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On the *Pseudomonocelis agilis* (Schultze, 1851) complex (Platyhelminthes: Proseriata), with description of two new species

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Abstract

Based on molecular, karyological, morphological analyses and cross-breeding experiments, the widespread proseriate *Pseudomonocelis agilis* turned out to consist of a complex of four species, two of which were unnamed. These two new species are described here: *P. occidentalis* n. sp. and *P. orientalis* n. sp. The two species are morphologically very similar. Both possess a reddish-brown pigment band in front of the statocyst, and are provided with paired external vaginae. The main difference between the two species is the bursa, which is placed vertically above the vaginal ducts in *P. occidentalis*, while it is more cranial and is provided with a long, horizontal bursal canal in *P. orientalis*. Their range is different: western and eastern Mediterranean for *P. occidentalis* and *P. orientalis* respectively. Furthermore, *P. agilis* is redescribed, and the original description of *P. cetinae* is integrated with the analysis of specimens from the type locality. A diagnostic key for the species of the genus *Pseudomonocelis* is provided.

Keywords: meiofauna, taxonomy, Mediterranean, biodiversità

Introduction

The majority of Proseriata, a species-rich taxon of Neoophoran Platyhelminthes, are unpigmented. In Europe, *Pseudomonocelis agilis* (Schultze, 1851) and *Pseudomonocelis cetinae* Meixner, 1943 (Proseriata: Monocelididae) are among the few exceptions. In fact, their body is yellowish owing to the presence of diffuse parenchymatic pigment. In addition, both species are provided with a conspicuous transverse band of concentrated reddish-brown pigment in front of the statocyst. *P. agilis* was described on specimens from the Baltic, while *P. cetinae* was described on specimens from the mouth of the river Cetina in the eastern Adriatic coast (Schultze 1851, Meixner 1943). Most of the later authors considered *P. cetinae* as a junior synonym of *P. agilis* (see Ax 2008, and references therein); with the exception of Schockaert & Martens (1987), who, given their broadly separated ranges, considered the two taxa as distinct species.

A recent application of integrative taxonomic techniques to the pigmented European species of *Pseudomonocelis*, including molecular, karyological, and morphological analyses on the Atlantic and Mediterranean populations as well as cross-breeding experiments, have revealed a far more

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diverse and complex scenario. In fact, in addition to *P. agilis* and *P. cetinae*, whose specific distinction was supported, the study also showed the presence of two further, undescribed species in the Mediterranean (Casu et al. 2009). These two new species are formally described in the present paper. An integration of the original descriptions of *P. agilis* and *P. cetinae* is provided as well.

Materials and methods

Specimens were collected in sandy habitats by scooping up the superficial layer of sediment or, in the case of *P. agilis*, from samples of algae. Extraction of the animals from the sediment was done with $MgCl_2$-decantation (Martens 1984). Morphological studies were performed on sectioned specimens, with the fixation and sectioning techniques routinely adopted for Proseriata (see Delogu et al. 2007, Casu et al. 2009). The karyotype was determined from acetic-orcein-stained spermatogonial mitoses, as described by Curini-Galletti et al. (1989). Relative lengths (r. l. = length of chromosome × 100/total length of haploid genome) and centrometric indices (c.i. = length of short arm × 100/length of entire chromosome) were obtained from measurements of camera lucida drawings of metaphase plates. The chromosome nomenclature employed is that of Levan et al. (1964): m = metacentric; sm = submetacentric; st = subtelocentric; t: acrocentric.

Type material is deposited in the collections of the Swedish Museum of Natural History (Stockholm, Sweden) (SMNH). Voucher material is deposited in the collection of the Zoological Museum of the University of Sassari (Italy) (CZM).

Abbreviations used in figures: **b**, bursa; **bc**, bursal canal; **br**, brain; **bs**, 'blindsack'; **co**, copulatory organ; **e**, eyespot; **fd**, female duct; **fg**, female glands; **fp**, female pore; **gl**, gut lumen; **mp**, male pore; **o**, ovary; **pg**, prostatic glands; **ph**, pharynx; **pp**, penis papilla; **r**, rhabdoid gland; **rp**, reddish-brown pigment band; **st**, statocyst; **t**, testis; **vd**, vaginal duct; **vi**, vitellaria; **vp**, vaginal pore; **wp**, white pigment band.

Taxonomic account

The genus *Pseudomonocelis* was introduced by Meixner (1943) and reviewed by Schockaert & Martens (1987). In both studies, the general characters of the genus were described extensively.

The genus includes species of Monocelididae with postpharyngeal ovaries, copulatory organ of the simplex type, and bursa and vagina(e), when present, in front of the copulatory organ. They may possess an accessory organ, provided or not with a stylet (Curini-Galletti 1997).

The species of the genus *Pseudomonocelis* have a deceptively 'simple' morphology, and discrimination among them may be challenging. The species-complex of *P. ophiocephala* (Schmidt, 1861) is exemplary at this regard, as it includes four cryptic species, which can be distinguished only on a molecular base, and partly on karyotype (Casu & Curini-Galletti 2006; see Appendix). On the contrary, the four species forming the *P. agilis* complex differ in details of pigmentation, and morphology of the genital organs (Casu et al. 2009). Therefore, these characters are emphasized in the following descriptions.

Fam. Monocelididae Hofsten, 1907
Subfam. Monocelidinae Midelburg, 1908
Genus *Pseudomonocelis* Meixner, 1943

Pseudomonocelis occidentalis n. sp.

(Figs. 1A–C, 2E)

Synonymy: *Pseudomonocelis* sp. nov. A (Casu et al. 2009)

Holotype: a mature specimen, sagittally-sectioned (SMNH 7647).

Type locality: Italy, Sardinia, Porto Pozzo (41°11'20.22" N; 9°17'11.03" E), lower intertidal in coarse sand and fine gravel, with fresh-water run-off (May 2004).

Additional material from the type locality: paratypes: two specimens sagittally-sectioned (SMNH 7648-7649); 27 specimens sagittally-sectioned (CZM 214-249); 20 specimens studied karyologically; 72 specimens used for molecular analyses (see Casu et al. 2009 for details).

Other localities: Tuscany: Giglio Island (42°21' 37.48" N; 10°55'12.94" E), inside the harbour, lower intertidal in coarse sand and fine gravel, with fresh-water run-off (June 2004), 30 specimens sagittally-sectioned (CZM 241–270); 20 specimens studied karyologically; 74 specimens used for mo-

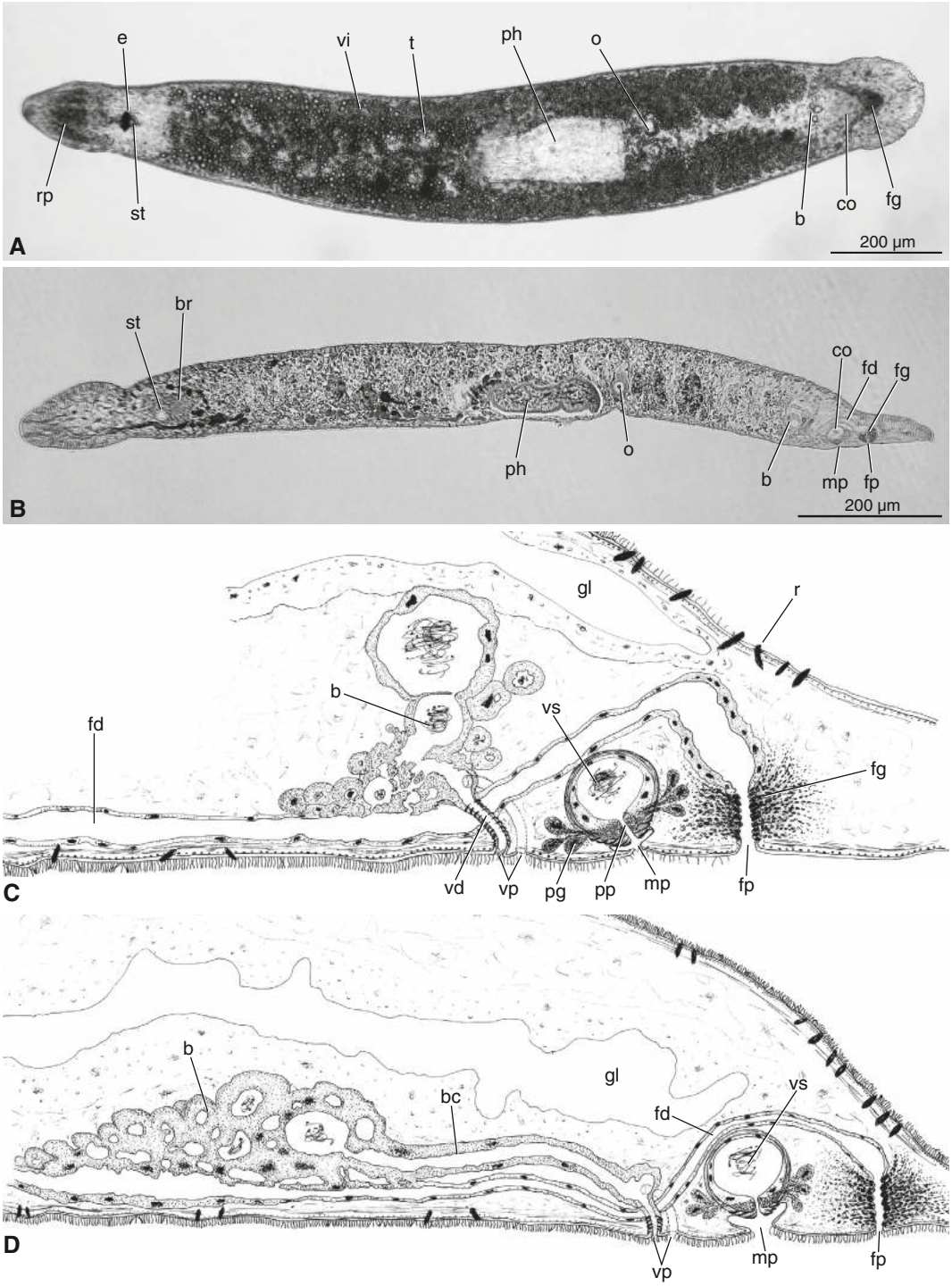

Fig. 1. A–C. *Pseudomonocelis occidentalis* n. sp. General organisation (**A**) and sagittal section (**B**) of specimens from the type locality. **C.** sagittal reconstruction of the genital organs. **D.** *P. orientalis* n. sp.: sagittal reconstruction of the genital organs. Scale bars: A, 200 µm; B, 150 µm.

lecular analyses (see Casu et al. 2009 for details). Tuscany: Castiglione della Pescaia, mouth of the S. Leopoldo drainage channel (42°43'50.94" N; 10°57'48.37" E), about 20 cm deep in fine to medium sand in brackish conditions, several specimens studied alive, two studied karyologically (March 1992). Sicily: Castellammare del Golfo, town beach, lower intertidal in medium sand, close to a fresh-water outlet. Several specimens studied alive, 10 studied karyologically (September 2005).

Etymology. The species is named after the geographical region where it is found, the Western (Latin: *occidentalis*) Mediterranean.

Description. Holotype 1.4 mm long. Live specimens appear yellowish due to parenchymatic pigment. With a brownish-red transverse pigment band in front of the statocyst, and with a pigmented eye spot. Rhabdoids about 10 µm long, densely packed dorsally, particularly numerous and evident at caudal and frontal ends. Epidermis ciliated, with the exception of the caudal end, posterior to the female pore. Cilia about 4 µm long, slightly longer ventrally than dorsally.

Male reproductive system. Numerous testes (15–40) in front of the pharynx. The two vasa deferentia enter the seminal vesicle anteriorly. The copulatory organ is globular, 32 µm high and 31 µm wide in the holotype, ranging 30.6 ± 0.7 µm and 30 ± 0.6 µm respectively in the sample of the type population), lined by a thin layer of spirally arranged muscles. Proximally, the seminal vesicle is lined by a thin epithelium with intra-epithelial nuclei; distally, the epithelium becomes high, and is pierced by the necks of prostate glands, whose bodies lie outside of the copulatory organ. The copulatory organ is provided with a short, weakly muscular penis papilla, about 9 µm long, provided with circular and longitudinal muscles externally, and a thick layer of prostatic tissue internally. Male antrum small and narrow, lined by a thin, unciliated epithelium with insunk nuclei, connected to the outside through the male pore.

Female reproductive system. Vitellaria lateral, extending posteriorly to the level of the genital organs; ovaries posterior to the pharynx. The very short oviducts fuse into a female duct, which is lined with an epithelium with intra-epitelial nuclei, and runs ventrally and posteriorly. In front of the copulatory organ, a large (about 80 µm wide, 75 µm high) bursa, of the resorbiens type, is present. It consists of numerous vacuoles, many of which contain sperm in the sectioned specimens, and extends vertically to abut the gut lining. Ventrally, the bursa is connected to two vaginal ducts, lying at both sides of the female duct, and opening ventrally in front of the male pore. The vaginae are lined with an epithelium with insunk nuclei, and surrounded by few, thin circular muscles. Although no clear connection between female duct and bursa could be observed, the dorsal lining of the female duct immediately below the bursa appears embedded in the bursal tissue, and connected to some of the vacuoles through which sperm may pass. From the bursa, the female duct lies dorsally from the copulatory organ. The holotype and most of the specimens sectioned show a distinct widening of the female duct immediately posterior to the copulatory organ, prior to its opening to the outside through the female pore, surrounded by eosinopholic, cement glands. This pore is situated about 50 µm posterior to the male pore.

Karyotype. With n = 3, and chromosomes slightly differing in size. Chromosomes I and III are acrocentric; Chromosome II metacentric, with low centromeric index. Karyometrical data:
Chrom. I r.l.: 36.05 ± 0.21; c.i.: 9.84 ± 0.47 (t);
Chrom. II r.l.: 33.17 ± 0.23; c.i.: 40.78 ± 0.36 (m);
Chrom. III r.l.: 30.60 ± 0.20; c.i.: 11.95 ± 0.53 (t);
(from Casu et al. 2009).

Remarks. Specimens from Giglio Island were slightly larger, averaging 1.7 mm in length, in comparison to the population from the type locality, with a larger copulatory organ, 33.1 ± 0.8 µm high and 34.2 ± 0.75 µm wide. The karyometrical data were not significantly different. The two populations had very low genetic distance (Nei's distance = 0.009), and were reproductively compatible (Casu et al. 2009). No molecular studies and crossbreeding experiments were performed on the other populations, and their attribution to *P. occidentalis* sp. nov. was based on pigmentation and karyotype. Karyometrical data of specimens from Castellamare del Golfo closely corresponded with those of specimens from Porto Pozzo and Giglio Island:
Chrom. I r.l.: 36.43 ± 0.22; c.i.: 8.57 ± 0.57 (t);
Chrom. II r.l.: 33.05 ± 0.21; c.i.: 41.75 ± 0.59 (m);
Chrom. III r.l.: 30.42 ± 0.27; c.i.: 10.66 ± 0.79 (t).

Only the presence in the set of one metacentric and two acrocentric pairs could be ascertained in specimens from Castiglione della Pescaia.

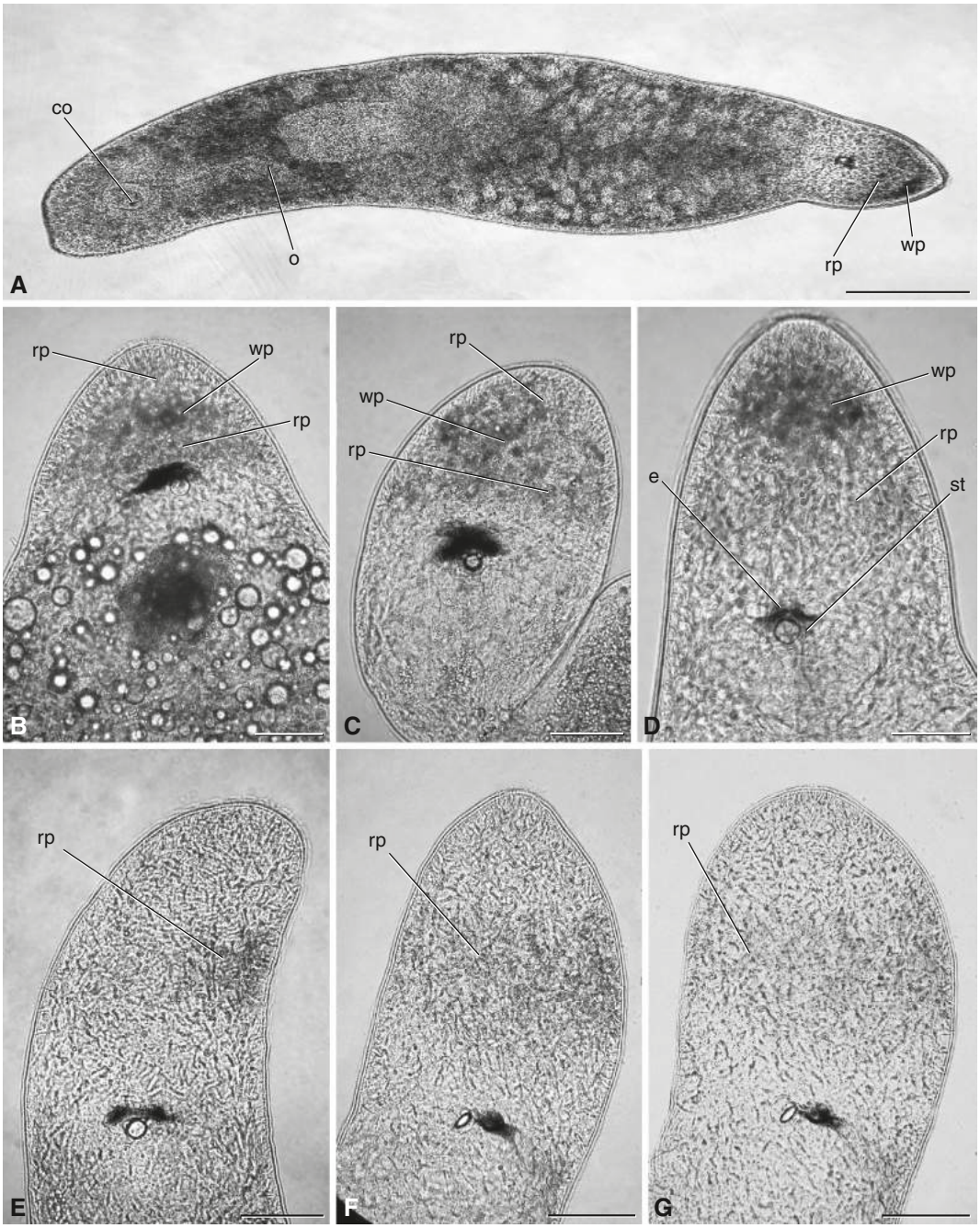

Fig. 2. Cephalic pigmentation of live specimens of *Pseudomonocelis cetinae* (**A, D**), *P. agilis* (**B, C**), *P. occidentalis* n. sp. (from Porto Pozzo) (**E**) and *P. orientalis* n. sp. (from Maliakòs) (**F, G**). Scale bars: A, 200 µm; B–F, 40 µm; G, 80 µm.

Given the west-Mediterranean distribution of *P. occidentalis* sp. nov., the specimens reported from the southern French lagoons (Canet, as *P. cetinae*, by Ax 1956 and the Grande Palun (Ca-

margue), as *P. agilis*, by Sopott-Ehlers 1993) may pertain to the new species. It should however be emphasized that, in the *P. agilis* complex, as in most members of the Monocelidinae, identification based exclusively on observations on semi – squashed live specimens may be unreliable.

Pseudomonocelis orientalis n. sp.
(Figs. 1D, 2F,G)

Synonymy: *Pseudomonocelis* sp. nov. B (Casu et al. 2009)

Holotype: a mature specimen sagittally sectioned (SMNH 7650)

Type locality: Greece, Gulf of Maliakòs, near Stylida (38°54'26.05" N; 22°37'22.25" E), lower intertidal in coarse sand, near a fresh-water oulet (March 2004).

Additional material from the type locality: paratypes: two specimens sagittally-sectioned (SMNH 7651–7652); 27 specimens sagittally-sectioned (CZM 271–297); 20 specimens studied karyologically; 72 specimens used for molecular analyses (see Casu et al. 2009 for details).

Other localities: Charaki, Rhodes Island, Greece (36°9'58.90" N; 28°5'52.49" E), lower intertidal in coarse sand and gravel, with fresh-water runoff (September 2003), 30 specimens sagittally-sectioned (CZM298–327); 20 specimens studied karyologically; 74 specimens used for molecular analyses (see Casu et al. 2009 for details).

Etymology: The species is named after its geographical range, the Eastern (Latin: *orientalis*) Mediterranean.

Description. Holotype 1.7 mm long. Type population averaging 1.5 mm long. Living animals identical in appearance and pigmentation to *P. occidentalis* n. sp. Epidermis ciliated near to the caudal tip, posterior to the female pore.

Male reproductive system. Copulatory organ globular, 39 µm high and 38 µm wide in the holotype, ranging 42.7 ± 1.4 µm and 37.7 ± 1.0 µm respectively in the sample of the type population. Proximally the seminal vesicle is lined with a thin epithelium with intra-epithelial nuclei. Distally, the epithelium is higher, and is pierced by the necks of prostate glands. Musculature of the bulb thin proximally, becoming progressively stronger distally. Penis papilla about 14 µm long, provided with circular and longitudinal muscles externally, and with a thick layer of prostatic tissue internally.

Female reproductive system. The short oviducts fuse into a female duct, which, for most of its length in front of the copulatory organ, is overlaid by a complex bursa-vagina system. The bursa is of the resorbiens type, about 200 µm wide and 45 µm high, and stretches backwards from the level of the oviducts. Similarly to the previous species, the lining of the female duct immediately below the bursa appears irregular and vacuolar. Posteriorly, the bursa is connected to a bursal duct, about 140 µm long, which runs dorsally and parallel to the female duct. It is lined by an epithelium with intra-epithelial nuclei, high and glandular, and is surrounded by a thin layer of circular musculature. In front of the copulatory organ, the bursal duct splits into two narrow, vaginal ducts, lined with an epithelium with insunk nuclei, which open ventrally in front of the male pore. The female duct runs posteriorly above the copulatory organ, and widens slightly prior to its opening behind the male pore though the female pore, surrounded by eosinophilous, cement glands and placed about 50 µm posterior to the male pore.

Karyotype. With n = 3, and chromosomes slightly differing in size. Chromosome I is submetacentric, at the border with the subtelocentric class; Chromosome II is metacentric; Chromosome III is subtelocentric. Karyometrical data:
Chrom. I r.l.: 37.09 ± 0.24; c.i.: 25.31 ± 1.02 (sm);
Chrom. II r.l.: 33.35 ± 0.21; c.i.: 44.78 ± 0.4 (m);
Chrom. III r.l.: 29.67 ± 0.27; c.i.: 16.04 ± 0.6 (st)
(from Casu et al. 2009)

Remarks. Specimens from Rhodes Island were larger, averaging 2 mm in length, in comparison to the type population, with a smaller copulatory organ, 38.1 ± 0.7 µm high and 35.9 ± 0.8 µm wide. The karyometrical data were not significantly different between the two populations, which, although fully interfertile, had a not negligible genetic distance (Nei's distance = 0.200) (Casu et al. 2009). The specimens studied from the two populations had a nearly identical bursa-vaginae system.

The specimens from the Bosporus reported by Ax (1959) as *P. agilis* may pertain to the new species. However, the caveats in relation to specific attributions based only on semi-squashed observations also apply in this case.

Pseudomonocelis agilis (Schultze, 1851)
(Figs. 2B,C, 3A,B)

Type material: not existing.

Type locality: Baltic sea: Greifswald (Germany).

Material studied: Helsingør, Denmark (56°2' 37.23" N; 12°36'52.48" E), inside the harbour, on seaweeds at low tide (September 2005), 11 specimens sagittally-sectioned (CZM 328-338); 20 specimens studied karyologically; 24 specimens used for molecular analyses (see Casu et al. 2009 for details).

Description. Fixed specimens averaging 1.7 mm in length. Live specimens yellowish; cephalic pigmentation consisting of a small, irregular reddish-brown pigment patch, close to the anterior tip of the body, followed posteriorly by a white transverse pigment band, appearing black in transmitted light, and by a brownish-red transverse pigment band, in front of the statocyst. With a pigmented eye spot.

Rhabdoids about 10 µm long, particularly evident at caudal and frontal ends. Epidermis ciliated, with the exception of the dorsal side of the caudal tip. Cilia 4–4.5 µm long, slightly longer ventrally than dorsally.

Male reproductive system. Numerous testes (15–40) in front of the pharynx. Copulatory organ ovoid, averaging 56 µm high and 43 µm wide. Proximally, the seminal vesicle is lined with a thin epithelium with intra-epithelial nuclei. Musculature of the bulb very thin proximally, becoming thicker proximally. The penis papilla, averaging 22 µm in length, is almost exclusively muscular, and is provided with thick layers of circular and longitudinal muscles. Male antrum small and narrow, lined by a thin, unciliated epithelium with insunk nuclei, connected to the outside through the male pore.

Female reproductive system. Vitellaria lateral, extending posteriorly to the level of the genital organs; ovaries posterior to the pharynx. Oviducts very short, leading to a small bursa, up to 25 µm wide, lined with an epithelium with intra-epithelial nuclei. The female duct runs posteriorly from the bursa. In its cranialmost portion, the female duct is surrounded by small vacuoles, some of which contain sperm. The degree of development of these vacuoles differs. They are barely noticeable in some specimens, while in others they reach and may even surround the bursa. The female duct is shaped irregularly, slightly swollen in parts. Posteriorly, the female duct runs dorsally to the copulatory organ and widens slightly prior to its opening through the female pore, surrounded by eosinophilic glands and situated about 20 µm posterior to the male opening.

Karyotype. With n = 3, and chromosomes slightly differing in size. Chromosome I is metacentric; Chromosome II is submetacentric; Chromosome III is subtelocentric. Karyometrical data:
Chrom. I r.l.: 38.9 ± 0.31; c.i.: 40.06 ± 0.63 (m);
Chrom. II r.l.: 32.27 ± 0.31; c.i.: 31.21 ± 0.52 (sm);
Chrom. III r.l.: 28.73 ± 0.43; c.i.: 16.89 ± 0.63 (st)
(from Casu et al., 2009).

Remarks. The original description of *Monocelis agilis* (Schultze, 1851), though concise, includes details on the cephalic pigmentation and the position of the ovaries, and allows proper generic attribution and species identification. *P. agilis* appears to be the only species of the complex present in northern Europe, and is known with certainty from the western Baltic, the Skagerrak, and the Nord-Ostsee Canal (Schultze 1851; Remane 1937; Ax 1952; Schütz & Kinne 1955; Schütz 1963, 1966; present paper). Confirmation of reports of *P. agilis* from Galicia (Norenã et al. 2007), Hebrides and Norway (Claparède 1861) would considerably widen the range of this species. However, particular caution should be taken when identifying the pigmented species of Monocelidinae in Western Europe. In that area, in fact, there are *Monocelis* species (i. e. *M. fusca* Örsted, 1843; *M.* cf. *longiceps* (Duges, 1830)) which could be misidentified as *P. agilis*, unless studied on live, semi – squashed specimens, or in sections. Owing to the lack of further information and voucher specimens, these latter reports are best regarded as unconfirmed.

Pseudomonocelis cetinae **Meixner, 1943**
(Figs. 2A,D, 3C,D)

Type material: not existing.

Type locality: Adriatic sea: Omiŝ (Croatia); mouth of the river Cetina.

Material studied: 12 specimens sagittally-sectioned (CZM 339–350); one specimen frontally-sectioned (CZM 351); 20 specimens studied karyologically; 73 specimens used for molecular analyses (see Casu et al. 2009 for details), all from the type locality (43°26'20.61"N; 16°41'6.08"E); lower intertidal in medium to coarse sand (October 2004).

Description. Fixed specimens averaging 1.9 mm in length. Live specimens yellowish; cephalic pigmentation consists of a white transverse pigment band, appearing black in transmitted light, and a brownish-red transverse pigment band, in front of the statocyst. A few, large specimens showed a very small, irregular pigment patch, close to the anterior tip of the body, similar to that present in *P. agilis*. With a pigmented eye spot, shaped as an inverted V. Cilia 3–3.5 µm long, slightly longer ventrally. Body entirely ciliated, a part from the dorsal side of the caudal tip. Ventrally, posterior to the female pore, cilia become shorter and sparser, and disappear just before the caudal tip. Rhabdoid glands about 12 µm, particularly evident at caudal and frontal ends.

Male reproductive system. Numerous testes (40–60) in front of the pharynx. Copulatory organ ovoid, comparatively large, averaging 76 µm high and 52 µm wide. Proximally, the seminal vesicle is lined with a thin epithelium with intra-epithelial nuclei. Musculature of the bulb thin proximally, becoming thicker distally. Penis papilla about 29 µm long, provided with very thin layers of circular and longitudinal muscles, and with a thick layer of prostatic tissue, which occupies most of the section of the papilla. Male antrum small, and unciliated.

Female reproductive system. Vitellaria lateral, extending posteriorly to the level of the genital organs; ovaries posterior to the pharynx. Oviducts very short. Bursa large, to 100 µm wide and 80 µm high, irregular in shape. In most mature specimens, the bursa is provided with a more or less conspicuous diverticulum, filled with sperm, parallel to the female canal ('blindsack' in Meixner 1943: p. 462, Fig. 3; 'bs' in Fig. 3C). The bursa is surrounded by numerous resorbing vacuoles. The female duct runs posteriorly from the bursa. It is large and irregularly swollen over most of its length. Posteriorly, the female duct runs dorsally to the copulatory organ, and opens through the female pore, surrounded by eosinophilic glands. The pore is situated about 60 µm posteriorly to the male opening.

Karyotype. With n = 3, and chromosomes slightly differing in size. Chromosome I and II are submetacentric; Chromosome III is subtelocentric. Karyometrical data
Chrom. I r.l.: 38.79 ± 0.29; c.i.: 27.97 ± 0.48 (sm);
Chrom. II r.l.: 32.46 ± 0.33; c.i.: 35.04 ± 0.38 (sm);
Chrom. III r.l.: 28.84 ± 0.31; c.i.: 15.13 ± 0.57 (st)
(from Casu et al. 2009).

Remarks. The species is only known from the type locality, at the mouth of the river Cetina, in an area heavily modified by the building of a pier. Research in the area by one of us (MCG) could confirm the presence of the species only in a few square meters of relatively unaffected shore.

Discussion

The presence of a species-complex within what was formerly considered the single, widespread species *Pseudomonocelis agilis* was first detected by an integrative approach, which revealed the existence of four highly divergent genotypic clusters, accompanied by karyological differences, with complete intersterility among the clusters tested (Casu et al. 2009). However, morphological analysis of the four species belonging to the complex revealed also marked differences in the anatomy of the female reproductive system.

Based on this feature, two species-pairs are present. *P. occidentalis* n. sp. and *P. orientalis* n. sp. are provided with two external vaginae. In *P. occidentalis*, however, the bursa extends vertically above the vaginal pores and is connected directly to the vaginal ducts (Fig. 1C). The bursa of *P. orientalis* is situated more cranially and is provided with a long, horizontal bursal canal, from which the paired vaginal ducts depart (Fig. 1D). In both species, no direct connection between bursa and the female duct could be traced. Thus, it is assumed that sperm passes through the resorbiens tissue which surrounds the bursa and the female duct.

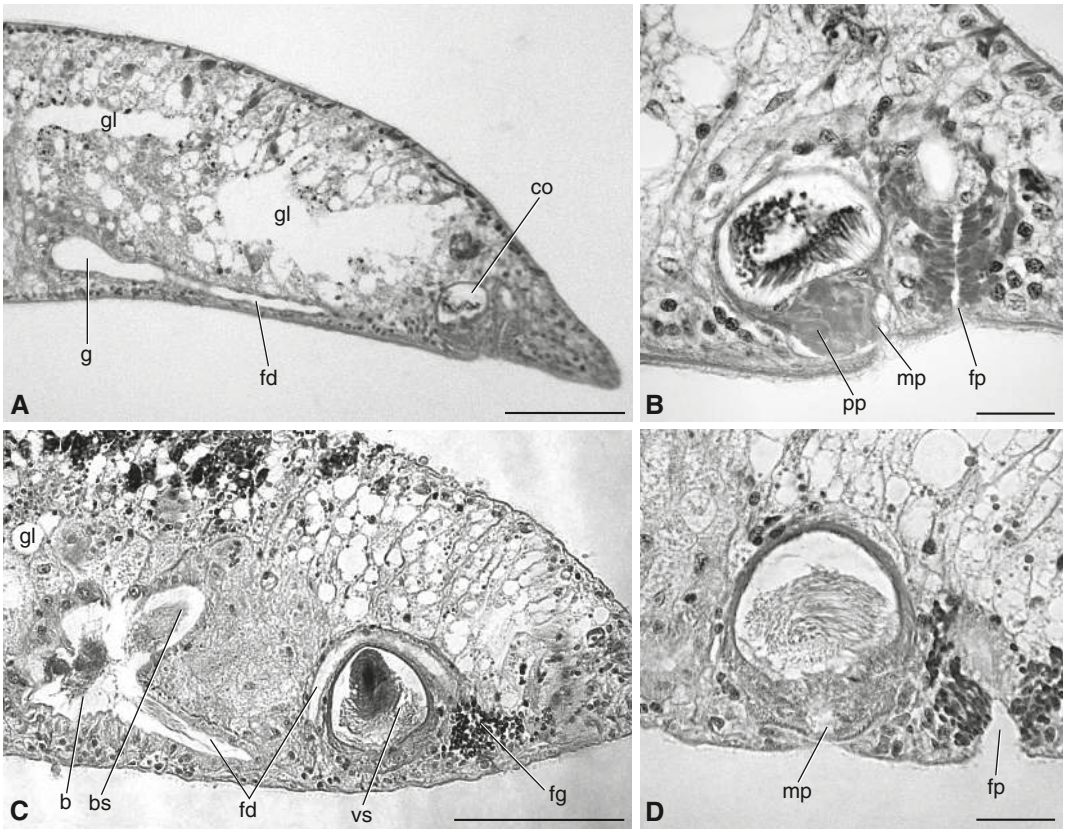

Fig. 3. Sagittal sections of the genital organs of *Pseudomonocelis agilis* (**A, B**) and *P. cetinae* (**C, D**). Scale bars: A, C, 100 µm; B, D, 25 µm.

On the contrary, *P. agilis* and *P. cetinae* do not have an external vagina in any stage examined, and their bursa is derived from the female duct. The two species, however, differ on the basis of the morphology of the bursa. This is very small, consisting of a slight, cranial widening of the female duct, in *P. agilis*; while it is considerably larger, with a posterior 'blindsack' parallel to the female duct, in *P. cetinae* (Meixner 1943; Fig. 3C). In addition, the two species-pairs also differ in pigmentation pattern. *P. agilis* and *P. cetinae*, in fact, have a white pigment band, which is absent in *P. occidentalis* and *P. orientalis* (Fig. 2).

The differences in bursal structure between *P. occidentalis* and *P. orientalis* do not allow to identify live, semi-squashed specimens. A clue to their identification can be found in their range: western and eastern Mediterranean for *P. occidentalis* and *P. orientalis* respectively. A similar separation is observed in the range of the avaginated species: *P. cetinae* occurs in the Adriatic Sea, whereas *P. agilis* occurs in North East Atlantic.

The results obtained from the molecular analyses revealed that the four pigmented species do not form a monophyletic group. *P. occidentalis* and *P. orientalis* cluster with the unpigmented *P. ophiocephala* cryptic species complex (Casu et al. 2009). These species indeed share two relevant, apomorphic features, namely the presence of paired, external vaginae and a bursa independent from the female duct (cf. Schockaert & Martens 1987). The bursa-female duct system of *P. ophiocephala* s.l. is comparable to that of *P. orientalis* (see Casu & Curini-Galletti 2006, Curini-Galletti & Casu 2005, Schockaert & Martens 1987, figs. 6, 7, pp. 111, 112). However, the bursa is shorter in *P. ophiocephala*, and does not extend backward to the level of the oviducts. It should be noted that the presence of two vaginae in the new species allows for an immediate distinction from the

extra-European species of *Pseudomonocelis*, that typically have a single vagina (see Appendix). The eastern Australian *P. hoplites* Curini-Galletti, 1997, which presumably has two vaginae, is an exception. However, the species is unpigmented, and has an accessory organ provided with a stylet (Curini-Galletti 1997). The avaginated species *P. agilis* and *P. cetinae* do not form a monophyletic group. Instead, they branch off independently at the base of the vaginated clade in the phylogram (Casu et al. 2009).

Indeed, phylogenetical reconstruction indicated that pigmentation is plesiomorphic in the genus (Casu et al. 2009). Pigmentation in Platyhelminthes is usually present in ephiphytic or semiplanktonic species, that are exposed to light. An adaptive value has been suggested for it, as a shield against excessive amounts of light, that could potentially damage the optic receptors (Armonies 1989). Pigmented *Pseudomonocelis* species occur in brackish conditions, where the animals tend to concentrate in the upper layer of the sediments, or on algae (Schockaert & Martens 1987; pers. obs.). As a consequence, they are exposed to higher levels of light in comparison to unpigmented, fully marine, interstitial species, such as *P. ophiocephala* s.l.

The fragmentation of a formerly wide-ranging species into discrete taxonomic units adds to the problem in the geographical distribution of meiofaunal organisms, which is particularly difficult to assess with certainty. In fact, distributions of the species of the complex appear limited, particularly in the case of *P. cetinae*, which is only known from a few square meters, on the central coast of the eastern Adriatic. Although distributional data may be biased by inadequate sampling effort, the fact that extensive research in the northern Adriatic and on both sides of the southern Adriatic and Ionian sea (Apulia, Western Greece) failed to reveal the species (MCG, pers. obs.) is suggesting a limited range. The limited ranges and strict ecological requirements make species of the *P. agilis* complex particularly vulnerable to alteration of littoral, brackish habitats.

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References

Armonies, W. (1989). Semiplanktonic Plathelminthes in the Wadden Sea. Marine Biology 101: 521–527.

Ax, P. (1952). Eine Brackwasser-Lebensgemeinschaft an Holzpfählen des Nord-Ostsee-Kanals. Kieler Meeresforschungen 8: 229–243.

— (1956). Les Turbellariés des étangs côtiers du littoral méditerranéen de la France meriodionale. Vie et Milieu Suppl. 5: 1–215.

— (1959). Zur Systematik, Ökologie und Tiergeographie der Turbellarienfauna in den ponto-kaspischen Brackwassermeeren. Zoologische Jahrbücher (Systematik, Ökologie und Geographie der Tiere) 87: 43–184.

— (2008). Plathelminthes aus Brackgewässern der Nordhalbkugel. Abhandlungen der Mathematisch-naturwissenschaftlichen Klasse, Akademie der Wissenschaften und der Literatur Mainz 1: 1–696.

Casu, M. & M. Curini-Galletti (2006). Genetic evidence for the existence of cryptic species in the mesopsammic flatworm *Pseudomonocelis ophiocephala* (Rhabditophora: Proseriata). Biological Journal of the Linnean Society 87: 553–576.

Casu, M., T. Lai, D. Sanna, P. Cossu & M. Curini-Galletti (2009). An integrative approach to the taxonomy of the pigmented European *Pseudomonocelis* Meixner, 1943 (Platyhelminthes: Proseriata). Biological Journal of the Linnean Society 98: 907–922.

Claparède, E. (1861). Récherches anatomiques sur les Annelídes, Turbellaríes, Opalines et Gregarines observes dans les Hebrides. Mémoires de la Société de Physique et d'Histoire Naturelle de Genève 16: 56–80, 124–148, 151–152.

Curini-Galletti, M. (1997). Contribution to the knowledge of the Proseriata (Platyhelminthes, Seriata) from eastern Australia: genera *Necia* Marcus, 1950 and *Pseudomonocelis* Meixner, 1938 (partim). Italian Journal of Zoology 64: 75–81.

Curini-Galletti, M. & M. Casu (2005). Contribution to the knowledge of the genus *Pseudomonocelis* Meixner, 1943 (Rhabditophora: Proseriata). Journal of Natural History 39: 2187–2201.

Curini-Galletti, M., I. Puccinelli & P. M. Martens (1989). Karyometrical analysis of ten species of the subfamily Monocelidinae (Proseriata, Platyhelminthes) with remarks on the karyological evolution of the Monocelididae. Genetica 78: 169–178.

Delogu, V. & M. Curini-Galletti (2007) New species of the genus *Parotoplana* Meixner, 1938 (Proseriata, Otoplanidae) from southern Apulia (Italy). Zootaxa 1529: 17–31.

Levan, A., K. Fredga & A. A. Sandberg (1964). Nomenclature for centrometric position on chromosomes. Hereditas 52: 201–220.

Martens, P. M. (1984). Comparison of three different extraction methods for Turbellaria. Marine Ecology Progress Series 14: 229–234.

Meixner, J. (1943). Über die Umbildung einer Turbellarienart nach Einwanderung aus dem Meere ins Süßwasser. Internationale Revue der gesamten Hydrobiologie und Hydrographie 43: 458–465.

Norenã, C., C. Damborenea, A. Faubel, & F. Brusa (2007). Composition of meiobenthonic Platyhelminthes from brackish environments of the Galician and Cantabrian coasts of Spain with the description of a new species of Djeziraia (Polycystididae, Kalyptorhynchia). Journal of Natural History 41: 1989–2005.

Remane, A. (1937). Die übrige Tierwelt. In: Die Schlei und ihre Fischereiwirtschaft, vol. 8, Neubauer, R. & S. Jaeckel (eds.). Schriften des Naturwissenschaftlichen Vereins für Schleswig-Holstein 22: 209–225.

Schockaert, E. R. & P. M. Martens (1987). Turbellaria from a Somalia. IV. The genus *Pseudomonocelis* Meixner, 1943. Monitore Zoologico Italiano 9: 101–115.

Schultze, M. S. (1851). Beiträge zur Naturgeschichte der Turbellarien. C. A. Koch's Verlagshandlung, Greifswald.

Schütz, L. (1963). Die Fauna der Fahrrine des NO-Kanals. Kieler Meeresforschungen 11, 110–135.

— (1966). Ökologische Untersuchungen über die Benthosfauna im Nordostseekanal. II. Autökologie der vagilen und hemisessilen Arten im Bewuchs der Pfähle: Mikro- und Mesofauna. Internationale Revue der gesamten Hydrobiologie und Hydrographie 51: 633–685.

Schütz, L. & O. Kinne (1955). Über die Mikro- und Makrofauna der Holzpfähle des Nord-Ostsee-Kanals und der Kieler Förde. Kieler Meeresforschungen 11: 110–135.

Sopott-Ehlers, B. (1993). Ultrastructural features of the pigmented eye-spot in *Pseudomonocelis agilis* (Platyhelminthes, Proseriata). Microfauna Marina 8: 77–88.

Appendix

Key to the genus *Pseudomonocelis* Meixner 1943

1 With accessory organ 2
– Without accessory organ 3

2 Accessory organ with stylet ..... *P. hoplites* Curini-Galletti, 1997
– Accessory organ without stylet ..... *P. cavernicola* Schockaert & Martens, 1987

3 Without vagina 4
– With one or two vaginae 5

4 With a small, cranial bursa; from NE Atlantic ..... *P. agilis* (Schultze, 1851)
– With a large bursa, from the Adriatic Sea ..... *P. cetinae* Meixner, 1943

5 With one vagina 6
– With two vaginae 8

6 Unpigmented ..... *P. caribbea* Curini-Galletti & Casu, 2005
– With a brownish pigment band in front of statocyst 7

7 With slender, feebly muscular penis papilla, from E. Australia ..... *P. schockaerti* Curini-Galletti & Cannon, 1995
– With stout, heavily muscular penis papilla, from E. Africa ..... *P. pardii* Schockaert & Martens, 1987

8 Unpigmented ..... *P ophiocephala* (Schmidt, 1861) complex*
– With a reddish-brown pigment band in front of statocyst 9

9 Bursa dorsal to the vaginae; no bursal canal; from W. Mediterranean ..... *P. occidentalis* n. sp.
– Bursa cranial; long bursal canal; from E. Mediterranean ..... *P. orientalis* n. sp.

* A complex of four cryptic species. Species descriptions have been based on non-morphological characters (karyotype, allozymic and RAPD patterns) (Casu & Curini-Galletti 2006). Species are partially distinct for habitat and range: *P. ophiocephala* is circum-Mediterranean; occurs in intertidal, well sorted, medium-to-coarse sand; *P. caputserpentis* Casu & Curini-Galletti, 2006 is restricted to the Corsican-Sardinian complex and to the adjacent Tuscan coast; it is exclusively found in reduced sediments beneath the 'banquette' of *Posidonia oceanica*; *P. caputdraconis* Casu & Curini-Galletti, 2006 is known from western Greece; occurs in mixed, silty sediments; *P. caputanguis* Casu & Curini-Galletti, 2006 is known from the northern Aegean Sea; occurs in mixed, silty sediments.