Redescription of two land planarian species of *Notogynaphallia* Ogren & Kawakatsu (Platyhelminthes, Tricladida, Geoplaninae) and confirmation of the heterogeneity of the genus

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ABSTRACT. *Notogynaphallia caissara* (E.M. Froehlich, 1955) and *N. fita* (Froehlich, 1959) are redescribed. Both species are included in the so-called group 2 of E.M. Froehlich & Leal-Zanchet (2003), in *Notogynaphallia,* and are provided with a cephalic retractor muscle derived from the ventral longitudinal subepidermic muscle layer. A muscle retractor does not exist in the type-species of the genus. This is strong evidence of the morphological heterogeneity within the genus, as E.M. Froehlich & Leal-Zanchet (2003) previously proposed mainly based on characters of the reproductive organs.
KEY WORDS. Cephalic retractor muscle; glandular organ; Neotropical Region; taxonomy; Terricola.

RESUMO. **Redescrição de duas espécies de *Notogynaphallia* Ogren & Kawakatsu (Platyhelminthes, Tricladida, Geoplaninae) e confirmação da heterogeneidade do gênero.** Redescrevem-se *N. caissara* (E.M. Froehlich, 1955) e *N. fita* (Froehlich, 1959), reunidas no chamado grupo 2 de E.M. Froehlich & Leal-Zanchet (2003), dentro de *Notogynaphallia.* Ambas as espécies possuem um músculo retrator cefálico derivado da musculatura subepidérmica longitudinal ventral. A presença deste retrator nas duas espécies, mas não na espécie-tipo do gênero, é uma evidência consistente da heterogeneidade morfológica do gênero, como previamente sugerido por E.M. Froehlich & Leal-Zanchet (2003) com base em características dos órgãos reprodutores.
PALAVRAS-CHAVE. Músculo retrator cefálico; órgão glandular; região neotropical; taxonomia; Terricola.

E.M. Froehlich & Leal-Zanchet (2003) have reported on the heterogeneity of the genus *Notogynaphallia* Ogren & Kawakatsu, 1990 (Geoplanidae) mainly in relation to certain characteristics of reproductive organs. The authors also subdivided the genus in two groups, identified as groups 1 and 2, according to morphological similarities.

According to Leal-Zanchet & E.M. Froehlich (2006) five of the eight species within group 2 constitute a complex sharing the following characteristics: elongated body, parallel margins and yellowish dorsum with five to seven dark longitudinal stripes, a long prostatic vesicle, a folded and typically 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. The species constituting this complex are *N. abundans* (Graff, 1899), *N. ceciliae* Froehlich & Leal-Zanchet, 2003, *N. ernesti* Leal-Zanchet & Froehlich, 2006, *N. graffi* Leal-Zanchet & Froehlich, 2006, and *N. caissara* (E.M. Froehlich, 1955), which together with *N. fita* (Froehlich, 1959), *N. muelleri* (Diesing, 1861) and *N. guaiana* Leal-Zanchet & Carbayo, 2001 constitute group 2. These three species differ from those of the complex by the color pattern of the dorsum: *N. fita*

presents four dark longitudinal stripes (plus a fifth one on the cephalic region), *N. muelleri* has either one medial black stripe plus a pair of lateral ferruginous ones or only one median black stripe, and *N. guaiana* is homogeneously grayish.

The value of cephalic structures in the subfamily Geoplaninae, to which *Notogynaphallia* belongs, has been stressed (Carbayo & Leal-Zanchet 2003, Carbayo 2005). On this basis, two species of the group 2 are herein redescribed, *N. caissara* and *N. fita*.

MATERIAL AND METHODS

Specimens were obtained on loan from E.M. Froehlich's Scientific Collection (EMF, Department of Zoology, Instituo de Biologia, Universidade de São Paulo), and Senckenbergischen Museum Frankfurt (SMF). A number of specimens were already sectioned when obtained on loan. Those preserved in alcohol were cut in tissue blocks, dehydrated in a graded series of ethanol, and treated with isopropyl alcohol and embedded in paraffin. Sagittal, transverse or horizontal 5 to 10 µm serial sections of either anterior region, the pharynx, the pre-pharyngeal region or the copulatory apparatus, were stained with Mallory/Cason and Masson/Goldner trichrome methods (Romeis 1989).

The remaining body portions were preserved in 70% ethanol. SMI (= mc:h, subepidermic musculature thickness: body height ratio) was calculated at pre-pharyngeal region, after FROEHLICH (1955). Drawings were prepared using a camera lucida.

TAXONOMIC SECTION

Notogynaphallia caissara (E.M. Froehlich, 1955)

Geoplana marginata Schirch, 1929: 35, Teresópolis; Riester, 1938: 29, Fazenda Boa Fé, Ribeirão Pires, São Paulo.

Geoplana caissara E.M. Froehlich, 1955a: 295, 1955b: 205; Froehlich, 1955: 267; Froehlich, 1956: 314, 331, Gávea Pequena, Rio de Janeiro; Froehlich, 1959: 219, 235.

nec Geoplana marginata Schultze & Müller, 1857: 63; Graff, 1899: 305; Marcus, 1951: 56.

Notogynaphallia caissara Ogren & Kawakatsu, 1990: 141.

Diagnosis. Body elongated and with 33.5 mm in maximum length when fixed, having parallel margins, and a yellowish dorsum with five dark dorsal stripes. A mesenchymal transverse muscle layer is located below the ventral nerve plate. The species has a very long prostatic vesicle, outside the common muscle coat, bifurcated proximally, with the paired branches lateral to the pharyngeal pocket. The efferent ducts open laterally into median region of prostatic vesicle. The female genital atrium is folded.

Distribution. Brazil: Barreira, Gávea Pequena, Teresópolis (RJ); Ribeirão Pires, Itanhaém, Ubatuba (SP).

Material examined. SMF Nr. 649 (938-947): Teresópolis (RJ), 28/III/1914, E. Bresslau *leg.* Pharynx and copulatory apparatus: sagittal sections on two slides (incomplete); anterior and posterior regions of the body in balsam on one slide. SMF Nr. 36 (938-947): Teresópolis (RJ), 01/IV/1929, E. Bresslau *leg.* Copulatory apparatus: sagittal sections on three slides (incomplete). SMF Nr. 137 (948-958): Teresópolis (RJ), 22/IV/1929, E. Bresslau *leg.* Pharynx and copulatory apparatus: sagittal sections on three slides (incomplete); anterior and posterior regions of the body in balsam on two slides. SMF Nr. 631 (923-937): Teresópolis (RJ), 25/III/1914, E. Bresslau *leg.* Pharynx: sagittal sections on two slides (incomplete); copulatory apparatus: sagittal sections on two slides (incomplete); intermediate region (probably the region posterior to the copulatory apparatus): transverse sections on 1 slide; anterior and posterior regions of the body in balsam on two slides. SMF Nr. 309 (923-937): Teresópolis (RJ), 07/I/1914, E. Bresslau, *leg.* Pharynx and copulatory apparatus: sagittal sections on two slides (incomplete); pre-pharyngeal region: transverse sections on one slide; anterior and posterior regions of the body in balsam on one slide. EMF Nr. 134b: Ubatuba (SP), 01/IX/1952, Froehlich & Froehlich, *leg.* Anterior region 1, from anterior tip to 4 mm behind: transverse sections on seven slides; anterior region 2, 10.5 mm long, beginning 4 mm behind the anterior tip: sagittal sections on 12 slides; pre-pharyngeal region: transverse sections on 8 slides; pharynx: sagittal sections on two slides (in-

complete); copulatory apparatus: sagittal sections on three slides (incomplete); posterior end of the body in 70% ethanol. EMF Nr. 134a: Ubatuba (SP), 02-07/IX/1951, Froehlich & Froehlich, *leg.* Pharynx: sagittal sections on one slide (incomplete); copulatory apparatus: sagittal sections on three slides (incomplete); remaining body in 70% ethanol. EMF Nr. 134c: Teresópolis (RJ), 15-16/VI/1954, Froehlich & Froehlich *leg.* Anterior region 1, from anterior tip to 4.5 mm behind: transverse sections on 10 slides; anterior region 2, 4.5 mm long, beginning 4.5 mm behind the anterior tip: sagittal sections on 8 slides; anterior region 3, 16.5 mm long, beginning 9 mm behind the anterior tip: sagittal sections on 33 slides; pre-pharyngeal region: transverse sections on 13 slides; region posterior to copulatory apparatus: sagittal sections on 12 slides; posterior end of the body in 70% ethanol. EMF Nr. 134d: Itanhaém (SP), 06/XI/1954, Froehlich & Froehlich, *leg.* Anterior region: sagittal sections on 8 slides. EMF Nr. 134e: Teresópolis (RJ), 15-16/VI/1954, Froehlich & Froehlich *leg.* Posterior half of the body: sagittal sections on three slides; Anterior half of the body in 70% ethanol (immature). EMF Nr. 134f: Teresópolis (RJ), 15-16/VI/1954, Froehlich & Froehlich *leg.* Anterior half of the body in 70% ethanol.

This redescription is, in general, in agreement with previous description made by E.M. FROEHLICH (1955a).

External morphology

Fixed specimens: *Notogynaphallia* of 33.5 mm in maximum length (Tab. I), slender (relative width: length, 6.0-6.7%) with parallel margins. The specimens have a rounded anterior tip and a pointed posterior end, while the cross-section of the body is elliptic. The color patterns of the dorsum is yellowish or grayish, with five dark brownish longitudinal stripes, approximately equidistant: one median (almost not visible in specimen EMF Nr. 134f), two paramedian and two lateral ones. The median stripe is the widest in specimens from Ubatuba (Fig. 1), but narrowest in those from Teresópolis (Fig. 2). All stripes discontinue at 2-3 mm from the anterior tip; only the two lateral ones reach the posterior end of the body where

Table I. Measurements (mm) of fixed specimens of *Notogynaphallia caissara*.

Specimen	EMF Nr.134a	EMF Nr. 134b
L (length)	33.5	31.5
W (width)	2.0	2.1
Height	0.7	0.8
Relative W: L	6.0%	6.7%
M (mouth - anterior body tip)	17.7	18.7
Relative M: L	52.8	59.6%
G (gonopore - anterior body tip)	23.8	23.3
Relative G: L	71.0%	74.0%
Creeping sole: body width	?	77%
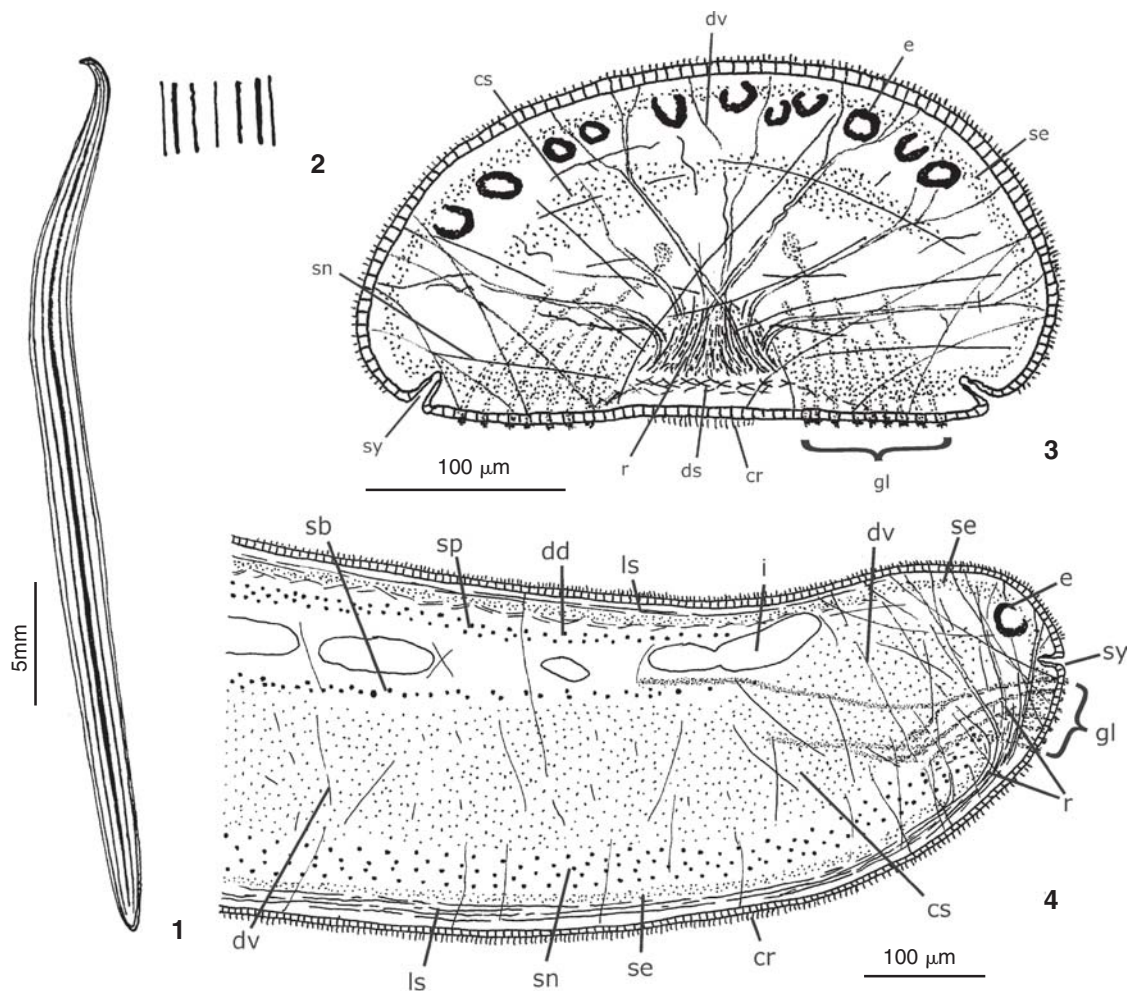
Figures 1-4. *Notogynaphallia caissara*: (1) a specimen from Ubatuba (SP) in dorsal view, after E.M. Froehlich (1955a); (2) dorsal pattern color of a specimen from Teresópolis (RJ), after E.M. Froehlich (1955a); (3) diagrammatic reconstruction of the cephalic retractor muscle in transverse section at 100 µm from anterior end of the body, specimen EMF Nr. 134c; (4) diagrammatic sagittal section of anterior part of the body of specimen EMF Nr. 134d. (cr) Creeping sole, (cs) central nervous system, (dd) diagonal mesenchymal muscles, (ds) diagonal subepidermic muscles, (dv) dorso-ventral mesenchymal muscles, (e) eyes, (gl) glandular organ, (i) intestine, (ls) longitudinal subepidermic muscles, (r) cephalic retractor muscle, (sb) sub-intestinal transverse mesenchymal muscles, (se) sub-muscular peripheral nerve net, (sn) sub-neural transverse mesenchymal muscles, (sp) supra-intestinal transverse mesenchymal muscles, (sy) sensory pit.

they converge; the other stripes become extinct at 1.5 mm from the tip. The ventral side is yellowish gray, marginally brown in the 3-5 mm of the anterior region (throughout the body length in specimen EMF Nr. 134d).

The eyes are monolobated, 30-37 µm in diameter, contouring marginally at the anterior tip, arranged in one or two irregular longitudinal rows. Four mm posteriad (equal to 13% of body length in specimen EMF Nr. 134b) the eyes become dorsal in distribution, occupying a band with a width equal to 1/4 of body width; posteriorly they become progressively marginal until the end of the body. The sensory pits are simple invaginations 17-31 µm deep, surrounding the whole cephalic region and extending posteriorly at a distance equivalent to 17.8% of body length (specimen EMF Nr. 134b).

The creeping sole is 69.0-77.3% of body width in the prepharyngeal region; towards the anterior tip, the sole narrows more intensely than the body and disappears close to the apex. The mouth is at a distance from the anterior tip equivalent to 52.8-59.6% of body length while the gonopore is 71.0-74.0%.

Internal morphology

Epidermis and subepidermic secretions. The epidermis is ciliated over the entire cephalic region, except in the glandu-

lar organ (Fig. 3). The organ is constituted by numerous secretory cells, the necks of which crossing the epithelium of the cephalic ventral side of the body. This epithelium is ca. 35 μm high, and 1.2x higher than that of the creeping sole. The outer edge of the glandular organ reaches the sensory border and is approximately 3 mm in length. The epithelial surface of the organ is U-shaped since its secretory cells do not cross the creeping sole. Four types of secretory cells constitute the glandular organ: cells with large xanthophilic granules, cells with erythrophilic granules, cells with cyanophilic granules and rhabditogen cells. The xanthophilic cells are the most apparent and differently from the other secretory cells, their necks are very long, extending posteriorly more than 3 mm. Body cells were not identified.

Three types of granulous secretory cells discharge onto all epidermis in the pre-pharyngeal region: erythrophilic, xanthophilic and cyanophilic, the latter are more abundant at the body margins. There is no glandular margin. Rhabditogen cells also open through dorsal and marginal epithelial surface.

Head musculature. The fibers of the ventral longitudinal subepidermic muscle layer are arranged in a different manner from that in the rest of the body (see below), forming a retractor muscle. At approximately 0.5 mm from the anterior end of the body the fibers concentrate progressively forward to the median region. At 0.1 mm from the anterior end the layer is 1.8x thicker than at 0.5 mm (Fig. 3). Closer to the anterior end of the body, bundles of 2-5 fibers detach from the retractor and successively continue obliquely to the dorsum, separating into single fibers before fixing on the basement membrane. Interestingly, part of the bundles from the left side of the body cross obliquely the sagittal plane and continue until affixing upon the basement membrane of the right side, and vice-versa (Fig. 4). The subepidermic circular layer is not apparent. The subepidermic diagonal layer is evident in the neighborhood of the retractor muscle. Dorso-ventral mesenchymal fibers are frequent, a number of them cross with those of the retractor. Other mesenchymal fibers unite the marginal epithelium with that of the glandular organ. The most developed mesenchymal muscle layer is the sub-neural. Its fibers anchor their extremities on the epidermis of the body side and of the ventral side, respectively. The dorsal diagonal muscle layer is weak, the supra-intestinal and sub-intestinal layers very weak (see below).

Musculature in the pre-pharyngeal region. The typical three layers of Geoplaninae (Tab. II): a subepithelial circular layer is followed by a diagonal with decussate bundles, and then a longitudinal layer also arranged in bundles. Close to the body margins the ventral longitudinal layer is 1.5x thicker than at the median region (Fig. 5). Furthermore, it is dorsally 1.5-2x thicker than ventrally. Subepidermic musculature thickness relative to body height (SMI), 12.7-20.2%. Mesenchymal muscle fibers are arranged in various directions, while the dorso-ventral ones are most abundant. Other mesenchymal fibers unite in four layers: a well developed dorsal diagonal layer with

Table II. Thickness of subepidermic musculature in the pre-pharyngeal region of *Notogynaphallia caissara*. The lowest and highest numbers of muscle fibers per bundle are given in parenthesis. SMI refers to subepidermic musculature thickness relative to body height.

Specimen	EMF Nr. 134b	EMF Nr. 134c
Dorsal circular	2.5 (1-2)	3.7 (1-2)
Dorsal diagonal	7.5 (3-5)	16.3 (3-6)
Dorsal longitudinal	45 (35-46)	105.0 (87-101)
Dorsal total	55.0	125.0
Ventral circular	2.5 (1-2)	2.5 (1-2)
Ventral diagonal	7.5 (2-4)	13.7 (4-8)
Ventral longitudinal	30.0 (40-49)	52.5 (46-71)
Ventral total	40.0	68.7
SMI	13%	20%

decussate fibers (38 mm thick), that is located under the dorsal sub-muscular peripheral nerve net, and three transverse ones, supra-intestinal (58 μm), sub-intestinal (48 μm), and sub-neural (= under the ventral nerve plate, 80 μm), respectively.

Digestive system. The mouth is located at the end of the first third of pharyngeal pocket. The pharynx is cylindrical (Fig. 6), with dorsal insertion posteriorly displaced, and distally folded. It occupies almost all the pharyngeal pocket. The esophagus is absent. The lining epithelium of the pharyngeal pocket is cuboidal to squamous, ciliated at the insertion of the pharynx; its musculature is composed of a one-fiber-thick circular subepithelial layer. The outer pharyngeal epithelium is cuboidal ciliated, and is crossed by abundant secretory cells with cyanophilic granules, mainly at the distal end of the pharynx. The outer musculature is composed of a longitudinal layer (2.5 μm), followed by a circular one (38 μm) with some interspersed longitudinal fibers. The inner pharyngeal epithelium is squamous to cuboidal, ciliated and crossed by scarce cyanophilic granulous secretory cells. The inner musculature consists of a longitudinal subepithelial layer (15 μm) followed by a circular layer (30 μm). Longitudinal and radial fibers comprise the mesenchym of the pharynx.

Male reproductive system. The testes are rounded, 170 μm in diameter, dorsal in position, under supra-intestinal muscle layer and intestinal diverticles, and partially between the diverticles (Fig. 5). They are arranged in an irregular row on each side of the body between the ovaries and the root of the pharynx. Most anterior and posterior testes are located, in relation to the anterior end, at 21.6 and 57.5% (specimen EMF Nr. 134b) of body length, respectively. A ductule communicates dorso-ventrally each testis with the efferent duct of the same body side. Efferent ducts run between fibers of the sub-intestinal layer; at the prostatic vesicle level they bend dorso-anteriorly to the sagittal plane, and open laterally into the median region of the prostatic vesicle (Fig. 7). The prostatic
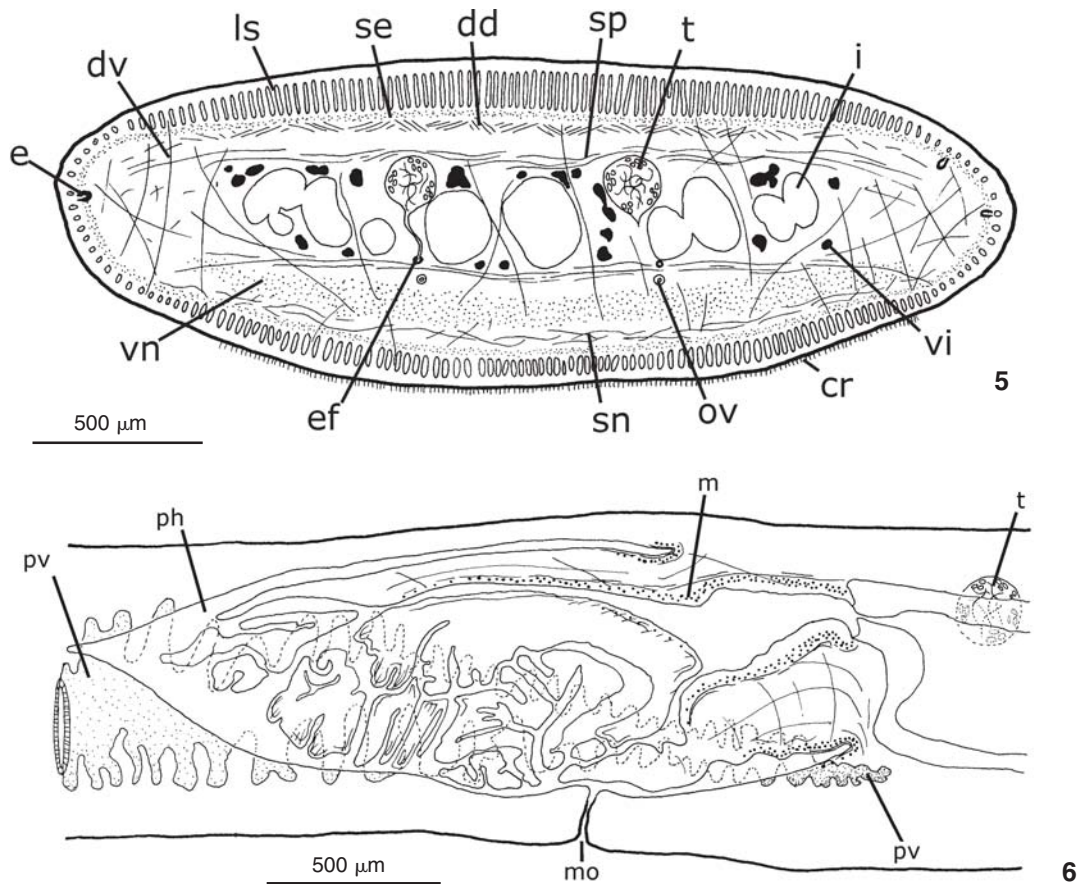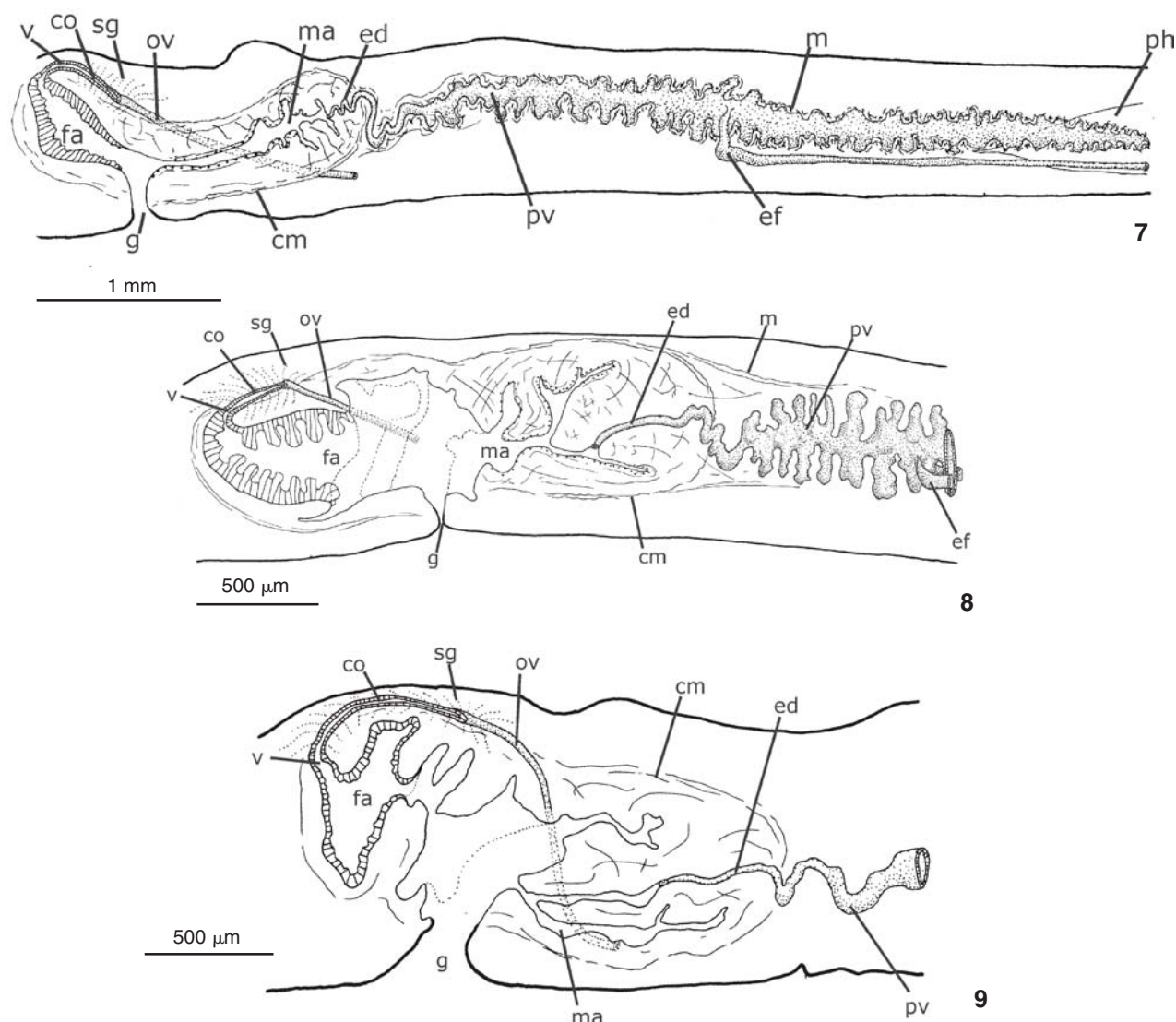
Figures 5-6. *Notogynaphallia caissara*: (5) diagrammatic transverse section of pre-pharyngeal region of specimen EMF Nr. 134d; (6) diagrammatic reconstruction of the pharynx in lateral view of specimen EMF Nr.134a. Note the anterior bifurcated portion of the prostatic vesicle, lateral to the pharyngeal pocket. (cr) Creeping sole, (dd) diagonal mesenchymal muscles, (dv) dorso-ventral mesenchymal muscles, (e) eyes, (ef) efferent duct, (i) intestine, (ls) longitudinal subepidermic muscles, (m) muscles, (mo) mouth, (ov) ovovitelline duct, (ph) pharyngeal pocket, (pv) prostatic vesicle, (se) sub-muscular peripheral nerve net, (sn) sub-neural transverse mesenchymal muscles, (sp) supra-intestinal transverse mesenchymal muscles, (t) testes, (vn) ventral nerve plate, (vi) vitellaria.

vesicle is a very long cavity (7-12x longer than wide), with diverticles, and forked in the first third. Its proximal portion can reach the root of the pharynx. The distal portion, being narrower, penetrates the common muscle coat and penis bulb and continues as an ejaculatory duct. It opens into the male atrium through a proximal fold of the atrium wall (Figs 7-9). This fold can acquire a penis-papilla shape (Figs 8-9). The cavity of the male atrium is ample and folded. An oblique fold can partially separate the male from the female atria.

Efferent ducts have a squamous to cuboidal ciliated epithelium; the muscularis is composed of a one-fiber-thick circular layer. The prostatic vesicle is composed of columnar ciliated epithelium. The erythrophilic granulous secretory cells open into prostatic vesicle; the muscularis is constituted of a subepithelial circular layer (20 µm) followed by scarce longitu-

dinal fibers at the distal portion. Dorso-ventral mesenchymal muscle fibers run between diverticles of prostatic vesicle. The ejaculatory duct has a cuboidal to columnar ciliated epithelium crossed by cyanophilic granulous secretory cells. The male atrium has a cuboidal to columnar non-ciliated epithelium and receives openings of the cyanophilic granulous secretory cells. The muscularis is composed of a subepithelial circular layer (7 µm) followed by a longitudinal layer (3 µm).

Female reproductive system. The ovaries are elongated (375 x 78 µm), ventral in position, between the sub-intestinal muscle layer and the ventral nerve plate. They lie at a distance from the anterior body end equal to 23.6 % of body length. Ovovitelline ducts arise from the second third of ovaries in its dorso-lateral (external) region and run posteriorly at the same height as the ovaries. Close to the gonopore region they curve obliquely to

Figures 7-9. *Notogynaphallia caissara*: (7) diagrammatic reconstruction of copulatory apparatus in lateral view of specimen SMF Nr. 631 (948-958); (8) diagrammatic reconstruction of the copulatory apparatus in lateral view of specimen EMF Nr.134a, prostatic vesicle continues directly from figure 6; (9) diagrammatic reconstruction of copulatory apparatus in lateral view of specimen SMF Nr. 649 (938-947). (cm) Common muscle coat, (co) common glandular ovovitelline duct, (ed) ejaculatory duct, (ef) efferent duct, (fa) female genital atrium, (g) gonopore, (m) muscles, (ma) male genital atrium, (ov) ovovitelline duct, (ph) pharyngeal pocket, (pv) prostatic vesicle, (sg) shell glands, (v) vagina.

the dorsum and the sagittal plane, and join together dorsally to the median or to the distal region of the female atrium (Figs 7-9). The common glandular ovovitelline duct is 6-13x longer than wide and runs posteriorly. The vagina is 1-3x longer than wide; it runs posteriorly downwards and opens into the dorso-proximal region of the ample female atrium. The female atrium is equal to 67-110% the length of male atrium.

The ovovitelline ducts have a cuboidal ciliated epithelium and openings of shell glands on the distal portion; the arrange-

ment of the muscle fibers is not distinguishable. The common glandular ovovitelline duct has a columnar ciliated epithelium with indistinguishable muscularis. The vagina is composed of columnar epithelium, traversed by openings of erythrophilic granulous secretory cells; the muscularis is composed of a subepithelial circular layer followed by a longitudinal layer. The female atrium proximally is comprised of tall columnar non-ciliated epithelium, and cuboidal to columnar close to the gonopore region. There are abundant openings of erythrophilic granu-

lous secretory cells, but scarce cyanophilic ones. The muscularis is constituted by a subepithelial longitudinal layer (5 µm) followed by an one-fiber-thick longitudinal layer.

Common muscular coat. It is constituted by a weak layer (10-20 µm) of longitudinal and diagonal fibers. It envelops a short distal portion of the prostatic vesicle, the male and female atria and the vagina (Figs 7-9).

Taxonomic note. Specimen SMF Nr. 112b (923-937), identified by RIESTER (1938) as *Geoplana marginata*, is immature and poorly preserved, so that identification was not possible. It consists of three slides: one slide with an intermediate region (without pharynx, nor copulatory apparatus) in balsam; one slide with transverse sections from probably the pre-pharyngeal region; and one slide with the anterior end of the body in balsam.

Notogynaphallia fita (Froehlich, 1959)

Geoplana fita Froehlich, 1959: 217; Froehlich, 1967: 159.
Notogynaphallia fita Ogren & Kawakatsu, 1990: 141.

Diagnosis. Body long, with 110 mm in maximum length when living, and very slender, having four longitudinal dark stripes over a yellowish dorsum, plus a fifth median one in the cephalic region. The pharyngeal pocket is twice the length of the pharynx. The prostatic vesicle is very long, and non-bifurcated. It lies outside the common muscle coat. Efferent ducts open into the distal portion of prostatic vesicle. The female atrium is ample and ovoid.

Locality. Brazil: Blumenau (Santa Catarina).

Material analyzed. All specimens are from Blumenau. EMF Nr. 193a: 23/VI/1953, Froehlich & Froehlich, *leg.* Anterior region 1, from anterior tip to 2.2 mm behind: transverse sections on three slides; anterior region 2, 9.5 mm long, beginning 2.2 mm behind the anterior tip: sagittal sections on nine slides; anterior region 3, 10 mm long, beginning 11.7 mm behind the anterior tip: sagittal sections on 11 slides; anterior region 4, 19.3 mm long, beginning 21.7 mm behind the anterior tip: horizontal sections on 17 slides; pre-pharyngeal region: transverse sections on seven slides; pharynx: sagittal sections on one slide (incomplete); copulatory apparatus: sagittal sections on 4 slides (incomplete); remaining body in ethanol 70°. EMF Nr. 193b: 23/VI/1953, Froehlich & Froehlich, *leg.* Anterior region 1, from anterior tip to 2 mm behind: sagittal sections on two slides; pre-pharyngeal region: transverse sections on six slides; pharynx: sagittal sections on six slides; copulatory apparatus: sagittal sections on 26 slides. EMF Nr. 193c: 23/VI/1953, Froehlich & Froehlich *leg.* Copulatory apparatus: sagittal sections on two slides (incomplete).

This redescription is, in general, in agreement with previous description made by FROEHLICH (1959).

External morphology

Fixed specimens: 94.5 mm in maximum length (Tab. III), very narrow (relative width: length, 2.0-2.8%) with parallel margins and rounded extremities (Fig. 10). The body is elliptic in cross section. The dorsum is yellowish in color, the ventral side is

Table III. Measurements (mm) of fixed specimens of *Notogynaphallia fita*.

| Specimen	EMF Nr. 193a	EMF Nr. 193b
L (length)	94.5	64.0
W (width)	1.9	1.8
Height	1.1	0.9
Relative W: L	2.0%	2.8%
M (mouth - anterior body tip)	64.0	40.0
Relative M: L	67.8%	62.5%
G (gonopore - anterior body tip)	80.0	50.0
Relative G: L	84.6%	78.1%
Creeping sole: body width	65%	?

yellow whitish. After FROEHLICH (1959), in life, "the color is light straw yellow, a little darker at the margins; at the cephalic region it acquires a ferruginous tint, also darker at the margins, and at the anterior tip it darkens even more, becoming brownish. On the back, there are two pairs of narrow longitudinal ferruginous stripes, a darker paramedian pair and a lighter sub marginal one. At the cephalic region there is a fine median dark line. All stripes end, without fusion with the others, near to the extremities of the body, except the sub marginal, which, one specimen excepted, unite at the posterior end. The ventral side is white" (Fig. 11).

The eyes are monolobated, 20-26 µm in diameter, without halos. They contour the anterior end of the body in a uniserial row and extend laterally until the posterior end. The sensory pits are simple invaginations, 27 µm deep, in a uniserial row contouring the anterior end of the body up to a distance equal to 3.5% of body length. The creeping sole is 65.3% of body width. Towards the anterior tip, the sole narrows more intensely than the body and disappears close to the apex. The mouth is at a distance from anterior tip equivalent to 64.0% of body length while the gonopore is 80.0%.

Internal morphology

Epidermis and subepidermic secretions. The epidermis is ciliated over the entire cephalic region, except in the glandular organ (Fig. 12). The organ is constituted by numerous secretory cells, the necks of which crossing the epithelium of the cephalic ventral side of the body. The epithelium of the organ is ca. 30 µm high, 1.5x higher than that of the creeping sole. The outer edge of the glandular organ reaches the sensory border and is approximately 2 mm in length. The epithelial surface of the organ is U-shaped since its secretory cells do not cross the creeping sole. Three types of secretory cells constitute the glandular organ: abundant cells with amorphous xanthophilic secretion, cells with fine erythrophilic granules and cells with cyanophilic granules. Granulous cyanophilic secretory cells and rhabditogen cells open also onto the dorsal epithelium.

In the pre-pharyngeal region, erythrophilic granulous secretory cells open throughout the entire body surface, being more abundant in the body margins, and scarce in the ventral
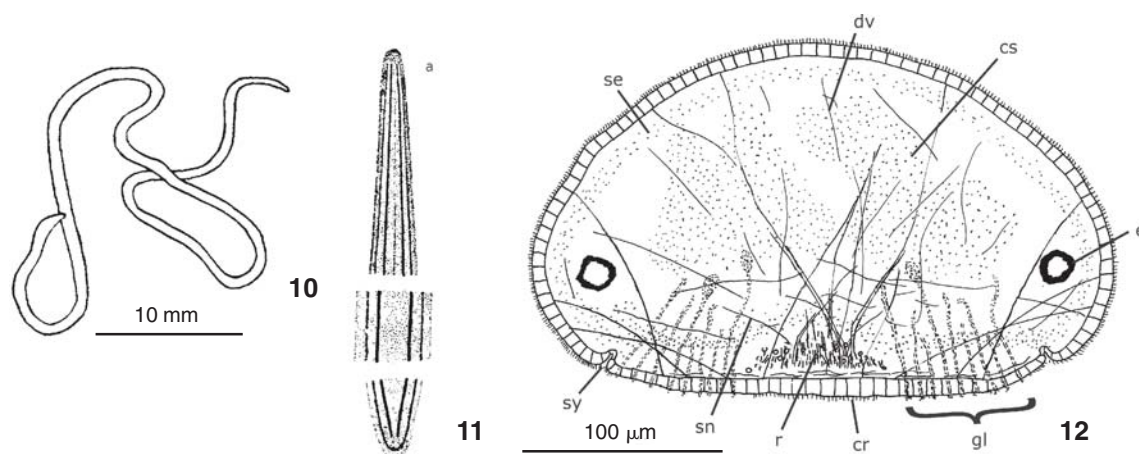
Figures 10-12. *Notogynaphallia fita*: (10) outline of a worm in dorsal view, after Froehlich (1959); (11) dorsal pattern color, after Froehlich (1959); (12) diagrammatic reconstruction of the cephalic retractor muscle in transverse section at 150 µm from anterior end of the body, specimen EMF Nr. 193b. (a) Anterior end of the body, (cr) creeping sole, (cs) central nervous system, (e) eyes, (dv) dorso-ventral mesenchymal muscles, (gl) glandular organ, (r) cephalic retractor muscle, (se) sub-muscular peripheral nerve net, (sn) sub-neural transverse mesenchymal muscles, (sy) sensory pit.

side. Abundant cyanophilic granulous secretory cells open through marginal region and scarcer dorsally. Scarce rhabditogen cells open only onto the dorsal epidermis. Without glandular margin.

Head musculature

The fibers of the ventral longitudinal subepidermic muscle layer are arranged in a different manner from that in the rest of the body (see below), forming a retractor muscle, similar to that of *N. caissara* in the arrangement of the fibers, but weaker. At 200 µm from anterior tip the layer becomes triangular-shaped in the cross-section (Fig. 12). Closer to the anterior end of the body some fibers and scattered bundles of 2-3 fibers orientate obliquely toward the epithelium of the dorsum and the body sides and fix on the basement membrane separately as isolated fibers. Part of the bundles from the left side of the body cross obliquely the sagittal plane and continue until affixing upon the basement membrane of the right side, and vice-versa. The subepidermic circular layer is not apparent. A number of fibers of the subepidermic diagonal layer are evident in the neighborhood of the retractor muscle. Dorso-ventral mesenchymal fibers are present, a number crossing with those of the retractor. Other mesenchymal fibers unite the marginal epithelium with that of the glandular organ. The most developed mesenchymal muscle layer is the sub-neural. Its fibers anchor their extremities on the epidermis of the body side and of the ventral side, respectively. The diagonal mesenchymal muscle layer is weak, the supraintestinal and subintestinal mesenchymal layers very weak (see below).

Musculature at the pre-pharyngeal region. The three typical subepidermic muscle layers of Geoplaninae (Tab. IV) comprises the subepidermic musculature: a circular one, followed

Table IV. Thickness of subepidermic musculature in the pre-pharyngeal region of *Notogynaphallia fita*. The lowest and highest numbers of muscle fibers per bundle are given in parenthesis. SMI refers to subepidermic musculature thickness relative to body height.

Specimen	EMF Nr. 193a
Dorsal circular	2.5 (1-2)
Dorsal diagonal	7.5 (2-4)
Dorsal longitudinal	52.5 (26-38)
Dorsal total	62.5
Ventral circular	2.5 (1-3)
Ventral diagonal	12.5 (3-5)
Ventral longitudinal	66.2 (33-52)
Ventral total	81.2
SMI	17%

by one of decussate bundles, then a layer of longitudinal fibers also arranged in bundles (Fig. 13). The latter is 1.3x thicker ventrally than dorsally. Mesenchymal musculature consists of muscle fibers arranged in various directions, mainly dorso-ventral (some of which join in bundles between intestinal diverticles) and diagonal. The first become more abundant towards the body margins and the latter in the sub-neural region. Other mesenchymal fibers gather in four layers (Fig. 13): a dorsal diagonal layer with decussate fibers, well developed (35 µm), under the dorsal sub-muscular peripheral nerve net, and three transverse ones, supra-intestinal (50 µm), sub-intestinal (6-12 µm, in bundles of 24-36 fibers), and sub-neural (30 µm), respectively.
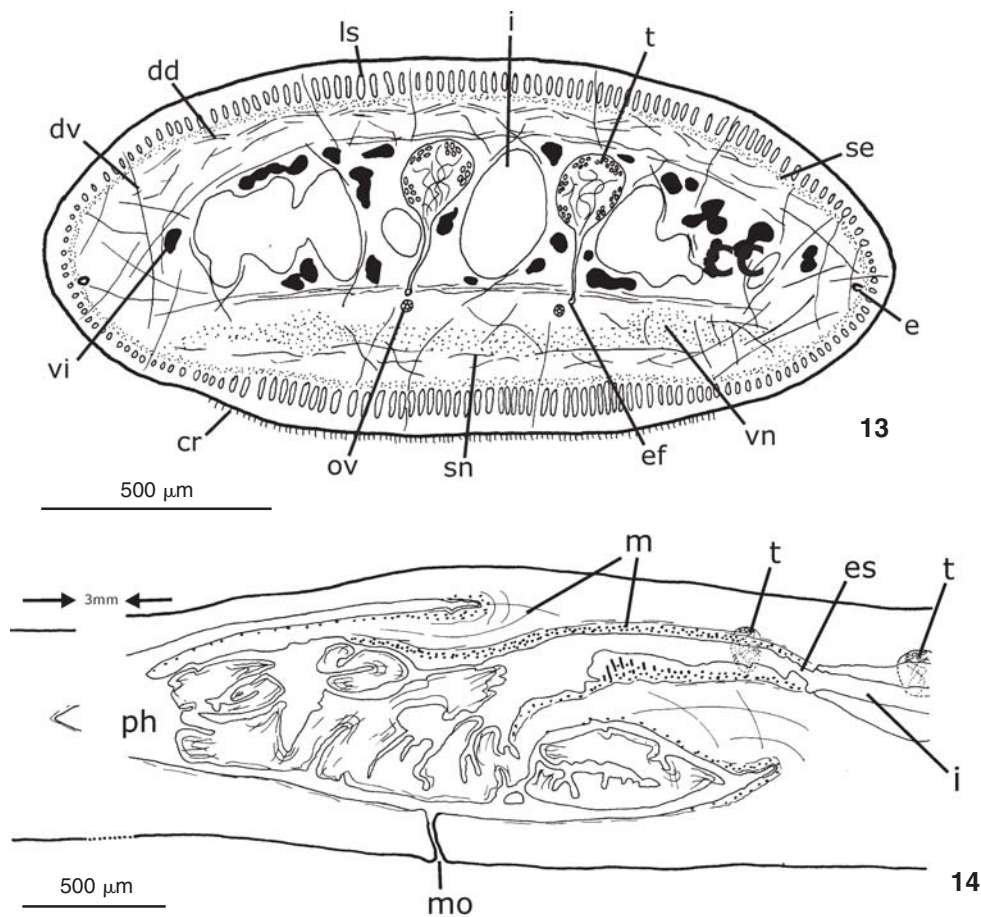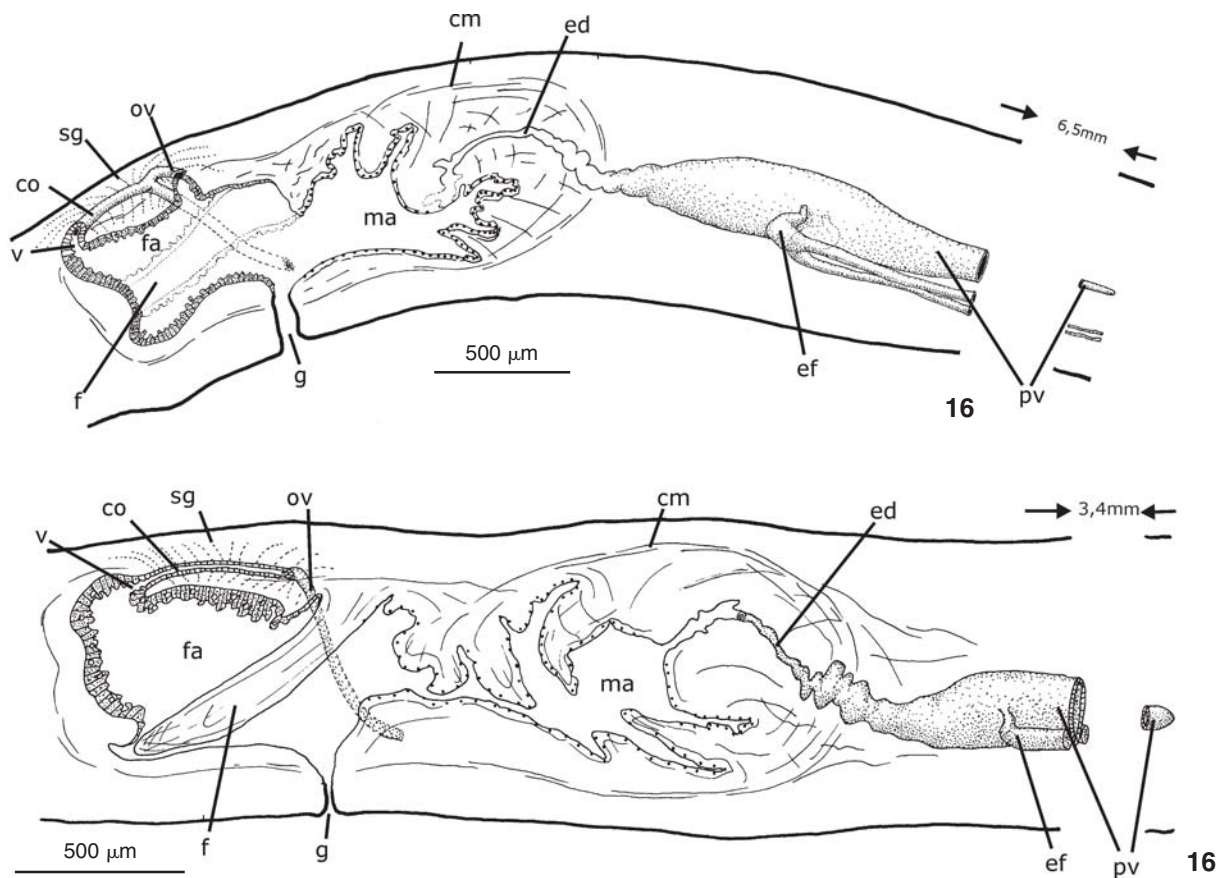
Figures 13-14. *Notogynaphallia fita*: (13) diagrammatic transverse section of pre-pharyngeal region of specimen EMF Nr. 193a; (14) diagrammatic reconstruction of the pharynx in lateral view of specimen EMF Nr. 193a. (cr) Creeping sole, (dd) diagonal mesenchymal muscles, (dv) dorso-ventral mesenchymal muscles, (e) eyes, (ef) efferent duct, (es) esophagus, (i) intestine, (ls) longitudinal subepidermic muscles, (m) muscles, (mo) mouth, (ph) pharyngeal pocket, (ov) ovovitelline duct, (se) sub-muscular peripheral nerve net, (sn) sub-neural transverse mesenchymal muscles, (t) testes, (vi) vitellaria, (vn) ventral nerve plate.

Digestive system. The mouth is located at the end of the first third of pharyngeal pocket. The pharynx is cylindrical to bell-shaped (Fig. 14), with dorsal insertion at the level of the mouth. It occupies one third of the pharyngeal pocket. The esophagus is very short with a length equal to 5.6% the length of the pharynx. The lining epithelium of the pharyngeal pocket is squamous non-ciliated; the musculature is composed of a thin circular subepithelial layer (5 μm). The outer pharyngeal epithelium is cuboidal ciliated, crossed by abundant granulous erythrophilic and granulous cyanophilic secretory cells. The outer musculature is composed of a longitudinal layer (2.5 μm), followed by a circular one (62 μm) with some interspersed longitudinal fibers in its innermost region. The inner pharyngeal epithelium is ciliated; the inner musculature consists of a circular subepithelial layer (16 μm) followed by a longitudinal one (3.5 μm). Longitudinal and radial fibers occur in the mesenchym of the pharynx.

Male reproductive system. The testes are ovoid shaped, 136 μm in height, under supra-intestinal muscle layer, and between intestinal branches (Fig. 13). They are arranged in an irregular row on each side of the body, from posteriorly to the ovaries to the pharyngeal root. The most anterior and the most posterior testes are located, at a distance, respectively, of 24.4 and 62.3% of body length, in relation to anterior end. A dorso-ventral ductule communicates each testis with the efferent duct of the same side. Efferent ducts run posteriorly between the fibers of the sub-intestinal layer, dorsally to the ovovitelline ducts. Distally they bend to the sagittal plane and open laterally into the distal region of the prostatic vesicle (Figs 15-16). The vesicle is a long tube-like cavity (4.2 x 0.5 mm in specimen EMF Nr. 193a; 8.2 x 0.6 mm in specimen EMF Nr. 193b). Close to the common musculature coat the vesicle becomes slightly sinuous and continues inside the muscle coat as the ejaculatory duct, sinuous

Figures 15-16. *Notogynaphallia fita*: (15) diagrammatic reconstruction of copulatory apparatus in lateral view of specimen EMF Nr. 193b; (16) diagrammatic reconstruction of copulatory apparatus in lateral view of specimen EMF Nr. 193a. (cm) Common muscle coat, (co) common glandular ovovitelline duct, (ed) ejaculatory duct, (ef) efferent duct, (f) atrial fold, (fa) female genital atrium, (g) gonopore, (ma) male genital atrium, (ov) ovovitelline duct, (pv) prostatic vesicle, (sg) shell glands, (v) vagina.

and dilated proximally, while canalicular distally. This duct opens into an irregular-shaped cavity which is inside an obliquely oriented fold of the proximal region of the male atrium. The distal region of the atrium is a spacious cavity with dorsal folds.

Efferent ducts are lined with a cuboidal ciliated epithelium. The muscularis is composed of a one-fiber-thick circular layer. The prostatic vesicle is composed of cuboidal to columnar densely ciliated epithelium, traversed by necks of erythrophilic granulous secretory cells, especially abundant in the more dilated region. The muscularis is composed of a subepithelial circular layer (11 μm) followed by longitudinal fibers at the distal portion. The ejaculatory duct is composed of cuboidal to columnar non-ciliated epithelium and crossed by two different types of acidophilic granulous secretory cells (abundant erythrophilic, scarce xanthophilic). The lining epithelium of the male atrium is columnar non-ciliated. The proximal half receives necks of erythrophilic granulous secretory cells, especially numerous close to the opening of the ejaculatory duct. The dorsal

distal half has xanthophilic granulous secretory cells and scarce erythrophilic, while the ventral distal half of atrium has two types of granulous secretory cells, cyanophilic and erythrophilic. The muscularis consists of a subepithelial circular layer (20 μm) followed by a longitudinal layer (15 μm). Scarce fibers are longitudinally and diagonally oriented in the stroma.

Female reproductive system. The ovaries are elongated (1.300 x 120 μm), ventral in position, and between the subintestinal mesenchymal muscle layer and the ventral nerve plate. They are located at a distance from the anterior body end equal to 18.5% of body length. Ovovitelline ducts arise from dorsal side of median region of ovaries, and run posteriorly at the same height of the ovaries. At the level of the gonopore, the ducts bend obliquely to the dorsum and the sagittal plane, joining together over the female atrium (Figs 15-16). The common glandular ovovitelline duct is 6-12 times longer than wide and run posteriorly. The vagina run postero-ventrally and opens into the dorso-proximal region of the female

atrium. The atrium is an ample and irregular-shaped cavity, with a distal lateral oblique fold. The female atrium is equal to 66% the length of male atrium.

Ovovitelline ducts consist of columnar ciliated epithelium. A short sub-terminal portion of the ducts receives openings of granulous xanthophilic secretory cells, and shell glands open into the terminal portion of the ducts. The muscularis is circular (10 μm). The common glandular ovovitelline duct is lined with cuboidal ciliated epithelium. The muscularis is longitudinal (10 μm). The vagina is composed of columnar ciliated epithelium, whose apical surface is irregular. The epithelium is pierced by necks of two types of granulous secretory cells, erythrophilic and cyanophil. The muscularis is circular (10 μm). The female atrium has a pseudo-stratified columnar epithelium, the apical surface of the epithelium is irregular. The lining epithelium is crossed by necks of three types of granulous secretory cells: erythrophilic, xanthophilic and cyanophilic, the latter cross only the dorsal epithelium. The muscularis (8 μm) is constituted by a layer seemingly diagonal, followed by a longitudinal one.

Common muscular coat. It is constituted by a layer (10 μm) of fibers arranged in several orientations. It envelops the male and female atria, and the vagina.

DISCUSSION

A diagnostic comparison between the species of the group 2, to which *N. caissara* and *N. fita* belong, was already made by E.M. Froehlich & Leal-Zanchet (2003). The species included in group 2 are "slender worms with longitudinal stripes on the dorsum; a long genital apparatus, with an extrabulbar vesicle, and atria, especially the male one, with folded walls. The female atrium, although smaller than the male one, always has an ample variously pleated cavity plus the female canal" (E.M. Froehlich & Leal-Zanchet 2003).

The two species are here comparatively discussed with genera also possessing a retractor muscle, namely *Supramontana* Carbayo & Leal-Zanchet, 2003, *Choeradoplana* Graff, 1899, *Cephaloflexa* Carbayo & Leal-Zanchet, 2003, and *Issoca* Froehlich, 1955. Three of the genera can be distinguished from *N. caissara* and *N. fita* by the following external features: *Issoca* species possess a spoon-shaped cephalic region, with the ventral side usually concave. In *Choeradoplana* and *Cephaloflexa* species the cephalic region is rolled upwards, and the eyes are absent at the anterior end of the body. Additionally, the cephalic region of *Choeradoplana* is laterally expanded and the ventral side provided with two glandular cushions, separated by a median groove. In *Cephaloflexa* the anterior third of the body narrows very gradually.

The most outstanding characteristic of the internal morphology is the organization of the retractor in *N. caissara* and *N. fita*. It differs from those of the other Geoplaninae genera. The retractor fibers in *Cephaloflexa* keep parallel to the antero-posterior body axis. In *Choeradoplana* the fibers are completely insunk in the mesenchym. In *Supramontana* bundles of retractor extend to body margins into horizontal planes. In *Issoca*, bundles of the retractor orientate obliquely to the epithelium of the dorsum and to that of the body sides; however, in this genus bundles from one side of the sagittal plane do not cross to the other side, like in *N. caissara* and *N. fita*.

The presence of a retractor muscle in *N. caissara* and *N. fita*, combined with the absence of it in the type-species of the genus, *N. plumbea* (Froehlich, 1956), is reliable evidence of the heterogeneity of the genus, as previously proposed by E.M. Froehlich & Leal-Zanchet (2003) based on other morphological characters. Furthermore, this evidence precludes maintaining the two species in *Notogynaphallia*, and their taxonomic status should be reviewed.

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