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Transfer of symbiotic bacteria in the mature oocyte of *Geodia cydonium* (Porifera, Demosponsgiae) : an ultrastructural study.

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Résumé : Les ovocytes mûrs de *Geodia cydonium* sont uniformément répartis dans le mésohyle et mesurent 30-40 µm ; ils ont un noyau (8-9 µm) nucléolé avec une chromatine diffuse. L'ovocyte a un contour irrégulier, dû à la présence de nombreux pseudopodes en mouvement dans le mésohyle. Il héberge dans son cytoplasme un grand nombre de bactéries qui proviennent du mésohyle et pénètrent par phagocytose sur toute la surface de l'ovocyte. Leur présence dans l'ovocyte semble assurer leur transmission au futur embryon.

Abstract : The astrophorid *Geodia cydonium*, an oviparous marine sponge, has small mature oocytes (30-40 µm) scattered in the mesohyl. The nucleus (8-9 µm) is nucleolated and exhibits fine-grained chromatin. The mature oocyte has an irregular surface because of the presence of numerous pseudopodia which protrude into the mesohyl. The most interesting aspect of the ultrastructural study is represented by the high number of bacteria which are present in the oocyte cytoplasm and which derive from the mesohyl. The import of the bacteria into the cell occurs by endocytosis. Their presence is interpreted as a means of transfer to the next embryo.

INTRODUCTION

Ultrastructural studies of the Demospongiae oocytes are quite recent and as such reference has been made to the following : Sciscioli *et al.* (1989, 1991) on *Erylus discophorus* and *Stelletta grubii*, Diaz *et al.* (1975) on *Suberites massa*, Gallissian and Vacelet (1976) on two species of the *Verongia (= Aplysina)* genus, Gaino (1980) on *Chondrilla nucula*, Gaino *et al.* (1986, 1987) on *Oscarella lobularis* and *Tethya citrina*, Saller (1988) on the freshwater sponge *Ephydatia fluviatilis*, Kaye (1991) on some commercial sponges of the genus *Spongia* and *Hippospongia.*

The general aspects of the sexual reproduction of Porifera are reported by Simpson (1984).

To supply a further contribution to the knowledge of the Astrophorids we have considered the mature oocyte of *Geodia cydonium* (Jameson), the reproductive cycle, sexual condition and oviparity of which are known from light microscopy studies (Scalera Liaci & Sciscioli, 1969).

MATERIALS AND METHODS

Specimens of *Geodia cydonium* were collected once a month for a two year period during dives in the Adriatic Sea near Bari, Italy. Immediately after collection the specimens were cut into small pieces and fixed in 2.5 % glutaraldehyde in cacodylate buffer (0.4 M) and sea water (pH 7.4). Brought to the laboratory, they were postfixed for 1 h in 1.0 % OsO_4 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 in distilled water and examined with a Zeiss EM 109 microscope.

RESULTS

Our observations regarded some ultrastructural aspects of the *Geodia cydonium* mature oocyte and the presence of bacteria inside the oocyte. The mesohyl of the sponge was full of mature oocytes in the synchronous developmental stage, during the period of higher reproductive activity (summer-autumn). Despite having examined numerous samples during the reproductive period, it proved to be impossible to study the different growth phases of the oocyte as the maturation is both synchronized and rapid. The mature oocytes were 30-40 µm in diameter; the nucleus, complete with nucleolus, had fine-grained chromatin and was 8-9 µm in diameter (Figs. 1, 2). Masses of chromatin were found inside the nucleus near the nuclear membrane (Fig. 3). A thin granular cytoplasmic zone in which dictyosomes were present was observed close to the nucleus. The cisternae of each dictyosome of the Golgi apparatus were widened towards the distal extremity where numerous small vesicles were detaching. These vesicles blending together gave rise to larger electron-transparent vesicles (Figs. 3, 4). Furthermore, some ergastoplasmic vesicles were observed (Fig. 5). Various inclusions were found in the remaining granular cytoplasmic matrix, which represents most of the cellular volume (Figs. 2, 3). Besides lipid inclusions (Fig. 2) it was possible to observe various yolk inclusions : some of these had circular sections, uniform and highly electron-dense content, some a clearer content and irregular form, and others a granular matrix (Fig. 6).The mitochondria had an electron-dense matrix in which the cristae were clearly visible and were clustered in various cytoplasmic areas (Figs. 2, 7). Phagosomes were present in the oocyte cytoplasm with irregular forms and heterogeneous electron-dense content (Fig. 7). Besides food vacuoles (Fig. 2), some vacuoles with lacunar and granular matrices were observed within which myelin-figures were visible (Fig. 6). The most interesting component inside the oocyte cytoplasm was the bacteria, belonging to different morphological types and placed in lacunar structures (Figs. 2, 7).

Numerous bacteria were present in the mesohyl of *Geodia cydonium* and their entry into the oocyte occurs by endocytosis which takes place on the whole cell surface. In fact, the formation of invaginations was observed in many areas of the surface near a bacterium. The bacteria were received in these invaginations and invagination vesicles formed (Figs. 2, 8).

Most of these bacteria were whole and some of them were in division (Fig. 10). The bacteria population within the mature oocyte was the same as that found in the mesohyl of the sponge.

Vacuolar structures with fibrillar contents were detected in the cortical portion of the oocyte. It was often possible to observe the presence of the bacteria in the same vacuolar structures (Fig. 10).

The oocytes in this stage of growth had irregular surfaces owing to the presence of numerous pseudopodia, some of which were considerably wide and protruding into the mesohyl (Fig. 1). The oocytes were separated from the surrounding mesohyl by collagen fibrils (Fig. 1).

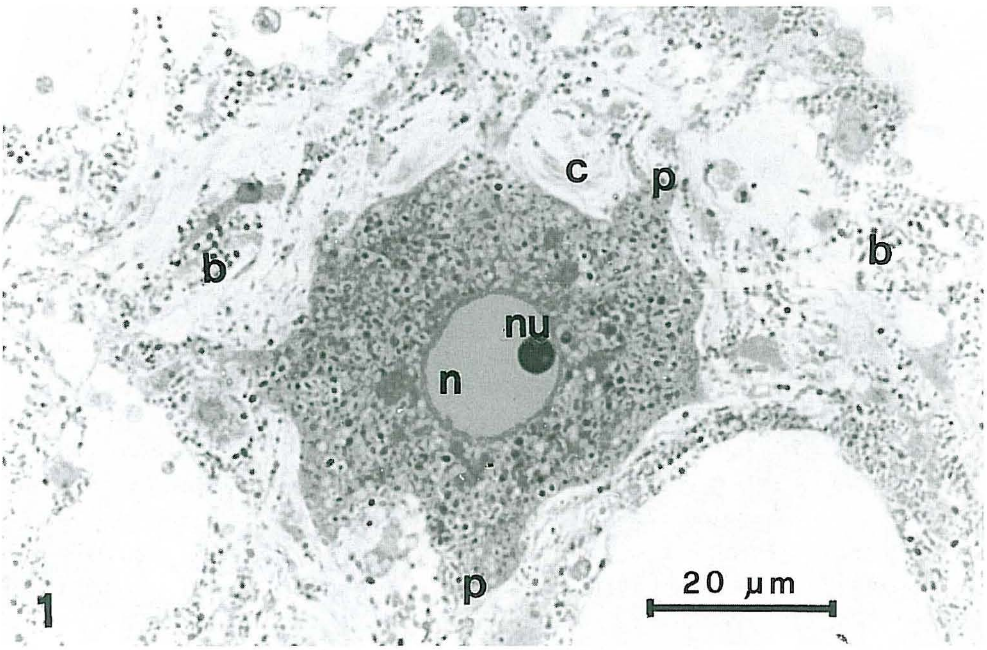
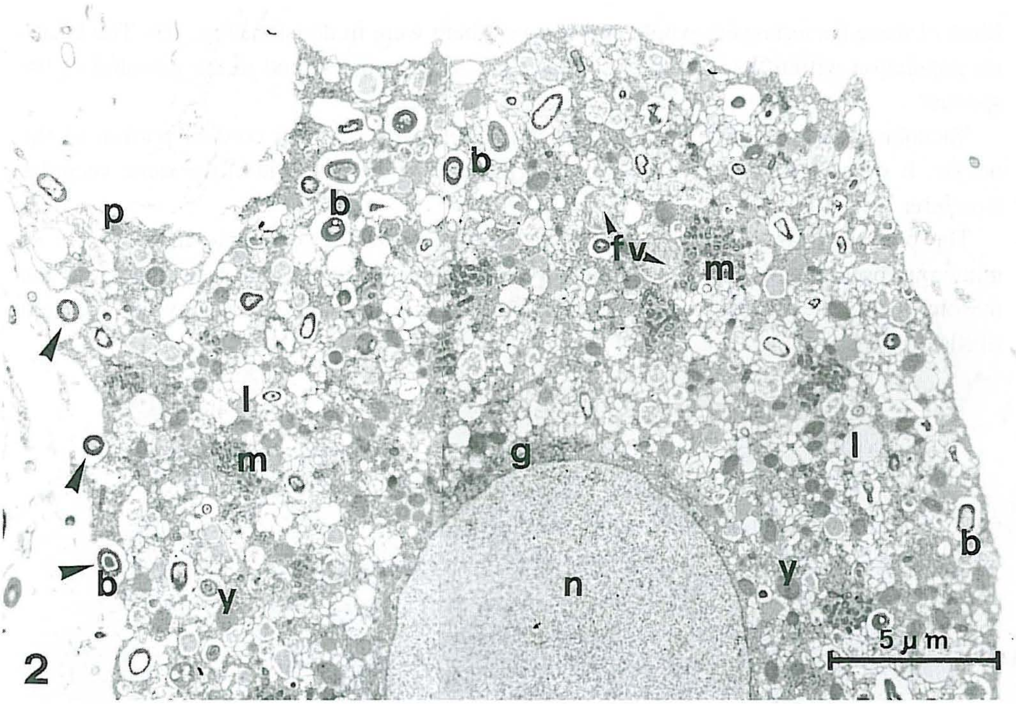
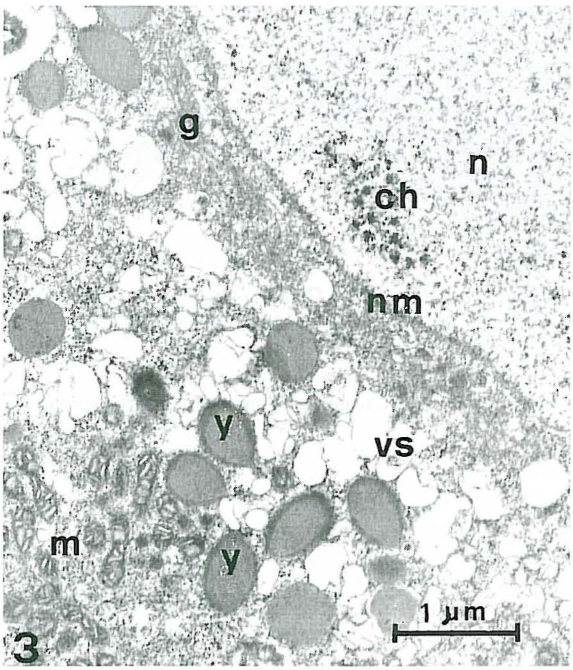


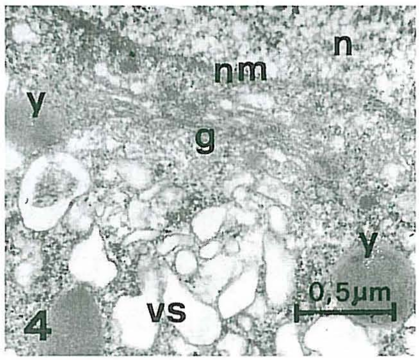
Fig. 1 : Semithin section showing a mature oocyte of *Geodia cydonium*. n, nucleus ; nu, nucleolus ; p, pseudopodia ; c, collagen ; b, bacteria.

DISCUSSION

The nutrients which serve for the elaboration of the yolk arrive in the oocyte by various processes : phagocytosis, pinocytosis, the presence of nurse and follicular cells, the capture of symbiotic bacteria. Furthermore, the oocyte is able to form reserve material by autosynthesis.







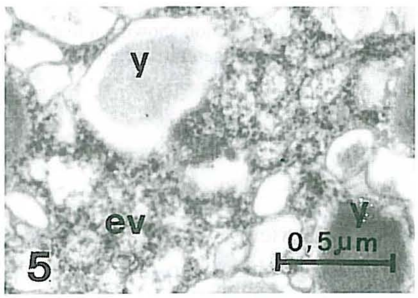
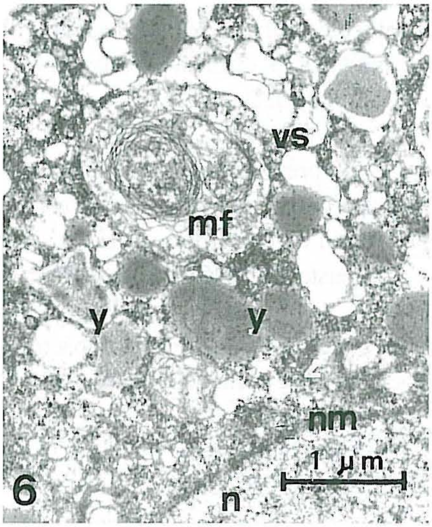


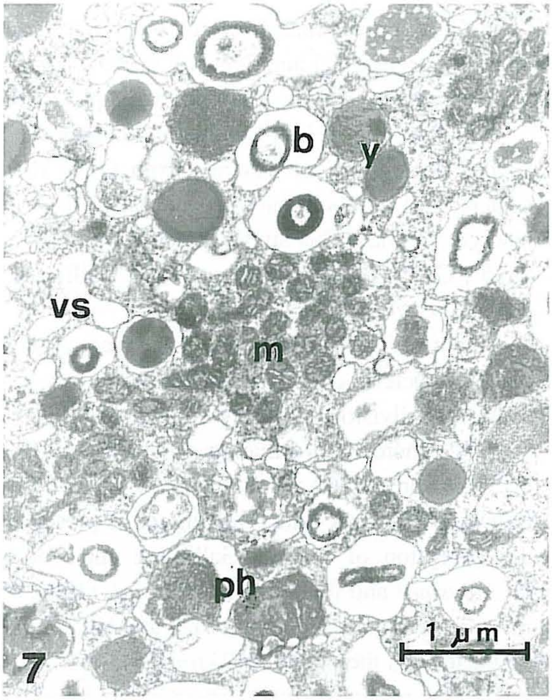
Fig. 2 : Portion of the *Geodia cydonium* oocyte. Bacteria (b) contained in vacuoles, mitochondria (m), food vacuoles (fv), lipid (l), electron-dense yolk inclusions (y), pseudopodia (p) are all evident in the granular cytoplasmic matrix. The arrowheads show the invagination of the oolemma near some bacteria. n, nucleus ; g, dictyosomes.

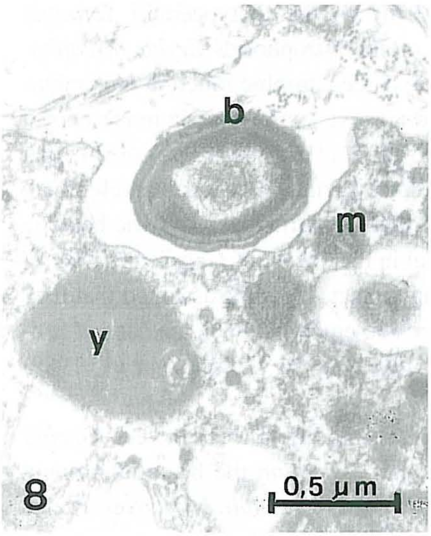
Fig. 3 : Micrograph showing a sector of a mature oocyte. n, nucleus ; nm, nuclear membrane ; ch, masses of chromatin ; g, dictyosomes ; m, mitochondria ; y, yolk inclusions ; vs, electron-transparent vesicles.

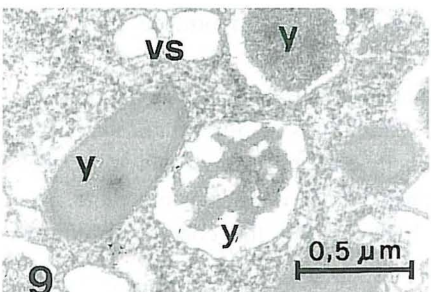
Fig. 4 : Detail of the oocyte cytoplasm. g, dictyosomes ; vs, vesicles ; y, yolk inclusions ; n, nucleus ; nm, nuclear membrane.

Fig. 5 : Detail of the oocyte cytoplasm in which ergastoplasmic vesicles (ev) and yolk inclusions (y) are visible.









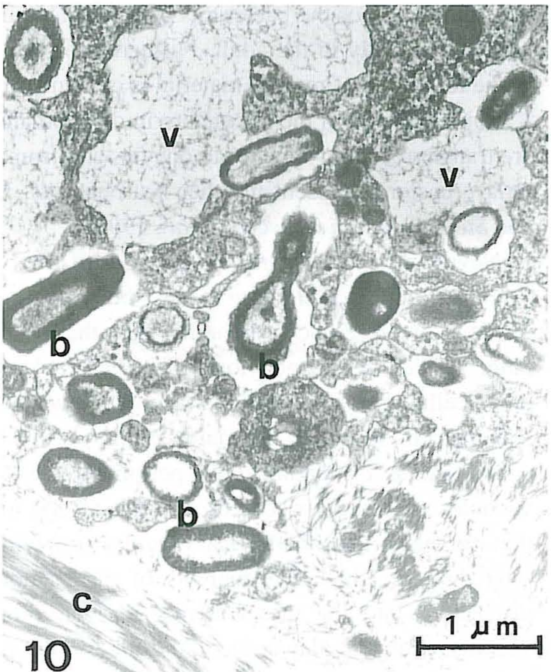


Fig. 6 : Myelin figure (mf) inside the ooplasm. n, nucleus ; nm, nuclear membrane ; vs, vesicles ; y, yolk inclusions.
Fig. 7 : Detail of the oocyte cytoplasm. m, groups of mitochondria ; b, bacteria ; y, yolk inclusions ; vs, vesicles ; ph, phagosomes.
Fig. 8 : Invagination of the plasmic membrane near a bacterium (b). m, mitochondria ; y, yolk inclusions.
Fig. 9 : Detail of the granular cytoplasmic matrix showing different types of yolk inclusions (y). vs, vesicles.
Fig. 10 : Periphery of a mature oocyte in which vacuoles with fibrillar content (v) are visible. b, bacteria ; c, collagen.

In *Geodia cydonium*, in which there are no nurse or follicular cells, as previously observed by Scalera Liaci and Sciscioli (1969), it is probable that the yolk originates partly from autosynthetic processes and partly from phagocytosis of symbiotic bacteria.

This does not exclude other storage methods.

The detachment of vesicles from Golgi dictyosomes, the presence of ergastoplasmic vesicles and a smooth endoplasmic reticulum suggest their participation in the formation of reserve material. This situation has already been pointed out in other Porifera (Diaz *et al.*, 1975 ; Gallissian and Vacelet, 1976 ; Gallissian, 1981 ; Aisenstadt and Korotkova, 1976 ; Sciscioli *et al.*, 1991).

The most interesting aspect of the mature oocyte of *Geodia cydonium* is related to bacteria. The bacteria enter the cell by the process of phagocytosis, but their number increases because of divisions that they undergo after their entry into the oocyte.

While there is information regarding the presence of symbiotic bacteria in the larvae (Lévi & Porte, 1962 ; Boury-Esnault, 1976 ; Lévi & Lévi, 1976 ; Vacelet, 1979 ; Kaye & Reiswig, 1991), the data referring to the presence of such bacteria in the oocyte is poor with the exception of that of Gallissian and Vacelet (1976) on the dictyoceratid *Verongia (= Aplysina)* and that of Sciscioli *et al.* (1989, 1991) on the astrophorids *Erylus discophorus* and *Stelletta grubii*. Gallissian and Vacelet (1976) exclude a trophic function of symbiotic bacteria in the oocyte of *Verongia*, however, we did observe some bacteria in the oocyte of *Geodia cydonium*, that were probably in digestion. Vacelet (1975) thinks it probable that the trophic role of the bacteria is of remarkable importance during embryonal development and subsequently in the adult. Furthermore, we assert that the participation of the bacteria in the formation of reserve material is certainly minimal in the oocyte of *Geodia cydonium*.

Some bacteria in the oocyte are in division and many intact. It can be assumed that they are transferred from sponges to oocytes and from oocytes to embryos. Such a means of bacterial transfer is present in other Astrophorids, *Erylus discophorus* (Sciscioli *et al.*, 1989) and *Stelletta grubii* (Sciscioli *et al.*, 1991).

Nevertheless, as regards the number of the bacteria some differences between *Geodia cydonium* and *Stelletta grubii* must be noted : in *Geodia cydonium* the bacterial amount inside the mature oocyte is higher than that observed in *Stelletta grubii*. Moreover, in this latter species the phagocytosis of bacteria takes place not only in the oocyte but also in other cellular elements. In *Geodia cydonium* this process has less importance since few somatic cells show any internal bacteria.

In *Geodia cydonium* some peripheral areas of the mature oocyte are occupied by vacuoles with fibrillar content. Franzen (1988) in his observations on *Scypha ciliata*, states that the membranes of fibrillar vacuoles fuse with the oocyte membrane producing an opening through which the contents of the vacuole, consisting of collagen precursors, are discharged into the mesenchyme. Furthermore, he believes that the contents of these vacuoles probably play a role in the adhesion of the larva to the substrate.

In our opinion these phenomena may also occur in *Geodia cydonium*. We do not exclude, however, that these vacuolar structures may originate from a process of capture of the collagen surrounding the oocyte. Thus it is probable that the bacteria phagocytosis is accompanied by that of part of the collagen. This hypothesis is supported by the observation of vacuoles containing fibrillar material and bacteria in the peripheral part of the oocyte.

Such vacuoles are also present in *Stelletta grubii* but to a greater extent. The very abundant collagen in *Stelletta grubii* in which it entirely separates the oocyte from the surrounding mesohyl, is not as thick in *Geodia cydonium* and has less numerous fibrils. In *Geodia cydonium* the presence of lophocytes was not observed in the collagen that surrounds the oocyte.

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REFERENCES

AISENSTADT, B. & G.P. KOROTKOVA, 1976. A study of oogenesis in marine sponge *Halisarca dujardini* Johnston (Dendroceratidae, Demospongiae). II. Phagocytic activity of the oocytes and vitellogenesis. *Tsitologiya*, 18 : 818-823.

BOURY-ESNAULT, N., 1976. Ultrastructure de la larve parenchymella d'*Hamigera hamigera* (Schmidt) (Demospongiae, Poeciloscleridae). *Cah. Biol. Mar.*, 27 : 9-20.

DIAZ, J.P., R. CONNES & J. PARIS, 1975. Étude ultrastructurale de l'ovogenèse d'une Démosponge : *Suberites massa* Nardo. *J. Microsc.*, 24 : 105-116.

FRANZEN, W., 1988. Oogenesis and larval development of *Scypha ciliata* (Porifera, Calcarea). *Zoomorphology*, 107 : 349-357.

GAINO, E., 1980. Indagine ultrastrutturale sugli ovociti maturi di *Chondrilla nucula* Schmidt (Porifera, Demospongiae). *Cah. Biol. Mar.*, 21 : 11-22.

GAINO, E., B. BURLANDO & P. BUFFA, 1986. Contribution to the study of egg development and derivation in *Oscarella lobularis* (Porifera, Demospongiae). *Int. J. Invertebr. Reprod.*, 9 : 59-69.

GAINO, E., B. BURLANDO, P. BUFFA & M. SARÀ, 1987. Ultrastructural study of the mature egg of *Tethya citrina* Sarà and Melone (Porifera, Demospongiae). *Gamete Research*, 16 : 259-265.

GALLISSIAN, M.F., 1981. Étude ultrastructurale de l'ovogenèse chez quelques éponges calcaires (Porifera, Calcarea). *Arch. Zool. Exp. Gen.*, 112 : 329-340.

GALLISSIAN, M.F. & J. VACELET, 1976. Ultrastructure de quelques stades de l'ovogenèse de spongiaires du genre *Verongia* (Dictyoceratida). *Ann. Sci. Nat. Zool. Biol. Anim.*, Série 18 : 381-404.

KAYE, H.R., 1991. Sexual reproduction in four Caribbean commercial sponges. II. Oogenesis and transfer of bacterial symbionts. *Int. J. Invertebr. Reprod.*, 19 (1) : 13-24.

KAYE, H.R. & H.M. REISWIG, 1991. Sexual reproduction in four Caribbean commercial sponges. III. Larval behaviour, settlement and metamorphosis. *Int. J. Invertebr. Reprod.*, 19 (1) : 25-35.

LÉVI, C. & P. LÉVI, 1976. Embryogenèse de *Chondrosia reniformis* (Nardo), démosponge ovipare, et transmission de bactéries symbiotiques. *Ann. Sci. Nat. Zool. Biol. Anim.*, 18 : 367-380.

LÉVI, C. & A. PORTE, 1962. Étude au microscope électronique de l'éponge *Oscarella lobularis* Schmidt et de sa larve amphiblastula. *Cah. Biol. Mar.*, 3 : 307-315.

MILLONIG, G., 1976. Laboratory manual of biological electron microscopy. Saviolo Ed Vercelli. 59 p.

SCALERA LIACI, L. & M. SCISCIOLI, 1969. La riproduzione sessuale di alcuni Tetractinellidi (Porifera). *Boll Zool.*, 36 : 61-70.

SCISCIOLI, M., L. SCALERA LIACI, E. LEPORE & M. GHERARDI, 1989. Indagine ultrastrutturale sugli ovociti di *Erylus discophorus* (Schmidt) (Porifera, Tetractinellida). *Oebalia*, 15-2 NS : 939-941.

SCISCIOLI, M., L. SCALERA LIACI, E. LEPORE, M. GHERARDI & T.L. SIMPSON, 1991. Ultrastructural study of the mature egg of the marine sponge *Stelletta grubii* (Porifera Demospongiae). *Molecular Reproduction and Development*, 28 : 346-350.

SALLER, U., 1988. Oogenesis and larval development of *Ephydatia fluviatilis* (Porifera Spongillidae). Zoomorphology, 108 : 23-28.

SIMPSON, T. L., 1984. Gamete, embryo, larval development. In T. L. Simpson (ed), The cell biology of sponges. Springer-Verlag, Berlin, Heidelberg, New York, Tokyo : 341-413.

VACELET, J., 1975. Étude en microscopie électronique de l'association entre bactéries et Spongiaires du genre *Verongia* (Dictyoceratida). *J. Microsc. biol. cell.*, 23 : 271-288.

VACELET, J., 1979. Quelques stades de la reproduction sexuée d'une éponge sphinctozoaire actuelle. In : *C. Lévi and N. Boury-Esnault (eds.), Biologie des Spongiaires, Colloq. Internat. CNRS, 291, CNRS,* Paris : 95-101.