

SUBTERRANEAN AQUATIC PLANARIANS OF SARDINIA, WITH A DISCUSSION ON THE PENIAL FLAGELLUM AND THE BURSAL CANAL SPHINCTER IN THE GENUS *DENDROCOELUM* (PLATYHELMINTHES, TRICLADIDA, DENDROCOELIDAE)

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Abstract: The paper provides the first detailed account on the taxonomic richness of the subterranean freshwater tricladids from Sardinia, including the description of four new species for the genera *Dendrocoelum* and *Phagocata*. New records for *Dugesia benazzii*, *Dugesia* sp., *Crenobia alpina*, and *Phagocata* sp. are also reported. The three new species of *Dendrocoelum* are the first reported for the island of Sardinia. These species display a bursal canal sphincter and a large adenodactyl with a characteristic anatomy with a zone of fine circular muscle fibers running through the mesenchyme of its papilla. A detailed analysis of the structure of the penial flagellum in the genus *Dendrocoelum* highlighted six main conditions, some of which have not been previously reported, in regard to the histology of the tip of the penis papilla and the extent of its inversion. The new species of *Phagocata* represents the first species recorded from Italy and the first anophthalmous species reported from Europe.

INTRODUCTION

The Mediterranean region has been identified as a biodiversity hotspot on a global scale, with the island of Sardinia as one of its regional hotspots (Médail and Quézel, 1999; Myers et al., 2000). Together with part of coastal Tuscany, the island belongs to the Sardinian stygofaunistic province (Pesce, 1985). The presence of a greatly diversified and ancient karst, dating to the Palaeozoic and Mesozoic periods of the Sardinian-Corsica plate, gave rise to a wide range of underground aquatic systems, ranging from inland and coastal caves to underground rivers (Pesce, 1985). Although more than 3000 terrestrial and marine karstic caves are registered in the Regional Speleological Register, the groundwater biodiversity of Sardinia is largely unknown. The literature on the taxonomic richness in the caves, springs, and wells of Sardinia is mainly focused on Crustacea (Lindberg, 1956; Stella, 1957; Puddu and Pirodda, 1973; Ruffo and Vigna Taglianti, 1975; Cassola, 1982; Pesce and Maggi, 1983; Pesce, 1985; Cottarelli et al., 1996; Argano et al., 1997). A few papers refer to freshwater Oligochaeta (Martinez-Ansemil and Sambugar, 2008), Gastropoda (Giusti and Castagnolo, 1983), Trichoptera (Moretti and Cianficconi, 1983; Cianficconi et al., 1998), and marine cave-dwelling fauna (see Manconi et al., 2009 and references therein).

Faunistic investigations of Sardinian epigeal waters, promoted first by Benazzi (1938) and subsequently further developed by Lepori and co-workers, highlighted a considerable taxonomic richness and endemism of the Tricladida (Lepori, 1951; Pala et al., 1980a,b,c, 1981, 1995,

1999, 2000; Casu et al., 1982; Stocchino et al., 2005). In contrast, very few data are available on the Sardinian groundwater tricladids, dating back to 1938 when Benazzi recorded *Atrioplanaria* sp. from a hydropetric habitat on limestone in north-western Sardinia (Benazzi, 1938). Other specimens, discovered in 1952 from Su Coloru Cave (northern Sardinia) and from an old mine (Baccu Arrosu, southwestern Sardinia), were assigned by the same author to *Atrioplanaria* sp. (Benazzi, 1982). Pala et al. (1980c) reported on *Crenobia alpina* Dana, 1776 from some springs on the Gennargentu Massif (central-eastern Sardinia). Apart from these data no new records of subterranean tricladids from the island have become available for a long period of time. Only recently have a few stygobiological studies on Sardinian tricladids been performed (Stocchino, 2003; Stocchino et al., 2008). The present paper provides a detailed account on the taxonomic richness of the subterranean tricladids from Sardinia, including the description of four new species and new records for several others.

MATERIALS AND METHODS

The specimens, collected during the period 2000–2010, were transferred to the laboratory, reared in shaded boxes in semi-dark conditions at 18 ± 2 °C and fed with fresh beef liver. For morphological study, specimens were fixed

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for 24 hours in Bouin's fluid, dehydrated in a graded ethanol series, cleared in toluene, and embedded in paraffin. Serial sections of specimens of the Puntadella Scmunica Spring were made at intervals of 5, 6, or 7 µm and stained with haematoxylin-eosin (Harris), Hansen's haematoxylin and eosin-orange, Dane Herman's tetrachrome, Mallory-Cason, or Pasini's reagent.

Serial sections of specimens from the Monte Majore Cave were made at intervals of 5 or 8 µm and stained in Dane Herman's tetrachrome or Mallory-Cason. Serial sections of specimens from Sa Ucca 'e su Peltusu Cave were made at intervals of 8 µm and were stained with Mallory-Cason. Serial sections of the specimen from the Cuccuru Tiria Cave were made at intervals of 5 µm and stained in Dane Herman's tetrachrome. Serial sections of specimens from the Su Cantaru Spring were made at intervals of 5 µm and stained in haematoxylin-eosin (Harris). Serial sections of specimens from the Gennargentu Massif spring were made at intervals of 8 µm and stained in Harris's haematoxylin-eosin. The material is deposited in the Netherlands Centre for Biodiversity Naturalis (section ZMA) and in the Giacinta A. Stocchino collection (CGAS), University of Sassari.

SYSTEMATIC ACCOUNT

(All abbreviations for labels in Figures 2–9 are listed in Table 1)

Order TRICLADIDA Lang, 1884
 Suborder CONTINENTICOLA Carranza, Littlewood, Clough, Ruiz-Trillo, Bagaña and Riutort, 1998
 Superfamily PLANARIOIDEA Stimpson, 1857
 Family DENDROCOELIDAE Hallez, 1892
 Genus *Dendrocoelum* Örsted, 1844
Dendrocoelum mariae Stocchino and Sluys sp. nov. (Table 2; Figs. 1, 2)

Material examined. All individuals (n = 20) were asexual at collection. The majority of the animals underwent a sexualization process after a year of rearing in the laboratory.

Holotype: ZMA V.Pl. 7100.1, sagittal sections on 17 slides, Punta della Scmunica, Asinara Island (41°05'35"N, 8°18'4"E), December 2000, coll. M. Piras and G.A. Stocchino.

Paratypes: ZMA V.Pl. 7100.2, ZMA V.Pl. 7100.3, ZMA V.Pl. 7100.4, *ibid.*, sagittal sections on 41 slides, 22 slides, 22 slides; ZMA V.Pl. 7100.5, *ibid.*, one set of transverse sections on 61 slides; CGAS Pla 1.1, *ibid.*, sagittal sections on 37 slides, immature specimen; CGAS Pla 1.2, *ibid.*, horizontal sections on 27 slides, not completely mature specimen.

Other material: ZMA V.Pl. 7101.1, ZMA V.Pl. 7101.2, two sets of sagittal sections on 5 slides and 19 slides, respectively, not completely mature specimens. Punta della Scmunica, Asinara Island (41°05'35"N 8°18'4"E) May 2005, coll. G.A. Stocchino; CGAS Pla 1.3, *ibid.* one

Table 1. Abbreviations used in figures.

Abbreviation	Term
<i>a</i>	adenodactyl
<i>ab</i>	bulb of the adenodactyl
<i>ap</i>	papilla of the adenodactyl
<i>bc</i>	bursal canal
<i>bg</i>	bulb glands
<i>ca</i>	common atrium
<i>cb</i>	copulatory bursa
<i>cm</i>	circular muscles
<i>co</i>	copulatory apparatus
<i>cod</i>	common oviduct
<i>cvs</i>	common vas deferens
<i>e</i>	eyes
<i>ed</i>	ejaculatory duct
<i>ep</i>	epithelium
<i>eep</i>	external epithelium
<i>f</i>	flagellum
<i>fcm</i>	fine circular muscles
<i>g</i>	gonopore
<i>h</i>	head
<i>ic</i>	intestinal caecum
<i>iep</i>	internal epithelium
<i>l</i>	lumen
<i>lm</i>	longitudinal muscles
<i>m</i>	musculature
<i>ma</i>	male atrium
<i>od</i>	oviduct
<i>pb</i>	penis bulb
<i>pg</i>	penis glands
<i>ph</i>	pharynx
<i>pl</i>	plug of cells
<i>pp</i>	penis papilla
<i>ppl</i>	penis papilla lumen
<i>s</i>	sphincter
<i>sg</i>	shell glands
<i>sp</i>	sperm
<i>spv</i>	spermiducal vesicles
<i>sv</i>	seminal vesicle
<i>vd</i>	vas deferens
<i>vdv</i>	vesicle of the vas deferens

set of sagittal sections on 12 slides; CGAS Pla 1.4–12 *ibid.*, nine sets of sagittal sections on 13 slides, 13 slides, 23 slides, 14 slides, 16 slides, 18 slides, 22 slides, 21 slides, 37 slides, respectively, not completely mature specimens. CGAS Pla 1.13–14, two sets of transverse sections on 21 slides and 46 slides, respectively, not completely mature specimens, Punta della Scmunica, Asinara Island (41°05'35"N 8°18'4"E) spring 2000, coll. M. Piras and G.A. Stocchino.

Etymology. The specific epithet refers to Professor emeritus Maria Pala (University of Sassari), in recognition of her contributions to the biology and systematics of freshwater triclads.

Table 2. Checklist of groundwater triclads from Sardinia.

Taxa	Caves	Springs	Locality	Coordinates		Reference
				Latitude	Longitude	
Dugesiidae Ball, 1974						
<i>Dugesia</i> Girard, 1850						
<i>Dugesia benazzii</i>		1	Su Cantaru Spring, Monte Albo	41°34'30"N	9°40'31"E	Present paper
<i>Dugesia</i> sp.		1	Sa Ucca 'e su Peltusu Cave	40°27'00"N	8°40'43"E	Present paper
Dendrocoelidae Hallez, 1892						
<i>Dendrocoelum</i> Örsted, 1844						
<i>Dendrocoelum mariae</i>		1	Punta della Scomunica Spring, Asinara Island	41°05'35"N	8°18'4"E	Present paper
<i>Dendrocoelum nuraghum</i>		1	Monte Majore Cave	40°30'51"N	8°36'37"E	Present paper
<i>Dendrocoelum vesiculosum</i>		1	Sa Ucca 'e su Peltusu Cave	40°27'00"N	8°40'43"E	Present paper
Planariidae Stimpson, 1857						
<i>Atrioplanaria</i> de Beauchamp, 1932						
<i>Atrioplanaria</i> sp.		1	Sassari surroundings	40°42'02"N	8°35'47"E	Benazzi, 1938
<i>Atrioplanaria</i> sp.		1	Su Coloru Cave	40°49'01"N	8°48'46"E	Benazzi, 1982
<i>Atrioplanaria</i> sp.		1	Baccu Arrosu Mine	39°9'36"N	8°45'15"E	Benazzi, 1982
<i>Phagocata</i> Leidy, 1857						
<i>Phagocata obscura</i>		1	Cuccuru Tiria Cave	39°19'25"N	8°34'29"E	Present paper
<i>Phagocata</i> sp.		1	Monte Majore Cave	40°30'51"N	8°36'37"E	Present paper
<i>Phagocata</i> sp.		1	Elighe Mannu Spring, Asinara Island	41°05'36"N	8°18'21"E	Present paper
<i>Phagocata</i> sp.		1	Alà dei Sardi	40°38'60"N	9°19'45"E	Present paper
<i>Crenobia</i> Kenk, 1930						
<i>Crenobia alpina</i> (Dana, 1766)		4	East Gennargentu Massif	40°09'24"N	9°15'16"E	Pala et al., 1980c
<i>C. alpina</i>		1	Su Sessene Spring, West Gennargentu Massif	40°01'40"N	9°12'1"E	Present paper

Habitat. From 2000 to 2005 (spring and winter) specimens of *D. mariae* were collected from a small perennial spring (at an altitude of ca. 350 m asl) on the eastern coast of Asinara Island (Fig. 1). The seasonal samplings highlighted the constant presence of planarians at this site. Substrate ranges from rocks to large boulders and sand. The spring is characterized by an extremely fluctuating flow, with minimum values during the summer. Water temperature ranges between 13 and 21.4 °C throughout the year; pH values range from 7 to 7.3. The water is rich in minerals (total dissolved solids ca. 600 mg L⁻¹) and is characterized by high values of chlorides, due to the salty aerosol blown in by wind. Dendrocoelidae were associated with a diverse invertebrate fauna, consisting of Gastropoda, Tubificidae, Ostracoda, Cyclopoida, Asellidae, Amphipoda, Hydracnida, Chironomidae, and the endemic anuran *Discoglossus sardus* Tschudi, 1837.

Geographical distribution. Endemic to Asinara Island and only known from the type locality.

Diagnosis. *Dendrocoelum mariae* is characterized by an adenodactyl that is much larger than the penis papilla, a copulatory apparatus far behind the pharyngeal pocket, the presence of a sphincter in the terminal tract of the bursal canal, and by the penis being in dorsal position and the adenodactyl located ventrally.

Description. Live animals are unpigmented, typically whitish, with a body size of 8 to 10 mm in length and a width ranging from ca. 2 mm in the central part of the body to less than 1 mm at the level of the head. The anterior end is truncated, with the middle part of the frontal margin convex, and is provided with a pair of rounded lateral lobes. Just behind the eyes there is a slight but clear constriction or neck that sets off the head from the rest of the body (Fig. 2A).

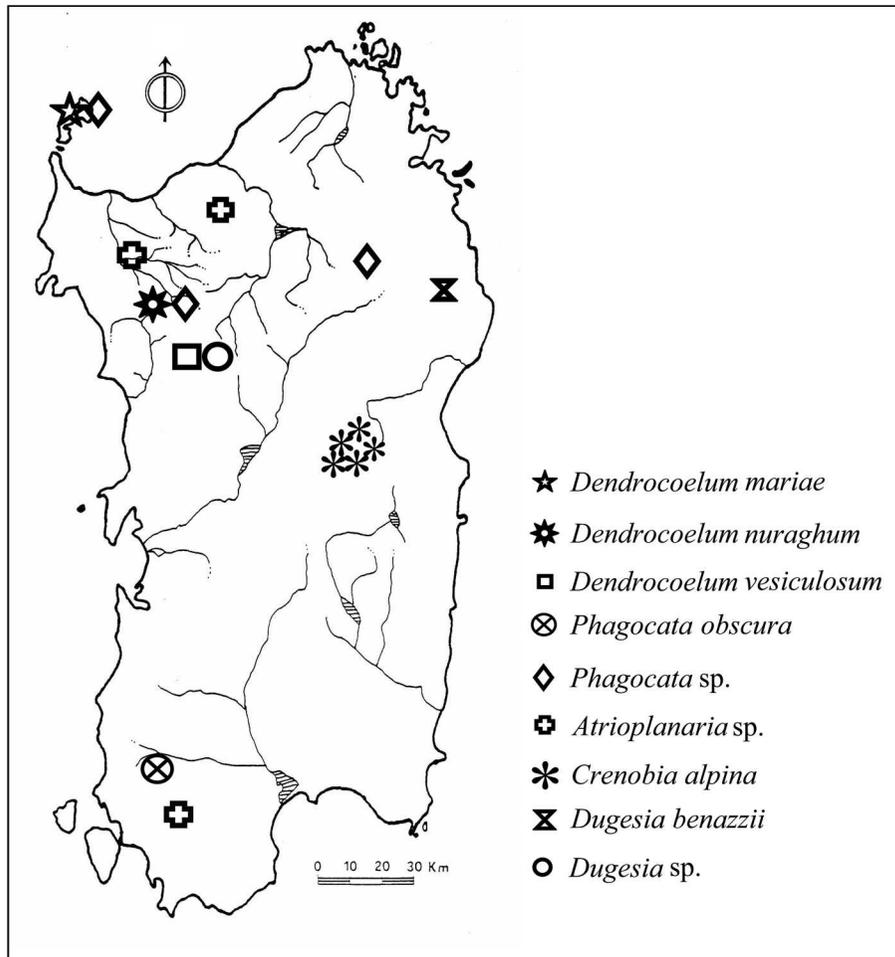


Figure 1. Geographic distribution of groundwater planarians in Sardinia. Data from Benazzi (1938, 1982), Pala et al. (1980c), and this study.

The two eyes are situated rather closely together. The distance between the eyes is slightly less than 1/4 of the width of the neck. Inter-ocular distance is smaller than the distance from each eye cup to the lateral margin, and the distance from the frontal margin to the eyes is greater than the distance to the lateral margin. Supernumerary eyes are often present, usually 2 or 3 on either side, sometimes arranged in a slightly crescent-shaped configuration.

The subterminal anterior adhesive organ, with a length comparable to the interocular distance, is moderately developed and consists of a shallow cup. The surface of the organ consists of a well-defined area of infranucleate epithelial cells pierced by numerous gland ducts. The cell bodies of the glands are scattered throughout the mesenchyme of the anterior part of the body. Two types of glands, strongly or weakly acidophilic, are recognizable with Dane Herman's tetrachrome, Pasini's reagent, and haematoxylin-eosin (Harris) stains. The musculature associated with this organ consists of a more strongly developed section of the ventral longitudinal body musculature.

The intestine is well visible, the anterior ramus reaches to a level just behind the neck and bears 13 to 15 branches on each side. The two posterior rami, each with 16 or 17 branches, usually converge behind the copulatory apparatus to form a common branch. The pharynx is located in the posterior half of the body and measures about 1/8 of the body length (Fig. 2A). Its internal muscle zone consists of a very thick layer of intermingled circular and longitudinal fibers. A subepithelial layer of longitudinal muscles followed by a layer of circular fibers forms the thin outer zone of muscles.

The two ovaries occur on the ventral side of the anterior region behind the brain. The ovaries are located at 1/5 of the distance between the brain and the root of the pharynx. The two oviducts originate from the dorsal part of the ovaries and are provided with a slight expansion at their anterior end, the tuba. The oviducts run posteriorly, fusing behind the copulatory apparatus to form a rather long common oviduct. The latter runs anteriorly to the right side of the opening of the bursal canal into the atrium. The common oviduct receives numerous openings of eosinophilic shell

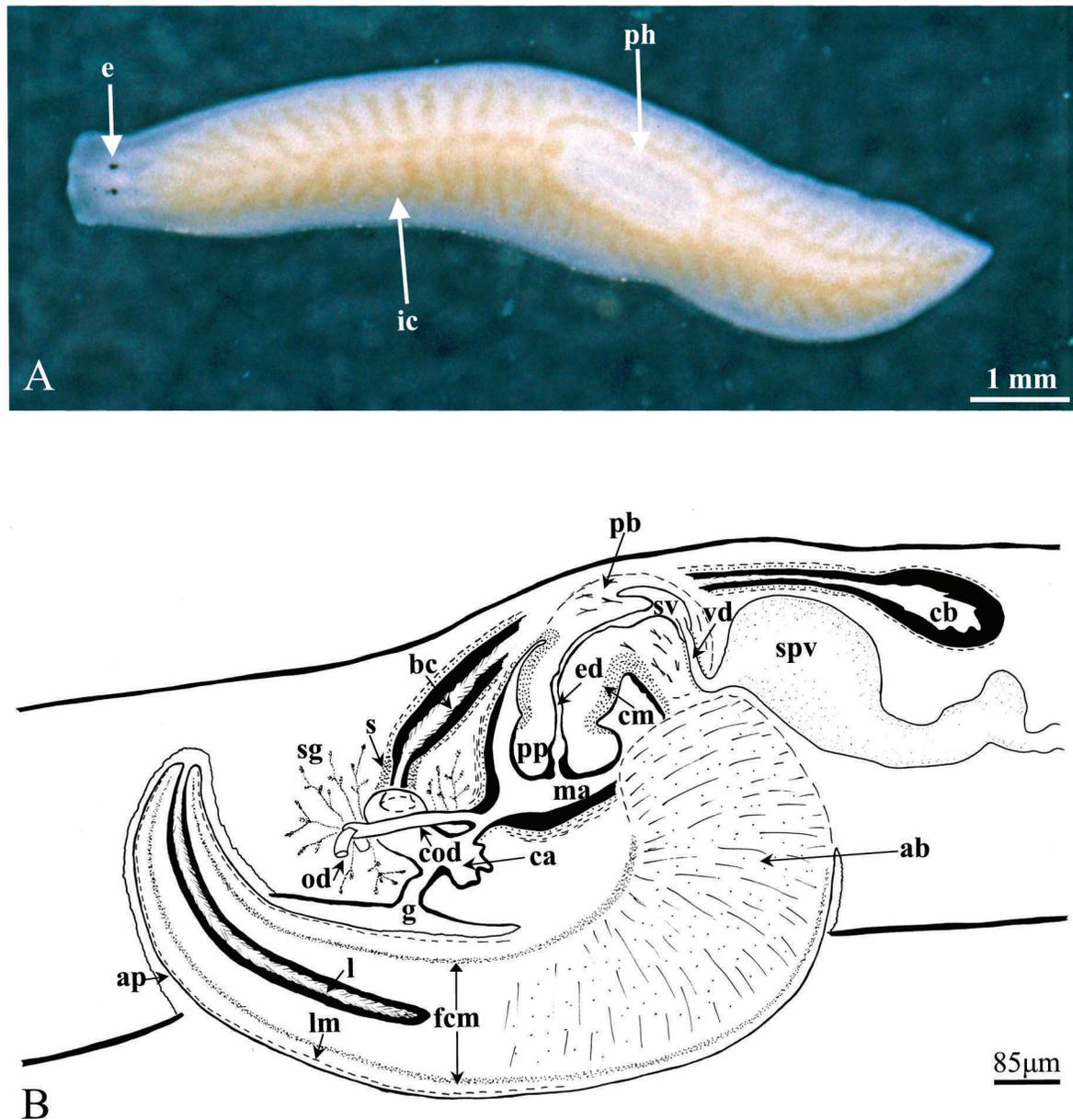


Figure 2. *Dendrocoelum mariae*. (A) habitus of a living specimen, (B) holotype ZMA V.Pl. 7100.1, sagittal reconstruction of the copulatory apparatus (anterior to the right).

glands along its entire length. The vitellaria are located between the intestinal diverticula and the testes.

The well-developed testes are numerous and essentially ventral in position (in one specimen some follicles are situated between the intestinal diverticula). The testes extend from a short distance behind the ovaries to the far posterior end of the body and are arranged on either side of the midline in two to three longitudinal zones. The sperm ducts form well-developed spermiducal vesicles, packed with sperm, between the mouth and the anterior level of the penis bulb (Fig. 2B).

The copulatory apparatus occupies the posterior half of the postpharyngeal region. The elongated, small copulatory

bursa is sac-shaped and situated in the dorsal part of the body. The bursa is lined with a high glandular epithelium and is surrounded by a layer of longitudinal muscles. The bursal canal runs posteriorly to the left of the penis and gradually widens, then turns ventrally and after narrowing again opens into the common atrium. The wall of the bursal canal consists of a nucleated epithelium with ciliated cells that gradually range from cuboidal in the proximal tract to cylindrical in the more distal section of the canal. The canal is surrounded by a subepithelial layer of circular muscles followed by a layer of longitudinal fibers. Just before the opening into the atrium the layer of circular muscles becomes thicker and thus forms a sphincter (Fig. 2B).

In the male copulatory apparatus the small, muscular penis bulb houses a seminal vesicle, devoid of a proper epithelium, that is surrounded by several interwoven layers of muscle. The vasa deferentia enter the seminal vesicle closely together from the antero-lateral sides and open symmetrically in its anterior part. The seminal vesicle communicates with the ejaculatory duct that opens at the tip of the small penis papilla. The penis papilla is slightly asymmetrical, with the ventral part somewhat larger than the dorsal one because of the dorsally displaced position of the ejaculatory duct. The penis papilla shows a division into two parts, a basal part with a thin epithelium underlain by a strong layer of circular muscles and a distal part covered with claviform epithelial cells with basal nuclei, but devoid of musculature. The two parts are separated by a constriction. The penis papilla has the same length as the penis bulb (Fig. 2B). In only one specimen (ZMA V.Pl. 7100.4) the penis papilla is twice as large as the penis bulb. In all examined specimens no penis glands were evident. The penis is located dorsally to the adenodactyl, while the bursal canal is situated to the left of the midline. The penis length is about 1/3 to 1/4 of the length of the adenodactyl depending on the state of contraction of the latter organ.

The adenodactyl is very large and consists of a free papilla and a well-developed bulbar part. The bulb is dorsal-ventral in orientation, and the adenodactyl makes a sharp posterior bend on the transition of the bulb to the papilla. In the majority of the examined mature specimens the adenodactyl is thrust out of the planarian body; in the holotype ZMA V.Pl. 7100.1 its tip is pushed into the parenchyma caudally to the copulatory apparatus (Fig. 2B). All of these conditions are very likely due to preservation artifacts. The bulb consists of rows of alternating longitudinal and circular muscles fibers. The lumen of the adenodactyl is lined by a layer of ciliated cells, and it is surrounded by a well-developed zone of glandular mesenchymatic tissue. Through this section of the mesenchyme runs a layer of circular muscle that is particularly evident in specimens ZMA V.Pl. 7100.4, ZMA V.Pl. 7100.5, and ZMA V.Pl. 7101.2. In the last-mentioned specimen, stained in Mallory-Cason, this layer of circular muscle is pale blue, whereas the intermingled muscles stain bright blue. Ectally to this zone of circular muscles runs a layer of longitudinal muscles fibers that stains red.

The male atrium is lined by a columnar epithelium, and it is surrounded by a subepithelial layer of circular muscles followed by three layers of longitudinal fibers. The male atrium communicates via a constriction with the common atrium. In this part it receives the opening of the common oviduct (Fig. 2B).

Discussion. The external morphology of *D. mariae* resembles that of *D. adenodactylosum* (Stanković and Komárek, 1927), both in the shape of the body and the frequent presence of supernumerary eyes. However, in *D. mariae* the eyes are arranged in two longitudinal, almost

straight rows, whereas in *D. adenodactylosum* they form two curved rows (see Kenk, 1978, p. 47).

Dendrocoelum mariae shares the presence of a very large adenodactyl, larger than the penis, with *D. adenodactylosum*, *D. maculatum* (Stanković and Komárek, 1927), *D. lacustre* (Stanković, 1938), *D. lychnidicum* (Stanković, 1969), *D. ochridense* (Stanković and Komárek, 1927), *D. sanctinaumi* (Stanković and Komárek, 1927), *D. minimum* Kenk, 1978, *D. nausicaae* (Stanković and Komárek, 1927), and *D. dani* Bromley, 1982. All of these species are endemic to the Lake Ohrid area, with the exception of *D. nausicaae* and *D. dani*. *Dendrocoelum nausicaae* is one of the most widely distributed species of the genus, together with *D. lacteum* Müller, 1774 and *D. album* (Steinmann, 1910), while *D. dani* is only known from northern Israel (Gourbault, 1972; Kenk, 1978; Bromley, 1982). Furthermore, *D. mariae* is similar to *D. adenodactylosum* and *D. maculatum* with respect to the anatomy of the adenodactyl. In the latter species the adenodactyl is characterized by the presence of a zone of fine circular muscle fibers that runs through the mesenchyme of the papilla. This particular adenodactyl anatomy, called the Balkan type, is also reported for *D. lacteum*, three recently described new species from Tunisia (Harrath et al., 2012), and the other two new species from Sardinia reported in the present paper. However, *D. mariae* differs from *D. adenodactylosum*, *D. maculatum*, *D. lacustre*, *D. lychnidicum*, *D. ochridense*, *D. sanctinaumi*, *D. nausicaae*, and *D. minimum* in the absence of the penis glands and the glandular field of tall epidermal cells surrounding the gonopore that is characteristic for these species. The glandular field around the gonopore and peculiarities in the histology of the oviducts (presence of numerous pear-shape cells) were considered by De Beauchamp (1931, 1932) to be distinguishing characteristics of the subgenus *Neodendrocoelum* Komárek, 1926. This subgenus comprises a homogeneous group of species with eyes, restricted to the Dinaric area and recorded chiefly from Lake Ohrid and its tributary streams and springs. According to Komárek (1926) all of these Balkan species are characterized by a large penis and a large adenodactyl, the latter being bigger than the penis, and by the presence of two atrial compartments. *Dendrocoelum mariae* differs from *D. dani* in the absence of a copulatory bursa in the latter that is replaced by bursal-intestinal communications (Bromley, 1982).

In most of the known species of the genus the copulatory apparatus is positioned just behind the pharyngeal pocket, while the penis and the adenodactyl in the majority of species are localized on the opposite sides of the body. In contrast, in *D. mariae* the copulatory apparatus is situated far behind the pharyngeal pocket, while the penis and the adenodactyl are always located dorsally and ventrally, respectively.

The new species bears a true sphincter in the terminal tract of the bursal canal. This prominent structure is mainly formed by several layers of circular muscle

surrounding a distinct narrowing of the bursal canal lumen (see separate discussion below).

DENDROCOELUM NURAGHUM STOCCHINO AND SLUYS SP. NOV.
(Table 2; Figs. 1, 3)

Material examined. Holotype: ZMA V.Pl. 7102.1, Monte Majore Cave (40°30'51"N, 8°36'37"E), 13 May 2009, coll. B. Cadeddu and P. Marcia, sagittal sections on 4 slides.

Other material: CGAS Pla 2.1, Monte Majore Cave (40°30'51"N, 8°36'37"E), April 2005, coll. P. Marcia and G. Tomasin, sagittal sections on 24 slides.

Etymology. The specific epithet refers to nuraghe, the typical Bronze Age megalithic edifice symbol of Sardinia.

Geographical distribution. Endemic to the Monte Majore Cave and only known from the type locality.

Habitat. Specimens of *D. nuraghum* were found in the Monte Majore Cave at ca. 530 m asl, under pebbles in a small streamlet at ca. 130 meters from the cave entrance in completely dark conditions (Fig. 1). Planarians were associated with *Proasellus* sp. (Asellidae). Although several sampling campaigns (20 in total) were held in a period of five years (2005 to 2010) only 4 planarians were found on three occasions during the spring (April, May). Only three of the four individuals collected were sexual; one of these sexual specimens did not survive under laboratory conditions. The immature, asexual specimen was processed for karyological analyses (not reported in this paper).

Diagnosis. *Dendrocoelum nuraghum* is characterized by the presence of a single common vas deferens, a large penis papilla with introverted apical part, a very large adenodactyl located to the left of the penis papilla, the presence of a sphincter in the terminal tract of the bursal canal, and by the posterior extension of the testes to only the level of the root of the pharynx.

Description. Live adult animals had a body size of ca. 8 mm in length and a width ranging from ca. 2 mm in the central part of the body to ca. 0.6 mm at the level of the head. They are unpigmented and fairly transparent, so that the intestinal branches, pharynx, and copulatory apparatus are clearly visible. The anterior end is truncated, with a convex mid-frontal margin, and is provided with a pair of rounded lateral lobes (Fig. 3A).

The two eyes are situated rather closely together. There is a slight neck-like constriction just behind the eyes, after which the body gradually widens, attaining its maximum width at the level of the pharynx and the copulatory apparatus. The distance between the eyes is 1/3 to 1/4 of the width of the neck. Inter-ocular distance is smaller than the distance from each eye cup to the lateral margin, and the distance from the frontal margin to the eyes is greater than the distance to the lateral margin.

The subterminal anterior adhesive organ has a length slightly greater than the inter-ocular distance and consists of a shallow cup. The surface of the organ consists of an area of infranucleated epithelial cells pierced by numerous gland ducts. The cell bodies of the very abundant glands

are scattered throughout the mesenchyme of the anterior part of the body. They pass above the brain and then above and under the intestinal diverticula, reaching up to the anterior surface of the ovaries. The musculature of the adhesive organ consists of a more strongly developed section of the usual ventral longitudinal body musculature.

The intestine is well visible, the anterior ramus reaches to a level just behind the neck and bears 7 or 8 branches on each side. The two posterior rami bear 16 or 17 branches. The pharynx is located in the posterior half of the body and measures about 1/7 of the body length (Fig. 3A). Its internal muscle zone consists of a thick layer of intermingled circular and longitudinal fibers. A subepithelial layer of longitudinal muscles followed by a layer of circular fibers forms the thin outer zone of muscles.

The two ovaries occur on the ventral side of the anterior region behind the brain. The ovaries are located at less than 1/3 of the distance between the brain and the root of the pharynx. The oviducts originate from the latero-dorsal part of the ovaries and are provided with a slight expansion at their anterior end, the tuba. The oviducts run posteriorly, converging behind the copulatory apparatus to form a single common oviduct. The latter runs anteriorly to the right side of the opening of the bursal canal and opens into the end part of the male atrium. The distal part of the oviducts and the first tract of the common oviduct receive the openings of the shell glands (Fig. 3B).

The numerous well-developed testes are essentially ventral in position, but some are situated in a dorsal position or in the middle of the body. Some follicles are oval in shape and occupy almost the entire dorso-ventral space of the body. The testes extend from a short distance behind the ovaries to the level of the root of the pharynx. The sperm ducts form very large spermiducal vesicles, packed with sperm, between the mouth and the anterior level of the penis bulb (Fig. 3B).

In the holotype (ZMA V.Pl. 7102.1) the copulatory apparatus is localized just behind the pharyngeal pocket, while in CGAS Pla 2.1 specimen the copulatory apparatus occupies the posterior half of the postpharyngeal region. The penis is located to the right, the bursal canal and the adenodactyl to the left of the midline. The copulatory bursa has the shape of a large sack that occupies almost the entire dorso-ventral diameter of the body. The bursa is lined with a tall glandular epithelium and is surrounded by a layer of longitudinal muscle. The bursal canal runs posteriorly to the left of the penis and gradually widens, then turns ventrally to open into the common atrium. The wall of the bursal canal consists of a nucleated epithelium with ciliated cells. The canal is surrounded by a layer of circular muscle, followed by a layer of longitudinal fibers. At a short distance from the opening in the common atrium the canal lumen reduces, while the circular muscle layer becomes very thick; this tract of the bursal canal is enveloped by a sphincter (Fig. 3C).

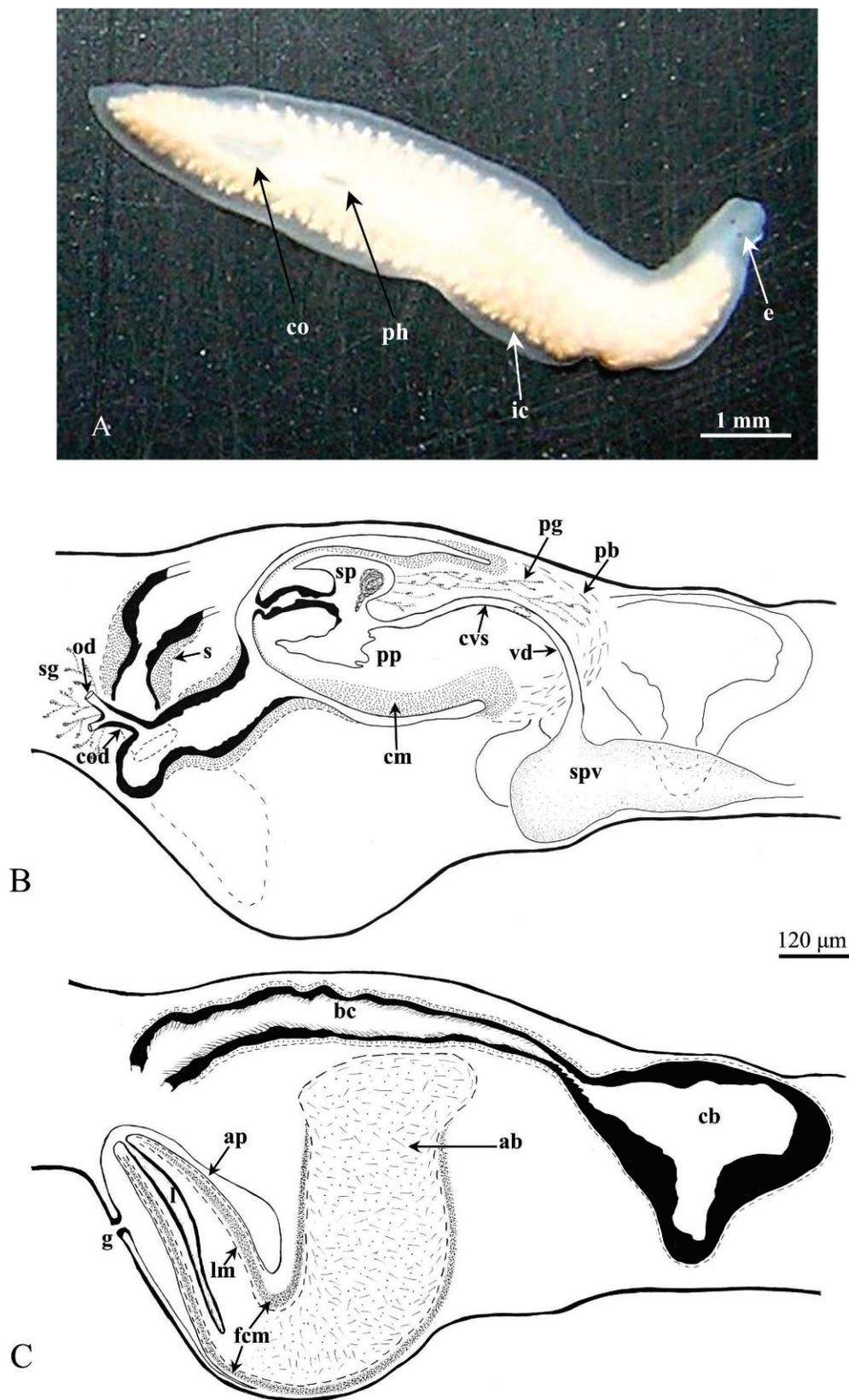


Figure 3. *Dendrocoelum nuraghum*. (A) habitus of a living specimen, (B-C), holotype ZMA V.Pl. 7102.1, sagittal reconstructions of the copulatory apparatus (anterior to the right).

In the male copulatory apparatus the muscular penis bulb is of moderate size. The vasa deferentia penetrate the penis bulb separately, but very close to each other, at its anterior part, and then they converge toward the midline of the penis bulb to form a common vas deferens that opens into the anterior part of the penis papilla lumen (Fig. 3B). The long penis papilla measures 3/4 of the total penis length. The penis length is ca. 1/2 of the adenodactyl length. The penis papilla is barrel-shaped in the holotype and finger-shaped in specimen CGAS Pla 2.1; the latter condition may be due to a preservation artifact.

The penis papilla is covered by a very thin epithelium at the basal part that becomes thicker at the apical part. The epithelium is underlined by a thick layer of circular muscle that is thicker in the ventral part and at the base of the papilla. In both specimens examined the apical part of the penis papilla lacks muscle layers and appears introverted into the very large papilla lumen like a pseudoflagellum (Fig. 3B; see discussion on flagellum below).

In the penis papilla lumen of the holotype a pack of sperm is present. The penis is rich in glands, which in the holotype are located in the penis bulb and in the dorsal part of the papilla. These glands open into the large penis lumen, which is full of secretion.

The adenodactyl is very large and consists of a free papilla and a well-developed, very muscular bulbar part (Fig. 3C). The free papilla is about 1/2 of the total adenodactyl length. The bulb starts in the dorsal part of the body and has a dorsal-ventral orientation. It consists of rows of intermingled longitudinal and circular muscles fibers covered by a thin layer of longitudinal fibers. Ectally to this thin longitudinal muscle layer runs a thick layer of fine circular muscle fibers that continues its course through the mesenchyme of the adenodactyl papilla (Fig. 3C). In specimen CGAS Pla 2.1, stained in Dane Herman's tetrachrome, this thick layer is grey and contrasts with the other muscular fibers, which stain bright yellow. In the holotype, stained in Mallory-Cason, this layer is pale blue, the longitudinal fibers stain red, and the intermingled muscles stain bright blue. The free papilla, which protrudes posteriorly and dorsally into the atrium, is characterized by a thin lining epithelium underlain by three layers of muscles: a subepithelial layer of longitudinal muscle, a thick layer of fine circular muscle fibers, and a thin inner layer of longitudinal muscle.

The male atrium consists of an anterior part that houses the penis papilla and a canal leading obliquely, in a postero-ventral direction, into the common genital atrium. This canal is lined by a thick epithelium and is surrounded by a strong layer of circular and longitudinal muscle fibers. The common atrium receives the opening of the bursal canal.

Discussion. *Dendrocoelum nuraghum* is characterized by the fusion of the two vasa deferentia into an intrapenial common duct. Within the genus *Dendrocoelum* this character is reported for only four other species: *D.*

jablancense (Stanković and Komárek, 1927), *D. puteale* Kenk, 1930, *D. kenki* De Beauchamp, 1937, and *D. constrictum* Harrath and Sluys, 2012. *Dendrocoelum nuraghum* differs from *D. puteale* and *D. kenki* because these are both anophthalmous species. *Dendrocoelum puteale* is characterized by an extrabulbar seminal vesicle that receives in its right part a common vas deferens, originating from the fusion of the two vasa deferentia at a level just behind the copulatory bursa (De Beauchamp, 1932). In *D. kenki* the vasa deferentia unite just before entering the penis bulb. Moreover, in this species the penis is in the middle, while the adenodactyl is on the right side of the body (De Beauchamp, 1937), in contrast to *D. nuraghum* in which the penis is localized on the right and the adenodactyl on the left of the midline of the body.

Dendrocoelum nuraghum is similar to *D. jablancense* because its vasa deferentia also separately penetrate the penis bulb and fuse to a common duct before opening into the penis papilla lumen. However, *D. nuraghum* differs from *D. jablancense* in the absence of the glandular field of tall epidermal cells that in the latter surrounds the gonopore. Moreover, the adenodactyl in *D. nuraghum* is on the left of the midline of the body, whereas in *D. jablancense* it is on the right. The last-mentioned species is also characterized by a double communication between the bursal canal and a common atrium (see Kenk, 1978), which is absent in *D. nuraghum*.

Dendrocoelum nuraghum is characterized by an irregular distribution of the testes (ventral, dorsal, and between the intestinal diverticula), which extend posteriorly to the level of the root of the pharynx. In contrast, in *D. puteale* and in *D. jablancense* the testes extend to the tail end and are rather dorsal and ventral, respectively. In *D. kenki* the testes are mainly ventral and extend posteriorly to the level of the copulatory bursa.

Dendrocoelum nuraghum has a distinct narrowing in its bursal canal, surrounded by a sphincter. This character is shared with two other Sardinian dendrocoelids (see below).

The large penis and large adenodactyl of *D. nuraghum* are shared with *D. adenodactylosum*, *D. maculatum*, *D. lacustre*, *D. lychnidicum*, *D. ochridense*, *D. sanctinaumi*, *D. minimum*, *D. nausicaae* and *D. dani*. *Dendrocoelum nuraghum* differs from *D. adenodactylosum*, *D. maculatum*, *D. lacustre*, *D. lychnidicum*, *D. ochridense*, *D. sanctinaumi*, *D. minimum*, and *D. nausicaae* in the absence of the glandular field around the genital pore that is present in all of these species.

DENDROCOELUM VESICULOSUM STOCCHINO AND SLUYS SP. NOV.
(Table 2; Figs. 1, 4, 5, 6)

Material examined. The population is characterized by the coexistence of two morphotypes: anophthalmous (morphotype 1) and two-eyed (morphotype 2) individuals.

Holotype: ZMA V.Pl. 7103.1, sagittal sections on 4 slides of a morphotype-2 animal, Sa Ucca è su Peltusu Cave (40°27'00"N, 8°40'43"E), 6 May 2009, coll. P. Marcia, B. Cadeddu, F. Stoch and G. Tomasin.

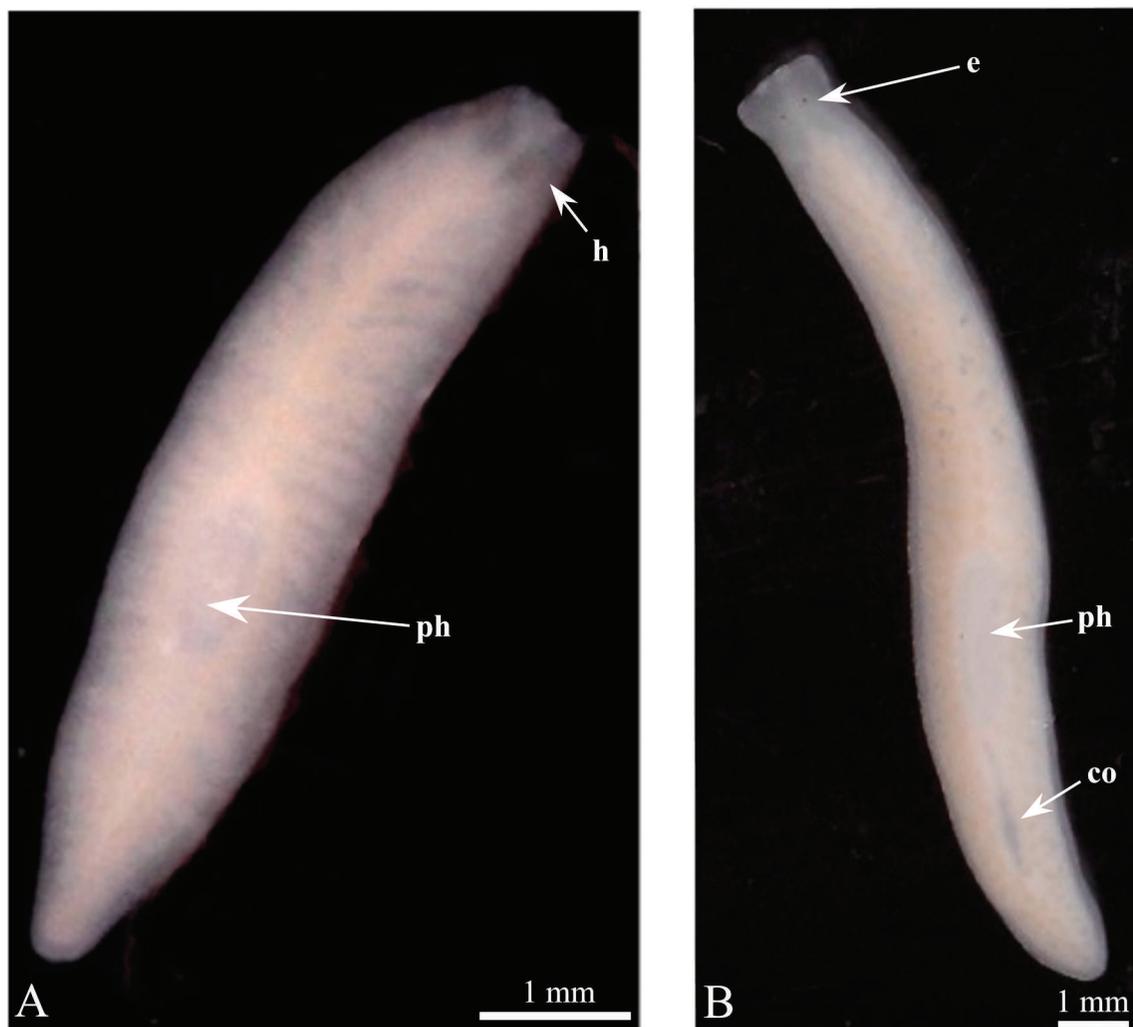


Figure 4. *Dendrocoelum vesiculosum*. Habitus of living specimens, (A) morphotype 1 characterized by the absence of eyes, (B) morphotype 2 with eyes.

Paratypes: CGAS Pla 3.1, *ibid.*, sagittal sections on 3 slides of a morphotype-2 animal; ZMA V.Pl. 7103.2, *ibid.*, sagittal sections on 3 slides of a morphotype-2 animal, ZMA V.Pl. 7103.3, *ibid.*, sagittal sections on 4 slides of a morphotype-1 animal.

Other material: CGAS Pla 3.2, sagittal sections on 6 slides of a morphotype-2 animal, not completely mature, Sa Ucca 'e su Peltusu Cave (40°27'00"N, 8°40'43"E) 3 September 2007, coll. P. Marcia and M. Fois; CGAS Pla 3.3, *ibid.*, sagittal sections on 13 slides, of a morphotype-1 animal, not completely mature; ZMA V.Pl. 7104.1, sagittal sections on 5 slides of a morphotype-1 animal, F1 offspring; ZMA V.Pl. 7105.1, one not completely mature morphotype-1 animal, resulting from the F2 offspring, sagittal sections on 3 slides.

Etymology. The specific epithet is derived from the Latin adjective *vesiculosus*, full of vesicles, and alludes to the presence of an expansion or vesicle in each vasa deferens.

Life cycle. Specimens of *Dendrocoelum vesiculosum* were collected in September 2007 and May 2009. On the first occasion two juveniles were collected, one specimen being anophthalmous and the other with two very small eyes. The life cycle of these individuals was observed for about three years under laboratory conditions. In this species the breeding period follows a seasonal cycle. From May to July the specimens developed a copulatory apparatus, while in summer-autumn they laid cocoons. In May 2008, after 7 months of rearing, a sexualization process was displayed in both specimens. In the following June the two animals produced 2 cocoons. From the only fertile cocoon five young anophthalmous planarians (F1) hatched after five weeks of development. Only three of these worms survived after 7 months of rearing (January 2009), and they became sexual in July 2009 and produced three cocoons during October 2009. From the only fertile cocoon four young anophthalmous planarians (F2) hatched after 4 weeks. In June 2010 the only surviving planarian became sexually mature.

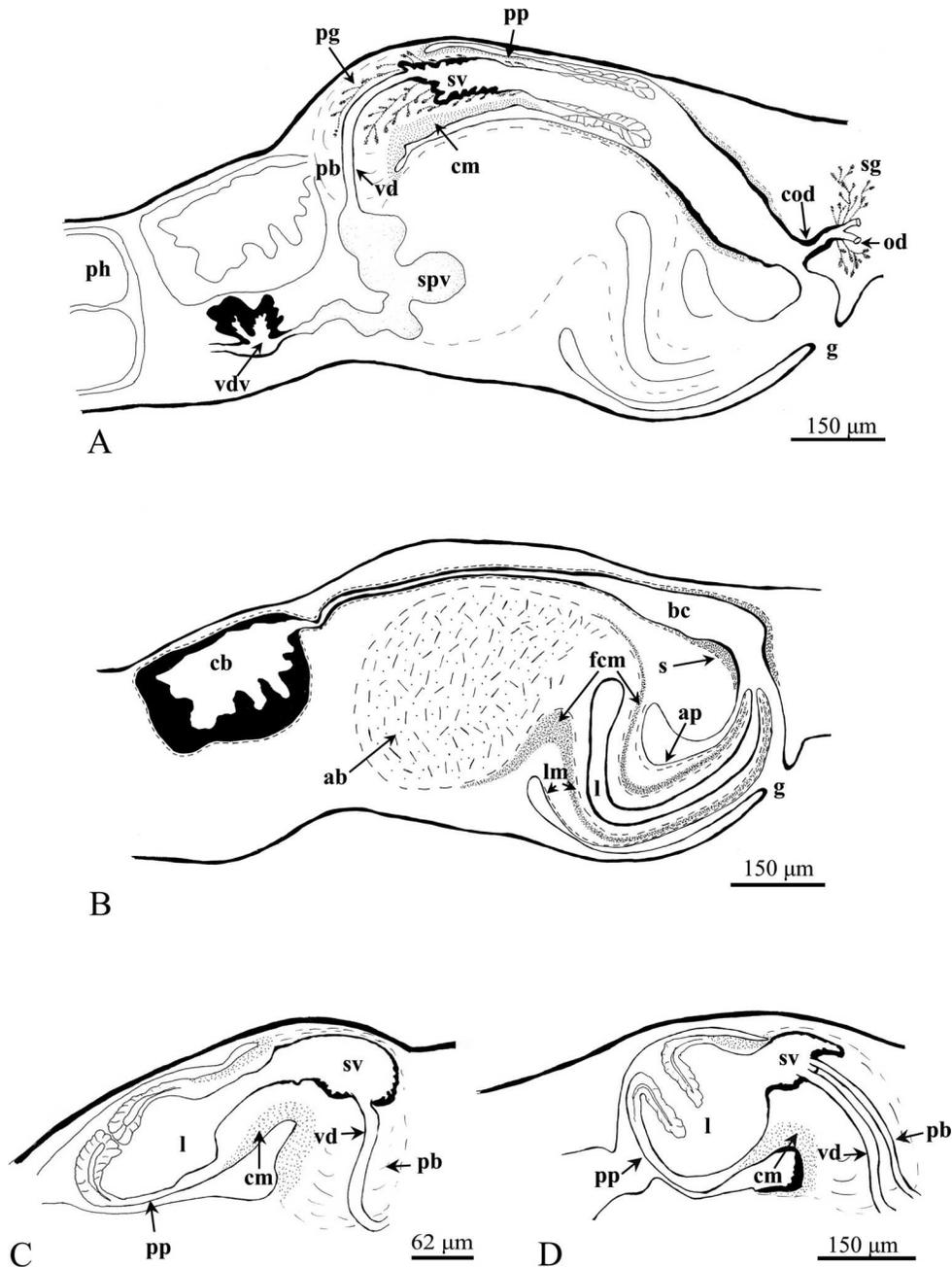


Figure 5. *Dendrocoelum vesiculosum*. (A-B) holotype ZMA V.Pl. 7103.1, sagittal reconstructions of the copulatory apparatus (anterior to the left), (C) CGAS Pla 3.1 sagittal reconstructions of the penis papilla (anterior to the right), (D) ZMA V.Pl. 7103.2, sagittal reconstructions of the penis papilla (anterior to the right).

The first two worms, i.e., the parents, were killed and preserved in July 2008, after the first offspring (F1) had been produced, one month after cocoon deposition. At that time, both gonads and the copulatory apparatus appeared to be in a state of regression, although abundant sperm were still present in the vasa deferentia. This condition of regression was particularly evident in the two-eyed specimen.

During the second collection, five specimens were captured, one sexual anophtalmous animal and four

with eyes, of which only two were sexually mature and eventually only one survived. The other two animals became sexually mature in January 2010, after eight months of rearing. All specimens were preserved at that time.

Habitat. Specimens of *Dendrocoelum vesiculosum* were found in totally dark conditions under pebbles in a small stream in the Sa Ucca 'e su Peltusu Cave at an altitude of ca. 560 m asl (Fig. 1). The dendrocoelids were associated with *Dugesia* sp.

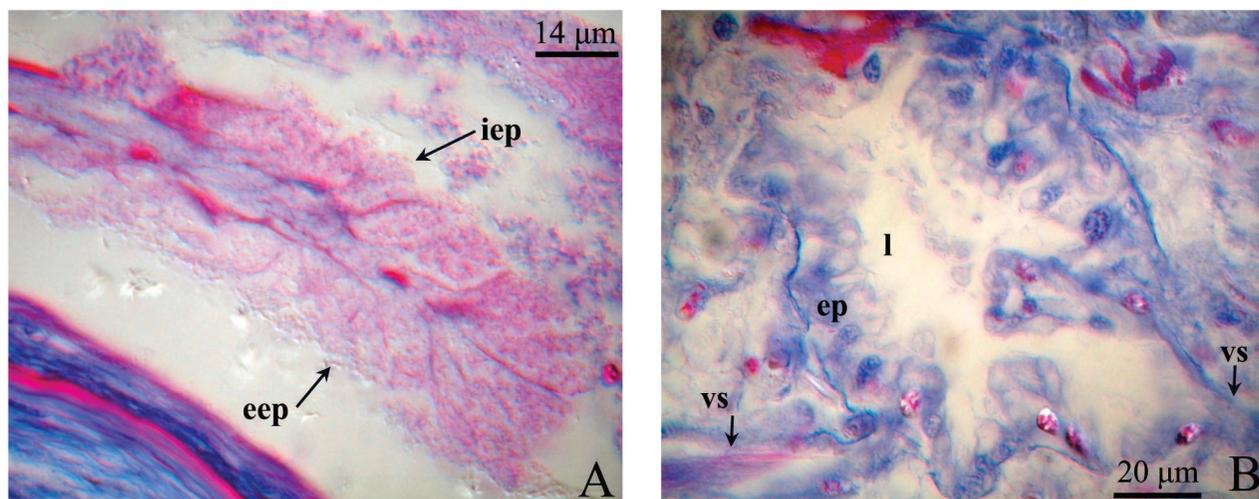


Figure 6. *Dendrocoelum vesiculosum*. (A) photomicrograph of the penis papilla tip showing the vacuolate secretory epithelium, (B) photomicrograph of the expansion/vesicle in the vas deferens.

Geographical distribution. Endemic to the Sa Ucca 'e su Peltusu Cave and only known from the type locality.

Diagnosis. *Dendrocoelum vesiculosum* is characterized by: the presence in the population of both anophtalmous and two-eyed individuals; a peculiar penis histology, with vacuolated cells lining the tip of the papilla; a convoluted adenodactyl; a sphincter in the terminal tract of the bursal canal; a very narrow horizontal tract of bursal canal; and the presence of a peculiar expansion in the vasa deferentia, different from the spermiducal vesicles.

Description. Live animals are unpigmented, typically whitish, with a body size of 8 to 10 mm in length and a width ranging from ca. 2 mm in the central part of the body to 1 mm at the level of the head. The anterior end is truncated with a pair of rounded lateral lobes (Fig. 4). In the ocular animals the eyes are small and placed not far from the frontal margin. Their distance from the frontal margin is slightly greater than the distance to the lateral margin. Inter-ocular distance is equal to the distance from each eye cup to the lateral margin. Just at level of eyes there is a slight constriction or neck that sets off the head from the rest of the body (Fig. 4B).

The anterior, subterminal adhesive organ, with a width slightly more than the inter-ocular distance, is feebly developed and consists of a small patch of epidermis with infranucleated cells that is pierced by numerous openings of glands ducts.

The pharynx is located in the posterior half of the body and measures somewhat less than 1/6 of the body length. The thin outer zone of muscles consists of a subepithelial layer of longitudinal muscle, followed by a layer of circular muscles. The inner epithelium is underlain by a thick layer of intermingled circular and longitudinal fibers.

The two ventral, paired ovaries are located at about 1/3 of the distance between the brain and the root of the

pharynx. The anterior-most sections of the oviducts are expanded to form a tuba. The oviducts run posteriorly, converge behind the copulatory apparatus, and unite to form a common oviduct that opens into the terminal tract of the male atrium. The common oviduct receives the openings of eosinophilic shell glands.

Well-developed rounded resorptive vesicles are present along the oviducts of all specimens examined. Each vesicle, characterized by the presence of vacuoles, communicates with the oviduct through a short, narrow duct (cf. Sluys, 1989). Sperm is present, both in the resorptive vesicles and in the short interconnecting ductules.

The well-developed, numerous testes are localized in both ventral and in dorsal position, while some follicles are situated in the middle of the body. The testes extend throughout the body from directly behind the ovaries to the tail end.

The sac-shaped copulatory bursa is situated just behind the pharynx. The bursa is lined with a tall glandular epithelium and is surrounded by a layer of longitudinal muscle. From the copulatory bursa the bursal canal runs posteriorly and horizontally to the left of the penis. This horizontal tract of the bursal canal is very narrow and is lined with a nucleated, ciliated epithelium, surrounded by only a thin layer of longitudinal muscle fibers. At the level of the insertion of the adenodactyl papilla the bursal canal widens, and from this point it is surrounded by a layer of circular muscle, followed by a layer of longitudinal fibers. Before opening into the atrium the bursal canal narrows again, while the circular muscles become thicker, thus forming a sphincter (Fig. 5B). This structure is most evident in those specimens in which the tip of the adenodactyl papilla is not deeply inserted into the bursal canal.

The well-developed penis bulb, formed by intermingled circular and longitudinal muscle fibers, houses a large

seminal vesicle that is lined by a vacuolated epithelium the cells of which protrude irregularly into the lumen (Fig. 5A).

In all specimens examined the two vasa deferentia run ventrally, and when the ducts arrive at the level of or just before the copulatory bursa they show a peculiar expansion of their dorsal wall, thus forming a large vesicle that can be either empty or full of sperm. The wall of this vesicle is lined by tall, claviform, vacuolated cells, very different from the rest of the ducts, which are lined with a thin epithelium (Figs 5A, 6B).

In specimen CGAS Pla 3.1 abundant sperm is present in the lumen of the two posterior intestinal branches. In the same specimen the right vas deferens shows at the level of the expansion a connection with the lumen of a posterior intestinal branch, most likely due to a preservation artifact.

After having given rise to an expansion, the vasa deferentia form well-developed spermiducal vesicles, packed with sperm. Thereafter the ducts curve to the dorsal side, in some cases (ZMA V.Pl. 7103.1, ZMA V.Pl. 7103.2, CGAS Pla 3.1) almost reaching the dorsal part of the body, and penetrate the penis bulb to open, closely together, into the antero-lateral section of the seminal vesicle. In specimens ZMA V.Pl. 7103.2 and ZMA V.Pl. 7103.3 the right vas deferens enters the penis bulb dorsally to the left one. The penis is rich in glands that open into the seminal vesicle and into the penis papilla lumen (Fig. 5A).

The penis papilla is characterized by a thick basal part and a thinner distal part. The basal part is covered with a flat epithelium that is underlain by a thick layer of circular muscle. The epithelium on the apical part consists of large, rounded, and vacuolated cells, with basal nuclei, very rich in secretion granules. This epithelium covers both the external and the internal distal part of the penis papilla (Figs 5A, 6A). In the holotype (ZMA V.Pl. 7103.1) sperm is present in the male atrium.

The penis papilla may assume different shapes. In the holotype and in ZMA V.Pl. 7103.3 it is considerably elongated and provided with a tunnel-shaped lumen (Fig. 5A). In CGAS Pla 3.1 the papilla is more contracted and therefore provided with a wider lumen (Fig. 5C). In ZMA V.Pl. 7103.2 the contraction and inversion of the tip is more advanced and thus forms a pseudo-flagellum (Fig. 5D).

The adenodactyl consists of a well-developed bulbar part and a free papilla. The bulb is very large and occupies almost the entire dorso-ventral space of the body (Fig. 5B). In all examined specimens the shape of the entire adenodactyl is sinuous, in that the bulb originates at the ventral region of the body and then gradually extends to the left with a dorso-lateral orientation. The adenodactyl papilla first has a dorso-ventral orientation and then makes a posteriorly directed bend, with the tip pointing into the terminal tract of the bursal canal. Due to the large size, the particular orientation, and the different states of contraction of the adenodactyl, the penis may be localized at

different positions. In specimens ZMA V.Pl. 7103.2, ZMA V.Pl. 7103.3, and ZMA V.Pl. 7104.1 the penis is located on the right side of the adenodactyl, whereas in CGAS Pla 3.1 and ZMA V.Pl. 7103.1 the penis is located on the left side of and dorsally to the adenodactyl, respectively.

The bulb of the adenodactyl consists of intermingled rows of longitudinal and circular muscle, bounded by a thin layer of longitudinal fibers. Ectally to this thin longitudinal muscle layer runs a thick layer of fine circular muscle fibers that continues its course through the mesenchyme of the adenodactyl papilla. Ectally to this zone of circular muscles runs a thin layer of longitudinal muscle fibers (Fig. 5B). In all examined specimens, stained in Mallory-Cason, this layer of fine circular fibers is pale blue, while the longitudinal fibers stain red and the intermingled muscles stain bright blue. No glands were observed to discharge into the lumen of the adenodactyl.

The male atrium is lined by a nucleated epithelium surrounded by a subepithelial layer of circular muscles, followed by a layer of longitudinal fibers. Just before opening in the common atrium the male atrium receives the opening of the common oviduct.

Discussion. In the course of our long-term study of the life cycle, during two generations (3 years), only anophthalmous offspring was observed. Partial or total loss of eyes may result from adaptation to the subterranean environment. A seasonal life cycle, as observed in *D. vesiculosum*, has been reported for only a few hypogean species, such as *Atrioplanaria delamarei* (Gourbault, 1972). The majority of subterranean triclads produce cocoons during the entire year (Gourbault, 1972).

The number of cocoons in *D. vesiculosum* is very low (2 or 3). Generally, the number of cocoons produced by hypogean species varies between species, but it is always considerably lower than in epigeic species (Gourbault, 1972). The fertility of the cocoons is in hypogean triclads also very variable between species (Gourbault, 1972). In *D. vesiculosum* the fertility was 30 to 50% and only 4 or 5 young hatched from each cocoon, in agreement with the general condition of hypogean triclads, in which generally fewer than eight young hatch from a cocoon.

The juveniles of *D. vesiculosum* became sexually mature 8 to 12 months after hatching, in contrast to *D. collini* (De Beauchamp, 1919), which needs 4 to 5 months, and *Phagocata vitta* juveniles that become sexual after 9 or 10 months (Gourbault, 1972). However, the low number of young hatching from the cocoons is counterbalanced by an increase of longevity. A specimen of *D. mariae* was still alive after 6 years, although laboratory conditions are less harsh than those in situ, for example, with respect to food availability. Our observations on the life history illustrate considerable slowing down of the cocoon deposition rhythm, as well as embryonic and post-embryonic development (K strategy) and are in agreement with the adaptive strategies typical of subterranean fauna (Juberthie

and Decu, 1994) and in particular of stygobiont triclads (Gourbault, 1994). This strategy ensures a more efficient management of a low energy budget.

Dendrocoelum vesiculosum shows a peculiar penis papilla histology, characterized by the presence of large, vacuolated cells that surround both the internal and the external wall of the tip of the penis papilla. This character is unique for this species (see discussion on flagellum below).

Another unique character for this new species is the presence of a peculiar dorsal expansion in the vasa deferentia. This expansion differs from the usual spermiducal vesicles and has not been described earlier for any other species of the genus *Dendrocoelum*.

Dendrocoelum vesiculosum is characterized by a large adenodactyl and by a sphincter in the terminal tract of the bursal canal. These characters are shared with two other Sardinian species (see discussion on sphincter below).

Family PLANARIIDAE Stimpson, 1857

Genus *Phagocata* Leidy, 1847

Phagocata obscura Stocchino and Sluys sp. nov.

(Table 2, Figs. 1, 7)

Material examined. Holotype: ZMA V.Pl. 7106.1, sagittal sections on 34 slides, Cuccuru Tiria Cave (39°19'25"N, 8°34'29"E), 28 March 2006, coll. P. Marcia.

Habitat. Specimens of *Phagocata obscura* were found under pebbles in a stream running in totally dark condition in the Cuccuru Tiria Cave (Fig. 1). Planarians were associated with *Stenasellus* sp. (Stenasellidae) and Oligochaeta.

Etymology. The specific epithet *obscura*, from the Latin adjective *obscurus* (dark, somber, hidden, invisible), alludes to both the cave habitat of the species and the fact that some details of its anatomy could not be discerned in the material available.

Geographical distribution. Endemic to the Cuccuru Tiria Cave and only known from the type locality.

Diagnosis. *Phagocata obscura* is characterized by the absence of eyes, a conical head, the presence of a well-developed penis bulb containing a spacious seminal vesicle, a plug of cells in the ejaculatory duct, a common vas deferens, and testes located mainly in prepharyngeal position.

Description. Live animals are unpigmented, typically whitish, with a body size in an elongated state of ca. 6 mm in length and a width ranging from 1 mm in the central part of the body to 0.5 mm at the level of the head. The anterior end is obtusely pointed and devoid of eyes (Fig. 7A). The pharynx is located in the posterior half of the body and measures about 1/5 of the body length.

The two ovaries are located at a short distance behind the brain; they are small and poorly developed. The oviducts run dorsally to the ventral cords and curve dorsally at the level of the penis bulb. The oviducts are still

visible at this point, but their continuation and subsequent communication with the female copulatory apparatus could not be discerned.

The numerous but poorly developed testes are closely packed together in ventral position. They begin at a considerable distance behind the ovaries and extend posteriorly to the level of the root of the pharynx. Some follicles may extend to halfway along the pharyngeal pocket.

The copulatory apparatus is located at some distance behind the pharyngeal pocket. The small sac-shaped copulatory bursa is lined with a tall epithelium and is surrounded by a layer of longitudinal muscle. From the bursa, a narrow bursal canal, lined with a squamous to cuboidal, nucleated epithelium, runs dorsally to the male atrium. Near the posterior end of the male atrium the canal curves ventrally to open into the common atrium (Fig. 7B).

The penis bulb is well-developed, with a length approximately equal to that of the penis papilla; its musculature is formed by intermingled longitudinal and circular muscles fibers.

A single vas deferens opens into the proximal, anterior section of the intrabulbar seminal vesicle. This duct may represent a common vas deferens, originating from the union of the two vasa deferentia in the anterior part of the penis bulb, but such could not be discerned in the sections.

The epithelium of the elongated, finger-shaped penis papilla is underlain by a thin layer of circular muscle fibers. The ejaculatory duct runs centrally through the penis and opens at the tip of the papilla. The ejaculatory duct is histologically differentiated into two sections. A short anterior portion is enlarged to form a relatively spacious seminal vesicle or intrabulbar cavity that is lined with tall secretory cells with basal nuclei and surrounded by a layer of longitudinal muscle. The posterior part of this cavity is lined with much taller cells, forming a plug that fills the entire lumen of the canal (Fig. 7B). The second, distal section of the ejaculatory duct is lined by an epithelium with cuboidal cells.

The male atrium is lined by tall nucleated cells, surrounded by a thick layer of circular muscle.

Discussion. *Phagocata obscura* is the first anophthalmous species of the genus reported in Europe. According to Kenk (1978) all European species of *Phagocata* are "two eyed planariids with a truncated head." This trait appears to be consistently present in all species that were subsequently described (cf. Sluys et al., 1995; Vila-Farrè et al., 2011).

The plug of cells in the ejaculatory duct is shared with a group of eleven species (cf. Sluys et al., 1995; Vila-Farrè et al., 2011): *P. albissima* (Vejdovský, 1883), *P. armeniaca*, *P. undulata*, *P. bosniaca*, *P. macedonica* (Stanković, 1926), *P. illyrica* (Komárek, 1919), *P. maculata*, *P. dalmatica* (Stanković and Komárek, 1927), *P. ochridana*, *P. stankovici*

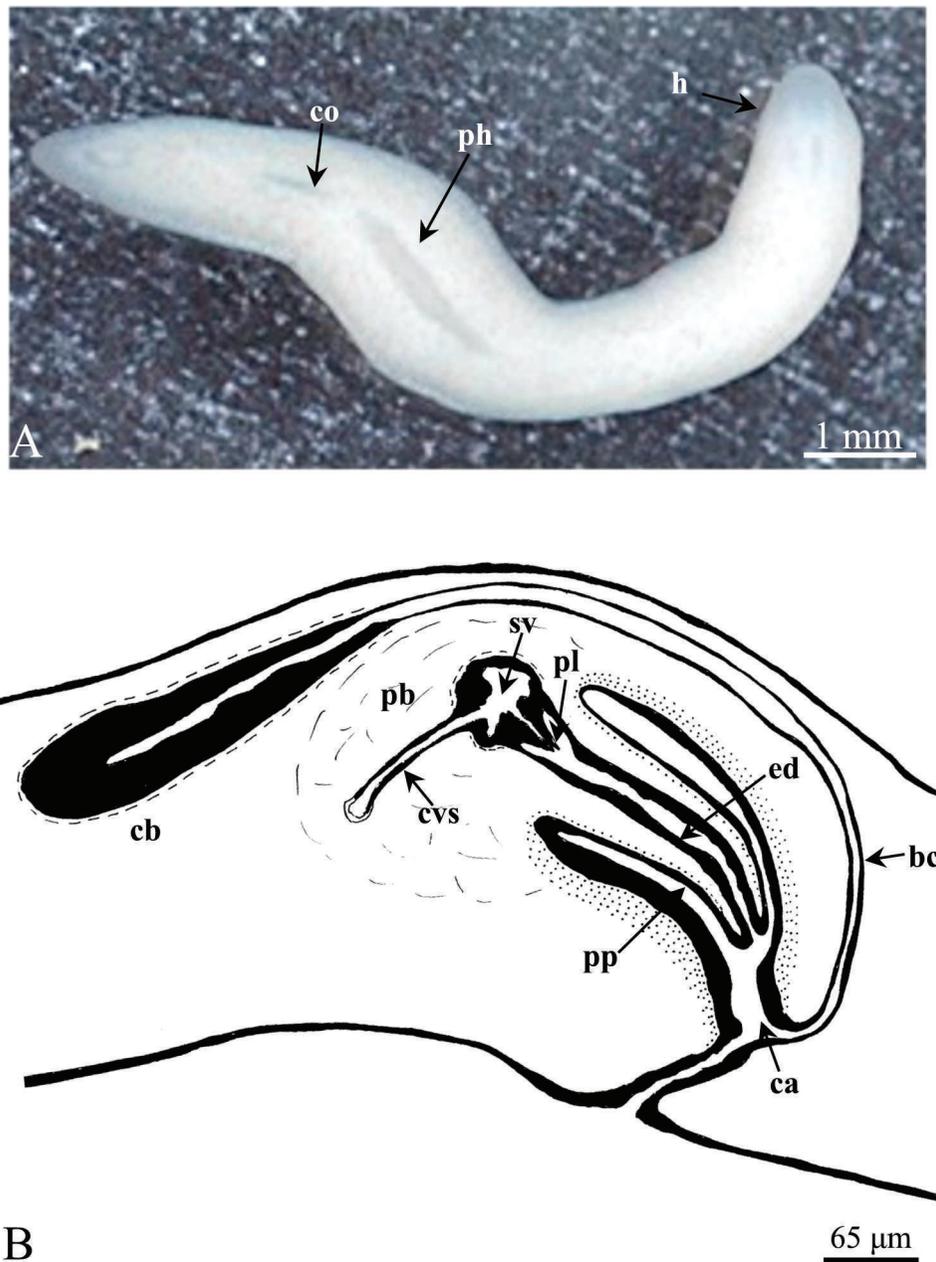


Figure 7. *Phagocata obscura*. (A) habitus of a living specimen, (B) holotype ZMA V.Pl. 7106.1, sagittal reconstruction of the copulatory apparatus (anterior to the left).

(Reisinger, 1960), and *P. hellenica* (Vila-Farré and Sluys, 2011). However, all of these species have two eyes.

Phagocata obscura shares its principally prepharyngeal testes with the North American species of *Phagocata*. In contrast to *P. obscura*, Palearctic species of *Phagocata* usually have their testes distributed through the entire body.

Although Stocchino et al. (2008) tentatively ascribed this material to *Atrioplanaria*, the present detailed morphological study highlighted that this is the first and only species of *Phagocata* recorded for Italy. Only *Phagocata* sp. was reported before now from Tuscany and Sardinia

(Benazzi, 1982; Stocchino, 2003). The few records of *P. vitta* reported for the Italian fauna (Bello et al., 1995; Ferreri, 1995) are not supported by a morphological account and should therefore be considered doubtful.

PHAGOCATA SP.

(Table 2; Figs. 1, 8A, B, D).

Asexual individuals from three populations were collected from the following three sites; absence of a fully developed copulatory apparatus prevented a more detailed assessment of their taxonomic status:

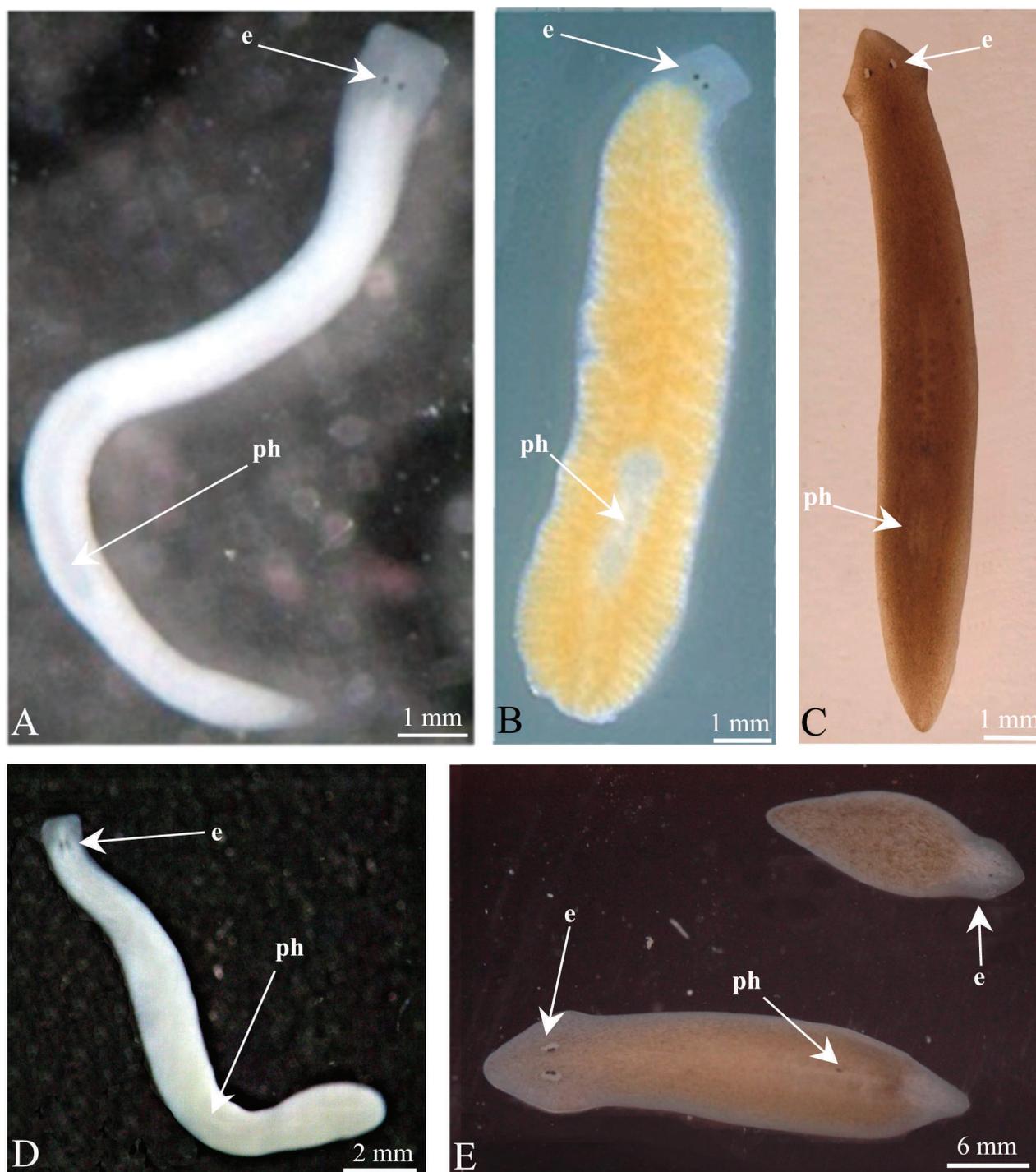


Figure 8. (A) *Phagocata* sp., habitus of a living specimen from the Monte Maggiore Cave, (B) *Phagocata* sp., habitus of a living specimen from the Elighe Mannu Spring, Asinara Island, (C) *Dugesia benazzii*, habitus of a living specimen from the Su Cantaru Spring (Monte Albo), (D) *Phagocata* sp., habitus of a living specimen from a spring at Alà dei Sardi, (E) *Dugesia* sp., habitus of live specimens from the Sa Ucca 'e su Peltusu Cave.

1) Monte Maggiore Cave (40°30'51"N, 8°36'37"E), coll. G.A. Stocchino and P. Marcia (Figs 1, 8A). Twenty-five specimens were collected during two occasions in June and July 2010. The specimens were found in a small pool with muddy bottom, originating from drip

water. The animals are whitish, very small and slender. The head is truncated, with two small eyes, situated close together and located far from the frontal margin. After more than one year of rearing they still did not show any sign of a sexualization process.

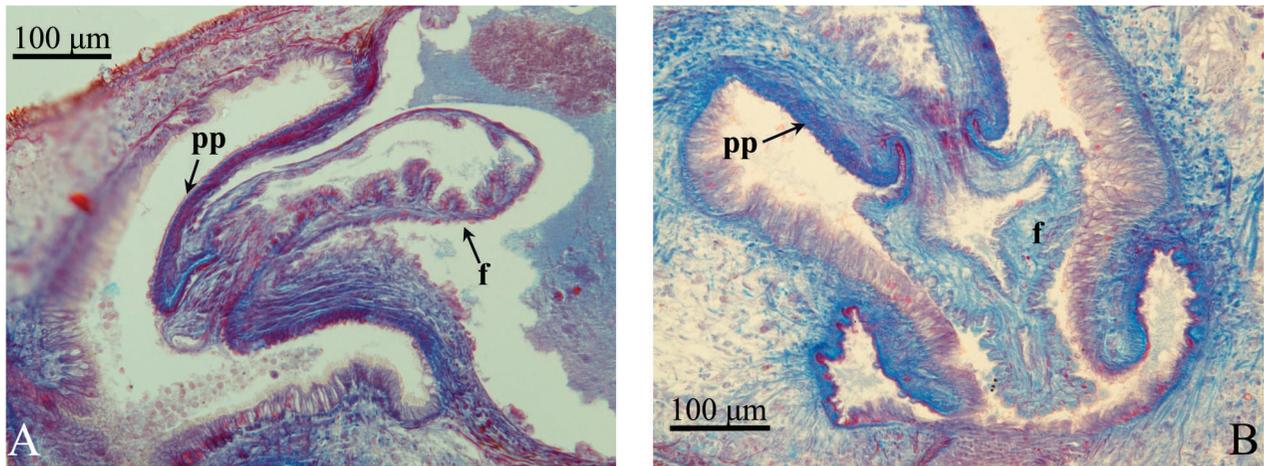


Figure 9. *Dendrocoelum lacteum* from the Ebro delta, Spain. (A) photomicrograph of an inverted, true flagellum (specimen RS 266 from the collections of NCB Naturalis-section ZMA), (B) photomicrograph of an everted, true flagellum (specimen RS 219).

- 2) Elighe Mannu Spring, Asinara Island (41°05'36"N, 8°18'21"E), December 2000–June 2002, coll. M. Piras and G.A. Stocchino (Figs 1, 8B). Eighteen asexual specimens were collected during eight collections made from December 2000 to June 2002. None of the specimens collected survived under laboratory conditions. The animals are unpigmented, with truncated head and with two small eyes located far from the frontal margin and situated close together. Planarians were associated with a diverse invertebrate fauna, including *Microdalyellia* sp. (Platyhelminthes, Dalyelliidae), *Ancylus* sp. (Gastropoda, Ancylidae), Planorbidae (Gastropoda, Pulmonata), Nematoda, Oligochaeta, Ostracoda, Cladocera, Cyclopoida, Hydracnida (Arachnida), Ephemeroptera, Dytiscidae, Hydrophilidae, Chironomidae, Culicidae, Ceratopogonidae, Polycentropodidae, and Rhyacophilidae.
- 3) Alà dei Sardi (40°38'60"N, 9°19'45"E), February 2010, coll. M. Fois (Figs 1, 8D). Just one individual was collected from a spring. The animal is unpigmented, whitish, with a body size in elongate state of ca. 7 mm in length and 1 mm in width. The head is truncated, with two eyes located far from the frontal margin. Each eye is accompanied by one small supernumerary eye.

GENUS *CRENOBIA* KENK, 1930
CRENOBIA ALPINA (DANA, 1766)
 (Table 2, Fig. 1)

Material examined. CGAS Pla 4.1-4, sagittal sections on 11 slides, 13 slides, 13 slides, 16 slides, respectively, immature specimens, Su Sessene Spring, 1368 m asl, western face of the Gennargentu Massif (40°01'40"N, 9°12'1"E), February 2004, coll. R. Fadda and M. Deiana (water temperature 7°C).

Comparative discussion. Unfortunately, all specimens collected were immature, i.e. without copulatory apparatus. Therefore, the animals were identified solely on the basis of external appearance and presence of only one

pharynx. The latter condition rules out the polypharyngeal forms *Crenobia alpina montenigrina* (Mrázek, 1904), *C. alpina anophthalma* (Mrázek, 1907), and *C. alpina teratophila* (Steinmann, 1908). This new record indicates a wider geographic distribution than previously known, although the species is restricted exclusively to the Gennargentu Massif springs, as reported earlier by Pala et al. (1980c).

Family DUGESIIDAE Ball, 1974
 Genus *Dugesia* Girard, 1850
Dugesia benazzii Lepori, 1951
 (Table 2; Figs. 1, 8C)

Material examined. ZMA V.Pl. 7107.1, one set of sagittal sections on 21 slides, ZMA V.Pl. 7107.2, one set of transverse sections on 37 slides, ZMA V.Pl. 7107.3, one set of horizontal sections on 7 slides, Su Cantaru Spring, Monte Albo (40°34'30"N, 9°40'31"E), 5 December 1998, coll. G.A. Stocchino and D. Salaris.

Comparative discussion. All specimens were sexual at collection. Identification of the species was based on histological study and karyological analysis. *Dugesia benazzii* is characterized by a finger-shaped penis papilla, a pointed diaphragm, a central course of the ejaculatory duct opening at the tip of the penis papilla, and symmetrical openings of the oviducts into the bursal canal. Specimens of *D. benazzii* from the Monte Albo Spring have a fold on the left of the penis papilla, like the majority of Sardinian populations (Lepori, 1951; Stocchino, unpublished data). The chromosomal number is $2n = 16$; $n = 8$. This is the first population of *D. benazzii* recorded from a Sardinian spring.

DUGESIA sp.
 (Table 2; Figs. 1, 8E)

Comparative discussion. Three fissiparous specimens were found under pebbles in a small stream running in the dark Sa Ucca 'e su Peltusu Cave (40°27'00"N, 8°40'43"E)

on 3 September 2007 (coll. P. Marcia and M. Fois), at an altitude of ca. 560 m asl (Fig. 1). Dugesiids were associated with *Dendrocoelum vesiculosum* (see above). After two months of rearing, 5 individuals were present, but they did not survive under laboratory conditions. This is the first record of dugesiids from a Sardinian cave.

GENERAL DISCUSSION

THE FLAGELLUM IN DENDROCOELID FLATWORMS

The term *flagellum* is often used *sensu lato* for the tip of a penis papilla that can be inverted, thus projecting into the lumen of the papilla. During the history of triclad taxonomy several attempts have been made to define the various states of inversion of the penis, notably how to differentiate between a so-called true flagellum and a pseudoflagellum. Komárek (1926) defined a true flagellum as a fold of the epithelium lining the internal surface of the penis papilla and mentioned as examples *Dendrocoelum lacteum* and *D. infernale* (Steinmann, 1907). Further, he mentioned *D. mrazeki* (Vejdovský, 1895) as an example of a flagellum that is merely formed by the invaginated distal part of the penis papilla. The same distinction had already been made by Steinmann (1909). He recognized that in most species the flagellum is “*nach innen gestülpt*” (folded inwards, inflected), whereas in *D. lacteum* and *D. infernale* the flagellum is “*umgestülpt*” (turned inside out). He also noted that in the flagellum of these two species the musculature is well-developed, notably the longitudinal muscles; the circular musculature is only weak.

Despite the studies of these workers, Hyman (1931, p. 318) considered presence or absence of an eversible flagellum to be an unreliable taxonomic character since “it has proved impossible to construct a precise morphological definition of the flagellum.”

Following up on the work of these earlier workers, however, we distinguish for the flagellum *s.l.* in the genus *Dendrocoelum* six main conditions in regard to the histology of the tip of the penis papilla and the extent of its inversion or invagination. Among our six conditions, the numbers 2, 3, and 4 correspond to the three main conditions distinguished earlier by De Beauchamp (1932).

- (1) Completely inverted penis papilla. In this case the entire penis papilla may be fully inverted or invaginated, e.g., *D. adenodactylosum*, *D. albidum* Kenk, 1978, *D. magnum* (Stanković, 1969), and *D. cf. beauchampi* (cf. Sluys and Benazzi, 1992).
- (2) Inverted tip of penis papilla. This condition refers to a histologically *homogeneous* penis (i.e., with no strong histological differences between the basal part and the apical part of the penis papilla) with an apical part that may be temporarily invaginated, as is the case in, for example, *D. mrazeki*.

- (3) Pseudoflagellum. This case is present in species with a penis papilla that can be divided into two parts, a basal part with a strong layer of circular muscle and a thin distal part with a few or no muscles and with an external epithelium (when it is everted) that is thicker than the basal part. In this case only the distal part of the papilla is invertible, while the basal part protrudes into the atrium. The pseudo-flagellum is more frequent when the apical part of the penis papilla is very thin with respect to the basal part. It is present, for example, in *D. maculatum*, *D. sanctinaumi*, *D. albidum*, *D. lacustre*, *D. minimum*, *D. translucidum* Kenk, 1978, *D. komareki* (Stanković, 1969), *D. nausicaae*, *D. remyi* De Beauchamp, 1926, *D. caspicum* Porfirjeva and Dyganova, 1973, *D. longipenis*, Komárek, 1916, and in the Sardinian *D. nuraghum* (this paper). A peculiar case is found in *D. tubuliferum* De Beauchamp, 1919, in which the apical part of the penis papilla has the shape of a tube, which may be considered to represent a pseudo-flagellum. A similar condition occurs in *D. hercynicum* Flösser, 1959 (cf. Sluys 2012).
- (4) True flagellum. This designates a tall, pleated section of the lining epithelium of the distal lumen of the penis papilla that may be everted or inverted, matching the definition of Komárek (1926) (Fig. 7). Moreover, the true flagellum is easily distinguished by the arrangement of its musculature, in that the circular fibers of the penis papilla reach only to the base of the flagellum and do not extend onto it. Therefore, the flagellum shows only longitudinal muscles underneath its tall, vacuolated epithelium. According to De Beauchamp (1932) and Gourbault (1972) the “true flagellum” is a character that only occurs in *D. lacteum* and *D. infernale*. Later, Kenk (1978) reported it also for *D. cruciferum* (Stanković, 1969). In *D. lacteum* the flagellum may become extraordinarily long, reaching the copulatory bursa of the partner and enveloping the sperms during copulation (cf. De Beauchamp, 1932, Fig. 36).
- (5) Inflated flagellum. The penis papilla is characterized by a basal thick part, with a squamous epithelium that is underlain by a strong layer of circular muscle, and a thinner distal part. The epithelium of this apical part is formed by large rounded vacuolated cells, with basal nuclei, very rich in secretion granules. This epithelium covers both the external and the internal apical part of the penis papilla. The penis papilla may assume different shapes, considerably elongated with a tunnel-shaped lumen, sac-shaped delimitating a wide lumen, or globular with its distal part inverted into a wider penis papilla lumen (like a pseudo-flagellum). This condition is present in the Sardinian species *D. vesiculosum* (this paper) and differs from the true flagellum because the vacuolated epithelium is present both on the outer and inner surface of the tip of the penis papilla (Fig. 6A).

- (6) Inflexible flagellum. The shape of this flagellum is independent of the degree of extension or contraction of the penis papilla and cannot be invaginated or inverted. It is present in the species *D. stenophallus* Codreanu and Bălcescu, 1967 and *D. nekoum* Sluys, 2012 (cf. Sluys, 2012).

SPHINCTER

All three new Sardinian *Dendrocoelum* species show around the terminal tract of the bursal canal a well-developed sphincter, i.e., an increase in the thickness of the circular muscle layer. Such a sphincter was first mentioned by De Beauchamp (1932) and, subsequently, by Kenk (1978) for *D. adenodactylosum*. However, examination of *D. adenodactylosum* material from the collections of the NCB Naturalis (section ZMA; specimens number RS157-1, 157-2), revealed that a well-developed coat of muscles is present on the vertically running part of the canal (i.e., after it has curved downwards to open into the atrium) consisting of alternating rows of longitudinal and circular muscle; the horizontal part of the bursal canal is merely surrounded by a layer of longitudinal muscle fibers. This was also correctly observed by De Beauchamp (1932, 1937), who described the vertical section to constitute a sphincter. However, it looks different from a proper sphincter, which probably induced Kenk (1978, p. 13) to refer to it as “sphincter”. Kenk (1978) reported the “sphincter” in *D. adenodactylosum* to be formed principally by circular muscles, contrary to our observations and those of De Beauchamp (1932). In our opinion, the difference between the musculature on the horizontal and vertical sections of the bursal canal merely gives the impression of the presence of a true sphincter. Furthermore, that this is not a true sphincter is also suggested by the fact that there is no distinct constriction of the lumen of the canal. All of this contrasts with the situation in the Sardinian species, which clearly show a true sphincter to be present in all three species, while the rest of the bursal canal is surrounded by a subepithelial layer of circular muscles, followed by a layer of longitudinal fibers.

A true sphincter is also present in, for example, *D. agile* De Beauchamp, 1932, *D. barbei* De Beauchamp, 1956, *D. atriostriatum* (Codreanu and Bălcescu, 1967), *D. caspicum*, *D. chujanum* Codreanu, 1943, *D. collini*, *D. cruciferum* (Stanković, 1969), *D. grimmi* Dyganova, 1983, *D. remyi*, and *D. romanodanubialis* (Codreanu, 1949).

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