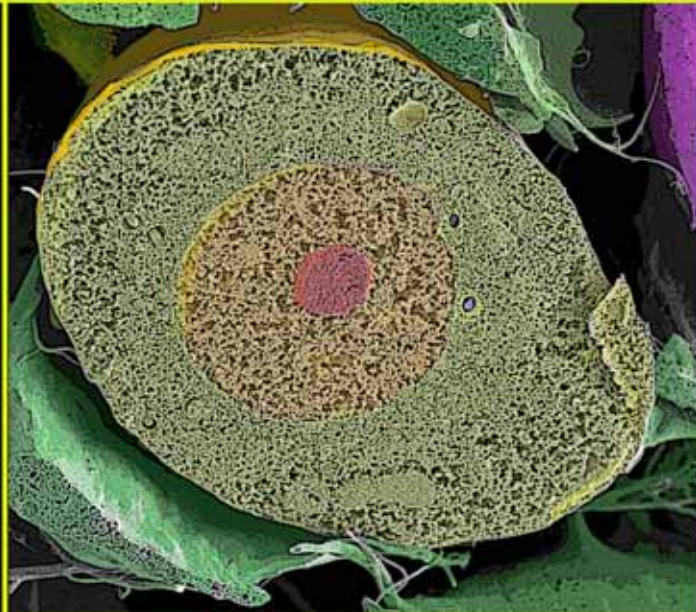


Departamento de Biología Celular  
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# Sexual reproduction in demosponges: ecological and evolutive implications

Reproducción sexual en demosponjas:  
implicaciones ecológicas y evolutivas



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# Chapter 3:

## ■ Introduction

Reproduction is still a relatively poorly known aspect of the sponge biology. However, some demosponge orders have been the object of intensive studies regarding multiple reproductive features. The reproductive biology of halichondrids (e.g., Evans 1977; Fell and Jacob 1979; Fell et al. 1979; Lewandroski and Fell 1981; Amano 1986; Barthel and Detmer 1990; Tanaka-Ichiara and Watanabe 1990; Witte and Barthel 1994; Witte et al. 1994; Woollacott 1999), homosclerophorids (e.g., Lévi 1953; Tuzet and Paris 1963, 1964; Gaino et al. 1986 a, b; Ereskovsky and Boury-Esnault 2002; Boury-Esnault et al. 2003), and haplosclerids (e.g., Tuzet 1930b, 1947; Meewis 1939; Fell 1969, 1976a, b; Elvin 1976; Saller and Weissenfels 1985; Fromont 1988; Paulus 1989; Woollacott 1993; Fromont and Bergquist 1994; Ereskovsky 1999; Ritson-Williams et al. 2004; Whalan et al. 2005) is the best studied among the demosponge orders, presumably because of their availability in the areas of study or their significance within the sponge phylogeny.

Lévi (1953) used the reproductive biology of the class Demospongiae to divide such class into two subclasses, depending on whether they were oviparous or viviparous. Oviparity was the rule in Tetractinomorpha and viviparity in Ceractinomorpha. However, during the 70s, oviparity was reported in few groups of ceractinomorphs, such as some haplosclerids (Scalera-Liaci et al. 1973a), verongids (Bergquist 1978), and more recently, in the poecilosclerid species *Neofibularia nolitangere* (Hoppe and Reichert 1987). Among Haplosclerida, Bergquist (1980) proposed a new order to contain those representatives of the order which were

oviparous: the order Petrosida. Currently, the order Petrosida is no longer accepted, but, in order to reflect the variety of developmental modes exhibited within haplosclerids, two suborders have been proposed: Haplosclerina and Petrosina (van Soest and Hooper 2002).

Even though the order Haplosclerida has been intensively studied (see above), most of the research on any feature concerning reproduction of the order has focused on freshwater sponges (Gilbert 1974; Harrison and Cowden 1975; van de Vyver and Willenz 1975; Saller and Weissenfels 1985; Paulus and Weissenfels 1986; Saller 1988; Paulus 1989; Gugel 2001). Therefore, there is little information about the reproductive biology of the suborder Petrosina. Chronologically, Scalera-Liaci et al. (1973a, 1975) pointed out the oviparity of *Petrosia ficiformis*, and reported its oogenesis to occur from spring to autumn (8 months), and its spermatogenesis within 15-20 days during the last part of the oogenic cycle. Fromont (1988) explored the gametogenic dynamics of *Xestospongia testudinaria* and its relationship with environmental stimuli, and found that only 5 days were required to complete spermatogenesis. Only a correlation between oocyte spawning and lunar cycle was established. Fromont (1994) found that 3 species of *Xestospongia* of the Great Barrier Reef were consistently oviparous species and contemporaneously gonochoristic; and Fromont and Bergquist (1994) investigated their reproductive timing finding long oogenic cycles of 5 months and rapid spermatogenesis (1 month). They also observed that oocyte spawning events were somehow related to lunar cycles. Lepore et al. (1995) described ultrastructurally the mature oocyte of *P. ficiformis*, with a brief description on the nourishment of the oocyte by nurse cells. Both Reiswig (1976) and Ritson-Williams et al. (2004) observed the oocyte spawning of *Xestospongia muta* in Caribbean waters, which occurred within a mucus coat, as described before by Fromont and Bergquist (1994). To date, information regarding the structure of sperm or the sperm spawning time is still lacking, although a sperm spawning prior to oocyte spawning have been suggested by Fromont and Bergquist (1994). Scarce observations of the embryogenesis and/or larval biology of the suborder have been recorded because of the high difficulty in the collection of data when fertilization takes place outside the sponge body. Fromont (1988) observed, under microscopical examination, some early embryos of *Xestospongia testudinaria* undergoing cell divisions; however, no further information was provided. Six years later Fromont and Bergquist (1994) collected some fertilized oocytes of *X. bergquistia* and

placed them in aquaria. After 4 days, white larvae measuring 160 x 98  $\mu\text{m}$  were found in the bottom of the aquaria. This was the only record of larvae within the entire suborder.

The scarce and incomplete information about gametogenesis of the suborder Petrosina in temperate waters led us to investigate the gametogenic dynamics and the ultrastructure of gametes of the most common littoral petrosinid of the Mediterranean: *Petrosia ficiformis*. For that purpose we used both light and electron microscopy, and made special effort on those reproductive features that remain unknown to date.

## ■ ■ Material and methods

### *Studied species*

The reproductive activity of *Petrosia ficiformis* was examined through the sampling of a population located in the sublittoral rocky bottoms of the coast between Blanes and Tossa de Mar (northwestern Mediterranean, Spain). *Petrosia ficiformis* belongs to the order Haplosclerida, suborder Petrosina. It displays different morphologies, from fig-shaped (ficiform) to irregularly globular with fused globes. It usually inhabits scarcely illuminated areas, such as crevices, caves, or shaded vertical walls. The photo-autotrophic symbionts located underneath the surface of the sponge provide the typical red-wine colour. Therefore, when the individuals inhabit caves or bad-illuminated areas are white because of the absence of symbionts.

### *Dynamics of abundance and size of reproductive elements*

We tagged 5 large individuals of the population and performed a long-term monitoring of their reproductive activity from October 2003 to October 2005, with monthly sample collections. Samples consisted of small tissue pieces of the sponges (approx. 1 x 0.5 x 0.5 cm) collected by scuba diving and using surgical scissors. Wounds caused by sampling were healed in 2-3 months, and in no case tissue collection caused death of the sampled individuals or perceivable functional damage. In 2004 and 2005, when samples from the tagged individuals revealed that gametogenesis activity was about to peak in the population -which happened in winter- we increased both

sampling frequency (10-day intervals) and number of sampled individuals (which depended on the availability of specimens in the area selected, and varied from 7 to 19). In 2006 we tagged 25 individuals (different from the first 5) in a particular location of Blanes and sampled them weekly from November 14 to December 2 in order to assess the sex-ratio of the population.

Tissue samples for optical microscopy were maintained in ambient seawater for transportation to the laboratory (1 to 2 h) and fixed in 4% formaldehyde in seawater for 24 h. Then, samples were desilicified with 5% hydrofluoric acid for 5 h, rinsed in distilled water, dehydrated through a graded ethanol series (70%, 96%, 100%), cleared in toluene, and embedded in paraffin to cut them into 5  $\mu\text{m}$ -thick sections using an Autocut Reichert-Jung microtome 2040. After deparaffining with xylene, sections were stained with Hematoxylin-PAS and observed through a Zeiss Axioplan II compound microscopy connected to a Spot Cooled Color digital camera. To count and measure the oocytes and spermatic cysts per tissue area unit, we took 3 pictures (x100) of each of 2 non-serial sections per individual. Pictures of tissue were taken at least 240  $\mu\text{m}$  from each other to avoid overlapping of reproductive products leading to overestimation. The 6 pictures provided a total surveyed area of 7  $\text{mm}^2$  per individual. Using the pictures of all samples we determined number of reproductively active sponges overtime. With 2003-2005 samples we estimated number of each reproductive element, and area and largest diameter of oocytes, using the public domain ImageJ Software (<http://rsb.info.nih.gov/ij/index.html>) on the digital histological images. Then, we estimated average density (mean number per unit area  $\pm$  SD), size and mesohyl occupancy (percentage area) for oocytes in the reproductive individuals. Because of the difficulty in estimating number of spermatic cysts, we only recorded their presence/absence.

We collected mature oocytes during the spawning event of December 2006, and they were placed in Petri dishes with unfiltered seawater in a temperature-controlled room (15°C). Their development was followed daily in the beginning and weekly after a few days.

#### *Reproductive activity vs. seawater temperature*

We measured seawater temperature ( $\pm$  0.5 °C) at the sampling sites monthly, placing the underwater thermometer (Suunto) close to the rocky walls where the

sponges grew. The potential relationship between temperature and reproductive activity was examined by plotting monthly temperature values versus estimated density (abundance per mm<sup>2</sup>) of oocytes.

#### *Ultrastructural study*

Transmission electron microscopy (TEM) was used when needed to describe the ultrastructure of gametes. The protocol is detailed in Chapter 2.

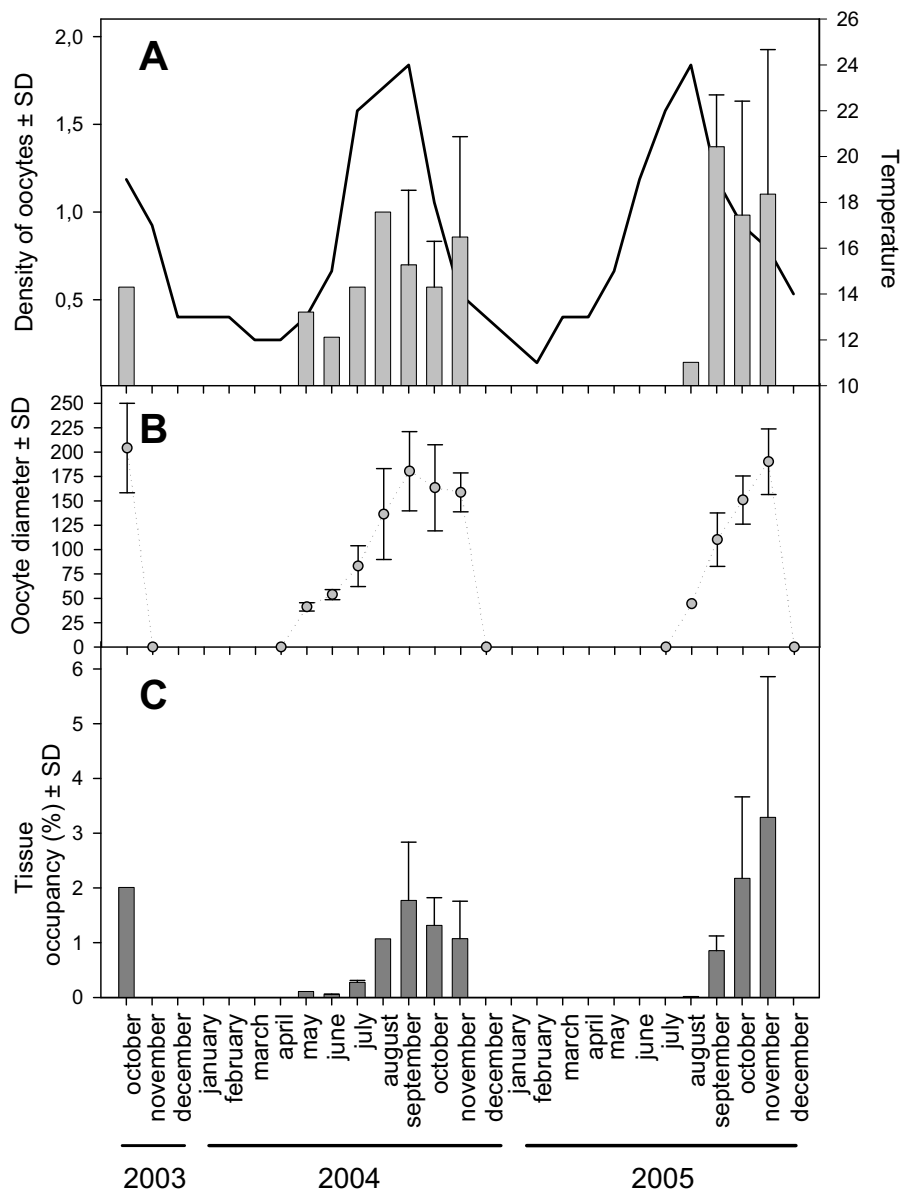
## ■ ■ **Results**

#### *Timing of reproduction and dynamics of abundance and size of gametes*

Histological sections of a total of 131 specimens were examined, finding that *Petrosia ficiformis* was a gonochoristic, oviparous species. Oogenesis developed during a variable period of 4 to 7 months that started in May or August, and ceased consistently in November (Fig. 1). Exhaustive samplings of 2004-2006 showed that the maximum percentage of individuals containing oocytes was 40%, being the average 20% (Fig. 2). Likewise, in 2006, 20% of the population was producing sperm and another 20% producing oocytes, which rendered a sex-ratio close to 1:1 (Fig. 2).

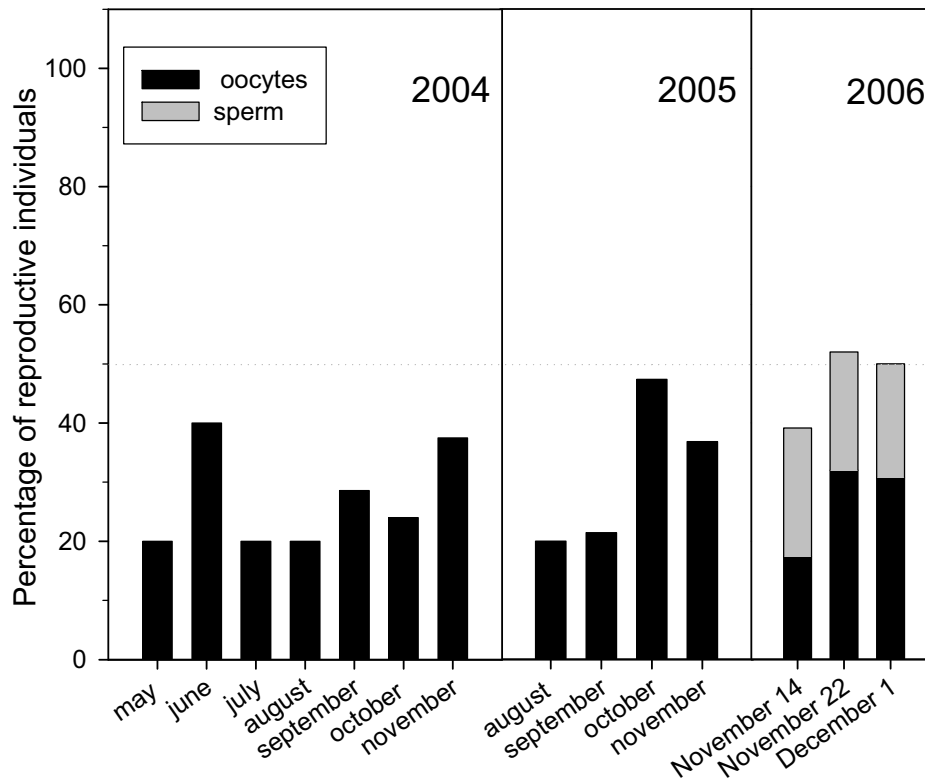
We referred hereafter oocytes as pre-vitellogenic and post-vitellogenic, and not primary and secondary oocytes, because of the difficulty in the observation of the emission of polar bodies. Smallest identifiable pre-vitellogenic oocytes were nucleolate, and measured approximately 30 µm in largest diameter (Fig. 3). They located highly spread in the choanosome of the sponge, but as oogenesis developed, large oocytes measuring approximately 200 µm in largest diameter (Fig. 3), clustered in groups. Oocyte growth was progressive and relatively rapid, since they increased a 500% in size in 3-5 months (Fig. 1).

During 2004, young oocytes appeared in May (in one out of the 5 tagged sponges) in moderately small quantities (0.43 oocytes mm<sup>-2</sup>) (Fig. 1). Production of oocytes was such low until August, when production rates duplicated (1 oocyte mm<sup>-2</sup>), and then remained around this value until the end of the oogenic cycle. However, during 2005, although the oogenic cycle started later in the course of the year (in August),



**Figure 1.** Density, diameter and tissue occupancy of oocytes in a tagged population of *Petrosia ficiformis* over a two-year cycle (October 2003–November 2005). **(A)** Average density mm<sup>-2</sup> (± SD) of oocytes in the sponge tissue. **(B)** Average diameter (± SD) of oocytes. **(C)** Average percentage of tissue occupancy (± SD) by oocytes.

production rates were higher, approximately  $1.5 \text{ oocytes mm}^{-2}$  (Fig. 1). Nevertheless, oogenesis ended in November in both years.

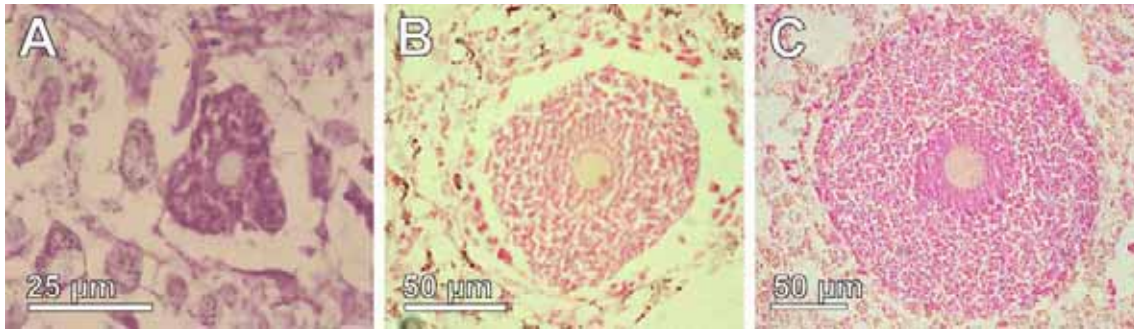


**Figure 2.** Abundance (%) of individuals containing reproductive products (oocytes and sperm) in 2004, 2005, and 2006.

In 2004, maximum tissue occupancy (percentage of tissue area occupied by oocytes) was  $1.8 \pm 1.1\%$ , and was recorded in September; however, maximum values in 2005 were recorded in November, being  $3.3 \pm 2.6\%$  (Fig. 1).

Despite intensive sampling from 2003 to 2005 spermatocysts were detected only during the sampling of 2006 (Fig. 2, 4). Spermatogenesis was a highly synchronized process at the population level. Spermatocysts with spermatogonia, measuring approximately  $60\text{-}80 \mu\text{m}$ , appeared in November 14. As spermatogenesis developed cysts did not enlarge at all, and, eventually, they disappeared in December 1. Thus, spermatogenesis was completed in about 2 weeks.





**Figure 3.** Oocyte growth. (A) Young pre-vitellogenic oocyte within the sponge tissue. (B) Mid-stage oocyte surrounded by nurse cells. Note the nucleolate nucleus. (C) Almost mature oocyte, still surrounded by nurse cells.

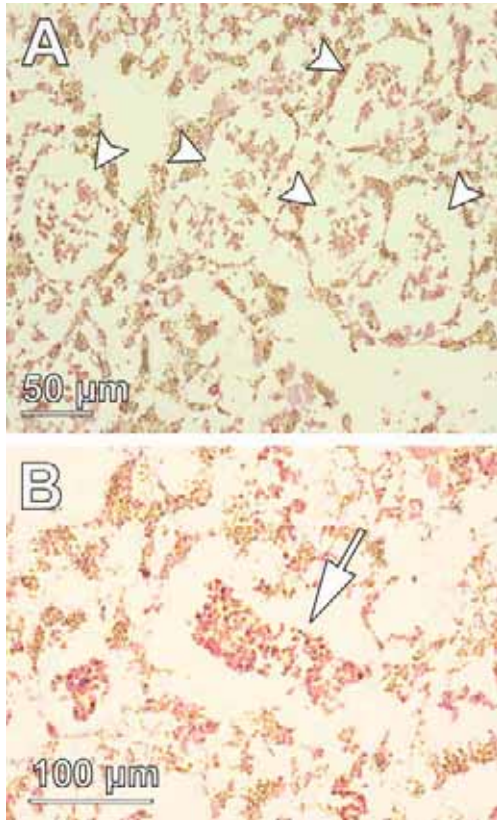
Egg spawning was observed during samplings of 2006. In November 22, most eggs located close to the canals or within them, presumably waiting for being released. Egg release occurred during December 1 and 2 (four and three days before full moon respectively). In the former day, at 12:00 a.m, 2 sponges (out of 35 observed) were observed releasing eggs. Individually-released eggs were white, without an obvious mucous sheath surrounding them. Release flow was almost continuous (100-200 per minute) with small pauses of 15-20''. After spawning, eggs, which showed negative buoyancy, were dispersed by the intense water current. Next day (December 2), at 11:30 a.m., 2 different sponges were spawning.

One of the sponges released 144 eggs per minute through its single osculum. The other one, showed a slower release flow (approximately 70 eggs per minute), but the spawning occurred through more than 1 osculum. During this dive, water was much calmer than the previous day. The following day, none of the sponges released oocytes.

Since samples of December 1 revealed that almost no sperm was within the sponge tissue (Fig. 2, 4) spawning time was inferred to occur, logically, prior to egg release.

#### *Reproductive activity vs. seawater temperature*

During 2004 oocytes appeared coincidentally with the seasonal temperature rising that occurred in May-June (Fig. 1). Although not observed, egg release occurred



**Figure 4.** Spermatic cysts. **(A)** Several spermatic cysts (arrow heads) within the choanosome of the sponge. **(B)** Detail of a spermatic cyst (arrow).

simultaneously to the temperature decline, in November. In 2005, however, oogenesis started with the maximum temperature value (24°C) in August, but also ceased in November, as the precedent year, resulting in an evident shorter cycle (Fig. 1).

In 2006, egg release was firstly observed during December 1 and 2. In those days temperature was 15°C.

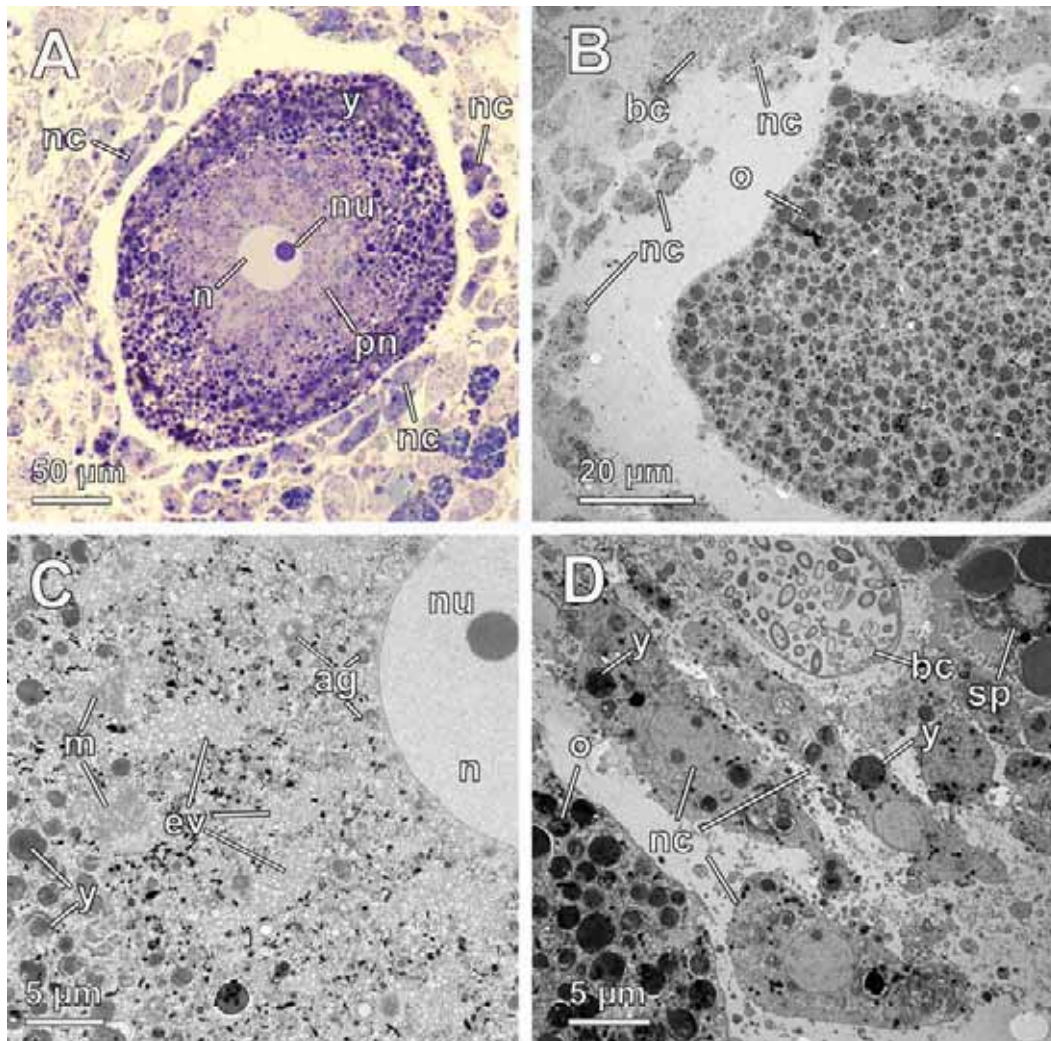
#### *Ultrastructural features of oogenesis and spermatogenesis*

##### **Oogenesis**

We failed to find young oocytes within the samples fixed for TEM, likely because of their scattered appearance in the tissue, scarcity and small size. Mid-stage oocytes (100-150 µm) were round to moderately irregular in shape (Fig. 5A-B), and appeared surrounded by numerous nurse cells (Fig. 5A-B, D). They showed a large

nucleolate nucleus (20  $\mu\text{m}$ ) surrounded by a wide perinuclear region (30-40  $\mu\text{m}$ ) devoid of inclusions (Fig. 5A, C) that contained multiple Golgi apparatus (Fig. 6A). Nuclear pores within the nuclear membrane were observed (Fig. 6A-B). The perinuclear region also contained small vacuoles with granular content close to the nuclear membrane (Fig. 6B), many accumulations of electron-clear vesicles measuring 0.25-0.35  $\mu\text{m}$  in diameter (Fig. 5C, 6C-D), and large clusters of approximately 30 mitochondria (Fig. 5C). Intermingled with the electron-clear vesicles, very small granules appeared (Fig. 6D), possibly free ribosomes. From the perinuclear region to the periphery of the oocyte, two different types of yolk inclusions were observed (Fig. 5B-C), even though they appeared to be different stages of the same type of granules: 1) those of a highly heterogeneous nature with granular content of different sizes (Fig. 6E), and 2) those with a more homogenous appearance, which content was interpreted as proteinaceous (Fig. 6F). Lipid droplets were found, but scarcely (not shown). Intermingled with yolk inclusions, we found glycogen granules (Fig. 6E) and also large clusters of mitochondria (Fig. 6F), although more scarcely than in the perinuclear region. Yolk inclusions, similar to those of the oocytes, appeared in the nucleolate nurse cells that were close to the oocyte membrane (Fig. 5D), suggesting a possible transmission to the oocyte. Apart from the numerous nurse cells that surrounded the oocyte, we found also bacteriocytes (Fig. 5B, D) and spherulous cells (Fig. 5D).

Released eggs were white and round, measuring 200-250  $\mu\text{m}$  (Fig. 7A), showing two small cells, interpreted as polar bodies, loosely attached to the egg surface (Fig. 7B). Semithin sections of those nucleolate eggs showed many yolk granules spread homogeneously throughout the cytoplasm, except an ovoid perinuclear region devoid of yolk (Fig. 7C). The nucleus contained finely diffused chromatin (Fig. 7D-E). Although several ultrathin sections were performed, we were unable to observe the nucleolus within the nucleus. Multilayered yolk granules were spread within most of the cytoplasm (Fig. 7E-F). Many microvilli occurred over the entire surface of the egg (Fig. 7E), and a thin layer of mucus surrounded the egg membrane (Fig. 7F). Small Golgi apparatus, comprised of small dyciosomes and few microvesicles, located intermingled with heterogeneous yolk granules in the periphery of the egg (Fig. 7G). Small groups of mitochondria located also in the periphery of the egg (Fig. 7H), but they were not clustering, as occurred in previous stages.



**Figure 5.** Mid-stage oocytes. **(A)** Semi-thin section of a mid-stage nucleolate (nu) nucleus (n) showing the perinuclear region (pn) devoid of yolk, and the periphery with high amounts of yolk granules (y). Note the multiple nurse cells (nc) that completely surround the oocyte. **(B)** Oocyte (o), that shows its irregular shape, surrounded by multiple nurse cells (nc) and bacteriocytes (bc). **(C)** Detail of the oocyte cytoplasm, showing the nucleolate (nu) nucleus, many Golgi apparatus close to the nuclear membrane (ag), accumulations of electron-clear vesicles (ev), and mitochondrial clusters (m). Towards the periphery of the oocyte yolk granules (y) appeared in high numbers. **(D)** Oocyte (o) approached by multiple nurse cells (nc) which are, as well, surrounded by bacteriocytes (bc) and spherulous cells (sp).

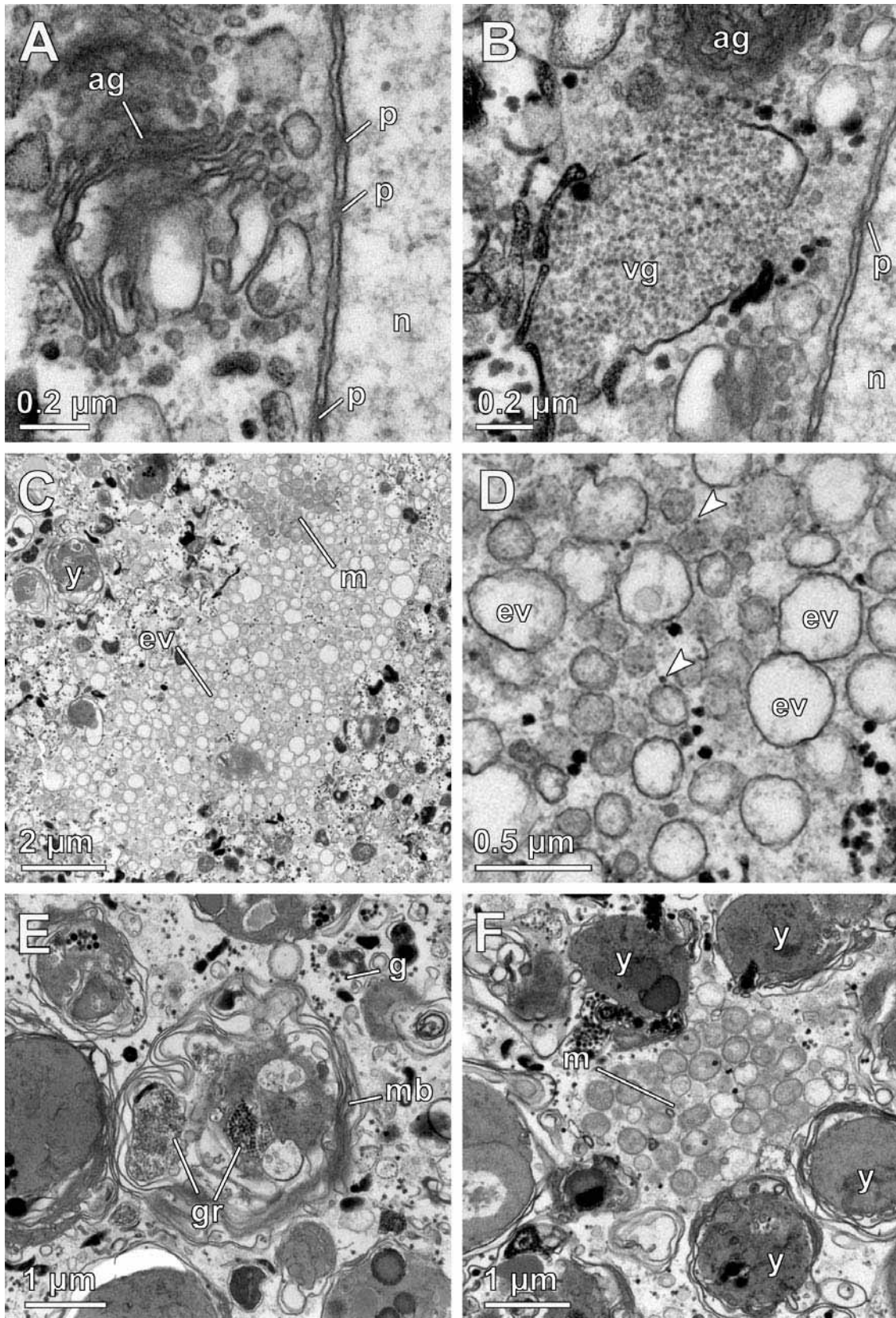
## Spermatogenesis

Swollen choanocytes were detected within regular flagellated chambers (Fig. 8A-B), showing similar features as choanocytes, i.e., similar nucleus size (2  $\mu\text{m}$ ), similar distribution and compaction of nuclear chromatin, digestive vacuoles, inclusions, collar, and a single flagellum, even though they were larger than regular ones (Fig. 8B). Such swollen choanocytes were interpreted as spermatogonia. They left the choanocyte chamber and move into the choanosome. Spermatogonia did not experienced the loss of the flagellum, since small groups of large flagellated cells, identified as primary spermatocytes were found close to choanocyte chambers (Fig. 8C). Primary spermatocytes measured approximately 3  $\mu\text{m}$  in largest diameter, and showed thread-like structures within their nuclei, that were interpreted as synaptonemal complexes (Fig. 8C-E). Their cytoplasm contained large digestive vacuoles and lipidic and other type of inclusions (Fig. 8D-E). The flagellum was anchored to a basal body (Fig. 8E) that lacked accessory centriole. Large groups of these primary spermatocytes were observed without any sort of follicle (Fig. 8F).

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**Figure 6.** Some features of mid-stage oocytes. **(A)** Golgi apparatus (ag) close to nucleus (n) of the oocyte. Along the nuclear membrane several pores (p) were observed. **(B)** Small vacuole of granular content (vg) and a Golgi apparatus (ag) in the vicinity of the nucleus (n), which showed nuclear pores (p) along the membrane. **(C)** Aggregations of electron-clear vesicles (ev) and mitochondrial clusters (m) within the perinuclear region. **(D)** Detail of electron-clear vesicles (ev) and free-ribosomes (arrowheads). **(E)** Multilayered (mb) heterogeneous yolk inclusion, showing its granular content (gr). Note the glycogen granules (g) scattered around the inclusions. **(F)** Mitochondrial cluster (m) within the cytoplasm, intermingled with yolk inclusions (y).

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No intermediate stages between primary spermatocytes and mature spermatozoa were found in the sponge tissue since spermatidogenesis and spermiogenesis were very rapid processes that presumably occurred in less than a week. Mature sperm was found in virtually empty spermatid cysts in December 1 (Fig. 9A-B) that located intermingled with large bacteriocytes. Those cysts were surrounded by flat follicle cells that showed, when attached, simple junctions (Fig. 9C-D). Long thin prolongations of bacteriocytes were found below the follicle cells (Fig. 9D). Free bacteria located in the lumen of the cysts (Fig. 9A).

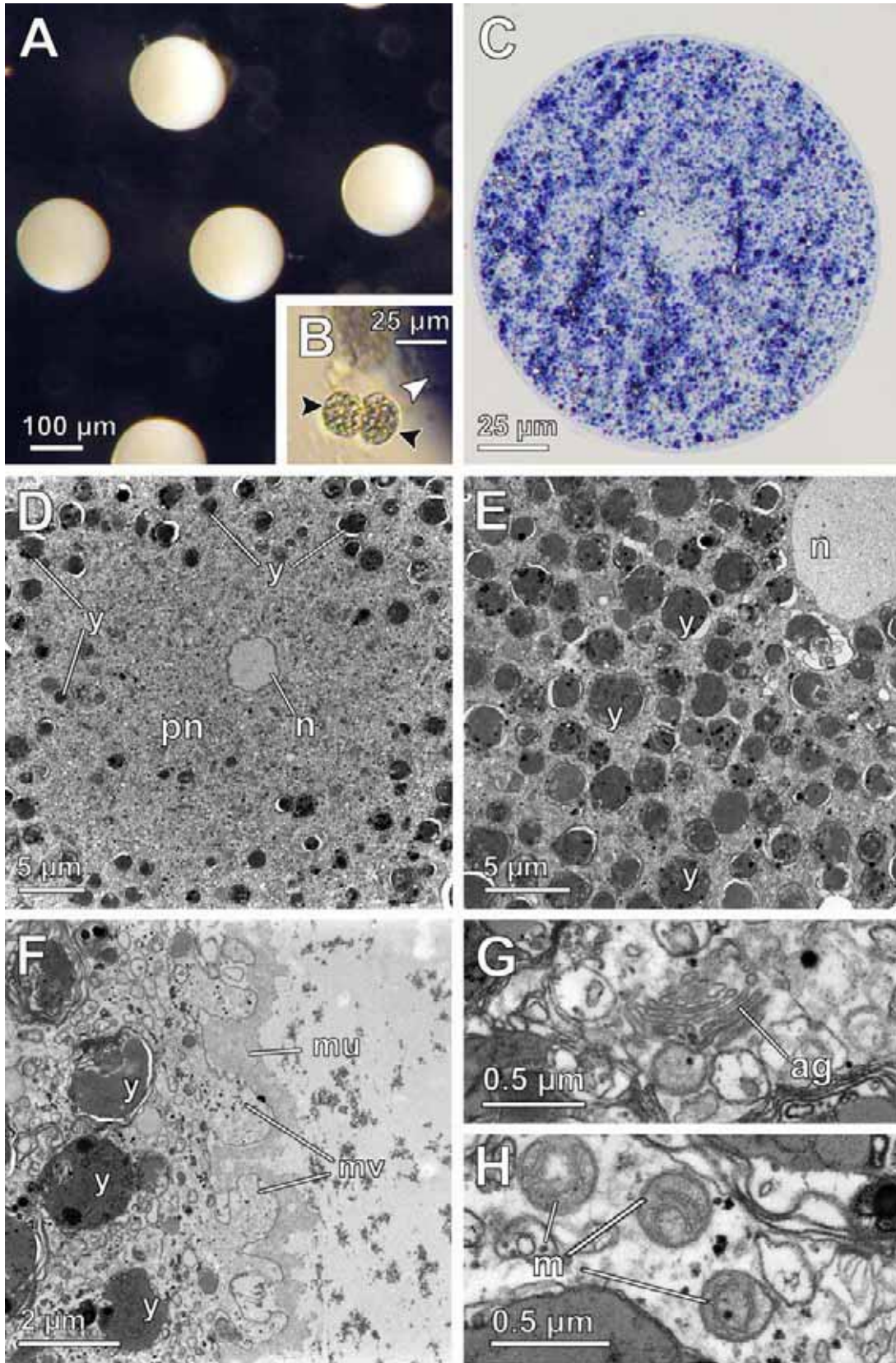
Mature spermatozoa were small flagellated cells (approximately 1  $\mu\text{m}$  in largest diameter) (Fig. 10A). The nucleus appeared highly condensed in the central part, with an ovoid less-condensed area surrounding it (Fig. 10B). The flagellum was anchored to a basal body that lacked accessory centriole (Fig. 10A). They were provided with 3 large mitochondria with well defined flat cristae and 15-20 round electron-dense proacrosomal vesicles, both organelle types located around the nucleus (Fig. 10A-C). Such proacrosomal vesicles were membrane-bound, measuring approximately 150 nm in diameter (Fig. 10D).

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**Figure. 7.** Released eggs. **(A)** Appearance of released eggs, few hours after the spawning. **(B)** Light micrograph showing the two polar bodies (black arrowheads) loosely attached to the egg surface (white arrowhead). **(C)** Semi-thin section of an egg showing the beginning of the perinuclear region in the center of the egg. **(D)** Central region of the egg showing the perinuclear region (pn) and the beginning of the nucleus (n). Surrounding the perinuclear region multiple yolk inclusions (y) can be found. **(E)** Micrograph, at a different level than Fig. 7C, of the nucleus (n) completely surrounded by yolk inclusions (y). **(F)** Periphery of the egg with numerous microvilli (mv) in the oolemma and multiple yolk inclusions (y) within the cytoplasm. Note the scant mucous sheath (mu) that covered the egg. **(G)** Small Golgi apparatus (ag) in the periphery of the egg. **(H)** Mitochondria (m), not clustering, located in the periphery of the egg.

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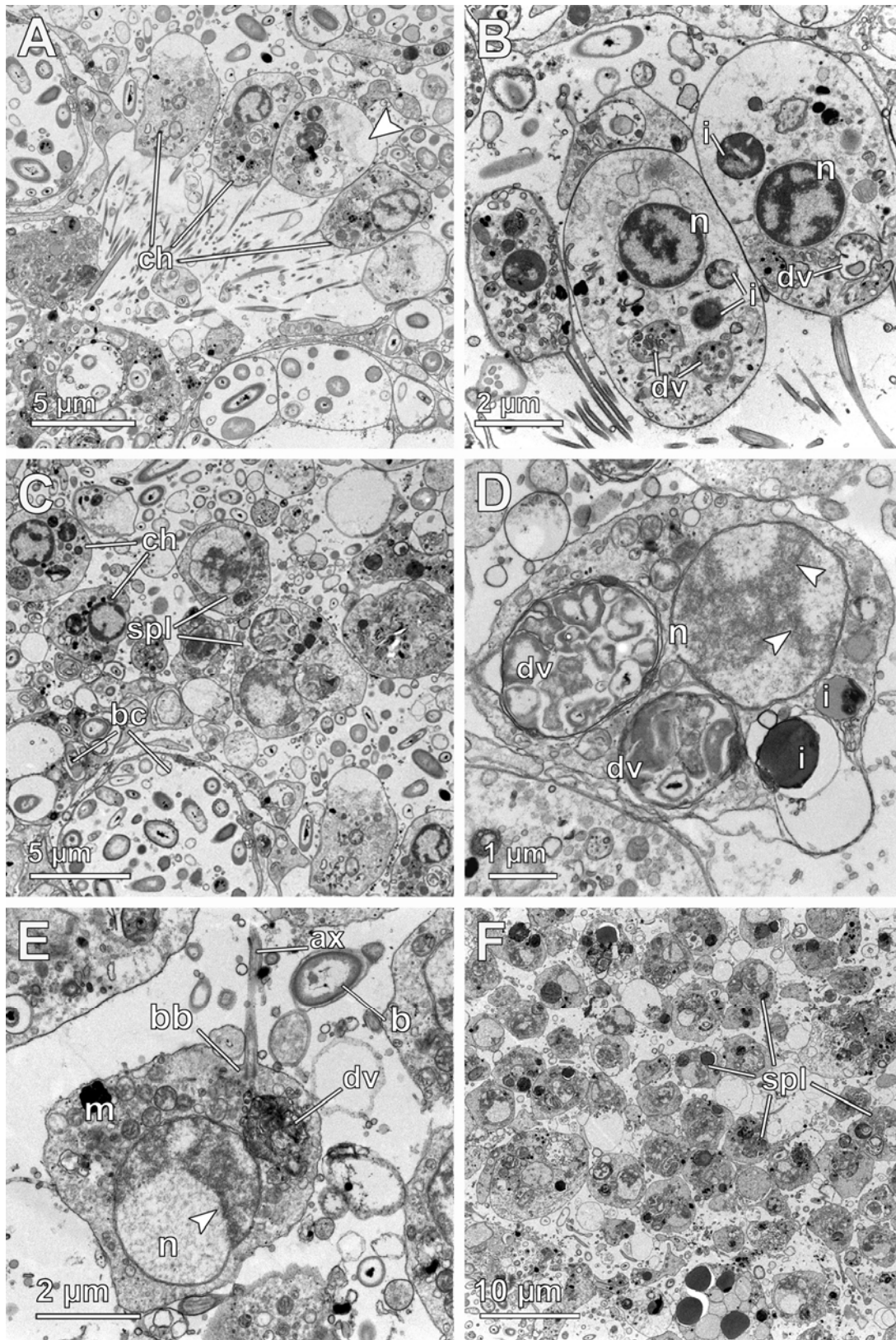
### Developmental observations

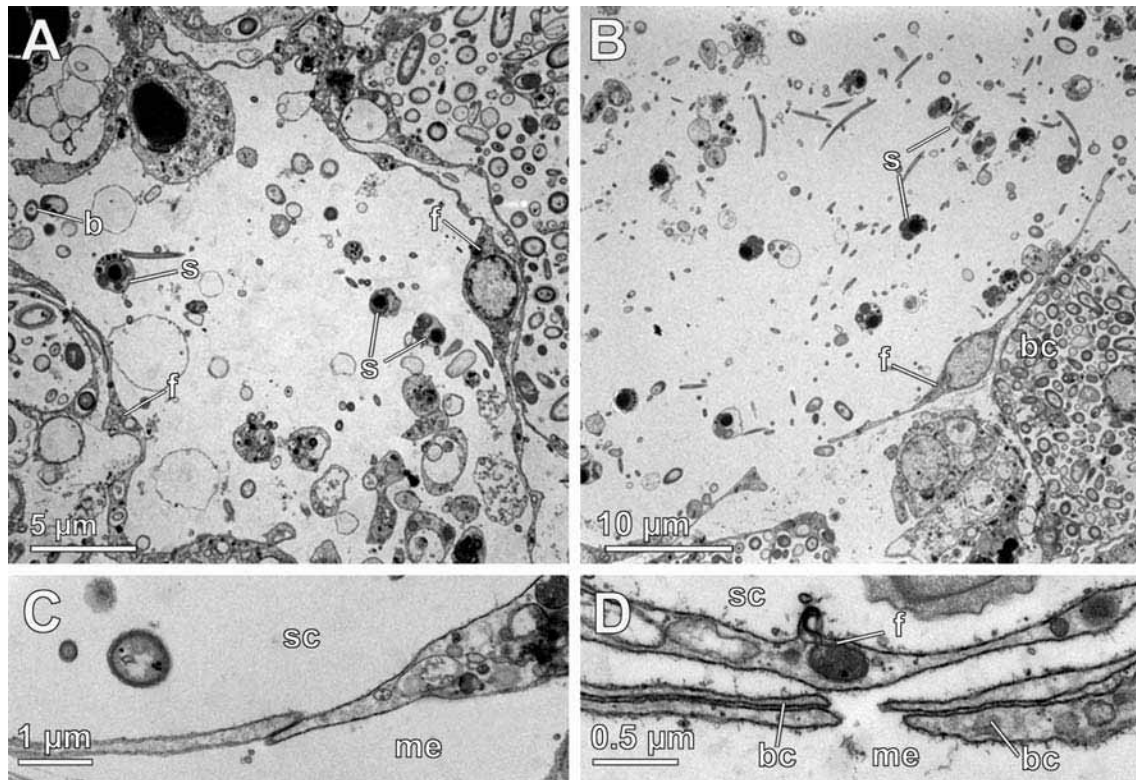
Most of the eggs placed in Petri dishes in December 1 (Fig. 10A) were degenerating after 2 days, but some of them started to cleave, showing an irregular pattern (Fig. 11A-B). Only 1% of them remained unclesaved and without any perceptible damage. After 4 days most of the eggs degenerated, less than 1% was still undivided but a small quantity settled in the bottom of Petri dishes (Fig. 11C). Settled sponges were white and measured 2-4 mm, displaying an irregular shape (Fig. 11C). After 15 days sponge juveniles were still alive and growing. Choanocyte chambers were evident in that period (Fig. 11D). After 3 months in some of the settled sponges small spicules appeared to project from the surface. In March 29, 2007 a small piece of tissue of one of the sponges was submerged in bleach, and 3 oxeas were found (not shown), in addition to many debris.

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**Figure 8.** Origin of sperm and cyst formation. **(A)** Choanocyte chamber with regular (ch) and swollen (arrowheads) choanocytes. **(B)** Swollen choanocytes showing normal features of choanocytes: nucleus (n) with partially condensed chromatin, digestive vacuoles (dv), and inclusions (i). **(C)** Spermatocytes I within the mesohyl, close to a choanocyte chamber (ch) and bacteriocytes (bc). **(D)** Spermatocyte I containing the nucleus (n) showing synaptonemal complexes (arrowheads), digestive vacuoles (dv), and incusions (i). **(E)** Spermatocyte I showing the nucleus (n) with synaptonemal complexes (arrowheads), several mitochondria (m), a degenerating digestive vacuole (dv), and the single flagellum (ax) which arose from the basal body (bb). Note free-living bacteria (b) in the vicinity of the spermatocyte. **(F)** Cyst of spermatocytes I (spI) without a definite follicle.

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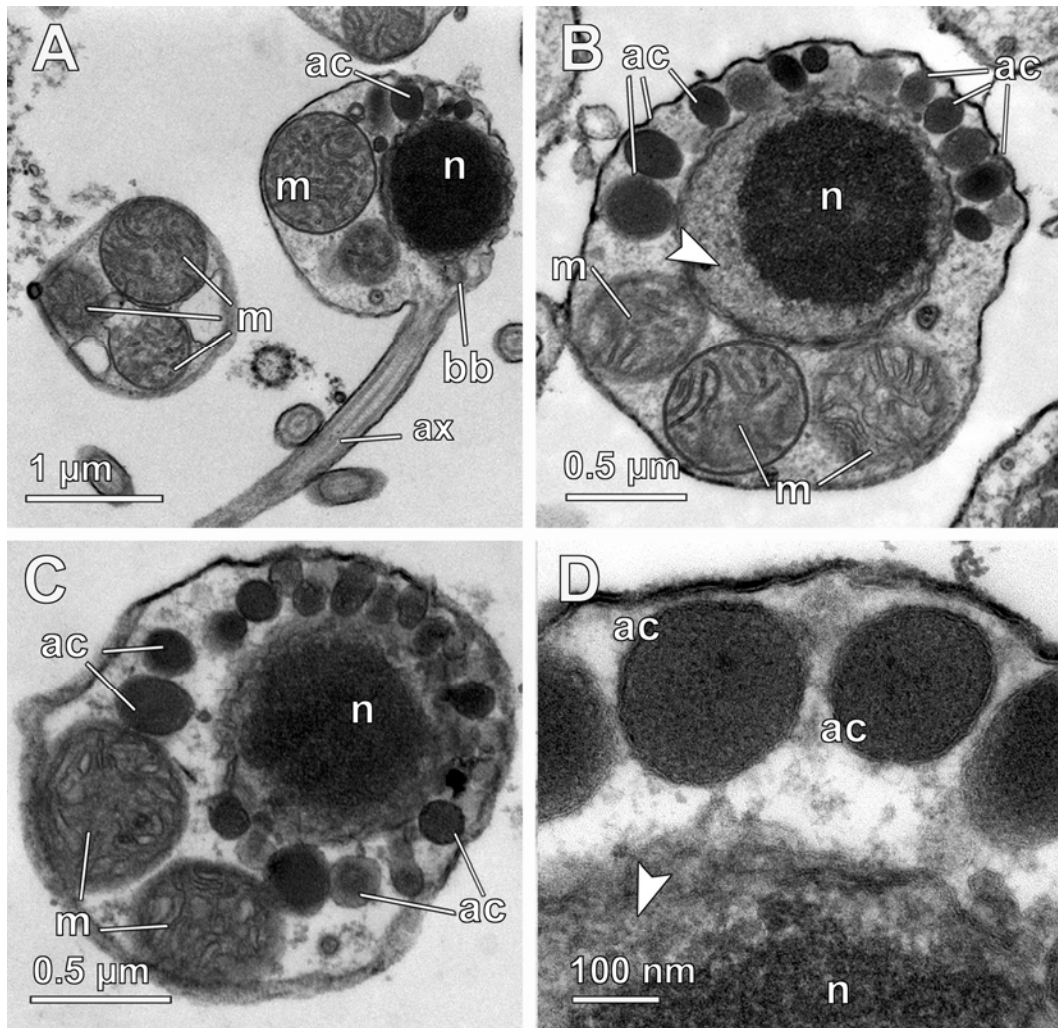




**Figure 9.** Envelopes of spermatogenic cysts with mature sperm. **(A-B)** Virtually empty spermatogenic cysts with mature sperm (s) and bacteria (b) surrounded by follicle cells (f) and bacteriocytes (bc). Note the space between two follicle cells in Fig. 9B. **(C)** Simple junction of two follicle cells, isolating the cyst (sc) from the mesohyl (me). **(D)** Prolongations of bacteriocytes (bc) below the follicle cells (f) that isolate the cyst (sc) from the mesohyl (me).

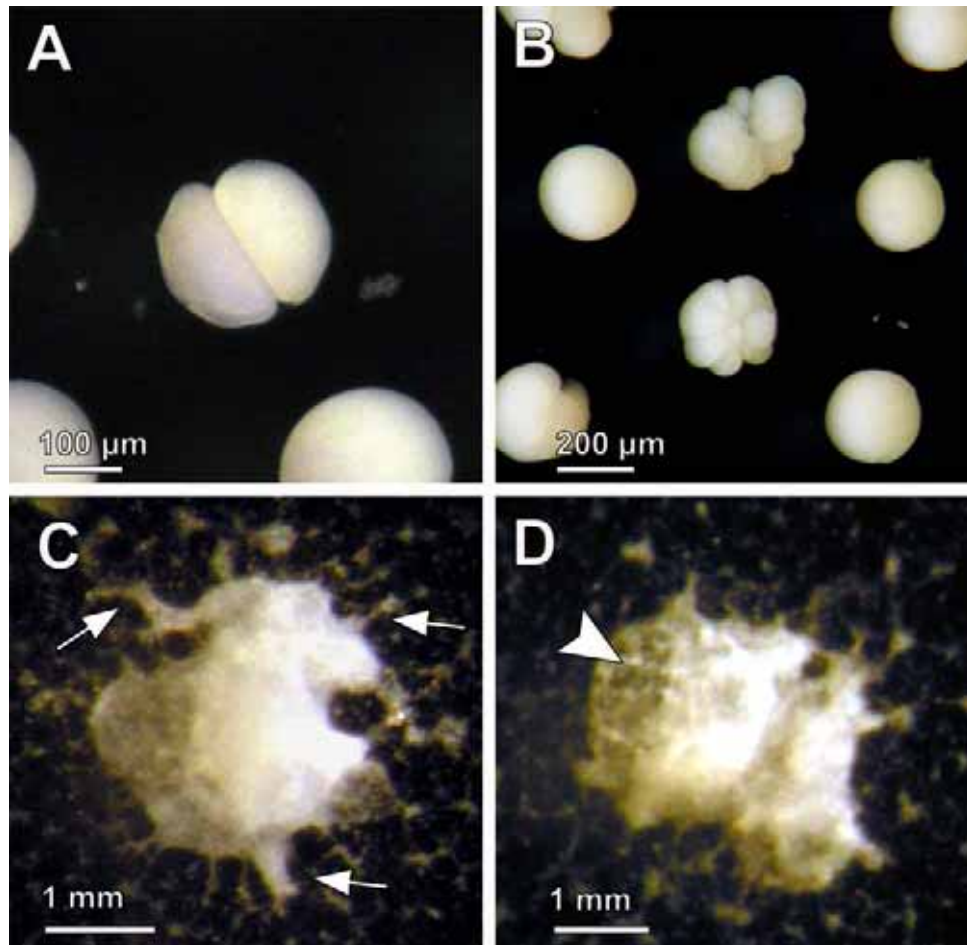
## ■ Discussion

The studied population of *Petrosia ficiformis* was reproductively active over a 4-7 month period that extended from May to November. These data about gametogenesis duration are similar to those reported in Scalera-Liaci et al. (1973a) and Scalera-Liaci and Sciscioli (1975), which described that the population was reproductively active during a period that spanned 8 months (from April to November). However, unlike them, during both years of study we found no reproductive elements until May. Nevertheless, we can not discard that the relatively small size and scarcity of oocytes hindered its detection in previous months. Shorter oogenesis (5-6 months), although



**Figure 10.** Structure of the mature spermatozoon. **(A)** General view of a mature spermatozoon containing a highly condensed nucleus (n), acrosomal vesicles (ac) surrounding the nucleus, 3 mitochondria (m), and a single basal body (bb) from which arose the flagellum (ax). **(B-C)** Spermatozoon with numerous acrosomal vesicles (ac) that completely surrounded the nucleus (n), which contained a non-condensed area (arrowhead) surrounding the highly condensed part of it, and 3 mitochondria (m). **(D)** Periphery of the spermatozoon showing the membrane-bounded nature of the acrosomal vesicles (ac). Note the non-condensed area (arrowhead) of the nucleus (n).

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**Figure 11.** Direct development. **(A)** 2-cell embryo, few hours after collection. **(B)** Cleaving embryos within the next 24 hours after collection. **(C)** Sponge juvenile after 4 days. Note the prolongations of the body (arrows) that indicates adhesion to the surface. **(D)** Sponge juvenile after 15 days, showing a clear area in which choanocyte chambers (arrowheads) are seen by transparency.

they can still be considered long, have been reported in other representatives of the suborder Petrosina, such as *Xestospongia bergquistia* and *X. testudinaria* (Fromont and Bergquist 1994). Similarly, long oogenesis (undergoing between 6 to 10 months) are experienced by some other temperate and tropical demosponges, like, for instance, *Haliclona permollis* (Elvin 1976), *Halichondria panicea* (Wapstra and van Soest 1987),

*Ircinia strobilina* (Hoppe 1988), and *Myxilla incrustans* (Ereskovsky 2000), although less frequently than short oogenesis (less than 4 months).

Oogenesis of *P. ficiformis* was coincidental with the warmest period of the year, starting simultaneously to water warming, and egg release occurred in December, after the seasonal decline of temperatures. In other petrosinids, oogenesis was also simultaneous with water warming, although in such cases the egg spawning occurred during maximum seawater temperatures (Fromont and Bergquist 1994), and not after the seasonal decline. Correlation between the temperature raising of seawater and gametogenesis has also been reported in many studies (e.g., Hartman 1958; Storr 1964; Fell 1974, 1976b; Scalera-Liaci and Sciscioli 1975; Johnson 1978; Tanaka-Ichiara and Watanabe 1990; Kaye and Reiswig 1991; Witte et al. 1994; Ereskovsky 2000; Mercurio et al. 2007).

Tissue occupancy rates by oocytes in *P. ficiformis* ranged from  $0.1 \pm 0.007$  to  $3.3 \pm 2.6$ . They were quite similar to those reported in other demosponges (Reiswig 1973; Corriero et al. 1996; Tsurumi and Reiswig 1997). Interestingly, *Petrosia*'s oocytes are amongst the largest recorded within the suborder (Fromont and Bergquist 1994), and also within the phylum (Fell 1983). Nevertheless, oocytes appeared in relatively small numbers (less than 2 oocytes  $\text{mm}^{-2}$ ), taking few space in the mesohyl. Subsequently, it appeared that scarce choanocyte chambers degenerated to allow the space required for the developing oocytes, and the regular structure of the mesohyl and choanosome remained stable, in contrast to what reported in other oviparous sponges, e.g., *Chodrilla australiensis* (Usher et al. 2004).

Oocytes contained the typical organelles, previously described by Lepore et al. (1995) in a mature oocyte: heterogeneous yolk granules, numerous electron-clear vesicles, Golgi dycytosomes in the periphery of the oocyte, and clusters of mitochondria. But, while in Lepore et al. (1995) oocytes measuring 140-160  $\mu\text{m}$  in diameter were described as mature oocytes, we found larger oocytes in the latest stages of development, reaching 250-300  $\mu\text{m}$  in diameter, as those described by Scalera-Liaci et al. (1973a).

Spermatogenesis took place synchronous and rapidly (2 weeks) in the population, as reported in most oviparous sponges (Reiswig 1983; Simpson 1984), and similarly to that found previously in *Petrosia ficiformis* by Scalera-Liaci et al. (1973a). Sperm production started during the last part of the seasonal temperature decline (last

week of November and first of December). Spermatogenesis triggered by water cooling has only been described in *Halichondria panicea* (Witte et al. 1994), *Halisarca dujardini*, *Myxilla incrustans* and *Iophon piceus* (Ereskovsky 2000), while warm temperatures are required in the majority of sponges for the onset of sperm production (see Simpson 1984 for a review).

Sperm of *Petrosia ficiformis* coincided with the “primitive” pattern of sperm suggested by Fawcett (1970) for marine and freshwater invertebrates which discharge their sperm into the water: round or conical sperm head, with a small acrosome, a single flagellum, and few large mitochondria arrayed around the proximal centriole. However, the sperm of *P. ficiformis* did not possess a small acrosome, but bore a relatively uncommon feature in sponge sperm but frequently reported in cnidarians (Hinsch and Clark 1973): proacrosomal vesicles (a total of 20). Proacrosomal vesicles are only well described in the sperm of *Suberites massa* (Diaz and Connes 1980). However, a true acrosome has been suggested in about one-third of the documented sperms of sponges (Reiswig 1983), but has been confirmed through electron microscopy only in homosclerophorid demosponges, *Oscarella lobularis* (Gaino et al. 1986b; Baccetti et al. 1986), *Pseudocortidium jarrei* (Boury-Esnault and Jamieson 1999), *Corticium candelabrum* (Riesgo et al. 2007), and the poecilosclerid *Crambe crambe* (Tripepi et al. 1984).

In December, 2006 *Petrosia ficiformis* started to release negatively buoyant eggs, as those reported in other oviparous species (e.g., Reiswig 1976; Bergquist 1978). The two polar bodies appeared attached to the egg surface, like in *Axinella damicornis* (Lévi 1950). The eggs were recently-fertilised oocytes, since they started to cleave few hours after spawning, under laboratory conditions. Egg spawning occurred few days before full moon. Spawning of *Neofibularia nolitangere* occurred around full moon (Hoppe and Reichert 1987). However, spawning is not always related with such clarity to moon phase. Whereas *Xestospongia testudinaria* -another petrosinid- showed a semilunar cycle of egg spawning (Fromont and Bergquist 1994; Ritson-Williams et al. 2004), egg spawning in *Agelas clathrodes* showed no apparent relationship to moon phase (Hoppe 1988).

In *Petrosia ficiformis* there was no larva; the zygote adhered to the substrate and developed directly into a small sponge. Among sponges, direct development is very uncommon, since most of the species possess a free-swimming larva (Maldonado and

Bergquist 2002; Maldonado 2004). We know only of 3 sponges that undergo direct development: *Tetilla serica* (Watanabe 1957, 1960), *Tetilla japonica* (Watanabe 1978), and *Stylocordyla borealis* (Sarà et al. 2002), and none of them is a haplosclerid, but all are demosponges. It is generally believed that free-living larvae are primitive in marine invertebrates and that the loss of larval stages is a derived condition (reviewed by Pechenik 1999). Although the pressures selecting for such loss are still unresolved, it appears that among animals with occurrence of clonality, selection has favoured retaining offspring near the parents (Jackson 1986). While the extensive dispersal provided by larval stages has many advantages (reduces competition for food or space among siblings and inbreeding, increases recolonization, etc), direct development carries implicit benefits. Among these benefits two arose as the most likely and intuitively approached: 1) implies settlement within the favourable parental habitat, and 2) decreases vulnerability to planktonic predators (Pechenik 1999). Other profits suggested are that larval stages may reduce juvenile fitness through delayed metamorphosis, and that nutritional and other stresses experienced by larvae can reduce postmetamorphic performance (Pechenik et al. 2006). Moreover, it appears that, although eggs displayed negative buoyancy, currents may disperse those eggs, and avoid the disadvantages of lacking a planktonic larva. Thus, the absence of larval stages in *P. ficiformis* accounts thereby for traits presumably more advantageous in their habitat, but because such implications have not been explored yet in sponges, everything we could add to the subject would be merely speculative.



