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Contribution to the knowledge of the genus *Pseudomonocelis* Meixner, 1943 (Rhabditophora: Proseriata)

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Abstract

A new species of the genus *Pseudomonocelis* (*P. caribbea* sp. n.) is described. It is distinguished from congeners by shape and size of the copulatory organ and relative positions of mouth and genital openings. It is the first species of the genus found in America. Sampling in east Africa (Zanzibar island) revealed two species, attributed to *P. pardii* and, tentatively, to *P. cavernicola*, both originally described from Somaliland. Morphological differences between Tanzanian and Somali specimens are reported. The Mediterranean *P. ophiocephala*, based on extensive morphological and karyological survey of 13 populations, is interpreted as constituting at least two sibling species, distinguishable solely on the basis of karyotype. Results support the notion that the present perception of the contribution of interstitial Rhabditophora to marine biodiversity may be unrepresentative.

Keywords: Marine biodiversity, Monocelididae, Platyhelminthes, sibling species

Introduction

The Proseriata is a diverse and species-rich taxon of free-living Rhabditophora. Albeit representatives of the group may be numerically abundant, characterizing entire communities (Remane 1933; Reise 1988), proseriates have received relatively little attention from systematists. As a result, species composition of the group in most areas of the planet (and especially in the tropics) is at present scanty or wholly unknown, de facto jeopardizing phylogenetic and biogeographical reconstructions.

The genus *Pseudomonocelis* Meixner, 1943 (Proseriata: Monocelididae) includes some of the numerically dominating flatworms of the upper infralittoral fringe in temperate and tropical areas (Murina 1981; Schockaert and Martens 1987). The genus is mainly characterized by the postpharyngeal position of the ovaries. However, displacement of the ovary from the plesiomorphic, pre-pharyngeal position may be a homoplasious feature for

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the species comprised at present in the genus, and its monophyly needs to be properly addressed, on a larger sample basis.

Over the years, records and finding of new species attributable to the genus *Pseudomonocelis* have been accumulated. Such miscellaneous notes are reported here, both as improvement of the knowledge on species morphology and biogeographical ranges, and as bases for further research.

Materials and methods

Specimens were collected in sandy habitats by scooping the superficial layer of sediment. Extraction of the animals from the sediment was through MgCl_2 decantation (see Martens 1984). For microscopical analysis, specimens were fixed in Bouin's fluid, embedded in 56°C paraplast, and serial sagittal sections were cut at 2–4 μm , stained with Mayer's haematoxylin and eosin and mounted in Depex. For the analysis of the interpopulational variability of *P. ophiocéphala*, 30 fully mature specimens per population were sectioned. In order to ensure comparable degree of contraction, animals were fixed after relaxation in an isotonic MgCl_2 solution. Measurements of morphological structures were taken by the same person (the senior author), at the same microscope, with an ocular micrometer.

Karyological techniques were as described by Curini-Galletti et al. (1989). Relative lengths (r.l.=length of chromosome $\times$ 100/total length of haploid genome) and centromeric indices (c.i.=length of short arm $\times$ 100/length of entire chromosome) were obtained from measurements of camera lucida drawings of at least 10 spermatogonial metaphase plates. Karyological analysis of populations of *P. ophiocéphala* was performed on 30 plates per population. Chromosome nomenclature follows Levan et al. (1964).

In order to test variability among populations of *P. ophiocéphala*, unifactorial ANOVA was performed on seven karyological and 22 morphological variables. "Population" factor was treated as fixed. Interspecific differences were deemed as significant with $P \leq 0.01$. Cochran's C-test was used to test assumption of homogeneity of variances. Percentage values were log-normal transformed (for variables: length of ventral cilia, maximum diameter of statocyst, number of inner circular fibres of the pharynx, thickness of epithelial lining of the seminal vesicle) to achieve homogeneity of variances. When appropriate, Student–Newman–Keuls (SNK) tests were used to separate means (at $\alpha=0.05$), following significant effects in the ANOVA (Underwood 1997).

The holotype of the new species is deposited in the collections of the Swedish Museum of Natural History (SMNH). The remaining material is stored in the Zoological Museum at the Dipartimento di Zoologia ed Antropologia Biologica, Università di Sassari.

The following abbreviations are used in the figures: b, bursa; cc, ciliated, outer region of the "horizontal canal"; co, copulatory organ; e, pigmented eyespots; fd, female duct; fg, female glands; fp, female pore; ga, gastrodermis; gg, eosinophilous gland; gl, gut lumen; ma, male antrum; mp, male pore; ov, ovary; pg, prostatoid glands; ph, pharynx; pi, pigment; pp, penis papilla; r, rhabdoid; sd, seminal duct; st, statocyst; t, testes; uc, unciliated, inner region of the "horizontal canal"; v, vagina; vd, vaginal duct; vi, vitellaria; vs, seminal vesicle.

Figure 1. *Pseudomonocelis caribbea* sp. n. (A) General organization of a live animal; (B) reconstruction of the genital organs from sagittal sections.

Family MONOCELIDIDAE Hofsten, 1907

Genus *Pseudomonocelis* Meixner, 1943

***Pseudomonocelis caribbea* sp. n.**

(Figures 1, 2A–C)

Material examined

Holotype: Belize: San Pedro (Ambergris Cay) (17°57'N, 87°55'W), beach at the northern end of the town, about 30 cm deep in well-sorted medium sand (August 1992); sagittally sectioned (SMNH type-5969). Other material: three specimens (same data as holotype) studied alive and processed for karyology.

Etymology

The specific epithet is coined after the geographical location of the type locality.

Description

The sectioned specimen is about 0.8 mm in length. Anterior end rounded, provided with numerous oily droplets and with two pigmented eyespots lateral to the statocyst. Posterior

Figure 2. (A–C) *Pseudomonocelis caribbea* sp. n.: (A) copulatory organ; (B) female genital organs; (C) spermatogonial mitosis. (D–G) *P. ophiocephala*, spermatogonial mitoses from different populations: (D) CAL; (E) HAI; (F) MLK; (G) OCC. (H, I) *P. cf. cavernicola*: live animals showing cephalic pigmentation. (L) *P. pardii*: section of the female genital region at the “horizontal canal” level. See text for abbreviations. Scale bar: 5 μ m (C–G); 10 μ m (A, B, H, L); 100 μ m (I).

end rounded, with numerous adhesive papillae. The epidermis, with depressed nuclei, is ciliated except at the caudal tip. Cilia are 3–4 μm long, slightly longer dorsally than ventrally. With numerous, elongate and comparatively large rhabdoid glands (12–15 μm long in sections), particularly evident in the caudal area. Large numbers of ovoid glands, whose necks pierce the epithelium, are present all over the body: they are particularly numerous dorsally at the caudal tip. These glands are about 11 μm long, with flat nucleated lining and very fine, granular eosinophilous content. The high number of these glands, as well as of rhabdoids, gives the animal's body a distinctive "opaque" appearance. Body musculature appears weakly developed, with one layer of external circular muscles, and very few layers (one to three) of inner longitudinal fibres.

The tubular pharynx is nearly in the middle of the body. It is almost twice as long as broad. Its epithelia are ciliated (external cilia about 1.5 μm long; luminal cilia about 2.5 μm long) apart from its most distal tip, where pharyngeal glands discharge. The luminal epithelium is also unciliated in a narrow proximal area, adjacent to the very short oesophagus (about one-eighth of the pharynx length). Musculature of the pharynx consists of weak outer longitudinal muscle layers and strong inner circular layers on both the external and luminal sides.

Male genital organs. Numerous testes (30–40) occur medially in front of the pharynx. The copulatory bulb, of the simplex type, is ovoid, 55 μm long and 28 μm wide in sections. It consists of a seminal vesicle and a narrowly pointed penis papilla, about 10 μm long. The seminal vesicle is lined by a very thin nucleated epithelium, which becomes glandular ("prostatoid") distally, with nuclei located outside the bulb. The muscular coating of the bulb is formed by inner circular and outer longitudinal muscles. It is very thin proximally (about 1 μm); distally, it thickens to about 3 μm . The male antrum is narrow and unciliated proximally; it opens to the exterior through a male pore located nearly halfway between vagina and female pore.

Female genital organs. Vitellaria extend from about the level of the first testes to in front of the copulatory bulb. Two ovaries lie ventro-laterally behind the pharynx. A small (about 25 μm wide in sections) vacuolar bursa, mostly of the resorbiens type, lies between the ovaries. From the bursa, a ventrally and posteriorly orientated, ciliated vaginal duct originates, surrounded by a coating of circular muscle fibres, particularly thick distally. The vagina opens to the outside nearly halfway between the mouth and the male pore. The common female duct is unciliated and runs from the postero-ventral part of the bursa to the female pore, which is surrounded by numerous female glands.

Karyology

With three isobrachial chromosomes, nearly even in length, in its haploid set. Karyotype formula: $15.5 \pm 0.4 \mu\text{m}$; $\text{NF}=6$; chromosome 1: 34.74 ± 1.44 ; 36.11 ± 0.89 (sm); chromosome 2: 34.12 ± 0.58 ; 43.12 ± 0.73 (m); chromosome 3: 31.14 ± 0.44 ; 36.09 ± 0.98 (sm).

Remarks

This is a minute, unpigmented *Pseudomonocelis* species without accessory organ, characterized by a markedly ovoid copulatory bulb, with a very weak muscular coating and a pointed penis papilla, unlike any known so far in the genus (cf. Schockaert and

Martens 1987; Curini-Galletti and Cannon 1995; Curini-Galletti 1997). Furthermore, it is the only species in the genus where ventral pores (i.e. mouth, vagina, male and female pore) appear nearly regularly spaced. Albeit eosinophilous glands, as those described above for the new species, are of common occurrence in the genus *Pseudomonocelis* (cf. Schockaert and Martens 1987), in none of the species studied by the authors are they so densely packed, especially dorsally and caudally, that the observation of anatomical details in semi-squashed specimens is somewhat obscured.

The female genital system of the new species appears similar to that of *P. cavernicola* Schockaert and Martens, 1987. The two species are, however, immediately recognizable as *P. cavernicola*, among other differences, has a unique accessory organ located between male and female pores, and a rounded copulatory bulb provided with an evenly thick muscular coating.

Pseudomonocelis caribbea n. sp. is the only species of the genus known so far from the entire American continent.

***Pseudomonocelis pardii* Schockaert and Martens, 1987**

(Figure 2L)

Material examined

Tanzania: Dongwe, east coast of Zanzibar island, upper intertidal in mixed sediment: silty medium-fine sand with shell fragments (January 1999). Two specimens sagittally sectioned; three specimens studied karyologically.

Description

Sectioned specimens closely correspond to the original diagnosis, based on specimens from the Somali coast (Djezira). The main differences are:

1. Cilia in *P. pardii* are described as “extremely densely packed and short (2–3 μm)” and “difficult to discern, even at high power magnification” (Schockaert and Martens 1987, p 104). They are longer (about 4 μm), with a “normal” density, in Tanzanian specimens.
2. *P. pardii* has been originally described as provided with a highly muscular, wholly unciliated horizontal canal connecting the vertical vaginal duct to a large bursa resorbiens (Schockaert and Martens 1987, p 110; Figure 2). In Tanzanian specimens, the “horizontal canal” is subdivided into two distinct, enlarged regions: an outer, ciliated portion, connected to the vaginal duct, and an inner, unciliated portion, connected to a comparatively smaller bursa resorbiens (Figure 2L). Both regions of the canal are provided with a coating of circular musculature.

Karyology

With three isobranchial chromosomes in its haploid set, with the third pair markedly smaller (nearly three-fifths) than the first. Karyotype formula: $13.3 \pm 0.3 \mu\text{m}$; NF=6; chromosome 1: 39.24 ± 1.27 ; 44.97 ± 0.77 (m); chromosome 2: 37.32 ± 0.84 ; 37.12 ± 0.95 (sm); chromosome 3: 23.44 ± 0.73 ; 41.69 ± 0.90 (m).

Remarks

Pseudomonocelis pardii is a large, stocky species, characterized by pigmentation and by the shape of the copulatory organ and of the complex vagina–bursa system (Schockaert and Martens 1987). A very similar species (*P. schockaerti*, Curini-Galletti and Cannon 1995) inhabits the “overlap zone” (southern Queensland–northern New South Wales) of eastern Australia, and a sister species relationship has been proposed for them (Curini-Galletti and Cannon 1995). The shape of the “horizontal canal” of the female system, as described for Tanzanian specimens referred here to *P. pardii*, recalls that of *P. schockaerti* (in which the canal is however a veritable bursa), consisting of an outer, ciliated region, and of an unciliated inner region with thick vacuolar walls (Curini-Galletti and Cannon 1995). Available data do not allow us to infer whether morphological differences between Somali and Tanzanian specimens are artefacts due to differential quality of histological mounts, allowing for more detailed observations, or reflect actual differences among populations to be inferred. Karyotype is at present only known for Tanzanian specimens: it is very similar to that of *P. schockaerti*, the main difference among sets being the centromeric index of chromosome 3, metacentric in Tanzanian specimens and acrocentric in *P. schockaerti* (Curini-Galletti and Cannon 1995). In particular, in no other *Pseudomonocelis* species is chromosome 3 so markedly smaller than the other two pairs. This feature may thus constitute a further synapomorphy for the two species.

***Pseudomonocelis* cf. *cavernicola* Schockaert and Martens, 1987**
(Figure 2H, I)

Material examined

Tanzania: Dongwe, east coast of Zanzibar island, upper intertidal in mixed sediment: silty medium-fine sand with shell fragments (January 1999). Four specimens sagittally sectioned; four specimens studied karyologically.

Description

The original description of *P. cavernicola*, based on specimens from the Somali coast (Hawadli, N of Mogadisciu), is particularly detailed. Only major differences from Schockaert and Martens (1987) are dealt with here:

- Somali specimens were unpigmented. Tanzanian specimens had an overall yellowish pigmentation, with a distinct, ovoid patch of dark brown pigment in front of the statocyst (Figure 2H, I).
- In Somali specimens, ventral ciliation begins just cranial of the accessory organ, with cilia 4–5 μm long, over all of the body. In Tanzanian specimens, ciliation begins anterior of the male pore, and ventral cilia, in the posterior region of the body, are particularly short (about 1–1.5 μm long) and sparse. Cilia progressively increase in density and length anteriorly, reaching 2.5–3 μm about midbody.
- In Somali specimens, vagina opens to the outside about midway between male pore and mouth. In Tanzanian specimens, distance between mouth and vagina is about one-third longer than distance between vagina and male pore.

- Somali specimens had a nearly spherical copulatory bulb, about 90 μm wide and 90 μm high, lined with a muscular sheath about 9 μm in diameter. Tanzanian specimens had a similarly shaped but smaller copulatory bulb, ranging from 40 to 60 μm in diameter and from 44 to 65 μm in height in the four specimens sectioned. Thickness of the muscular coating ranged from 5 to 13 μm .

Karyology

With three isobrachial chromosomes in its haploid set. Karyotype formula: $10.1 \pm 0.5 \mu\text{m}$; $\text{NF}=6$; chromosome 1: 37.01 ± 0.38 ; 47.54 ± 0.41 (m); chromosome 2: 34.83 ± 0.52 ; 41.87 ± 1.11 (m); chromosome 3: 28.16 ± 0.37 ; 45.89 ± 0.97 (m).

Remarks

Somali and Tanzanian specimens present obvious similarities, especially in the presence of an unarmed accessory organ, located halfway between male and female pores, which closely agrees in morphological details and size in the two populations. There is also a general correspondence in the morphology of the reproductive organs, although the copulatory bulb appears consistently larger in Somali specimens. With respect to congenetics, both populations show a reduction of the ventral ciliation, particularly evident in Tanzanian specimens, where the area around the male pore is unciliated, and cilia are much sparser and shorter on both ventral and dorsal sides. The most obvious difference between the two populations lies in the pigmentation of Tanzanian specimens. Presence of a reddish brown pigment girdle in the cephalic area is a widespread feature in the genus *Pseudomonocelis*, probably arisen independently in different biogeographical areas (*P. cetinae* Meixner, 1943 and *P. agilis* (Schultze, 1851) in the Atlanto-Mediterranean region; *P. pardii*, *P. schockaerti* and two undescribed species (cf. Curini-Galletti and Cannon 1995) in the Indo-Pacific region). In none of the species above, however, has an intra- or inter-population variability in the expression of the pigmentation ever been documented. Interestingly, the unpigmented Somali specimens were collected in a coastal cave, where reduction of light may have inhibited the expression of pigmentation. It is, however, worth noting that the population collected at Dongwe (Tanzania) has been cultured in our laboratory ever since, far from any direct sunlight, and depigmented specimens have never been observed in cultures. Lack of knowledge on the intraspecific variability of *P. cavernicola*, both within the type locality and in neighbouring, extra-cave habitats, prevents any further inference on the taxonomic attribution of Tanzanian specimens.

Pseudomonocelis ophiocephala (O. Schmidt, 1861)

(Figures 2D–G, 3; Tables I–III)

The species, common and widespread in the Mediterranean in shallow-water habitats (Murina 1981), is comparatively well known, and its morphology has been discussed at length by Meixner (1943) and Schockaert and Martens (1987). Thus, it may hardly appear to need reconsideration. However, karyological assays in NW Corsica revealed differences in karyotype among neighbouring populations, apparently without intergrades. This stimulated a karyological and morphological analysis of populations attributable to *P. ophiocephala*, on a wider geographical basis.

Figure 3. Sampling localities of study populations of *Pseudomonocelis ophiocephala*. See text for abbreviations.

Table I. Karyometric data (means $\pm$ standard deviations) of *Pseudomonocelis ophiocephala*, and results of ANOVA (bottom).

	Chromosome 1		Chromosome 2		Chromosome 3		Haploid genome length (μ m)
	r.l.	c.i.	r.l.	c.i.	r.l.	c.i.	
OCC	37.96 $\pm$ 0.36	24.88 $\pm$ 0.61	34.85 $\pm$ 0.46	34.54 $\pm$ 0.32	27.20 $\pm$ 0.52	16.02 $\pm$ 0.45	13.38 $\pm$ 0.21
CVI	37.86 $\pm$ 0.26	12.31 $\pm$ 0.40	33.49 $\pm$ 0.41	39.16 $\pm$ 0.47	28.68 $\pm$ 0.37	14.30 $\pm$ 0.44	12.69 $\pm$ 0.11
PTO	37.43 $\pm$ 0.26	10.44 $\pm$ 0.35	33.25 $\pm$ 0.44	38.97 $\pm$ 0.29	28.70 $\pm$ 0.34	13.17 $\pm$ 0.34	12.52 $\pm$ 0.18
BIO	38.05 $\pm$ 0.32	25.42 $\pm$ 0.44	34.46 $\pm$ 0.53	35.09 $\pm$ 0.43	27.40 $\pm$ 0.59	14.62 $\pm$ 0.44	12.72 $\pm$ 0.19
PFE	37.01 $\pm$ 0.31	10.93 $\pm$ 0.32	33.26 $\pm$ 0.42	40.47 $\pm$ 0.38	29.73 $\pm$ 0.40	13.19 $\pm$ 0.53	12.31 $\pm$ 0.21
LIV	37.93 $\pm$ 0.22	11.75 $\pm$ 0.41	33.34 $\pm$ 0.45	38.98 $\pm$ 0.29	28.73 $\pm$ 0.30	13.84 $\pm$ 0.42	12.44 $\pm$ 0.18
STR	37.26 $\pm$ 0.24	11.65 $\pm$ 0.42	33.48 $\pm$ 0.33	40.07 $\pm$ 0.35	29.21 $\pm$ 0.57	13.83 $\pm$ 0.55	12.53 $\pm$ 0.17
COR	37.48 $\pm$ 0.27	10.99 $\pm$ 0.45	33.76 $\pm$ 0.34	40.57 $\pm$ 0.39	28.84 $\pm$ 0.30	13.41 $\pm$ 0.54	12.25 $\pm$ 0.22
GOU	37.52 $\pm$ 0.36	26.30 $\pm$ 0.44	34.35 $\pm$ 0.31	34.22 $\pm$ 0.42	28.13 $\pm$ 0.57	14.59 $\pm$ 0.39	12.44 $\pm$ 0.20
PAT	37.93 $\pm$ 0.26	26.96 $\pm$ 0.47	33.99 $\pm$ 0.27	35.83 $\pm$ 0.38	28.06 $\pm$ 0.37	14.24 $\pm$ 0.46	12.64 $\pm$ 0.16
PIR	37.34 $\pm$ 0.35	12.36 $\pm$ 0.41	33.36 $\pm$ 0.39	39.51 $\pm$ 0.37	27.18 $\pm$ 0.43	12.96 $\pm$ 0.49	12.96 $\pm$ 0.18
MLK	37.73 $\pm$ 0.28	26.23 $\pm$ 0.42	33.91 $\pm$ 0.36	35.37 $\pm$ 0.46	28.28 $\pm$ 0.34	14.10 $\pm$ 0.37	12.31 $\pm$ 0.14
NMI	38.16 $\pm$ 0.39	27.26 $\pm$ 0.45	34.67 $\pm$ 0.42	35.68 $\pm$ 0.33	27.07 $\pm$ 0.50	13.36 $\pm$ 0.53	12.24 $\pm$ 0.18
HAI	37.85 $\pm$ 0.29	12.76 $\pm$ 0.21	34.05 $\pm$ 0.36	39.70 $\pm$ 0.35	28.10 $\pm$ 0.39	12.60 $\pm$ 0.54	12.71 $\pm$ 0.22
$F_{[13,406]}$	2.091	314.191	2.012	39.073	1.574	3.463	4.032
P	0.015	0.0000	0.016	0.0000	0.091	0.0000	0.0000

See text for population abbreviations.

Populations studied

Thirteen populations, spanning the Mediterranean, were sampled (Figure 3). Unless specified, populations were studied both karyologically and morphologically.

France: Corsica: Ocelluccia cove (OCC), reduced medium sand beneath stranded masses of *P. oceanica* leaves (“banquette”) (May 1996); Calvi (CVI), harbour, medium-coarse sand (May 1996). Italy: Pto Torres (PTO), harbour, at the mouth of the river Mannu, medium-coarse sand (karyology only) (September 2000); Biodola cove (BIO), medium-coarse sand beneath a *P. oceanica* “banquette” (karyology only) (April 1999); Portoferraio bay (PFE), medium-coarse sand (karyology only) (April 1999); Livorno (LIV): harbour, medium-coarse sand (karyology only) (July 1996); Porto Cesareo, La Strea (STR), sheltered medium sand (March 2000). Greece: Corfu, Yacht Club harbour (COR), coarse sand (December 1997); Corfu, Goubià (GOU), very sheltered silty medium-fine sand (December 1997); Patràs (PAT), about 5 km south of town, sheltered outlet of an artificial channel, silty medium sand (September 1998); Piraeus, Glyfada (PIR), medium-coarse sand (September 1998); Maliakòs (MLK), harbour, silty coarse sand (September 1998); Nea Mihaniona (NMI), harbour, silty coarse sand (September 1998).

Israel: Haifa (HAI), small fishing harbour just north of Tel Shiqmona, medium-coarse sand (October 1997).

Results

Karyology

Relative lengths of corresponding chromosomes did not significantly differ among populations (Table I). Differences in centromeric indices were on the contrary highly significant, and allowed the recognition of two groups of populations, characterized by

Table II. Biometrical data (means $\pm$ standard deviations) of *Pseudomonocelis ophiocephala*, and results of ANOVA (right).

	OCC	CVI	STR	COR	GOU	PAT	PIR	MLK	NMI	HAI	$F_{[9,290]}$	P
(1)	2.18 $\pm$ 0.04	2.2 $\pm$ 0.04	2.2 $\pm$ 0.05	2.76 $\pm$ 0.05	2.19 $\pm$ 0.05	2.62 $\pm$ 0.05	2.16 $\pm$ 0.05	2.27 $\pm$ 0.05	2.28 $\pm$ 0.06	1.98 $\pm$ 0.04	24.34	0.0000
(2)	35.67 $\pm$ 0.75	33.47 $\pm$ 0.75	36.93 $\pm$ 0.95	51.46 $\pm$ 0.69	35.8 $\pm$ 1	39.8 $\pm$ 0.96	35 $\pm$ 1.13	40.8 $\pm$ 0.8	44.07 $\pm$ 1.45	47.2 $\pm$ 0.87	37.6	0.0000
(3)	38.07 $\pm$ 0.82	34.5 $\pm$ 0.91	37 $\pm$ 0.6	41.67 $\pm$ 1.06	41.1 $\pm$ 1	44.3 $\pm$ 0.7	38.63 $\pm$ 0.55	33.3 $\pm$ 0.7	41.43 $\pm$ 0.85	34.07 $\pm$ 0.9	20.13	0.0000
(4)	39.97 $\pm$ 0.97	33.97 $\pm$ 1	36.97 $\pm$ 0.84	37.6 $\pm$ 0.65	37.9 $\pm$ 0.8	41.6 $\pm$ 0.7	36.27 $\pm$ 0.57	35.9 $\pm$ 0.7	40.57 $\pm$ 1.07	32.93 $\pm$ 0.59	11.47	0.0000
(5)	13.4 $\pm$ 0.54	12.03 $\pm$ 0.43	14.47 $\pm$ 0.45	17.47 $\pm$ 0.39	13.3 $\pm$ 0.4	8.9 $\pm$ 0.5	12.4 $\pm$ 0.47	11.3 $\pm$ 0.5	15.47 $\pm$ 0.47	10.97 $\pm$ 0.39	28.28	0.0000
(6)	1.8 $\pm$ 0.1	1.7 $\pm$ 0.11	1.4 $\pm$ 0.11	2 $\pm$ 0.11	2.5 $\pm$ 0.1	2.2 $\pm$ 0.1	3.4 $\pm$ 0.1	1.6 $\pm$ 0.1	1.7 $\pm$ 0.1	1.2 $\pm$ 0.1	36.65	0.0000
(7)	15.67 $\pm$ 0.78	14.5 $\pm$ 0.94	15.17 $\pm$ 0.78	20.33 $\pm$ 0.74	13.3 $\pm$ 0.7	19 $\pm$ 1	1.77 $\pm$ 0.08	2.07 $\pm$ 0.08	1.72 $\pm$ 0.06	1.15 $\pm$ 0.11	12.18	0.0000
(8)	1.23 $\pm$ 0.07	0.7 $\pm$ 0.08	0.77 $\pm$ 0.12	0.88 $\pm$ 0.07	1.4 $\pm$ 0.1	1.1 $\pm$ 0.1	1.03 $\pm$ 0.08	0.9 $\pm$ 0.05	0.75 $\pm$ 0.08	0.9 $\pm$ 0.07	7.9	0.0000
(9)	2.78 $\pm$ 0.1	2.57 $\pm$ 0.08	2.63 $\pm$ 0.09	3.8 $\pm$ 0.88	2.2 $\pm$ 0.1	2.4 $\pm$ 0.1	3.32 $\pm$ 0.09	2.47 $\pm$ 0.08	2.65 $\pm$ 0.05	3 $\pm$ 1.3	29.04	0.0000
(10)	4 $\pm$ 0.1	3.35 $\pm$ 0.11	3 $\pm$ 0.07	3.8 $\pm$ 0.08	2.6 $\pm$ 0.1	2.6 $\pm$ 0.05	3.05 $\pm$ 0.08	2.44 $\pm$ 0.14	3.32 $\pm$ 0.09	2.98 $\pm$ 0.07	30.23	0.0000
(11)	258.03 $\pm$ 8.1	339.4 $\pm$ 9.81	327.3 $\pm$ 9.61	397.74 $\pm$ 7.5	269.3 $\pm$ 8.42	263.54 $\pm$ 7.6	320.33 $\pm$ 8.5	335 $\pm$ 7.45	334.7 $\pm$ 8.65	390.3 $\pm$ 7.5	34.34	0.0000
(12)	17.97 $\pm$ 0.6	18.2 $\pm$ 0.49	18.6 $\pm$ 0.59	23.1 $\pm$ 0.58	20 $\pm$ 0.66	18.6 $\pm$ 0.7	19.47 $\pm$ 0.5	18.9 $\pm$ 0.61	18.03 $\pm$ 0.41	21.9 $\pm$ 0.72	8.53	0.0000
(13)	4.13 $\pm$ 0.74	4.87 $\pm$ 0.6	4.9 $\pm$ 0.51	3.67 $\pm$ 0.44	4.03 $\pm$ 0.31	3.9 $\pm$ 0.5	4.2 $\pm$ 0.99	3.6 $\pm$ 0.32	3.83 $\pm$ 0.83	2.7 $\pm$ 0.56	20.58	0.0000
(14)	4.8 $\pm$ 0.37	5 $\pm$ 0.48	4.4 $\pm$ 0.45	3.8 $\pm$ 0.44	4.4 $\pm$ 0.3	4.2 $\pm$ 0.3	4.5 $\pm$ 0.81	3.87 $\pm$ 0.26	3.73 $\pm$ 0.34	3.83 $\pm$ 0.35	13.57	0.0000
(15)	3.1 $\pm$ 0.08	29 $\pm$ 0.59	25.17 $\pm$ 0.68	29.33 $\pm$ 0.58	3 $\pm$ 0.1	2.9 $\pm$ 0.6	3 $\pm$ 0.08	29.67 $\pm$ 0.63	2.8 $\pm$ 0.05	2.95 $\pm$ 0.07	5.39	0.0000
(16)	1.85 $\pm$ 0.02	2.05 $\pm$ 0.05	2.05 $\pm$ 0.07	2.22 $\pm$ 0.07	2.1 $\pm$ 0.1	2 $\pm$ 0.05	2.22 $\pm$ 0.04	2.05 $\pm$ 0.05	1.98 $\pm$ 0.06	1.83 $\pm$ 0.05		
(17)	19.9 $\pm$ 0.37	21.37 $\pm$ 0.30	21.5 $\pm$ 0.24	24.1 $\pm$ 0.33	21.27 $\pm$ 0.3	20.1 $\pm$ 0.4	22.1 $\pm$ 0.33	23.37 $\pm$ 0.44	21.07 $\pm$ 0.27	22.06 $\pm$ 0.3	14.75	0.0000
(18)	26.5 $\pm$ 0.52	28.5 $\pm$ 0.66	30.37 $\pm$ 0.82	35.5 $\pm$ 0.99	33.9 $\pm$ 0.69	36.1 $\pm$ 0.75	29.76 $\pm$ 0.69	34.2 $\pm$ 0.72	32.9 $\pm$ 0.76	27.97 $\pm$ 0.51	21.78	0.0000
(19)	17.8 $\pm$ 0.51	15.5 $\pm$ 0.46	12.1 $\pm$ 0.42	17.07 $\pm$ 0.52	17.4 $\pm$ 0.4	13 $\pm$ 0.4	17.03 $\pm$ 0.35	17.03 $\pm$ 0.56	14.8 $\pm$ 0.39	16.97 $\pm$ 0.39	20.05	0.0000
(20)	16.3 $\pm$ 0.35	15.17 $\pm$ 0.29	14.77 $\pm$ 0.35	18.5 $\pm$ 0.32	15.4 $\pm$ 0.3	16.9 $\pm$ 0.3	16.23 $\pm$ 0.28	16.8 $\pm$ 0.37	16.43 $\pm$ 0.33	15.03 $\pm$ 0.32	12.08	0.0000
(21)	13.8 $\pm$ 0.3	12.97 $\pm$ 0.28	13.33 $\pm$ 0.24	15.5 $\pm$ 0.23	13.5 $\pm$ 0.1	14.7 $\pm$ 0.2	13.13 $\pm$ 0.27	13.27 $\pm$ 0.25	13.9 $\pm$ 0.34	11.43 $\pm$ 0.29	16.48	0.0000
(22)	0.94 $\pm$ 0.02	1.02 $\pm$ 0.02	1 $\pm$ 0.02	1.11 $\pm$ 0.02	1.08 $\pm$ 0.02	1.08 $\pm$ 0.02	1.05 $\pm$ 0.02	0.92 $\pm$ 0.01	1.02 $\pm$ 0.02	1.05 $\pm$ 0.02	8.74	0.0000
(23)	12.76 $\pm$ 0.35	11.93 $\pm$ 0.36	12.27 $\pm$ 0.37	11.27 $\pm$ 0.29	12.47 $\pm$ 0.36	11.27 $\pm$ 0.31	12.33 $\pm$ 0.38	11.77 $\pm$ 0.2	12.5 $\pm$ 0.32	11.67 $\pm$ 0.29	2.21	0.0133

Values, if not specified, are in μm . Characters are as follows: (1) total length (in mm); (2) distance between male and female pores; height (3) and width (4) of the copulatory organ; (5) length of penis papilla; thickness of proximal (6) and lateral (7) muscle sheath of the copulatory bulb; (8) thickness of the epithelial lining of the seminal vesicle; length of dorsal (9) and ventral (10) body cilia; (11) length of pharynx; (12) length of unciliated apex of pharynx; number of outer (13) and inner (14) distal circular muscle fibres of the pharynx ($\times 10 \mu\text{m}$); length of inner (15) and outer (16) cilia of pharynx; (17) diameter of statocyst; (18) maximum diameter of neuropil; (19) thickness of ganglionic lobes of brain; length of cephalic (20) and body (21) rhabdoids; (22) height/width ratio of copulatory organ; (23) body rhabdoids/total length ratio. See text for population abbreviations.

Table III. Student–Newman–Keuls (SNK) tests on significantly different karyological (above) and morphological (below) parameters.

c.i. Chromosome 1	PTO=PFE=COR=STR=LIV=CVI=PIR=HAI<<OCC=BIO=MLK=GOU=PAT=NMI
c.i. Chromosome 2	GOU=OCC=BIO=MLK=NMI=PAT<<PTO=LIV=CVI=PIR=HAI=STR=PFE=COR
c.i. Chromosome 3	na
Genome length	NMI=COR=PFE=MLK=GOU=LIV=PTO=STR=PAT=CVI=HAI=BIO=PIR<OCC
(1)	CVI=PIR=OCC=GOU=STR<PAT=MLK<NMI<HAI<<COR
(2)	MLK=HAI=CVI<STR=OCC=PIR<GOU=NMI=COR<PAT
(3)	na
(4)	HAI<PIR=OCC=GOU=STR=CVI=MLK=NMI<<PAT<COR
(5)	PAT<<HAI=MLK=CVI=PIR=GOU=OCC=STR=NMI<<COR
(6)	OCC=PAT=GOU<<PIR=STR=NMI=MLK=CVI<<HAI=COR
(7)	HAI=STR=MLK=NMI=CVI=OCC=COR=PAT=GOU<<PIR
(8)	na
(9)	GOU=PAT=MLK=CVI=STR=NMI=OCC=HAI<<PIR<<COR
(10)	MLK=GOU=PAT<HAI=STR=PIR=NMI=CVI<<COR=OCC
(11)	OCC=NMI=CVI=STR=PAT=MLK=PIR=GOU<HAI=COR
(12)	OCC=PAT<NMI=GOU=CVI=STR=HAI=PIR<MLK=COR
(13)	HAI<<MLK=COR=NMI=PAT=GOU=OCC=PIR<<CVI=STR
(14)	na
(15)	OCC=HAI=CVI=PIR=STR<NMI=GOU=MLK=COR=PAT
(16)	STR=PAT<<NMI=CVI=HAI=MLK=PIR=COR=GOU=OCC
(17)	STR=HAI=CVI=GOU=PIR=OCC=NMI=MLK=PAT<<COR
(18)	STR<<NMI=CVI=COR=HAI=PAT=MLK=PIR=GOU=OCC
(20)	CVI=NMI=STR=COR=HAI=MLK=PIR=PAT=OCC<GOU
(21)	HAI<<CVI=PIR=MLK=STR=GOU=OCC=NMI=PAT<COR
(22)	MLK=OCC<STR=NMI=CVI=PIR=HAI=PAT=GOU=COR

See legend of Table II for coding of morphological characters. See text for population abbreviations. na, no alternative hypotheses could be identified.

different chromosome sets (Table III). One set (henceforth called karyotype A), present in CVI, PTO, LIV, PFE, STR, COR, PIR, and HAI, showed chromosome 1 acrocentric with high index value, and chromosome 2 metacentric with a low value (Figure 2D, E). The other set (karyotype B), present in OCC, BIO, GOU, PAT, MLK, and NMI, was characterized by chromosome 1 at the border between subtelo- and submetacentric, and chromosome 2 submetacentric with a high index value (Figure 2F, G). Chromosome 3, albeit significantly different in the sample, did not allow recognition of discrete subsets of populations. Differences in haploid genome length were due to a single population (OCC), significantly larger than the others (Table III).

Morphology

Twenty-one morphological characters, plus the ratio between height and width of the copulatory bulb, were analysed (see legend of Table II for details). The character “length of outer cilia of pharynx” did not pass the Cochran’s C-test, and was excluded from further analyses. All other characters displayed significant differences among populations (Table II). For a few characters (width of copulatory organ, thickness of lateral muscle sheath of the bulb, number of inner circular fibres of pharynx), discrete groupings among populations could not be identified (Table III). When clusters of populations were

evidenced (distance between male and female pore, height of copulatory organ, diameter of statocyst, length of ventral cilia, length of pharynx, maximum diameter of neuropil), they were neither internally consistent, nor geographically structured, and in no instances was there a correspondence with the two clusters of populations separated by karyometry. For most characters, significant differences among populations (total body length, ratio height/width of the copulatory organ, length of penis papilla, thickness of proximal muscle sheath of the bulb, thickness of the epithelial lining of the seminal vesicle, length of dorsal cilia, length of inner cilia of pharynx, length of the unciliated apex of pharynx, number of outer circular fibres of pharynx, thickness of ganglionic lobes of brain, length of cephalic and body rhabdoids) were exclusively due to few (one to three) diverging populations (Table III). COR, the population attaining the largest size, was, in most instances, the outlier. However, the second largest population (PAT) did not show a similar pattern. In order to avoid a possible size effect, all analyses were re-run, and each morphological variable was divided by the absolute length of the specimen considered. Similar to previous analyses, results were highly significant (data not shown), apart from length of body rhabdoids, which, in this case, displayed no significant differences among populations (character 23 in Table II).

Remarks

The 13 populations examined spanned most of the species' distribution. The species, in fact, has never been reported in the westernmost Mediterranean, and research on Spanish coasts by the senior author were unsuccessful.

Karyometric data neatly split the sample of populations into two distinct clusters. Populations showing karyotype A span the Mediterranean, from Corsica (CVI) to Israel (HAI). Populations with karyotype B were found in three distinct areas: the Sardinian–Corsican complex, including Elba Is. (OCC, BIO), as well as west (GOU, PAT) and east (MLK, NMI) coasts of Greece. Remarkably, populations with different karyotypes were collected in beaches only a few kilometres apart, as in Corsica (CVI, OCC), Elba (PFE, BIO) and Corfu (COR, GOU). In no instances were specimens with intermediate karyotypes found. Such a clear-cut situation is not paralleled by the results of the morphological analysis, where not only each parameter differed significantly across samples, but clusters of populations were conflicting, and never consistent with the two karyological clusters. The singularity of significantly different populations, in cases showing the same karyotype, and occurring in the same geographical area, needs consideration. Though particular care has been devoted to remove artefacts due to differential treatment of specimens, a degree of deformation of some morphological structures may accidentally occur during fixation, and bias the overall result. However, for most populations, redundant numbers of individuals were sectioned, and obviously deformed specimens were discarded from the sample. On the other hand, given the limited dispersal power of proseriates (Curini-Galletti 2001), morphological differences may hint at a genetical heterogeneity among populations. In any case, results strongly suggest the existence of at least two sibling species, distinct in karyotype, within the taxon *P. ophiocéphala*. There are clear indications of habitat separation among siblings, as populations with karyotype A occur in higher energy, medium-coarse sand, while populations with karyotype B were found in low-energy habitats. Differential habitat selection among populations with karyotype B (reduced sediment beneath stranded *P. oceanica* leaves for the westernmost populations; silty coarse sand for the Greek populations) stimulates further studies aimed at assessing their conspecificity.

Discussion

The randomness of most of the samples from which specimens dealt with here have been isolated suggests that species of the genus *Pseudomonocelis* may be far more common and widespread, especially in intertropical habitats, than hitherto considered. The poor state of our knowledge of tropical proseriates, with vast areas of the planet still unsampled, makes it plausible that present diversity of the genus (with only eight species described) is severely unrepresentative of its actual contribution to marine biodiversity. Furthermore, the lack of any sclerotized structures, which are most often helpful in species identification in Proseriata, raises the suspicion that cryptic diversity may be present within the few taxa described. Available data do not allow assessment of whether differences between Somali and Tanzanian populations—about 1100 km apart—have to be considered as intraspecific variability, or, especially in the case of *P. cf. cavernicola*, assumed to be due to interspecific differentiation. On the contrary, more exhaustive data on the Mediterranean *P. ophiocephala* support the hypothesis of the presence of sibling species, with different habitat requirements, in the taxon. If confirmed by further genetic studies presently in progress (M. Casu and M. Curini-Galletti, in preparation), this case is particularly disturbing, since not even a particularly detailed biometrical analysis allowed discrimination among species, and raises questions on the actual extent of our knowledge of the biodiversity of interstitial flatworms, which may be much higher than perceived at present. The fact that morphology alone may not be enough for safe taxonomic attribution should be taken into account when comparing populations of taxa with few diagnostic morphological characters (such as Monocelidinae with simplex copulatory organ), wide biogeographical ranges and catholic habitat choice.

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