Taxonomy of subtidal marine Polyclads from Tabarka (northwest Tunisia) with remarks on their habitat preferences

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Abstract
The Polyclad fauna of the northwest Tunisian coasts is still unexplored. This is the first investigation of Polyclad flatworms collected by scuba diving in Tunisian waters. Three species belonging to three different families and three different genera are reported and re-described. Echinoplana celerrima (Haswell, 1907) was found to feed on the ascidian Ciona intestinalis (Linnaeus, 1767) at 20 m of depth and Thysanozoon brocchii (Risso, 1818) was observed to eat on sponges Dysidea tupha (Martens, 1824) at 16 m of depth. Prosthiostomum siphunculus (Delle Chiaje) was found creeping on stones at 29 m of depth. Some anatomical data of living specimen of P. siphunculus and fixed specimens of T. brocchii were provided for the first time.

Key words: Free-living marine flatworms, Tunisia, scuba diving, Ecology.

Introduction
Polyclads are almost exclusively free-living marine flatworms (Prudhoe, 1985; Newman and Cannon, 2003). Only one species of the genus Limnostylochus (Bock, 1913) is known to be of the brackish waters of Borneo (Stummer-Traunfels, 1902). Polyclads are primarily benthic but due to their very thin and flattened dorso-ventrally bodies, few species were shown to be pelagic (Palombi, 1924; Faubel, 1984; Prudhoe, 1985). They are known to be predators of commercial bivalves (Galleni et al., 1980; Gammoudi et al., 2016a). On the other hand, investigation has shown that polyclads are pharmacologically interesting as their tissues contain toxins and bioactive compounds (Miyazawa et al., 1987; Schupp et al., 1999, 2009). They are mainly inhabitants of the sub-littoral zone extending in depth to the edge of continental shelf (Prudhoe, 1985). Some species are found in deep-sea waters (Prudhoe, 1985; Novell, 2001; Quiroga et al., 2006).

Despite the importance of these flatworms in different fields, they have received relatively little systematic and ecological attention. In fact, polyclads have proven to be a difficult group for taxonomical investigations due to the difficulty of the sample collection and histological preparation. They have extremely fragile nature and they tend to autolyze in stressful conditions.

The order Polycladida is subdivided in two suborders Acotylea and Cotylea based on the absence and presence of a cotyl or a sucker behind female genital pore (Lang, 1884). In Tunisia, All Polyclads species were reported from shallow waters, in sand or mud, among algae and under stones. The only deeper site (2.5 m) where specimens collected was located in Bizerta lagoon (Gammoudi et al., 2009; Gammoudi and Tekaya, 2011).
No polyclad species were reported from the shallow waters of Tabarka (North West of Tunisia) up to date.

The aim of this work is to contribute to the study of biodiversity and horizontal/vertical repartition of Polyclad flatworms in Tunisian waters offering some new ecological data.

Material and Methods

Study area and sampling sites: The study was carried out in August 2015 in Cap Tabarka (36°58’0” N and 8°45’0” E), a tapering piece of land projecting into a body of water, less prominent than a cape (Fig. 1a,b).

Sampling: Animals were collected by scuba diving using a transparent plastic bottle, the bottom of which is provided with a fine mesh net. Once the worms enter the bottle, they are captured by closing the lid.

Identification: After being photographed using a Nikon Coolpix camera, specimens were fixed in Bouin Hollande fluid, embedded in paraffin and subsequently serially sectioned at 7μm. The serial sections were stained with eosin and blue of toluidine.

The classification model adopted is the one of Faubel (1983, 1984).

Figure 1. Map of the sampling location (Scale bar a=100km, b=125km).
Results

Systematic account

One acotylean and two cotylean species belonging to three different families were collected. Redescription of these species is provided.

**Acotylea** Lang, 1884
Leptoplanoeida Faubel, 1984
Gnesiocerotidae Marcus and Marcus, 1966 emend. Prudhoe, 1982
Echinoplana Haswell, 1907

**Echinoplana celerrima** Haswell, 1907

*General features.* The body is elongated oval shaped. Tentacles are wanting. The color of dorsal surface is brownish (Fig. 2a). The length can reach 40 mm. Tentacular and cerebral eyes form two anterior symmetric clusters. The formers are larger and more posterior than cerebral eyes (Fig. 2b). Ventral surface is white and transparent. The ruffled pharynx is visible and shows 12 to 16 lateral folds.

*Reproductive apparatus.* Testes are mainly ventral and dispersed between digestive ramifications. They form a small follicles enwrapped each one by e basal membrane. Each testis contains different stages of germs cells (Fig. 2f). Ovaries are less numerous and located mainly in dorsal parenchyma. The seminal vesicle occurs just behind pharyngeal cavity. The main feature of copulatory apparatus is the presence of brown line located in the last third of body which correspond to the cuticularized cirrus. A comb-shaped corrugated surface is present between the two genital pores (Fig. 2c). Two vas deferens lie in both sides of pharynx enter the oval shaped seminal vesicle located behind the pharyngeal cavity. The seminal vesicle is close to ventral wall and communicates with a tubular prostatic vesicle of interpolated type. This vesicle have internal lining provided with radial folds and characterized by a very thick muscular wall consisting of an external layer of longitudinal fibres and a well-developed internal layer of circular fibers. The prostatic vesicle is connected to the eversible cirrus armed with sclerotized spines (Fig. 2d, e).

The vagina is formed by three sections, the vagina externa, the vagina media, and the vagina interna. The former is narrow. The second is known as shell chamber and receive secretions of shell glands. The vagina interna receive the uteri filled with eggs (Fig. 2g).

*Habitat.* Four specimens of *Echinoplana celerrima* were collected from a depth of 20 m. All specimens were found to be associated with the very abundant tunicate *Ciona intestinalis*. Other associated animals are presented by the sea urchin *Paracentrotus lividus* (Lamarck, 1816) and the starfish *Asterina gibbosa* (Pennant, 1777).

**Cotylea** Lang, 1884
Eurypletoidea Faubel, 1984
Prosthiostomidae Lang, 1884
Prosthiostomum Quatrefages, 1845

**Prosthiostomum siphunculus** (Delle Chiaje, 1822) Lang, 1884

*General features.* The worm is of elongated oval shape rounded anteriorly and tiped posteriorly. The dorsal surface is smooth brownish (Fig. 3a). Tubular pharynx is located in the second fourth of body. Usually, the animal protrudes its pharynx as a reaction to fixation. Marginal eyes form a semi circle band located in the anterior part of body (Fig. 3b). Cerebral eyes are larger than marginal ones and form two clusters included in the semi circle formed by the marginal eyes (Fig. 3b). The ventral surface is whitish transparent and shows the tubular muscular pharynx, two genital pores and a well developed sucker located 1mm behind female genital pore.

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Figure 2. Echinoplana celerrima. a Dorsal view. Scale bar = 1mm. b Cerebral ce and tentacular eyes te. Scale bar = 1mm. c Section through comb-shaped corrugated surface cs. Scale bar = 1mm. d Section through cirrus sac cs showing cirrus provided with sclerotized spines arrows. Scale bar = 1mm. Cirrus lumen cl e details of sclerotized spines arrows. Scale bar = 1mm. f Follicular testis showing different germ cells. Scale bar = 10µ. g Section through uterus filled with eggs arrows. Scale bar = 10µ.
Figure 3. *Prothiostomum siphunculus*  

**a** Dorsal view. Scale bar = 1mm.  
**b** Anterior region showing the arrangement of eyes. Note the presence of marginal eyes *me* and two clusters of cerebral eyes *ce*. Scale bar =10μ.  
**c** Ventral view of anterior region showing the paired prostatic vesicles *pv*. Prostatic canal *pc*. Scale bar =10μ.  
**d** Ventral view of anterior region showing the prominent stylet *st*. Male gonopore *mg*, prostatic canal *pc*. Scale bar = 10μ.  
**e** Section through male apparatus. Prostatic vesicle *pv*, male atrium *ma*, male gonopore *mg*, stylet *st*, median intestine *mi*. Scale bar = 10μ.  
**f** Section through female genital apparatus, note the presence of cement pouch *cp* in the vagina. Female gonopore *fg*, main intestine *mi*. Scale bar =10μ.

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Reproductive apparatus. Testes are numerous and are scattered mainly in ventral parenchyma more than dorsal one. The ovaries are bigger in size and lie more or less dorsally. The male gonopore leads to a horizontal male antrum housing the pointed strong stylet (Fig. 3d, e). The usual eosinophilous granulations indicative of prostatic secretions are observed in the wall around the stylet supported by small penis papillae (Fig. 3e). The latter receive the ejaculatory duct from seminal vesicle and the two prostatic ducts from paired free prostatic vesicles (accessory vesicles) (Fig. 3c). The two vesicles are round muscular bodies with narrow lumen and muscularized wall formed by circular fibres (Fig. 3c).

The main feature of the female genital apparatus is the presence of cement pouch characteristic of sub-order Cotylea (Fig. 3f). The vagina is oriented vertically and is connected with two uteri. The sucker is well developed and lies 1 mm behind female genital pore (Fig. 3e).

Habitat. The single specimen of Prosthiostomum siphunculus was found creeping on a stone from a depth of 29m. The associated fauna included the sea urchin Paracentrotus lividus, the sea cucumber Holothuria (Roweothuria) polii (Delle Chiaje, 1824) and the polychaete Protula tubularia (Montagu, 1803). Thysanozoon brocchii (Risso, 1818) Grube, 1840

General features. This polyclad is broadly oval shaped. It uses undulations of its body margins to swim actively. The dorsal face is cream to brownish and provided with several black papillae. The number and size of papillae decrease going from median region to the marginal zone (Fig. 4a). Some papillae are provided with a white spots. Tentacles are developed and appear as expansion of anterior margin. They contain two clusters of marginal eyes (Fig. 4c). Behind marginal eyes, are located in the median region, the two semi-circular clusters of cerebral eyes. The ventral surface is whitish and transparent and shows a ruffled pharynx located in the first third of body. In ventral surface of fixed specimens, different prominent structures are located in the anterior half of body (Fig. 4b). The mouth opens in the posterior part of pharyngeal cavity. Two symmetric male gonopores are situated in the same horizontal level and in both side of median line of body. A unique female gonopore lies behind the two male gonopores. Posteriorly, is located a developed sucker characteristic of cotylea sub-order (Fig. 4b).

Reproductive apparatus. The testes follicles are numerous and located more in the ventral parenchyma then in dorsal one. The ovaries are less numerous and bigger in size than testes (Fig. 4e). They are located in the dorsal and median parenchyma. There are two male copulatory apparatus. Each male gonopore leads to a male atrium in which is projected a penis papillae armed with stylet. The penis papillae are surrounded by a penis sheath. The ejaculatory duct leads to elongated seminal vesicle filled usually with sperm (Fig. 4d). A free prostatic vesicle is connected to the ejaculatory duct via a narrow prostatic canal. The eosinophilous secretions are detected in the two prostatic canals and in male atrium. The single female apparatus comprise a narrow female atrium that extends vertically to form a widened chamber corresponding to cement pouch characteristic of cotylea sub-order. The vagina curve then anteriorly where is located the entrance of oviducts to form a section oriented anteriorly.

Habitat. The two specimens of Thysanozoon brocchii were collected from a depth of 16 m among a dense sea grass meadows of of Posidonia oceanica (Linnaeus) Delile extended in rocky substrate. These flatworms were found associated with the sponge Dysidea tupha, the sea urchin Paracentrotus lividus, the crustacean Achaearans cranchii Leach, 1817 and the bryozoans Electra posidoniae Gautier, 1954. Thysanozoon brocchii is a good swimmer. It move using undulations of its flat body.
Figure 4. *Thysanozoon brocchii* a Dorsal view. Scale bar = 1mm. b Anterior dorsal region showing marginal tentacular eyes *te*. Scale bar=200µ. c Ventral view in fixed specimen showing the mouth *m*, the two male gonopores *mg*, the female gonopore *fg* and the developed sucker *s*. Scale bar = 100µ. d Section through the elongated seminal vesicle *sv*. Scale bar = 100µ. e Section through ovary *ov*. Scale bar =100µ.
Discussion

While we have presented new data to polyclad flatworms distribution in Tunisian waters, we increased our understanding of their subtidal habitats in Tunisia. It is the first investigation of polyclads flatworms sampled by scuba diving. Here we show that ascidians belong to the associated fauna of the polyclad flatworm *Echinoplana celerrima*. One of collected specimens was observed to enter the siphon of tunicate *Ciona intestinalis*. Based on literature, ascidians have been shown to be preys of polyclads (Kubanek et al., 1995; Perez-Portela and Turon, 2007; Noreña et al., 2014) therefore we suggest a trophic relation between the acotylean *Echinoplana celerrima* and the ascidian *Ciona intestinalis*. On the other hand, the cotylean *Thysanozoon brocchii* was observed to feed on sponge *Dysidea tupha*. The flatworm was seen to protrude its ruffled pharynx and attack the sponge.

In our work we cited the tubiculous polychaete *Protula tubularia* as belonging to the associated fauna of both *Prosthiostomum siphunculus* and *Thysanozoon brocchii*. The tubiculous polychaete (of the genus *Spirographis*) were previously signalized as associated fauna of these two polyclads (Lang 1884).

In this work, some anatomical data were illustrated for the first time. The paired prostatic vesicles typical for the genus *Prosthiostomum* were showed in living animals. On the other hand, fixed specimens of *Thysanozoon brocchii* have been useful to show the paired male gonopores, the single female gonopore, the sucker and the mouth.

The depth from which were collected our two specimens of *Thysanozoon brocchii* is almost the same with the specimens were found by Novell (2001) (up to 15 m depth). A similar information was offered by Lang (1884) who only noticed that his specimens were collected from a relatively shallow waters. On the other hand, *Prosthiostomum siphunculus* was found at 29 m in the present study, which is different from Novell (2001) who found his specimens until to 15 m. Lang (1884) collected *P. siphunculus* from 80 m of depth. Although *Echinoplana celerrima* was found more in shallow waters in previous studies along Tunisian coasts (Gammoudi and Tekaya, 2012; Gammoudi et al., 2009; Gammoudi et al., 2012; Gammoudi et al., 2016b), the species was sampled at 20 m of depth for the first time in the study area. It should be noticed that our findings were obtained after two scuba diving, the diversity and ecological aspects of the polyclads fauna in Tunisian waters will be better achieved if studies will include more stations and depths in the area, especially lagoonar ecosystem.

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References


