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A new species of the genus *Oscarella* (Porifera: Homosclerophorida: Plakinidae) from the North-West Pacific

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Abstract: A new species, *Oscarella kamchatkensis* sp. nov., is described from the upper sublittoral in the north-western Pacific (coastal waters of Kamchatka, Avacha Gulf) at depths between 10 to 23 m from stones or boulders. It is characterized by its orange-yellow colour, lumpy, lobate surface, and soft slimy consistency. The shape of sponge is irregular and consists of numerous small cushions. The new species differs clearly from all previously described *Oscarella* spp. in its external morphology, cell composition and endobiotic bacteria. It has three particular kinds of cells with inclusions (one type of spherulous and two types of granular cells), and three morphotypes of endobiont bacteria. The anatomy and cytology are described and compared to that of other *Oscarella* species.

Résumé : *Une nouvelle espèce du genre* Oscarella *(Porifera : Homosclerophorida : Plakinidae) du nord-ouest Pacifique.* Une espèce nouvelle de Spongiaires de genre *Oscarella, O. kamchatkensis* sp. nov., est décrite dans la zone subtidale supérieure du Pacifique nord-ouest (eaux côtières de Kamchatka, Golfe d'Avacha) à des profondeurs comprises entre 10 et 23 m sur des pierres ou des gros cailloux. Cette espèce est caractérisée par sa couleur orange-jaune, sa surface grumeleuse et microlobée et par sa consistance molle et muqueuse. La forme est irrégulière et se compose de nombreux petits coussins. La nouvelle espèce diffère clairement de toutes les *Oscarella* spp. décrites auparavant par sa morphologie externe, ainsi que sa composition en cellules et en bactéries symbiotiques. Il y a trois types particuliers de cellules avec des inclusions (un type de cellules sphéruleuses et deux types de cellules granuleuses) et trois morphotypes de bactéries symbiotiques. L'anatomie et la cytologie sont décrites et comparées à celles d'autres espèces d'*Oscarella*.

Keywords: Taxonomy ● Plakinidae ● *Oscarella* ● New species ● Kamchatka ● Cytology

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Introduction

The Homosclerophorida Dendy, 1905 presently contains one family, the Plakinidae Schulze, 1880 that contains seven genera (Muricy & Diaz, 2002). All genera have a more or less worldwide distribution with the exception of the genus *Pseudocorticium* Boury-Esnault et al., 1995. Sponges belonging to genus *Oscarella* Vosmaer, 1884 are often small, fragile and always lack spicules and a fibrous skeleton, making them difficult to identify. Attempts to define species of *Oscarella* by morphological features have led to a confusing reduction of species names. However, more suitable methods for characterization of species in this genus are histological, ultrastructural and embryological (Boury-Esnault et al., 1992; Muricy et al., 1996; Bergquist & Kelly, 2004; Muricy & Pearse, 2004; Ereskovsky, 2006). 14 species of the genus *Oscarella* are listed in the World Porifera Database (van Soest et al., 2008). Two of these species, *O. carmela* Muricy & Pearse, 2004 and *O. malakhovi* Ereskovsky, 2006 were described from the North Pacific, two from the Indo-Pacific: *O. nigraviolacea* Bergquist & Kelly, 2004 and *O. stillans* Bergquist & Kelly, 2004. There is only one previous record of the genus in the northwestern part of the Pacific, *Oscarella lobularis* (Schmidt, 1862) reported by Koltun (1966). The present paper describes *Oscarella kamchatkensis* sp. nov. from the upper sublittoral of this area.

Material and methods

Specimens were collected in October 2007 and October 2008 from Starichkov Island (Avacha Gulf, Pacific coast of Kamchatka, 52°46.360'N-158°36.925'E and 52°46.760'N-158°37.332'E) from rocks and boulders at a depth of 10-23 m (Fig. 1). Sponges were photographed underwater in the field with a Nikon F80 film camera in Sea&Sea housing, and in the aquarium with a Nikon D70 digital camera. Small boulders with the sponges were collected and kept in an aquarium with refrigerated water. This allowed us to conduct a detailed study on living specimens and take high-resolution photographs of the specimens in the aquarium. Small pieces of the sponges were collected with a scalpel and immediately fixed in 2.5% glutaraldehyde (25% glutaraldehyde diluted and buffered with seawater), post-fixed in 1% osmium tetroxide and embedded in araldite resin for semithin and ultrathin sections. Semithin sections were stained with toluidin blue, observed by light microscopy (LM), and photographed with a Leica DMLB digital camera. Ultrathin sections were stained with uranyl acetate, contrasted with lead citrate and observed with LEO 910 and LEO 912 transmission electron microscopes (TEM). For scanning electron microscopy (SEM), the specimens were fractured in liquid nitrogen, critical-point-dried, sputter-coated with gold-palladium, and observed under a Hitachi S570 SEM. To verify that the specimens represented a new species, original descriptions and available semi-thin sections, TEM and SEM micrographs for 7 other *Oscarella* species were consulted. Available taxonomic characters from all species that were considered were compiled in a Table 2 for comparison. Cell and cellular structures dimensions are given as minimum-mean-maximum (min-**mean**-max) elsewhere. The specimens were transferred to 70% ethanol for long-term storage; vouchers are deposited at the Zoological Institute of Russian Academy of Sciences in Saint-Petersburg, Russia (ZIN RAS).

Results

PHYLUM PORIFERA Grant, 1836
Class Demospongiae Sollas, 1885
Order Homosclerophorida Dendy, 1905
Family Plakinidae Schulze, 1880
Genus *Oscarella* Vosmaer, 1884

Type species

Halisarca lobularis Schmidt, 1862 (by monotypy). [*Oscaria*] Vosmaer, 1881: 163 (preocc. by *Oscaria* Gray,

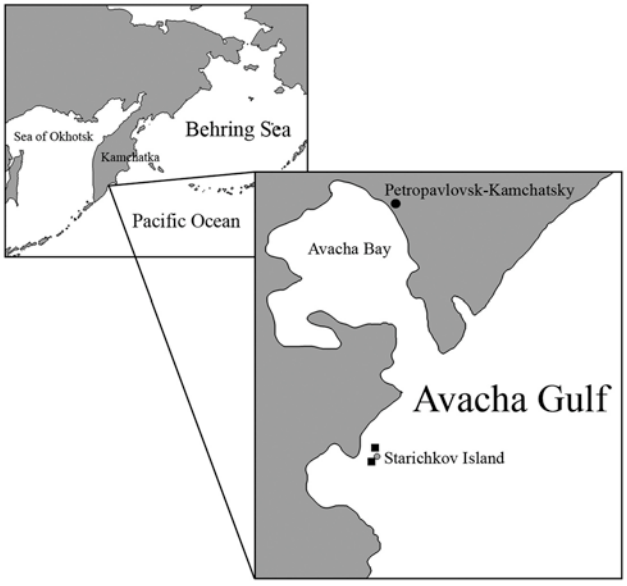
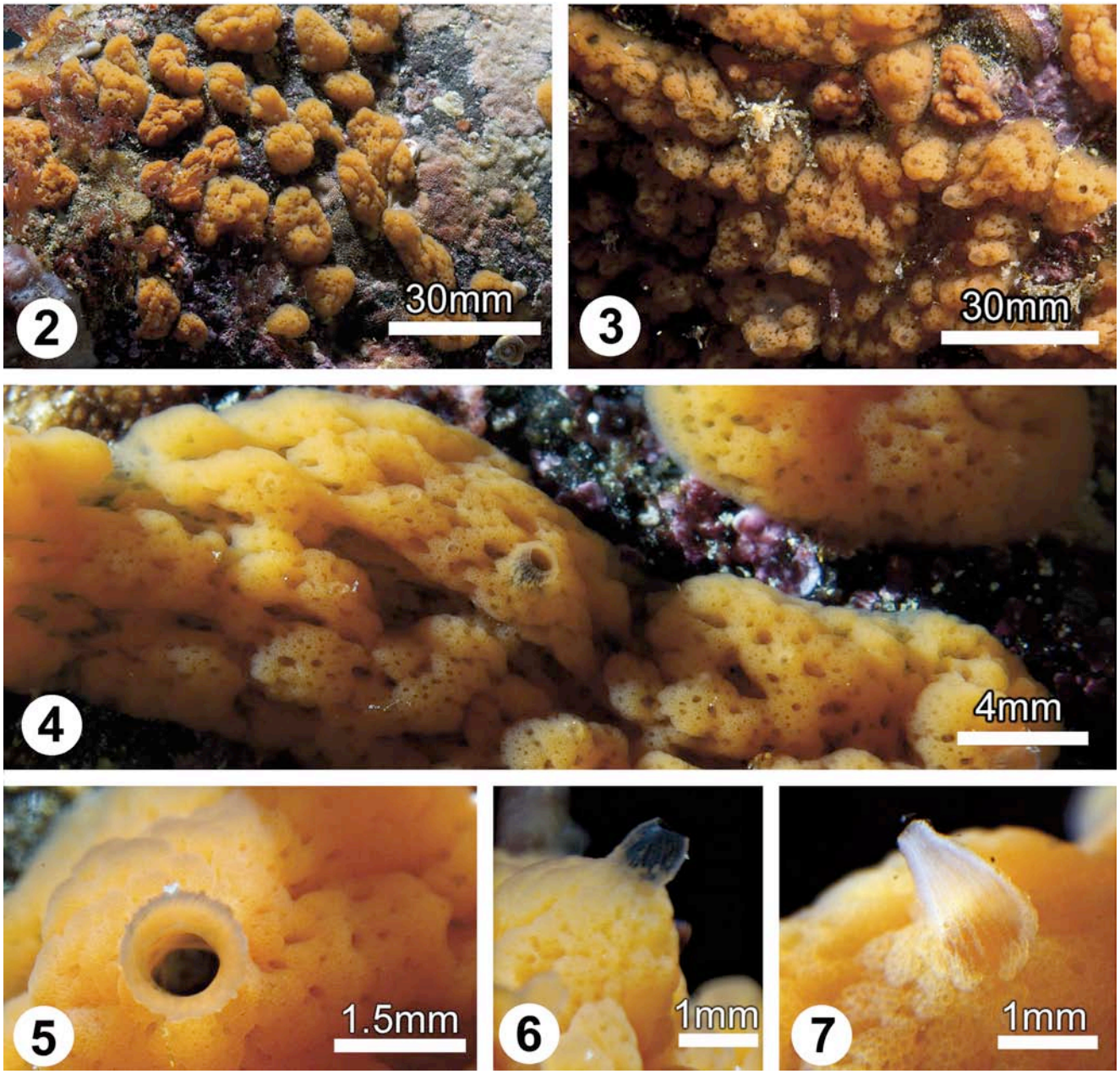


Figure 1. *Oscarella kamchatkensis* sp. nov. Location of the collection sites of in the Avacha Gulf (coastal waters of Kamchatka, north-western Pacific).

Figure 1. *Oscarella kamchatkensis* sp. nov. Localisation des sites de récolte dans le Golfe d'Avacha (eaux côtières de Kamchatka, Pacifique du nord-ouest).



Figures 2-7. *Oscarella kamchatkensis* sp. nov. External morphology *in vivo* and *in situ*. **2 & 3.** Underwater photographs of typical specimens on a stone, taken at 17 m depth. The characteristic lumpy, microlobated surface is clearly visible. **4.** Detail of the sponge surface. **5-7.** Photographs of oscular tubes of a sponge in the aquarium.

Figures 2-7. *Oscarella kamchatkensis* sp. nov. Morphologie externe *in vivo* et *in situ*. **2 & 3.** Photos sous-marines des exemplaires typiques sur une pierre, faites à 17 m de profondeur ; la surface caractéristique grumeleuse et microlobée est bien visible. **4.** Détail de la surface de l'éponge. **5-7.** Photos des tubes osculaires des éponges en aquarium.

1873 - Reptilia); *Oscarella* Vosmaer, 1884: pl. 8 (explanation); 1887: 326 (nom. nov. for *Oscaria* Vosmaer). *Octavella* Tuzet and Paris, 1964: 88.

Diagnosis (Muricy & Diaz, 2002)

Plakinidae without skeleton, with thinly encrusting to lobate shape. Thin ectosome (< 100 µm), often limited to pinacoderm; true cortex absent. Mesohyl poorly developed, with a proportion of mesohyl to chambers varying from 0.5:1 to 1.2:1. The aquiferous system with sylleibid organization, with spherical, eurypylous choanocyte chambers uniformly arranged around large, regular exhalant canals, and with large basal cavity.

Oscarella kamchatkensis sp. nov.
(Figs 2-27)

Material examined

Holotype ZIN RAS 11058 - Russia, Pacific Ocean, Avacha Gulf, Starichkov Island, (52°46.360'N-158°36.925'E), 21-23 m, on stones and boulders, 18.10.2008, collector N.P. Sanamyan. Paratypes: ZIN RAS 11059 - (4 specimens): the same locality; ZIN RAS 11060 - (3 specimens): the same locality, 18.10.2008, collector N.P. Sanamyan; ZIN RAS 11061 - (4 specimens): Russia, Pacific Ocean, Avacha Gulf, Starichkov Island, left of Karaulny Cliff, (52°46.760'N-158°37.332'E), 10-11 m, on boulder, 18.10.2008, collector N.P. Sanamyan.

Etymology

The species name is derived from the region where it was discovered.

Diagnosis

Shallow-water *Oscarella* from upper subtidal stones or boulder biocoenoses; orange or orange-yellow in colour, with lumpy, lobate surface and soft slimy consistency; containing three particular kinds of cells with inclusions (two kinds of granular cells and one kind of spherulous cell with granular paracrystalline inclusions), and three morphotypes of endobiont bacteria.

Description

Morphology. The sponge has an irregular form and consists of numerous small cushions which may be well separated from each other (Figs 2 & 3) or sometimes are more crowded and forming large continuous individuals (Fig. 3). The cushions are small and low, typically 1-2 cm diameter and about 3-5 mm high, but on underwater photographs some specimens, especially growing on undersurfaces of stones, appear to be higher, probably up to 8-10 mm (Fig. 3). The appearance of the cushions depends significantly on the external condition of the sponge: They may be well inflated (as often seen in natural conditions in the sea), or, in response to mechanical disturbance, may slowly contract and become much thinner with a more rugose surface. The colour is rather constant, orange or orange-yellow. The surface is irregular, microlobate (Figs 4 & 5). Lobes are small, round, and irregular. The surface is corrugated and perforated by abundant inhalant ostia, 18-27 µm in diameter. Oscular tubes are not numerous, typically one per cushion, occasionally more than one. They are about 1-2 mm in height and 1 mm in diameter, thin-walled, almost transparent, tapering toward the end (Figs 6 & 7) or with a flared-out margin (Fig. 5). The body is soft but rather elastic, easily torn when removed from substrate. The sponge does not extrude much slime when exposed on air.

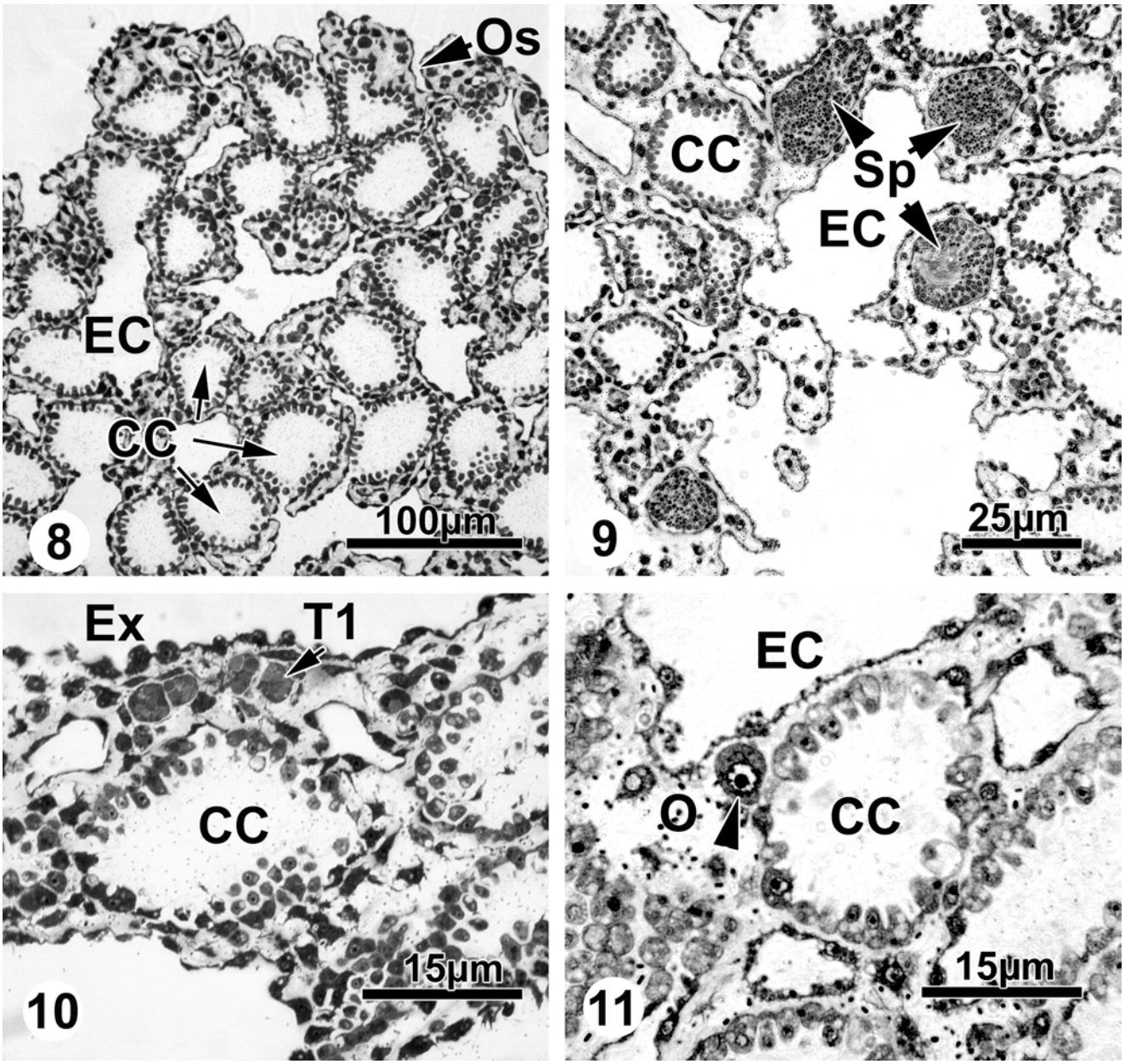
Anatomy. Spicules and a fibre skeleton are absent. The ectosome is fine, only 4-25 µm thick (Figs 8 & 10). Inhalant canals are 5-12 µm in diameter. Within the choanosome, the choanocyte chambers are ovoid to spherical, eurypylous, and 15-23 µm in diameter (Figs 8-11). Exhalant canals (Fig. 8) run toward a well-developed system of basal exhalant cavities 15-70 µm in diameter, and lead to the oscula. The basal part of the sponge, composed of mesohyl, is devoid of aquiferous system elements. It is mainly comprised of cells type 3 and occasionally of cells type 1 (see below).

Cytology. **Choanocytes** have irregular, pyramidal to ovoid cell bodies 3.1-**3.8**-4 µm wide at the central part and 4.6-**5.2**-6 µm high (Figs 12 & 14). The collar measures 1.9-**2.2**-2.4 µm in width and is composed of about 29-**32**-34 microvilli. The nucleus is central or rarely basal, ovoid, about 1.6x2.6 µm in dimension, sometimes with a nucleolus. The cytoplasm usually contains phagosomes 0.5-**1.0**-2 µm in diameter, smaller digestive vacuoles, osmiophilic inclusions and mitochondria. The adjacent choanocytes are in contact with each other at their central or basal parts. The cells have short pseudopodia.

Apopylar cells (Figs 13 & 15) are roughly triangular in cross section, 7.4-**8.2**-9.6 µm wide and 3.2-**3.4**-3.7 µm high. Their nuclei are apical, ovoid, and up to 1.6 µm in diameter. The cells possess small lateral pseudopodia. The cytoplasm contains phagosomes and small osmiophilic inclusions.

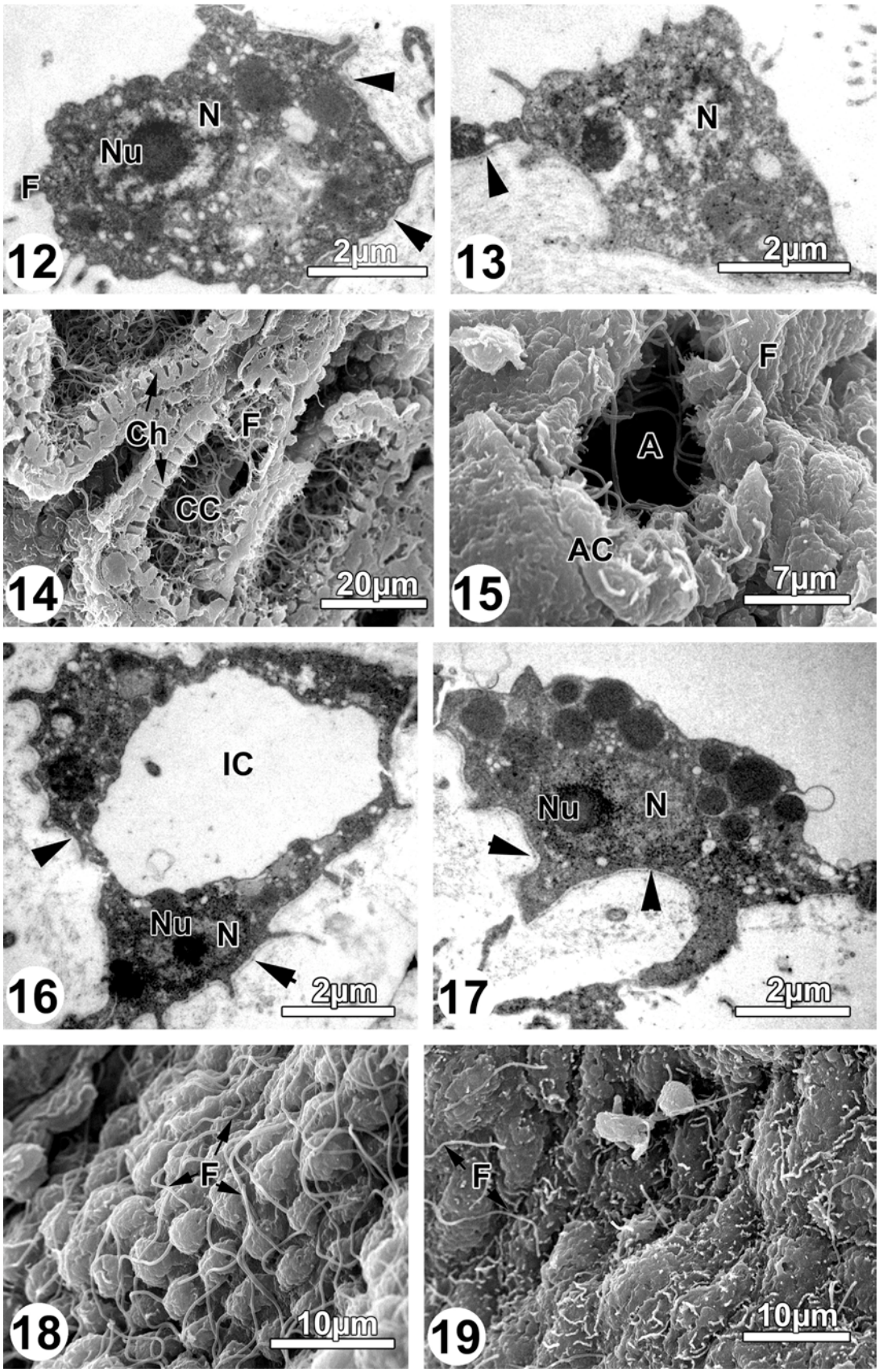
Endopinacocytes (Figs 16 & 18) are flat, flagellated, 6.2-**8.7**-9.5 µm wide by 1.9-**2.5**-2.6 µm high. Their nuclei are ovoid, approximately 1.1x2.5 µm in dimension with a nucleolus. They often show irregular, thin cytoplasm projections at their basal part. The cytoplasm contains numerous osmiophilic inclusions and phagosomes of 0.4-**0.6**-0.8 µm in diameter.

Exopinacocytes (Figs 17 & 19) are similar to the endopinacocytes.



Figures 8-11. *Oscarella kamchatkensis* sp. nov. General anatomy of *Oscarella kamchatkensis* sp. nov. observed by light microscopy **8.** The arrangement of choanocyte chambers (CC) around exhalant canals (EC). **9.** The choanosome of a male sponge with spermatocysts (Sp) near the exhalant canal. **10.** The ectosomal part of a sponge with Type 1 spherulous cells. **11.** Young oocyte (O) in the mesohyl. Abbreviations: CC - choanocyte chamber; EC - exhalant canal; Ex - exopinacoderm; O - oocyte; Os - ostium; Sp - spermatocysts; T1 - Type 1 spherulous cell.

Figures 8-11. *Oscarella kamchatkensis* sp. nov. Microscopie optique de l'anatomie générale. **8.** L'arrangement des chambres choanocytaires (CC) autour des canaux exhalants (EC). **9.** Le choanosome d'une éponge mâle avec les spermatocystes (Sp) près du canal exhalant. **10.** La partie ectosomale d'une éponge avec les cellules sphéruleuses de type 1. **11.** Jeune oocyte (O) dans le mésohyle. Abréviations : CC - chambre choanocytaire ; EC -canal exhalant ; Ex - exopinacoderme ; O - oocyte ; Os - ostium ; Sp - spermatocystes ; T1 - cellule sphéruleuse de type 1.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Figures 12-19. *Oscarella kamchatkensis* sp. nov. Epithelial cells. **12.** TEM micrograph of a choanocyte. **13.** TEM micrograph of an apopylar cell. **14.** SEM micrograph of choanocyte chambers (CC) with choanocytes (Ch). **15.** SEM micrograph of an apopyle (A) with apopylar cell (AC). **16.** TEM micrograph of three endopinacocytes, forming an inhalant canal (IC). **17.** TEM micrograph of an exopinacocyte. **18.** SEM micrograph of endopinacoderm. **19.** SEM micrograph of exopinacoderm. Arrowheads - basement membrane. Abbreviations: A - apopyle; AC - apopylar cell; CC - choanocyte chamber; Ch - choanocytes; F - flagellum; IC - inchalant canal; N - nucleus; Nu - nucleolus.

Figures 12-19. *Oscarella kamchatkensis* sp. nov. Cellules épithéliales. **12.** Micrographie MET d'une choanocyte. **13.** Micrographie MET d'une cellule apopylaire. **14.** Micrographie MEB des chambres choanocytaires (CC) avec choanocytes (Ch). **15.** Micrographie MEB d'une apopyle (A) avec les cellules apopylaires (AC). **16.** Micrographie MET de trois endopinacocytes, formant le canal inhalant (IC). **17.** Micrographie MET d'une exopinacocyte. **18.** Micrographie MEB d'endopinacoderme. **19.** Micrographie MEB d'exopinacoderme. Têtes de flèche - membrane basale. Abréviations : A - apopyle ; AC - cellule apopylaire ; CC - chambre choanocytaire ; Ch - choanocyte ; F - flagelle ; IC - canal inchalant ; N - nucléus ; Nu - nucléoles.

A thin, irregular layer of glycocalyx covers the surface of the exopinacocytes, endopinacocytes, choanocytes and apopylar cells. Choanoderm and pinacoderm are lined by a basement membrane, which is a continuous, 17-22 nm thick layer of condensed collagen microfibrils (Figs. 12, 13, 16 & 17).

Three types of cells with inclusions were observed within the mesohyl, without any special localization:

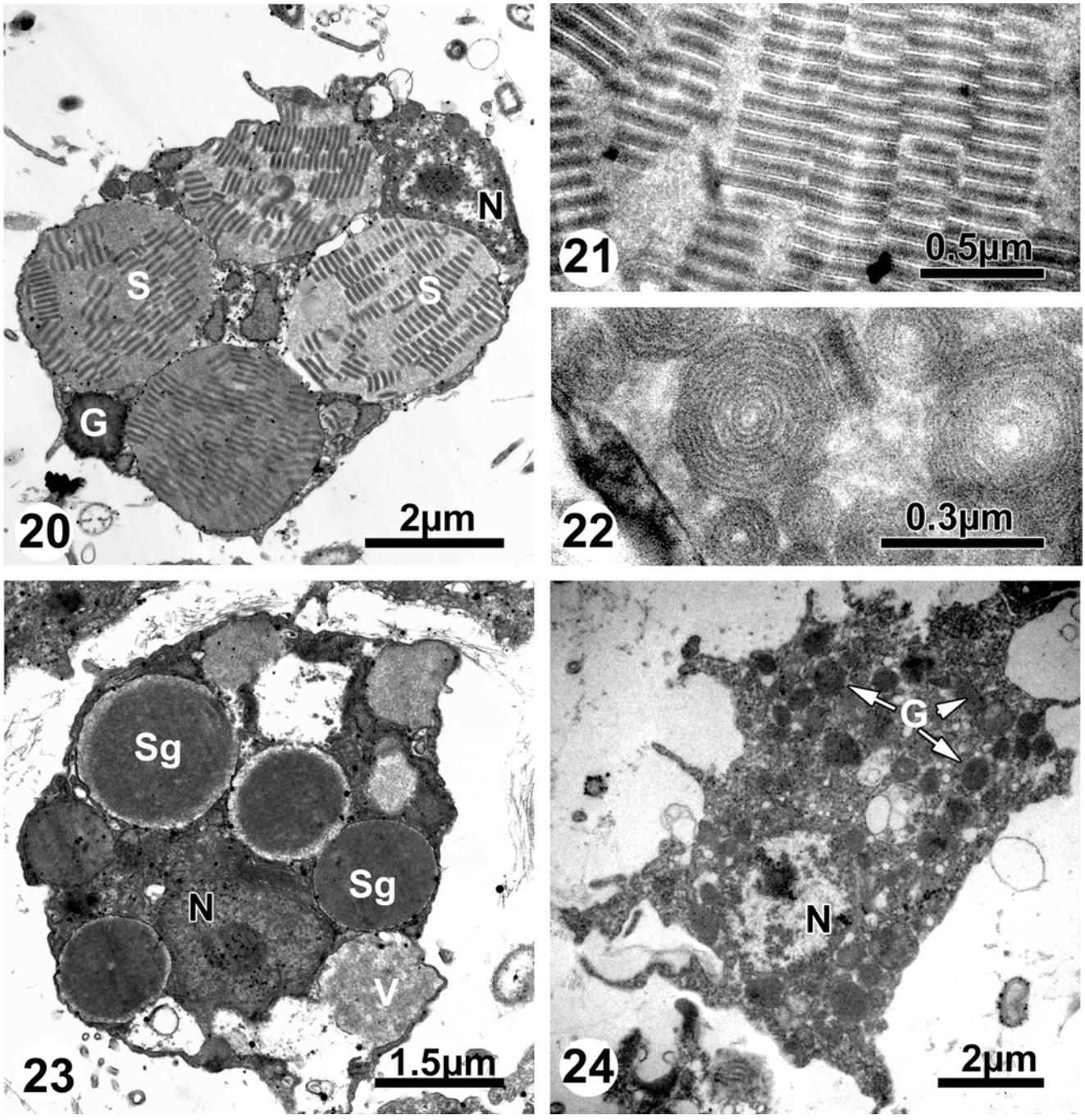
Type 1: Spherulous cells with paracrystalline inclusions (Figs 10 & 20-22). They are ovoid or rarely spherical cells that are 5.5-**7.6**-9.9 µm long and 4.5-**5.9**-6.9 µm in diameter, with nucleolated nucleus 2.2 µm in diameter. The cytoplasm is almost completely filled with 2-7 spherical heterogeneous inclusions 1.5-**2.1**-3.9 µ m in diameter, composed of paracrystalline elements included in the homogenous matrix. The paracrystalline elements are ovoid or cylindrical in longitudinal section and transversally round (0.5-**0.9**-1.1 µm long and 0.12-**0.3**-0.5 µm in diameter). These elements are composed of fibrils arranged in a transverse banding pattern with alternating dark and clear bands (Table 1). In cross section the paracrystalline elements are organized in spiral lines. The cytoplasm can also contain 4-8 spherical granules (0.3-**0.6**-1.1 µm in diameter) with electron-dense homogenous inclusions. Mitochondria are rare.

Type 2: Granular cells with large granular inclusions (Fig. 23). They are ovoid or round cells (4.3-**5.1**-6.0 µm in diameter) with nucleolated nucleus (about 2.3 µm in diameter). The Golgi complex is situated near the nucleus. The cytoplasm is filled with two types of inclusions. The first are large spherical granules (4-8 per cell), 0.5-**0.8**-1.6 µm in diameter, with electron-dense, homogeneous contents. Often these inclusions have a ring of semi-transparent (grey) material at their periphery. The cytoplasm also contains spherical or oval electron-transparent vacuoles with rare inclusions, 0.8-**1.0**-1.5 µm in diameter, many small, electron-transparent vacuoles and some mitochondria.

Type 3: Microgranular cells (Fig. 24). They are ovoid to irregular, 5.9-**6.3**-6.8 µ m long and about 4.4 µ m in diameter. The nucleus (2.5 µm in diameter) is nucleolated. The Golgi complex is situated near the nucleus. The cytoplasm is filled with 8-15 electron-dense, homogeneous granules, 0.2-**0.4**-1.0 µ m in diameter. In the cytoplasm there are rare electron-transparent vacuoles 0.4-**0.6**-0.9 µm in diameter. Other special inclusions are absent.

Table 1. Comparative characteristics of spherulous cells with paracrystalline inclusions in Homoscleromorpha species.
Tableau 1. Comparaison des cellules sphéruleuses avec les inclusions paracristallines chez les Homosclérómorphes.

Species	Cell diam (µm)	Inclusions (spherules) diam (µm)	Shape	Dimensions (µm) Wide	Dimensions (µm) Long	Fibrils Cross section (nm) Dark	Fibrils Cross section (nm) Clear	Fibrils Longitudinal section (nm) Dark	Fibrils Longitudinal section (nm) Clear
O. kamchatkensis	5-8	1.5-3.9	Cylindrical to ovoid	0.12-0.4	0.5-1.1	12	20	20	80
O. microlobata	8-12	0.8-4.5	Cylindrical to ovoid	0.17-0.3	0.5-2	10	20	15	70
O. imperialis	8-13	2-4.5	Cylindrical to ovoid	0.17-0.3	0.5-2	10	20	15	70
Pseudocorticium jarrei	12-18	Up to 4	Cylindrical to ovoid	0.2-0.3	About 1.3	?	20	20	90



Figures 20-24. *Oscarella kamchatkensis* sp. nov. TEM micrographs of the cells of the mesohyl. **20.** Spherulous cell - type 1 - with paracrystalline inclusions (S). **21.** Parallel section of paracrystalline inclusions. **22.** Perpendicular section of paracrystalline inclusions. **23.** Granular cell - type 2 - with big granular inclusions (Sg) and vacuoles (V). **24.** Microgranular cell - type 3. Abbreviations: G - granule; N - nucleus; S - paracristalline inclusions; Sg - granular inclusions; V - vacuole.

Figures 20-24. *Oscarella kamchatkensis* sp. nov. Micrographies MET des cellules du mésohyle. **20.** Une cellule sphéruleuse - le type 1 - avec les inclusions paracristallines (S). **21.** Section parallèle d'inclusions paracristallines. **22.** Section perpendiculaire d'inclusions paracristallines. **23.** Une cellule granuleuse - le type 2 - avec de grandes inclusions granuleuses (Sg) et vacuoles (V). **24.** Une cellule microgranuleuse - le type 3. Abréviations : G - grain ; N - nucléus ; S - inclusions paracristallines ; Sg - inclusions granuleuses ; V - vacuole.

Archaeocytes were not detected.

Symbiotic bacteria (Figs 25-27)

There are three morphotypes of bacterial symbionts in *Oscarella kamchatkensis* sp. nov. All of them are present in the sponge mesohyl and are relatively numerous. They have different shapes, sizes and some intracellular characters, but cell wall and structure of the nucleoid region are similar in all morphotypes.

Bacteria of morphotype *a* are rodlike, 1.0-1.1 µm in length and 0.24-**0.25**-0.26 µm in diameter (Fig. 25). The cell wall has two distinct membranes and can be described as Gram-negative one. There is visible periplasmic space between the membranes. The granular cytoplasm layer is very narrow on the cell periphery and up to 0.15 µm wide on the distal cell ends. The nucleoid region is well developed, electron transparent and with an irregular network of filaments. Most of the bacteria of this morphotype have transparent oval inclusions close to the distal parts of the cell.

The shape of morphotype *b* symbionts is ovoid, 0.3-0.4 by 1.0-1.1 µm (Fig. 26). Their cell walls are the same as in morphotype *a*, however, the outer membrane is undulating. The zone of cytoplasm is granular, 0.1 µm thick. The nucleoid region is big and filamentous. In some cells the filaments can join together.

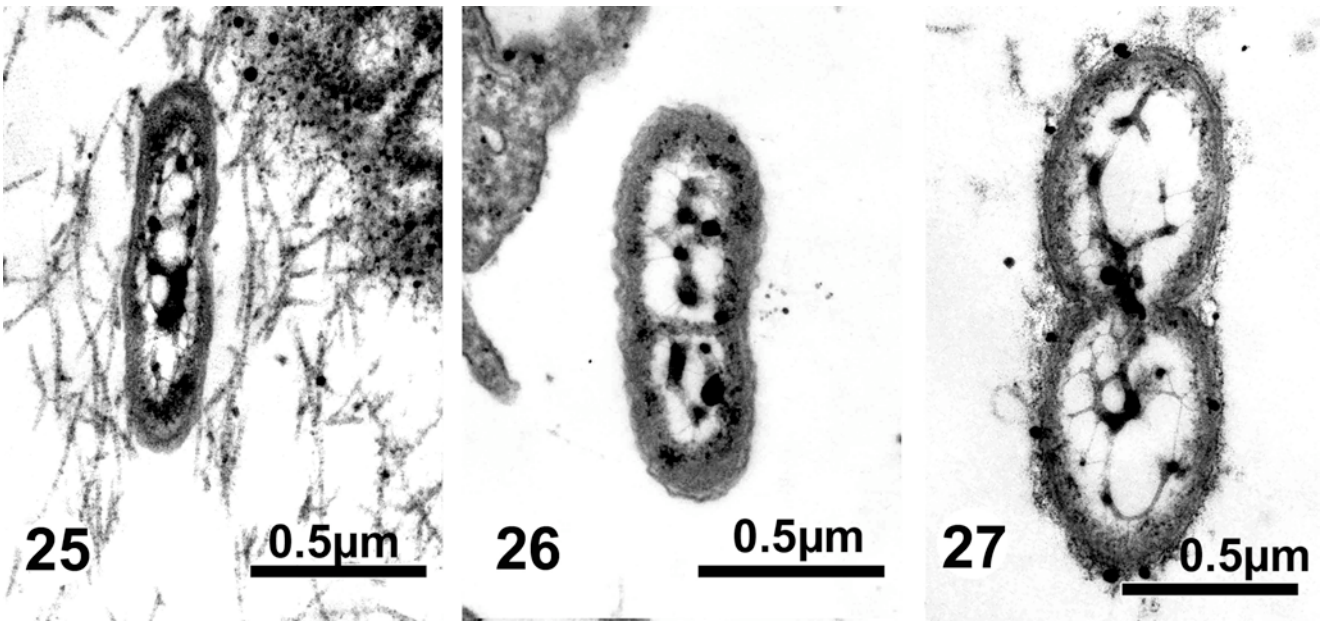
Bacterial symbionts of morphotype *c* are oval, 0.5-0.6 µm in smaller diameter and 1.3-**1.4**-1.5 µm in length (Fig. 27). This bacterial morphotype is Gram-negative. The width of the granular cytoplasm zone is about 0.1 µm above the cell perimeter. The nucleoid region has dark filaments of various thicknesses.

Reproduction

Because we have samples only from October 2007 and 2008, we do not know the reproductive cycle of the new species. Nevertheless, all observed individuals had gametes. According to our results the new species is dioecious. Oocytes were rare and at early stages of oogenesis, before yolk accumulation (Fig. 11). In contrast, the mesohyl of male individuals was filled with spermatocysts at different stages of spermatogenesis (Fig. 9).

Locality and habitat

Oscarella kamchatkensis sp. nov. occurs in depths > 10 m, but appears to be more abundant deeper, at about 20 m or more. The depth around 20 m in the studied region is characterized by a rapid change of benthic fauna that is obviously connected to decreasing temperatures. Even in summer the temperature at and below 20 m is about 2-4°C and appears to be rather constant, while shallower it is



Figures 25-27. *Oscarella kamchatkensis* sp. nov. TEM micrographs of endosymbiotic bacteria. **25.** Bacteria type *a*. **26.** Bacteria type *b*. **27.** Bacteria type *c*.

Figures 25-27. *Oscarella kamchatkensis* sp. nov. Micrographies MET des bactéries endosymbiotiques. **25**. Bactéries de type *a*. **26**. Bactéries de type *b*. **27**. Bactéries de type *c*.

Table 2. Main morphological, anatomical, cytological and ecological characters of valid *Oscarella* species.
Tableau 2. Caractéristiques morphologiques, anatomiques, cytologiques et écologiques principales des espèces valides d'*Oscarella*.

	O. lobularis	***O. tuberculata***	***O. viridis***	***O. microlobata***	***O. imperialis***	***O. stillans***	***O. nigraviolacea***	***O. carmela***	***O. malakhovi***	***O. Kamchatkensis***
Locality	Mediterranean	Mediterranean	Mediterranean	Mediterranean	Mediterranean	Indo-Pacific	Indo-Pacific	N-E Pacific	N-W Pacific	N-W Pacific
Habitat	Vertical walls	Walls, entrance of caves	Semi-obscure caves	Semi-obscure caves	Vertical walls	Vertical walls	Underside of plate coral	Boulders	Bivalve shells, stones	Boulders, rocks
Depth (m)	5 - 15	4 - 35	6 - 15	12 - 15	12 - 20	12	6	Intertidal	0.4 - 5 m	10 - 23 m
Colour	Pink-rose	Variable	Light-green	Light-brown	Yellowish-white	Dark honey yellow	Dark violet, almost black	Light brown to rusty orange	Pinky-beige to yellow	Orange-yellow
Consistency	Soft	Cartilaginous	Very soft, fragile	Soft, fragile	Soft	Collagenous	Soft, limb, mushy	Extremely soft, slimy	Soft, slimy	Soft, slimy
Surface	Smooth	Wrinkled	Rugose	Rugose	Rugose	Very smooth	Inflated, bubbly	Bumpy, microlobate	Lumpy or undulated, microlobate	Lumpy, microlobate
Shape	Thick lobate	Thick lobate	Thin lobate	Thin lobate	Thick lobate	Series of fused thin tubes	Lobate, convoluted	Thin microlobate	Thin microlobate	Thin lobate
Ectosome thickness (µm)	5 - 50	5 - 90	10 - 50	10 - 50	15 - 50	25-30	20-30	5-10	8 - 15	4 - 25
Chamber diameter (µm)	35-90	40-75	30-75	40-75	45-80	17x30	30-40	25-65	12-33	15 - 23
Sylleibid canal system	+	+	+	+	+	?	?	+	+	+
Eurypylous chambers	+	+	+	+	+	?	+	+	+	+
Archaeocytes	Very rare	Common	Common	Very rare	Common	?	?	Common	Rare	Not detected
Vacuolar cells	Two types	One type	No	One type	Two types	?	?	One type	One type	No
Granular cells	No	No	One type	No	No	?	?	One type	One type	Two types
Spherulous cells	No	No	No	Two types	One type	?	?	No	No	One type
Types of bacteria	3	2	2	4	3	?	?	2	2	3
References	Schmidt 1862; Boury-Esnault et al., 1992	Schmidt 1868; Boury-Esnault et al., 1992	Muricy et al., 1996	Muricy et al., 1996	Muricy et al., 1996	Bergquist & Kelly, 2004	Bergquist & Kelly, 2004	Muricy & Pearse, 2004	Ereskovsky, 2006	Present study

typically about 7-10°C or warmer. Several specimens were observed growing on calcareous tubes of serpulid worms (Polychaeta), solitary ascidians (*Dendrodoa aggregata*) and crabs (*Hias* sp.).

Discussion

Among 14 species listed in the World Porifera Database (van Soest et al., 2008) the genus *Oscarella* includes 9 valid species which are known from different oceans around the world (Table 2). Four species occur in the Pacific: two in the Indo-Pacific - *O. nigraviolacea* and *O. stillans* (Bergquist & Kelly, 2004), one species from the Eastern Pacific (California) - *O. carmela* (Muricy & Pearse, 2004), and one - *O. malakhovi* - in the northwestern Sea of Japan (Ereskovsky, 2006). *O. lobularis,* identified by Koltun (1966) from the eastern coast of the former USSR (Bering, Okhotsk, Japan Seas and Pacific coasts of the Kuril Islands) is unrecognizable: firstly, by reason of bad description without any histological and cytological details; secondly, because of inappropriate for cytological investigations fixations (ethanol) of the samples.

The species of *Oscarella* have neither an inorganic skeleton nor an organic one, and they appear homogeneous in histological sections. The differences among species are mostly represented by external traits such as a color, consistency, and aspect of the surface, which are very subjective to describe (Boury-Esnault et al., 1992; Muricy et al., 1996; Muricy & Diaz, 2002; Bergquist & Kelly, 2004; Muricy & Pearse, 2004; Ereskovsky, 2006). That is why more unusual characters, such as the types of cells with inclusions and the morphology of symbiotic bacteria, are critical for species identification. Cells with inclusions are special sponge cells with different cytoplasm inclusions, most of which have unknown functions (e.g. Simpson, 1984). They are abundant and diverse in species of *Oscarella*, and each species has a particular set of such cells (Boury-Esnault et al., 1992; Muricy et al., 1996; Muricy & Pearse, 2004; Ereskovsky, 2006). It is, therefore, important that *Oscarella* specimens are properly preserved for cytological study, e.g. in glutaraldehyde, which allows accurate observation with TEM and identification of cells with inclusions. Molecular methods (e.g., allozyme electrophoresis, DNA sequencing) may also help in the definition of species boundaries in *Oscarella*. Nevertheless, it is important to note, that cytological characters are stable enough during a species' life cycle (Ereskovsky et al., unpubl.).

In comparison with northern Pacific species (*O. malakhovi* and *O. carmela*) *Oscarella kamchatkensis* sp. nov. is unique in its orange-yellow colour. The surface aspects of the sponge (lumpy, smooth, microlobate) are helpful, but the species cannot be separated from others on that basis alone. The new species is encrusting, of irregular shape, with soft, slimy consistency, with lobes being round and irregular. *Oscarella carmela* Muricy & Pearse, 2004 differs from *O. kamchatkensis* sp. nov. by its relatively big oscular tubes and its variability of colour and surface characters: It displays a light brown to tan or dull orange colour and a smooth to microlobate surface. The principal distinction of *O. malakhovi* from the new species is its thinly encrusting shape of 1-2 mm in thickness, and the colour from pinky-beige to yellow.

At cytological level *O. kamchatkensis* sp. nov. differs from *O. carmela* and *O. malakhovi* by the presence of three cell types with inclusions (two granular cells and one spherulous cell with granular paracrystalline inclusions) and by the absence of the vacuolar cells. The archaeocytes present in most *Oscarella* species were not detected in *O. kamchatkensis* sp. nov. This cell type is also very rare in *O. lobularis*, *O. microlobata* and *O. malakhovi*.

Granular cells of *O. kamchatkensis* sp. nov. contain two types of inclusions absent in the cytoplasm of granular cells (Type 1) of *O. carmela.* Granular cells of *O. malakhovi* have oval electron-dense granules that are never present in granular cells of both types in the new species. Microgranular cells of *O. kamchatkensis* sp. nov. are unique and have not any analogous cells in other *Oscarella* species.

Another unusual cell type, characteristic of *O. kamchatkensis* sp. nov. is the spherulous cell with paracrystalline inclusions. Spherulous cells are cells filled with large round spherules that occupy almost the entire cytoplasm (Boury-Esnault & Rützler, 1997). This cell type is very rare in *Oscarella* spp. – it was previously described in only two species: *O. imperialis* and *O. microlobata*. Spherulous cells of both species have paracrystalline inclusions. This cell type is also characteristic for other homoscleromorph species without skeleton - *Pseudocorticium jarrei* Boury-Esnault et al., 1995 - and has never been described in other homoscleromorphs and even in other sponges. This similarity in ultrastructural features of paracrystalline inclusions in these species is interesting and may have implications for the same role of these cells in aspicular Plakinidae (Table 1).

The morphology of symbiotic bacteria is another useful character for species identification in *Oscarella*. Different sponge species from one genus can possess various bacterial morphotypes (Boury-Esnault et al., 1992; Muricy et al., 1996 & 1999; Muricy & Pearse, 2004; Ereskovsky, 2006; Hentschel et al., 2006; Taylor et al., 2007). Symbiotic bacteria have been found in the mesohyl of all species of Homoscleromorpha studied to date, and the morphology of the symbiotic bacterial populations has been shown to be species specific (Boury-Esnault et al., 1992 & 1995, Muricy et al., 1996 & 1999; Vishnyakov & Ereskovsky, 2009). *Oscarella kamchatkensis* sp. nov. has a distinct set

of symbiotic bacteria that includes cells of three morphotypes. According to ultrastructural data the symbionts are Gram-negative with a clear filamentous nucleoid region and a dark granular peripheral layer of cytoplasm. Their shapes and dimensions make it possible to recognize them easily.

Two other species of genus *Oscarella* from the Pacific, *O. carmela* and *O. malakhovi*, have their species-specific sets of prokaryotic symbionts (Muricy & Pearse, 2004; Ereskovsky, 2006). Symbionts of *O. carmela* (types B1 and B2) resemble symbiotic bacteria of *O. kamchatkensis* sp. nov. in morphology and size (morphotypes *c* and *a*, respectively). Nonetheless, differences in cell wall outline for symbionts of B1 in *O. carmela* and *c* types in *O. kamchatkensis* sp. nov. and dissimilar internal content of symbionts B2 (*O. carmela*) and *a* (*O. kamchatkensis* sp. nov.) suggest different bacterial species. Symbionts of *O. malakhovi* differ significantly from those of *O. carmela* and *O. kamchatkensis* sp. nov. (Ereskovsky, 2006).

The absence of skeleton, that provides a main morphological character for sponge taxonomy in most other groups, explains why during 130 years the species *Oscarella lobularis* was considered as the only species of the genus, displaying different morphotypes (cream, yellowish, red, brown, blue, violet, or even green; Topsent, 1895) and consistencies, and having a nearly cosmopolitan distribution. In fact, *Oscarella* appears to be one of the best examples in sponges of a single, "cosmopolitan" species which turned out to be a highly diversified complex of cryptic species (Ereskovsky et al., 2009). At present 14 *Oscarella* species are known. Many of these species were described and redescribed using ultrastructural and genetic methods. Complementary tools are necessary for investigating *Oscarella* species diversity: a combination of molecular markers, biochemical fingerprints, enzyme analyses, occurrence and characters of symbiotic bacteria, and new morphological characters. Therefore, the specific discrimination of an *Oscarella* species can be ensured by different approaches, thus facilitating its recognition by a large scientific community.

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