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Cleavage pattern in Oscarella species (Porifera, Demospongiae, Homoscleromorpha): transmission of maternal cells and symbiotic bacteria

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The cleavage and the first stages of development of larvae in five *Oscarella* species, from the north-west Mediterranean, *O. lobularis*, *O. tuberculata*, *O. imperialis*, *O. microlobata* and *Oscarella* sp., are investigated. Eggs are isolecithal, and rich in yolk inclusions. The cleavage is holoblastic, equal and asynchronous. During cleavage, there is no central cavity (blastocoel). The result of cleavage is the formation of a solid morula constituted by equal blastomeres. The polarity of the blastomeres is not expressed prior to the beginning of larva differentiation, i.e. approximately up to 64-cell stage. The superficial membrane of the blastomeres shows numerous filopodia which form blastomere contact. Symbiotic bacteria and cells with inclusions of the maternal mesohyl are present in the intercellular spaces of the embryo from the beginning of cleavage. Whereas maternal symbiotic bacteria are present in the embryo of the five species studied, maternal cells with inclusions are absent from two species (*O. tuberculata* and *O. microlobata*). The most original feature of early development in the genus *Oscarella* is the formation of a coeloblastula larva from a morula due to the progressive migration of the internal cells to the periphery.

KEYWORDS: Cleavage, ultrastructure, maternal cells, symbiotic bacteria, sponge, *Oscarella*, Mediterranean sea.

Introduction

Cleavage is an important stage in animal embryogenesis. Although well known in some phyla (Annelida, Echinodermata etc.), it has been poorly described in others such as Porifera. The pattern of cleavage may be indicative of phylogenetic relationships (Ivanova-Kasas, 1995; Brusca *et al*., 1997) and enhanced knowledge of it in

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different poriferan clades may bring new insights to the phylogenetic history of this controversial phylum (Borchiellini *et al*., 2001).

The members of the subclass Homoscleromorpha (Demospongiae) have been considered as the simplest representatives of the Porifera (Lévi, 1956, 1970) due to their simple anatomic organization. Lévi and Porte (1962) considered *Oscarella* Vosmaer, 1887 as the almost ideal prototype of sponges on the basis of the reduced mesohyl, resulting in the sponge tissue almost entirely made up of two epithelia—choanoderm and pinacoderm—and the absence of skeleton. More recently, however, the presence of both an acrosome in spermatozoids (Baccetti *et al.*, 1986; Boury-Esnault and Jamieson, 1999) and a basal membrane (Boute *et al.*, 1996) points to a higher evolutionary level than that expected from the simple organization in this subclass. Cytological studies have been recently undertaken on Mediterranean species from the genera *Oscarella*, *Corticium* Schmidt, 1862, *Plakina* Schulze, 1880, *Pseudocorticium* Boury-Esnault et al., 1995 and some cell features have been used as diagnostic characters for taxonomy. Symbiotic bacteria have been found in the mesohyl of all species of Homoscleromorpha studied so far and the composition of the symbiotic bacterial population has been shown to be species-specific (Boury-Esnault *et al.*, 1984, 1992, 1995; Muricy *et al.*, 1996, 1999).

By the end of the 19th century the embryology of *Oscarella* had been extensively studied due to the simplicity of the organization which might represent the prototype of development pattern for not only Porifera, but also the whole Metazoa (Giard, 1873; Carter, 1874; Barrois, 1876; Schulze, 1877, 1880, 1881; Metschnikoff, 1879; Heider, 1886). Subsequently, only two studies have been published on the reproduction and the larva of *Oscarella* (Meewis, 1938; Tuzet and Paris, 1964). Ultrastructural studies have been limited to the description of the mature larva (Lévi and Porte, 1962), egg development (Gaino *et al.*, 1986a) and spermatogenesis (Baccetti *et al.*, 1986; Gaino *et al.*, 1986b). The Homoscleromorpha larva has been termed an 'amphiblastula' since the earliest studies although its morphogenesis is not homologous at all to that of the larva of the Calcaronea (Brien, 1967; Borojevic, 1970). To avoid confusion between the two types of larva a new name has recently been proposed for the homoscleromorph larva, i.e. 'cinctoblastula' (Boury-Esnault and Rützler, 1997).

We describe here the cleavage pattern from mature egg to the beginning of larval development and the way by which symbiotic bacteria and somatic cells are transmitted to the egg or the embryos in five species of *Oscarella* through light, transmission and scanning electron microscopy (TEM and SEM).

Materials and methods

Five Mediterranean species of *Oscarella* have been studied, *Oscarella tuberculata* (Schmidt, 1868), *O. lobularis* (Schmidt, 1862), *O. imperialis* Muricy *et al*., 1996, *O. microlobata* Muricy *et al.*, 1996 and *Oscarella* sp. which is probably a new, undescribed species from Medes Islands (NE of Spain).

The sponges were collected by Scuba diving from June to August 1999 and in August 2000, in the western Mediterranean Sea, at depths of 5–25 m (figure 1; table 1).

Pieces of each specimen were fixed *in situ* or immediately after collection. For SEM the fixative used was a mixture of 2% OsO_4 and saturated mercury chloride (5/1) (Johnston and Hildemann, 1982). For TEM a standard fixation method was used: glutaraldehyde 2.5% in a mixture of 0.4 M cacodylate buffer and sea water

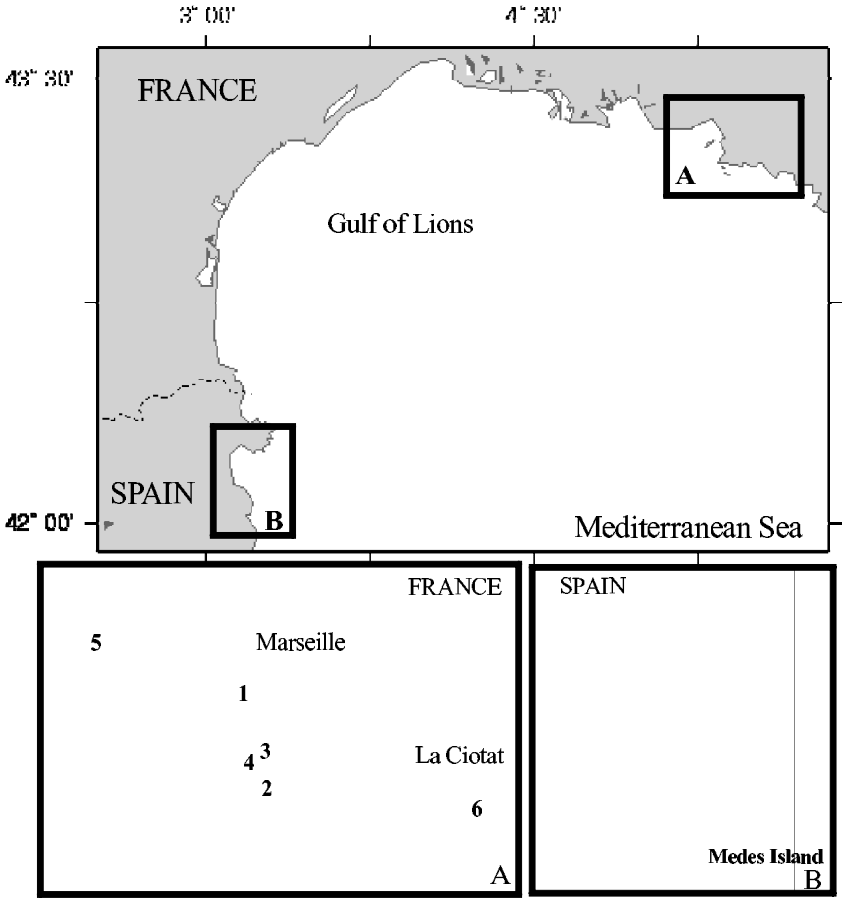


FIG. 1. Map of the north-west Mediterranean coast showing the localities (A and B) of the sites of collection of the five species of *Oscarella*. (A) Details of the Provence coast; 1, Endoume Cave; 2, Riou Archipelago; 3, Jarre Island; 4, Jarre Cave; 5, La Vesse; 6, Cap Fauconnières. (B) Detail of the Catalan coast with the locality of the Medes Islands.

Table 1. Stations and depth of *Oscarella* species collection in the north-west Mediterranean. The numbers refer to the map (figure 1).

Stations/species	*tuberculata*	*lobularis*	*imperialis*	*microlobata*	*Oscarella* sp.
Endoume Cave (1)	5–7 m	–	–		–
Riou Archipelago (2)	8–10 m	22–25 m	–		–
Jarre Island (3)	7–9 m	20–24 m	22–25 m		–
Jarre Cave (4)				10–12 m	
La Vesse (5)	8–13 m	–	–		–
Cap Fauconnières (6)	8–10 m	13–15 m	–		–
Medes Islands	13–17 m	15–17 m	–		15–18 m

(4 vol.: 5 vol.; 1120 mOsm) and post-fixation in 2% OsO_4 in sea water (Boury-Esnault *et al.*, 1984). For light microscopy, specimens were fixed in Bouin and embedded in paraffin. Paraffin sections were stained with Mayer's haematoxylin and

semi-thin sections were stained with toluidine blue. Thin sections, contrasted with uranyl acetate and lead citrate, were observed under a JEM-7 TEM. For SEM, the specimens were fractured in liquid nitrogen, critical-point dried, sputter-coated with gold-palladium, and observed under a Hitachi S570 SEM.

Results

In the five species of *Oscarella* investigated, mature eggs are ovoid and are located in the basal or central parts of the choanosome. Their size varies slightly among the five species (table 2). The nucleus is central, ovoid and contains one or two small nucleoli. The eggs are rich in heterogeneous yolk inclusions of different size (0.7–9 μm in diameter) (figure 2). They are isolecithal although a higher concentration of small yolk granules was observed close to the nucleus (figure 3).

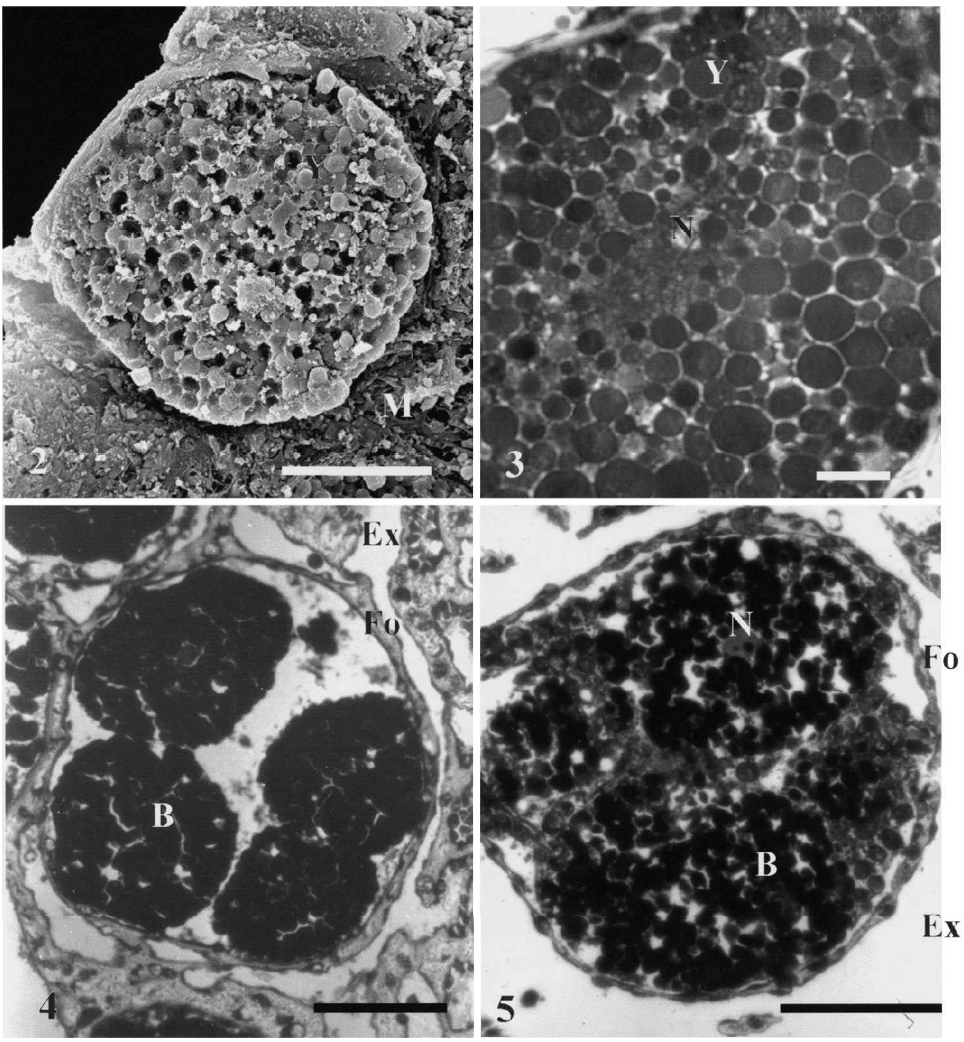
Cleavage is holoblastic, equal and synchronous during the two first divisions. As polar bodies were not found, it is impossible to know the polarity of the first and second furrows of divisions. The first division leads to the formation of two identical blastomeres with a central nucleus. The second division is also equal and synchronous, and appears to be perpendicular to the first one. The four blastomeres are slightly ovoid in shape, and their size is variable. They have a nucleus of about 8 μm in diameter and a nucleolus of 1.6–3.3 μm (table 2). They always form a tetrahedron figure (figures 4, 5). From the third division, cleavage becomes asynchronous (stage 5–6 blastomeres) (figure 6). At this stage, the blastomeres are densely joined to each other, and have a polygonal shape (table 2; figure 7). At the 16-cell stage, the blastomeres show a spherical shape with a central nucleolated nucleus (table 2; figure 8). During the fourth and fifth divisions, the mitotic spindles are perpendicular to the embryo surface. At this stage, a typical morula is formed in all *Oscarella* species studied (figure 9).

Up to the 64-blastomere stage, there is neither embryo polarity nor differentiation of blastomeres. At this stage, the blastomeres are always spherical and rich in heterogeneous yolk granules (table 2) which are irregularly distributed (figures 10–13). Adjacent blastomeres are always linked by filopodia (figure 14). A true cavity of cleavage is absent, although extensive intercellular spaces appear at the end of cleavage in all species (figures 10–12).

Approximately at the 64-cell stage, the beginning of the differentiation of the superficial flagellated layer occurs. The blastomeres close to the surface of the morula divide more actively, their size greatly decreases and they become ovoid. Their nucleus is close to the cell membrane (figures 16, 17). The size of yolk granules in the cytoplasm decreases gradually. The long axis of the cells is perpendicular to the

Table 2. Dimensions of eggs and blastomeres of *Oscarella* species at different stages of cleavage.

	O. tuberculata	*O. lobularis*	*O. imperialis*	*O. microlobata*	*Oscarella* sp.
Eggs	134–146	141–159	150–180		144–149
Blast. stage 4		76			90
Blast. stage 8		62			52
Blast. stage 16		45	37		
Blast. stage 48	35	33			
Blast. stage 64	22	28			

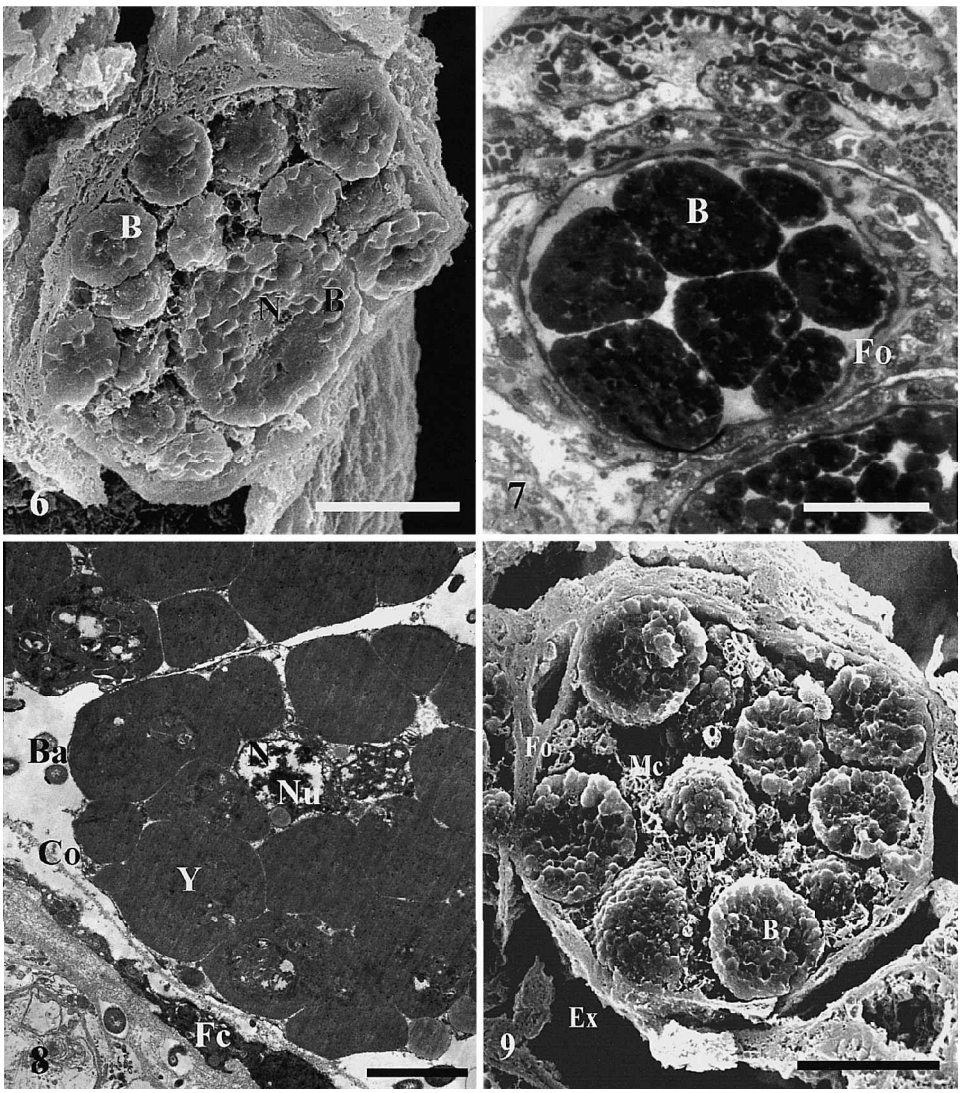


FIGS 2–5. (2) Egg of *O. tuberculata* (SEM). M, mesohyl; Y, yolk granules. Scale bar: 50 μm. (3) Semi-thin section through the central part of the egg *O. imperialis*. N, nucleus; Y, yolk granules. Scale bar: 10 μm. (4) Semi-thin section through the stage four blastomeres (B) of *O. lobularis*. Fo, follicle; Ex, exhalant canal. Scale bar: 50 μm. (5) Semi-thin section through the stage four blastomeres (B) of *Oscarella*. sp. N, nucleus; Fo, follicle; Ex, exhalant canal. Scale bar: 50 μm.

embryo surface. Due to the migration of internal cells to the surface the intercellular spaces turn into a large cavity (figure 15).

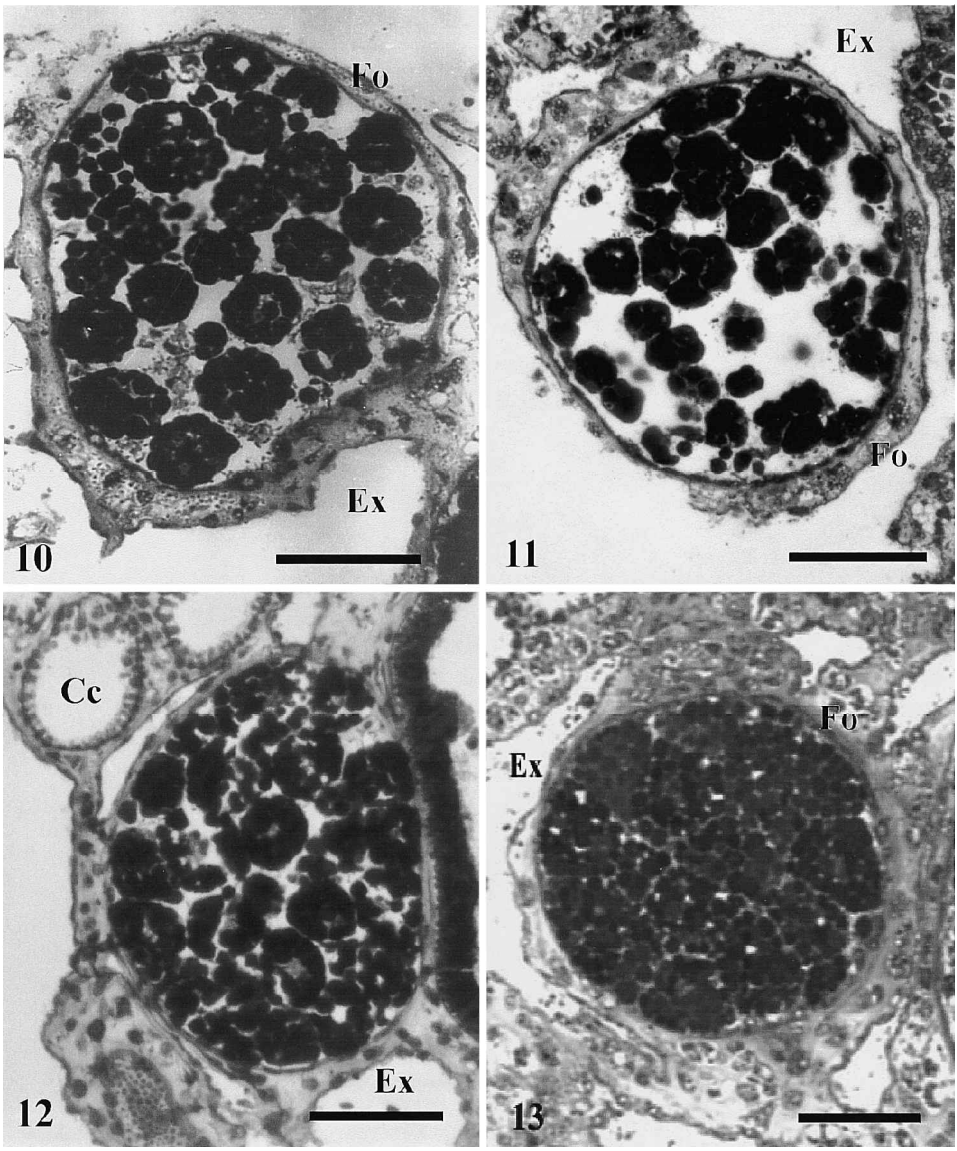
At the late stages of egg vitellogenesis, a follicle formed by endopinacocytes completely surrounds mature eggs and embryos (figure 18). A complex network of filopodia connects mature eggs or embryos and follicle cells (figure 14).

Maternal cells (cells with inclusions) migrate from mesohyl into the space between mature eggs to follicle cells in *O. lobularis*, *O. imperialis* and *Oscarella* sp. The same migration occurs for symbiotic bacteria in the five species studied (figures 9, 18–25). The migrating cells have a structure similar to that of cells of the adult. In *O. lobularis*



FIGS 6–9. (6) Asynchronous cleavage in the embryos of *O. lobularis* at the stage 16 blastomeres (B) (SEM). N, nucleus. Scale bar: 50 μm. (7) Semi-thin section through the cleaving embryos of *O. imperialis* at the stage 10 blastomeres (B). Fo, follicle. Scale bar: 50 μm. (8) Blastomere of the embryo of *O. imperialis* at the stage 16 blastomeres (TEM). N, nucleus; Nu, nucleolus; Y, yolk granule; Ba, symbiotic bacteria; Fc, follicular cells; Co, collagen layer of the follicle. Scale bar: 5 μm. (9) Cleaving embryos of *O. lobularis* at the stage 24 blastomeres (B) (SEM). Fo, follicle; Mc, maternal cells; Ex, exhalant canal. Scale bar: 50 μm.

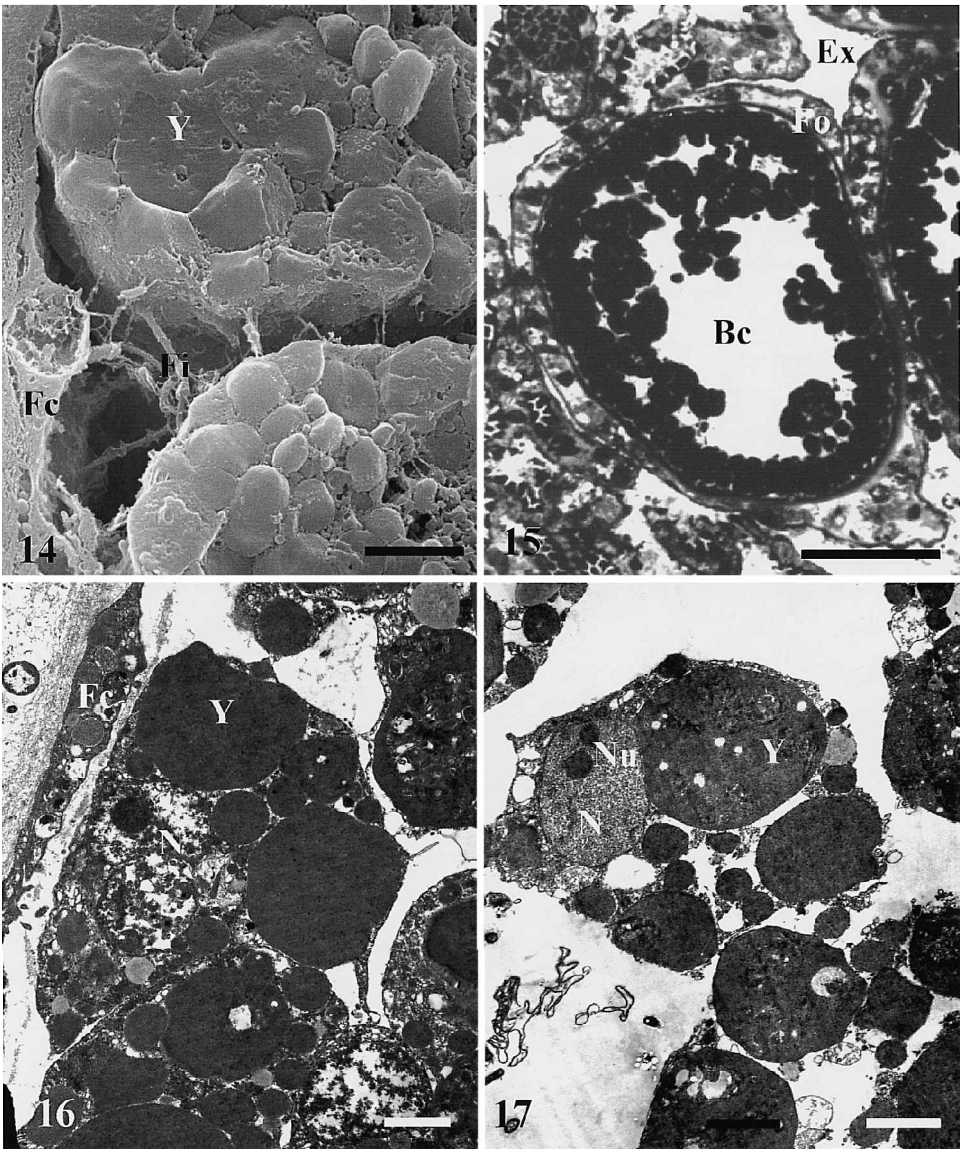
they belong to type I vacuolar cells (figures 19, 20) (Boury-Esnault *et al.*, 1992), in *O. imperialis* to type II spherulous cells (Muricy *et al.*, 1996) and in *Oscarella* sp. they are archaeocytes, vacuolar cells or globular cells. In all species studied, symbiotic bacteria, identical to those present in the mesohyl of the parent, are also present in the space between egg and follicle. They belong to one, two, five and six morphotypes



FIGS 10–13. (10) Semi-thin section through the morula of *O. lobularis*. Fo, follicle; Ex, exhalant canal. Scale bar: 50 μm. (11) Semi-thin section through the morula of *O. imperialis*. Fo, follicle; Ex, exhalant canal. Scale bar: 50 μm. (12) Semi-thin section through the morula of *O. tuberculata*. Cc, choanocyte chamber; Ex, exhalant canal. Scale bar: 50 μm. (13) Semi-thin section through the morula of *O. microlobata*. Fo, follicle; Ex, exhalant canal. Scale bar: 50 μm.

in *O. tuberculata*, *O. imperialis*, *O. lobularis*, *Oscarella* sp. and *O. microlobata*, respectively.

During the cleavage process, maternal cells and symbiotic bacteria penetrate into the intercellular spaces of the embryo (figures 18–23). They are located in the intercellular spaces and between the follicle and embryo. The number of symbiotic bacteria increases due to divisions during embryo development (figure 18). Inside

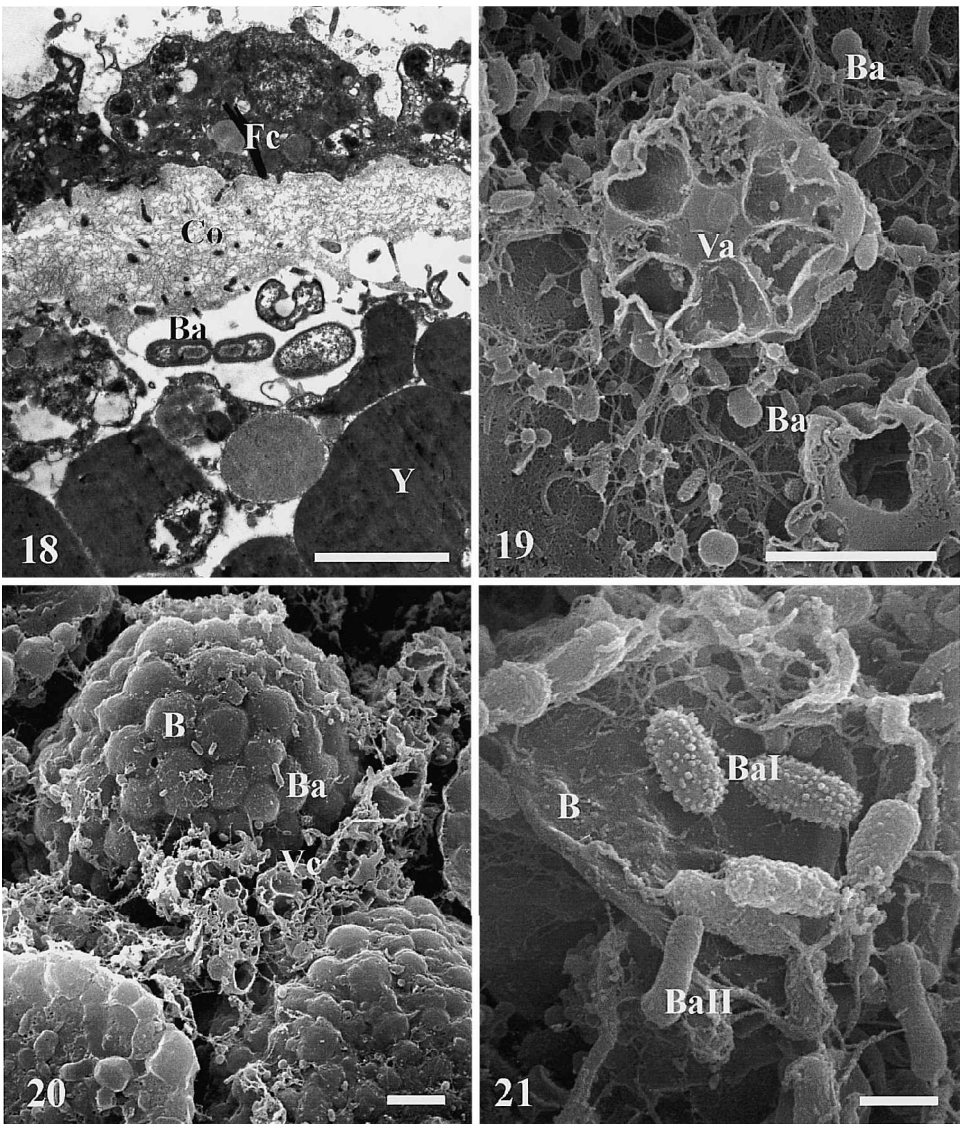


FIGS 14–17. (14) Peripheral blastomeres of the morula of *O. lobularis* (SEM). Fi, filopodia; Y, yolk granules; Fc, follicular cell. Scale bar: 5 μm. (15) Semi-thin section through the morula of *O. tuberculata* during migration of internal cells to the surface of the embryos. Bc, blastocoel; Fo, follicle; Ex, exhalant canal. Scale bar: 50 μm. (16) Peripheral blastomeres of the morula of *O. imperialis* (TEM). N, nucleus; Y, yolk granules; Fc, follicular cell. Scale bar: 2 μm. (17) Internal blastomeres of the morula of *O. lobularis* (TEM). N, nucleus; Nu, nucleolus; Y, yolk granules. Scale bar: 2 μm.

the embryos, the maternal cells and the symbiotic bacteria are included in a complex network of filopodia.

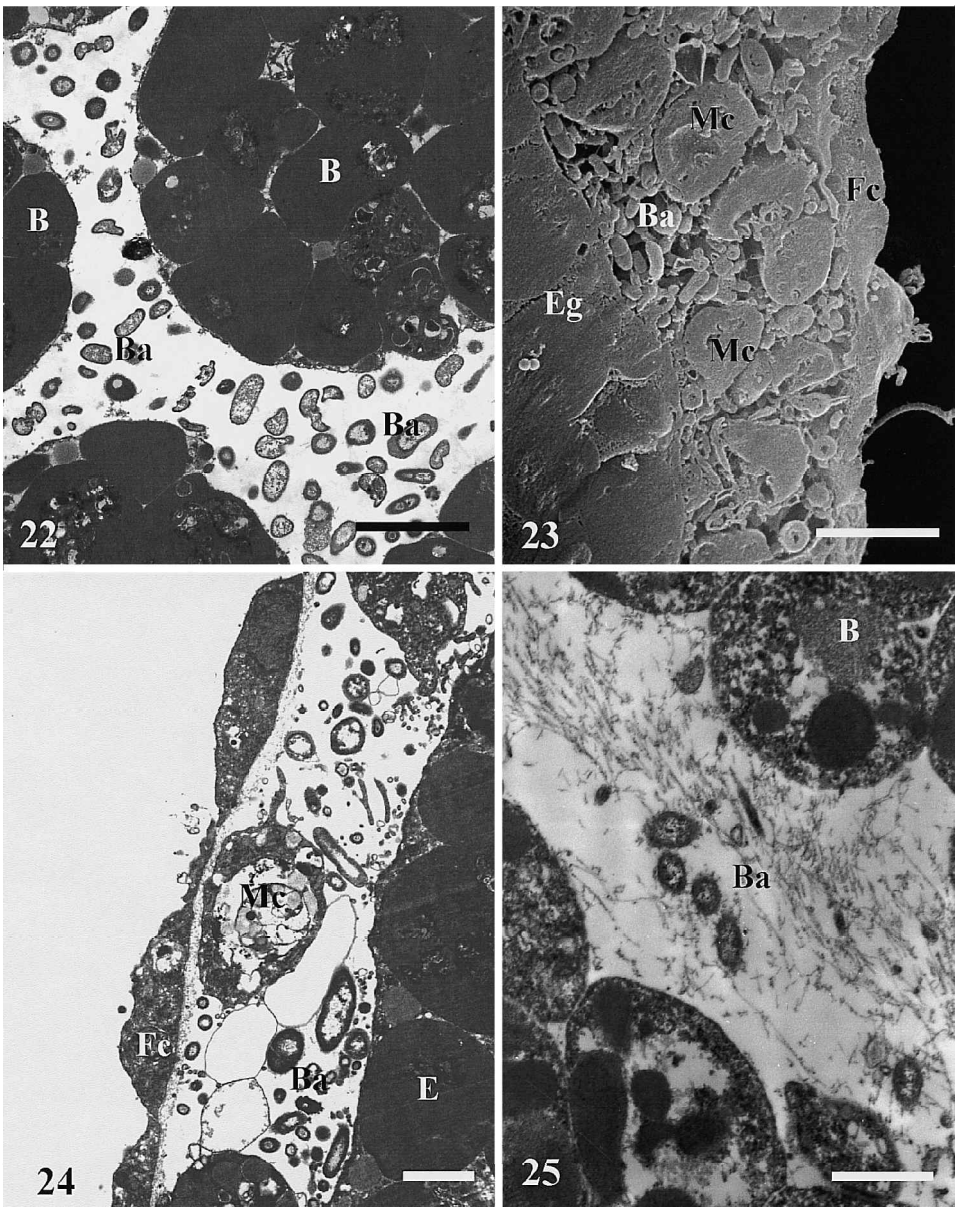
Discussion

The mature eggs of all studied *Oscarella* species were located in the basal parts of the maternal sponges. This is a characteristic localization for all



FIGS 18–21. (18) Follicle of the egg of *O. imperialis* (TEM). Fc, follicular cells; Co, internal layer of collagen; Ba, dividing symbiotic bacteria; Y, yolk granules of the egg. Scale bar: 2 μm. (19) Vacuolar cells type I (Vc) and symbiotic bacteria (Ba) inside the mesohyl of an adult specimen of *O. lobularis* (SEM). Scale bar: 5 μm. (20) Vacuolar cells type I (Vc) and symbiotic bacteria (Ba) inside a cleaving embryos of *O. lobularis* (SEM). B, blastomeres. Scale bar: 5 μm. (21) Symbiotic bacteria of two morphological types (BaI, BaII) inside the cleaving embryos of *O. lobularis* (SEM). B, blastomere. Scale bar: 1 μm.

Homoscleromorpha species studied so far (Barrois, 1876; Schulze, 1877, 1880, 1881; Heider, 1886; Meewis, 1938; Tuzet and Paris, 1964). They are isolecithal as in most sponge species (for a review, see Fell, 1983) and the egg nucleus occupies a central position.



FIGS 22–25. (22) Symbiotic bacteria (Ba) inside the cleaving embryos of *O. imperialis* (TEM). B, blastomeres. Scale bar: 2 μm. (23) Symbiotic bacteria (Ba) and maternal cells (Mc) between the layer of follicular cells (Fc) and the mature egg (Eg) of *Oscarella* sp. (SEM). Scale bar: 5 μm. (24) Symbiotic bacteria (Ba) of different morphotypes and maternal cells (Mc) between the layer of follicular cells (Fc) and the cleaving embryos (E) of *Oscarella* sp. (SEM). Scale bar: 2 μm. (25) Symbiotic bacteria (Ba) of one morphotype inside the morula of *O. tuberculata* (TEM). B, blastomeres. Scale bar: 1 μm.

Cleavage is holoblastic, as in all animals with an isolecithal egg (Brusca *et al.*, 1997).

Cleavage in these five *Oscarella*, as well as in previously studied homoscleromorph species [i.e., *Plakina monolopha* Schulze, 1880 and *Corticium candelabrum* Schmidt, 1862 (Carter, 1874; Barrois, 1876; Schulze, 1877, 1880, 1881; Meewis, 1938; Tuzet and Paris, 1964)], is equal, and asynchronous from the third division, and gives rise to a regular solid morula (stereoblastula). Adjacent blastomeres are in close contact by means of filopodia. These blastomere contacts through filopodia have been suggested to be a type of cell-to-cell adhesion complex in embryos of many animals: mouse (Calarco, 1975), sea-urchin (Schroeder, 1978), octocorals (Benayahu *et al.*, 1989; Dahan and Benayahu, 1998), *Hydra* (Martin *et al.*, 1997), *Amphioxus* (Hirakow and Kajita, 1990) and Porifera [*Halisarca dujardini* Johnston, 1842 (Ereskovsky, personal observations)].

There is no polarity in the embryo until the 64-cell stage. This stage corresponds to the end of the cleavage and the beginning of the differentiation of the larval flagellated epithelium. Extensive intercellular spaces also appeared at the end of the cleavage. This stage corresponds to the beginning of the formation of a coeloblastula larva named 'cinctoblastula' (Boury-Esnault and Rützler, 1997).

The cinctoblastula is formed by the progressive migration of internal morula cells to the periphery and, consequently, the formation of a monolayered larva with a central cavity where maternal cells and symbiotic bacteria remain. This centrifugal migration of cells from the centre to the periphery is unique not only for Porifera, but also for Metazoa. It is a unique case of cell migration from the centre to the periphery of a stereoblastula to form a coeloblastula. This phenomenon is exactly the opposite of centripetal migration, which occurs by multipolar ingression during gastrulation in some cnidarians (Ivanova-Kasas, 1995; Brusca *et al.*, 1997) and might be considered as a multipolar 'egression'.

The embryogenesis of all viviparous sponges takes place in a special embryonic envelope or follicle, which is formed by flattened cells of choanocyte origin, e.g. *H. dujardini* (Korotkova and Apalkova, 1975) and some Calcaronea (Duboscq and Tuzet, 1937), or of pinacocyte and collencyte origin, e.g. *Hymeniacidon heliophila* Parker, 1910 or *Suberites massa* Nardo, 1847 (Simpson, 1984). In the five species of *Oscarella* investigated, the follicle is formed from endopinacocytes.

During early embryogenesis of *Oscarella* species, cells with inclusions and symbiotic bacteria, characteristic of the mesohyl of adult sponges, are incorporated in the embryos, intermingled in the net made by filopodia. The bacteria inside embryos and morula of *Oscarella* species show no signs of digestion, and many of them undergo division. The incorporation of somatic cells and symbiotic bacteria to embryos has been described in a relatively high number of sponge species, and could be more frequent than normally reported in text-books. To date, symbiotic bacteria and/or maternal cells have been found in embryos or larvae of Demospongiae, Calcispongia and Hexactinellida: Demospongiae—*Halisarca nahantensis* Chen, 1976 and *H. dujardini* [Chen, 1976; Korotkova and Ermolina, 1986; Ereskovsky and Gonobobleva, 2000]; *Iophon piceus* (Vosmaer, 1881) [Ereskovsky, 1986]; *Microciona prolifera* (Ellis and Solander, 1786) [Simpson, 1968]; *Vaceletia crypta* (Vacelet, 1977) [Vacelet, 1979]; *Spongia barbara* Duchassaing and Michelotti, 1864, *S. graminea* Hyatt, 1876, *Hippospongia lachne* Laubenfels, 1936 [Kaye, 1991]; *Hemectyon ferox* Duchassaing and Michelotti, 1864 [Reiswig, 1976]; *Cladorhiza* sp. [Vacelet *et al.*, 1995, 1996]; *Alectona wallichii* (Carter, 1874) and *A. mesatlantica* Vacelet, 1999

[Vacelet, 1999]; *Cliona celata* Grant, 1841 [Warburton, 1961]; *Aplysina cavernicola* (Vacelet, 1959) [Vacelet, 1975; Gallissian and Vacelet, 1976]; *Chondrosia reniformis* Nardo, 1847 (Lévi and Lévi, 1976). CALCISPONGIA— *Grantia compressa* (Fabricius, 1780) [Lufty, 1957; Gallissian, 1983]; *Sycon ciliatum* (Fabricius, 1780) [Franzen, 1988]. HEXACTINELLIDA— *Oopsacas minuta* Topsent, 1927 [Boury-Esnault *et al.*, 1999].

Symbiotic bacteria inside larvae of *O. lobularis* were first described by Lévi and Porte (1962). The authors assumed that the symbionts penetrate the larva in the late stages of development. However, our data show that the penetration of symbiotic bacteria through the follicle occurs during late oogenesis and that they penetrate the intercellular cavities of embryos during cleavage. The stage in which the penetration of symbiotic bacteria or maternal cells occurs varies in the different sponge species. It occurs during oogenesis in *Oscarella* species, and in the demosponges *Cliona celata* (Warburton, 1961), *Aplysina cavernicola* (Gallissian and Vacelet, 1976), *Stelletta grubii* Schmidt, 1862 (Sciscioli *et al.*, 1991) and *Chondrosia reniformis* (Lévi and Lévi, 1976), at the beginning of cleavage in *Alectona*, and during late stages of the formation of the larva in *Halisarca* (Sizova and Ereskovsky, 1997; Ereskovsky and Gonobobleva, 2000), Dictyoceratida (Kaye, 1991) and in the Calcispongia *Grantia compressa* (Gallissian, 1983). For the five species studied here, there is always a vertical transfer of the symbiotic bacteria whereas the transmission of somatic cells occurs only in *O. lobularis*, *O. imperialis* and *Oscarella* sp.

From the taxonomic point of view, these observations are consistent with those made on the adult cytology and which have recently led to descriptions of new species (Muricy *et al.*, 1996) and confirm the presence of at least five *Oscarella* species in the Mediterranean Sea. For example, in developing embryos and larvae of *O. tuberculata* the parent cells with inclusions are always absent. On the basis of this feature it is possible to assume that the studies on the development of *Oscarella* (*Halisarca*) *lobularis* by previous authors (Giard, 1873; Carter, 1874; Barrois, 1876; Schulze, 1877; Heider, 1886; Meewis, 1938; Lévi and Porte, 1962), were in fact done on *O. tuberculata*, which has been confused with *O. lobularis* (Boury-Esnault *et al.*, 1992) for over a century.

Vertical mode of symbiont transmission seems to be a general rule for Porifera with symbiotic bacteria. Direct transmission of somatic cells was observed only in three species of the five *Oscarella* studied here. Therefore it is not a generic character but could be used as a discriminating character among *Oscarella* species.

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