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Contribution to the knowledge of the Proseriata (Platyhelminthes: Rhabditophora) from southeast Brazil

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Abstract The composition of the taxon Proseriata (Platyhelminthes) in the São Sebastião area (S.E. Brazil) is presented and discussed. The area was extensively studied by E. Marcus, who described a large number of species, most of which have not been found thereafter. Of the 29 species found, 16 have been described by Marcus. The new findings allowed in many cases integration of the original descriptions, of particular interest in the case of the type species of genera: *Minona evelinae*, *Duplominona mica*, *Mesoda gabriellae*, *Pystrix thelura*, *Meidiama lutheri*, *Phylosirtis eumeca*, *Vannuccia martae*. Nine of the species found are new, and could be formally described: *Monocelis non-scripta* nov. sp., *Pseudomonocelis paulista* nov. sp., *Duplominona brasiliensis* nov. sp., *Archiloa polycirrus* nov. sp., *Archimonocelis marci* nov. sp., *Pseudosyrtis cebimari* nov. sp., *Parotoplanina antaliformis* nov. sp., *Monostichoplana fonsecai* nov. sp., *Nematoplana mirabilis* nov. sp. One species is tentatively identified as *Itaspis evelinae*. Most of the new species came from the few subtidal samples examined, where specimens of further undescribed species, whose material was inadequate for formal description, were also found. The diversity of S.E. Brazil Proseriata appears thus far from being exhaustively known.

Keywords Microturbellaria · Meiofauna · Marine biodiversity · Taxonomy

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Introduction

Thanks to intensive research of E. Marcus (1946, 1948, 1949, 1950, 1951, 1952, 1954a), southeast Brazil has long been the only area outside Europe where the composition of the taxon Proseriata (Platyhelminthes) was known in some detail. Marcus described 24 species of Proseriata: although extensive, Marcus's original descriptions left taxonomic problems open. The lack, in many instances, of detailed information on sclerotised structures, which are most often the key discriminating factor in Proseriata (Curini-Galletti 2001), made species identifications problematic. To make things worse, Marcus's original material, deposited in the collections of the Swedish Museum of Natural History (Stockholm), does not yield further information. Furthermore, very few of Marcus's species were found thereafter (Hooge and Rocha 2011), and the phylogenetical position of his many monotypic genera has not yet been assessed with the contribution of molecular information.

The workshop on "Taxonomy and Diversity of Marine Meiofauna, Brazil", held at CEBIMar, the Centre for Marine Biology of the University of São Paulo in São Sebastião in 2012 (28 October-9 November 2012), offered extensive sampling opportunities, and thus increased chances to find Marcus's species. Although not entirely successful, due to technical problems (see below), the sampling campaign allowed the finding of numerous species. Besides many of Marcus's species, several of the species found were new to science, improving significantly the knowledge of the taxon Proseriata in the area.

Material and methods

Sampling and morphological study

Samples were collected manually by scooping up the superficial layer of sediment. Subtidal samples were collected by

scuba-divers. Extraction of the animals from the sediment was accomplished using $MgCl_2$ decantation (Martens 1984). Each specimen was first studied alive by slight squeezing under the cover slip. For microscopical analysis, the material was fixed in Bouin's fluid, embedded in Paraplast (60ºC), serial sagittal sections were obtained at 4-μm intervals, stained with Hansen's haematoxylin and eosin-orange and mounted in Eukitt.

Unfortunately, the Bouin's fluid available did not enable a good level of fixation, leading to loss of entire vials. Even when some degree of fixation was attained, only rarely was this of a desirable level. This shortcoming affected particularly some, rare species, whose anatomy could not be understood entirely, or which could not be described at all.

Position of sclerotised structures ('spines') in the cirrus is described as observed in the inverted condition. When the cirrus is everted, what is described as proximal in the text becomes distal, and this may cause confusion.

Even when not explicitly stated, whenever possible anterior parts of the specimens studied, or whole specimens, were stored in ethanol for future molecular analysis.

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).

Karyology

The karyotype was determined from acetic orcein-stained spermatogonial mitoses, as described by Curini-Galletti et al. (1989). Relative lengths (r.l. = length of chromosome × 100/total length of haploid genome) and centromeric indices (c.i. = length of short arm × 100/length of entire chromosome) were obtained from measurements of the camera lucida drawings of metaphase plates. The chromosome nomenclature used was that of Levan et al. (1964) (*m* metacentric, *sm* submetacentric, *st* subtelocentric, *t* acrocentric). In many cases, since the acetic acid solution causes tissue maceration, karyological techniques allow more detailed observation of sclerotised pieces. Therefore, they have been employed even when there was not enough time for a colchicine treatment. After karyological study, slides, if relevant, were made permanent by lactophenol.

Abbreviations used in figures

aci accessory cirrus, *ag* adhesive glands, *ar* muscular "annular rim", *as* accessory stylet, *b* bursa, *br* brain, *cci* copulatory cirrus, *ci* chorda intestinalis, *cgp* common genital pore, *ci* cirrus, *cn* cnidae, *co* copulatory organ, *e* eye, *fd* female duct, *fp* female pore, *ga* common genital atrium, *gid* genito-intestinal duct, *gl* gut lumen, *go* "glandular organ", *lbd* left bursal duct, *ma* male atrium, *ml* muscular lining, *mp* male pore, *od* oviduct, *ov* ovary, *pg* prostatic glands, *ph* pharynx, *po* prostatoid organ, *pop* prostatoid organ pore, *pp* penis papilla, *ppb* postpenial bursa, *pd* prostatic ducts, *pv* prostatic vesicle, *rbd* right bursal duct, *rfd* resorbiens tract of the female duct, *rh* rhabdoid gland, *sd* spermiduct, *st* stylet, *sv* seminal vesicle, *t* testis, *v* vagina, *vd* vaginal duct, *vi* vitellaria

Taxonomic section

Order Proseriata Meixner, 1938

Suborder Lithophora Steinböck, 1925

Fam Monocelididae Hofsten, 1907

***Minona evelinae* Marcus, 1946**

(Figure 1A)

Type locality. S.E. Brazil, Guarujá (Santos), shelly sand (Marcus 1946).

Material examined. S.E. Brazil, São Sebastião, CEBIMar, beach in front of station, intertidal to about 1 m deep, medium/fine sand (10.28.2012): numerous specimens observed alive, one specimen frontally sectioned (CZM 538), seven specimens studied karyologically. S.E. Brazil, Ilhabela, mouth of river close to Ferryboat landing, intertidal (10.30.2012): one specimen studied karyologically. Marcus's type material (eight slides: SMNH 108235–108242).

Remarks. Being the type of the genus *Minona* Marcus, 1946, the species has a particular interest. The specimens from the type locality were described with two pigmented eye-spots; according to Marcus (1946), the pigment may be lacking unilaterally or bilaterally, resulting in totally unpigmented specimens. This latter condition was the only one ascertained in specimens from the São Sebastião–Ilhabela area. Although some variability in the intensity/morphology of the ocular pigmented shield has already been reported in Monocelididae (see Curini-Galletti and Mura 1998), its lack in entire populations is definitely unusual. However, for all other characters, the numerous specimens found corresponded to Marcus's detailed original description. The species is characteristic for its immediately post-pharyngeal bursa-vagina complex, the ovoid copulatory bulb, with a long, strongly muscular inverted penis, internally ciliated. The prostatoid organ has its own pore; it bears a stylet 30.5±3.1 μm long (N=8).

Karyotype. With haploid number n=3, and all chromosomes metacentric (Fig. 1A). Chromosome 1: r.l. = 37.51±2.02; c.i. = 47.1±1.23 (m); Chromosome 2: r.l. = 34.21±1.67; c.i. = 44.17±2.62 (m); Chromosome 3: r.l. = 28.27±0.96; c.i. = 47.37±1.93 (m) (based on nine plates).

***Minona divae* Marcus, 1951**

Type locality. S.E. Brazil, Baía de Guanabara, Ilha de Paquetá. Intertidal in medium sand with shell fragments (Marcus 1951).

Material examined. S.E. Brazil, São Sebastião, Toque Toque Grande, intertidal in medium sand (10.28.2012): one specimen observed alive and stored in ethanol for molecular studies.

Other localities. Panama, Playa Langosta (Colon) (9°28'24.58" N; 79°43'35.21" O), coarse coralline sand

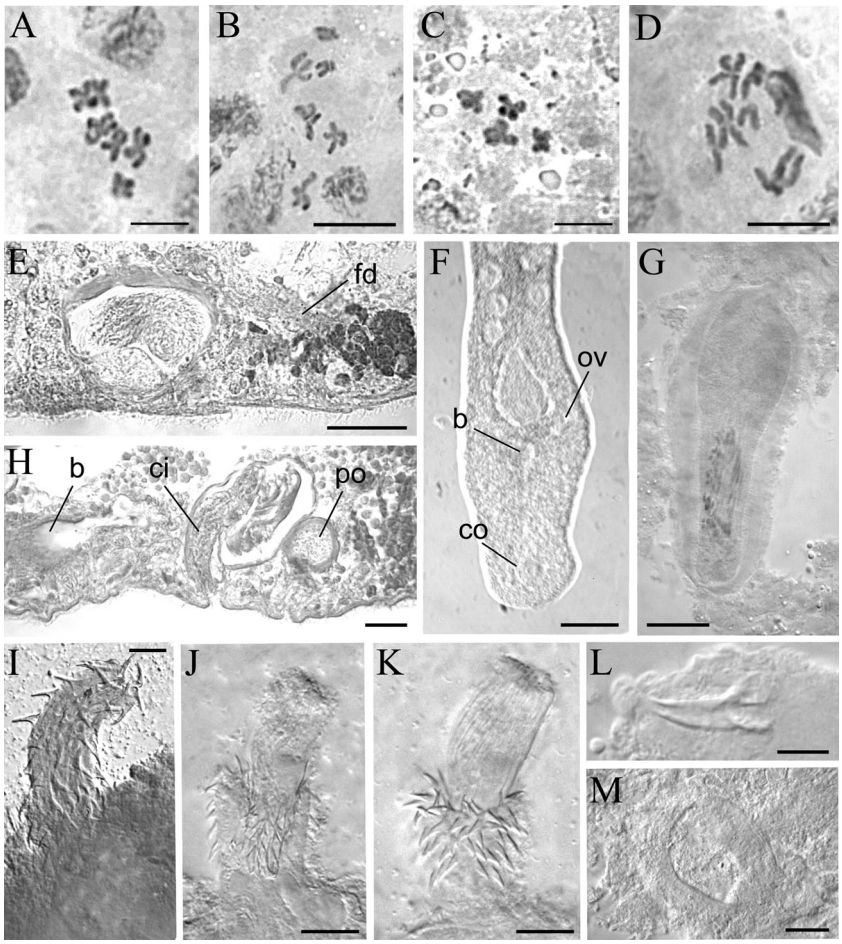

Fig. 1 **A-D**: Spermatogonial metaphase plates of *Minona evelinae* (**A**), *Monocelis tabira* (**B**), *Monocelis non-scripta* nov. sp. (**C**), *Duplominona tridens* (**D**). **E**: *Monocelis non-scripta* nov. sp.; sagittal section of genital area. **F**, **G**: *Pseudomonocelis paulista* nov. sp.; posterior body in a living specimen (*f*); copulatory organ from a karyological slide (**G**). **H**, **I**: *Duplominona mica*: sagittal section of genital area (**H**); semi-everted cirrus (**I**). **J-M**: *Duplominona brasiliensis* nov. sp.; semi-everted cirrus, at different focus (**J**, **K**); accessory prostatoid stylet (**L**); muscular 'annular rim' of vagina from whole mount (**M**). *Scale bars*: A-D; L, M=5 μm; I-K=10 μm; E, H, G=20 μm; F=100 μm

among rocks, intertidal to about 30 cm deep (10.2012): one specimen observed alive and stored in ethanol for molecular studies.

Remarks. See Marcus (1951) for extended description. The species is extremely characteristic for the presence of two vaginae, each connected to its own bursa. However, it was very rare in our samplings, with only one specimen found. The gross anatomy of the single Panamian specimen closely corresponded to Marcus's description. If confirmed by molecular data, the finding would extend the range of the species to the Caribbean.

***Monocelis tabira* Marcus, 1950**

(Figure 1B)

Type locality. S.E. Brazil, Baía de Santos (Marcus 1950).

Material examined. S.E. Brazil, São Sebastião, CEBIMar, beach in front of station, intertidal to about 1 m deep, medium/fine sand (10.28.2012): numerous specimens observed alive; five specimens sagittally sectioned (CZM 539–543), six specimens studied karyologically. São Sebastião, Araçá Bay, intertidal in medium sand (10.29.2012): numerous specimens studied alive.

Other localities. S.E. Brazil, Ponta Sta. Tereza (Ilhabela), baìa de Guanabara (Rio de Janeiro) (Marcus 1950). Bermuda, Flatts Inlet, lower intertidal, mixed medium-fine sand (7.30.1992): one specimen observed alive and studied karyologically; Bayley's Bay, lower intertidal, mixed medium-fine sand (8.01.1992): two specimens observed alive and studied karyologically. Puerto Rico, La Parguera, Magueyes Is., −10/-30 cm deep, sheltered beach among mangroves, silty medium sand (see Curini-Galletti 1991).

Remarks. The species was the only monocelidid in the São Sebastião-Ilhabela area with pigmented eye-spots, and thus immediately recognisable. The description given by Marcus (1950) is detailed, and could be confirmed by observations on the numerous specimens found during the workshop. In living specimens, however, many dot-like rhabdoids glands could be easily observed: they are explicitly reported as lacking in Marcus's (1950) original description. Dot-like rhabdoids were also present in specimens from Puerto Rico (Curini-Galletti 1991), and Bermuda.

Karyotype: with n=3 (Fig. 1B). Chromosome 1: r.l. = 39.53±2.16; c.i. = 42.35±2.23 (m); Chromosome 2: r.l. = 35.59±1.41; c.i. = 30.14±0.33 (sm); Chromosome 3: r.l. = 24.48±2.85; c.i. = 31.82±7.19 (sm) (based on four plates). Three specimens had 1–3 small, supernumerary chromosomes. Specimens from Bermuda had the first two pairs of

chromosomes more even in size, and Chromosome 3 more heterobrachial than Brazilian specimens: Chromosome 1: r.l. = 35.92±3.4; c.i. = 43.04±3.98 (m); Chromosome 2: r.l. = 34.82±2.36; c.i. = 27.32±4.28 (sm); Chromosome 3: r.l. = 29.29±1.75; c.i. = 18.66±4.03 (st) (based on six plates).

Notes. There are indications that the species may have a wide range: specimens from Puerto Rico appeared morphologically identical to Brazilian specimens, and shared the same karyotype (Curini-Galletti 1991). Bermudian specimens, although morphologically indistinguishable, showed a somewhat different karyotype, and deserve to be studied in more detail. The species was reported from the Falkland Is. by Westblad (1952), but the accuracy of the identification has been contested by Karling (1966a) and Marcus (1954b).

***Monocelis non-scripta* nov. sp**.

(Figures 1C, E and 2A, G)

Holotype. S.E. Brazil, Ilhabela, mouth of river close to Ferryboat landing, intertidal in brackish conditions (10.30.2012): one specimen sagittally sectioned (SMNH Type-8592).

Other material. Same locality, one specimen sagittally sectioned (Paratype, CZM 544), two specimens studied karyologically.

Etymology. The specific epithet refers to the lack of diagnostic features of the species (from latin *non scriptus*: unmarked).

Description. A rather large *Monocelis*: living specimens are 2–3 mm long, colourless, opaque, without pigmented eyespots (Fig. 2A). Epithelium with insunk nuclei, ciliated (cilia about 3–4 μm long) apart from the dorsal side of the caudal tip. With numerous, comma-shaped rhabdoids, slightly smaller than statolyth diameter. Posterior end with numerous adhesive glands. Subepidermal musculature poorly developed.

With a comparatively short pharynx in the second half of body. It is ciliated externally, and internally in its distal half. Oesophagus about one-third the total length of pharynx.

Male genital system. Few testes (10–12) in one irregular median row, in front of the pharynx. The copulatory organ is nearly spherical (43 μm wide, 40 μm high) (Figs. 1E and 2G). It consists of a globular seminal vesicle, surrounded by a muscular layer, with inner circular and outer longitudinal muscles, up to 5 μm thick proximally. Proximally, the seminal vesicle is lined by a thin epithelium with intra-epithelial nuclei; distally, the epithelium becomes high, and is pierced by the necks of prostate glands, whose bodies lie outside the bulb. The copulatory organ is provided with a very short, muscular penis papilla, lined internally with a thick layer of secreting epithelium. Male atrium extremely small; it opens to the outside through a narrow pore.

Female genital system. Ovaria just in front of pharynx. Vitellaria in the mid area of body. Oviducts join just behind pharynx into a female duct. This duct is swollen, lined with an irregular, high epithelium, and opens to the outside posterior to the male bulb through the female pore, surrounded by female glands (Figs. 1E and Fig. 2G). Neither in living nor in sectioned specimens a bursa-vagina complex was observed.

Karyotype. With n=2, and two metacentric chromosomes, of the same size (Fig. 1C). Chromosome 1: r.l. = 56.44±1.91; c.i. = 47.66±0.78 (m); Chromosome 2: r.l. = 43.56±1.91; c.i. = 45.65±2.27 (m) (based on six plates).

Diagnosis. Unpigmented *Monocelis* with 10–12 testes. Spherical copulatory organ (about 40 μm across), with a muscular coating up to 5 μm thick, and a poorly developed penis papilla. Without bursa or vagina; female duct irregularly swollen. Karyotype with haploid number n=2.

Remarks. The genus *Monocelis* Ehrenberg, 1831 is particularly problematic. Most of numerous species (about 30 recognised as valid, see Tyler and Artois 2013; Tyler et al. 2006–2012) of the genus, in fact, lack any sclerotised structure in their simple copulatory organ, and mostly differ for characters of the female system, or, rarely, for pigmentation. The specimens here described fit this case, lacking any obvious distinguishing feature. They were clearly different from *M. tabira* (see above), the only *Monocelis* species known so far for eastern South America. Yet, they proved of difficult interpretation. Lack of bursa and vagina were the only identification clues: their absence, in fact, is not a common feature in the genus, and it is shared with few species only. Interestingly, two of these species come from western South America, and both were described by Marcus (1954b). *Monocelis pardus* (Marcus, 1954) has two pigmented eyespots, absent in the new species, more (about 30) testes, and a somewhat differently proportioned copulatory organ (30 μm wide and 40 μm high). The species, furthermore, is brown overall due to the presence of pigment in the parenchyma. *Monocelis psilus* (Marcus, 1954) is small (less than 1 mm long), and has the musculature of the copulatory bulb extremely thick proximally: it makes nearly half the height of the copulatory bulb itself; its female duct is not swollen. The species has been described as without rhabdoids (Marcus 1954b). Although Marcus's observations on lack of rhabdoids may not be particularly reliable (see above, under *M. tabira*), the abundant, long, comma-shaped rhabdoids of the new species can hardly escape notice.

The other species lacking a vagina are *Monocelis galapagoensis* Ax & Ax, 1977, from Galapagos Is., which however has a copulatory bulb much higher than wider, a ciliated atrium, and a bursa of the resorbiens type (Ax and Ax 1977); *Monocelis pictocephala* Martens & Curini-Galletti, 1989, from Sulawesi, which has cephalic pigment and a post-penial bursa (Martens and Curini-Galletti 1989), and *Monocelis parvula* Curini-Galletti & Mura, 1998, from the western Mediterranean. This latter species is particularly similar to the new species for features of the female system and morphology of the copulatory bulb (see Curini-Galletti and Mura 1998): it differs, however, due to its reduced size,

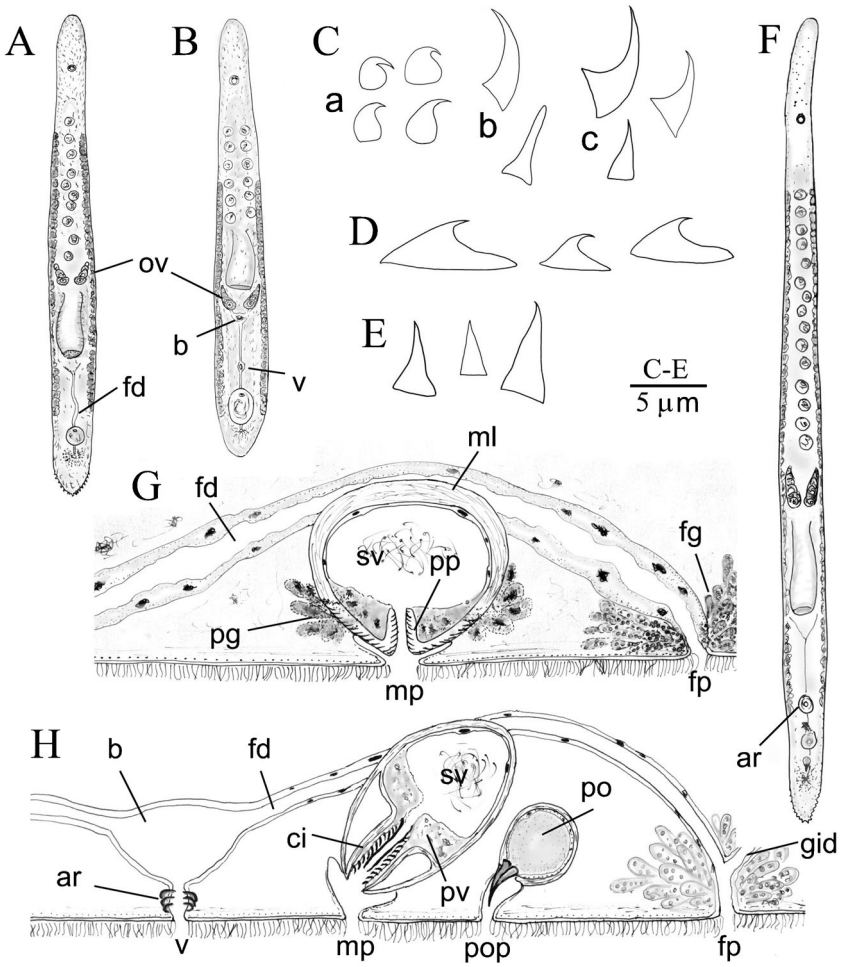

Fig. 2 **A**, **B**, **F**: General organisation of live specimens: *Monocelis non-scripta* nov. sp. (**A**); *Pseudomonocelis paulista* nov. sp. (**B**); *Duplominona brasiliensis* nov. sp. (**F**). **C**-**E**: morphology of cirrus spines of *Duplominona mica* [**C**: proximal spines (*a*); median spines (*b*); distal spines (*c*)]; *Duplominona tridens* (**D**); *Duplominona brasiliensis* nov. sp. (**E**). **G**, **H**: sagittal recostruction of the genital area of *Monocelis non-scripta* nov. sp. (**G**); *Duplominona brasiliensis* nov. sp. (**H**)

smaller than 1 mm, which affects all morphological features, from epidermal cilia, up to 2 µm long only, to the size of the copulatory bulb, which is about 25 µm wide and 20 µm high. The number of testes is also lower (up to four in a median row), and the karyotype is different: its haploid number is n=3, with a chromosome pair much larger than the others.

The Brazilian specimens, therefore, could not be accommodated into any of the described species. Furthermore, a karyotype with n=2, as found in the new species, is particularly rare in the genus *Monocelis*: so far, it is only known for *Monocelis nexilis* Curini-Galletti & Cannon, 1996, an eastern Australian species with bursa and vagina, strongly muscular distal portion of the copulatory bulb, and provided with pigmented eye-spots. The species is, therefore, here described as new.

Given the rarity of the character elsewhere, the prevalence of *Monocelis* species without external vagina, and in most cases even without a defined bursa, around South America, is remarkable, and hints at a common ancestry of these species.

***Pseudomonocelis paulista* nov. sp.**

(Figures 1F, G and 2B)

Holotype. S.E. Brazil, São Sebastião, Itaçucê, 7 m deep in shell gravel (10.30.2012): one whole mount with two specimens, one of which was chosen as holotype (SMNH Type-8595).

Other material. São Sebastião, CEBIMar, beach in front of station, about 3 m deep, medium sand with shell fragments (10.28.2012): one specimen sagittally sectioned (CZM 545), one specimen studied karyologically. São Sebastião, Guaecá (10.30.2012): one specimen observed alive, intertidal in gravelly sand. Ubatuba, Praia Vermelha, subtidal among rocks (11.06.2012): one specimen sagittally sectioned (CZM 546).

Etymology. From the nickname of the inhabitants of the state of São Paulo, where the animals occur.

Description. Very small, about 1 mm long, agile (Fig. 2B). Epithelium with insunk nuclei, cilia long 5 µm. Rhabdoids comma-shaped, longer than statolyth diameter, very numerous, so that living animals appear opaque. Anterior end with oily droplets. Posterior end squarish, with adhesive glands. Pharynx submedian, tubular. It is ciliated externally (cilia 2.5 µm long), and internally (cilia 3 µm long); oesophagus small.

Male genital system. With 10–18 testes, arranged in two more or less regular rows, in front of pharynx. Copulatory bulb placed close to the posterior end of body, elongate, lined with a muscular sheath 2–4 µm across proximally, and 10 µm

across distally. With a long inverted penis papilla. In living, lightly squeezed specimens, the bulb is about 60–70 μm in its longest axis; in hardly squeezed specimens, with everted penis papillae, the bulb attained a length of 100–110 μm, with a maximum diameter of 43 μm (Fig. 1G).

Female genital system. Vitellaria lateral to testes, running posteriorly to the level of the copulatory bulb. Position of ovaries varied in the living specimens observed: in three specimens the ovaria were immediately postpharyngeal (Fig. 1F), two specimens had them lateral to pharynx, and in one specimen they were just anterior to the pharynx. The two oviducts join into a small, immediately postpharyngeal bursa, from which the female duct originates. It runs above the copulatory bulb to open into a female pore, placed near to the caudal end. A vagina has been observed in front of the male pore (Fig. 2B).

Karyotype. With n=3: Chromosome 1: r.l. = 39.33; c.i. = 44.64 (m); Chromosome 2: r.l. = 31.8; c.i. = 23.75 (st); Chromosome 3: r.l. = 28.86; c.i. = 34.79 (sm) (based on one plate).

Diagnosis. Unpigmented *Pseudomonocelis*, about 1 mm long, with 10–18 testes. With very elongate copulatory bulb, lined with a thick muscular coating, and with eversible penis papilla. Ovaria postpharyngeal (may vary position); with bursa and vagina. Karyotype with haploid number $n=3$.

Remarks. Only a few specimens of this species were found, and observations were limited—particularly on sections, which were of poor quality. The position of ovaries, which, to some extent, varied among specimens observed alive, was puzzling, and, to my experience, quite unusual. However, in most specimens they lied posterior-lateral to the pharynx. The postpharyngeal position of the ovaries is the key diagnostic feature of the genus *Pseudomonocelis* Meixner 1943. The genus includes nine species, mostly from the Atlanto-Mediterranean region, with a few known from the Indo-Pacific, and one from Belize (Casu et al. 2011). This latter species, *Pseudomonocelis caribbea* Curini-Galletti & Casu, 2005, is particularly similar to the new species for general morphology and size of the copulatory bulb, and for morphology of the female system. However, the Caribbean species is larger, has more numerous testes (30–40), has pigmented eyespots, and appears opaque due to the presence of densely packed rhabdoids and numerous subepidermal eosinophylous glands, which are lacking in the new species. Furthermore, the muscular lining of the bulb is much weaker proximally, attaining 1 μm at most. The karyotype is also different: in *P. caribbea*, Chromosome 2 is metacentric and nearly of the same size of Chromosome 1; it is subtelocentric and appreciably smaller than Chromosome 1 in the new species.

The other species of *Pseudomonocelis* have an accessory organ (provided or not with a stylet (*P. hoplites* Curini-Galletti, 1997, *P. cavernicola* Schockaert & Martens 1987; *P. paupercula* Curini-Galletti, Casu & Lai, 2011), double vaginae (*P. ophiocephala* (Schmidt, 1861) complex, see Casu et al. 2009), pigmented cephalic region, without vagina (*P. cetinae* Meixner, 1943, *P. agilis* (Schultze, 1851) or with a complex bursa-vaginal system (*P. pardii* Schockaert & Martens, 1987, *P. schockaerti* Curini-Galletti & Cannon, 1995) (Casu et al. 2011). Furthermore, none of the *Pseudomonocelis* species described so far is as tiny, and agile, as the new species. It is therefore apparent that the specimens belong to a new species: the biogeographical interest of this finding (it is the first species of the genus *Pseudomonocelis* known from South America) stimulated to its description, albeit poor was the material available.

***Duplominona mica* (Marcus, 1951)**

(Figures 1H, I and 2C)

Type locality. S.E. Brazil, "Ilha de São Sebastião" (Ilhabela), in fine and in coarse sand, at a depth of 3–5 m (Marcus 1951).

Material examined. S.E. Brazil, São Sebastião, Araçá Bay, intertidal in medium sand (10.29.2012): four specimens sagittally sectioned (CZM 547–550), two specimens studied karyologically; Ilhabela, Parcel de Julião, 5 m deep, medium sand (10.31.2012): two specimens sagittally sectioned (CZM 551–552), one whole mount (CZM 553), three specimens studied karyologically.

Remarks. Another species of particular interest, as it is the type of the genus *Duplominona* Karling, 1966. Marcus (1951) merely described the cirrus lumen as 'narrowed by strong cuticular aculei'. His figure (Marcus *op.cit*., p. 119) shows a *Duplominona* with a long cirrus, almost as long as the whole copulatory bulb, provided with numerous sclerotised spines. Among the numerous species of *Duplominona* found at São Sebastião (see below), only one matched this description (cf Fig. 1H).

The specimens found had a cirrus 90–130 μm long, 10–12 μm wide, bearing 15–20 spirally arranged rows of spines (Fig. 1I). Morphology and size of these spines change gradually along the length of the cirrus. Proximally, spines are small, up to 2.5 μm high, and broad (up to 3 μm wide at their basis), with a long, pointed, downturned distal tip. Progressively, they become larger (about 3 μm high and 3.5 μm wide), with a shorter, recurve apex (Fig. 2C *a*). At about half the length of the cirrus, spines are even larger (5–6 μm high; 4–5 μm wide), distinctly recurved and acutely pointed. More distally, the spines become straight and narrower (up to 2 μm wide at their basis) (Fig. 2C *b*). At the top of the inverted cirrus, spines are slightly recurved, 4–7.5 μm long, 2.5–3.5 μm wide (Fig. 2C *c*). Accessory stylet small, about 15–18 μm long.

***Duplominona tridens* (Marcus, 1954)**

(Figures 1D, 2D and 3A)

Type locality. S.E. Brazil, "Ilha de São Sebastião" (Ilhabela), in coarse sand with algal debris (Marcus 1954a, b).

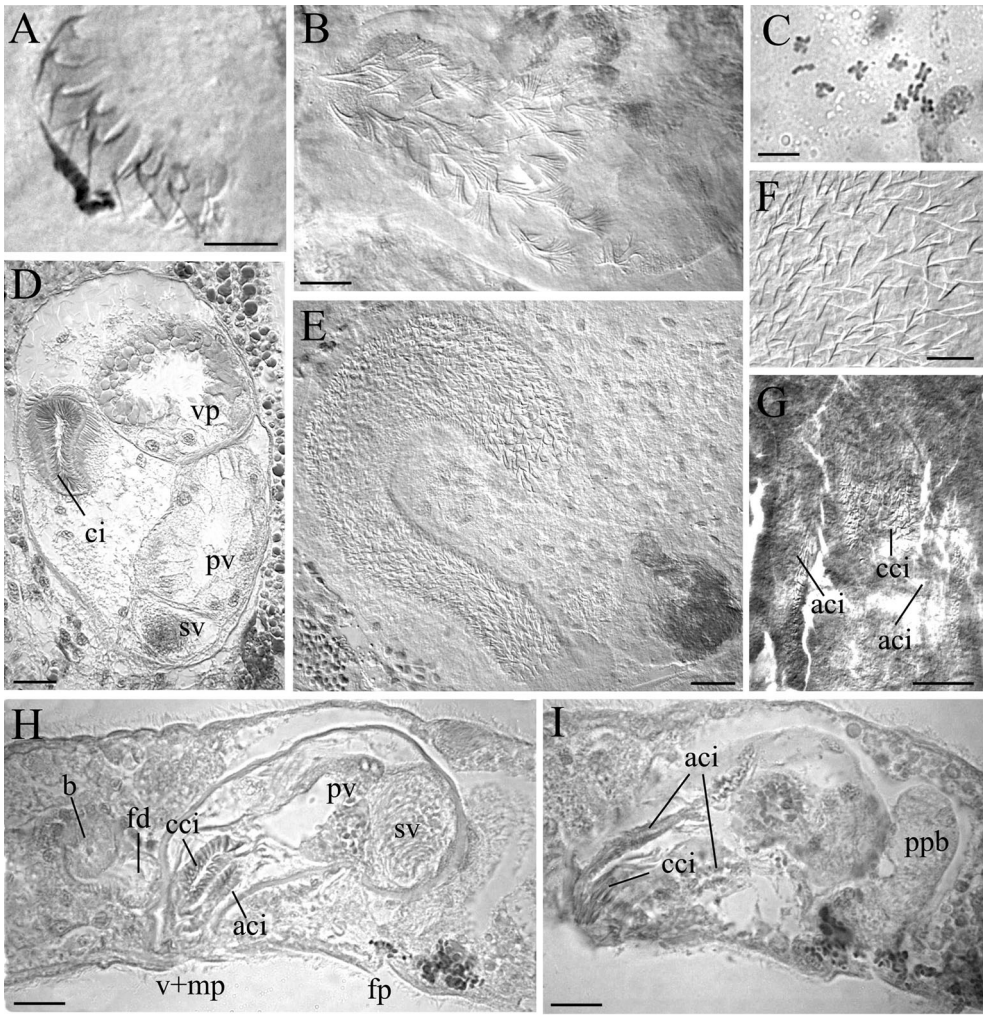

Fig. 3 **A**: *Duplominona tridens*; cirrus spines. **B**, **C**: *Mesoda gabriellae*; cirrus spines (**B**); spermatogonial metaphase plate (**C**). **D-F**: *Inaloa scalopura*; sagittal section of copulatory organ (**D**); cirrus (**E**); proximal spines (**F**). **G-I**: *Archiloa polycirrus* nov. sp.; cirri in a whole mount (**G**); sagittal sections of genital area, at different levels (**H**, **I**). *Scale bars*: A, C=5 μm; B, F, D=10 μm; E, G-I=20 μm

Material examined. S.E. Brazil, São Sebastião, CEBIMar, beach in front of station, about 3 m deep, medium sand with shell fragments (10.28.2012): several specimens observed alive, five studied karyologically, one whole mount (CZM-554). São Sebastião, Itaçucê , 7 m deep in shell gravel (10.30.2012): one specimen sagittally sectioned (CZM 555), several specimens observed alive. Marcus's type material (three slides: SMNH 108244–108246).

Remarks. One of the most distinctive proseriates of São Sebastião, immediately recognisable for its caudal region, split into three even-sized 'toes', provided with numerous adhesive glands. The original description of Marcus (1954a) was somehow puzzling, as the species was described as with a 'naked' cirrus, devoid of spines. To my knowledge, a cirrus of the duplex type entirely without sclerotised structures is unique among Monocelididae. However, although not reported in the description, Marcus's figure (1954a) hints at the presence of a few small spines at the very tip of cirrus. The specimens found during the workshop showed consistently a girdle of small spines topping the cirrus, and the original description of the species may thus be integrated with the new observations.

The copulatory bulb is small, globular, 35 μm high. With few spines, about 12, arranged in a single row apically, different in shape and size (Figs. 2D and 3A). Five to six of these spines are large, 2.3-3 μm high, with a characteristically broad basis, up to 7.5 μm wide, with a pointed, slightly recurve distal tip. A few spines are proportionally taller, with a narrower basis (4.5-5 μm). The rest of the spines are much smaller, from 2.5 μm high and 4 μm broad, similar in shape to the previous, to triangular, 1.5 μm high and 1.5 μm wide, with only a slightly recurve apex. With a large, ovoid prostatic bulb, 75 μm across, with a straight stylet, narrow, pointed, 25 μm long sclerotised, with a small (about 5 μm across) basis.

Karyotype with n=3 and basic for the genus *Duplominona* (see Martens et al. 1989): Chromosome 1: r.l. = 50.72±0.97; c.i. = 45.07±1.74 (m); Chromosome 2: r.l. = 33.43±1.78; c.i. = 47.50±0.26 (m); Chromosome 3: r.l. = 15.40±1.23; c.i. = 7.15±2.32 (t) (based on five plates) (Fig. 1D).

Notes. Specimens of the genus *Duplominona* with three 'toes', identical in their external morphology to *D. tridens*, have been found both in the Caribbean and in the Pacific coast of Panama. However, they clearly differ from Brazilian specimens for the presence of many more spines, differently shaped, in the cirrus (own unpubl. data). It is therefore open to further, molecular investigation if they constitute a monophyletic group within the genus *Duplominona*, or the splitting of the caudal region is an independently evolved adaptation. It is, however, peculiar that this morphological feature has been so far only found in the American continent (pers. obs.)

***Duplominona brasiliensis* nov. sp**.

(Figures 1J-M and 2E, F, H)

Holotype: S.E. Brazil, Ilhabela, Fome beach, 12 m deep, medium-coarse sand (11.08.2012): one specimen sagittally sectioned (SMNH Type-8593).

Other material: same locality, one whole mount (Paratype, CZM 556).

Etymology: the specific epithet refers to the geographic area of finding.

Description. A very elongate, filiform *Duplominona*, about 4 mm long (Fig. 2F). Unpigmented; with small rhabdoids, irregular in shape, and oily droplets in front of the statocyst. Epithelium with insunk nuclei, ciliated (cilia about 3 μm long). Subepithelial muscle layers thin. Pharynx long, tubular, in the posterior third of body. It is ciliated externally, and internally in its distal three-quarters, with inner cilia 5 μm long and outer cilia 2 μm long. Oesophagus very small, less than 1/15th of the length of pharynx. Pharynx musculature reversed compared with the rest of the body.

Male genital system. With about 15 testes in a single row. Copulatory organ of the duplex type, with a small, ovoid bulb. With a small seminal vesicle separated by a cellular diaphragm from a prostatic vesicle. Cirrus about 65 μm long, with only the distal two-thirds spiny, and provided with an inner, tubular stylet (Fig. 1J, K). Spines arranged in about 25 rows. The most proximal spines are 4 μm long, straight, with an oblique basis 2 μm broad. They become smaller (to about 2 μm) and straighter further up along the cirrus. Distal spines larger, triangular, 1.5-2.5 μm broad at basis, and 4.5-5 μm long, pointed and slightly recurved at the top (Fig. 2E). The stylet is a poorly sclerotised tube, 35 μm long, 12 μm wide, with distinct longitudinal furrows. It is brought internally to the cirrus in the inverted condition: when the cirrus is fully everted, it tops the cirrus distally (Fig. 2J, K).

Prostatoid organ with an ovoid bulb, 25 μm in its maximum diameter, with a slender stylet 16 μm long, of which only 4 μm pertain to the basis (Fig. 1L). The prostatoid organ opens to the outside with its own pore, posterior to the male pore.

Female genital system. Ovaries very close to each other in front of pharynx. Vitellaria seen as thin lateral lines, in the midbody. The oviducts fuse into a female duct just behind the pharynx. In front of the copulatory organ, the female duct widens to form a small bursa, 10–25 μm across. This bursa is connected ventrally to a short and wide vaginal duct, which opens into an external vagina. The vaginal duct is surrounded by a very strong muscular anular rim, 3–5 μm thick, presumably acting as a sphincter (Fig. 1M). The female duct opens exteriorly into a female pore, surrounded with female glands, posterior to the prostatoid organ pore. A genito-intestinal duct is present (Fig. 2H).

Diagnosis. Unpigmented filiform *Duplominona* with about 15 testes in a single row and four distinct genital pores. Ovoid copulatory organ with a cirrus provided with spines in its distal two-thirds, with about 25 rows of triangular spines varying from 2 to 5 μm long along the cirrus length, and with a poorly sclerotised, furrowed stylet, about 35 μm long. With a slender accessory stylet, about 16 μm long. With a small bursa; vaginal duct short and broad, surrounded by a thick muscular rim. With genito-intestinal duct.

Remarks. With 24 species described, the genus *Duplominona* is very large for Proseriate standards (see Tyler and Artois 2013; Tyler et al. 2006–2012). Among these, species provided with a tubular copulatory stylet are only eight, and most of them come from the Pacific Ocean. Two of these species come from south Sulawesi: *D. samalonae* Martens & Curini-Galletti, 1989, has a very narrow stylet about 100 μm long; *D. axi* Martens & Curini-Galletti, 1989 lacks spines, and the prostatoid stylet opens into the male pore; furthermore it lacks a vagina (Martens and Curini-Galletti 1989). *D. stilifera* Sopott-Ehlers & Ax, 1985 from Washington (USA) has an extremely narrow, recurved stylet, over 60 μm long, and few spines, arranged in about three rows. Species of the *D. galapagoensis* group (which includes four species from Galapagos Is.: *D. galapagoensis* Ax & Ax, 1977; *D. karlingi* Ax & Ax, 1977, *D. krameri* Ax & Ax, 1977, and *D. sieversi* Ax & Ax, 1977) share a unique combination of characters: the simultaneous presence of combined male and vaginal pore and of combined female and prostatoid pore (Sopott-Ehlers and Ax 1985; Ax and Ax 1977).

The new species is most similar to the only Atlantic species of this group: *D. septentrionalis* Martens 1983. This species, however, has a shorter stylet, 25 μm long; fewer (4–6) rows of longer (ranging 6–8 μm) and more slender (diameter of basis 2 μm) spines than the new species (Martens 1983).

Notes. At least two other *Duplominona* species have been found. In both cases, material was inadequate for a formal description.

***Duplominona* sp. 1**

Material examined. S.E. Brazil, São Sebastião, Itaçucê, 7 m deep in shell gravel (10.30.2012): one specimen studied karyologically; São Sebastião, CEBIMAR, beach in front of station, about 3 deep, medium sand with shell fragments (10.28.2012): one specimen studied karyologically.

Description. A small, unpigmented species. With about 20 testes. With a small cirrus, only 17 μm long, provided with 5–6 rows of closely packed spines. Spines are fine and slender, longer distally (to about 4 μm) than proximally (about 2.5 μm). Prostatoid organ with a bulb about 40 μm in diameter, and a stylet about 19 μm long. It opens to the outside through its own pore. With an external vagina.

***Duplominona* sp. 2**

Material examined. S.E. Brazil, Ilhabela, Fome beach, 12 m deep, medium-coarse sand (11.08.2012): one specimen damaged during observation.

Description. A very large, thick *Duplominona*, quite unlike any found in the area. With a comparatively small cirrus. Prostatoid organ with a very long stylet (over 25 μm long), forwards oriented.

***Archiloa polycirrus* nov. sp**.

(Figures 3G-I and 4A, I)

Holotype. S.E. Brazil, Ilhabela, Fome beach, 12 m deep, medium-coarse sand (11.08.2012): one specimen sagittally sectioned (SMNH Type-8594).

Other material. Same locality, two whole mounts (Paratypes: CZM 557–558), one specimen studied karyologically, made permanent with lactophenol (CZM 559).

Etymology: the specific epithet, a noun used in apposition, refers to the presence of several (from the Greek, 'polùs') cirri in the copulatory organ of the species.

Description. A small, slender species: living animals about 1.5 mm (Fig. 4A). Unpigmented. Epithelium ciliated (cilia about 2–3 μm long), apart from a small dorso-caudal area. Epithelium very thin (about 1 μm thick), overlying an evident basal lamina. Nuclei, given the extreme thinness of the epithelium, presumably insunk overall. However, the presence of a few, more densely stained areas within the epithelium, may hint at the presence of at least some scattered intraepithelial nuclei (see also species below). With few large clusters of rhabdoids, about 20 μm long, particularly numerous caudally. Subepidermal musculature consisting of a thin layer of circular musculature, and an almost equally thin layer of longitudinal fibres, seen as a thin line parallel to the basal lamina in sagittal sections. Pharynx comparatively short, somewhat bell-shaped, at the beginning of the posterior half of body.

Male genital system. With 8–10 testes arranged in two irregular rows in the anterior part of body. Copulatory organ of the duplex type, about 85 μm long and 30 μm high, entirely surrounded by an outer muscular sheath (Fig. 4I). The seminal vesicle occupies the most proximal region of the bulb. It is lined by a thin epithelium abutting the outer muscular sheath of the bulb. It is separated from the ectal prostatic vesicle by an incomplete cellular diaphragm. The somewhat spherical prostatic vesicle is lined by a muscular sheath, clearly separated from the outer sheath of the bulb. The secretion of the prostatic vesicle appears to be of two different types, with coarser and finer granules respectively—in at least one section, these appear to be drained into the ejaculatory duct by two channels. The long ejaculatory duct is surrounded by the continuation of the (inner) muscular sheath surrounding the prostatic vesicle, and, in the inverted condition, is provided internally with numerous spines. The spiny portion of the cirrus is about 60 μm long. It consists of about 30 rows of densely arranged spines (about 15 per row). In the inverted cirrus, the most proximal spines are broadly triangular, 4 μm high and 4 μm wide at basis. The spines become slightly larger and more slender distally: around the middle of the cirrus they are about 5 μm high, 3 μm wide at the basis, and slightly curved distally; the distalmost spines are large, triangular: 5–6 μm long, 2–3 μm broad at basis.

In addition to this (copulatory) cirrus, two accessory cirri are present, arranged symmetrically above and below the copulatory cirrus (Figs. 3G-I and 4I). The ventral accessory cirrus is about 70 μm long, similar in morphology to the copulatory cirrus; its spines are arranged in about 15 rows. They are triangular, 2–3 μm high and 2 μm wide, with a recurved distal tip. The most proximal spines of this cirrus progressively decrease in size to about 0.5 μm, till they disappear. The dorsal accessory cirrus is much narrower, up to 3 μm in section. It is provided with numerous spines. Distally, they start as a line of very small triangular spines, about 0.5 μm long. They become larger proximally, and, in the middle part, there are about 20 acutely triangular spines about 3 μm long. At the proximal end of this cirrus, they progressively decrease in size, till they are again about 0.5 μm or less and progressively vanish.

These two accessory cirri enter proximally into the prostatic vesicle. Distally, they run parallel to the copulatory cirrus, and open with the latter into the atrium. Around the opening of these cirri, the outer sheath of the bulb forms a strong muscular rim of circular fibres.

Female genital system. With two ovaries immediately behind the testes, and at some distance in front of pharynx. Vitellaria entirely posterior to ovaries: part in front (about 3–4 follicles per side) and a much larger part behind the pharynx. Posterior to the pharynx, the female duct appears entirely consisting of a thick, vacular, resorbiens tissue, entirely occupying the duct itself (Fig. 4I). These vacuoles become progressively larger caudally, and contain sperm at various degrees of degeneration. Just in front of the copulatory organ, an almost spherical structure is present, about 40 μm across with a strong muscular coating, 4–5 μm thick (Fig. 3H). Internally, it is lined with pseudociliated epithelium. Given the position, this spherical structure is interpreted as a bursa. However, no connections could be seen anteriorly with the resorbiens female duct. A vaginal duct can be seen just below the bursa; however, no connections with it could be traced: rather, it seems connected with the posteriormost portion of the resorbiens female duct. The vagina (interna) joins the outlet of the copulatory organ into a small atrium, which opens to the outside through a single genital pore. At its posterior end, the

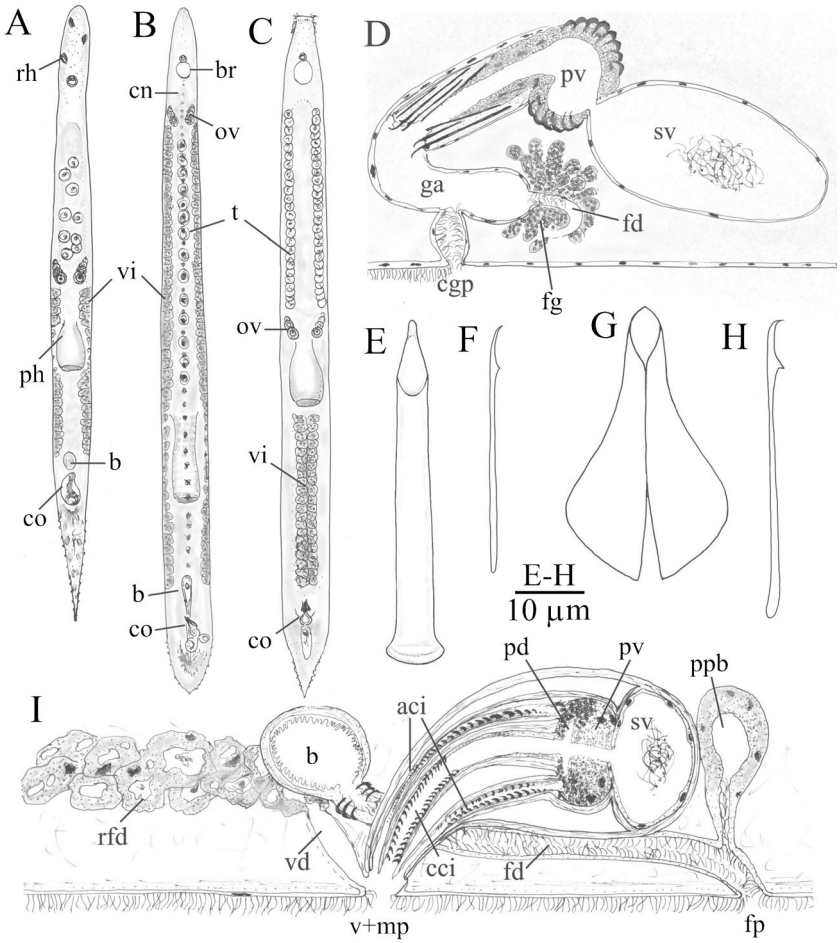

Fig. 4 General organisation of live specimens: *Archiloa polycirrus* nov. sp. (**A**); *Archimonocelis marci* nov. sp. (**B**); *Pseudosyrtis cebimari* nov. sp. (**C**). **D**: *Pseudosyrtis cebimari* nov. sp.; sagittal recostruction of the genital area . **E**, **F**: *Archimonocelis marci* nov. sp.; stylet (**E**); spine of girdle (**F**). **G**, **H**: *Pseudosyrtis cebimari* nov. sp.; stylet (**G**); spine of girdle (**H**). **I**: *Archiloa polycirrus* nov. sp.; sagittal recostruction of the genital area

spherical bursa is connected to a wide, ciliated 'posterior' female duct, which runs parallel and laterally to the copulatory organ, and opens posteriorly into a female pore surrounded by female glands. No clear, direct connection could be traced between the 'anterior', vacuolar female duct, and this 'posterior' female duct. Distally, the posterior female duct is connected to a post-penial bursa (Fig. 3I). The bursal duct is ciliated, and leads to an ovoid bursa, lined with an unciliated epithelium which, given its intense staining, is presumably glandular.

Kayotype. With n=4, and one large metacentric, two medium-sized (one metacentric, one acrocentric), and one smaller, acrocentric chromosome. One plate was measurable: Chromosome 1: r.l. = 34.77; c.i. = 47.44 (m); Chromosome 2: r.l. = 27.18; c.i. = 14.02 (st); Chromosome 3: r.l. = 23.39; c.i. = 43.24 (m); Chromosome 4: r.l. = 14.67; c.i. = 5.17 (t).

Diagnosis. Unpigmented *Archiloa*-like species with vitellaria posterior to ovaria, and mostly behind the pharynx. Copulatory organ with a copulatory spiny cirrus and two accessory spiny cirri. With a long, vacuolar 'anterior' female duct, and a small prepenial bursa, connected to a 'posterior' female duct. With a vagina interna. Vaginal duct connected to the 'anterior' female duct. With a postpenial bursa, connected to the female pore. Karyotype with n=4.

Remarks. The species appears particularly puzzling, partly for its outstanding autapomorphic character (no species of Monocelididae is known to present three cirri), and partly for the difficulty to reconstruct its internal anatomy on a single sectioned specimen, which, although of decent quality, did not yield all the desirable information. Details of the female system are indeed not exactly clear, and some connections may have been missed, nor has the position of the epithelial nuclei been assessed with certainty.

The species clearly belongs to the *Archiloa* group, which includes several genera whose monophyletism is uncertain (see Martens and Curini-Galletti 1994, for a thorough discussion and genus diagnoses).

The species shares some characters with the three European species of the genus *Archiloa* De Beauchamp, 1910 (see Ax (2008) for the possible synonimy of *A. rivularis* De Beauchamp, 1910 and *A. westbladi* Ax, 1954, which should be carefully evaluated, given the differences in their karyotypes (Curini-Galletti and Martens, unpubl. data):

1. The presence of an accessory cirrus
2. A combined male and vagina genital pore
3. A bursa not connected anteriorly with the female duct

4. Prostatic and seminal vesicles not filling out the bulb
5. A karyotype with $n=4$

However, in the new species the accessory cirri are more than one, the prepenial bursa is connected posteriorly to a female duct, and a postpenial bursa is present.

The new species also shares character with four, north European species of *Archilopsis* Meixner, 1938:

1. Prostatic glands discharging into the ejaculatory duct via two prostatic ducts
2. Presence of a postpenial bursa
3. Characters shared also with *Archiloa*: vagina interna, prostatic vesicle not filling the bulb

However, in the genus *Archilopsis* the vagina interna is connected postpenially with the female duct; species have chromosome number $n=5$, with mostly heterobrachial chromosomes, and no accessory cirrus is present. It is also fair to say that the 'prostatic ducts' in the new species could be noticed in one section only, and their presence needs to be confirmed on more material.

The two species of *Tajikina* Martens & Curini-Galletti, 1994 from Japan are somehow comparable with the new species for the presence of vagina interna, and postpenial bursa: however, their vaginal duct is very long, the prepenial bursa is interpolated among the female duct, and their haploid chromosome number is $n=2$. Species of the genus *Inaloa* Martens & Curini-Galletti, 1994 (two European and one Brazilian species, but see below) have separated male and vaginal pores, an extremely long cirrus and a long vaginal duct; bursa is prepenial and interpolated; haploid chromosome number is $n=4$. The numerous species of *Archilina* Ax, 1959 are similar to species of *Inaloa*, but have much shorter cirrus and vaginal duct.

The arrangement of testes, ovaria and vitellaria (mostly postpharyngeal) observed in the new species is identical to the two, northern European species of the genus *Monocelopsis* Ax, 1951. These species have a resorbiens female duct connected to a prepenial bursa, and a small postpenial bursa. However, in the two species the vagina opens independently from the male pore, the prepenial bursa is interpolated among the female duct, the prostatic and seminal vesicles fill out the bulb completely, no accessory cirri are present, and their karyotype has a haploid number of $n=5$, with most chromosomes metacentric. To complete the information, see also the discussion of the genera *Mesoda* Marcus, 1949 and *Pistrix* Marcus, 1951, below.

The generic placement of the new species appears therefore problematic, and the erection of a new genus to accommodate it might be justified. However, there has not been any serious attempt so far to evaluate the monophyly of the genera in the *Archiloa* complex. In addition, the examination of the single sectioned specimen left some uncertainties on the actual morphology of the female system. Therefore, although not entirely fitting the diagnosis, the species is, at least provisionally, attributed to the genus *Archiloa*, the oldest of all them, lest to add further confusion with the erection of a new genus.

***Mesoda gabriellae* Marcus** 1949

(Figure 3B, C)

Type locality. S.E. Brazil, "Ilha de São Sebastião" (Ilhabela), in fine sand (Marcus 1949).

Material examined. S.E. Brazil, Ilhabela, Perequê, sand bar, medium-fine sand (10.30.2012): one specimen sagittally sectioned (CZM 560); three specimens studied karyologically. Marcus's type material (ten slides: SMNH 108807–108816).

Remarks. Boreocelis-like in external appearance. The most peculiar trait of its morphology is the presence of a wholly intranucleated epithelium, as opposed to the vast majority of the Monocelididae, where it is infranucleated (with nuclei sunk below the basal lamina). The specimen sectioned was not of the best quality, and it shows a situation somehow comparable to the previous species, with very thin epithelium, and a few elliptic areas more heavily pigmented, which may represent some widely scattered intraepithelial nuclei. In Marcus's type material, there are four slides with specimens sectioned. Each slide contains the sections of very many specimens, all mixed together, and it is frustrating to try to follow a single specimen even in contiguous sections. In some cases, it is obvious that mistakes in identification have happened: among the specimens sectioned there are even members of the Otoplanidae, with unciliated dorsum, and dot-like rhabdoids. Some specimens have clearly intranucleated epithelium, but appear immature, and cilia length is far too long to pertain to *M. gabriellae*. Some specimens, however, belong clearly to the intended species. The quality of fixation and staining is indeed very good; however, the problem of the position of epithelial nuclei is not completely solved. In fact, in some of the specimens the epithelium is clearly intranucleated. In others, the situation appears similar to my sectioned specimen. This may even correspond to age: my specimen was very small, while at least some of Marcus's specimens were decidedly larger. We may conclude that at least a large part of the epithelium of the species is intranucleated, but this is best observed in large specimens, and in slides of excellent quality. In any case, the new specimens found gave more detailed information on the fine morphology of the sclerotised pieces. In fact, the spines of the copulatory organs were only vaguely mentioned by Marcus (1949), and his whole mounts do not allow any further information. In the two specimens examined, there are about 40 spines, arranged in six longitudinal rows. The spines are triangular, poorly sclerified, with an obtuse point, and with longitudinal furrows (Fig. 3B). Distal spines are very large, up to 13 μm high, and 8 μm broad at the basis. They progressively decrease in size: the most proximal spines range 3.5-6 μm.

Karyotype peculiar: $n=5$, with three metacentric chromosome pairs and one submetacentric pair almost of the same

size, and one acrocentric pair, almost half the size of the other chromosomes (Fig. 3C). Chromosome 1: r.l. = 24.61±1.08; c.i. = 47.01±1.11 (m); Chromosome 2: r.l. = 22.33±0.99; c.i. = 46.18±1.93 (m); Chromosome 3: r.l. = 20.71±1.33; c.i. = 25.83±2.70 (sm); Chromosome 4: r.l. = 20.03±1.16; c.i. = 45.09±1.53 (m); Chromosome 5: r.l. = 11.70±2.43; c.i. = 5.68±2.25 (t). Based on seven plates.

Notes. Although the similarity of the general organisation of the genital organs of *M. gabriellae* and species of *Archilina* has been underlined (see Martens and Curini-Galletti 1994), the former species appears quite distinct for morphology of the epithelium, presence of postpenial bursa, and prostatic and seminal vesicle not filling out the bulb; karyotype is also different. It is worth mentioning that Sopott (1972) described *Mesoda septentrionalis* from the North Sea, on the basis of the intraepithelial nuclei of at least part of the epidermis. The species was later transferred to the genus *Monocelopsis* Ax, 1951 by Martens and Curini-Galletti (1995). Indeed, the genera *Mesoda* and *Monocelopsis* share several characters, such as the sequential arrangement of testes-ovaria-vitellaria, with most of the latter being post-pharyngeal, a small postpenial bursa, five chromosomes, mostly metacentric, in their haploid set, and epithelium at least in part intranucleated (see Martens and Curini-Galletti 1995). It is hoped that molecular information may help to disentangle the present, almost inextricable taxonomic situation of the *Archiloa* genus-group.

***Pistryx thelura* Marcus, 1951**

Type locality. S.E. Brazil: "Canal de São Sebastião", in fine sand, in the tidal zone (Marcus 1951).

Material examined. S.E. Brazil, Ilhabela, Praia de Julião, intertidal in medium sand (10.31.2012): one specimen sagittally sectioned (CZM 561). Marcus's type material (eight slides: SMNH 108270–108278).

Remarks. Both the sectioned specimen and Marcus's type material show the characters of the genus, i.e. the epitelium with insunk nuclei, and the absence of postpenial bursa. This latter character is the main anatomical difference with *Mesoda gabriellae*, and the reason driving Marcus (1951) to establish a monospecific genus for it. The genus *Pistryx* has been synonymised with *Mesoda* by Martens and Curini-Galletti, 1994. Given the status of present knowledge of the *Archiloa*-genus group, any taxonomic decision seems preposterous, and is postponed until after a much needed thorough phylogenetic analysis of the group.

***Inaloa scalopura* (Marcus, 1949)**

(Figure 3D-F)

Type locality. S.E. Brazil, São Vicente (Baía de Santos): brackish conditions among mangrove roots (Marcus 1949)

Material examined. S.E. Brazil, São Sebastião, Araçá Bay, intertidal in medium sand (10.29.2012): numerous specimens observed alive, 12 specimens sagittally sectioned (CZM 562–573), two whole mounts (CZM 574–575), six specimens studied karyologically. Ilhabela, mouth of river close to Ferryboat landing, intertidal in brackish conditions (10.30.2012): two whole mounts (CZM 576–577), one specimen studied karyologically.

Other localities. SE Brazil, Ilhabela, brackish conditions at the mouth of a creek (Marcus 1949).

Description. The abundant material found allows for some integrations to Marcus's (1949) original description. The species has a very small seminal vesicle (Fig. 3D). On the contrary, the prostatic vesicle is unusually elongate, and split into two portions: the basal portion is lined with few, large, columnar cells, with basal nuclei. They appear poorly secretory at best, and do not stain comparably to cells of 'standard' prostatic vesicles elsewhere in the Monocelididae. A strong muscular diaphragm separates this basal part from a portion (called 'vesicular part' by Marcus 1949), which is large, globular and lined with muscular cells. The cirrus is very long, ranging 400–790 μm, and bears at least 120 rows of densely packed spines (about 20 spines per row) (Fig. 3E). Proximally, there is small patch of about 15 isolated spines: some of them are small, acutely triangular (3 μm high, 2.5 μm at the basis); most are larger (7–10 μm high, 5–6 μm wide at the basis), rapidly tapering into an acutely pointed distal tip. In the rest of the cirrus, the proximal spines range 7–12 μm high, with a basis 5.5 -7.5 μm, tapering into a long pointed, generally straight, distal tip (Fig. 3F). Spines gradually change morphology along the length of the cirrus. In the central and distal portion they are distinctly narrower (6–10.5 μm high, 2.5-4.5 μm broad at the basis). The extreme rim of the cirrus has spines of varying size (2–6 μm high), similar in morphology to the proximal spines.

Karyotype: n=4, with one large metacentric chromosome pair, and three small acrocentric pairs. Chromosome 1: r.l. = 48.35±1.06; c.i. = 45.77±0.74 (m); Chromosome 2: r.l. = 20.33±0.58; c.i. = 3.89±1.38 (t); Chromosome 3: r.l. = 17.15±0.38; c.i. = 11.05±3.25 (t); Chromosome 4: r.l. = 14.58±0.20; c.i. = 5.98±2.35 (t). Based on four plates.

Remarks. The species had a troubled taxonomic history: Marcus (1949) attributed it to the genus *Monocelis*; Karling (1966a) transferred the species to the genus *Archiloa*, and Martens and Curini-Galletti (1994) placed it into the genus *Inaloa*. However, when observed on actual material, the acritical attribution of this species (as well as of the previous species of the *Archiloa*-group) to boreal genera seems debatable at least. The construction of the copulatory bulb, and especially of the 'prostatic vesicles', of *I. scalopura* are in fact unique among Proseriata, and stress, if further necessary, the need for a thorough phylogenetical re-examination of the whole *Archiloa* group.

***Monotoplana* cf *diorchis* Meixner, 1938**

Material examined. S.E. Brazil, Ilhabela, near Praia Mansa, 15 m deep, fine sand (11.08.2012): one specimen

sagittally sectioned (CZM 578), one specimen studied karyologically.

Description. Small animals, about 1–1.5 mm long, ovoid in shape, very active and agile, almost continuously free swimming in the petri dish. Very distinctive for the presence of parenchymatous red pigment overall. With a patch of stiff cilia anteriorly. Epithelium intranucleated. Pharynx very large, tubular, about one-quarter of the total length of the animal, with the inner epithelium glandular in its proximal third. In the two specimens found, only one copulatory organ could be clearly seen.

Karyotype. With n=4, with one large metacentric pair, two submetacentric pairs almost of the same length, and one acrocentric pair markedly smaller than the others. One plate was measurable: Chromosome 1: r.l. = 32.81; c.i. = 47.02 (m); Chromosome 2: r.l. = 23.45; c.i. = 45.47 (m); Chromosome 3: r.l. = 22.91; c.i. = 32.89 (sm); Chromosome 4: r.l. = 21.45; c.i. = 13.74 (st).

Remarks. One of the most peculiar species of Proseriata, not only for its shape and pigmentation but also for the presence of two testes only and of a row of copulatory organs in different stages of maturity. The species was originally placed in the Archimonocelidinae Meixner 1938 due to the presence of a glandular lumen of pharynx and of intranucleated epithelium. Then, the monotypic family Monotoplanidae Ax 1958 was erected for it (Ax 1958). The first molecular study of the Proseriata (Littlewood et al. 2000) revealed its close links to the Monocelididae, de-emphasising the numerous autopomorphies of the taxon, and adding to the suspicions that the position of epithelial nuclei may not be of particular phylogenetic weight.

A small, red *Monotoplana* species is present in almost any subtidal area in the world, in sediments with at least some silt (pers. obs.). They differ for chromosome number and morphology (see Willems et al. 2009) and number of copulatory organs. Clearly, we are facing a complex of species, yet to be studied in detail.

Fam. Archimonocelidae Meixner 1938

***Archimonocelis marci* nov. sp.**

(Figures 4B, E, F and 5A)

Holotype. S.E. Brazil, São Sebastião, CEBIMar, beach in front of station, about 3 m deep, medium sand with shell fragments (10.28.2012): whole mount with four specimens, one of them chosen as holotype, the others designed as paratypes (SMNH Type-8596).

Other material. Same locality, two specimens sagittally sectioned (Paratypes, CZM 579–580), five specimens studied karyologically. Ilhabela, Fome beach, 12 m deep, medium-coarse sand (11.08.2012): one specimen studied karyologically.

Etymology. The specific epithet honours Prof. Ernesto Marcus (1893–1968), for his outstanding contribution to the knowledge of free-living Platyhelminthes of South America.

Description. A rather small *Archimonocelis* (holotype about 2.9 mm long), slender, agile (Fig. 4B). Epithelium thin, with intraepitelial nuclei, ciliated (cilia 3–4 μm long). Cilia decrease in size in the dorso-posterior part of body; the caudal portion is devoid of cilia in a small dorsal patch. With one dorsal, median row of cysts. In the anterior part of body, these contain a single large nematocyst, and many very small ones. Posteriorly, cysts contain more nematocysts, with more numerous large ones. The gut does not form any 'chorda intestinalis'. It contains numerous nematocysts posteriorly.

Pharynx in the posterior half of body, tubular, long and narrow in living specimens. In fixed, contracted specimens, it ranges 320–330 μm long. Epithelium ciliated externally (cilia about 2 μm long). It is unciliated at tip, and for most of its inner side, except for an isolated, distal patch of cilia. Proximally, a well developed oesophagus with glands is present. Mouth lined with an unciliated, intranucleated epithelium.

Male genital system. With about 20 testes in a median row, running from posterior to the ovaries to in front of pharynx. With two seminal vesicles, lined with a thin epithelium, and containing few sperm. The seminal vesicles enter distally the prostatic vesicle, which is pyriform in shape, 60–70 μm long, and lined with a well evident musculature. Distally, it is connected to the copulatory stylet, surrounded with a girdle of spines (Fig. 5A). The stylet is a tubular structure, 41–58 μm long, straight, and pointed, with a proximal and a distal opening (Fig. 4E). The distal opening is oblique, 11–14.5 μm long, and 4–6 μm in diameter, and ends into an obtuse distal tip, appreciable in squeezed, karyological slides. The diameter of the tubular part of the stylet is about 5.5 μm distally, and broadens basally to 6–7.5 μm. The proximal opening is slightly inflated, 7–10.5 μm broad, and is surrounded by a slightly thickened rim, 1.5-2.5 μm high. Proximally, the stylet is connected to two thin companion spines, about 40 μm long, with a pointed distal tip, whose presence is only appreciable in good quality karyological slides.

Spines of girdle fine and numerous, difficult to count and observe in detail on lactophenol slides. Details can only be appreciated in karyological slides (Fig. 5A). They are 41–45 in number, 34–40 μm long and thin—the stem is about 1 μm in diameter (Fig. 4F). The shortest spines appear to be closest to the stylet, while the longest are at the opposite side. The distal tips are thickened, harpoon shaped, widening basally into an apophysis about 1.5 μm in diameter, and narrowing into a slightly curved distal tip. The apophysis is placed at about 7 μm from the distal tip in the shortest spines, and at 8–8.5 μm from the tip in the longest spines.

Female genital system. With two post-cephalic ovaries. Vitellaria in two lateral rows posterior to ovaria, consisting of about 35 follicles each. Of these, about 8 follicles are placed posterior to the pharynx. The oviduct fuse posterior to the pharynx to form a long straight female duct, surronded by a strong musculature. The female duct is slightly enlarged anteriorly, in a structure comparable to the bursa of other

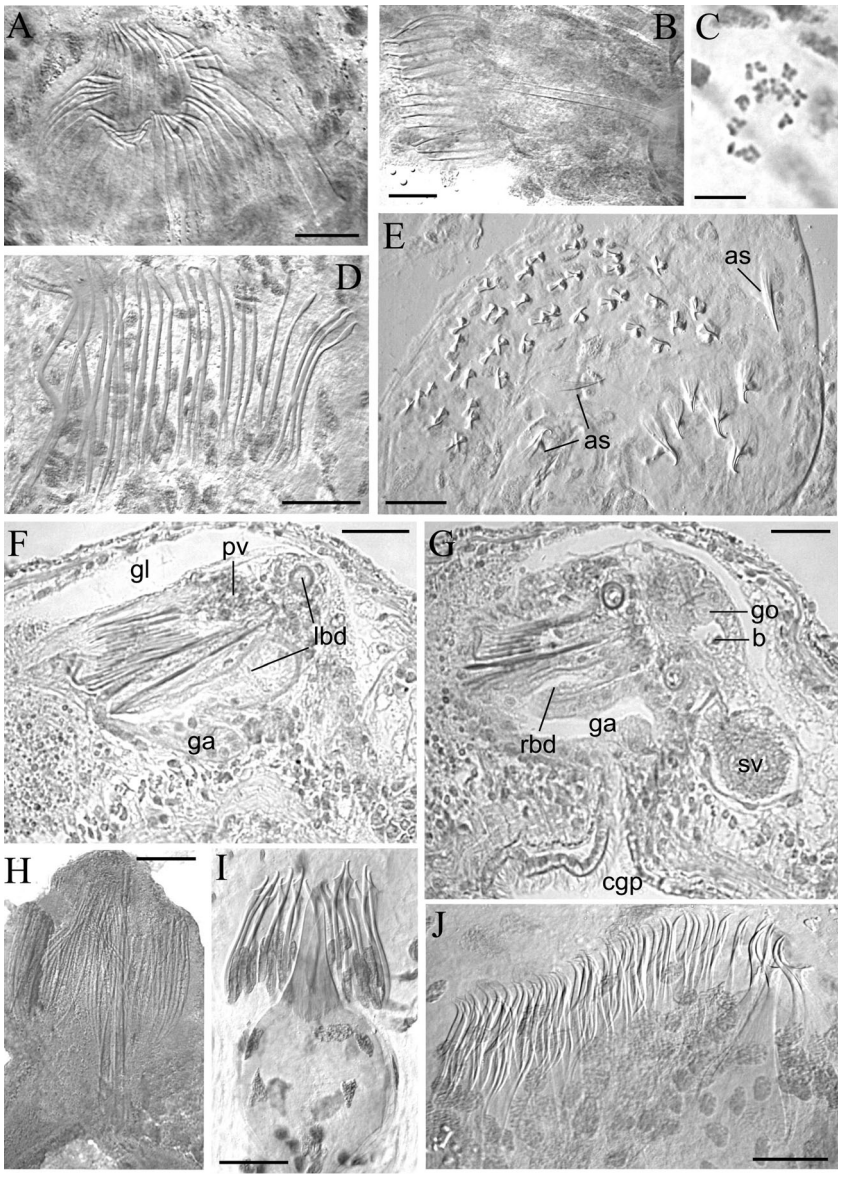

Fig. 5 **A**: *Archimonocelis marci* nov. sp.; sclerotised pieces (stylet and girdle of spines) of copulatory organ. **B**, **C**: *Meidiama lutheri*; sclerotised copulatory structures (**B**); spermatogonial metaphase plate (**C**). **D**: *Parotoplana moya*; sclerotised copulatory structures. **E**: *Vannuccia martae*, sclerotised structures of cirrus. **F**-**H**: *Parotoplanina antaliformis* nov. sp. sagittal sections of genital area, at different levels (**F**, **G**); copulatory structures (**H**). **I**: *Pseudosyrtis cebimari* nov. sp.; sclerotised copulatory structures. **J**: ? *Itaspis evelinae*: sclerotised copulatory structures. *Scale bars*: A-C=10 μm; D-G, I, J=20 μm; E, H=30 μm

Archimonocelis species; however, no sperm nor a vagina could be observed. The female duct runs posteriorly to the male copulatory organ, and opens to the outside via a female pore, surrounded by female glands.

Karyotype. With n=10, and most chromosomes acrocentric. Chromosome 1: r.l. = 15.41±1.74; c.i. = 8.95±1.98 (t); Chromosome 2: r.l. = 12.43±0.18; c.i. = 13.96±0.05 (st); Chromosome 3: r.l. = 11.53±1.26; c.i. = 41.18±3.09 (m); Chromosome 4: r.l. = 10.57±0.96; c.i. = 43.03±0.43 (m); Chromosome 5: r.l. = 10.01±0.08; c.i. = 8.69±3.81 (t); Chromosome 6: r.l. = 9.32±0.06; c.i. = 10.41±3.12 (s); Chromosome 7: r.l. = 8.91±0.03; c.i. = 15.0±4.01 (st); Chromosome 8: r.l. = 8.0±0.36; c.i. = 4.83±2.83 (t); Chromosome 9: r.l. = 7.61±0.11; c.i. = 8.18±3.63 (t); Chromosome 10: r.l. = 6.16±0.07; c.i. = 11.6±4.49 (t) (based on three plates).

Diagnosis. Species of *Archimonocelis* with two seminal vesicles. With a straight, tubular stylet, 41–58 μm long, with a straight, very slightly inflated proximal aperture and an obtuse distal tip, provided with an oblique, distal opening. With two slender companion spines, attached basally to stylet. With a girdle of 41–45 spines 34–40 μm long, 1 μm broad, shorter close to stylet, and provided with a slightly falcate apical tip and a subterminal apophysis, placed at 7–8.5 μm from the distal tip. With n=10, and most chromosomes heterobrachial.

Remarks. The genus *Archimonocelis* Meixner, 1938 is very large and diverse, with 25 species described, mostly from warm temperate-tropical areas (see Tyler and Artois 2013; Tyler et al. 2006–2012). However, very few species have been described from the American continents, and none from South America. The new species belongs to the group of species

without accessory girdle, and with spines almost identical in size and morphology.

Of them, *A. monicae* Martens & Curini-Galletti, 1993, from the Red Sea, has fewer (11–16) spines, thicker and with proportionally shorter distal tip, and a karyotype with $n=7$.

A. carmelitana Martens & Curini-Galletti, 1993, from the Mediterranean, has a longer (about 100 μm long) stylet, with many more (over 100) thin spines, without distinct apophyses, and a karyotype with $n=9$;

A. koinocistis Karling, 1966, from Bergen area (Norway), has an acuminate stylet, progressively decreasing in diameter along its length, and spines with a characteristically 'bifurcated' apex;

A. bathycola Karling, 1966, also from Bergen, has a slender, acutely pointed stylet, similar in morphology to the previous species, and about 20 spines less than half the length of the stylet;

A. coronata Karling, 1966, from Bodega Bay (West USA) has a stylet characteristically recurved distally, and spines less than one-third the length of the stylet (see Martens and Curini-Galletti 1993 for extended descriptions of these species).

The species which appears more similar to the new species is *A. scopulicola* Curini-Galletti, Delogu, Campus & Casu, 2007 from the Mediterranean. Indeed, the two species appear to differ basically in the number and size of spines (*A. scopulicola* has fewer [18] and smaller spines, 30–35 μm long, 1–2 μm broad, with proportionally shorter distal tips) and in the presence of a somewhat obtuse tip of the stylet and of a proximal rim in the new species. Furthermore, companion spines are not present in *A. scopulicola*. The two species share the same haploid chromosome number $n=10$, but chromosomes in *A. scopulicola* are markedly less heterobrachial (Curini-Galletti et al. 2007).

Fam Meidiamidae Schockaert, Curini-Galletti, De Ridder, Volonterio & Artois, 2009

***Meidiama lutheri* Marcus, 1946**

(Figure 5B, C)

Type locality. S.E. Brazil, Guarujá (Santos): shelly sand (Marcus 1946).

Material examined. S.E. Brazil, Ilhabela, Praia de Julião, intertidal in medium sand (10.31.2012): two specimens studied karyologically.

Description. The only mature specimen found had a tubular stylet, 75 μm long. The stylet is basically a gutter-shaped structure, with the edges fused for most of its length. They are separated at both proximal and distal openings, which appear longitudinally split. This split is particularly evident at the basis, where it is about 8 μm long. Proximal opening 14 μm wide, narrowing distally into a tube about 5 μm in diameter. The tube narrows slightly distally, and the distal opening is 3.5 μm wide. The stylet is clearly straight in the karyological slide studied (Fig. 5B): however, this may be an artefact due to compression: it appeared slightly recurved in the living specimen. The stylet is surrounded by a girdle formed by 14 spines, 26–27 μm long, with poorly sclerotised basis. They vary in morphology: close to the stylet they are stouter (stem about 2.5 μm in diameter), with a small, distinct, slightly upturned apophysis about 1.2 μm long placed at 6.5 μm from the slightly hooked distal tip. Opposite to the stylet they are thinner (stem up to 1.5 μm across), with a smaller, barely noticeable apophysis, at about 5 μm from tip.

Karyotype. With $n=6$ and mostly evenly sized, isobrachial chromosomes (Fig. 5C). One plate was measurable: Chromosome 1: r.l. = 18.75; c.i. = 31.35 (sm); Chromosome 2: r.l. = 18.74; c.i. = 48.15 (m); Chromosome 3: r.l. = 16.66; c.i. = 33.33 (sm); Chromosome 4: r.l. = 16.66; c.i. = 47.92 (m); Chromosome 5: r.l. = 14.57; c.i. = 43.41 (m); Chromosome 6: r.l. = 14.57; c.i. = 33.63 (sm).

Remarks. The genus *Meidiama* Marcus, 1946, of which *M. lutheri* is the type species, includes three species only, all from South America. Of these, *M. lutheri* and *M. uruguayensis* Schockaert, Curini-Galletti, De Ridder, Volonterio & Artois, 2009 are most similar. The two species differ in the number of spines (9–14 in *M. lutheri*, 19–21 in *M. uruguayensis*). The size of the stylet is similar, 60–75 μm and 80–90 μm long respectively. The distal opening is the key distinguishing feature: it is basically round, with a short longitudinal split, in *M. lutheri*, while it bears a variably ornamented 'crest' (a sharp lamina running from the opening for a short distance longitudinally outside the stylet) in *M. uruguayensis* (Schockaert et al. 2009). *M. schockaerti* Martens & Curini-Galletti, 1993, from Punta Arenas (Argentina), has a straight stylet 60 μm long, surrounded by 20 spines 45–50 μm long (Martens and Curini-Galletti 1993).

Fam. Coelogynoporidae Karling, 1966

***Vannuccia martae* Marcus 1948**

(Figure 5E)

Type locality. S.E. Brazil, Guarujá (Santos): shelly sand (Marcus 1948).

Material examined. S.E. Brazil, São Sebastião, Guaecá, gravelly sand (10.30.2012): one specimen sagittally sectioned (CZM 581), one whole mount (CZM 582), one specimen studied karyologically.

Description. The habitus and general anatomy of the species is described in detail by Marcus (1948). The specimens found had a cirrus provided with three accessory stylets (Fig. 5E). The cirrus bears 46–48 spines; 40–42 of these spines are similar in morphology, and are arranged in 9 girdles. The most proximal of these spines are about 11 μm high, and 2.5-3 μm wide at their basis, with a pointed and curved distal tip, and a comparatively very large, flap-like apophysis, almost triangular in shape, 4 μm long, and 3.5 μm wide at most; it is placed at about 4 μm from the distal tip. More distally, spines become progressively larger, to 12 μm high, 5 μm wide at their basis, with a downward sloping apophysis less triangular in appearance, with a rounded distal end.

At its distal end, the cirrus is provided with 6 larger spines. The smallest of them are more proximal: they are 21 μm high, with a bulbous basis, 11 μm wide, tapering abruptly into a slightly hooked distal end, and with a rounded apophysis 4.5 μm long placed at about 8 μm from the distal end. The distalmost spines are 30 μm high, with a 6-μm long apophysis at about 10 μm from distal tip.

The three accessory stylets are connected to the proximal basis of the cirrus by long, strong muscles, enabling them to move around the cirrus itself. Two of these stylets are placed laterally to the cirrus, and are identical in shape: they are sharply pointed, conoidal structures, 30 μm long and with a maximum diameter of 9 μm. The third accessory stylet is placed above the cirrus, and more similar in morphology to the cirrus spines: it is 31 μm long, tapering into a distinctly curved distal tip, and with a sloping downwards apophysis, 6 μm long, placed at about 6 μm from the distal tip.

Remarks. The sclerotised structures of the specimens found correspond almost in every detail to Marcus's description and drawings (1948: Fig. 63, p. 223). In particular, the number and peculiar morphology of the spines, with their large, flap-like apophysis, and the distinction in size among proximal and distalmost spines, clearly correspond to what was observed in the specimen found. Marcus (1948) also described the two conoidal accessory stylets. However, he considered them as part of the cirrus, and apparently failed to notice their independent connection to long muscle bundles. The third accessory stylet is not reported in the original description: however, this is similar in shape to the distalmost spines of the cirrus, and might have been mistaken for one of them. Indeed, it is particularly difficult to evidence it in the whole mount, where all the accessory stylets are intermingled with the cirrus spines.

The acknowledgment of the presence of accessory stylets in *Vannuccia martae* (the type species of the genus *Vannuccia* Marcus, 1948) has taxonomic implications. Faubel & Rohde (1998), in fact, based on the description by Marcus (1948), erected the new genus *Stilivannuccia* Faubel and Rhode, 1998, in order to include species with accessory stylets. The presence of these stylets in the type species of *Vannuccia* clearly makes *Stilivannuccia* a junior synonym of *Vannuccia*.

Fam. Otoplanidae Hallez, 1892

***Philosyrtis eumeca* Marcus, 1950**

Type locality. S.E. Brazil, Ilha Porchat, Baía de Santos, in fine sand with shell fragments (Marcus 1950).

Material examined. S.E. Brazil, São Sebastião, Toque Toque Grande, intertidal in medium sand (10.28.2012): one whole mount (CZM 583). SE Brazil, Guaratuba, medium sand with shell fragments (10.23.2012): one whole mount (CZM 584).

Remarks. The taxon *Philosyrtis monotoides* Giard, 1904 had a troubled history. First described as an aberrant Gastrotrich (Giard 1904), it was later transferred into the Otoplanidae (Remane 1926). Meixner (1938) described a second species in the genus (*Philosyrtis germanica* Meixner, 1938); and Marcus (1950) attributed a further new species (*P. eumeca*) to the genus *Philosyrtis* on the basis of the perceived similarity of his species with *P. germanica*. However, Giard's description and drawing (1904) are based on an unrecognisable juvenile Otoplanid, with no hint of sexual maturity, and his taxon should be considered as *nomen dubium*. Furthermore, *Phylosirtis germanica* was later transferred to the genus *Kataplana* Ax, 1956. Marcus's species cannot be included into this genus, as, among other relevant differences, *K. germanica* is characterised by the presence of a secondary bursa, which opens to the outside through an independent pore (Ax 1956). A reasonable course of action would be to introduce a new genus name to accommodate Marcus's species (which is at present considered as the type species of the genus *Phylosirtis*, see Tyler et al. 2006–2012) and the others currently attributed to *Philosyrtis*. However, this would require a thorough revision of the Parotoplaninae, which has not yet been accomplished. In any case, the finding of *Philosyrtis eumeca* is of particular interest, as the specimens stored in alcohol may yield precious phylogenetic information. As usual with the related species, the tiny, very active juveniles were common in sediments, while mature individuals were much less frequent. The adult specimens found corresponded closely to Marcus's (1950) original detailed description.

***Parotoplana moya* Marcus, 1949**

(Figure 5D)

Type locality. S.E. Brazil, "Ilha de São Sebastião" (Ilhabela), in coarse sand (Marcus 1949).

Material examined. S.E. Brazil, Ilhabela, Perequê, sand bar, medium-fine sand (10.30.2012): one specimen studied karyologically.

Description. See Marcus (1950) for a detailed description of the anatomy of the species. The specimen found had a sclerotised copulatory apparatus formed by numerous spines, arranged into two distinct girdles. The largest girdle contains 17 spines, ranging in length 50–60 μm, with a stem 1–1.5 μm in diameter. The falcate distal tip of these spines is about 2 μm at its maximum width, which is placed at 4.5-5 μm from the tip. The largest spines have a small (0.7-1 μm long) apophysis at about 4 μm from the distal tip. The other girdle is formed by 6 needle-shaped spines, which are straight, pointed, with a diameter of 1.5 μm, and a length of 67–72 μm. In the squeezed mount studied, these two contiguous girdles appear fused: they were observed as separated in the living specimen.

***Pseudosyrtis cebimari* nov. sp.**

(Figures 4C, D, G, H and 5I)

Holotype. S.E. Brazil, Caraguatatuba, Ilha do Tamanduá, 15 m deep, shelly sand (10.31.2012): one specimen sagittally sectioned (SMNH Type-8598).

Other material. Same locality, seven specimens sagittally sectioned (Paratypes, CZM 585–591), two whole mounts (Paratypes, CZM 585–591), three specimens studied karyologically.

Etymology. The specific epithet refers to the CEBIMar, the Centre for Marine Biology of the University of São Paulo, Brazil, where present research was carried out.

Description. An elongate species, about 3 mm long, flattened, ribbon shaped, only slightly tapering caudally (Fig. 4C). Anterior end truncated, provided with stiff sensorial cilia, and a ciliated furrow. Caudal end with adhesive glands. Epithelium intranucleated, ciliated only in a median, ventral band; cilia about 5 μm long. With numerous, minute, dot-like rhabdoids, arranged in longitudinal rows. Subepidermal musculature particularly developed ventrally.

Pharynx submedian, closer to the anterior end. It is short and tubular, and it is held horizontally. In living animals, it may modify its shape, and become more or less elongate; however, it never becomes collar-shaped, nor vertically oriented. The pharynx is ciliated both on its outer and inner surfaces: inner cilia are 4 μm long, outer cilia 2 μm long.

Male genital system. With 44–50 testes in front of pharynx, arranged in two rows laterally to the gut. The copulatory bulb is formed by a seminal vesicle and a nearly spherical prostatic vesicle, provided with a stylet surrounded by a girdle of spines (Fig. 5I). The seminal vesicle is long, lined by a thin epithelium, and surrounded by a muscular coating, which is very thin proximally, and becomes much thicker distally. The prostatic vesicle is small, 35 μm across in the whole mounts, and about 50 μm in the squeezed specimens. It is surrounded by a thick muscular coating (Fig. 4D). The stylet is a funnel-shaped structure (Fig. 4G). It is 41–43 μm high, with a diameter at the basis of 20–28 μm. The distal opening is 3–6 μm across. In unsqueezed whole mounts, it is about 30 μm long, and 14 μm wide at basis. The stylet is surrounded by 17–25 spines. They are 40–44 μm long, with a stem 1.5-2.2 μm wide, and with a sickle-shaped distal tip. At about 7 μm from the tip, a broad (to 3.5 μm wide), somewhat triangular apophysis is placed (Fig. 4H). The stylet opens into the anterior part of the common genital atrium.

Female genital system. With two ovaries just in front of pharynx. With numerous (about 50) vitellarian follicles entirely post-pharyngeal, arranged into two submedian rows, ending just in front of the copulatory organ. The female duct opens into the common genital atrium, in its posterior portion, and is surrounded by numerous female glands (Fig. 4D). The bursa could not be detected. The common atrium is lined by an unciliated flattened epithelium, and opens ventrally to the outside via the common genital pore.

Diagnosis. Ribbon-shaped Parotoplaninae with intraepithelial nuclei and tubular pharynx. With a small, spherical prostatic vesicle, and a funnel-shaped stylet, 30–43 μm long, surrounded by 17–25 falcate spines, 40–44 μm long, provided by a triangular apophysis. Vitellaria entirely post-pharyngeal.

Remarks. Although clearly belonging to the subfamily Parotoplaninae (see Casu et al. 2014, for an extensive discussion on this taxon), the generic attribution of the species is problematic. Also in this case, the quality of fixation (and consequently of the sections) is far from optimal. However, at least in some of the slides, the epithelium was adequately fixed, and the (intraepithelial) position of nuclei could be evidenced. The main problem concerns the presence or not of a bursa, which could not be observed in any of the sectioned material, or in living animals. It is therefore assumed that the bursa is lacking in the species.

The only genus of Parotoplaninae with intraepithelial nuclei, without bursa, with a stylet and with a prostatic vesicle not entirely surrounded by the sclerotised pieces of the copulatory organ is *Pseudosyrtis* Ax, 1956, which includes four species: three come from brackish waters of the Mediterranean and Atlantic coasts of Europe, and one (*P. calcaris* Sopott-Ehlers, 1976) from an exposed beach in Gran Canaria. All of them share with the new species the entirely postpharyngeal position of the vitellaria, and the presence of a central stylet surrounded by falcate spines. The latter species seems particularly similar to the new species, as both have a pharynx ciliated at both sides (it is unciliated externally in the other species). *P. calcaris*, however, clearly differs for the presence of two large spines, flanking the stylet and its surrounding spines, which, furthermore, are only four.

It is questionable whether all these species constitute a monophyletic group, but this cannot be resolved with the information available. In any case, it has at least a biogeographic sense that the two most similar species appear to be those at the two sides of the Atlantic, and the only ones living in marine conditions.

***Parotoplanina antaliformis* nov. sp.**

(Figures 5F-H and 6A-G)

Holotype. S.E. Brazil, São Sebastião, Itaçucê, 7 m deep in shell gravel (10.30.2012): one specimen sagittally sectioned (SMNH Type-8599).

Other material. Same locality, one specimen sagittally sectioned (Paratype, CZM 594), one whole mount (Paratype, CZM 595), two specimens studied karyologically, made permanent with lactophenol (CZM 596–597).

Etymology. The specific epithet refers to the presence in the new species of a long, recurved, tubular stylet, similar in shape to species of the genus *Antalis* Adams & Adams, 1858 (Mollusca: Scaphopoda).

Description. A flattened, medium-sized (about 3 mm long) species (Fig. 6A). Head with ciliated furrow apically, and sensorial bristles. Posterior end fan-shaped, provided with numerous adhesive glands. Rhabdoids rod-shaped, 10–15 μm long, arranged in few irregular rows. Body ciliated

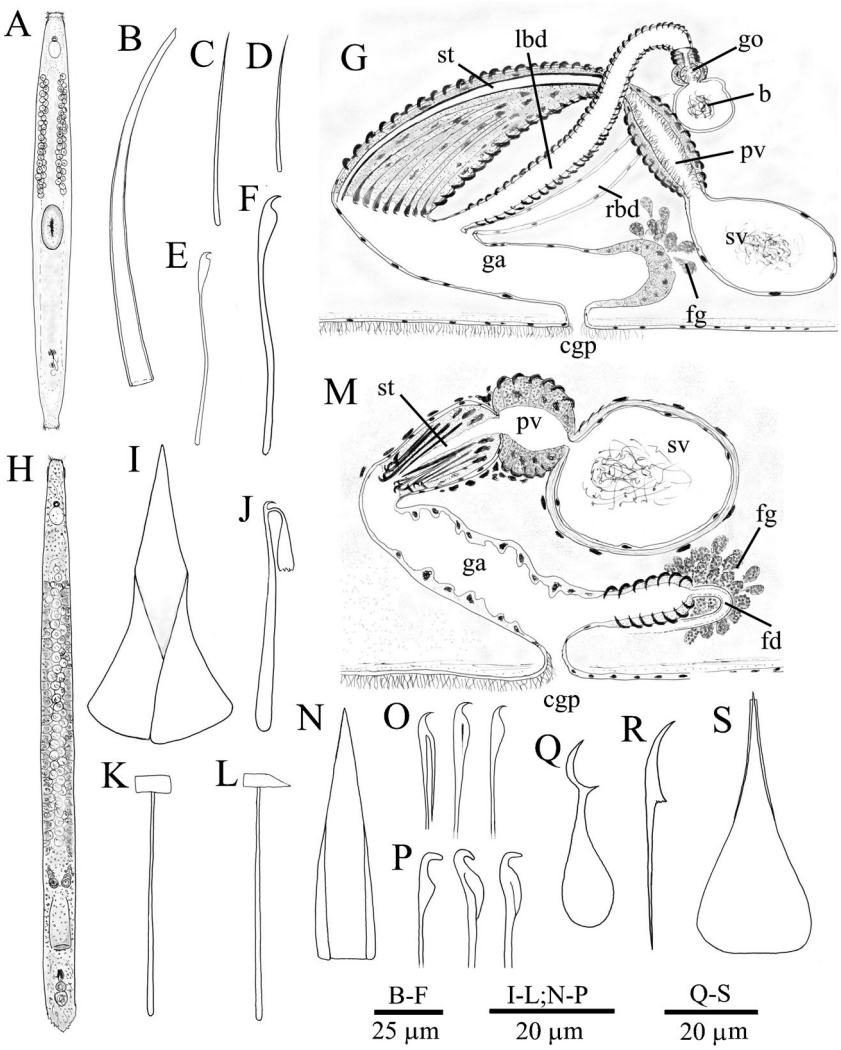

Fig. 6 **A**–**G**: *Parotoplanina antaliformis* nov. sp.: general organisation of a live specimen (**A**); stylet (**B**); spines (**C**-**F**, see text for explaination); sagittal recostruction of the genital area (**G**). **H**-**M**: *Monostichoplana fonsecai* nov. sp.: general organisation of a live specimen (**H**); stylet (**I**); spines (**J**-**L**, see text for explaination); sagittal recostruction of the genital area (**M**). **N**, **O**: *Kata leroda*; stylet (**N**); distal parts of spines of girdle (**O**). **P**: *Kata evelinae*; distal parts of spines of girdle. **Q**-**S**: ? *Itaspis evelinae*: stylet (**S**); spines (**Q**, **R**, see text for explanation)

ventrally, with cilia up to 7 μm in length, ending a short distance behind the genital opening. Epithelium intranucleated, both in ciliated and unciliated areas.

Body musculature consisting of an outer layer of circular musculature, very thick caudally, and a thick, especially ventrally, inner layer of longitudinal musculature.

Pharynx in the middle of the body, collar shaped, vertically oriented, ciliated externally and internally (outer cilia 3–5 μm long; inner cilia 3–4 μm long). With very numerous eosinophilous pharyngeal gland, discharging through the unciliated tip of the pharynx.

Male genital system. With numerous testes (about 60) arranged in two irregular, lateral rows in front of the pharynx. Copulatory organ with a small, nearly spherical seminal vesicle, a long, narrow prostatic vesicle, and a complex sclerotised apparatus, with spines and stylet (Fig. 5H). The seminal vesicle is lined with a thin epithelium. The prostatic vesicle is surrounded by a thick sheath of circular muscles, and lined internally with a ciliated, glandular epithelium. A prostatic, glandular tissue can be traced for the length of the ejaculatory duct, inside the sclerotised apparatus (Fig. 6G). The stylet is tubular, 130–180 μm long, narrow (8–13.5 μm in diameter at its basis, and 2–3.5 μm at its distal end), and more or less curved depending presumably of body contraction. The proximal opening is straight, while the distal opening is oblique (Fig. 6B). The stylet is flanked by two pairs of needle-shaped spines, progressively tapering into a fine, straight distal end. These are about 50 μm (Fig. 6D) and 70 μm long (Fig. 6C) respectively, and up to 2 μm in their maximum, proximal diameter. Ventrally to the stylet and its flanking spines, about 62 spines are present. They are arranged in two girdles—the inner consists of about 30 spines, varying in length from those closest to the stylet, which are about 132 μm, to about 90 μm. They are thin, with the diameter of their stem up to 1.5 μm, and their distal end widens to form a falcate, broad tip, 13–16 μm long (Fig. 6E). The outer girdle consists of about 32 spines, ranging 73–80 μm in length, with a stem about 2.5 μm in diameter, and a somewhat shorter and broader distal end than

the previous spines (Fig. 6F). The copulatory organ ends in the male (anterior) portion of the common atrium, lined with a robust, basically circular, musculature.

Female genital system. Vitellaria and ovaria not observed in living specimens, some of which were clearly immature in the female line. In one sectioned specimen, vitellaria could be observed posterior to pharynx. Female duct traceable with difficulty: a few female glands are present in the posterior part of the common atrium, and it is supposed that the outlet of the female duct is there. The epithelium lining the atrium in this caudal, female portion is higher than in the rest of the atrium, and presumably glandular. Just posterior to the outlet of the male system, the openings of two ducts could be observed (Figs. 5F, G and 6G). These ducts lie almost parallel for most of their lengths, and run anteriorly at the two sides of the prostatic vesicle, were a small bursa is placed. One of these ducts (the right-hand sided in the sagittal sections) is broader, and surrounded by a thick layer of circular musculature. Anteriorly, it is connected to a structure similar in morphology to the 'glandular organ' of some *Parotoplana* species (see Delogu et al. 2008): a globular structure surrounded by a cubic epithelium which opens into a thin walled, irregularly spherical bursa, filled with sperm (Fig. 5G). The other duct is narrower, not surrounded by an appreciable muscular coating, and can be traced anteriorly to close proximity of the bursa, although no connections could be seen with certainty. The common atrium opens ventrally into the common genital pore.

Diagnosis. A species of Parotoplaninae with intranucleated epithelium, collar-shaped pharynx ciliated at both sides. Copulatory organ with a slender stylet, 130–180 μm long, surrounded by about 62 slender spines (73–32 μm long) with a falcate distal tip, arranged in two concentric girdles. With two 'bursal' canals, and a small bursa anterior to the copulatory organ.

Remarks. A further taxonomically extremely problematic species. In this case, uncertainties derive from the difficulty to understand the female system in suboptimal slides, and in particular to follow the route of the female duct. Furthermore, the specimens found were not completely mature in the female line. In the description above, the outlet of the female duct is postulated to be in the usual position as in the rest of the Parotoplaninae, as suggested by the presence of the (very few) female glands. However, this is not based on solid evidence.

In any case, the only Parotoplaninae with two canals, running anteriorly from the genital atrium to meet a bursa placed above the copulatory organ, is *Parotoplanina geminoductus* Ax, 1956, from the North Sea. In this species, however, the ciliated epithelium has insunk nuclei, and both canals are identical in shape, and act as bursal ducts, and the female pore opens in the posterior portion of the genital atrium, where the epithelium is high and glandular. The species lacks a stylet, although its slender spines are somewhat comparable to the new species, as they are of two types: needle-shaped and with falcate tips (Ax 1956). The actual extent of the differences in anatomy of the two species, however, should be checked on more and better material than what is available at present. The species is clearly undescribed: although not entirely satisfactorily, it is here attributed to the genus *Parotoplanina* Ax, 1956.

Parotoplaninae sp.

(Figure 7H)

Material examined. S.E. Brazil, Caraguatatuba, Ilha do Tamanduá, 15 m deep, shelly sand (10.31.2012): one whole mount (CZM 613).

Description. A small species, slender, with a long adhesive tail. With long rhabdoids, evident in the cephalic region. Pharynx collar-shaped, submedian. With seven pairs of testes, neatly arranged in the anterior part of body. Copulatory organ with a short prostatic vesicle, entirely confined within the sclerotised apparatus. This is formed by 15 spines, about 90 μm long, with a small apophysis at about 25 μm from distal tip, which is very slender, whip-like. Two ovaries just in front of pharynx. Vitellaria (7–8 follicles per side) entirely postpharyngeal.

Notes. Based on details of pharynx, and organisation of gonads, the species appears to be a member of the Parotoplaninae. However, without anatomical information based on sections, its generic attribution is impossible, and the species cannot be described at the moment. It is reported here to document the diversity of S.E. Brazilian Otoplanidae.

***Monostichoplana fonsecai* nov. sp.**

(Figures 6H-M and 7B, C, E, F)

Holotype. S.E. Brazil, Caraguatatuba, Ilha do Tamanduá, 15 m deep, shelly sand (10.31.2012): one whole mount (SMNH Type-8597).

Other material. Same locality: one whole mount (Paratype, CZM 598); three specimens sagittally sectioned (Paratypes, CZM 599–601); one specimen studied karyologically.

Etymology. The species is dedicated to Dr. Gustavo Fonseca, the organiser of the workshop "Taxonomy and Diversity of Marine Meiofauna" (2012), which greatly contributed to the knowledge of Brazilian meiofauna.

Description. A narrowly elongate species, about 5 mm long (Fig. 6H). Head with a tuft of stiff, sensorial cilia. Body ciliation (cilia about 6 μm long) restricted to a creeping sole, ending at the level of the genital opening. Ciliated epithelium with insunk nuclei; the unciliated areas have intraepithelial nuclei. With very long rhabdoids; adhesive glands numerous at the caudal end, and scattered along sides of body. Living animals present numerous opaque, spherical 'glands', particularly evident in the cephalic region. In sections, these appear as globules, about 5 μm in diameter, with homogeneous content, located within the parenchyme. The state of fixation does not allow to appreciate whether these structures are

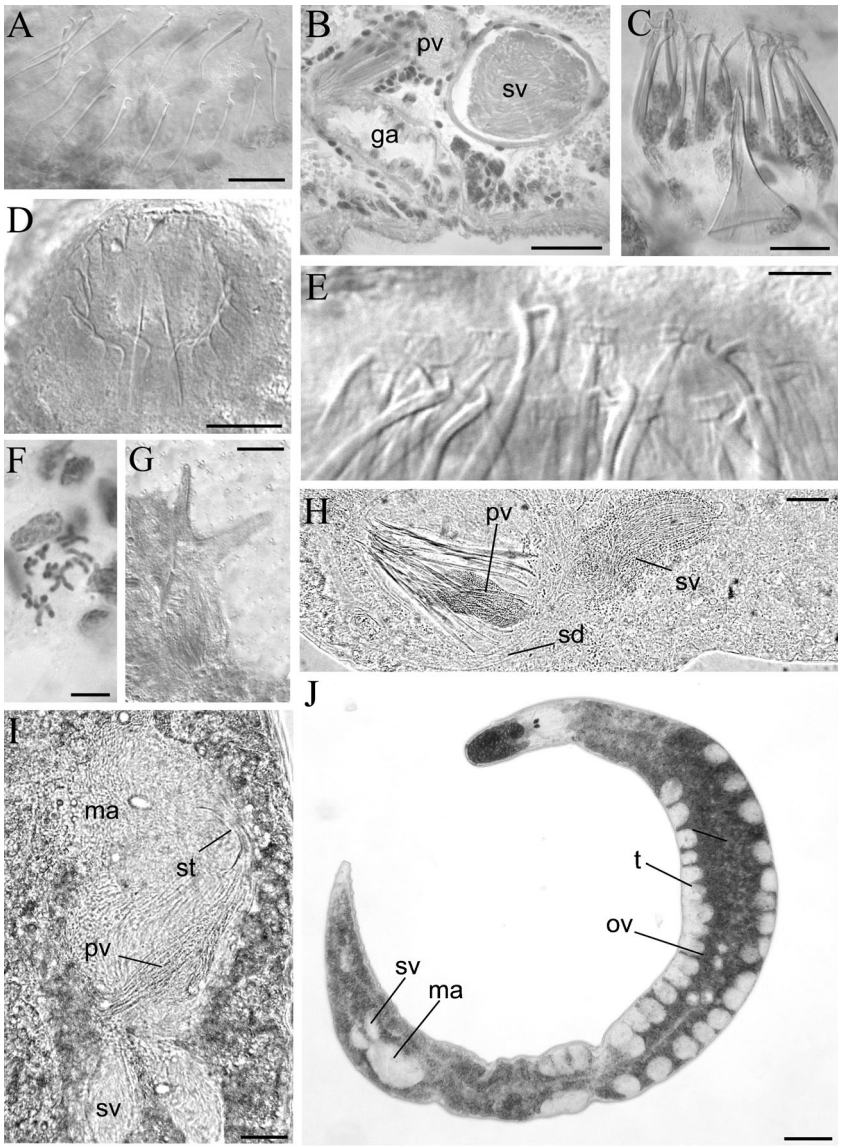

Fig. 7 **A**: *Kata evelinae*; sclerotised copulatory structures. **B**, **C**, **E**, **F**: *Monostichoplana fonsecai* nov. sp.; sagittal section of genital area (**B**); sclerotised copulatory structures (**C**, **E**: detail); spermatogonial metaphase plate (**F**). **D**: *Kata leroda*; sclerotised copulatory structures. **G**: *Nematoplana asita*; stylet from whole mount. **H**: Parotoplaninae sp.; genital organs from a live specimen. **I**, **J**: *Nematoplana mirabilis* nov. sp.; copulatory area from a live specimen (**I**); mature living specimen (**J**). *Scale bars*: F= 5 μm; E=10 μm; A-D, G-I= 20 μm; J=100 μm

intracellular; in no instance was a connection to the outside apparent.

Pharynx elongated, horizontal, placed close to the posterior end of body. It is ciliated both internally and externally (inner cilia 4 μm long, outer cilia 3 μm long). With very numerous pharyngeal glands, ending into the unciliated distal tip of pharynx. With a very developed proximal oesophagus, almost half the size of the pharynx.

Male genital system. With about 30 testes irregularly placed in a median row, in front of pharynx. Copulatory bulb close behind pharynx, consisting of a large seminal vesicle, a prostatic vesicle and a sclerotised apparatus consisting of a stylet surrounded by needles (Fig. 7C). The seminal vesicle is long, wide, with a muscular coating that progressively thickens distally. The small, round prostatic vesicle is surrounded by a thick muscle coating, and lined internally with a secretory epithelium (Figs. 6M and 7B). The stylet is about 50 μm high, and 23–30 μm wide at its basis (Fig. 6I). It is a funnel-shaped, with rims more or less overlapping proximally, for a height of 20–28 μm, above which it consists of an acutely pointed lamina. Eighteen spines are present, arranged into two close rings around the stylet. The outer ring is formed by six halberd-shaped spines (Fig. 6J), 36–39 μm long, with a stem up to 3.8 μm wide at its basis, and progressively narrowing distally to about 1 μm near the distal end, which is formed by a short, recurved distal tip, approximately 1.5-2 μm high and wide, and by a long apophysis which departs orthogonally from the stem for about 2.5 μm, and then turns sharply downwards and becomes parallel to the stem. This downturned part is banner-like, 7–10 μm long, and widens distally till 7 μm wide. These spines are in close contact and somewhat flanked by the spines of the inner ring. These are 12

in number, 37–40 μm long, thinner than the previous (less than 2 μm wide at the basis, narrowing to less than 1 μm below the apex), and provided with a rectangular apex, about 3–5 μm long and 2–4.5 μm wide (Fig. 6K and 7E). In some the spines the apex is longer, up to 6 μm, and slightly pointed at one side. These spines with longer apices appear to be arranged at the two sides of the girdle (Fig. 6L).

The copulatory organ opens into a common genital atrium, lined with a high, unciliated, intranucleated epithelium.

Female genital system. With two ovaries just in front of pharynx. Vitellaria in two irregular rows, placed laterally in front of pharynx, and in close contact with testes. Bursa absent. The female duct ends into the posteriormost part of the common genital atrium, surrounded with female glands (Fig. 6M). This part of the atrium is narrow and tubular and is surrounded by a thick coating of circular muscles. The atrium opens ventrally into the common genital pore.

Karyotype: with n=4. With two large metacentric pairs, and two smaller metacentric pairs (Fig. 7F). Chromosome 1: r.l. = 36.51±1.23; c.i. = 45.14±0.74 (m); Chromosome 2: r.l. = 27.81±0.2; c.i. = 44.95±2.86 (m); Chromosome 3: r.l. = 19.32±1.08; c.i. = 42.73±4.01 (m); Chromosome 4: r.l. = 16.36±0.34; c.i. = 42.47±2.34 (m) (based on four plates).

Diagnosis. Monostichoplana species with a funnel-like stylet 47 μm high and 23–30 μm wide, surrounded by 18 spines: 6 of them are stouter, falcate, 36–39 μm long, with a long, downturned, banner-like apophysis; 12 are more slender, without apophyses, and with a more or less rectangular apex. With haploid number n=4, and two large and two smaller chromosome pairs.

Remarks. The species fits into the genus *Monostichoplana* Ax, 1956 for details of body ciliation, position of epithelial nuclei, morphology, ciliation, and position of pharynx, as well for the general organisation of male and female genital systems (see Ax 1956). The genus includes only two species from Europe, although several species, mostly from the Mediterranean, are still undescribed (own unpubl. data). *M. fonsecai* nov. sp. is unique for the morphology of its spines: no species in the genus, in fact, is known to present spines with a rectangular apex. In *M. filum* (Meixner, 1938), two rings with different types of spines are present: the outer ring is formed by 8–12 spines, comparable to the halberd-shaped spines of the new species, but with very narrow, filiform apophyses; the inner ring is made by 16–24 narrow spines, with an acutely pointed apex, and provided with a very long and slender, downturned apophysis (Ax et al. 1978). *M. tenuissima* Ax, 1956 has spines of one type only, similar to those of the outer ring of *M. filum* (Ax, 1956). Furthermore, none of these species presents the globular, subepitelial 'inclusions' of the new species. On the contrary, subepidermal glands are present in species of two distinct genera: *Notocaryoplana* Steinböck, 1935, and *Notocaryoturbella* Lanfranchi, 1969. These are globular, and their content gives a distinctive yellowish colour to the body. However, in contrast with the new species, their glandular nature is apparent, and their necks pierce the epithelium (Ax 1956, p. 206). Furthermore, their content is extracted during fixation, and appear 'empty' in sections. However, these species are extremely similar to species of *Monostichoplana* for most other regards, including, body shape, pharynx morphology and position, and genital systems. The sclerotised system of these species consists in a funnel-shaped stylet, surrounded by a ring of spines, similar in morphology to the spines of the outer ring of *M. filum*. The type species of *Notocaryoplana* (*N. arctica* Steinböck, 1935) is distinct for the ovaries fused medially (Ax 1956, p. 206); these are unfused in *N. geminofollicularis* Tajika, 1983, and in *Notocaryoturbella bigermaria* Lanfranchi, 1969. The necessity of three distinct genera for these closely similar species is questionable, and should be further scrutinised.

? ***Itaspis evelinae*** **Marcus, 1952**

(Figures 5J and 6Q-S)

Type locality. S.E. Brazil, Ubatuba (praia do Cruzeiro): intertidal in coarse sand (Marcus 1952)

Material examined. S.E. Brazil, São Sebastião, Itaçucê, 7 m deep in shell gravel (10.30.2012): two specimens sagittally sectioned (CZM 602–603); two whole mounts (CZM 604–605), five specimens studied karyologically. S.E. Brazil, Caraguatatuba, Ilha do Tamanduá, 15 m deep, shelly sand (10.31.2012): one whole mount (CZM 606).

Remarks. The specimens found corresponded to the general morphology of *Itaspis evelinae*, as described by Marcus (1952, Pl. 20, Figs. 115–123): a flat, broadly ovate species, with a large, bell-shaped horizontal pharynx, and gut branching among testes follicles; furthermore, the sclerotised apparatus (see below) does not seem to differ significantly. However, in the specimens found vitellaria were entirely postpharyngeal, while they are along the sides of body in *I. evelinae*; in none of them was more than a single pair of ovaries seen, while in *I. evelinae* there are 2–3 pairs of ovaries. Furthermore, the most striking feature of *I. evelinae*, viz. the two immediately postcephalic bursae, connected to very long, lateral ovovitelloducts, could not be seen. However, the animals were opaque, due to food content and large rhabdoid glands, and the observations on the few mature specimens were particularly difficult. Fixation was very poor, and sections are of bad quality.

The sclerotised apparatus of the specimens included a stylet, and numerous spines (Fig. 5J). The conoidal stylet, straight in most specimens, is 53–59 μm long, and tapers from the proximal opening, 25–35 μm across, to a pointed distal end. This distal end is noticeably split: one of the ends is truncated (2–3.5 μm wide), while the other tapers into an acute point (Fig. 6S). The stylet is flanked by 4–6 spines, 43–44 μm long, with a broad basis, 6.5-12 μm wide, tapering to 1.5-1.8 μm close to the distal end, which is recurve, hook-

shaped, and provided with a small (2.5 μm) apophysis placed at about 10 μm from the distal tip (Fig. 6Q).

The rest of the spines are arranged in a U-shaped complex, consisting of 50–80 spines (Figs. 5J and 6R). Close to the stylet, they are small, 17–25 μm long, with a thick stem (1.5-2.5 μm in diameter), and a very slightly falcate distal end, and provided with a small, ill-defined apophysis at a 6–11 μm from the distal tip. Farther on, the spines become progressively longer, to 40 μm, very thin, and with longer (to 15 μm) distal ends. Those farthest from the stylet are even longer (to 65 μm), and similarly thin.

According to Marcus (1952), the about 40 spines have two types of distal end—part of them have a falcate tip and a marked apophysis and part a slightly recurved apex with a ill defined apophysis; the stylet is distally split into two 'needles'. The similarities with what seen in the sampled specimens are therefore remarkable, also taking into account that the details here described, and the exact number of the numerous, overlapping spines, can only be appreciated in squeezed karyological slides.

The exact identification of the specimens remains unsettled, till more adequate material is studied. It is, however, worthy of mention that their general morphology and, apart from details, the shape, number and organisation of the sclerotised apparatus closely correspond also to species of the genus *Pseudorthoplana* Ax, Weidemann and Ehlers, 1978, from Europe. The basic difference among the genera is the lack in the latter of post-cephalic bursae, and of multiplication of ovaries. From the above, it is apparent that the relationships among species of the two genera need to be thoroughly assessed.

***Kata evelinae* Marcus, 1949**

(Figures 6P and 7A)

Type locality. S.E. Brazil, "Ilha de São Sebastião" (Ilhabela), in sand (Marcus 1949).

Material examined. S.E. Brazil, Ilhabela. Pitangueiras, intertidal in granules (11.08.2012): two specimens sagittally sectioned (CZM 606–607), 2 specimens studied karyologically.

***Kata leroda* Marcus 1950**

(Figures 6N, O and 7D)

Type locality. S.E. Brazil: "Ilha de São Sebastião" (Ilhabela), in fine sand (Marcus 1950).

Material examined. S.E. Brazil, São Sebastião, Toque Toque Grande, intertidal in medium sand (10.28.2012): two whole mounts (CZM 608–609); three specimens studied karyologically. São Sebastião, Araçá Bay, intertidal in medium sand (10.29.2012): four specimens studied karyologically

Remarks. Both the species described by Marcus (1949, 1950) in the genus *Kata* Marcus, 1949 were found. They were easy to discriminate: *K. evelinae* is strikingly large for an otoplanid, and mature specimens are over 4 times the length of adult *K. leroda*. In addition to details of the sclerotised structures (see below), *K. evelinae* is distinct for the presence of two vaginae, opening posterior to the common genital pore, while *K. leroda* has one vagina only. However, this feature may be treated with some caution as a discriminating character. Some variability in the latter species has in fact been observed: the vaginal opening may be transversally elongated, and often split into two (in one observed case, even three) very close openings, with ragged edges. The two species differ for habitat preference: *K. leroda* was common along the continental coast of São Sebastião, occurring intertidally in medium sand, while *K. evelinae* was only found on the outer coast of Ilhabela, on a high energy beach.

The genus *Kata* (type species: *Kata evelinae*) is widespread in the tropics and in the southern hemisphere, and includes numerous, very similar species (see Tyler and Artois 2013; Tyler et al. 2006–2012; unpubl. data). Based on the new material found, a redescription of the sclerotised apparatus of these species may thus be necessary:

K. evelinae: without stylet. With a girdle formed by 10-17 spines, 48–56 μm in length, with a thin stem, 1–1.5 μm across. Distal end markedly hook-shaped; apophysis at about 5 μm from the distal tip, 2–3 μm long, obtusely triangular. In the largest, apparently more formed, spines, the apophysis becomes more conspicuous and a carved longitudinal furrow, separating it from the stem, progressively appears (Figs. 6P and 7A).

K. leroda: with a gutter-shaped stylet, 40–50 μm long, 11–15 μm wide at its basis, parallel sided for about half of its length, and then tapering into a point distally. In squeezed specimens, the stylet appears as a flat lamina. With a girdle formed by 11–18 spines, 12–22 μm long, with a stem about 1 μm wide, and with a hooked distal end. Apophysis similar to that of the previous species: however, the furrow further deepens, and in many spines the apophysis is is long (5–10 μm), thin, and runs parallel to the stem (Figs. 6N, O and 7D).

Suborder Unguiphora Sopott-Ehlers, 1985

Fam. Nematoplanidae Meixner, 1938

***Nematoplana asita* Marcus 1950**

(Figures 7G and 8A, B, E)

Type locality. S.E. Brazil, "Ilha de São Sebastião" (Ilhabela), in sand (Marcus 1950).

Material examined. S.E. Brazil, São Sebastião, Toque Toque Grande, intertidal in medium sand (10.28.2012): two whole mounts (CZM 610–611); two specimens studied karyologically.

Description. The original description of the stylet of *N. asita* is somehow puzzling (see Curini-Galletti and Martens 1992). No other *Nematoplana*, in fact, has a stylet with a broad, straight basis, from which a continuously and regularly narrowing tube departs, curving at about 130-150°, and ending distally into a spike

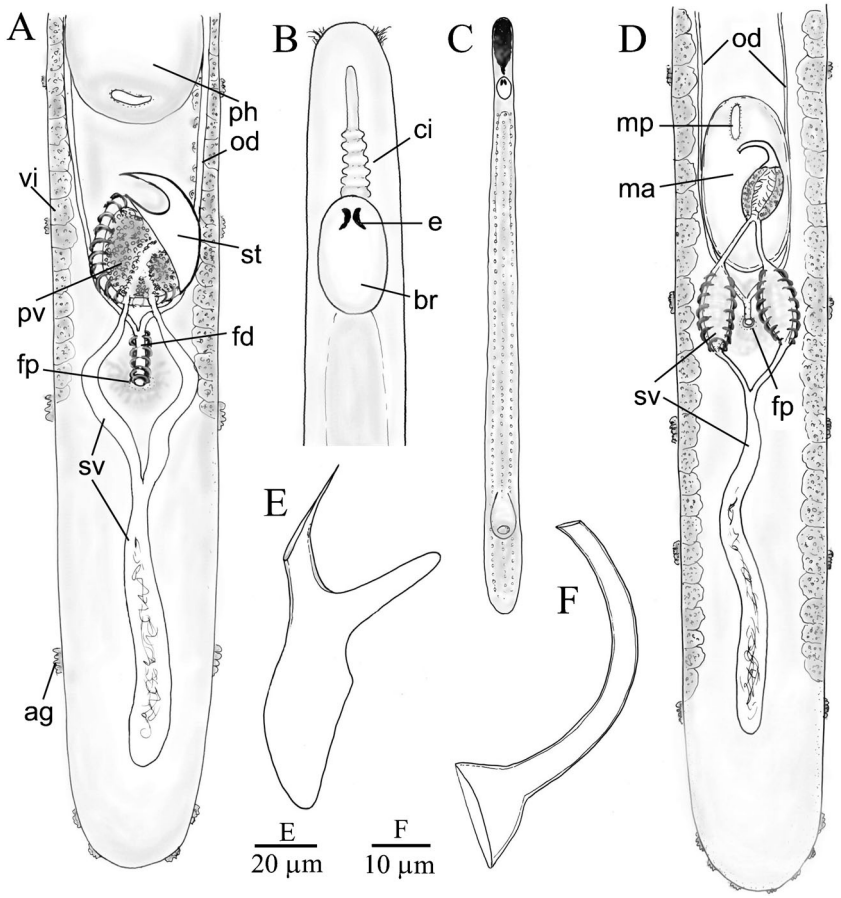

Fig. 8 **A**, **B**, **E**: *Nematoplana asita*; organisation of the genital area (**A**); cephalic region (**B**); stylet (**E**). **C**-**D**, **F**: *Nematoplana mirabilis* nov. sp.; juvenile specimen (**C**); organisation of the genital area (**D**); stylet (**F**)

(Marcus 1950, Pl. 201). Indeed, the living specimens observed showed exactly the morphology described by Marcus (Fig. 8A).

However, when macerated with acetic acid and squeezed, the full details of the stylet could be revealed (Figs. 7G and 8E). In fact, the stylet is very poorly sclerotised, and this may account of its shape when, in vivo, is connected to thick bundles of muscles. The stylet is 50–61 µm in length, and is provided with a straight, pointed distal end 23–25 µm long, where the distal opening is placed. A long, straight, obtusely pointed apophysis, 30–35 µm long, and 9 µm wide at its basis is placed at 7–8 µm from the distal tip. The proximal portion of the stylet is rounded, and about 30 µm wide; the proximal opening runs for most of its length ventrally. The morphology of the stylet is thus comparable to that of most species of *Nematoplana* (see Curini-Galletti and Martens, 1992), from which *N. asita* is basically differs for the shape of the apophysis, which is unusually long and broad at its basis.

***Nematoplana mirabilis* nov. sp.**

(Figures 7I, J and 8C, D, F)

Holotype. S.E. Brazil, São Sebastião, Toque Toque Grande, intertidal in medium sand (10.28.2012): one mature specimen sagittally sectioned (SMNH Type-8600).

Other material. Same locality: one whole mount (Paratype: CZM 612), numerous juveniles studied alive.

Etymology. The specific epithet derives from latin *mirabilis*: admirable, and refers to the peculiar features of the life cycle of the new species, as well as to its unusual pigmentation.

Description. Small (up to 2–3 mm long) for the genus, agile, fast moving species. Cephalic area provided with a pigment band, white in reflected and black in transmitted light, starting from the brain and progressively broadening apically (Fig. 7J). With two pigmented eyes within the brain case. Epithelium intranucleated, wholly ciliated (cilia about 7 µm long). With numerous adhesive glands in the posterior body. Body musculature with both circular and longitudinal components weak.

Immature specimens had three longitudinal rows of isolated nematocysts, neatly spaced along the length of the body, and a shortly elongate pharynx in the posterior part of body (Fig. 8C). Mature specimens were distinctly smaller than most immatures, without any trace of pharynx, and lacking the longitudinal rows of nematocysts (Fig. 7J). In these specimens, the gut could still be traced, although with difficulty, and the presence of some nematocysts in the gut could be noticed.

Male genital system. About 20 testes regularly arranged in two lateral rows, in front of the copulatory organ, which consists of a bulb, with a stylet, and two seminal vesicles (Fig. 7I). The bulb is over 100 μm long and 30 μm broad, with thick muscular walls. It is placed inside a wide atrium, provided with a large male pore in its anterior part. The bulb is lined internally with a ciliated epithelium; proximally, the epithelium is high and strongly glandular. The bulb is connected to two seminal vesicles (Figs. 7I and 8D). The 'intra-atrial' trait of the seminal vesicles is narrow, and internally ciliated. Just outside the atrium, the seminal vesicles widen and are surrounded by a thick coating of circular muscles. Caudally, the muscular coating disappears abruptly, and the seminal vesicles appear narrower, and lined by a thin epithelium. The two vesicles fuse caudally into a long, median single seminal vesicle.

The stylet is a recurved tube, about 50 μm long, with a slightly flaring proximal opening 20 μm wide and 8 μm high; the tube is 3 μm in diameter, and ends into a slightly oblique distal tip (Fig. 8F).

Female genital system. With 4–5 isolated oocites, more or less regularly arranged into a single line between the caudal testes (Fig. 7J). Vitellaria only observed caudal to the copulatory organ. The ciliated oviducts fuse just behind the bulb into an extremely short female duct, which opens through the female pore among the seminal vesicles (Fig. 8D).

Diagnosis. A small species of *Nematoplana*, with a cephalic pigment band, white in reflected light, and with pigmented eyes. Without chorda intestinalis. Juveniles with three longitudinal rows of nematocysts, and a short horizontal pharynx. Adult specimens without pharynx and nematocysts. With a large male atrium, and a recurved, tubular stylet about 50 μm long. With two seminal vesicles fused caudally. With few, isolated oocites in a single row.

Remarks. Immature specimens were frequently encountered during the workshop, and raised particular attention for their morphology and pigmentation. On the contrary, only two mature specimens were found, which allowed to place the animals into the genus *Nematoplana* Meixner, 1938: the seminal vesicles fused posteriorly are in fact an important apomorphic feature of the genus (Curini-Galletti and Martens 1992).

The species is immediately recognisable as new. A recurved tubular stylet similar to that of the new species is only found *Nematoplana rubra* Curini-Galletti, Oggiano & Casu, 2002, a comparably peculiar species from eastern Australia, crimson-red for the presence of pigment in the parenchyme, and with rows of sponge spiculae instead of nematocysts. The single line of oocytes is rare as well, and was found in several small sized species from eastern Australia, and is possibly linked to small adult size (Curini-Galletti et al. 2002). Cephalic pigmentation and presence of eyes make the species similar to *N. nigrocapitula* Ax, 1966, which however is much larger, and, at least in mature specimens from the Galapagos Is. attributed to the taxon (Ax and Ax 1974), with a completely different morphology of stylet.

Degeneration of the pharynx is not unusual in Nematoplanidae. In species of *Ezoplana* Tajika, 1982, and in *Nematoplana ezoplanoides* Curini-Galletti, Oggiano & Casu, 2002, two pharynges are present in at least part of the life cycle: the posterior one degenerates while a new pharynx forms anteriorly to it (Tajika 1982; Curini-Galletti et al. 2002). In other cases, the worm stops feeding altogether, and the pharynx disappears completely; apparently, this is linked to the attainment of female maturity (Marcus 1950, Sopott 1972; Ax and Ax 1974; Ehlers and Ehlers 1980). This latter case applies to the new species. The consequences of cessation of nutrition at maturity are indeed visible: the species feeds, at least partially, on cnidarians, and stores dorsally the nematocysts of the prey as a defensive weapon (see Karling 1966b). The lack of continuous supply may either cause degeneration of nematocysts, or, more likely, the worm utilises them as food—in pharynx-less adults, nematocysts, in various states of degeneration, are seen in the gut, and they completely lack the regular rows of nematocysts seen in juveniles. Furthermore, adults are appreciably smaller than juveniles, and it is likely that they may actually resorb body tissues, paralleling a process well known in starved planarians (Gonzalez-Estevez et al. 2012).

Conclusions

Of the 24 species of Proseriata described by Marcus, 16 species (including the tentative identification of *Itaspis evelinae*) were found. Among the 'missing' species, some were originally found in specific habitats, which were not sampled during the workshop: *Necia sopha* Marcus, 1950, was found on algae; *Peraclistus itaipus* Marcus, 1950, was found inside tubes of Terebellidae (Polychaeta), and *Promonotus erinaceus* Marcus, 1950 and *P. villacae* Marcus, 1949 were both caught by bait at the mouth of rivers, in waters with very low salinity content (Marcus 1949, 1950). *Nematoplana naia* Marcus, 1949 is only known from Santos Bay, west of our sampled area. The three remaining species (*Vannuccia talea* Marcus, 1954; *Tabaota curiosa* Marcus, 1950, and *Togarma evelinae* Marcus, 1949) are known to occur in intertidal coarse sand on exposed beaches. The habitat has not been intensively sampled during the workshop. It is however possible that the juvenile Unguiphora found, which could not be assigned to *N. asita* nor *N. mirabilis* sp. nov., might indeed belong to either *Tabaota curiosa* or *Togarma evelinae*: in both species the discriminating features only develop at maturity.

With the possible exceptions of *Minona divae* and *Monocelis tabira*, none of the diversified S.E. Brazilian Proseriate fauna has been found elsewhere. Proseriata are

indeed known to have limited distribution ranges (Curini-Galletti et al. 2012), and the contribution of the taxon to marine biodiversity is still very incompletely known (see Appeltans et al. 2012). It is therefore regrettable that technical problems with the fixatives used did not allow a more detailed description of the diversity of the taxon in the area.

Finally, it is remarkable that most of the new species came from the few subtidal samples taken during the workshop. It is thus quite likely that our knowledge on S.E. Brazil Proseriata is still very incomplete, with a large subtidal diversity almost unscratched, leaving ample possibility for further research on the taxon in the area.

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