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A new haplosclerid sponge species from the Red Sea

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A new species of *Chalinula* (Haplosclerida: Chalinidae), *C. saudiensis*, is described from the coral reefs of the Red Sea off Jeddah. The new species is remarkable in its vivid blue colour and its bioactivity. Its description includes cytological features in transmission electron microscopy.

INTRODUCTION

Although the sponge fauna of the Red Sea has been extensively studied (Keller, 1889, 1891; Row, 1910, 1911) and more recently (Lévi, 1958, 1965), the survey of its coral reefs by SCUBA divers has demonstrated that many conspicuous species are still undescribed or not recorded from this area. Conversely, some remarkable species which were apparently common one century ago now appear to be absent, indicating possible long term changes in the sponge fauna.

During a study of marine natural products, a sponge remarkable both for its bioactivity and vivid blue colour was collected in the Red Sea off Jeddah. It belongs to an apparently undescribed species of the order Haplosclerida. The present paper gives the description, with an emphasis on the cytological features, which are important in the distinction of species in this order. The order Haplosclerida is one of the most difficult in the Demospongiae, because of the number of species, the small number of morphological characters available and the subtle nature of some skeletal features. The ultrastructure of the cellular components, especially the so-called 'spherulous cells' could prove good additional characters.

The sponge belongs to the genus *Chalinula* Schmidt, 1868 (=*Acervochalina*, Ridley, 1884), which is apparently poorly represented in the Indo-Pacific region, but which could be more common than apparent due to the taxonomic confusion in chalinid sponges.

MATERIALS AND METHODS

The sponges were collected by SCUBA diving on the outer reef off Jeddah. The largest fragments of the specimens were preserved in methanol. Voucher specimens have been fixed in formalin for a few days and stored in ethanol.

For cytology in light and transmission electron microscopy (TEM), the specimens were fixed in glutaraldehyde 2.5% in a mixture of 0.4M cacodylate buffer and seawater (4 vol: 5 vol). They were post fixed 2 h in 2% osmium tetroxide in seawater, dehydrated through an alcohol series, and embedded in Araldite. Semi-thin sections were stained with toluidine blue. Thin sections, contrasted with uranyl acetate and lead citrate, were observed under a Zeiss EM 912 transmission electron microscope.

For the study of spicules, the tissue was digested by boiling in nitric acid. The dissociated spicules were separated by filtration on 0.1 μm Cyclopore membrane (Reiswig & Browman, 1987), sputter-coated with gold-palladium, then observed under a Hitachi S570 scanning electron microscope (SEM). Spicule sizes were measured by light microscopy from 25 mature spicules in each specimen. The skeletal architecture was studied by light microscopy either on hand-cut tangential sections of the ectosome and perpendicular sections of the choanosome, or on thick polished sections obtained by sawing specimens embedded in Araldite with a low speed saw using a diamond wafering blade, and wet-ground with abrasive paper. For SEM, thick hand-cut sections were partially etched with sodium hypochlorite until clearing of the fibre skeleton.

SYSTEMATICS

Class DEMOSPONGIAE
Order HAPLOSCLERIDA
Family CHALINIDAE Gray, 1867
Genus *Chalinula* Schmidt, 1868

Type species

Chalinula renieroides Schmidt, 1868.

Definition

Chalinidae with choanosomal skeleton reticulation of primary and secondary lines; secondary lines more than one spicule in length. Ectosomal skeleton absent. Oxeas short, ranging from vestigial to cigar-shaped. Spongin usually abundant, with high variation within species. No microscleres. Consistency spongy, elastic, or very soft and limp. Colour brown or purplish (from Weerdt, 2000).

Chalinula saudiensis sp. n.

Type material and material examined

Three specimens have been collected and deposited as type material in the Muséum National d'Histoire Naturelle in Paris (MNHN):

Holotype. MNHN, no. D JV 66: (SP083199.01, Jeddah, outer reef, 21°46.633′N 38°52.015′E, 31 August 1999, 20 m on a dead coral, coll. J. Vacelet, formalin).

Paratypes. MNHN, no. D JV 67: (SP082999.02, Jeddah, outer reef, 21°46.543′N 38°53.533′E, 20 m, 29 August 1999, coll. A. Al-Sofyani, formalin).

MNHN, no. D JV 68: (SP102598.04, Jeddah, outer reef, 21°45.953′N 38°54.863′E, 30 m, 25 October 1998, formalin).

Etymology

From Saudi Arabia.

Locality and habitat

All the specimens have been collected in the Red Sea off Jeddah, Saudi Arabia, from the outer reef at Black Coral Canyon, at ~20–30 m in depth, on the upper surface of dead coral heads, close to black coral trees *Antipathes*.

Morphology

Sponge (Figure 1) thickly encrusting, 10–20 mm in thickness, with irregular round outlines, attached only in some places to the substratum from which it is easy to separate. The holotype SP083199.01 and the specimen SP102598.04 were 30 cm in maximum extension. Specimen SP082999.02 is a smaller thick sheet lying parallel to the substratum to which it is attached by a short, thick peduncle, with the oscules located on the upper surface. Colour vivid blue in life, light brown in alcohol. Consistency soft but resilient.

Oscules, from 2 to 6 mm in diameter, with slightly raised margin, numerous on the upper surface. Both the upper and under surfaces display subdermal cavities, 600–700 μm in diameter, regularly disposed 1 to 2 mm apart. Ostia, 15–40 μm in diameter, clustered in a thin ectosome above subdermal cavities.

The skeleton (Figure 2A,B) is a reticulation of primary and secondary fibres. The primary fibres are loosely reticulated and principally ascending, ending at the surface in a faint hispidation. Most are 20 to 35 μm in diameter cored with one to three spicules, although some may be up to 60 μm thick and cored with up to five spicules. The largest primary fibres often reinforce the wall of the main exhalant canals which lead to the oscules. The secondary fibres, 10–25 μm in thickness, are unispicular and generally more than one spicule long. They connect the primary fibres in a reticulation with irregular, triangular or quadrangular meshes, 100–250 μm wide. There is no special ectosomal skeleton, but the ends of the ascending fibres pierce the surface, with one or two protruding spicules (Figure 2A,B). The spongin is rather abundant around the spicule lines. It is made of a feltwork of fine collagen fibrils, with an irregular lamination (Figures 2F & 3A). The axial filament of the spicule is hexagonal in section.

Spicules (Figure 2C,D): oxea slightly curved with truncated ends, most often irregular, with small swellings and a rounded tip (nearly strongylote). The overall size is 110–181 μm/1.5–4.5 μm in range. Mature spicules inside the fibres have similar size and shape in the three specimens (Table 1), although the specimen SP102598.04 has a larger quantity of thinner spicules, probably immature. These immature spicules, which could be as small as 60/0.5 μm, are abundant in the living tissue, as usual in chalinid sponges.

The choanosome is rather lacunose, with a low density mesohyl and a high number of choanocyte chambers

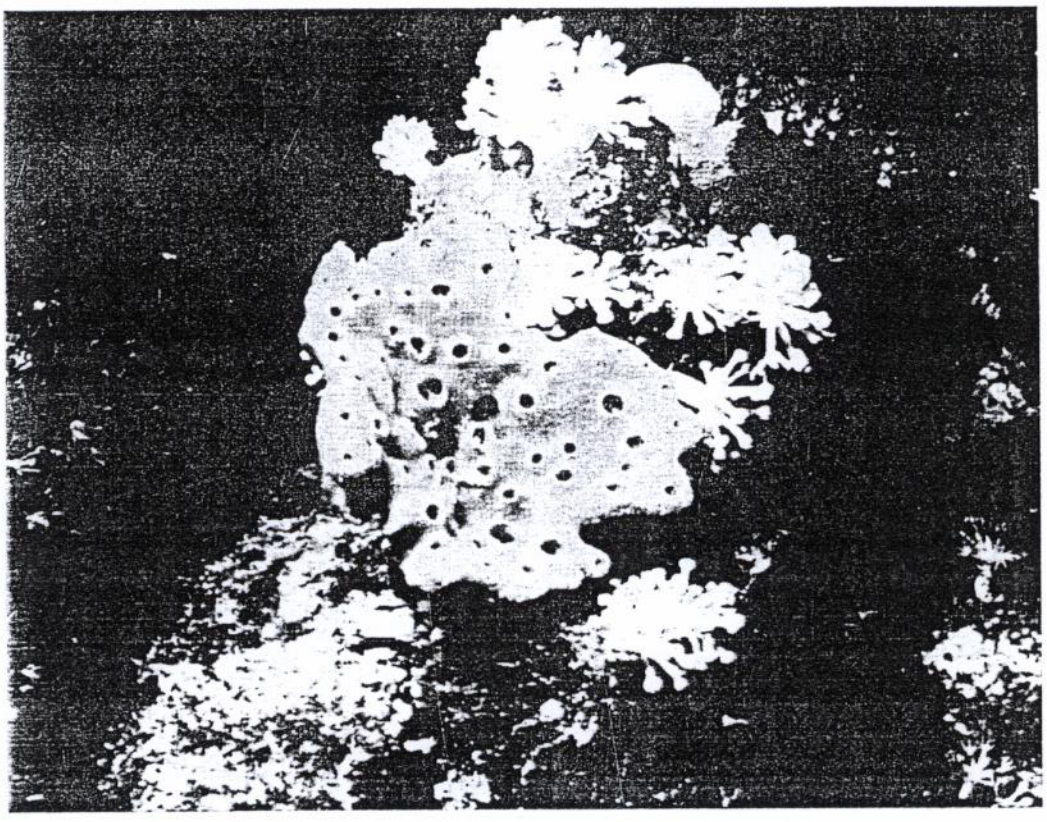

Figure 1. *Chalinula saudiensis* sp. n. Underwater photograph of specimen SP082999.02.

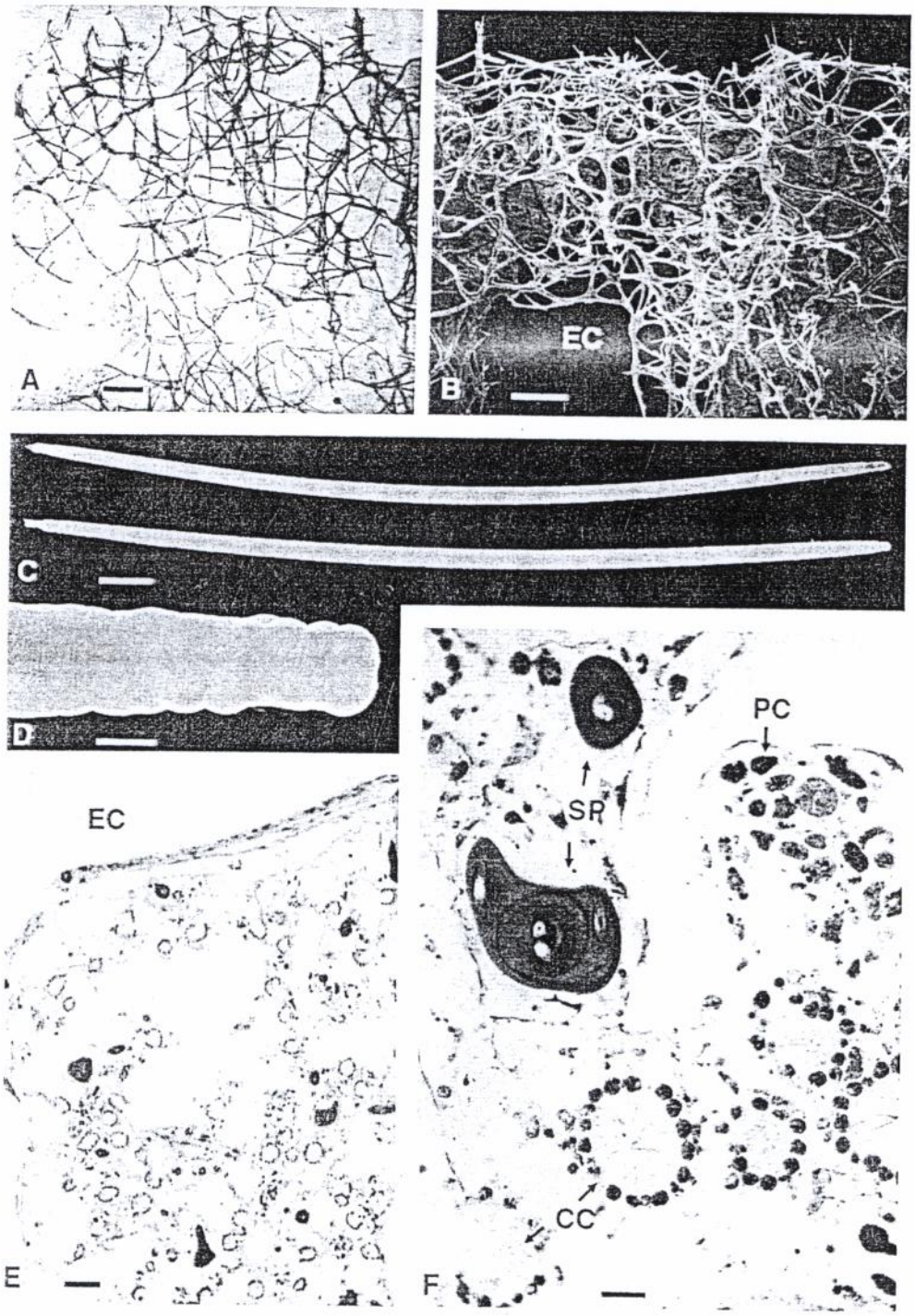

Figure 2. *Chalinula saudiensis* sp. n. (A) Perpendicular section through the skeleton; (B) SEM photograph, perpendicular section near the surface and a large exhalant canal; (C&D) SEM views of spicules; (E&F) semi-thin section, choanosome organization near a large exhalant canal. CC, choanocyte chamber; EC, exhalant canal; SP, spongin fibre; PC, cluster of pigment cells. Scale bars: A&B, 150 μm; C&F, 10 μm; D, 1 μm; E, 50 μm.

(Figure 2E,F). The intercellular collagen fibrils and the symbiotic bacteria have an irregular distribution, being more concentrated in some places. Choanocyte chambers (Figures 2E,F & 3B) are 25 to 30 μm in diameter. Most chambers belong to the 'hanging type' of choanocyte chamber characteristic of many haplosclerids (Johnston & Hildemann, 1982; Langenbruch & Scalera-Liaci, 1990). They are not embedded in the mesohyl tissue, but are directly exposed by a large part of their surface in the inhalant canals. Their outer surface is covered by pinacocytes, although this cover is often incomplete, possibly due to poor preservation of the pinacocyte. They have a large apopyle approximately 10 μm wide.

Several parasite barnacles (*Acasta* sp.) are present inside the canals of the lower surface in specimen SP083199.01.

Cytology

Choanocytes (Figure 3B) are approximately cube-shaped, 3–4 μm in height, with a nucleus 1.2–1.5 μm in diameter. Most contain dense heterogeneous inclusions,

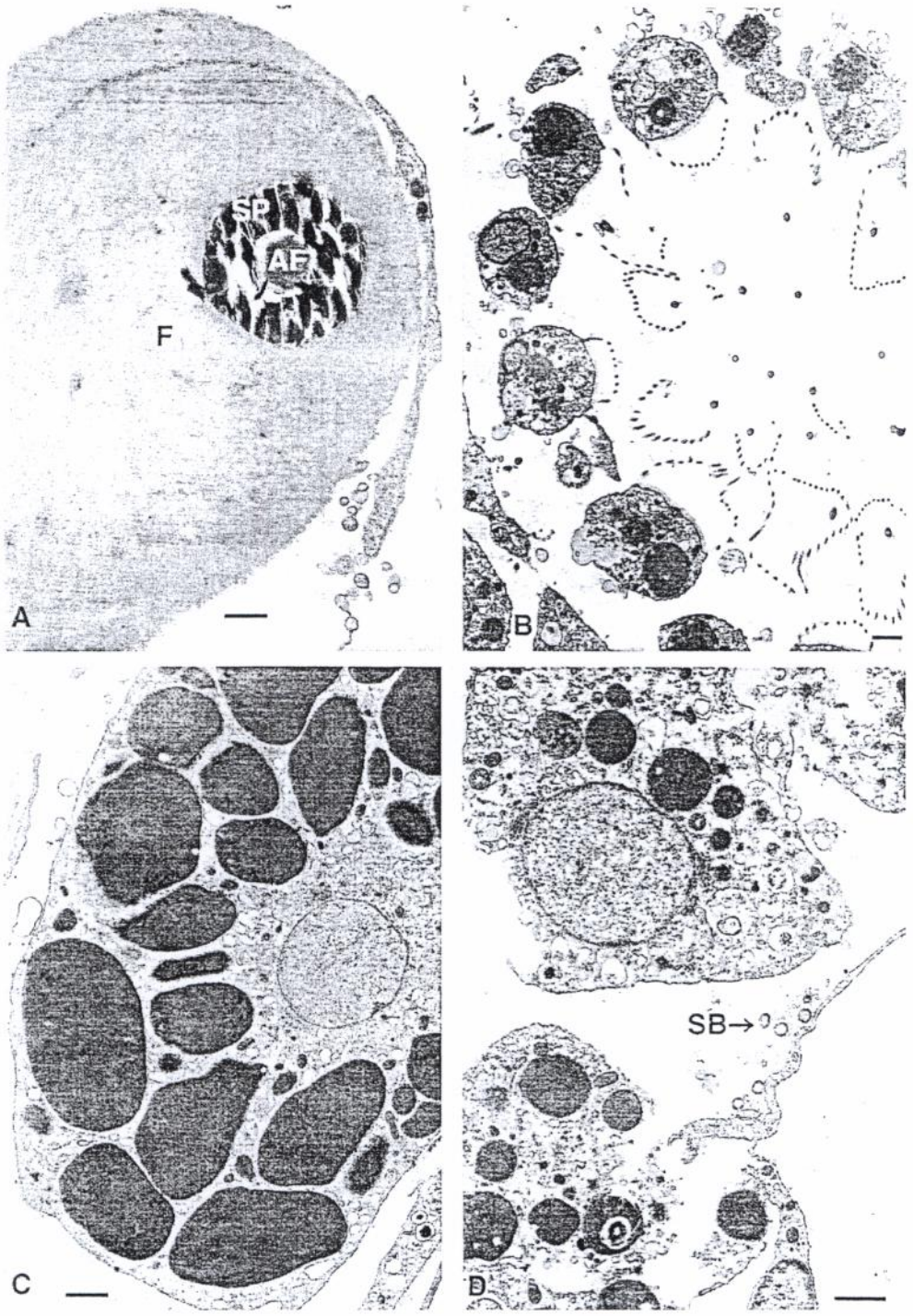

Figure 3. *Chalinula saudiensis* sp. n. (A) Spongin fibre and spicule; (B) choanocyte chamber; (C) spherulous cells; (D) pigment cells. AF, axial filament; SB, symbiotic bacteria in a concentration of collagen fibrils; F, fibre; SP, spicule. Scale bars: A, 0.5 μm; B–D, 1 μm.

1–1.5 μm in diameter. The number of microvilli in the collar varies from 35 to 39. There is no periflagellar sleeve and the flagellar vanes are reduced or absent. A dense glycocalyx layer covers the cell surface inside the collar (Figure 3B).

The non-flagellated endopinacocytes and the archaeocytes, with a nucleolate nucleus, have no special character. Some elongate cells line the main exhalant canals (Figure 2E).

The spherulous cells (Figure 3C) are spherical or elongated cells, 20–25 μm in diameter, up to 40 on 10 μm when elongated. They contain dense, homogeneous spherules, spherical or polygonal in shape, usually 2.5 μm in diameter but varying from 0.2–4.2 μm, which occupy nearly all of the cytoplasm volume. The nucleus, approximately 3 μm in diameter, is anucleolate. A rough endoplasmic reticulum with enlarged vesicles is well-developed. Spherulous cells are most abundant around the exhalant canals, especially near the osculum, in the layer of elongate cells around the exhalant canals.

The mesohyl also contains cells 10–12.5 μm in diameter with a nucleolate nucleus (3 μm), containing various

Table 1. *Spicule size in μm (N=25).*

	Length	Width
SP102598.04	110–180 ($\overline{X}$=144.58 ±7.26)	1.5–4.5 ($\overline{X}$=2.36 ±0.32)
SP082999.02	127–181 ($\overline{X}$=155.80 ±5.42)	3–4.5 ($\overline{X}$=3.68 ±0.15)
SP083199.01	122–165 ($\overline{X}$=149.18 ±4.1)	2.5–3.75 ($\overline{X}$=3.02 ±0.2)

inclusions including dense heterogeneous spherules, 0.6–1.5 μm in diameter (Figures 2F & 3D). These inclusions, which resemble those of the choanocytes, are purple in colour when observed *in toto* in specimens preserved in formalin and disappear in alcohol. These cells are probably the pigment cells responsible for the bright blue colour of the sponge.

Symbiotic bacteria (Figure 3D) are remarkably few in number. They are extracellular and localized in a few areas of the mesohyl where collagen fibrils accumulate. They belong to a single morphotype, rod-like cells, 0.25–0.3 μm in diameter, 1.1 μm in maximum length on thin sections, with an outer wall displaying an undulated outline. Specimen SP082999.02 contains a large number of red filaments. 10 μm in diameter, lying on the surface or included in the superficial tissue, which are cyanobacteria probably belonging to the genus *Oscillatoria* (identification T. Le Campion).

Reproduction

The specimen SP083199.01 contains numerous embryos clustered in the central part of the choanosome. In the same cluster, the embryos, 400 to 500 μm in diameter, are in various stages of development, from a morula with few blastomeres to a nearly mature parenchymella covered by a convoluted layer of ciliated cells. The parenchymella is devoid of larval spicule and of choanocyte chambers.

DISCUSSION

The skeleton of this sponge is typical of *Chalinula*, with secondary fibres most often more than one spicule long. The range of variation of the skeletal characters is quite high, which seems to be normal in this genus (Weerdt, 2000). Examination of the available type material has recently shown that *Acervochalina* Ridley, 1884 to which such sponges have been often referred is a junior synonym of *Chalinula* (Weerdt, 2000), which is used here. The genus is poorly documented in the Indian Ocean region, without any recorded species having such a distinctive blue colour. However, the description of a new species of haplosclerid must always be made with caution, especially in the Indo-Pacific, due to the confusion of the taxonomy in this order both at species and genus level and to the high species diversity. The distinctive colour is not a good criterion for a comparison with the known species, as most ancient descriptions do not indicate the colour in life, and as the unique blue colour of the new species is lost after preservation, precluding its conservation on type specimens. The sponge has accordingly been compared to all the descriptions of the haplosclerids from the Red Sea and more generally from the Indo-Pacific. Its external shape, skeletal architecture and spicule features do not match any described species.

The only similarity has been found with a '*Callyspongia* sp.' figured on an underwater photograph from the Maldive Islands (Erhardt & Moosleitner, 1995). This sponge which has been identified on an underwater photograph without examination of the skeletal character, is so similar in shape, colour and general appearance to the new *Chalinula* that the presence of the new species in the Maldive Islands appears likely.

In the vicinity of Jeddah, the sponge appears to be uncommon and localized on the outer reef, as only three specimens have been found during extensive research prompted by the discovery of the interest of its natural products. It has not been recorded during a survey of the sponge fauna of Obhor Creek (Sarà et al., 1979) and during an extensive survey of the sponge fauna near Jeddah in 1983 (J.V., unpublished data).

The distinctive blue colour is not linked to the presence of prokaryotic symbionts, as it is sometimes the case in non-haplosclerid blue sponges. The colour seems to be localized in the inclusions of some of the spherulous cells and possibly also of choanocytes.

The sponge displays very strong antiviral activity and cytotoxicity and contains several already known bioactive compounds such as renieramycin A (Frincke & Faulkner, 1982) and shaagrockol C (Isaacs & Kashman, 1992). Other bioactive compounds are presently under study. Several related metabolites have been described from blue or purple haplosclerids from other regions (Frincke & Faulkner, 1982; Weerdt et al., 1999; Devijver et al., 2000) or even from the Red Sea for *Toxiclona toxius* (Isaacs & Kashman, 1992). These sponges are clearly differentiated by their morphological characters and in most cases by their geographical distribution and overall chemical composition.

The bioactive compounds of this sponge are most probably not produced by the symbiotic bacteria, which are rare in the tissue. The association with a filamentous cyanobacterium that appears non specific is inconstant and the specimen harbouring this associate has similar compounds than those without cyanobacteria.

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