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# A NEW SPECIES OF *HALISCARCA* (PORIFERA, DEMOSPONGIAE) FROM THE CARIBBEAN, WITH REMARKS ON THE CYTOLOGY AND AFFINITIES OF THE GENUS.

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ABSTRACT

A new *Halisarca, H. caerulea n. sp.*, is described and its ultrastructural characters are compared with those of *H. dujardini* Johnston and *H. ectofibrosa* Vacelet *et al.* These sponges seem to be characterised at the ultrastructural level by a granulo-fibrillar matrix in which are embedded tight bundles of collagen fibrils and the cellular elements. This matrix originates from disintegration of spherulous cells. Although a somewhat similar structure has been observed in the family Chondrosiidae, the affinities of the Halisarcidae remain uncertain.

## INTRODUCTION

Difficult taxonomic problems are encountered when dealing with sponges without a skeleton, as the conventional basis of species distinction is here lacking. At a high taxonomic level, the affinities of these sponges may be clear when they have close relatives with a skeleton. This is the case with genera such as *Oscarella* within the Homosclerophorida or *Hexadella* within the Ceractinomorpha Dendroceratida. A more puzzling situation is found when these sponges have no obvious affinities with sponges with a skeleton. Thus, the Chondrosiidae may be classified in the Tetractinomorpha as *incertae sedis* (Lévi, 1973), or within the Hadromerida (according to the presence of asteroid spicules in *Chondrilla* and *Chondrillastra)* (Bergquist, 1978), or within the Astrophorida (Simpson *et al*, 1985). The Halisarcidae, considered as a monogeneric family, are classified in the Ceractinomorpha Dendroceratida "only because there is no clear indication of any other affinities" (Bergquist & Wells, 1983). In these sponges, characterized by long tubular, branched choanocyte-chambers, the ectosome structure, the cytology and the reproductive features allowed the distinction of closely related species, which are almost impossible to distinguish by the usual criteria (Topsent, 1893; Lévi, 1956; Chen, 1976), but did not indicate the affinities of the family at the ordinal or even subclass level. Chemotaxonomic investigations did not provide more information on their relationships (Bergquist & Wells, 1983), but showed that they have no affinities with the Aplysillidae, a family of the Dendroceratida to which they have often been compared.

In the present paper, we will try, while describing a new species in *Halisarca,* to investigate if an ultrastructural study could provide other criteria at the specific level and clear up the problem of the evolutionary relationships of the family Halisarcidae.

## MATERIAL AND METHODS

The sponges were collected by SCUBA diving during field trips in the French Antillae ("missions Corantilles" in 1981 and 1983-84) for *H. caefulea* n.sp. and in New Caledonia (ATP PUD no. 3393 of CNRS) in 1978 for *H. ectofibrosa.* Specimens of *H. dujardini* were fixed by Dr. W. C. Jones in Great Britain (Menai Straits) and *Chondrillastra* sp. was collected in Jeddah in the Red Sea (Mission Océanographique Française au Moyen Orient).

The fixations were made in 10% neutral formalin and the specimens were then kept in spirit. Some histochemical tests (PAS – Alcian blue at pH2, toluidine blue at pH 4.5) were performed on histological sections. For electron microscopy, the fixations were made in 2.5% glutaraldehyde in sea water for 20 to 24 h. The specimens were then rinsed in sea water, post-fixed in osmium tetroxide (2% in sea water) for

2h and embedded in araldite.

## RESULTS

Description of *Halisarca caerulea* n.sp. Holotype: Museum National d'Histoire Naturelle, Paris, no. L.B.I.M.D. JV47. Type locality: Martinique, baie of Fort de France (Gros Ilet), 5 m, coll. J. Vacelet.

Localities: This sponge is fairly common in Martinique and Guadeloupe, between 2 and 15 m, on subvertical surfaces such as dead corals, mostly in pure and open water conditions, but also in lagoons or in a muddy environment.

— Guadeloupe:— Grand Cul de Sac Marin, outer reef (15m, 14/3/1981 and 9/1/1984), lagoon (6m, 10/1/1984).

- Sainte Anne (12m, 17/4/1984).

— Martinique:— Baie of Fort de France, Gros Ilet (3 - 9m in a muddy environment, 22/12/1984, holotype).

- Pointe Burgaus (3m, 2/1/1984)
- Pointe Vetiver (3 m, 5/1/1984)
- Anse Figuier (2-3 m, 24/12/1983)
- Sainte Luce (15 m, 25/12/1983)
- Ilet Madame on the east coast (9 m, 3/1/1984)

Description (fig. 1-11): Thinly incrusting sponge, 1.2 to 1.5 mm in thickness. The largest observed specimen covered a surface of about 50 cm by 25 cm. Surface smooth, somewhat slimy. Consistency fleshy. Colour: all the living specimens were of a very distinctive blue, varying according to underwater photographs from 20 A 7 to 21 A 7 of the Methuen Handbook of Colour (Kornerup & Wanscher, 1983). The sponge is yellowish grey in spirit. The surface is covered by a thin, resistant skin, which is detachable. This skin is reinforced by a regular reticulation of bundles of collagen fibrils (Fig. 3), 40 to 50 $\mu$m thick, which gives a trellis-like appearance. The meshes are about 50 $\mu$m in diameter, and one ostiole, 10 to 15 $\mu$m in diameter, is often visible in the centre. Spherulous cells are accumulated in the meshes and under the skin. The oscula are about 2.5 to 3.5 mm in diameter (according to measurements made on underwater photographs). Each osculum is the centre of a conspicuous star-shaped canal system, composed of short, branching superficial canals. These systems are very regularly distributed at the surface of the sponge and separated from each other by a smooth area (fig. 1). After fixation, the oscula are not visible, but the star-shaped canal systems are still apparent, although contracted.

Histology and cytology (fig. 2-11): The cytological organisation is typical of the genus. The sponge is basically composed of a fibrous cortical layer and of a choanosome with canals, elongated choanocyte chambers and bundles of collagen fibrils.

All the components of the tissue, i.e. bundles of collagen fibrils, choanocyte chambers, spherulous cells and other cells, are dispersed in a ground substance, which is made of aggregates of a granulo-fibrillar material. This matrix, which never contains isolated collagen fibrils, displays a strong metachromasy when stained by toluidin blue and is stained by Alcian blue. It is probably made of glycosaminoglycan. This ground substance is of variable density according to the area of the sponge. The maximum density is observed at the transitional zone between the cortex and the choanosome, where are located most of the degenerating spherulous cells which will be described later.

The cortex is bordered on its outer side by a thin exopinacoderm, 0.2 to 0.3 $\mu$m in thickness, made of T-shaped pinacocytes. This cortex, which is resistant, is 25 to 80 $\mu$m in thickness. It is made of an interlacement of bundles of collagen fibrils which, in surface view, gives it its trellis-like appearance. The bundles are mostly acellular, with the exception of some star-shaped collencytes. The whole cortex is crossed by prolongations of T-shaped pinacocytes (Boury-Esnault, 1973), the cell bodies of which are located at its inner limit. Spherulous cells are remarkably abundant at the junction between the cortex and the choanosome, and may appear as a continuous layer in surface view of the detached cortex.

The choanocyte chambers are tubular and sometimes branched. Their maximum length, according to sections, is at least 200 $\mu$m. They are about 25 $\mu$m in diameter. The choanocytes are small, irregularly shaped cells, 4-5 $\mu$m high and 2.5 -3 $\mu$m wide, with a nucleus 2 $\mu$m in diameter. The nucleus often displays a small nucleolus, 0.5 $\mu$m in diameter. A cytoplasmic expansion is often visible on each side of the collar. The basal part of the cell is frequently anchored by long pseudopodia in the low density matrix which extends between the chambers and the collagen bundles. The apopyle seems to be bordered by an apopylar cell (Boury-Esnault *et al.*, 1984), which is difficult to recognize on sections. This cell is intermediate between choanocytes and the endopinacocytes of exhalant canals. It is more flattened than choanocytes, but possesses a flagellum and a fringe of microvilli on the side toward the chamber. The cytoplasmic inclusions are similar to those of choanocytes.

The canals are lined by non flagellated, fusiform endopinacocytes, containing numerous



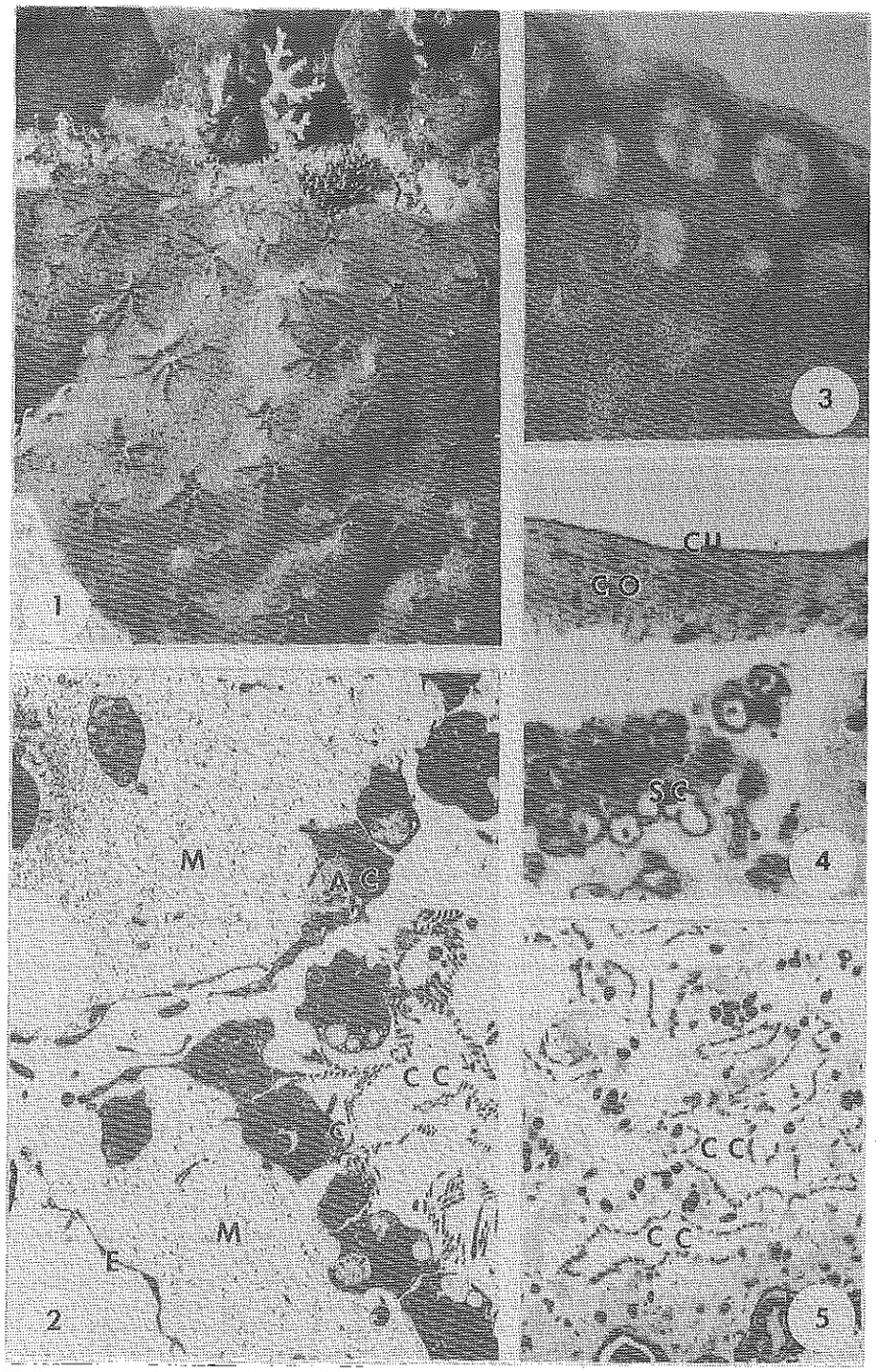


Fig. 1 — *Halisarca caerulea n. sp. in situ.* Note the star-shaped exhalant systems and the trellis-like appearance of the surface. Approximately X 0.75.
Fig. 2 — Choanpcyte chamber (CC) with choanocyte (C), apopylar cell (AC) and endopinacocyte (E). M: Matrix or ground substance. X 3,200.
Fig. 3 — Superficial skin, with a reticulation of bundles of collagen fibrils. X 188.
Fig. 4 — Semi-thin section. CO: cortex: CU: cuticle: SC: degenerating spherulous cells. X 470.
Fig. 5 — Semi-thin section in the choanosome. CC: choanocyte chamber. X 188

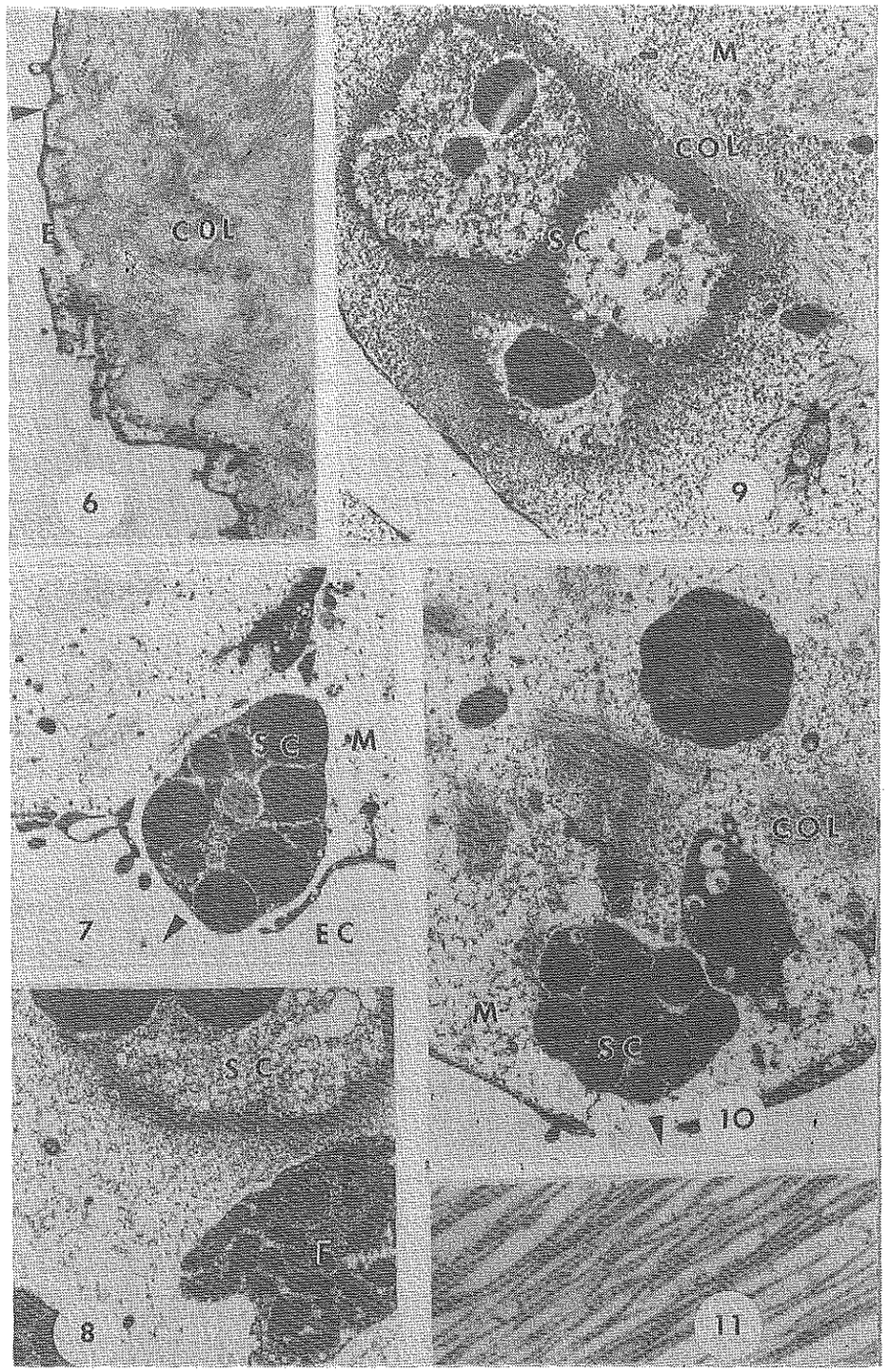


Fig. 6 – Endopinacoderm. E: endopinacocyte: COL: bundles of collagen fibrils: arrow: matrix. X, 4,500.
Fig. 7 – A spherulous cell (SC) extruded into an exhalant canal (EC). M: matrix. X 5,600.
Fig. 8 – A disintegrating spherulous cell (SC). F: fuchsinophil cell. X. 6,840.
Fig. 9 – Disintegrating spherulous cells (SC). COL: bundles of collagen fibrils: M: matrix. X 4,500.
Fig. 10 – A spherulous cell (SC) extruded into an exhalant canal. COL: bundles of collagen fibrils: M: matrix. X 4,500.
Fig. 11 – Collagen fibrils. X 85,000.



phagosomes like the choanocytes. The lateral expansions of the pinacocytes, 0.3 to 0.5 $\mu$m thick, develop small cell processes toward the lumen of the canal, and are anchored in the matrix by pseudopodia (fig. 6). Canals are usually surrounded by large collagen bundles, which are separated from the pinacoderm by a more or less developed area of granulo-fibrillar matrix.

Cells with inclusions are present in large numbers. Some of them (fig. 8), which are not very common, possess elongated, heterogeneous inclusions, 1.5 /0.5 $\mu$m: their cytoplasm is well developed and contains mitochondria and a Golgi apparatus. These cells, which measure 6 to 8 $\mu$m, with a nucleolate nucleus, 1.8 to 2.8 $\mu$m in diameter, seem to correspond to the fuchsinophil cells described by Lévi (1956) in *Halisarca dujardini*.

True spherulous cells are more abundant. They are about 12 $\mu$m in diameter, with a nucleolate nucleus of 2$\mu$m. Their cytoplasm is filled by large, round inclusions, 3 to 4 $\mu$m in diameter, which are dense and homogeneous. The inclusions are not dissolved during paraffin embedding after formalin fixation, and are strongly PAS positive. These spherulous cells seem to have two different fates. Some of them, which have a non degenerative appearance, are extruded into the exhalant canals (fig. 7, 10) through gaps between the endopinacocytes. Others disintegrate into the tissue, and apparently discharge the components of the granulo-fibrillar matrix (fig. 4, 8, 9). This interpretation is sustained by a number of intermediate stages between normal spherulous cells and entirely disintegrated cells surrounded by a dense layer of matrix. Some cells display, side by side, intact, dark spherules and a spherule formed by a granular material which seems to be extruded into the neighbouring tissue. The cell membrane has disappeared in this place, but is still marked by a denser area of the granular material (fig.8). Most often, the whole cell undergoes disintegration and the cellular organites are not recognizable. The place corresponding to the cell is then a clear alveolus of about 10 $\mu$m, in which some cellular remains are still visible (fig. 9). These alveoli are bordered by a granular, strongly metachromatic material, which is concentrated for 1 to 1.5 $\mu$m, then progressively dispersed to become the granulo-fibrillar ground substance in which the whole cells and collagen elements are embedded. In some areas, these degenerating cells are abundant, and the whole tissue displays a special, alveolar aspect (fig. 4). Such areas have been found mostly at the junction between the cortex and the chanosome, or beneath the fibrous layer surrounding exhalant canals. The development of this alveolar area is variable according to the specimen studied.

Bundles of collagen fibrils are abundant in the whole tissue. They are particularly well developed in the cortex and around large exhalant canals. The larger attain 40 $\mu$m in diameter. Their collagen fibrils lie mostly in parallel and are closely packed. These fibrils (fig. 11), about 16 nm in diameter, display a faint transverse periodicity and belong to the smooth type defined by Garrone (1978). The bundles are embedded in the granulo-fibrillar matrix, the visible material of which remains external to the bundle.

Symbiotic, intercellular bacteria are present in the tissue. They are always few in number and belong to a single morphological type, i.e. small rods, 0.25 $\mu$m in diameter.

No reproductive stage was observed.

## DISCUSSION

The genus *Halisarca* appears to be represented in the West Indies only by *H. purpura* Little, 1963, from Florida. This species differs from *H. caerulea* by its purple colour, the absence of the star-shaped canals (which are very conspicuous in the new species, even in spirit specimens), and by the thickness of the cortex. Bergquist & Hartman (1969) indicated the presence of a new species in the West Indies, but did not describe it. Van Soest (1981) gave a short description of a species from Curaçao, without naming it: this sponge does possess the characteristic star-shaped canal system, and although light purple, may be conspecific with *H. caerulea*.

This new species is clearly distinct from the type species of the genus *H. dujardini* Johnston, and from its twin species (*H. sputum* Topsent, *H. metschnikovi* Lévi, *H. nahatensis* Chen) by its colour, the structure and thickness of the cortex and the oscula. However, *H. caerulea* appears to have close relatives in the Indopacific. Its colour is similar to that of *H. ectofibrosa* Vacelet *et al.*. (1976) from Madagascar, which however has no star-shaped exhalant canals and has a cortex reinforced by stronger collagen bundles. An apparently undescribed species from Djibouti displays a similar cortex and star-shaped exhalant systems, and differs from *H. caerulea* only by its cream colour.

The ultrastructural study of *H. caerulea* displays some unusual features. The most prominent is the separation between the collagen bundles and a ground substance, in which the

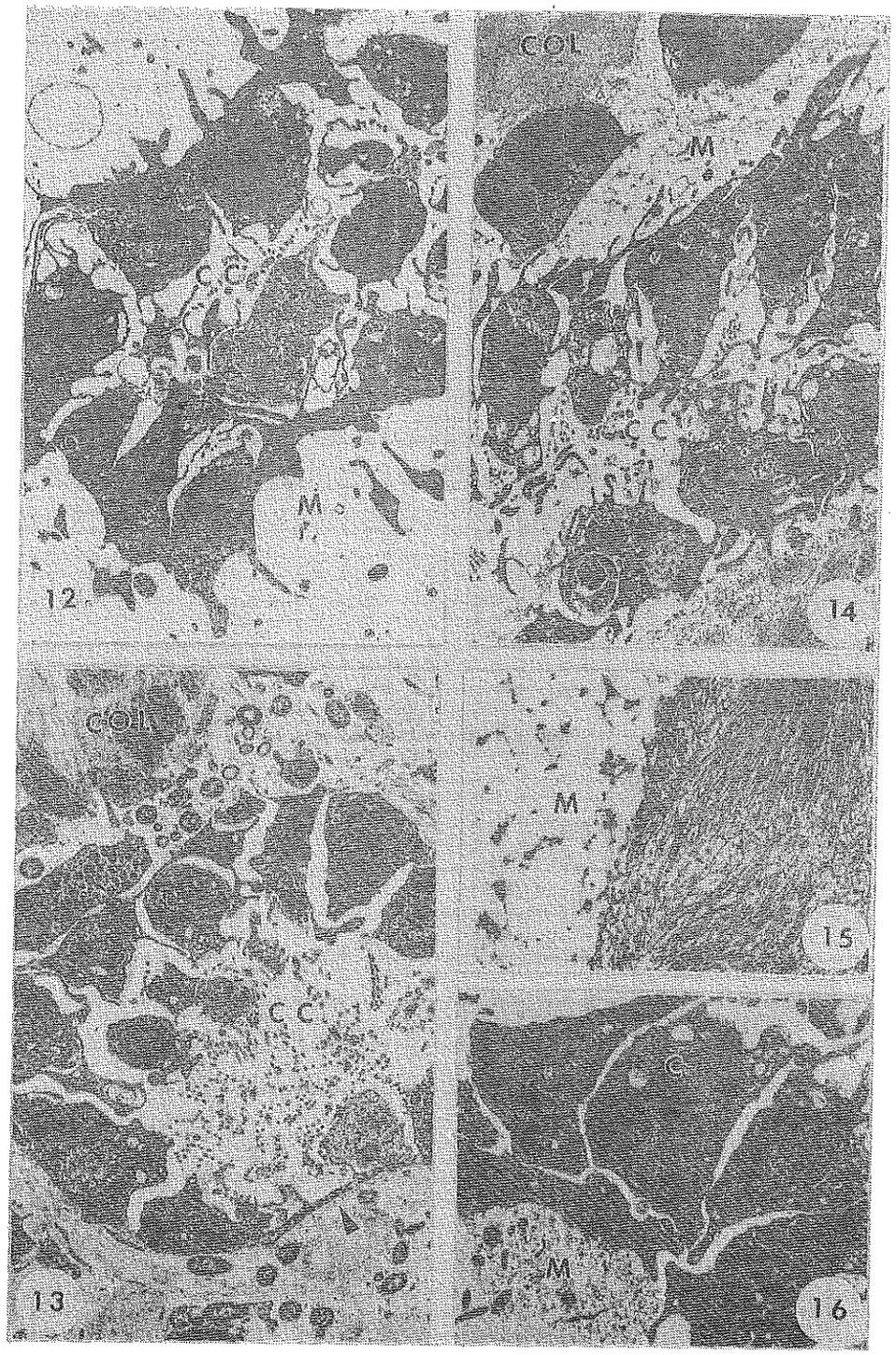


Fig. 12 – Choanocyte chamber (CC) of *Halisarca dujardini*. M: low density matrix. X 3,500.
Fig. 13 – Choanocyte chamber (CC) of *Chondrillastra sp.* COL: bundles of collagen fibrils; arrow: low density matrix. X 3,800.
Fig. 14 – Choanocyte chamber (CC) of *Halisarca ectofibrosa*. Note the anchorage of the basal part of choanocytes by long pseudopodia into the low density matrix (M). COL: bundles of collagen. X 3,800.
Fig. 15 – Low density matrix (M) and collagen fibrils of *Halisarca ectofibrosa*. X 52,000.
Fig. 16 – Low density matrix (M) of *Chondrillastra sp.* underneath choanocytes (C). X 9,000.



choanocytes and the pinacocytes are anchored by pseudopodia. This ground substance is a granulo-fibrillar matrix which seems to be formed by degenerating spherulous cells. These peculiarities are added to the well known peculiar anatomy of the choanocyte chambers and to a strong development of a collagenous cortex. Two questions arise: (1) Are these peculiarities to be found in other members of the genus? (2) Do they exist in other sponges?

(1) For the first point, we have found a similar organisation in an *Halisarca* from New Caledonia, which is very similar and probably conspecific with *H. ectofibrosa* from Madagascar, and in *H. dujardini*. *H. ectofibrosa* (fig. 14, 15) has a thick cortex reinforced by a reticulation of collagen bundles, which are up to 400 $\mu$m thick and thus stronger than in *H. caerulea*. The long, branching choanocyte chambers are separated from the choanosomal bundles of collagen fibrils by an area of a granulo-fibrillar ground substance. The choanocytes are anchored in this matrix by long pseudopodia; they differ from those of *H. caerulea* only by a greater development of their pseudopodia. Their nucleus is nucleolated and their cytoplasm contains numerous large, spherical inclusions. The spherulous cells, although similar, do not display pictures of degenerescence as clear as those of the new species. *H. dujardini* (fig.12) is also very similar. However, the cortex is considerably thinner (10-12 $\mu$m), and does not contain trellis-like bundles of collagen. The granulo-fibrillar, metachromatic area between choanocyte chambers and collagen bundles does exist but is thinner than in the two tropical species. The spherulous cells belong to three different kinds, 10 to 12 $\mu$m in diameter, which differ by their inclusions. No disintegration stages were observed. In this species, which does not cover large surfaces, the development of collagen is less important, both in the cortex and in the choanosome, but the general organisation is the same.

(2) For the second point, a number of observations indicate that in many sponges, spherulous cells or spumous cells are involved in the secretion of an intercellular matrix in which collagen fibrils are embedded (see Simpson, 1984 for a review). But this matrix, made of glycosaminoglycan, is not clearly separated from the other components of the mesohyle, according to the published observations. However, an exception may be Garrone's observation (1978) in *Chondrosia reinformis*, where a condensation of a granulo-fibrillar matrix appears to be in part distinct from the collagen bundles (although some collagen fibrils do occur in this matrix, unlike *Halisarca*). We found such a disjunction in another member of the family Chondrosiidae, *Chondrillastra sp.* from the Red Sea (unpublished observations). In this species (fig. 13, 16), the choanocyte chambers are surrounded by an area of matrix devoid of collagen fibrils, in which the nucleolate choanocytes are anchored by basal pseudopodia. Another interesting point is the observation by Gaino & Pronzato (1983) of spherulous cells in *Chrondrilla nucula* degenerating in a way which reminds one of the secretion of the matrix in *H. caeulea* A similar organization has been found in the Mediterranean *Chondrilla nucula* by Simpson & Langenbruch (personal communication).

This matrix displays some morphological similarities with the ground substance of vertebrate cartilages. Cartilage-like tissues have been described in cnidarians, although they have not been subjected to chemical analysis, and in a number of other invertebrate phyla (Person & Philpott, 1969), but never in the Porifera. It would be of interest to compare more thoroughly the morphology and the biochemistry of this matrix of *Halisarca* with those of true cartilages.

## CONCLUSIONS

The genus *Halisarca* appears to have some unusual ultrastructural features, which confirm its uniqueness. The anatomical characters do not justify classification within the Dendroceratida, which was already inconsistent with the biochemical evidence (Bergquist & Wells, 1983). Some interesting similarities appear here with the Chondrosiidae, i.e. a strong development of collagen bundles, a similar mode of formation of a ground matrix, and a very peculiar disjunction between both cellular and collagen elements and the metachromatic matrix. It may be of interest to investigate more thoroughly the relationship between the families Chondrosiidae and Halisarcidae, which are widely separated within modern classifications, but which have been united in the same family by ancient authors such as Carter (1881) on the basis of the absence of skeleton and of the subsequent development of an "elastic tissue".

However, it must be kept in mind that these two families differ widely in the anatomy of choanocyte chambers and by the reproductive characters. The peculiar organization of the mesohyle described here may be related to the development of the collagen bundles, which itself

may be related to the absence of a skeleton in both families more than to phylogenetic relationships.


## ACKNOWLEDGEMENTS

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# BIOGEOGRAPHIC AND TAXONOMIC NOTES ON SOME EASTERN ATLANTIC SPONGES


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## ABSTRACT

New records and revisory remarks are presented on the following Eastern Atlantic species: *Tedania anhelans* (new to the Atlantic coasts of France, with a review of North Atlantic *Tedania* species), *Iotroatd spinosa* (new record, with a review of birotuliferous Poecilosclerids), *Spanioplon armaturum* (new for the Norwegian coast, with a review of its distribution), *Hymedesmia coriacea* (revision), *Ulosa stuposa* (revision), and *Halicnemia patera* (new to the Irish fauna, with a review of micracanthoxea-bearing Desmoxyids).


## INTRODUCTION

Sponges are among the few remaining animal groups which present problems in field recognition and identification, even in such well-researched areas as Western Europe. Recently efforts have been made to remedy this, and now there is cooperation between sponge taxonomists throughout Europe. This will result in taxonomic problems no longer being attacked on a regional basis, but in species and genera being studied throughout their ranges. The present paper is only a modest contribution in this direction. It concerns the presentation of a series of unrelated new records and observations on Eastern Atlantic species, a harvest of several trips in various parts of Europe. These data justify some revisory remarks on the genera concerned and discussions of some distribution patterns.

The material is preserved and incorporated in the collections of the Zoologisch Museum Amsterdam (ZMA). The following persons and institutions contributed to the results: Mr. Matt Murphy (Sherkin Marine Station), Miss S.M. Stone (British Museum (Natural History), London), Drs. W.H. de Weerdt (Amsterdam), Dr. O.S. Tendal (Copenhagen), Dr. T. Brattegard (Bergen, Norway), Drs M. Wapstrapa (Amsterdam), Drs M. Reichert (Amsterdam), Drs M. - J. Leloup (Amsterdam), Dr. L. Cabioch (Roscoff) and Drs J.C. den Hartog (Rijksmuseum van Natuurlijke Historie at Leiden, RMNH).

I. ***Tedania anhelans***, new to the Atlantic coasts of France.

Order Poecilosclerida
Family Myxillidae
Genus *Tedania* Gray, 1867
*Tedania anhelans* (Lieberkuhn, 1859). Pl. I Figs 1 - 4, Text-fig. 1.
Restricted synonomy:
*Tedania digitata* (Schmidt, 1862)
*Tedania nigrescens* (Schmidt, 1862)
*Tedania toxicalis* sensu Arroyo, *et al.*, 1976 (not *T. toxicalis* De Laubenfels, 1930).

Material: ZMA POR. 4863, Chateau du Taurau, Baie de Morlaix, north coast of Brittany, France, dredged at 40m, 19 - IV - 1982.
Description: a massive specimen, 8cm long, 4cm wide and 6cm high, with conical oscular elevation; next to these there are several thinner, blind fistulae. Oscula 4mm in diameter. Surface smooth. Consistency soft, compressible, easily damaged. Colour: reddish brown. Ectosome: tangential bundles of ectosomal tylotes are strewn at random in the dermis. Choanosome: the skeleton consists of multispicular columns of styles; the reticulate plan is largely obscured, but next to numerous irregularly arranged styles, there are single styles making up a ladder-like reticulum in between primary skeletal columns. Spicules: ectosomal tylotes with microspined apices: 75 - 205 by 3.5 - 5μm; choanosomal styles: 215 - 230 by 7 - 9μm; onychaetes: 120 - 160 by 1μm. Distribu-
Distribution: Mediterranean, Portugal, Galicia, Canary Islands, West Africa, Azores.
Discussion: the morphology and spiculation of the present specimen is in complete accordance with descriptions from the Mediterranean (e.g. Boury-Esnault, 1969, 1971; Pulitzer-Finali, 1978), and from Portugal and Northern Spain (e.g. Arndt, 1941 as *T. suctoria;* Solorzano & Babio, 1980), thus making it clear that the present record is more or less continuously linked with the distributio-