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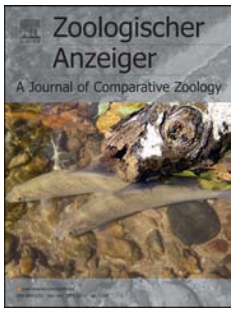

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Ilana Rossi, Jéssica Cardoso Peres, Lisandro Negrete, Ana Leal-Zanchet

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INCREASING THE SPECIES DIVERSITY OF THE GENUS *Paraba* (PLATYHELMINTHES) WITH THE DESCRIPTION OF TWO NEW SPECIES FROM THE SOUTHERN ATLANTIC FOREST

Ilana Rossi[1,2,3], Jéssica Cardoso Peres[2], Lisandro Negrete[4,5] & Ana Leal-Zanchet[1,2]*

[1]Programa de Pós-Graduação em Biologia, Universidade do Vale do Rio dos Sinos – UNISINOS, São Leopoldo, RS 93022-000, Brazil, [2]Instituto de Pesquisas de Planárias, Universidade do Vale do Rio dos Sinos – UNISINOS, São Leopoldo, RS 93022-000, Brazil, [3]Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Sul - Campus Vacaria, Vacaria, RS 95200-000, Brazil and [4]División Zoología Invertebrados, Facultad de Ciencias Naturales y Museo (UNLP), Boulevard 120 & 61, B1900CHX, La Plata, Buenos Aires, Argentina. [5]CONICET – Consejo Nacional de Investigaciones Científicas y Técnicas, La Plata, Buenos Aires, Argentina

* Corresponding author: zanchet@unisinos.br

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Abstract

The genus *Paraba* Carbayo et al., 2013 currently includes 16 species of land flatworms, with a distribution restricted to the Brazilian biomes Atlantic Forest and the Cerrado. However, recent ecological studies and inventories carried out in natural areas of southern Brazil and northeastern Argentina indicate the existence of species of the genus that are not yet described. In this study, we describe two new species of *Paraba* from areas of the southern portion of the Atlantic Forest, located in the Iguassu River Drainage Basin: *Paraba smaragdina* sp. nov., showing a disjunct distribution with records in Argentina and Brazil, and *Paraba iguassuensis* sp. nov. with records in the southern Brazilian state of Parana. Both

species can be distinguished from their congeners, as well from each other, by colour pattern of the body combined with anatomical details of the copulatory apparatus. This contribution increases the species diversity of the genus in southern South America.

Key words: Tricladida, Geoplaninae, land flatworms, taxonomy, Neotropical region

1. Introduction

The Atlantic Forest has been considered a hotspot of land flatworm diversity due to the record of a large number of species of the subfamily Geoplaninae, which has a Neotropical distribution (Sluys, 1999; Baptista et al., 2006; Fick et al., 2006; Álvarez-Prezas et al., 2014). Despite the large number of known species, knowledge about the diversity of Geoplaninae has been constantly expanded, with recent descriptions of new genera and species. The subfamily currently consists of 25 genera, among them *Paraba* (Carbayo et al., 2013; Leal-Zanchet and Marques, 2018; Negrete et al., 2019).

The genus *Paraba* was proposed based on molecular analyses supported by morphological features. All the 16 species of *Paraba* show cylindrical pharynx, male atrium with a protrusible penis papilla, extrabulbar prostatic vesicle, and rounded female atrium lined by an epithelium with multi-layered aspect as main diagnostic characters (Carbayo et al., 2013; Amaral et al., 2019; Silva and Carbayo, 2020).

Species of *Paraba* have been recorded in southern, southeastern and northeastern Brazil and northeastern Argentina (Fig. 1), in areas of the Atlantic Forest and the Cerrado biomes (Carbayo et al., 2013; Negrete and Brusa, 2017; Amaral et al., 2019). Only four of these species occur in the southern Atlantic Forest (Graff, 1899; Froehlich, 1959; Leal-Zanchet and Carbayo, 2001; Baptista and Leal-Zanchet, 2005; Leal-Zanchet and Matos, 2011; Negrete and

Brusa, 2017). Nevertheless, many ecological studies and inventories in this region indicated the existence of undescribed species of *Paraba* (Baptista et al., 2006; Fick et al., 2006; Amaral et al., 2014; Negrete et al., 2014). Two of these studies recorded about 50 new species of land flatworms in Brazilian and Argentine areas, of which at least four belong to *Paraba* (Negrete et al., 2014; Rossi, 2016). In this paper, we describe two of these species, one of them occurring in Brazil and Argentina, thus increasing the species diversity of this genus.

2. Material and Methods

Land planarians were collected during the day by direct sampling in leaf litter and under fallen logs and stones, and during the night, when they are more active, by direct observation of the ground with a head lantern. Sampling was carried out in four areas located in the Iguassu River Drainage Basin, namely three areas in southern Brazil (states of Paraná and Santa Catarina) and a fourth area in northern Argentina (Misiones province) (Fig. 1). *Paraba smaragdina* sp. nov. was collected in two protected areas, as follows: Três Barras National Forest (26°10'S; 50°18'W), in Três Barras, state of Santa Catarina, and Campo Anexo Manuel Belgrano (26°02'S; 53°47'W), in San Antonio, Misiones province. *Paraba iguassuensis* sp. nov. was sampled in two areas of the state of Paraná, viz. a private protected area, the Araucaria Natural Heritage Private Reserve (26°23'S; 51°22'W), in General Carneiro, and an unprotected area that belongs to the Federal University of Paraná (25°25'S; 49°15'W), in Curitiba.

Live specimens were photographed and analysed regarding colour pattern and body shape and dimensions. Afterwards, specimens were euthanised with boiling water and fixed in neutral formalin 10% and subsequently preserved in 70% ethanol. Methods described by Rossi et al. (2015) were used for histological processing of material and analysis of external

and internal characters. The material was sectioned at intervals of 6–8 µm and stained with Masson's trichrome method or haematoxylin and eosin (Romeis, 1989).

The ratio of the height of the cutaneous musculature to the height of the body (mc:h index in C. G. Froehlich 1955) was determined in the median region of a transverse section of the pre-pharyngeal region. Mesenchymal muscle fibres were counted in transverse sections of the same region.

Type-material is deposited in the Museu de Zoologia da Universidade do Vale do Rio dos Sinos, São Leopoldo, state of Rio Grande do Sul, Brazil (MZU), the Helminthological Collection of Museu de Zoologia da Universidade de São Paulo, state of São Paulo, Brazil (MZUSP), and the Invertebrate Collection of Museo de La Plata (MLP), Argentina.

3. Results

Taxonomic part

Family Geoplanidae Stimpson, 1857

Subfamily Geoplaninae Stimpson, 1857

Genus *Paraba* Carbayo et al., 2013

3.1. *Paraba smaragdina* Rossi, Negrete & Leal-Zanchet, sp. nov.

LSID: zoobank.org:act:0413DFBB-06A8-4D02-9CE0-E9DFCC0945A1

Geoplana sp. 7: Negrete, Colpo & Brusa, 2014

Paraba sp. 1: Rossi, 2016

3.1.1. Etymology

The specific epithet derives from the Latin *smaragdus* ("emerald") + *inus* ("pertaining to"), in reference to the emerald-green dorsal colour of the holotype.

3.1.2. Type-material

Holotype: MZUSP PL.2188: J.A.L. Braccini, *coll*. 29. July 2014: Três Barras (Três Barras National Forest), state of Santa Catarina, Brazil – anterior tip: transverse sections on 15 slides; anterior region at the level of the ovaries: sagittal sections on 14 slides; pre-pharyngeal region: transverse sections on 10 slides; pharynx and copulatory apparatus: sagittal sections on 20 slides.

Paratypes: MZU PL.00306: I. Rossi, *coll*. 02. June 2015: Três Barras (Três Barras National Forest), state of Santa Catarina, Brazil – pre-pharyngeal region: transverse sections on 6 slides; pharynx and copulatory apparatus: sagittal sections on 22 slides. **MZU PL.00307:** I. Rossi, *coll.* 02. June 2015: Três Barras (Três Barras National Forest), state of Santa Catarina, Brazil – anterior tip: transverse sections on 13 slides; anterior region at the level of the ovaries: sagittal sections on 25 slides; pre-pharyngeal region: transverse sections on nine slides; pharynx and copulatory apparatus: sagittal sections on 27 slides. **MZU PL.00308:** J. A. L. Braccini, *coll*. 02. June 2015: Três Barras (Três Barras National Forest), state of Santa Catarina, Brazil – anterior tip: transverse sections on 15 slides; anterior region at the level of the ovaries: sagittal sections on 14 slides; pre-pharyngeal region: transverse sections on 10 slides; pharynx and copulatory apparatus: sagittal sections on 20 slides. **MZU PL.00309:** I. Rossi, *coll.* 02. June 2015: Três Barras (Três Barras National Forest), state of Santa Catarina, Brazil – pre-pharyngeal region: transverse sections on 12 slides; copulatory apparatus: horizontal sections on 16 slides. **MLP He-6487:** L. Negrete, *coll.* 05. November 2010: Campo Anexo Manuel Belgrano (CAMB), Misiones province, Argentina – anterior tip and anterior region at the level of the ovaries: sagittal sections on 16 slides; pre-pharyngeal region: transverse sections on 5 slides; pharynx and copulatory apparatus: sagittal sections on 20 slides.

3.1.3. Diagnosis

Species of *Paraba* with dark-green to dark-brown dorsal colour; eyes marginal without clear halos; pharynx cylindrical; prostatic vesicle with two portions: an oval-elongated extrabulbar proximal portion with lumen restricted to a narrow canal with irregularly folded walls and a tubular, slightly sinuous, intrabulbar distal portion; penis papilla conical with some asymmetries, with small folds in their insertions; common glandular ovovitelline duct long; constriction between male and female atria.

3.1.4. Description

3.1.4.1. External features

Body elongated with parallel margins, slightly subcylindrical to oval in cross section; both anterior and posterior tip posterior tip rounded (Fig. 2A–C). Maximum length, when creeping, reached 25 mm and after fixation was 22.5 mm. Mouth and gonopore at posterior third of body in average (Table 1).

Alive, dorsal colour pattern varies from dark-green with slightly reddish anterior tip to dark-brown (Fig. 2A–C). Ventral surface light-gray or light-brown. After fixation, dorsal colour becomes dark-brown, lighter in the first five millimeters of the body and ventral surface becomes lighter.

Eyes monolobate and without clear halos. Diameter of pigment cups 30–45 μm. They are initially uniserial, surrounding the anterior tip. After the second millimetre, they become pluriserial restricted to the body margins (maximum width of about 10% on each side of the body width). Eyes remain marginal over the entire body, becoming less numerous towards posterior tip (Fig. 3).

3.1.4.2. Sensory organs, epidermis and body musculatures

Sensory pits (Fig. 4A–B), as simple invaginations (30–50 μm), contour anterior tip and occur ventromarginally in an irregular, single row in the first 5 mm of the body (approximately 22% of body length, in the holotype). Creeping sole occupies the whole body width in pre-pharyngeal region (Fig. 4C, E).

Three types of glands discharge through whole epidermis of pre-pharyngeal region, without forming a glandular margin: numerous rhabditogen glands with xanthophil secretion, erythrophil glands with fine secretion and scarcer cyanophil glands with fine secretion (Fig. 4C–E). Cyanophil glands are more abundant in ventral epidermis and erythrophil glands in dorsal epidermis (Fig. 4D–E). Glands discharging through the anterior tip of the body are similar to those of the pre-pharyngeal region. (Fig. 4A–B).

Cutaneous musculature with the usual three layers in Geoplaninae (circular, oblique and longitudinal), with longitudinal layer organized in thick discrete bundles (Fig. 4D–E, Table 2). Musculature slightly thicker laterally than medially and becoming progressively thinner towards body margins. Ventral musculature with similar thickness as the dorsal musculature or sometimes thicker than the dorsal musculature at the sagittal plane in the pre-pharyngeal region, being about two times higher than the epidermis (Table 2). In relation to the body height, cutaneous musculature thinner in the pre-pharyngeal region than in the cephalic region, gradually diminishing its thickness towards the anterior tip (Fig. 4A). Mc:h varying between 9% and 13% (Table 2).

Mesenchymal musculature (Fig. 4C–E) poorly developed, mainly composed of three layers: (1) dorsal subcutaneous, located close to the cutaneous longitudinal muscle bundles, with oblique decussate fibres (about 1–3 fibres thick); (2) supra-intestinal transverse (about 2–4 fibres thick); and (3) sub-intestinal transverse (about 3–6 fibres thick). In addition, there are scattered dorsoventral fibres and ventral subcutaneous oblique fibres. The mesenchymal

musculature of the anterior region of the body is slightly less developed than in the pre-pharyngeal (Fig. 4A).

3.1.4.3. Pharynx

Pharynx cylindrical and short, about 6–8% of body length, with dorsal insertion slightly shifted posteriorly, located in the anterior third of the pharyngeal pouch. The pharynx occupies 70–90% of the pharyngeal pouch. Mouth located in the posterior half of the pharyngeal pouch (Fig. 4F). Oesophagus inconspicuous in the holotype and paratype MZU PL.00308 probably due to contraction. Paratypes MZU PL.00307, MZU PL.00306 and MLP He-6487 have a short oesophagus (130–200 μm), corresponding to an oesophagus: pharynx ratio of 8–11%.

Pharynx and pharyngeal lumen lined by ciliated, cuboidal epithelium with insunk nuclei. Pharyngeal glands constituted by four gland types: numerous erythrophil glands with fine granules, cyanophil glands of two types (finely granular and amorphous secretion) and less numerous xanthophil glands with amorphous secretion. Outer pharyngeal musculature (3–6 μm thick) comprised of a subepithelial layer of longitudinal muscles, followed by a circular layer. Inner pharyngeal musculature (30–50 μm thick) composed of a thick subepithelial layer with circular fibres, mixed with longitudinal fibres. Both musculatures become thinner towards pharyngeal tip.

3.1.4.4. Reproductive organs

Testes arranged in two irregular rows on either side of the body, and located beneath the dorsal transverse mesenchymal muscles, between intestinal branches (Fig. 4C–D). They arise about the same transverse level as the ovaries in the anterior fourth of the body and extend to near the root of the pharynx (Table 1). Sperm ducts dorso-medial to ovovitelline

ducts in pre-pharyngeal region (Fig. 4C). They form spermiducal vesicles anteriorly to pharynx. Distally, spermiducal vesicles extend to penis bulb, bend anteriorly, ascend and penetrate the lateral wall of the proximal portion of the prostatic vesicle (Fig. 5A–B). Prostatic vesicle unpaired (Figs. 5A–B, 6A, 7A, 8B), consisting of two portions: an oval-elongated extrabulbar proximal portion with lumen restricted to a narrow canal with irregularly folded walls, and a tubular slightly sinuous intrabulbar distal portion (Figs. 5A–B, 7A, 8B). The proximal portion of the prostatic vesicle shows lateral expansions that are larger in the holotype (Fig. 6A) and paratype MZU PL.00308 and smaller in the other paratypes. In the paratypes MZU PL.00308, MZU PL.00306 and MLP.He-6487, the proximal portion of the prostatic vesicle is globose, showing a wider lumen with less folded walls in the latter (Fig. 8B). Ejaculatory duct almost straight, bending ventrally to open into the ventral surface of the penis papilla in the holotype (Figs. 5A–B, 6A, 7B). In the paratypes the ejaculatory duct is less curved, ending close to or at the tip of the penis papilla (Fig. 8A). Male atrium occupied by a large and conical penis papilla. Penis papilla with some asymmetries showing small folds close to their insertions (Figs. 5A–B, 6A–B, 8A, Table 1). In the holotype the tip of the penis papilla is folded.

Sperm ducts lined with ciliated, cuboidal epithelium and coated with a thin muscularis (about 3 μm thick) constituted of interwoven circular and longitudinal fibres. Prostatic vesicle lined with ciliated, tall columnar epithelium. Muscle coat of prostatic vesicle (50–120 μm thick) comprises longitudinal, circular and oblique intermingled fibres. Numerous glands with erythrophil secretion of two types pierce the prostatic epithelium: one with coarsely granular, heavily stained secretion and the other with finely granular slightly stained secretion. Such glands become scarcer in the distal, tubular portion of the prostatic vesicle. Ejaculatory duct lined with ciliated, columnar epithelium and coated with a thin longitudinal subepithelial

layer and a subjacent circular layer (about 9 µm). This duct receives amorphous, slightly cyanophil secretion.

Penis papilla and male atrium lined with non-ciliated, cuboidal to columnar epithelium (6–20 µm high) with abundant openings of erythrophil glands with finely granular secretion, besides scarcer xanthophil glands with finely granular secretion and cyanophil glands with amorphous secretion. Erythrophil glands scarcer in the male atrium walls. Muscularis of penis papilla and male atrium (8–20 µm thick) constituted of a subepithelial circular layer followed by a subjacent longitudinal layer.

Vitellaria (Fig. 4C–E, G) scattered between intestinal branches, more developed in the holotype and in paratype MZU PL.00308 than in paratypes MZU PL.00307, MZU PL. 00309, MZU PL.00306, and MLP He-6487. Ovaries ovoid (Fig. 4G), with approximately the same width and length, measuring 0.3 mm in the anteroposterior axis. They are partially embedded in the ventral nerve plate, in the anterior fourth of the body (Table 1). Ovovitelline ducts emerge dorsally from the median third of the ovaries (Fig. 4G) and run posteriorly immediately above the nerve plate. Ascending portion of ovovitelline ducts located anterior to the gonopore. Common glandular ovovitelline duct long (500–700 µm), located dorsally to the female atrium (Figs. 5A–B, 6A, 7C, 8C). The female genital duct is a short, dorso-anteriorly curved diverticulum of the female atrium (Figs. 7C, 8C). Female atrium oval-elongate, with lumen restricted to a narrow central canal. Length of female atrium about half that of male atrium (Figs. 5A–B, 6A, 8A, Table 1).

Ovovitelline ducts and common ovovitelline duct lined with ciliated, cuboidal to columnar epithelium and covered with intermingled circular and longitudinal muscle fibres (about 10 µm thick). Numerous shell glands with xanthophil secretion empty into common glandular ovovitelline duct as well as into distal third of the ascending portion of the ovovitelline ducts (Figs. 5A–B, 6A, 7C, 8C). Female genital duct and female atrium lined by

epithelium of stratified appearance (50–100 μm high) with some lacunae, lower in the female duct (15–30 μm high), excepting the most distal part of the female atrium, which is lined by a columnar epithelium. Abundant cyanophil and erythrophil glands, both with finely granular secretion, open into the female duct and atrium. Muscularis of female duct and atrium (about 12–30 μm thick) composed of interwoven longitudinal and circular fibres.

Gonoduct slightly inclined forward at the sagittal plane. A constriction separates male and female atria, leaving a narrow communication between them (Figs. 5A, 6A). Gonoduct lined with ciliated, columnar epithelium, receiving the openings of numerous erythrophil with finely granular secretion and cyanophil glands with amorphous secretion, besides rhabditogen glands with xanthophil secretion. Muscularis of gonoduct comprised of a subepithelial layer of circular fibres, followed by a longitudinal layer. The common muscle coat, which is composed of intermingled circular, longitudinal and oblique fibres, is thin along both male and female atria.

3.1.4.5. Variability

Despite the observed variations in colour pattern in live specimens, they all share a similar general anatomy. Variations observed in the prostatic vesicle may be due to diverse physiological and/or maturation stages among type-specimens. For instance, the holotype and paratype MZU PL.00308 seem to be more mature, since their vitellaria are more developed than in the other paratypes. Other variations in the anatomy observed in the holotype, such as the folded tip of the penis papilla, the marked curvature of the ejaculatory duct and absence of oesophagus are probably caused by the contraction of this specimen.

3.2. *Paraba iguassuensis* Peres, Rossi & Leal-Zanchet, sp. nov.

LSID: zoobank.org:act:FD63CEE2-0C1A-426A-9D35-8DABD9B08B90

3.2.1. Etymology: The specific epithet refers to the to the region where the type-specimens were collected, the Iguassu River Drainage Basin.

3.2.2. Type-material

Holotype: MZUSP PL.2189: I. Rossi, *coll*. 06. June 2015: General Carneiro (Araucaria Natural Heritage Private Reserve), state of Paraná, Brazil – anterior tip: transverse sections on 7 slides; anterior region at the level of the ovaries: sagittal sections on 12 slides; pre-pharyngeal region: transverse sections on 5 slides; pharynx and copulatory apparatus: sagittal sections on 18 slides.

Paratypes: MZU PL.00310: I. Rossi, *coll*. 06. June 2015: General Carneiro (Araucaria Natural Heritage Private Reserve), state of Paraná, Brazil – pre-pharyngeal region: transverse sections on 8 slides; copulatory apparatus: horizontal sections on 31 slides. **MZU PL.00311:** I. Rossi, *coll.* 13. May 2014: Curitiba (Campus of the Federal University of Parana), state of Paraná, Brazil – anterior region: transverse sections on 26 slides; pharynx and copulatory apparatus: sagittal sections on 18 slides.

3.2.3. Diagnosis: Species of *Paraba* with dark-brown dorsal colour and light-yellow median stripe; eyes marginal without clear halos; pharynx cylindrical; prostatic vesicle with a globose extrabulbar proximal portion, with folded walls and a relatively broad lumen that becomes narrower laterally, and a short tubular intrabulbar distal portion; penis papilla cylindrical; common glandular ovovitelline duct long; ventral fold in the distal region of the female atrium.

3.2.4. Description

3.2.4.1. External features

Body elongated with parallel margins; both anterior and posterior tip rounded (Fig. 9A–B). When creeping, maximum length reached 28 mm. After fixation, the maximum length was 17 mm. Mouth and gonopore at posterior third of body in average (Table 3).

Alive, dark-brown dorsum with light-yellow median stripe with defined boundaries, and greyish margins (Fig. 9A–B). Paratype MZU PL.00311 presents black dorsum with a thin light-green median stripe, besides reddish colour in the anterior tip. Light brown to dark-grey ventral surface (Fig. 9B). After fixation, light-yellow median stripe becomes whitish and occupies the maximum width of 0.3 mm (13% of the body width), becoming slightly narrower toward anterior tip and wider over the pharynx and the copulatory apparatus (Figs. 9A, 10).

Eyes monolobate, without clear halos. Diameter of pigment cups 12–40 µm. They surround the anterior tip and maintain their uniserial pattern and position at the body margins over the entire body length, becoming less numerous towards posterior tip (Fig. 10).

3.2.4.2. Sensory organs, epidermis and body musculatures

Sensory pits (Fig. 11A–B), as simple invaginations (25–70 µm), contour anterior tip and occur ventromarginally in two irregular rows in in the first 3 mm of the body (approximately 17% of body length, in holotype). Creeping sole occupies the whole body width in pre-pharyngeal region (Fig. 11C, E).

Four types of glands discharge through whole epidermis of the pre-pharyngeal region, without forming a glandular margin: numerous rhabditogen glands with xanthophil secretion, cyanophil glands with amorphous secretion besides xanthophil and erythrophil glands with finely granular secretion (Fig. 11C–E). Openings of cyanophil glands are more abundant

through the ventral epidermis. Glands discharging through the anterior tip of the body are similar to those of the pre-pharyngeal region (Fig. 11A).

Cutaneous musculature with the usual three layers observed in Geoplaninae (circular, oblique and longitudinal), with longitudinal layer organized in thick discrete bundles (Fig. 11C–E, Table 4). Musculature slightly thicker laterally than medially becoming progressively thinner towards body margins. Ventral musculature slightly thicker than the dorsal musculature at the sagittal plane in the pre-pharyngeal region, being about three times thicker than the epidermis (Table 4). In relation to the body height, cutaneous musculature thinner in the pre-pharyngeal region than in the cephalic region, gradually diminishing its thickness towards anterior tip (Fig. 11A). Mc:h varying between 13% and 16% (Table 4).

Mesenchymal musculature (Fig. 11C–E) poorly developed, mainly composed of three layers: (1) dorsal subcutaneous, located close to the cutaneous musculature, with oblique, decussate fibres (about 4–5 fibres thick); (2) supra-intestinal transverse (about 5–6 fibres thick); (3) sub-intestinal transverse (about 3–5 fibres thick). In addition, there are numerous dorsoventral fibres and scattered ventral subcutaneous oblique fibres. The mesenchymal musculature is slightly less developed in the anterior region of the body than in the pre-pharyngeal (Fig. 11A).

3.2.4.3. Pharynx

Pharynx cylindrical, about 11% of body length, with dorsal insertion shifted posteriorly. The pharynx occupies almost the entire pharyngeal pouch. Mouth located in the posterior third of pharyngeal pouch (Fig. 11F). Oesophagus short (50–60 µm), with folded walls. Oesophagus: pharynx ratio 3–5%.

Pharynx and pharyngeal lumen lined by ciliated, cuboidal epithelium with insunk nuclei. Pharyngeal glands constituted by three gland types: numerous xanthophil and

erythrophil glands, both with coarsely granular secretion, beside cyanophil glands with amorphous secretion. Outer pharyngeal musculature (3–10 μm thick) comprised of a subepithelial layer of circular muscles, followed by a longitudinal layer with intermingled circular fibres. Inner pharyngeal musculature (20–40 μm thick) composed of a thick subepithelial layer with circular fibres, followed by a longitudinal layer with intermingled circular fibres. Both musculatures become thinner towards pharyngeal tip.

3.2.4.4. Reproductive organs

Testes arranged in two irregular rows on either side of the body, located beneath the dorsal transverse mesenchymal muscles, between intestinal branches (Fig. 11C–D). They arise slightly posteriorly to the ovaries, in the anterior fourth of the body, and extend to near the root of the pharynx (Table 3). Sperm ducts medial to ovovitelline ducts in the pre-pharyngeal region (Fig. 11C, E). They form spermiducal vesicles laterally to pharynx. Distally, spermiducal vesicles extend towards the penis bulb and bend anteriorly to penetrate in the lateral wall of the proximal portion of the prostatic vesicle (Figs. 12A–B, 13B). Prostatic vesicle unpaired (Figs. 12A–B, 13A–B, 14A) and consisting of two portions: a globose extrabulbar proximal portion and a short tubular, intrabulbar distal portion. The extrabulbar portion shows folded walls and a relatively broad lumen (Fig. 14A) that becomes narrower laterally (Fig. 13A). Ejaculatory duct almost straight, opening at the tip of penis papilla (Fig. 12A–B). Male atrium with slightly folded walls and occupied by a cylindrical penis papilla with some asymmetries, such as dorsal side thicker than ventral side (Figs. 12A–B, 13A–B, Table 3).

Sperm ducts lined with ciliated, cuboidal epithelium and coated with a thin muscularis (about 4 μm thick) constituted of interwoven circular and longitudinal fibres. Prostatic vesicle lined with ciliated, tall columnar epithelium, with abundant openings of erythrophil glands

with coarsely granular secretion and sparse openings of cyanophil glands with amorphous secretion as well as of xanthophil glands with finely granular secretion. Muscularis of prostatic vesicle (100–190 μm thick) comprises longitudinal, circular and oblique intermingled fibres. Ejaculatory duct lined with ciliated, cuboidal epithelium, receiving cyanophil glands with amorphous secretion. Muscle coat of ejaculatory duct (about 5 μm) comprises longitudinal and circular intermingled fibres.

Penis papilla and male atrium lined with non-ciliated, cuboidal to columnar epithelium with abundant openings of erythrophil and xanthophil glands with finely granular secretion, besides cyanophil glands with amorphous secretion. Openings of the erythrophil glands are more numerous close to the ventral insertion of the penis papilla, as well as in the ventral and lateral walls of the male atrium. Muscularis of penis papilla and atrium (6–20 μm thick) constituted of a thick subepithelial layer with circular fibres followed by a subjacent longitudinal layer.

Vitellaria (Fig. 11C–E, G) not fully developed in the holotype, and poorly developed in paratypes MZU PL.00310 and MZU PL.00311, situated between intestinal branches. Ovaries ovoid or oval-elongate (Fig. 11G), with approximately ½ times longer than wide, measuring 0.3 mm in the anteroposterior axis. They are located dorsal to the ventral nerve plate, in the anterior fourth of the body (Table 3). Ovovitelline ducts emerge dorsally from the median third of the ovaries (Fig. 11G) and run posteriorly immediately above the nerve plate. Ascending portion of ovovitelline ducts located anterior to the gonopore. Common glandular ovovitelline duct long (300–500 μm), located dorsally to female atrium (Figs. 12A–B, 14B). The female genital duct is a short diverticulum dorso-anteriorly curved of the female atrium. Female atrium oval-elongate, with lumen restricted to a narrow central canal. Folds protrude from the dorsal and ventral walls of the male and female atria, separating them. Length of female atrium about two-thirds that of male atrium (Figs. 12A–B, 13A–B, 14B, Table 3).

Ovovitelline ducts and common ovovitelline duct lined with ciliated, cuboidal epithelium and covered with intermingled circular and longitudinal muscle fibres (about 7 μm). Shell glands with xanthophil secretion empty into the common glandular ovovitelline duct as well as into the distal third of the ascending portion of the ovovitelline ducts (Figs. 12A–B, 14B). Female genital duct and atrium lined by tall epithelium of stratified appearance (100–250 μm high), with abundant openings of cyanophil with amorphous secretion and weakly stained, erythrophil glands with finely granular secretion. Abundant cyanophil secretion occupies the lumen of the female genital duct, the proximal portion of the female atrium and the distal portion of the common ovovitelline duct. The folds that separate the female and male atria receives abundant openings from heavily stained cyanophil glands with amorphous secretion and xanthophil glands with finely granular secretion. Muscularis of female duct and atrium (7–12 μm thick) composed of interwoven longitudinal and circular fibres.

Gonoduct slightly inclined forward at the sagittal plane, lined with ciliated, columnar epithelium, receiving the openings of numerous rhabditogen glands with xanthophil secretion, besides scarce xanthophil and erythrophil glands with finely granular secretion, and cyanophil glands with amorphous secretion. Muscularis of gonoduct comprised of a subepithelial layer of circular fibres, followed by a longitudinal layer. Common muscle coat thin, with circular, longitudinal and oblique intermingled fibres.

3.3. Notes on ecology and distribution

Paraba smaragdina sp. nov. occurred in its type locality, Três Barras National Forest (Brazil), in areas of *Araucaria* moist forest characterized by the dominance of *Bromelia antiachantha* Bentol. in the understory (Rossi and Leal-Zanchet, 2017), showing moderate abundance (n=8) during both day and night samplings (Rossi, 2016). In CAMB (Argentina), the only specimen was found in an area of *Araucaria* moist forest, in the vicinity of

plantations of exotic species (e.g. *Pinus* spp. and *Eucalyptus* sp.). The two localities are located approximately 400 km apart (Fig. 1), without relevant geographical barriers among them.

The type locality of *Paraba iguassuensis* sp. nov. is the Araucaria Natural Heritage Private Reserve, in a site of Araucaria moist forest showing an initial stage of regeneration with poorly developed understory (Rossi and Leal-Zanchet, 2017). The species was also collected in remnants of *Araucaria* moist forest in the Federal University of Paraná, showing low abundance in both localities (Rossi, 2016).

4. Discussion

The two new species described herein can easily be assigned to the genus *Paraba* by presenting its diagnostic features, such as a protrusible and conical penis papilla and the female atrium lined with epithelium of multilayered aspect (Carbayo et al., 2013).

In the following comparative discussion, a combination of external features and anatomical characteristics of the reproductive system is used to distinguish species. Both new species described herein show marginal eyes and an unforked prostatic vesicle. Thus, they share superficial similarities with eight other species of *Paraba*: *P. bresslaui* (Schirch, 1929), *P. cassula* (Froehlich, 1955), *P. goettei* (Schirch, 1929), *P. incognita* (Riester, 1938), *P. phocaica* (Marcus 1951), *P. piriana* (Almeida & Carbayo, 2012), *P. preta* (Riester, 1938) and *P. suva* (Froehlich, 1959), to which they are compared below.

Regarding the external morphology, *Paraba smaragdina* sp. nov., by having a homogeneous dorsal coloration, can be differentiated from seven of these species, namely *P. bresslaui*, *P. cassula*, *P. goettei*, *P. incognita*, *P. phocaica*, *P. piriana*, and *P. suva* (Schirch, 1929; Riester, 1938; Marcus, 1951; Froehlich, 1955; Froehlich, 1959; Almeida et al., 2012; Silva and Carbayo, 2020), besides *P. iguassuensis* sp. nov., which show a striped dorsal

pattern. *Paraba smaragdina* sp. nov. can also be differentiated from *P. preta,* which has a homogeneous dorsal colour pattern in life, with a dorsal, narrower stripe becoming visible after fixation (Riester, 1938).

Paraba iguassuensis sp. nov., which shows a single median stripe along the dorsal surface, differs in colour pattern from *P. suva* and *P. phocaica* that have irregular stripes or dots over the dorsal surface, and *P. preta* that has a homogeneous dorsal coloration (Riester, 1938; Froehlich, 1959; Marcus, 1951). It can also be distinguished from *P. cassula* and *P. piriana* which have a dorsal colour pattern with transverse stripes (Froehlich, 1955; Almeida et al., 2012). The dorsal pattern of *P. iguassuensis* sp. nov. is similar to that of *P. bresslaui*, *P. goettei* and *P. incognita* (Schirch, 1929; Riester, 1938). However, *P. incognita* has pluriserial eyes, different from *P. iguassuensis* sp. nov., which shows uniserial eyes along the body length.

Regarding the copulatory apparatus, by presenting a large and globose or oval-elongated extrabulbar portion of the prostatic vesicle, the two new species differ from *P. bresslaui*, *P. incognita, P. phocaica, P. piriana* and *P. cassula*. In contrast, the prostatic vesicle of *P. incognita* has a shorter and rounded extrabulbar portion, whereas *P. phocaica, P. piriana* and *P. cassula* show a tubular or more elongated extrabulbar portion (Riester, 1938; Marcus, 1951; Froehlich, 1955; Almeida et al., 2012). In addition, the prostatic vesicle is vertically oriented in *P. bresslaui, P. phocaica* and *P. piriana* (Marcus, 1951; Almeida et al., 2012; Silva and Carbayo, 2020), contrasting with the horizontal position of the prostatic vesicle in *P. smaragdina* sp. nov. and *P. iguassuensis* sp. nov.

Paraba smaragdina sp. nov. and *P. iguassuensis* sp. nov. show similarities regarding the shape of the prostatic vesicle with *P. goettei, P. suva* and *P. preta*. However, in *P. smaragdina* sp. nov. and *P. iguassuensis* sp. nov. the proximal portion of the prostatic vesicle shows a single lumen, without subdivisions, whereas in *P. goettei, P. suva* and *P. preta* it is

formed by two globular expansions separated by a constriction (Riester, 1938; Froehlich, 1955; Froehlich, 1959). Additionally, these two globular expansions, in *P. goettei* and *P. suva*, differ histologically through glandular secretions (Froehlich, 1955; Froehlich 1959), which do not occur in *P. smaragdina* sp. nov. and *P. iguassuensis* sp. nov.

Besides differences related to the prostatic vesicle, four species included in the comparative discussion stand apart from *P. smaragdina* sp. nov. and *P. iguassuensis* sp. nov. by a combination of other characteristics of the copulatory apparatus. *Paraba incognita* shows a more compact copulatory apparatus and an oblique penis papilla (Riester, 1938). *Paraba suva* and *P. phocaica* have a more elongated male atrium and a relatively shorter penis papilla (Marcus, 1951; Froehlich, 1959). *Paraba goettei* shows the common ovovitelline duct opening into the distal portion of the female atrium (Froehlich, 1955).

The copulatory apparatus of the two new species differ regarding the shape of the prostatic vesicle, the opening of the ejaculatory duct and the folds in the male and female atria. The prostatic vesicle in *P. smaragdina* sp. nov. presents an oval-elongated proximal portion, with lateral expansions, whereas *P. iguassuensis* sp. nov. has a globose proximal portion, whose lumen narrows laterally. The ejaculatory duct in *P. iguassuensis* sp. nov. opens at the tip of the penis papilla, differing from that of *P. smaragdina* sp. nov., which is ventrally curved. In addition, the atria are separated by a constriction in *P. smaragdina* sp. nov., whereas in *P. iguassuensis* sp. nov. the atria are separated by dorsal and ventral folds of the female and male atria.

The distribution of the genus *Paraba* mainly includes areas of southeastern and southern Brazil, besides recent records from northeastern Argentina and northeastern Brazil (Carbayo et al., 2013; Negrete and Brusa, 2017; Amaral et al., 2019). Most species are known only from their type-localities, but *Paraba multicolor*, the type-species, shows a broad distribution with records in northeastern Argentina and southeastern and southern Brazil,

occurring mainly in man-disturbed areas (Graff, 1899; Leal-Zanchet and Matos, 2011; Negrete and Brusa, 2017). In contrast, *P. smaragdina* sp. nov. presents a disjunct distribution with records in forest remnants from south Brazil and northeastern Argentina. Despite the considerable distance among the two sites where *P. smaragdina* sp. nov. was recorded, the conspecificity of the specimens from southern Brazil and northeastern Argentina is supported by characters regarding the eye and colour pattern, pharyngeal anatomy, as well as anatomical and histological details of the reproductive organs.

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Figure 1. Distribution range of the genus *Paraba* in areas of Atlantic Forest and Cerrado biomes in Brazil and northeastern Argentina.

Figure 2. *Paraba smaragdina* sp. nov., photograph of type-specimens in dorsal view: (A) Holotype; (B) Paratype MZU PL.00309; (C) Paratype MLP He-6487. Anterior tip to the left.

Figure 3. *Paraba smaragdina* sp. nov., holotype: eye pattern. Anterior tip to the left. (e) eyes.

Figure 4. *Paraba smaragdina* sp. nov., holotype (A–E, G) and paratype MZU PL.00307 (F), photomicrographs of transverse (A–E) and sagittal sections (F–G; anterior tip to the left): (A) anterior region of body; (B) detail of anterior region of body; (C) pre-pharyngeal region; (D) detail of dorsal surface of pre-pharyngeal region; (E) detail of ventral surface of pre-pharyngeal region; (F) pharynx; (G) ovary. (cg) cyanophil glands; (cs) creeping sole; (de) dorsal epidermis; (di) dorsal insertion of pharynx; (dm) dorsal cutaneous musculature; (dsm) dorsal subcutaneous mesenchymatic musculature; (e) eyes; (eg) erythrophil glands; (i) intestine; (im) internal musculature; (lu) pharyngeal lumen; (m) mouth; (n) nerveplate; (o) ovary; (oe) oesophagus; (om) outer musculature; (ov) ovovitelline ducts; (pp) pharyngeal pouch; (rg) rhabditogen glands; (sbm) sub-intestinal transverse mesenchymatic musculature; (sd) sperm ducts; (sp) sensory pit; (spm) supra-intestinal transverse mesenchymatic musculature; (t) testes; (v) vitelline folicles; (vi) ventral insertion of pharynx; (vm) ventral cutaneous musculature.

Figure 5. *Paraba smaragdina* sp. nov., (A) sagittal composite reconstruction of copulatory apparatus of the holotype; (B) horizontal composite reconstruction of copulatory apparatus of paratype MZU PL.00309. Anterior tip to the left. (cmc) common muscle coat; (cov) common glandular ovovitelline duct; (eg) erythrophil glands; (ej) ejaculatory duct; (f) fold; (fa) female atrium; (fc) female canal; (go) gonopore; (ma) male atrium; (ov) ovovitelline ducts; (p) penis papilla; (pv) prostatic vesicle; (sg) shell glands; (sv) spermiducal vesicle.

Figure 6. *Paraba smaragdina* sp. nov., photomicrographs of the copulatory apparatus: (A) holotype in sagittal section; (B) paratype MZU PL.00309 in horizontal section. Anterior tip to the left. (cmc) common muscle coat; (cov) common glandular ovovitelline duct; (ej)ejaculatory duct; (fa) female atrium; (fc) female canal; (go) gonopore; (ma) male atrium; (ov) ovovitelline ducts; (p) penis papilla; (pv) prostatic vesicle; (sg) shell glands; (sv) spermiducal vesicle. The prostatic vesicle in (A) was parasagittally sectioned, showing a lateral expansion (arrow).

Figure 7. *Paraba smaragdina* sp. nov., photomicrographs of the copulatory apparatus in horizontal sections (A) and sagittal sections (B–C): (A) prostatic vesicle of paratype MZU PL.00309; (B) male atrium of the holotype; (C) female duct and common glandular ovovitelline duct of the holotype. Anterior tip to the left. (cov) common glandular ovovitelline duct; (ej) ejaculatory duct; (fa) female atrium; (fc) female canal; (ma) male atrium; (pv) prostatic vesicle; (sg) shell glands.

Figure 8. *Paraba smaragdina* sp. nov., photomicrographs of the copulatory apparatus in sagittal sections of paratype MLP He-6487: (A) detail at the level of gonopore, (B) prostatic vesicle, (C) female duct and common glandular ovovitelline duct. Anterior tip to the left. (cmc) common muscle coat; (cov) common glandular ovovitelline duct; (ej) ejaculatory duct; (f) fold; (fa) female atrium; (fc) female canal; (go) gonopore; (i) intestine; (ma) male atrium; (ov) ovovitelline ducts; (p) penis papilla; (pp) pharyngeal pouch; (pv) prostatic vesicle; (sg) shell glands; (sv) spermiducal vesicle.

Figure 9. *Paraba iguassuensis* sp. nov.: (A–B) photograph of the holotype in dorsal view. Anterior tip to the left.

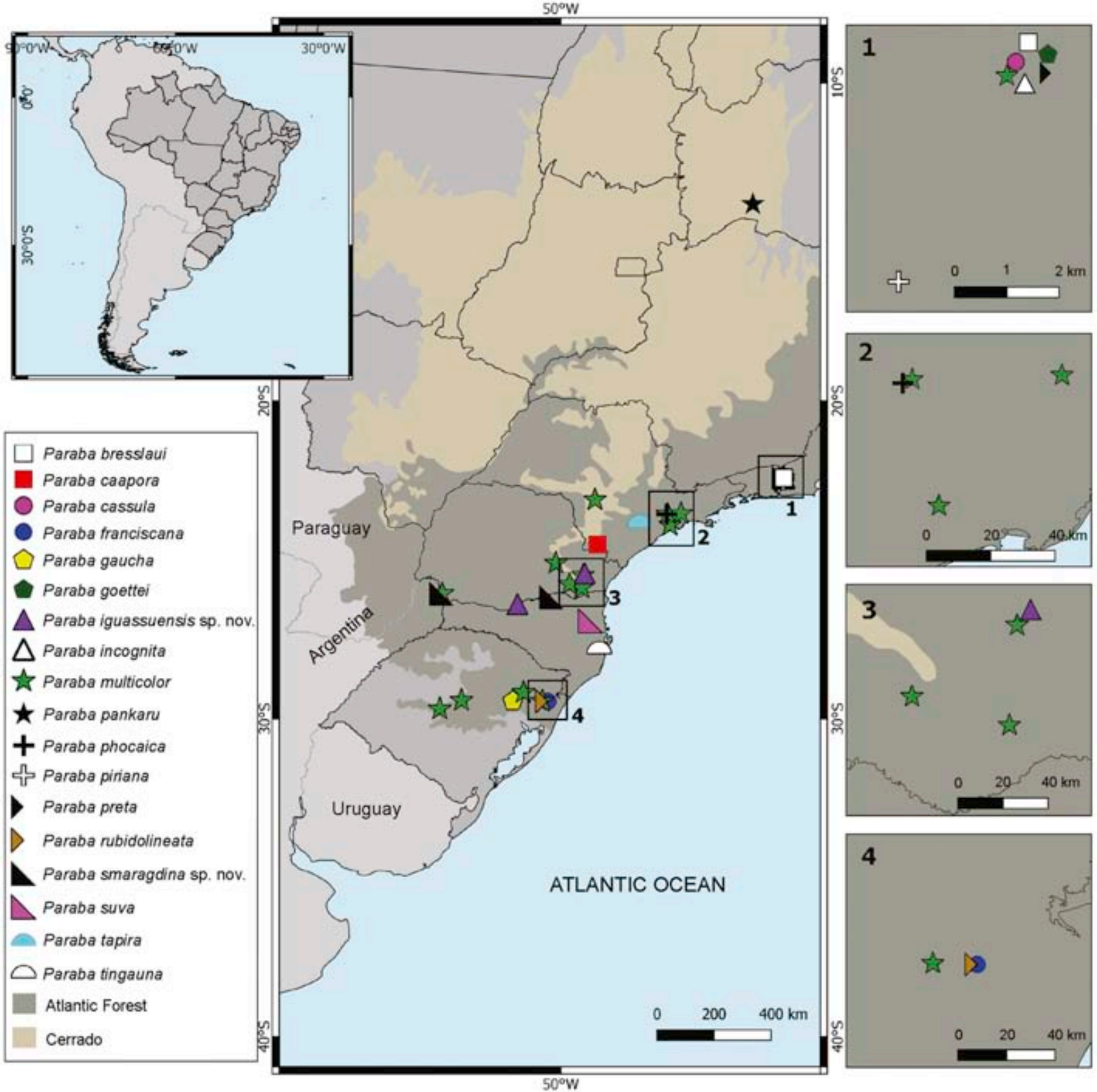
Figure 10. *Paraba iguassuensis* sp. nov., holotype: dorsal pattern of pigmentation and eye pattern of the holotype. Anterior tip to the left. (e) eyes; (ms) median stripe.

Figure 11. *Paraba iguassuensis* sp. nov., holotype, photomicrographs of transverse (A–E) and sagittal sections (F–G; anterior tip to the left): (A) anterior region of body; (B) detail of the anterior region of body of the holotype; (C) pre-pharyngeal region; (D) detail of dorsal surface of pre-pharyngeal region; (E) detail of ventral surface of pre-pharyngeal region; (F) pharynx and (G) ovary. (cg) cyanophil glands; (cs) creeping sole; (de) dorsal epidermis; (di) dorsal insertion of pharynx; (dm) dorsal cutaneous musculature; (dsm) dorsal subcutaneous mesenchymatic musculature; (e) eyes; (i) intestine; (im) internal musculature; (lu) pharyngeal lumen; (m) mouth; (n) nerve plate; (o) ovary; (oe) oesophagus; (om) outer musculature; (ov) ovovitelline ducts; (pp) pharyngeal pouch; (rg) rhabditogen glands; (sbm) sub-intestinal transverse mesenchymatic musculature; (sd) sperm ducts; (sp) sensory pit; (spm) supra-intestinal transverse mesenchymatic musculature; (t) testes; (v) vitelline folicles; (vi) ventral insertion of pharynx; (vm) ventral cutaneous musculature.

Figure 12. *Paraba iguassuensis* sp. nov.: (A) sagittal composite reconstruction of copulatory apparatus of the holotype; (B) horizontal composite reconstruction of copulatory apparatus of paratype MZU PL.00310. Anterior tip to the left. (cmc) common muscle coat; (cov) common glandular ovovitelline duct; (eg) erythrophil glands; (ej) ejaculatory duct; (f) fold; (fa) female atrium; (fc) female canal; (go) gonopore; (ma) male atrium; (ov) ovovitelline ducts; (p) penis papilla; (pv) prostatic vesicle; (sg) shell glands; (sv) spermiducal vesicle.

Figure 13. *Paraba iguassuensis* sp. nov., photomicrographs of the copulatory apparatus: (A) holotype in sagittal section; (B) paratype MZU PL.00310 in horizontal section. Anterior tip to the left. (cmc) common muscle coat; (ej) ejaculatory duct; (f) fold; (fa) female atrium; (go) gonopore; (ma) male atrium; (ov) ovovitelline ducts; (p) penis papilla; (pp) pharyngeal pouch; (pv) prostatic vesicle; (sg) shell glands; (sv) spermiducal vesicle. The prostatic vesicle in (A) was parasagittally sectioned.

Figure 14. *Paraba iguassuensis* sp. nov., photomicrographs of the copulatory apparatus of the holotype: (A) detail of prostatic vesicle; (B) detail of female atrium and common glandular ovovitelline duct. Anterior tip to the left. (cov) common glandular ovovitelline duct; (fa) female atrium; (fc) female canal; (ma) male atrium; (pv) prostatic vesicle; (sg) shell glands. The arrow indicates cyanophil secretion filling the lumen of the female atrium.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Paraba incognita
Paraba multicolor
Paraba pankaru
Paraba phocaica
Paraba piriana
Paraba preta
Paraba rubidolineata
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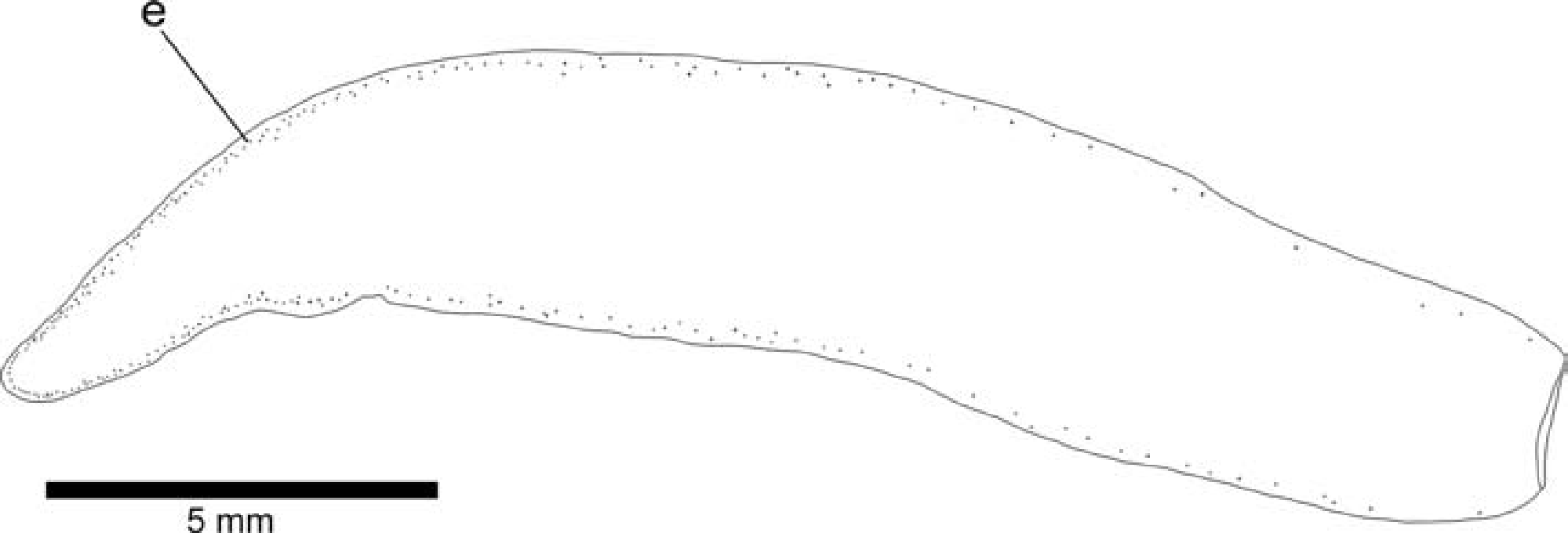
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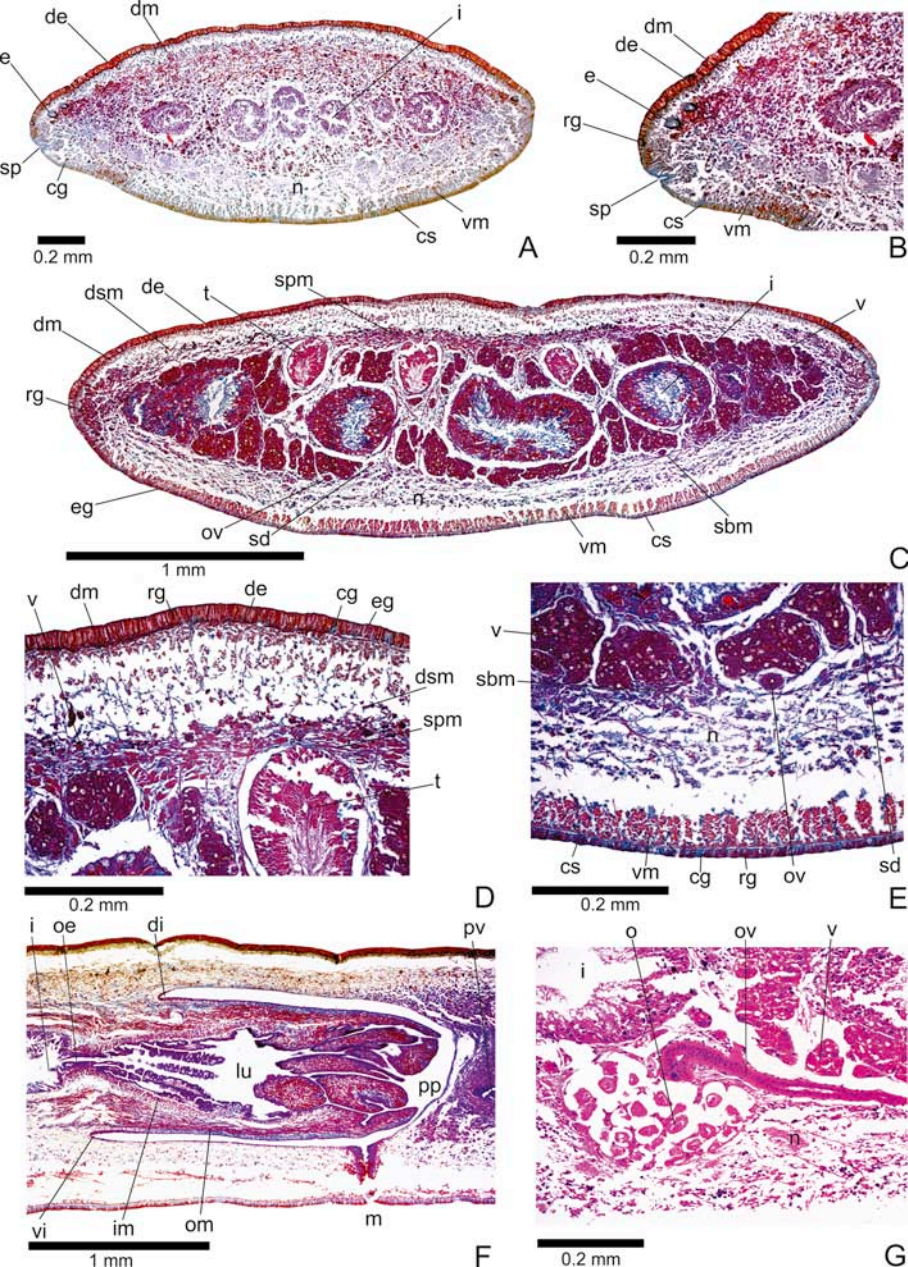
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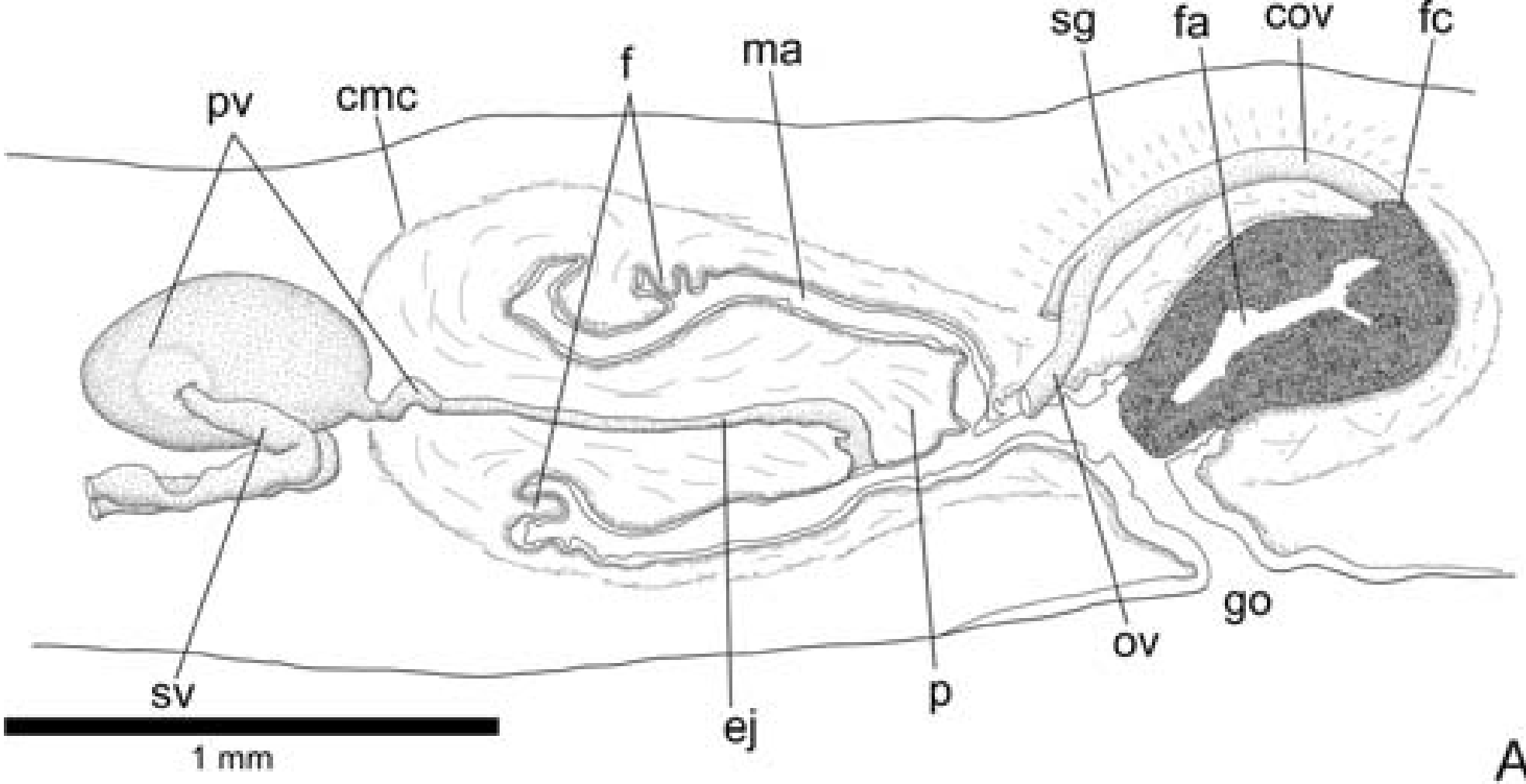
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1 mm
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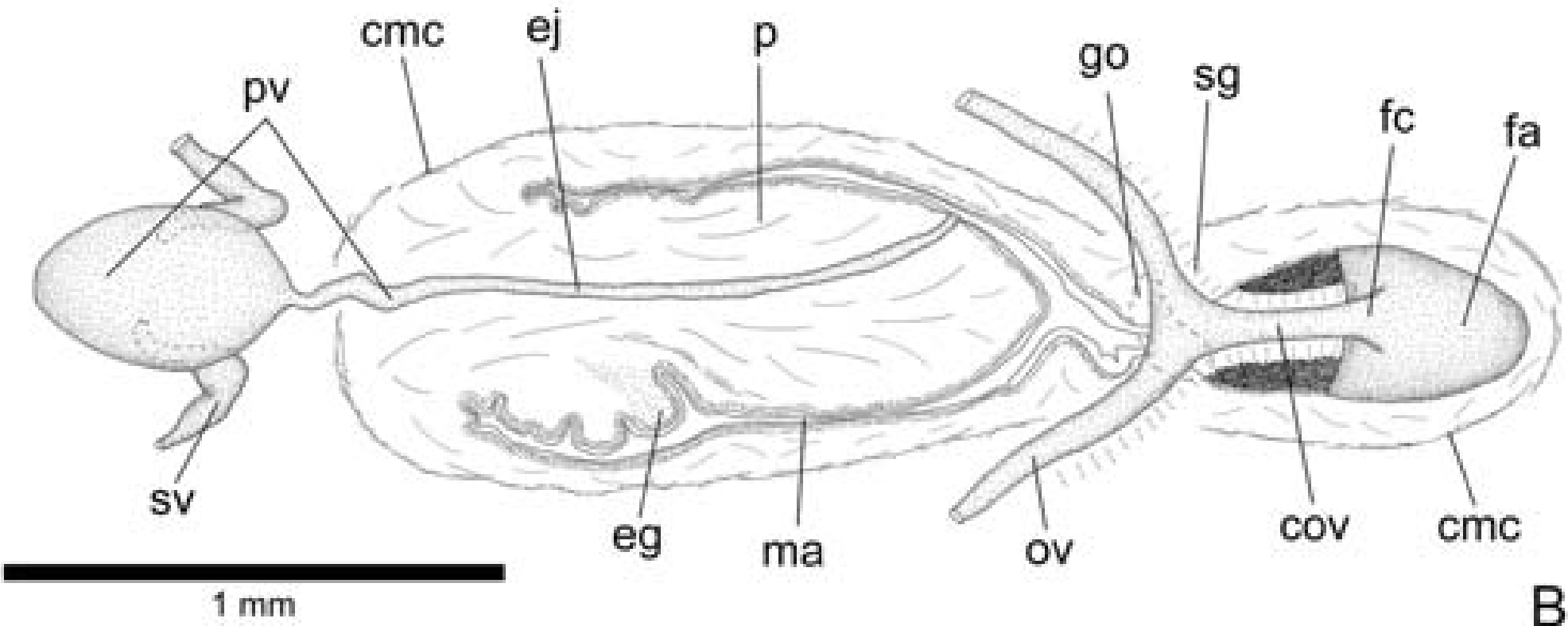
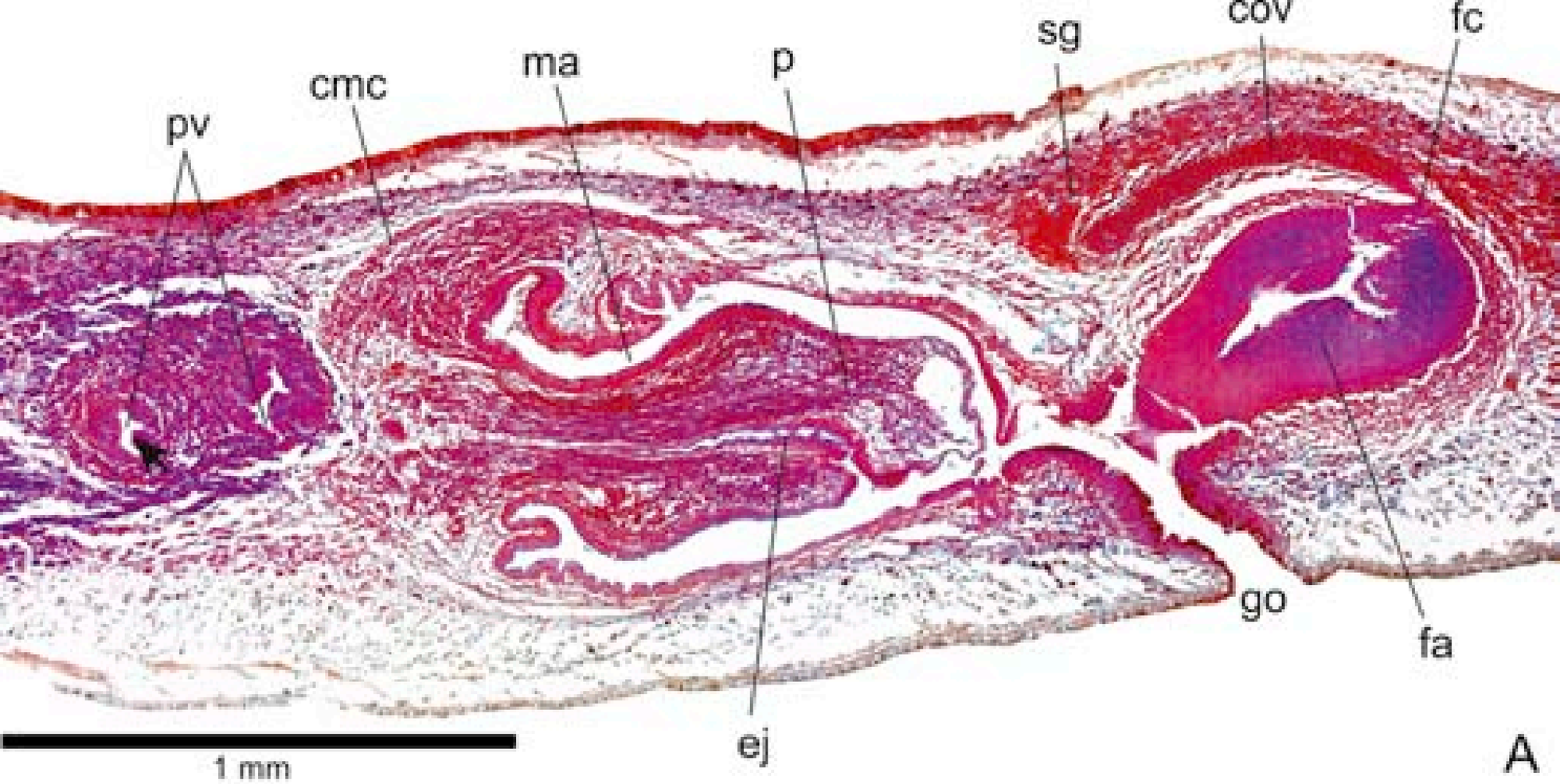
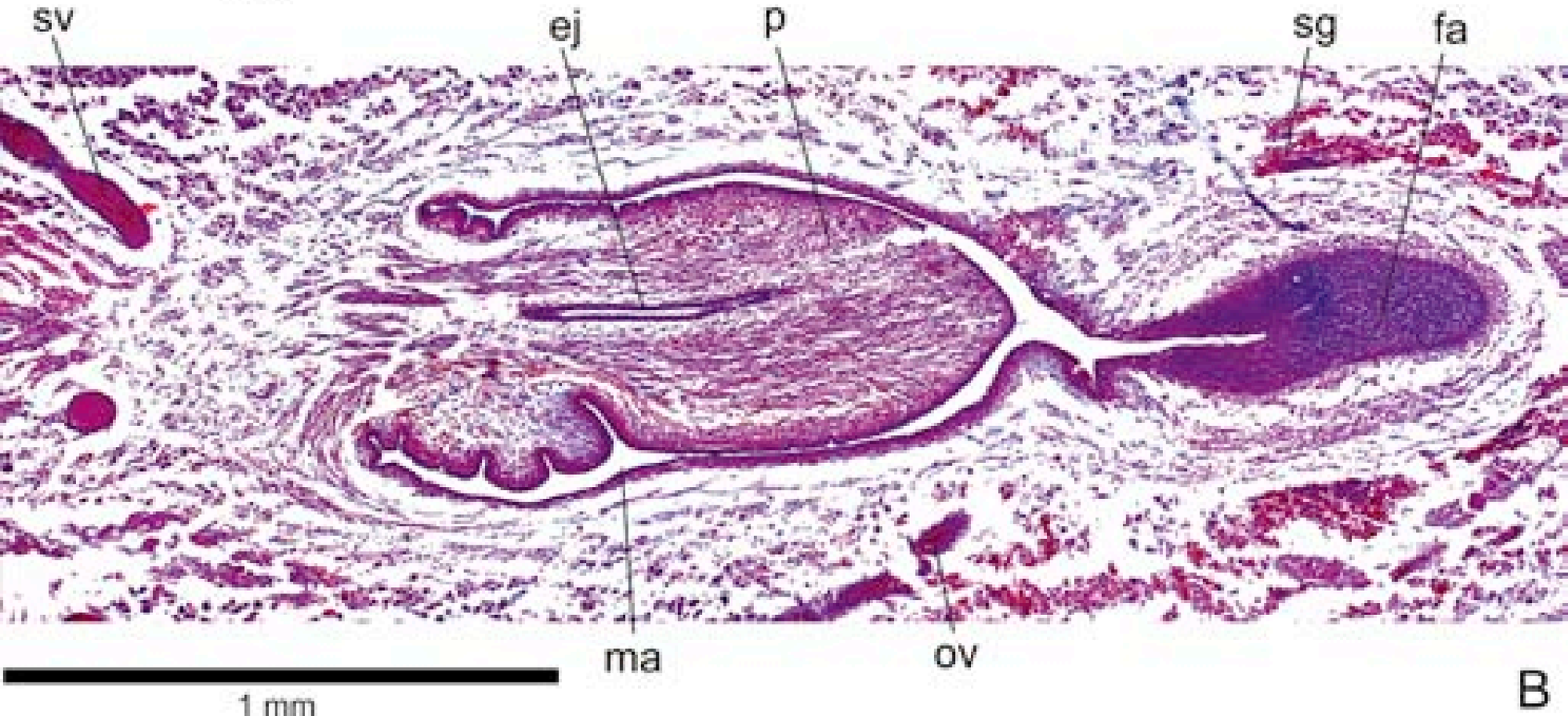
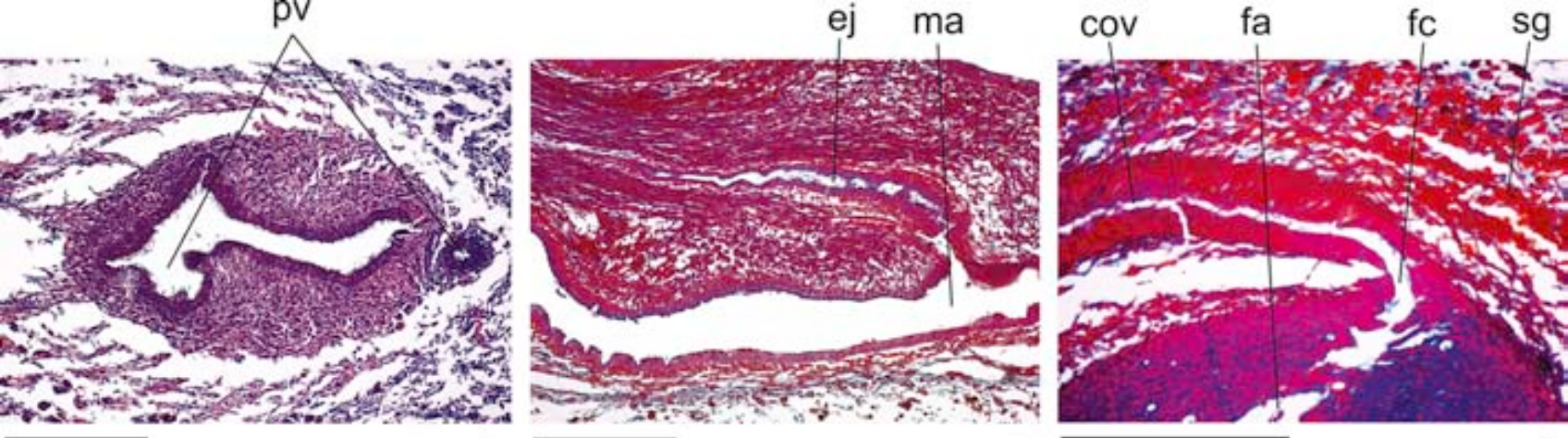
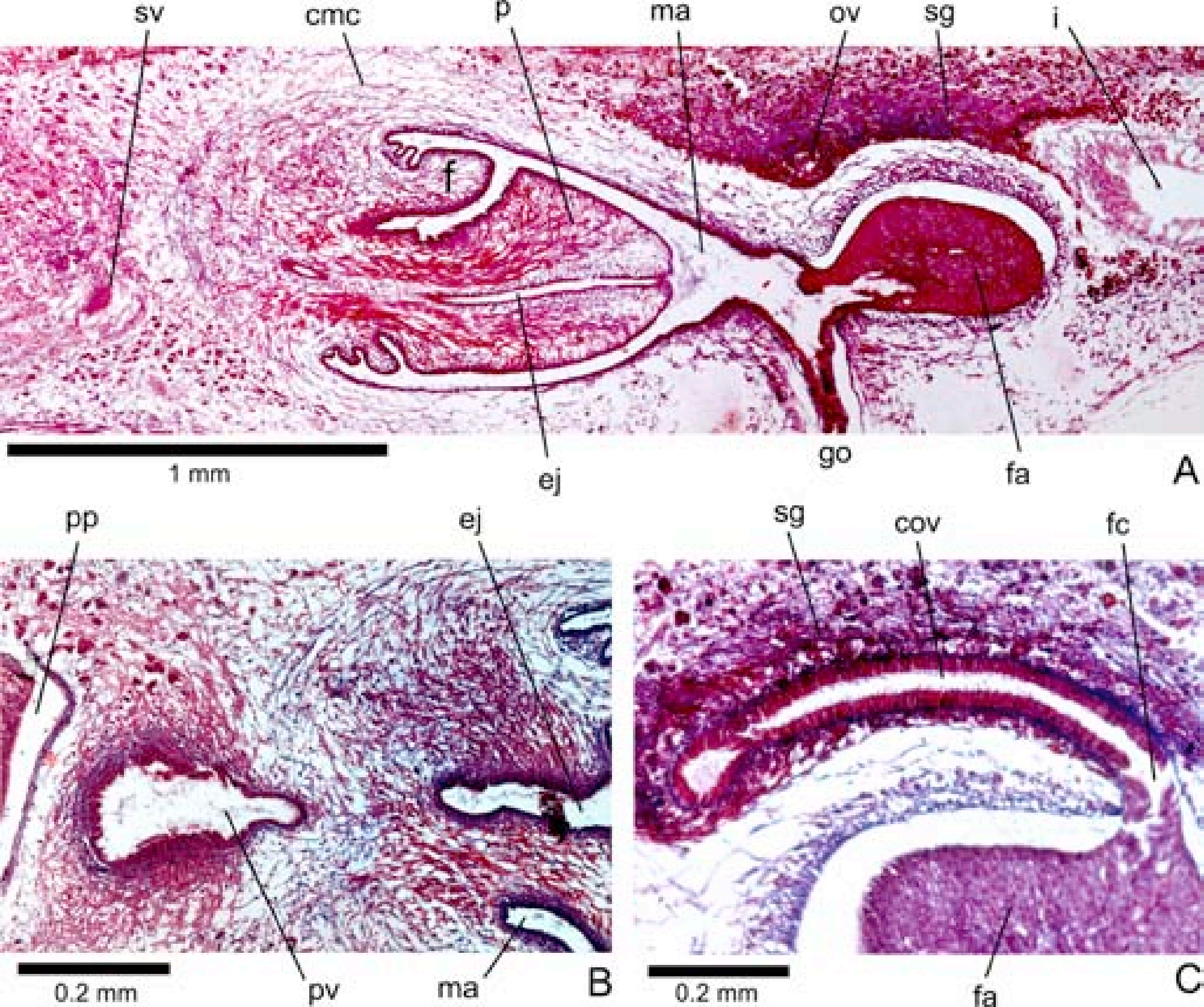
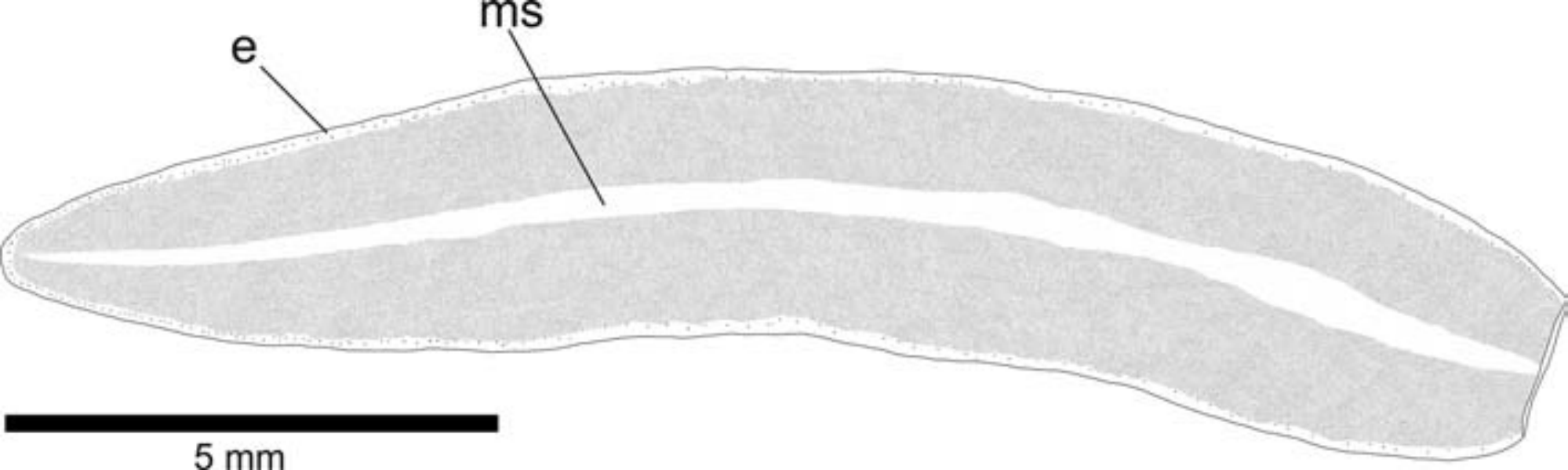

cmc
ej
p
go
sg
pv
fc
fa
sv
eg
ma
ov
cov
cmc
1 mm
B


pv
cmc
ma
p
sg
cov
fc
go
fa
ej
1 mm
A


sv
ej
p
sg
fa
ma
ov
1 mm
B

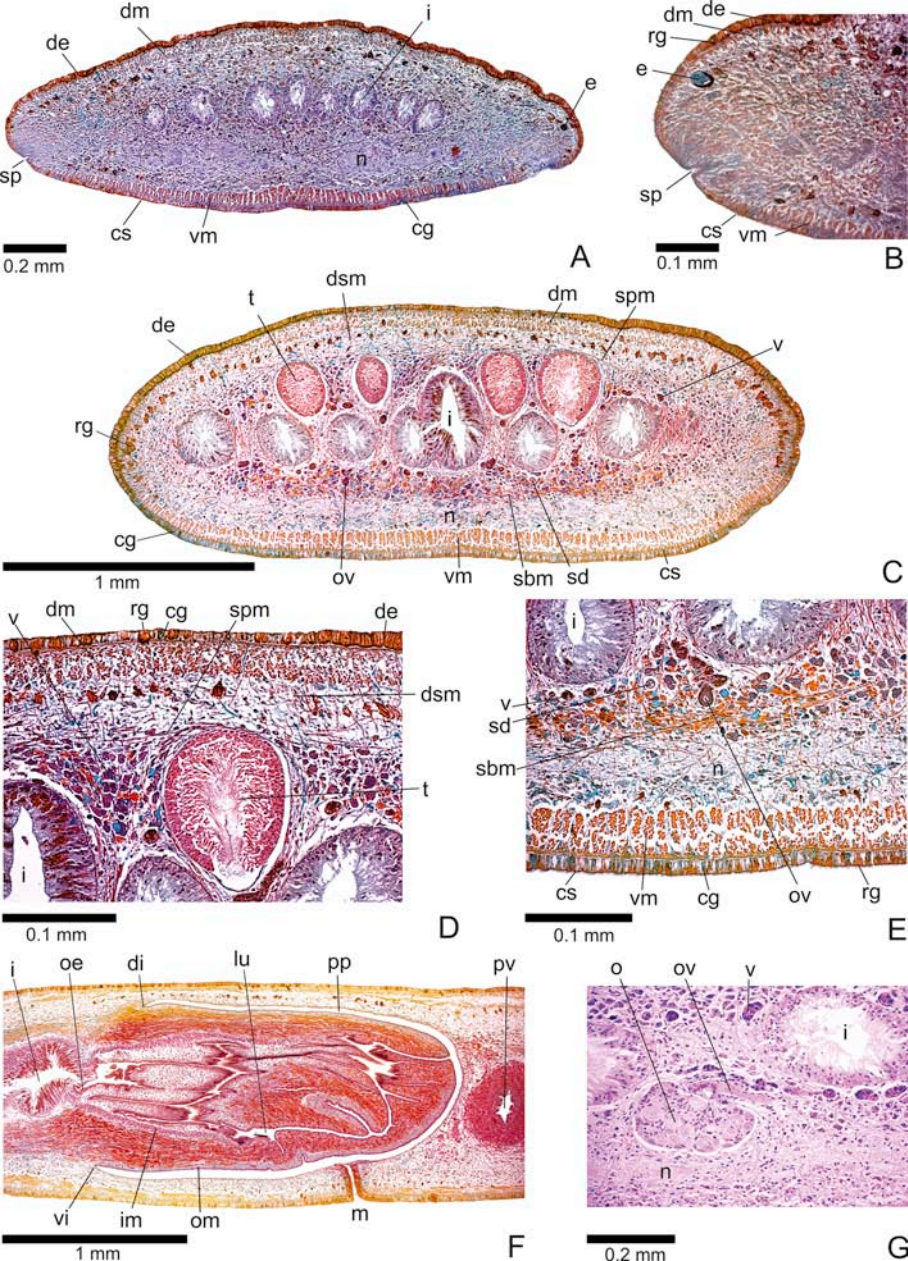

pv
ej
ma
cov
fa
fc
sg
0.2 mm
A
0.2 mm
B
0.1mm
C

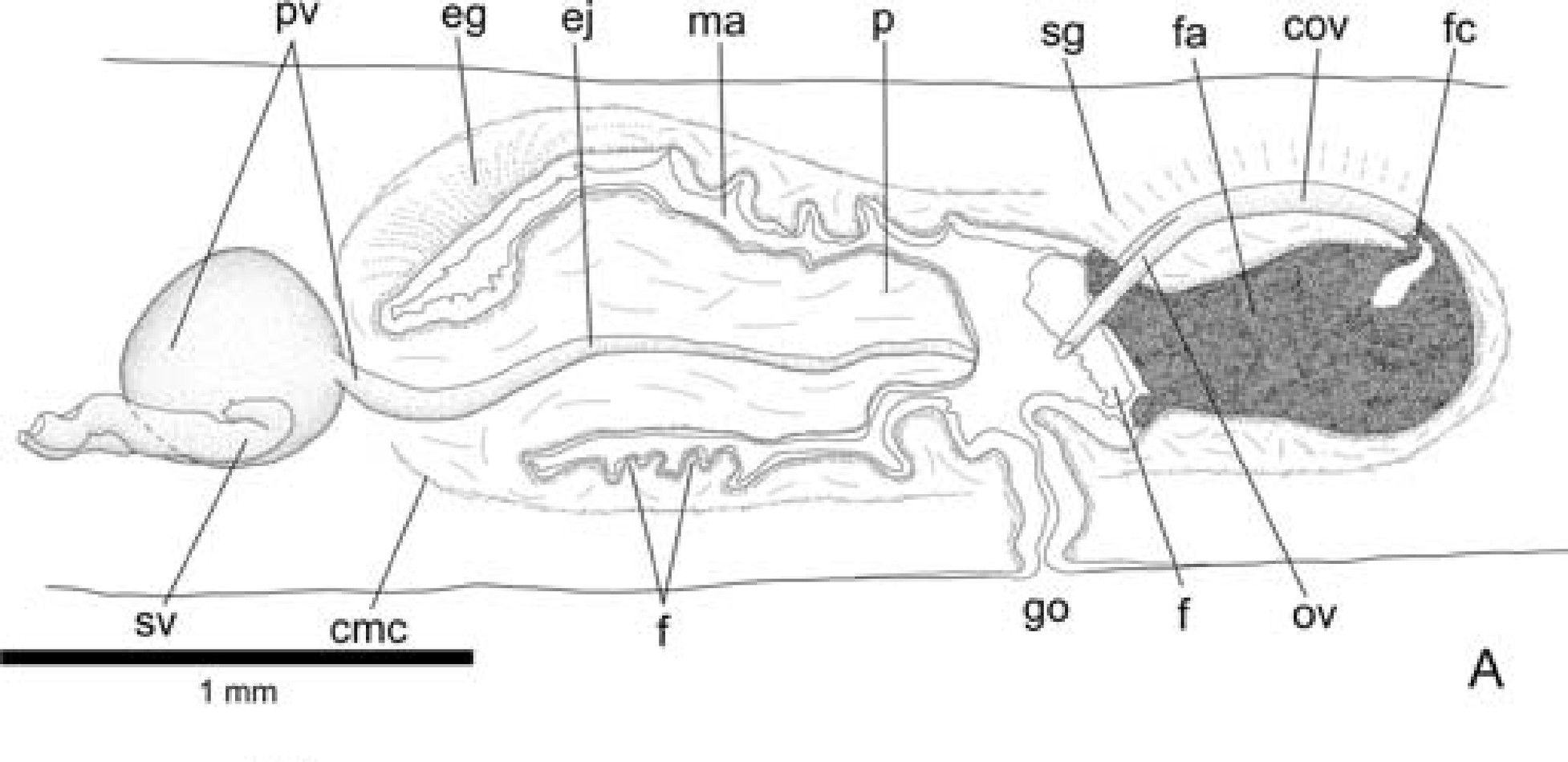

sv
cmc
p
ma
ov
sg
i
f
1 mm
ej
go
fa
A
pp
ej
0.2 mm
pv
ma
B
sg
cov
fc
0.2 mm
fa
C

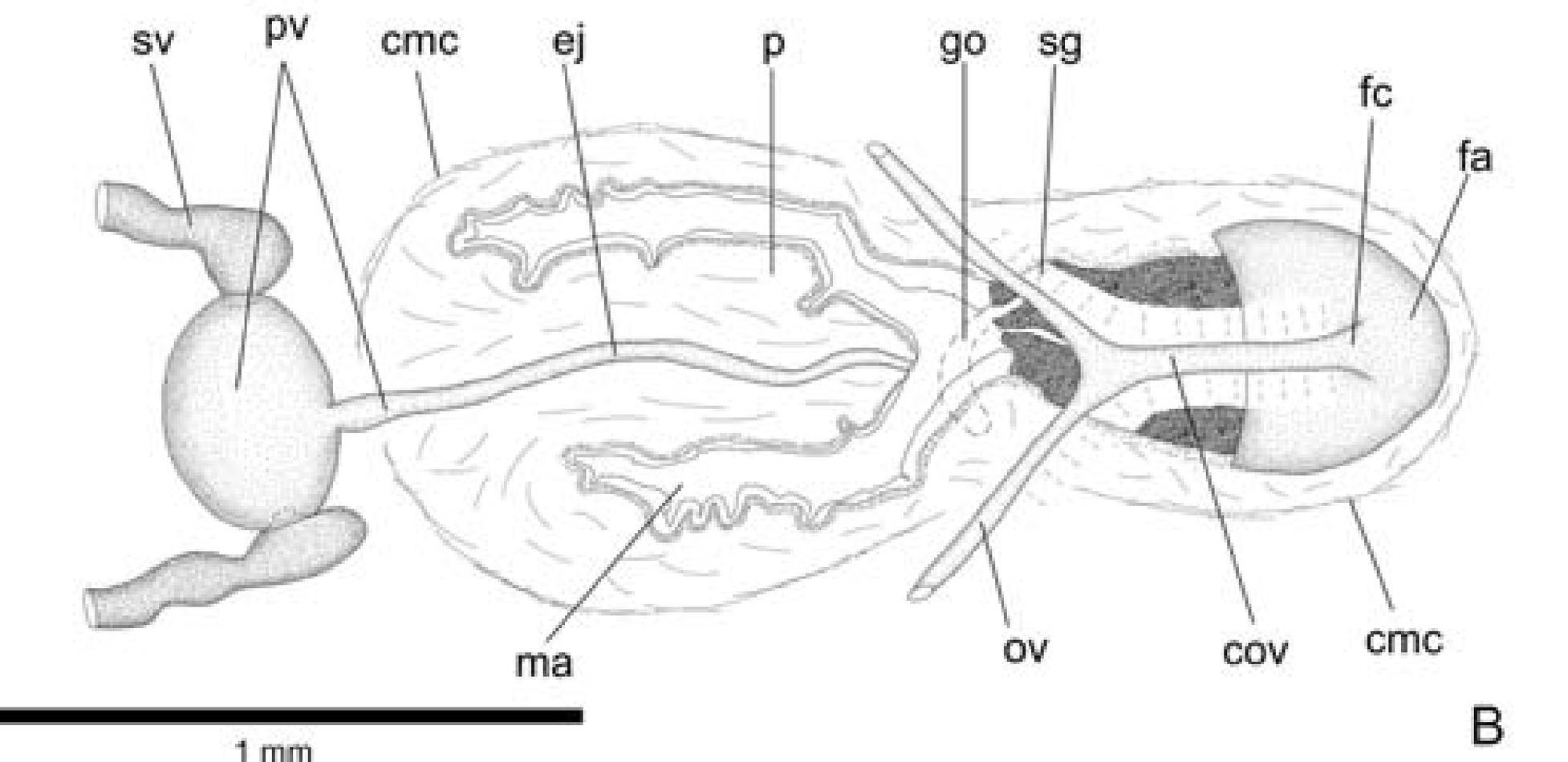

e
ms
5 mm

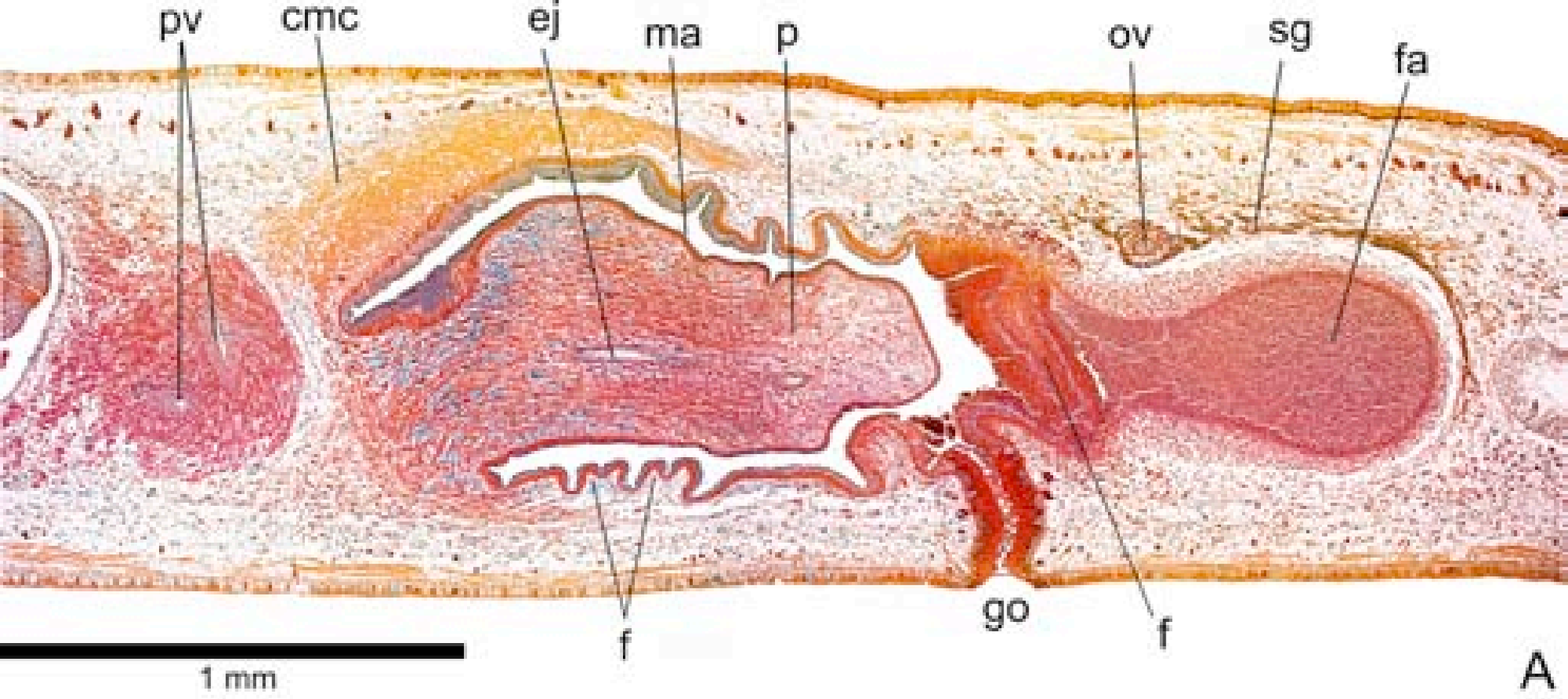

5 mm
A

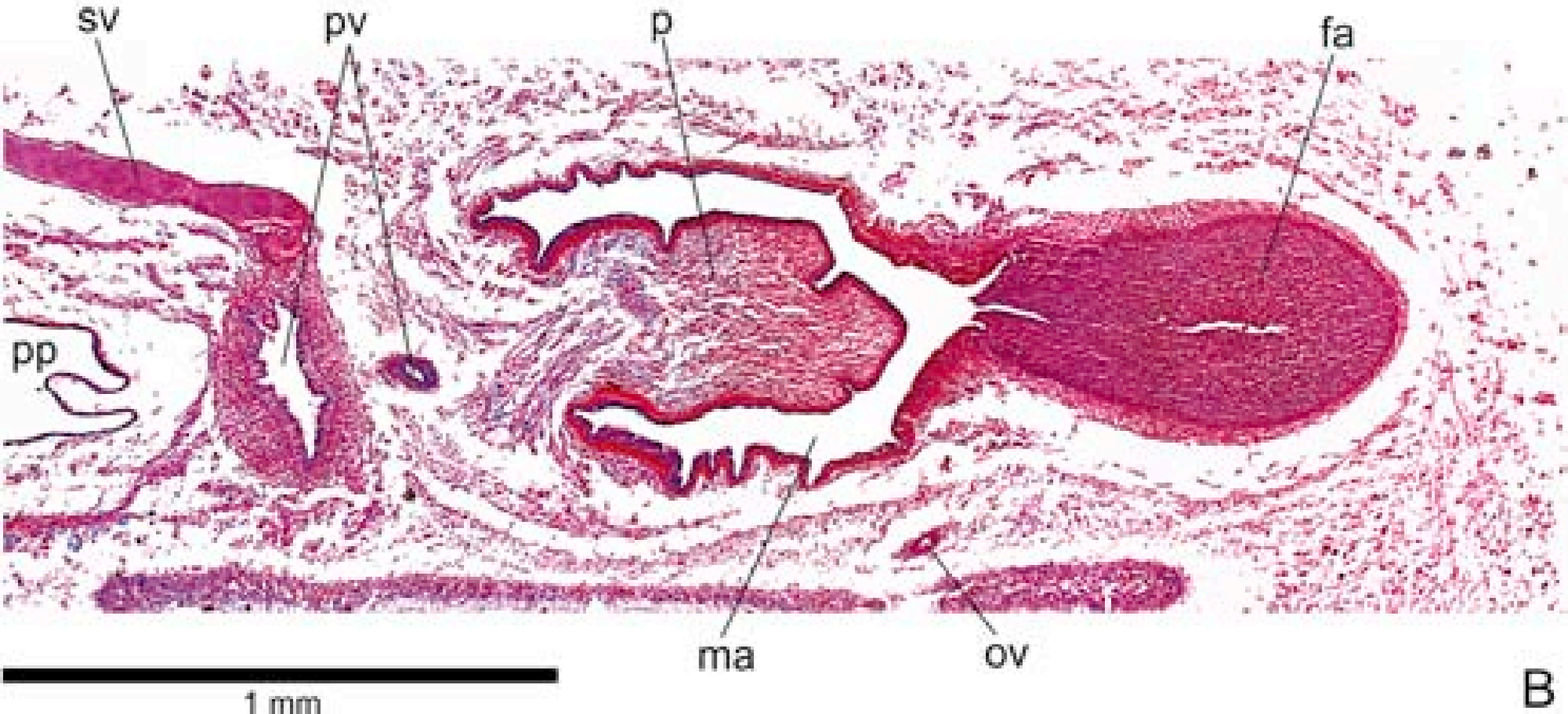

5 mm
B

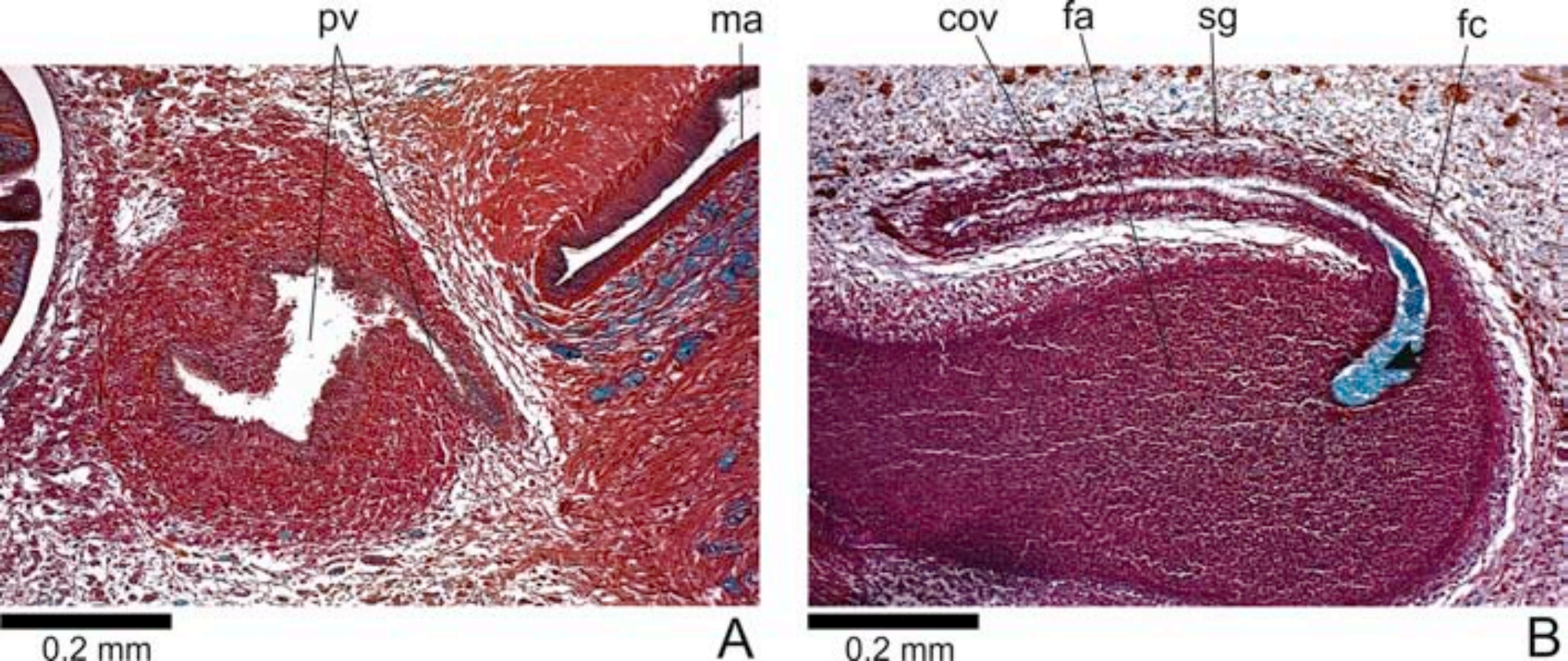

dm
de
i
e
sp
n
cs
vm
cg
0.2 mm
A
dm
de
rg
e
sp
cs
vm
0.1 mm
B
t
dsm
dm
spm
de
v
rg
i
n
cg
ov
vm
sbm
sd
cs
1 mm
C
v
dm
rg
cg
spm
de
dsm
t
i
0.1 mm
D
i
v
sd
sbm
n
cs
vm
cg
ov
rg
0.1 mm
E
i
oe
di
lu
pp
pv
vi
im
om
m
1 mm
F
o
ov
v
i
n
0.2 mm
G


pv
eg
ej
ma
p
sg
fa
cov
fc
sv
cmc
f
go
f
ov
1 mm
A


sv
pv
cmc
ej
p
go
sg
fc
fa
ma
ov
cov
cmc
1 mm
B


pv
cmc
ej
ma
p
ov
sg
fa
f
go
f
1 mm
A


sv
pv
p
fa
pp
ma
ov
1 mm
B


pv
ma
cov
fa
sg
fc
0,2 mm
A
0,2 mm
B

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: