



The ultrastructure of the mature oocyte and the nurse cells of the ceractinomorpha *Petrosia ficiformis*.

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Abstract: *Petrosia ficiformis* (Poiret), one of the Demospongiae Ceractinomorpha, is an oviparous species having relatively large oocytes (140-160 μm) and a nucleolated nucleus. The perinuclear region is devoid of yolk inclusions and contains an elevated number of dictyosomes and ergastoplasmic lamellae as well as numerous mitochondrial clusters. In comparison the other parts of the cytoplasm are rich in variously organized inclusions. The oocyte is surrounded by archeocyte-type nurse cells. Cell contacts via pseudopods on both cell surfaces, and spherical electron-dense corpuscles in spaces between nurse cells and oocyte have been seen. It is suggested that in *Petrosia ficiformis*, because of the presence of the nurse cells, the majority of the yolk material is derived from these cells and is then subsequently elaborated. A part of the yolk is also formed through the autotrophic activity of the oocyte itself.

Résumé : *Petrosia ficiformis* (Poiret) (Demospongiae, Ceractinomorpha) est une espèce ovipare caractérisée par des ovocytes volumineux (140-160 μm) avec un noyau nucléolé. La région périnucléaire du cytoplasme de l'ovocyte est sans inclusions vitellines ; on y observe dictyosomes, lamelles ergastoplasmiques et nombreux amas de mitochondries. La région périphérique du cytoplasme est au contraire très riche en inclusions vitellines. L'ovocyte est entouré de cellules nourricières de type archéocytaire. Des observations ultrastructurales montrent que des pseudopodes établissent des contacts entre l'ovocyte et les cellules nourricières et que des corpuscules denses aux électrons sont présents dans les espaces intercellulaires. Ces observations suggèrent que les cellules nourricières procurent l'essentiel des éléments du matériel vitellin qui est ensuite élaboré par l'ovocyte. Une partie du vitellus est également directement synthétisé par l'ovocyte lui-même.

Keywords: *Petrosia ficiformis*, Porifera, oocyte, nurse cells, ultrastructure.

Introduction

The Demospongiae are subdivided into two subclasses: Tetractinomorpha and Ceractinomorpha. This classification was proposed by Lévi in 1956 based on the criteria of oviparity and viviparity but was discredited by the discovery of oviparous species in the subclass Ceractinomorpha previously considered viviparous. This oviparity, according to

Bergquist (1980 a; 1980 b), not only involves single species but the entire orders Verongida and Petrosida.

Despite this, for the present study of the ceractinomorpha *Petrosia ficiformis*, we will continue to subdivide the Demospongiae into Tetractinomorpha and Ceractinomorpha until the situation has been clarified (Van Soest, 1991).

The majority of observations on the oogenesis of Ceractinomorpha are light microscope observations. Ultrastructural studies have concerned the genera *Aplysina* (*Verongia*) (Gallissian and Vacelet, 1976), *Hippospongia* and *Spongia* (Kaye, 1991; Kaye and Reiswig, 1991).

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The only ultrastructural observations made for *Petrosia ficiformis* concern the choanocyte chambers (Langenbruch *et al.*, 1985) and the architecture of the canal systems (Bavestrello *et al.*, 1988) and the interactions between prokaryotic cells and bacteriocytes (Bigliardi *et al.*, 1993).

The sexual reproduction of *Petrosia ficiformis* has been studied previously by Scalera Liaci *et al.* (1973). Such light microscope observations have brought to light some differences between the behaviour of *Petrosia ficiformis* and other Ceractinomorpha Haplosclerida such as *Haliciona elegans*, *Adocia varia* and *Gellius fibulatus*. From this work it emerges that, aside from the gonochorism present in all the species examined, *Petrosia ficiformis* is an oviparous sponge having large oocytes surrounded by nurse cells. For these reasons we decided to reexamine *Petrosia ficiformis* on the ultrastructural level to better understand the morphological organization of the oocyte and its relationship to the nurse cells.

Materials and methods

Specimens of *Petrosia ficiformis* were collected once a month for a two year period during dives in the Adriatic Sea near Bari, Southern Italy. Immediately after collection the specimens were cut into small pieces and fixed in 2.5 % glutaraldehyde in cacodylate buffer (0.4 M) in sea water (pH 7.4). In the laboratory, they were postfixed for 1 h in 1.0 % OsO₄ in sea water at 4° C. After rinsing in sea water, desilification was carried out by immersion in 5.0% hydrofluoric acid for 1.5 h. Specimens were then rinsed in sea water, dehydrated in acetone, and embedded in a mixture of Epon-Araldite (according to Millonig, 1976).

Semithin sections of thickness 1.0 µm were heat stained with toluidine blue-borate. Thin sections were contrasted with 5.0 % uranyl acetate in 50 % ethanol and lead citrate and examined with a Zeiss EM 109 microscope.

Despite collections being carried out in two consecutive years it was not possible to observe in detail the various phases of oogenesis due to the rapid growth of the germ cells. Furthermore, the lack of gonads made it difficult to identify the various stages of oogenesis. For this reasons we have confined our ultrastructural observations to a description of the oocyte at what we interpret to be the end of the first meiotic prophase at which vitellogenesis is completed.

Results

Petrosia ficiformis is an oviparous species that reproduces during the summer months. It is characterized by relatively large oocytes (140-160 µm in diameter) contained in a follicle made up of numerous archeocytes (Figs. 1, 2).

Even at the level of semithin sections it is possible to observe a net separation between the perinuclear region (about 40 µm in thickness) which is poor in inclusions and

the remaining cytoplasm (about 30 µm in thickness) rich in reserve material (Fig. 1).

Under ultrastructural observation the nucleus (about 20 µm in diameter) appears vesicular with finely diffused chromatin. Close to the nuclear membrane, in the area of the nucleolus, chromatin masses are visible. The passage of electron-dense material across the pores of the nuclear membrane can be observed (Figs. 3, 4). Two nucleoli are present and often possess electron-dense and granular regions. The perinuclear cytoplasmic region devoid of yolk inclusions, has a granular matrix, numerous electron-transparent vesicles and many Golgi dictyosomes, the majority of which are situated in the vicinity of the nuclear membrane (Fig. 4). Dictyosomes are also found some distance from the nucleus but are completely absent in regions rich in inclusions. The flattened saccules which make up the dictyosomes take on an arc shape, with small vesicles associated with their periphery which may join together to give rise to larger vesicles (Fig. 5).

Ergastoplasmic lamellae with electron-dense contents, numerous transparent vesicles and mitochondria were also found in the region poor in yolk inclusions (Fig. 6). The highly vesiculated cytoplasm is probably a fixation artefact. The mitochondria are grouped together and, at times, are arranged concentrically within the groups. An accumulation of granular material can be observed between mitochondria (Fig. 7).

The oocyte region in which the yolk inclusions are completely absent gradually changes to a peripheral region where the inclusions are very numerous (Fig. 8). This latter region extends as far as the oolemma and is characterized by the presence of a large number of spherical electron-dense inclusions. These inclusions are not homogeneous, in fact the majority contain internal inclusions of different densities while some also contain irregular electron-transparent areas; only in a few are granular contents intermixed with elongated electron-dense bodies (Figs. 9, 10). Numerous small irregular electron-dense bodies surround large yolk inclusions and may fuse with the yolk during growth (Fig. 12). Lipid globules are small and rare (Fig. 13).

At this phase of growth, the *Petrosia ficiformis* oocyte is surrounded by archeocytes. However, from light microscope observations, these archeocytes are known to disappear prior to oocyte release (August - September) (Fig. 11). The archeocytes, that are distributed throughout the mesohyl of the sponge, display the same features as nurse cells: a nucleus with chromatin masses, granular cytoplasm containing small vacuoles and spherical electron-dense inclusions of various sizes (Fig. 12). Some archeocytes collect near oocytes and modify their shape to conform to that of the surface of the oocyte. As a result of TEM observations, cell contacts have been seen between archeocytes and the oocyte via small pseudopods on both cell surfaces (Fig. 12).

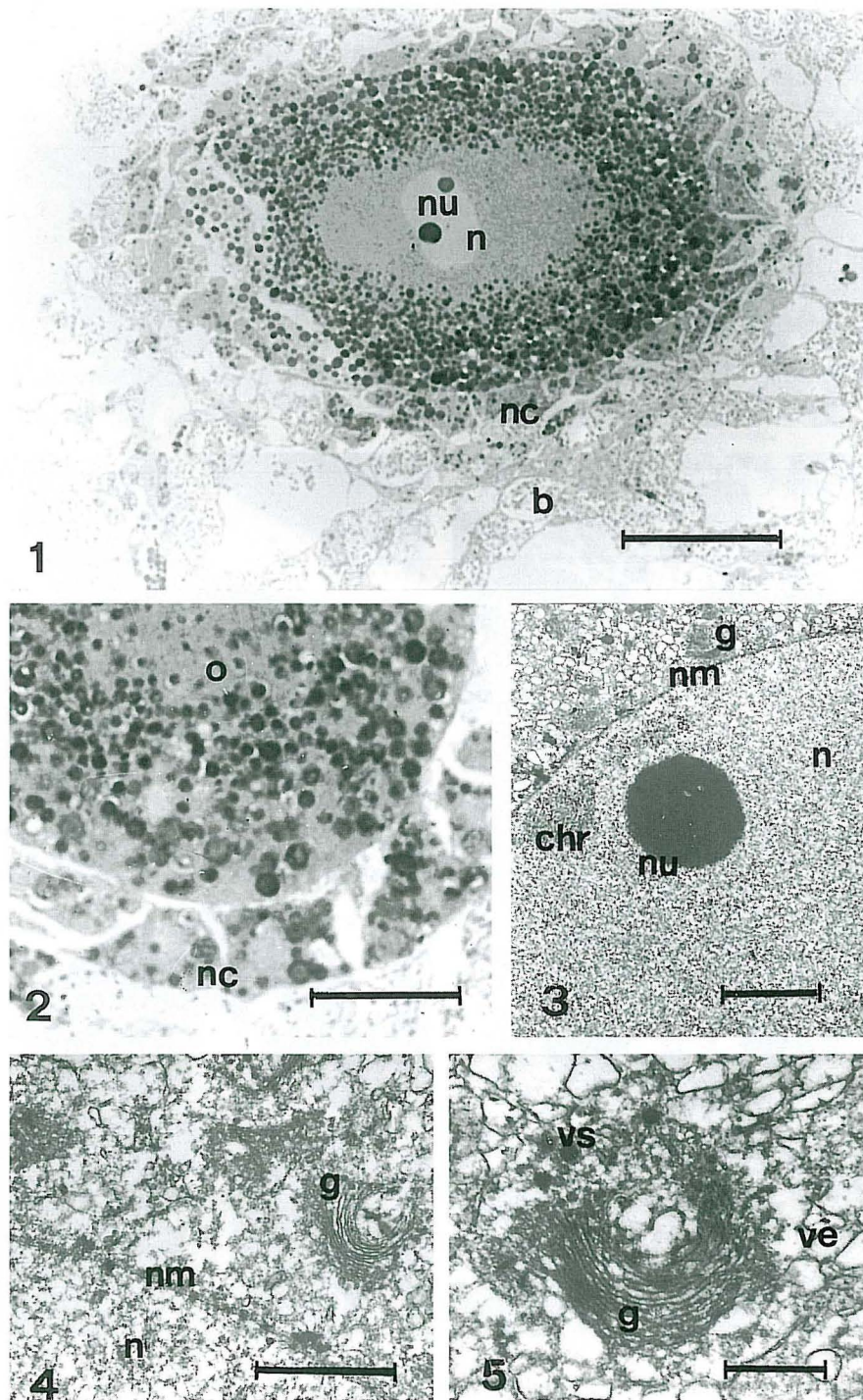


Fig. 1. Semithin section of an oocyte of *Petrosia ficiformis*, surrounded by nurse cells-like archeocytes. Note, round the nucleolated nucleus, the clear division between the area poor in cytoplasmic inclusions and the remaining oocyte region, rich in yolk. n, nucleus; nu, nucleoli; nc, nurse cells; b, bacteriocytes. Scale bar: 50 μ m.

Fig. 2. Semithin section of an oocyte portion, in which the archeocyte envelope is well evident. o, oocyte; nc, nurse cells. Scale bar: 20 μ m.

Figs. 3, 4. Micrographies of oocyte relative to nuclear and perinuclear portions. n, nucleus; nu, nucleolus; chr, chromatin masses; nm, nuclear membrane; g, dictyosomes. Scale bars in fig. 3: 2 μ m; in fig. 4: 1 μ m.

Fig. 5. Arc-shaped dictyosome (g) in the perinuclear area. The detachment of vesicles (vs) from the dictyosome periphery, is evident. ve, electron-transparent vesicles. Scale bar: 0.5 μ m.

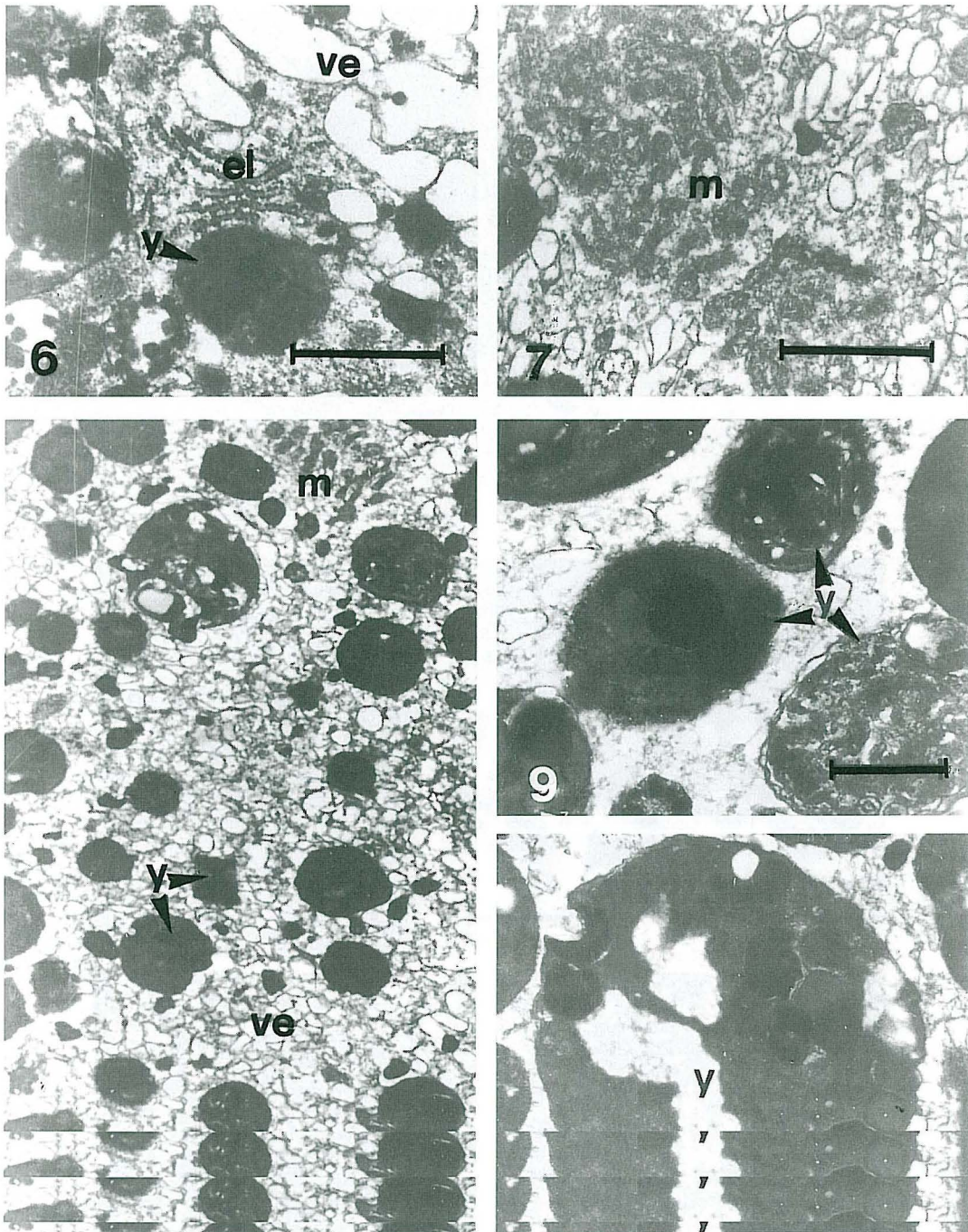


Fig. 6. Micrography showing ergastoplasmic lamellae (el). ve, electron-transparent vesicles; y, yolk. Scale bar: 1 μ m.

Fig. 7. Detail of ooplasm showing a cluster of mitochondria (m). Scale bar: 1 μ m.

Fig. 8. Cytoplasmic oocyte area with vesicles (ve) and yolk inclusions (y). m, mitochondria. Scale bar: 2 μ m.

Figs. 9, 10. Micrographies of yolk inclusions (y) with different structures. Scale bars in fig. 9: 0.5 μ m; in fig. 10: 1 μ m.

Spherical electron-dense corpuscles, which could represent material provided by the archeocyte to the oocyte, are often found in spaces between the nurse cells and the oocyte (Fig. 13).

Discussion

The *Petrosia ficiformis* oocyte is surrounded by a follicular epithelium made up of a number of somatic cells of the archeocyte type. Simpson (1984) reports that the termino-

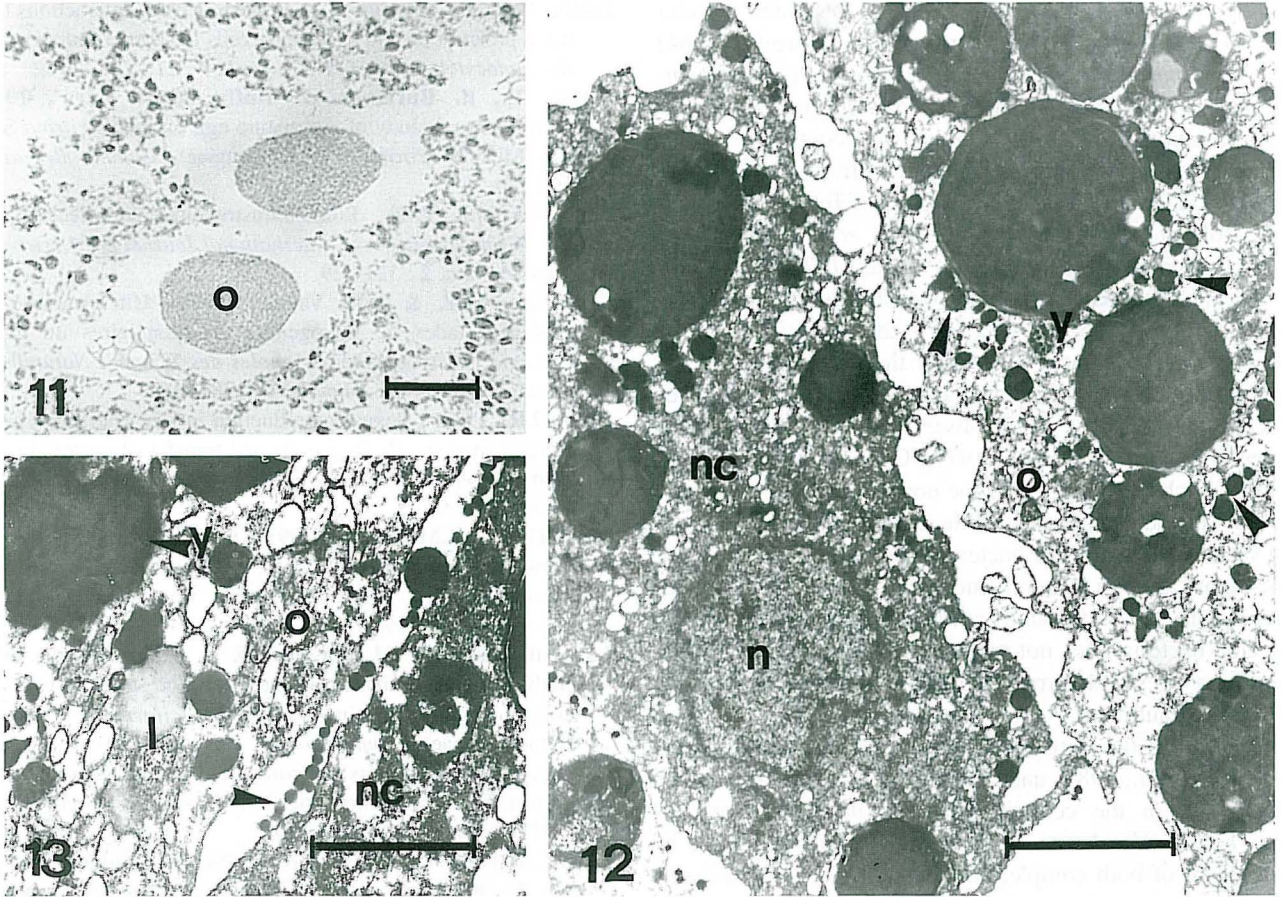


Fig. 11. Paraffin section in which the oocytes (o), migrating to in the excurrent canals at spawning are no longer surrounded by the archeocyte envelope. Scale bar: 100 μ m.

Fig. 12. Micrograph showing contacts between a nurse cell (nc) and an oocyte (o). In the oocyte small electron-dense bodies (arrows) surround large yolk inclusions (y). n, nucleus. Scale bar: 2 μ m.

Fig. 13. Small electron-dense bodies (arrow) are also present in the intercellular space between a nurse cell (nc) and an oocyte (o). y, yolk; l, lipid. Scale bar: 1 μ m.

logy commonly used for all somatic cells, whether associated temporarily or long term with the growth of the oocyte, is that of the nurse cells and the same terminology is used here.

As reported by Simpson (1984) there are various types of interaction that occur between nurse cells and oocytes during growth. One of these is represented by the phagocytosis of the nurse cells on the part of the oocyte. In other cases the nurse cells remain close to the oocyte until later when they transfer material to the embryo during its growth. Another type of interaction involves the liberation of material by the nurse cells into the perioocyte space and the subsequent capture of the material by the oocyte.

In *Chondrosia reniformis*, Lévi and Lévi (1976) state that the follicular cells that surround the oocyte have the function of transferring symbiotic bacteria and probably other material as well. However, in *Aplysina (Verongia) caverni-*

cola the role played by the spherulous cells that surround the oocyte is not clear (Gallissian and Vacelet, 1976).

It should be remembered that in *Petrosia ficiformis* the nurse cells have an important role in the nutrition of the oocyte since they establish cell contacts with oocytes and permit the transfer of substances which very likely are used in the synthesis of the yolk.

To this type of transfer can be added the possibility of capture by the oocyte of material released by the nurse cells into the intercellular space. It can be suggested that the bulk of the material for the yolk reaches the oocyte from the exterior and is subsequently transformed. Another part of the yolk is also derived from the activity of the oocyte itself. This activity is indicated by the extrusion of nuclear material in the oocyte cytoplasm, the elaboration of the Golgi dictyosomes, the presence of ergastoplasmic lamellae and mitochondrial clustering. Observations on the autosynthetic

activity have also been made by Gallissian and Vacelet (1976) in *Aplysina (Verongia) cavernicola*, Vacelet (1964) in *Petrobiona massillana*, Gallissian (1980) in *Grantia compressa* and Sciscioli *et al.* (1991) in *Stelletta grubii*.

It is important to emphasize that the oocyte of *Petrosia ficiformis* is devoid of bacteria which are present in large numbers in the oocytes of some species of Tetractinomorpha (Sciscioli *et al.*, 1989, 1991; Gaino *et al.*, 1987) and Ceractinomorpha (Gallissian and Vacelet, 1976).

Taking this into consideration we suggest two possibilities. First, the absence of bacteria in the *Petrosia ficiformis* oocyte could be related to the fact that in this species the bacteria are localized inside of specialized cells, the bacteriocytes, and are only rarely observed outside the bacteriocytes (Vacelet and Donadey, 1977). Consequently phagocytosis of such free bacteria by the oocytes can hardly occur.

Our second view is that, while it is accepted that in several species the symbiotic bacteria which are digested by the oocyte are used in the construction of the yolk (Gallissian and Vacelet, 1976; Sciscioli *et al.*, 1991), the phagocytosis of such bacteria does not occur in *Petrosia ficiformis*, because the oocyte is surrounded by nurse cells that provide the bulk of nutrients.

Whilst in the tetractinomorpha *Erylus discophorus* (Sciscioli *et al.*, 1989) and *Stelletta grubii* (Sciscioli *et al.*, 1991) and in the ceractinomorpha *Aplysina (Verongia) cavernicola* (Vacelet and Donadey, 1977) the presence in the oocyte of both complete bacteria and those in division assures their transfer into the embryo, in *Petrosia ficiformis* the absence of oocyte bacteria excludes their transmission from the maternal sponge to the embryo. At this point we can only hypothesize that the bacterial introduction occurs after the release of the egg as stated by Lévi and Lévi (1976) for *Chondrosia reniformis*.

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