

# Occurrence of somatic cells within the spermatogenic cysts of demosponges: A discussion of their role

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Received 31 January 2008; accepted 31 March 2008

Available online 23 May 2008

## Abstract

During ultrastructural studies on the spermatogenesis of two demosponges, *Raspaciona aculeata* and *Petrosia ficiformis*, somatic cells were detected within their spermatogenic cysts. In *R. aculeata* large, amoeboid somatic cells, presumably derived from follicle cells, were found inside cysts filled with secondary spermatocytes. These cells were actively phagocytosing spermatocytes. Such phagocytosis could be directed to eliminate aberrant spermatogenic cells, maintain appropriate cell number within the cysts, or recapture energetic reserves originally allocated to the reproductive process via phagocytosis of spermatocytes. In *P. ficiformis*, somatic round cells were observed only after the spawning event, phagocytosing unspawned sperm in nearly emptied spermatogenic cysts. Unlike in *R. aculeata*, these cells were more similar to archaeocytes (common cells of the demosponge mesohyl with phagocytic activity) than to follicle cells. Phagocytosis of spermatogenic cells and unspawned spermatozoa by somatic cells from the wall of the testes is a well-known process in vertebrates and many invertebrates. Occurrence of somatic cells in the spermatogenic cysts of sponges, whose function appears to be partially analogous to that of Sertoli cells, reveals that sponges possess cellular mechanisms to regulate testis functioning that are equivalent to those in higher metazoans.

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**Keywords:** Sexual reproduction; Spermatogenesis; Spermatozoa; Phagocytosis; Sertoli-like cells; Porifera

## 1. Introduction

In many cases among invertebrates and vertebrates, the totality of produced sperm is never released during spawning (e.g., Jørgensen and Lützen, 1997; Quintana et al., 2004; Kalachev and Reunov, 2005). Phagocytosis of unspawned sperm and/or resorption of the whole testis are two processes taking place in the spawned testes of most animals. Such a phagocytic activity is carried out by somatic cells. In many animal groups, these cells have been identified as Sertoli cells (Hinsch, 1980; Buckland-Nicks and Chia, 1986; Jørgensen and Lützen, 1997), while in others they have been referred to as coelomocytes, amoebocytes, and macrophages (Pacey and Bentley, 1992; Kalachev and Reunov, 2005). There are examples in both vertebrates (Griswold, 1995, 1998; Nakanishi and Shiratsuchi, 2004) and invertebrates

(Buckland-Nicks and Chia, 1986; O'Donovan and Abraham, 1987; Jørgensen and Lützen, 1997; Guraya, 1995) that these cells phagocytose not only unspawned sperm but also sperm precursors (i.e., spermatogonia, spermatocytes, spermatids) and mature spermatozoa that are identified as aberrant, as well as cytoplasmic bodies residual from spermatogenesis. Among marine invertebrates, such phagocytic processes have been reported in cnidarians (Kutznetsov et al., 2001), ctenophores (Franc, 1973), plathelminths (O'Donovan and Abraham, 1987), bivalves (Erkan and Sousa, 2002), gastropods (Buckland-Nicks and Chia, 1986), arthropods (Koulish and Kramer, 1986; Hinsch, 1993), echinoderms (Chia and Buckland-Nicks, 1987; Reunov et al., 2004; Kalachev and Reunov, 2005), ascidians (Jørgensen and Lützen, 1997), and cephalochordates (Holland and Holland, 1989). Nevertheless, in these animals the phagocytic cells have not always been called Sertoli cells, but “accessory cells”, “auxiliary cells”, “nutritive phagocytes” or “wall cells”. In order to simplify terminology, many authors pro-

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pose to use the term Sertoli cells (e.g., Hinsch, 1980; Buckland-Nicks and Chia, 1986; Erkan and Sousa, 2002) to refer to those cells with a function analogous to that of Sertoli cells of mammals (Hess and França, 2005). In general, the complexity of morphological organization of Sertoli cells is closely related to the complexity of the testicular architecture in animals (Guraya, 1995).

Some authors consider that Sertoli cells are needed for a correct progression of gametogenesis in many animals (Griswold, 2005). Sertoli cells usually locate in the walls of the testes and seminiferous tubules (Hess and França, 2005), but in some cases they can detach and migrate into the lumen (Jørgensen and Lützen, 1997; Ramofafia et al., 2003). When in the walls of the testes, Sertoli cells provide crucial factors (e.g., regulatory proteins: peptide growth factors, hormones, etc.) that facilitate the successful progression of germ cells into spermatozoa (Guraya, 1995; Griswold, 1995, 1998; Hess and França, 2005). This facilitation may be in the form of physical support by creating a blood–testis barrier, or biochemical stimulation by providing growth factors and/or nutrients (Buckland-Nicks and Chia, 1986; Guraya, 1995; Griswold, 1998). In addition, not only are aberrant or degenerating spermatogenic cells phagocytosed by Sertoli cells but also regular sperm cells to control germ cell numbers in testis (Buckland-Nicks and Chia, 1986; Griswold, 1995; Jørgensen and Lützen, 1997; Hess and França, 2005). Furthermore, the compounds and energy derived from phagocytosed residual cytoplasmic bodies and germ cells are also believed to be recycled and reused to facilitate proliferation and development of the remaining spermatogenic cells (Guraya, 1997).

Coelomocytes, amoebocytes, and macrophages, which are cells usually involved in elimination of unwanted cellular and non-cellular material from the coelom of invertebrates (Dhainaut and Porchet-Henneré, 1989), can additionally play in bivalves (Vaschenko et al., 1997) and polychaetes (Pacey and Bentley, 1992) the phagocytic function that Sertoli cells play in higher animals.

Sponges are gonad-lacking organisms, with no definite line of germ cells (Bergquist, 1978; Simpson, 1984; Boury-Esnault and Jamieson, 1999). Two different types of somatic cells have been postulated as the origin of spermatogonia. These cells are either archaeocytes – amoeboid totipotent cells – (e.g., Hoppe and Reichert, 1987; Riesgo et al., 2007a) or, more frequently, choanocytes – flagellated collar cells involved in particle capture and sponge feeding – (see Reiswig, 1983 and Boury-Esnault and Jamieson, 1999 for reviews). Spermatogenesis takes place in spermatocysts (spermatocysts) which develop within the internal tissue of sponges – i.e., choanosome or mesohyl depending on species – (Reiswig, 1983; Simpson, 1984). Spermatocysts are generally, but not always, surrounded by follicle cells apparently derived from adult cells, that in most cases are pinacocytes (pseudo-epithelial cells) or archaeocytes (Boury-Esnault and Jamieson, 1999). Sperm release has been observed in few demosponges, often being a population synchronous event in

most gonochoristic, oviparous species (Reiswig, 1970, 1976; Lévi and Lévi, 1976; Hoppe and Reichert, 1987; Ritson-Williams et al., 2004), and somewhat asynchronic in brooding sponges (Riesgo et al., 2007b). The fate of unspawned sperm in Porifera has rarely been approached so far. Diaz and Connes (1980) suggested the possibility that follicle cells of the spermatocysts of the demosponge *Aplysilla rosea* may eliminate abnormal sperm by phagocytosis, although the issue was not further investigated. Nevertheless, the involvement of accessory somatic cells in sperm removal is still thought not to occur in Porifera (Guraya, 1995). In the current study, we investigate the occurrence of somatic cells within late-stages of spermatocyst development and immediately after spawning in two oviparous gonochoristic demosponge species, *Raspaciona aculeata* and *Petrosia ficiformis*.

## 2. Materials and methods

For long-term monitoring of spermatogenesis in *R. aculeata* and *P. ficiformis*, we tagged and sampled individuals growing in a sublittoral rocky community of the Catalan coast (Spain) in the northwestern Mediterranean (41°11'18"N, 2°45'2"W). Five tagged individuals of *R. aculeata* were sampled monthly from January 2004 to September 2005. From September to November 2005, we increased number of sampled individuals ( $N=13$ ) and they were collected fortnightly. Likewise, five individuals of *P. ficiformis* were tagged and collected monthly from October 2003 to December 2006. Number of sampled individuals increased to 19 in November 2005 and to 25 in November–December 2006, and sampling frequency became fortnightly.

Using scuba and surgical scissors small tissue pieces (approximately 0.7 cm × 0.5 cm × 0.3 cm of *R. aculeata* and 1 cm × 1 cm × 0.5 cm of *P. ficiformis*) from each sponge were collected at each sampling time. Tissue samples were divided into two pieces, one assigned to light microscopy and the other to electron microscopy. Samples for electron microscopy were pre-fixed in 2.5% glutaraldehyde in 0.2 M Millonig's phosphate buffer (MPB) and 1.4 M sodium chloride, and stored for a couple of weeks until further examination according to the results obtained by light microscopy.

Tissue samples for light microscopy were maintained in ambient seawater for transportation to the laboratory and fixed within 2 h after collection 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, cleared in toluene, and embedded in paraffin to cut them into 5 μm-thick sections with an Autocut Reichert-Jung microtome 2040. After deparaffining with xylene, sections were stained with Hematoxylin-PAS, and studied through a Zeiss Axioplan II compound microscope. When sections of both species revealed spermatogenic activity we underwent the post-fixation of selected samples for TEM (transmission electron microscopy).

For electron microscopy, samples were then rinsed with MPB for 40 min, post-fixed in 2% osmium tetroxide in MPB, dehydrated in a graded acetone series, and embedded in Spurr's resin. Ultrathin sections obtained with an Ultracut Reichert-Jung ultramicrotome were mounted on gold grids and stained with 2% uranyl acetate for 30 min, then with lead citrate for 10 min. Observations were conducted with a JEOL 1010 transmission electron microscope (TEM) operating at 80 kV and provided with a Gatan module for acquisition of digital images.

### 3. Results

#### 3.1. *R. aculeata*

Spermatogenesis in *R. aculeata* took place during October in 2004, and during October and November in 2005. Spermatic cysts containing spermatocytes II were examined for somatic cells by TEM in November 2005 (time dynamics of spermatogenesis is explained in details in Riesgo and Maldonado, 2008). Cysts were approximately 200  $\mu\text{m}$  in maximum diameter (Fig. 1), lined by a single layer of follicle cells. Follicle cells were flat, measuring approximately 15–20  $\mu\text{m}$  in longest axis (Fig. 2). They showed usually a nucleolate nucleus, many phagosomes of heterogeneous nature, mitochondria, and a large Golgi apparatus with lamellae oriented parallel to the external nuclear membrane (Fig. 2). Junctions between follicle cells were simple (apposition) (Fig. 3) with no detectable membrane specialization. In some instances, there were narrow passages between follicle cells (up to 10  $\mu\text{m}$ ) (Figs. 1 and 4), which could be either fixation artifacts or true spaces. Follicle cells appeared to enter the cysts occasionally (Fig. 5).

Inside the cysts, some somatic cells were found intermingled with spermatocytes II (which measured up to 2  $\mu\text{m}$  in diameter). Although they were amoeboid (Figs. 1, 6–8), their internal organization strongly resembled that of follicle cells. Like follicle cells, they possessed a round nucleolate nucleus measuring approximately 3–4  $\mu\text{m}$  (Figs. 5–7), multiple phagosomes of heterogeneous nature (which appeared to be lipidic in many cases) (Figs. 5 and 6), many electron-clear vesicles (Figs. 5–8), dictyosomes (Fig. 5), and mitochondria (Fig. 7). These somatic cells were seen developing pseudopodia to presumably engulf spermatocytes (Fig. 6) and also containing engulfed secondary spermatocytes in large vesicles (Fig. 7). Some of the free bacteria occurring within the cysts (Fig. 6) were also found in large vesicles within the cytoplasm of the somatic cells (Fig. 8), along with residual cytoplasmic bodies exocytosed by spermatocytes (Fig. 8).

Additionally, other amoeboid cells (approximately 15  $\mu\text{m}$  in their largest diameter) were occasionally found close to spermatocysts in the mesohyl of the sponges, having incorporated spermatocytes within their cytoplasm (Figs. 9 and 10). These cells were somewhat different from the somatic cells found within the cysts. They had a large nucleus (approx-

imately 3.5  $\mu\text{m}$ ), apparently anucleolate (Fig. 9), with finely dispersed chromatin (Fig. 10). They contained few phagosomes, a Golgi apparatus attached to the external nuclear membrane, and some vesicles containing lipid droplets (Figs. 9 and 10).

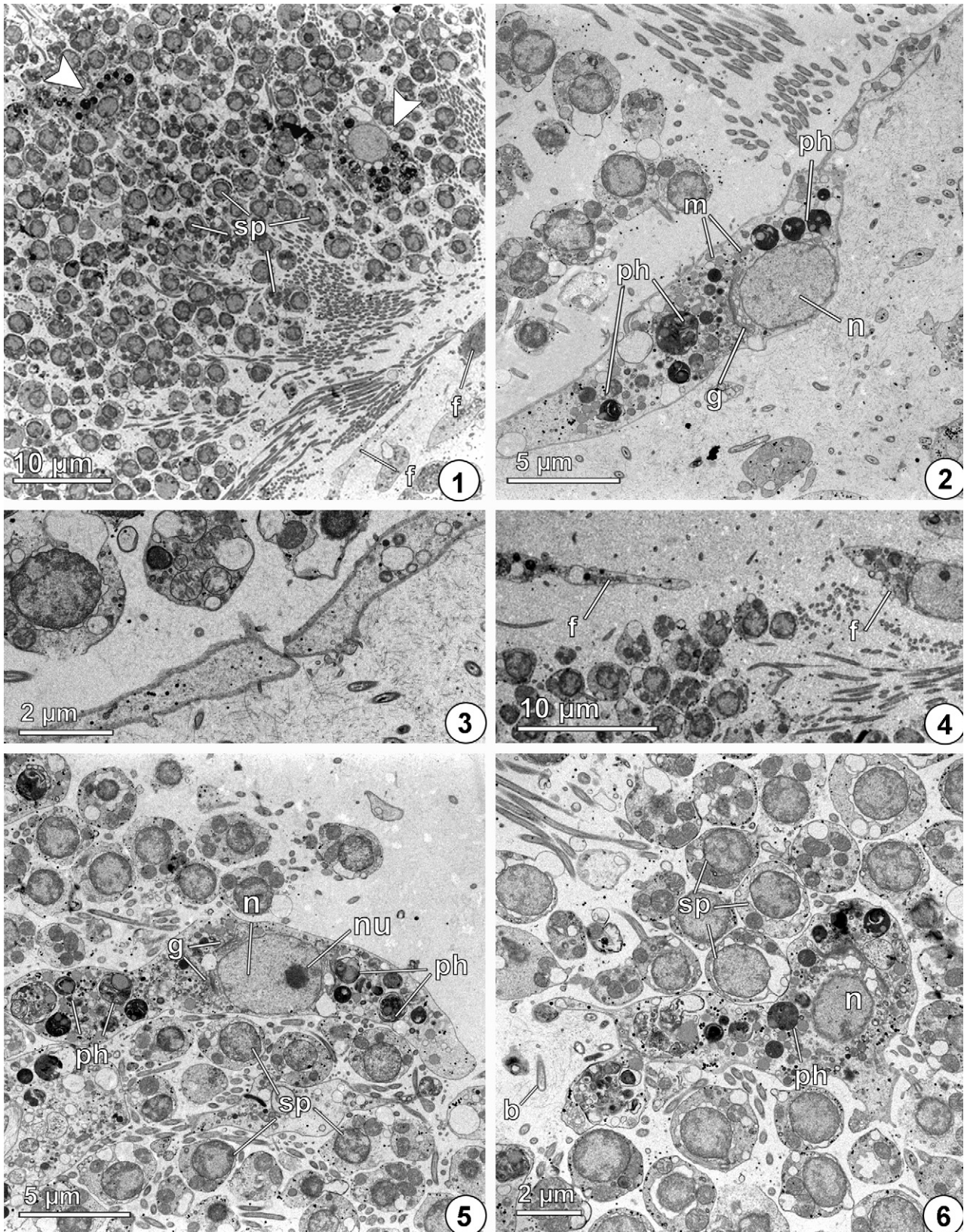
#### 3.2. *P. ficiformis*

We examined ultrastructurally spermatic cysts for somatic cells during the entire spermatogenic period and immediately after the spawning event occurred in November 2006. Somatic cells were found within spermatocysts only after the spawning. At this stage, cysts were lax, lined by a discontinuous layer of cells (Fig. 11), which were joined by interdigitate junctions and contained scarce and dispersed spermatozoa. Small somatic cells (approximately 5  $\mu\text{m}$ ) containing spermatozoa were found within the spermatic cysts (Figs. 11–14). These cells were amoeboid to round cells (Figs. 11–14), showing a nucleolate nucleus (not shown). Numerous phagosomes of heterogeneous nature were observed within their cytoplasm in different stages of digestion (Figs. 13 and 14). Large vesicles also occurred in their cytoplasm, in many cases containing phagocytosed bacteria (Fig. 12), as well as large electron-clear vesicles apparently empty (Figs. 12 and 13). These somatic cells located around unspawned spermatozoa (Fig. 11), and sometimes were seen containing engulfed sperm (Figs. 12–15). They often emitted pseudopodia, presumably indicating intense phagocytic activity of unspawned spermatozoa (Figs. 12–14). Similar cells, in both size and morphology, were detected in the mesohyl of the sponge (Fig. 15) containing spermatogenic cells partially digested. These cells were also round to amoeboid, had a nucleolate nucleus, and numerous phagosomes of heterogeneous nature (Fig. 15).

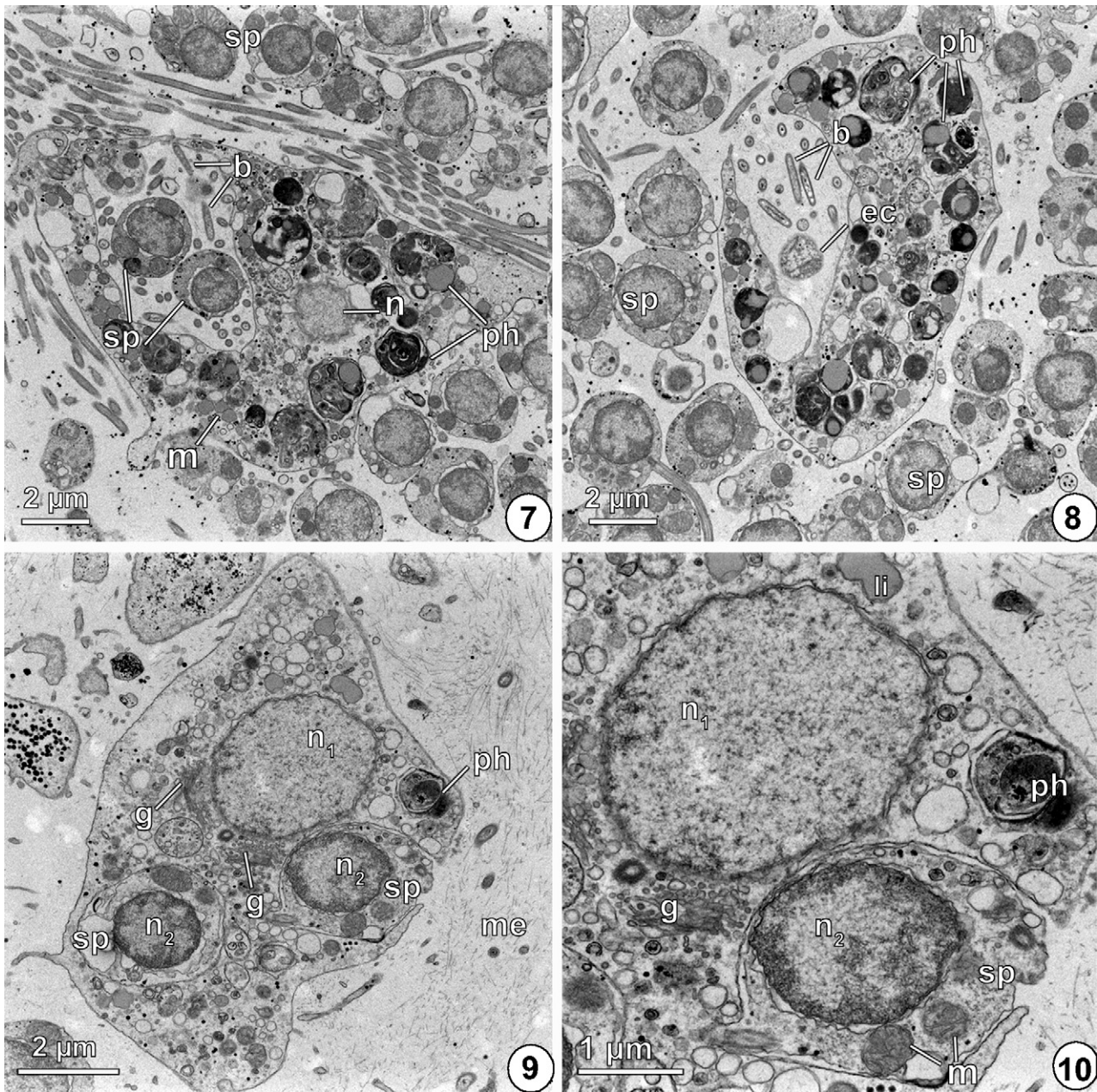
Additionally, we found some large bacteriocytes (approximately 12  $\mu\text{m}$  in largest diameter) also containing engulfed unspawned spermatozoa (Fig. 16), which located in the mesohyl around spermatic cysts. Phagocytosed spermatozoon was in large cell pockets that also contained bacteria (Fig. 16).

### 4. Discussion

In marine invertebrates, removal of unsuccessful gametes (i.e., unfertilized oocytes and aberrant or unspawned spermatozoa) may proceed either by resorption (cell degeneration) or phagocytosis. Resorption of oocytes is a widespread process among many invertebrates, being documented in cnidarians (Szmant-Froelich et al., 1980; Kruger and Schleyer, 1998; Neves and Pires, 2002), nemertines (Bierne, 1983), acanthocephalans (Crompton, 1983), molluscs (Jong-Brink et al., 1983; Dorange and Le Pennec, 1989; Fabioux et al., 2005), polychaetes (Olive et al., 1981), tardigrades (Bertolani, 1983), and brachiopods (Chuang, 1983). Nevertheless, phagocytosis of unfertilized oocytes has only been observed in sipunculans (Rice, 1983) and crustaceans



Figs. 1–6. (1) Spermatogenic cyst of *R. aculeata* containing secondary spermatocytes (sp) and somatic cells (arrow heads). Note the space between the follicle cells (f) that enclosed the cyst. (2) *R. aculeata*. Follicle cell lining the spermatogenic cyst showing an oval nucleus (n), a well-developed Golgi apparatus (g), many mitochondria (m), and multiple phagosomes (ph) within the cytoplasm. (3) *R. aculeata*. Simple junction between two follicle cells surrounding the spermatogenic cyst. (4) *R. aculeata*. Follicle cells (f) surrounding the spermatogenic cyst. Note the space between both cells. (5) *R. aculeata*. Follicle cell migrating into the

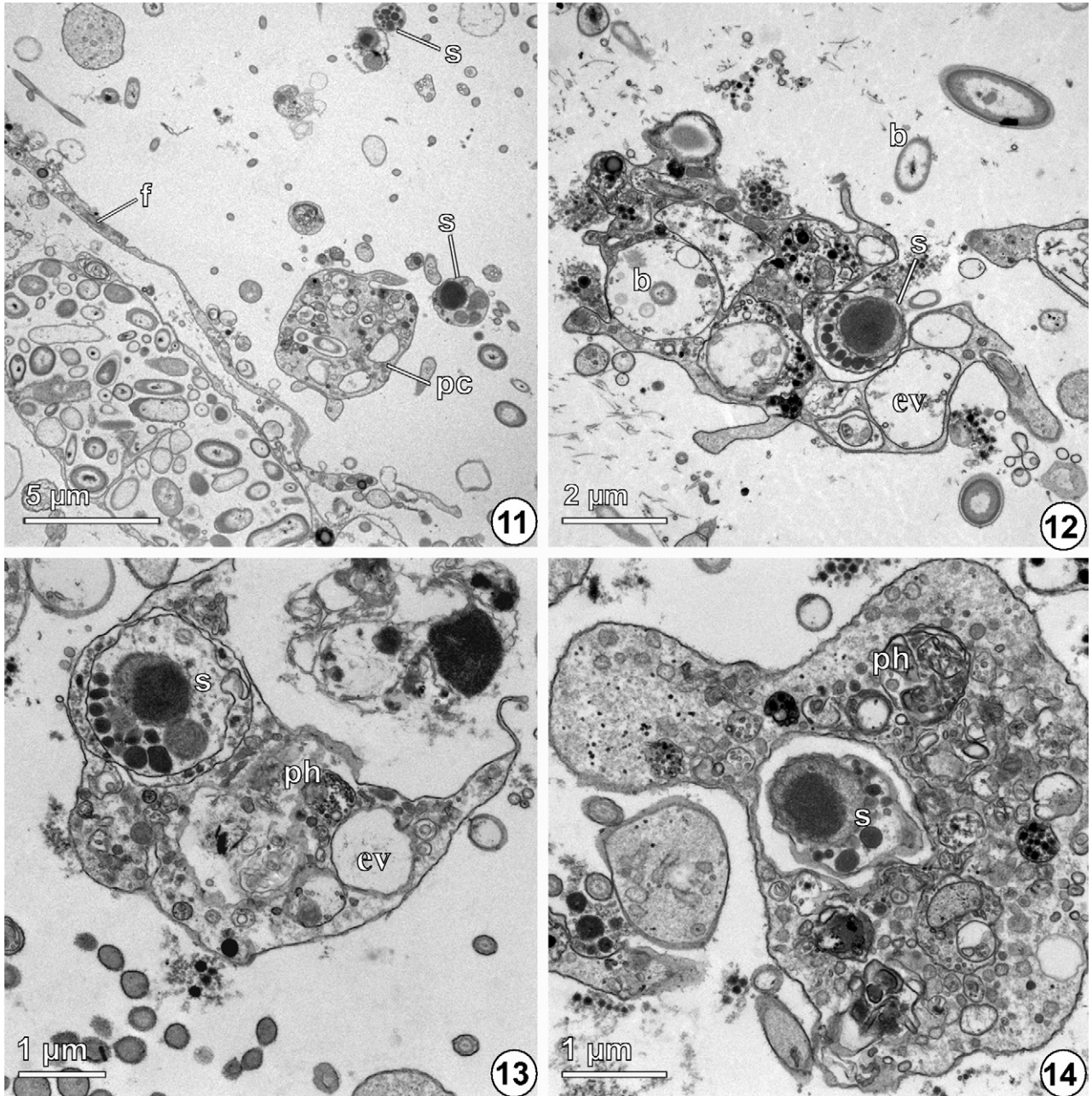


Figs. 7–10. (7) *R. aculeata*. Somatic cell within a spermatic cyst phagocytosing secondary spermatocytes (sp) and bacteria (b). Note the nucleus (n), numerous phagosomes (ph), and mitochondria (m) within the cytoplasm. (8) *R. aculeata*. Somatic cell inside a spermatic cyst engulfing free bacteria (b) and residual cytoplasmic bodies (ec) jettisoned by secondary spermatocytes. (9) *R. aculeata*. Phagocytic cell in the mesohyl of the sponge (me) engulfing two secondary spermatocytes (sp). In the cytoplasm of the phagocytic cell, it can be observed the nucleus ( $n_1$ ), several Golgi apparatus (g), and phagosomes (ph). Note the chromatin condensations attached to the inner nuclear membrane of the secondary spermatocyte's nucleus ( $n_2$ ). (10) *R. aculeata*. Magnification of 9, showing the phagocytic cell's nucleus ( $n_1$ ), the Golgi apparatus (g), and a phagosome (ph). Note the nucleus ( $n_2$ ) and two mitochondria (m) of the secondary spermatocyte (sp).

(Adiyodi and Subramoniam, 1983). In contrast to that found for oocytes, phagocytosis of sperm (unspawned or abnormal) is more common than simply sperm degeneration (Buckland-Nicks and Chia, 1986; O'Donovan and Abraham, 1987; Chia and Buckland-Nicks, 1987; Holland and Holland, 1989;

Jørgensen and Lützen, 1997; Reunov et al., 2004). Sperm phagocytosis is usually carried out by somatic cells which are in most cases accessory cells located in either the testis wall – generally called Sertoli cells – (Buckland-Nicks and Chia, 1986; Erkan and Sousa, 2002) or the lumen of the

spermatic cyst that contained secondary spermatocytes (sp). Note the nucleolate (nu) nucleus (n) of the cell, the dictyosomes (g), and many phagosomes within the cytoplasm (ph). (6) *R. aculeata*. Somatic cell intermingled with secondary spermatocytes (sp) inside the spermatic cyst. Note the nucleus (n) and multiple phagosomes (ph) in the cytoplasm. Free bacteria (b) can be found in the lumen of the cyst.

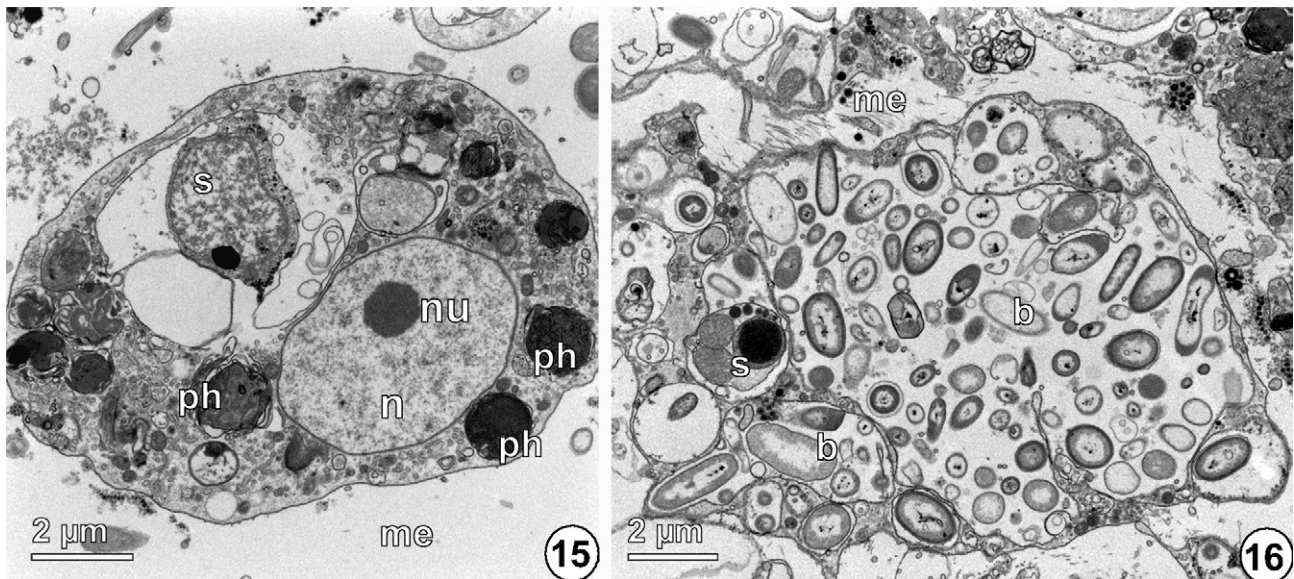


Figs. 11–14. (11) *P. ficiformis*. View of a virtually empty spermatic cyst lined by a single cellular follicle (f), containing few mature spermatozoan (s), and a somatic cell (pc). (12) *P. ficiformis*. Somatic cell within a nearly disassembled spermatic cyst, engulfing a mature spermatozoan (s) and containing electron-clear vesicles (ev). Note the occurrence of free bacteria (b) in the lumen of the cyst, and in a phagocytic vesicle of the cell. (13) *P. ficiformis*. Somatic cell within a cyst showing an engulfed spermatozoan (s) in a large vesicle of its cytoplasm. Note the heterogeneous appearance of the pseudo-digested content of a phagocytic vesicle (ph) and the electron-clear vesicle (ev). (14) *P. ficiformis*. Somatic cell within a cyst containing a phagocytosed spermatozoan (s) in a large vesicle and many phagosomes (ph).

testes (Hinsch, 1980; Pacey and Bentley, 1992; Jørgensen and Lützen, 1997; Vaschenko et al., 1997; Ramofafia et al., 2003; Kalachev and Reunov, 2005).

In sponges, the fate of waste gametes unspawned or unfertilized oocytes has been investigated in some cases. Degeneration and resorption of unfertilized oocytes have been reported in *Grantia compressa* (Duboscq and Tuzet, 1937), *Halisarca dujardini* (Lévi, 1956), *Sycon raphanus*

(Colussi, 1958), *Hymeniacidon sanguinea* (Sarà, 1961), *Petrobiona massiliana* (Vacelet, 1964), *Haliclona ecbasis* (Fell, 1969), *Spongilla lacustris* (Gilbert, 1974), *Verongia cavernicola* and *Verongia aerophoba* (Gallissian and Vacelet, 1976), *Suberites massa* (Diaz, 1979) and *Haliclondria okadai* (Tanaka-Ichiara and Watanabe, 1990). In *Haliclona permollis* oocytes were found to be further numerous than embryos, suggesting an important oosorption after



Figs. 15 and 16. (15) *P. ficiformis*. Phagocytic cell in the mesohyl of the sponge, containing a pseudo-digested spermatogenic cell (s). Note the nucleolate (nu) nucleus (n) of the phagocytic cell and the many phagosomes (ph) within its cytoplasm. (16) *P. ficiformis*. Bacteriocyte in the mesohyl (me) in the vicinity of an empty spermatogenic cyst showing large cell pockets charged with bacteria (b) and a mature spermatozoan (s).

fertilization (Elvin, 1976). In *Haliclona loosanooffi* (Fell, 1976) and *Corticium candelabrum* (Riesgo et al., 2007b) production of new oocytes was documented simultaneously to maturation and fertilization of mature oocytes, being the new oocytes presumably resorbed or used as food for the latter ones.

Unlike for oocytes, the issue of sperm resorption during spermatogenesis and after spawning has never been thoroughly investigated in sponges. Diaz and Connes (1980), in their ultrastructural study of the spermatogenesis of *Aplysilla rosea*, suggested that follicle cells could remove unspawned spermatozoa by phagocytosis. Likewise, Barthel and Detmer (1990) noticed that the follicle cells of empty spermatogenic cysts of *Halichondria panicea* contained phagocytic vesicles, and interpreted them as phagosomes that would support their archaeocytic origin. Nevertheless, those phagosomes could well be the result of sperm phagocytosis.

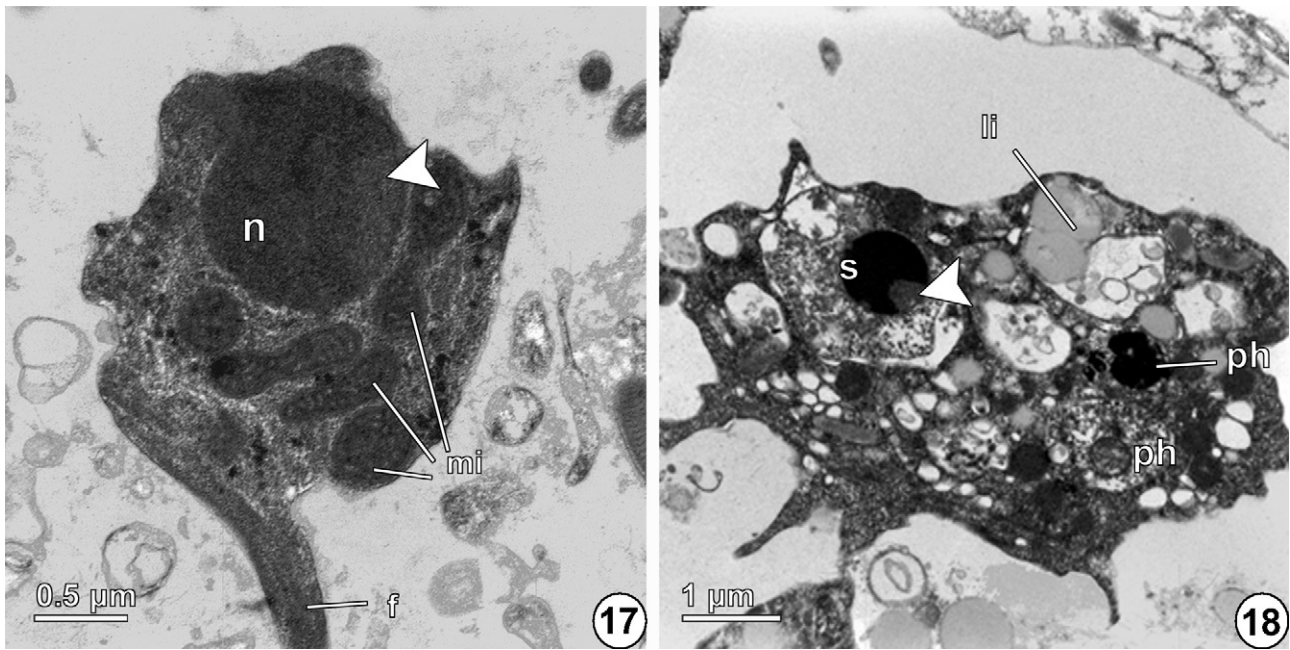
Somatic cells found within the cysts of *R. aculeata* appear to be follicle cells that detach from the follicular epithelium and enter the lumen of the cysts. In addition, we observed that some archaeocyte-like cells in the mesohyl also contained phagocytosed spermatozoa, even though they were outside the cysts. Therefore, we cannot discard that archaeocytes can also enter the cysts to phagocytose spermatozoa. Alternatively, secondary spermatozoa could have escaped from the cysts to the mesohyl, and once there, they could have been phagocytosed by those archaeocyte-like cells. The objective of such sperm removal remains unclear, although the elimination of damaged or abnormally developing spermatozoa emerges as a plausible explanation.

In *P. ficiformis*, the function of somatic cells within the spermatogenic cysts was fairly different. They were actively removing mature spermatozoa that had not been spawned.

In this case, the somatic cells found within the cysts were more similar to sponge archaeocytes than to follicle cells. Archaeocytes, typical constituents of demosponge mesohyl, are totipotent (undifferentiated) amoeboid cells with a nucleolate nucleus and evident motility and phagocytic activity (Simpson, 1984). Among other functions, these cells have been pointed out as the most probable origin of oocytes in many demosponges, and also of follicle cells of both oocytes and spermatocysts (Simpson, 1984). Since they are motile cells, their occurrence in the lumen of spermatogenic cysts would not be surprising. Moreover, the junctions between follicle cells of the spermatogenic cysts of *P. ficiformis* are not tight and leave occasional passages, which may facilitate penetration of archaeocytes into the lumen of cysts.

Phagocytosis of unspawned sperm may be a common process among demosponges. It has also been noted in *Chondrilla nucula*. After a massive sperm spawning in a Bahamian population (Maldonado, personal observations), samples of male individuals were fixed and examined by TEM. Phagocytic activity of archaeocyte-like cells over unspawned sperm (Fig. 17) located within the mesohyl was obvious (Fig. 18). The fact that aberrant sperm has been found in *Chondrilla australiensis* prior and after spawning (Usher et al., 2004), supports the hypothesis that somatic cells of the genus *Chondrilla* may also be involved in elimination of both unspawned and aberrant sperm cells by phagocytosis.

The present study suggests that sponge somatic cells found inside the spermatogenic cysts of demosponges are involved in both elimination of presumably aberrant spermatozoa during spermatogenesis and removal of unspawned sperm. Nevertheless, although we assume that the spermatozoa phagocytosed within the cysts of *R. aculeata* were abnormal, other hypotheses, in which sperm abnormality does



Figs. 17 and 18. (17) *Chondrilla nucula*. Unspawned mature spermatozoon found within the mesohyl of the male. Note the clearer region (arrowhead) within the nucleus (nu), the multiple mitochondria (mi), and the single flagellum (f). (18) *C. nucula*. Archaeocyte-like cell showing in the cytoplasm a phagocytosed spermatozoon (s) with a clear region in the nucleus (arrowhead), lipid granules (li), and phagosomes (ph) in different digestion stages.

not concur, may also be taken into account to explain these observations. *R. aculeata* is an oviparous externally fertilising demosponge. We noticed that spermatogenesis causes serious disruption of the histological organization of the mesohyl in male individuals because choanocytes transdifferentiate into spermatogonia. This phenomenon has been previously reported for many other sponges (Tanaka-Ichiara and Watanabe, 1990; Tsurumi and Reisswig, 1997; Ereskovsky, 2000). Mesohyl disruption implies temporary loss of nearly all choanocyte chambers, which is the main via for capturing bacteria and particulate material for feeding (Simpson, 1984). The presumable starvation suffered by males during spermatogenesis may lead to situations of sperm self-predation, in order to regain critical nutritive reserves to palliate starvation. Although the direct costs of sperm production are poorly understood in invertebrates and vertebrates (Wedell et al., 2002), it has been observed that sometimes sperm production reduces life-span, as in *Caenorhabditis elegans* (Van Voorhies, 1992), or produces a great loss of body-mass, as detected in the adder *Vipera berus* (Olsson et al., 1997). Thus, cellular actions aimed to recapture some reserves and palliate starvation should not be surprising. An additional explanation accounting for self-predation of spermatocytes in Porifera may be derived from previous work by Griswold (1995, 1998, 2005) on vertebrates. This author postulated the essential role of Sertoli cells during spermatogenesis to prevent proliferation of sperm cells beyond an unsatisfactory threshold density by phagocytosing them. In *Hydra* testis, apoptotic spermatogonia and spermatocytes are removed by the epithelial cells that line the testis (Kutznetsov et al., 2001).

Although such cell removal was initially interpreted as a “quality control” developed by such cells, the authors did not discard Griswold’s alternative explanation for their findings. Apoptosis of sperm cells and subsequent removal from the testis could be directed to achieve the precise homeostasis for each sperm cell by maintaining a proper ratio between differentiating spermatogonia and epithelial cells. A similar explanation can not be ruled out in the case of *R. aculeata*.

The occurrence of cellular mechanisms for putatively eliminating abnormal spermatocytes – to ensure an appropriate sperm density within the cysts, and/or to recycle sperm precursors (spermatogonia, spermatocytes, spermatids) or unspawned spermatozoa – come to the still incomplete list of complex features displayed by sponges that are widely known in higher invertebrates. Furthermore, it suggests that cellular processes aimed to regulate testis functioning in higher metazoans have their equivalent in sponges. Although these mechanisms do not attain the complexity displayed by those based on Sertoli cells, their functionality appears to be considered analogous.

#### Acknowledgements

We thank Nùria Cortadellas and Almudena García for their valuable help in processing TEM samples. This study was supported by two grants from the Spanish Ministry for Science and Education (MCYT-BMC2002–01228; MEC-CTM2005–05366/MAR).



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