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*Contribution to the knowledge of the family
Otoplanidae Hallez, 1892 (Rhabditophora,
Proseriata) in the Mediterranean*

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Abstract

A growing awareness is rising that present perception of marine biodiversity is inadequate, and based upon unrepresentative data. The inadequacy of knowledge on taxonomy and distribution of marine organisms is particularly acute for interstitial meiofauna, especially for 'soft bodied' taxa, which necessitate of observations on both living and fixed specimens. Among these groups, information on Platyhelminthes, a species-rich, ubiquitous and diverse taxon, is at present far from adequate, and is entirely absent for entire biogeographical regions. In this scenario, my research aimed to gather data in the composition and distribution of a family of Platyhelminthes Proseriata, the Otoplanidae Hallez, 1892, in biogeographically significant sectors along the coasts of Mediterranean. The most striking results of a series of sampling campaigns is the great number of new Otoplanidae species found. In this work fifteen new species, belonging to the genus *Parotoplana* Meixner, 1938 are formally described: *P. pythagorae* Delogu & Curini-Galletti, 2007; *P. spathifera* Delogu & Curini-Galletti, 2007; *P. terpsichore* Delogu & Curini-Galletti, 2007; *P. jondelii* Delogu & Curini-Galletti, 2007; *P. cucullata* Delogu et al., 2008; *P. fretigaditani* Delogu et al., 2008; *P. varispinosa* Delogu et al., 2008; *P. mastigophora* Delogu et al., 2008; *P. geminispina* sp. n., *P. crassispina* sp. n., *P. carthagoensis* sp. n., *P. ichnusae* sp. n., *P. pulchrispina* sp. n., *P. obtusispina* sp. n. and *P. axii* sp. n.

Introduction

Following the United Nations Conference on Environment and Development (UNCED) held at Rio de Janeiro in 1992, the value of biological diversity has been recognised also by governments and numerous national and international plans were launched to investigate global biodiversity both in terrestrial and marine environment. Traditionally marine environment is considered a low specific diversity habitat but at present a growing awareness is rising that this perception of marine biodiversity is inadequate and based upon unrepresentative data (Bouchet *et al.* 2002, Sala 2002). The inadequacy of data on taxonomy and distribution of marine organisms is particularly acute for interstitial meiofauna, where information may be absent altogether for entire biogeographical regions (Kennedy and Jacoby 1999). The lack of data about interstitial meiofauna is particularly worrisome if we consider in terrestrial habitat, the highest values of species diversity falls in the size class of 1-2 mm (Siemann *et al.* 1996) which, in the sea, is mostly represented by meiofaunal taxa. Knowledge of taxa belonging to the so-called “soft bodied” meiofauna, which necessitate observations on both living and fixed specimens, is particularly limited. Among meiofaunal taxa Platyhelminthes are most important for their contribution both in number of species and individuals. Their abundance is generally related to the granulometry of the sediments; they are well represented in sandy sediments (7 - 25% of total meiofauna in density), where their diversity, as well as the biomass, may reach and even exceed that of Nematodes. Moreover in high-coarse grained sediments, Platyhelminthes may represent the 95% of total meiofauna (Remane 1933, Radziejewska & Stankowska-Radziun 1979, Martens & Schockaert 1986). Information on Platyhelminthes is at present far from adequate and it is considered one of the taxa (as well as Polychaeta, Olygochaeta, and Nematoda), that especially needs new identification guides and revised taxonomic lists (Costello *et al.* 2006). In fact, only few areas are relatively well known, especially northern Europe and the Galápagos Islands (Ax & Schmidt 1973, Armonies & Reise 2000), with little and taxonomically limited research elsewhere. Recent evident suggest that marine-interstitial flatworms have limited geographical ranges (Casu & Curini-Galletti 2006) presumably linked to their lack of dispersal stage. For this reason these are the groups where modification in ranges and species composition, caused by phenomena of global change, could be more easily detectable. However, the absence of basic knowledge of most taxa for entire biogeographical regions has always prevented them utilisation for environmental monitoring. Furthermore the poor state of taxonomic knowledge may lead species to undetected cases of “silent extinction”.

Among Platyhelminthes, Proseriata (Neophora), represent a taxon of high interest. Proseriata is a diverse and species-rich taxon of small (1-10 mm long), mostly free-living Platyhelminthes. They are particularly common in coastal habitats, from shallow water to the upper intertidal (Sopott 1972, 1973; Reise 1984, 1988). In some cases, they may be among the dominating organisms of interstitial assemblages, e.g., the Otoplanidae in the high-energy intertidal zone (Remane 1933). Many proseriates occupy narrow ecological niches. As a consequence, they show low α -diversity, i.e., in any given locality, each sediment type usually harbours a limited number of species. However larger scale data of proseriates suggest that γ -diversity of the group may be particularly high. In fact, different marine areas harbour quite distinct proseriate faunas. In Europe, North Atlantic has only the 7.5% of species in common with the Mediterranean (Curini-Galletti 2001), on the other hand among prosobranch gastropods, a group renowned as good biogeographical descriptors (Briggs 1974), shared species constitute about 18% of the mediterranean species (Poppe & Goto 1991). This implies that proseriates may experience physical and climate barriers to distribution to a finer degree than most macrofaunal taxa, resulting in “small-scale” biogeography, and ample opportunities for allopatric speciation.

In this scenario, my research aimed to gather data in the composition and distribution of a family of Proseriata, the Otoplanidae Hallez, 1892, in the Mediterranean.

The family Otoplanidae is the dominant taxon in the surf zone of the high energy beaches (the “Otoplana-zone” of Remane, 1933) (Reise 1988; Curini-Galletti 2001). It is known from marine environments of the North Atlantic (and adjacent seas: North Sea and Baltic Sea)(Ax 1956, 1959; Ax & Sopott-Ehlers 1987; Karling 1973; Giard 1904; Graff 1913; Luther 1960; Martens & Schockaert 1981; Meixner 1938; Sopott 1972; Sopott-Ehlers 1976, 1985; Sopott-Ehlers & Ehlers 1980), the Pacific Ocean (Karling 1964; Ax & Ax 1967; Tajika 1938a-b-c, 1984), the Atlantic coast of South America (Marcus 1949, 1950, 1952), the Galápagos Islands (Ax & Ax 1974), and the eastern Australia (Miller & Faubel 2003). Only five species has been reported from the freshwater or brackish-water environments (Riemann 1965; Gieysztor 1938; Ax 1951; Ax & Armonies 1990; Noreña et al., 2005). In Mediterranean only few areas, the Gulf of Lion, the Gulf of Naples and the Tuscany coast, has been studied (Du Plessis, 1889; Ax 1956; Ax, Weidemann & Ehlers 1978; Lanfranchi 1969, 1978). Recently only two new species (*Otoplana oxypina* Lanfranchi & Melai 2007; *Parotoplana rosignana* Lanfranchi & Melai 2008) have been reported for Mediterranean (Lanfranchi & Melai 2007, 2008), excluding my contributions.

During the three years of the Doctorate, a series of sampling campaigns has been undertaken, aimed to gather data on species composition and distribution of Proseriata, in biogeographically significant sectors along the coasts of Mediterranean and, particularly, I have dealt with Otoplanidae species. The sampling areas were been:

- 1 Porto Cesareo – Italy, Ionian sea;
- 2 Capo Caccia-I.la Piana – Italy, Tyrrhenian sea;
- 3 Tuscany coasts – Italy, Tyrrhenian sea;
- 4 Miramare-Gulf of Trieste – Italy, Adriatic sea;
- 5 Archipelago of La Maddalena (National Park) – Italy, Tyrrhenian sea;
- 6 Gulf of Catania – Italy; Ionian sea;
- 7 Cres Island – Croatia, Adriatic sea;
- 8 Coasts of Southern Spain, Alboran Sea;
- 9 Coasts of Tunisia, Gulf of Gâbes;
- 10 Banyuls sur mer – France, Gulf of Lion.

The most striking results of these campaigns is the great number of Otoplanidae species found: about 80 species, most of them new! Furthermore, most species show much more restricted distribution than hitherto supposed. In fact many appear exclusive of single geographical sectors, if not single localities! The remarkable number of new species found reveals the amount of work which still needs to be performed before an adequate knowledge of the biodiversity of interstitial flatworms of the Mediterranean is attained. In fact many sectors are still unsampled and, the species inventory, is presumably far from being definitive. The almost total absence of species shared among central-east Mediterranean, northern Adriatic, Ionian sea, Alboran sea, west coast of Europe and the North African coastline, suggests that the ‘ traditional ’ biogeographical regions based on macrofaunal organisms might not apply to meiofauna. The restricted range of most species (in some cases, apparently limited to single brackish lagoons) is particularly noteworthy, and may constitute a previously undetected challenge for conservation, which is most important in Mediterranean whose marine biodiversity is, actually, undergoing rapid alteration because of a process of “tropicalization” (Bianchi & Morri 2003; Bianchi 2007), or rather the occurrence and spread of warm-water species due to different causes, such as Atlantic influx, lessepsian migration, introductions by humans and sea warming. The results of the sampling campaigns stress, if necessary, the potential interest (on issues spanning from biodiversity to biogeography, patterns of speciation, conservation) of the study of meiofauna – unfortunately, too often neglected (even by granting agencies...) in favour of macrofaunal taxa.

Because of the large number of new species found belonging to different taxa, I focused my studies, primarily on the genus *Parotoplana* Meixner, 1938. The genus *Parotoplana* Meixner, 1938 is characterized by the presence of a usually collar-shaped pharynx, entirely ciliated with the exception of its distal tip; serial arrangement of gonads (two rows of testes in front of the prepharyngeal ovaries; two rows of vitellaria behind the ovaries), and a female apparatus with paired oviducts which can join up into a female duct behind the pharynx or distally, before reaching the common genital atrium; bursa opening independently into the common genital atrium, between the openings of the male and female systems (Ax 1956). Fifteen new species are formally described.

Systematic.

The family Otoplanidae was established by Hallez (1892) which raised to the rank of family the genus *Otoplana* described by Du Plessis in 1889 and included it in the order Tricladida, suborder Maricola. Few years later, the genus *Otoplana* was excluded from the previous order by Vejdovsky (1895), and placed with the genus *Bothrioplana* Braun, 1881 inside the family Bothrioplanidae. After the monographic work done by Graff (1908, 1913) and the discovery of the new genus *Bothriomolus* Hallez, 1909, the family Otoplanidae was re-established again. While Graff supported them as an independent family, Hallez grouped the genera *Otoplana* and *Bothriomolus* in the subfamily Otoplaninae, family Bothrioplanidae (Hallez 1910). Later, Hofsten (1918) divided the group Alloecoela Crossocoela + Cyclocoela in four distinct families: Monocelididae, Otomesostomatidae, Otoplanidae and Bothrioplanidae. The only attempt of a division of the family Otoplanidae was done by Palombi (1926), after the description of the species *Digenobothrium inerme* Palombi 1926. He divided the family in two subfamilies:

1. subfamily Otoplaninae; genus *Otoplana*
2. subfamily Bothriomolinae, genera *Bothriomolus* and *Digenobothrium*.

However, Marcus (1949), proved on the basis of different characteristics such as the epithelium of the body completely ciliated, male and female gonopores separate and the copulatory organ without spines, that the genus *Digenobothrium* did not belong to the family Otoplanidae and placed it in the subfamily Promonotinae, family Monocelididae.

In 1956, the family Otoplanidae was characterise and discussed in detail by Peter Ax in the only monography of the family existing today, in which, in addition to the Subfamily Otoplaninae Hallez, 1892 three new subfamilies were established:

1. subfamily Archotoplaninae Ax, 1956;
2. subfamily Bulbotoplaninae Ax, 1956;
3. subfamily Parotoplaninae Ax, 1956.

Ax included in the family Otoplanidae, numerous new species on the basis of two main characteristics:

1. the restriction of ciliation to the ventral creeping sole and to small dorso-lateral areas of the anterior end; the exception is the subfamily Archotoplaninae which lack ciliation in the cephalic region and the caudal area of the copulatory organs only;
2. the presence of a common gonopore.

No other important taxonomic and morphological studies of the family have been done after Ax's monography since, 2003.

In 2003 Miller and Faubel, in fact, after the discovery of the species *Cirroplana mansi* Miller & Faubel, 2003, which is provided of a cirrus instead of a sclerotized apparatus in association with the male copulatory organ, established the new subfamily Cirroplaninae and emended the family diagnosis of the Otoplanidae (which included like diagnostic feature the presence of the sclerotized apparatus) to encompass the new subfamily Cirroplaninae. Furthermore the diagnosis of the subfamily established by Ax, except for the subfamily Archotoplaninae, was emended and a new identification key of the genera of the subfamily Otoplaninae was done to include the new genus *Lauraplana* Miller & Faubel, 2003 which presented all the classic diagnostic features of the subfamily Otoplaninae (see the diagnosis of the subfamily below) with the exception of the separate gonopore.

The following is the updated diagnosis of the family Otoplanidae and the complete keys to the genera of the subfamily Otoplaninae (Miller & Faubel, 2003) and Parotoplaninae (with the insertion of taxa described after Ax, 1956).

Family Otoplanidae Hallez, 1892

Diagnosis. – Proseriata with characteristic otoplanid head and lateral ciliated pits. **With penial stylet complex or cirrus armed with thorns**; seminal vesicle caudal or lying above the male opening. If sclerotized present, always consisting of needles an often with additional median funnel-like tube. Opening of male and female ducts through a single common pore (**exception being *Lauraplana* Miller & Faubel, 2003**). Ciliation of body restricted to the ventral creeping sole and to small dorso-lateral areas of the forebody, the exception being species of the Archotoplaninae.

Diagnosis by Ax, (1956), emendation by Miller & Faubel (2003) in bold.

Type genus. – *Otoplana* Du Plessis, 1889.

Key to the subfamilies of the Otoplanidae :

- 1 body totally covered with cilia.....Archotoplaninae Ax, 1956
 - body with cilia only on the ventral side.....2
 2 pharynx bulbous, reversed inner muscle layers.....Bulbotoplaninae Ax, 1956
 - pharynx with normal muscle layers.....3
 3 male copulatory organ with sclerotized stylet and/or spines.....4
 - **male copulatory organ without spines but with armed cirrus.....**
**Cirroplaninae Miller & Faubel, 2003**
 4 pharynx long, cylindrical, horizontal; with cement and shell glands.....
Otoplaninae Hallez, 1910
 - pharynx short, collar-like, diagonally or perpendicularly orientated to the ventral
 side; only a uniform shell gland complex present.....Parotoplaninae Ax, 1956

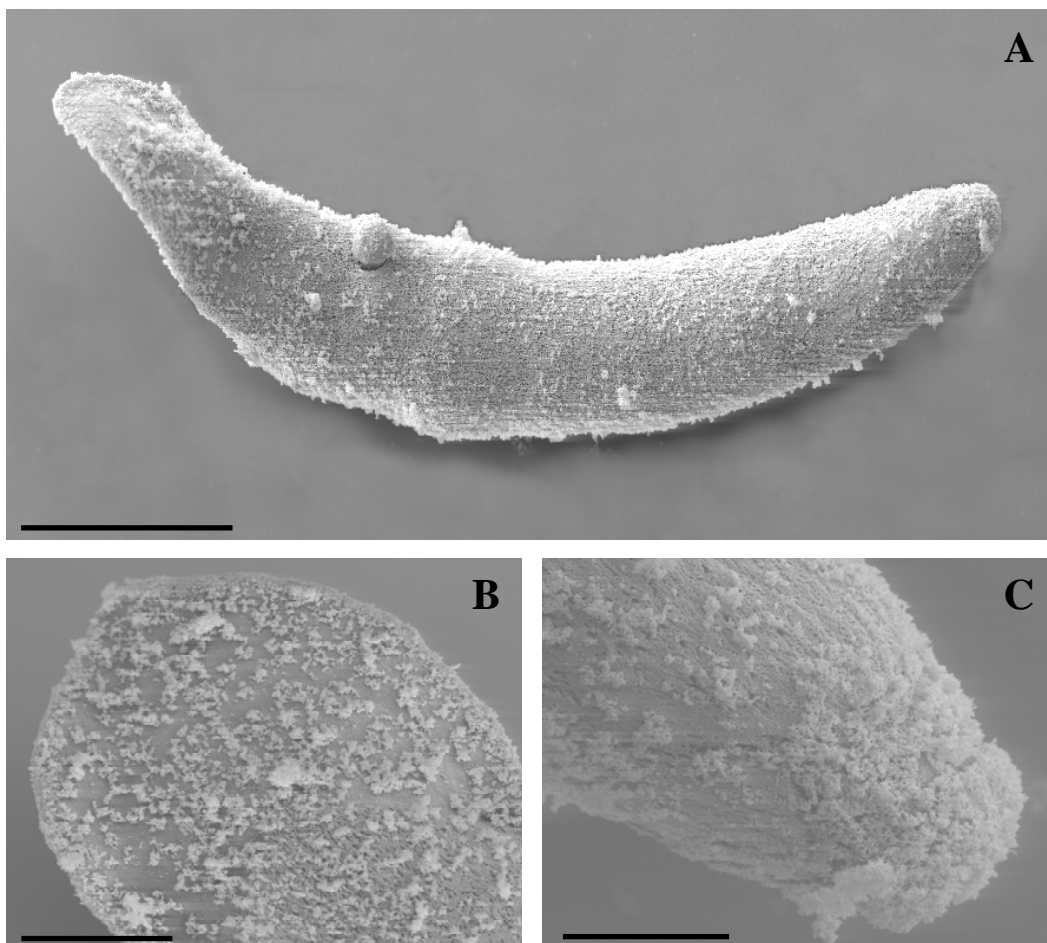


Figure 1. *Archotoplana holotricha* Ax, 1956. A.: Scanning electronic fotomicrograph of the external morphology, scale 250 μm .; B. Caudal end, scale 100 μm ; C. Cephalic end, scale 50 μm .

Subfamily Archotoplaninae Ax, 1956

Diagnosis. Otoplanidae with body totally covered with cilia. Ciliation absent in the head region and in the caudal area of the copulatory organ only. With cylindrical pharynx, horizontally oriented. Cement and shell glands differentiated.

Type and only genus of the subfamily: *Archotoplana* Ax, 1956 (figs. 1A-B-C).

Subfamily Bulbotoplaninae Ax, 1956

Diagnosis. Otoplanidae with a highly specialised pharynx. Pharynx short and barrel-like, ciliated outside, with grasping bulge and reversed muscle layers (circular muscles immediately beneath the epithelium layer, deeper longitudinal muscles). Accessory genital glands differentiated into cement and shell glands. **Common gonopore.**

Diagnosis by Ax, (1956), emendation by Miller & Faubel (2003) in bold.

Type and only genus of the subfamily: *Bulbotoplana* Ax, 1956.

Subfamily Otoplaninae Hallez, 1910

Diagnosis. Otoplanidae with cylindrical pharynx in horizontal plane. Pharynx ciliated outside and inside (exception, *Otoplanella baltica* with a short pharynx ciliated only externally). Intestine above pharynx narrowed to a small tube (exception, *Orthoplana foliacea*). Accessory genital glands differentiated into cement and shell glands. With common gonopore or **separate genital pores.**

Diagnosis by Ax, (1956), emendation by Miller & Faubel (2003) in bold.

Type genus: *Otoplana* Du Plessis, 1889 (figs. 2A-B-C).

Key to the genera of Otoplaninae:

- | | | |
|---|--|---|
| 1 | common genital pore present..... | 2 |
| - | male and female gonopores separate..... | <i>Lauraplana</i> Miller & Faubel, 2003 |
| 2 | in addition to the common genital pore, no additional pore present..... | 8 |
| - | in addition to the common genital pore, 1 or 2 accessory pores developed..... | 3 |
| 3 | accessory pores of the female system..... | 5 |
| - | accessory pores of the male system..... | 4 |
| 4 | seminal bursa present..... | <i>Otoplana</i> Du Plessis, 1889 |
| - | seminal bursa lacking..... | <i>Zygotoplana</i> Tajika, 1983 |
| 5 | accessory pores of the female system (3); vaginal pores on the ventral side..... | 7 |

- 1 or 2 vaginal pores on the dorsal side.....6
- 6 paired or unpaired primary bursa, additional vesicles absent*Kata* Marcus, 1949
- primary bursa and numerous bursal vesicles.....*Pluribursaeplana* Ax & Ax, 1967
- 7 vaginal pores on the ventral side (5); with prepharyngeal bursa.....
.....*Xenotoplana* Ax, Weidemann & Ehlers, 1978
- without bursa but with pregenital vagina.....*Alaskaplana* Ax & Armonies, 1990
- 8 in addition to the common genital pore no additional pores present (1); without
primary seminal bursa.....10
- primary seminal bursa which opens to the genital atrium.....9
- 9 primary bursa enters genital atrium from behind.....*Itaspiella* Ax, 1956
- primary bursa enters genital atrium via vagina from in front.....
.....*Americanaplana* Ax & Ax, 1967
- 10 primary seminal bursa absent (8); stylet apparatus with funnel-like tube and
needles.....12
- stylet apparatus without funnel-like tube but with needles.....11
- 11 glands surrounding distal germovitelloduct.....*Serpentiaplana* Karling, 1964
- glands at distal germovitelloduct and genital pore.....*Bothriomolus* Hallez, 1909
- 12 stylet apparatus with funnel like tube and needles (10); bilateral ovaries and
vitellaria.....14
- single germaria.....13
- 13 testes follicles, vitellaria and germaria in single median line anterior to pharynx.....
.....*Monostichoplana* Ax, 1956
- testes follicles and germaria single, vitellaria bilateral.....
.....*Notocaryoplanella* Ax, 1956
- 14 bilateral ovaries and vitellaria (12); ovaries single at each side.....15
- ovaries increased to 2-3 on each side.....*Itaspis* Marcus, 1952
- 15 bilateral testes follicles.....18
- testes follicles in median line.....16
- 16 prepharyngeal testes follicles in median line.....17
- prepharyngeal testes follicles in single, clustered complex.....
.....*Pseudorthoplana* Ax, Weidemann & Ehlers, 1978
- 17 pharynx in mid-body.....*Otoplanella* Ax, 1956
- pharynx caudal close to copulatory complex.....*Notocaryoplana* Steinböck, 1935
- 18 bilateral testes follicles (15); with germovitelloducts uniting caudally of genital

atrium to a common duct.....	20
- with germovitelloducts uniting anteriorly to genital atrium to common duct.....	19
19 bilateral germovitelloducts in whole length of body, branching behind the pharynx to unite to common duct.....	<i>Paradoxoplana</i> Ax, 1956
- with germovitelloducts uniting anteriorly to pharynx to a common duct.....	
.....	<i>Notocaryoturbella</i> Lanfranchi, 1969
20 with germovitelloducts uniting caudally of the genital atrium to a common duct (18); stylet apparatus with one type of needles.....	<i>Dicoelandropora</i> Ax, 1956
- stylet apparatus with two types of needles.....	
.....	<i>Napoliplana</i> Ax, Weidemann & Ehlers, 1978

Note to the key.

Orthoplana Steinböck, 1932 incertae sedis

In 1956, Ax provisionally placed *Orthoplana mediterranea* Ax, 1956 and *Orthoplana borealis* (Steinböck, 1935) in the genus *Orthoplana*. However nothing is known of the female characteristics and the two species differ from each other in several characters (Ax, 1956). For this reason the genus *Orthoplana* is not included by Miller & Faubel in the key presented above.

Danubia antipa (An Der Lan, 1962)

On the basis of *Otoplana antipa* An Der Lan, 1962, Mack-Fira (1970) established the genus *Danubia* without giving a diagnosis. As the description of *O. antipa* made by An Der Lan (1962), is very poor in taxonomy and morphology, the genus *Danubia* could not be considered by authors in the determination key of the genera of Otoplaninae.

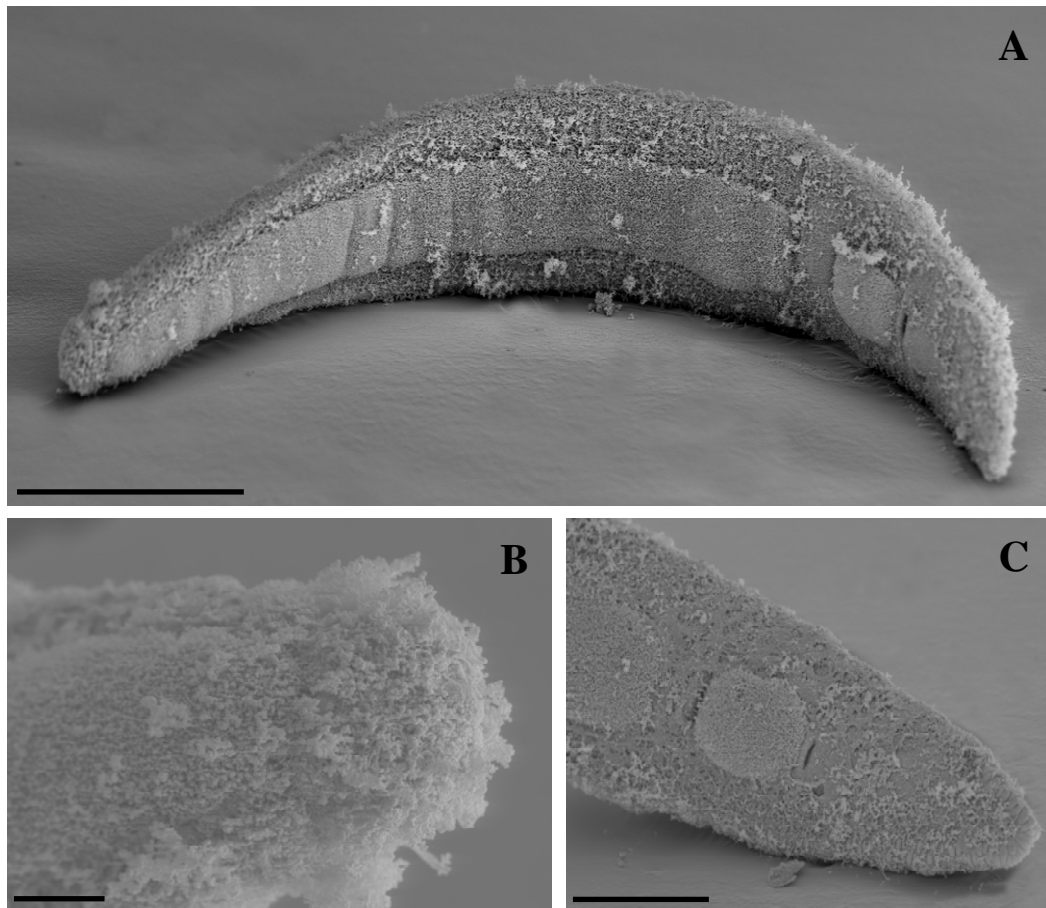


Figure 2. *Otoplana* sp.: A. Scanning electronic fotomicrograph of the external morphology, scale 200 µm.; B. Cephalic end, scale 20 µm; C. Particular of caudal end in which are well visible the mouth and the genital pore, scale 100 µm.

Subfamily Cirroplaninae Miller & Faubel, 2003

Diagnosis. Otoplanidae with adenal nuclei of the epithelia over the entire body. Ciliation restricted to the creeping sole. Pharynx bulbous like, diagonally orientated in relation to the ventral surface, in mid-body, with normal muscles layers (longitudinal outer muscles, directly beneath the epithelium, and circular inner muscles). Pharynx ciliated internally and externally. Male copulatory organ with cirrus armed with small thorns. Paired seminal vesicle, no seminal bursa. Testis follicles unpaired in a median line anterior to the pharynx; vitellaria paired along the length of the body from behind the brain to anterior the male copulatory organs. Common gonopore.

Type and only genus of the subfamily: *Cirroplana* Miller & Faubel, 2003.

Subfamily Parotoplaninae Ax, 1956

Diagnosis. Otoplanidae with short collar-like pharynx diagonally or vertically oriented in relation to the ventral side. Pharynx always internally ciliated, outside often without cilia. Intestine above the pharynx with wide lumen. Accessory genital glands compact at the opening of the germovitelloducts into the genital atrium (exception, *Pseudosyrtris* Ax, 1956).

With common gonopore.

Diagnosis by Ax, (1956), emendation by Miller & Faubel (2003) in bold.

Type genus: *Parotoplana* Meixner, 1938 (figs. 3A-B-C).

Key to the genera of Parotoplaninae:

- 1 epithelium with intraepithelial nuclei in all body or with insunk nuclei in the creeping sole and in the anterior end.....2
 - epithelium with insunk nuclei in all body.....12
- 2 prepharyngeal testes follicles.....3
 - postpharyngeal testes follicles.....*Polyrhabdoplana* Ax & Ax, 1967
- 3 seminal bursa present.....5
 - seminal bursa lacking.....4
- 4 sclerotized apparatus with central stylet and a girdle of spines equal like each other; separated prostatic vesicle and sclerotized apparatus.....*Pseudosyrtris* Ax, 1956
 - sclerotized apparatus without a central stylet but with a girdle of spines of different morphologies. Connected prostatic vesicle and sclerotized apparatus.....*Philosyrtris* Giard, 1904
- 5 with primary bursa which opens to the genital atrium through one or two bursal canal.....6
 - with secondary bursa, anterior or posterior to the male copulatory organ, which opens to the outside through an independent pore.....10
- 6 bursal canal which opens to the genital atrium anterior to the copulatory organ, internally ciliated. With spermatic ducts connected to the ovaries.....*Praebursoplana* Ax, 1956
 - bursal canal which opens to the genital atrium posterior to the copulatory organ; without spermatic ducts.....7
- 7 bursal canal connected to the middle part of the genital atrium, unpaired germovitelloduct opening behind.....8
 - bursal canal connected to the posterior part of the genital atrium, germovitelloduct opening before.....*Postbursoplana* Ax, 1956

- 8 epithelium with intraepithelial nuclei in all body.....*Parotoplana* Meixner, 1938
 - epithelium with insunk nuclei in the ciliated parts of the body.....9
 9 with unpaired bursal canal; ovaries placed in the anterior part of the body behind
 the cephalic area; pharynx internally ciliated.....*Parotoplanella* Ax, 1956
 - with paired bursal canal; ovaries placed in the middle of the body in front of the
 pharynx; pharynx both internally and externally ciliated.....*Parotoplanina* Ax, 1956
 10 secondary bursa unpaired, posterior to the copulatory organ.....11
 - secondary bursa paired, between pharynx and copulatory organ, which open to the
 outside through two independent pores. With spermatid ducts connected to the
 ovaries.....*Triporoplana* Ax, 1956
 11 epithelium with intraepithelial nuclei in all body; pharynx both internally and
 externally ciliated.....*Kataplana* Ax, 1956
 - epithelium with insunk nuclei in the ciliated parts of the body; pharynx internally
 ciliated.....*Otoplanidia* Meixner, 1938
 12 epithelium with insunk nuclei in all body; with bursa of resorbiens type dorsal to
 the copulatory organ.....*Galapagoplana* Ax & Ax, 1974
 - epithelium with insunk nuclei in all body; without bursa.....*Paraplana* Ax, 1956

Note to the key

In the description of the genus *Paraplana* Ax, 1956, the author indicated as a diagnostic character the presence of a vesicular organ opening independently to the outside posterior to the copulatory organ, and suggested the possibility that this organ could be a seminal bursa but the exiguous sample did not allow to confirm the hypothesis. For this reason, awaiting new data, I have considered the genus lacking of bursa in the key presented above.

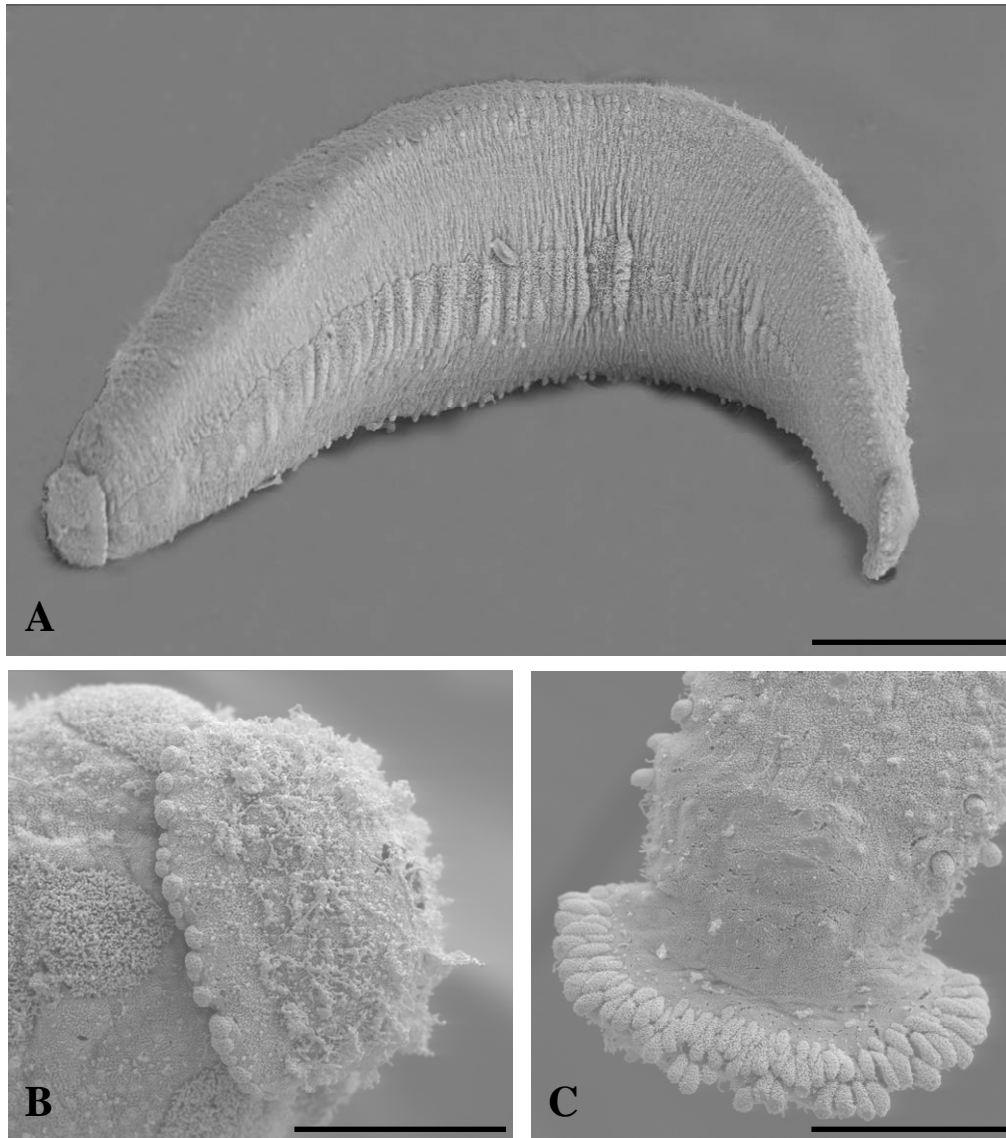


Figure 3. *Parotoplana* sp.: A. Scanning electronic fotomicrograph of the external morphology, scale 200 μm .; B. Particular of the cephalic end, scale 50 μm ; C. Caudal end provided of numerous adhesive glands, scale 50 μm .

Recently molecular data supported the monophyly of the Otoplanidae (Littlewood *et al.*, 2000; Curini-Galletti, 2001) however in a morphological tree, *Archotoplana holotricha* Ax, 1956 which is the type of the genus, did not cluster with the rest of Otoplanidae. *Archotoplana*, in fact, lacks some of the obvious apomorphies of the Otoplanidae (ciliated creeping sole, sensory pits with thick sensory bristles)(Ax, 1956). Basis on this work, Ax (2008) in the monography of the Platyhelminthes of brackish water of the north-hemisphere, underlines the position of the genus *Archotoplana* which shows the plesiomorphic character of the body totally covered with cilia and suggest the name Euotoplanida to define the rest of Otoplanidae which show the apomorphic characters (ciliated creeping sole, sensory pits with thick sensory bristles). These results confirm the necessity of future research both to

understand the fluidity of supra generic arrangement and to describe new species. The future description of new species is particularly important to understand the morphological variety of the taxon which, actually, is based on poor data. Furthermore, the contribution of new molecular data is desirable and necessary to verify the actual status of subfamilies and genera.

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New species of the genus *Parotoplana* Meixner, 1938 (Proseriata, Otoplanidae) from southern Apulia (Italy)

Zootaxa

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Abstract

A survey of the genus *Parotoplana* Meixner, 1938 in southern Apulia (Italy) resulted in the finding of six species, four of which are new, and are formally described here. *P. pythagorae* sp. n. is distinguished by the presence of sharply triangular apices of companion spines of the stylet. *P. spathifera* sp. n. has the lowest number of spines (eight) known in the genus, arranged in four pairs of different morphology. *P. terpsichore* sp. n. differs from *P. multispinosa* Ax, 1956 by the number and shape of spines. *P. jondelii* sp. n. has unique broad spines and is equipped with bursal spines. The karyotypes of *P. pythagorae* sp. n. (n=6), *P. spathifera* sp. n. (n=9) and *P. jondelii* sp. n. (n=6) are described. The remaining two species belong to the species-group of *P. renatae* Ax, 1956 / *P. macrostyla* Lanfranchi, 1978, and their identification on the basis of the limited sample available is problematical. The finding of numerous new species in an area as comparatively well studied as the central Mediterranean bears witness to our poor knowledge of the biodiversity of marine interstitial flatworms.

Key words: taxonomy, marine biodiversity, meiofauna, Mediterranean, *Parotoplana*, new species

Introduction

The Proseriata is a diverse and species-rich taxon of neophoran Platyhelminthes. They are particularly common in marine, coarse sediments; one family in particular, the Otoplanidae, is the dominant taxon in the surf zone of high-energy beaches (the '*Otoplana*- zone' of Remane, 1933) (Reise, 1988; Curini-Galletti, 2001). However, the overall complexity of the study of interstitial Platyhelminthes (which includes observations of living organisms as well as of sections to appreciate the fine details of their complex reproductive system) has so far jeopardized a detailed knowledge of their ecological role and contribution to marine biodiversity. Furthermore, as is the case for many interstitial flatworms, most species are only known from their type locality. Present data do not allow to decide whether this may indeed point to the existence of narrow distributional ranges, or rather only reflect an inadequacy of samplings.

Recently, under the framework of the project 'BIOIMPA' ('Biodiversity of Inconspicuous Organisms in Italian Marine Protected Areas'), a series of sampling campaigns has been undertaken, aimed to gather data on species composition and distribution of selected meiofauna taxa along the coasts of Italy. Not unexpectedly, samples yielded a vast number of new species of interstitial Platyhelminthes, which are currently in the course of description. Members of the genus *Parotoplana* Meixner, 1938 (Proseriata: Otoplanidae) were particularly well represented in the samples. The genus is characterized by the presence of a collar-shaped pharynx in the middle of the body, a serial arrangement of gonads (two rows of testes in front of the prepharyngeal ovaries; two rows of vitellaria behind the ovaries), and a female apparatus with a bursa opening independently into the common genital atrium, between the openings of the male system and the common oviduct, which arises from the post-pharyngeal fusion of the germovitelloducts (Ax, 1956).

In this first contribution, the composition of the genus *Parotoplana* in the marine protected area of Porto Cesareo and surroundings (southern Apulia, Mediterranean Sea) is presented and discussed.

Material and methods

Specimens were collected in sandy habitats by scooping up the superficial layer of sediment. Extraction of the animals from the sediment was with $MgCl_2$ decantation (Martens, 1984). Each species was first studied alive by slight squeezing under the cover slip. Preservation techniques routinely adopted for Proseriata were used (see Martens et al., 1989); whole mounts were made with lactophenol. For microscopic analysis material was fixed in Bouin's fluid, embedded in 60°C Paraplast, and serial sagittal sections were cut at 4 μm , stained with Hansen's haematoxylin and eosin-orange and mounted in Eukitt. When not otherwise stated, single morphological measurements given in species description are based on the holotypes.

The karyotype was determined from acetic orcein stained spermatogonial mitoses, as described by Curini Galletti et al. (1989). Relative lengths ($r.l. = \text{length of chromosome} \times 100 / \text{total length of haploid genome}$) and centrometric indices ($c.i. = \text{length of short arm} \times 100 / \text{length of entire chromosome}$) were obtained from measurements of camera lucida drawings of metaphase plates. The fundamental number (FN) (*i.e.* the number of chromosome arms in the karyotype) is derived according to Matthey (1949) and the chromosome nomenclature employed is 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 material, when present, is stored 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; ca: common atrium; cm: circular musculature; co: copulatory organ; fd: female duct; gc: glandular cells; go: glandular organ; gp: genital pore; ma: male antrum; msa: male sclerotized apparatus; nhe: nucleated high epithelium; o: oviduct; ov: ovary; ph: pharynx; pv: prostatic vesicle; sg: shell glands; sv: seminal vesicle; te: testis; vi: vitellaria.

Results

Family Otoplanidae Hallez, 1892

Genus *Parotoplana* Meixner, 1938

Parotoplana pythagorae sp. n.

(Figs. 1 A–C; Figs. 6 A–C)

Holotype: one whole mount (SMNH 6661).

Type locality: Apulia, Italy: Porto Cesareo (Lecce), Bacino Grande Bay, loc. Scala di Furno (lat. 40°16'26"N, long. 17°52'53.68"E), slightly silty coarse sand among rocks, lower intertidal in the *Otoplanazone*. May 2005.

Additional material: Paratype (SMNH 6662): one specimen from the type locality, sagittally sectioned. Six specimens sagittally sectioned (CZM-34/39); two karyological slides (CZM-40,41); two whole mounts (CZM-42,43), all from the type locality.

Etymology: the species name relates to the presence, in the sclerotized structures, of peculiar right-angled triangles, to which Pythagoras' (Samos, 571-496 B.C.) most famous theorem refers.

Description. Living specimens agile, colourless and semi-transparent, of average size for the genus: the holotype, an adult worm, is about 1.3 mm long in fixed condition. The anterior end is clearly marked and pro-

vided with sensory bristles, and, ventrally, with a few adhesive papillae. The posterior end is fan-shaped and provided with very numerous adhesive papillae (Fig. 1 C). The encapsulated, oval shaped brain (about 60 μm long) abuts on a statocyst (about 21 μm in diameter).

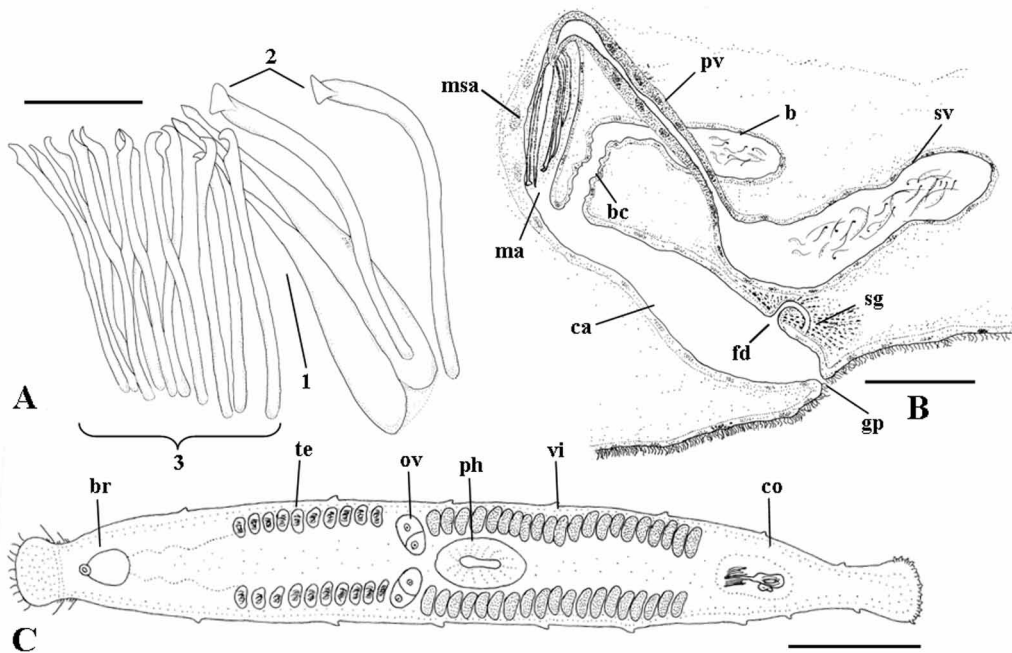


FIGURE 1. *Parotoplana pythagorae* sp. n. A, Sclerotized apparatus. Scale = 30 μm . B, Reconstruction of the genital organs from sagittal sections. Scale = 50 μm . C, General organisation of a live animal. Scale = 200 μm .

Epithelium with intraepithelial nuclei. Ciliation restricted to a sensorial groove in the anterior end, and, ventrally, to a creeping sole that extends from the anterior end to posterior to the genital pore. The ciliated epithelium is markedly higher than the non-ciliated epithelium. Cilia up to 7 μm in length. Rhabdoids (up to 15 μm in length) are numerous dorsally and arranged in longitudinal rows.

The gut extends posteriorly nearly to the caudal end and anteriorly to the cephalic area. The nearly median pharynx is collar-shaped; its nucleated epithelium is ciliated except for a small area at the distal tip. Pharyngeal cilia are about 3 μm long. No esophageal area could be seen. The pharyngeal cavity opens to the outside through the mouth, which is surrounded by a ring of circular muscles.

Male genital organs. Testes, located anterior to the ovaries, consist of two lateral rows of about ten follicles each (Fig. 1 C). The copulatory organ consists of a sacciform seminal vesicle (about 105 μm long), connected distally to a prostatic vesicle (Figs. 1 B; 6 A, C). The seminal vesicle is lined by a nucleated epithelium. The prostatic vesicle is narrowly elongate and its nucleated epithelium is non ciliated. It is distally connected to the sclerotized apparatus (Figs. 1 A; 6 B) which consists of a central stylet (1), two companion spines (2) and a girdle of smaller spines (3). The stylet (95 μm long in the holotype, range 93–95 μm in the sample) wraps around the distal portion of the ejaculatory duct. In well-squeezed specimens, it is evident that the seemingly tubiform structure seen in semi-squashed living specimens consists of two spines connected for most of their length by a feebly sclerotized lamina. These spines are straight and their distal end is acutely rhomboidal. Two companion spines are placed laterally to the stylet. These spines are 96–98 μm long, slightly wider than the other spines, with distinctly triangular apices. Stylet and companion spines are surrounded by a girdle of 12 slender spines (69 to 76 μm long) with sickle shaped apices, provided with a somewhat obtuse subterminal tooth. The sclerotized apparatus is located within the male antrum, lined by a nucleated epithelium, which opens into the anterior portion of the common atrium.

Female genital organs. Two ovaries (Fig. 1 C) are located anterior to the pharynx. Two rows of yolk follicles (vitellaria) are present posterior to the ovaries to the level of the copulatory organ. In the sectioned material, it is possible to trace the presence of the female common duct only in its distal portion, at its opening into the common atrium, where it is surrounded by numerous shell glands (Fig. 1 B). The bursa (Figs. 1 B; 6 A), which is comparatively large and obvious in living specimens, opens into the common atrium close to the opening of the male system, by means of a wide canal about 70 μm long in the sectioned paratype, lined by an epithelium which appears irregular in shape, with numerous folds. The bursa, which is filled with sperm in all specimens sectioned, is lined by a low, nucleated epithelium. Male antrum, female duct and bursal canal open into a vast common genital atrium, which is lined by non ciliated, nucleated epithelium, surrounded by well developed longitudinal muscles. It opens to the outside through the common genital pore.

Karyotype. Chromosome number: $n = 6$; $FN = 12$. All chromosomes are isobrachial; the first two pairs are more than twice the size of the remaining chromosomes (Fig. 8 B). Chrom. I = r.l.: 28.49 ± 0.68 ; c.i.: 46.06 ± 0.55 (m); Chrom. II = r.l.: 27.80 ± 0.21 ; c.i.: 44.04 ± 0.66 (m); Chrom. III = r.l.: 12.3 ± 0.59 ; c.i.: 35.8 ± 3.01 (sm); Chrom. IV = r.l.: 11.99 ± 0.76 ; c.i.: 46.73 ± 0.49 (m); Chrom. V = r.l.: 9.94 ± 0.18 ; c.i.: 30.06 ± 0.7 (sm); Chrom. VI = r.l.: 9.46 ± 0.46 ; c.i.: 36.86 ± 2.66 (sm) (based on the measurements of five spermatogonial plates).

Remarks. Among the species of *Parotoplana* provided with a stylet (*P. renatae*; *P. papii* Ax 1956; *P. procerostyla* Ax 1956; *P. pacifica* Ax & Ax 1967; *P. macrostyla* Lanfranchi 1978 and *P. uncinata* Lanfranchi 1978) only the Mediterranean *P. renatae* and *P. macrostyla* share with *P. pythagorae* **sp. n.** a general arrangement of spination consisting of a central stylet, two flanking companion spines and a girdle of smaller spines. Size of the sclerotized structures is comparable: the stylet is about 84–104 μm in *P. renatae*, 87–90 μm in *P. macrostyla* and 93–95 μm in the new species; companion spines reach respectively 100 μm (*P. renatae*), 87–90 μm (*P. macrostyla*) and 96–98 μm (*P. pythagorae* **sp.n.**) in length; girdle spines are about 71–77 μm long in *P. renatae*, 74–77 μm in *P. macrostyla* and 69 to 76 μm in *P. pythagorae* **sp.n.** However, in none of the aforementioned species, are the companion spines provided with a distinctly triangular apex, easily appreciable even in semi-squashed living specimens. On the contrary, the apex of the companion spines is sickle-shaped in *P. macrostyla* and *P. renatae* (Ax, 1956, Fig. 158, pg. 225), which is provided with a triangular, laminar stylet. The stylet of *P. macrostyla* has a split apex (Lanfranchi, 1978, Fig. 1 B, pg. 250), and thus somewhat similar to the new species. However, in *P. macrostyla* the common female duct is virtually not existing, and the two oviducts join just at their opening into the common atrium (Lanfranchi, 1978, Fig. 3, pg. 250). On the contrary, the existence of a common female duct could be observed in all the sectioned specimens of *P. pythagorae* **sp. n.**

The only species of *Parotoplana* whose karyotype is known is *P. macrostyla*. The karyotype of *P. pythagorae* **sp. n.** appears very similar for number and relative size of chromosomes. However, in this species all chromosomes are isobrachial, whereas in *P. macrostyla* Chrom. VI is subtelocentric, with low centromeric index (Curini-Galletti et al., 1984).

Diagnosis: Species of *Parotoplana* with a wide bursal canal, lined with a corrugated epithelium. The sclerotized apparatus of the copulatory organ consists of a stylet (93–95 μm long); two companion spines with triangular apex (96–98 μm long) and a girdle of 12 spines, provided with sickle shaped apices and subterminal, obtuse tooth (69–76 μm long). Karyotype with $n = 6$, and all chromosomes isobrachial.

***Parotoplana spathifera* sp. n.**

(Figs. 2 A–B; Figs. 6 D–E)

Holotype: one whole mount (SMNH 6663).

Type locality: Apulia, Italy: Porto Cesareo (Lecce), Torre Scianuli (lat. 40°14'3.72"N, long. 17°54'35.77"E), about 7 m deep in coarse sand among rocks, May 2005.

Additional material: Paratype (SMNH 6664): one specimen from the type locality, sagittally sectioned. Seven specimens sagittally sectioned (CZM-44/50); one karyological slide (CZM-51); three whole mounts (CZM-52/54), all from the type locality.

Etymology: the specific epithet refers to the shape of one pair of spines in the sclerotized organ, the apex of which somewhat resembles the spathe of an arum lily (fam. Araceae).

Description. The holotype is an adult worm, about 1.5 mm long in fixed condition. The anterior and the fan-shaped posterior end are provided with numerous adhesive papillae. Short tactile bristles are present at the cephalic end. Encapsulated, oval shaped brain 55 μm long in the paratype; statocyst about 20 μm in diameter. The nucleated epithelium is ciliated ventrally (with cilia about 6 μm long), forming a creeping sole, which runs from the anterior end to behind the genital pore.

Numerous rhabdoids (up to 20 μm long) are scattered all over the body. Subepidermal longitudinal musculature well developed ventrally. The collar shaped pharynx (about 80 μm long) is located in the second half of the body. It is entirely ciliated (cilia about 3,5 μm long), with the exception of its distal tip.

Male genital organs. Two lateral rows of testes (about 34 in each row) are present anterior to the ovaries. The copulatory organ consists of a large sacciform seminal vesicle (76 μm in length in the paratype), which is distally connected to an elongate prostatic vesicle (Figs. 2 B; 6 E). The seminal vesicle has a nucleated epithelium and it is provided with a layer of well-developed circular musculature. The prostatic vesicle, lined by a glandular, non-ciliated epithelium, is distally connected to the sclerotized apparatus (Figs. 2 B; 6 E) which consists of four pairs of spines, symmetrically arranged (Figs. 2 A; 6 D):

1. two median spines with pointed apices (100 μm long in the holotype, ranging 100–102 μm);
2. two spines (94–95 μm long; range: 93–95 μm) with sickle shaped apices, with pointed distal end and a subterminal tooth;
3. two spines (90–92 μm long; range: 89–92 μm) with recurve apices, provided with a feebly sclerotized, distal subtriangular lamina;
4. two spines (70–72 μm long; range 70–74 μm) with slightly sickle shaped apices and obtuse distal tip.

The male antrum is lined with a nucleated epithelium; it opens into the anterior part of the common genital atrium.

Female genital organs. Two ovaries anterior to the pharynx. Two rows of vitellaria extend from posterior to the ovaries to anterior of the copulatory organ. About five vitellarium vesicles are present anterior to the pharynx and 17 behind it. The ovoid bursa is lined by a nucleated epithelium (Figs. 2 B; 6 E). It opens into the common atrium through a wide, straight canal with a low, nucleated epithelium.

The oviducts are ciliated. They join distally into an extremely short, ciliated, female duct. This female duct is partly surrounded by few, very large glandular cells (the 'glandular organ') and by the female glands (Figs. 2 B; 6 E). It opens into the common genital atrium. The epithelium of the atrium is high and nucleated. Adjacent to the 'glandular organ', the epithelium is formed by glandular cells, with very fine, basophilous secretion.

Karyotype. Chromosome number: $n = 9$; $FN = 16$. Chromosome pairs differ noticeably in size and centromeric index. Chrom. I is a large metacentric; Chrom. II is medium sized, at the border between submetacentric and subtelocentric. The remaining pairs can be arranged in a decreasing series; Chrom. IX is very small, about 1/9th the size of the largest pair. The only plate suitable for karyometric analysis yielded the following data: Chrom. I = r.l.: 27.9; c.i.: 45.07 (m); Chrom. II = r.l.: 16.13; c.i.: 25.4 (sm); Chrom. III = r.l.: 12.6; c.i.: 28.87 (sm); Chrom. IV = r.l.: 10.22; c.i.: 45.12 (m); Chrom. V = r.l.: 9.3; c.i.: 41.05 (m); Chrom. VI = r.l.: 7.92; c.i.: 17.14 (st); Chrom. VII = r.l.: 7.26; c.i.: 14.83 (st); Chrom. VIII = r.l.: 6.72; c.i.: 28.34 (sm); Chrom. IX = r.l.: 3.28; c.i.: 33.33 (sm).

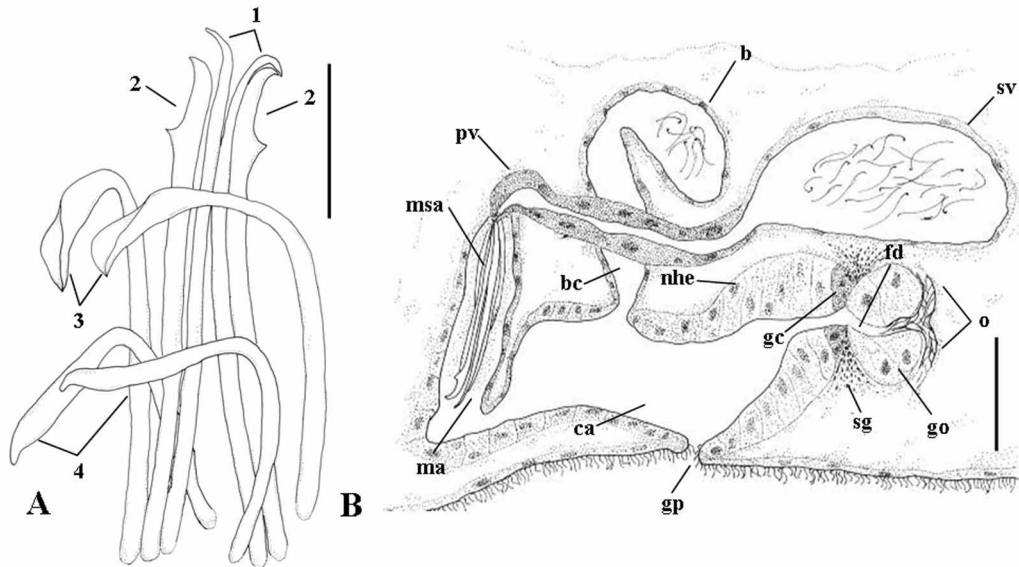


FIGURE 2. *Parotoplana spathifera* sp. n. A, Sclerotized apparatus. Scale = 30 μ m. B, Reconstruction of the genital organs from sagittal sections. Scale = 60 μ m. Abbreviations are the same as Fig 1.

Remarks. Except for the new species, species of *Parotoplana* lacking a central stylet include *P. capitata* Ax 1956, *P. primitiva* Ax 1956, *P. multispinosa* Ax 1956, *P. moya* Marcus 1949, *P. turgida* Ax & Ax 1974, *P. bermudensis* Ax & Sopott-Ehlers 1987, *P. lata* Ax & Sopott-Ehlers 1987, *P. subtilis* Ax & Sopott-Ehlers 1987, *P. mollis* Ax & Sopott-Ehlers 1987, and *P. bicupa* Sopott-Ehlers 1976. Only *P. spathifera* sp. n. however has a sclerotized apparatus consisting of four pairs of spines, with each pair markedly different in shape from each other. Furthermore, it has the lowest number of spines (eight) known for the genus *Parotoplana*. Only *P. turgida* has a sclerotized apparatus of less than 10 spines. In this species, however, the spination consists of two larger and seven distinctly smaller spines; all spines have sickle shaped apices (Ax & Ax, 1974).

The 'glandular organ' seen in the female genital system of the new species is similar to that described for *P. capitata* (Ax, 1956, fig. 150, pg. 220) and for *P. uncinata* (Lanfranchi 1978, fig. 7, pg. 254). In *P. capitata*, however, a much longer female duct is present. The distal fusion of the oviducts, as seen in the new species, is a rare character in the genus *Parotoplana*, and only reported from *P. macrostyla* and *P. uncinata* (Lanfranchi, 1978). Furthermore, in both these species the epithelium lining the female portion of the common atrium is high and glandular, similarly to *P. spathifera* sp. n. The morphology of the sclerotized structures in the species above is however quite distinct, as both *P. macrostyla* and *P. uncinata* have a central stylet, and a girdle of numerous spines with the same morphology. Any phylogenetic inference based on the shared presence of the 'glandular organ' in these species should therefore be deferred until a thorough revision of the genus is accomplished.

P. spathifera sp. n. is the only species of *Parotoplana* so far known with $n = 9$. The small size of many chromosome pairs and their low centromeric index suggest that the high haploid number is derived through a series of fissioning processes. Robertsonian mechanisms of chromosome fission and fusion have already been documented in Proseriata, and appear to be a widespread pattern of karyological evolution in the group (Curini-Galletti et al., 1989).

Diagnosis: Species of *Parotoplana* with a glandular organ surrounding the outlet of the extremely short female duct in the common atrium. The sclerotized apparatus consists of two median spines with pointed apices (100–102 μ m long); two spines (93–95 μ m) with sickle shaped apices, with pointed distal end and a sub-terminal tooth; two spines (89–91 μ m) with spathe-shaped apices; two spines (70–74 μ m) with sickle shaped apices and rounded distal tip. Karyotype with $n = 9$, with chromosome pairs markedly differing in size.

***Parotoplana jondelii* sp. n.**

(Figs. 3 A–B; Figs. 7 A–B)

Holotype: one whole mount (SMNH 6666).**Type locality:** Apulia, Italy: Santa Maria di Leuca (Lecce), cave 'la Principessa' (lat. 39°47'58.50"N, long. 18°22'27.86"E), about 5 m deep in medium to coarse sand, May 2005.**Additional material:** two specimens from the type locality studied alive, one prepared as whole mount (CZM-55) and one sagittally sectioned (CZM-56)**Etymology:** This species is dedicated to Prof. Dr. Ulf Jondelius (Sweden) in recognition of his contribution to the study of Platyhelminthes, and for his kind patience in dealing with the enthusiasm of the first author in identifying meiofaunal taxa.**Description.** Holotype about 1 mm long in fixed conditions. External morphology similar to the other species of the genus, with clearly marked anterior end and fan-shaped caudal end. Limited observations of the internal anatomy could be retrieved from the poor-quality sectioned specimen. The encapsulated oval shaped brain (60 µm long) abuts on the statocyst. Rhabdoids (up to 9 µm in length) are present dorsally and ventrally at both ends of the body. The creeping sole (cilia up to 6,5 µm long) extends from the anterior end to the genital pore. The ciliated epithelium is distinctly higher than the surrounding, non-ciliated epithelium.

The subepidermal longitudinal musculature is particularly well developed ventrally.

The holotype showed a moderately elongate, tubiform pharynx, horizontally oriented. The other two specimens, on the contrary, showed a more typical collar-shaped pharynx.

Male genital organs. With two rows of about eight testes each in front of the ovaries. The male copulatory organ consists of a sacciform seminal vesicle, a comparatively short prostatic vesicle (Fig. 3 B) lined by a ciliated epithelium, a sclerotized apparatus (Figs. 3 A; 7 A) consisting of 18 spines in both the whole mounts.

Five types of spines, arranged symmetrically into a girdle, can be recognized:

- 1) two very narrow and straight spines, 75–79 µm long in the holotype;
- 2) four broad spines, 75–83 µm long, with slightly recurve, bulbous apices provided with a tooth placed at the basis of the distal third of the spine;
- 3) four broad spines, 70–80 µm long, narrowing distally into acute apices without any subterminal tooth;
- 4) two broad spines (62–65 µm long), with sickle shaped apices and marked subterminal tooth;
- 5) six spines similar in morphology to the previous, but distinctly smaller (53–55 µm long).

In specimen CZM 55, the morphology of the spines was essentially similar, although sizes were somewhat reduced (group 1 = 60 µm; group 2 = 65 µm; group 3 = 65–67 µm; group 4 = 59–60 µm; group 5 = 52–57 µm). In this specimen, most spines appeared feebly sclerotized basally, presumably due to an early stage of maturity. According to the position of spines in the whole mounts, spines of group 1 seem to be median, and probably act as a functional stylet; spines of group 3 appear to flank the previous ones, and the other spines are symmetrically arranged at their sides.

The male antrum has a nucleated epithelium, and opens into the common atrium.

Female genital organs. With two ovaries anterior to the pharynx. Two rows of numerous vitellaria are present posterior to the ovaries. In preparations of semi-squashed living specimens, a bursa provided with a distinct bursal canal was observed. In sections, the morphology of the bursal canal is obscured by the strong circular musculature that enwraps it entirely. The ovoid bursa shows dorsally a resorbiens portion. In the whole mount, the presence of numerous bursal spines (Fig. 7 B), arranged into two blocks, could be seen. These spines are sharply triangular in shape (up to 4–5 µm long), feebly sclerotized, and appear as basal lamina derivatives. No bursal spines were found in CZM-55 or in the sectioned specimen (CZM-56).

Karyotype. Chromosome number: $n = 6$; $FN = 12$. All chromosomes are isobrachial; the first three pairs are distinctly larger than the remaining pairs. The only plate suitable for karyometric analysis yielded the following data: Chrom. I = r.l.: 25.55; c.i.: 47.47 (m); Chrom. II = r.l.: 23.99; c.i.: 49.52 (m); Chrom. III = r.l.:

23.65; c.i.: 41.26 (m); Chrom. IV = r.l.: 10.4; c.i.: 33.33 (sm); Chrom. V = r.l.: 9.47; c.i.: 37.12 (sm); Chrom. VI = r.l.: 6.93; c.i.: 38.19 (m).

Remarks. The limited sample of *P. jondelii* sp. n. appears heterogeneous. The holotype, which is presumably a mature specimen, showed an elongated pharynx, and the presence of bursal spines, characters that were absent in the other two specimens. However, CZM 55, given the incomplete sclerotization of copulatory spines, was clearly at an early phase of maturity. Furthermore, due to the contraction of the different muscles of the pharynx, a collar shaped pharynx can be held horizontally, at least for a short time (pers. obs.), and may thus not be a specific character. *P. jondelii* sp. n. appears nonetheless unique in the genus, for the details of its sclerotized apparatus. None of the known species, has spines as broad, or with markedly blunt apices, as the ones found in the new species. *P. jondelii* sp. n. is the second species of *Parotoplana* known with a tubiform pharynx (but see above comment). The other species, *P. pacifica*, was described by Ax & Ax (1967) and belongs to the species group with a central stylet, which is absent in the new species. The new species also shows the presence of bursal spines. This character is shared with two species only: *P. capitata* (type of the genus *Parotoplana*) and *P. procerostyla* (Ax, 1956). Both these species have sickle-shaped, slender spines; the latter, in addition, is provided with an elongate, tubular stylet. It should be however mentioned that the presence of bursal spines is not easily appreciable, except on well-squeezed mounts and the character might be more widespread than presently acknowledged.

The karyotype of *P. jondelii* sp. n., albeit basic in number ($n = 6$), is nonetheless distinct for the presence of three pairs of large chromosomes. All the species with the same haploid number have only two distinctly larger pairs (Curini-Galletti et al., 1984; present paper).

Diagnosis: *Parotoplana* provided with triangular bursal spines and with a sclerotized apparatus consisting of a girdle of 18 spines: two narrow median spines, four submedian broad spines, with slightly hooked apices, and 12 broad spines, differing for the development of subterminal teeth and the shape of the apex (blunt to hooked). Karyotype with $n = 9$, with three chromosome pairs markedly larger than the others.

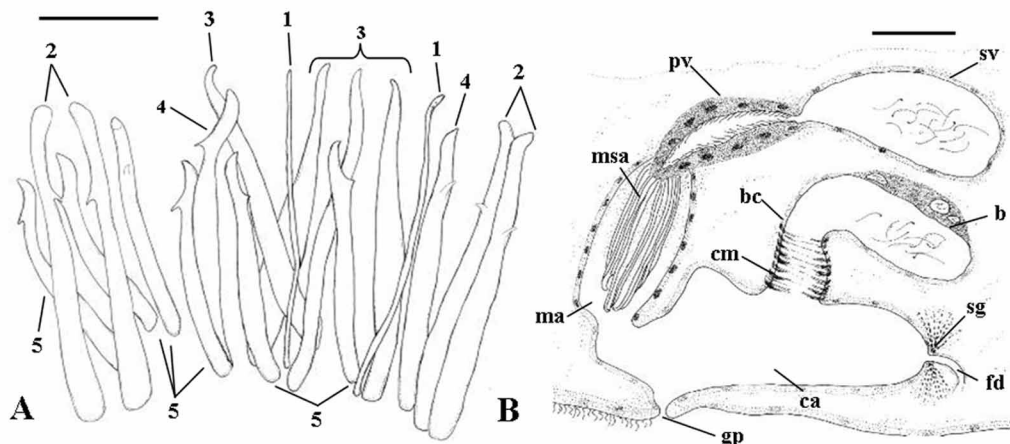


FIGURE 3. *Parotoplana jondelii* sp. n. A, Sclerotized apparatus. Scale = 30 μ m. B, Reconstruction of the genital organs from sagittal sections. Scale = 50 μ m. Abbreviations are the same as Fig 1.

***Parotoplana terpsichore* sp. n.**

(Figs. 4 A–B; Fig. 7 D)

Holotype: one whole mount (SMNH 6665).

Type locality: Apulia, Italy: Porto Cesareo (Lecce), in front of the harbour (lat. 40°14'59.44"N, long. 17°53'31.22"E), about 22 m deep, medium-fine sand, May 2005.

Etymology: the specific epithet refers to the muse Terpsichore, usually portrayed as playing a lyre, similarly shaped to the sclerotized organs of the new species.

Description. Limited observations could be performed on the only specimen found. Size, general morphology of the body and arrangement of organs, similar to the other species of the genus (Fig. 4 A). With two rows of testes (8-9 in each row) anterior to the ovaries, and numerous vitellaria in two rows posterior to the ovaries and extending laterally from the pharynx to the genital organ. A bursa filled with sperm could be observed.

With an ovoid seminal vesicle, and a comparatively short prostatic vesicle, connected to the sclerotized apparatus, consisting of numerous (48) spines (Figs. 4 B; 7 D) which can be classified into four groups:

1) four median, nearly straight, broad spines ranging 86–90 μm , narrowing distally and provided with acutely pointed apices;

2) two groups of nine spines each, placed laterally to the previous spines, with acutely pointed apices, without a subterminal tooth. In each group, the most proximal spines are shorter (about 70 μm long). They progressively become longer (up to 83 μm long), narrower, and straighter laterally;

3) two groups of 12 spines each, lateral to the previous. In each group, the spines are arranged into a girde, with the six most proximal spines (about 67 μm long), provided with very elongate, sickle shaped apices, and a distinct subterminal tooth, and the six marginal spines (about 60 μm long), provided with a shorter apex;

4) two additional spines appear placed above all the others. These are 68 μm long, straight, and as broad as the median spines. Their apex forms a spike, and is provided with a distinct subterminal tooth.

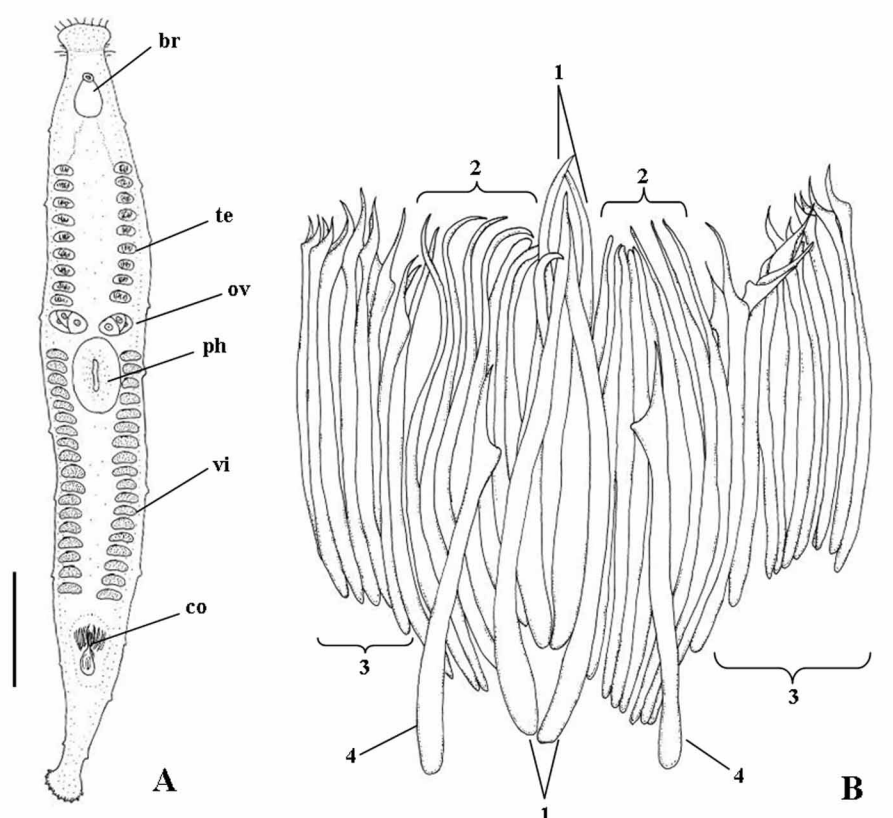


FIGURE 4. *Parotoplana terpsichore* sp. n. A, General organisation of a live animal. Scale = 300 μm . B, Sclerotized apparatus. Scale = 30 μm . Abbreviations are the same as Fig 1.

Remarks. Although only one specimen of *P. terpsichore* sp. n. has been found, its features were nonetheless so distinctive as to warrant a species description. The complex, sclerotized structures of the new species appear comparable uniquely to that of the western Mediterranean *P. multispinosa* (cf Ax, 1956, Fig. 173, pg.

232). Both species show a very large number of spines (48 in *P. terpsichore* **sp. n.**, 38 in *P. multispinosa*) and a similar general arrangement of the copulatory structures, but differ in details of spination. In *P. multispinosa*, the median spines consist of one larger, (78 μm long), and one smaller, straighter and narrower pair, (60 μm long). Furthermore, in this species there is no distinction in size and morphology of the lateral spines (groups 2 and 3 in *P. terpsichore* **sp.n.**), which all have sickle shaped apices with a subterminal tooth. The two additional spines are comparatively much longer, about 76 μm long.

Diagnosis: *Parotoplana* with a sclerotized apparatus consisting of four median, nearly straight, comparatively broad spines (86–90 μm long); two groups of nine narrower spines each, symmetrically arranged laterally to the previous, without subterminal tooth and becoming straighter laterally; two further groups of 12 spines each with sickle-shaped apex and a subterminal tooth (60–65 μm long), and two straight spines, 68 μm long, placed above all the others, with pointed apex and a subterminal tooth.

In addition to the previous species, two specimens of problematic identification were found:

***Parotoplana* sp. A**

(Figs. 5 A–B; Fig. 7 C)

Material: one whole mount (CZM 57). Apulia, Italy: Porto Cesareo (Lecce), La Strea (lat. 40°15'34.26"N, long. 17°53'47.63"E), slightly silty fine sand among rocks, about 1 m deep (*Otoplana* zone), May 2005.

Description. General morphology of the body and arrangement of organs similar to the other species of the genus (Fig. 5A). Sclerotized apparatus (Figs. 5 B; 7 C) consisting of:

- 1) a central stylet composed of a triangular lamina (83 μm long), well sclerotized only at its margin and at the very slightly forked apex;
- 2) two companion spines (80 μm long), with poorly developed sickle shaped apices and obtuse subterminal teeth;
- 3) 12 spines (55 to 60 μm) with sickle shaped apices and obtuse subterminal teeth.

Karyotype. Chromosome number: $n = 6$; $FN = 11$. With chromosome pairs I–V metacentric; Chrom. VI subtelocentric, with a high centromeric index. Chroms. I and II distinctly larger than the remaining pairs. The only plate suitable for karyometric analysis yielded the following data: Chrom. I = r.l.: 28.74; c.i.: 47.53 (m); Chrom. II = r.l.: 27.3; c.i.: 35.71 (sm); Chrom. III = r.l.: 13.14; c.i.: 44.5 (m); Chrom. IV = r.l.: 11.7; c.i.: 33.33 (sm); Chrom. V = r.l.: 10.72; c.i.: 30 (sm); Chrom. VI = r.l.: 8.38; c.i.: 23.64 (st).

***Parotoplana* sp. B.**

(Fig. 5 C; Fig. 7 E)

Material: one whole mount (CZM 58). Apulia, Italy: Porto Cesareo (Lecce) Bacino Grande bay, loc. Scala di Furno (lat. 40°16'26"N, long. 17°52'53.68"E), slightly silty coarse sand among rocks, lower intertidal in the *Otoplana*-zone. May 2005.

Description. General morphology of the body and arrangement of organs similar to the other species of the genus. Sclerotized apparatus (Fig. 5 C) consisting of:

- 1) a central plate-like stylet (115 μm long) with distinctly forked apex;
- 2) two straight companion spines (105–106 μm long), slightly swollen distally, and forming a markedly bulbous distal point;
- 3) two spines (82 μm) with sickle shaped apices, obtuse distal tips, and with subterminal, narrow and elongate teeth;

4) eight spines (75 to 78 μm long) with sickle shaped apices, pointed distal tip, and obtuse subterminal teeth.

Karyotype. Chromosome number: $n = 6$; $\text{FN} = 12$. All chromosomes are isobrachial; Chrom. VI is submetacentric with very low index. The first two pairs are distinctly larger than the remaining chromosomes. Chrom. I = r.l.: 32.23 ± 2.85 ; c.i.: 46.97 ± 0.24 (m); Chrom. II = r.l.: 27.46 ± 2.95 ; c.i.: 38.46 ± 3.08 (m); Chrom. III = r.l.: 12.82 ± 0.38 ; c.i.: 41.93 ± 0.39 (m); Chrom. IV = r.l.: 10.47 ± 0.18 ; c.i.: 37.11 ± 2.48 (sm); Chrom. V = r.l.: 8.75 ± 1.05 ; c.i.: 44.49 ± 3.22 (m); Chrom. VI = r.l.: 8.23 ± 0.38 ; c.i.: 26.93 ± 1.69 (sm) (based on the measurements of three spermatogonial plates).

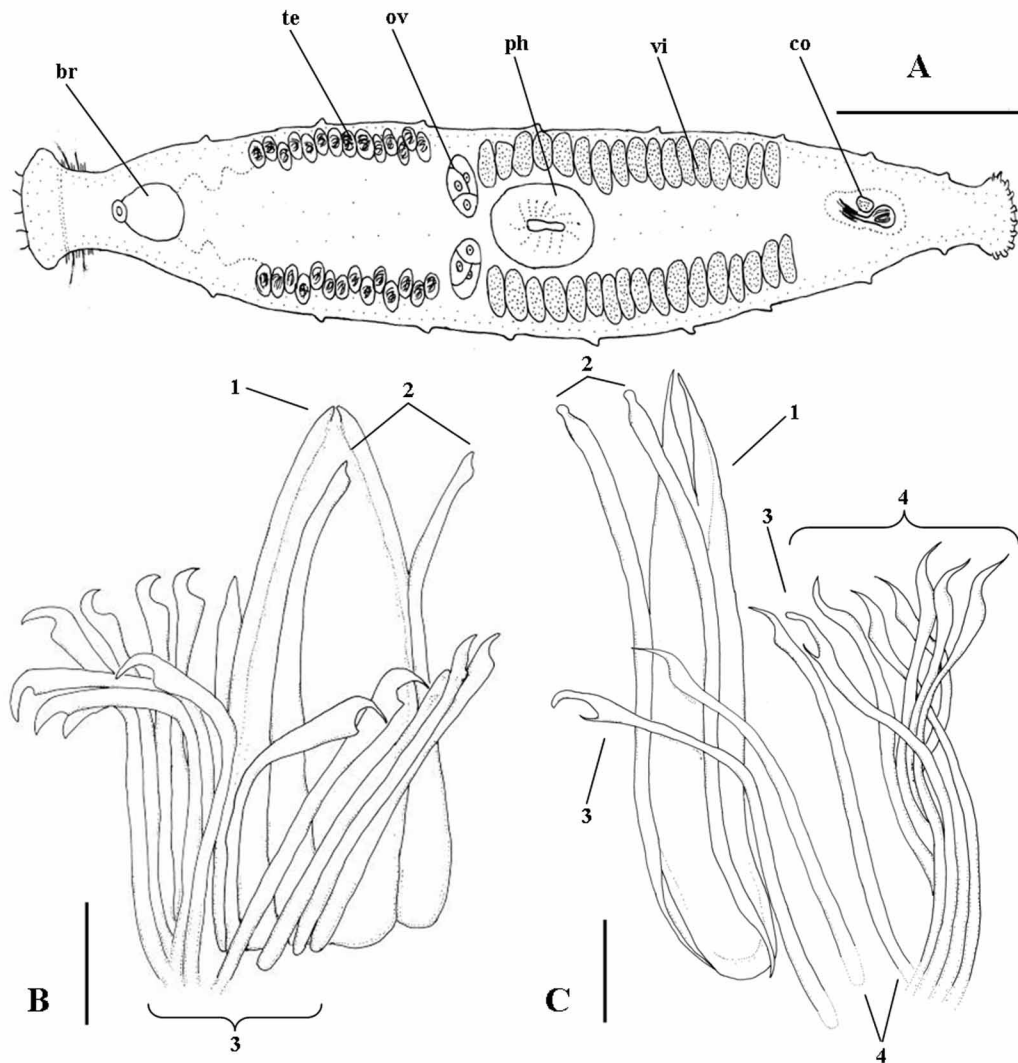


FIGURE 5. *Parotoplana* sp. A. A, General organisation of a live animal. Scale = 300 μm . B, Sclerotized apparatus. Scale = 15 μm . *Parotoplana* sp. B, C, Sclerotized apparatus. Scale = 15 μm . Abbreviations are the same as Fig 1

Remarks. The two specimens, although similar in the general arrangement of the sclerotized structures and karyotype, do not appear to be conspecific. In fact, they differ in the number of spines (14 in *Parotoplana* sp. A, 12 in *Parotoplana* sp. B) and for their size (consistently larger in *Parotoplana* sp. B). Furthermore, their stylet is different (plate-like in *Parotoplana* sp. A; distally split in *Parotoplana* sp. B), as is the shape of the companion spines, forming a unique bulbous apex in *Parotoplana* sp. B. This latter specimen also showed a peculiar morphology of the first, larger pair of spines of the girdle, namely an exceptionally long and thin subterminal tooth.

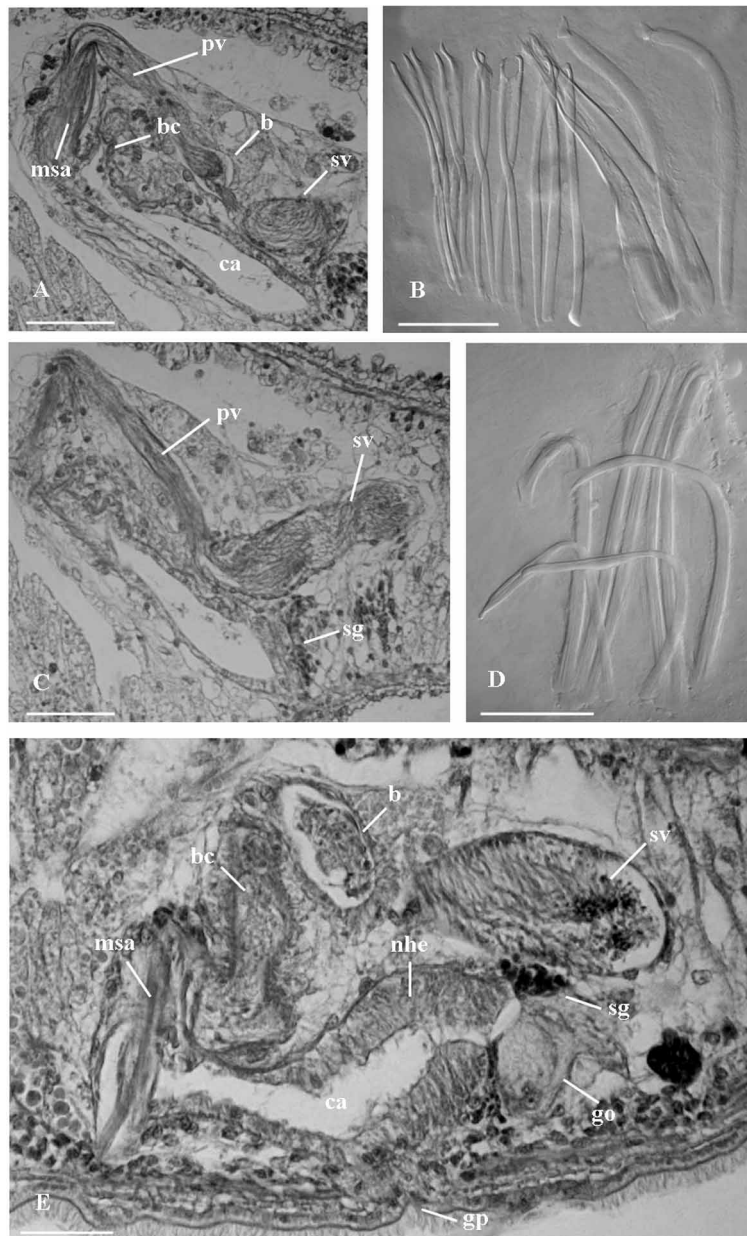


FIGURE 6. *Parotoplana pythagorae* **sp. n.** A, Sagittal section (paratype). Scale = 50 μ m. B, Sclerotized apparatus (holotype). Scale = 30 μ m. C, Sagittal section of the paratype. Scale = 50 μ m. *Parotoplana spathifera* **sp. n.** D, Sclerotized apparatus (holotype). Scale = 30 μ m. E, Sagittal section (paratype). Scale = 30 μ m. Abbreviations are the same as Fig 1.

Among the species found in southern Apulia, the two specimens are similar to *P. pythagorae* **sp. n.** in number and arrangement of spines. This species however has a forked stylet, patently formed by two spines joined by a thin membrane; the companion spines are distinctly broader than the others and provided with characteristic apical triangles, and the girdle, though similar to *Parotoplana* sp. A, is formed by larger spines. The comparatively large sample of *P. pythagorae* **sp. n.** examined showed constant characters, and was syntopic with *Parotoplana* sp. B.

The two specimens appear to be very similar to two Mediterranean species, *P. macrostyla* from the Ligurian Sea and *P. renatae* from the Gulf of Lyon, because of the presence of a median, laminar stylet, two larger companion spines, and a girdle of 10–13 spines. According to Lanfranchi (1978), the discriminating feature between *P. renatae* and *P. macrostyla* is the absence of a common female duct in the latter. In *P. macrostyla*,

the oviducts join just before their opening into the common genital atrium – a situation similar to *P. spathifera* **sp. n.** described above. However, it must be noted that the distal joining of the oviducts is impossible to appreciate on semi-squashed living specimens, and easily overlooked in less than very high quality sections. *P. renatae*, on the other hand, is described as provided with a triangular stylet, whereas *P. macrostyla* has a distally forked stylet. However, the shape of the stylet can be unequivocally assessed only in strongly squeezed mounts. Based on available data, one might therefore question whether *P. renatae* and *P. macrostyla* are indeed two different species. The problem will be only solved following a detailed study of further specimens from the type localities.

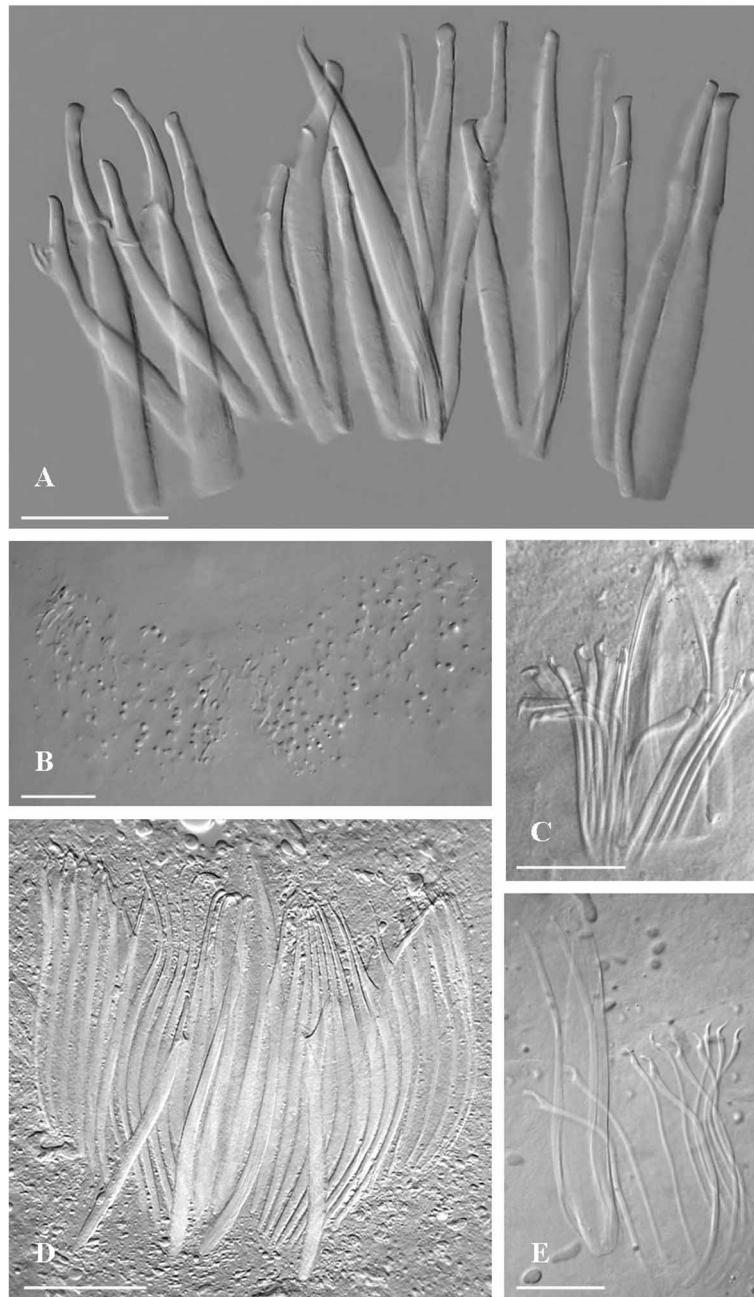


FIGURE 7. *Parotoplana jondelii* **sp. n.** A, Sclerotized apparatus (holotype). Scale = 30 μ m. B, Bursal spines (holotype). Scale = 30 μ m. *Parotoplana* sp. A. C, Sclerotized apparatus (CZM 57). Scale = 30 μ m. *Parotoplana terpsichore* **sp. n.** D, Sclerotized apparatus (holotype). Scale = 30 μ m. *Parotoplana* sp. B. E, Sclerotized apparatus (CZM 58). Scale = 30 μ m.

The two specimens from Porto Cesareo seem to add further complexity to the question. In fact, at least *Parotoplana* sp. B, though similar to *P. macrostyla* for the shape of stylet, differs in the unique morphology of companion spines and the two largest spines of the girdle. *Parotoplana* sp. A has a triangular plate-like stylet, similar to that described for *P. renatae*, but differs for the less acutely pointed companion spines and the hooked shape of most of the girdle spines. Both specimens show a karyotype indistinguishable to that of *P. macrostyla* (cf. Curini-Galletti et al., 1984) for the general morphology of chromosomes, and for the shape of the smallest pair (Chrom. VI), which is distinctly less isobrachial than the others (ranging from submetacentric with very low index to subtelocentric).

The complexity of the taxonomy of the group, the paucity of the material available for study, and the lack of histological sections, refrain us from any further taxonomic inference on the two specimens.

Discussion

The number of species of *Parotoplana* found in southern Apulia (four new species, formally described here, and two problematic specimens that may represent additional species) exceeds by a third all previously known species (16) in the rest of the world. Most researchers have so far dealt with details of spination as the main character for discriminating species in the genus. Our results confirm that these details are indeed of paramount importance in species discrimination, and that the degree of morphological differentiation among species is even larger than hitherto suspected. In fact, some of the new species have features – such as the very low number of spines of different shapes, arranged in pairs, as seen in *P. spathifera* sp. n., spines of peculiar shape as in *P. pythagorae* sp. n., or broad spines as found in *P. jondelii* sp. n. – that widen the range of morphological variability known in the genus. However, the need of high quality, strongly squeezed mounts needs to be emphasized as details may remain hidden in slightly squeezed living and whole-mounted specimens, where the spines appear tightly grouped.

In addition to spination, other morphological features are of potentially great interest for species discrimination, such as the presence of bursal spines in *P. jondelii* sp.n. This character is present in the type species of the genus *Parotoplana*, *P. capitata*, and in *P. procerostyla* (Ax, 1956), but has never been reported for other species. We suspect that this character, which is very difficult to see in both living and fixed material, may be more widespread than hitherto considered, and in future studies should be examined carefully. Other potentially interesting features for systematics include the karyotype, the presence of the ‘glandular organ’ at the outlet of the female duct into the genital atrium (as seen in *P. spathifera* sp.n.), the distal fusion of the oviducts, the topography of gonads (in particular, the position of the ovary, which can be more or less anterior to the pharynx) and the shape of the pharynx itself. Some caution is, however, necessary for the latter two characters, since their intrapopulational variability should be tested carefully, before its use as a discriminating tool. In fact, both seem to present variations, possibly linked to the age of specimens (cf. discussion of *P. jondelii* sp. n. above).

All the characters listed above are of potential phylogenetic interest. However, a reconstruction of phylogenetic relationships within the genus has not been attempted so far, and we deem it as premature at the moment. In fact, the finding of a high number of new species in a comparatively small area suggests that the taxonomic knowledge of the genus is far from adequate, and it is clear that much more taxonomical work is necessary before any further analysis is undertaken. In this regard we note that BIOIMPA campaigns in other sectors of the Mediterranean Sea have revealed a considerable number of new species of *Parotoplana*, which are currently being described.

The observed presence in the Apulian sample of sister-species relationships with other Mediterranean species is suggestive of an intra-basin speciation, which may be at the basis of the high number of species of *Parotoplana* in the Mediterranean Sea. At the moment, this should be considered a working hypothesis, to be tested in future research on the taxon.

Acknowledgements

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II

The genera *Parotoplana* Meixner, 1938 and *Parotoplanella* Ax, 1956 (Platyhelminthes: Proseriata) in southern Spain

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The genera *Parotoplana* Meixner, 1938 and *Parotoplanella* Ax, 1956 (Platyhelminthes: Proseriata) in southern Spain

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Collecting in southern Spain yielded five species of *Parotoplana* and one species of *Parotoplanella* (Proseriata: Otoplanidae). Four species of *Parotoplana* proved to be new to science. *Parotoplana cucullata* sp. n. is distinguished by the shape of the lateral spines in the copulatory organ and in the presence of a “glandular organ” around the distal portion of the female duct. *Parotoplana fretigaditani* sp. n. is similar to *P. procerostyla* Ax, 1956 in the general arrangement of spines, but their fine morphology is distinctive. *Parotoplana varispinosa* sp. n. differs from *P. capitata* Ax, 1956 in the shape and number of spines. *Parotoplana mastigophora* sp. n. has a unique morphology of the stylet, and the spines of the girdle markedly differ in size and shape. The karyotype of *P. cucullata* sp. n. (n=6) is described. In addition, *Parotoplana primitiva* and *Parotoplanella heterorhabditica*, previously known from the Atlantic coast of France and north-west Mediterranean, respectively, were found. The high proportion of new species demonstrates the present poor state of knowledge of interstitial meiofauna.

Keywords: *Otoplanidae*; taxonomy; marine biodiversity; marine biogeography; meiofauna; new species.

Introduction

Present knowledge of marine biodiversity is deemed unsatisfactory (Bouchet et al. 2002; Sala 2002). The inadequacy of data on the taxonomy and distribution of marine organisms is particularly acute for interstitial meiofauna, where information may be absent altogether for entire biogeographical regions (Kennedy and Jacoby 1999). Knowledge of taxa belonging to the so-called “soft bodied” meiofauna, which necessitate observations on both living and fixed specimens, is particularly limited. Among these groups, information on Platyhelminthes, a species-rich, ubiquitous and diverse taxon, is, at present, adequate only for a few areas (notably northern Europe and the Galapagos Islands, see Ax and Schmidt 1973, Armonies and Reise 2000), with little and taxonomically limited research elsewhere. Recent evidence suggesting that marine interstitial flatworms have limited geographical ranges (Casu and Curini-Galletti 2006) is particularly worrisome, as these sedentary organisms may be particularly affected by global climate change which, given the poor state of taxonomic knowledge, may lead to undetected cases of “silent extinction”.

Recently, within the framework of the project BIOIMPA (Biodiversity of Inconspicuous Organisms in Italian Marine Protected Areas), a series of sampling campaigns was conducted in the Mediterranean, aimed to gather data on the composition and distribution of a taxon of Platyhelminthes, the Proseriata. Among

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the areas sampled, southern Spain yielded a particularly rich, diverse, and mostly yet undescribed, proseriate fauna. The present contribution deals with two genera, *Parotoplana* Meixner, 1938 and *Parotoplanella* Ax, 1956 (Otoplanidae). The genus *Parotoplana* is species-rich in the Mediterranean (see Delogu and Curini-Galletti 2007), and five species have been found in southern Spain, four of which are new to science. Rather unusually for the genus, which is often represented by vast numbers of individuals in samples, only a few specimens were collected, and most species are described here on the basis of single specimens. However, discrimination among species of *Parotoplana* can be in most instances based on details of the sclerotized apparatus of the copulatory organ, which is easily appreciable in diaphanized whole mounts (cf. Delogu and Curini-Galletti 2007). The often unique and peculiar morphologies found, and the present, complete lack of information on the composition of the Proseriata in the area, stimulated the preparation of the present contribution, notwithstanding the obvious limitations of the sample.

In addition, the first attempt to draw phylogenetic relationships within the genus *Parotoplana* has been made. The analysis was limited by the uneven level of species descriptions; several species, in fact, are only known from whole mounts. Therefore, characters potentially phylogenetically informative (e.g. presence of bursal spines, distal fusion of oviducts, presence of a glandular organ in the female duct) (Delogu and Curini-Galletti 2007), could not be taken into consideration, as these can only be appreciated on sectioned material. The results, although certainly far from being conclusive, helped nonetheless, to offer some insights into phylogenetic relationships of the new species.

Material and methods

Specimens were collected in sandy habitats by scooping up the superficial layer of sediment. Extraction of the animals from the sediment was with $MgCl_2$ decantation (Martens 1984). Each species was first studied alive, slightly squeezed beneath a cover-slip. Preservation techniques routinely adopted for Proseriata were used (see Martens et al. 1989b); whole mounts were made with lactophenol. For microscopical analysis, material was fixed in Bouin's fluid, embedded in 60°C Paraplast and serial sagittal sections cut at 4 μm , were 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. (1989). Relative lengths (r.l.=length of chromosome x 100/total length of haploid genome) and centrometric indices (c.i.=length of short arm x 100/length of entire chromosome) were obtained from measurements of camera lucida drawings of metaphase plates. The fundamental number (FN) (i.e. the number of chromosome arms in the karyotype) is derived according to Matthey (1949) and the chromosome nomenclature employed is that of Levan et al. (1964): m=metacentric; sm=submetacentric.

Type material is stored in the collections of the Swedish Museum of Natural History (Stockholm, Sweden) (SMNH). Additional material, when present, is stored in the collection of the Zoological Museum of the University of Sassari (Italy) (ZMC).

Cladistic analysis was based on the following morphological characters:

- 1) anterior position of the ovaries;
- 2) numerous (>10) vitellaria anterior to the pharynx;

- 3) stylet:
 - (0) absent;
 - (1) two spines unfused;
 - (2) gutter shaped, formed by the fusion of two spines;
 - (3) with gutter shaped basis and tubular distal portion;
 - (4) entirely tubular;
- 4) lateral spine(s):
 - (0) absent;
 - (1) one;
 - (2) two;
 - (3) three;
 - (4) four or more;
- 5) morphology of lateral spine(s):
 - (0) absent;
 - (1) spines identical;
 - (2) spines different;
- 6) girdle spines:
 - (0) all with the same morphology;
 - (1) with one pair with different morphology;
 - (2) with several groups showing different morphologies;
 - (3) with spines gradually changing morphology;
- 7) numerous girdle spines (>20);
- 8) testes behind ovaries;
- 9) testes within vitellaria.

Except in the case of multistate characters, absence of the character was scored as 0, presence as 1. All characters were unweighted and unordered, with the exception of character 3, which was ordered (option: Wagner). *Parotoplanella progermaria* Ax, 1956 was chosen as the outgroup. The matrix (table 1) was edited in MacClade (Maddison and Maddison 1992), and the parsimony analysis performed in Paup 4.0b (Swofford 1998). Heuristic search (with stepwise-addition option) was applied, and all minimal trees were kept. Bremer support values were calculated with AutoDecay (Eriksson 1998).

Abbreviations used in figures: b: bursa; bc: bursal canal; br: brain; ca: common atrium; cm: circular musculature; co: copulatory organ; fd: female duct; go: glandular organ; gp: genital pore; ma: male antrum; msa: male sclerotized apparatus; nhe: nucleated high epithelium; ov: ovary; ph: pharynx; pv: prostatic vesicle; r: rhabdoid; sg: shell glands; st: stylet; sv: seminal vesicle; te: testis; vi: vitellaria.

Results

Phylogenetic relationships

The analysis yielded 21,328 most parsimonious trees (tree length=35 steps; consistency index=0.5143; retention index=0.6852). The 50% majority rule consensus is reported in Figure 5. However, it should be noted that Bremer support

Table 1. Character matrix of the species of the genus *Parotoplana*; *Parotoplanella progermaria* is chosen as the outgroup.

Characters	1	2	3	4	5	6	7	8	9
<i>Parotoplanella progermanica</i>	1	1	0	2	1	0	0	1	1
<i>Parotoplana bermudensis</i>	0	0	0	2	1	0	0	0	0
<i>Parotoplana lata</i>	0	0	0	2	1	0	0	0	0
<i>Parotoplana subtilis</i>	0	1	0	2	1	0	0	0	0
<i>Parotoplana mollis</i>	0	0	0	1	1	0	0	0	0
<i>Parotoplana capitata</i>	0	0	0	3	2	3	0	0	0
<i>Parotoplana moya</i>	0	0	0	4	1	0	0	0	0
<i>Parotoplana primitiva</i>	0	0	0	4	1	0	0	0	0
<i>Parotoplana renatae</i>	0	0	2	2	1	0	0	0	0
<i>Parotoplana papii</i>	1	1	2	0	0	0	0	0	0
<i>Parotoplana procerostyla</i>	0	0	3	3	1	2	0	0	0
<i>Parotoplana multispinosa</i>	0	0	0	4	2	0	1	0	0
<i>Parotoplana pythagorae</i>	0	0	2	2	2	0	0	0	0
<i>Parotoplana spathifera</i>	0	0	0	0	0	2	0	0	0
<i>Parotoplana jondelii</i>	0	0	0	0	0	2	0	0	0
<i>Parotoplana terpsichores</i>	0	0	0	4	2	2	1	0	0
<i>Parotoplana bicupa</i>	1	1	1	2	1	3	1	0	0
<i>Parotoplana macrostyla</i>	0	0	2	2	1	0	0	0	0
<i>Parotoplana uncinata</i>	0	0	2	2	1	0	0	0	0
<i>Parotoplana pacifica</i>	0	0	4	4	1	0	1	0	0
<i>Parotoplana turgida</i>	0	0	0	2	1	0	0	0	0
<i>Parotoplana cucullata</i>	0	0	2	2	1	1	0	0	0
<i>Parotoplana fretigaditani</i>	0	0	3	3	2	2	0	0	0
<i>Parotoplana varispinosa</i>	0	0	0	3	1	3	0	0	0
<i>Parotoplana mastigophora</i>	0	0	3	0	0	3	0	0	0

values are very low throughout, and the tree should be considered only as the best phylogenetic hypothesis available at the moment.

Taxonomy

New species

Family OTOPLANIDAE Hallez, 1892

Subfamily PAROTOPLANINAE Ax, 1956

Genus *Parotoplana* Meixner, 1938

The genus *Parotoplana* Meixner, 1938 is characterized by the presence of a collar-shaped pharynx, entirely ciliated with the exception of its distal tip; serial arrangement of gonads (two rows of testes in front of the prepharyngeal ovaries; two rows of vitellaria behind the ovaries); a female apparatus with bursa opening independently into the common genital atrium, between the openings of the male system and of the common oviduct, which arises from the post-pharyngeal fusion of the germovitello-ducts (Ax 1956).

***Parotoplana cucullata* sp. n.**

(Figures 1 A–C, 3A, 5)

Holotype. One whole mount (SMNH 6786).

Type locality. Spain, Tarifa, Mediterranean side (lat. 36°0'42.38"N, long. 5°35'45.09"); intertidal, silty medium sand among rocks. October 2005.

Paratype. A specimen from the type locality, sagittally sectioned (SMNH 6787).

Additional material. Thirteen specimens sagittally sectioned (CZM 71-83); three karyological slides (CZM 84-86); three whole mounts (CZM 87-89), all from the type locality.

Etymology. The specific epithet is based on the presence, in the sclerotized apparatus of the new species, of two spines whose apices resemble a monk's hood (Latin: *cucullus*)

Description

The holotype is an adult worm, about 1.5 mm long in fixed condition. With a clearly marked anterior end, provided with sensory bristles, and a fan-shaped caudal end, with numerous adhesive papillae (Figure 1A). The encapsulated, oval-shaped brain is about 64 µm long, 50 µm wide, and abuts on a statocyst, 18 µm in diameter. Epithelium with intraepithelial nuclei. Ciliation restricted to a sensory groove in the anterior end, and, ventrally, to a creeping sole which extends from the anterior end to behind the genital pore. Cilia up to 5 µm in length. With numerous rhabdoids, narrowly elongate, up to 20 µm in length, arranged in longitudinal rows.

The gut extends posteriorly nearly to the caudal end and anteriorly to the cephalic area. The nearly median pharynx is collar-shaped; its nucleated epithelium is ciliated except for a small area at the distal tip where the pharyngeal glands, whose body is located outside the pharynx, discharge. A very small oesophagus is present. The pharyngeal cavity opens to the outside through the mouth, which is surrounded by a ring of circular muscles. A strong subepidermal longitudinal musculature is present both dorsally and ventrally.

Male genital organ. With two lateral rows of about 30 testes each, anterior to the ovaries (Figure 1A). The male copulatory organ consists of a sacciform seminal vesicle lined by a thin nucleated epithelium, an extremely elongate prostate vesicle, about 33 µm in diameter, lined internally by a ciliated epithelium, and a sclerotized apparatus consisting of a central stylet, two lateral spines and a girdle of 16 spines (Figures 1B–C, 3A).

The stylet, 80–90 µm long (holotype: 90 µm), is groove shaped, provided with a slender apex, and wraps around the distal portion of the ejaculatory duct. In well-squeezed mounts its morphology reveals its origin from two broad, thick spines,

connected by a feebly-sclerotized lamina. The two lateral (companion) spines are 80–84 μm long, with comparatively broad stems (about 5 μm thick) and very slender, arrow-shaped apices, provided with a feebly sclerotized, hood-shaped distal envelope (Figures 1C: 1, 3A). Among the 16 spines of the girdle, the two most proximal to the stylet are 72–75 μm long, with a comparatively broader stem (about 3 μm thick), and are provided with distinctly globose apices (Figures 1C: 2, 3A). The remaining 14 spines are thinner (stem 1–2 μm broad) and 60–75 μm in length (Figures 1C: 3, 3A). Their distal tips are falcate; they are provided with an obtuse, laminar subterminal tooth.

The sclerotized apparatus is located within the male antrum, lined by a nucleated epithelium, which opens into the anterior portion of the common atrium.

Female genital organs. Two ovaries lie anterior to the pharynx (Figure 1A). Two rows of vitellaria stretch from posterior to the ovaries to anterior to the copulatory organ. The oviducts fuse behind the pharynx to form a common female duct, which runs posteriorly lateral to the genital pore, and bends sharply upwards to enter the common genital atrium at its posterior end. The common female duct is lined by a ciliated epithelium; its distalmost tract, at its opening into the genital atrium, is surrounded by few, very large glandular cells (the “glandular organ”) and by the female glands (Figure 1B).

The bursa + vagina system opens independently into the genital atrium, close to the outlet of the male copulatory organ. It consists of a small bursa (about 30 μm in its widest axis in sections) of the resorbiens type, lined by a high, vacuolar epithelium. Sperm could be seen inside some of the vacuoles. The bursa is connected to the common atrium through a long and narrow bursal canal (about 90 μm in length) lined by a low, nucleated epithelium. The bursal canal is surrounded by a layer of well developed circular muscles.

The common genital atrium opens to the outside through a large genital pore. The epithelium of the atrium is high and nucleated, especially the portion adjacent to the glandular organ.

Karyotype. With $n=6$, and $FN=12$. The karyotype has two chromosome pairs distinctly larger than the others, which are rather small and rather even in size. Chromosomes are meta-submetacentric; the smallest pair is at the lower limit of the submetacentric class. The measurement of nine spermatogonial plates yielded the following karyometrical data: Chrom. I: $r.l.=30.11 \pm 1.47$; $c.i.=45.51 \pm 2.87$ (m); Chrom. II: $r.l.=25.18 \pm 0.68$; $c.i.=44.89 \pm 1.56$ (m); Chrom. III: $r.l.=12.6 \pm 1.32$; $c.i.=45.75 \pm 2.54$ (m); Chrom. IV: $r.l.=11.65 \pm 0.94$; $c.i.=36.89 \pm 2.17$ (sm); Chrom. V: $r.l.=10.83 \pm 1.13$; $c.i.=45.15 \pm 2.71$ (m); Chrom. VI: $r.l.=9.61 \pm 0.87$; $c.i.=25.12 \pm 4.15$ (sm).

Remarks

The presence of a central stylet in the sclerotized structures of the copulatory organ is a very rare feature in the subfamily Parotoplaninae, absent in the vast majority of the genera (see Ax 1956). It is considered a synapomorphic feature for a group of species of *Parotoplanea* (cf. Figure 5) which, besides the new species and two further new

species described below, includes *P. renatae* Ax, 1956; *P. papii* Ax, 1956; *P. procerostyla* Ax, 1956; *P. pacifica* Ax and Ax, 1967; *P. macrostyla* Lanfranchi, 1978, *P. uncinata* Lanfranchi, 1978 and *P. pythagorae* Delogu and Curini-Galletti, 2007. Among these species, only *P. renatae*, *P. macrostyla*, *P. uncinata* and *P. pythagorae* (all from the Mediterranean Sea) share with *P. cucullata* sp. n. a general arrangement of spination consisting of a central, groove-shaped stylet, two lateral companion spines and a girdle of spines (Ax 1956, Figure 158; Delogu and Curini-Galletti 2007, Figure 1A; Lanfranchi 1978, Figures 1B, 6B). However, only the new species has lateral spines provided with arrow-shaped apices surrounded by a feebly-sclerotized lamina similar to a hood. Furthermore, no other species has a pair of spines in the girdle provided with globular apices.

Parotoplana cucullata sp. n. shares with *P. uncinata* the presence of a “glandular organ” in the female genital system (see Lanfranchi 1978, Figure 7: 254). This character has been reported only for two other species: *P. capitata* Ax, 1956, and *P. spathifera* Delogu and Curini-Galletti, 2007, both clearly distinct from the new species for the lack of central stylet. However, the organ can be appreciated only in sections, and present information is too limited to decide whether the character is homoplasous, or its presence in the genus *Parotoplana* is more widespread than known at present.

The karyotype of the new species is very similar to those of *P. renatae* and *P. pythagorae* (Curini-Galletti et al. 1984; Delogu and Curini-Galletti 2007). The slight differences observed in the centromeric indices of the smallest pair (at the upper limit of the submetacentric class in *P. pythagorae*, at the lower limits of the subtelocentric class in *P. renatae* and submetacentric in the new species) should be treated cautiously, as they may reflect the intrinsic difficulty in measuring very small chromosomes.

Parotoplana cucullata sp. n. was very common in lower intertidal samples collected in the Tarifa area, on the Gibraltar strait. It was not found elsewhere.

Diagnosis

Species of *Parotoplana* provided with a sclerotized apparatus consisting of a central stylet (80–90 µm long), two lateral spines with arrow-shaped apices surrounded by feebly-sclerotized, hood-shaped envelopes and a girdle made of two spines (72–75 µm long) with globular apices, and 14 thinner spines (60–75 µm long), with sickle-shaped apices and obtuse subterminal teeth. With a glandular organ surrounding the outlet of the female duct into the common genital atrium. Karyotype with $n=6$; $FN=12$.

***Parotoplana fretigaditani* sp. n.**
(Figures 1D, 2A, 3C–E, 5)

Holotype. One whole mount (SMNH 6785).

Type locality. Spain, Tarifa (lat. 36°0'42.38"N, long. 5°35'45.09"W); intertidal, silty medium sand among rocks. October 2005.

Etymology. The specific epithet refers to the geographic area (the Strait of Gibraltar, Latin: *fretum gaditanum*) where the species was found.

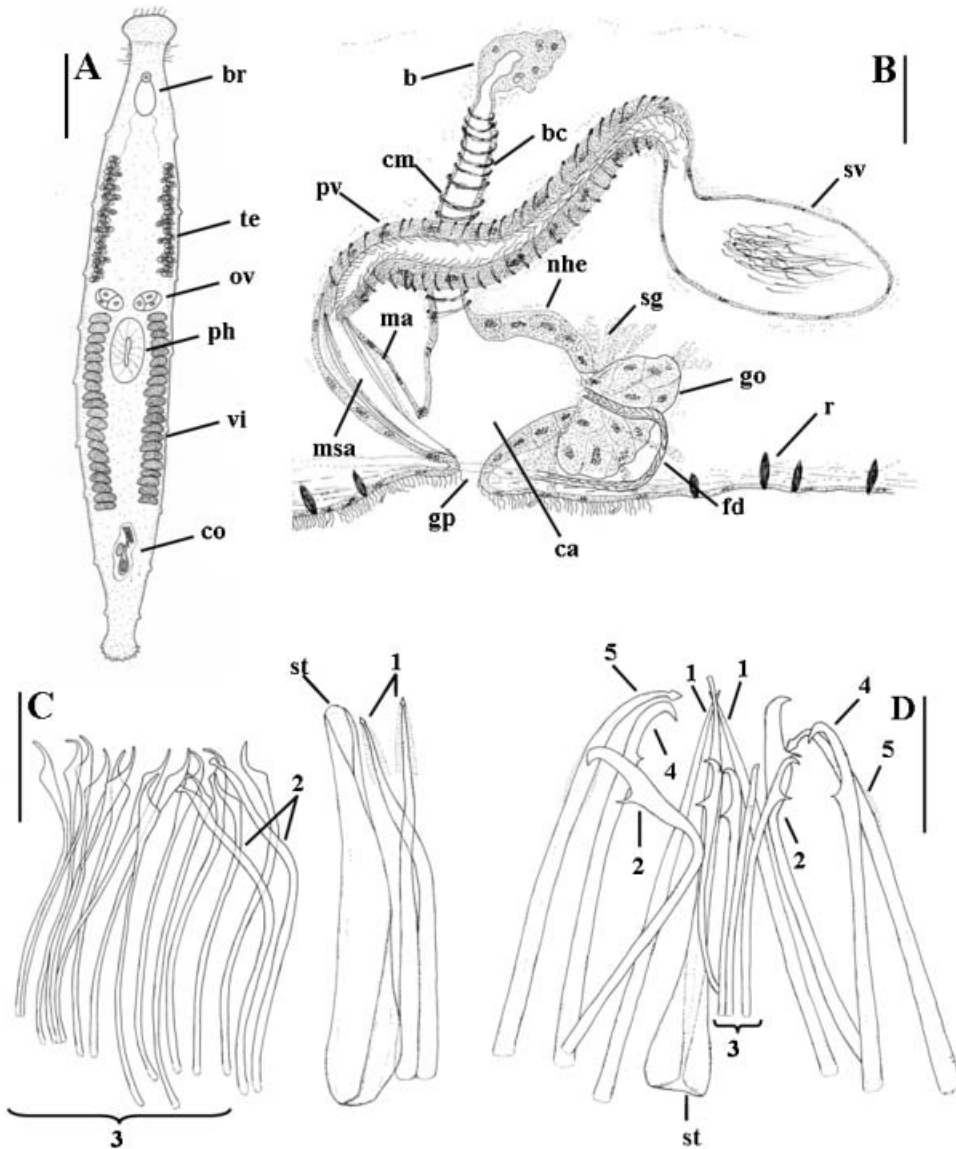


Figure 1. *Parotoplana cucullata* sp. n. A, general organisation of a live animal. Scale=200 μ m. B, reconstruction of the genital organs from sagittal sections. Scale=40 μ m. C, sclerotized apparatus. Scale=30 μ m. *Parotoplana fretigaditani* sp. n. D, sclerotized apparatus. Scale=30 μ m.

Description

A comparatively large and broad *Parotoplana*: the fixed holotype is about 2.2 mm long. General morphology of the body and arrangement of organs, similar to the other species of the genus. Dorsally, the narrowly elongate rhabdoids are arranged in eight to ten longitudinal rows. Pharynx in the middle of the body.

Male genital organs. With two rows of about ten testes each, anterior to the pharynx. The male copulatory organ consists of a sacciform seminal vesicle, about 120 μm long, a prostatic vesicle, 200 μm long, and a sclerotized apparatus consisting of a stylet surrounded by 12 spines (Figures 1D, 3C–D).

The stylet is elongate, about 85 μm long. It has a swollen, gutter-shaped base, 15 μm wide (Figure 2A). About 15 μm above the basis, the stylet narrows to a width of 3–5 μm . The truncated distal tip is about 1.5 μm wide.

The spines are arranged symmetrically at the two sides of the stylet (Figures 1D, 3C), and consist of:

- 1) two needle-shaped spines, straight and slender, 87–88 μm long, with a base about 4 μm broad, and pointed apices;
- 2) two spines, 80–83 μm long and 3 μm broad at their bases, provided with broad (to 6 μm wide), falcate apices, and with distinct, narrowly-elongate subterminal teeth;
- 3) four smaller spines, 58–60 μm long and about 2.5 μm broad at their bases, with narrower (to 3 μm wide) falcate apices and with subterminal teeth;
- 4) two spines, 89–93 μm long and 4 μm broad at their bases, with recurvate, narrow, falcate apices, provided with poorly-developed teeth;
- 5) two spines 90–91 μm long and 4 μm broad at their bases, provided with an irregularly crenulated fringe at the base of the distal third of their lengths, resulting from sites of attachment of longitudinal muscles, and with distinctive, spade-shaped apices (Figure 3E).

Female genital organs. With two ovaries anterior to the pharynx, and two rows of vitellaria extending posterior to the ovaries to in front of the copulatory organ.

The bursa + vagina system could be observed on the living specimen before fixation. It consisted of a small, ovoid bursa, connected to the common atrium through a long and narrow canal, surrounded by a thick layer of circular muscles.

Remarks

Among the species of *Parotoplana* provided with a stylet, only *P. procerostyla* Ax, 1956 from the northern Mediterranean (Gulf of Lyon) shares with *P. fretigaditani* sp. n. a similar general arrangement of spination consisting of a central stylet and spines of different morphologies, symmetrically arranged at its sides. This arrangement is a synapomorphy for the two species (Figure 5). However, the stylet, although similar, is longer (120 μm high) with an acutely-pointed apex in *P. procerostyla*. This species, furthermore, has only three types of spines:

- 1) four spines (110 μm long), with falcate apices and subterminal teeth, comparable to groups 2 and 4 of *P. fretigaditani* sp. n.;
- 2) six straight, very thin needle-shaped spines, with slightly recurvate apices (102 μm);
- 3) four longer, outer spines, 135 μm in length, with slightly recurvate apices.

Parotoplana. fretigaditani has thus a group of small spines, with falcate apices and subterminal teeth (group 3), absent in *P. procerostyla*. Furthermore, the characteristic outer spines of *P. fretigaditani*, swollen in their distal third, and with

spade-shaped apices, are markedly different from the longer, narrower, needle-shaped outer spines of *P. procerostyla*, which are provided with pointed, slightly falcate distal tips (cf. Ax 1956, Figure 167).

Diagnosis

Parotoplana species with a sclerotized apparatus consisting of a central stylet and five groups of spines, symmetrically arranged:

- 1) two needle-shaped spines, 87–88 μm long;
- 2) two spines with broadly falcate apices, and well-developed subterminal teeth, 80–83 μm long;
- 3) four smaller spines with narrowly falcate apices and well-developed subterminal teeth, 58–60 μm long;
- 4) two spines with narrowly-falcate apices and poorly-developed teeth, 89–93 μm long;
- 5) two spines swollen in their distal third, and with spade-shaped apices, 90–91 μm long.

Parotoplana mastigophora sp. n.

(Figures 2C, 4B, 5)

Holotype. One whole mount (SMNH 6789).

Type locality. Spain. Benajarafe (lat. 36°42'54.38"N, long. 4°10'49.95"W); about 5 m deep, fine sand. October 2005.

Etymology. The specific epithet refers to the peculiar stylet of the new species, shaped like a whip (Greek: *mastix*).

Description

A very small *Parotoplana* (the fixed holotype is about 1.2 mm long), slender and elongate. With a poorly-developed adhesive area at the caudal end. With large, elongate rhabdoids, arranged in few, irregular longitudinal rows. The median pharynx is collar shaped.

Male genital organs. With two rows of seven to eight testes each anterior to the ovaries. The male copulatory organ consists of an elongate seminal vesicle, an ovoid prostatic vesicle (67 μm long, 25 μm high), surrounded by a thick outer layer of circular muscles, and a sclerotized apparatus consisting of a stylet surrounded by 18 spines (Figures 2C, 4B). The stylet is an elongate, inverted cone, 122 μm long. Its basis (diameter 15 μm) is gutter shaped. The edges of the gutter fuse at about one-third of the length of the stylet, and coalesce into a narrow, tubular structure about 2.5 μm broad. The distal portion of the stylet narrows to about 1 μm in diameter, and the apex is truncated.

The spines of the girdle sharply increase in size from those most proximal to the stylet (dorsal) to those ventral to the stylet. The five ventral spines are about 5 μm

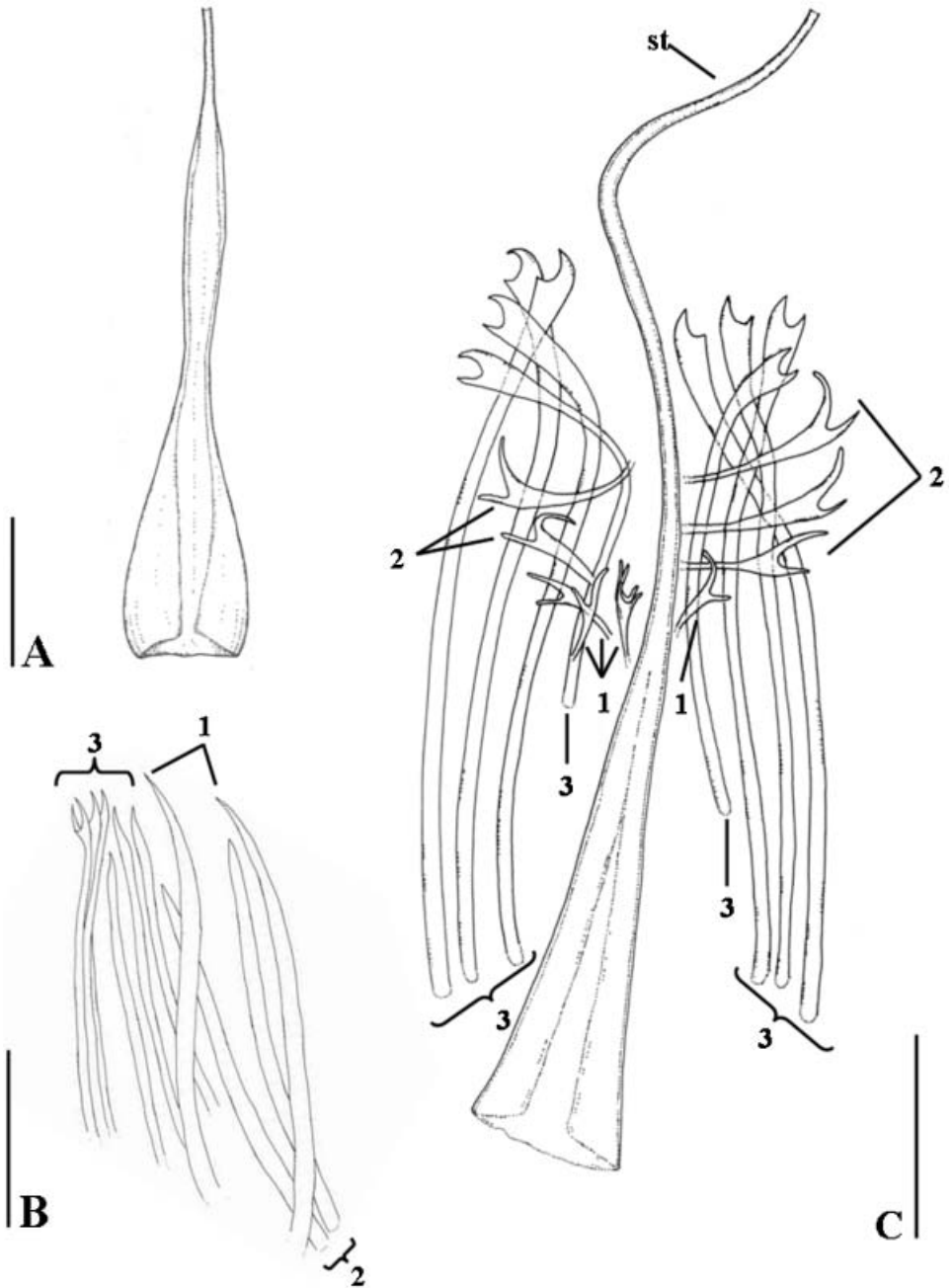


Figure 2. *Parotoplana fretigaditani* sp. n. A, stylet. Scale=20 μ m. *Parotoplana varispinosa* sp. n. B, sclerotized apparatus. Scale=20 μ m. *Parotoplana mastigophora* sp. n. C, sclerotized apparatus. Scale=20 μ m.

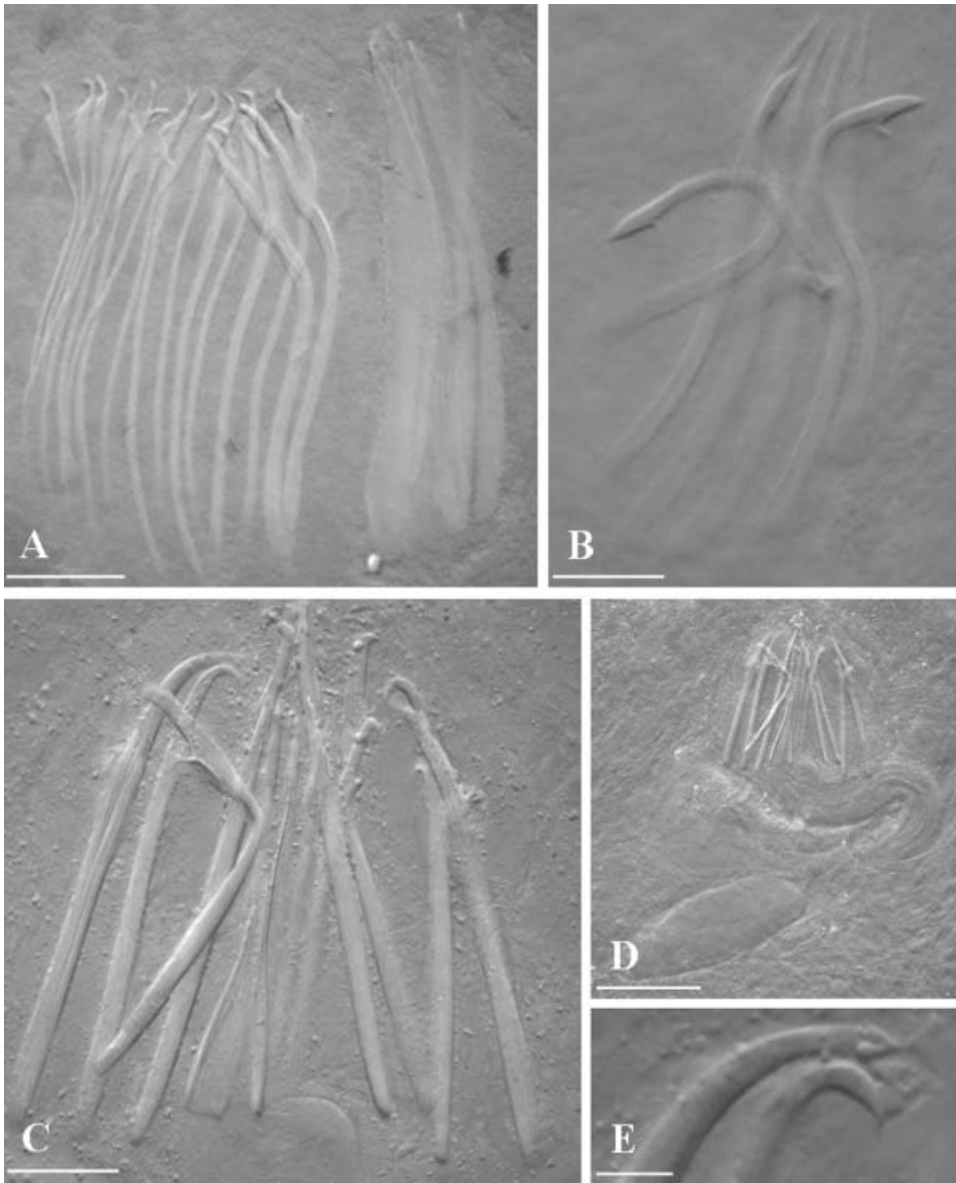


Figure 3. *Parotoplana cucullata* sp. n. A, sclerotized apparatus (holotype). Scale=20 μ m. *Parotoplanella heterorhabditica*. B, sclerotized apparatus. Scale=10 μ m. *Parotoplana fretigaditani* sp. n. C, sclerotized apparatus (holotype). Scale=20 μ m. D, male genital organs (holotype). Scale=60 μ m. E, detail of the spade shaped apex (holotype). Scale=3 μ m.

long, and are provided with slender distal tips, with very elongate subterminal teeth (Figure 2C: 1). Dorsally, these are followed by five longer (up to 20 μ m) spines, with progressively longer subterminal teeth (Figure 2C: 2). In the eight most dorsal spines (46–75 μ m long) the distal tips are shorter, broader and distinctly recurvate, and the subterminal teeth become progressively smaller (Figure 2C: 3).

Female genital organs. With two ovaries anterior to the pharynx, and two rows of vitellaria extending posterior to the ovaries to anterior to the copulatory organ.

With a small, ovoid bursa, about 40 µm wide, opening dorsally in the common atrium through a long bursal canal.

Remarks

Parotoplana mastigophora sp. n. belongs to the group of species with a central stylet. However, the morphology of its sclerotized apparatus is unique, and does not allow further inferences on its phylogenetic relationships (see Figure 5). None of the known species of *Parotoplana*, in fact, has a similarly elongate, whip-shaped stylet, nor has girdle spines so markedly different in shape and morphology. The species thus appears very characteristic, and markedly distinct from congeners.

The single mature specimen found came from the only subtidal station sampled in southern Spain. Its distribution could therefore be wider than documented at present.

Diagnosis

Parotoplana with a sclerotized apparatus consisting of an extremely elongate central stylet, 122 µm long, and a girdle consisting of 18 spines, ranging from 5 µm (with elongate subterminal teeth) to 75 µm (with comparatively small teeth, and recurvate apices) in length.

Parotoplana varispinosa sp. n.
(Figures 2B, 4A, 5)

Holotype. One whole mount (SMNH 6788).

Type locality. Spain. Playa del Pinillo, Marbella (lat. 36°30'0.65"N, long. 4°55'48.10"W); lower intertidal in the *Otoplana*-zone, medium sand between rocks. October 2005.

Etymology. The specific epithet refers to the presence of different (Latin: *varius*) kinds of spines in the sclerotized apparatus.

Description

A large *Parotoplana* (the fixed holotype is about 2 mm long) with the general morphology of the body similar to most other species of the genus.

Male genital organs. With two rows of about six testes each, anterior to the ovaries. The male copulatory organ consists of an elongate seminal vesicle (about 60 µm long), a tubular prostatic vesicle (45 µm in length) and a sclerotized apparatus consisting of 11 spines. These are arranged into a central group made of two different types of spines (Figures 2B, 4A):

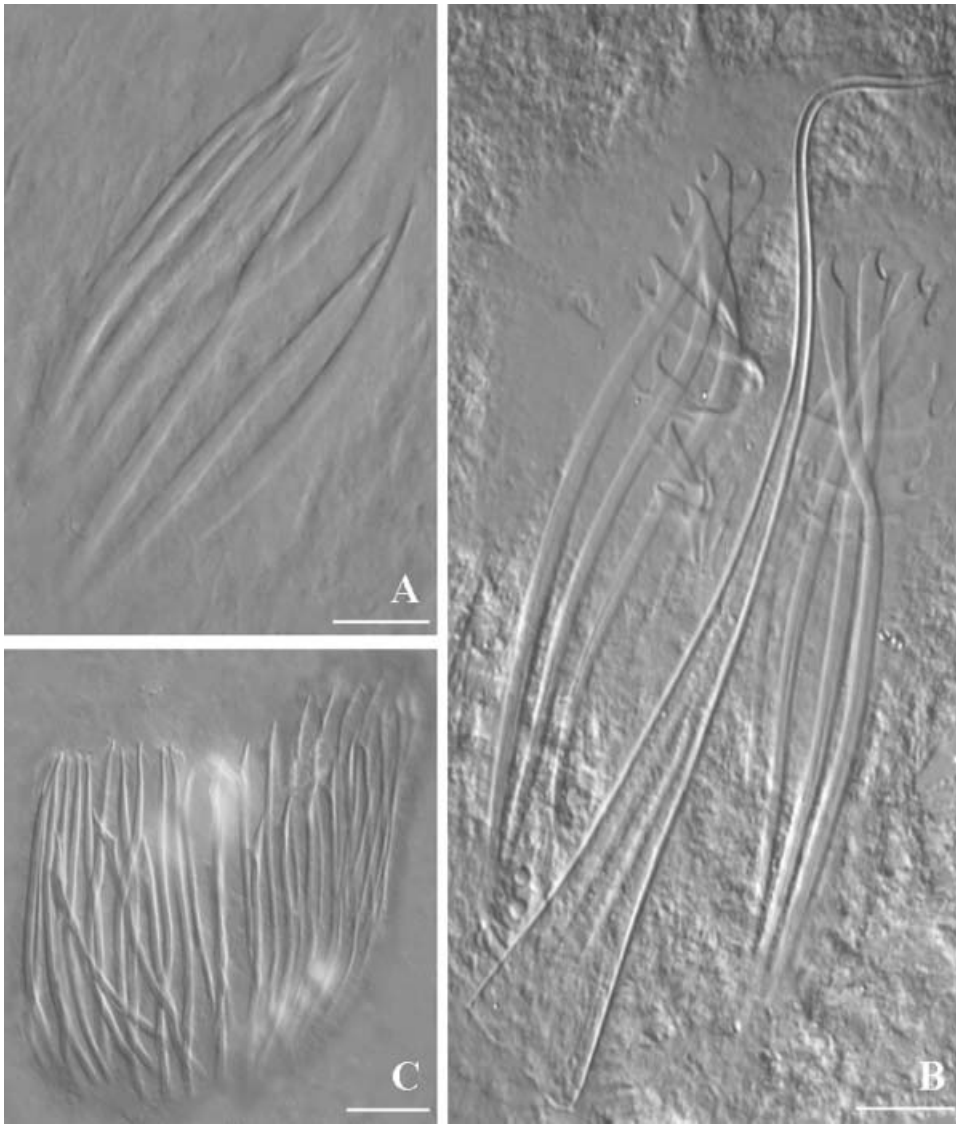


Figure 4. *Parotoplana varispinosa* sp. n. A, sclerotized apparatus (holotype). Scale=10 μ m. *Parotoplana mastigophora* sp. n. B, sclerotized apparatus (holotype). Scale=10 μ m. *Parotoplana primitiva*. C, sclerotized apparatus. Scale=10 μ m.

- 1) two larger spines, 49–51 μ m long, acutely pointed distally;
- 2) two smaller spines, 45–46 μ m long, with a more obtuse distal tip. This central group is partially surrounded by a girdle, consisting of seven spines, 40–45 μ m long (Figure 2B: 3). The morphology of the spines of the girdle varies: the most dorsal spines have slightly falcate apices, with a hint of a subterminal tooth; the most ventral spines are provided with more recurvate apices, and well-developed subterminal teeth.

Female genital organs. With two ovaries anterior to the pharynx, and two rows of vitellaria extending behind the ovaries to anterior to the copulatory organ.

Observations on the living specimen showed the existence of a small bursa, opening independently into the common atrium.

Remarks

Several species of *Parotoplana* have a sclerotized apparatus provided with a group of few spines, presumably acting as functional copulatory stylet, partially or completely surrounded by a girdle of spines. This morphology is plesiomorphic for the genus *Parotoplana* (see Figure 5), and it is found in most genera and species of the Parotoplaninae (cf. Ax 1956). Species with these characters are often confusingly similar (cf. Ax and Sopott-Ehlers 1987). Our phylogenetic analysis (Figure 5) points to a sister species relationship of the new species with *P. capitata* Ax, 1956, a species ranging from Baltic Sea south to the west coast of France (Arcachon) (Ax 1956). The two species share the presence of a central group of four spines, and a gradual change in the morphology of the spines of the girdle. However, the four central spines are needle shaped in the new species, while one pair has falcate, recurvate distal tips, and is provided with distinct subterminal teeth, in *P. capitata* (see Ax 1956, Figure 140). The girdle differs in the number of spines: 16–19 in *P. capitata* and only seven in the new species. Furthermore *P. capitata* is provided with bursal spines, which are absent in *P. varispinosa* sp. n. Differences in morphology thus appear sufficient to warrant specific distinction of the Mediterranean specimen.

Diagnosis

Parotoplana with a sclerotized apparatus consisting of a group of four needle-shaped spines, 45–51 µm long, and a girdle of seven spines, 40–45 µm long; those most proximal to the central group have slightly recurvate apices, progressively becoming more falcate and with more developed subterminal teeth in the distalmost (ventral) spines.

New records

***Parotoplana primitiva* Ax, 1956**

(Figures 4C, 5)

Material examined

Playa el Palo, Malaga (lat. 36°43'3.62"N, long. 4°21'29.06"W); about 2 m deep, fine sand. Five specimens sagittally sectioned (CZM 90–94); one karyological slide, made permanent (CZM 95); two whole mounts (CZM 96–97). October 2005.

Playa les Marines, Bolaga (lat. 37°11'17.03"N, long. 1°48'55.68"W); about 1 m deep, medium sand. Whole mount (CZM 98). October 2005.

Tarifa, Atlantic side (lat. 36°0'31.06"N, long. 5°36'33.06") about 50 cm deep, medium sand. Whole mount (CZM 99). October 2005.

Description

For general shape and organisation, see the original description (Ax 1956).

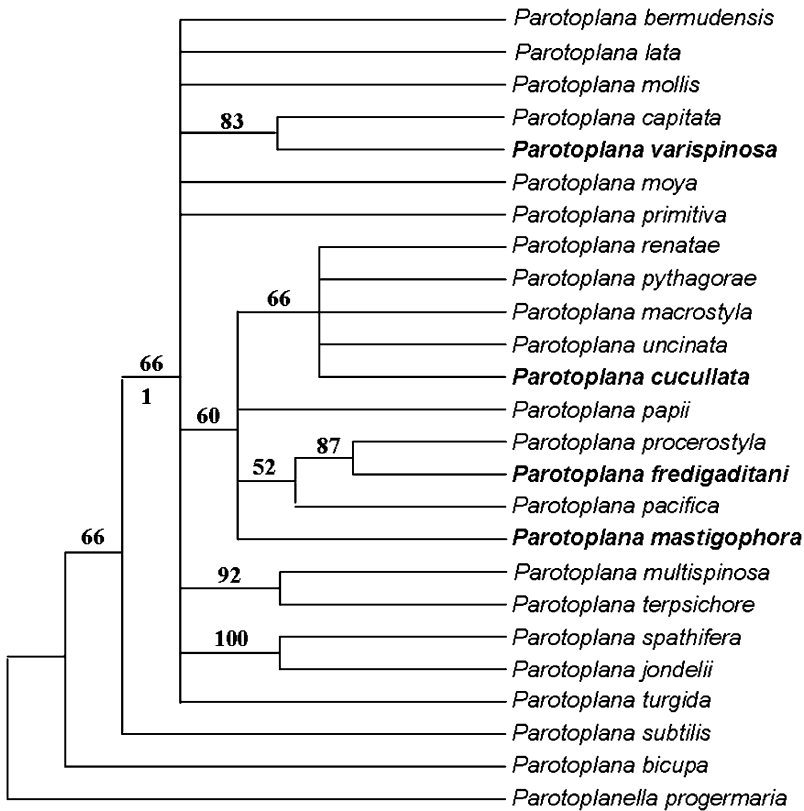


Figure 5. Cladogram depicting the phylogenetical relationships among *Parotoplana* species. 50% majority rule and, when present, Bremer support values are indicated at nodes. New species in bold.

Male genital organs. The Spanish specimens presented two lateral rows of testes (about 11 in each row) anterior to the ovaries. Copulatory organ consisting of a sacciform seminal vesicle, distally connected to a prostatic vesicle, about 60 μm long. Prostatic vesicle lined by a glandular, non-ciliated epithelium. It is distally connected to the sclerotized apparatus, consisting of two concentric girdles of spines. The outer girdle consists of 22–23 spines with sickle-shaped apices; dorsal spines are broader and shorter (about 40–43 μm), spines become progressively longer and slender (about 50–52 μm long) ventrally. The inner girdle consists of 10–13 needle-shaped spines, 48–53 μm long.

Karyotype. With $n=5$, and $FN=10$. The haploid set consists of three comparatively large chromosomes, one chromosome intermediate in size, and one very small chromosome. Chromosomes are meta-submetacentric; the smallest is at the lower limit of the submetacentric class. The measurement of four spermatogonial plates yielded the following karyometrical data: Chrom. I: $r.l.=27.27 \pm 0.44$; $c.i.=45.95 \pm 1.76$ (m); Chrom. II: $r.l.=25.03 \pm 1.19$; $c.i.=41.38 \pm 1.88$ (m); Chrom.

III: r.l.= 23.16 ± 0.3 ; c.i.= 35.6 ± 0.92 (sm); Chrom. IV: r.l.= 16.94 ± 1.09 ; c.i.= 37.19 ± 1.27 (sm); Chrom. V: r.l.= 7.58 ± 0.03 ; c.i.= 28.97 ± 0.75 (sm).

Remarks

The species was described from specimens from the Atlantic coast of France (Bay of Arcachon) (Ax 1956), the only locality where the species had been found so far. Anatomical details, as appreciable in sectioned specimens, and size and morphology of the sclerotized structures, appear practically identical in French and Spanish specimens, allowing the assumption that only a single species is concerned, whose range thus encompasses the western Mediterranean. *Parotoplana primitiva* widespread on the southern coast of Spain, from Bologa to Tarifa.

The karyotype of the species is quite distinctive. The chromosome number $n=5$ is in fact the lowest known so far for the family Otoplanidae (cf. Delogu and Curini-Galletti 2007). The absence of a pair of chromosomes with respect to the basic set for the Otoplanidae (cf. Martens et al. 1989a) and the presence of a third pair of large metacentric chromosomes suggest a Robertsonian mechanism of chromosome fusion, involving two of the small chromosome pairs, that may be at the basis of the karyotype evolution of *P. primitiva*. Similar processes have already been extensively documented for Proseriata (Curini-Galletti et al. 1989).

Genus *Parotoplanella* Ax, 1956

General morphology similar to the genus *Parotoplana* Ax, 1956. Characterized by the anterior position of the ovaries, which are intercalated among testes, and by the vitellaria which, anteriorly, lie laterally to testes. Epithelium of the ciliated creeping sole with depressed nuclei. Outer epithelium of the pharynx non ciliated.

Parotoplanella heterorhabditica Lanfranchi, 1969 (Figure 3B)

Locality. Spain, St. Pola (lat. $38^{\circ}11'10.67''N$, long $0^{\circ}35'53.26''W$); lower intertidal in the *Otoplana*-zone, medium sand.

Material examined. One whole mount (SMNH 6784).

Description

For general shape and organisation, see the original description (Lanfranchi 1969). The specimen found had a male copulatory organ consisting of a small, globular seminal vesicle connected to a short, ovoid prostate vesicle, and a sclerotized apparatus consisting of seven spines, divided into two groups:

- 1) four straight, needle-shaped spines, with pointed apices, ranging from 49–51 μm in length;
- 2) three shorter spines, 38–40 μm long, with swollen, pointed apices, and provided with well-developed, elongate subterminal teeth.

Remarks

The species was described from specimens from the Ligurian Sea (northern Tuscany, Italy) (Lanfranchi 1969). The specimen found corresponds closely, for general arrangement of organs, and size and morphology of the sclerotized structures, to the original description.

General remarks

Previous results of the BIOIMPA campaigns concerned the composition of the genus *Parotoplana* in a marine protected area of the central Mediterranean (southern Apulia, Italy), where four new species were found (Delogu and Curini-Galletti 2007). The new records bring the number of Mediterranean species to 14, of which only one (*P. primitiva*) is known to occur outside the basin (present note). Our knowledge of the composition of the genus in the Mediterranean is however far from being exhaustive, as BIOIMPA campaigns, in other sectors of the Italian coasts, have revealed a considerable number of new species, which are still in the course of being described.

At the present stage of knowledge, most species of *Parotoplana* appear exclusive to their type locality and surroundings, and whenever different geographic sectors are sampled, a new suite of species is found. This obviously has to do with the geographic scale of the sampling design of the BIOIMPA project, which was indeed planned with the aim to maximize the discovery of new species, with the selection of stations in different sectors of the Mediterranean. However, the congruence of the pattern found so far (new species in each area, with few or no overlap in species composition among stations) points indeed to the existence of limited distribution ranges. Ecological differences among sectors of the Mediterranean (cf. Pérès and Picard 1964), coupled with the short life cycles and lack of dispersal phases of interstitial Platyhelminthes may be at the basis of present species diversity. It is indeed reasonable to assume that interstitial organisms in general may have scales for allopatric speciation different from those of macrofaunal organisms (cf. Casu and Curini-Galletti 2006) raising questions about the global contribution to marine biodiversity of meiofaunal taxa, which is probably severely underestimated at present.

In this general context, the fauna of southern Spain is of particular interest for its geographic position. Among the species whose phylogenetic relationships could be traced, two (*P. fredigatidani* sp. n. and *P. cucullata* sp. n.) are sister species of Mediterranean taxa, while *P. varispinosa* sp. n. is the sister species of an Atlantic taxon. The two species whose ranges extend beyond the coasts of southern Spain show a similar pattern, with one species known from western France (*P. primitiva*) and one from the northern Mediterranean (*Parotoplanella heterorhabditica*). Therefore, the presence, in southern Spain, of an overlap zone between Atlantic and Mediterranean faunas, well documented in macrofaunal groups, with an Atlantic component often conspicuous at the Gibraltar sill, which progressively disappears towards the eastern borders of the Alboran Sea (see, amongst others Harmelin and D'hondt 1993; Maldonado and Uriz 1995; Abello et al. 2002; Arroyo et al. 2006 and references therein), appears to apply to meiobenthic flatworms as well. Only rarely have Alboran endemics been found in macrofauna, and, when present, interpreted as Tethyan relicts of Premessinian biota (Boury-Esnault et al.

1992). Although biased by the poor overall knowledge of south-western Europe and west African meiofauna, present data suggest that, for interstitial Platyhelminthes, the presence of species endemic to the Alboran sea may on the contrary be relevant.

Finally, there are suggestions that the same ecological constraints which apply to the Atlantic component of the macrofauna, whose eastward penetration appears in many instances limited by Palos Cape, off south-east Spain (Abello et al. 2002), may affect meiofauna distributions as well. Species of Proseriata found in the only station sampled north of Palos Cape (St. Pola) were in most instances different from the suite of species found in the stations located in the Alboran Sea (unpl. data). In the case of the genera considered here, one species only was found at St. Pola, namely *Parotoplanella heterorhabditica*, a northern Mediterranean species absent from the Alboran Sea. However, hints about biogeography and patterns of biodiversity raised by the present study inevitably call for renewed and extensive work of taxonomic research.

Acknowledgements

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III

Otoplanidae (Platyhelminthes, Proseriata) from the northern Adriatic Sea

Submitted to *Meiofauna Marina*

V. Delogu & M. Curini-Galletti

Otoplanidae (Platyhelminthes, Proseriata) from the northern Adriatic Sea

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Keywords: meiofauna, taxonomy, biodiversity, Mediterranean

Abstract

The composition of the family Otoplanidae (Platyhelminthes) in samplings performed in the northern Adriatic Sea is described and discussed. Only five species belonging to the family were found. The prevalence of silty substrates in the area is deemed responsible for the paucity of species, confirming the strict ecological requirement of most representatives of the family for clean, coarse-grained sediments. Of the few species found, one is described as new: *Parotoplana geminispina* sp. n. The sclerotized apparatus of the male copulatory organ of the new species is characterised by a central group of three pairs of spines fused at their bases; a lateral pair of unfused, tubular spines may be also present. Additional morphological and karyological information are given on *Parotoplana rosignana* Lanfranchi & Melai, 2008, recently described for the west coast of Italy. An identification key to the Mediterranean species of the genus *Parotoplana* is given.

Introduction

During the years 2005 and 2006, within the framework of the project BIOIMPA (“Biodiversity of Inconspicuous Organisms in Italian Marine Protected Areas”), a series of sampling campaigns was performed in western Mediterranean, aimed to gather data on selected taxa of meiofauna (Platyhelminthes, Nemertodermatida, Gastrotricha, Rotifera, Tardigrada). Platyhelminthes in particular were noticeable for the large number of new species found, so far published only in part (Curini-Galletti et al. 2007; Delogu & Curini-Galletti 2007; Delogu et al. 2008).

A study of the specimens belonging to the family Otoplanidae (Platyhelminthes: Proseriata) found in the BIOIMPA samples is currently undertaken by the first author. The Otoplanidae is a large and diverse group, cosmopolitan in distribution, and characteristic of high energy beaches, where they may be among the dominant organisms (Remane 1933; Reise 1988). They are, on the contrary, rare or absent in silty substrates (Curini-Galletti 2001).

Samplings in the northern Adriatic Sea, both on Italian and Croatian coasts, revealed only few representatives of the family. The sample, although very limited, has some interest, as it offers some new insights both on biodiversity and biogeography of the family in Mediterranean. It is presented and discussed here.

Material and methods

BIOIMPA samplings in the northern Adriatic were centred on the Miramare Marine Protected Area (Gulf of Trieste), and spanned the area from Caorle (Italy) to

Cres Island (Gulf of Kvarner) (Croatia). However, specimens belonging to the Otoplanidae were found only in the following stations:

- Cres Island (Croatia);
- Brestova (Croatia);
- mouth of the river Isonzo (Italy).

Specimens were collected in sandy habitats by scooping up the superficial layer of sediment. Extraction of the animals from the sediment was with $MgCl_2$ decantation (Martens 1984). Each species was first studied alive by slight squeezing under the cover-slip. Preservation techniques routinely adopted for Proseriata were used (see Martens et al. 1989); whole mounts were made with lactophenol. For microscopical analysis material was fixed in Bouin's fluid, embedded in 60°C Paraplast and serial sagittal sections were cut at 4 μm , 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. (1989). Relative lengths (r. l. = length of chromosome \times 100/total length of haploid genome) and centrometric indices (c. i. = length of short arm \times 100/length of entire chromosome) were obtained from measurements of camera lucida drawings of metaphase plates. Karyotype formula as follows: haploid genome absolute length in μm ; fundamental number; relative length and centromere index of each chromosome; chromosome nomenclature between parentheses (m = metacentric; sm = submetacentric; st = subtelocentric; t = acrocentric). The fundamental number (FN) (i.e the number of chromosome arms in the karyotype) is derived according to Matthey (1949) and the chromosome nomenclature employed is that of Levan et al. (1964).

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).

Results

Taxonomy

Family OTOPLANIDAE Hallez, 1892 **Subfamily PAROTOPLANINAE Ax, 1956**

Genus Parotoplana Meixner, 1938

The genus *Parotoplana* Meixner, 1938 is characterized by the presence of a collar-shaped or shortly cylindrical pharynx entirely ciliated with the exception of its distal tip; serial arrangement of gonads (two rows of testes in front of the prepharyngeal ovaries; two rows of vitellaria behind the ovaries); a female apparatus with bursa opening independently into the common genital atrium, between the openings of the male system and of the common oviduct, which arises from the post-pharyngeal fusion of the germovitelloducts, only very rarely unfused (cf Lanfranchi 1978).

Parotoplana geminispina sp. n.
(Figs.1; 4)

Holotype: one whole mount (SMNH 7565).

Type locality: Giardinelli. La Maddalena Is. (lat. 41°13'55.73"N, long. 9°26'47.48"E), very fine gravelly very coarse sand, about 7 m deep.

Etymology: the specific epithet refers to the presence of twin spines (from latin *geminus*: twin, paired; and *spina*: spine).

Additional material: one specimen from the type locality (CZM 101). One specimen (CZM 102) from Cres Island (Croatia); "Lina" wreck (lat. 45°06'05.96"N, long. 14°16'40.47"E); about 43 m deep in slightly silty medium sand; July 2006. Two specimens (CZM 103-104), from Capo Caccia (Sardinia); Archi Cave ((lat. 40°34'10.71"N, long. 8°13'43.93"E), about 10 m deep in coarse sand.

Description

A comparatively large species (fixed specimens about 1.3 mm long), flattened and ribbon-shaped in appearance. General morphology of the body and arrangement of organs, similar to the other species of the genus. With two rows of 7-10 testes anterior to the ovaries, which precede the numerous vitellaria follicles, almost equally distributed anterior and posterior to the pharynx and extending to the genital organs.

The pharynx is located in the posterior half of the body, and appears variable in shape - from elongate to collar-shaped - depending from specimens and degree of squashing during observation.

The male copulatory organ consists of a sacciform seminal vesicle (about 80 µm long in fixed whole mounts), an elongate prostatic vesicle (90 µm long) and a sclerotized apparatus consisting of a central group of spines and a variable number of lateral spines.

The central group consists of three pairs of spines. These spines are fused basally, so that the group remained connected even in hardly squeezed karyological slides. The three pairs have markedly different morphology:

1: the central pair of spines is provided with an acuminate tip, swollen at its basis and falcate distally. Their length ranges 130-135 µm in the type population; 105-112 µm in the specimens from Capo Caccia, and 108 -109 µm in the specimen from Cres Is. Their width ranges 7-9.5 µm.

2: two spines taper distally into an acuminate, slightly recurve tip. The distal third of these spines is wrapped by a thin but distinctly sclerotized lamina, narrower basally, and broader distally. Their length ranges 128-140 µm in the type population, and 103-115 and 108 µm in the specimens from Capo Caccia and Cres Is., respectively.

3: two straight, thick spines (about 6-8 µm broad) are fused medially up to more than half their length. Distally they split, and taper till the obtuse distal tip, provided with a basal tooth which is fused with the corresponding structure of the twin spine. These spines are 130-143 µm long in the type population, and 105-121 µm and 110-112 µm in the specimens from Capo Caccia and Cres Is., respectively.

In addition to the central group, the two specimens from the type locality present an additional pair of lateral, unfused spines (group 4 in fig. 1C). These spines are 106-110 µm long, straight, gutter shaped and provided with rounded bases and acute, pen-nib shaped distal tips. The specimen from Cres Is. had only one of these spines, 103 µm long. None were present in the two specimens from Capo Caccia.

During limited observations on the female genital system on semisquashed specimens, a bursa filled with sperms, lateral to the male copulatory system, and similar to that of most *Parotoplana* species, could be detected.

Karyotype. One specimen from Capo Caccia had $n = 8$. Karyotype formula: 21.31 μm ; NF = 12; Chrom. I: 29.37; 44.05 (m); Chrom. II: 14.11; 22.98 (st); Chrom. III: 11.81; 27 (sm); Chrom. IV: 10.05; 23.59 (st); Chrom. V: 9.73; 39.05 (m); Chrom. VI: 8.89; 23.22 (st); Chrom. VII: 8.37; 40.3 (m); Chrom. VIII: 7.58; 16.25 (t) (based on the measurement of two plates). The karyotype is distinctive, for the overall large size of chromosomes and the presence of a metacentric Chrom. I, markedly larger than the remaining chromosomes. Chrom. II is almost half the size of Chrom. I, and subtelocentric with high index.

Remarks

The unusual specimen from the northern Adriatic is unquestionably similar to specimens collected in western Mediterranean, in north-west (Capo Caccia) and north-east (La Maddalena) Sardinia. Due to the bizarre central group of spines, there are no problems in recognizing these specimens as different from any species described so far. None of the known species of the genus, in fact, has copulatory spines fused basally into a single structure. The specimens are also characteristic for the low number of spines, arranged into pairs.

Only *P. spathifera* Delogu & Curini-Galletti, 2007, originally described from southern Italy (Porto Cesareo, Apulia), and known at present from scattered localities of the western Mediterranean, including northern Sardinia (own unpubl. data) has a sclerotized apparatus consisting of few (four) pairs of spines, with each pair markedly different in shape from each other (see appendix). Morphology and number of the spine pairs however differs. Among the major differences, *P. spathifera* has a central pair of thin, almost needle shaped spines (see Delogu & Curini-Galletti 2007), lacking in *P. geminispina* n. sp., nor is any spine in *P. spathifera* even remotely comparable to the straight, rod-shaped, distally fused spines of pair 3 of the new species. The lateral group of spines of *P. spathifera*, whose apices are provided with a feebly sclerotized, distal subtriangular lamina, indeed recalls the peculiar spines of pair 2 of the *P. geminispina* n. sp., where, however, the lamina is much more developed and broadly ovate.

The karyotype of *P. geminispina* n. sp, also, shares characters with that of *P. spathifera*. Albeit the latter has a different chromosome number ($n=9$) (Delogu & Curini-Galletti 2007)., both species have sets characterized by the presence of a comparatively very large metacentric chromosome, a chromosome II somewhat intermediate in size, at the border between submetacentric and subtelocentric, and with the rest of chromosomes of the set decidedly smaller.

The characters “few pairs of twin spines”, and “chromosome sets with a disproportionally large, metacentric Chrom. I” appear very rare, and not plesiomorphic, for the genus *Parotoplana* (cf Delogu et al. 2008). They are possible synapomorphies shared by *P. geminispina* n. sp. and *P. spathifera*, which can thus be considered as sister-species.

Notwithstanding the differences in size in individuals from the type locality and the rest of the sample studied, conspecificity of the northern Adriatic and northern Sardinia specimens is apparent from the shape of the central, copulatory group – the three pairs of spines have indeed almost identical morphology in the sample studied. More puzzling is, on the contrary, the presence of the additional, unfused pair of spines, laterally symmetrical to the central group, present in the type population (figs. 1C; 4A). The single specimen from the northern Adriatic had only one of these spines, while none could be found in the sample from north western Sardinia (Capo Caccia).

While the variability in the number of spines is known for many otoplanid species, the presence or absence of an entire pair seems a rather extreme case. However, the Adriatic specimen, with one spine, strengthens the hypothesis of an inter- and intra-population variability of their presence, whose full extent may be appreciable only in a wider sample than that available at present.

With the description of *P. geminispina* n. sp., the number of *Parotoplana* species

known at the present from the Mediterranean rises to 16. The identification key of these species is presented in the appendix, where emphasis has been given to characters appreciable in semi-squashed specimens. However, in few instances, identification is possible only on sectioned material (as in the case of *P. macrostyla* Lanfranchi, 1978).

Diagnosis: Species of *Parotoplana* provided with a male sclerotized apparatus consisting of six spines fused at their bases: two spines with pointed apices (about 108-135 μm long); two spines (108-140 μm long) tapering distally and provided with a distal, flattened lamina; two spines (105-143 μm long) with obtuse apices and subterminal tooth, fused for most of their length. May possess or not 1 to 2 additional, unfused tubular spines, 103-110 μm long. With $n=8$, and one metacentric chromosome pair distinctly larger than the other chromosomes of the set.

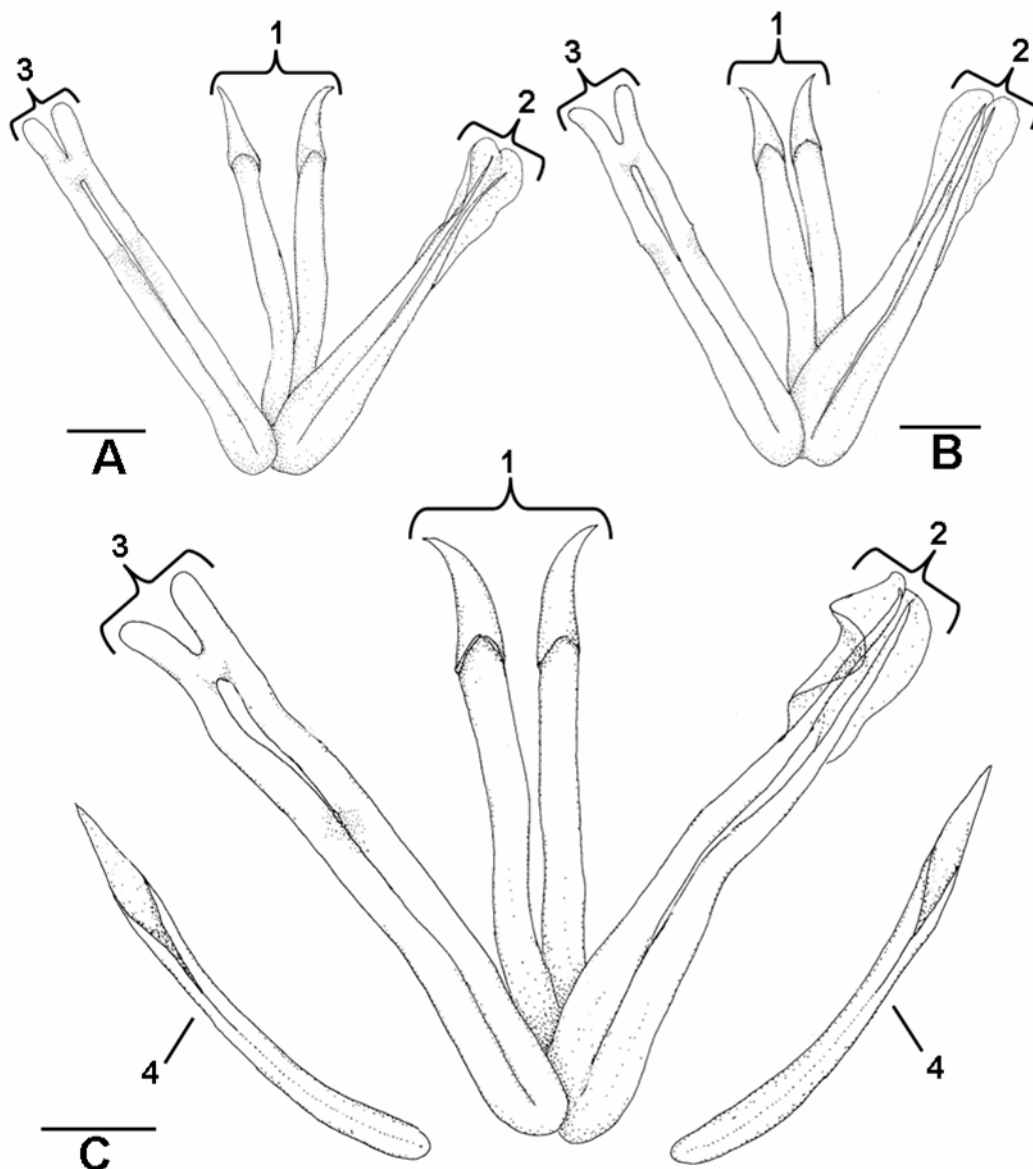


Figure 1: *Parotoplana geminispina* sp. n. Sclerotized apparatus of specimens from: A) Cres Is. (CZM 102), B) Capo Caccia (CZM 103), C) La Maddalena (Holotype, SMNH 7565). Scale bar = 20 μm .

Parotoplana rosignana Lanfranchi & Melai, 2008
(Figs.2; 5C-D)

Material examined

Italy: Castiglioncello (LI), La Caletta (type locality) (lat. 43°24'02.42"N, long. 10°25'22.18"E); mixed, coarse sand, from the lower intertidal to about -20 cm deep, July 2006; two specimens used for karyology and conserved as whole mounts (CZM 105-106).

Croatia: Cres Island, (lat. 45°06'11.43"N, long. 14°16'47.74"E); lower intertidal gravel, July 2006, five specimens sagittally sectioned (CZM 107-111); three karyological slides (CZM 112-114); two whole mounts (CZM 115-116).

Description

The species was recently described (Lanfranchi & Melai, 2008) without indication of a type locality. The new material from the northern Adriatic and from the first locality given for the species by Lanfranchi and Melai (2008) ("Castiglioncello in Caletta Beach", here designated as type locality of the species) and the availability of sectioned specimens allows an integration of the original description, as follows.

General morphology of the body and arrangement of organs, similar to the other species of the genus. Epithelium with intraepithelial nuclei. Ciliation restricted to a sensorial groove in the anterior end, and, ventrally, to a creeping sole which extends from the anterior end to the genital pore. Cilia up to 6 µm in length. The gut extends posteriorly nearly to the caudal end and anteriorly to the cephalic area. The nearly median pharynx is collar-shaped; its nucleated epithelium is ciliated except for a small area at the distal tip. Pharyngeal cilia are about 3 µm long.

Male genital system. Two rows of 7-10 testes each are located anterior to the ovaries. The male copulatory organ consists of a sacciform seminal vesicle (about 30 µm in length and 33 µm in diameter in specimens from the northern Adriatic) connected distally to a prostatic vesicle, about 20 µm wide. The seminal vesicle is lined by an extremely thin epithelium, becoming higher distally. The prostatic vesicle is lined by an unciliated, high, nucleated epithelium, and is surrounded by a thick layer of circular muscles. The prostatic vesicle is distally connected to the sclerotized apparatus which consists of a stylet surrounded by 12 spines.

The pen-nib shaped stylet (1 in figs. 2A,B) wraps the distal portion of the ciliated ejaculatory duct; it ranges 65-72 µm in length in the sample from northern Adriatic, and 87-88 in specimens from the type locality; it is 12-13 µm and about 16 µm wide, respectively, at its basis. It is formed by two gutter shaped spines. These spines, at about half of their length, above the swollen basis, taper abruptly into a narrow (3-5 µm wide), straight distal tip, distinctly truncated distally.

The 12 spines are arranged symmetrically at the two sides of the stylet, and consist of four groups with different morphology (figs 2A,B):

2: two spines, close to the stylet, 70-80 µm long in specimens from the northern Adriatic and 94-96 in specimens from the type locality with hook-shaped, falcate apices and well developed, thin and elongate, subterminal teeth. Their stalk is 4-6 µm broad;

3: two spines, with smaller, sickle-shaped apices, ranging 71-77 µm and 96-99 µm respectively in the two samples examined. These spines have a thinner stalk (3-5 µm wide), which broadens distally into an obtuse subterminal tooth, to which traces of the attachment of longitudinal muscles, which may give the impression of the presence of a sclerotized prominence, can be seen;

4: four slender spines, ventral to the stylet. These are comparatively very small in the Adriatic sample, ranging 42-59 µm, with a thin stalk, about 1.5 µm broad, and with slender sickle-shaped apices provided with a pointed subterminal tooth. They are

distinctly larger, ranging 95-118 μm in the sample from the type locality, with a comparatively wider distal portion below the subterminal tooth.

Lateral to the spines above, which surround the stylet, there are two pairs of symmetrically arranged, larger spines, which include:

5: two spines 80-97 μm long in the Adriatic and 108-117 μm long in the Tyrrhenian sample, with sickle-shaped apices provided with poorly developed subterminal tooth;

6: two spines, 90-101 μm and 120-121 μm long respectively, provided with flattened, elongate, spatulated apices and with a small, obtuse tooth located at about 1/3 of their length from the apex.

The sclerotized apparatus is located in the male antrum, lined by a nucleated epithelium, which opens into the male, anterior portion of the common atrium. This portion is surrounded by a uniform layer of circular musculature.

Female genital organs. Two ovaries are located in front of the pharynx. Two rows of vitellaria are present posterior to the ovaries to the level of the copulatory organ. In the sectioned material, it is possible to trace the presence of the female common duct only in its distal portion where it is surrounded by numerous shell glands. It opens into the common genital atrium at its posterior end. The common female duct is lined by a unciliated epithelium, and is surrounded by numerous female glands in its distal portion. The epithelium close to the opening of the female duct into the common atrium is distinctly swollen, irregular, and glandular in appearance.

The bursa opens into the common atrium through a long bursal canal, surrounded by strong circular muscles in its distal portion. Proximally, this bursal canal widens and is surrounded by an irregular, glandular epithelium. The distal portion of the bursal canal is much narrower, and is surrounded by a thick layer of circular muscles. This portion is provided internally with a peculiar bursal nozzle. It is funnel shaped, 40-60 μm long, with a flaring, outer rim, and fits entirely the lumen of the canal.

The bursa consists of an anterior, ovoid region, lined by a high epithelium about 30 μm high, connected to an inner portion, lined by a vacuolated epithelium of the resorbiens type, which abuts the gut.

Close to the opening of the bursal canal into the common atrium, the outlet of a smaller duct could be traced in all the specimens sectioned. It is surrounded by very numerous glands with a fine-grained content, whose secretion apparently discharges into the atrium.

The common genital atrium, which is lined by a thin nucleated epithelium, opens to the outside through a common genital pore.

Karyotype. With $n = 7$. Karyotype formula: $10.77 + 1.27 \mu\text{m} ; 11$; Chrom. I: 25.23 ± 0.72 ; 46.29 ± 1.65 (m); Chrom. II: 22.29 ± 1.28 ; 45.36 ± 1.11 (m); Chrom. III: 14.59 ± 0.62 ; 26.37 ± 4.98 (sm); Chrom. IV: 11.78 ± 0.66 ; 24.37 ± 6.29 (st); Chrom. V: 9.79 ± 0.95 ; 15.46 ± 9.08 (st); Chrom. VI: 8.88 ± 0.44 ; 26 ± 9.53 (sm); Chrom. VII: 7.7 ± 0.66 ; 23.32 ± 11.58 (st) (based on the measurement of five plates from two specimens of the type locality). The karyotype is characterized by a small genome length, and the presence of two distinctly larger metacentric chromosomes, about three times the size of the smallest chromosomes of the set.

Remarks

In the original description of *P. rosignana*, the stylet is summarily described as a "central, funnel shaped sting", without indication of its being formed by two clearly distinct spines; the two pairs of central spines (groups 2 and 3 above) are described as identical in morphology, both provided with very slender, long basal tooth; and the

outer pair of spines (group 6 above) is described as arcuate, without a submedian tooth. Furthermore, no mention whatsoever is done to the obvious, peculiar, trumpet-shaped sclerotized bursal nozzle. This description thus departs from the morphology of the specimens found in the northern Adriatic; and could have supported a non conspecificity of the two populations. However, a check on the type population of *P. rosignana* revealed morphological details much more in agreement with the specimens from Cres Is., with differences in size of the spines interpretable as inter-population variability, as in the case of *P. geminispina* n. sp. above.

The scattered reports of *P. rosignana* from the Ligurian and Adriatic sea suggest a continuous distribution along at least the Italian coastline. As deducible from the identification key in appendix, it is most similar to *P. procerostyla* from north west Mediterranean, and *P. fretigaditani* from the Strait of Gibraltar area (Ax 1956; Delogu et al. 2008). The characters shared by the three species, in particular the very slender stylet formed by two spines, do not appear plesiomorphic for the genus *Parotoplana* (see Delogu et al. 2008), and support their close phylogenetical relationships. Within the group, *P. rosignana* could be hypothesized as the sister species of *P. procerostyla*, based on the shared presence of a bursal nozzle, a rare feature in the genus and in the whole family Otoplanidae.

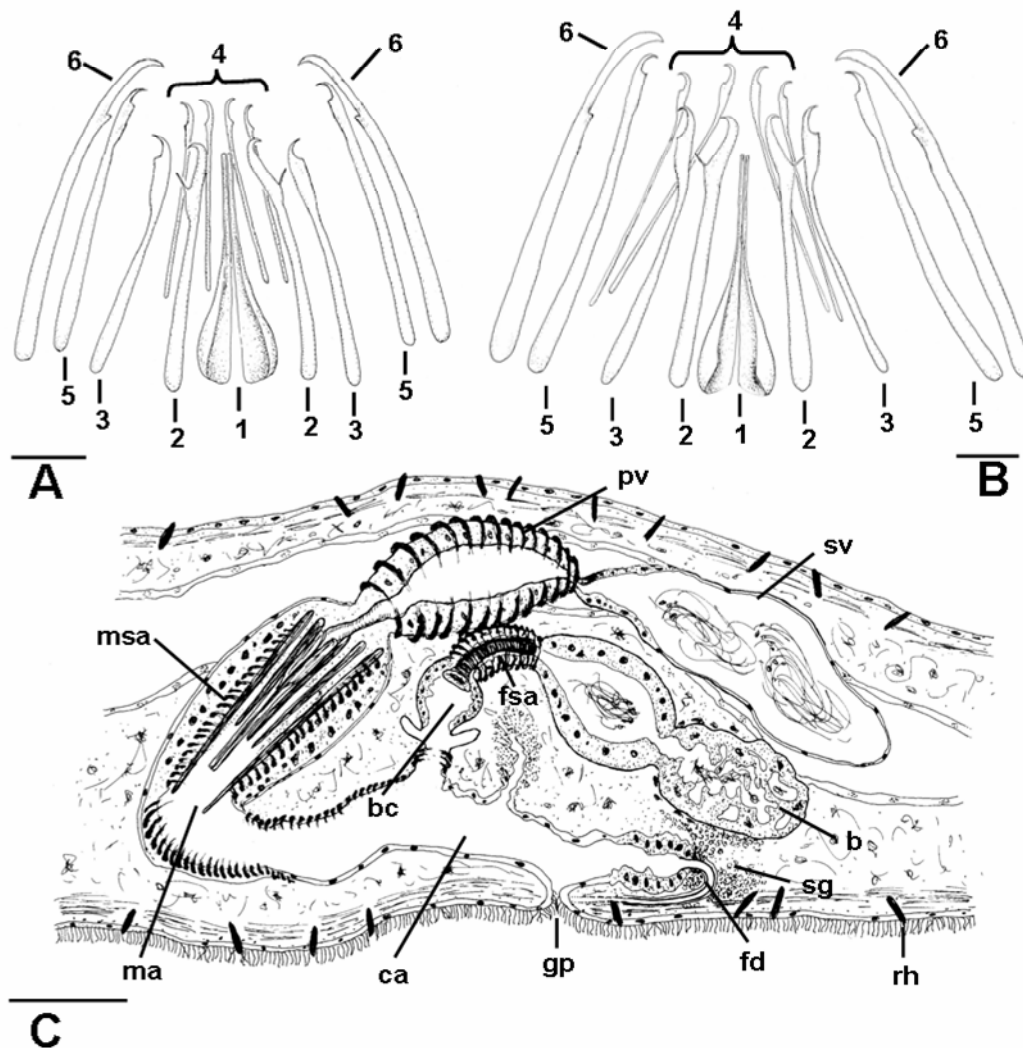


Figure 2: *Parotoplana rosignana* Lanfranchi & Melai, 2008. Sclerotized apparatus of specimens from: A) Cres Is. (CZM 112), B) Castiglioncello (type locality)(CZM 105). C): reconstruction of the genital organs from sagittal sections of specimens from Cres Is. A,B: scale bar = 20 μm. C: scale bar = 50 μm.

Xenotoplana acus Ax, Weidemann & Ehlers, 1978
(Figs.3; 5A,B)

Material examined

Croatia: Cres Island, small cave near "Lina" wreck (lat. 45°06'05.96"N, long. 14°16'40.47"E), in slightly silty fine-medium sand, about 5m deep; one whole mount (CZM 117).

Italy: Punta Ala, Castiglione della Pescaia (Gr), near Torre Hidalgo (lat. 42°48'26.78"N, long. 10°44'33.41"E), slightly silty medium-coarse sand, two whole mounts (CZM 118-119). Budelli Is., La Maddalena archipelago, Il Cavaliere beach. (lat. 41°17'7.48"N, long. 9°21'36.26"E), slightly very fine gravelly medium sand, about 8 m deep, one whole mount (CZM 120).

Description

The diagnostic features of the genus (vaginal opening and the conspicuous vaginal duct) are easily appreciable even in living, gently squeezed specimens. The detailed original description of *X. acus* (Ax et al., 1978; type locality: Villa Galotti, Gulf of Naples) allows immediate identification of the specimen from Cres Is. The species is characterised by a flat stylet, surrounded by different groups of spines.

These sclerotized structures, in the specimens from the northern Adriatic, were as follows:

1: pin-shaped stylet, flat, slightly gutter-shaped medially, with an obtuse distal tip, 75 µm long, and 15 µm wide at its basis, surrounded by two pairs of spines

2: two longer spines, about 60 µm in length, with a thin stalk, 3 µm wide, sickle-shaped apex, and a long subterminal prominence, basally oriented, and eventually fused with the stalk

3: a shorter and stockier pair, 49 µm long with a stalk 4 µm wide, with the apex similar to the previous pair, but the subterminal, basally oriented and fused prominence more developed.

4: a group of three slender, ventral spines, about 43 µm long, with acute sickle-shaped apices and sharp subterminal tooth;

5: one pair of outer spines 58-62 µm long, with a recurve, gutter-shaped apex, ending into an acute tip. Their bases, about 6 µm broad, is sulcate; the median groove is particularly marked, and the basis appears as split at its middle.

Remarks

Overall, the specimen found at Cres Is. shows close similarity with the drawings and description of *Xenotoplana acus* (Ax et al. 1978). Among the few differences, the higher degree of development of the subterminal emergence of pairs 2 and 3, which, however, appears variable in the sample examined from western Italy and Sardinia and may be linked to interpopulational variability and/or different maturity stages. The outer pair of spines is drawn by Ax et al. (1978, pg. 126 fig. 6B), with acutely pointed bases, and rounded, sulcate apices, split in the middle. These are obviously the same spines as described above, drawn upturned, as the pointed apices, as usual in the Otoplanidae, are forwards directed. In reality, these spines appear to be obliquely oriented in living organisms, and one (as in the specimen in fig. 5A) or both may be upturned in hardly squeezed specimens.

Although this are the first additional reports of the species since its original description, *X. acus* appears to be common and widespread in central Mediterranean, mostly in high energy, subtidal coarse sediments (unpublished data).

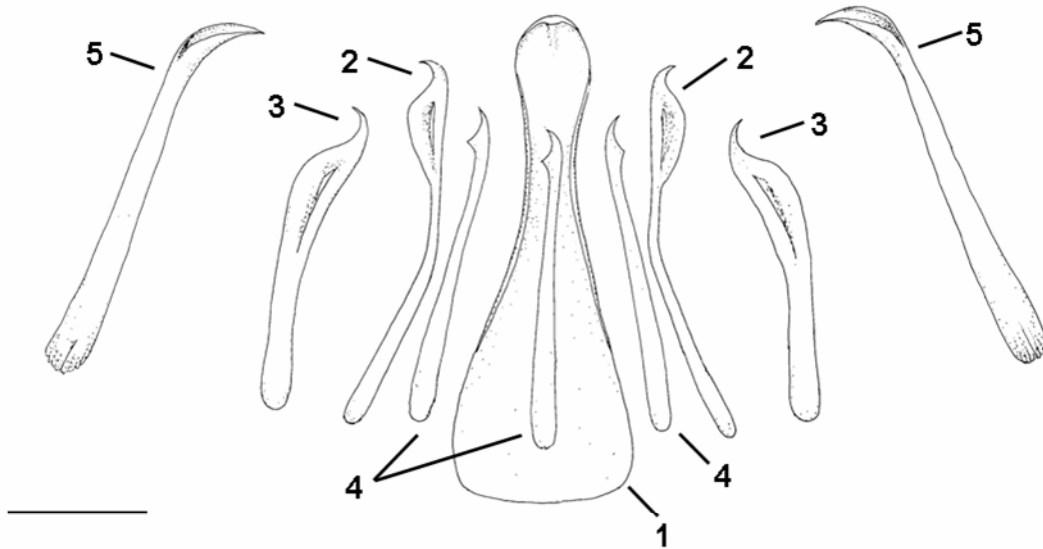


Figure 3: *Xenotoplana acus* Ax, Weidemann & Ehlers, 1978. Sclerotized apparatus. Scale bar = 20 μm .

Monostichoplana n. sp.

Material examined

One specimen from Cres Island (Croatia); "Lina" wreck (lat. 45°06'05.96"N, long. 14°16'40.47"E); medium- fine slightly silty sand with shell fragments, about 38m deep.

The specimen found showed clear differences in the male sclerotized apparatus from the only species of the genus known so far for the Mediterranean, *Monostichoplana filum neapolitana* Ax, Weidemann & Ehlers, 1978. It will be described in a taxonomic revision of the genus *Monostichoplana*, currently in progress (Delogu & Curini-Galletti in prep.).

Postbursoplana (?) sp.

Material examined

Italy: mouth of the river Isonzo (lat. 45°43'26.08"N, long. 13°33'04.84"E); one specimen in silty medium sand, with extremely high densities of the bivalve *Lentidium mediterraneum* (Costa O.G., 1829).

Croatia: Brestova (lat. 45°08'37.23"N, long. 14°13'27.07"E); two specimens in intertidal gravel.

A few, immature specimens of a small otoplanid, were found in shallow waters of the northern Adriatic in two stations. While the general body shape and arrangement of organs suggested their inclusion in the genus *Postbursoplana* Ax, 1956, found in similar habitats in most of the Mediterranean (Ax 1956; Lanfranchi 1969; pers.obs.) the lack of mature individuals precluded any further characterisation of these specimens.



Figure 4: *Parotoplana geminispina* sp. n. Sclerotized apparatus of specimens from: A) La Maddalena (Holotype, SMNH 7565), B) Capo Caccia (CZM 103), C) Cres Is. (CZM 102). Scale bar = 20 μ m.

Discussion

If anything, the list of the Otoplanidae found in the Northern Adriatic is remarkable for the paucity of species, and confirms the strict ecological requirement of the family for clean, coarse grained sediments. In the Gulf of Trieste, in fact, all the sediments sampled contained a more or less heavy amount of silt, and even the *Otoplana*-zone (see Remane, 1933) was remarkably deprived of its namesake – an extremely unusual occurrence for the Mediterranean. In such environments, other Proseriata, namely representatives of the Monocelididae, more typical of sheltered, silty substrates (Reise, 1988; Curini-Galletti 2001) were found in great abundance and diversity (unpublished data). The only samples where the

Otoplanidae were represented, although with low diversity, were those collected in the stations located in the Gulf of Kvarner (Croatia), where the sediments, due to higher hydrodynamism of the area, contained a smaller silty fraction.

The sample found raises a few additional points, of more general application in the field of the study of interstitial Platyhelminthes:

1) since most papers deal with the description of new species, usually known from the type locality only, the topic of differentiation among allopatric populations is very rarely addressed. Data presented here point that differences in size and details of morphology of sclerotized structures, and even of the number of spines, may be a significant component of intra-specific variability in Proseriata. This degree of allopatric divergence should be carefully considered when comparing specimens sampled in far away locations, and, whenever possible, combined with molecular data;

2) the case of *Parotoplana rosignana* treated above, if necessary, stresses the need for a careful description of the material on which new species are based, including observations on hardly squeezed material, where all details of the sclerotized structures can be retrieved. Only in this way, in fact, other researchers can be allowed a proper basis on which identification of their findings may be based.

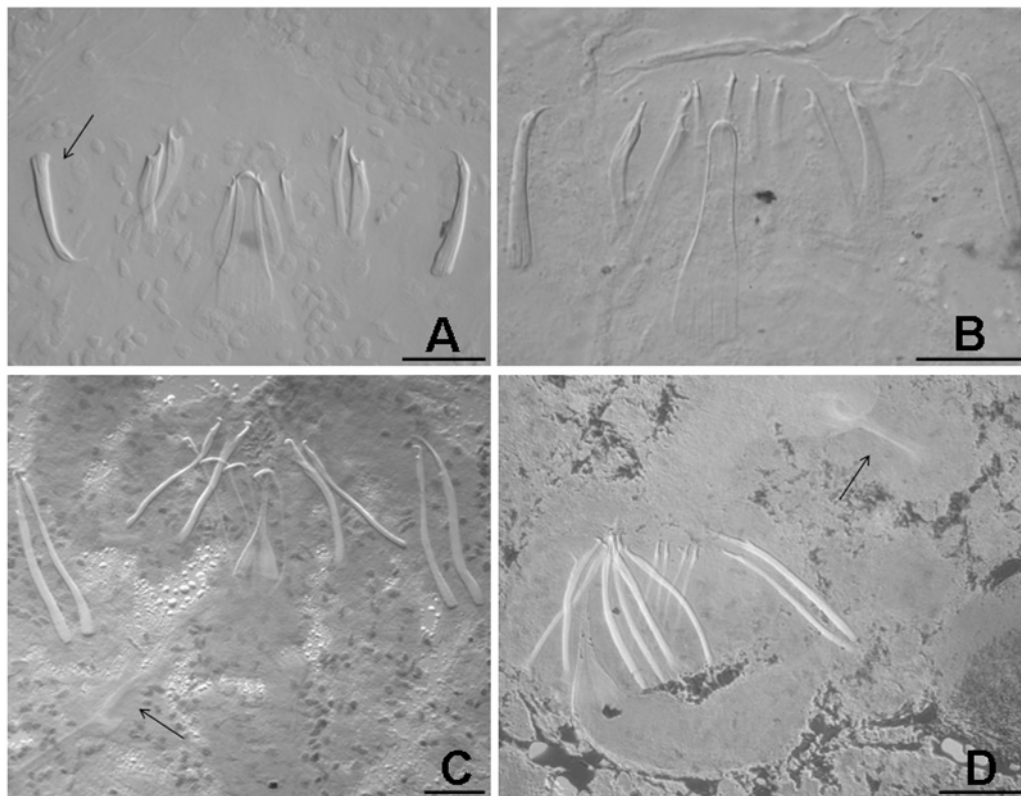


Figure 5: A, B. *Xenotoplana acus* Ax, Weidemann & Ehlers, 1978. Sclerotized apparatus of specimens from A) La Maddalena (CZM 120)(arrow points the upturned outer spine), B) Cres Is. (CZM 117). Scale bar = 30 μ m. C, D. *Parotoplana rosignana* Lanfranchi & Melai, 2008. Sclerotized apparatus of specimens from: C) Castiglioncello (type locality)(CZM 105), D) Cres Is. (CZM 112). Arrows point the bursal nozzles. Scale bar = 40 μ m.

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Appendix

Key to the Mediterranean *Parotoplana* species:

- 1 Male sclerotized apparatus provided with a central stylet.....2
 - Without central stylet.....10
- 2 With a girdle of spines.....3
 - With different types of spines, symmetrically arranged in pairs or groups at the two sides of the stylet.....8
- 3 With companion spines.....4
 - Without companion spines.....*P. mastigophora* Delogu *et al.*, 2008
- 4 With a girdle of one type of spines.....5
 - With a girdle of two or more types of spines.....*P. pythagorae* Delogu & Curini-Galletti, 2007
- 5 With common germovitelloduct.....6
 - With paired germovitelloducts.....7
- 6 With female glandular organ.....*P. cucullata* Delogu *et al.*, 2008
 - Without female glandular organ.....*P. renatae* Ax, 1956
- 7 With female glandular organ*P. uncinata* Lanfranchi, 1978
 - Without female glandular organ.....*P. macrostyla* Lanfranchi, 1978
- 8 With bursal nozzle.....9
 - Without bursal nozzle.....*P. fretigaditani* Delogu *et al.*, 2008
- 9 With three types of spines.....*P. procerostyla* Ax, 1956
 - With more of three types of spines.....*P. rosignana* Lanfranchi & Melai, 2008
- 10 With bursal nozzle.....*P. jondelii* Delogu & Curini-Galletti, 2007
 - Without bursal nozzle.....11
- 11 With less than 20 spines.....12
 - With more than 20 spines.....14
- 12 With spines arranged in a central group and a girdle.....*P. varispinosa* Delogu *et al.*, 2008
 - With spines arranged in pairs13
- 13 With broad spines, mostly fused at their bases.....*P. geminispina* sp.n.
 - With all spines unfused at their bases...*P. spathifera* Delogu & Curini-Galletti, 2007
- 14 With spines arranged in two concentric girdles.....*P. primitiva* Ax, 1956
 - With spines arranged in a central group, surrounded by a girdle of spines.....15
- 15 With less than 40 spines.....*P. multispinosa* Ax, 1956
 - With more than 40 spines.....*P. terpsichore* Delogu & Curini-Galletti, 2007

IV

A Mediterranean radiation of interstitial flatworms: the *Parotoplana jondelii* (Platyhelminthes: Proseriata) species group

Manuscript

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**A Mediterranean radiation of interstitial flatworms: the
Parotoplana jondelii (Platyhelminthes: Proseriata) species group.**

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Keywords: meiofauna, taxonomy, *Parotoplana*, Mediterranean

Abstract

Five new *Parotoplana* species (Proseriata: Otoplanidae), belonging to the *P. jondelii* complex, are described from the Mediterranean. They are characterized by the presence of unusually broad spines, arranged into a girdle around the distal portion of the ejaculatory duct, with a dorsal pair of spines distinctly different from the rest of the girdle, and at least two pairs of needle shaped ventral spines. The five new species (*P. crassispina* sp. n., *P. carthagoensis* sp. n., *P. pulchrispina* sp. n., *P. obtusispina* sp. n., *P. axii* sp. n., *P. ichnusae* sp. n.) are distinguished by number and shape of the copulatory spines, and presence/absence of bursal nozzles. The *P. jondelii* complex is widespread in central Mediterranean, with most species showing limited, non overlapping range.

Introduction

Traditionally marine environment was considered a low specific diversity habitat but at present a growing awareness is rising that this perception of marine biodiversity is inadequate and based upon unrepresentative data (Bouchet *et al.* 2002, Sala 2002). The inadequacy of data on taxonomy and distribution of marine organisms is particularly acute for interstitial meiofauna, where information may be absent altogether for entire biogeographical regions (Kennedy and Jacoby 1999). Among these groups, information on Platyhelminthes, a species-rich, ubiquitous and diverse taxon, is, at present, far from adequate and it is considered one of the taxa (as well as Polychaeta, Olygochaeta, and Nematoda), that especially needs new identification guides and revised taxonomic lists (Costello *et al.* 2006). In fact, only few areas are relatively well known, especially northern Europe and the Galápagos Islands (Ax & Schmidt 1973, Armonies & Reise 2000), with little and taxonomically limited research elsewhere. In this scenario, under the sponsorship of the project PRIN-2004 BIOIMPA (Biodiversity of Inconspicuous Organisms in Marine Protected Areas), a series of sampling campaigns was carried out along the Mediterranean coasts, aimed to gather data on the composition and distribution of a taxon of Platyhelminthes, the

Proseriata. Among Proseriata, the family Otoplanidae is the dominant taxon in the surf zone of the high energy beaches (the “Otoplana-zone” of Remane, 1933) (Reise 1988; Curini-Galletti 2001), and, the genus *Parotoplana* Meixner, 1938 is particularly well represented in the samples. The present contribution is focused on a group of *Parotoplana* species which are all provided with a sclerotized apparatus consisting of broad and flat spines, the “jondelii-complex”. The name of this group refers to the first species with these particular spines found. *Parotoplana jondelii* Delogu & Curini-Galletti, 2007 was described for the coasts of Southern Apulia (Italy) (Delogu & Curini-Galletti, 2007), and at that time, it appeared the only species in the genus provided with these kinds of particular spines. Here six new species belonging to the group are described. In order to facilitate future identification of species within the group, we give an overview of the general morphology of spines of all species.

Material and methods

Specimens were collected in sandy habitats by scooping up the superficial layer of sediment. Extraction of the animals from the sediment was with $MgCl_2$ decantation (Martens, 1984). Each species was first studied alive by slight squeezing under the cover-slip. Preservation techniques routinely adopted for Proseriata were used (see Martens et al., 1989); whole mounts were made with lactophenol. For microscopical analysis material was fixed in Bouin’s fluid, embedded in 60°C Paraplast and serial sagittal sections were cut at 4 μm , 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.* (1989). Relative lengths (r. l. = length of chromosome x 100/total length of haploid genome) and centrometric indices (c. i. = length of short arm x 100/length of entire chromosome) were obtained from measurements of camera lucida drawings of metaphase plates. The fundamental number (FN) (i.e the number of chromosome arms in the karyotype) is derived according to Matthey (1949) and the chromosome nomenclature employed is that of Levan et al. (1964): m = metacentric; sm = submetacentric.

Type materials is stored in the collections of the Swedish Museum of Natural History (Stockholm, Sweden)(SMNH). Additional material, when present, is stored in the collection of the Zoological Museum of the University of Sassari (Italy)(CZM).

General morphology

In all species, spines are arranged into a girdle which wraps around the distal portion of the ejaculatory duct. In the girdle, it is possible to distinguish the dorsal side and the ventral side. In order to render the discrimination of the species more comprehensible, spines are classified in different groups:

Dorsal spines

Group 1. – thinner straight spines;

Group 2. – central spines usually with falcate or slightly falcate apices (the exception is *Parotoplana pulchripina* sp. n. whose apices are straight), provided of a teeth placed far from the distal tip (distance: 12 to 22 μm);

Group 3. – broad straight spines;

Ventral spines

Group 4. – spines with sickle-shaped apices;

Group 5. – numerous spines (six or more with the exception of *P. carthagoensis* sp. n. in which they are four or five), usually with sickle-shaped apices and subterminal teeth (the exception is *P. obtusispina* sp. n. which is provided of straight spines). These spines may be smaller than the previous or with a larger sickle-shaped apices;

Group 6. – spines with different kind of morphologies.

Taxonomy

Family OTOPLANIDAE Hallez, 1892

Subfamily PAROTOPLANINAE Ax, 1956

Genus *Parotoplana* Meixner, 1938

Parotoplana jondelii Delogu & Curini-Galletti, 2007

For general shape and organisation, see the original description (Delogu & Curini-Galletti, 2007)

The male sclerotized apparatus consists of five types of spines, arranged symmetrically into a girdle (see Delogu & Curini-Galletti, 2007: fig. 3A pg. 24; 7A pg. 29):

1) two very narrow and straight spines, 75–79 μm long in the holotype;

2) four broad spines, 75–83 μm long, with slightly recurve, bulbous apices provided with a tooth placed at the basis of the distal third of the spine;

3) four broad spines, 70–80 μm long, narrowing distally into acute apices without any subterminal tooth;

4) two broad spines (62–65 μm long), with sickle shaped apices and marked subterminal tooth;

5) six spines similar in morphology to the previous, but distinctly smaller (53–55 μm long).

In the whole mount, the presence of numerous bursal spines (see Delogu & Curini-Galletti, 2007: fig. 7B pg. 29):, arranged into two blocks, could be seen. These spines are sharply triangular in shape (up to 4–5 μm long), feebly sclerotized, and appear as basal lamina derivatives.

New species

Parotoplana crassispina sp. n.

(Figs. 1A - C)

Holotype: one whole mount (SMNH 7568).

Type locality: Capezzòlo (Tuscany) (42°45'55.29"N, long. 10°51'46.11"E); in intertidal medium sand. March 2007

Paratype: one specimen sagittally sectioned (SMNH 7569). Torre Civette (Tuscany) (lat. 42°50'41.70"N, long. 10°46'30.34"E), in intertidal medium sand. March 2007.

Additional material: Capezzòlo (Tuscany). Five specimens sagittally sectioned (CZM 121-125) and four karyological slides made permanent with lactophenol (CZM 126-129).

Torre Civette (Tuscany). 14 specimens sagittally sectioned (CZM 129-142) and eight karyological slides made permanent with lactophenol (CZM 143-150).

Le Bombarde beach (Sardinia) (lat. 40°35'2.59"N, long. 8°15'37.36"E), in medium-fine sand, about 1 m deep. One specimen sagittally sectioned (CZM 151) and five karyological slides made permanent with lactophenol (CZM 152-156).

Archi cave (Sardinia) (lat. 40°34'10.71"N, long. 8°13'43.93"E), in medium sand, about 10 m deep. One specimen made as whole mounth (CZM 157).

Girin (Sardinia) (lat. 39°6'56.68"N, long. 8°18'33.33"E), in medium sand. Three specimens made as whole mount (CZM 158-160).

Canet beach (France), in medium-fine sand, about 2 m deep. Five specimens

made as whole mount (CZM 161-165).

Etymology: from latin *crassus*, fat – with reference to the peculiar, broad spines of the new species.

Description

Living specimen of average size of the genus; adult worms up to 1.5 mm long. External morphology similar to the other species of the genus, with clearly marked anterior end and fan-shaped caudal end. The encapsulated oval-shaped brain is about 45 μm in its widest axis and abuts on a statocyst about 15 μm in diameter. The epithelium is nucleated. Dorsally, the narrowly elongate rhabdoids (up to 12 μm long) are arranged in about 15 irregular rows. Ciliation restricted to a sensory groove in the anterior end and, ventrally to a creeping sole extending from the anterior end to the genital pore. Cilia up to 6 μm in length. The collar shaped pharynx is located in the middle of the body. It is entirely ciliated (cilia about 3 μm long), with the exception of its distal tip. The pharyngeal cavity opens to the outside through the mouth which is surrounded by a ring of circular muscles.

Male genital organs. Two lateral rows of few testis (5 to 9 each row) are present anterior to the ovaries. The copulatory organ consists of an ovoid seminal vesicle (about 37 μm long) which is lined by a thin nucleated epithelium, a prostatic vesicle (about 40 μm in length) internally ciliated which is distally connected to the sclerotized apparatus. The sclerotized apparatus consists of 14 spines, arranged symmetrically into a girdle (Fig. 1A):

Group 1: two thin, straight spines (Holotype: 56-60 μm ; range: 46-60 μm) with straight apices and blunt distal tip. Stem 2-3 μm broad;

Group 2: two spines with slightly falcate apices (Holotype: 58-59 μm long; range 47-60 μm) and rounded distal tip. They are provided with a large, plate teeth, variably in size (Holotype: 15 μm long and 3 μm wide; range: 8-20 μm in length and 2-3 μm wide). The distance between the teeth and the distal tip is about 16 to 22 μm . Stem from 4.5 to 7 μm broad;

Group 3: two broad, straight spines (Holotype: 59 μm ; range: 48-60 μm) with straight apices and blunt distal tip. Stem 4-6 μm broad;

Group 4: two spines (Holotype: 41-44 μm ; range: 36-46 μm) with sickle shaped apices, pointed distal tip and small teeth. Distance between teeth and tip about 6 μm . Stem 5-6 μm broad;

Group 5: six broad spines (Holotype: 57-60 μm ; range: 47-60 μm) with falcate apices and small button shaped distal tip. They are provided with a wide teeth at about 10-15 μm from the tip. Stem up to 7 μm broad.

Group 6: absent.

Female genital organs. Two ovaries lie anterior to the pharynx. Two rows of numerous vitellaria (20 to 30 each row) extend from posterior to the ovaries to anterior to the copulatory organ. A small bursa (about 20 μm in diameter) opens into the common atrium through a short bursal canal (about 22 μm). It is provided with numerous triangular bursal spines (1 to 5 μm in length), arranged in few parallel rows (up to 6) (Fig. 1C).

Diagnosis. *Parotoplana* species with the sclerotized apparatus consisting of five types of spines:

1. two thin, straight spines with straight apices and blunt distal tip, 46-60 μm long;
2. two spines with slightly falcate apices, round distal tip and large, plate teeth, 47-60 μm long;
3. two broad, straight spines with straight apices and blunt distal tip, 48-60 μm long;
4. two spines with sickle shaped apices, pointed distal tip and small teeth, 36-46 μm ;
5. six broad spines with falcate apices, small button shaped distal tip and broad teeth, 47-60 μm long.

Triangular bursal nozzles, from 1 to 5 μm in length.

***Parotoplana carthagoensis* sp. n.**

(Figs. 1B - D)

Holotype: one specimen sagittally sectioned (SMNH 7566).

Paratype: One specimen prepared as a whole mount (SMNH 7567).

Type locality: Sidi bou said (Tunis), (lat. 36°51'59.62"N, long. 10°20'52.74"E).
March 2008.

Additional material: nine specimens sagittally sectioned (CZM 166-174) and two karyological slides (CZM 175-176).

Etymology: the name refers to the type locality, adjacent to the ruins of the mighty city of Carthago.

Description

The holotype is an adult worm about 1 mm long in fixed conditions. The anterior end is clearly marked and provided with sensory bristles and, ventrally, with a few adhesive papillae. The fan-shaped caudal end is equipped with numerous adhesive papillae. The encapsulated, oval-shaped brain is about 64 μm in diameter, and abuts on a statocyst (diameter: 15 μm). Epithelium with intraepithelial nuclei. With numerous rhabdoids, up to 11 μm in length, arranged dorsally in 15 irregular, longitudinal rows. Ciliation restricted to a sensorial groove in the anterior end and, ventrally, to a creeping sole extending from the anterior end to the genital pore. Cilia up to 5 μm in length. The gut extends anteriorly to the cephalic area, and posteriorly nearly to the caudal end. The pharynx is collar-shaped; its epithelium is completely ciliated except for a small area at the distal tip. The pharyngeal cavity opens to the outside through the mouth, which is surrounded by a ring of circular muscles.

Male genital organ. With two rows of testis anterior to the ovaries. The copulatory organ consists of an ovoid seminal vesicle (about 30 μm in length; diameter: 15 μm), a short prostatic vesicle (20 μm) lined internally by a nucleated epithelium. It is distally connected to a sclerotized apparatus.

The sclerotized apparatus consists of 14 spines (13 in specimen CZM 175, 15 in specimen CZM 176) symmetrically arranged (Fig. 1B):

Group 1: two (only one in CZM 175) straight, thin spines (range 38-45 μm) with straight apices and blunt distal tip. Stem 1 μm broad;

Group 2: two spines (range 40-45 μm in length) with falcate apices and button shaped distal tip; very small teeth at about 12 μm from the tip. Stem 3 μm broad, distally blunt;

Group 3: two straight, wide spines (43-50 μm long) with straight apices and blunt distal tip. Stem 4-5 μm broad;

Group 4: two spines (37-45 μm in length) with falcate apices and pointed distal tip. They are provided with small pointed teeth, located at about 8 μm from the tip. Stem about 3 μm thick;

Group 5: four spines (five in specimen CZM 176) (range 37-41 μm in length) with falcate apices and button shaped distal tip. Provided with short broad teeth at about 9 μm from the tip. Stem 3 to 4 μm thick;

Group 6: two broad straight spines (40-47 μm) with button-shaped distal tip and provided with a slight thickening at about 14 μm from the tip. Stem up to 4.5 μm broad.

Female genital organ. With two ovaries anterior to the pharynx. Two rows of vitellaria stretch from posterior to the ovaries to anterior to the copulatory organ. The ovoid small bursa (about 20 μm in its widest axis in sections) is lined with a nucleated epithelium. It is connected to the common atrium through a short bursal canal (20-30 μm long). It is provided with numerous triangular bursal nozzles (2 to 3 μm in length), that in some cases, may be fused as a large tube (Fig. 1D) .

Diagnosis. Parotoplana species with sclerotized apparatus consisting of six types of spines:

1. one or two thin straight spines with straight apices and blunt distal tip, 38-45 μm long;
2. two spines with falcate apices, button-shaped tip, small teeth, 40-45 μm long;
3. two broad straight spines with straight apices and blunt distal tip, 43-50 μm long;
4. two spines with falcate pointed apices and small pointed teeth, 37-45 μm long;
5. four or five spines with falcate apices, button-shaped distal tip and short broad teeth, 37-41 μm long;
6. two broad straight spines with button-shaped distal tip and slight thickening at 14 μm from the tip, 40-47 μm long.

Bursal canal provided with triangular bursal spines (2-3 μm long), which can be fused as a large tube.

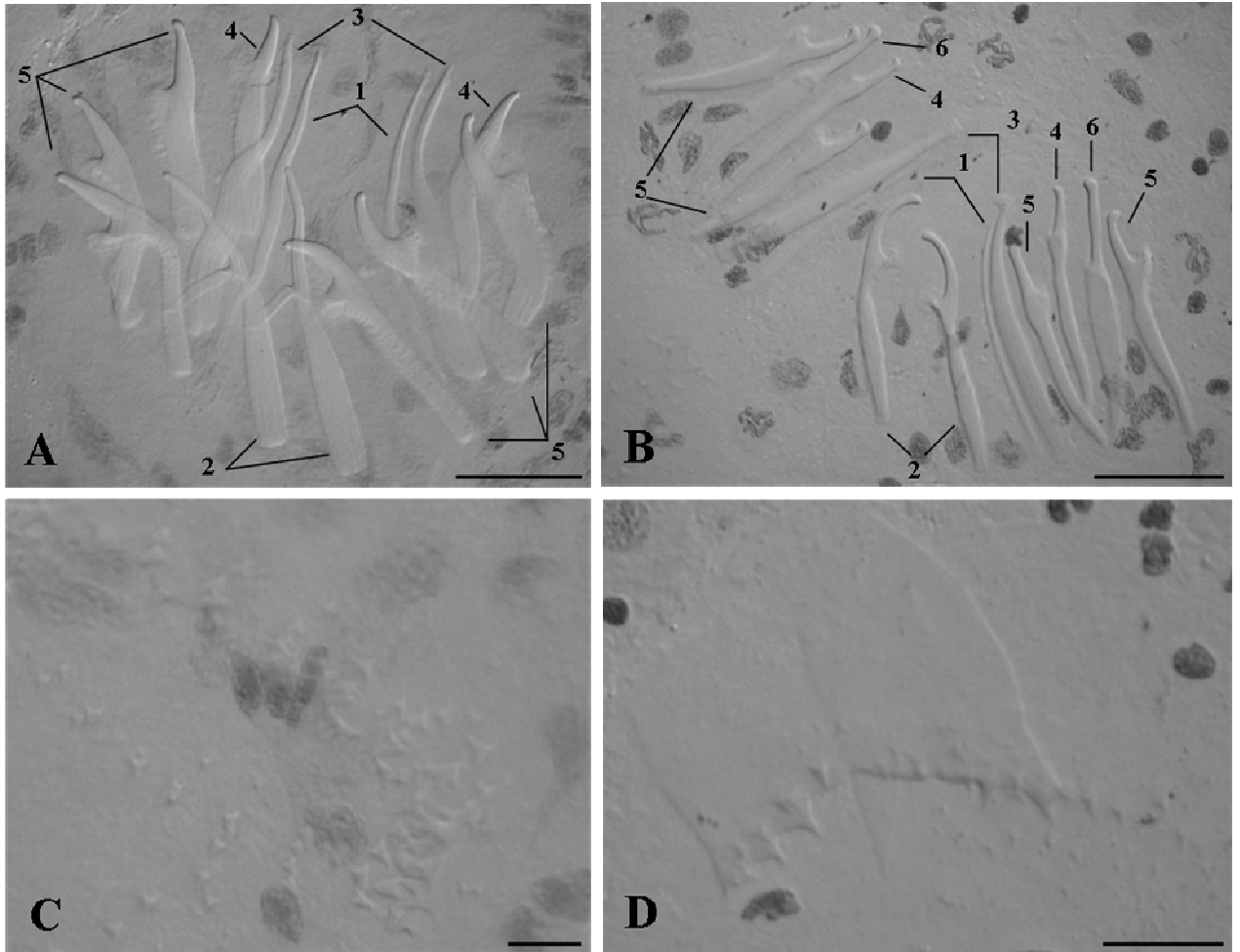


Figure 1. *Parotoplana crassispina* sp. n.: A. male sclerotized apparatus, scale 20 µm – C. bursal nozzles, scale 10 µm; *Parotoplana carthagoensis* sp. n.: B. male sclerotized apparatus, scale 20 µm – D. bursal nozzles, scale 10 µm.

***Parotoplana ichnusae* sp. n.**

(Fig. 2A)

Holotype: one karyological slide made permanent with lactophenol (SMNH 7570).

Type locality: Le Bombarde beach (Sardinia) (lat. 40°35'1.50"N, long. 8°15'38.05"E), in medium-fine sand, about 2 m deep.

Additional material: Porto Alabe (Sardinia) (lat. 40°14'46.87"N, long. 8°28'28.49"E), in intertidal medium sand. One karyological slides made permanent with lactophenol (CZM 177).

Etymology: the name refers to the latin name of Sardinia.

Description

Size, general morphology of the body and arrangement of organs, similar to the

other species of the genus. About 20 rows of rhabdoids are present dorsally. Collar-shaped pharynx in the middle of the body.

Male genital organs. Two rows of about ten testis each are located anterior to the ovaries. The male copulatory organ consist of a sacciform seminal vesicle, a prostatic vesicle and a sclerotized apparatus consisting of 16 spines. Six groups of spines, can be recognized:

Group 1: two thin, straight spines (holotype: 63 μm ; range 61-65 μm) with straight apices and blunt distal tip. Stem about 1.5 μm broad;

Group 2: two slender spines (holotype: 77-78 μm ; range 70-78 μm) with falcate apices, obtuse distal tip, and provided with well developed teeth, distally blunt. The distance between apices and teeth is about 12 μm . Stem about 4 μm broad, distally obtuse;

Group 3: two broad, straight spines (holotype 60-62 μm ; range 60-65 μm) with straight apices and blunt distal tip. Stem about 4 μm thick;

Group 4: two broad spines (holotype 60-62 μm ; range 60-65 μm) with falcate apices and small button shaped distal tip; with a slight thickening at 12 μm from the distal tip. Stem 6 μm broad;

Group 5: six broad spines (holotype 63 -79 μm ; range 63-79 μm) with falcate apices and button shaped distal tip. Very broad teeth located at about 22-25 μm from the distal tip. Stem up to 8 μm , distally obtuse;

Group 6: two broad spines (holotype 60-63 μm ; range 60-65 μm) with falcate apices with button shaped distal tip and short, broad teeth. The distance between distal tip and teeth is 19 to 21 μm . Stem about 7 μm broad, distally truncated.

Female genital organs. Two rows of about 28 vitellaria posterior to the ovaries and extending laterally : 20 anterior to the pharynx, 3 at the same level of the pharynx and about five posterior. The bursa could be observed on the living specimen before fixation. It is connected to the common atrium through a canal surrounded by a thick layer of circular muscles.

Diagnosis. Species of *Parotoplana* with the sclerotized apparatus consisting of six types of spines symmetrically arranged:

1. two thin, straight spines, 61-65 μm long;
2. two slender spines with falcate apices, blunt distal tip and teeth distally blunt, 70-78 μm long;
3. two broad, straight spines, 60-65 μm long;
4. two broad spines with falcate apices, small button shaped tip and a slight thickening, 60-65 μm long;

5. six spines with falcate apices, button shaped tip and broad teeth. Stem distally pointed, 63-79 μm long;

6. two spines with falcate apices, button shaped tip and broad teeth. Stem distally truncated, 60-65 μm long.

***Parotoplana pulchripina* sp. n.**

(Fig. 2B)

Holotype: one karyological slide made permanent with lactophenol (SMNH 7571).

Type locality: Le Bombarde beach (Sardinia) (lat. 40°35'1.50"N, long. 8°15'38.05"E), in medium-fine sand, about 2 m deep.

Additional material: One karyological slides made permanent with lactophenol (CZM 178).

Etymology: the name refers to the fine, elegant morphology of the spines (latin *pulcher* = fine).

Description

Size, general morphology of the body and arrangement of organs similar to the other species of the genus. The collar-shaped pharynx is located in the first half of the body and its epithelium is entirely ciliated with the exception of the distal tip. The internal cilia are more longer (4 μm) then the external (2 μm). Few rhabdoids are scattered both ventrally and dorsally.

Male genital organs . Two rows of few testis (about five in each row) anterior to the ovaries. The male copulatory organ consists of a seminal vesicle, a prostatic vesicle and a sclerotized apparatus. The sclerotized apparatus consist of numerous spines (22 in the holotype and 20 in the specimen CZM 158):

Group 1: two thin straight spines (Holotype: 46-49 μm ; CZM 158: 51-52 μm) with straight apices and pointed distal tip. Stem 2-3 μm broad;

Group 2: four straight spines (Holotype: 48-50 μm ; CZM 158: 50-53 μm) with pointed apices and small thin teeth. The distance between the tip and the attachment of teeth is about 13 μm . Stem about 3 μm broad;

Group 3: two broad straight spines (Holotype: 55-56 μm ; CZM 158: 51-52 μm) with straight apices and pointed distal tip. Stem 4-5 μm broad;

Group 4: two spines (Holotype: 49-51 μm ; CZM 158: 45-49 μm) with broadly sickle shaped apices and button shaped distal tip. Thin, distally pointed, subterminal teeth. Stem 2.5 μm broad;

Group 5: twelve spines (ten in specimen CZM 158) (Holotype: 39-44 μm ; CZM 158: 40-54 μm) similar in morphology to the previous, but distinctly smaller. Stem 1.5 μm broad.

Group 6: absent.

Female genital organs. Two ovaries posterior to the testis. Numerous vitellaria are arranged in two rows extending laterally posterior to the ovaries to anterior to the genital organs. A small bursa could be observed.

Diagnosis. Species of *Parotoplana* with the sclerotized apparatus consisting of five types of spines:

1. two thin straight spines with pointed apices, 46-52 μm long;
2. four straight spines with pointed apices and subterminal teeth, 48-53 μm long;
3. two broad straight spines with pointed apices, 51-56 μm long;
4. two spines with broadly sickle shaped apices distally button shaped, subterminal teeth, 45-51 μm long;
5. ten to twelve spines with thin sickle shaped apices distally button shaped, subterminal teeth, 39-54 μm long.

***Parotoplana obtusispina* sp. n.**

(Fig. 2C)

Holotype: one karyological slide made permanent with lactophenol (SMNH 7572).

Type locality: St. Cyprien beach (lat. 42°37'45.97"N, long. 3°2'11.55"E), in medium sand, about 2 m deep. August 2008.

Additional material: three karyological slides made permanent with lactophenol (CZM 179-181).

Etymology: the name refers to the blunt (latin *obtusus*) apices of the spines.

Description

Size and general morphology of the body similar to the other species of the genus.

Male genital organs. Two rows of about ten testis each are located anterior to the ovaries. In the living specimen it could be observed a sacciform seminal vesicle connected

distally with a large prostatic vesicle. The sclerotized apparatus consists of 14 spines (15 in specimen CZM 159) arranged in four different types:

Group 1: two comparatively thin straight spines (Holotype: 70-72 μm ; range: 69-73 μm) with rounded apices. Stem about 3 μm broad;

Group 2: absent;

Group 3: two broad straight spines (Holotype: 71-73 μm ; range: 70-73 μm) with slightly hooked blunt apices. Stem about 6.5 μm broad;

Group 4: two broad spines (Holotype: 64-66 μm ; range: 63-66 μm) with button shaped apices. Stem about 4.5 μm broad;

Group 5: eight thin spines (nine in CZM 159) (Holotype: 60-61 μm ; range: 51-61 μm) with button shaped apices. Stem about 3 μm broad.

Group 6: absent.

Female genital organs. Two ovaries lie behind the testis. Two rows of vitellaria extending from posterior to the ovaries to anterior to the genital organs. Observations on the living specimen showed the existence of a small bursa opening independently in the common atrium.

Diagnosis. Parotoplana species with the sclerotized apparatus consisting of: two thin straight spines with rounded apices (1), 69-73 μm long; two broad straight spines with slightly hooked apices (3), 70-73 μm long; two broad spines with button shaped apices (4), 63-66 μm long; eight or nine thin spines with button shaped apices (5), 51-61 μm long.

***Parotoplana axii* sp.n.**

(Fig 2D)

Holotype: one karyological slide made permanent with lactophenol (SMNH 7573).

Type locality: Agnone (Sicily) (lat. 37°18'38.03"N, long. 15°6'24.63"E); coarse volcanic sand; mid littoral.

Etymology: the species is dedicated to dr. prof. Peter Ax, for his longlasting contribution to the study of interstitial flatworms, and of Otoplanidae in particular.

Description

Limited observations could be performed on the only specimen found. Size, general morphology of the body and arrangement of organs, similar to the other species of the genus.

Male genital organs. Two rows of about five testis each anterior to the ovaries. The male copulatory organ consists of a sacciform seminal vesicle, a short prostatic vesicle distally connected with the sclerotized apparatus. The latter consists of 16 spines symmetrically arranged into a girdle:

Group 1: absent;

Group 2: two spines (49-51 μm) with falcate apices and pointed distal tip. Small pointed teeth at about 13 μm from the tip. Stem about 3 μm broad;

Group 3: four straight spines (50-51 μm in length) with slight curved apices and blunt distal tip. Stem about 3 μm broad;

Group 4: absent;

Group 5: 10 spines (34-45 μm in length) with sickle shaped apices and pointed distal tip. Small teeth located at 7 to 9 μm from the tip. In this group of spines it is possible to appreciate a gradient of size. Stem about 2 μm broad.

Group 6: absent.

Female genital organs. With two ovaries anterior to the pharynx and numerous vitellaria in two rows posterior to the ovaries and extending laterally from the pharynx to the genital organ. In the living specimen, a bursa filled with sperm could be observed.

Diagnosis. *Parotoplana* species with a sclerotized apparatus consisting of 16 spines belonging to three groups: two spines with pointed falcate apices and small pointed teeth (2) (49-51 μm); four straight spines with blunt slightly curved apices (3) (50-51 μm) and 10 spines with pointed sickle-shaped apices (5) (34-45 μm).

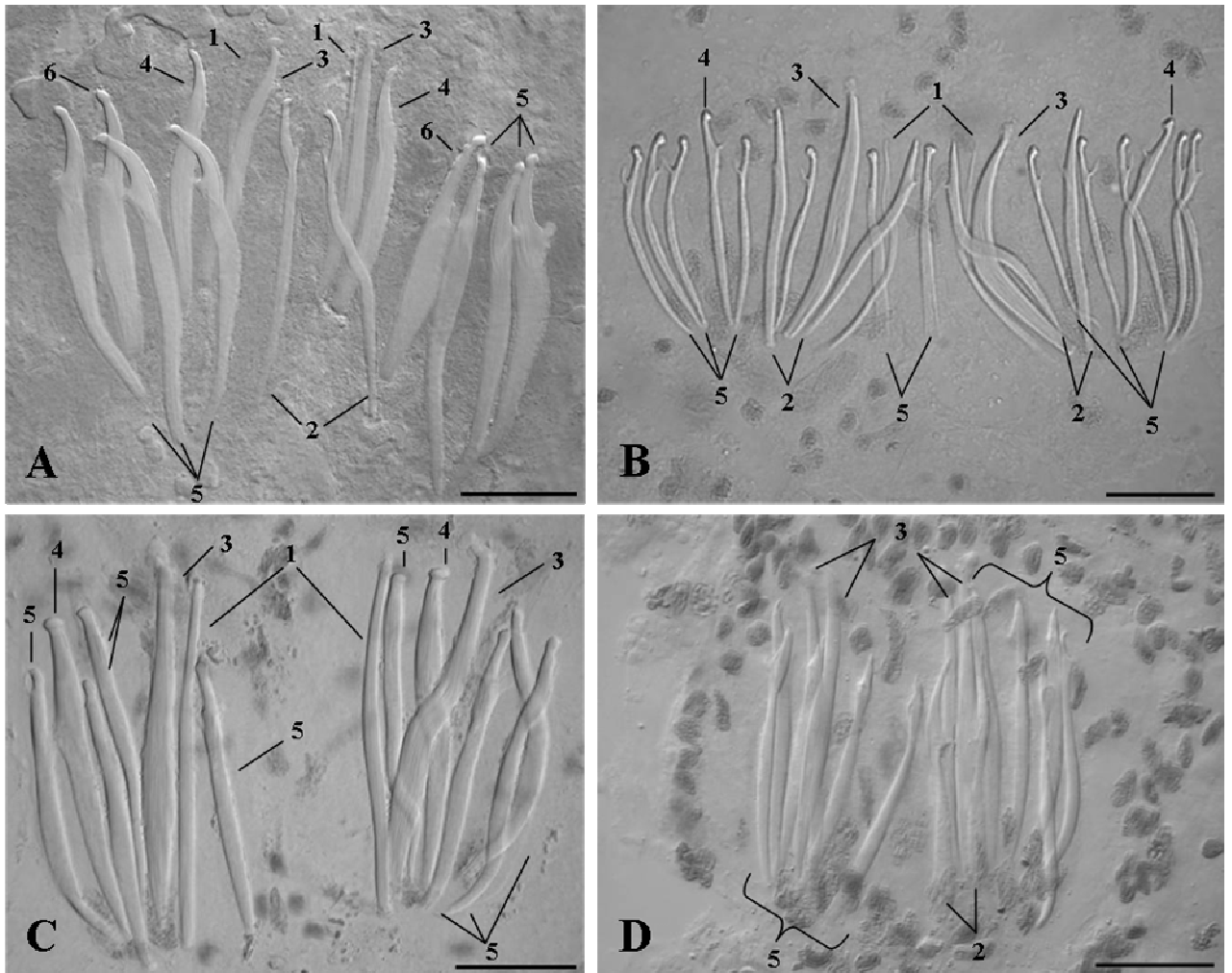


Figure 2. Male sclerotized apparatus: A. *P. ichnusae* sp. n.; B. *P. pulchripina* sp. n.; C. *P. obtusispina* sp. n.; D. *P. axii* sp. n.; scale 20 μ m.

Appendix

Key to the species

1. With bursal nozzles.....2
- Without bursal nozzles.....4
2. With six different groups of spines.....*P. carthagoensis* sp. n.
- With less than six groups of spines.....3
3. With less than 10 dorsal spines: two thin straight spines (Group 1) (38-45 μ m long), two spines with slightly falcate apices, round distal tip and large, plate teeth (Group 2) (47-60 μ m long), two broad straight spines in (Group 3) (48-60 μ m long).....*P. crassispina* sp. n.

- With more than ten dorsal spines: two thin straight spines (Group 1) (75–79 μm long), four spines with slightly recurve apices, bulbous distal tip and small pointed teeth (Group 2) (75–83 μm long),. four broad straight spines (Group 3) (70–80 μm long)*P. jondelii* Delogu & Curini-Galletti, 2007
- 4. With three groups of spines.....*P. axii* sp. n.
- With more than three groups of spines.....5
- 5. With all spines without subterminal teeth.....*P. obtusispina* sp. n.
- With spines provided of subterminal teeth.....6
- 6. With five groups of spines: three types of dorsal spines (Groups 1-2-3), and two types of ventral spines (Groups 4-5).....*P. pulchrispina* sp. n.
- With six groups of spines: three types of dorsal spines (Groups 1-2-3) and three types of ventral spines (Groups 4-5-6).....*P. ichnusae* sp. n.

Remarks

The *jondelii*-group is a morphologically homogeneous group, containing seven species which all have a male sclerotized apparatus with broad spines similarly arranged. The first species of *Parotoplana* belonging to this group, whose name derive from it, was described by Delogu & Curini-Galletti (2007, figs. 3A – B, pg. 24; fig. 7A – B, pg. 29) for Southern Apulia. The first inside discriminating character among the species of the group is the presence of bursal nozzles. Only two new species *P. carthagoensis* sp. n. and *P. crassispina* sp. n., share with *P. jondelii* Delogu & Curini-Galletti, 2007, the presence of bursal nozzles. However, *P. carthagoensis* sp. n. has Group 6 of spines which is absent both in *P. crassispina* sp. n. and *P. jondelii*, furthermore it differs to *P. jondelii* in number of spines (13 to 15 in *P. carthagoensis* sp. n. and 18 in *P. jondelii*). Group 2 in *P. carthagoensis* sp. n. consists of two spines (40-45 μm long), with falcate apices, button shaped tip, and small teeth placed at about 12 μm from the tip, while in *P. jondelii* this group is composed of four spines (75–83 μm long) with slightly recurve, bulbous apices provided with a tooth placed at the basis of the distal third of the spine. Group 3 of spines, in *P. jondelii*, is represented by four broad straight spines (range 70-80 μm in length) with pointed distal tip whereas in *P. carthagoensis* sp. n. group 3 consists of two broad straight spines (range 43-50 μm in length) with blunt distal tip. Group 5 in *P. jondelii* consists of six spines (53-55 μm) with sickle shaped apices, pointed distal tip and marked subterminal pointed teeth instead of four broad spines (37-41 μm) with

falcate apices, button shaped distal tip and broad short teeth, which are present in *P. carthagoensis* sp. n.

P. crassispina sp. n. has a similar number of spines as *P. carthagoensis* sp. n. (14 in *P. crassispina* sp. n. and 13 to 15 in *P. carthagoensis* sp. n.) and differ from *P. jondelii* whose sclerotized apparatus consists of 18 spines. Group 2 of spines in *P. crassispina* sp. n. consists of two spines (47-60 µm), the same number of spines of *P. carthagoensis* sp. n. (40-45 µm) but with different morphologies: in *P. crassispina* sp. n. they are provided with characteristic large and plate teeth (up to 20 µm in length), markedly different of that present in *P. carthagoensis* sp. n. which are very small and short. Group 5 is formed from six spines both in *P. crassispina* sp. n. and in *P. jondelii*, similar in size (53-55 µm in *P. jondelii* and 47-60 µm), but different for the morphologies of the apices which are sickle-shaped distally pointed with a small teeth in *P. jondelii*, and falcate distally button shaped with a long and broad teeth in *P. crassispina* sp. n.

Among the species lacking of bursal nozzles (*P. ichnusae* sp. n., *P. axii* sp. n., *P. obtusispina* sp. n. and *P. pulchrispina* sp. n.), *P. ichnusae* sp. n. is provided of all six groups of spines thus like *P. carthagoensis* sp. n. Spines of Group 2 in *P. ichnusae* sp. n. differ of that in *P. carthagoensis* sp. n. in size (70-78 µm in *P. ichnusae* sp. n. and 40-45 µm in *P. carthagoensis* sp. n.), and for details in the shape of apices which have an obtuse distal tip and a well developed teeth at their basis in *P. ichnusae* sp. n., and a button shaped distal tip and a small teeth in *P. carthagoensis* sp. n. Spines of Group 6 (60-65 µm long in *P. ichnusae* sp. n., 40-47 µm in *P. carthagoensis* sp. n.) have falcate, distally button-shaped, apices and broad teeth in *P. ichnusae* sp. n., while in *P. carthagoensis* sp. n., they have straight apices without any teeth.

The sclerotized apparatus of *P. pulchrispina* sp. n. shows the largest number of spines (20 to 22 spines) of the *jondelii*-group. Both *P. pulchrispina* n. sp. and *P. jondelii*, have Group 2 of spines made of four spines however they show a different morphology of the apices and different size: pointed and straight in *P. pulchrispina* sp. n., bulbous and slightly recurve in *P. jondelii*; the length of spines is 48-53 µm in *P. pulchrispina* sp. n. and 75-83 µm in *P. jondelii*. Group 6 is made of spines with sickle-shaped apices both in *P. jondelii* and *P. pulchrispina* sp. n. but in the latter, they are thinner and about double in the number.

P. obtusispina sp. n. is the only species of this group which lack of Group 2 of spines. Furthermore, none of the species of the group shows a sclerotized apparatus made of spines which all have an obtuse apices and lack of subterminal teeth as in *P. obtusispina* sp. n.

Among the *jondelii*-group species, *P. axii* sp. n. is the only which has the sclerotized apparatus made of three groups of spines (Group 2-3-5).

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Italian Summary

L'ambiente marino è stato tradizionalmente considerato un habitat a bassa diversità specifica, ma, attualmente è emersa la consapevolezza che la percezione della biodiversità marina sia inadeguata e basata su dati non rappresentativi. La scarsità di conoscenze sulla tassonomia e la distribuzione degli organismi marini è particolarmente acuta per la meiofauna interstiziale di cui mancano totalmente informazioni per intere regioni biogeografiche. La mancanza di dati sulla meiofauna interstiziale è particolarmente preoccupante se si considera che in ambiente terrestre e dulciacquicolo, il più alto valore di diversità specifica ricade nella classe dimensionale attorno ad 1-2 mm, che in ambiente marino è rappresentata principalmente da organismi della meiofauna. Recentemente sono state individuate proprio nei taxa della meiofauna marina le maggiori lacune sistematiche e biogeografiche tra tutti gli organismi della fauna italiana. Tra i gruppi di tale comparto faunistico, la cui sistematica e biogeografia sono meno conosciute, si inseriscono a pieno titolo i Rhabditophora (Platyhelminthes). I plattelminti sono importanti costituenti delle comunità meiobentoniche. La loro abbondanza è in genere correlata con la granulometria: scarsamente rappresentati in sedimenti siltosi, raggiungono densità comprese tra 7 e 25% della meiofauna totale in ambienti sabbiosi sino a raggiungere il 95% in ambienti grossolani ad alta energia. Nonostante la loro importanza nella strutturazione delle comunità meiobentoniche, le attuali informazioni sulla tassonomia e distribuzione dei Platyhelminthes sono inadeguate ed è considerato uno dei taxa, così come i Polychaeta, Oligochaeta e Nematoda, che maggiormente necessita di guide identificative e di check-list aggiornate. Recentemente è stato evidenziato come spesso i plattelminti presentino un'areale di distribuzione ristretto, rappresentato talvolta da una sola località, probabilmente dovuto alla loro taglia e all'assenza di fasi larvali dispersive. Per questa ragione essi sono uno dei gruppi in cui le modificazioni di distribuzione e composizione delle specie dovuti a fenomeni di "global change", potrebbero essere maggiormente visibili ma, la scarsità di informazioni, se non addirittura assenza, relative alla maggior parte dei taxa per intere regioni biogeografiche, ne ha impedito l'utilizzo in programmi di monitoraggio ambientale. I Proseriata (Neophora), rappresentano uno dei taxon di maggiore interesse fra i Platyhelminthes; ricco di specie particolarmente comuni in ambiente costiero dove occupano nicchie ecologiche ristrette e per questo presentano una bassa diversità α e, spesso ogni tipo di sedimento ospita un numero ristretto di specie.

In questo scenario, lo scopo di questa ricerca è stato quello di raccogliere dati sulla composizione e distribuzione di una famiglia di proseriati, gli Otoplanidae Hallez, 1892, nel Mediterraneo. Gli Otoplanidae rappresentano il taxon dominante nella fascia intertidale di

ambienti grossolani ad alta energia. Relativamente ben studiati nell'Oceano Atlantico, nel Mar del Nord, nell'Oceano pacifico, nelle isole Galápagos e in Australia, in Mediterraneo sono state oggetto di studio poche aree quali, il Golfo del Leone, il Golfo di Napoli e la costa toscana. Durante il mio Dottorato, sono state effettuate una serie di campagne di campionamento lungo le coste del Mediterraneo ed in particolare nell'AMP di Porto Cesareo e il Golfo di Catania nel mar Ionio; nell'AMP di Capo Caccia – I.Ia Piana, nel PN dell'Arcipelago di La Maddalena e le coste della Toscana nel mar Tirreno; nell' AMP Miramare – Golfo di Trieste e l'Isola di Cherso (Croazia) nel mar Adriatico; nella costa meridionale della Spagna (Mare d'Alboran); nelle coste della Tunisia sino al Golfo di Gâbes ed, infine, nel Golfo del Leone (Banyuls sur mer – Francia). Il risultato più sorprendente di queste campagne è stato l'elevato numero di specie di otoplanidi trovate, circa 80, la maggior parte delle quali nuove. A causa del grande numero di nuove specie trovate appartenenti a diversi generi, ho iniziato il mio lavoro di studio e descrizione, con le specie appartenenti al genere *Parotoplana* Meixner, 1938. Gli organismi ascrivibili al genere *Parotoplana*, sono caratterizzati dalla presenza di un faringe tipicamente a collaretto interamente ciliato ad eccezione dell'estremità distale; da un arrangiamento seriale delle gonadi (testicoli – ovari – vitellari); ovidotti pari che possono fondersi in un dotto femminile comune al livello del faringe o a ridosso dell'ingresso dell'atrio comune; presenza di una bursa copulatrice che sbocca indipendentemente nell'atrio comune tra l'apertura dell'apparato genitale maschile e di quello femminile. Quindici nuove specie sono formalmente descritte:

P. pythagorae Delogu & Curini-Galletti, 2007;

P. spathifera Delogu & Curini-Galletti, 2007;

P. terpsichore Delogu & Curini-Galletti, 2007;

P. jondelii Delogu & Curini-Galletti, 2007;

P. cucullata Delogu *et al.*, 2008;

P. fretigaditani Delogu *et al.*, 2008;

P. varispinosa Delogu *et al.*, 2008;

P. mastigophora Delogu *et al.*, 2008;

P. geminispina sp. n.,

P. crassispina sp. n.,

P. carthagoensis sp. n.,

P. ichnusae sp. n.,

P. pulchrispina sp. n.,

P. obtusispina sp. n.;

P. axii sp. n.

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