Suborders Acotylea and Cotylea (Polycladida): Study on morphological, ecological and reproductive features of some representative species from Tunisian coasts (Mediterranean)

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Abstract

The aim of this work is to provide some important morphological, ecological and reproductive features of 8 polyclad species from Tunisian waters belonging to Acotylea: *Echinoplena celerrima* Haswell, 1907, *Leptoplena mediterranea* (Bock, 1913), *Discocelis tigrina* (Blanchard, 1847) and *Imogine mediterranea* (Galleni, 1976) and Cotylea: *Thysanozoon brocchii* (Risso, 1818), *Prosthiostomum siphunculus* (Delle Chiaje, 1822), *Yungia aurantiaca* (Delle Chiaje, 1822) and *Prostheceraeus moseleyi* (Lang, 1884). New data on distribution of some species are added. Moreover, morphological data are provided for the first time in living specimens of *D. tigrina*. Based on our specimens, we confirm characterization of the two sub-orders Acotylea and Cotylea that have been already made in previous studies. Function of attachment organs in polyclads is discussed. On the other hand, data dealing with associated fauna are offered for all species. The two acotyleans *E. celerrima* and *I. mediterranea* were seen to cover their egg plates practicing thereby a parental care. This work could be a baseline for future taxonomic and behavioural investigations.

Key words: free-living flatworms; morphology; reproduction; Turbellarians; Tunisia.

Introduction

Members of the order Polycladida are known to have a highly branched intestine (Hyman 1951). The subdivision of order in two suborders Acotylea and Cotylea was made by Lang (1884) based on the absence and presence of true ventral sucker behind female genital pore. The word “cotyl” means sucker (Newman & Cannon 2003). The true sucker is a protuberance consisting of modified epithelium covering a very thin basement membrane and a thick muscular lamella (Prudhoe 1985). The almost 800 described polyclad species (Martín-Durán & Egger 2012) are divided into 450 acotyleans and 350 cotyleans. For identification of polyclad species, external morphology is not sufficient; study of reproductive system is needed (Faubel 1983, 1984; Prudhoe 1985). The acotyleans families (e.g.: Stylochidae, Planoceridae) are known to be voracious predators of barnacles (Branscomb 1976), and mussels (Galleni et al. 1980; Gammoudi et al. 2017a in press), rock oysters, pearl oysters and giant clams (Newman & Cannon 1993), scallops (Heasman et al. 1998) and edible oysters (Littlewood & Marsbe 1990; Chintala & Kennedy 1993; Jennings & Newman...
On the other hand, cotyleans include colourful species exhibiting striking colour patterns. Hyman (1954), Prudhoe (1985, 1989) and Newman & Cannon (1995) deduced that colour and colour patterns are a good criteria for identification of cotyleans. This suborder includes species elaborating a chemical toxins and bioactive compounds (Schupp et al. 1999; Proksch et al. 2003; Ritson-Williams et al. 2006).

In the literature, the oldest taxonomic work discussing differences between the two suborders Acotylea and Cotylea is the one of Lang (1884). Further works of Bock (1913, 1922), Hallez (1913), Marcus and Marcus (1966) tried to characterize the two suborders. Faubel (1983, 1984) discussed in his works the differentiation of two orders and reserved the entire part II of his monograph to cotyleans (Faubel 1984). Prudhoe (1985) mentioned the differences between two orders. Recently, comparison of male gamete at ultrastructural level was made by Liana & Litvaitis (2007, 2010a, 2010b).

One of the most prominent characters of suborder Cotylea is the indirect development of its entire known species. Furthermore, all the cotyleans derive from metamorphosis of a Müller’s larva which is not the case of suborder Acotylea, in which there are the two modalities: direct and indirect development via larvae of Götte, Müller or Kato (Prudhoe 1985).

In Tunisian waters, 14 polyclads species (9 acotyleans and 5 cotyleans) were recorded to date (Gammoudi et al. 2009, 2011; Gammoudi & Tekaya 2012; Gammoudi et al. 2017b in press).

The aim of the present work is to provide morphological and developmental data of 4 species belonging to Acotylea and 4 species belonging to Cotylea collected from Tunisian waters and to improve our knowledge on their comparative anatomy.

**Material and methods**

Specimens were sampled between March 2012 and May 2016. They were hand-collected using a soft paintbrush. *Yungia aurantiaca* was 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. Location, depth and habitat of each species are given in Table 1.

After collecting animals in the field, they were transferred to transparent boxes filled with sea water. Boxes were maintained at darkness at an average temperature of 20 °C.

The external and internal morphology of living worms was observed and photos were taken using a Nikon CoolPix 4500 camera.

Mature animals were fixed in Bouin’s fixative, embedded in paraffin, sectioned sagittally (6–8 µm) and sections stained in eosin and toluidine blue. For determination of development type, eggs plates of each species were removed from boxes walls and bottoms and then placed in separate recipients filled with sea water. Hatched juveniles and larvae were observed using a stereomicroscope.

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

**Results**

4 acotylean and 4 cotylean species were examined. For each species, a brief description is offered with ecological, taxonomic, or reproduction remark where necessary. The list of species and their correspondent taxonomic position are presented in Table 2.

**Echinoplana celerrima** Haswell, 1907

**Morphological features.** Specimens are brownish. Tentacles are absent. Cerebral and tentacular eyes form two elongated paramedian bands (Fig. 1A). Ventral surface is whitish transparent and shows the ruffled pharynx, the two sperm ducts and the two uteri. A corrugated surface is present between male and female genital pores (Fig. 2A). It is formed by a whitish area provided with a number of ventral wall deformations or horizontal ventral folds. The largest folds are located in the middle of whitish area.

**Reproductive features.** The male copulatory apparatus is provided with cirrus armed with sclerotized spines. Prostatic vesicle is of interpolated type. Seminal vesicle is ventral. The female copulatory apparatus is
formed by a narrow vagina formed by three section, external vagina or female atrium, median vagina or shell chamber where open several shell glands and internal vagina where are connected separately the two uteri. Ovaries are dispersed mainly in the ventral parenchyma between digestive ramifications. Histological sections of female gonads show a ventral germinative zone containing young oocytes and dorsal growing zone containing growing oocytes (Fig. 3A).

**Reproductive behaviour.** During animal culture, specimens were seen to cover their egg plates with their bodies. Even after being moved to other area of the same container, they returned again to cover their eggs.

**Associated fauna.** Specimens were found to be associated with proseriates (marine free-living flatworms) and sea urchin *Paracentrotus lividus* (Lamarck, 1816).

**Table 1.** The list of locations of collected species with location features.

| Species                      | Location                           | Depth | Habitat               |
|------------------------------|------------------------------------|-------|-----------------------|
| *Echinoplana celerrima*      | Lake of Tunis (36°48’ 24.40"N; 10°14’ 54.53”E); Monastir (35°45 ’ 46.00”N; 10°50’ 28.26”E); | 0.5m  | Under stones           |
| (Haswell, 1907)              |                                     |       |                       |
| *Discocelis tigrina*         | Lake of Tunis (36°48’ 24.40”N; 10°14’ 54.53”E); Hergla (36°01 ’ 59.19”N; 10°30’ 33.59”E); | 5m    | Among oysters          |
| (Blanchard, 1847) Lang, 1884 |                                     |       | *Crassostrea gigas*    |
| *Leptoplana mediterranea*    | Lake of Tunis                       | 0.5m  | Under stones           |
| (Bock, 1913) Gammoudi et al, | (36°48’ 24.40”N; 10°14’ 54.53”E)    |       |                       |
| *Imogine mediterranea*       | Bizerta lagoon                     | 4.5m  | Among mussels          |
| (Galleni, 1976) Jennings &  | (37°13’19.29”N, 9°55’49.94”E).      |       | *Mytilus galloprovincialis* |
| Newman, 1996                 |                                     |       |                       |
| *Prostheceraeus moseleyi*    | Bizerta lagoon                     | 4.5m  | Among ascidians        |
| Lang, 1884                   | (37°13’19.29”N, 9°55’49.94”E.).     |       | *Styela plicata*       |
| *Thysanozoon brocchii*       | Ghar el meleh Lagoon (37°09’.40.  | 1m    | Among algae            |
| (Risso, 1818) Grube, 1840    | 47”N, 10° 13’ 21.45”E).            |       |                       |
| *Prosthiostomum siphunculus* | Port de Prince (36° 52’ 25,87” N ; 10° | 1m    | Under stones           |
| (Delle Chiaje, 1822) Lang, 1884 | 39° 57.06° E).                     |       |                       |
| *Yungia aurantiaca*          | Korbous                            | 8m    | Among *Posidonia*      |
| (Delle Chiaje, 1822) Lang, 1884 | (36° 49’ 00,86” N; 10° 34’ 00,81” E) |       | *oceanica*             |

**Leptoplana mediterranea** (Bock, 1913) Gammoudi et al, 2012

**Morphological features.** The dorsal surface is dull in coloration, yellowish to brownish. Tentacular eyes and cerebral eyes form two dark clusters (Fig. 1B). Marginal eyes and tentacles are lacking. Pharynx is ruffled and opens in a mouth located almost in the half of ventral surface. A genital pit typical for the genus *Leptoplana* is located between the two genital pores. It is a kind of wall ventral depression. In histological sections, it appears as epidermal invagination devoid of muscle layers.
Table 2. Taxonomic position of listed species.

| Order       | Sub-order | Superfamily   | Family   | Genus       | Species                          |
|-------------|-----------|---------------|----------|-------------|----------------------------------|
| Acotylea    | Lang, 1884| Leptopanoidea | Gnesioceridae | Echinoplana | Echinoplana celerrima          |
|             |           |               | Marcus & Marcus, 1966 |           |                                  |
| Polycladida | Lang, 1884| Ilyplanoidea  | Leptoplana | Echinoplana | Leptopoma mediterranea (Bock, 1913) Gammoudi et al, 2012 |
|             |           |               | Stimpson, 1857 | Ehrenberg, 1831 |                                 |
|             |           |               | Discocelida | Discocelis | Discocelis tigrina (Blanchard, 1847) Lang, 1884 |
|             |           |               | Laidlaw, 1903 | Ehrenberg, 1836 |                                 |
|             |           | Stylochoidea  | Stylochidae | Imogine     | Imogine mediterranea (Galleni, 1976) Jennings & Newman 1996 |
|             | Poche, 1926|               | Stimpson, 1857 | Girard, 1853 |                                 |
| Cotylea     | Lang, 1884| Pseudoceroidea| Yungia    | Lang, 1884 | Yungia aurantiaca (Delle Chiaje, 1822) Lang, 1884 |
|             |           |               | Pseudocerota |           |                                  |
|             | Faubel, 1984| Faubel, 1884 | Lang, 1884 |             |                                  |
|             |           | Eurypleotide  | Prostheceraeus | Prostheceraeus | Prostheceraeus moseleyi Lang, 1884 |
|             | Faubel, 1844|               | Lang, 1884 | Schmarda, 1859 |                                |
|             |           | Prothiostomidae| Prostheceraeus | Prostheceraeus | Prostheceraeus moseleyi Lang, 1884 |
|             |           |               | Lang, 1884 | Quatrefages, 1845 |                              |

**Reproductive features.** The whole copulatory apparatus is located in the posterior part of body. Male copulatory apparatus is provided with interpolated tubular prostatic vesicle with a ventral diverticulum. This vesicle communicates with oval shaped seminal vesicle that receives a common sperm duct (Fig. 3B). The female apparatus is formed by a vagina that extends dorsally then caudally.

**Developmental model.** The development is of direct type and the juveniles possess twelve eyes distributed in two groups: four anterior pairs and two posterior pairs. The body is flattened dorsoventrally and completely ciliated (Fig. 5A).

**Associated fauna.** Specimens were found among tunicates *Ciona intestinalis* (Linnaeus, 1767) and cnidarians *Anemonia sulcata* (Pennant, 1777).

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

**Morphological features.** The body is oval shaped with a posterior end more pointed than the anterior. The length of specimens can reach 25 mm. Dorsal face is cream to white. Many small spots of brown color are present throughout the dorsal surface and are more concentrated on the median line. Tentacles are lacking. Tentacular and cerebral eyes form two black clusters (Fig. 1C). Marginal eyes are distributed along the two anterior thirds of body. Observation of whitish transparent ventral face shows a huge ruffled pharynx (Fig. 4A).
POLYCLAD SPECIES FROM TUNISIAN COASTS

**Figure 1.** Anterior dorsal region in A: *Echinoplana celerrima*. See the tentacular (te) and cerebral eyes (ce) forming two paramedian bands, scale bar: 0.5 mm. B: *Letoplanara mediterranea*. See clusters of tentacular (te) and cerebral eyes (ce), scale bar: 0.5 mm. C: *Discocelis tigrina*. Note the presence of cerebral and tentacular eyes clusters, scale bar: 0.5 mm. D: *Imaginere mediterranea*. Note the presence of two everted nuchal tentacles (arrows), scale bar: 0.5 mm. E: *Yungia aurantiaca*. See the two marginal tentacles (arrows) and cerebral eyes spots (arrowhead), scale bar: 1 mm. F: *Thysanozoon brocchii*. Dorsal faces provided with papillae and developed marginal tentacles (arrows), scale bar: 1 mm. G: *Prostheceraeus moseleyi*. Note the presence of marginal tentacles (arrows) and yellow marginal line, scale bar: 1 mm. H: *Prosthiostomum siphunculus*. The cerebral eyes (ce) are not included in the semi circle formed by marginal eyes (me), scale bar: 1 mm.

**Reproductive features.** A pair of uteri and a pair of vasa deferentia are visible by transparency in living specimens extending on either side of the pharynx (Fig. 4A). A common genital pore located in the second fourth of body. Pyriform prostato id organs provided with muscular wall are seen also by transparency. The female genital apparatus is provided with a horse-shoes shaped Lang’s vesicle (Fig. 4A).

Histological sections show the common antrum, the prostato id organs and the vagina (Fig. 4B). The diagrammatic reconstruction of copulatory apparatus show the male apparatus formed by the penis and prostato id organs projected in a common antrum. The genital pore is common. The female apparatus is formed by vagina and Lang’s vesicle (Fig. 4C).

**Associated fauna.** They were found essentially among oysters *Crassostrea gigas* (Thunberg, 1793). It is likely that the flatworms are feeding on the bivalves.

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

**Morphological features.** The body is relatively thick and fleshy. The dorsal face is white containing several brown spots. It is almost the only species that could be handled without using a paint brush. Two retractile nuchal tentacles are present (Fig. 1D). Tentacular and cerebral eyes are present. Marginal eyes form a row along the entire body margin. The pharynx is voluminous and opens in a mouth located in the half of body.

**Reproductive features.** The copulatory apparatus is located in the last fourth of body. The sperm ducts open into a tripartite anchor shaped seminal vesicle connected to a free prostatic vesicle by a prostatic canal. The ejaculatory duct is narrow and leads to a male genital pore located 0.5 mm anterior to the female pore. The latter leads to a vagina provided with epithelial folds.

**Developmental study.** The development is indirect and a four-lobed Götte’s larva hatches. It is provided with 3 eyes (Fig. 5B).
**Associated fauna.** All specimens were found among mussels *Mytilus galloprovincialis* (Lamarck, 1819).

**Yungia aurantiaca (Delle Chiaje, 1822) Lang, 1884**

**Morphological features.** The length reaches 45 mm. The body is oval shaped. The colour of the dorsal face is orange with presence of white spots dispersed throughout its entirety. Two well-developed marginal tentacles are present (Fig. 1E). The ventral surface is whitish and shows the mouth, the male and female gonopores and the very developed true sucker. The cerebral eyes form two semicircular spots. The tentacular eyes are also present but they are more spaced than the cerebral.

**Reproductive features.** The male copulatory apparatus show a free prostatic vesicle and elongated seminal vesicle. The vagina is provided with a cement pouch. The two gonopores are separated. A true well developed sucker is present behind female genital pore. It is formed by a protuberance armed with muscular layer and modified epithelium.

According to our observation, the animal used its sucker to attach itself to a substrate fighting against strong action of waves.
**Associated fauna.** These flatworms are usually found associated with bryozoans and the tunicate *Aplidium conicum* (Olivi, 1792).

**Thysanozoon brocchii (Risso, 1818) Grube, 1840**

**Morphological features.** Body is oval. Length can reach 40 mm. Two prominent marginal tentacles are present (Fig. 1F). The pharynx is ruffled and located in the anterior half of body. Dorsal face is provided with papillae (Fig. 1F) and ventral one is smooth. A well developed sucker is located behind the mouth and genital pores. According to our observation, as in *Yungia aurantiaca*, the animal used this structure to attach itself to a substrate fighting against strong action of waves.

**Reproductive features.** This species have two male copulatory apparatus, indeed there exists a pair of seminal vesicles, a pair of vesicles prostatic, a pair of ejaculatory ducts and a pair of male gonopores. The latter are located on the same horizontal line. Both of copulatory apparatus are functional as sperm is seen inside both seminal vesicles and vasa deferentia. The prostatic vesicles are of free-type. Each ejaculatory duct leads to a penis papilla armed with a stylet. The testes are usually ventral. The ovaries are located in dorsal parenchyma. Both uteri are filled with eggs. The vagina is oriented anteriorly and is provided with uterine ramifications. A widening of vertical section of vagina surrounded by glands is noted, this is the cement pouch characteristic representatives of the Suborder Cotylea.

Histological sections show a dorsal germinative zone containing young oocytes and ventral growing zone containing growing oocytes (Fig. 3B).

**Associated fauna.** The associated fauna included the sea urchin *Paracentrotus lividus* (Lamarck, 1816), the crustacean *Achaeus cranchii* Leach, 1817 and the starfish *Asterina gibbosa* (Pennant, 1777).
Figure 4. Copulatory apparatus in *Discocelis tigrina*. A: ventral view showing by transparency the ruffled pharynx (ph), the two uteri (ut), the two vasa deferentia, the common gonopore (cg), the common atrium (ca), the prostatoid organs (po) and the Lang’s vesicle (Lv), scale bar: 0.5 mm. B: Section through common copulatory com; mon atrium (ca) showing prostatoid organs (po), vagina (vg), uterus (ut) and vas deferens (vd) scale bar: 0.1 mm C: Diagrammatic reconstruction of copulatory apparatus. common gonopore (cg), common atrium (ca), ejaculatory duct (ed), Lang’s vesicle (Lv), Penis (p) and prostatoid organs (po), scale bar: 0.1 mm.
Prostheceraeus moseleyi Lang, 1884

*Morphological features.* Its general colour is milky white, greyish, speckled with white spots and numerous small dark spots, violet or black slightly more elongated in the middle of the body. The edge of the worm is a bit corrugated and bordered with orange-brown and yellow. Two marginal tentacles are present (Fig. 1G). Two clusters of cerebral eyes are present. Marginal tentacular eyes occur. The ventral surface shows clearly a well developed true sucker characteristic of the suborder Cotylea. The mouth lies in the anterior hind of pharyngeal cavity.

*Reproductive features.* Like all cotyleans, the copulatory apparatus is oriented posteriorly. The two vasa deferentia open separately in an elongated seminal vesicle. The ejaculatory canal communicates with a free prostatic vesicle via a short prostatic canal. The prostatic vesicle is provided with a smooth glandular epithelium. It is surrounded by an inner layer of longitudinal fibers muscle and a thicker outer of circular fibers. The ejaculatory canal leads to an unarmed penis papilla. The latter opens at the outside by a gonopore located at the last third of animal body.

The vagina shows a cement pouch characteristic of the Cotylea suborder. It extends vertically and then anteriorly to form the internal vagina. The internal part of vagina is provided with small vesicles serving for storage of eggs called uterine vesicles.

*Associated fauna.* This species was found associated with the tunicate *Styela plicata* (Lesueur, 1823).
Prosthiostomum siphunculus (Delle Chiaje, 1822) Lang, 1884

Morphological features. The body is elongated rounded anteriorly and pointed posteriorly. The length can reach 28 mm. Dorsal face is smooth and shows clearly the tubular pharynx. The tentacles are lacking (Fig. 1H). Marginal, cerebral and tentacular eyes are present. Ventral face is transparent and shows tubular pharynx, vasa deferentia and uteri. A developed true sucker is present behind genital pores (Fig. 3C).

Reproductive features. Testes are mainly ventral and ovaries are dispersed dorsally. A pair of free prostatic vesicles of free-type is present. The unique seminal vesicle receives the two prostatic canals and communicates with the ejaculatory duct which leads to a penis papillae armed with a stylet. The two gonopores are separated. The vagina shows a widening, the cement pouch and then curve dorsally where it receives the uteri.

Developmental model. The development is indirect and a ciliated Müller's larva hatches (Fig. 5C, D). It is provided with eight lobes.

Associated fauna. Specimens were found to be associated with the sea urchin Paracentrotus lividus (Lamarck, 1816).

Discussion

In this work, 8 polyclads species belonging to the two suborders Acotylea and Cotylea were described. Some anatomical data were shown for the first time such as Lang’s vesicle and accessory prostatic organs known as prostatoid organs in living specimens of Discocelis tigrina.

New data dealing with distribution of polyclad species in Tunisian waters were provided. D. tigrina and Echinoplana celerrima were signalized respectively from the coasts of Hergla and Monastir for the first time. Monastir station is considered hitherto to be the most southern Tunisian site of polyclads. It has to note that the south eastern coasts of Tunisia still be completely unexplored.

On the other hand, during this study it was possible to detect some differences in morphology, developmental models of acotyleans and cotyleans and then to confirm the observation already made by Lang (1884), Laidlaw (1903), Marcus & Marcus (1966) and more extensively by Faubel (1983, 1984) and Prudhoe (1985).

The main morphological difference between the two suborders is the presence of true sucker ‘cotyl’ behind female genital pore in Cotylea. With respect to the function of this sucker, it is not yet known (Prudhoe, 1985). According to our observation, the animal used this structure to attach itself to a substrate fighting against strong action of waves. The same behavior was described by Jokiel & Townsley (1974) in Prosthiostomum sp. The author concluded that the ability to withstand a high rate of water movement probably is an adaptation of the worm to wave turbulence as organ of attachment; the animal uses this organ to be attached to a substrate. On the other hand, Jennings (1957) mentioned that the cotylean Cycloporus papillosus was seen attached to the surface of the colony of Botryllus schlosseri by its ventral sucker and extract whole zooids. The same behaviour was signalized by Jokiel & Townsley (1974) in Prosthiostomum sp. which attaches to the coral Montipora verrucosa. Therefore, the true sucker is an organ that can be used also for feeding. The true sucker of cotyleans differs from others pseudosuckers of acotyleans mainly by being armed with muscular layers and then by its localization. It differs from genital pit described in species of genus Leptoplana, L. mediterranea and corrugated surface of E. celerrima which are located between the two genital pores. These two structures are probably implied in copulation process (Gammoudi et al. 2012a) In some Cestoplanidae a sucker could be present behind female genital pore but in the hind most end of the body contrary to cotyleans provided with a sucker located more or less in the middle region of the body (Prudhoe, 1985).

Study of development showed a direct development in L. mediterranea (acotylean) with a twelve eyed juvenile (Fig. 5A) and indirect development in both Imogene mediterranea (acotylean) and Prosthiostomum siphunculus (cotylean) with Götte's larva (Fig. 5B) and Müller's larva (Fig. 5C, D) respectively. Gammoudi et al. (2012b) deduced that the influence of the temperature on the speed of
development is connected with the mode of development: in directly developing polyclads, temperature changes have less pronounced effects on developmental time than in indirectly developing polyclads.

At ultrastructural level and based on bibliographic data, studies of male and female reproductive system has shown a dorsal disposition of ovarian germinative zone in the acotyleans *Pleioplana atomata* and *E. celerrima* (Liana & Litvaitis 2009; Gammoudi et al. 2016a) whereas this zone is ventral in the cotyleans *P. siphunculus* (Gammoudi et al. 2016b). The same applies to Hallez (1911) who showed a ventral position of germinative zone in the cotyleans *Enterogonimus aureus*, *Laidlawia trigonopora* and *Stylochooides albus*. Here in our work we confirm this disposition using histological section of ovaries, dorsal position of germinative zone in the acotylean *Echinoplana celerrima* and ventral in the cotylean *Thysanozoon brocchii* (Fig. 3A, B). It seems that disposition of ovarian germinative and growing zone is typical for each suborders. On the other hand, ultrastructural investigations of male gamete revealed that in Acotylea, nucleus of spermatozoon is located in posterior part of shaft and flagella are attached to the sperm shaft (Liana & Litvaitis 2007; Liana & Litvaitis 2010a, 2010b) whereas in Cotylea, spermatozoon possess elongated nucleus extending almost along the entire sperm body. Flagella are free and are not in close contact with the sperm shaft (Liana & Litvaitis 2007; Gammoudi et al. 2016c; Liana & Litvaitis 2010b).

In this study, we showed that *E. celerrima* and *I. mediterranea* covered their egg plates. This parental care has been described previously in *E. celerrima* (Johnston & Lee 2008; Rawlinson et al. 2008), and in *I. mediterranea* by Galleni et al. (1980). This strategy has been also shown in other acotyleans (Pearse & Wharton 1938; Rzhepishevskij 1979; Murina et al. 1995; Merory & Newman 2005; Rawlinson et al. 2008). Rawlinson et al. (2008) considered that this strategy may be used as protection against potential predators.

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