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Occurrence of five-rayed spicules in a calcareous sponge: *Sycon pentactinalis* sp. nov. (Porifera: Calcarea)

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Abstract: We describe for the first time the presence of five-rayed spicules in a calcareous sponge *Sycon pentactinalis* sp. nov. We named these spicules pentactines, following the names of other calcareous spicules: diactines, triactines and tetractines. The pentactines show an almost simultaneous formation of their actines and behave as single calcite crystals under polarised light. The discovery of this five-rayed spicule contradicts Woodland´s theory of the sclerocyte affinity, illustrating that it is possible to produce spicule mineralization with more than four sclerocytes in extant Calcarea. Several hypotheses for pentactine formation are proposed.

Résumé : *Existence de spicules à cinq actines dans une éponge calcaire :* Sycon pentactinalis *sp. nov. (Porifera : Calcarea).* Nous décrivons la présence de spicules à cinq actines dans l´éponge calcaire *Sycon pentactinalis* sp. nov. Nous avons nommé ces spicules des pentactines, suivant les noms des autres spicules calcaires: diactines, triactines et tétractines. La formation des cinq actines semble quasi simultanée et les pentactines présentent sous lumière polarisée les caractéristiques d'un seul cristal de calcite. La découverte de spicules pentactines contredit la théorie de Woodland sur l'affinité de sclérocytes et montre la possibilité d'une minéralisation stable sous l'activité de plus de quatre sclérocytes chez les éponges calcaires récentes. Plusieurs hypothèses sur la formation des pentactines sont proposées.

Keywords: Calcarea ● *Sycon pentactinalis* ● Pentactine ● Spicule

Introduction

Calcareous sponges (Porifera: Calcarea) are characterized by the presence of calcium carbonate spicules to which a rigid calcareous skeleton may be added (Borojevic et al., 1990). Several categories of spicules have been described in extant Calcarea. Nevertheless, these categories are mere variations of the basal diactine and triactine plans, resulting in only three kinds of spicules in both subclasses Calcinea and Calcaronea: diactines, triactines and tetractines. Ontogenetically, triactines are the first spicules to be produced by the Calcinea, whereas the Calcaronea form

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diactines first (Manuel et al., 2002). Diactines have a rod shape, but represent two actines formed in opposite directions from the initial nucleation site. Conversely, triactines that are found in the vast majority of calcareous sponges have the three axes laid in the same plane. The majority of calcareous sponge body-plans clearly corresponds to a tubular organization, being simple in the asconoid ones, and generating ramified or anastomosing structures in the more complex syconoid or leuconoid ones. The plane of the basal triactine spicule is laid within the plane of the tube wall. The fourth actine of tetractines is perpendicular to the basal triactine system. The tube wall is asymmetrical: the luminal side of the tubes harbours choanoderm or pinacoderm of the exhalant system, whilst the abluminal side stays in contact with the external pinacoderm or the mesohyl. The two sides of the triactine-system in the tube wall are thus not equivalent. The fourth actine grows on the luminal side only, and never on the abluminal side of the spicule. Hence, the fourth actine penetrates into the central cavity delimited by the tube, corresponding to the choanocoel or to the lumen of the exhalant cavities such as excurrent canals and atrium.

Calcareous spicules are produced inside an intercellular space delimited by a number of cells that corresponds to the number of actines of the spicule to be secreted. Proteins secreted into this space guide the formation of the spicule calcite crystal (Ledger, 1975; Ledger & Jones, 1977; Aizenberg et al., 1995; Aizenberg et al., 2003).

In the present study, five-rayed spicules (pentactines) are found to occur for the first time within the extant Calcarea, and a new species of *Sycon* is described.

Materials and methods

Specimens of *Sycon pentactinalis* sp. nov. were collected by SCUBA in São Sebastião (São Paulo State, Brazil; 23°49.65'S, 45°25.36'W). They were fixed and preserved in 70% ethanol.

Preparations of spicule slides and sections followed standard procedures (Wörheide & Hooper, 1999). Mounting medium was Entellan (Merck, Brazil).

For micrometric analyses, the width at the base of each actine and the length from tip to base was measured. Whenever the length of the basal actines varied, the largest actine was measured. Pentactine measurements were restricted to the largest and the shortest actines, since it was not always possible to further categorize the actines. Measurements were made using an ocular micrometre. The results are presented in tabular form, featuring length (minimum, mean, maximum and standard deviation – s.d.), width (mean and s.d.) and the sample size (n) (Table 1). Photo-micrographies were taken with a digital camera using a Zeiss Axioscope microscope.

Scanning electron microscopy was performed in a Jeol 5310 microscope. Digital images were acquired using the SEMA software. Samples were also observed in a Zeiss-Axioplan light microscope, under the bright field and polarization light modes.

Results

Systematics

Class CALCAREA Bowerbank
Subclass CALCARONEA Bidder
Order LEUCOSOLENIDA Hartman
Family SYCETTIDAE Dendy
Genus *Sycon* Risso

Type species – Sycon humboldti Risso, 1826 (by subsequent designation; Dendy 1892)

Sycon pentactinalis sp. nov.

Institutional abbreviation. MNRJ - Museu Nacional do Rio de Janeiro, Brazil. CEBIMAR - Centro de Biologia Marinha, Universidade de São Paulo (USP), São Sebastião, São Paulo, Brazil.
Type material. MNRJ 1716 (holotype/alcohol); MNRJ 9646, MNRJ 9647, MNRJ 9648, MNRJ 9649 (paratypes/slides). Collected by F. L. Silveira (1997), 2 m depth.
Etymology. Derived from the presence of pentactines.
Type Locality. CEBIMAR, São Sebastião, São Paulo, Brazil (23° 49.65' S, 45° 25.36' W).

Diagnosis

Sycettidae with radial tubes partially or fully coalescent; distal cones are decorated by tufts of diactines. The inhalant canals are generally well defined between the radial tubes and are often closed at the distal end by a membrane that is perforated by ostia, devoid of a skeleton. There is no continuous cortex covering to the distal ends of the radial tubes. Skeleton of the atrium and of the tubes is composed of triactines, tetractines and/or pentactines (*emend*. from Borojevic et al., 2002a).

Morphology

The specimens were white in life and in alcohol. The largest specimen is 0.9 cm long and 0.5 cm wide (Fig. 1). The radially organized body is very fragile.

The osculum is apical, surrounded by a simple crown of trichoxeas of only one type, supported by an organized skeleton of sagittal triactines disposed parallel to each

Table 1. *Sycon pentactinalis* sp. nov. Measurements of the spicules of the holotype MNRJ 1716.
Tableau 1. *Sycon pentactinalis* sp. nov. Mesures des spicules de l'holotype MNRJ 1716.

Spicule	Actines	Length (µm) min	mean	s.d.	max	Width (µm) mean	s.d.	n
Diactines		392.0	486.9	67.6	640.0	9.6	3.3	30
Triactines of the cone	Paired	48.0	98.5	21.4	128.0	4.5	1.4	30
	Unpaired	48.0	94.0	16.4	120.0	5.2	1.9	30
Tubar triactines	Paired	48.0	97.2	20.4	140.0	4.5	1.4	30
	Unpaired	60.0	130.3	26.3	180.0	5.9	2.0	30
Tubar tetractines	Paired	40.0	96.8	24.6	144.0	5.6	2.0	30
	Unpaired	72.0	131.7	29.7	200.0	6.8	1.9	30
	Apical	8.0	23.9	7.9	40.0	4.4	1.2	30
Tubar pentactines	Shortest	4.0	28.4	26.0	84.0	4.9	1.8	10
	Largest	60.0	106.8	23.2	132.0	5.2	1.9	10
Subatrial triactines	Paired	64.0	103.1	18.3	140.0	6.3	2.0	30
	Unpaired	68.0	132.8	28.4	196.0	6.4	2.0	30
Atrial tetractines	Paired	36.0	101.7	32.9	160.0	6.4	2.0	30
	Unpaired	20.0	77.5	48.2	180.0	6.5	2.0	30
	Apical	20.0	50.3	13.6	80.0	7.7	1.8	30

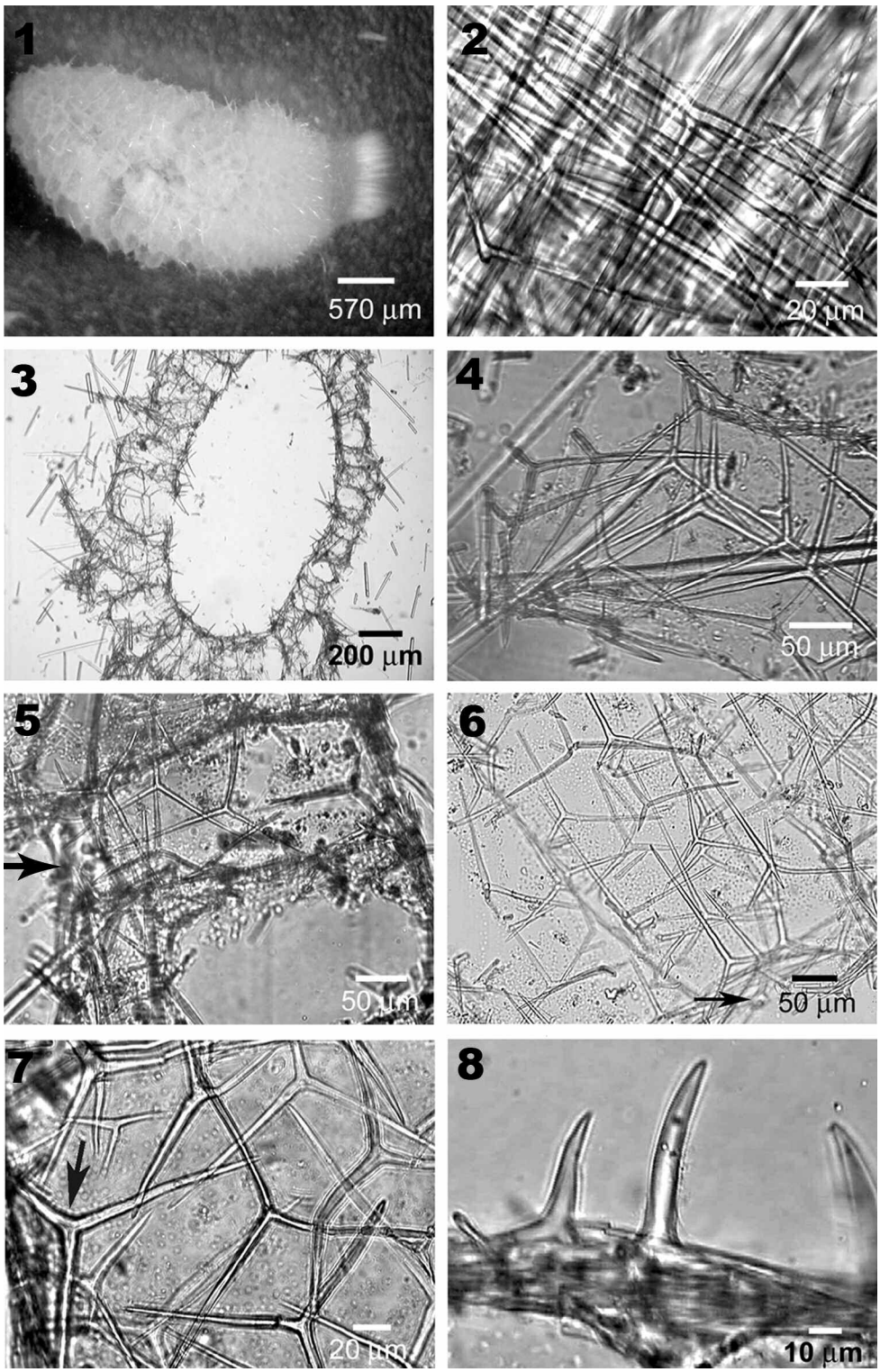
other, with the unpaired actine directed towards the basal region (Fig. 2). A suboscular region is present between the base of the crown and the first choanocyte chambers. At this region, the sagittal triactines are gradually substituted by disorganized and contorted triactines, tetractines and pentactines. The atrium is central and large, with a very thin wall (Fig. 3). The radial tubes are coalescent, with small tufts of 5 to 10 diactines, protruding through the distal cones (Fig. 4). The proximal side of diactines occasionally reaches close to the atrial skeleton. The maximal angle between the diactines and the axis of the cones is approximately 45°. The cones are supported by triactines that are highly variable in shape, sometimes becoming similar to pseudosagittal triactines. Their longer paired actine, however, is not necessarily directed centripetally, and they do not form a distinct layer of spicules in the distal part of the tubar skeleton, such as observed in Heteropiidae. The distal parts of the cones are supported by the unpaired actine of triactines, which never crosses the surface. The inhalant canals between the tubes are closed by a thin membrane, sometimes supported in part by actines of spicules of the distal cones. The canals cross the entire width of the wall in regions in which the wall is thin (Fig. 5). The tubar skeleton contains several spicules that range from the atrial side to the distal cone. Although it is not organized in regular rows of tubar triactines, such as observed in many other *Sycon* species, it keeps a preferential orientation, reminiscent of an articulate choanoskeleton. The tubar skeleton is composed of triactines, tetractines and pentactines with variable shapes and contorted actines (Fig. 6). In the tube walls, triactines and the actines of the triradiate basal system of tetractines and pentactines lay inside the wall, projecting only the apical actine(s) i.e. the fourth/fifth actines (of the pentactines) into the tube lumen. It is also common to observe triactines that sustain the wall between the tubes with their paired actines, and project the unpaired actine into the tube lumen. The apical actine of the tetractines and occasionally the fourth and/or the fifth actines of the pentactines are curved towards the distal cones. The subatrial skeleton is composed of sagittal triactines and very few tetractines that project the unpaired actine, which is longer than the paired ones, towards the distal cones (Fig. 7). These spicules are easily differentiated by their sagittal shape and by their actines that are straight and not contorted. The atrial skeleton is thin (approximately 20 µm), composed of large sagittal tetractines, disposed side by side, and few triactines (Fig. 8). Their apical actines are straight or curved. These actines are frequently thicker than the basal ones and they are projected into the atrium. Triactines are the most abundant spicules in *Sycon pentactinalis* sp. nov.

Spicules

Trichoxeas of the perioscular crown: The diametre of these thin spicules reaches only 3 µm, and their length could not be accurately determined since they were always broken.

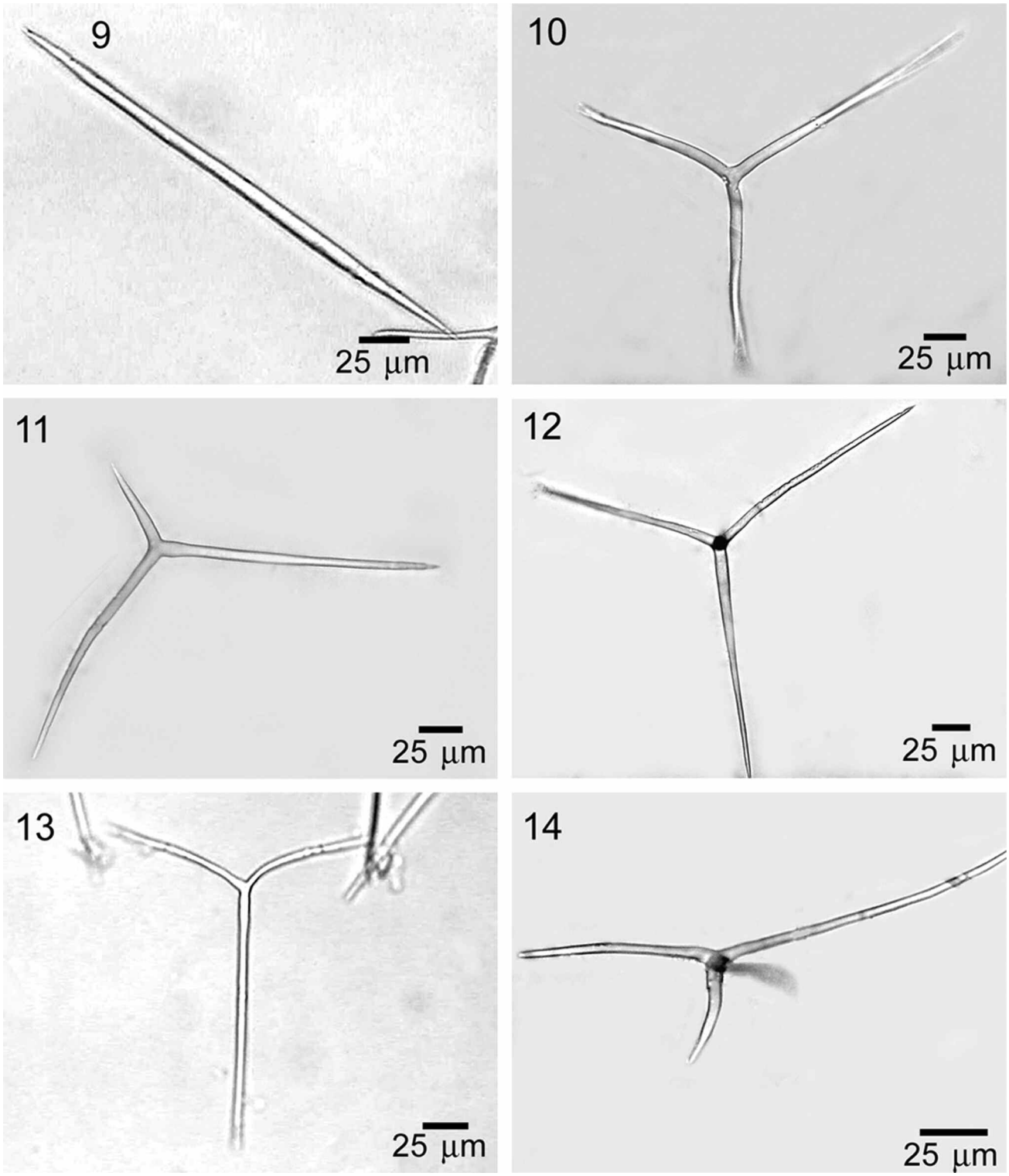
Diactines of the distal cones: These spicules vary from giant to common size diactines. They are straight and fusiform, with sharp tips (Fig. 9). Tufts of spicules are anchored in the distal cones and penetrate into the choanosome, sometimes almost reaching the atrium.

Triactines of the distal cones: Their shape is almost regular (equiradiate and equiangular), sometimes looking



Figures 1-8. *Sycon pentactinalis* sp. nov. **1.** External morphology of preserved holotype. **2.** Detail of the skeleton below the osculum. **3.** Cross-section of a specimen. **4.** Detail of a distal cone. **5.** Radial tubes and an inhalant canal (arrow). **6.** Articulated tubar skeleton (arrow pointing to the atrium). **7.** Subatrial skeleton (arrow). **8.** Atrial skeleton showing apical actines projected into the atrium.

Figures 1-8. *Sycon pentactinalis* sp. nov. **1.** Morphologie externe, holotype préservé. **2.** Détail du squelette sous l'oscule. **3.** Coupe transversale d'un spécimen. **4.** Détail du cône distal. **5.** Tubes radiaires et canal inhalant (flèche). **6.** Tubes radiaires avec squelette articulé (la flèche indique l'atrium). **7.** Squelette subatrial (flèche). **8.** Squelette atrial avec les actines apicaux projetées dans l'atrium.



Figures 9-14. *Sycon pentactinalis* sp. nov. **9.** Diactine of the distal cones. **10.** Triactines of the distal cones. **11.** Triactine of the tubar skeleton. **12.** Tetractine of the tubar skeleton. **13.** Subatrial triactine. **14.** Atrial tetractine.

Figures 9-14. *Sycon pentactinalis* sp. nov. **9.** Diactine des cônes distaux. **10.** Triactines des cônes distaux. **11.** Triactine des tubes radiaires. **12.** Tétractine des tubes radiaires. **13.** Triactine subatrial. **14.** Tétractine atrial.

like pseudosagittal spicules. Their actines are straight or curved, and their shape is slightly conical. Their paired actines are frequently curved, accompanying the shape of the distal cones (Fig. 10).

Tubar triactines: These spicules are, as all other spicules of the choanosome, very irregular, and even contorted. Actines are slightly conical and sharp. They vary from almost regular (equiradiate and equiangular) to sagittal. Sometimes, one of the paired actines is shorter than the other, and the unpaired actine is even shorter and curved. It frequently penetrates into the tubes (Fig. 11).

Tubar tetractines: These spicules are similar to the tubar triactines, except for the presence of the apical actine. The apical actine is sharp, curved and shorter than the basal ones. It penetrates into the tubes (Fig. 12).

Tubar and suboscular pentactines: These spicules are not abundant. They have a highly variable shape and are very irregular and contorted. Their basal triactine system is similar to tetractines, but they develop a fifth actine. In some pentactines, the two apical actines share side-by-side their origin, on the luminal side of the spicule, being both anchored at the centre of the triactine system. The fourth and the fifth actine are in this case placed lateral to the axis of the unpaired actine, bifurcating from the spicule centre and following approximately the direction of the paired actines in an angle smaller than 90°, which is typical for the fourth actine of tetractines (Figs 15 & 16). Conversely, in some pentactines, the five actines rise from the spicule centre apparently in random directions, and the basal triactine system cannot be recognized anymore (Figs 17 & 18). In this case, they acquire the form of "polyactines", with low restriction of the direction of actine growth from the spicule centre (Fig. 17).

Subatrial triactines: These spicules are strongly sagittal, sometimes with a curve between the paired actines. The unpaired actine is always longer than the paired ones, pointing towards the distal cones. Their actines are straight with sharp tips (Fig. 13).

Atrial tetractines and triactines: These tetractines are larger and thicker than the tubar tetractines. They are strongly sagittal and their apical actine penetrates the atrium. The apical actine is conical, sometimes curved, smooth and sharp and it is frequently thicker than the basal actines (Fig. 14). Triactines are also present. They are strongly sagittal and have the unpaired actine much shorter than the paired ones.

Polarizing Light Microscopy and SEM

Under the polarizing light microscope, all regions of the pentactines, as well as of the other spicule types, presented the same maximum intensity of transmitted light and extinction positions, indicating that the spicule is a monocrystal (Figs 19 & 20). SEM analyses illustrate that the junction between the actines has no discontinuities (Fig. 18).

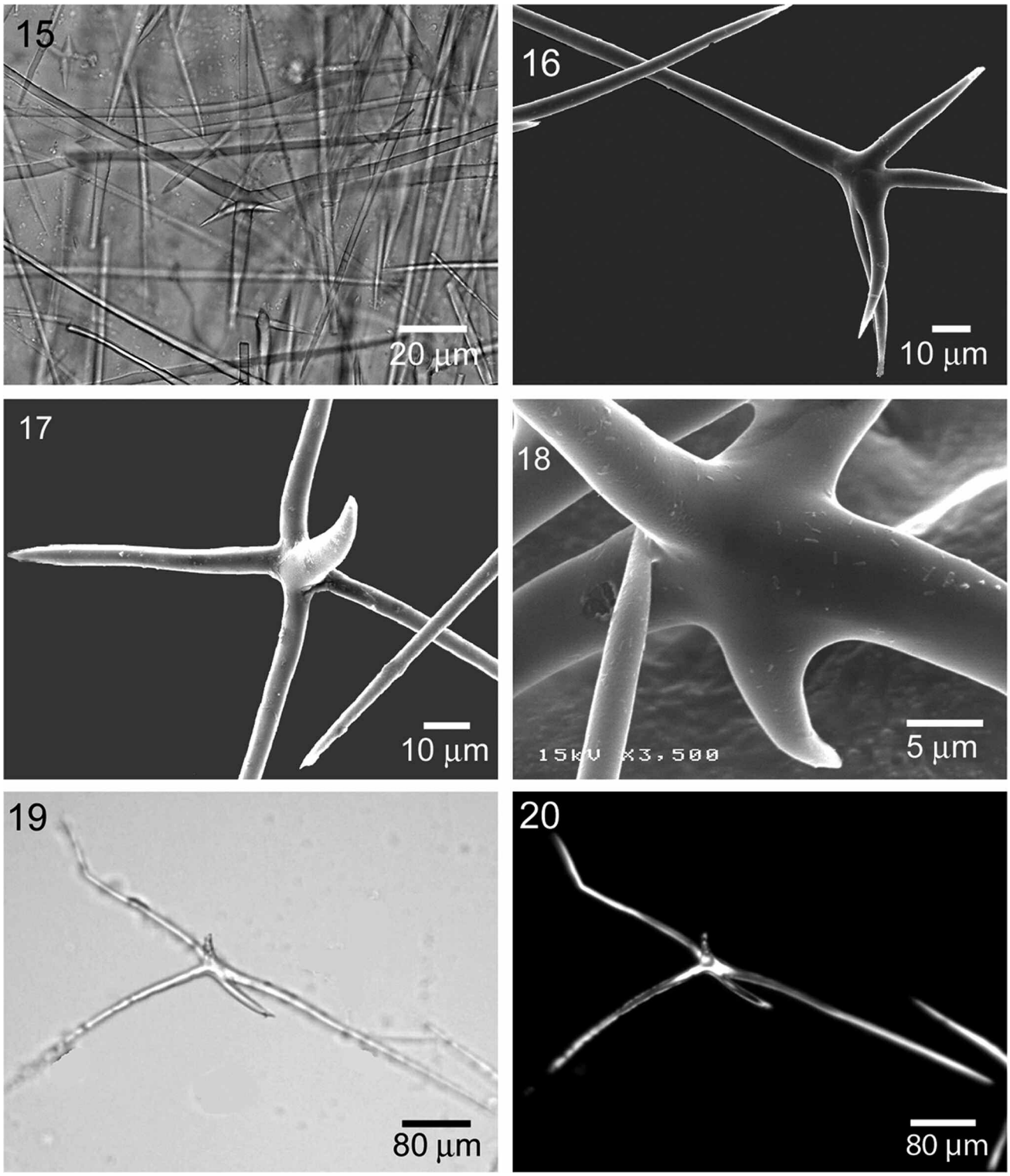
Ecology

The water temperature in São Sebastião ranges from 13 to 30°C, and the warm waters are preponderant. Hydrodynamics are weak, but they become stronger when S/SW wind blows. The five specimens of *Sycon pentactinalis* sp. nov. were collected in a rocky substrate almost vertical, protected from direct light, in a depth of 2 m.

Remarks

All specimens were found in the same locality. Although they were not apparently linked by stolons, neither had a common basal region, this does not preclude that they may belong to a single clone. Similar specimens were not collected at other periods, and they were not found in other localities, preventing a more extensive study of the variability within the species. No other *Sycon* species similar to the here described one is known, even neglecting the presence of pentactines, but this may be due to our poor knowledge of South Atlantic Calcarea. Only four species of *Sycon* have been referred to Brazil: *S. ampulla* (Haeckel, 1872), *S. brasiliense* Borojevic, 1971, *S. vigilans* Sarà & Gaino, 1971 and *S. frustulosum* Borojevic & Peixinho, 1976. *S. brasiliense* and *S. frustulosum*, which were originally described from Brazil (Borojevic, 1971, Borojevic & Peixinho, 1976), differ from *S. pentactinalis* sp. nov. mainly by the shape of diactines. In *S. pentactinalis* sp. nov., diactines are fusiform, while they are lanceolate in *S. brasiliense*, and jagged in *S. frustulosum*. *S. ampulla* has been previously reported from Brazil by Haeckel (1872). It differs from our species by the size of spicules and absence of tetractines forming the tubar skeleton. The specimens cited to the Brazilian coast by Borojevic & Peixinho (1976) as *S. vigilans* may be distinguished from *S. pentactinalis* sp. nov. by the spicules size, by the presence of tetractines in the subatrial skeleton and of a stalk.

We find convenient to describe the present set of specimens as a new species, in order to draw attention to the particularity of pentactines, but further studies will be required to set up the limits of this species. The same is true for its attribution to the genus *Sycon*. Recent molecular studies have suggested that *Sycon* may be a polyphyletic genus (Manuel et al., 2004). This is a plausible proposal, since *Sycon* is a very broad genus, defined by several negative characters (absence of cortex, absence of a distinct row of pseudosagittal spicules in a distal part of radial tubes, absence of chiactines, etc.), but there is at present no consistent proposal based on other criteria for better definition of the scope of this genus.



Figures 15-20. *Sycon pentactinalis* sp. nov. **15.** Pentactine with a bifurcated apical actine (Light Microscope). **16.** Pentactine with one longer actine (SEM). **17.** Pentactine with four facial actines and a fifth apical actine (SEM). **18.** Detail of the centre of a pentactine in SEM. **19.** Bright field image of a pentactine. **20.** Pentactine in a Polarizing Light Microscope.

Figures 15-20. *Sycon pentactinalis* sp. nov. **15.** Pentactine avec l'actine apicale bifurquée (Microscope optique). **16.** Pentactine avec une actine plus longue (MEB). **17.** Pentactine avec quatre actines faciales et une cinquième actine apicale (MEB). **18.** Détail du centre de la pentactine au MEB. **19.** Microscopie en champ clair d'une pentactine. **20.** Pentactine au Microscope sous lumière polarisante.

Discussion

Physical processes involved in the formation of calcareous spicules were described in detail by several authors (Minchin, 1898a & b; Jones, 1967; Ledger & Jones, 1977). Nonetheless, it remains unclear why extant calcareous sponges could not secrete spicules with more than four actines. In siliceous sponges, the production of spicules begins and when spicules are small enough is completed inside the sclerocytes. Their form and ornamentation are guided by the central proteinaceous filament produced by each sclerocyte before mineralization. Conversely, calcareous spicules are produced inside a closed intercellular space, delimited by several sclerocytes. The proteins that guide spicule formation in Calcarea are thus secreted into this space, and are a product of the synthetic activity of several cells. Although calcareous spicules do not show a central organic filament, *in vitro* studies have shown that the proteinaceous component of calcareous spicules is both required and sufficient to confer to the spicule its typical form (Aizenberg et al., 1995; Aizenberg et al., 2003). This information is coded in the species genome, and a complex regulatory network is probably at play in controlling expression of these genes.

Simultaneously, the spicule form in Calcarea is rigorously dependent upon its position in the sponge wall. This means that either the expression of genes that guide spicule formation is determined by positional information, or by epigenetic modifications of proteins secreted into the intercellular space dependent upon local conditions or physical constraints. Jones (1961) has already described that the angle of tubar triactines and tetractines in *Leucosolenia* is dependent upon the spicule-position in the tube, corresponding potentially to the local structural tension, being thus controlled by epigenetic phenomena. Nevertheless, these were only form variation of the same spicule type in an asconoid tube. In more complex Calcarea, each different spicule type has a very well defined position in the sponge body. Recently, presence of genes that are associated in higher animals with the positional information has been described in Calcarea, but their relationship with the spicule form, or even their general function in Calcarea are at present unknown (Manuel & Le Parco, 2000).

It is common knowledge that calcareous sponges produce triactines using simultaneously one sclerocyte for each actine, which move from the central to apical position along the nascent actines. In addition, the apical actine of the tetractines is secreted by the fourth sclerocyte that arrives when basal actines have already started to be secreted, and a space is available at the central point of the triactine system to anchor the fourth actine (Minchin, 1898a). The abluminal central side of the triactine system is apparently not available, since no actine is formed in this direction, and the reasons for this restriction are at present unknown.

Since tetractines behave as a single calcite crystal, the nucleation point of the three actines that form the basal triactine system must be used also for nucleation of the fourth actine. Woodland (1905) proposed that this type of secretion is directly related to the sclerocytes affinity. Accordingly, up to four cells would work together in order to produce spicule mineralization with stability in extant Calcarea, although the fourth cell must wait until the other three cells start to secrete their actines. Theoretically, the impossibility to form a fifth actine could be a matter of impediment in the sclerocytes affinity. We believe, however, that the impediment to form higher numbers of actines is more likely to physical than to affinity mechanisms. If the apical actine can be formed because the fourth sclerocyte arrives when there is enough space for it at the centre of the spicule, the presence of a fifth or even more actines could be possible when this space is free, provided the availability of the crystal nucleation origin.

The same maximum intensity of transmitted light and extinction positions were observed in all actines of pentactines under the polarizing light microscope (Figs 19 & 20). This means that pentactines behave as single crystals under crossed polarizers. Moreover, the absence of discontinuities in the joint between actines indicates that they are formed from the same nucleating origin (Fig. 18). However, different from triactines and tetractines, pentactines do not have a standard spatial morphology or even a regular geometry. This fact may be a consequence of a less stringent control of the use of the spatial centre and of the nucleation point for additional actines, including the potential use of the abluminal side of the centre as observed in pentactines with the polyactine morphology (Fig. 17). This may be perceived as a "loss of function" rather than a "gain of function", since the restrictions associated with the use of the nucleation point are looser in pentactines than in other spicules. Apparently, this is not a consequence of a general modification or mutation of proteins that guide spicule formation in the space delimited by sclerocytes, since in *Sycon pentactinalis* sp. nov. the subatrial spicules have a regular geometry similar to all other Calcaronea. The spicule form follows thus the positional information, with loose restriction of the nucleation point in the tubar choanoskeleton and more rigorous restriction in the subatrial and atrial regions, with exception of the suboscular atrial skeleton. The paucity of extant Calcarea with such a loss of strict spatial organization of spicules may be related to the fact that it is unlikely that such spicules may be used as modules for constructing a highly organized skeleton such as that present in *Sycon*, generating a selective pressure against formation of irregular spicules with more than four actines in Calcarea.

Differently of what occurs in extant Calcarea, pentactines are frequent in Hexactinellida and in fossil Heteractinida (Pickett, 2002). A recent study of Eiffeliidae has proposed that these two groups represent a common root from which both siliceous and calcareous sponges have evolved (Botting & Butterfield, 2005). In these groups, pentactines are derived from typical hexactine spicules that have one of their actines much reduced or rudimental, and the remaining actines are arranged in three mutually perpendicular planes. In *S. pentactinalis* sp. nov., pentactines are derived from triactines that are laid in the same plan, while the fourth and the fifth actines are produced into the tubar lumen. Alternatively, as shown in the present study, they may acquire the form of polyactines, in which the nucleation spicule centre can provide condition for forming five or more actines. Such spicules are frequent in the Wewokellidae family of Heteractinida. Admitting now that polyactines can be derived from spicules such as observed in extant Calcarea, the genus *Wewokella* seems to be close to the order Baerida (Borojevic et al., 2002b). Besides polyactines, it is characterized by a typical internal reticulate skeleton composed of large triactines, with small tetractines that have the typical basal triactine system in one plane, and the apical fourth actine similar to pugioles, as well as a thick cortex composed of small spicules, typical of several Baeridae (Pickett, 2002). These characteristics are fully compatible with Baerida, and the relationship between the genus *Wewokella* and Baerida should be further studied.

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