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## State of knowledge of the Acotylea (Polycladida, Platyhelminthes) from the Mediterranean coasts of Spain: new records and new species

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### Abstract

Along the Mediterranean coast of the Iberian Peninsula, great species diversity is thought to exist, but our knowledge of Iberian polyclads is, in fact, very limited. This study contributes to the Polycladida (Platyhelminthes) of the Iberian Peninsula, in particular those of the Mediterranean coast. Nine species, mainly new species or new records, are described. *Imogine stellae* sp. nov. from Mar Menor (Murcia, Spain) is described, while *I. mediterranea* Galleni, 1976 is recorded for the first time in Spain. The genus *Planocera* Blainville, 1828 within the Mediterranean basin is reviewed: *Planocera graffi* Lang, 1879 is redescribed, and its synonymisation with *Planocera pellucida* (Mertens, 1833) considered. Also, the genus *Notoplanella* Bock, 1931 is represented by two species in Spain, *N. inarmata* Bock, 1931 type species, from Formentera Island and *N. estelae* sp. nov., from Mar Menor. *Trigonoporus cephalophthalmus* Lang, 1884 is rediscovered after the description of Lang (1884). *Stylochus neapolitanus* (Delle Chiaje, 1841–1844) Lang, 1884 is recorded and *S. pilidium* (Goette, 1881) is also redescribed, and *Leptoplana mediterranea* (Bock, 1913) is newly recorded for the Iberian Peninsula.

**Key words:** Polycladida, marine flatworms, Iberian Peninsula, Mediterranean Sea, distribution, taxonomy

### Introduction

*Yungia dicquemari* (Risso 1818) Lang 1884 and *Thysanozoon brocchi* (Risso 1818) Grube 1840 are possibly the first reliable records for polyclads within the Mediterranean basin, although originally described within the phylum Mollusca as *Tergipes dicquemari* Risso, 1818 and *Tergipes brocchi* Risso, 1818. Shortly thereafter, Stefano Delle Chiaje (1822–1829) wrote an exhaustive study on invertebrates of the Kingdom of Naples. Several polyclad species were described, but all were included in the genus *Planaria*, which was in the order Tricladida at the time. Delle Chiaje wrote a similar study for Sicily in 1841 in which all of the described polyclad species were also included in the genus *Planaria*.

In addition to the works of Delle Chiaje, Adolf Eduard Grube (1840) produced a compilation describing marine invertebrates (mainly Actinia, Echinodermata and Vermes) of the Mediterranean and Adriatic Seas. In this work, several polyclad species belonging to the genera *Stylochus*, *Leptoplana* and *Thysanozoon* were briefly mentioned. Later, Jean Louis Armand de Quatrefages (1845) published an impressive study describing several genera including *Tricelis* Quatrefages, 1845 (currently *Cestoplana* Lang, 1884), *Polyclelis* Quatrefages, 1845 (including several species that nowadays belong to other genera of Polycladida), *Prosthiostomum* Quatrefages, 1845, *Proceros* Quatrefages, 1845 (currently *Pseudoceros* Lang, 1884), *Eolidiceros* Quatrefages, 1845 (currently *Thysanozoon* Grube, 1840) and *Stylochus* Ehrenberg, 1831.

In 1884, Arnold Lang published a detailed study of polyclads from the Mediterranean Sea, particularly those found in the Gulf of Naples. Lang's study marked a turning point in the field of polyclad taxonomy, as most of his discoveries and results are still considered essential for systematic studies of this group within the Mediterranean. Subsequent studies are highly specific or restricted because only a small number of species from localised regions

were described. However, these studies are essential for understanding the distribution and biodiversity of Mediterranean polyclads. These works include the description of *Cestoplana raffaelei* Ranzi, 1928 (currently *Acestoplana raffaelei* (Ranzi, 1928)) from the Gulf of Naples, a review of polyclads from Sebastopol by Jakubova (1909), especially his studies of the family Stylochidae, contributions by Ludwig van Graff (1886, 1889) on the Polycladida of the Adriatic Sea, which includes a description of *Enantia spinifera* Graff, 1889 (Family Enantiidae Graff, 1889), an extremely peculiar species (with cuticular spines along the body edge) that was found by that author in the summer of 1876, but has never since been recaptured, and more recently, a revision of the genus *Leptoplana* within the Mediterranean Sea (Gammoudi *et al.* 2012).

For the Iberian Peninsula, high invertebrate species diversity along the Mediterranean coast is recognised (Hofrichter 2003), but our knowledge of Iberian polyclads is, in fact, very limited. The first comprehensive study of the order Polycladida in the Iberian Peninsula is the thesis of Carles Novell (2003) in which 22 different polyclad species found along the Catalan coast were recorded. Since then, few records for Iberian polyclads have been published, though these include studies in which *Echinoplana celerrima* Haswell, 1907 is mentioned for the first time (e.g., Gammoudi and Tekaya 2012).

In this study, we focus on the acotylean genera and species from the Mediterranean coast of the Iberian Peninsula, specifically the coastline from Castellón to Almuñecar (Granada), including Mar Menor (Murcia, Spain) and various islands (Fig. 1, Table 1).

## Material and methods

Specimens of *Imogine stellae* sp. nov. were collected between and underneath old bricks and rubble covered with periphyton and algae on the eastern seashore of Mar Menor, Murcia, ( $37^{\circ}46'16.10''N$ ,  $0^{\circ}45'02.50''W$ ) in August 2011. The specimens were found in shallow warm waters from 0.2 to 0.4 metres deep and water temperatures of approximately  $30^{\circ}C$ .

Specimens of *Planocera graffi* were collected on the seashore of Calpe at ‘Raco Beach’ ( $38^{\circ}38'07.26''N$ ,  $0^{\circ}04'15.28''E$ ), and near Cabo de Palos ( $37^{\circ}37'49.11''N$ ,  $0^{\circ}41'46.38''W$ ) from waters 2 to 5 metres deep and a water temperature of  $20^{\circ}C$ . They were found next to a *Posidonia oceanica* meadow, underneath rocks covered with green epiphytic algae that were sitting on a sandy bottom.

Specimens were collected live, placed into plastic bottles and then transported to the laboratory. Animals were fixed in Bouin’s fluid, following the Newman and Cannon method (Newman and Cannon 2003). Photographs were taken of both live and fixed animals to retain maximum information regarding shape, colouration and internal anatomy.

*Notoplanella estelae* sp. nov., *Leptoplana mediterranea*, *Imogine mediterranea*, *Stylochus neapolitanus* and *Stylochus pilidium* were collected in Mar Menor (Murcia) during 2006, as part of the project titled: “Food web reply to nutrient inputs in a coastal lagoon (EUTRO-COST)” (Ecology and Management of Coastal Marine Ecosystems research group, University of Murcia. Principal Investigator: Angel Pérez-Ruzafa).

*Notoplanella inarmata* and *Trigonoporus cephalophthalmus* were collected over two periods, during Iberian Fauna oceanographic campaigns III and IV in 1994 and 1996, respectively. Iberian Fauna oceanographic campaign III (June–July 1994) occurred in the Balearics and Columbretes Islands, while Iberian Fauna oceanographic campaign IV (June–July 1996) took place in the Columbretes and Hormigas Islands and Alborán Island. Samples were obtained by SCUBA diving in depths from 0 to 44 metres, and from dredges up to 400 metres deep. In Mar Menor, rocky surfaces were sampled by total scratching, while soft bottoms were sampled by dredging. Specimens were fixed with 4% formaldehyde and preserved in 70% ethanol.

Specimens were subsequently embedded in paraplast, serially sectioned at intervals between 7 to 10  $\mu m$  and stained with AZAN (trichrome staining method). Reconstructions of internal anatomy were derived from serial sagittal sections. Measurements were determined from fixed material.

Holotypes were deposited into the Invertebrate Collection of the Museo Nacional de Ciencias Naturales de Madrid (MNCN) in Spain.

**TABLE 1.** Acotylean species of the Mediterranean shores of the Iberian Peninsula.

Species	Sites	Coordinates	Collection	Substrate	Depth (m)
<i>Discocelis tigrina</i> (Blanchard, 1847) Lang, 1884	Catalan coast	41°22' N-02°11' E; 41°40' N-02°48' E; 42°17' N-03°16' E;	SCUBA diving	Rocks, algae	0 - 18
<i>Pleioptana atomata</i> (O. F. Müller, 1776) Faubel, 1983	Catalan coast	41°50' N-03°08' E 41°40' N-02°48' E	SCUBA diving	Rocks, <i>Codium</i>	0 - 15
<i>Notoplana vitrea</i> (Lang, 1884) Bock, 1913	Catalan coast	41°40' N-02°48' E	SCUBA diving	Rocks	15
<i>Complana agilis</i> (Lang, 1884)	Catalan coast	41°40' N-02°48' E	SCUBA diving	Rocks, algae, <i>Posidonia oceanica</i>	7 - 12
<i>Hoploplana villosa</i> (Lang, 1884) Laidlaw, 1902	Catalan coast	41°40' N-02°48' E	SCUBA diving	Algae	9
<i>Notoplana inarmata</i> Bock, 1931	Formentera Island (12 miles East)	38°45' N-01°18' E; 38°43' N-01°18' E	Dredging	Rocks	126 - 134
<i>Notoplana estelae</i> sp. n.	Lo Pagán; (Mar Menor, Murcia)	37°48' N-00°47' W	SCUBA diving	Rocks	0.2 - 1
<i>Leptoplana mediterranea</i> Bock, 1913	Isla del Ciervo, Lo Pagán, Los Urrutias; (Mar Menor, Murcia)	37°48' N-00°47' W; 37°40' N-00°49' W; 37°39' N-00°44' W;	SCUBA diving, dredging	Clays, <i>Caulerpa prolifera</i>	2
<i>Trigonoporus cephalophthalmus</i> Lang, 1884	Columbretes Islands, Hormigas Islands, Alborán Island (3 miles North)	39°52' N-00°40' E; 37°39' N-00°38' W; 35°58' N-02°58' W	SCUBA diving	Rocks	14.3; 23.5; 36
<i>Planocera pellicula</i> , Mertens, 1833	Calpe (Alicante, Spain) at the 'Raco beach'; Cabo de Palos (Murcia, Spain)	38° 38'N-0°04' E	SCUBA diving	Underneath rock covered with green epiphytic algae	2 - 5
<i>Planocera ceratommata</i> (Palombi, 1936) Faubel 1983	Catalan coast	41°40' N-02°48' E; 41°53' N-03°11' E	SCUBA diving	Rocks, <i>Codium</i>	17
<i>Imagine mediterranea</i> Galleni, 1976	Los Urrutias; Mar Menor (Murcia, Spain)	37°40' N-00°49' W	SCUBA diving	Rocks	0.2 - 0.5
<i>Imagine stellae</i> sp. nov.	El Estacio; Mar Menor (Murcia, Spain)	37°46'N-0°45'W	SCUBA diving	Old brick and rubble covered with periphyton	0.2 - 0.5
<i>Stylochus neapolitanus</i> (Delle Chiaje, 1841-1844) Lang, 1884	Los Urrutias; Mar Menor (Murcia, Spain)	37°40' N-00°49' W	SCUBA diving	Rocks, <i>Caulerpa prolifera</i>	0.2 - 2
<i>Stylochus pilidium</i> (Goette, 1881)	Los Urrutias; Mar Menor, (Murcia, Spain)	37°40' N-00°49' W	Dredging	<i>Caulerpa prolifera</i>	2

## Systematics

**Suborder ACOTYLEA Lang, 1884**

**Superfamily LEPTOPLANOIDEA Faubel, 1984**

**Family CRYPTOCELIDAE Laidlaw, 1903**

**Genus *Notoplanella* Bock, 1931**

***Notoplanella inarmata* Bock, 1931**

Figs. 1 (1), 2, 3

**Material examined.** One mature specimen sagittally sectioned, collected in Formentera Island from rocky substrate found between 126–134 metres deep.

**Morphological remarks.** This specimen is consistent with Bock's description of *Notoplanella inarmata*. Unfortunately, the description was realized only on fixed material, therefore the information about the colour is scarce. The fixed individual presents an oblong-oval body shape, lacking tentacles and whitish-grey background colouration with reddish-brown speckles arranged in lines, diverging from the centre to the margins. Marginal band and pharynx region free of pigment spots (Fig. 2A). Paired cerebral and tentacular eye clusters present (Fig. 2B, 3A). Pharynx ruffled central, oral pore at the posterior end (Fig. 2C).

Within the male reproductive system (Fig. 2D), a narrow duct connects the seminal vesicle to the prostatic vesicle (Fig. 3B). The prostatic vesicle is interpolated, enlarged, with numerous secretory ducts. Unarmed penis papilla (Fig. 3C). The female apparatus consists of a muscular external vagina, with the common oviduct clearly differentiated and without Lang's vesicle. Unlike to the description of *N. inarmata* from Cape Town (Bock 1931), both gonopores, female and male, are well separated (Fig. 2D), and the musculature of the body wall surrounding the female genital pore (Fig. 3D) is considerably thickened. This feature was also recognised by Prudhoe (1989), when he examined specimen from Cape Province dredged in 19 metres deep, also from Danger Point and Saldanha Bay.

**Distribution.** South Africa South Africa (Simon's Bay, Bock 1931; False Bay, Day *et al.* 1970; Cape Province, Danger Point, Saldanha Bay, Prudhoe 1989). This is the first record for the Mediterranean Sea.

***Notoplanella estelae* sp. nov.**

Figs. 1 (2), 4, 5

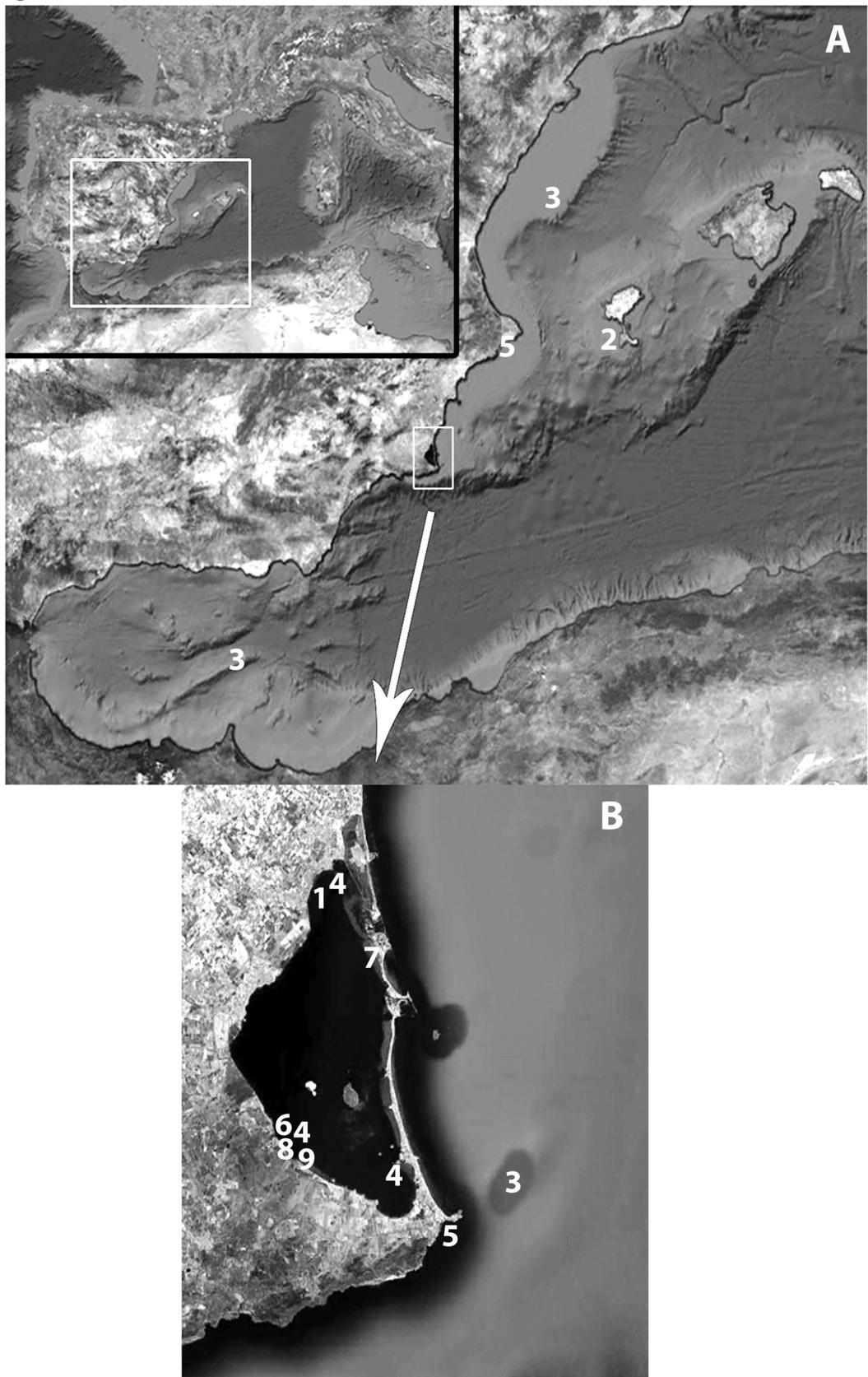
**Type locality.** Lo Pagán, Mar Menor, 37°48' N, 00°47' W. Collected by SCUBA diving; from rocky substrate found between 0.2–1 metres deep; Fig. 1 (2).

**Type material.** Holotype: one mature specimen sagittally sectioned; MNCN 4.01/200 to MNCN 4.01/215 (15 slides). Paratype: one mature specimen sagittally sectioned; MNCN 4.01/216- MNCN 4.01/231 (16 slides). Leg. Angel Pérez-Ruzafa.

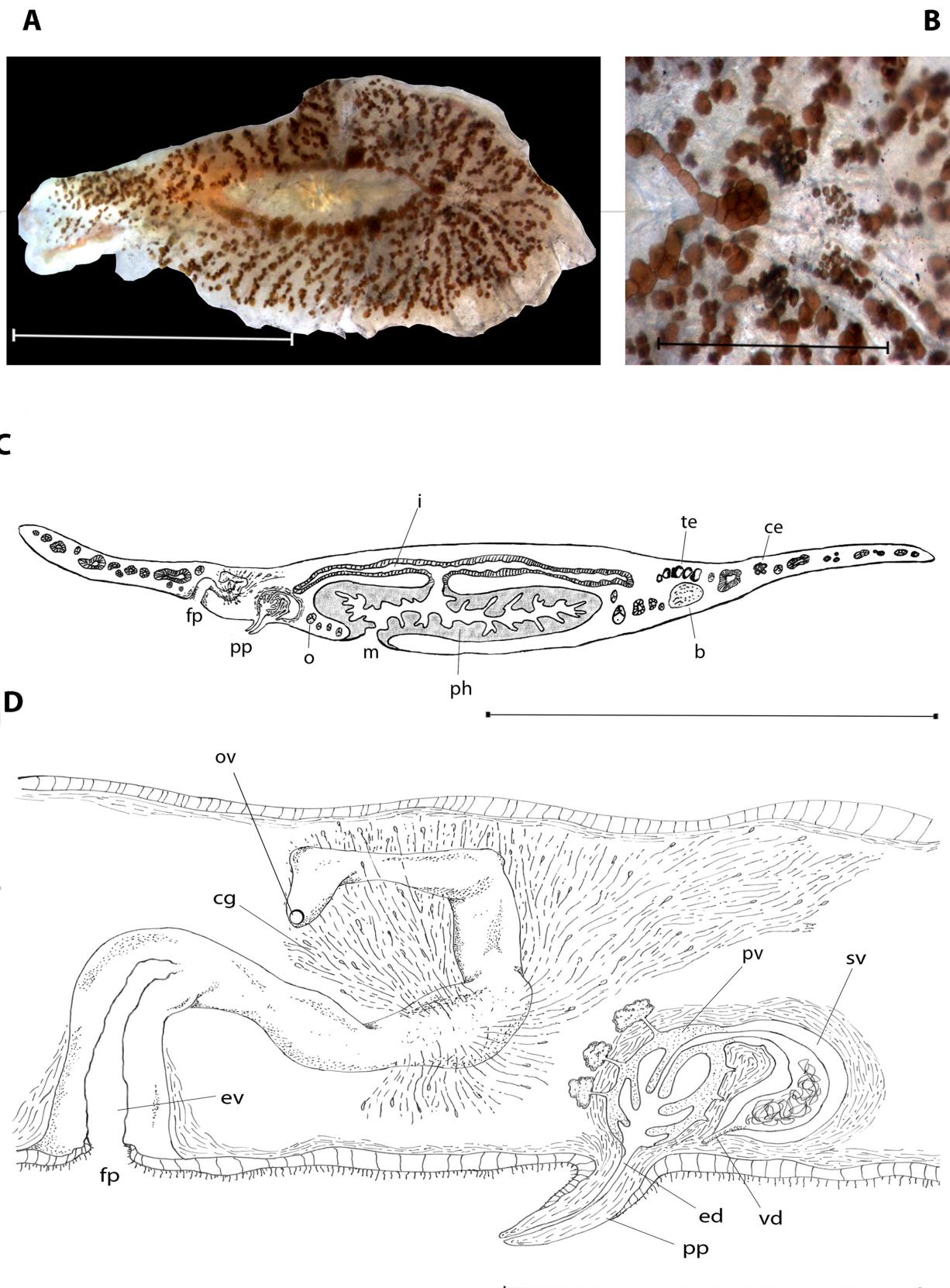
**Material examined.** Two mature (MNCN 4.01/200 to MNCN 4.01/215 (15 slides) and MNCN 4.01/216- MNCN 4.01/231 (16 slides)) and two juvenile specimens sagittally sectioned. The material is deposited in the invertebrate collection of Museo Nacional de Ciencias Naturales de Madrid (Spain). Collected by Angel Pérez-Ruzafa.

**Etymology.** The specific epithet is dedicated to Estela Barroso, girlfriend of the first author.

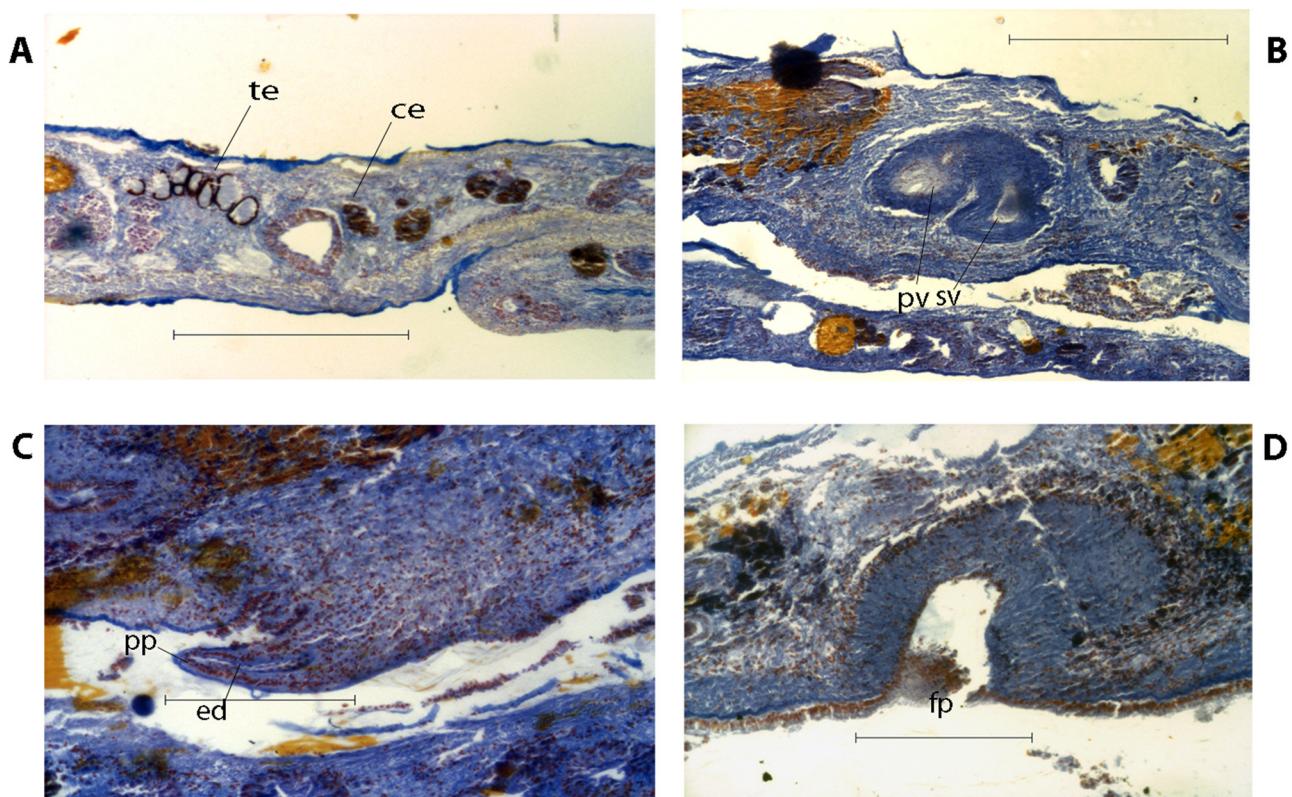
**Diagnosis.** *Notoplanella* with numerous dark spots on the dorsal surface, except on the median line. With nuchal tentacles. Tentacular and cerebral eyes present. A short duct links the seminal and the prostatic vesicles and shows a dilatation before the prostatic vesicle. Large penis papilla. Female system with a small Lang's vesicle. Gonopores separated.



**FIGURE 1.** Distribution map of examined specimens. General views of **A** the eastern coast of the Iberian Peninsula, **B** Mar Menor (Murcia). Numbers: *Notoplanella inarmata* (1), *Notoplanella estelae* sp. nov. (2), *Trigonoporus cephalophthalmus* (3), *Leptoplana mediterranea* (4), *Planocera graffi* (5), *Imogine mediterranea* (6), *Imogine stellae* sp. nov. (7), *Stylochus neapolitanus* (8), *Stylochus pilidium* (9).



**FIGURE 2.** *Notoplanellea inarmata* (Bock, 1931). **A** Habitus; **B** eyes; **C** sagittal reconstruction of the whole animal (anterior to the right); **D** sagittal reconstruction of the male and female genital systems (anterior to the right). Abbreviations: b, brain; ce, cerebral eyes; cg, cement glands; ed, ejaculatory duct; ev, external vagina; fp, female pore; i, intestine; m, mouth; o, ovary; ovd, pharynx; pp, penis papilla; pv, prostatic vesicle; sv, seminal vesicle; te, tentacular eyes; vd, vas deferens. Scale bars: **A** 5mm, **B** 500µm, **C, D**, 200µm.



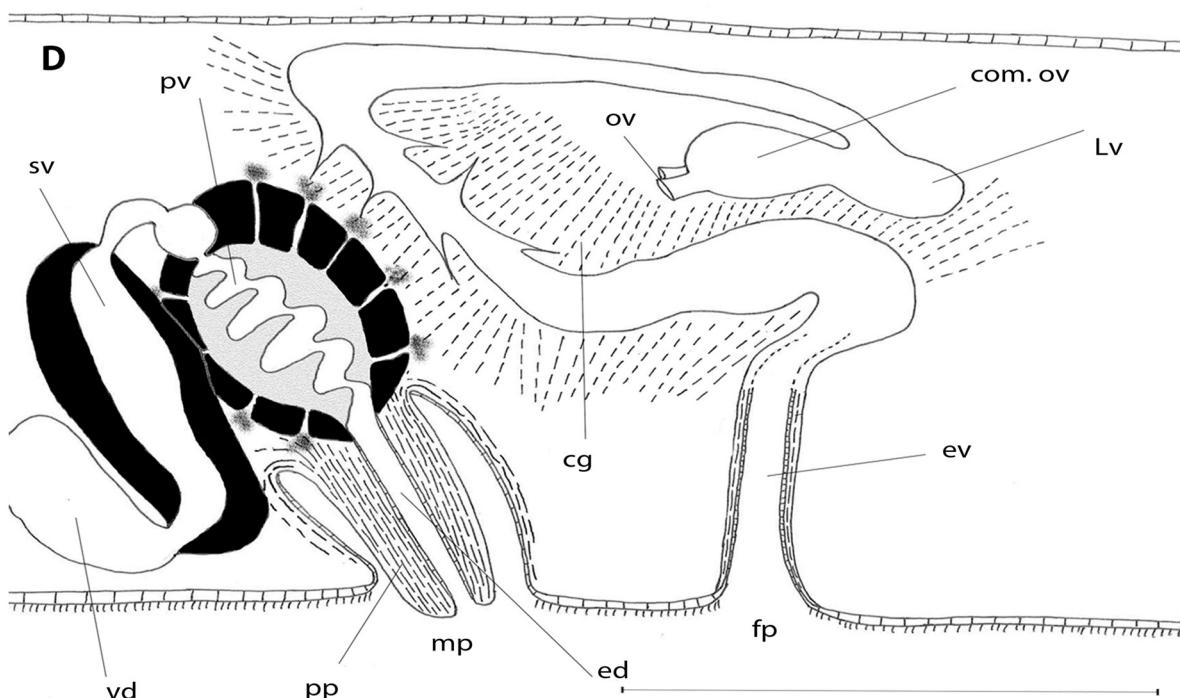
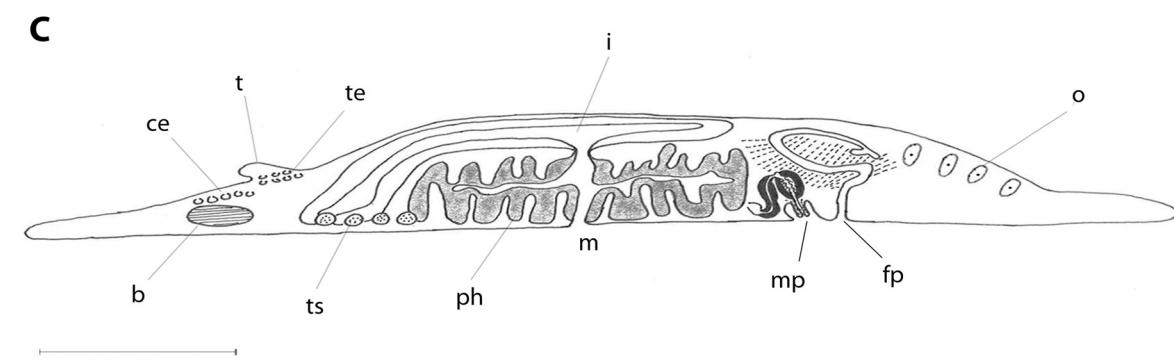
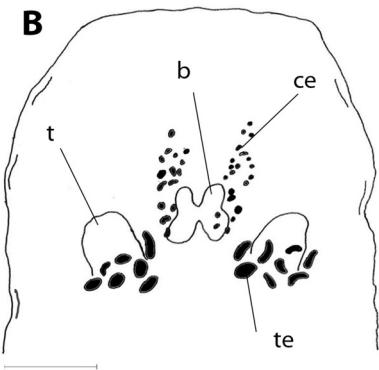
**FIGURE 3.** Histological sections of *Notoplanella inarmata* (Bock, 1931). **A** eyes; **B** male copulatory complex; **C** penis papilla; **D** external female reproductive system. Abbreviations: ce, cerebral eyes; ed, ejaculatory duct; fp, female pore; pp, penis papilla; pv, prostatic vesicle; sv, seminal vesicle; te, tentacular eyes. Scale bars: **A** 500µm, **B, C, D** 200µm.

**Description.** Fixed specimen is 7 mm long by 2 mm wide. Body elongated, anteriorly truncated, lateral margins almost parallel, posteriorly slightly rounded. Ground colour pale, with numerous smaller and larger dark spots on the dorsal surface, except along above the pharynx and the genital region. Sometimes the smaller spots arranged in small interconnected groups. Marginal band free of spots (Fig. 4A). Small nuchal tentacles present. Tentacular eyes primarily at the bases and some inside the tentacles (Figs. 4B, 5A). The cerebral and pre-cerebral eyes form a single and elongated undifferentiated group; marginal or frontal eyes lacking. Tentacular eyes larger than cerebral ones. Bilobated brain with two symmetrical lobes (Fig. 4B). The pharynx occupies the middle third of the body, mouth opening at the medial region (Fig. 4C).

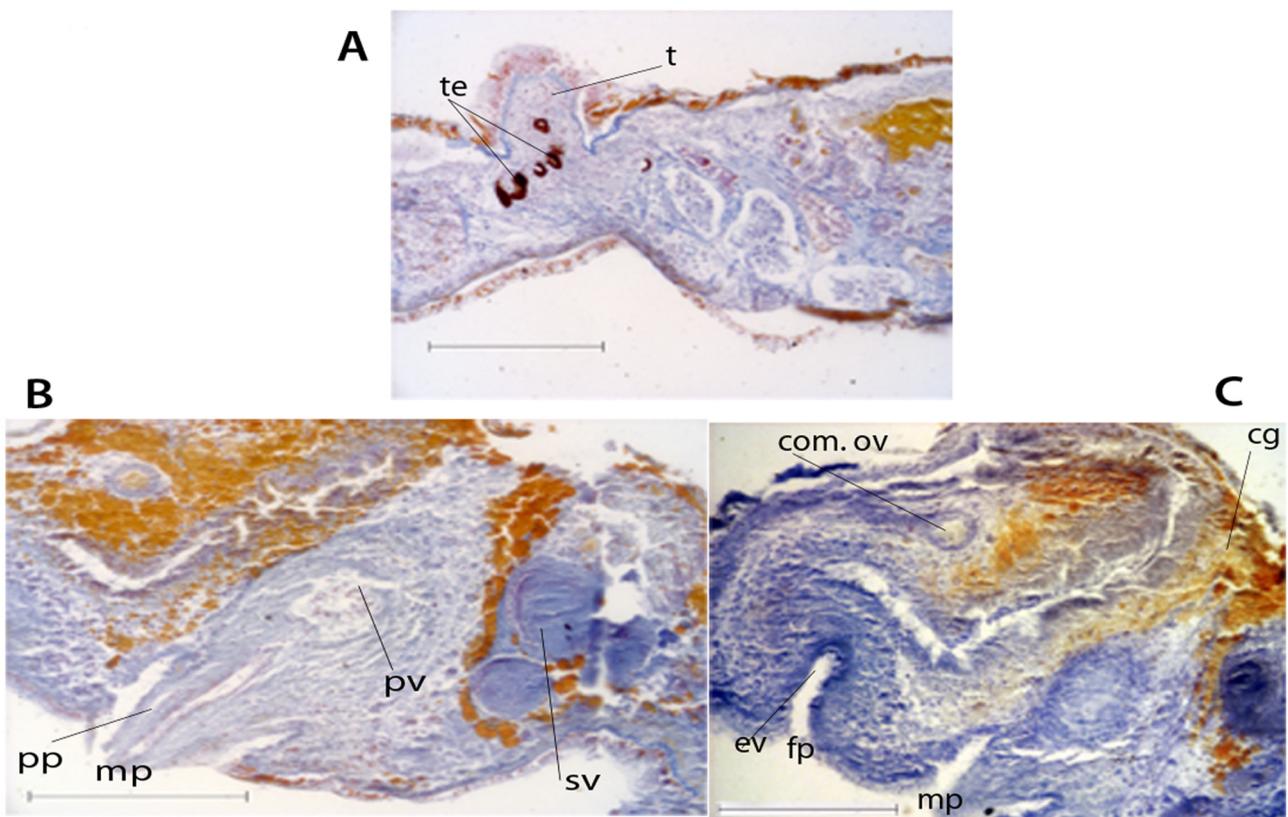
Testes and ovaries extend on both sides of the body; testes ventral and ovaries dorsal. Gonopores separated. Male copulatory apparatus, directed backwards, begins almost immediately behind the pharyngeal cavity. Deferent ducts become wider before entering ventrally, describing an arc, into the seminal vesicle (Fig. 4D). Seminal vesicle with thick muscular walls. Elliptic prostatic vesicle (65 µm in length and 40µm in width) interpolated, with a strong muscular envelope and inner epithelium folded, with eosinophilic secretion. Seminal and prostatic vesicle connected by a short narrow duct that widens at the end. Ejaculatory duct straight, with undifferentiated inner epithelium. Large and unarmed penis papilla, conical-shaped, housed in a non-ciliated atrium (Fig. 5B).

Female system with vestigial Lang's vesicle. Oviducts lead together into an unpaired organ, anterior to the poorly developed Lang's vesicle. The narrow proximal tract of the vagina runs forwards, turns backwards, then becomes wider and receives secretions from well developed cement glands. The external vagina is short and narrow, and runs straight down to the gonopore (Figs. 4D, 5C).

**Discussion.** The genus *Notoplanella* has the only species *Notoplanella inarmata* from South Africa Bock, 1931. With the discovering and description of *N. estelae*, the genus has currently increased to two species. The specimen captured in Mar Menor clearly belongs to the genus *Notoplanella*. It exhibits characteristics of the genus, such as an oval-shaped body, paired cerebral and tentacular eye clusters, a central pharynx, a true seminal vesicle connected by a narrow duct to a large elliptic and interpolated prostatic vesicle with strong muscle layers, an unarmed penis papilla, a weak muscular vagina and a common oviduct (Bock 1931).



**FIGURE 4.** *Notoplanella estelae* sp. nov. **A** Habitus; **B** dorsal view of the anterior region; **C** sagittal reconstruction of the whole animal (anterior to the left); **D** sagittal reconstruction of male and female genital systems (anterior to the left). Abbreviations: b, brain; ce, cerebral eyes; cg, cement glands; com. ov, common oviduct; ed, ejaculatory duct; ev, external vagina; fp, female pore; i, intestine; Lv, Lang's vesicle; m, mouth; mp, male pore; o, ovary; ov, oviduct; ph, pharynx; pp, penis papilla; pv, prostatic vesicle; sv, seminal vesicle; t, tentacle; te, tentacular eyes; ts, testes; vd, vas deferens. Scale bars: **A** 2mm, **B** 500µm, **C** 1mm, **D** 200µm.



**FIGURE 5.** Histological sections of *Notoplanella estelae* sp. nov. **A** Tentacle region and eyes (anterior to the left); **B** male copulatory complex (anterior to the right); **C** female reproductive system (anterior to the right). Abbreviations: cg, cement glands; com. ov, common oviduct; ev, external vagina; fp, female pore; mp, male pore; pp, penis papilla; pv, prostatic vesicle; t, tentacle; te, tentacular eyes; sv, seminal vesicle. Scale bars: **A** 100 $\mu$ m, **B, C** 200 $\mu$ m.

*Notoplanella estelae* sp. nov., in contrast to *N. inarmata*, possesses nuchal tentacles, located slightly behind the brain. The brain is bilobated in both species; however, in *N. inarmata*, the anterior end of the lobes is pointed and larger than the posterior end (Bock 1931), while in *N. estelae*, the lobes are rounded in both extremes and the posterior end is larger than the anterior. Both species of the genus also have centrally-located pharynges, although in *N. inarmata*, the oral pore opens at the posterior end of the pharynx, while in *N. estelae*, it opens at the centre.

The main differences in the reproductive system largely correspond to the male complex. The characteristic duct between the seminal and prostatic vesicles is much shorter in *N. estelae* and enlarges before entering the prostatic vesicle. In addition, the prostatic vesicle is smaller and the penis papilla is much larger in *N. estelae* than in *N. inarmata*. The only difference in the female system between these two species is the presence of a vestigial Lang's vesicle in *N. estelae*.

In the description of *N. inarmata*, Bock (1931) asserts that the opening of the oviducts suggests its ancestor had a Lang's vesicle and, furthermore, that the structure of the proximal portion of the vagina can only be explained owing to this character. Thus, the presence of a vestigial Lang's vesicle in *N. estelae* is compatible with belonging to the genus *Notoplanella*.

The presence of nuchal tentacles and a vestigial Lang's vesicle in *N. estelae* requires the addition of those characters to the genus' diagnosis. Therefore, the genus diagnosis is now as follows:

**Diagnosis** (Bock 1931) **emended**: Cryptocelidae with oblong-oval body shape, with or without tentacles. Paired cerebral and tentacular eye clusters present. Pharynx central. True seminal vesicle connected to the prostatic vesicle by a narrow duct. Interpolated large prostatic vesicle, elliptic, with numerous secretory ducts. Unarmed penis papilla. Weak muscular vagina, with or without a vestigial Lang's vesicle, with the common oviduct clearly differentiated.

## Family LEPTOPLANIDAE Stimpson, 1857

### Genus *Leptopiana* Ehrenberg, 1831

#### *Leptopiana mediterranea* (Bock, 1913)

Synonymous: *Leptopiana tremellaris* forma *mediterranea* Bock, 1913

**Material examined.** Six mature specimens, collected in Isla del Ciervo, Lo Pagán and Los Urrutias (Mar Menor, Murcia). From clays and *Caulerpa prolifera* (Chlorophyta) found two metres deep; Fig. 1 (4).

**Morphological remarks.** As described in Gammoudi *et al.* (2012), the position and structure of the proximal chamber of the prostatic vesicle, and its ventral attachment to the seminal vesicle, are similar in all six specimens and confirms that our exemplars belong to *L. mediterranea*, supporting the hypothesis that *L. mediterranea* is the only representative of the genus in the Mediterranean Sea (Gammoudi *et al.* 2012).

**Distribution.** Well documented in the Mediterranean Sea (although also reported as *Leptopiana tremellaris*) by Grube 1840 (Palermo); Lang 1884 (Naples); Lo Bianco 1888, 1899 (Naples); Pruvot 1897 (Gulf of Lyon); Micoletzky 1910 (Trieste); Palombi 1928 (Port Said), 1936 (Naples); Steinböck 1933 (Adriatic Sea); Arndt 1943 (Naples); Galleni & Gremigni 1989 (Italian coasts); Novell 2003 (Catalonia); Gammoudi *et al.* 2012 (Tunisia); and here for Mar Menor (Murcia, Spain).

## Superfamily STYLOCHOIDEA Poche, 1926

### Family CALLIOPLANIDAE Hyman, 1953

#### Genus *Trigonoporus* Lang, 1884

##### *Trigonoporus cephalophthalmus* Lang, 1884

Figs. 1 (3), 6, 7

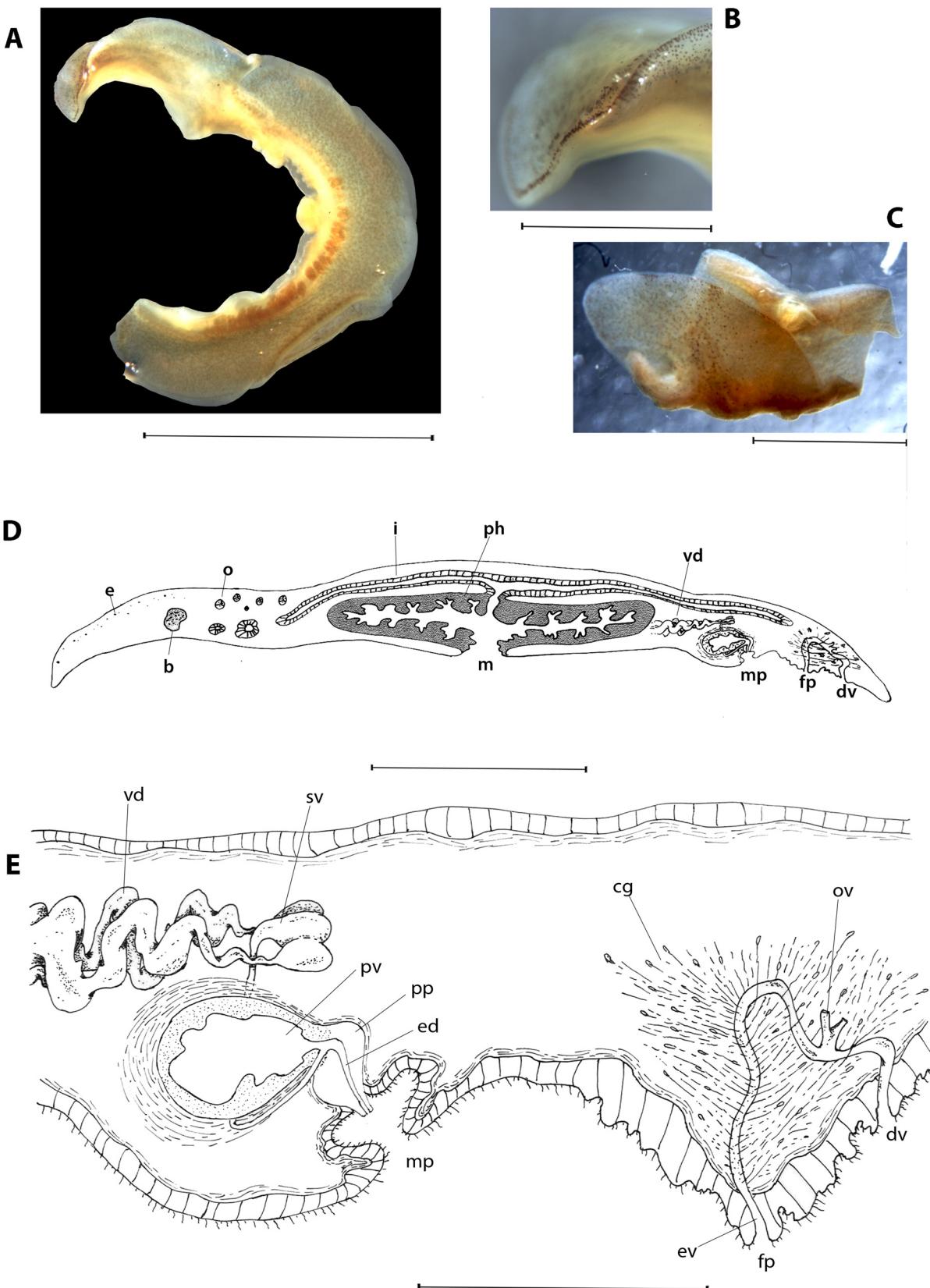
**Material examined.** Three mature specimens, collected in the Columbretes and Hormigas Islands and Alborán Island from rocky substrate found at 14.3, 23.5 and 36 metres deep, respectively for each location; Fig. 1 (3).

**Description.** Fixed specimen is approximately 2 cm long by 5 mm wide. Very elongated body. Colour whitish-yellow, partially lost during conservation, with numerous and small brown spots (Fig. 6A). Region between the brain and the anterior margin strewn with numerous small eyes spreading out in a fan-like manner (Fig. 6B). Marginal eyes arranged along the frontal margin of the body. Tentacles and tentacular eyes lacking. Brain located about 2 mm from the anterior margin (Figs. 6C, 7C). Ruffled pharynx situated in the middle third of the body with the oral pore opening in the centre of it. Ovaries dorsal. Separated gonopores (Fig. 6C).

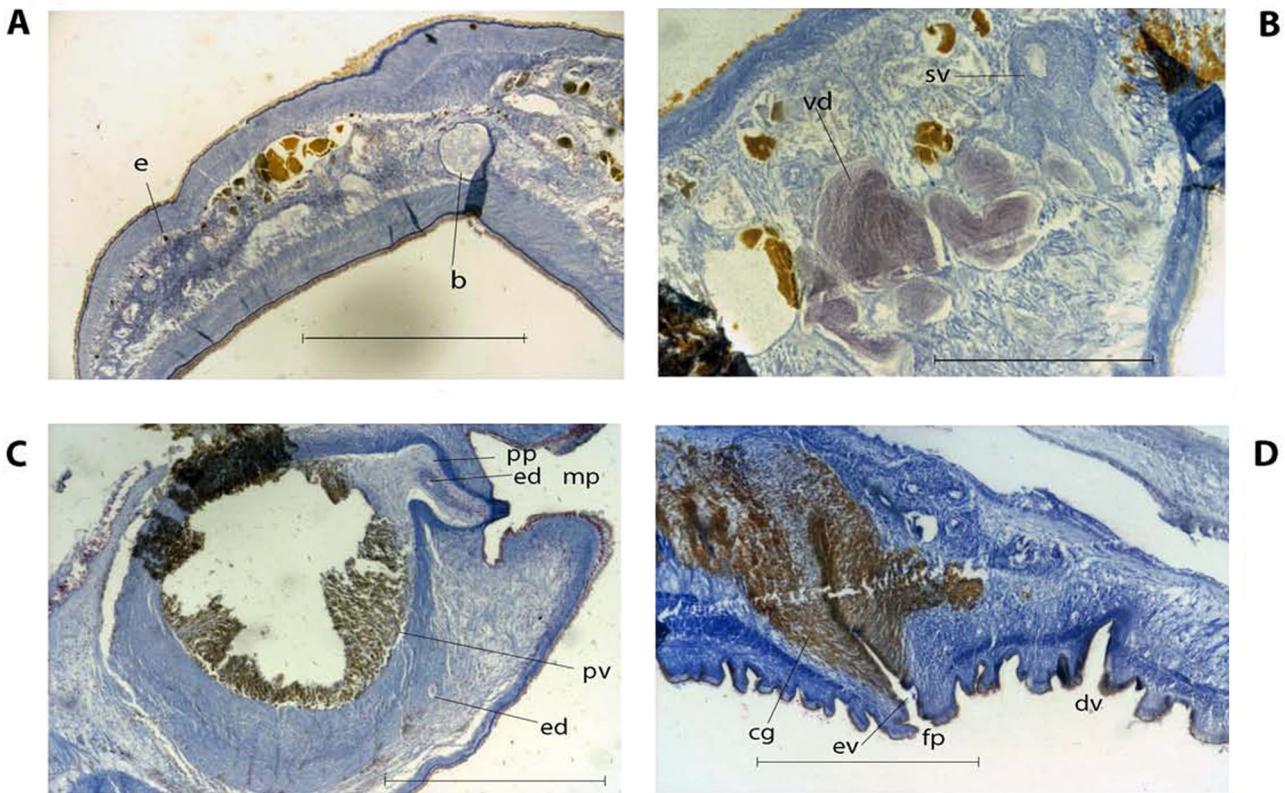
Male copulatory complex directed backwards, near the pharyngeal cavity (Fig. 6D). *Vasa deferentia* occupy the middle third of the body due to being dilated, and open laterally into a tubular seminal vesicle thickened in the junctions region (Figs. 6D, 7D). The ejaculatory duct runs ventrally from the seminal vesicle to the proximal end of the prostatic vesicle. The prostatic vesicle has a strong muscular envelope and is lined with a smooth and folded glandular epithelium (Fig. 7B). The penis papilla, small and unarmed, is conical-shaped, with a strong muscular envelope, housed in a wide, ciliated atrium.

The epidermis around the female pore appears corrugated, forming an adhesive pad (Figs. 6D, 7A). The female system consists of an external and internal vagina. The short narrow external vagina runs from the gonopore upwards to the internal vagina. The internal vagina ascends from the proximal region of the external vagina, curves downwards and backwards and, after receiving the oviducts, continues to the *ductus vaginalis*. Oviducts lead separately into the middle of the internal vagina. Proximal tract of the internal vagina runs forwards, receiving secretions from the well developed cement glands. Lang's vesicle lacking. The *ductus vaginalis* opens to the exterior behind the female pore.

**Distribution.** *Trigonoporus cephalophthalmus* has only been found once in the Gulf of Naples, Italy (Lang 1884). This is the first record for this species both outside of the Gulf of Naples and for the Iberian Peninsula.



**FIGURE 6.** *Trigonoporus cephalophthalmus* (Lang, 1884). **A** Habitus; **B, C** eyes; **D** sagittal reconstruction of the whole animal (anterior to the left); **E** sagittal reconstruction of the male and female genital systems (anterior to the left). Abbreviations: b, brain; cg, cement glands; dv, *ductus vaginalis*; e, eyes; ed, ejaculatory duct; ev, external vagina; fp, female pore; i, intestine; m, mouth; mp, male pore; o, ovary; ov, oviduct; ph, pharynx; pp, penis papilla; pv, prostatic vesicle; sv, seminal vesicle; vd, *vas deferens*. Scale bars: **A** 1cm, **B** 2mm, **C** 5mm, **D** 5mm, **E** 500 $\mu$ m.



**FIGURE 7.** Histological sections of *Trigonoporus cephalophthalmus* (Lang, 1884). **A** eyes; **B** vas deferens and seminal vesicle; **C** prostatic vesicle and penis papilla; **D** female reproductive system. Anterior to the left in all figures. Abbreviations: b, brain; cg, cement glands, d, ductus vaginalis; e, eyes; ed, ejaculatory duct; ev, external vagina; fp, female pore; mp, male pore; pp, penis papilla; pv, prostatic vesicle; sv, seminal vesicle; vd, vas deferens. Scale bars: **A** 1mm, **B, C, D** 500µm.

## Family PLANOCERIDAE Lang, 1884

### Genus *Planocera* Blainville, 1828

#### *Planocera graffi* Lang, 1879

Figs. 1 (5), 9, 10

**Type locality.** Gulf of Naples, associated with other invertebrates such as *Balanus*, *Halichondria*, *Penares* and *Lithodomus* (Lang 1879, 1884, Tyler *et al.* 2012).

**Type material.** not available.

**Material examined.** One mature specimen from Cabo de Palos (Murcia, Spain) and two mature specimens from littoral areas near Calpe (Alicante, Spain) were studied. One mature specimen from Calpe was sectioned sagittally and mounted on 50 slides (MNCN 4.01/130 - MNCN 4.01/180). The specimen from Cabo de Palos and the second specimen from Calpe were stored in alcohol 70%.

**Localities:** Cala Racó, Calpe, Alicante ( $38^{\circ}38.07'N$ ,  $0^{\circ}04.46'E$ ) Spain; Cabo Palos, Murcia, Spain ( $37^{\circ}37.39'N$ ,  $0^{\circ}42.25'W$ ); Fig. 1 (5).

**Other material.** *Planocera pellucida* (Mertens, 1833) Oersted, 1844 Zoologisches Institut und Zoologisches Museum der Universität Hamburg, slides: V 13167/1-V13167/18 (Atlantic Ocean, pelagic); V 131171/1-V13171/15 (Atlantic Ocean, pelagic); V 13169/1-V13169/37 (Atlantic Ocean); V 13177/1-V13177/11 (Atlantic Ocean) and 5 specimens in alcohol: V13131 (Cape Verde Islands).

**Description.** The specimen (Fig. 9A–D) has a broadly oval, almost round, form with slightly ruffled margins, 20 mm long by 15 mm wide (measurements from fixed specimen). The ground colour is yellow-orange, with a reddish net-like accumulation of pigment granules at the body midline along the intestine branches. The terminal

ramifications on the dorsal side and the margin of the body sometimes reflect a white colour. The body appearance is fleshy, but very transparent and frail in appearance. Two conspicuous conical tentacles located near the brain, but far from the margins. Numerous tentacular eyes at the base of each tentacle. Several small cerebral eyes present anterior as well as posterior to the tentacles, being more abundant behind the tentacles. A broad ruffled pharynx occupies the central region of the body (Fig. 9E).

In general, the organisation of the copulatory complex (Fig. 9F) agrees with the detailed descriptions of Lang (1884). Nevertheless, some additional details can be discerned in our specimens. For example, the *vasa deferentia* dilate and become the spermiducal bulbs before entering proximally into the muscular cirrus bulb. The prostatic vesicle opens together with the spermiducal bulbs at the distal end of the male bulb. The prostatic vesicle is lined with a glandular epithelium forming the characteristic tubular chambers. The ejaculatory duct is wide and short. The cirrus is composed of distal spines and teeth that progressively increase in size towards the genital atrium. The rather short male genital atrium is bottle-shaped. The entire male complex is surrounded by thick muscle layers. The female apparatus does not show remarkable differences from Lang's (1884) description of the species. Briefly, it consists of a muscular external vagina or *vagina bulbosa*, surrounded by muscle layers, which is lined with a delicate cuboidal, glandular epithelium at the distal end; in some sections, this epithelium shows fringe-like extensions. The internal vagina first runs anteriorly and then posteriorly. Numerous cement glands open in the distal section of the internal vagina. Proximally, the two oviducts join the internal vagina and Lang's vesicle is either entirely lacking or only present as a vestigial vesicle.

**Discussion.** The genus *Planocera* Blainville, 1828 shows a wide, almost cosmopolitan, distribution (Fig. 8). From the Mediterranean basin, three species have been reported: *Planocera folia* Grube, 1840, *Planocera graffi* Lang, 1879 and *Planocera ceratommata* (Palombi, 1936).

*Planocera graffi* was first described for the Gulf of Naples (Lang 1879, 1884), and also reported for the islands of São Vicente (Cape Verde) (Laidlaw 1903) and the Catalan coast (Novell 2003).

*Planocera folia* was originally described for Palermo by Grube (1840), and later cited for Barwick Bay, USA (Apalachee Bay?) by Johnston (1865). Unfortunately, the internal anatomy of *P. folia* is unknown, thus making it impossible to compare with other species of the genus. Ludwig von Graff (1904) last cited *P. folia*, merely as the record of Grube (1840); therefore, the last real record of *P. folia* appears to be in 1865 by George Johnston. For the Mediterranean, the only record is the original description of Grube (1840).

*Planocera ceratommata* was originally described by Palombi (1936) for Cape Town (South Africa), but more recently has been cited by Novell (2003) for the Catalan coast.

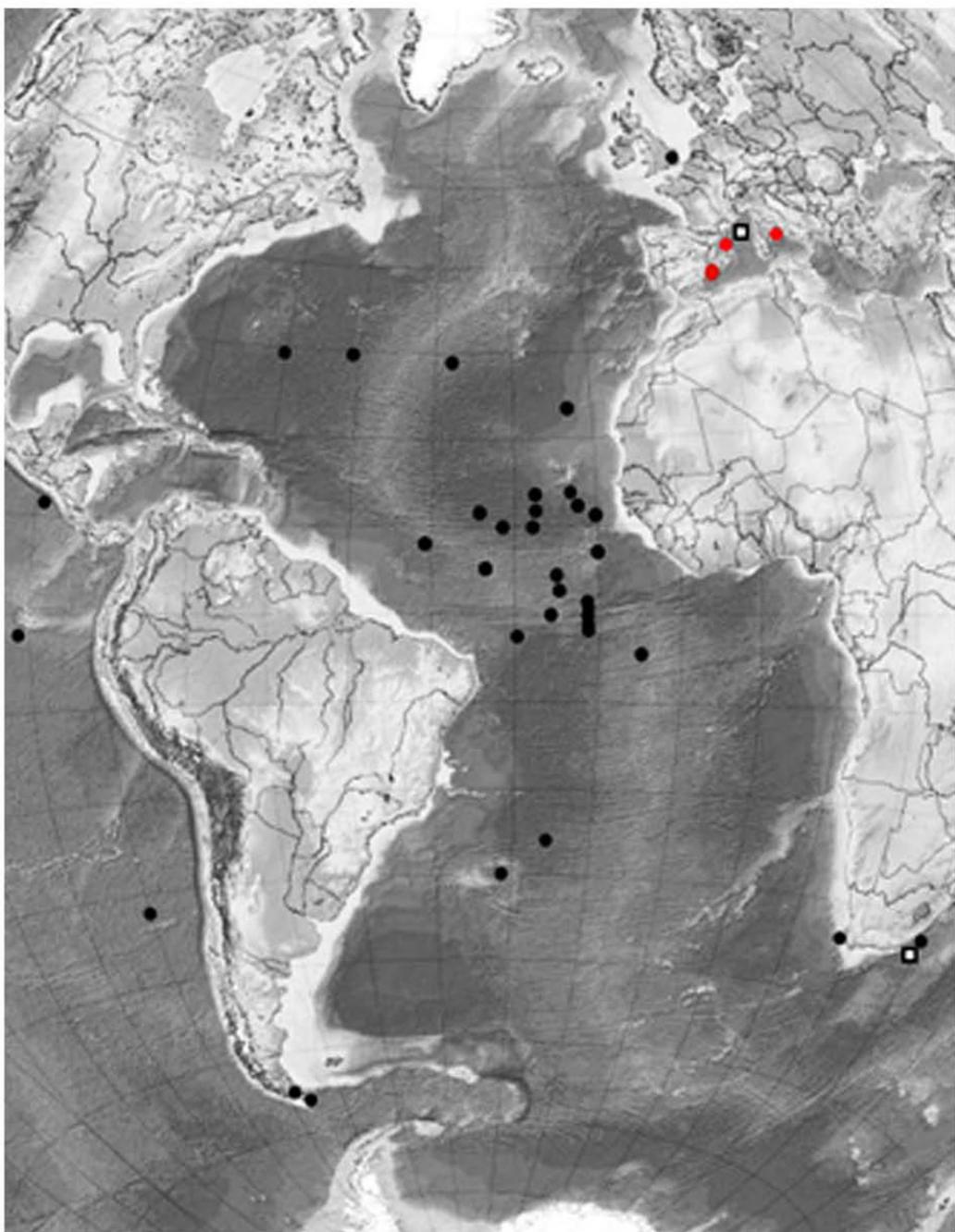
Although Lang (1879, 1884) could not compare *Planocera graffi* with *P. pellucida* (Mertens, 1833) as the description of *P. pellucida* does not include internal anatomical features, he did comment on the great similarity between *P. graffi* and *P. pelagica* (Moseley, 1877), which only differ in the shape of the ejaculatory duct (tortuous in *P. pelagica*). Years later, Faubel (1983) synonymised *P. pelagica* with *P. pellucida* (Mertens, 1833).

Bock (1913) and Faubel (1983) described *P. pellucida* as oval, tapering posteriorly and *P. graffi* as rounded, sometimes broader than long. However, Prudhoe (1985) reported that *P. pellucida* could show both body shapes, slightly elongated or rounded. This variation in body shape is, in our opinion and after a careful reading of the original description, the only reported difference between both species. An anatomical comparison between both species was necessary. Therefore, specimens of *P. pellucida* from the invertebrate collection of the Zoological Museum of Hamburg, captured in the Atlantic shores, were compared with our specimens from the Mediterranean coasts. The results of this comparative study (Fig. 10) are the following: the cirrus of the male copulatory organ shows the same shape and type of spines in all specimens, the disposition and localisation of the prostatic and seminal vesicle is the same and the *vasa deferentia* show identical trajectories. It is evident that there are no significant morphological differences between the two species and a synonymization is justified. Therefore, we propose that *P. graffi* is synonymous with *P. pellucida* with the consequent expansion of the distribution of *P. pellucida* within the Mediterranean Sea.

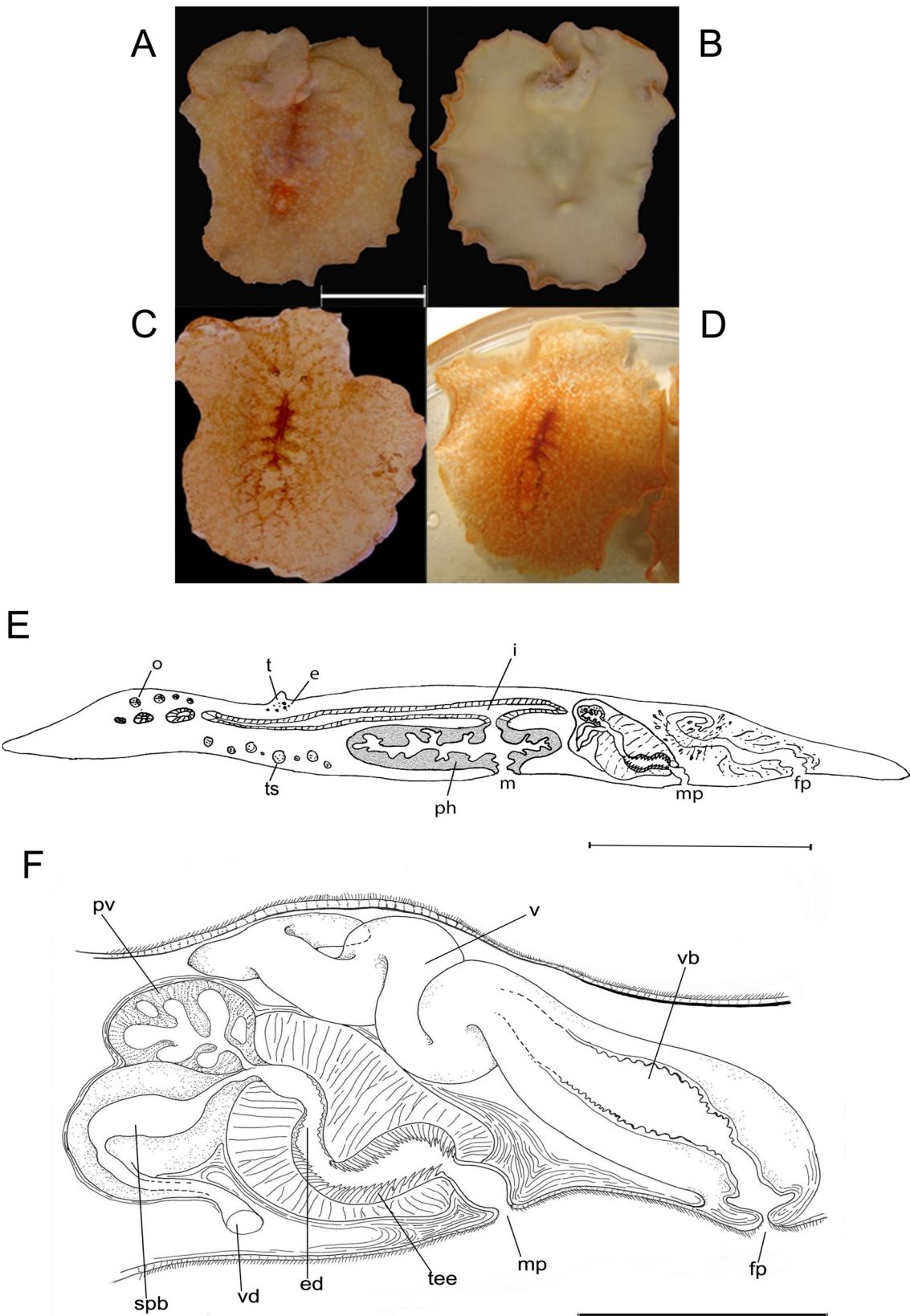
With respect to the third *Planocera* species cited for the Mediterranean shores, *P. ceratommata* (Palombi, 1936), this species is actually the most cited species of the genus *Planocera* within the Mediterranean Sea, although it was originally described for Still Bay, Cape Town (South Africa) (Palombi 1936).

Unfortunately, the original material of *P. ceratommata* is missing, but numerous similarities between this species and *P. pellucida* suggest a possible case of synonymy; although, some differences must also be considered, that do not allow a synonymization. The most significant variation between *P. ceratommata* from South Africa and our

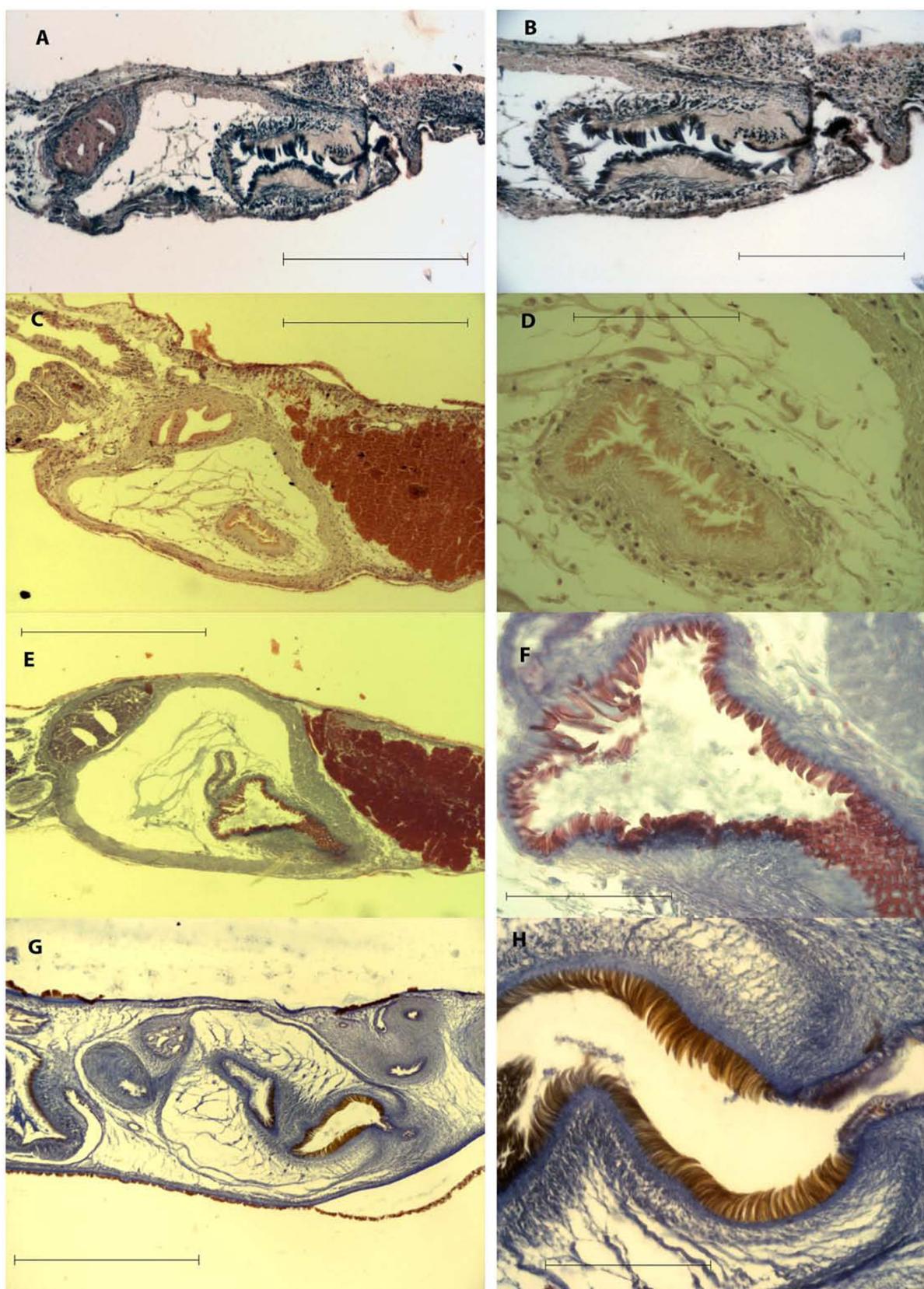
material lies in the arrangement of the eyes. In *P. pellucida*, the tentacular eyes surround the tentacles in an orderly (stereotypical) manner, whereas in *P. ceratommata* the arrangement of the eyes is irregular (Palombi, 1936; fig. 17). A similar pattern is also observed for the cerebral eyes. Another difference concerns the organisation of the prostatic vesicle. According to Palombi (1936), the copulatory organ “È formato de una grossa vescicola glandulare granulosa avvolta di una rica fasci muscolare costituita di fibri longitudinale e circolari” (“It is formed by a large glandular prostatic vesicle involved by well developed bundles of muscles formed by longitudinal and circular fibers”); this description, together with the figure (Palombi 1936, fig. 18), illustrate a prostatic vesicle that is different from the vesicle in *P. pellucida* described by Bock (1913). Nevertheless, we assume that many of the records of *P. ceratommata* for the Mediterranean in fact concern *P. pellucida*.



**FIGURE 8.** Map of known localities of *Planocera pellucida* (black dots), *Planocera ceratommata* (white squares) and *Planocera graffi* (red dots).



**FIGURE 9.** *Planocera graffi* (Lang, 1879). **A** Dorsal and **B** ventral views of fixed specimen; **C** dorsal view of a cleared animal; **D** dorsal view of a live animal; **E** sagittal reconstruction of the whole animal (anterior to the left); **F** sagittal reconstruction of male and female genital systems (anterior to the left). Abbreviations: e, eyes; ed, ejaculatory duct; fp, female pore; m, mouth; mp, male pore; o, ovary; ph, pharynx; pv, prostatic vesicle; spb, spermiducal bulb; t, tentacle; ts, testes; tee, teeth; v, vagina; vb, *vagina bulbosa*; vd, *vas deferens*. Scale bars: **A**, **B**, **C**, **D** 7mm, **E** 500µm, **F** 500µm.



**FIGURE 10.** *Planocera pellucida* (Mertens, 1833) of the Atlantic coast. A–B specimen V13167/1-V13167/18 (Iron-hematoxylin-eosin stain), A male copulatory organ, B detail of cirrus and teeth; C–D specimen V13171/1-V13171/15 (Pasini staining), C male copulatory organ, D detail of cirrus; E–F specimen V13169/1-V13169/37 (Pasini staining), E male copulatory organ, F detail of cirrus; G–H specimen of the Mediterranean coast (Azan staining), G male copulatory organ, H detail of cirrus. Anterior to the left in all figures. Scale bars: A, C, E, G 500µm, B, D 200µm, F, H 100µm.

## Family STYLOCHIDAE, Stimpson, 1857

### Genus *Imogine* Girard, 1853

#### *Imogine mediterranea* Galleni, 1976

**Synonymous:** *Stylochus mediterraneus* Galleni, 1976.

**Material examined.** One mature specimen, collected in Los Urrutias (Mar Menor, Murcia). From rocky substrate found between 0.2 to 0.5 metres deep; Fig. 1 (6).

**Morphological remarks.** The specimen shows very few frontal eyes. The marginal eyes are present only in the anterior half of the body and not in the whole body margin as originally described for *Imogine mediterranea* (Galleni, 1976). However, the internal anatomy of the reproductive organs of *I. mediterranea* coincides with the original description and figure of Galleni (1976). This difference in the distribution of marginal eyes may be related to the size of the specimen: mature worms are approximately 3 cm long, and this specimen, though mature, is only 12 mm long.

**Distribution.** *Imogine mediterranea* has been found in several areas of the northern Mediterranean coast (Italy: Livorno, La Spezia, Pisa (Galleni 1974) and Liguria (Wenzel *et al.* 1992); Croatia: Istria (Bytinski-Salz 1935)) and now in the western Mediterranean coast in Mar Menor.

#### *Imogine stellae* sp. nov.

Figs. 1 (7), 11, 12; Table 2

**Type locality.** Mar Menor (Murcia, Spain) (37°46.16'N, 0°45.02'W); Fig. 1 (7).

**Type material.** Holotype one mature specimen sectioned sagittally. MNCN 4.01/181 - MNCN 4.01/199 (18 slides).

**Material examined.** 14 live specimens from the eastern seashore of Mar Menor (Murcia, Spain) were studied; of these, four mature specimens were sectioned sagittally and mounted on slides. The material is deposited in the invertebrate collection of Museo Nacional de Ciencias Naturales de Madrid (Spain).

**Etymology.** The specific epithet is dedicated to Estrella Fernandez-Despiau, who collected the species.

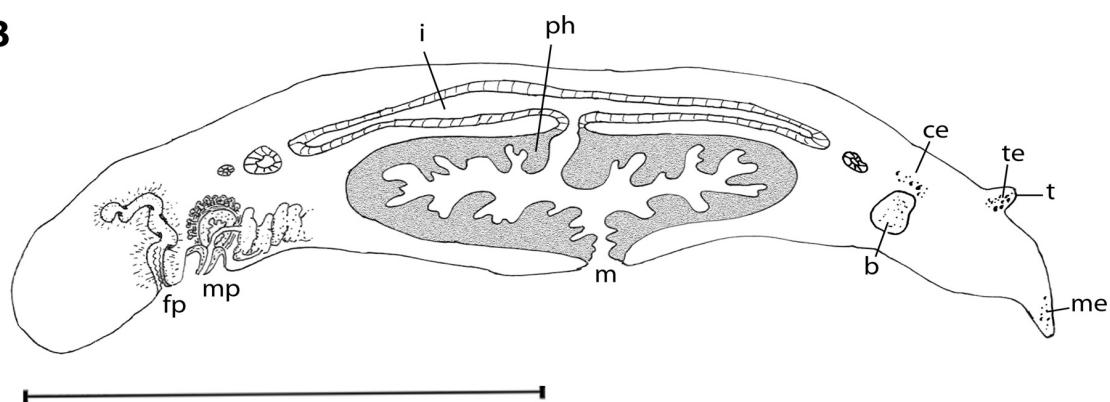
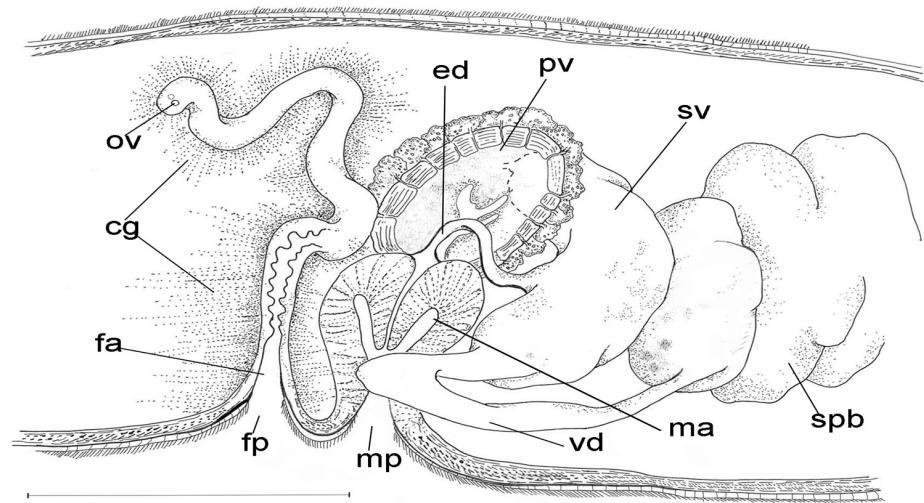
**Diagnosis.** *Imogine* with elongated body, without marked undulations; pigmentation brownish with dark brown spots, less dense at the margins and midline; nuchal tentacles present; tentacular, marginal and cerebral eyes present; anchor-shaped seminal vesicle opens at the base of the prostatic vesicle; prostatic vesicle “djiboutiensis” type; male and female atrium not ciliated; male and female gonopore close together, but clearly separated.

**Description.** Body elongated with rounded anterior and posterior ends, 12 mm long and 5.5 mm wide, tapering slightly posteriorly. Body margins without marked undulations. Nuchal tentacles located in the anterior one-fifth of the animal. Dorsal pigmentation brownish with dark brown spots, turning pale and less dense towards the margins and midline (Fig. 11A). Tentacular, marginal and cerebral eyes present. Tentacular eyes denser at the inner margins of the nuchal tentacles (Fig. 12A). Cerebral eyes form two elongated clusters. Marginal eyes small, arranged around the anterior end of the body margin. Ventral surface light yellow. Pharynx located in the anterior half of the body. Oral pore located at one-third of the body length. Main digestive trunk extends forwards and backwards of the pharyngeal cavity (Fig. 11B).

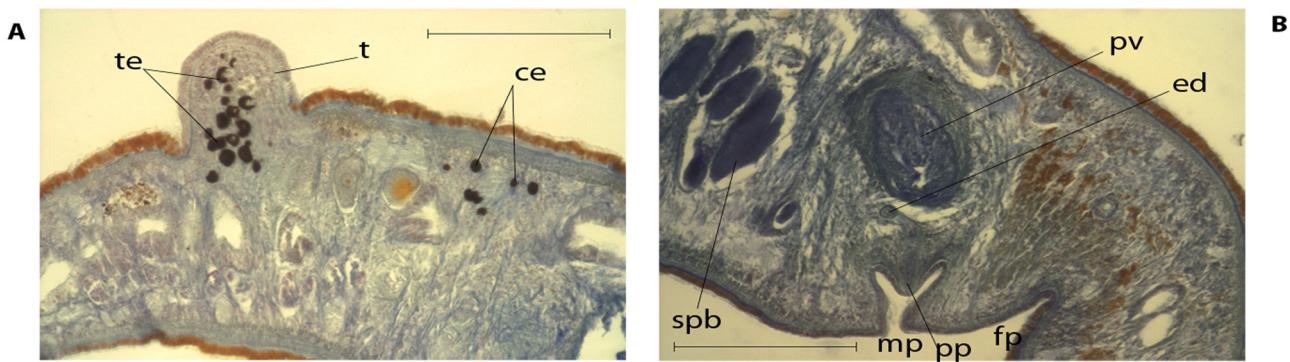
Reproductive system (Figs. 11C, 12B): The male system is located in the posterior part of the animal and consists of well developed spermiducal bulbs, a true anchor-shaped seminal vesicle, a musculo-glandular ovoid prostatic vesicle, a penis papilla, crossed by a straight ejaculatory duct and an atrium. The conspicuous spermiducal bulbs are located behind the anchor-shaped seminal vesicle. The *vasa deferentia* ducts are wide, becoming even wider towards the seminal vesicle, finally entering laterally into the vesicle. The characteristic seminal vesicle opens through a curved duct directly at the base of the prostatic vesicle. This duct subsequently becomes the ejaculatory duct. The prostatic vesicle shows, according to Faubel 1983, the characteristic shape of the “djiboutiensis” type: a small lumen with an external muscle layer and internal glandular lining, and surrounded by numerous prostatic glands. The conspicuous muscle layer at the distal end of the ejaculatory duct forms the penis papilla. The male atrium is narrow and not ciliated.

**TABLE 2.** Species belonging to the genus *Imogine* grouped after four conspicuous characters: male and female genital pore, the opening of the prostatic duct, the longitude of the penis papilla and the distal dilatation of the female atrium.

Species of <i>Imogine</i>	Character	Male and female genital pore	Opening prostatic duct in Ejac. duct	Longitude penis papilla	Distal dilatation of female atrium
1	<i>stellae</i>	sp.nov. Kato, 1944	together	proximal	Triangular dilation
	<i>hamanensis</i>	Bock, 1913	together	short	Without dilations
	<i>hyalina</i>	Laidlaw, 1904	together	medium	Without dilations
	<i>ceylanica</i>	Kato, 1944	together	long	Without dilations
	<i>izuensis</i>	Jennings & Newman, 1996	together	long	Light triangular
	<i>meganae</i>	(Bock, 1931) Marcus, 1947	together	/medium	Without dilations
	<i>sixtini</i>	(Schmarda, 1859)	together	short	Without dilations
	<i>megalops</i>	Palombi, 1940	distal	long	Without dilations
	<i>minima</i>	(Girard, 1853)	proximal	short	Without dilations
	<i>nebulosa</i>	Yeri & Kaburaki, 1918	distal	long	Without dilations
2	<i>rutilis</i>	Kato, 1944	together	short	With dilation
	<i>miyadaii</i>	Sluys, Faubel, Rajagopal, & van der Velde, 2005	common	long	Without dilation
	<i>necopinata</i>	Hyman, 1953	common	long	Without dilation
	<i>tripartita</i>	Kato, 1944	common	short	Without dilation
	<i>uniporus</i>	Bois-Reymond Marcus, 1958	common	short	Without dilation
	<i>cata</i>	Kato, 1937	separated	short	Without dilation
	<i>aomori</i>	Willey, 1897	No data	long	Without dilation
	<i>arenosa</i>	Hyman, 1953	No data	No data	No data
	<i>exigua</i>	Yeri & Kaburaki, 1918	separated	short	No data
	<i>ijimai</i>	Jennings & Newman, 1996	separated	short	?
3	<i>kimae</i>	Lee, Beal & Johnston, 2005	separated	long	With dilation
	<i>lateontere</i>	Jennings & Newman, 1996	separated	short	With dilation
	<i>lesteri</i>	Bock, 1925	separated	long	Triangulate dilation
	<i>marmorea</i>	Jennings & Newman, 1996	separated	long	Without dilations
	<i>migrathi</i>	(Galleni, 1976)	separated	long	Without dilations
	<i>mediterranea</i>	Buhnes, 2010	separated	long	Little dilatation
	<i>meliherthani</i>	Girard, 1853	separated	short	Without dilations
	<i>oculifera</i>	Bock, 1913	separated	long	With dilatation
	<i>orientalis</i>	Jennings & Newman, 1996	separated	medium	Without dilations
	<i>pardalonus</i>	Hyman, 1940	separated	medium	Without dilations
4	<i>pulcher</i>	Bois-Reymond Marcus, 1965	separated	medium	With dilation
	<i>refertia</i>	Kato, 1937	separated	long	Without dilations
	<i>speciosa</i>	Marcus, 1952	separated	long	Without dilations
	<i>tica</i>	(Verriil, 1882)	separated	long	With dilation
	<i>zebra</i>	Ehrenberg, 1831	separated	short	Without dilations
	<i>suzensis</i>				Elongated dilations

**A****B****C**

**FIGURE 11.** *Imogine stellae* sp. nov. **A** Habitus; **B** sagittal reconstruction of the whole animal (anterior to the right); **C** sagittal reconstruction of male and female genital systems (anterior to the right). Anterior to the left in all figures. Abbreviations: b, brain; ce, cerebral eyes; cg, cement glands; ed, ejaculatory duct; fa, female atrium; fp, female pore; i, intestine; m, mouth; ma, male atrium; me, marginal eyes; mp, male pore; ov, oviduct; ph, pharynx; pv, prostatic vesicle; spb, spermiducal bulbs; t, tentacle; te, tentacular eyes; sv, seminal vesicle; vd, vas deferens. Scale bars: **A**, **B** 6 mm, **C** 200 µm.



**FIGURE 12.** Histological sections of *Imogine stellae* sp. nov. **A** tentacles region and **B** male copulatory organ (anterior to the left). Abbreviations: ce, cerebral eyes; ed, ejaculatory duct; fp, female pore; mp, male pore; pp, penis papilla; pv, prostatic vesicle; spb, spermiducal bulb; t, tentacle; te, tentacular eyes. Scale bars: **A** 100 $\mu$ m, **B** 200 $\mu$ m.

The female system is located close to the male copulatory organ. Female and male gonopores are in close proximity, but clearly separated. The paired oviducts enter separately at the proximal end of the internal vagina. In preserved specimens, the vagina runs from the dorso-lateral side of the body to the medial axis, then bends anteriorly and runs ventrally towards the female pore; this last portion is known as the external vagina. The female atrium is narrow, conical and not ciliated. Numerous cement glands are located along the entire female system, but are open mainly in the external vagina. Lang's vesicle is absent.

**Discussion.** Due to the presence of an anchor-shaped seminal vesicle and a strong muscular penis papilla, *Imogine stellae* sp. nov. belongs to the genus *Imogine*. However, with a comparative study of highly characteristic features of the 34 known *Imogine* species (Jennings & Newman 1996; Bulnes 2010; and Table 2 of this study), we can distinguish three major groups based on four main features; viz. the location of the male and female gonopores, the junction of the sperm duct with the prostatic duct, the shape and size of the penis papilla, and the distal shape of the female external vagina.

The first group (Table 2) contains species characterised by the presence of two separate, yet physically close, gonopores: *Imogine stellae* sp. nov.; *I. hamanensis* (Kato, 1944); *I. hyalina* (Bock, 1913); *I. ceylanica* (Laidlaw, 1904); *I. izuensis* (Kato, 1944); *I. meganae* Jennings & Newman, 1996; *I. sixteeni* (Bock, 1931); *I. megalops* (Schmarda, 1859); *I. minima* (Palombi, 1940); *I. nebulosa* (Girard, 1853); *I. rutilis* (Yeri & Kaburaki, 1918) (Table 2). However, *I. stellae* sp. nov. can be differentiated from the other species of the group by its proximal junction of the prostatic and sperm ducts, a character also shared with *I. minima*; the other species of this group show either medial or distal junctions. Other differences are found in the male and female systems. The female gonopore and distal region of the external vagina of *I. stellae* sp. nov. present a remarkable dilatation, while the penis papilla appears longer than in other species. These characters distinguish *I. stellae* sp. nov. from *I. minima*, in which the distal external vagina lacks dilatations and the penis papilla is very short in comparison. Nevertheless, these characters must be carefully treated as their size may be influenced by the state of relaxation or contraction of the animal during fixation (Meixner 1907).

The second group comprises *Imogine miyadai* Kato, 1944; *I. necopinata* Sluys, 2005; *I. tripartitus* (Hyman, 1953); *I. uniporus* (Kato, 1944); and *I. catus* (Bois-Reymond Marcus, 1958). This group is characterised by a common gonopore and an external vagina without dilatations. Differences between species concern the location of the fusion of the prostatic and sperm duct and the shape of the penis papilla.

The third group contains the largest number of species (Table 2) and is mainly characterised by the marked separation of the female and male gonopores. Species of this group show different types of prostatic and sperm ducts junctions, as well as varying sizes and shapes of penis papillae and external vaginas. Species of this group include *Imogine aomori* (Kato, 1937); *I. arenosa* Willey, 1897; *I. exigua* (Hyman, 1953); *I. ijima* (Yeri & Kaburaki, 1918); *I. kimae* Jennings & Newman, 1996; *I. lateotentare* Lee, Beal & Johnston, 2005; *I. lesteri* Jennings & Newman, 1996; *I. marmorea* (Bock, 1925); *I. mcgrathi* Jennings & Newman, 1996; *I. mediterranea* (Galleni, 1976); *I. melihertani* Bulnes, 2010; *I. oculifera* Girard, 1853; *I. orientalis* (Bock, 1913); *I. pardalotus* (Jennings & Newman, 1996); *I. pulcher* (Hyman, 1940); *I. referta* (Bois-Reymond Marcus, 1965); *I. speciosa* (Kato, 1937); *I. tica* (Marcus, 1952); and *I. zebra* (Verrill, 1882). Species of this group can be differentiated by the pattern of

pigmentation, size of the tentacles, disposition of the tentacular, cerebral and marginal eyes and distribution of the cement glands around the female canal (Jennings & Newman 1996, Bulnes 2010).

In relation to the Mediterranean basin, aim of our research, only two other *Imogine* species have been previously described from the Mediterranean basin: *I. mediterranea* (Galleni, 1976) and *I. melihertani* Bulnes, 2010 (both members of the third group). *Imogine mediterranea* (Galleni, 1976) is known from the Italian and Tunisian coasts (Galleni 1976, Gammoudi *et al.* 2009), while *I. melihertani* has been reported from the western coast of the Aegean Sea (Bulnes 2010). *Imogine stellae sp. nov.* differs from *I. mediterranea* in the location of the two gonopores, which are clearly separated in *I. mediterranea* and close together in *I. stellae sp. nov.*. Another conspicuous difference in *I. stellae sp. nov.* is the opening of the seminal vesicle at the caudal base of the prostatic vesicle, whereas in *I. mediterranea* and *I. melihertani*, the ejaculatory duct receives the prostatic duct at a medial region. Furthermore, the *vasa deferentia* and spermiducal bulbs are more prominent and well developed in *I. stellae sp. nov.* compared to *I. mediterranea*, although this may be due to differences in developmental states.

The shape of the female system also shows clear differences between these three Mediterranean species. In *I. stellae sp. nov.*, the female pore is wide, while the atrium is pyramid-like and lacks cilia, whereas in *I. mediterranea* and *I. melihertani*, the female pore is small, and the atrium is ciliated in *I. melihertani*. The location of the cement glands along the internal and external vagina is similar in *I. stellae sp. nov.* and *I. mediterranea*, but differs in *I. melihertani*, in which the cement glands extend along the entire female canal.

## Genus *Stylochus* Ehrenberg, 1831

### *Stylochus neapolitanus* (Delle Chiaje, 1841–1844) Lang, 1884

**Synonymous:** *Planaria neapolitana* Delle Chiaje, 1941.

**Material examined.** Two mature specimens, collected in Los Urrutias (Mar Menor, Murcia). From rocky substrate and *Caulerpa prolifera* found between 0.2 to 2 metres deep; Fig. 1 (8).

**Morphological remarks.** Male copulatory system with simple seminal vesicle; the ejaculatory duct runs straight to the base of the prostatic vesicle, where it joins the prostatic duct and forms the penis papilla. Gonopores separated. Simple female system without Lang's vesicle. The specimens agree with the description and figure of Lang's *Stylochus neapolitanus* (1884), except for one difference: *S. neapolitanus* only has marginal eyes in the anterior edge, but one of these specimens have them around the entire body edge. Such phenotypic variation in the distribution of the eye spots have been reported by Meixner (1907).

**Distribution.** Western Mediterranean Sea: Delle Chiaje 1841 (Sicily); Lang 1884 (Naples); Novell 2003 (Cap de Creus). Laidlaw (1906) cited it for the Cape Verde Islands, but Palombi (1939) later assigned that specimen to *Stylochus castaneus*.

### *Stylochus pilidium* (Goette, 1881)

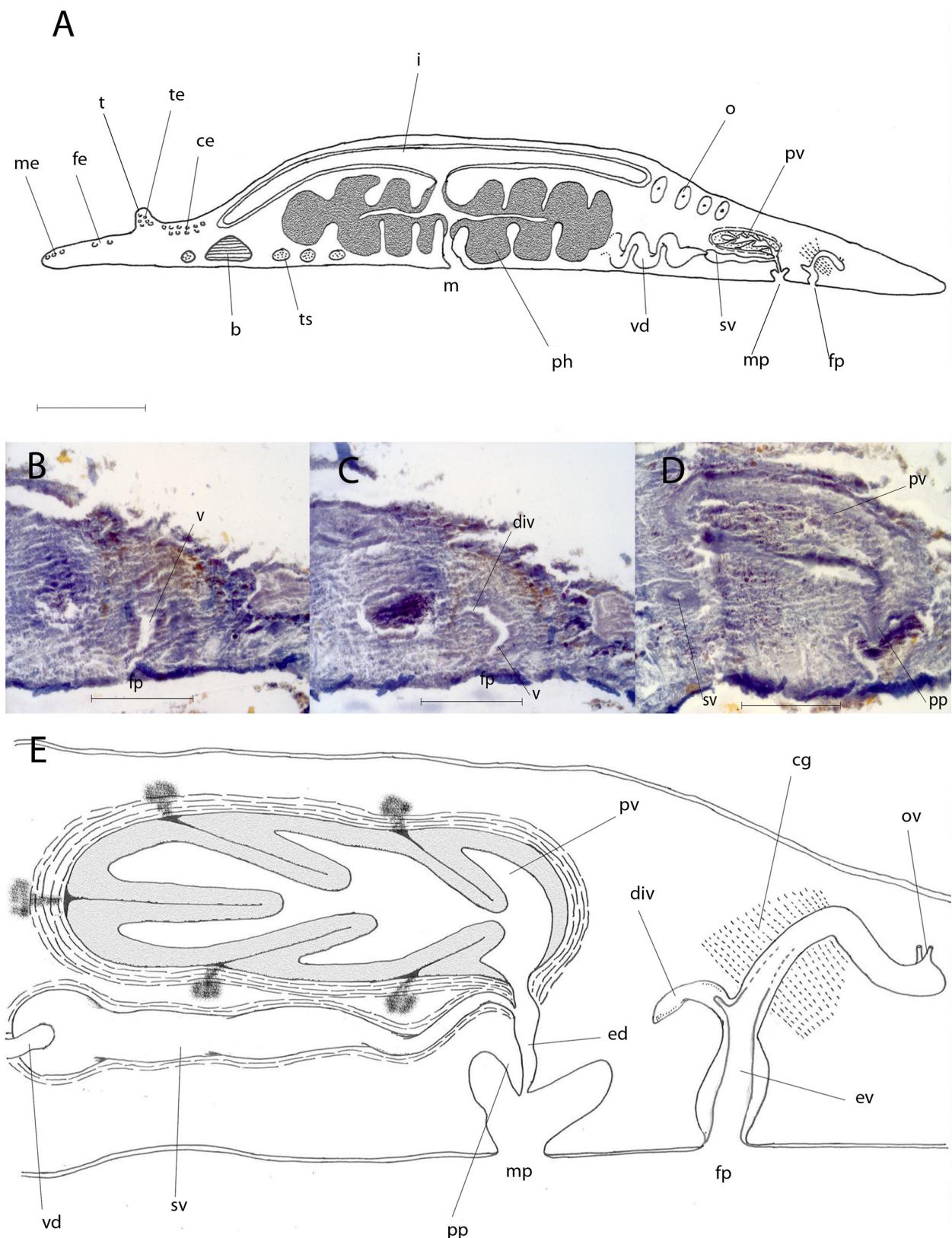
Figs. 1 (9), 13

**Synonyms:** *Planaria neapolitana* Delle Chiaje, 1841 in Goette, 1878; *Stylochopsis pilidium* Goette, 1881.

**Material examined.** One mature specimen, collected in Los Urrutias (Mar Menor, Murcia). From *Caulerpa prolifera* found two metres deep; Fig. 1 (9).

**Description.** Fixed specimen about 10 mm long and 7 mm wide. Colour lost during conservation. Well developed nuchal tentacles, with numerous internal eyes. Numerous cerebral eyes behind the tentacles, arranged in two longitudinal clusters. Large marginal eyes arranged around the first third of the body edge. Few frontal eyes. The muscular pharynx is located in the middle third of the body. Mouth opening in the centre (Fig. 13A).

Gonopores separated and located in the last third of the body. Testes ventral. Male copulatory apparatus with simple seminal vesicle, which has weak muscular walls. Spermiducal bulbs developed. Prostatic vesicle elongated (225 µm in length and 75 µm in width) and well developed, dorsal to the seminal vesicle, with thick muscular envelope. The prostatic duct joins the ejaculatory duct at the base of the prostatic vesicle (Figs. 13B, 13E). The ejaculatory duct runs straight to the tip of a short conical penis papilla housed in a ciliated atrium.



**FIGURE 13.** *Stylochus pilidium* (Goette, 1881). A Sagittal reconstruction of the whole animal anterior to the left); B–D histological sections, B male copulatory complex, C, D female reproductive system; E sagittal reconstruction of male and female genital systems (anterior to the left). Abbreviations: b, brain; ce, cerebral eyes; cg, cement glands; div, diverticulum; ed, ejaculatory duct; fe, frontal eyes; fp, female pore; i, intestine; m, mouth; me, marginal eyes; mp, male pore; o, ovary; ov, oviduct; ph, pharynx; pp, penis papilla; pv, prostatic vesicle; sv, seminal vesicle; t, tentacle; ts, testes; te, tentacular eyes; v, vagina; vd, vas deferens. Scale bars: A 1.5mm, B, C, D 200 $\mu$ m.

Ovaries dorsal. Female system lacks Lang's vesicle (Figs. 13C, 13E). The oviducts enter separately. Proximal tract of female duct, the *vagina interna* or internal vagina, extending forwards, first anterior-dorsally and then anterior-ventrally. The last tract, which is known as external vagina or *vagina externa*, runs straight down, with strong muscular walls, wider than the proximal tract. Narrow, forward-oriented diverticulum at the top of the external vagina (Fig. 13D), not described for any species of the genus *Stylochus*.

**Discussion.** Given that descriptions of *Stylochus pilidium* are rare, we aim to provide a detailed description of this species.

Goette (1881) first designed this species as *Planaria neapolitana*, but later identified it as *Stylochopsis pilidium*. In his original description, *Stylochopsis pilidium* was only compared to *Stylochus neapolitanus*. *Stylochus* and *Stylochopsis* are both cited by other authors (Lang 1884, Meixner 1907), however, these identifications were made based only on external morphology. Novell (2003) was the first to describe its internal anatomy.

*Stylochus pilidium* is clearly similar to *S. neapolitanus* as both species share the following characters: the junction of the ejaculatory and prostatic duct, the shape and size of the penis papilla and the outline of the seminal vesicle and of the female system. Nonetheless, although in previous descriptions (Lang 1884; Meixner 1907), *S. pilidium* and *S. neapolitanus* are described as quite similar or even equal, some characters are different in both species: *S. pilidium* shows separated gonopores, which in *S. neapolitanus* are common, and the distance from the female gonopore to the posterior end is greater and the prostatic vesicle is longer in *S. pilidium* than in *S. neapolitanus*.

**Distribution.** West Mediterranean Sea: Naples (Lang 1884); Sitges, Catalonia (Novell 2003); Mar Menor, Murcia (this paper).

## Conclusions

Based on the presence and distribution of the studied species, four types of distribution patterns are observed on the Mediterranean shores of the Iberian Peninsula. Generally, species either are endemic to the Mediterranean (group A) or show a nearly cosmopolitan distribution (group C). Between these opposing groups, we find intermediate models (group B). The fourth group consists of species thought to have been introduced (group D). One important factor that influences polyclad distribution is dispersion. Some species have indirect development with a planktonic larval stage (Götte, Müller and Kato's larvae; Gammoudi *et al.* 2011), and frequently use plants, algae meadows (Plehn 1896, Bock 1913) and vessel hulls for dispersal (Bock 1931).

A) Endemic species. The following species may be considered endemic in the Mediterranean:

### *Notoplana vitrea* (Lang, 1884) Bock, 1913

*Notoplana vitrea* is considered an endemic species of the Mediterranean Sea, cited from the Gulf of Naples (Lang 1884), the Gulf of Triest (Micoletzky 1910) and Catalonia (Novell 2003). To date, *N. vitrea* has never been cited for eastern coasts of the Mediterranean.

### *Trigonoporus cephalophthalmus* Lang, 1884

This species was first described by Lang (1884). Although other *Trigonoporus* species have been reported for localities outside of the Mediterranean (Tyler *et al.* 2012), *T. cephalophthalmus* has, to date, never again been cited. Only one exemplar *T. cephalophthalmus* was previously collected in the Isle of Gaiola in the Gulf of Naples (Lang 1884). In this study, only one exemplar for each locality in the islands of Columbretes, Hormigas and Alboran (Fig 1 (3)) was also collected, showing that this species is very rare.

### *Comoplana agilis* (Lang, 1884) Faubel, 1983

Originally described as *Stylochoplana agilis* Lang 1884, this species was reported for the first time for the Gulf of Naples (Lang 1884) and later for the coast of Catalonia (Novell 2003). In this study, *C. agilis* was not found at any of the sampled localities, but based on existing information, we consider *C. agilis* an endemic species of the Mediterranean Sea.

*Leptoplana mediterranea* (O. F. Müller, 1773) Bock, 1913

*Leptoplana mediterranea* is a endemic species of the Mediterranean Sea and its distribution extends mainly throughout the western Mediterranean basin (Gammoudi *et al.* 2012); to date, it has not been cited for the eastern Mediterranean coast.

*Imogine mediterranea* (Galleni, 1976) Jennings & Newman, 1996

The genus *Imogine* Girard, 1853 is a relatively new genus for the Mediterranean Sea, though known since nineteenth century for the western Pacific (e.g. Japan, Australia, Thailand) and western Atlantic (e.g. USA, Costa Rica, Brazil) coasts. *Imogine* is nearly unknown for the European Atlantic coast, with the exception of *I. necopinata* Sluys, 2005 (Sluys *et al.* 2005) from the North Sea canal in The Netherlands.

In the Mediterranean Sea, *Imogine* has only been reported twice: *I. mediterranea* (Galleni, 1976) and *I. melihertani* Bulnes, 2010. Within the Iberian Peninsula, *I. mediterranea* (Galleni, 1976) was found for the first time in the present study.

The lack of *Imogine* records in the well known occidental shores of the Mediterranean (Galleni 1976), suggests that this genus recently extend its distribution within the Mediterranean and possesses a high degree of speciation.

*Stylochus neapolitanus* (Delle Chiaje, 1841–1844) Lang, 1884

Since 1884, *S. neapolitanus* has been well known for the occidental coasts of the Mediterranean but, to date, has not been cited in the eastern Mediterranean coast. Laidlaw (1906) cited *S. neapolitanus* for the Cape Verde Islands, but he also considered this record dubious (pg. 707, Laidlaw 1906). Therefore, at present, *S. neapolitanus* is considered an endemic species of the Mediterranean.

*Stylochus pilidium* (Goette, 1881) Lang, 1884

Currently, *S. pilidium* is only known for the western Mediterranean coast and thus can be considered characteristic or endemic for the Mediterranean Sea.

*Notoplanella estelae* sp. nov. and *Imogine stellae* sp. nov. are considered endemic for the Mediterranean until proven otherwise.

B) Species mainly known for Mediterranean waters, but that have also been cited in other regions.

*Discocelis tigrina* (Blanchard, 1847) Lang, 1884

*Discocelis tigrina* was first cited for the coast of Sicily (Blanchard 1847), then subsequently for the Gulf of Naples (Lang 1884) and the coast of Catalonia (Novell 2003). Also recorded for Mauritania by Palombi (1939) although, this is the only record for *D. tigrina* outside of the Mediterranean. *Discocelis tigrina* did not appear in sampling and/or collection material in this study.

*Hoploplana villosa* (Lang, 1884) Laidlaw, 1902

*Hoploplana villosa* was originally described as *Planocera villosa* by Lang (1884) for the Gulf of Naples, specifically for Nisida Island. It was also recorded once from Susaki (Izu, Japan) by Kato (1937) and from Catalonia by Novell (2003).

C) Species that have been cited in several regions and thus have a cosmopolitan distribution.

*Pleioplana atomata* (O. F. Müller, 1776) Faubel, 1983

Although cited by Novell (2003) for the coast of Catalonia, this species was not found in the southern Mediterranean shores of the Iberian Peninsula in this study. It was also cited by Palombi (1928) for the Canal of Suez. This species is considered cosmopolitan because it is also common along the North Atlantic coasts of Europe and North America (Tyler *et al.* 2012).

*Planocera pellucida* (Mertens, 1833)

With the synonymization of *P. graffi* Lang, 1884 with *P. pellucida* (Mertens, 1833), the distribution of this

species, which is already known for both the northern and southern coasts of the Atlantic Ocean (Bock 1913, 1931), as well as some locations for Japan, has extended to include the Mediterranean Sea.

#### D) Species suspected to have been introduced.

This section lists the species thought to have been introduced based on their sporadic appearance and divergent sampling localities.

##### *Notoplanella inarmata* Bock, 1931

*Notoplanella inarmata* is known for different localities near Cape Town (Bock 1931, Day 1970, Prudhoe 1989), but it has never been captured in the Mediterranean until now. However, localities between Cape Town and the Iberian Peninsula were not sampled, therefore we cannot be certain it is an introduced species. In South Africa and Formentera, the specimens were collected by dredging (0–134 metres). This finding, together with *Notoplanella estelae* sp. nov., is the first record of *Notoplanella* for the Mediterranean Sea.

##### *Planocera ceratommata* (Palombi, 1936) Faubel, 1983

Palombi (1936) originally described this species from South Africa (Still Bay) as *Planocerodes ceratommata*; Novell (2003) also cited this species for the Catalan coasts. As well as *P. ceratommata* is frequently photographed in the shores of the Mediterranean (e.g. see <http://fotosubmallorca.blogspot.com.es/2010/02/opistobranquio-no-clasificado.html>; <http://bitxosdelmediterrani.blogspot.com/2008/10/planoceros-sp.html>; [http://www.cibsub.com/bioespecie\\_es-planocera\\_ceratommata-36639](http://www.cibsub.com/bioespecie_es-planocera_ceratommata-36639); [http://doris.ffessm.fr/fiche2.asp?fiche\\_numero=1090](http://doris.ffessm.fr/fiche2.asp?fiche_numero=1090)). Although the identification is somewhat dubious, because it is only based on the external anatomy and this genus needs for the determination at species level the study of the internal anatomy.

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