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## New records of cotylean flatworms (Platyhelminthes: Polycladida: Rhabditophora) from coastal habitats of Israel

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### Abstract

Seven new records of cotylean flatworms belonging to two families and four genera were identified from Israel collected in the eastern Mediterranean Sea and the northern Red Sea. The family Pseudocerotidae was represented by three genera and six species (*Pseudoceros duplicinctus*, *Pseudobiceros apricus*, *Pseudobiceros damawan*, *Pseudobiceros murinus*, *Pseudobiceros stellae*, and *Thysanozoon broccchii*), followed by the Euryleptidae with a single species (*Maritigrella fuscopunctata*). *Pseudoceros duplicinctus* is recognized as a senior synonym of *Pseudoceros prudhoei* and color and pattern variations are reported for *Maritigrella fuscopunctata*. In addition, the presence of *Pericelis byerleyana* in the northern Gulf of Aqaba is confirmed, illustrated, and properly described with morphological and molecular data. *Pseudoceros duplicinctus* and *Pseudobiceros stellae* represent new geographic records for the Mediterranean Sea and *Pseudobiceros apricus* and *Pseudobiceros damawan* are new for the Red Sea. Detailed morphological analysis with emphasis on the color and pattern was applied for species identifications including high quality photographs of live and fixed animals. Partial sequences of the 28S ribosomal DNA (rDNA) of four of the species were obtained and deposited in GenBank with accession numbers provided with the corresponding species description. Our results represent a significant taxonomic contribution for the diversity and distribution of the Polycladida in Israel pointing out the importance for more integrative and comprehensive surveys in these two regions to advance the knowledge of marine biodiversity worldwide and along both Israeli coasts.

**Key words:** Polyclads, Pseudocerotidae, Euryleptidae, eastern Mediterranean coast, Gulf of Aqaba, zoogeographical records

### Introduction

The order Polycladida currently contains about 850 valid species Tyler *et al.* (2006–2018) of marine free-living platyhelminths that are globally distributed, living in a wide range of habitats from the intertidal to the deep-sea (Newman & Cannon 2003; Quiroga *et al.* 2006, 2008). The group is divided in two suborders comprising approximately 380 cotyleans and 470 acotyleans with the greatest diversity in tropical regions such as the wider Caribbean (Stummer-Traunfels 1933; Hyman 1939, 1955a, b; Marcus & Marcus 1968; Quiroga *et al.* 2004; Bolaños *et al.* 2007) and the Indo-Pacific (Hyman 1954, 1955c, 1959; Prudhoe 1989, Newman & Cannon 1994, 1997, 1998, 2003, 2005; Apte & Pitale 2011; Bolaños *et al.* 2016).

In the Mediterranean Sea, the polyclad fauna is known mainly from Italy (Delle Chiaje 1822; Grube 1840; Quatrefages 1845; Lang 1884; Galleni 1972, 1974, 1976, 1978; Curini-Galletti *et al.* 2008), Tunisia (Gammoudi *et al.* 2009; Gammoudi *et al.* 2011), and Spain (Novell 2001; Marquina *et al.* 2014) with more than 80 described species. Specifically for the eastern Mediterranean basin, polyclad records are rather scarce. To date, 16 species have been reported from Egypt (Palombi 1928; Steinböck 1937; Prudhoe 1989), Greece (Curini-Galletti *et al.* 2008), Turkey (Bulnes *et al.* 2009; Bulnes 2010), and Israel (Prudhoe 1989; Curini-Galletti & Campus 2007; Curini-Galletti *et al.* 2008). Likewise, polyclad records for the Red Sea are limited to approximately 40 species,

mainly described from locations in the northern Red Sea such as the Gulf of Suez (Leuckart 1828; Ehrenberg 1831; Boutan 1892), Suez Canal (Palombi 1928), Gulf of Aqaba (Prudhoe 1952, 1989), and the Egyptian Red Sea (Hurghada: Melouk 1940, 1941 and El Qoseir: Meyer 1922). Most of these records are based on a single, poorly preserved specimen representing incomplete and ambiguous descriptions.

Particularly for the Israeli coasts, including the eastern Mediterranean and northern Red Sea, the polyclad knowledge is restricted to only eight species, *Imogine orientalis* BOCK, 1913, *Notoplana cotylifera* MEIXNER, 1907, *Echinoplana celerrima* HASWELL, 1907, *Neoplanocera steueri* (STEINBÖCK, 1937), *Pericelis byerleyana* (COLLINGWOOD, 1876), *Lurymare drygalski* (BOCK, 1913), *Theama mediterranea* CURINI-GALLETTI, CAMPUS & DELOGU, 2008, and the first Lessepsian migrant, *Boninia neotethydis* CURINI-GALLETTI & CAMPUS, 2007. Additionally, few photographic records without any taxonomic information are known (Dafni 2009). The current study describes seven new records of cotylean flatworms in two regions of the Israeli coast: the eastern Mediterranean Sea and the northern Red Sea, including high quality photographs of live and fixed animals, along with DNA sequences of the D1-D6 expansion segments of the 28S ribosomal DNA (rDNA) for some of the species. In addition to these new records, the presence of *Pericelis byerleyana* for the northern Gulf of Aqaba (Eilat), Red Sea is confirmed and properly described and illustrated after almost 28 years of being briefly mentioned by Prudhoe (1989).

Much of the diversity of cotylean polyclads involves complex variation in color and patterns. Newman & Cannon (1994, 1997, 1998) categorized these features into six and eight groups for the genera *Pseudobiceros* and *Pseudoceros*, respectively. This categorization has been for the most part reliable and has served as one of the main diagnostic characters for identifications at species level. However, color polymorphism among individuals of the same species are now more frequently encountered (Bolaños *et al.* 2016) and cases of species complex (Litvaitis *et al.* 2010) are waiting to be resolved. Here, we discuss color variation in some cotylean species, recognize *Pseudoceros duplicinctus* PRUDHOE, 1989 as a senior synonym of *Pseudoceros prudhoei* NEWMAN & CANNON, 1994 based on its color pattern, and present an example of species complex for the euryleptid *Maritigrella fuscopunctata* (PRUDHOE, 1978). We also present molecular data to validate our findings pointing out the importance to carry out more integrative studies combining classical taxonomy and molecular approaches. This study not only shows that the polyclad fauna has been overlooked in Israel but also indicates that a higher diversity of marine flatworms for the sampled areas can be expected. This represents the first and most complete taxonomic report of the order Polycladida from the Israeli coasts, also resulting in the first polyclad museum collection for the country.

## Material and methods

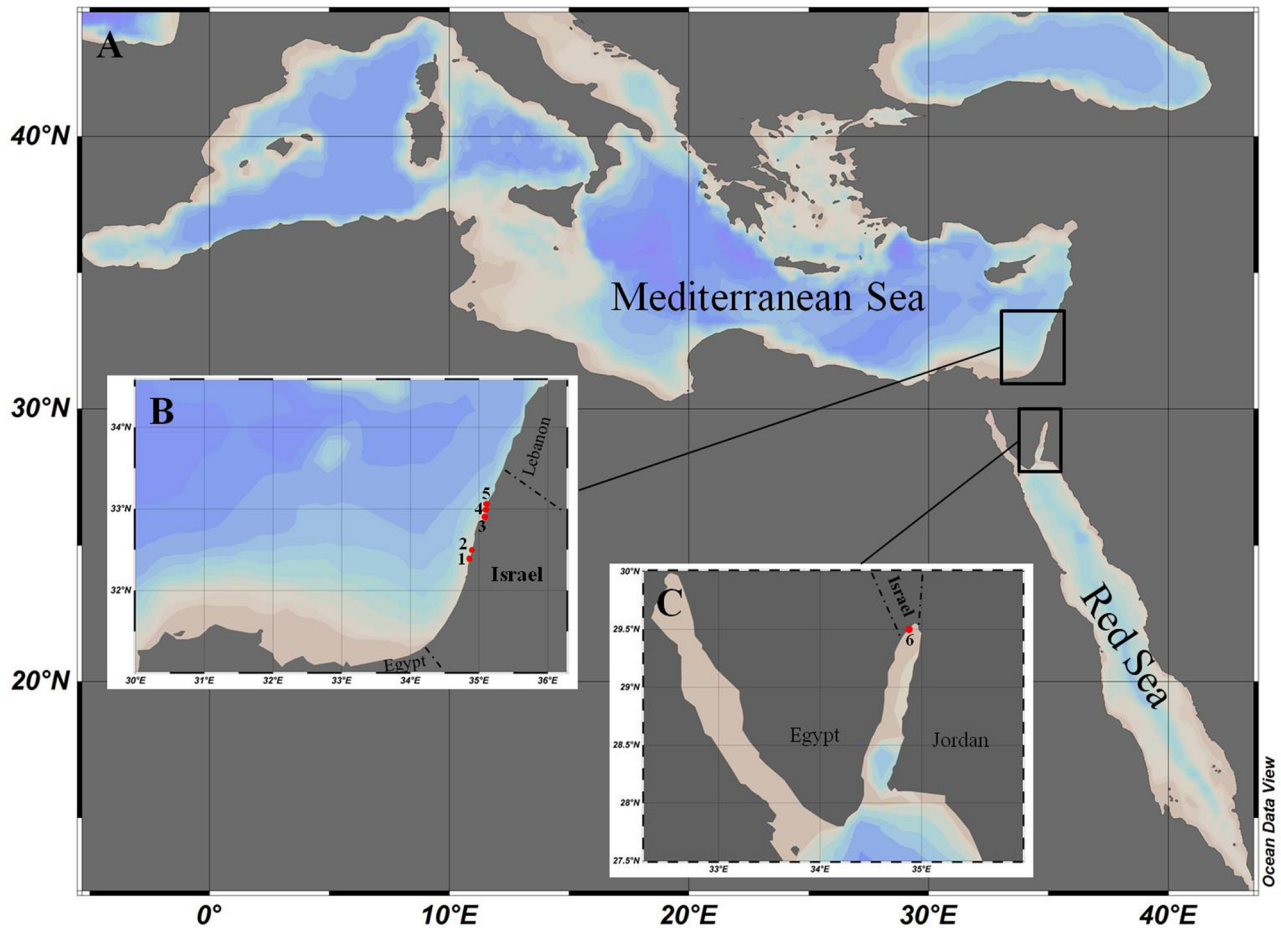
Specimens were collected along the intertidal (rocky shore habitat) and subtidal zone of the Israeli Mediterranean and the northern Gulf of Aqaba (Red Sea) including the shallow reefs in the latter (Fig.1), by using a soft paint brush to remove animals from the substrate. Specific locations, habitat information, and geo-references are given with each species description. In the laboratory, the animals were photographed alive, measured (length x width mm), fixed on a block of frozen 10% formalin and then preserved in 70% ethanol for histological analysis (Bolaños *et al.* 2007).

Taxonomic identifications follow the classification system proposed by Newman & Cannon (1994) and Faubel (1984) and were based mainly on color and color patterns and other external morphological features such as shape of pseudotentacles, type of pharynx, eyespot, and number of male gonopores (Newman & Cannon 1994, 1997, 1998). The material was deposited in the Steinhardt Museum of Natural History, Israel National Center for Biodiversity Studies at Tel Aviv University, and includes collection numbers preceded by ZMTAU-VR (Table 1).

For *Pseudoceros duplicinctus*, *Pseudobiceros stellae*, *Maritigrella fuscopunctata*, and *Pericelis byerleyana*, a small piece of tissue was dissected immediately after collection and preserved in 95% ethanol for DNA analysis. DNA was extracted using the DNEasy Blood and Tissue kit (Qiagen Inc., Valencia, CA). Primer targets for this study included the D1-D6 expansion segments (~1500 bp) of the nuclear 28S rDNA gene (Litvaitis *et al.* 1996; Sonnenberg *et al.* 2007).

DNA amplifications followed the protocol outlined in Litvaitis & Newman (2001). Amplicons were gel-purified and sent to a commercial lab for sequencing (Eurofins Genomics, Louisville, Kentucky, USA).

Sequencing occurred in both directions. Trace files were edited using GENEIOUS v. 5.6.3 (Kearse *et al.* 2012). GenBank accession numbers are provided with individual species descriptions. The sequences of *P. byerleyana* and *M. fuscopunctata* were compared to available sequences in GenBank using a MAFFT (v. 7.157) alignment (Katoh & Standley 2013) as implemented in GENEIOUS.



**FIGURE 1.** Map showing the collecting sites. (A) General view of the Mediterranean and Red Sea regions. (B) Close up of the Israeli coast in the eastern Mediterranean Sea indicating the collecting sites; 1: Mikhmoret; 2: Sdot Yam; 3: Nahariya; 4: Acre; 5: Achziv. (C) Close-up of the Israeli coast in the northern Gulf of Aqaba indicating the collecting site; 6: Inter University Institute for Marine Sciences in Eilat (IUI). The map was generated by Ocean data view, Version 4.7.

## Systematics

### Clade Rhabditophora Ehlers, 1986

### Order Polycladida Lang, 1884

### Suborder Cotylea Lang, 1884

### Superfamily Pseudoceroidea Faubel, 1984

### Family Pseudocerotidae Lang, 1884

### Genus *Pseudoceros* Lang, 1884

***Pseudoceros duplicinctus* Prudhoe, 1989**

(Fig. 2)

**Synonyms:** *Pseudoceros prudhoei* (Newman & Cannon 1994, 1998, 2003, 2005; Gosliner *et al.* 1996; Apte & Pitale 2011, Dixit & Raghunathan 2013; Marquina *et al.* 2015); *Pseudoceros cf. prudhoei* (Maghsoudlou & Rahimian 2014).

**Material examined and locality:** a) One mature specimen (30x10 mm live, ZMTAU-VR25166) preserved in ethanol 70%. Collected at Mikhmoret, Israeli eastern Mediterranean Sea (32° 24' N, 34° 52' E) on 1 June 2015.

b) One mature specimen (28x10 mm, fixed, ZMTAU-VR25167; GenBank ID: MH047292) preserved in ethanol 70%. Collected at Achziv, Israeli eastern Mediterranean Sea (33° 2' N, 35° 6' E) on 8 December 2015.

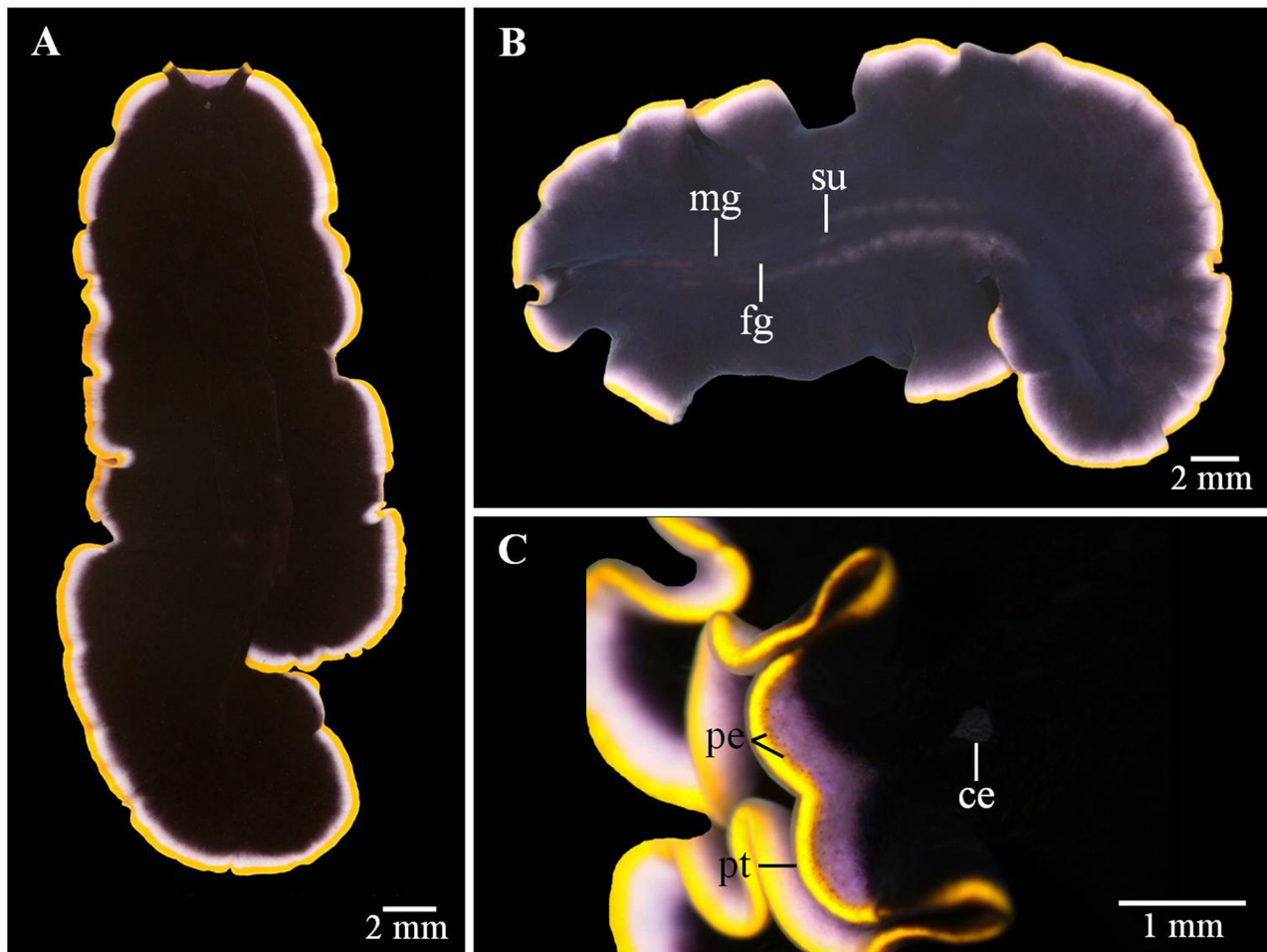
**Habitat:** Specimens found in the rocky shore, intertidally under rocks (1–2 m depth), and in subtidal rocky reef habitats (5 m depth).

**TABLE 1.** List of polyclad flatworms collected in the Israeli coasts, eastern Mediterranean Sea (E-MS) and northern Red Sea (N-RS) including location sites, habitat and depth, museum collection number, and GenBank accession numbers. \*: species previously found in Israel; †: new records for the Mediterranean Sea; ‡: new records for the Red Sea.

Species	Region	Site	Habitat and depth	Museum Collection number ZMTAU-VR	GenBank Accession number
<i>Pseudocerotidae</i> Lang, 1884					
<i>Pseudoceros</i> Lang, 1884					
† <i>Pseudoceros duplicinctus</i> Prudhoe, 1989	E-MS	Mikhmoret Achziv	rocky reef habitats (4–5 m) rocky shore (1–2 m)	25166 25167	MH047292
<i>Pseudobiceros</i> Faubel, 1984					
‡ <i>Pseudobiceros apricus</i> Newman & Cannon, 1994	N-RS	Eilat	rocky shore (1 m)	25165	---
‡ <i>Pseudobiceros damawan</i> Newman & Cannon, 1994	N-RS	Eilat	coral reef habitats (2–3 m)	25164	---
<i>Pseudobiceros murinus</i> Newman & Cannon, 1997	N-RS	Eilat	rocky shore (1 m)	25134, 25135	---
† <i>Pseudobiceros stellae</i> Newman & Cannon, 1994	E-MS	Mikhmoret	rocky shore (1 m)	25179	MH047293
<i>Thysanozoon</i> Grube, 1840					
<i>Thysanozoon brocchii</i> (Risso 1818)	E-MS	Sdot Yam	rocky shore (1 m)	25137, 25138, 25139	---
<i>Pericelidae</i> Laidlaw, 1902					
<i>Pericelis</i> Laidlaw, 1902					
* <i>Pericelis byerleyana</i> (Collingwood, 1876)	N-RS	Eilat	coral reef habitats (12–15 m)	25162, 25163	MH047291
<i>Euryleptidae</i> Lang, 1884					
<i>Maritigrella</i> Newman & Cannon, 2000					
<i>Maritigrella fuscopunctata</i> (Prudhoe, 1977)	E-MS	Nahariya Acre	rocky reef habitats (4–5 m) rocky shore (1–2 m)	25169, 25170, 25171	MH047290

**Distribution:** Inhaca Island, Mozambique (Type locality) (Prudhoe 1989); Heron Island, Great Barrier Reef, Australia and Madang, Papua New Guinea (Newman & Cannon 1994); Lizard Island, Australia (Newman & Cannon 1998, Marquina *et al.* 2015); Maldives, Micronesia, Philippines, Marshal Island, Japan, Hawaii, and Kenia (Gosliner *et al.* 1996; Newman *et al.* 2003; Newman & Cannon 2005); Kavaratti Island, Andaman and Nicobar Islands, India (Apte & Pitale 2011; Dixit & Raghunathan 2013); and Qeshm Island, Iran Maghsoudlou & Rahimian (2014). In this study specimens were found along the Israeli Mediterranean coast.

**External morphology.** Oval and elongated body with a slightly folded margin (Fig. 2A). Dorsal background velvety black with two distinct marginal bands surrounding the entire body. The inner band is wide and white followed by a narrow yellow margin (Fig. 2A). The ventral side has the same pattern (Fig. 2B). Simple black pseudotentacles with yellow tips but without the extension of the inner white band (Fig. 2A). Dorsal pseudotentacular eyes arranged in two scattered lines between the pseudotentacles. Small cluster of cerebral eyes located in a clear area with an inverted heart shape (Fig. 2C). Ruffled pharynx with complex folds located anteriorly. Separate gonopores. Single male gonopore located posterior to the pharynx followed by a close female gonopore. Small sucker posterior to the female gonopore (Fig. 2B).



**FIGURE 2.** *Pseudoceros duplicinctus*. (A) Dorsal view, *in vivo*. (B) Ventral view of the live specimen showing the reproductive structures and sucker. (C) Close-up of the anterior region showing the pseudotentacles, pseudotentacular eyes and cerebral eyes. ce: cerebral eyes; fg: female gonopore; mg: male gonopore; pe: pseudotentacular eyes; pt: pseudotentacles; su: sucker.

**Taxonomic remarks.** *Pseudoceros duplicinctus* is one of the many early polyclad records that has remained ignored in the literature. As a result, the new species *Pseudoceros prudhoei* was created by Newman & Cannon (1994) without considering the resemblance in color and pattern between both species. Since then, different putative morphotypes of *P. prudhoei* have been recorded (Newman & Cannon 2005; Apte & Pitale 2011; Dixit & Raghunathan 2013; Maghsoudlou & Rahimian 2014; Marquina *et al.* 2015), but neither completely matches the original diagnosis (Table 2) nor refers to the similarity with *P. duplicinctus*. *P. prudhoei* only differs from *P. duplicinctus* by the yellow outer marginal band instead of orange. Because Prudhoe (1989) based his description on a single preserved specimen and a water-color painting of the specimen when alive, we consider this difference inappropriate to treat *P. duplicinctus* and *P. prudhoei* as two separate species.

The specimens found in Israel vary from the original description of *P. duplicinctus* by the presence of a velvety black dorsal surface and a white inner marginal band instead of a dark brown background and a pale blue margin. This is not surprising since it is widely known that color is greatly affected by the content of the intestinal branches,

geographic location, habitat, and overall animal health (Newman & Cannon 1994; 2003; 2005; Bahia *et al.* 2014; Bolaños *et al.* 2016). In fact, Marquina *et al.* (2015) noticed that the inner band of a presumed morphotype of *P. prudhoei* can fade from bluish grey to whitish and the dorsal background varies from dark to light brown. This variation was also observed in another specimen from India (Apte & Pitale 2011) and even in pictures associated with the original description of *P. prudhoei* (Newman & Cannon 1994; 2003). Additional records identified as *Pseudoceros* cf. *prudhoei* from Iran, also showed the velvety black and white inner margin as seen in our specimens as well as another record from India (Newman & Cannon 2005; Dixit & Raghunathan 2013; Maghsoudlou & Rahimian 2014). Interestingly, a common feature of all the above-mentioned reports, including the specimens in this study, is the lack of numerous dark brown microdots over the whole dorsal surface described by Newman & Cannon (1994) as a distinctive character for *P. prudhoei* (Table 2). Based on these observations, it is likely that *P. duplicinctus* represents another pseudocerotid conflicting species with a high level of color variation and therefore, we consider that all the morphotypes previously described as *P. prudhoei* are junior synonyms of *P. duplicinctus*. Our statement is supported by a molecular analysis of our specimen and two other morphotypes from Australia using nucleotide sequences of the D1-D2 expansion segment of the 28S rDNA gene which revealed very little differences among individuals (M. Litvaitis, pers.comm).

*Pseudoceros depiliktabub* NEWMAN & CANNON, 1994 is another species that closely resembles *P. duplicinctus*. However, *P. depiliktabub* is considered to have three marginal bands: an inner dark green a middle yellow-cream, and an outer orange rim. Despite being described with an orange margin as *P. duplicinctus*, additional photographic records show that *P. depiliktabub* can also display a yellow rim with white dots scattered over the dorsal surface (Newman & Cannon 2003; 2005). Despite their close similarity in color and pattern, at this point it is not possible to make final conclusions about the synonymy of these two species due to the lack of molecular data and additional reports of *P. depiliktabub*. Molecular studies are needed to validate and confirm species identities. However, we are contributing with our sequence for *P. duplicinctus* (MH047292), as a reference for future comparisons that help in resolving this issue.

Finally, *P. duplicinctus* is known from the Indian and the Indo-Pacific Oceans and to date, there are no records for other regions. Therefore, *P. duplicinctus* represents the first record for the Mediterranean Sea, adding to the list of non-indigenous platyhelminthes for the area together with *Boninia neotethydis* CURINI-GALLETI & CAMPUS, 2007 and *Maritigrella fuscopunctata* NEWMAN & CANNON, 2000 (Curini-Galletti & Campus 2007; Crocetta *et al.* 2015). This species has also been sighted in different locations on the Tel Aviv coast, indicating that *P. duplicinctus* might be established on the Israeli Mediterranean coast.

**TABLE 2.** Comparative table of color variation for *Pseudoceros duplicinctus* and different morphotypes attributed to *Pseudoceros prudhoei*.

Species	Background Color	Dots on dorsal surface	Inner Band	Outer Band	Pseudotentacles	Reference
<i>Pseudoceros duplicinctus</i>	Dark brown, lighter along the median line	Not mentioned	Pale blue	Orange	Brownish tipped with orange	Prudhoe 1989
<i>Pseudoceros prudhoei</i> (current holotype)	Brown-orange	Dark brown microdots	Sky blue or light purple	Yellow or cream	Brownish bordered with some blue and tipped with yellow	Newman & Cannon 1994; Newman & Cannon 2003
<i>Pseudoceros prudhoei</i> (Morphotype 1)	Velvety black	Absent	White	Yellow	Black tipped with yellow	Newman & Cannon 2005; Maghsoudlou & Rahimian 2014; this study
<i>Pseudoceros prudhoei</i> (Morphotype 2)	Brown-orange	Absent	Bluish grey-whitish	Yellow	Brownish bordered with inner blue and outer yellow	Newman & Cannon 2005; Marquina <i>et al.</i> 2015; Apte & Pitale 2011
<i>Pseudoceros prudhoei</i> (Morphotype 3)	Dark brown-black	White dots	White	Yellow	Dark brown tipped with yellow	Dixit & Raghunathan 2013

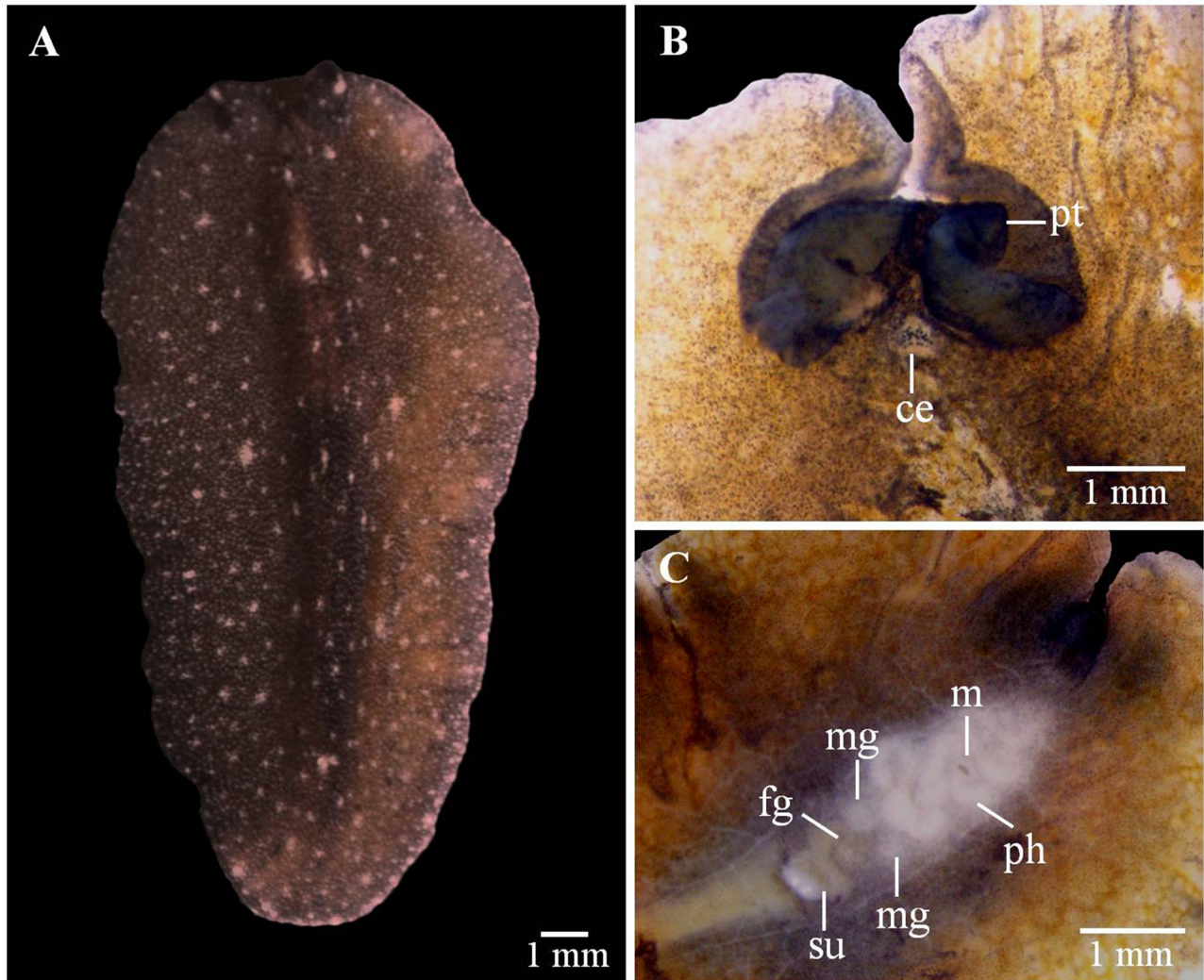
Genus *Pseudobiceros* Faubel, 1984

*Pseudobiceros apricus* Newman & Cannon, 1994  
(Fig. 3)

**Material examined and locality:** One mature specimen (17x10 mm, live, ZMTAU-VR25165) preserved in ethanol 70%. Collected at the Inter University Institute for Marine Sciences (IUI), Eilat, Israel, northern Gulf of Aqaba, Red Sea (29° 30.211' N, 34° 55.068' E) on 10 December 2013.

**Habitat:** Specimen found on the rocky shore, under rocks during low tide (1 m depth).

**Distribution:** Heron Island, Great Barrier Reef, Australia (Type locality) (Newman & Cannon 1994). In this study, the specimen was found in Eilat, northern Gulf of Aqaba, Red Sea.



**FIGURE 3.** *Pseudobiceros apricus*. (A) Dorsal view, *in vivo*. (B) Close-up of the anterior region in a preserved animal showing the pseudotentacles and cerebral eyes. (C) Ventral view of the preserved specimen showing the pharynx, reproductive structures, and sucker. ce: cerebral eyes; fg: female gonopore; m: mouth; mg: male gonopore; ph: pharynx; pt: pseudotentacles; su: sucker.

**External morphology.** Oval and elongated body, slightly tapered posteriorly (Fig. 3A). Translucent dorsal surface with blackish-dark brown coloration. Numerous small white dots scattered over the entire dorsal surface with bigger white dots forming irregular and spaced clusters. Raised dorsal midline, darker in coloration. White dots of different size around the margin (Fig. 3A). Pseudotentacles square-like, slightly ruffled, dark black with white tips and small white dots in between (Fig. 3A, B). Unrecognizable pseudotentacular eyes due to the black pigment. Small cerebral eyespot located in a clear area (Fig. 3B). Ruffled pharynx with simple folds located

anteriorly (Fig. 3C). Two separate male gonopores located posterior and on each side of the pharynx. Female gonopore located in the midline, posterior to the male gonopores. Presence of a conspicuous sucker behind the female gonopore (Fig. 3C).

**Taxonomic remarks.** *Pseudobiceros apricus* was originally described by Newman & Cannon (1994) as having a transparent orange background with white microdots and a black marginal band with white dots. The specimen found in Israel differs from the original description by having a brownish-black dorsal surface and the absence of a black marginal band. Color and pattern variations for *P. apricus* were later revealed by the same authors, presenting photographic records of translucent dark brown to black background animals without the black marginal band (Newman & Cannon 2003, p.81; 2005). This second morphotype resembles our specimen. Again, we inferred that the variation in the dorsal coloration is due to differences in habitat and intestinal content. In addition, it is possible that the dark overall pigment of the body makes the black margin indistinguishable in some cases.

*P. apricus* is included within the color pattern group 3 characterized by spots and dots (Newman & Cannon 1994, 1997). In this group, only *Pseudobiceros bajae* (HYMAN, 1953) and *Pseudobiceros stellae* NEWMAN & CANNON, 1994 also exhibit translucent dark background with small scattered white dots. Although *P. bajae* has similar square, ruffled pseudotentacles, it does not have white pseudotentacular tips. Also, it differs from *P. apricus* by the absence of white dots bordering the margin and the individual, evenly spaced small dots do not form clusters. On the other hand, *P. stellae* has a similar configuration of dots and clusters as *P. apricus* but unlike this species, clusters in *P. stellae* are arranged in a flower-like pattern, the marginal band is not clear, and the pseudotentacles are highly developed. *P. apricus* is a rare species, infrequently sighted and has been only recorded in Australia. *P. apricus* is recorded for the first time in the Red Sea and Israel.

#### ***Pseudobiceros damawan* Newman & Cannon, 1994**

(Fig. 4)

**Material examined and locality:** One mature specimen (35x18 mm, live, ZMTAU-VR25164) preserved in ethanol 70%. Collected at the Inter University Institute for Marine Sciences (IUI), Eilat, Israel, northern Gulf of Aqaba, Red Sea (29° 30.211' N, 34° 55.068' E) on 18 March 2014.

**Habitat:** Specimen found subtidally in coral reef habitats, under rocks (2–3 m depth).

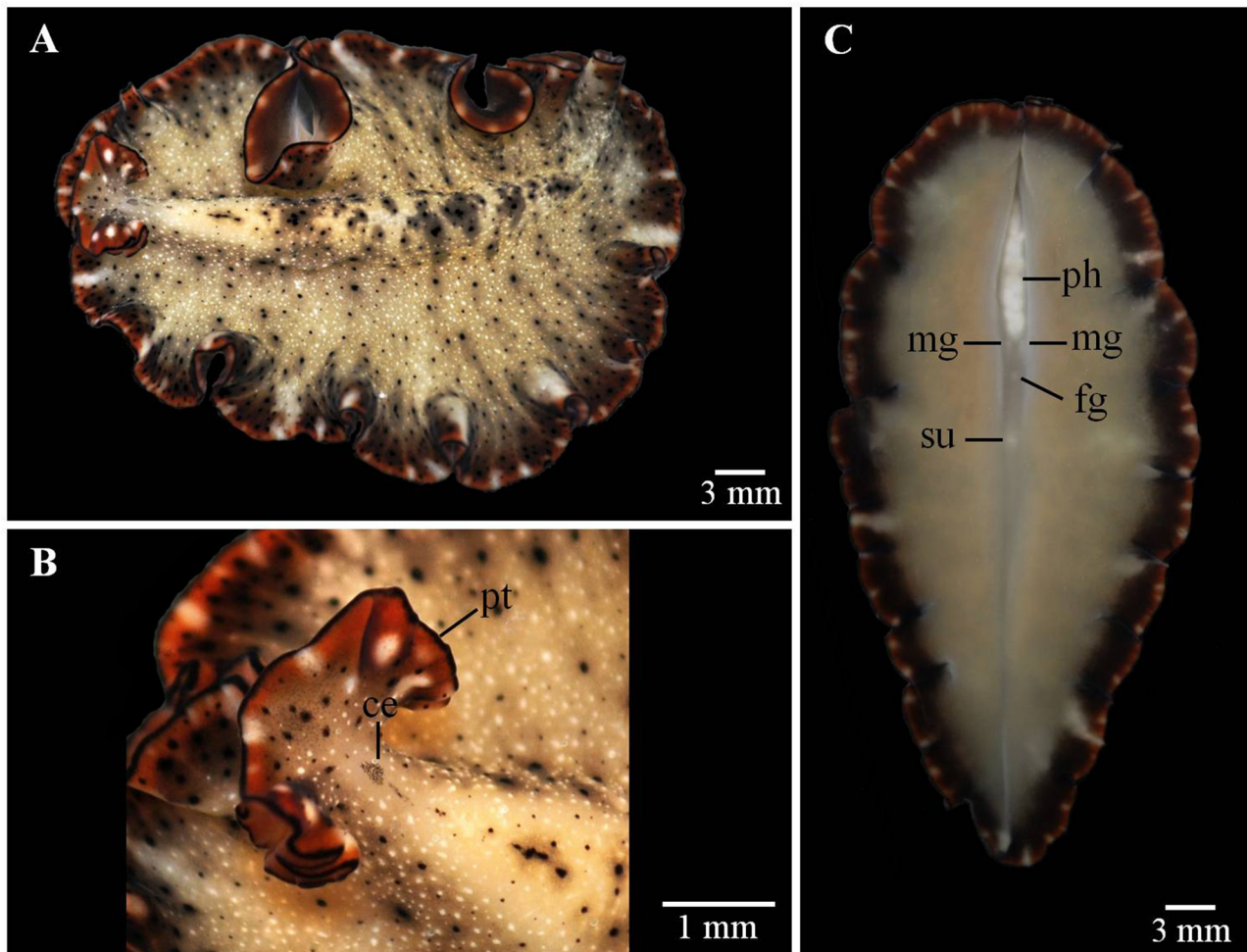
**Distribution:** Laing Island, Madang, Papua New Guinea (Type locality) and Heron Island, Great Barrier Reef, Australia (Newman & Cannon 1994); Coral Bay, Australia (Newman & Cannon 1997); Guam, Micronesia (Newman *et al.* 2003); Indonesia and South Africa (Newman & Cannon 2005); Andaman, Nicobar Island, India (Sreeraj & Raghunathan 2011); and Kusu Island, Singapore (Bolaños *et al.* 2016). In this study, the specimen was found in Eilat, northern Gulf of Aqaba, Red Sea.

**External morphology.** Oval and elongated body with deep folds of the margin (Fig. 4A). Mottled white-cream background with shadows of grey towards the margin. Small white dots over the entire dorsal surface and spaced black spots of different sizes. Raised dorsal midline with shadows of grey surrounding the black spots. Presence of an orange submarginal band with a black hue in its inner area around the entire body. The orange submarginal band is interrupted by white stripes and spots. Extremely narrow black rim (Figs. 4A, B). The ventral side is evenly cream with the same marginal pattern as the dorsal surface (Fig. 4C). Squared pseudotentacles with deep lateral folds and an orange band with white spots and black rim as the rest of the body (Fig. 4B). Clusters of numerous pseudotentacular eyes. Round cerebral eyespot with numerous eyes (Fig. 4B). Thin ruffled pharynx with simple folds located anteriorly (Fig. 4C). Two separate male gonopores located posterior and on each side of the pharynx. Female gonopore located in the midline posterior to the male gonopores and small sucker behind the female gonopore (Fig. 4C).

**Taxonomic remarks.** *Pseudobiceros damawan* was recently described for Singapore by Bolaños *et al.* (2016) who reported a morphotype with some slight color variation compared to the specimens from Australia and India (Newman & Cannon 1994; 1997; Sreeraj & Raghunathan 2011). Animals from Singapore exhibit a dark coloration along the inner side of the orange marginal band but the orange pigment along the midline is lacking. These features were also seen in the specimen from Israel. The same authors emended the diagnosis for this species due to its close similarity with *P. murinus*. However, *P. murinus* has a transparent rim and a white triangle between its pseudotentacles, which are both absent in *P. damawan*. The presence of a mottled dorsal surface varying from grey



and white to light or dark brown and a conspicuous narrow black rim with an interrupted orange marginal band allowed us to identify our specimen as *P. damawan*. Similar colored species within the same coloring group are *Pseudobiceros brogani* NEWMAN & CANNON, 1997 and *Pseudobiceros fulvogriseus* (HYMAN, 1959) (for detailed comparisons see taxonomic remarks for *P. murinus* below and Bolaños *et al.* 2016). *P. damawan* represents a new record for the Red Sea and Israel.



**FIGURE 4.** *Pseudobiceros damawan*. (A) Dorsal view, *in vivo*. (B) Close-up of the anterior region showing the pseudotentacles and cerebral eyes. (C) Ventral view of the live specimen showing the pharynx, sucker, and the reproductive structures. ce: cerebral eyes; fg: female gonopore; mg: male gonopore; ph: pharynx; pt: pseudotentacles; su: sucker.

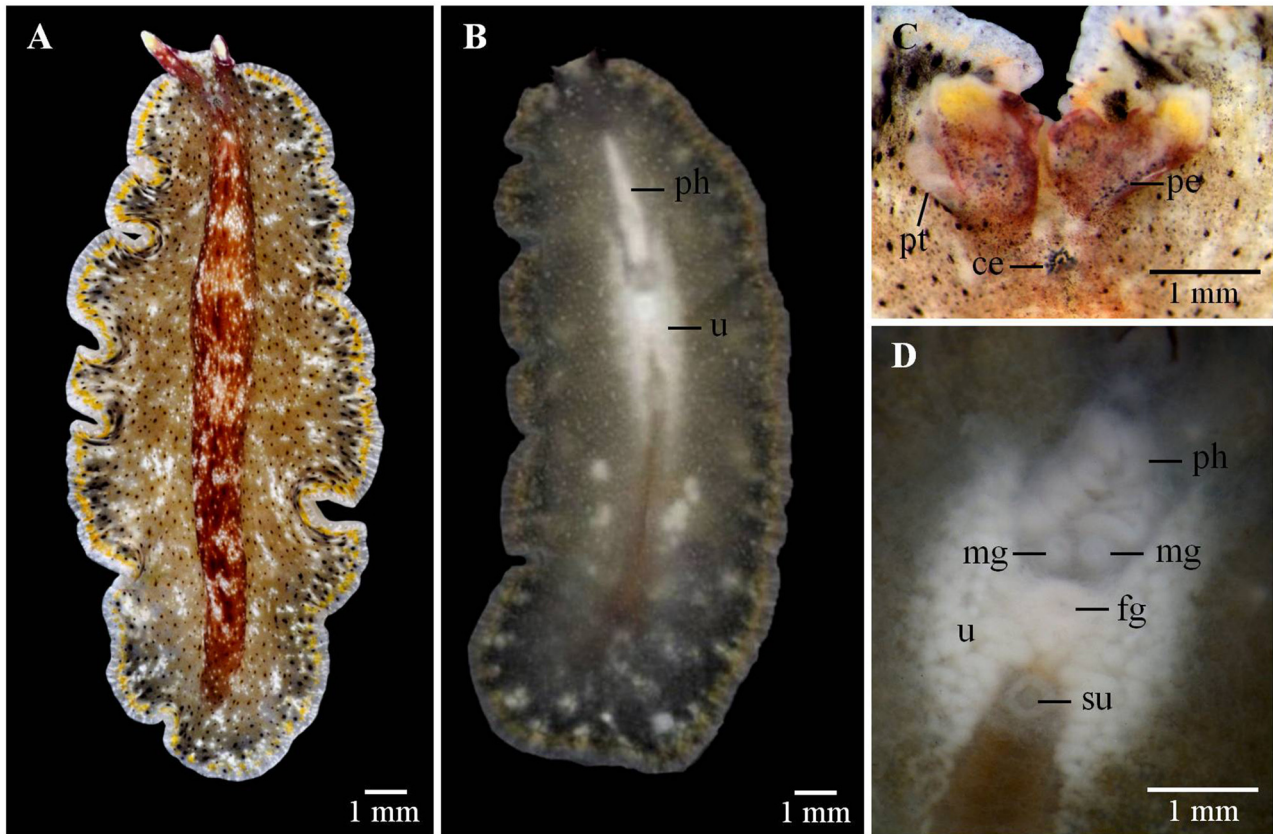
***Pseudobiceros murinus* Newman & Cannon, 1997**

(Fig. 5)

**Material examined and locality:** Two mature specimens collected at the Inter University Institute for Marine Sciences (IUI), Eilat, Israel, northern Gulf of Aqaba, Red Sea (29° 30.211' N, 34° 55.068' E). a) One specimen (15x7 mm, live, ZMTAU-VR25134), as sagittal sections of the reproductive structures (8 slides). The remaining portion of the specimen preserved in Ethanol 70%. Collecting date on 1 November 2013. b) One specimen (17x8 mm, live, ZMTAU-VR25135), preserved in ethanol 70%. Collecting date on 1 November 2013.

**Habitat:** Specimens found on the rocky shore during low tide (1 m depth), under rocks dominated principally by ascidians and sponges.

**Distribution:** Madang, Papua New Guinea (Type locality) and Lizard Island, Great Barrier Reef, Australia (Newman & Cannon 1997); Guam and Yap Islands, Micronesia, and the Red Sea (no information of the exact location) (Newman & Cannon 2003; 2005); and Kavaratti Island, India (Apte & Pitale 2011). In this study, the specimens were found in Eilat, northern Gulf of Aqaba, Red Sea.



**FIGURE 5.** *Pseudobiceros murinus*. (A, B) Dorsal and ventral view, *in vivo*. (C) Close-up of the anterior region of a preserved animal showing the pseudotentacles, pseudotentacular eyes, and cerebral eyes. (D) Close-up of the ventral side of a preserved animal showing the pharynx, reproductive structures, and sucker. ce: cerebral eyes; fg: female gonopore; mg: male gonopore; pe: pseudotentacular eyes; ph: pharynx; pt: pseudotentacles; su: sucker; u: uteri.

**External anatomy.** Oval and elongated body with some deep folds of the margin (Fig. 5A). Dorsal background grey to olive green or brownish-green with small black spots evenly distributed over the entire dorsal surface. Few blotches of white scattered around the body. Conspicuous raised dorsal midline with a bright reddish-brown coloration extending to the pseudotentacles. Presence of a fine yellow submarginal band with a dark hue in its inner area surrounding the entire body. Clear rim marked with white spots (Fig. 5A). Ventral side translucent grey with opaque white dots and visible yellow line (Fig. 5B). Pointed, long, erected ear-like pseudotentacles with yellowish-white tips and red tinge at the base. A triangle of white dots between the pseudotentacles (Fig. 5A, C). Small clusters of dorsal and ventral pseudotentacular eyes. Cerebral eyespot horseshoe-shaped located in a clear area (Fig. 5C). Ruffled pharynx with simple folds located anteriorly (Figs. 5B, D). Two separate male gonopores, one close to the other located posterior to the pharynx. Female gonopore located in the midline, posterior to the male gonopores. Conspicuous sucker behind the female gonopore (Fig. 5D).

**Taxonomic remarks.** *Pseudobiceros murinus* is included into the color group 4 which is characterized by a mottled pattern (Newman & Cannon 1994; 1997). This group includes six species of which *P. fulvogriseus* and *Pseudobiceros gardineri* (LAIDLAW, 1902) also show a mottled grayish background with pointed pseudotentacles. Although *P. gardineri* also exhibits black dots, it lacks the yellow submarginal band. Similarly, *P. fulvogriseus* lacks the yellow band and the black spots present in *P. murinus*. As mentioned above, *P. damawan* closely resembles *P. murinus*. However, *P. damawan* has a wide orange submarginal band interrupted with white dots and a conspicuous black rim instead of a fine yellow submarginal band with a clear rim as *P. murinus*. The pseudotentacles of *P. murinus* also differ from the three species mentioned before. However, *Pseudobiceros mikros* NEWMAN & CANNON, 1997 exhibits the same pseudotentacle shape and color pattern as *P. murinus* but in contrast, *P. mikros* has a yellow rim and white submarginal band.

Newman & Cannon (2005) mentioned that the color of this species is variable depending on food in the gut

and presented three different photographic records for this species. Likewise, Apte & Pitale (2011) reported a morphotype with a lighter dorsal background, more white blotches, a thicker orange-yellowish submarginal band, and purplish-pink pseudotentacles instead of red. Also, the dark coloration associated with the inner area of the yellow band was absent. According to Newman & Cannon (2005), *P. murinus* has been seen in the Red Sea but there is no additional information, a formal description or specific location. Therefore, this species represents the first record for Eilat, northern Gulf of Aqaba, Red Sea, Israel.

### ***Pseudobiceros stellae* Newman & Cannon, 1994**

(Fig. 6)

**Material examined and locality:** One mature specimen (27x13 mm, live, ZMTAU-VR25179; GenBank ID: MH047293) preserved in ethanol 70%, collected at Mikhmoret, Israeli eastern Mediterranean Sea (32° 24' N, 34° 52' E) on 22 May 2016.

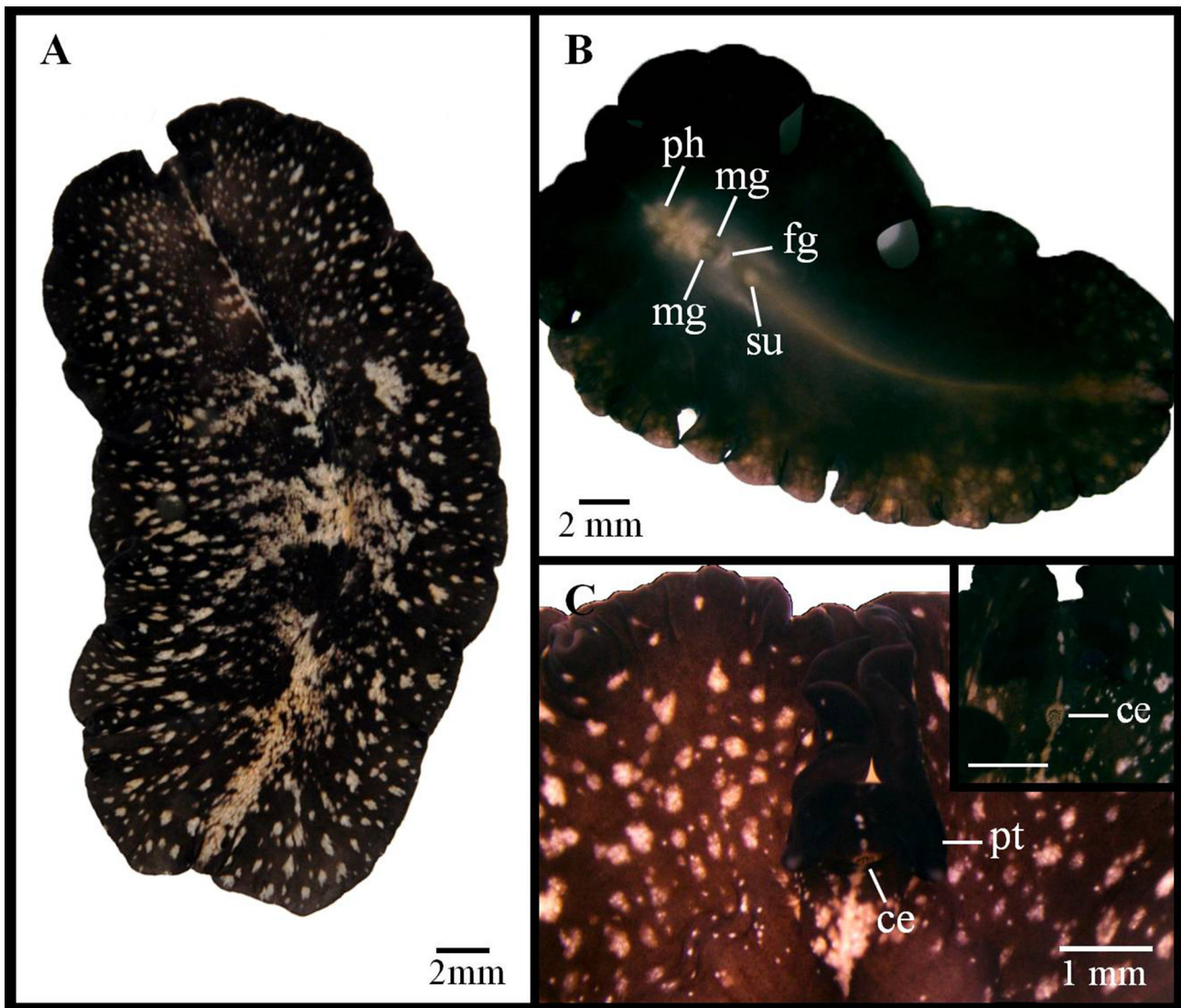
**Habitat:** Specimen found on the rocky shore, under rocks during low tide (1 m depth).

**Distribution:** Heron Island (Type locality), Tree Island, Great Barrier Reef, Australia and Madang Island, Papua New Guinea (Newman & Cannon 1994). Lizard Island and Coral Bay, Australia (Newman & Cannon 1997); Hawaii, Galapagos Island, Indonesia, Marshall Island, and possibly the Red Sea (Poulter 1987; Newman & Cannon 2005); Kavaratti Island (Apte & Pitale 2011) and Nancowry Island, India (Sreeraj & Raghunathan 2015); Singapore (Ong *et al.* 2015). In this study, the specimen was found at Mikhmoret, Eastern Mediterranean Sea, Israel.

**External anatomy.** Oval and elongated body with ruffled margin (Fig. 6A, B). Dorsal surface black with numerous white dots distributed over the entire body. Some dots are small and are more evenly distributed than the larger ones that aggregate and form large white clusters. Raised dorsal midline with larger patches of white close to each other, forming a wide irregular band posteriorly. A fine line of white microdots at the anterior end (Fig. 6A). Ventral side black, translucent towards the margin (Fig. 6B). Square, lateral ruffled pseudotentacles, black in coloration with white tips (Figs. 6C). Indistinguishable pseudotentacular eyes due to the dark pigment. Cerebral eyespot located in a clear oval area (Fig. 6C). Short and ruffled pharynx with simple folds located anteriorly (Fig. 6B). Two conspicuous male gonopores located posterior to the pharynx. Female gonopore located in the midline close to the male gonopores. Presence of a large sucker behind the female gonopore (Fig. 6B).

**Taxonomic remarks.** *Pseudobiceros stellae*, *Pseudobiceros bajae*, and *Pseudobiceros caribbensis* BOLAÑOS, QUIROGA & LITVAITIS, 2007 comprised a group of species with dark background and white spots over the dorsal surface. However, *P. stellae* has a “flower-like” organization of the white dots not seen in any of the other two species. Although our specimen displays such particular arrangement, the clusters are not regularly distributed as mentioned in the original description. In addition, our animal exhibits large white blotches along the midline that have not been observed in previous reports (Poulter 1987; Newman & Cannon 1994; Apte & Pitale 2011; Sreeraj & Raghunathan 2015; Ong *et al.* 2015). Therefore, we considered that this feature could be related to the specific condition of the collected specimen (excessive clustering of dots or depigmentation) rather than a case of color variation.

Two other species with similar color pattern are *Pseudoceros josei* NEWMAN & CANNON, 1998 and the morphotype of *Pseudoceros maximus* LANG, 1884 reported for Tunisia (Gammoudi & Tekaya 2012; Gammoudi *et al.* 2011, 2017). However, these are characterized by a single male gonopore instead of two as seen in *P. stellae*. Because researchers frequently fail to present detailed observations of the ventral side of the animal, polyclad taxonomy is replete with misidentifications, synonyms, and erroneous geographic distribution records. For instance, Newman & Cannon (2005) indicated that *P. stellae* has been reported in the Red Sea. However, no specific location, photographic record or additional information was provided. Hence, at this point it is not possible to confirm that *P. stellae* is distributed in both the Mediterranean and the Red Sea, potentially representing another Lessepsian species. This highlights the importance of a thorough examination of actual specimens and the avoidance of describing polyclad species based only on photographic records.



**FIGURE 6.** *Pseudobiceros stellae*. (A) Dorsal view, *in vivo*. (B) Ventral view of the live specimen showing the pharynx, reproductive structures, and sucker. (C) Close-up of the anterior region showing the pseudotentacles and cerebral eyes. Inset showing the shape of the cerebral eyespot. Scale bar: 1mm. ce: cerebral eyes; fg: female gonopore; mg: male gonopore; ph: pharynx; pt: pseudotentacles; su: sucker.

## Genus *Thysanozoon* Grube, 1840

### *Thysanozoon brocchii* (Risso 1818)

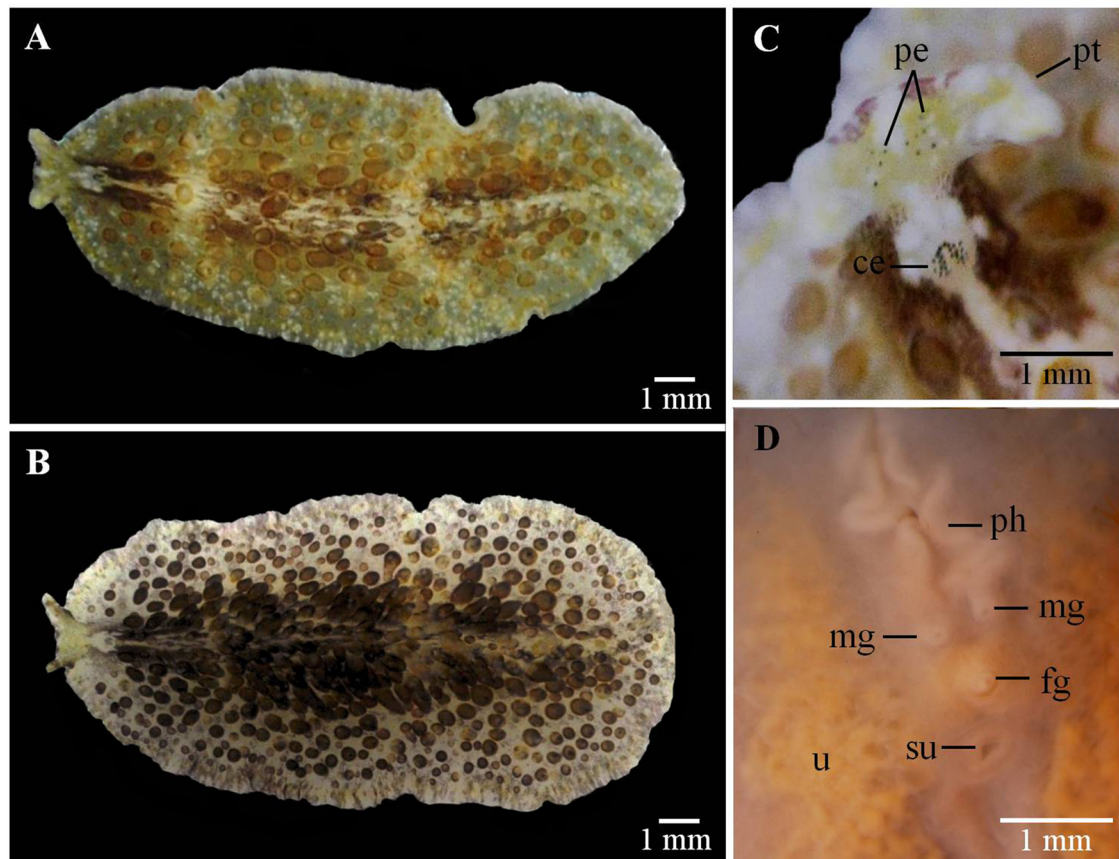
(Fig. 7)

**Synonyms:** *Tergipes brocchii* Risso, 1818; *Planaria brocchii* (Risso) Risso, 1826; *Planaria tuberculata* Delle Chiaje, 1828; *Planaria verrucosa* Delle Chiaje, 1829; *Stylochus papillosus* Diesing, 1836; *Thysanozoon diesingii* Grube, 1840; *Thysanozoon papillosum* (Diesing) Grube, 1840; *Thysanozoon tuberculatum* (Delle Chiaje) Grube, 1840; *Planaria dicquemaris* Delle Chiaje, 1841; *Planaria dicquemaris* var. *verrucosa* (Delle Chiaje, 1829) Delle Chiaje, 1841; *Thysanozoon dicquemaris* (Delle Chiaje) Örsted, 1844; *Eolidiceros panormus* Quatrefages, 1845; *Eolidiceros brocchii* (Risso) Quatrefages, 1845; *Thysanozoon panormus* (Quatrefages) Diesing, 1850; *Thysanozoon fockei* Diesing, 1850; *Thysanozoon* spec. Schultze, 1854; *Planeolis panormus* (Quatrefages) Stimpson, 1857; *Thysanozoon* spec. Moseley, 1877; *Thysanozoon brocchii* var. *cruciatum* Laidlaw, 1906; *Thysanozoon lagidium* Marcus, 1949.

**Material examined and locality:** Three mature specimens collected at Sdot-Yam, off Caesarea, Israeli eastern Mediterranean Sea (29° 30' N, 34° 55' E). a) One specimen (16x12 mm, live, ZMTAU-VR25137). Reproductive

structures sagittally sectioned but no anatomical information from histological sections was obtained. The remaining portion of the specimen preserved in ethanol 70%. Collected on 8 July 2013. b) One specimen (17x13 mm, live, ZMTAU-VR25138), preserved in ethanol 70%. Collected on 8 July 2013. c) One specimen (16x13 mm, live, ZMTAU-VR25139), preserved in ethanol 70%. Collected on 20 August 2013.

**Habitat:** Specimens found on the rocky shore, during low tide, under rocks dominated principally by ascidians (1 m depth).



**FIGURE 7.** *Thysanozoon brocchii*. (A, B) Dorsal view, *in vivo* showing color variation. (C) Close-up of the anterior region showing the pseudotentacles and the pseudotentacular and cerebral eyes. (D) Close-up of the ventral view of a preserved specimen showing the pharynx, reproductive structures, and sucker. ce: cerebral eyes; fg: female gonopore; mg: male gonopore; pe: pseudotentacular eyes ph: pharynx; pt: pseudotentacles; su: sucker; u: uteri.

**Distribution:** Cosmopolitan species found in Naples, Italy, Western Mediterranean Sea (Type locality) (Risso 1818; Lang 1884); Tunisia (Gammoudi *et al.* 2011, 2017; Gammoudi & Tekaya 2012); Egypt, eastern Mediterranean Sea (Palombi 1928); East London, South Africa, Indian Ocean, and Rio de Oro, West coast of Africa, Atlantic Ocean (Palombi 1939a, b); Canary Islands (Vera *et al.* 2009); Iberian Peninsula (Noreña *et al.* 2014); Caribbean Sea (Quiroga *et al.* 2004); Brazil (Bahia *et al.* 2012, 2014, 2015); Argentina (Brusa *et al.* 2009; Bulnes *et al.* 2011); Japan (Yeri & Kaburaki 1918); Borneo, Vietnam, New Zealand, and England (Prudhoe 1989); Persian Gulf and Gulf of Oman, Iran (Maghsoudlou & Rahimian 2014); West Coast of India (Pitale & Apte 2017). In this study, the specimens were found at Sdot-Yam, eastern Mediterranean Sea, Israel.

**External anatomy.** Oval body with numerous papillae over the dorsal surface. Longer, thicker and more abundant papillae along the midline, decreasing in size and number towards the margin and becoming completely absent in the border (Fig. 7A, B). Dorsal background variable in color, ranging from yellow to light brown and dark brown to black. Presence of white dots extending towards the margin. A white longitudinal median line and some specimens with two transversal white lines formed by white pigment and lighter colored papillae (Fig. 7A). A faint yellow submarginal line bordering the entire body is present. The papillae along the midline line are darker than those close to the margin. Presence of a clear rim dotted with white (Figs. 7A, B). The ventral side is whitish-grey. Pointed, ear-like, pale yellow with white tip pseudotentacles, scattered with white and brown spots and a pinkish

tinge at the margin (Fig. 7C). Cerebral eyes arranged in horseshoe shape. Small clusters of few pseudotentacular eyes (Fig. 7C). Short ruffled pharynx located anteriorly (Fig. 7D). Two conspicuous male gonopores close to each other located posteriorly to the pharynx. A prominent female gonopore in the midline, posterior and close to the male gonopores. Sucker located behind the female gonopore (Fig. 7D).

**Taxonomic remarks.** *Thysanozoon brocchii* (RISSO 1818) is the type species of the genus but it represents perhaps, the most doubtful species within the suborder Cotylea in terms of taxonomic identifications. The problem goes back to its original description as a nudibranch *Tergipes brocchii* (RISSO 1818). The author failed in presenting figures and detailed information of the newly described species; thus, to date, uncertainty even exists at the phylum level for this first original record. Based on the presence of dorsal papillae, Grube (1840) erected the genus *Thysanozoon*, made the new combination *Thysanozoon brocchi*, and created simultaneously the new species *Thysanozoon diesingii* GRUBE 1840. However, during those 22 years before this new combination, multiple species with apparent papillae (called “tubercles” in the old literature) on the dorsal surface were described. Likewise, after the creation of the genus, numerous new species within the taxon have been erected (see synonyms above). To date, *T. brocchii* has approximately 20 synonyms and many other still remain as *Incertae sedis* (Faubel 1984). Sadly, most of these records are unsatisfactory and ambiguous descriptions hindering the appropriate comparison and accurate placement of the species.

Another aspect that has contributed to the problem is the lack of internal and external morphological characters. The relative homogeneity of the reproductive system has limited researchers to mainly using differences in color and shape of dorsal papilla to distinguish *Thysanozoon* related-species. However, we know that this is not a good practice due to the high degree of color variation and cases of species complexes seen in pseudocerotids (Litvaitis *et al.* 2010; Bolaños *et al.* 2016). Again, DNA-based methods are needed for future studies to investigate the significance of these morphological details. Unfortunately, molecular and additional morphological information from most, if not all of the old records is not possible to obtain due to missing holotypes, misidentifications, and poor specimen preservation.

Based on the number of synonyms, *T. brocchii* is considered a cosmopolitan species and it has been largely accepted that the color greatly varies according to its geographic location. The specimens found in this study are yellowish-brown and dark brown which is consistent with color descriptions of previous reports (Marcus & Marcus 1968; Brusa *et al.* 2009; Bulnes *et al.* 2011; Bahía *et al.* 2014). In addition, our specimens exhibit a clear rim and an extremely delicate yellow submarginal band encircling the entire body. Differently, Bahía *et al.* (2015) mentioned that specimens from another locality in the Mediterranean show a red marginal band. Likewise, specimens reported for India showed a pink tint at the periphery (Pitale & Apte 2017). Maghsoudlou & Rahimian (2014) indicated a purple band for animals from Iran. Brusa *et al.* (2009) reported a thin and discontinuous black line delineating the body in the specimens from Argentina; however, this feature was not observed in animals from a nearby location (Bulnes *et al.* 2011) nor for animals from other localities. Brown dots on the pseudotentacles are apparently a general feature for this species (Brusa *et al.* 2009; Bulnes *et al.* 2011; Bahía *et al.* 2012, 2014, 2015). *T. brocchi* is a common species in the Mediterranean Sea and it represents a new record for the Israeli Mediterranean coast.

## Family Pericelidae Laidlaw, 1902

### Genus *Pericelis* Laidlaw, 1902

#### *Pericelis byerleyana* (Collingwood, 1876)

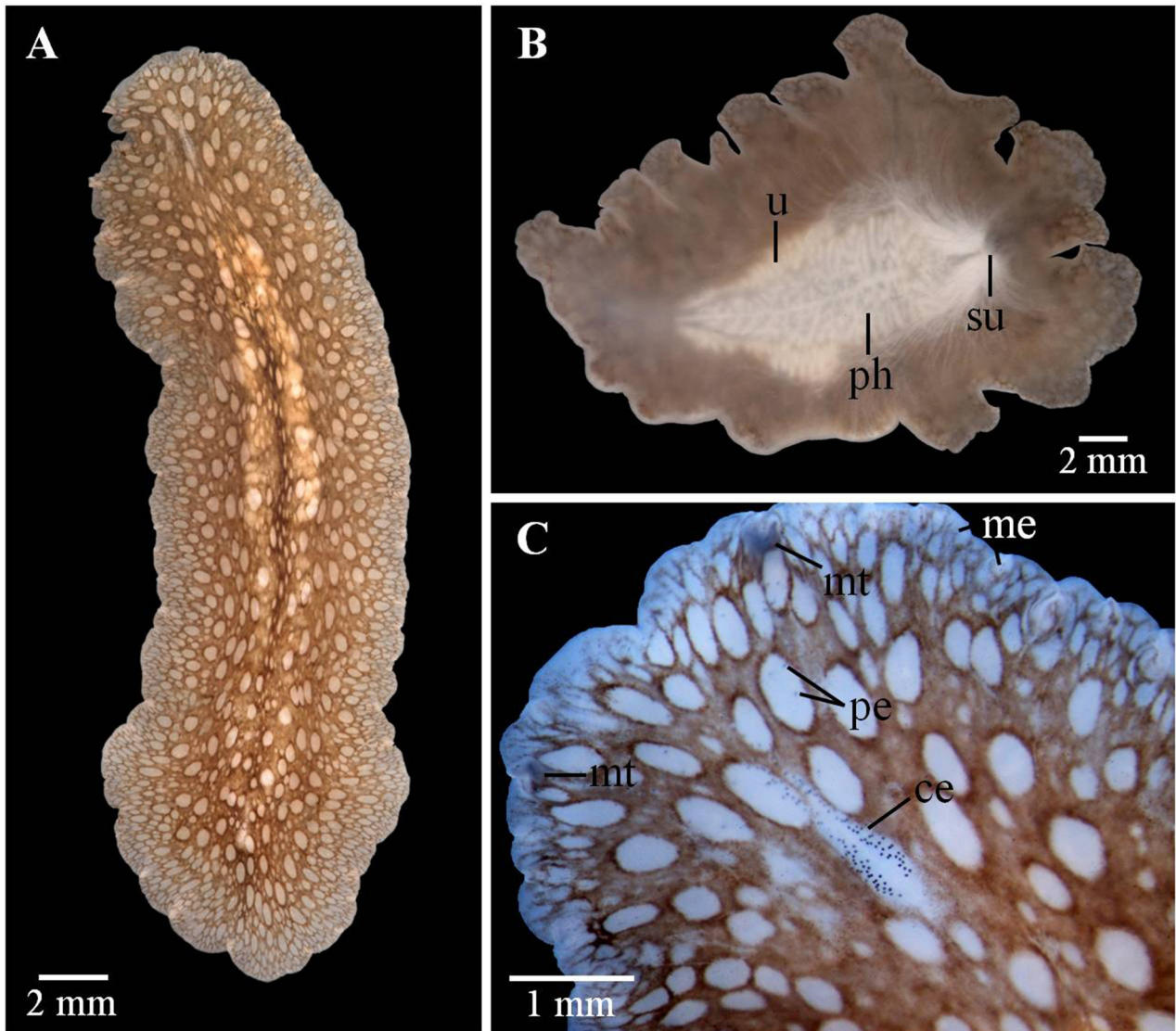
(Figs. 8)

**Synonyms:** *Typhlolepta byerleyana* Collingwood, 1876; *Pericelis* cf. *hymanae* Newman & Cannon, 2003; *Pericelis* sp. 3 Newman & Cannon, 2003.

**Material examined and locality:** Two mature specimens collected at the Inter University Institute for Marine Sciences (IUI), Eilat, Israel, northern Gulf of Aqaba, Red Sea (29° 30.211' N, 34° 55.068' E). a) One specimen (29x14 mm, ZMTAU-VR25162), preserved in ethanol 70%. Collecting date on 21 March 2013. b) One specimen (26x13 mm, ZMTAU-VR25163, (GenBank ID: MH047291), preserved in ethanol 70%. Collecting date on 29 May 2015.

**Habitat:** Specimens found in reef habitats (12–15 m depth).

**Distribution:** Pulo Barundum, Borneo, Pacific Ocean (Type locality) (Collingwood, 1876). Maldives, Laccadive Islands & Mauritius Island, Indian Ocean (Laidlaw 1902; Palombi 1938); Gulf of Tadjoura, Djibouti, Gulf of Aden, Somalia (Meixner 1907); Rotuma Island, Fiji (Laidlaw 1903); Micronesia (Kato 1943; Newman *et al.* 2003); Japan (Kato 1944); Australia and Indonesia (Kato 1944; Prudhoe 1989); Eilat, northern Gulf of Aqaba, Red Sea (Prudhoe 1989). In this study, specimens were found in Eilat in accordance to the locality given by Prudhoe (1989).



**FIGURE 8.** *Pericelis byerleyana*. (A) Dorsal view, *in vivo*. (B) Ventral view of the live specimen showing the pharynx, uteri, and sucker. (C) Close-up of the anterior region showing the pseudoentacles and the cerebral, pre-cerebral, and marginal eyes; ce: cerebral eyes; me: marginal eyes; mt: marginal tentacles; pe: pre-cerebral eyes; ph: pharynx; su: sucker; u: uteri.

**External anatomy.** Large, oval, and elongated body with ruffled margins. Dorsal surface light brown with well-defined roundish cream circles of different sizes forming a reticulate pattern. Presence of smaller circles between the interstices of the larger. Midline with a darker brown coloration and larger and more spaced rings becoming smaller and more numerous toward the margin (Fig. 8A). Light brown ventral surface lacking the reticulate pattern (Fig. 8B). Indistinct, small pseudotentacles formed by simple folds of the anterior margin and with numerous pseudotentacular eyes at the tip (Fig. 8C). Cerebral eyes arranged in two elongated and separated clusters located in a clear area. More numerous eyes posteriorly extending to thin lines of less and more scattered eyes anteriorly (Figs. 8C). Marginal eyes surrounding the entire body. In the anterior area, few pre-cerebral eyes are

also present (Fig. 8C). A large ruffled pharynx, highly branched located centrally (Fig. 8B). Separate male and female gonopores. The male gonopore is located immediately after the pharynx and the female pore close to male gonopore. Small sucker located posteriorly and close to the female gonopore (Fig. 8B).

**Taxonomic remarks.** Currently, the genus *Pericelis* contains four valid species (Faubel 1984; Tyler *et al.* 2006–2018), mainly distinguished by the color and pattern. Of these, *P. byerleyana*, *P. cata*, and *P. orbicularis*, share a similar reticulated brown pattern. However, *P. cata* differs from *P. byerleyana* by the conspicuous pseudotentacles with black tips, patchy background with irregular-shaped white circles, and a few black spots scattered over the dorsal surface. On the other hand, *P. orbicularis* has a dark brown pigment forming a loose network pattern instead of well-defined white rings as seen in *P. byerleyana*. Moreover, *P. orbicularis* has a more developed sucker. Despite their morphological similarity, a comparison based on available molecular data for *P. cata* (EU679114), *P. orbicularis* (EU679116), and *P. byerleyana* (MH047291), using about 950 base pairs of the 28S rDNA gene, revealed species-specific changes allowing us to clearly distinguish three different species (Table 3).

**TABLE 3.** Distance matrix for members of the genus *Pericelis* showing percentage of nucleotide similarity among sequences.

	<i>Pericelis cata</i> (EU6791141)	<i>Pericelis orbicularis</i> (EU6791161)	<i>Pericelis byerleyana</i> (MH047291)
<i>Pericelis cata</i> (EU6791141)	-	96.2	95.9
<i>Pericelis orbicularis</i> (EU6791161)	96.2	-	97.9
<i>Pericelis byerleyana</i> (MH047291)	95.9	97.9	-

In addition, *P. byerleyana* has been frequently found in the Indian Ocean and the Indo-Pacific region whereas *P. cata* and *P. orbicularis* have been found in the Atlantic Ocean including the Caribbean and the Gulf of Mexico (Schmarda 1859; Hyman 1955b; Marcus & Marcus 1968; Quiroga *et al.* 2004). A previous record of *P. byerleyana* was poorly presented by Prudhoe (1989) for Israel. Thus, we present a detailed description of the species based on the external morphology, high quality photographs, and molecular sequence data (MH047291) for future comparisons with other pericelid species.

## Superfamily Euryleptoidea Faubel, 1984

### Family Euryleptidae Lang, 1884

#### Genus *Maritigrella* Newman & Cannon, 2000

#### *Maritigrella fuscopunctata* (Prudhoe, 1978) Newman & Cannon, 2000

(Fig. 9)

**Synonyms:** *Pseudoceros fuscopunctatus* (Prudhoe 1978); *Eurylepta fuscopunctatus* (Gosliner *et al.* 1996); *Eurylepta* sp.3 (Gosliner *et al.* 1996); *Maritigrella makranica* (Maghsoudlou & Rahimian 2014).

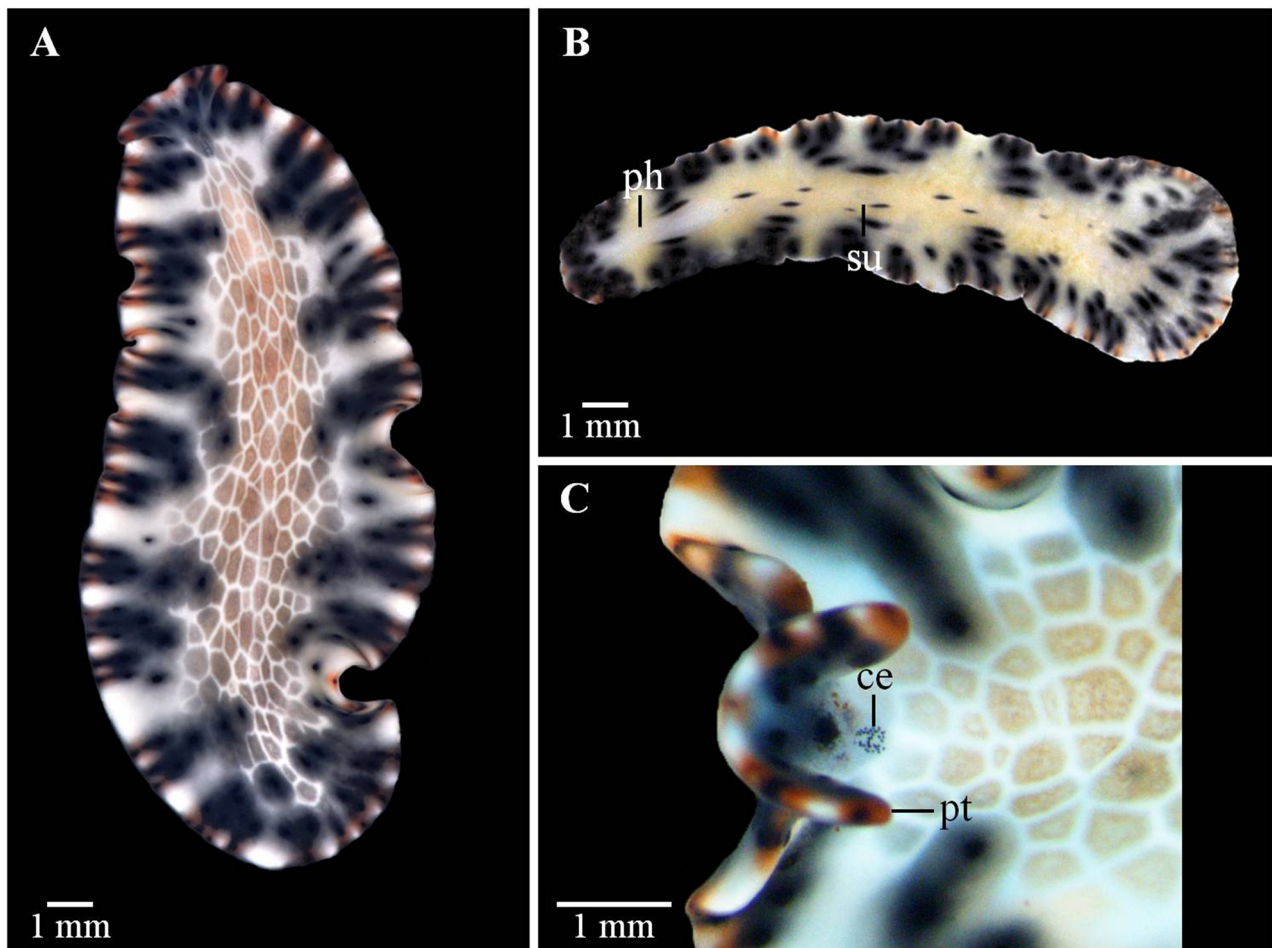
**Material examined and locality:** a) One mature specimen (12x6 mm, live, ZMTAU-VR 25169) preserved in ethanol 70%. Collected at Nahariya, Israeli eastern Mediterranean Sea (33° 00' N, 35° 05' E) on 12 October 2013. b) One specimen (14x7 mm, live, ZMTAU-VR25170), preserved in ethanol 70%. Collected at Acre, Eastern Mediterranean Sea (32° 55' N, 35° 04' E) on 16 June 2014. c) One specimen (10x4 mm, live, ZMTAU-VR25171; (GenBank ID: MH047290), preserved in ethanol 95%. Collected at Acre, Israeli eastern Mediterranean Sea (32° 55' N, 35° 04' E) on 16 June 2014.

**Habitat:** Specimens found subtidally in rocky reef habitats (4–5 m depth) and intertidally on the rocky shore, under rocks (1–2 m depth).



**Distribution:** Dunsborough and Broome, Western Australia (Type locality) (Prudhoe 1978); Heron Island and Lizard Island, Australia (Newman & Cannon 2000); Madang, Papua New Guinea; Maldives, Indonesia, Micronesia, Philippines, Marshal Island, Japan, Hawaii, Tanzania (Gosliner *et al.* 1996; Newman *et al.* 2003; Newman & Cannon 2005); Kavarratti Island, India (Apte & Pitale 2011); Gulf of Oman, Iran (Maghsoudlou & Rahimian 2014); Malta, Italy (Crocetta *et al.* 2015; Vella *et al.* 2016). In this study, the specimens were found along the Israeli Mediterranean coast.

**External anatomy.** Oval and elongated body with a ruffled margin (Fig. 9A). Dorsal background white-cream with black spots variable in size, forming irregular transverse rows perpendicular to and around the margin. Transverse rows surrounded by a greyish-black shade and stained with a vivid orange pigment at the margin. Few small, individual black spots scattered between the transverse rows. Large pale orange-brownish spots arranged in a honeycomb pattern in the midline running longitudinally from the level of the cerebral eyespot to the posterior edge of the body (Fig. 9A). The ventral side exhibits the same pattern as the dorsal surface with few black spots scattered in the median area instead of the honeycomb (Fig. 9B). Marginal tentacles with the same pattern of the body margin. Black spots surrounded by a black shadow and orange stains at the edge (Figs. 9A, C). Several eyes scattered between the marginal tentacles, cerebral eyes arranged in two elongated clusters (Fig. 9C). Small tubular pharynx located anteriorly (Fig. 9B). Separate male and female gonopores. The single male gonopore is located immediately after the pharynx and the female pore close to male gonopore. Conspicuous sucker located posteriorly and well separated from the female gonopore (Fig. 9B).



**FIGURE 9.** *Maritigrella fuscopunctata*. (A) Dorsal view, *in vivo*. (B) Ventral view of the live specimen showing the pharynx and sucker. (C) Close-up of the anterior region showing the marginal tentacles and cerebral eyes. ce: cerebral eyes; ph: pharynx; pt: pseudotentacles; su: sucker.

**Taxonomic remarks.** The genus *Maritigrella* includes ten valid species (Newman & Cannon 2000; Tyler *et al.* 2006–2018) characterized by a tubular pharynx, well-developed marginal tentacles, and a conspicuous lined or striped pattern (Newman & Cannon 2000). Originally described as *Pseudoceros* (Prudhoe 1978), then moved to

*Eurylepta* (Gosliner *et al.* 1996), and finally placed within *Maritigrella*, this species is distinguished by the transverse rows formed by dots, the orange-brown honeycomb pattern along the midline, and the dark and purple-violet pigment associated with the transverse lines (Newman & Cannon 2000). The specimens found in Israel agreed with the general diagnostic pattern for the species but slightly differs in the color encircling the black spots, being dark grey close to the mid-body and orange at the margin (Fig. 9). Crocetta *et al.* (2015) and Vella *et al.* (2016) reported *M. fuscopunctata* from another location in the Mediterranean, in which the orange pigment forms an interrupted marginal band. Although our specimens have orange pigment at the margin, a distinct band is not evident due to a more spaced grouping of the transverse rows.

A geographically more distant and morphologically different morphotype was described from India (Apte & Pitale 2011), exhibiting a solid orange band at the margin instead of an interrupted band or separated orange stains as mentioned above for the Mediterranean worms. In addition, the Indian specimen has the margin bordered by regularly organized black dots with larger dots more scattered over the dorsal surface than forming well-defined transverse rows (Apte & Pitale 2011). The same morphotype was also reported by Maghsoudlou & Rahimian (2014) for the Gulf of Oman in Iran. Differently, these authors considered such discrepancies significant enough for the creation of the new species *Maritigrella makranica* MAGHSOUDLOU & RAHIMIAN, 2014. While species identifications within the genus exclusively rely on color and pattern, variation also occurs in euryleptids (Bahía *et al.* 2014) as it has also been largely discussed for pseudocerotids (See Bolaños *et al.* 2016). Despite color differences, the basic pattern remains nearly constant between these morphotypes.

Maghsoudlou & Rahimian (2014) stated as major differences between both species the presence of black spots scattered over the dorsal surface and the dark-grey hue surrounding the transverse black dots towards the midline, which is orange towards the margin in *M. makranica*, and the lack of a distinct orange submarginal band and the reticulated brownish honeycomb pattern extending over the entire dorsal surface in *M. fuscopunctata*. First, the specimens collected in our study and several web-based photographic records show that some morphotypes have black dots scattered over the dorsal surface (Figs. 9A, B; Newman & Cannon 2005: Photos 6-11; Cobb *et al.* 2003-2017; Tan 2008; Ling 2009; Charpin 2004-2016; Crocetta *et al.* 2015: Fig. 7a-c, p. 689; Vella *et al.* 2016: Fig. 2a-c, p. 123). In fact, it is commonly called punctuated worm or dark-spotted flatworm (Tan 2008; Charpin 2004-2016; Vella *et al.* 2016). Second, it was possible to recognize variants for the pigment surrounding the black spots: a purple-violet (Newman & Cannon 2000: Fig. 22, p. 203; Harasti 2003), brownish-orange (Tan 2008; Charpin 2004-2016); brownish-orange medially and dark grey towards the margin (Newman & Cannon 2005: Photo 9; Vella *et al.* 2016: Fig. 2a, p.123), dark grey-blackish (Newman & Cannon 2005: Photo 10; Vella *et al.* 2016: Fig. 2a, b, p. 123; this study), in addition to the description for *M. makranica*. Third, the marginal band can be orange or purple as well as interrupted, solid or patchy (Duncan *et al.* 2015-2017; Harasti 2003; Cobb *et al.* 2003-2017; Newman & Cannon 2005: Photos 9, 10; Boyer 2009-2017; Crocetta *et al.* 2015; Aya 2015; Vella *et al.* 2016: Fig. 2a-e, p. 123). Finally, the honeycomb pattern is consistent in all the specimens and variation in length and width has no taxonomic significance. The rim delineated by more organized black spots observed in *M. makranica* is also observed in the specimens from Malta in the Mediterranean (Vella *et al.* 2016), Indonesia (Aya 2015), and Hawaii (Duncan *et al.* 2015-2017). The remarkable mixture of morphological characters observed in several formal and informally documented records for *M. fuscopunctata* clearly support the low taxonomic weight of the differential characters selected for *M. makranica*, and are considered insufficient for the creation of a new species. Therefore, it is highly likely that *M. makranica* represents a junior synonym of *M. fuscopunctata*.

A similar case is that of *Maritigrella ocellata* NEWMAN & CANNON, 2000 which differs from *M. fuscopunctata* mainly by the absence of the reticulated orange pigment along the midline region. Another difference mentioned by Newman & Cannon (2000) is the more abundant number of black spots surrounded by an orange hue instead of purple-violet as *M. fuscopunctata*. While we considered the presence of an orange pigment in a honeycomb pattern an important character for species distinction, number of dots and small differences in color are subjective and unreliable features for species distinction. Prudhoe (1978) presented a diagram of a cleared specimen of *M. fuscopunctata* (Fig. 5a, p. 596) in which the pattern perfectly fits with the description for *M. ocellata*. In fact, the author stated that only by examining a color-transparency of the cleared animal when alive, allowed him to place both specimens under the same species. The foregoing opens the possibility of a potential synonymy between *M. fuscopunctata* and *M. ocellata* and raises the question if the lack of orange-brown pigment in the medial region is due to immaturity (juvenile worm) or the homeostatic condition (health, nutrition, reproductive success) of each individual. Additionally, several records for *M. fuscopunctata* have been sighted in different locations in the Indo-pacific unlike *M. ocellata* where no other reports exist since the creation of the species.

Finally, we compare available sequences in GenBank from the specimens found in Malta (KU674837-9) with the homolog sequence of our specimen from Israel (MH047290). Our analysis revealed a close match of almost the full-length of the sequences (~984bp), which allowed us to confirm that these variants of *M. fuscopunctata* inhabiting different locations in the Mediterranean correspond to the same species (Table 4). Additionally, our analysis validated the hypothesis that small color and pattern variations in *M. fuscopunctata* such as pigment around the black spots and a solid, interrupted or spotted orange margin may occur and are not sufficient for the creation of a new species. However, a more thorough molecular analysis is needed to determine if *M. fuscopunctata* is another species complex. *M. fuscopunctata* is recorded for the first time in the Israeli eastern Mediterranean Sea.

**TABLE 4.** Distance matrix for different morphotypes of *Maritigrella fuscopunctata* found in the Mediterranean Sea showing percentage of nucleotide similarity among sequences.

	<i>Maritigrella fuscopunctata</i> 1 Malta (KU674837)	<i>Maritigrella fuscopunctata</i> 2 Malta (KU674838)	<i>Maritigrella fuscopunctata</i> 3 Malta (KU674839)	<i>Maritigrella fuscopunctata</i> Israel (MH047290)
<i>Maritigrella fuscopunctata</i> 1 Malta (KU674837)	-	99.8	99.8	99.4
<i>Maritigrella fuscopunctata</i> 2 Malta (KU674838)	99.8	-	99.9	99.6
<i>Maritigrella fuscopunctata</i> 3 Malta (KU674839)	99.8	99.9	-	99.5
<i>Maritigrella fuscopunctata</i> Israel (MH047290)	99.4	99.6	99.5	-

## Discussion

The current study is the most detailed taxonomic work on polyclad flatworms of two regions of the Israeli coast, the eastern Mediterranean and northern Red Sea, adding seven new records for the country. Among the species described here, *Pseudobiceros damawan* and *Pseudobiceros apricus* are new records for the Red Sea. These findings suggest that species richness in the Red Sea has not been adequately assessed, particularly since the Red Sea has been considered a ‘hotspot’ of biodiversity for several invertebrates such as mollusks, crustaceans, and corals (Moretzsohn & McShane 2004; Roberts *et al.* 2002). Further surveys are needed to determine the polyclad species composition and expand the knowledge of polyclad fauna in this region.

*Pseudoceros duplicinctus* and *Pseudobiceros stellae* represent new records for the Mediterranean Sea. These species have only been recorded for the Indo-Pacific Ocean (Poulter 1987; Newman & Cannon 1994, 1997, 1998, 2003, 2005; Apte & Pilate 2011; Dixit & Raghunathan 2013, Maghsoudlou & Rahimian 2014; Marquina *et al.* 2015; Ong *et al.* 2015; Sreeraj & Raghunathan 2015) and thus, represent non-indigenous species for the area. The introduction of those polyclad species into the Mediterranean Sea might have been through the Suez Canal, which allows the entry of Indo-Pacific and Eritrean fauna (Galil & Zenetos 2002). Likewise, intense maritime activities in the Mediterranean provides the possibility to introduce species into the region through the discharge of ballast water (Bianchi & Morri 2000). The increasing number of non-indigenous polyclad species in the region emphasizes the need for future taxonomic and ecological studies of this taxon.

The species described here exhibit brilliant coloration and diverse color patterns which are distinctive features of species belonging to Pseudocerotidae and Euryleptidae (Newman & Cannon 1994, 1997, 1998, 2000). Although those two families seem to be close relatives, they are differentiated by the shape of the pharynx and pseudotentacles. In both cases, the organization of the male and female reproductive systems is homogeneous, hence the color and patterns are used to distinguish among species (Newman & Cannon 1994, 1997, 1998, 2000; Litvaitis & Newman 2001). While in this study we followed the traditional classification system established by Faubel (1984) and Newman & Cannon (1994), alternative phylogenies based on molecular and morphological characters have been recently presented (Aguado *et al.* 2017; Bahia *et al.* 2017).

Taxonomic studies have shown that polyclads present a large variation in color and pattern and it might be

related to the content of the intestinal branches, geographic location, and habitat (Newman & Cannon 1994; 1997; 2000; 2003; Bahia *et al.* 2014; Bolaños *et al.* 2016). For instance, individuals of the same species recorded in different locations or habitats can show differences in color as observed in the species described in this study. This variability has created confusion for species identification and therefore, the use of molecular analysis is needed to validate differences in color and color pattern to distinguish species complexes (Litvaitis *et al.* 2010). This is particularly true not only in pseudocerotids but also in other families such as Euryleptidae which also exhibit a broad color variation as seen here for *Maritigrella fuscopuntata*. Moreover, it is important that taxonomic studies on polyclads combine molecular and morphological data for accurate species identifications.

The results of this study demonstrate that the polyclad diversity in the two studied regions of the Israeli coast call for further studies of their fauna. The current findings significantly contribute to the knowledge of this neglected group of marine worms both in the eastern Mediterranean and northern Red Sea, raising the number of records from 8 to 15, and thus represent a major contribution to the study of polyclad biodiversity in the country.

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