10 slides; pre-pharyngeal region in two fragments: transversal sections on 16 slides; pharynx: sagittal sections on 5 slides; copulatory apparatus: sagittal sections on 9 slides; MZU PL.00053: F. Carbayo, leg. 03.III.98, São Francisco de Paula/RS – preserved in ethanol 70°; MZU PL.00054: M. Cardoso, leg. 04.V.98, São Francisco de Paula/RS, preserved in ethanol 70°; MZU PL.00055: I.A. Fick, leg. 11.II.99, São Francisco de Paula/RS, pre-pharyngeal region: transversal sections on 3 slides; pharynx: sagittal sections on 6 slides; copulatory apparatus: sagittal sections on 8 slides; MZU PL.00056: I.A. Fick, leg. 13.V.99, São Francisco de Paula/RS, copulatory apparatus: horizontal sections on 4 slides; MZU PL.00057: F. Carbayo, leg. 13.V.99, São Francisco de Paula/RS, copulatory apparatus: sagittal sections on 10 slides; MZU PL.00058: A.M. Leal-Zanchet, coll. 26.VII.2000, Salvador do Sul/RS – preserved in ethanol 70°.

Type-locality

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

Distribution

Rio Grande do Sul (São Francisco de Paula, Salvador do Sul, Três Coroas, Taquara, São Leopoldo).

Diagnosis

Dorsum gold-yellow with five black well-delimited longitudinal stripes; median and lateral ones thin, paramedian wide; eyes dorsal, with clear halos when in paramedian stripes; without glandular margin; mc:h, 13-15%; pharynx cylindrical with dorsal insertion posteriorly displaced, folded margins; foremost testes anterior to ovaries, most posterior ones near root of pharynx; efferent ducts open into median third of prostatic vesicle; prostatic vesicle extrabulbar, long, spacious; male atrium, relatively short, almost filled by large annular ental fold separating from general male cavity a restrict, intra-antral, cavity with irregular contour; ejaculatory duct with two histologically distinct portions, opening through a small projection into bottommost part of intra-antral cavity; oviducts emerging dorsally from anterior or median third of ovaries, and ascending anteriorly to gonopore; common glandular oviduct dorsal to female atrium, long, with few openings of shell glands on proximal third; vagina directed dorsally and forwards; female atrium long, highly folded, approx. as long as male atrium.

External morphology

Body elongate with parallel margins, anterior end obtuse and posterior pointed. When crawling, maximal length reaches 50mm (Table 2). Mouth distance from anterior tip varying from 52% to 67% relative to body length, gonopore from 75% to 86% (Table 2). Alive, dorsum gold-yellow, becoming cream in some preserved worms. Dorsally, five black longitudinal stripes, one median, two paramedian and two lateral (Figs 2, 14). In paratype MZUSP PL.175, median stripe begins at 1.5mm

from anterior tip (ca. 7.5% of body length), paramedian at 0.3mm (ca. 1.5% of body length), and lateral at 3.0mm (ca. 15% of body length). Median stripe extends up to 0.9mm (ca. 95% of body length), paramedian and lateral stripes up to 0.3mm (ca. 98% of body length) from poste-

rior tip. All stripes well delimited, median and lateral ones thin (approx. 0.05mm or 3% of body width), and paramedian stripes comparatively very wide (approx. 0.3mm or 19% of body width) (Fig. 14). Venter whitish or yellowish.

TABLE 2

Measurements, in mm, of type-specimens of *N. graffi* sp. nov. - : not measured; * : After fixation; ** Exemplar with empty pharyngeal pouch (without the pharynx); 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 as related to body length.

	paratype MZUSP PL.175	holotype MZUSP PL.176	paratype MZUSP PL.177	paratype MZU PL.00051	paratype MZU PL.00052**	paratype MZU PL.00053	paratype MZU PL.00054	paratype MZU PL.00055	paratype MZU PL.00056	paratype MZU PL.00057	paratype MZU PL.00058
Maximum length in extension	28	45	45	-	34	26	22	47	43	35	50
Maximum width in extension	1.0	2.0	2.0	-	1.5	1.5	1.5	1.0	1.0	2.0	2.0
Length at rest	12	35	20	-	-	14	12	32	18	20	-
Maximum width at rest	2.0	3.5	3.0	-	-	2.0	2.0	2.0	2.0	3.0	4.0
Length*	20	41	37	27	30	21	16	32	25	28	36
Width*	1.5	2.0	2.0	2.0	1.5	1.5	1.5	2.0	2.0	<2.0	2.5
DM*	12 (60%)	24 (58%)	23 (62%)	14 (52%)	18 (60%)	14 (67%)	8 (50%)	19 (59%)	16 (64%)	16 (57%)	23 (64%)
DG*	16 (80%)	33 (80%)	29 (78%)	21 (78%)	23 (77%)	18 (86%)	10 (62%)	25 (78%)	20 (80%)	21 (75%)	29 (80%)
DMG*	4.0	9.0	6.0	7.0	5.0	4.0	2.0	6.0	4.0	5.0	6.0
DPVP*	-	1.7	-	0.7	0.4	-	-	-	-	-	-
Creeping sole	-	83%	-	-	-	-	-	85%	-	-	-
Ovaries	-	7.0 (22%)	-	-	7.0 (17%)	-	-	-	-	-	-
Anteriormost testes	-	6.0 (19%)	-	-	5.4 (14%)	-	-	-	-	-	-
Posteriormost testes	-	23 (55%)	-	-	17.4 (58%)	-	-	-	-	-	-
Prostatic vesicle	-	2.5	2.8	2.0	2.0	-	-	-	-	-	-
Male atrium	-	0.9	0.6	0.6	0.9	-	-	-	-	-	-
Female atrium	-	1.0	0.6	0.7	0.8	-	-	-	-	-	-

In paratype MZUSP PL.175, eyes uniseriably surround anterior tip, and become pluriserial immediately after. Between 2.5mm and 9.0mm (approx. 12% and 45% of body length) behind anterior end, they extend from body margins up to paramedian stripes, and may invade the latter acquiring clear halos (Fig. 14). Subsequently, up to posterior end, they become scarce and limited to body margins.

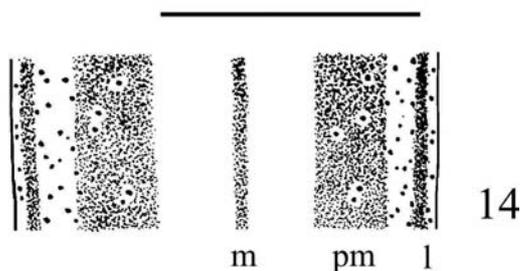


Fig. 14. – Detail of colour pattern of preserved specimen of *N. graffi* sp. nov. (paratype MZUSP PL.175). (l) lateral stripe, (m) median stripe, (pm) paramedian stripe. Scale bar : 1mm.

Epidermis and musculature at pre-pharyngeal region

Creeping sole, 83% to 85% of body width (Table 2).

Three types of secretory cells open through dorsal epidermis and body margins : (1) cells numerous with coarse erythrophil secretion; (2) cells less frequent with cyanophil amorphous secretion; (3) rhabditogen cells with xanthophil secretion. A fourth secretory cell type with fine chromophob granular secretion opens near body margins. Creeping sole receives less numerous secretory cells of three types : scarce cells with fine weakly erythrophil granular secretion; cells with cyanophil amorphous secretion; and rhabditogen cells. Glandular margin is absent.

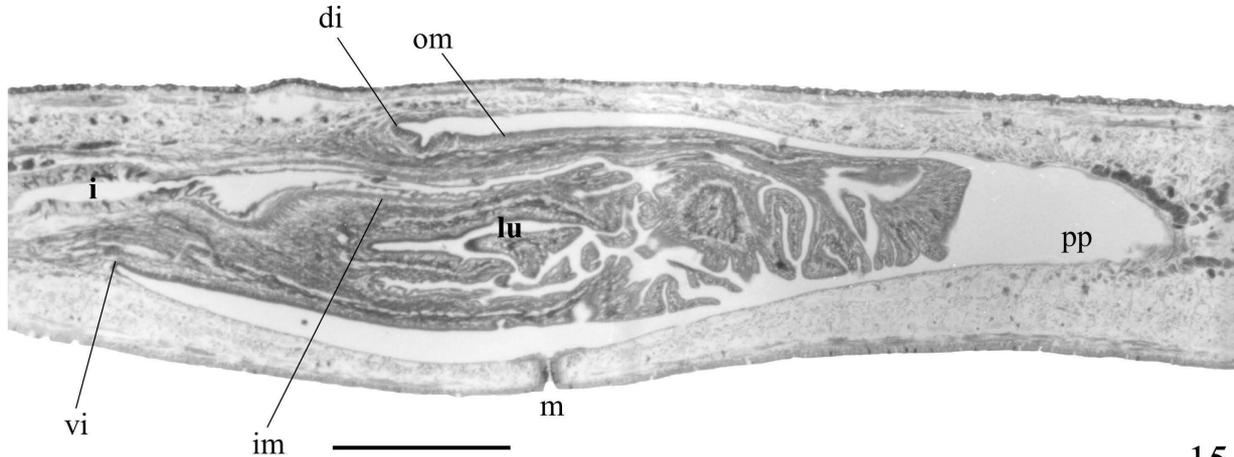
Cutaneous musculature with constitution similar to that described for *N. ernesti*, being, however, laterally in pre-pharyngeal sections, as high as medially. Mc:h 13% to 15% (Table 4). Mesenchymatic musculature as in *N. ernesti*.

Pharynx

Pharynx (Fig. 15) of cylindrical type with dorsal insertion posteriorly displaced, but still in anterior third of pha-

ryngeal pouch, and with folded margins. Mouth in median third of pharyngeal pouch : posterior to dorsal insertion. No esophagus. Pharyngeal glands with cell bodies located in mesenchyme, mainly anterior and posterior to pharyngeal pouch. Three secretory cell types : (1) cells with

densely arranged, xanthophil granulous secretion; (2) cells with strongly erythrophil granulous secretion; and (3) cells with cyanophil amorphous secretion. Outer and inner pharyngeal musculatures as in *N. ernesti*, but inner one less developed.



15

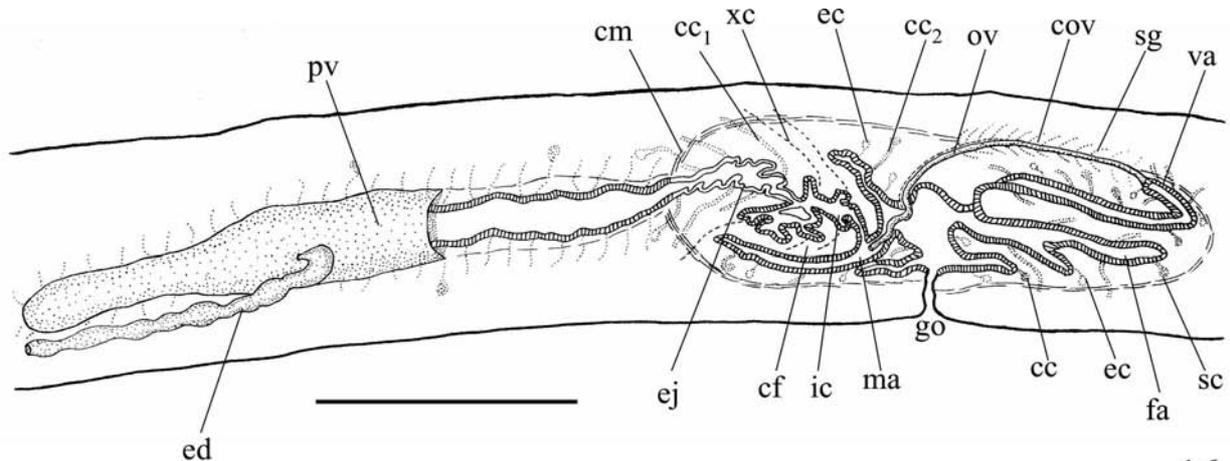
Fig. 15. – Pharynx of *N. graffi* sp. nov. (holotype). Sagittal section. (di) dorsal insertion, (i) intestine, (im) internal musculature, (lu) pharyngeal lumen, (m) mouth, (om) outer musculature, (pp) pharyngeal pouch, (vi) ventral insertion. Scale bar : 500µm.

Reproductive apparatus

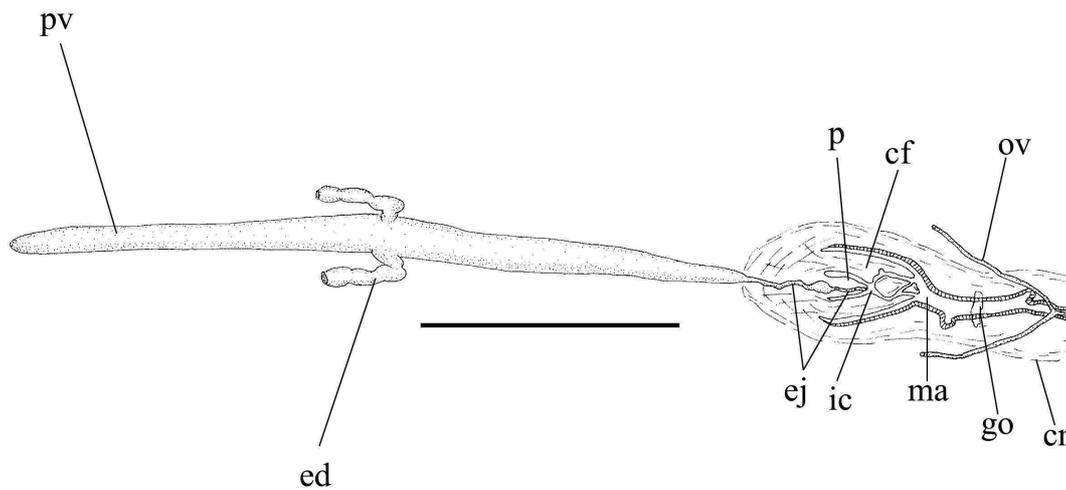
Testes beginning anteriorly to ovaries and extending up to near root of the pharynx (Table 2). Pre-pharyngeally, efferent ducts dorsal to oviducts, sometimes laterally displaced. Behind pharynx forming false seminal vesicles and opening laterally into median third of prostatic vesicle (Figs 16-19).

Extrabulbar prostatic vesicle elongate (Table 2), with spacious cavity (Figs 16-19). Proximal portion not reaching pharyngeal pouch; unforked in holotype and most analysed specimens, except for paratype 172 which a short ental furcation (0.4mm or approx. 1/5 of total length of vesicle). Lining epithelium columnar ciliated, irregular

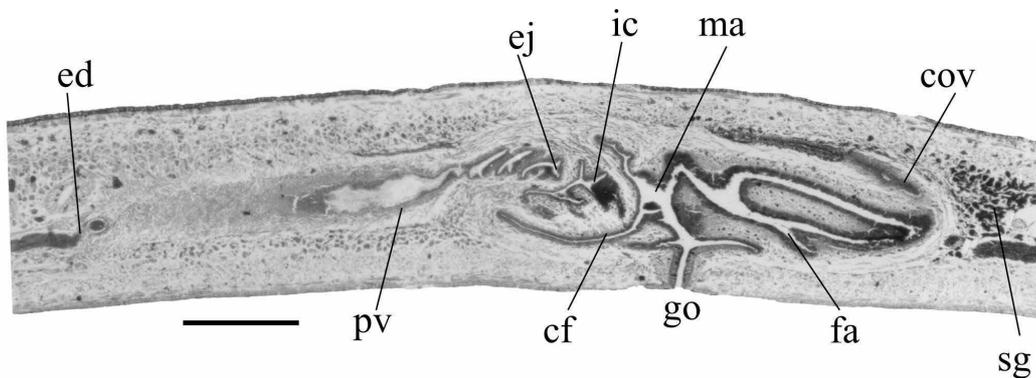
in height, receiving numerous fine-grained, erythrophil glands with polygonal cell bodies in surrounding mesenchyme. Muscularis well developed, mainly at the proximal half (35µm thick), at the distal half diminishing up to 24µm; composed of interwoven circular, oblique and longitudinal fibers. Approaching penis bulb, prostatic vesicle narrows and gives rise to sinuous ejaculatory duct, lined with columnar ciliated epithelium, higher in proximal portion, weakly cyanophil glands, with cell bodies extrabulbar around prostatic vesicle, opening into both. Coating muscularis, consisting of mixed circular and longitudinal fibers, weakly developed but thicker proximally (9µm) than distally (2µm).



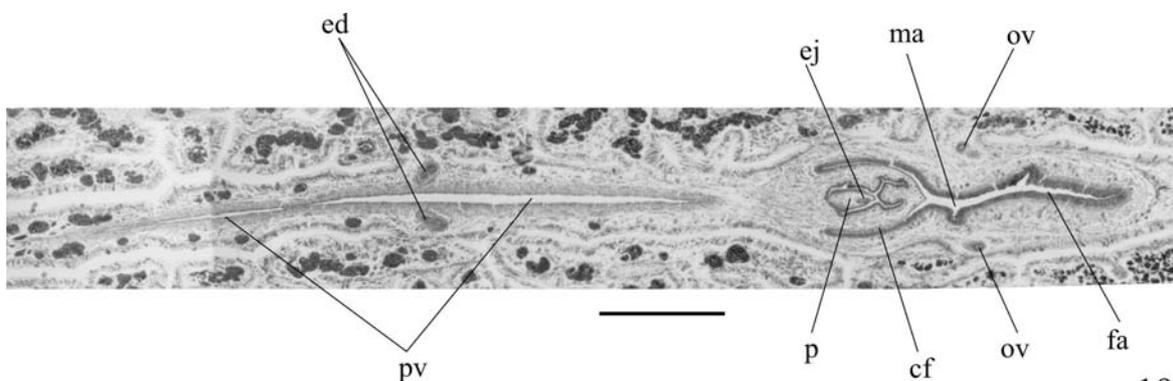
16



Figs 16-17. – Diagrammatic composite reconstructions of copulatory apparatus of *N. graffi* sp. nov. : (16) from sagittal sections (holotype); (17) from horizontal sections (paratype MZUSP PL.177). (cc) cyanophil secretory cells, (cc₁) cyanophil secretory cells opening into the ejaculatory duct, (cc₂) cyanophil secretory cells opening into the male atrium, (cf) circular fold, (cm) common muscle coat, (cov) common glandular oviduct, (ec) erythrophil secretory cells, (ed) efferent duct, (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ic) intra-antral cavity, (ma) male atrium, (ov) oviducts, (p) projection into the intra-antral cavity, (pv) prostatic vesicle, (sc) secretory cells with ill-defined stained secretion, (sg) shell glands, (va) vagina, (xc) xanthophil secretory cells. Scale bar : 1mm.



18



19

Figs 18-19. – Copulatory apparatus of *N. graffi* sp. nov. : (18) holotype in sagittal section; (19) paratype MZUSP PL.177 in horizontal section. (cov) common glandular oviduct, (cf) circular fold, (ed) efferent duct, (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ic) intra-antral cavity, (ma) male atrium, (ov) oviducts, (p) projection into the intra-antral cavity, (pv) prostatic vesicle, (sg) shell glands. Scale bar : 500µm. In fig. (18), section shows only the posterior third of the prostatic vesicle.

Relatively short male atrium (Table 2) mainly filled by a large annular fold that, arising from its bottom, encloses an intra-antral restricted cavity, with irregular contour and folded walls (Figs 16-19). Ejaculatory duct opens into a small projection of the bottommost part of intra-antral cavity. In paratypes MZU PL.00051 and MZUSP PL.177, both fixed after copulation (24h and 2h, respectively), this projection is more in evidence (Fig. 19). In two incompletely mature worms (paratypes MZU PL.00056 and MZU PL.00057), the annular fold is elongate, cylindrical, encircling a canalicular intra-antral cavity extending as a direct continuation of the ejaculatory duct. Transition from ejaculatory duct to canalicular intra-antral cavity very clear due to their different linings.

Epithelial lining of male atrium, circular fold and enclosed cavity included, with low columnar non-ciliated with erythrophil cytoplasm and xanthophil apical portion. Two types of secretory cells empty through the epithelium: (1) cells containing strongly erythrophil granulous secretion with short necks and subepithelial bodies; (2) cyanophil cells with bodies internal to muscle coat. A third type of gland, with weakly xanthophil granulous secretion and bodies in mesenchyme external to bulb, enter the circular fold and open through its epithelial lining. Muscularis of male atrium (10-12 μ m) less developed in the circular fold (6-7 μ m); composed of circular subepithelial and longitudinal subjacent layer, partially intermixed in some places.

Oviducts emerging dorsally from anterior or median third of ovaries, lead backwards immediately dorsal to nerve plate. Before gonopore, oviducts rise through an S-shaped path, postero-mediad inclined, to unite and continue dorsally to female atrium as common glandular oviduct (Figs 16-18). Common glandular oviduct long, with first two thirds horizontal and distal one slightly descendent (Figs 16-17). Lining epithelium of paired oviducts cubical to columnar ciliated, of common oviduct columnar ciliated; muscle coat of paired and common oviducts mainly with circular fibers. Shell glands open into distal ascending portion of paired and in common oviducts, becoming fewer towards distal portion of the latter.

Vagina continues dorsally and forward-directed from posterior end of female atrium (Figs 16-17). Female atrium nearly as long as male one (Table 2, Figs 17-18), with long folds that, traversing diagonally the whole cavity, ligate ventral and dorsal walls, or unite with folds of ventral distal wall of male atrium.

Epithelium lining vagina and female atrium tall columnar non-ciliated with xanthophil apical portion and pierced by erythrophil cells with short necks and subepithelial bodies, and very numerous cells with a cyanophil amorphous secretion and bodies in mesenchyme external to common muscle coat. Female atrium additionally receives a third type of gland cells with fine-grained and ill-defined staining secretion, bodies internal to common muscle coat, and distally more numerous. Muscularis constituted of intermixed circular and longitudinal muscle fibers, less developed in female atrium (8 μ m thick) than in male one.

Gonopore canal vertical (Figs 16, 18), slightly inclined forwards.

Common muscle coat (14 μ m and 19-28 μ m thick, respectively around female and male atria) with circular, longitudinal and oblique fibers. Between atrial muscularis and common muscle coat, a stroma with muscle fibers variously oriented.

Notogynaphallia abundans (Graff, 1899)

Geoplana marginata var. *abundans* Graff, 1899 (Tafel V, Fig. 30: external aspect). Localization of original material unknown

Geoplana abundans: Froehlich, 1959

Notogynaphallia abundans: comb. nov. Ogren, Kawakatsu & E.M. Froehlich, 1992

Nec *Geoplana abundans*: Almeida, Yamada & E.M. Froehlich, 1991

Material examined

EMF Nr. 331a: J. Hauser, leg. 26.IX.55, São Leopoldo/ RS, copulatory apparatus sectioned in two fragments: sagittal sections on 4 slides; EMF Nr. 331b: J. Hauser, leg. 10.X.55, São Leopoldo/ RS, copulatory apparatus: sagittal sections on 2 slides; MZUSP PL.178: F. Carbayo, leg. 14.XII.97, Boa Vista, Poço das Antas/ RS, copulatory apparatus sectioned in two fragments, 1: sagittal sections on 19 slides, and 2: sagittal sections on 7 slides; MZU PL.00059: F. Carbayo, leg. 18.X.96, São Sebastião do Cai/ RS, copulatory apparatus: sagittal sections on 46 slides; MZU PL.00060: D.C. Vara, leg. 06.III.97, Campo Bom/ RS, copulatory apparatus: sagittal sections on 10 slides; MZU PL.00061: W.H. Santos & L. Bonneau, leg. 19.VIII.97, São Leopoldo/ RS, anterior region level with ovaries: sagittal sections on 16 slides; pre-pharyngeal region: transversal sections on 8 slides; pharynx: sagittal sections on 13 slides; copulatory apparatus: horizontal sections on 37 slides; MZU PL.00062: W.H. Santos & L. Bonneau, leg. 19.VIII.97, São Leopoldo/ RS, copulatory apparatus: sagittal sections on 18 slides; MZU PL.00063: M. Cardoso, leg. 13.X.97, Novo Hamburgo/ RS, preserved in ethanol 70°; MZU PL.00064: M. Cardoso, leg. 21.X.97, Novo Hamburgo/ RS, anterior region level with ovaries: sagittal sections on 12 slides; pre-pharyngeal region: transversal sections on 4 slides; pharynx: sagittal sections on 14 slides; copulatory apparatus: sagittal sections on 14 slides; MZU PL.00065: M. Cardoso, leg. 21.X.97, Novo Hamburgo/ RS, pharynx: sagittal sections on 12 slides; copulatory apparatus: sagittal sections on 8 slides; MZU PL.00066: M. Cardoso, leg. 16.X.97, Novo Hamburgo/ RS, preserved in ethanol 70°; MZU PL.00067: I.A. Fick, leg. 02.VIII.98, Glorinha/ RS, copulatory apparatus: horizontal sections on 35 slides; MZU PL.00068: F. Carbayo, leg. 25.VIII.98, São Leopoldo/ RS, anterior region level with ovaries in two fragments – sagittal sections on 24 slides; pre-pharyngeal region: transversal sections on 10 slides; pharynx: sagittal sections on 26 slides; copulatory apparatus: sagittal sections on 49 slides; MZU PL.00069: M. Cardoso, leg. 22.XI.99, Salvador do Sul/ RS, pharynx and copulatory apparatus: horizontal sections on 29 slides.

Type-locality

Taquara, state of Rio Grande do Sul (RS), Brazil.

Distribution

Rio Grande do Sul (Poço das Antas, Salvador do Sul, Tupandi, Taquara, Novo Hamburgo, Campo Bom, São Leopoldo, Glorinha) – Brazil

Diagnosis

Dorsum cream, yellowish or pale brownish with seven longitudinal dark brown stripes; median, the thinnest; lateral and submarginal, the darkest; paramedian and lateral often poorly delimited; eyes dorsal, without clear halos except those in submarginal stripes; glandular margin absent; mc :h, 12-13%; pharynx bell-shaped with folded margins; foremost testes anterior to ovaries, most posterior anterior to root of pharynx; efferent ducts opening into median third of prostatic vesicle; prostatic vesicle extrabulbar, long and laterally sinuous, proximal portion, most often unforked, exceeding a little the posterior end of pharyngeal pouch; male atrium, elongate, highly folded with proximal wall histologically differentiated; oviducts arising from dorsal side of median third of ovaries, and rising behind gonopore; common glandular oviduct dorsal to female atrium; vagina short, dorso-anteriorly directed; female atrium, long and folded; male atrium length, 2.5 to 4.0 times that of female atrium.

External morphology

Body elongate with parallel margins, anterior end obtuse and posterior pointed. When creeping, maximal length may reach 60mm (Table 3). Mouth distance from anterior tip varies from 54% to 62% relatively to body length, gonopore from 70% to 81% (Table 3). Dorsum cream, yellowish or pale brownish with seven dark brown longitudinal stripes, one median, two paramedian, two lateral, two submarginal; lateral and submarginal being the darkest (Figs 3, 20). Venter cream. In specimen MZU PL.00063, median stripe begins at 1.5mm from anterior tip, a little behind the others which begin between 0.7 and 1.0mm (between 2 and 3% of body length). Stripes are not evenly distributed throughout dorsum width : interval between paramedian and lateral stripes being the narrowest, sometimes near virtual. Near posterior end (approx. 1mm or ca. 97% of body length), on each side of body, paramedian and lateral stripes converge and finish; median stripe ends at approximately the same level, whereas submarginal stripes of both sides converge and lead to posterior tip. Median stripe is the thinnest (approx. 0.05mm or 2% of body width), followed by paramedian and submarginal (approx. 0.07mm or 4% of body width), and lateral (approx. 0.15mm or 8% of body width) stripes (Fig. 20).

TABLE 3

Measurements, in mm, of type-specimens of *N. abundans* (Graff, 1899). - : not measured; * : After fixation; DG : distance of gonopore from anterior end; DM : distance of mouth from anterior end; DMG : distance between mouth and gonopore.

** This exemplar had the posterior end damaged. The numbers given in parentheses represent the position as related to body length.

	MZUSP PL.178	MZU PL.00059**	MZU PL.00060	MZU PL.00061	MZU PL.00062	MZU PL.00063	MZU PL.00064	MZU PL.00065	MZU PL.00066	MZU PL.00067	MZU PL.00068	MZU PL.00069
Maximum length in extension	45	45	>30	35	50	38	58	52	42	60	-	58
Maximum width in extension	2.5	3.0	1.5	2.0	2.0	2.0	2.5	2.5	2.0	2.5	-	2.0
Length at rest	-	-	-	-	-	-	-	-	-	35	-	32
Maximum width at rest	-	-	-	-	-	-	-	-	-	4	-	4.0
Length*	33	38**	20	44	34	29	49	33	34	54	53	42
Width*	3	3.0	2.0	3.0	4.0	2.5	3.0	3.0	2.5	3.5	3	3
DM*	18.5	23 (-)	11	26	20.5	17	29	18	21	33	30	25
	(56%)		(55%)	(59%)	(60%)	(59%)	(59%)	(54%)	(62%)	(61%)	(57%)	(59%)
DG*	26	34.5 (-)	14	34	26.5	22	39	25.5	27	42	40	34
	(79%)		(70%)	(77%)	(78%)	(76%)	(80%)	(77%)	(79%)	(78%)	(76%)	(81%)
DMG*	7.5	11.5	3.0	8.0	6.0	5.0	10	7.5	6.0	9.0	10	9.0
Creeping sole	-	-	-	73%	-	-	68%	-	-	-	70%	-
Ovaries	-	-	-	-	-	-	10.5	-	-	-	13.5	-
							(21%)				(25%)	
Anteriormost testes	-	-	-	-	-	-	12	-	-	-	14	-
							(24%)				(26%)	
Posteriormost testes	-	-	-	-	-	-	28	-	-	-	28	-
							(57%)				(53%)	
Prostatic vesicle	2.7	>3.3	1.4	-	2.3	-	4.6	3.3	-	-	4.3	-
Male atrium	2.5	4.4	1.7	-	2.7	-	2.7	2.2	-	-	3.4	-
Female atrium	0.7	1.0	0.6	-	0.7	-	0.8	0.6	-	-	1.0	-

Eyes, initially marginal and uniserial, surrounding anterior end. In specimen MZU PL.00063, become pluri-serial between 1.5 and 3.5mm from tip (approx. 5% and

12% of body length). Following, up to approx. 10mm from anterior tip (ca. 34% of body length), they spread as far as between submarginal and lateral stripes (Fig. 20),

being here the most numerous. Backwards become limited to body margins, occurring up to posterior tip. Those in submarginal stripes are surrounded by clear halos (Fig. 20).

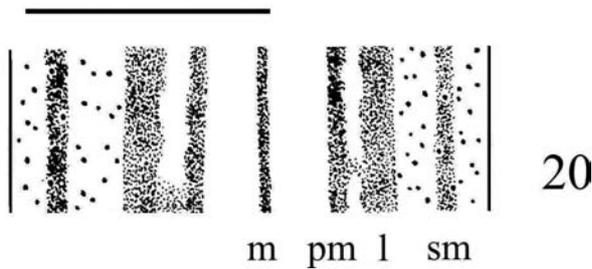


Fig. 20. – Detail of the colour pattern of a preserved specimen of *N. abundans* (specimen MZU PL.00063). (l) lateral stripe, (m) median stripe, (pm) paramedian stripe, (sm) submarginal stripe. Scale bar : 1mm.

Epidermis and musculature at pre-pharyngeal region

Creeping sole, 68% to 73% of body width (Table 3).

Three types of secretory cells discharge through dorsal epidermis and body margins : (1) abundant cells with coarse erythrophil secretion; (2) few cells with cyanophil amorphous secretion; (3) rhabditogen cells with xanthophil secretion, less frequent dorsally when compared with those of first two species, however, very numerous through body margins. Creeping sole receives cells with a coarse erythrophil secretion, very numerous cells with amorphous cyanophil secretion and few rhabditogen cells. Glandular margin absent.

Cutaneous musculature with similar constitution as in precedent species; mc :h 12% to 13% (Table 4). Mesenchymatic musculature as described for *N. ernesti*, sub-intestinal transversal layer being, however, thicker (12 fibers thick).

TABLE 4

Table 4. Cutaneous musculature in the median region of a transversal section of the pre-pharyngeal region and ratio of the height of the cutaneous musculature to the height of the body (mc :h index) of specimens of *N. ernesti*, *N. graffi* and *N. abundans*.

	<i>N. ernesti</i>				<i>N. graffi</i>		<i>N. abundans</i>				
	paratype EMF 595b	paratype EMF 677	paratype EMF 761b	paratype EMF 927	paratype MZU PL.00047	paratype MZU PL.00048	paratype MZU PL.00052	paratype MZU PL.00055	specimen MZU PL.00061	specimen MZU PL.00064	specimen MZU PL.00068
Circular ventral	2	3	2	2	2	2	2	2	2	2	2
Oblique ventral	10	18	10	9	10	10	7	10	11	10	18
Longitudinal ventral	49	67	60	65	56	60	30	42	50	70	62
Ventral total	61	88	72	76	68	72	39	54	63	82	82
Circular dorsal	2	2	2	2	2	2	2	2	3	3	2
Oblique dorsal	9	13	11	10	8	11	6	9	8	7	10
Longitudinal dorsal	54	72	71	60	75	95	31	40	42	31	36
Dorsal total	65	85	84	72	85	108	39	51	53	41	48
mc :h	15%	18%	18%	17%	20%	17%	13%	15%	12%	13%	13%

Pharynx

Pharynx bell-shaped with folded margins. Mouth in median third of pharyngeal pouch, on the same transversal level of dorsal insertion or slightly posterior (Fig. 21). No esophagus. Pharyngeal glands as those of *N. ernesti*. Outer and inner pharyngeal muscle layers similar to those of *N. ernesti* except for outer longitudinal layer, more developed in *N. abundans* (8µm thick).

Reproductive apparatus

Foremost testes anterior to ovaries; most posterior ones, anterior to pharynx (Table 3). Efferent ducts run dorsally to oviducts, laterally displaced in some points, and enter prostatic vesicle in median third (Figs 22-23). Form false seminal vesicles from pharynx level or slightly posterior. Lining epithelium cuboidal ciliated.

Prostatic vesicle, long (Table 3) and laterally sinuous, extends anteriorly a little beyond and ventrally to posterior end of pharyngeal pouch, without forking in most analysed specimens (Figs 22-23). In specimen 376, however, proximal portion, corresponding to 1/6 of the whole length of the organ, is forked. Lining epithelium, columnar to pseudostratified ciliated, receives abundant erythrophil granulous secretion from secretory cells with bodies lying in mesenchyme around or anterior to vesicle. Muscularis (31µm thick) constituted of longitudinal sub-epithelial layer, followed by circular fibers. Entering bulbar muscular coat, prostatic vesicle gradually narrows and becomes more sinuous (Fig. 22), forming ejaculatory duct which, after short course, ascends, describing an arc before opening dorsally into proximal portion of male atrium. Ejaculatory duct lined with columnar ciliated epithelium and coated with weakly developed muscularis (ca. 7µm thick). There are no secretory cells opening into ejaculatory duct.

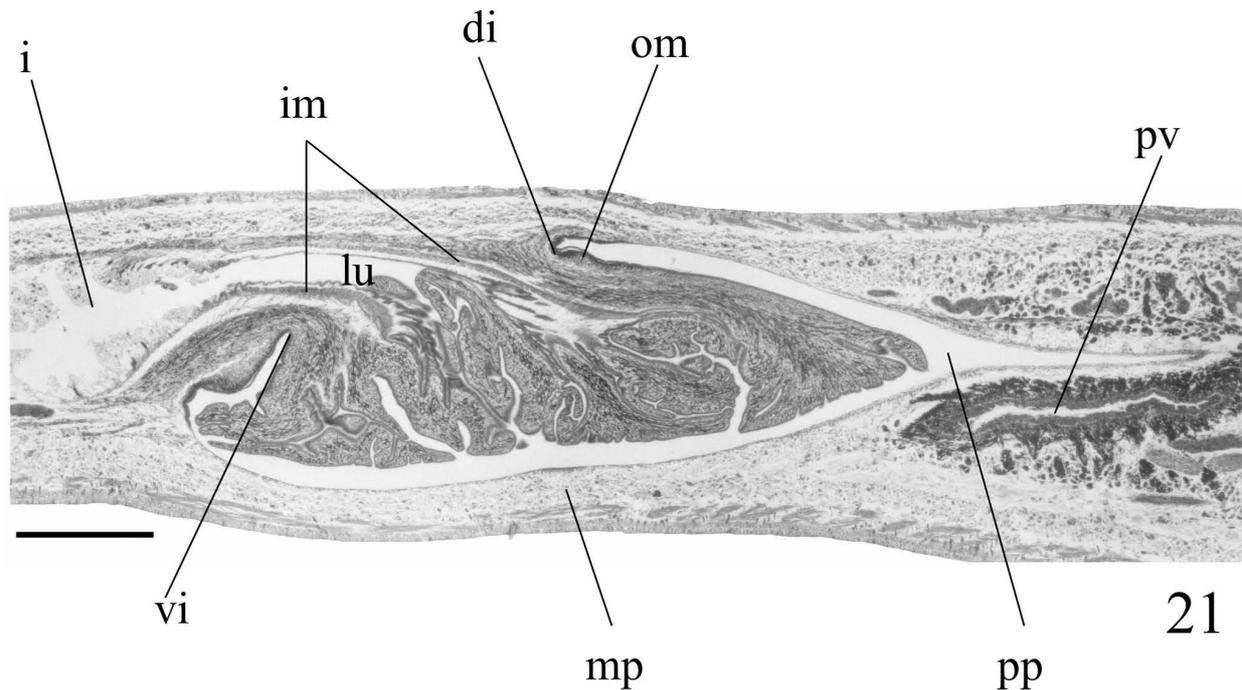


Fig. 21. – Pharynx of *N. abundans* (specimen MZU PL.00068). Sagittal section. (di) dorsal insertion, (i) intestine, (im) internal musculature, (lu) pharyngeal lumen, (mp) mouth's position, (om) outer musculature, (pp) pharyngeal pouch, (pv) prostatic vesicle, (vi) ventral insertion. Scale bar : 500 μ m.

Male atrial cavity elongate (Table 3) with very irregular contour as a consequence of its highly folded walls (Figs 22-24). In most specimens it is entally very dorsoventrally narrowed due to a wide, complex, asymmetrical fold, variously shaped in different specimens and occupying variable extension (from one fifth to one half) of male atrium (Figs 22-25). Lining of male atrium columnar non-ciliated epithelium of irregular height, and free surface differentiated, being more strongly or more weakly stained. Those of ental fold with cuboidal epithelium penetrated by two secretory cell types which are restricted to it : (1) cells with densely arranged, granulous cyanophil secretion; (2) cells with densely arranged, granulous erythrophil secretion. However, mostly of male atrial wall receives three other secretory cell types : (1) cells with weakly cyanophil amorphous secretion, distally more abundant; (2) cells with fine xanthophil secretion; (3) less frequent cells with coarse xanthophil secretion. Bodies of all secretory cells in mesenchyme, anteriorly or laterally to copulatory apparatus. Muscularis well developed (ca. 46-48 μ m) with subepithelial layer of circular fibers, followed by, and partially intermixing with, longitudinal fibers. On ental fold muscularis mainly composed of longitudinal fibers intermixed with some circular ones, and crossed by radial fibers from the stroma between muscularis and common muscle coat.

Oviducts arise from dorsal side of median third of ovaries and run posteriorly, immediately above nerve plate. Behind gonopore, oviducts proceed dorsomedial, unite above female atrium forming common glandular oviduct which continues backwards, slightly inclined ventrally, to enter into vagina (Figs 22-23). Cells of epithelium lining oviducts and common glandular oviduct columnar

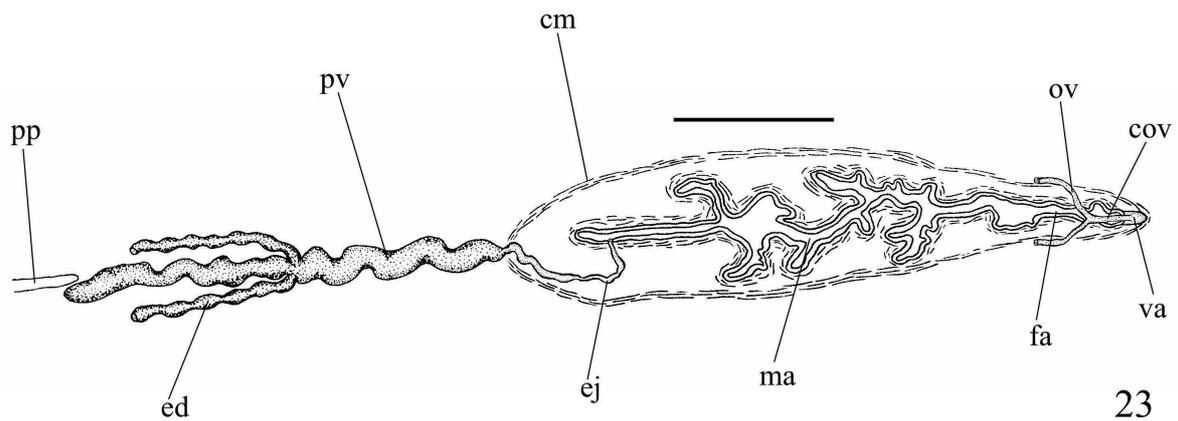
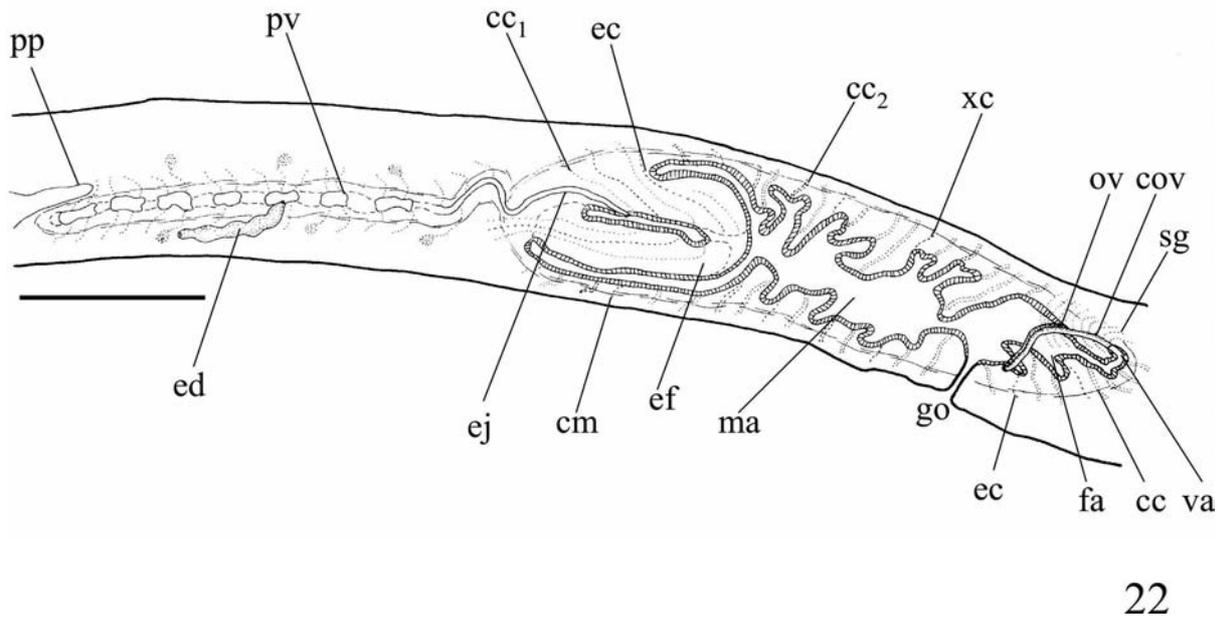
ciliated; that on paired oviducts, with basal nuclei and, mainly in apical half, cyanophil cytoplasm. Muscle coat of oviducts constituted of circular fibers, that of common glandular oviduct of mixed circular and longitudinal fibers. Shell glands opening into distal ascending portion of paired oviducts, besides into common glandular oviduct.

Vagina short arising from dorsal ental wall of female atrium, slightly curved forward (Figs 22, 24). Female cavity with intensely folded walls; some folds arising from ental wall traverse the whole cavity to unite with folds from wall of male atrial near to gonopore (Fig. 24). Length of female atrium (Table 3) equal to one third or one quarter of male atrial length.

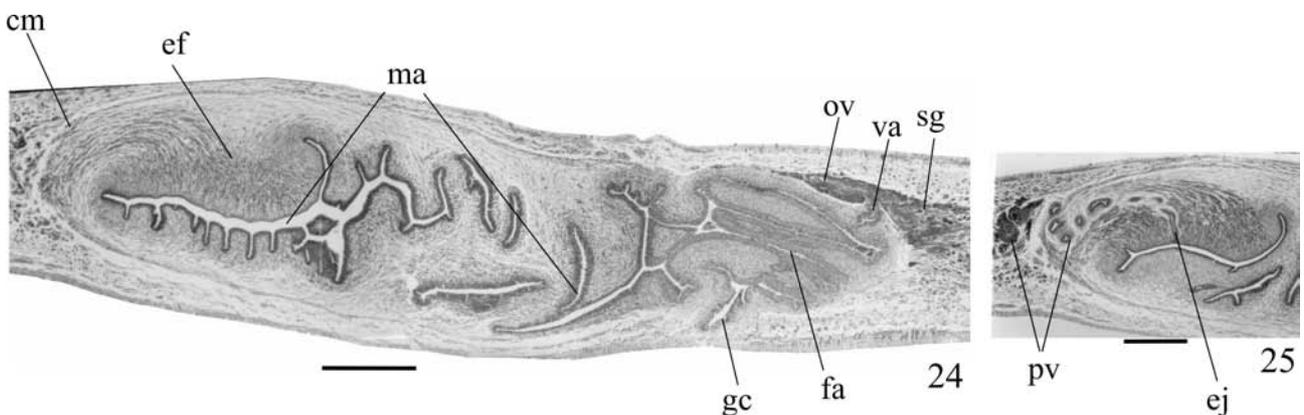
Vagina and atrium lined with tall columnar epithelium, distally ciliated in the vagina and with apical surface weakly erythrophil in the atrium. Glands with cyanophil amorphous secretion discharge into whole epithelium, numerous in atrium and fewer towards distal end of vagina. A second type of gland; less numerous, with erythrophil granulous secretion, empties into the atrium. Cell bodies of both gland types lateral or posterior to female atrium. Muscularis consisting of circular layer with some longitudinal subjacent fibers in vagina, and of layer (approx. 20 μ m thick) with loosely arranged circular and longitudinal fibers in atrium.

Gonopore canal slightly inclined backwards (Figs 22, 24).

Common muscle coat well developed around male atrium (19-22 μ m thick), and very thin (2 μ m thick) at level of female one. Between muscularis and common muscle coat, a stroma with muscle fibers variously oriented.



Figs 22-23. – Diagrammatic composite reconstructions of copulatory apparatus of *N. abundans* : (22) from sagittal sections (specimen MZUSP PL.178); (23) from horizontal sections (specimen MZU PL.00069). (cc) cyanophil secretory cells, (cc₁) cyanophil secretory cells opening into the ejaculatory duct, (cc₂) cyanophil secretory cells opening into the male atrium, (cm) common muscle coat, (cov) common glandular oviduct, (ec) erythrophil secretory cells, (ed) efferent duct, (ef) ental fold, (ej) ejaculatory duct, (fa) female atrium, (go) gonopore, (ma) male atrium, (ov) oviducts, (pp) pharyngeal pouch, (pv) prostatic vesicle, (sg) shell glands, (va) vagina, (xc) xanthophil secretory cells. Scale bar : 1mm.



Figs 24-25. – Copulatory apparatus of *N. abundans* (specimen MZU PL.00068) in sagittal section : (24) overall view; (25) detail of proximal portion of male atrium. (cm) common muscle coat, (ef) ental fold, (ej) ejaculatory duct, (fa) female atrium, (gc) gonopore canal, (ma) male atrium, (ov) oviduct, (sg) shell glands, (pv) prostatic vesicle, (va) vagina. Scale bar : 500µm.

DISCUSSION

When describing *N. ceciliae* FROEHLICH & LEAL-ZANCHET (2003) commented on the heterogeneity of the species in the genus *Notogynaphallia* and distinguished, in a first general approach, two groups. One of them included, besides *N. guaiana* Leal-Zanchet & Carbayo, 2001, *N. muelleri* (Diesing, 1861), *N. fita* (Froehlich, 1959), and *N. caissara* (E.M. Froehlich, 1955), also *N. ceciliae* and the three species herein described.

Regarding external morphology, and as already shown for *N. ceciliae*, the first three species are readily separable from *N. ernesti*, *N. graffi*, *N. abundans* and *N. caissara*. *N. guaiana* shows a plainly grey dorsum and exclusively marginal eyes. *N. fita* has a much larger size, exclusively marginal eyes, and dorsum with four stripes along the major part of body. *N. muelleri* has a greater length and leanness and a dorsum with only one or three stripes; when with three stripes the lateral ones are always ferruginous, never brownish or black as the median.

Of the remaining five species of the group, *N. abundans* is the only one with seven dark stripes, whereas the other four have five. The latter species, despite their striking external similarities, can be separated from each other by details such as the relative width of the different stripes, arrangement of stripes relative to body width and procedure of stripes near the extremities of body, specially the caudal one.

In *N. ceciliae* stripes, all of similar width run closer to each other, and nearer the median line of body, than in *N. caissara*, *N. ernesti* and *N. graffi*, so as to leave the widest band of ground-colour on body margins, as compared to the three latter species. Besides, all stripes end at the same distance from the posterior extremity without converging. In *N. caissara* from Rio de Janeiro all stripes are of similar width, the lateral being darker and slightly wider; in specimens from Ubatuba, however, the median stripe is some five times wider than the others. Median and paramedian stripes end, at the same level, a little before both extremities; lateral ones, as a continuous line, contour both extremities. As already commented by MARCUS (1951) when comparing specimens from V. Atlântica and São Paulo, in *N. ernesti* width of paramedians and lateral stripes are characteristically variable according to worm provenance, the former being the widest in worms from V. Atlântica, and the latter the widest in most specimens from São Paulo. Specimens from Curitiba and S. Francisco de Paula, studied herein, are similar, respectively, to those from V. Atlântica and S. Paulo. Procedure of stripes regarding body ends also varies in different specimens, except for the lateral, which are always convergent towards caudal end. However, in no specimen lateral stripes are continuous around both extremities, as in *N. caissara*. This detail constitutes the only constant external difference between *N. ernesti* from S. Paulo and *N. caissara* from Rio de Janeiro. This might explain why Marcus, despite important incongruities he verified and commented on, between Riester's description and drawing of copulatory apparatus and copulatory apparatus of his own worms, surprisingly decided on the conspecificity of his and Riester's material. Finally, in *N. graffi* the stripes are very densely pigmented and sharply delimited as in none

of the five-striped species dealt with above. Paramedian stripes are consistently some four to six times as wide as median and lateral ones. GRAFF (1899) described and drew the intense darkness and the greater width of the paramedian stripes, which was registered and commented on by Marcus as this did not occur in his own worms. He might have attributed it to geographical variation since at that time *N. ernesti* was not known from Rio Grande do Sul. In *N. graffi*, all stripes finish at slightly different levels from both extremities, but it is the only among the five-striped species in which paramedian and lateral stripes converge on each side of body before posterior end.

Eye distribution throughout body length is similar in the four species of the complex. In *N. abundans*, *N. ernesti* and *N. graffi* they become pluriserial at approx. 1mm (2 to 5% of body length) from the anterior end, and in *N. ceciliae* at approx. 4mm (ca. 14% of body length) from the tip; thereafter they become dorsal and are abundant up to 9 to 12mm (20 to 45% of body length) from the anterior extremity. In *N. caissara* eye distribution is also similar.

GRAFF (1899, p. 333) described a slender longitudinally striped species, *G. bohlsi*, from Asuncion, Paraguay, based on two fragments without either of the extremities. The stage of development was not commented upon, but its colour pattern was described as a median dark line and two grey bands, one at each side, on a yellowish background; both margins of grey bands, the external and the internal, the latter twice as wide as the first, were darker. Judging from Fig. 17 (Taf. VII), they were also rather clearly delimited. Continuing, GRAFF (1899) commented that if the grey between them were fainter one would be able to consider the worm as being five striped, although to him the fragments "sind so charakteristisch gezeichnet, das es nicht schwer sein wird, danach die Art wiederzuerkennen" (show a very peculiar pattern, so that it will be not difficult to recognize the species). MARCUS (1951), however, suggested that *G. bohlsi* was probably a synonym of *G. marginata* sensu Graff. Nevertheless, additionally to the peculiarities of its colour pattern, the provenance of *G. bohlsi* does not seem to be consistent with the distribution of *N. graffi* or that of any of the five striped species discussed herein, taking into account the high degree of endemism generally presented by land planarians. So the assignment of the species by OGREN & KAWAKATSU (1990) to their Geoplaninae genus *Pseudogeoplana*, "collective group to temporarily assign species inquirendae and nomina dubia" (l.c., p. 90), seems a more adequate procedure.

Internal anatomy

Due to its female atrium being almost completely filled by a multilayered lining epithelium, *N. guaiana* once more stands apart from the striped species of group 2 of *Notogynaphallia* (FROEHLICH & LEAL-ZANCHET, 2003).

The seven species with a striped dorsum, regarding anatomy, can be assembled as follows: *N. graffi*, *N. caissara* and *N. fita* on the one side, *N. ernesti* and *N. muelleri* on the other, while *N. abundans* and *N. ceciliae* combine, each in their own way, characters similar to those of both groups.

N. graffi, *N. caissara* and *N. fita* have in common a very long prostatic vesicle with a comparatively narrow cavity; efferent ducts entering laterally into the prostatic vesicle. The male atrium is not very long, approximately of the same size as the female one, with folded walls, and a large penial papilla-like fold around the opening of the ejaculatory duct.

Regarding *N. graffi*, there are some important differences between Graff's description and drawing of the copulatory apparatus, and that of the worms herein studied. GRAFF (1899) described and drew the vesicle as an elongate structure with an ample cavity and thin, richly folded walls. He considered it originated as a fusion of the efferent ducts, and so, as an unpaired false seminal vesicle (1899, p. 165). In seven specimens, studied herein, the wall – epithelial lining plus muscularis – of the vesicle, although not very thick, is much thicker than those of the efferent ducts, something never observed in false vesicles of any other species. The vesicle in the present specimens is undoubtedly a prostatic vesicle receiving plenty of erythrophil glands. Besides in none of our specimens is it a through continuation of efferent ducts as in Graff's drawing, but, instead, as above-stated, receives each of them, on either side. Still regarding the vesicle, in present specimens it is not "falteneiche" (very folded) as in Graff's one, but with rather smooth walls. It is important to remark here that the poor histological conditions of Graff's material, as commented by him, could explain, at least in part, the important differences discussed above.

With regard to other characteristics of the copulatory apparatus, they are in agreement with those described by GRAFF (1899). It seems that there are only, in some points, divergences in the interpretation of certain structures. Thus the penial papilla described by Graff is here considered an ental ring-like fold of the male atrial wall, around the opening of the ejaculatory duct. The latter is ciliated while, like the rest of the atrial wall, the narrow cavity delimited by the fold is not. So it is not an extension of the duct as Graff considered albeit having observed the histological differences.

N. caissara, one of the species mistakenly identified as *G. marginata* Shultze & Müller (LEAL-ZANCHET & FROELICH, 2001), was described as possessing a penial papilla, thus placing the species in an ambiguous position inside *Notogynaphallia*. After the original description, some other specimens from one of the original localities were sectioned, the entire material of this species being restudied for this paper. This leads to the conclusion that the "papila penial" is actually a projection involving a variable extension of the proximal atrial wall, with irregular invaginations on its surface, in one of which the ejaculatory duct empties. As a consequence, any possible doubt about the assignment of the species to *Notogynaphallia* is removed. Further, *N. caissara* belongs to the species complex herein discussed, and is the sole species of the complex not yet found southwards from S. Paulo state. The main differences between *N. caissara* and *N. graffi* concern the form and extension of the prostatic vesicle, which in the former is very long, extending anteriorly up to the posterior extremity of the pharyngeal pocket, where it forks and continues, even arriving in some of the worms, probably depending on the grade of maturity, at

the level of the ventral insertion of the pharynx. Its walls are so intricately folded, mainly in more mature specimens, that, in most of the longitudinal sections, it appears as a large agglomerate of cross-sectioned canals. The vesicle of *N. graffi* is long, and surpasses slightly the end of pharyngeal pocket, but it is not forked, and its walls are smooth.

N. fita, although the most divergent of the three species with regard to the external morphology, presents a very similar copulatory apparatus. The principal difference is the localization of the ejaculatory entrance into the distal third of the prostatic vesicle, near the penis bulb, and not into the median third, rather far from the bulb, as in *N. graffi* and *N. caissara*. The prostatic vesicle is also tubular and very long, but, does not reach the pharyngeal pouch; besides it is not forked and its walls are smooth. Another outstanding difference between the three species is that the pharynx is cylindrical in *N. graffi* and *N. caissara* but bell-shaped in *N. fita*.

In the second assemblage of species, the prostatic vesicle is a voluminous and compact organ, without branches or diverticula, and much shorter than in the species of first group. Its muscle coat is rather thick and dense, being heavily traversed by the neck of gland cells. The efferent ducts, after turning dorsoanteriorly, penetrate it not laterally into the median or ectal portion but into the ental portion, terminal or subterminally. The male atrium is very long; at least twice as long as the very short female atrium, and frequently more.

In most specimens of *N. ernesti* the opening of efferent ducts into the vesicle is subterminal, located ventrally (specimens from states of São Paulo and Paraná) or dorsally (specimens from São Francisco de Paula). In *N. muelleri* the entrance is terminal in the characteristically upturned proximal portion of the prostatic vesicle. In mature worms of both species the male atrium is comparatively more intensively and deeply pleated in *N. ernesti* than in *N. muelleri*. The walls of the gonopore canal are smooth in all specimens of *N. ernesti*, but deeply pleated in the three sectioned specimens of *N. muelleri*. The pharynx of *N. muelleri* is bell-shaped with pleated margins, and the dorsal insertion is situated on the same transversal level as the mouth. That of *N. ernesti*, here described as bell-shaped, was described by Marcus as of the cylindrical type, approaching the bell type. He depicted its dorsal insertion slightly anterior to the mouth, as in most specimens herein studied.

With such a similar internal anatomy, a fact already remarked by FROELICH (1959), who described the anatomy of *N. muelleri* for the first time, the separation of these two species relies heavily on the externally observable differences, mainly colour pattern, and behaviour. FROELICH (1959) described *N. muelleri* as "a lively species, with quick reactions when stimulated", and the second author remarked in her field notes, together with drawings of living worms, that when touched these worms quickly shorten. A similar behaviour was never observed in *N. ernesti* by both authors in worms from Rio Grande do Sul, and neither from worms from São Paulo.

MARCUS (1951) sectioned and studied two specimens of what he considered conspecific with Graff's *G. marginata*, one of which with a ring-like fold of the ental wall of

the male atrium around the opening of the ejaculatory duct, similar to that described herein for the specimen from Valinhos. He described the whole copulatory apparatus as in a stage of evaginate penial papilla. Thus, he considered the fold a transitory structure formed by the eversion of the ental atrial wall for transferring spermatozoa during copulation. A similar fold was not found in any of the three sectioned worms of *N. muelleri*, which does not mean, of course, that its copulatory apparatus does not work in the same way on the proper occasion. However, in one of the worms, there is a cluster of spermatozoa plus eosinophil secretion fixed to the ventral wall of the male atrium, near the gonopore. The structure was well described by FROEHLICH (1959), who considered it a spermatophore. A similar structure has been also described for *Notogynaphallia sexstriata* (FROEHLICH, 1956), for *Amaga righii* (FROEHLICH & FROEHLICH, 1972) and for *Choeradoplana iheringi* Graff, 1899, in which a similar cluster of spermatozoa was also seen free in the atrial cavity (LEAL-ZANCHET & SOUZA, 2003; SOUZA & LEAL-ZANCHET, 2004). The two last mentioned species are also devoid of a penial papilla and have a long pleated male atrium. Another interesting point worthy of note is that in all these species the cluster of spermatozoa is fixed on the ventral wall of the atrium, near the gonopore. These facts appear to indicate that in *N. muelleri*, as well as in these other three species, the transference of spermatozoa would be accomplished through the eversion of the distal wall instead of the proximal wall of the male atrium.

In the discussion of his *G. marginata*, MARCUS (1951) commented that the drawings of the genital apparatus presented by him were concordant with those of RIESTER (1938) and GRAFF (1899) only with respect to general topography, as an analysis of the details unveiled inexplicable incongruities. In spite of this, he concluded the conspecificity of the three species. E.M. FROEHLICH (1955) separated Riester's and Marcus' species; the separation of Graff's and Marcus' species is accomplished herein.

The principal differences between *N. ernesti* and *N. graffi* appear immediately by the herein assignment of them to distinct well-characterised groups. Some of the main similarities between both species, as pointed out by MARCUS (1951), do not prevail after the present study of several new specimens of Graff's species. In this manner, the penial papilla in GRAFF's figure (1899, Text-fig. 36) was interpreted by MARCUS (1951) as also being a transitory structure as that in his own material, and so, contrary to what was concluded in the present work. Although not being a true penial papilla, the fold around the opening of the ejaculatory duct of *N. graffi* is present in all studied specimens. Another similarity according to MARCUS (1951), the entrance of the efferent ducts, localized by GRAFF (1899) on the proximal end of the prostatic vesicle, is also in disagreement with what was shown herein. RIESTER (1938), who studied Graff's slides and commented on its poor histological conditions, had already emended Graff's description regarding this point.

In *N. abundans* the prostatic vesicle is similar to that of *N. graffi*: a long branchless tube in most specimens, with a well-developed muscularis, and receiving the efferent ducts approximately in the same position. However, dif-

ferently from that of *N. graffi*, it is laterally sinuous and extends anteriorly beyond the posterior end of the pharyngeal pouch in all studied specimens. The male atrium, in turn similar to that of *N. ernesti* and *N. muelleri*, is very long, at least twice as long as the female one. Again, resembling species of the first assemblage, there is a large fold in the proximal wall of the male atrium, delimiting a small cavity, where the ejaculatory duct opens, from the rest of the atrium. In *N. abundans*, this fold is variously shaped in different specimens and occupies a comparatively minor portion of the atrial cavity. The pharynx of *N. abundans* is bell-shaped as in *N. fita* and in both species of the second assemblage.

In *N. ceciliae* the prostatic vesicle is a long tube as that of species of the first assemblage and that of *N. abundans*. It approaches that of *N. caissara*, being anteriorly forked and extended beyond the end of the pharyngeal pouch, although considerably less so than in *N. caissara*. That of *N. abundans* also shortly surpasses the end of the pharyngeal pouch but is not forked in most specimens. In *N. graffi* and *N. fita* the vesicle stands at a distance from the pharyngeal pouch and is not forked. Concerning the atrial cavities, *N. ceciliae* very much resembles species of the second assemblage; the atria have a similar general structure, besides similar size and relative proportions. The pharynx is bell-shaped as that of *N. fita*, *N. abundans*, *N. ernesti* and *N. muelleri*, the dorsal insertion lying in the median third of the pouch, on the same transversal level as the mouth. Nevertheless, *N. ceciliae* is distinct from the other five striped species, as well as from the remaining species of *Notogynaphallia* gathered in group 2 of FROEHLICH & LEAL-ZANCHET (2003), by its distally branched efferent ducts, each branch opening separately into the vesicle, a very rare trait in the Terricola.

To conclude, what GRAFF (1899), RIESTER (1938) and MARCUS (1951) considered as *G. marginata* SCHULTZE & MÜLLER, 1857, currently, after E.M. FROEHLICH (1955), and the present study, corresponds, to three species: respectively *N. graffi*, herein described; *N. caissara* (FROEHLICH, 1955); and *N. ernesti*, herein described. Further, these three species plus *N. abundans*, the anatomy of which was herein described for the first time, and *N. ceciliae* FROEHLICH & LEAL-ZANCHET, 2003, constitutes a complex of species with the characteristics presented in the introduction.

Geoplana marginata Schultze & Müller was transferred by OGREN et al. (1992) to *Pseudogeoplana*, as its anatomy remains unknown.

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