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Sabussowia ronaldi sp. nov. (Platyhelminthes: Tricladida: Maricola), a new Mediterranean species and its life cycle

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Abstract

Sabussowia ronaldi sp. nov. is described from the Mediterranean Sea. It differs from its congeners, *S. dioica* and *S. wilhelmii*, for the presence of two testes, ventrally placed, instead of numerous (up to 90), dorsal testes. The bursal canal is short and non-ciliated in *S. ronaldi*, whereas it is long and ciliated in *S. dioica* and *S. wilhelmii*. Furthermore, *S. dioica* is a larger, gonochoristic species. Karyotype is different, with n = 7 in *S. dioica*, n = 6 in *S. ronaldi* n. sp. In *S. wilhelmii* the vasa deferentia fuse just before entering into the penis papilla, while they form a long common duct in the new species. *S. ronaldi* resembles *Cerbussowia cerrutii* in general topography of genital organs, and number and arrangement of testes. However, it differs in the presence of an unarmed penis papilla, and in the morphology of the ovaries. Cultured *Sabussowia ronaldi* sp. nov. specimens from the type locality had a life span of 71.7 ± 11.9 days (n = 20). Animals laid cocoons when about 2 mm long; cocoons were ovoid and unstalked; hatchlings emerged 28–35 days after deposition. Fertility was low, averaging 7.5 ± 3.5 offsprings per pair (n = 10). The relationships of the new species, and of the genus *Sabussowia* within the Cercyroidea, are discussed.

Key words: Tricladida, Maricola, new species, taxonomy, marine biodiversity

Introduction

Species of marine triclads (Platyhelminthes, Tricladida, Maricola) are in general relatively rare and often overlooked by researchers (Sluys & Kawakatsu 2005). Consequently, knowledge of diversity and distribution of the group is limited. The western Mediterranean Sea, with seven species of marine triclads, is one of the most species-rich areas of the world (Vila-Farrè et al. 2009). Recently, during sampling campaigns performed in Western Mediterranean under the sponsorship of the project BIOIMPA ("Biodiversity of Inconspicuous Organisms in Marine Protected Areas"), specimens of a minute marine triclad were found in subtidal samples collected in northern Sardinia (Italy). The study of these specimens revealed that they belong to a yet undescribed species, which is described and discussed below.

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Material and methods

Samples were collected by scuba diving by scooping up the superficial layer of sediment. Extraction of the animals from the sediment was accomplished using $MgCl_2$ decantation (see Martens, 1984). The animals were kept in the laboratory at a temperature of 18 ± 1 °C, and fed weekly with crushed specimens of *Sphaeroma* sp. (Crustacea: Isopoda).

The study of the life cycle of the species was based on ten pairs of newborn specimens. Each pair was placed in a 20 cc container, at the same laboratory conditions as the stock population. Once a week, specimens were counted, and the laying of cocoons and the emergence of offsprings were recorded. For microscopical analysis, mature specimens were fixed in Bouin's fluid, and embedded in 60 °C Paraplast. Serial sagittal sections were cut at 4 µm intervals, stained with Hansen's haematoxylin and eosin-orange and mounted in Eukitt.

The karyotype was determined from acetic orcein-stained spermatogonial mitoses, as described by Curini-Galletti et al. (1985). Relative lengths (r.l. = length of chromosome × 100/total length of haploid genome) and centromeric indices (c.i. = length of short arm × 100/length of entire chromosome) were obtained from measurements of the camera lucida drawings of metaphasic plates. Chromosome nomenclature used was that of Levan et al. (1964) (m = metacentric; sm = submetacentric; st = subtelocentric). Type material is stored in the collections of the Swedish Museum of Natural History (Stockholm, Sweden) (SMNH). Additional voucher material is stored in the collection of the Zoological Museum of the University of Sassari (Italy) (CZM).

Abbreviations used in figures: **b**, bursa; **cm**, circular musculature; **el**, eye lens; **in**, intestine; **lm**, longitudinal musculature; **od**, oviducts; **ov**, ovaries; **pp**, penis; **ph**, pharynx; **sd**, spermiducal duct; **sg**, shell glands; **sv**, seminal vesicle; **te**, testis; **vd**, vas deferens; **vi**, vitellaria.

Results

Superfamily *Cercyroidea* Sluys, 1989
Family Cercyridae Böhmig, 1906
Genus *Sabussowia* Böhmig, 1906

Sabussowia ronaldi sp. nov.

Type material. Holotype, one sagittally sectioned specimen (SMNH 7951); paratypes, 14 sagittally sectioned specimens (CZM 195–208), three horizontally sectioned specimens (CZM 209–211); two specimens used for karyology, all from the type locality.

Additional material. Two specimens sagittally sectioned; (CZM 212–213); three specimens used for karyology all from Sardinia, Capo Caccia, 'Il Porticato' cave (40°33'37.11" N, 8°9'50.60" E), about 24 m deep, in medium sand (September 2005).

Type locality. Sardinia: La Maddalena island, Cala Lunga, (41°15'31.28" N, 9°25'52.46" E), about 30 m deep, in medium sand (September 2009).

Etymology. The species is dedicated to Prof. Ronald Sluys (Netherlands) in recognition of his contribution to the study of marine triclads.

Diagnosis. *S. ronaldi* sp. nov. is characterized by the presence of (1) two large ovoid testes, (2) an unarmed penis papilla, (3) U-shaped ovaries and (4) lensed eyes with three retinal cells.

Description. Living, sexually mature specimens up to 3 mm in length. The body shape changes according to movements and muscular contraction: broadly ovate when resting, specimens become narrowly elongate anteriorly when moving; with a rounded posterior end (Fig. 1B–E). Unpigmented; the dorsal surface appears irregularly brownish due to gut content and the presence of numerous vitellaria. The external epithelium is ciliated; cilia are about 3.5 µm in length. However, the observation of details of body ciliation was hampered by the thickness of the mucous layer that covers the epidermis. Adhesive glands are present ventrally, at both anterior and posterior tip of the body.

The subepidermal musculature is well developed, especially at the ventral side.

With two eyes. The pigment cup is about 20 µm in diameter; three retinal cells are present in each cup. An oval lens is situated in the dorsal

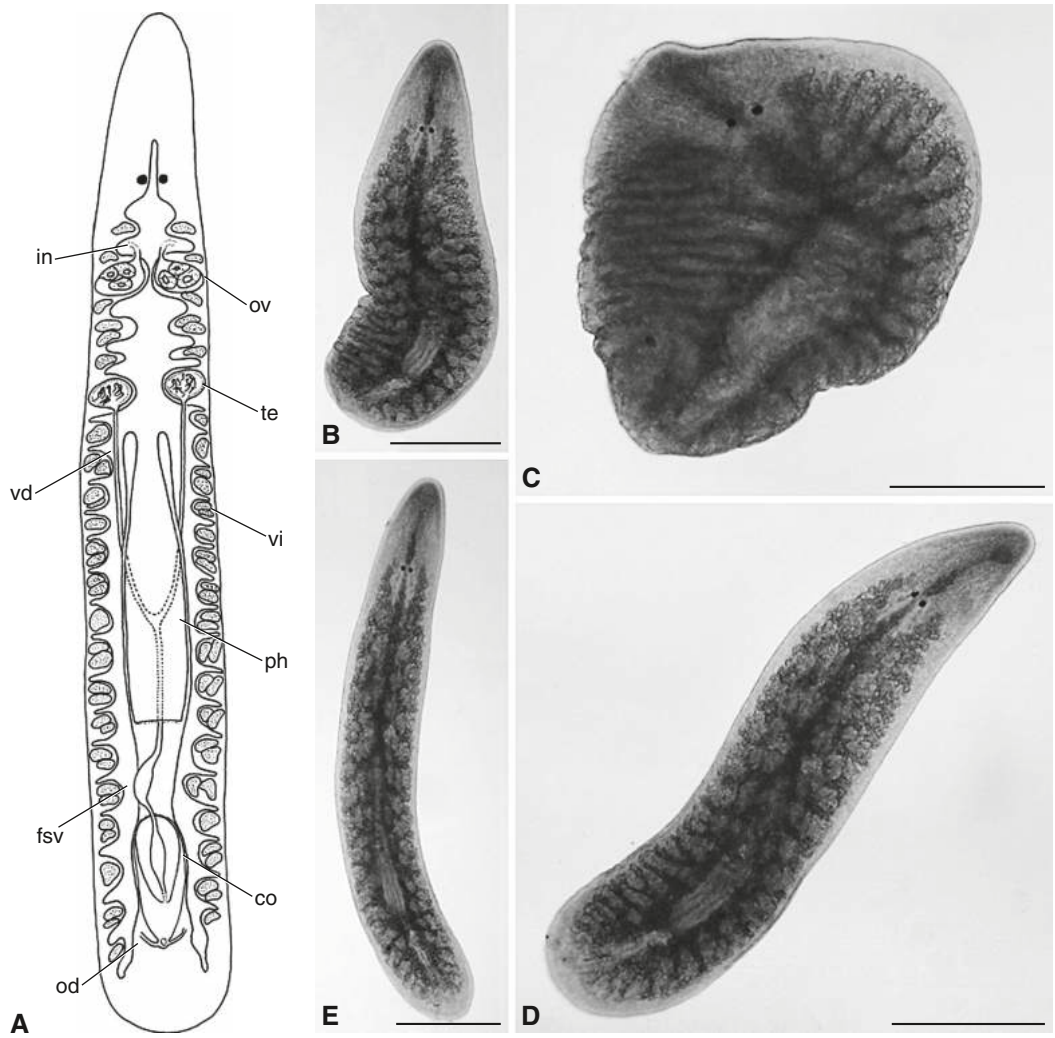

Fig. 1. *Sabussowia ronaldi* sp. nov. **A.** General organisation of a live animal. **B–E.** Pictures from live animals. Scale bars = 500 µm.

opening of the pigment cup and protrudes outside the cup itself (Fig. 3A). The lens stains differently from the surrounding tissue, appearing bright red in sections.

The pharynx (about 250 µm long in the holotype) is of the plicated type, and is located in the middle of the body. It is ciliated internally and externally, with the exception of the distal tip. The pharynx musculature is of the planariid type (see Kenk 1930, Sluys 1989): beneath the outer epithelium there is an outer layer of longitudinal muscle fibers, followed by an inner layer of circular fibers; while beneath the inner epithelium there is an outer layer of circular fibers and an inner layer of longitudinal fibers.

The anterior branch of the intestine extends anterior to the eyes, forming a preocellar diverticulum.

Male reproductive system. Two comparatively large, ovoid testes (about 95 µm long at their greatest width in the holotype), are present ventrally, anterior to the pharynx. At the level of the pharyngeal pouch, the two vasa deferentia join into a long common duct. This duct runs ventrally and widens in front of the penis bulb to form a spermiducal vesicle, which narrows

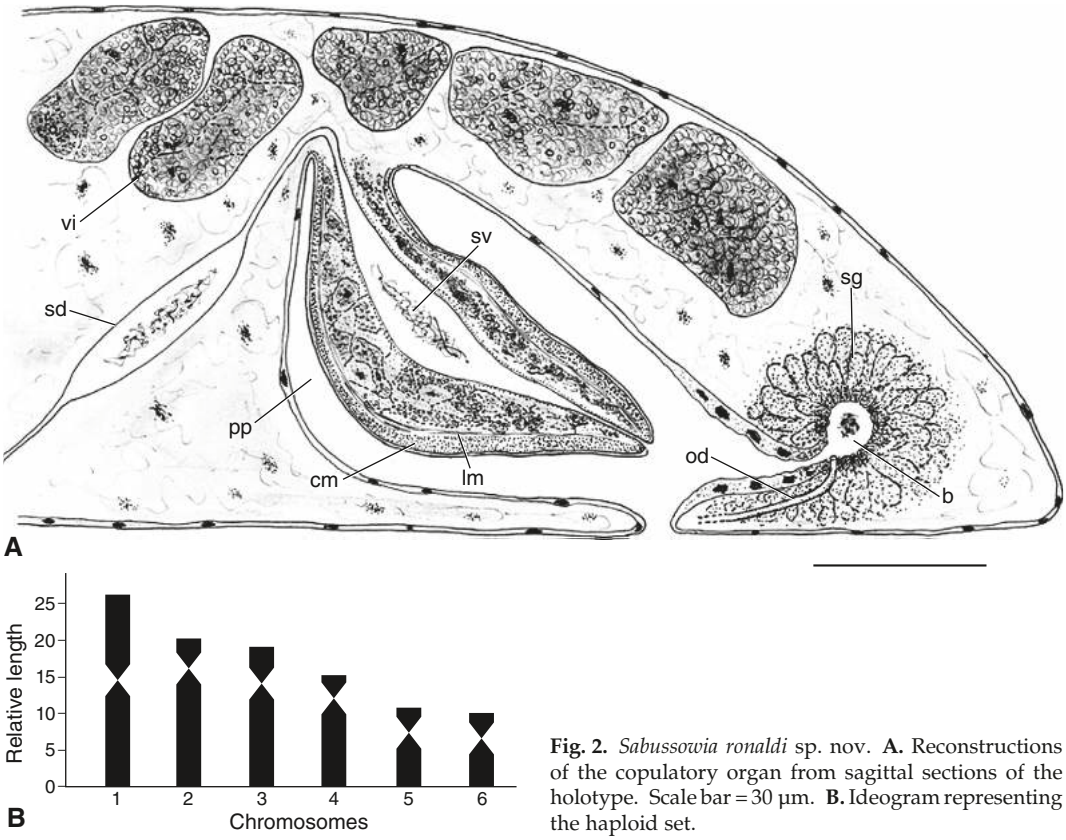

Fig. 2. *Sabussowia ronaldi* sp. nov. **A.** Reconstructions of the copulatory organ from sagittal sections of the holotype. Scale bar = 30 µm. **B.** Ideogram representing the haploid set.

before entering into the penis bulb. Inside the bulb a large seminal vesicle is present. It is connected to the ejaculatory duct.

The male copulatory complex is about 95 µm long in the holotype (range: 95–125 µm) (Figs. 2A, 3C). Its shape and position are strongly determined by fixation. In most specimens, the penis is oriented parallel to the dorsal and ventral body surfaces. In specimens CZM 203–206, however, it has a dorso-ventral orientation. The penis consists of a globular bulb (about 65 µm in diameter in the holotype), and a conical penis papilla. The latter is provided with a thick layer of subepidermal circular musculature (fig. 2B) followed by a much thinner layer of inner longitudinal muscles. The conical papilla is about 50 µm long in the holotype (range: 50–58 µm). The ejaculatory duct is surrounded by a thin layer of circular muscles. Two different kinds of secretions could be discerned in the parenchyma of the penis papilla: (1) a coarse-grained erytrophilic secretion, and (2) a fine-grained cyanophilic secretion. However, it was not possible to ascertain where these secretions are discharged. The penis papilla protrudes into the common atrium, which is lined with an unciliated infranucleated epithelium.

Female reproductive system. The numerous vitellaria occupy the entire space between the dorsal and the ventral body surface, extending from directly behind the ovaries to the caudal end. Most of the vitellaria are positioned laterally, where they fill the available space between the intestinal branches; only few are irregularly scattered in the rest of the body.

Two ovaries (about 100 µm broad at their greatest width in the holotype) lie dorsally to the ventral nerve cords, at about one-third of the distance between the brain and the root of the pharynx. The ovaries are U-shaped (Fig. 3D) and contain oocytes at different stages of development. The germ centre is located posteriorly, at the ventral side. The oviducts arise from the anterior portion of the ovaries. The oviducts curve posteriorly and run backwards to the level of the

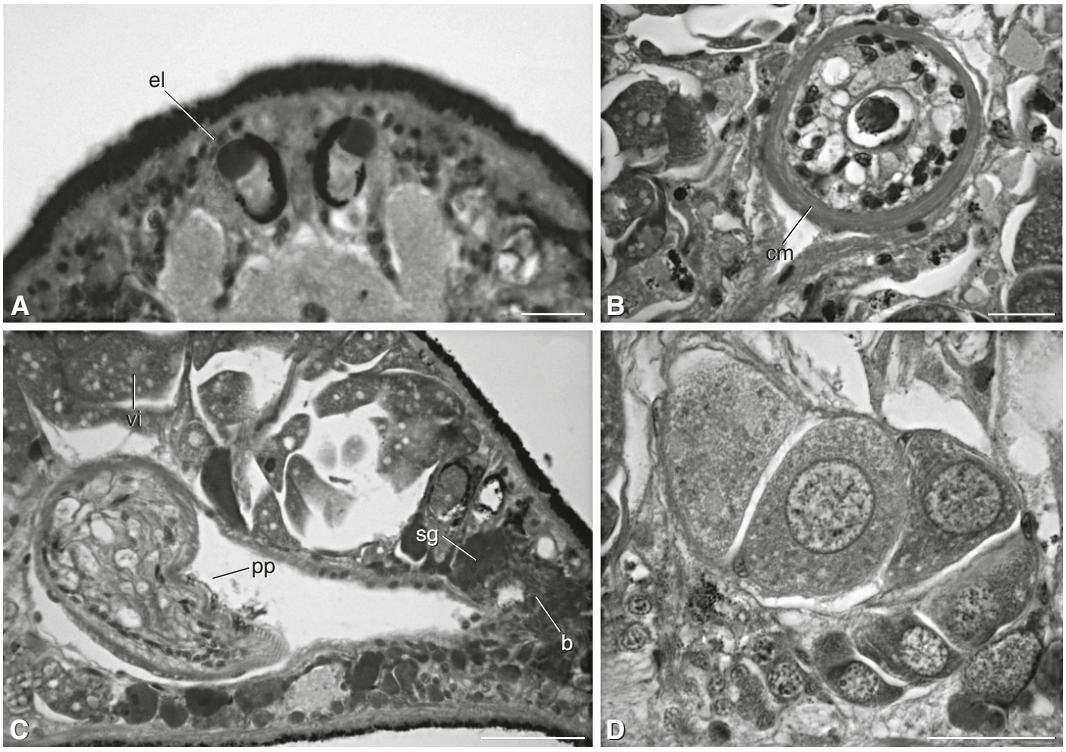

Fig. 3. *Sabussowia ronaldi* sp. nov. **A.** Horizontal section of lensed eyes (CZM 211). **B.** Horizontal section of the penis papilla (CZM 211). **C.** Holotype. Sagittal section of genital organs; caudal end on the right. **D.** Sagittal section of the U-shaped ovaria (CZM 208); caudal end on the right. Scale bar A, C, D, 30 µm; B, 15 µm.

copulatory organ, where they enter the bursal canal separately. The oviducts are surrounded by a thin unciliated epithelium. The small bursa is lined by an unciliated epithelium with intra-epithelial nuclei, and contained sperm in many of the animals sectioned. This bursa is connected to the common atrium through a short bursal canal, lined with an unciliated epithelium, higher than the rest of the common atrium. Both the bursa and the bursal canal are surrounded by shell glands.

Karyology. With n = 6 (Fig. 2B). Chromosomes I to IV are distinctly larger that the remaining two pairs. Chromosomes I and VI are metacentric; the rest of chromosomes are at the border between submetacentric and subtelocentric. Karyometrical data of specimens from the type locality:

Chrom. I r.l.: 25.69 + 1.02; c.i.: 46.04 + 1.33 (m);
Chrom. II r.l.: 19.88 + 1.46; c.i.: 20.27 + 2.20 (st);
Chrom. III r.l.: 18.27 + 0.57; c.i.: 26.28 + 4.08 (sm);
Chrom. IV r.l.: 15.09 + 0.71; c.i.: 20.51 + 3.81 (st);
Chrom. V r.l.: 10.34 + 0.34; c.i.: 26.44 + 4.55 (sm);
Chrom. VI r.l.: 10.05 + 0.30; c.i.: 39.78 + 2.46 (m); (based on 5 spermatogonial plates).

Specimens from Capo Caccia had similar karymetrical data:

Chrom. I r.l.: 25.13 + 1.19; c.i.: 42.72 + 2.06 (m);
Chrom. II r.l.: 19.02 + 1.77; c.i.: 18.25 + 2.15 (st);
Chrom. III r.l.: 18.02 + 0.59; c.i.: 24.58 + 3.81 (st);
Chrom. IV r.l.: 16.09 + 0.51; c.i.: 20.33 + 4.55 (st);
Chrom. V r.l.: 11.11 + 1.23; c.i.: 22.07 + 6.68 (st);
Chrom. VI r.l.: 10.63 + 1.33; c.i.: 32.86 + 6.78 (m); (based on 6 spermatogonial plates).

Life cycle. Specimens from the type locality had a short life span in culture (71.7 ± 11.9 days, n = 20). Animals laid cocoons when about 2 mm long. Cocoons were about 375 µm at their greatest width, ovoid and unstalked. Hatchlings (one to two per cocoon) emerged from the cocoons 28–35 days after deposition. The number of offspring hatching from a cocoon is the lowest known for a marine triclad. In the congeneric *S. dioica* one to eight, usually four, juveniles may hatch from a

single cocoon (Tekaya et al. 1999), while there are up to nine hatchlings in *Bdelloura candida* (Girard 1850) (Sluys 1989). Fertility was low, averaging 7.5 ± 3.5 offsprings per pair (n = 10).

Remarks. During BIOIMPA campaigns, which spanned the western and central Mediterranean Sea, specimens of the new species were found exclusively in two sites, both located in northern Sardinia. These sites are comparable in depth and sediment texture, and both are in areas with high hydrodynamism. Once isolated, the animals showed good tolerance of laboratory conditions, and were easily maintained, although they showed a tendency to crawl above the water level, and eventually die because of dehydration.

Discussion

The presence of a long, extra-bulbar common vas deferens, and the position of the ovaries at some distance behind the brain, place the new species into the superfamily Cercyroidea (Sluys 1989).

The Cercyroidea consist of three families: Cercyridae, Centrovarioplanidae Westblad, 1952, and Meixneridae Westblad, 1952 – this latter considered of incertae sedis by Sluys (1989). The diagnostic features of the Centrovarioplanidae (above all, the presence of an inverted penis papilla ending into a glandular seminal sac) and of the Meixneridae (among the most obvious, the distal portion of the ejaculatory duct provided with sclerotised spines) are absent in the new species. Conversely, the presence of a compartmentalised germ centre points to its position into the taxon Cercyrini Sluys, 1989, within the family Cercyridae.

Among the Cercyrini, the new species fits into the genus *Sabussowia*, which is represented by hermaphroditic or dioecious Cercyrini without a penis stylet and with ovaries directly behind the brain or located at some distance posterior to the latter (Sluys 1989).

Other genera of Cercyrini with Mediterranean representatives have features not present in the new species: *Cerbussowia* Wilhelmi, 1909 has a penis stylet, while *Cercyra* Schmidt, 1861 shows a penis stylet and a pigmented cephalic band (Sluys 1989). The other genera of the tribe Cercyrini include species with (1) the copulatory bursa anterior to the copulatory organ (*Probursa* Hyman, 1944, from eastern North America), (2) a musculo-glandular organ with a sclerotized tip and ovaries posterior to the pharyngeal cavity (*Pacifides* Holmquist & Karling, 1972, from the Indo-Pacific), or (3) adhesive disks (*Puiteca* du Bois-Reymond Marcus, 1955, from Brazil and Australia).

Until now, the genus *Sabussowia* included only two species, *S. dioica* (Claparède, 1863) from the Mediterranean and *S. wilhelmii* Ball, 1973 from East Canada (Sluys 1989, Vila-Farrè et al. 2009).

The new species differs from its congeners in the presence of only two ventral testes, instead of numerous (up to 90) dorsal testes. The bursal canal is short and unciliated in *S. ronaldi*, whereas it is long and ciliated in *S. dioica* and *S. wilhelmii* (Sluys 1989).

Furthermore, *S. dioica* is a larger (up to 8 mm long), gonochoristic species (Rieger et al. 1991, Tekaya et al. 1997). In this species the penis bulb and the papilla are well separated by a thick area of concentric musculature, while its copulatory bursa is lined with a high vacuolated, and infranucleated epithelium. In addition, its karyotype is different, with n = 7 and Chromosome I being distinctly larger than the rest of the set (Charbagi-Barbirou & Tekaya 2009). In contrast, in *S. ronaldi* Chromosome I is only slightly larger than Chromosomes II-IV.

S. wilhelmii is a simultaneous hermaphroditic species, as is *S. ronaldi*. However, the bursa of *S. wilhelmii* is large and irregular in shape, whereas it is small and roundish in the new species. Furthermore, in *S. wilhelmii* the vasa deferentia fuse shortly before entering the penis papilla (see Sluys 1989, fig. 81, p. 118), whereas in *S. ronaldi* they form a long common duct at the level of the pharynx (Fig. 1A).

The new species resembles *Cerbussowia cerrutii* Wilhelmi, 1909, which occurs in the Mediterranean. In fact, the two species have the same number and arrangement of testes, as well as a similar general topography of the genital organs (Sluys 1989: figs. 70–73, pp. 110–111). The basic difference between the two species is the presence of a penis stylet in *C. cerrutii*, which justifies its placement in a different genus according to the current taxonomy of the Maricola (Sluys 1989). Furthermore, in this species the germ centre is situated in a characteristic, tube-shaped sac. In contrast to *S. ronaldi*, specimens of *C. cerrutii* present a musculo-glandular organ (Sluys 1989), which, however, may be absent in some populations (Tekaya et al. 1996).

The U-shaped morphology of the ovaries of the new species is unique in the superfamily Cercyroidea. Within the Maricola only two species, both belonging to the superfamily Bdellouridea, share this feature with *S. ronaldi*, viz. *Nerpa evelinae* Marcus, 1948 and *Pentacoelum fucoideum* Westblad, 1935 (Sluys 1989).

S. ronaldi is the second known species of the superfamily Cercyridae that is provided with eye lenses. The other species, *Stummeria marginata* (Hallez 1906) has peculiar, large eye cups containing many small retinal cells. On the contrary, lensed eyes with a morphology similar to that of the new species can be found in species of the superfamily Bdellouroidea (Sluys 1989). Furthermore, the eye morphology observed in the new species is similar to that found in a species of the superfamily Procerodoidea, viz. *Procerodes lactea* Ijima & Karaburaki, 1916. Sluys (1989) considered the feature "lensed eyes" as an apomorphic character for the superfamily Bdellouroidea, and its presence in *Procerodes lactea* and *Stummeria marginata* as a result of parallel evolution. Therefore, the finding of the 'bdellouroid' type of lensed eye in a species of the superfamily Cercyroidea may have phylogenetical relevance, as the character may be more widespread in the Maricola than hitherto known.

It is apparent from the discussion above that the new species presents a somewhat puzzling combination of characters, making its systematic placement in the current classification of the Maricola difficult. A reassessment of the classification of the group and a scrutiny of the validity of monotypic genera, such as *Cerbussowia*, appear thus highly desirable.

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