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SPONGES WITHOUT SKELETON: A NEW MEDITERRANEAN GENUS OF HOMOSCLEROMORPHA (PORIFERA, DEMOSPONGIAE)

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ABSTRACT

A new genus of aspiculate homoscleromorph, *Pseudocorticium,* is described along with the new species *P. jarrei* found in a littoral cave located in the western Mediterranean Sea (France). The anatomy and cell composition are described and compared to that of species of the other homoscleromorph genus without skeleton, *Oscarella,* and to genera with skeleton, *Corticium* and *Plakina.* The new sponge differs from *Oscarella* in its morphology, anatomy, and cytology. An emended definition of the genus *Oscarella* is proposed. Anatomical and cytological characters carry a high informative content and should be described for a greater number of sponge species.

Keywords: new genus *Pseudocorticium,* cytology, ecology, anatomy, Mediterranean, demosponges.

INTRODUCTION

Porifera is one of the major animal phyla for which the classification is still unresolved at all taxonomic levels. Sponge systematics has traditionally been based mainly on morphological and skeletal characters. These characters long proved to give insufficient information on phylogenetic relationships, generating a search for new, potentially informative taxonomic criteria.

A fruitful period for sponge systematics began in the 1960's, based on an evaluation of the phylogenetic information content of embryological, cytological, ultrastructural, biochemical, and molecular characters at all taxonomic levels (Lévi 1956, Bergquist & Hartman 1969, Bergquist & Hogg 1969, Bergquist et al. 1980, 1984, 1986, Bergquist & Wells 1983, Boury-Esnault et al. 1984, 1990, 1992,

Lawson et al. 1984, Solé-Cava & Thorpe 1987, Hooper et al. 1992, Lafay et al. 1992, Solé-Cava et al. 1992). Not only have such studies demonstrated the great utility of new classes of characters, but most of them have also concluded as to the inadequacy of traditional classification.

In contrast to classes Calcarea and Hexactinellida, where a spicule skeleton is always present, several species of Demospongiae are devoid of a figured skeleton of fibres or spicules. Skeleton-lacking genera *Oscarella* Vosmaer, 1884, *Chondrosia* Nardo, 1833, *Halisarca* Johnston, 1842, *Bajalus* Lendenfeld, 1885 and *Hexadella* Topsent, 1896, still constitute a major problem in sponge classification. On the basis of anatomical, cytological, and reproductive characters, *Chondrosia* has been included in the family Chondrosiidae Topsent, 1895, with unresolved relationships within Tetractinomorpha, along with *Chondrilla* Schmidt, 1862, *Chondrillastra* Topsent, 1918 and *Thymosia* Topsent, 1895. The characters of the aquiferous system of *Hexadella* are those of the family Aplysillidae (Ceractinomorpha, Dendroceratida) Topsent, 1896. *Bajalus* is classified in the family Ianthellidae (Verongida) Hyatt, 1875 (Bergquist, 1980). *Halisarca* is the only genus of the family Halisarcidae, which is considered as incertae sedis within Ceractinomorpha (Bergquist 1980, Boury-Esnault et al. 1990).

The subclass Homoscleromorpha, containing very simple "primitive" sponges that may consist of little more than the two fundamental layers of pinacocytes and choanocytes (Lévi 1970), includes species without skeleton that have been isolated in *Oscarella,* family Oscarellidae. The type species of *Oscarella, O. lobularis* (Schmidt, 1862), is well-characterized among the other homoscleromorphs not only by the absence of skeleton but also by the morphology and by the organization of the aquiferous system.

The subclass Homoscleromorpha has been divided into two families: 1) Oscarellidae Lendenfeld, 1887 without skeleton and including two genera (*Oscarella* and the dubious *Octavella* Tuzet and Paris, 1963); and 2) Plakinidae Schulze, 1880 with spicule skeleton and including such genera as *Plakina* Schulze, 1880; *Corticium* Schmidt, 1862; *Plakortis* Schulze, 1880; *Plakinastrella* Schulze, 1880; and *Placinolopha* Topsent, 1897.

We have found a new Mediterranean homoscleromorph, devoid of spiculate skeleton but displaying other characters of the family Plakinidae. This sponge is so similar in external aspect to the plakinid *Corticium candelabrum* that, before histological studies, it was wrongly interpreted as an aspiculate morph in cave habitats of this species common outside caves. In a previous paper (Solé-Cava et al. 1992), the genetic divergence between homoscleromorphs with and without skeleton was assessed using enzyme electrophoresis. The allozyme pattern of the undescribed aspiculate species was compared to two species of the aspiculate genus *Oscarella* and to the closely related spiculate species *Corticium candelabrum* Schmidt, 1862. Despite the absence of skeleton in both *Oscarella* and the new species, the genetic identity of the new species was found to be closer to *C. candelabrum*

than to both *Oscarella* species (Solé-Cava et al. 1992). Consequently, the two families Plakinidae and Oscarellidae, distinguished only by the presence or absence of skeleton, have been synonymized as Plakinidae as previously suggested by Lévi (1953). More information is clearly needed to clarify the relationships between these species.

Diaz and van Soest (1994) pointed out that a phylogenetic analysis of the homoscleromorphs is presently hampered by two main problems (i) there is restricted knowledge on the less common plakinid genera such as *Plakinastrella* and *Placinolopha;* (ii) outgroup relationships of homoscleromorphs are still uncertain, especially in view of the widely debated ordinal and subclass classification of the Demospongiae. We thus refrain from making a cladistic analysis of the Plakinidae until more information is available. The morphology, anatomy, cytology, and ultrastructure of the new homoscleromorph without skeleton are described and compared to closely related species of the genera *Oscarella* (Boury-Esnault et al. 1984, 1992), *Plakina* (Donadey 1979) and *Corticium* (Boury-Esnault et al. 1984) in order to answer two questions: Are the allozyme patterns supported by cytological and anatomical differences? Does a clear generic distinction exist between the new species and *Oscarella?*

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MATERIAL AND METHODS

Samples of the new aspiculate species were collected by means of scuba diving from 1984 to 1993 in the Riou Archipelago (Marseille, France) (Fig. 1), from a large population found in the darkest part of the Jarre cave about 50 m from the entrance and at a depth of 15 m (Fig. 2).

For cytological work, 15 specimens collected at different periods of the year were fixed as previously described (Boury-Esnault et al. 1984): glutaraldehyde 2.5% in a mixture of 0.4M cacodylate buffer and sea water (4 vol.: 5 vol.; 1120 mOsm) and postfixed in 2% osmium tetroxide in sea water. For light and transmission electron microscopy (TEM) the specimens were embedded in Araldite. Semi-thin sections were stained with toluidine blue. Thin sections, contrasted with uranyl acetate and lead citrate, were observed under a transmission electron microscope (Hitachi Hu 600). Critical-point-dried specimens were observed under a scanning electron microscope after cryofracture in liquid nitrogen and sputter-coating with gold-palladium. Chamber volume and number of choanocytes per chamber were estimated by the indirect method of Rasmont & Rozenfeld (1981).

RESULTS

Systematics

Class Demospongiae Sollas, 1885; Subclass Homoscleromorpha Lévi, 1973; Order Homosclerophorida Dendy, 1905; Family Plakinidae Schulze, 1880.

Diagnosis. "Demospongiae with a skeleton, if present, formed by a combination of relatively small calthrops and/or derivatives (diods and triods), generally arranged uniformly in the sponge body, usually surrounding the aquiferous system in a very regular "alveolar way". Choanocyte chambers with 300-500 choanocytes, usually eurypilous, occasionally aphodal" (Diaz & van Soest 1994). Larvae are of a unique type (cinctoblastulae) (Borojevic et al. in press).

Pseudocorticium new genus

Diagnosis. Plakinidae without skeleton, with a well-developed ectosome, a leuconoid organization of the aquiferous system, diplodal choanocyte chambers. Proportion of mesohyl to chambers greater than 2:1.

Pseudocorticium jarrei new species

Holotype: Museum national d'Histoire naturelle de Paris, MNHN-NBE-94.1. Paratypes: Universidade federal do Rio de Janeiro, UFRJ-POR T-5 and T-6.

Derivatio nominis: the genus name is due to external similarity with *Corticium candelabrum.* The species name is derived from the Jarre Island, located in the Archipelago of Riou (Marseille, Fig. 1), on which is located the cave where the sponge lives.

Description

Morphology. The sponge is thickly encrusting to lobate (Figs 3, 4). Lobes can reach 12 cm in length and 2 cm in diameter, and hang down from the walls and the ceiling of the cave. The surface of the base is 3-10 cm wide and 0.5-3 cm thick. Colour in vivo is cream (colours 3A4, 4A3 and 5A2 in Methuen Handbook of Colour (Kornerup & Wanscher 1978)); in alcohol it varies from yellowish white to brownish grey (colours 1A2, 2A2, 4B4, 7B2 and 7C2). Pigment is present only in the choanosome. Consistency is firm and cartilaginous.

The surface is smooth and slippery but corrugated, folded with irregular depressions 0.1-1 cm long by 1-2 mm deep (Fig. 4). Abundant inhalant ostia 10-20 μm in diameter are located at the free surface or inside the depressions. Surface 2- to 5-mm-diameter exhalant canals lead to 0.5- to 1-cm diameter oscula. Oscula are located at the extremity or on the sides of the lobes, 0.5 cm high above the sur-

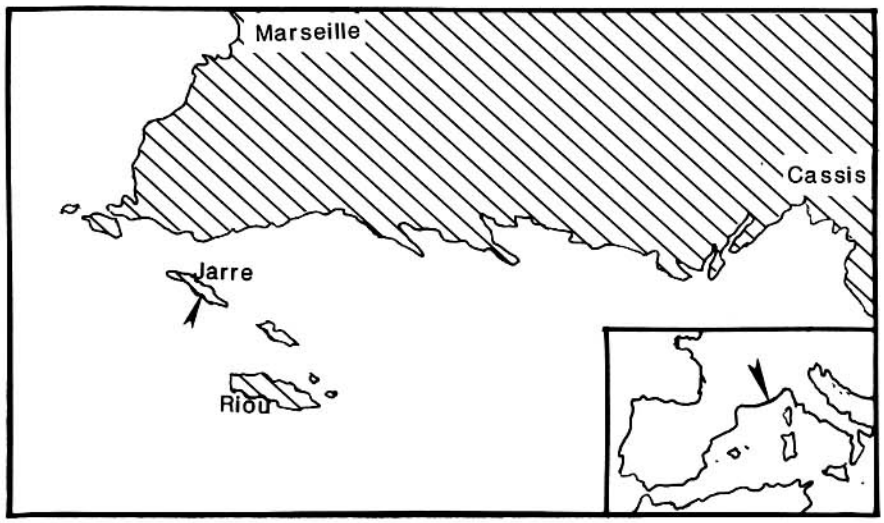

Fig. 1. Map of the region between Marseille and Cassis with the location of the Riou Archipelago and Jarre Island. Arrow indicates the Jarre cave. The inset shows the location of Marseille on the northern coast of the Mediterranean.

face, and are surrounded by a slightly transparent oscular rim. Within the lobes there is usually a central exhalant canal 1-3 mm in diameter.

General organisation. The ectosome is 50 to 350 μm thick (Fig. 5), and externally limited by exopinacocytes. It is composed of a dense, homogeneous collagenous matrix, with spherulous cells, abundant collencytes, and scarce bacteria.

Within the choanosome, the choanocyte chambers are ovoid to irregular, diplodal (Fig. 6), and 25-*42*-60 μm in diameter. An estimated volume of up to 90,000 μm^3 has been calculated, with about 300 choanocytes per chamber. The inhalant canals ramify into prosodi 3-12 μm in diameter and 10-45 μm long. Water enters the choanocyte chambers through one to three prosodi, each terminating in 3- to 12-μm-diameter prosopyles, and leaves the chambers through an 11- to 25-μm-wide apopyle (Fig. 7), surrounded by apopylar cells and leading to an aphodus 20 to 40 μm long. In light microscopy, the choanosomal mesohyl

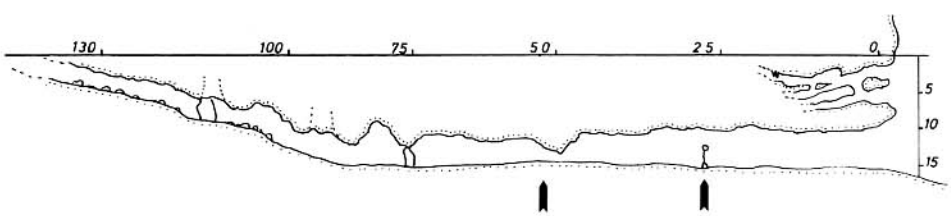

Fig. 2. Jarre Island cave seen in vertical section. The population of *Pseudocorticium jarrei* appears at 25 m from the entrance and reaches its highest density at 50 m (between vertical arrows).

has a granular appearance due to the presence of abundant bacteria; it also contains collencytes, archaeocytes and cells with inclusions. Choanocyte chambers and canals are surrounded by a clear, 1- to 6-μm-thick, relatively bacteria-free layer. A dense 20-nm-thick layer of fibrils forms a basement-membrane-like layer lining both choanoderm and pinacoderm. Among different individuals, the proportion in volume of mesohyl to choanocyte chambers in the choanosome varies between 2.2 and 2.8: 1. Spicule and fibre skeleton are absent.

Cytology. Choanocytes are truncated to pyramidal, 3-6 μm high, and 3-5 μm wide. In TEM after fixation with glutaraldehyde/OsO_4, they are in contact only at their base, the rest of the cells being separated by a space of approximately 0.6 μm. In SEM after fixation in a mixture of OsO_4-$HgCl_2$ the choanocytes appear cylindrical and are in contact all along the lateral sides. Choanocytes lie on the basement-membrane-like layer or are anchored in the mesohyl by basal 0.2 μm-long pseudopodia (Figs 7-8). The 4.5-μm-high, 2.5-μm-wide collar is composed of 25-28 microvilli. The nucleolated 2-μm-diameter nucleus is apical. A double centriole is located at the base of the flagellum and a Golgi apparatus is observed near the nucleus. The cytoplasm also contains mitochondria, 0.7-μm-diameter phagosomes, and very often a 1- to 2-μm vacuole with fibrillar inclusions.

Apopylar cells in sections are triangular, up to 2.5-3.0 μm long with a 1.5-μm-long ovoid nucleus. The cytoplasm contains more or less numerous osmiophilic 0.4 μm-diameter inclusions. They form a ring around the apopyle (Figs 6-7), connecting choanocytes and apopinacocytes.

Exopinacocytes (Fig. 9) are irregular, about 8 μm high and 10 μm wide, with a short flagellum. The nucleolated 2- to 3-μm-diameter nucleus is located in the basal part of the cell, which is deeply inserted in the mesohyl by pseudopodia. The shape of the cells is intermediate between flat and T-shaped pinacocytes. The cytoplasm contains about 10 phagosomes (1 μm in diameter), and 5-10 homogeneous osmiophilic inclusions (0.5-1.5 μm in diameter).

Endopinacocytes (Fig. 10) are fusiform or oval, about 5 by 10 μm, with a nucleolated 2.5-μm-diameter nucleus. On the canal surface, they have a 5-μm-

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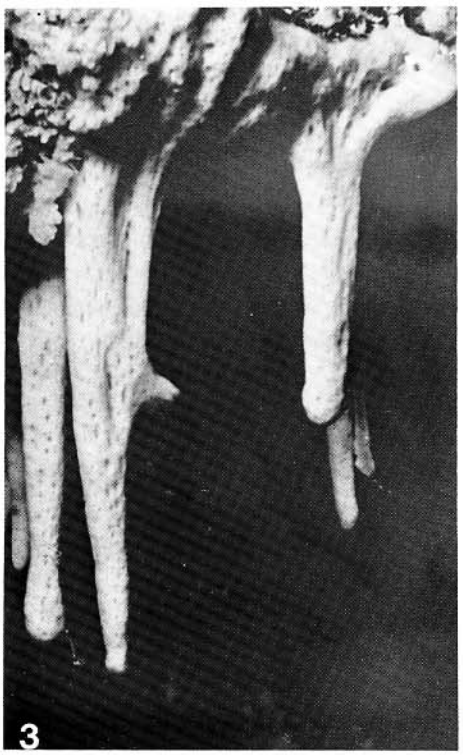
Fig. 3. *Pseudocorticium jarrei* population hanging from the ceiling of the Jarre Island cave. The length of the specimens approximates 6-12 cm. Fig. 4. In situ close-up showing exhalant canals leading to an osculum and the inhalant depressions of the surface. Scale = 1 cm. Fig. 5. Transverse section through the cortex and the choanosome. An inhalant canal and an inhalant ostiole are visible. SEM, scale = 150 μm. Fig. 6. Semi-thin section of the choanosome showing the diplodal arrangement of the choanocyte chamber, scale = 30 μm. Abbreviations: A: apopyle; Ac: apopylar cell; Ap: aphodus; Ba: bacteria type a; Bb: bacteria type b; Bc: bacteria type c; Bd: bacteria type d; Be: bacteria type e; C: choanocyte chamber; CH: choanosome; ch: choanocyte; Co: cortex; Ex: exhalant canal; F: flagellum; G: Golgi apparatus; h: heterogeneous inclusion, i: osmiophilic inclusion; Id: inhalant depression; In: inhalant canal; M: mesohyl; m: mitochondria; mv: microvilli; N: nucleus; Nu: nucleolus; O: ostiole; Os: osculum; P: prosodus; Pa: paracrystalline inclusion; Pi: pinacocyte; S: surface; V: vacuole. Arrow: basement-membrane-like layer.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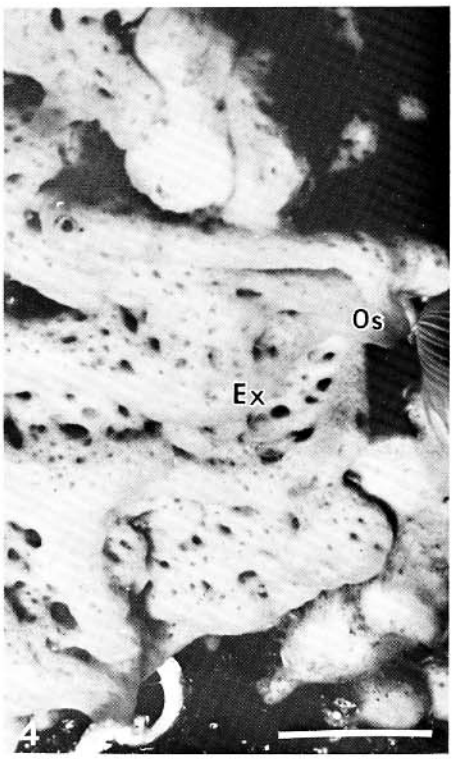
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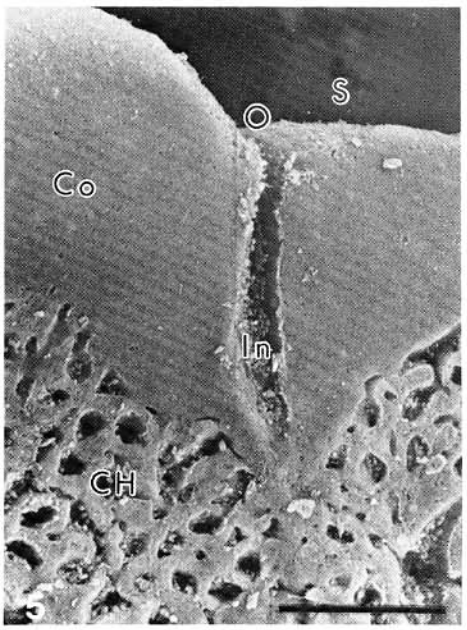
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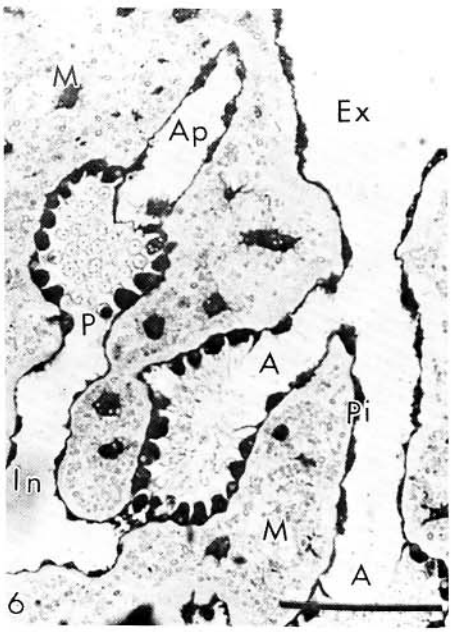
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long flagellum, and on the basal surface they may be anchored in the mesohyl by pseudopodia. The cytoplasm contains the same inclusions as the exopinacocytes. The free surface of the endopinacocytes is covered by a thin (0.2-0.5 μm thick) glycocalyx layer. The apopinacocytes bear small microvilli in addition to the flagellum.

Abundant star-shaped cells (Fig. 11) are dispersed in the mesohyl. They are amoeboid cells 6-13 μm long with a nucleolated 2- to 3-μm diameter nucleus and long, thin filopodia. Numerous homogeneous, osmiophilic cytoplasmic inclusions, 0.5-1.5 μm in diameter, are present. These cells are very active, with clearly visible mitochondria, well-developed ergastoplasm, and Golgi apparatus, as well as 1- to 1.5-μm-diameter phagosomes. They often phagocytose symbiotic bacteria, thus displaying the function of both collencytes and archaeocytes.

Four different types of cells with inclusions are distinguishable within the mesohyl:

• Type 1: ovoid (12-18 μm) spherulous cells (Fig. 12) with nucleolated 2-μm-diameter nucleus. The cytoplasm is filled with inclusions up to 4 μm in diameter. TEM shows these inclusions to be made up of an osmiophilic electron-dense matrix in which are embedded paracrystalline elements up to 0.2-0.3 by 1.3 μm, which show a transverse banding pattern with dark 90-nm-wide bands separated by clear 20-nm-wide bands and a longitudinal striation of 20 nm. In cross sections, the paracrystalline elements are organized in spiral lines. In the mature stage, both matrix and membrane may disappear, resulting in the dispersion of the paracrystalline content in the cytoplasm.

• Type 2: ovoid to irregular (7-10 μm) cells with a 1.5-μm-diameter nucleus and irregular homogeneous inclusions (Fig. 13). An active dictyosome is well-developed within the cytoplasm and near the nucleus. The young cells are in fact composed of more than 50 small spherical inclusions (0.3-0.6 μm), which have a tendency to fuse together to give irregular >4-μm-long inclusions. The inclusions may either be dispersed in the cytoplasm or form a ring around the nucleus. In some senescent cells, 0.2-μm-long, 30-nm-diameter rods are dispersed in the cytoplasm. After the release of the inclusions within the mesohyl, the cell (Fig. 14) is reduced to an homogeneous ring of cytoplasm with a nucleus and an active dictyosome.

• Type 3: ovoid cells, 6-12 μm long (Fig. 15), with nucleolated 2 μm-diameter nucleus. They contain about 20 osmiophilic 1.8-μm-diameter inclusions and 1 to 6 clear vacuoles 3-4 μm in diameter that reduce the cytoplasm to thin strands. They are located mainly in the ectosome, close to the surface or canals.

• Type 4: ovoid cells (Fig. 16), about 5 by 9 μm, with a 2.5-μm-diameter nucleus. The cytoplasm is filled by a single, large (5 μm) osmiophilic inclusion and several small ones 0.6 μm in diameter. The ergastoplasm is very active and seems to secrete these small inclusions. Mitochondria are highly visible.

Bacteria (Figs 17-18) are abundant in the choanosome, with at least five differ-

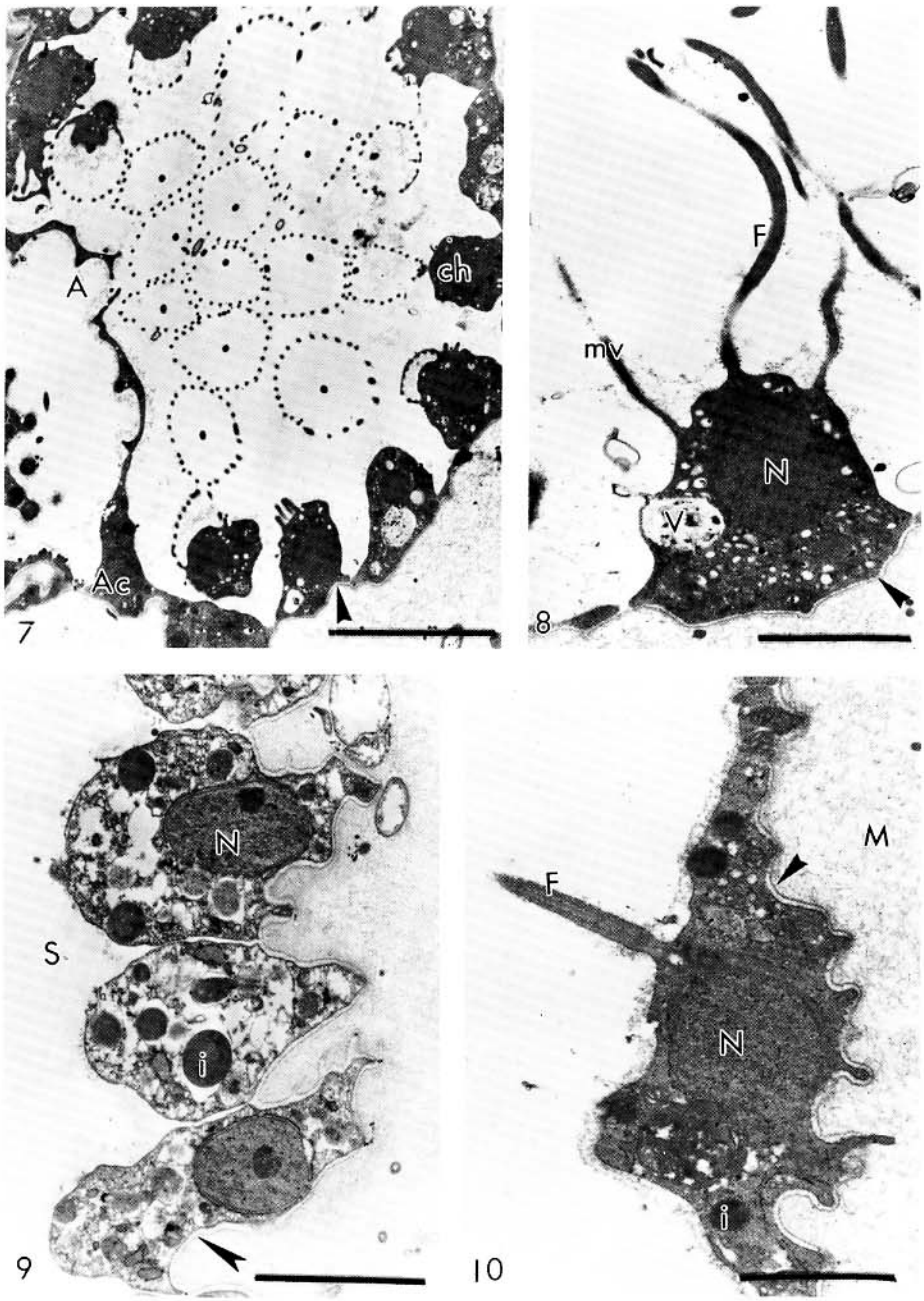
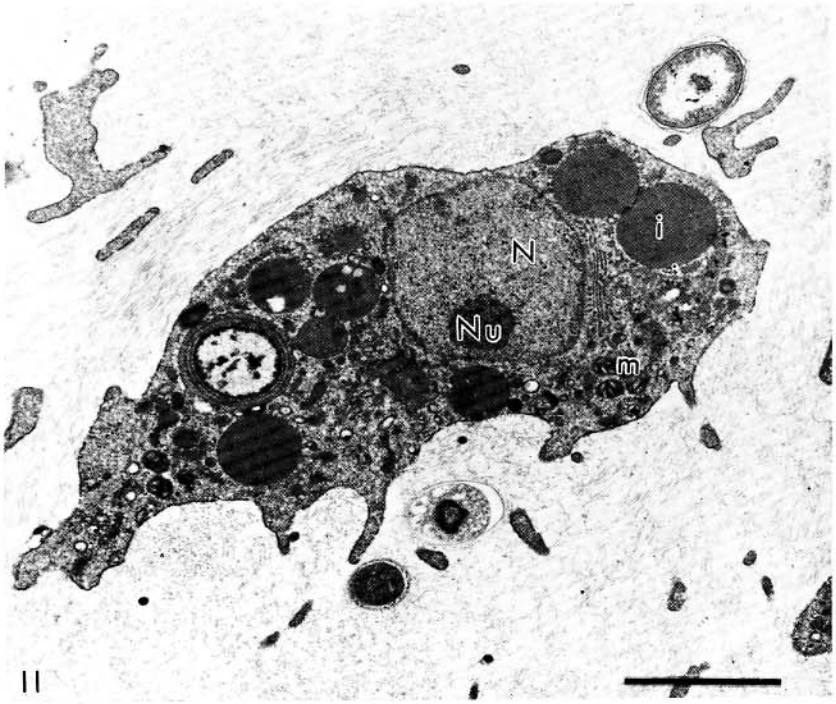


Fig. 7. Choanocyte chamber with the apopyle. TEM, scale = 5.5 μm. Fig. 8. Detail of a choanocyte. TEM, scale = 2.3 μm. Fig. 9. Exopinacocytes. TEM, scale = 2.2 μm. Fig. 10. Endopinacocyte. TEM, scale = 1.6 μm. Abbreviations: see Figs 3-6.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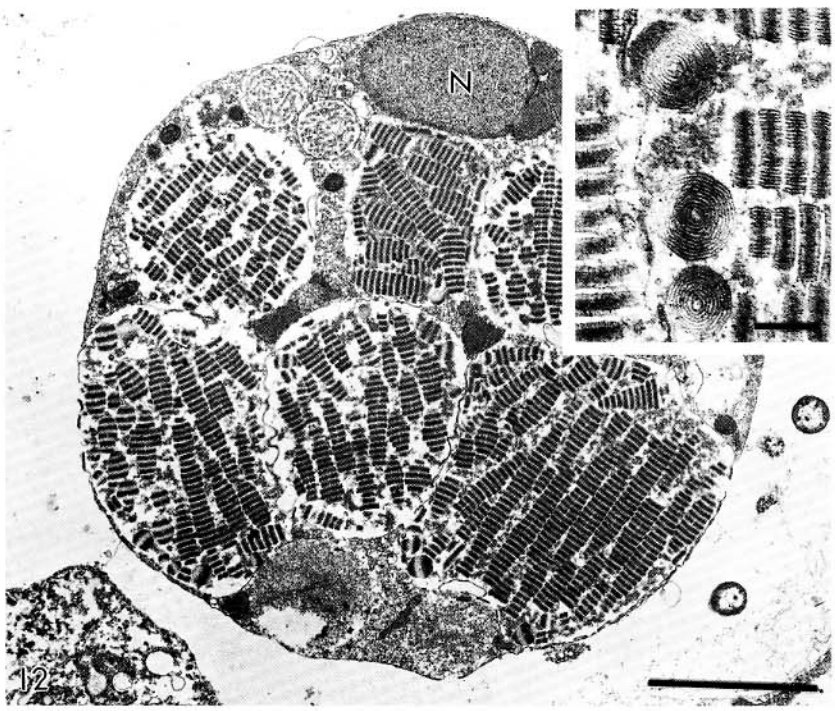
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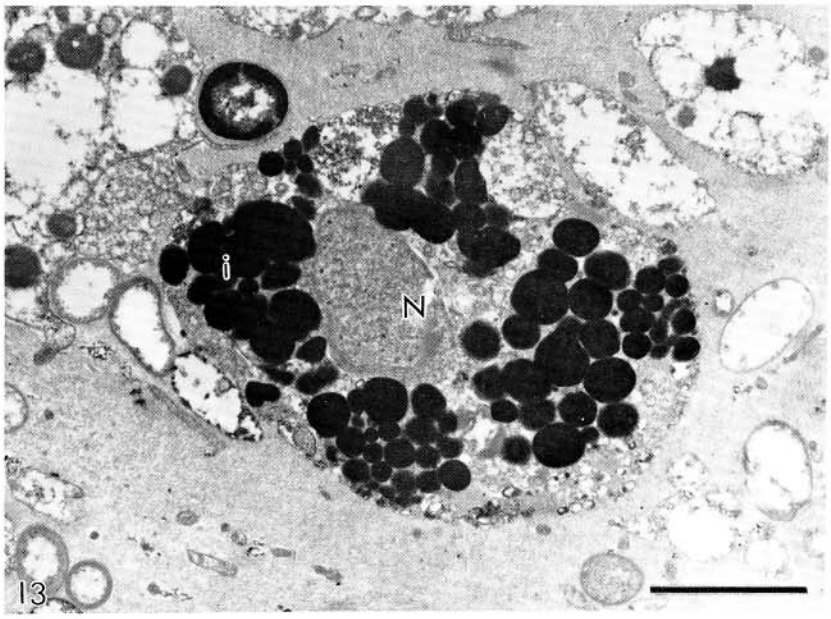


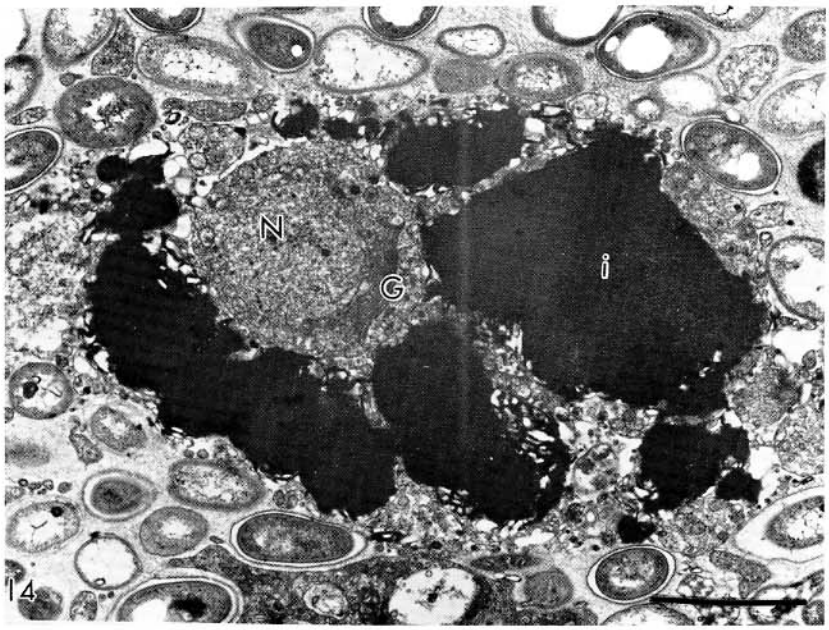
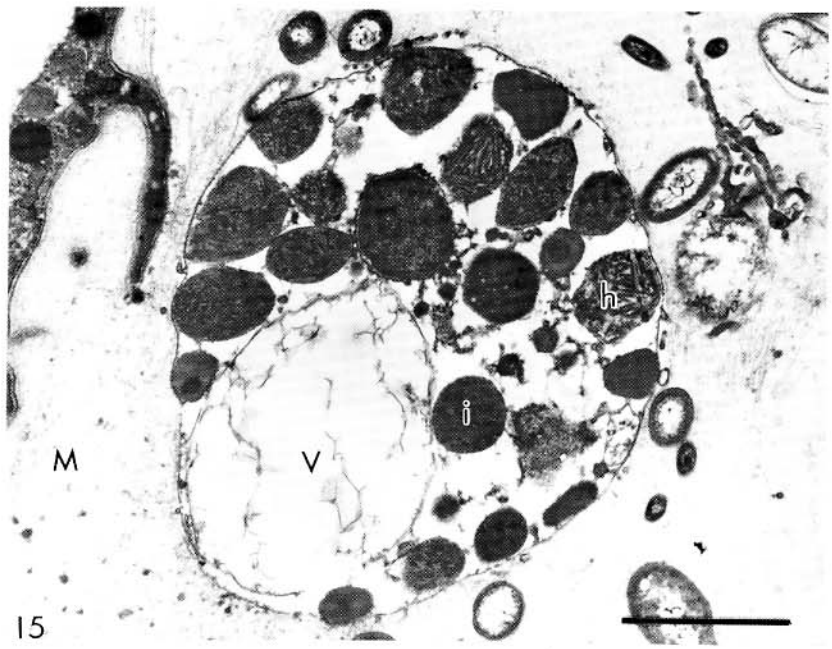


Fig. 13. Cell-type 2 with osmiophilic inclusions. TEM, scale = 2.2 μm. Fig. 14. Senescent cell-type 2. TEM, scale = 1.7 μm. Abbreviations: see Figs 3-6.

← Fig. 11. Star-shaped cell. TEM, scale = 1.9 μm. Fig. 12. Cell-type 1 with paracrystalline inclusions, scale = 1.8 μm. Inset: detail of paracrystalline inclusions. TEM, scale = 0.3 μm. Abbreviations: see Figs 3-6.



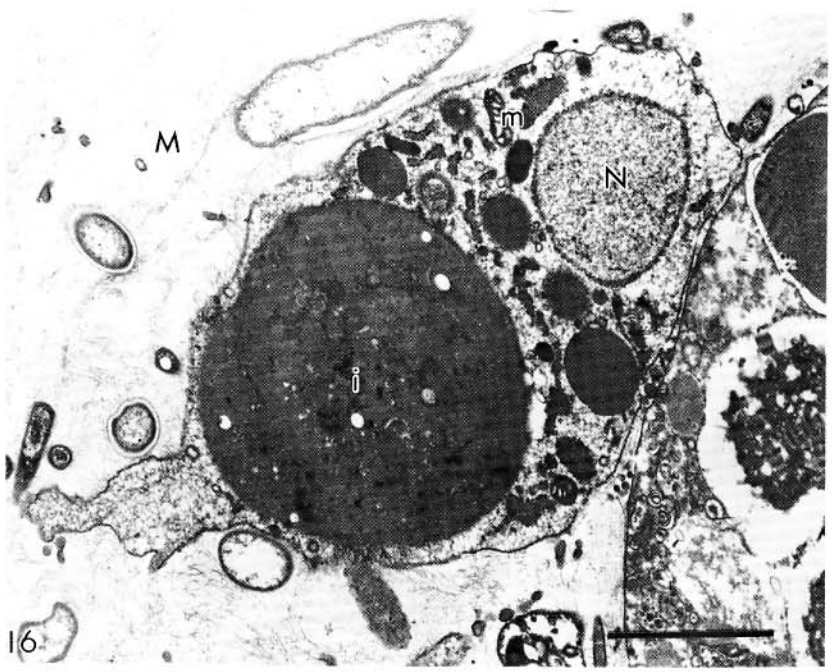


Fig. 15. Cell-type 3 with inclusions and vacuoles. TEM, scale = 2.3 μm. Fig. 16. Cell-type 4 with one large inclusion. TEM, scale = 1.9 μm. Abbreviations: see Figs 3-6.

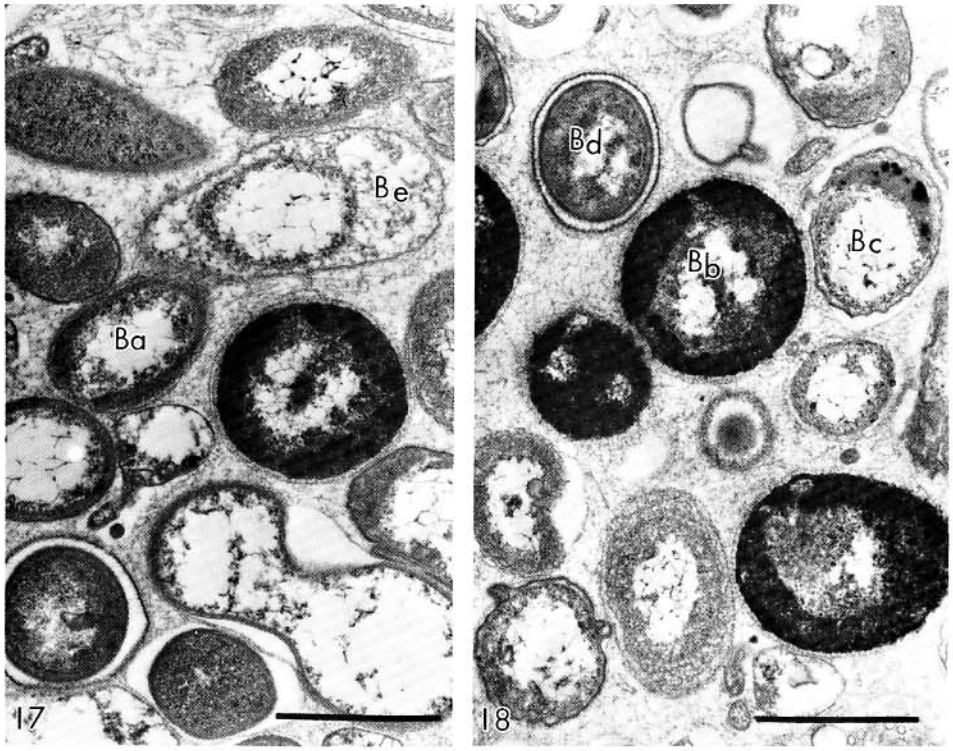


Fig. 17. Bacteria. types a and e. TEM, scale = 0.8 μm. Fig. 18. Bacteria. types b, c, d. TEM, scale = 0.9 μm. Abbreviations: see Figs 3-6.

ent morphological types. They are rare or absent both in the ectosome and in thin areas (1-6 μm) immediately adjacent to choanocytes and pinacocytes, and are in direct contact with the collagen fibrils of the mesohyl.

• Type a: bacteria 1-1.5 by 0.6-0.7 μm, with a 7-nm-thick cytoplasmic membrane, a zone interpreted as periplasm and varying from 50 to 100 nm in thickness, and two dense external zones separated by a 7-nm clear zone. The cytoplasm is less dense than the periplasm and contains numerous ribosomes.

• Type b: ovoid bacteria, about 0.8 by 1.7 μm and well-characterized by the dense aspect of the zone interpreted as periplasm. The cell wall is made up of two double membranes separated by a 22-nm clear space; the outer membranes are about 11 nm thick and the denser inner membranes 17 nm thick. The two layers of the inner membranes are separated by an irregular osmiophilic zone interpreted as a periplasm, as much as 220 nm thick in some places. The innermost membrane is interpreted as the cytoplasmic membrane. The cytoplasm, 170-300 nm thick, contains osmiophilic ribosomes. The nuclear region has a very low density and a diameter of about 400 nm.

• Type c: bacteria 0.7-0.8 by 1-1.5 μm, characterized by a crescent-shaped zone, about 170 nm thick at the widest part, that is interpreted as periplasm. They are surrounded by an irregular, wavy, 22-nm-thick double membrane, separated from another double 9-nm membrane by a clear, 20- to 40-nm-thick zone. The crescent-shaped periplasm is found between the inner double and the cytoplasmic membranes. Osmiophilic inclusions 20-40 nm in diameter are often visible in the periplasm. The cytoplasm, the thickness of which varies from 65 to 130 nm, contains numerous ribosomes. The nuclear zone is about 0.5 μm in diameter.

• Type d: bacteria 0.7-0.8 by 1-1.6 μm, characterized by the radial aspect of the wall. From outside to inside, the wall is made up of: 1) a very thin outer membrane; 2) a 9-nm-thick and electron-dense inner one; 3) a clear space, 43-87 nm thick and crossed by radial structures; and 4) three 22-nm-thick membranes, the innermost being interpreted as the cytoplasmic membrane. The cytoplasm is well-developed and the nuclear zone considerably reduced.

• Type e: ovoid bacteria, 0.7-1.1 by 0.9-1.6 μm, enveloped by a double external membrane. The zone interpreted as periplasm is from 80 to 400 nm thick and made of loose fibrillar material. The off-centred spherical cytoplasm and the nuclear zone both have a diameter of about 0.4-0.7 μm. The 55-nm-thick cytoplasm is denser than the periplasm.

Reproduction. Reproductive elements (ovocytes, embryos, and spermatic cysts) were found in specimens collected from June to November, and will be described in a further paper. The sponge is a simultaneous hermaphrodite that incubates cinctoblastula larvae.

Ecology and distribution

Pseudocorticium jarrei has been found so far in a single cave hollowed out in limestone on the south side of Jarre Island (Riou Archipelago, Marseille). The topography of the "Jarre Island cave" has been described by Fichez (1989) (Fig. 2). It is a tunnel of karstic origin including two zones separated by a narrow passage. The first is 60 m long, 5-7 m wide and 1-4 m high, ascending from 19 to 15 m in depth. The bottom is covered by a muddy sediment. The second part, 70 m in length, is a narrow tunnel, ascending from 15 to 3 m in depth, with a bottom alternately composed of mud and of sand or gravel. The end of the tunnel, at 130 m from the entrance, is too narrow for exploration and may or may not communicate with the north coast of the island through fissures, thus bringing about partial renewal of the water in the cave.

The sponge is located in the first zone at between 25 m and 50 m from the entrance. It is the dominant element of the sessile fauna found on the walls and ceiling (Fig. 3) of the section between 30 m and 40 m from the entrance. It has not been observed in the narrow tunnel inhabited by several other types of sponge, in-

cluding other homoscleromorphs (undescribed *Oscarella* and *Plakina* presently under study). The sponge has never been found in any other cave, despite extensive surveys in the area of Marseille and other parts of the Mediterranean.

DISCUSSION

This species clearly belongs to Homoscleromorpha, as demonstrated by the incubated cinctoblastula larvae (Borojevic et al. in press) and the presence both of a basement-membrane-like layer lining the choanoderm and pinacoderm (Humbert-David & Garrone 1993) and of flagellated exo- and endopinacocytes with osmiophilic inclusions. However, its relationships with other homoscleromorph genera need to be carefully examined, since it can be considered to belong either to the genus *Corticium,* because of high genetic identity with *Corticium candelabrum* (Solé-Cava et al. 1992), or to the genus *Oscarella,* currently defined by the absence of skeleton (Lévi 1956, 1973, Bergquist 1978).

Table 1 gives the most important characters of the genera *Oscarella, Plakina, Corticium* and *Pseudocorticium.* Two different patterns of aquiferous system occur in these genera. The aquiferous system of *Oscarella* consists of radial invaginations with one layer of choanocyte chambers (Schulze 1877), which calls to mind the sylleibeid organization of some Calcarea. In *Plakina* the organization is quite similar, but in some species a beginning of corticalization occurs without disturbing the sylleibeid organization, at least near the surface (Schulze 1880). In *Corticium* and *Pseudocorticium,* a regular arrangement of choanocyte chambers along invaginations is no longer visible, and corticalization and a true leuconoid organization exist. The ectosome of *Corticium* and *Pseudocorticium* is about 200 μm thick against 50 μm in some *Plakina* and only 5 μm in *Oscarella* (Table 1). There is a regular increase in the proportion of the volume of the mesohyl to the volume of the choanocyte chambers, from *Oscarella* and *Plakina,* where the mesohyl represents a smaller volume than the choanocyte chambers (0.7:1), to *Corticium,* where the volume of mesohyl and choanocyte chambers are equivalent (1:1), and finally to *P. jarrei,* where the volume of the mesohyl is greater than the choanocyte chambers (2.5:1). The choanocyte chambers are eurypylous in *Oscarella* and *Plakina* but aphodal or diplodal in *Corticium* and *Pseudocorticium.*

The cytology of *Pseudocorticium jarrei* differs from *Plakina* and *Corticium* in the presence of four different types of spherulous cells, absent in both the other genera. The star-shaped cells, which may cumulate the functions of both collencytes and archaeocytes, seem to play an important role in the control of symbiotic bacteria populations. *Pseudocorticium jarrei* differs from the described *Oscarella* both in the presence of spherulous cells and in the type of vacuolar cells.

A new definition for the genus *Oscarella* is needed to differentiate it from the new genus *Pseudocorticium.* We propose the following diagnosis, which may be

modified when more is known about its cytology and anatomy [several Mediterranean species from the genus will be described in further papers (Muricy et al. in prep.)].

Genus *Oscarella* Vosmaer, 1884

Diagnosis. Plakinidae without skeleton, with poorly developed ectosome essentially limited to the pinacoderm, a sylleibeid organization of the aquiferous system, eurypylous choanocyte chambers. Proportion of mesohyl to chambers less than 1:1.

The narrow distribution of *P. jarrei* raises an interesting question: Why is it remarkably abundant within a limited area of the Jarre Island cave, when it is absent in a number of nearby caves displaying similar conditions? Such a distribution is unusual. A local speciation from a species such as *Corticium candelabrum* is not a likely explanation, given the significant differences between the two sponges and the relatively short period of time between the last transgression and the immersion of the cave (approximately 8000 years). It appears more likely that the population originated from an exceptional colonization by the larvae of some other unknown population living in caves or under overhangs in a nearby deep-sea canyon (Cassidaigne canyon).

The subclass Homoscleromorpha, with its few skeletal characters, is a perfect example for demonstrating the particular usefulness of cytological parameters not only in the systematics of sponges in which skeletal characters are insufficient or absent (Vacelet & Donadey 1987), but also as important complementary information on skeleton-bearing species (Simpson 1968, Pomponi 1976, De Vos et al. 1991). Unfortunately, the widespread use of such characters is presently hampered by the small number of adequately described species. Just as in recent works that have demonstrated a correspondence between cytological criteria and allozyme patterns on the one hand (Boury-Esnault et al. 1992), and between cytological criteria and skeletal organisation on the other (Boury-Esnault et al. 1994), this study suggests once more that cell composition and arrangement are representative of the sponge organisation as a whole, and may be indicative of relationships and evolutionary trends among sponge taxa. Homoscleromorpha is generally considered to contain only a few species. More thorough observations and the use of non-skeletal characters show that its diversity – at least in the Mediterranean Sea – has, in fact, been considerably underestimated.

REFERENCES

Bergquist, P. R., 1978. Sponges. – Hutchinson & Co., London.

Bergquist, P. R., 1980. A revision of the supraspecific classification of the orders Dictyoceratida, Dendroceratida, and Verongida (class Demospongiae). – N. Z. J. Zool. 7: 443-503.

Table 1. Main reproductive, anatomical and cytological characters of *Pseudocorticium jarrei, Corticium candelabrum, Plakina trilopha, Oscarella lobularis* and *O. tuberculata.*

	Pseudocorticium	*Corticium*	*Plakina*	*Oscarella lobularis*	*Oscarella tuberculata*
larva	cinctoblastula	cinctoblastula	cinctoblastula	cinctoblastula	cinctoblastula
basement-membrane	+	+	+	+	+
aquiferous system	leuconoid	leuconoid	sylleibeid	sylleibeid	sylleibeid
choanocyte chambers	diplodal	aphodal	eurypilous	eurypilous	eurypilous
choanocytes/ chamber	300	300	250	500	400
proportion mesohyl: chambers	2.5:1	1:1	0.7:1	0.5:1	0.5:1
ectosome thickness	90-350 μm	100-300 μm	40-80 μm	5-10 μm	< 5 μm
exopinacocytes	flagellate, osm. incl.	flagellate, osm. incl.	flagellate, osm. incl.	flagellate, osm. incl.	flagellate, osm. incl.
endopinacocytes	flagellate, osm. incl.	flagellate, osm. incl.	flagellate, osm. incl.	flagellate, osm. incl.	flagellate, osm. incl.
apopylar cells	flagellate, osm. incl.	flagellate, osm. incl.	flagellate, osm. incl.	flagellate, osm. incl.	flagellate, osm. incl.
bacteria	abundant, several types	abundant, several types	abundant, several types	abundant, one type	scarce one type
collagen	dense	dense	loose	loose	dense
spiculocyte	-	+	+	-	-
archaeocytes	+	+	+	-	+
spherulous cells	3	-	-	-	-
large vacuolar cells	-	-	-	+	+
ovoid vacuolar cells	+	+	-	+	-

osm.incl. = osmiophilic inclusions.

Bergquist, P. R. & W. D. Hartman, 1969. Free amino acid patterns and the classification of the Demospongiae. - Mar. Biol. **3**: 247-268.

Bergquist, P. R., W. Hofheinz & G. Oesterhelt, 1980. Sterol composition and the classification of the Demospongiae. - Biochem. Syst. Ecol. **8**: 423-435.

Bergquist, P. R. & J. J. Hogg, 1969. Free amino acid patterns in Demospongiae; a biochemical approach to sponge classification. - Cah. Biol. mar. **10**: 205-220.

Bergquist, P. R., A. Lavis & R. C. Cambie, 1986. Sterol composition and classification of the Porifera. - Biochem. Syst. Ecol. **14**: 105-112.

Bergquist, P. R., M. P. Lawson, A. Lavis & R. C. Cambie, 1984. Fatty acid composition and the classification of the Porifera. - Biochem. Syst. Ecol. **12**: 63-84.

Bergquist, P. R. & R. J. Wells, 1983. Chemotaxonomy of the Porifera: The development and current status of the field. - *In* P. J. Scheuer (ed.): Marine Natural Products: Chemical and Biological Perspectives, vol. 5: 1-50. - Academic Press, London.

Borojevic, R., N. Boury-Esnault, R. Desqueyroux-Faundez, C. Lévi, M. Pansini, K. Rützler, R. van Soest, S. Stone, M.-J. Uriz & J. Vacelet, in press. - *In* N. Boury-Esnault & K. Rützler (eds): Thesaurus of terms for sponges. Smithsonian Institution Press, Washington.

Boury-Esnault, N., L. De Vos, C. Donadey & J. Vacelet, 1984. Comparative study of the choanosome of Porifera: I. The Homoscleromorpha. - J. Morphol. **180**: 3-17.

Boury-Esnault, N., L. De Vos, C. Donadey & J. Vacelet, 1990. Ultrastructure of choanosome and sponge classification. - *In* K. Rützler (ed.): New Perspectives in Sponge Biology: 237-244. Smithsonian Institution Press, Washington.

Boury-Esnault, N., E. Hajdu, M. Klautau, M. Custodio & R. Borojevic, 1994. The value of cytological criteria in distinguishing sponges at the species level: example of the genus Polymastia. - Can. J. Zool. **72**: 795-804.

Boury-Esnault, N., A. M. Solé-Cava & J. P. Thorpe, 1992. Genetic and cytological divergence between colour morphs of the Mediterranean sponge *Oscarella lobularis* Schmidt (Porifera, Demospongiae, Oscarellidae). - J. nat. Hist. **26**: 271-284.

De Vos, L., K. Rützler, N. Boury-Esnault, C. Donadey & J. Vacelet, 1991. - Atlas of sponge morphology. - Smithsonian Institution Press, Washington.

Diaz, M. C. & R. W. M. van Soest, 1994. The Plakinidae: a systematic review. - *In* R. W. M. van Soest, Th. M. G. van Kempen & J. C. Braekman (eds): Sponge in time and space: 93-110. A. A. Balkema, Rotterdam.

Donadey, C., 1979. Contribution à l'étude cytologique de deux démosponges homosclérophorides: *Oscarella lobularis* (Schmidt) et *Plakina trilopha* Schulze. - *In* C. Lévi & N. Boury-Esnault (eds): Biologie des Spongiaires. - Colloques internationaux CNRS, vol. 291: 165-172. CNRS, Paris.

Fichez, R., 1989. Phénomènes d'oligotrophie en milieu aphotique. Etude des grottes sous-marines, comparaison avec les milieux profonds et bilans énergétiques. - Doctorat ès Sciences Thesis, Université d'Aix-Marseille II, Marseille, France.

Hooper, J. N. A., R. J. Capon, C. P. Keenan, D. L. Parry & N. Smit, 1992. Chemotaxonomy of marine sponges: families Microcionidae, Raspailiidae and Axinellidae, and their relationships with other families in the orders Poecilosclerida and Axinellida (Porifera: Demospongiae). - Invertebr. Taxon. **6**: 261-301.

Humbert-David, N. & R. Garrone, 1993. A six-armed tenascin-like protein extracted from the Porifera *Oscarella tuberculata* (Homosclerophorida). - Eur. J. Biochem. **6**: 255-260.

Johnston, G., 1842. - A history of British sponges and lithophytes. - Edinburgh.

Kornerup, A. & J. H. Wanscher, 1978. - Methuen Handbook of Colour, 3rd ed. - Methuen, London.

Lafay, B., N. Boury-Esnault, J. Vacelet & R. Christen, 1992. An analysis of partial 28S ribosomal RNA sequences suggests early radiations of sponges. - Biosystems **28**: 139-151.

Lawson, M. P., P. R. Bergquist & R. C. Cambie, 1984. Fatty acid composition and the classification of the Porifera. - Biochem. Syst. Ecol. **12**: 375-393.

Lendenfeld, R. von, 1885. A monograph of the Australian sponges. Parts 4, 5, 6. - Proc. Linn. Soc. New South Wales **10**: 3-22, 282-325, 481-553.

Lendenfeld, R. von, 1887. On the systematic position and classification of sponges. - Proc. zool. Soc. London: 558-662.

Lévi, C., 1953. Description de *Plakortis nigra* nov. sp. et remarques sur les Plakinidae (Démosponges). - Bull. Mus. natn. Hist. nat., Paris Sér. 2 **25**: 320-328.

Lévi, C., 1956. Etude des *Halisarca* de Roscoff. Embryologie et systématique des démosponges. - Arch. Zool. exp. gén. **93**: 1-184.

Lévi, C., 1970. Les cellules des éponges. - *In* W. G. Fry (ed.): Biology of the Porifera, Symposia of the zoological Society of London, vol. 25: 353-364. Academic Press, London.

Lévi, C., 1973. Systématique de la classe des Demospongiaria (Démosponges). - *In* P. P. Grassé (ed.): Spongiares, Traité de Zoologie, vol. 3(1): 577-632. Masson & C°, Paris.

Nardo, G. D., 1833. Auszug aus einen neuen System der Spongiarien, wornach bereits die Aufstellung in der Universitäts-Sammlung zu Padua gemacht ist. - Isis: 519-523.

Pomponi, S. A., 1976. A cytological study of the Haliclonidae and the Callyspongiidae (Porifera, Demospongiae, Haplosclerida). - *In* F. W. Harrison & R. R. Cowden (eds): Aspects of Sponge Biology: 215-235. Academic Press, New York.

Rasmont, R. & F. Rozenfeld, 1981. Etude micro-cinématographique de la formation des chambres choanocytaires chez une éponge d'eau douce. - Ann. Soc. r. Zool. Belg. **111**: 33-44.

Schmidt, O., 1862. - Die Spongien des Adriatischen Meeres. - Wilhelm Engelmann, Leipzig.

Schulze, F. E., 1877. Untersuchungen über den Bau und die Entwicklung der Spongien. Die Gattung *Halisarca*. - Z. wiss. Zool. **28**: 1-48.

Schulze, F. E., 1880. Untersuchungen über den Bau und die Entwicklung der Spongien. Die Plakiniden. - Z. wiss. Zool. **34**: 407-451.

Simpson, T. L., 1968. The structure and function of sponge cells: new criteria for the taxonomy of Poecilosclerid sponges (Demospongiae). - Bull. Peabody Mus. nat. Hist. **25**: 1-142.

Solé-Cava, A. M., N. Boury-Esnault, J. Vacelet & J. P. Thorpe, 1992. Biochemical genetic divergence and systematics in sponges of the genera *Corticium* and *Oscarella* (Demospongiae: Homoscleromorpha) in the Mediterranean Sea. - Mar. Biol. **113**: 299-304.

Solé-Cava, A. M. & J. P. Thorpe, 1987. The use of electrophoresis in sponge taxonomy. - *In* J. Vacelet & N. Boury-Esnault (eds): Taxonomy of Porifera from the N. E. Atlantic and Mediterranean Sea, NATO ASI Series, Series G, vol. 13: 243-258. Springer-Verlag, Berlin.

Topsent, E., 1895. Etude monographique des Spongiaires de France. II. Carnosa. - Arch. Zool. exp. gén. **3**: 493-590.

Topsent, E., 1896. Matériaux pour servir à l'étude de la faune des Spongiaires de France. - Mém. Soc. zool. France **9**: 113-133.

Topsent, E., 1897. Spongiaires de la baie d'Amboine. - Rev. suisse Zool. **4**: 421-487.

Topsent, E., 1918. Eponges de San Thomé. Essai sur les genres *Spirastrella, Donatia* et *Chondrilla*. - Arch. Zool. exp. gén. **57**: 535-618.

Tuzet, O. & J. Paris, 1963. *Octavella galangaui* n.g. n.sp., Demospongiae, Oscarellidae, de Banyuls-sur-Mer. - Vie Milieu **14**: 71-89.

Vacelet, J. & C. Donadey, 1987. A new species of *Halisarca* (Porifera, Demospongiae) from the Caribbean, with remarks on the cytology and affinities of the genus. - *In* W. C. Jones (ed.): European contributions to the taxonomy of sponges, Publication of the Sherkin Island Marine Station, vol. 1: 5-12. Litho Press Co., Midleton, Ireland.

Vosmaer, G. C. J., 1884. Porifera. - *In* H. G. Bronn (ed.): Die Klassen und Ordnungen des Thierreichs: 65-176.