



“Dalyellioida” (Platyhelminthes, Rhabdocoela) from the Río de la Plata estuary in Argentina, with the description of two new species of *Gieysztoria*

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Abstract

Two new species of *Gieysztoria* are described, along with the first record of two species of this genus in Argentina. *Gieysztoria atalaya* n. sp. has a sclerotic stylet with a window on one of its sides and a belt bearing three types of spines. One hollow spine, originated under the window, is basally broad and tapers towards its blunt distal end. This spine separates the other two groups of spines, one of approximately ten thick spines situated on the edge of the belt, and the other arranged in several rows. The proximal row bears approximately 10 triangular spines, broad at the base and rapidly tapering. *Gieysztoria matilde* n. sp. has a sclerotic stylet with a belt formed by a fibrous framework and a circular window. A central projection arising under this window bears a large blade-shaped hollow spine. This spine separates two major groups of thin spines, one formed by several parallel rows of hollow, acicular, very thin spines, the other comprising hollow and slightly thicker spines with broader base, arranged in several irregular rows. *Gieysztoria evelinae* (Marcus 1946) Luther 1955, *Gieysztoria hymanae* (Marcus 1946) Luther 1955 and *Gieysztoria falx* Brusa, Damborenea and Noreña 2003 are reported for the second time after their original description. *Nygulus evelinae* Marcus 1954 is cited for the first time for Argentina; until now, this species was known only for the estuary of Itanhaen River in Bahia de Santos, Brazil.

Key words: Dalyelliidae, *Gieysztoria*, Graffillidae, *Nygulus*, turbellarian, benthos

Introduction

The “Dalyellioida” are a group of small rhabdocoel Platyhelminthes, comprising both free-living and parasitic forms; the group is considered polyphyletic (Willems *et al.* 2006). The Dalyelliidae is one of the richest and most diverse families, containing mostly free-living species, with approximately 170 species described from primarily freshwater environments (Cannon 1986). The genus *Gieysztoria* is the most speciose of this family, with about 70 species (Tyler *et al.* 2006). Twenty-five of these species are known from South America, most of them from Brazil, and only 8 have been described for Argentina (Noreña-Janssen 1995; Brusa *et al.* 2003; Damborenea *et al.* 2007).

The Graffillidae is a smaller family of parasitic and free-living species that inhabit aquatic environments. The subfamily Pseudograffillinae comprises three genera of free-living marine and brackish species. The single species of the monospecific genus *Nygulus*, *N. evelinae* Marcus 1954, is known only for the Southern Hemisphere; it is the only species of the subfamily recorded in brackish environments from the Brazilian coast.

Research on the microturbellarian fauna of Argentina is still at an early stage. Few regions within Argentina have been explored, and only poorly at that (Böhmig 1902; Brugni 1993; Trochine *et al.* 2006). Nevertheless, studies carried out in areas such as the floodplain of the Middle Paraná River or areas associated with the

Río de la Plata (south Buenos Aires city) are potential sources of new and essential information on the systematics of this taxon within South America (Noreña-Janssen 1995; Brusa *et al.* 2003; Brusa 2006a; b; Noreña *et al.* 2005; Damborenea *et al.* 2007).

In this work, we describe two new species of *Gieysztoria* from Argentina, and report two *Gieysztoria* species originally known from Brazil, but now found in Argentina for the first time. In addition, *Gieysztoria falx* Brusa, Damborenea and Noreña 2003 is reported for the second time after its original description. *Nygulus evelinae* is cited for the first time for Argentina; up to the present, this species had only been recorded in the estuary of Itanhaen River in Bahia de Santos, Brazil (Marcus 1954).

Materials and methods

Seasonal sampling was performed from August 2000 to January 2004 at two sample sites located in the Argentine littoral of the Río de la Plata. The localities were Atalaya (35°00'53.6"S–57°32'3.3"W) and Punta Piedras (35°21'23.1"S–57°10'22.52"W), in Buenos Aires province. Both the littoral benthos of the river and the floating vegetation of associated environments were sampled at Punta Piedras, while only the second type of environment was sampled at Atalaya. The non-benthic habitats were located near the littoral river zone, in an area covered with abundant floating vegetation at Atalaya, and in a lotic environment (La Matilde stream) at Punta Piedras.

Benthos samples were collected with corer tubes 4.5 centimeters in diameter along a transect from upper tidal level (UTL) to lower tidal level (LTL). The first two centimeters of sediment were taken to the laboratory for *in vivo* observation. Samples from vegetated areas were taken using 40–80 µm mesh size hand nets. All samples were transported *in vivo* to the laboratory in plastic bags. The following environmental parameters were recorded at each site: salinity, conductivity, TDS, dissolved O₂, O₂ saturation and temperature. Substrate type and associated fauna were also recorded (tables 1–2).

TABLE 1. Environmental data of the Punta Piedras locality. Type of substrate and associated fauna are also provided.

Punta Piedras	Salinity (‰)	Conductivity (µS)	TDS (mg/l)	pH	O ₂ (mg/l)	O ₂ sat.(%)	Water T° (°C)	Type of substrate	Associated fauna
02/08/2000***	#	2750	1350	#	#	#	13	Caliche-clay/muddy*	NC
12/02/2001***	2.5	1171	587	7.94	#	#	27.5	Caliche-clay/muddy*	NC
24/08/2001***	4	2110	#	7.89	10.05	116	20.7	Caliche-clay/muddy*	NC
05/12/2001	5	9650	4790	9.46	10.22	142	33.1	Caliche-clay/muddy*	Polychaeta, Nematoda, Gastropoda
04/04/2002***	4	5080	2600	8.9	5.5	100	27	Caliche-clay/muddy*	NC
05/03/2003***	1.5	757	368	7.63	8.8	115	28.7	Caliche-clay/muddy*	Acari, Copepoda, Cladocera, Ostracoda, Nematoda, Oligochaeta

not quantified; NC: not considered; * from Darrigran (1999); *** dates at which turbellarians were found.

The microturbellarians were separated and observed *in vivo* by squash preparations in the laboratory. Then they were fixed in Bouin's fixative, cut sagittally into 4 µm thick sections, and stained by Azan method. Some specimens were mounted *in toto* in polyvinyl-lactophenol for study of the male stylet. Photomicrographs were taken with a Zeiss Axioplan 2 Microscope. Nomarski's interference contrast filters were used for

photomicrographs of stylet. Other specimens were dissected; their stylets were extracted, left to air dry and later metallized for observation under scanning electron microscope (SEM) JEOL 100. The materials are deposited in the Invertebrate Zoology Collection of Museo de La Plata (MLP).

TABLE 2. Sample date, environmental data and vegetation type for La Matilde stream and Atalaya localities.

La Matilde	Salinity (‰)	Conductivity (µS)	TDS (mg/l)	pH	O ₂ (mg/l)	O ₂ sat. (‰)	Water T° (°C)	Type of vegetation
12/02/2001***	#	#	#	#	#	#	#	Lemnaceae
24/08/2001	1.5	387	#	6.4	4.13	42.9	16.3	NC
05/12/2001	0	1439	725	7.7	1.9	23.2	24.7	few Lemnaceae, in decomposition
05/03/2003***	0.5	1813	918	7.14	0.28	3.2	28.4	A lot of Lemnaceae, in decomposition
19/04/2003		1171	580	7.02	0.48	4.6	16.5	<i>Lemna giba</i>
Atalaya								
31/03/2000	#	#	#	#	#	#	#	<i>Pistia stratiotes</i> and Lemnaceae
05/03/2001***	0	358	181	5.73	#	#	26	<i>Salvinia biloba</i> and <i>Limnobium spongia laevigatum</i>
02/08/2001***	0	879	441	6.28	0.28	2.4	11	<i>Pistia stratiotes</i> , <i>Limnobium spongia laevigatum</i> , <i>Salvinia biloba</i> and Lemnaceae
02/02/2002***	0	571	278	6.25	#	#	24	<i>Pistia stratiotes</i> , <i>Salvinia biloba</i> and <i>Hydrocotyle ranunculoides</i>
04/04/2002***	1	1266	629	6.7	0.1	1	16.8	<i>Pistia stratiotes</i> , <i>Salvinia biloba</i> and <i>Hydrocotyle ranunculoides</i>
25/06/2002	#	501	278	7.05	#	#	5.8	<i>Salvinia biloba</i> and <i>Hydrocotyle ranunculoides</i>
21/02/2003***	#	397	180	6.5	1.1	11.5	17.6	<i>Pistia stratiotes</i> and <i>Salvinia biloba</i>
10/04/2003***	1.5	438	220	7.03	6.19	62.2	16.7	<i>Pistia stratiotes</i>
27/01/2004***	2	5490**	2750**	6.81	0.1	2	29.2	<i>Pistia stratiotes</i> and <i>Salvinia biloba</i> **

not quantified; NC: not considered; * from Darrigran (1999); ** the communication between the sample place and the Ro de la Plata was modified during the study. This provoked changes in water and vegetation quality; *** dates at which turbellarians were found.

Results

Family Dalyelliidae Graff 1908

Genus *Gieysztoria* Ruebush & Hayes 1939

Gieysztoria atalaya n. sp.

Figures 1, 7C–F

Material. Holotype: one specimen *in toto* mounted in polyvinyl-lactophenol MLP 5420, Atalaya 21–02–03.

Paratypes: Four specimens, sagittally cut MLP 5340, 5341, Atalaya 05–03–01, 02–02–02. One specimen *in toto* mounted in polyvinyl-lactophenol, MLP 5421, Atalaya 10–04–03.

Other material: Fifteen specimens, sagittally cut MLP 5342, 5343, 5344, 5723, 5724, Atalaya 05–03–01, 02–02–02, 04–04–02, 21–02–03, 10–04–03.

Type locality. Atalaya (35°00′53.6″S–57°32′3.3″W), Buenos Aires province, Argentina.

Etymology. The species name refers to the type locality.

Description. Live adult specimens 430–575 µm long and 95–115 µm wide. Anterior end rounded. Five or six caudal papillae at abruptly tapering posterior end. Rhabdites arranged in groups of 3–4 along body. Rigid cilia at anterior and posterior ends of body longer than those on remaining epidermis.

Pharynx *in vivo* 130 µm long and 100 µm wide, with four dorsal papillae at anterior margin of pharynx in contrast to other *Gieysztoria* species.

Broad nerve tracts from brain to anterior region observed in sagittal sections. Conspicuous nucleus evident at peripheral anterior and posterior regions of brain. Black eyes formed by very numerous pigment spheres arranged in a kidney-shaped structure.

Male reproductive system formed by two non-compact testes situated in posterior body region. Spermatozooids observed at periphery of testes. Vasa deferentia projecting from rostral portion of testes toward anterior region and apically or subapically entering seminal vesicle; the latter relatively small and continuous with similar-sized prostate vesicle.

Sclerotic stylet approximately 62 µm long and 30 µm wide, with well-defined fibrous-like proximal belt showing a clear window on one side (figure 1A, C). Belt open at side opposite window. Three types of spines originating from belt. A) One spine (spine “a” in figure 1C, length 23 µm) issuing from under window – broad at the base and tapering towards blunt distal end. Spine hollow, situated between the other two groups of spines. B) On one side of stylet, a group of approximately ten thick spines (spines “b” in figure 1C), originating from belt margin, all of similar shape and length. Very thin spines scattered among them. C) On other side of spine “a”, a third group of spines (spines “c” in figure 1C) arranged in several rows forming a “brush”. Approximately 10 spines in proximal row, with broad base and rapidly tapering so as to present triangular shape (figure 1C).

Female reproductive system comprising an ovary located on right side of body and dorsal to intestine. Oocytes arranged in two or three series at proximal part of ovary, and uniserially at distal part (figure 1D). Ovary continued into a short oviduct; oviduct widening to form a seminal receptacle connected to uterus by a long common duct. Uterus with thick walls, curved to open into the genital atrium. Egg in uterus large (greater diameter 152 µm, lesser diameter 93 µm), oval, with operculum at one of its ends. Vitellaria smooth (figure 1B); anteriorly reaching base of pharynx and ventral at this level; becoming dorsal at posterior end, there merging to enter the common duct. Bursa copulatrix with very thick muscular walls, opening into common duct. Gonopore surrounded by a strong sphincter and cement glands.

Discussion. Based mainly on the configuration and shape of the male stylet, *G. atalaya* could be compared with three species: *Gieysztoria pavimentata* (Beklemishev 1926) Luther 1955, *G. virgulifera* (Plotnikov 1906) Luther 1955 and *G. beltrani* (Gieysztor 1931) Luther 1955. *Gieysztoria pavimentata* is the species with the most similar stylet to that of the new species. *G. pavimentata* was considered by Luther (1955) as belonging to the Inaequales group, subgroup Fenestrate. Both species possess a robust spine, but in *G. atalaya* this spine occupies a central position on the girdle (spine “a” figure 1C) whereas in *G. pavimentata* it is located on the right side of the stylet (spine “d” of Luther 1955, figure 41 G, H and 42B). In addition to the different location, the central spine of *G. atalaya* is clearly larger and differs in shape from the rest of spines, which is not the case of spine “d” (after Luther 1955) in *G. pavimentata*.

Furthermore, the constellation of the smaller spines of *G. pavimentata* is different from that of *G. atalaya*. *G. pavimentata* bears 10 spines on the girdle edge, followed by 2 or 3 irregular rows of spines on the wall of the copulatory organ. In contrast, the spines of *G. atalaya* are arranged in two groups, the first group (spines

“b”) formed by about 10 spines and the second group (spines “c”) formed by three or four rows of thin spines with similar shape, but different length.

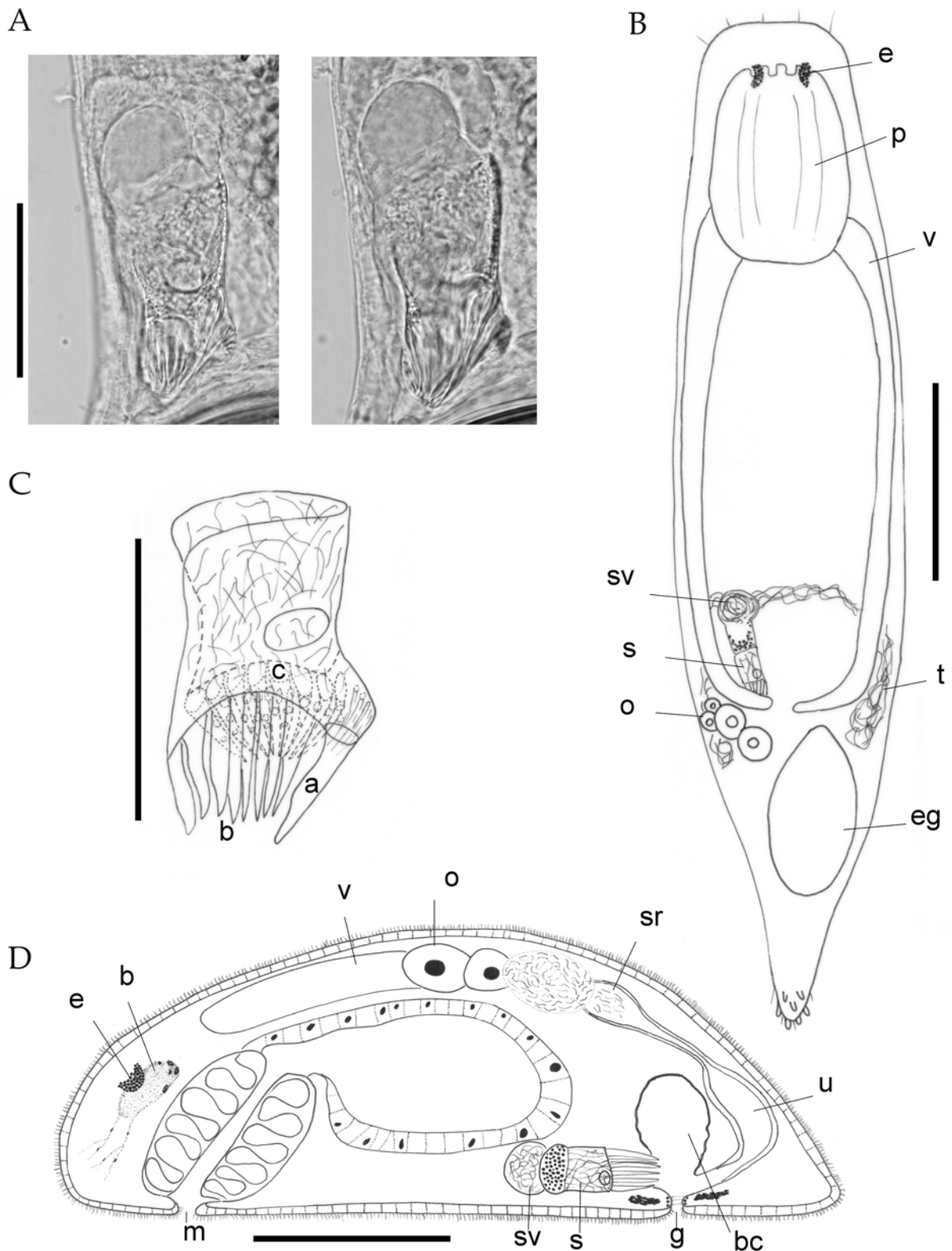


FIGURE 1. *Giesztoria atalaya* n. sp. A, photograph of the stylet. B, dorsal general view *in vivo*. C, reconstruction of the stylet (a, b, and c indicate the three types of spines). D, sagittal reconstruction. Scales A and C: 50 μ m. Scales B and D: 100 μ m. For abbreviations see Figure 8.

The stylets of *Gieysztoria beltrani* and *G. virgulifera* are also similar to that of *G. atalaya*; both have a belt with a middle window and a large spine issuing from under it. In *G. beltrani* the large spine under the window (Gieysztor 1931; Luther 1955) bears small spines, contrasting with the smooth large spine of *G. atalaya*. In *G. beltrani*, the rest of the spines are grouped at the sides of the large spine, with six spines on one side and a variable number on the other. *G. virgulifera* has two groups of spines at both sides of the smooth large central spine, similarly to the condition in *G. atalaya*, but the groups differ in number and shape of the spines. In *G. virgulifera* the first group is formed by 4–7 spines on one side and only two robust, scimitar-shaped spines, on the other (Luther 1955).

Gieysztoria matilde n. sp.

Figure 2

Material. Holotype: One specimen mounted in toto in polyvinyl–lactophenol, MLP 5345, La Matilde stream 12–02–01, 05–03–03.

Type locality. La Matilde stream, Buenos Aires province, Argentina.

Etymology. The species name refers to the type locality.

Description. Specimens fixed and mounted in polyvinyl–lactophenol are 495 μm long and 150 μm wide. Eyes large, interocular distance similar to distance between eye and body margin.

Body rounded at anterior end and tapering towards posterior end (figure 2B). In fixed specimens, pharynx is 150 μm long and 67 μm wide.

Male reproductive system with seminal vesicle located in front of copulatory apparatus, diameter of seminal vesicle similar to stylet length. Sclerotic stylet 54–65 μm long. Proximal region (proximal belt) formed by a fibrous framework with a dorsal discontinuity. A circular window, sometimes traversed by a few fibers, located in this fibrous region (figure 2A, C). A central projection arising under this window bears a large blade-shaped hollow spine, 25 μm long. This spine separates two major groups of thin spines; one comprising several parallel rows of hollow, acicular, very thin spines, the other group located on the opposite side, formed by hollow and slightly thicker spines with broader bases, arranged in several irregular rows.

Discussion. Due to the paucity of available specimens, our description of this species is based almost exclusively on its stylet structure. *G. matilde* n. sp. is most similar to *Gieysztoria therapaina* (Marcus 1946) Luther 1955 (Inaequales, Fenestratae). However, in the latter species the stylet is 33 μm long, almost half the length of the stylet in the new species (54–65 μm). In *G. matilde* the large central spine is about half the length of the stylet, whereas in *G. therapaina* the comparable spine (“b” in Marcus 1946; Luther 1955) is about 2/3 of the length of the stylet. *G. therapaina* has four different types of spines (Marcus 1946), whereas *G. matilde* n. sp. has only three types of spines. *G. matilde* n. sp. also lacks what Marcus (1946) described in *G. therapaina* as “pente pedunculado”, a projection originating at the distal part of the belt from under the window and bearing several smaller spines on its apical portion. These two species also differ in characteristics of the smaller spines; in *G. therapaina*, one of the groups is formed by thin spines arranged in a single row, whereas in *G. matilde* n. sp., the two groups of smaller spines are arranged in several rows.

Marcus (1946) mentioned that *G. therapaina* specimens were 700 μm long, somewhat longer than the specimens studied here. The same author indicated, among other features of *G. therapaina*, the small eyes (vs. large in the new species described here) and the pharynx which is 180 μm long and 120 μm in diameter (vs. much smaller in the new species).

Gieysztoria evelinae (Marcus 1946) Luther 1955

Figures 3, 4, 8A–D

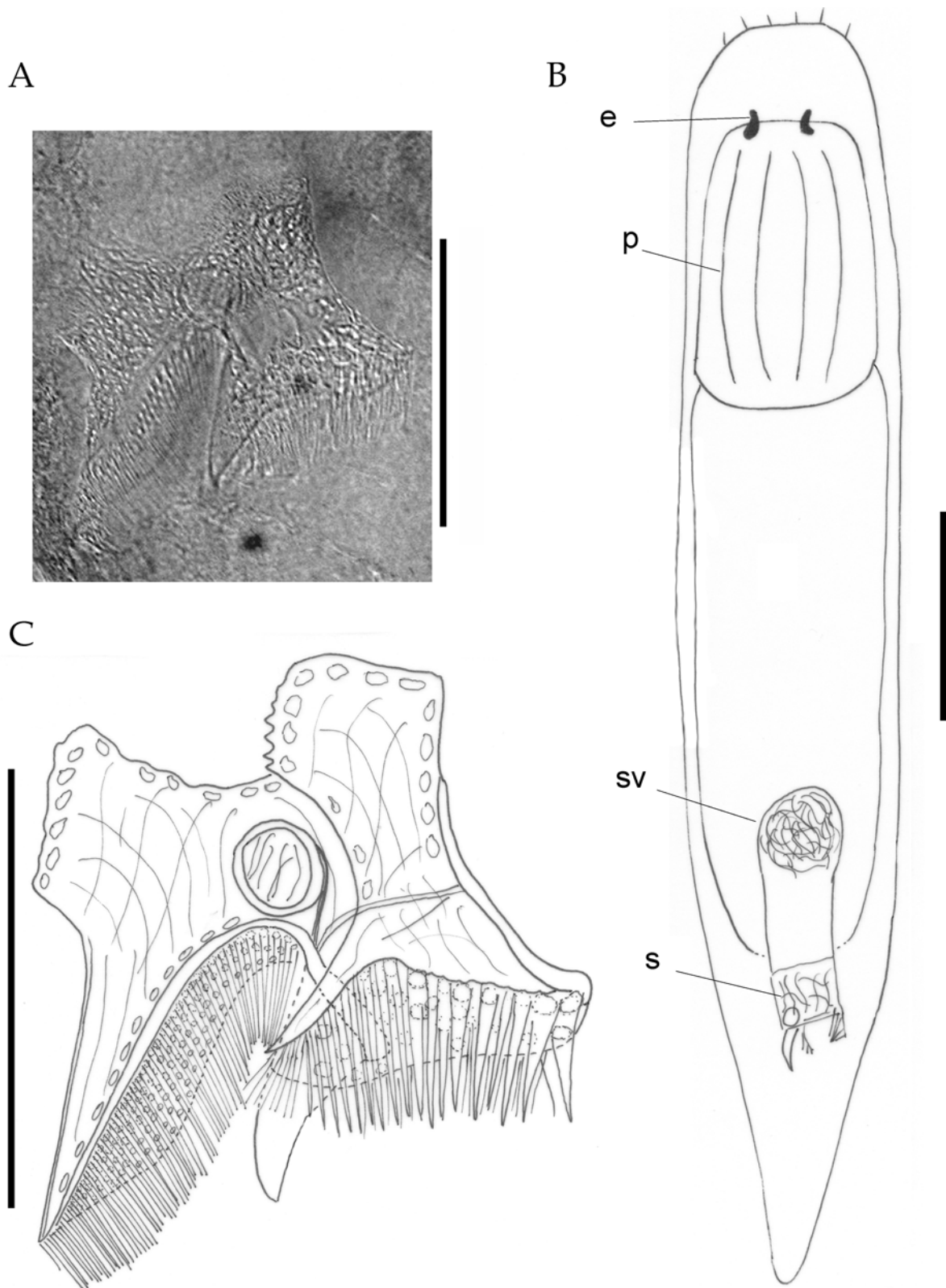


FIGURE 2. *Gieystoria matilde* n. sp. A, photographs of the stylet. B, general view of adult specimen *in vivo*. C, diagram of stylet. Scale A and C: 50 μ m. Scale B: 100 μ m. For abbreviations see Figure 8.

Material and locality. Specimens studied *in vivo* by squash method. Twenty specimens sectioned in the sagittal plane (MLP 5356, 5357, 5358), and one stylet examined under scanning electron microscope, Atalaya (05-03-01, 02-08-01), and La Matilde stream (05-03-03). This is the first record of this species in Argentina.

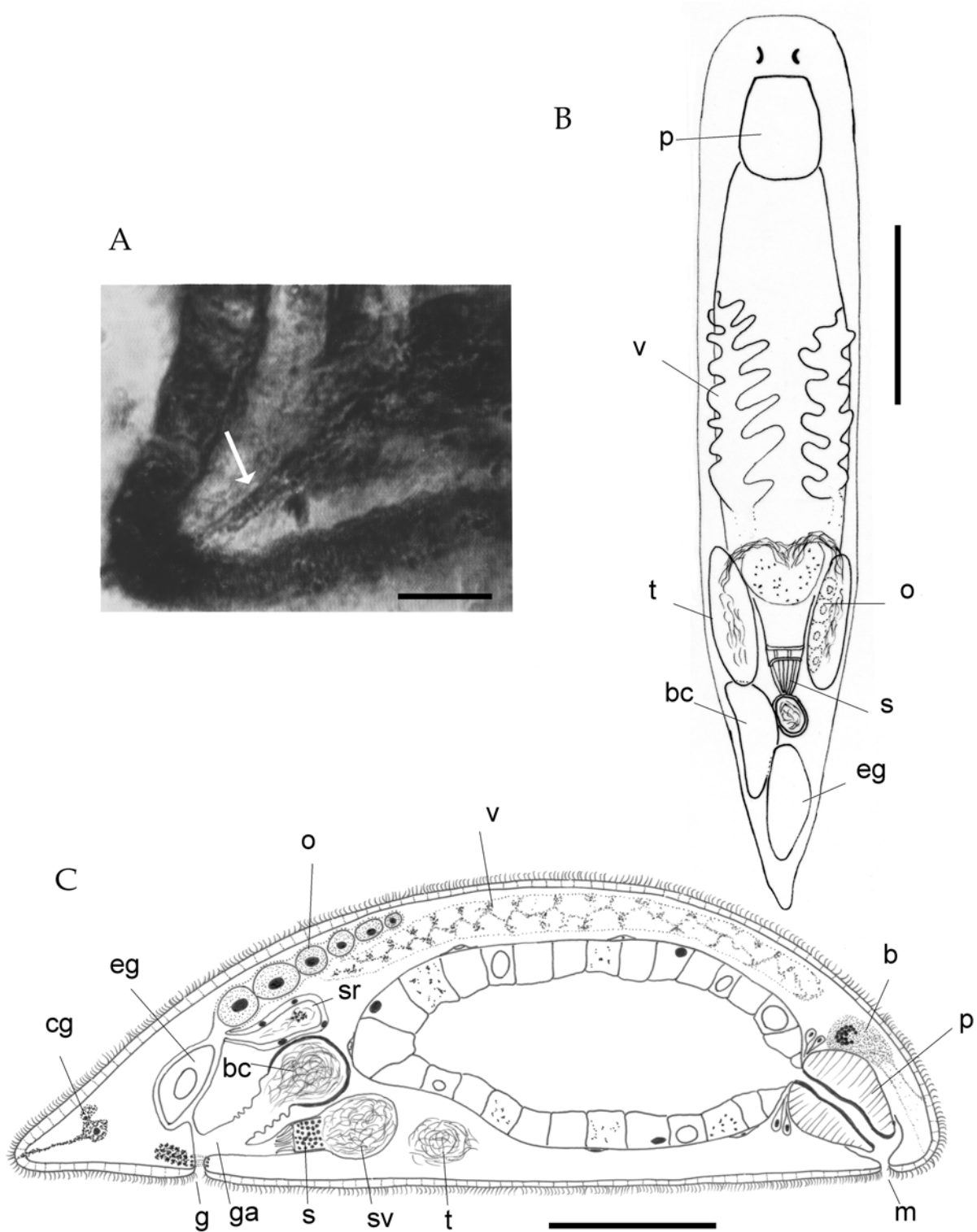


FIGURE 3. *Gięsztorja evelinae*. A, photograph of the caudal region (arrow indicates duct of the caudal glands). B, general ventral view *in vivo*. C, sagittal reconstruction. Scale A: 10 m. Scale B: 200 m. Scale C: 100 m. For abbreviations see Figure 8.

Comments. The morphology of the specimens studied here is similar to the description provided by Marcus (1946) for specimens from Brazil. However, subtle differences between both populations can be noted. The fixed adult specimens studied here are 483–583 μm long and 283–316 μm wide. The coloration pattern of

adult specimens -orange color and accumulations of dark pigments, especially in the pharynx and posterior body- is similar to the pattern described by Marcus (1946).

The long rigid cilia at the anterior and posterior ends of the body, and the posterior adhesive papillae (figure 3A), are similar to those described by Marcus (1946). However, despite the well developed papillae, Marcus (1946) indicated that the specimens did not attach to the substrate by means of them. This comment does not agree with the observations made during the present work, in which specimens were recorded attached to the substrate by their posterior end.

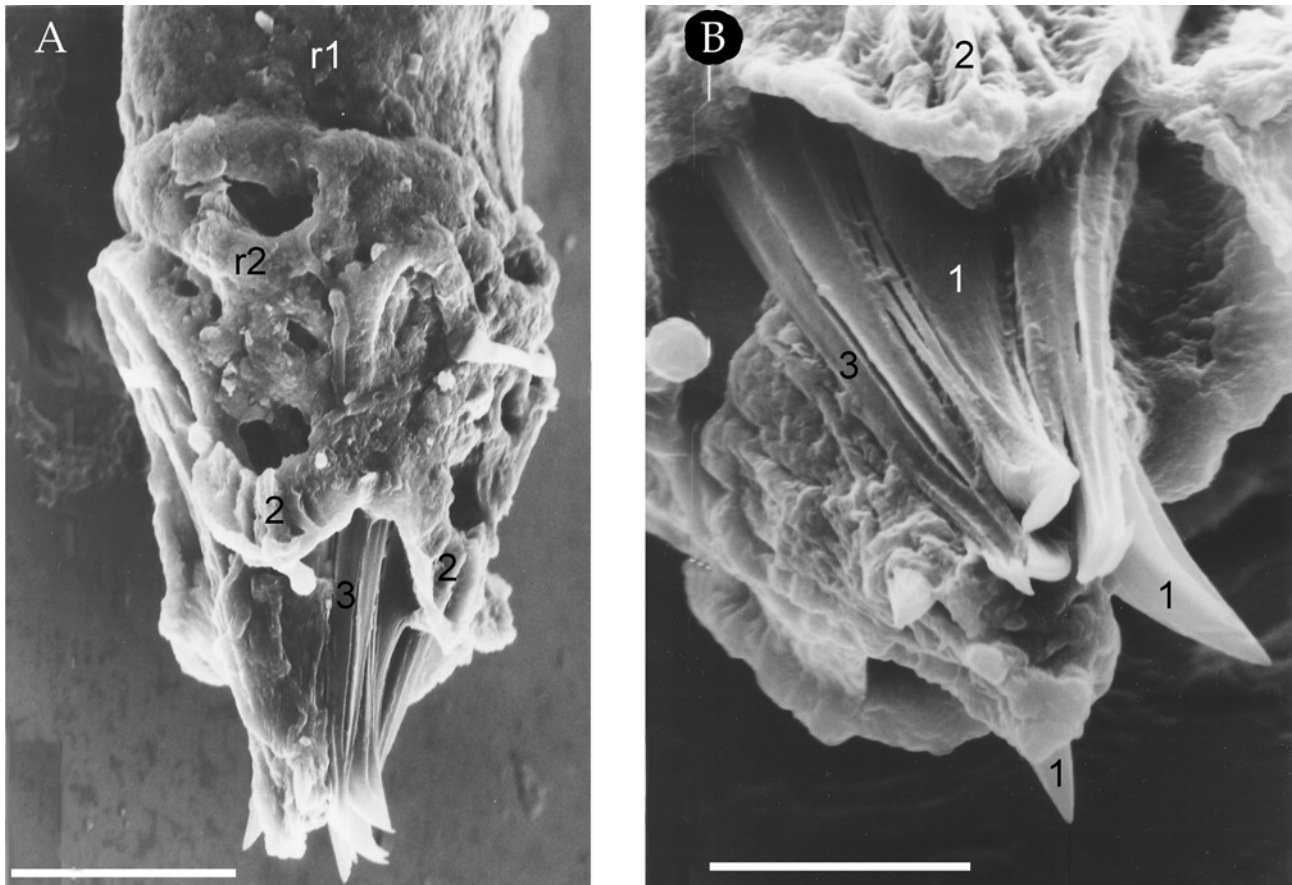


FIGURE 4. *Gieysztoria evelinae*. Microphotograph of the stylet, (SEM). A, general view. B, detail of the area with spines. r1, r2, indicate de rings of the stylet, and numbers 1–3 indicate the type of spines. Scale A: 20 μm . Scale B: 1 μm .

The pharynx of fixed studied specimens was 64 μm long. No pharyngeal papillae were present (figure 3B–C). Marcus (1946) mentioned three types of glands associated with the pharynx: buccal eosinophilic (acidophilic) pharyngeal glands, salivary granulose basophilic glands, and glands with their cellular bodies sunk into the parenchyma at the posterior portion of the pharynx. In the specimens studied here, no such differentiation was possible, and only glands in the pharynx body were observed. On the contrary, large eosinophilic glands, which correspond to “Minot’s gland cells” according to their position and secretory characteristics (Marcus 1946; Meixner 1915), were observed at the transition between pharynx and intestine, while Marcus (1946) found such cells in the deep intestine.

The testes, vasa deferentia, seminal vesicle, prostate vesicle and ejaculatory duct of these specimens are as described by Marcus (1946). The sclerotic stylet is formed by two rings (r1 and r2 in figure 4A) joined by bridges of variable shape. Four strong hollow spines (1 in figure 4B) with broad triangular outward-curved base (2 in figure 4) arise from the distal ring. The bases of these spines are sieve to allow insertion of abductor muscles. These four strong spines are separated by arcs, each of which bears seven to nine longer fine spines (3 in figure 4). The sclerotic apparatus is 90–100 μm in total length, and the spines occupy 50–60 μm . These

features and measurements agree with the description and figures of Marcus (1946). The sclerotic apparatus is surrounded by a sheath connecting with the atrium. Marcus (1946) mentioned a sphincter between the penis sheath and the genital atrium, but such a structure was not observed in our specimens.

The observed morphology of the vitellaria, ovary and seminal receptacle agree with Marcus' description. Marcus (1946) mentioned some vacuoles within the wall of the seminal receptacle, which were not observed in the material studied here. The eggs were ellipsoid and triangular in shape. The operculum described for the Brazilian specimens was absent in our materials.

Previously known distribution. Brazil. São Paulo (Marcus 1946). Corôa lagoon, Canindé district in São Paulo city; in tributaries of the Pinheiros River and in streams of the Cantareira Mountain. The exact locality in São Paulo city where the material was collected has disappeared due to the expansion of the city. It is worth noting that this is the first citation of this species since its original description.

***Gieysztoria hymanae* (Marcus 1946) Luther 1955**

Figures 5, 7A, B

Material and locality. Live specimens studied by squash method. Two specimens sectioned sagittally MLP 5359. Atalaya (2-8-01; 27-1-04). This is the first record of the species in Argentina.

Comments. The specimens studied here are similar to those described by Marcus (1946) from Brazil. However, subtle differences between both populations can be observed. Fixed studied specimens were 250–290 µm long, with the pharynx 85 µm long and 57 µm wide. Within the male genital system, Marcus (1946) mentioned that the vasa deferentia arose from the rostral region of the testes and the seminal vesicle was small, in contrast with the morphology observed in our materials in which the vasa deferentia issue from the medial-posterior region of testes, first extending anteriorly and then merging together before entering a large seminal vesicle at its medial region (figure 5B).

The morphology and size of the stylet agree with Marcus' description (Marcus 1946). The stylet was 60 µm in total length, with a belt with two rings joined by several bridges (figure 5A). It bears four thick spines with three groups of 4–6 thin spines interspersed, and two thin spines on each side between the thick lateral spines and the opening of the belt, all arising from the distal ring in a single row.

In specimens observed *in vivo*, spermatozoids are found in the peripheral region of the testes, while none are present in the central region.

The morphology of ovary, seminal receptacle and vitellaria agree with Marcus' descriptions. According to Marcus (1946), the bursa copulatrix of some specimens has a pseudocuticle formed by the basilar walls of the epithelium, which was not observed in our material. On the other hand, a sinuosity was observed in the peduncle of the bursa, and a pair of lateral projections in the main region of the bursa (figure 5C). These were very evident in our material, but are not mentioned in the original description of this species.

Previously known distribution. Brazil: São Paulo, a stream near Pirajussára River and a marsh in Brooklyn Paulista (Marcus 1946). This last locality where the material was originally collected has disappeared because of the expansion of São Paulo city. It is worth noting that this is the first record of this species since its description.

***Gieysztoria falx* Brusa, Damborenea and Noreña 2003**

Material and locality. Specimens studied *in vivo* by squash method. Atalaya on 2-8-01 and La Matilde stream on 5-3-03. Sagittal sections of one specimen deposited in the Invertebrate Zoology Collection of the Museo de La Plata, MLP 5355.

Comments. The morphology of the specimens studied here is similar to the description of specimens studied by Brusa *et al.* (2003) from Los Talas, Berisso, Buenos Aires, Argentina.

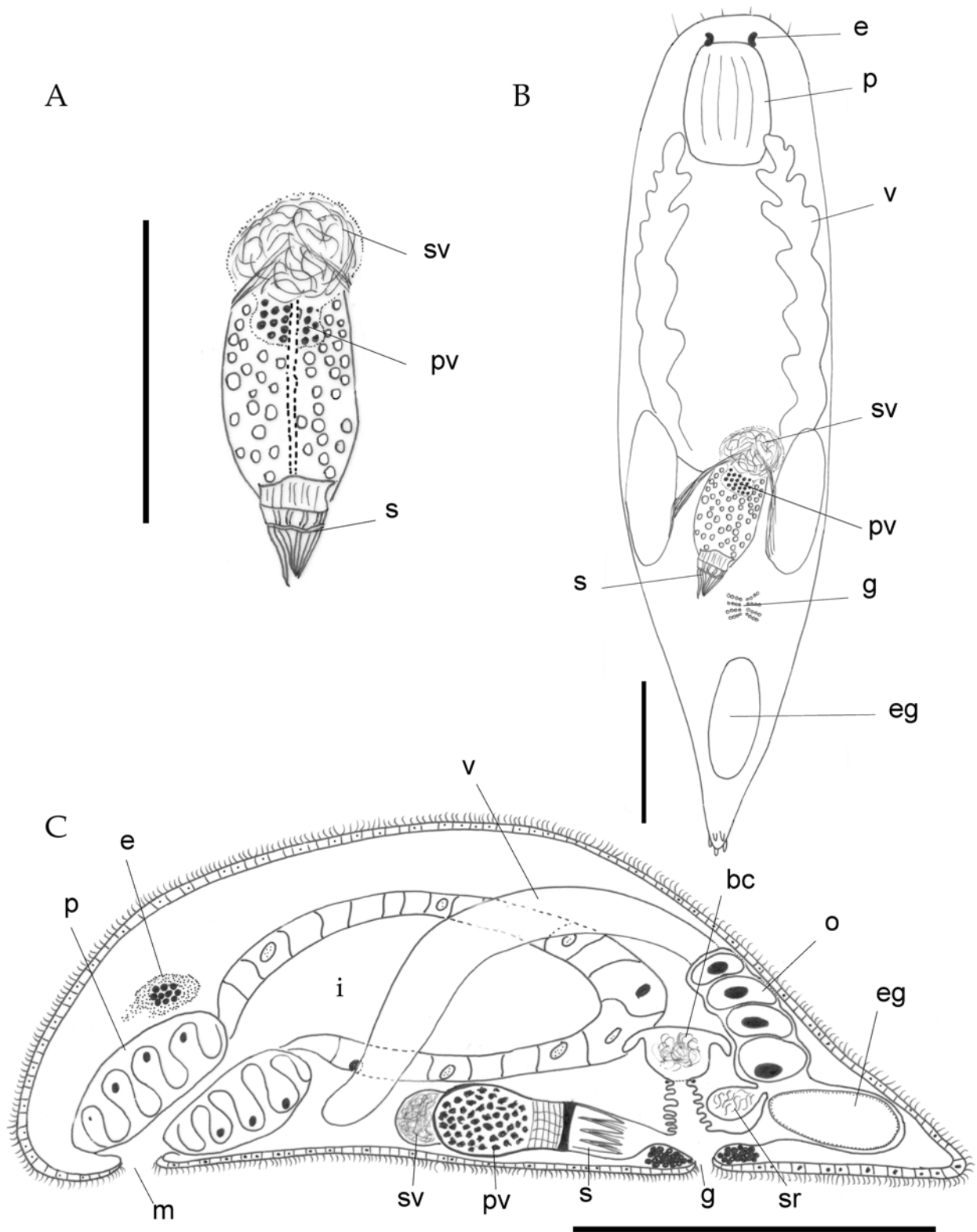


FIGURE 5. *Giaysztoria hymanae*. A, detail of the copulatory apparatus. B, general view *in vivo*. C, sagittal reconstruction. Scale A–C: 100 μ m. For abbreviations see Figure 8.

Family Graffillidae Graff 1908

Subfamily Pseudograffillinae Meixner 1938

Genus *Nyulgus* Marcus 1954

Nyulgus evelinae Marcus 1954

Figures 6, 8E.

Material and locality. Specimens *in vivo* studied by squash method. Twelve specimens sectioned in the sagittal plane, MLP 5365, 5366, Punta Piedras (2-8-00, 12-2-01, 24-8-01; 4-4-02; 5-3-03).

Comments. Live adult specimens were 480 μm long and 95–115 μm wide, and fixed specimens were 200 μm long. Marcus (1954) mentioned that the largest specimens were 500 μm long and 200 μm wide, which represents a slightly larger size compared to the specimens studied here.

The epithelium of the Río de la Plata specimens was 4 μm thick, with a well developed basal membrane and very numerous small rhabdites. The first two features are coincident with Marcus' description (Marcus 1954); however, this author mentioned the absence of rhabdites in the epithelium. The parenchyma of live specimens contains symbiotic algae, greenish brown in color. Marcus (1954) mentioned that the distribution of the symbionts in the parenchyma is variable; however in the studied material the algae were concentrated on the dorsum, from the pharynx to the posterior body. The brain is large, almost the same size as the pharynx. The eyes are located in the anterior region, with large pigment granules arranged around three visual cells (Figure 6C), in agreement with Marcus' description.

The mouth is subterminal, with a sphincter. The buccal tube has ciliated epithelium surrounded by muscles, expanded to form the pharyngeal bursa. The pharynx was 80 μm long and 40 μm wide in live Brazilian specimens (Marcus 1954), while the pharynx of Argentine specimens is 44 μm long. In the same work, this author mentioned that the pharyngeal nuclei are located in the oesophagus, while the nuclei observed in the pharynx correspond to the pharyngeal glands and myoblasts. The pharynx presents a triradiate lumen, continued into the intestine. The latter bears a crown of "Minot's gland cells" at its cephalic end.

The male reproductive system consists of two lateral testes located in posterior half of body (figure 6B); in contrast, Marcus (1954) mentioned that the testes are at pharynx level. One efferent duct issues from the caudal end of each testis, connecting with the seminal vesicle posterior to the pharynx. The ejaculatory duct crosses over the penis papilla, which is wrapped by a thin trumpet-shaped sclerotic stylet. The base of the stylet is formed by two rings; it is 38 μm long and 16 μm wide at the level of these rings, and 7.5 μm wide at its expanded distal end (figure 6A). The stylet illustrated by Marcus (1954) is slightly shorter than the ones studied here (approximately 30 μm long and 12 μm wide proximally, after Marcus 1954, figure 35). The stylet reaches the male region of the atrium rostrally. The atrium opens to the exterior through a mid-ventral gonopore, surrounded by a sphincter.

The morphology of the female reproductive system agrees with the original description (Marcus 1954), comprising a pair of ovaries located near the caudal body, and partially covered by two dorsal vitellaria. The germinative region of the ovaries has rostro-ventral position, and the oocytes become mature towards the caudo-dorsal region. The oocytes are arranged biserially in the germination zone, and uniserially at mature stage. Two short ovovitelloducts open into the female region of atrium. The proximal atrium functions as uterus. Spermatozooids were frequently observed in both uterus and bursa; these structures are separated by a sphincter. The bursa is thin in young specimens and communicates with the intestine; this connection disappears in adults. The bursa functions as *bursa resorbiens* and the uterus acts as seminal receptacle (Marcus 1954). A gland, sometimes lobulate, with eosinophilic secretions, opens on the ventro-caudal wall of the uterus.

Previously known distribution. Brazil: mangrove swamp at the estuary of Itanhaen River (50 km southwest from Santos) (Marcus 1954). This is the first record of this species in Argentina, as well as the first citation after its original description.

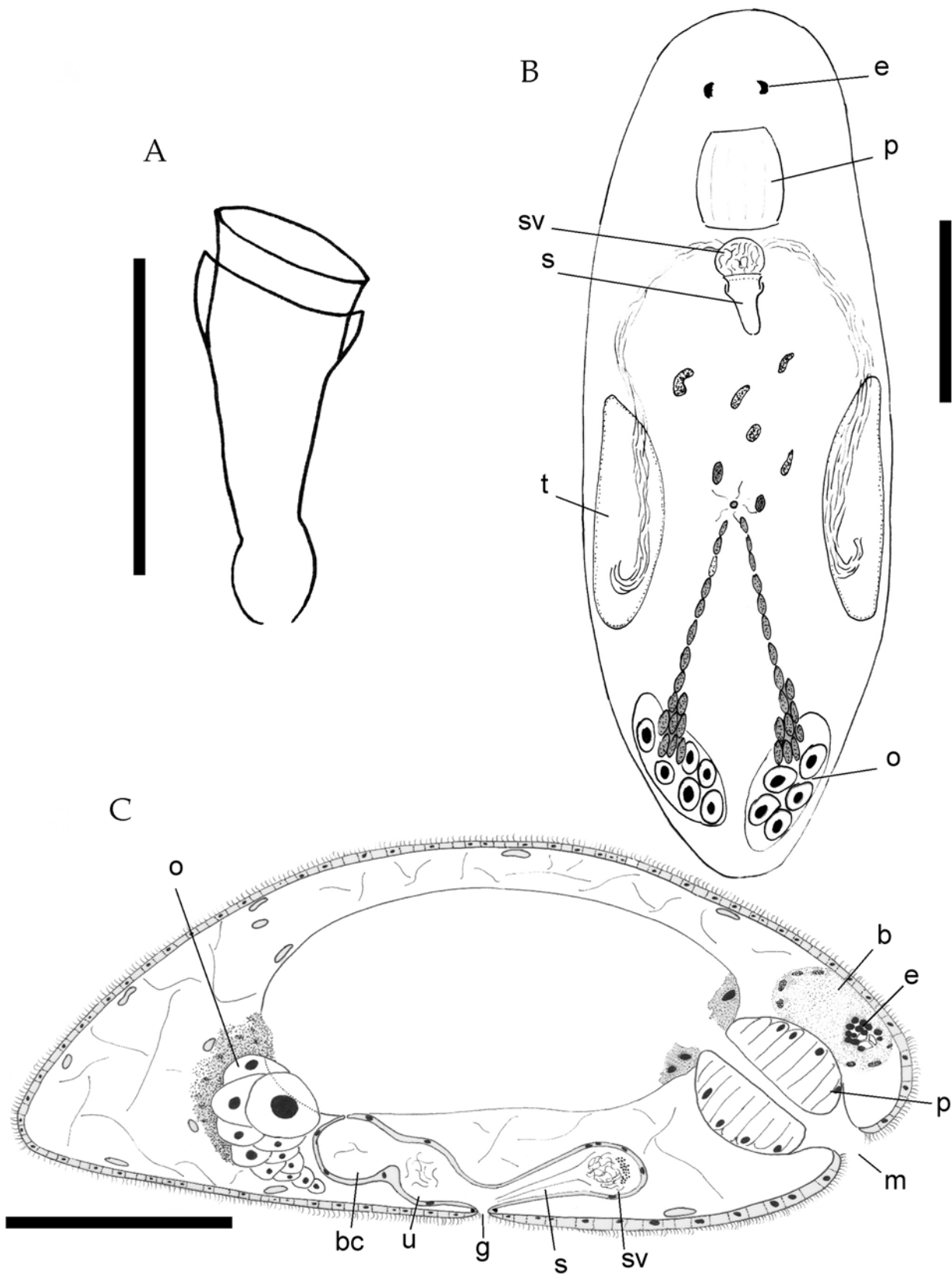


FIGURE 6. *Nygulgus evelinae*. A, stilet. B, general view. C, sagittal reconstruction. Scale A: 30 μ m. Scale B and C: 100 μ m. For abbreviations see Figure 8.

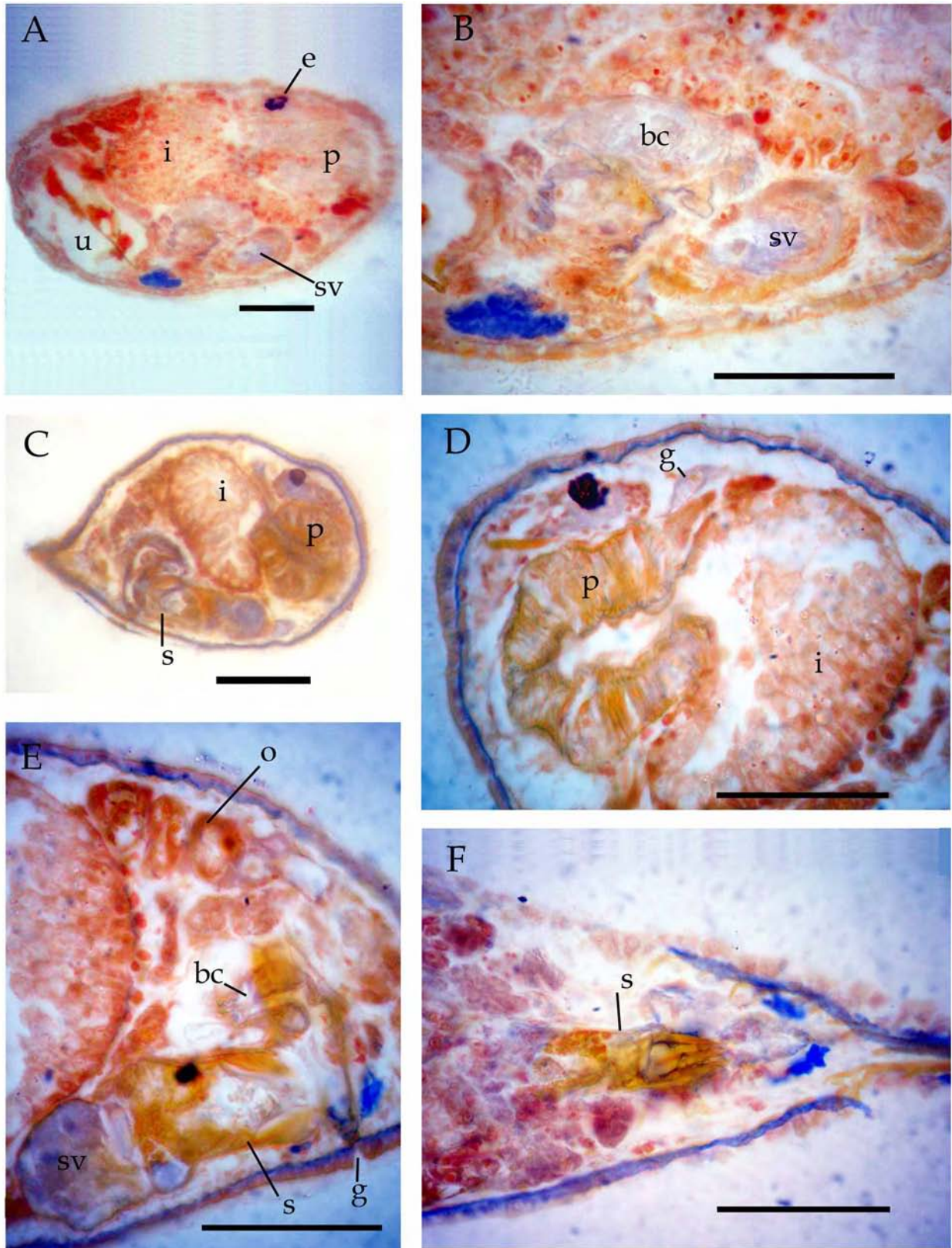


FIGURE 7. Microphotographs of sagittal sections of *Giaysztoria hymanae*. A, general view. B, detail of genital region. Microphotographs of *Giaysztoria atalaya* **sp. n.** Sagittal sections. C, general view. D, detail of digestive system. E, detail of genital region. F: horizontal section of stylet region. Scale A–F: 50 μ m. For abbreviations see Figure 8.

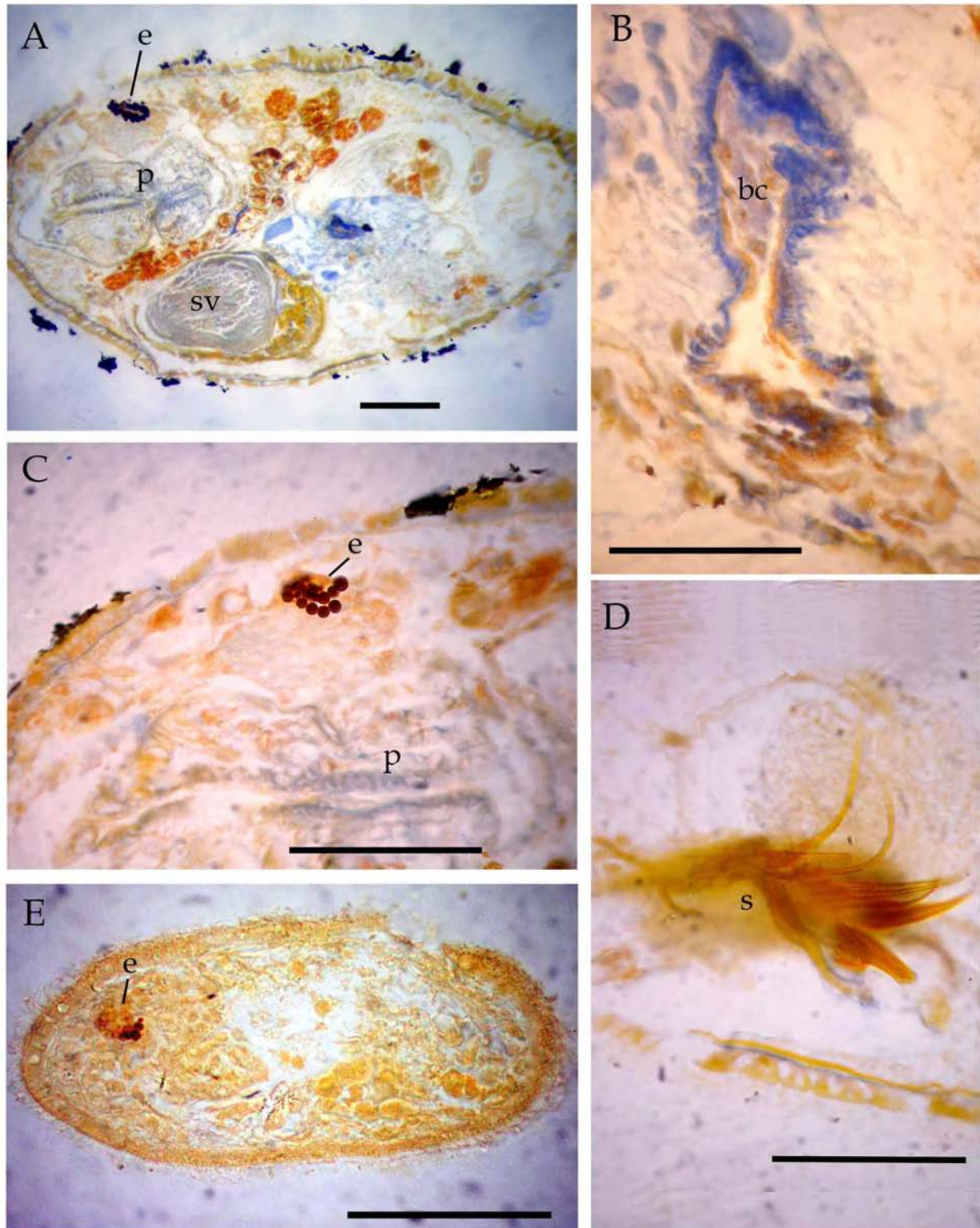


FIGURE 8. Microphotograph of sagittal sections of *Gieysztoria evelinae*. A, general view. B, detail of bursa copulatrix. C, detail of anterior region. D, detail of stylet. Microphotographs of sagittal sections of *Nygulgus evelinae*. E, general view. Scale A–E: 50 μm . b: brain, bc: bursa copulatrix, cg: caudal glands, e: eye, eg: egg, g: gonopore, ga: genital atrium, i: intestine, m: mouth, o: ovary, p: pharynx, pv: prostate vesicle, s: stylet, sr: seminal receptacle, sv: seminal vesicle, t: teste, u: uterus, v: vitellaria.

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