

**Morphological structure of healthy and unhealthy *Stylophora pistillata*  
from Gulf of Suez, Red Sea**

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**ABSTRACT**

This study discusses the morphological characteristics of tissue and skeleton of *Stylophora pistillata* coral species, living on the reef patch at 3-5 m depth of El-Ain El-Sukhna area, Gulf of Suez, Red Sea, Egypt. Live tissue and skeleton imaging for *S.pistillata* were performed by using scanning electron microscopy (SEM) in order to understand the ultrastructural characteristics before doing any further molecular or genomic studies. Colonies showed numerous polyps, linked together by a common tissue usually referred to as the coenosarcs. Corallites were rounded in shape, regularly distributed along the polyps and linked together by the coenosteum ornamented with coenosteal spines. Aggregates of aragonite crystals were observed adjacent to coenosteal spines on the tissue. The skeleton located beneath the calcicoblastic ectoderm of the polyp or the coenosarc. The surface of the skeleton was bearing shallow pits which might represent desmocyte attachment scars. Some polyps showed the presence of microborers as cyanobacteria, endolithic algae, and foraminifera which causing bioerosion in form of microscopic holes into the surface of the polyps which lead to damage of the living tissue and the skeleton. Bioerosion removes calcium carbonate of the skeleton which would weaken the coral skeletal framework and could reduce the mechanical stability of the reef.

**Key words:** Morphology, Scleractinian coral, *Stylophora pistillata*, Microborers, scanning electron microscopy.

**INTRODUCTION**

The branching coral *Stylophora pistillata* (Esper,1797; Anthozoa: Zoantharia: Scleractinia: Pocilloporidae), one of the most abundant hermatypic corals along the coasts of the Red Sea, found in shallow waters down to at least 60 m<sup>(1)</sup>. It has been used for many years as a model species for coral biological studies. *S. pistillata* is one of the tramp species of the coral world. Larvae readily attach themselves to floating pumice, or sometimes pieces of wood, where they can grow into colonies several centimeters across. These colonies may be transported hundreds, even thousands of kilometers and can produce more larvae en route. It is therefore no wonder the species is so widely distributed.

Corals calcify faster than most other animals and outpace inorganic calcification rates on the reef by a factor of more than 100<sup>(2)</sup>. In doing so, they control the tempo of the biomineralization in reef communities<sup>(3)</sup>. The mineralogy of aragonitic skeleton of scleractinian corals was investigated in great detail<sup>(4)</sup>. The building blocks of the skeleton are formed of thin aragonite crystals or fibres (0.04–0.05 mm in diameter), which set up in a tri-dimensional structure. Organisms exert an exceptional control over the polymorphism, orientation and morphology of their mineral components through a series of biochemical processes generally included under the term biomineralization<sup>(5, 6)</sup>. It is generally recognized that the biomineralization process involves several steps: the fabrication of a hydrophobic solid organic substrate; the nucleation of crystalline materials associated with specific

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polyanionic macromolecules that cover the internal wall of the organic scaffold; the crystal growth, controlled by new secretions of polyanionic macromolecules; the termination of the process by secretion of inhibitory macromolecules<sup>(7)</sup>.

A building block grows into a vertical spine called trabecula; groups of trabeculae form the septa, the primary macroscopic structure of the coral skeleton, arranged inside the skeleton in a radial way, which is species specific<sup>(8)</sup>. At the centre of each spine there is the centre of calcification (COC, nucleation centre), from which the aragonite fibres grow. Inside the COCs granular sub-micronic crystals grouped in 2–4 mm nuclear packets are located<sup>(9,10)</sup>.

Understanding the mechanisms of biomineralization in hermatypic scleractinians requires knowledge of both the organism and the calcareous skeleton. Since the 1970s, researchers have understood the importance of comparative studies on the morphology of the skeleton and the histology of the skeletogenic tissues<sup>(9, 11)</sup>. Recent studies, however, have focused either on the properties of the calcifying tissue or on the properties of the skeleton, and only three studies have linked these two aspects at the microscopic level of the calcifying interface<sup>(12)</sup>. Corals have a range of morphological and behavioral characteristics that affect the likelihood of a polyp encountering a food particle and various studies have demonstrated relationships between water movement, feeding efficiency and different branch spacing of ramified corals<sup>(13)</sup>. Additionally, certain corals with large polyps may have problems feeding in high flow due to the difficulty of keeping their polyps extended<sup>(14)</sup>. Corals grow indeterminately<sup>(15)</sup> and thus interactions between neighboring colonies are common. These interactions may not always involve actual contact and/or competition, but they will often result in some alteration of growth trajectories<sup>(16)</sup>. Coral skeletons constitute the basis of coral reefs, the world-largest bioconstruction, and are widely used for several purposes ranging from taxonomy, records of environmental information<sup>(17)</sup> or as bioimplants for bone surgery<sup>(18)</sup>. The anatomy of corals is described in Chevalier (1987)<sup>(19)</sup>, and Fautin and Mariscal (1991)<sup>(11)</sup>. It is generally compared to a bag attached by its basis to the skeleton located outside the animal, with an oral tissue facing the seawater and an aboral tissue facing the skeleton. Each tissue is indeed composed of two epithelial cell layers, named epidermis (but generally referred as ectoderm) and gastrodermis (generally referred as endoderm), respectively, for the external and internal layers<sup>(20)</sup>. Coral reef maintenance depends on the balance between constructive and destructive forces. Constructive forces are mainly calcification and growth of corals and encrusting coralline algae. Destructive forces comprise physical, chemical, and biological erosion. Bioerosion is considered as the main force of reef degradation because physical erosion (storms) is temporary and localized, and chemical erosion is considered as negligible due to the actual ocean chemistry<sup>(21)</sup>. The concept of bioerosion was introduced by Neumann (1966)<sup>(22)</sup>. It includes biocorrosion, which refers to destruction of carbonates by chemical means, and bioabrasion which refers to mechanical removal of carbonates by organisms<sup>(23)</sup>. The microboring organisms penetrate actively into carbonate substrates by dissolving them, whereas macroborers, depending on the organisms, penetrate into the substrates using chemical and mechanical means. The mechanisms of penetration by those different organisms have not been completely resolved. Hutchings (1986)<sup>(24)</sup> and Tribollet (2008a)<sup>(25)</sup> reviewed different hypotheses referring to the mechanisms of penetration into substrates used by internal bioeroders, such as calcium pumps in boring cyanobacteria<sup>(26)</sup> and etching cells in boring sponges<sup>(27)</sup>.

Microborers are phototrophic and organotrophic microorganisms<sup>(25)</sup>. Microboring phototrophs are prokaryotic cyanobacteria and eukaryotic chlorophytes and rhodophytes. Organotrophs (heterotrophs) are fungi, foraminifera, and other mostly unidentified prokaryotic and eukaryotic light-independent microorganisms. The colonization of live coral

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skeletons is a selective process requiring a positive phototropic growth orientation and a rate of growth and carbonate penetration equal to or exceeding that of skeletal calcification<sup>(28)</sup>. Microborers with such ability comprise cyanobacteria, such as *Plectonema terebrans* and the chlorophyte *Ostreobium quekettii*, and less frequently, the Conchocelis stages of Bangial rhodophytes<sup>(29)</sup>. Among light-independent organotrophs, fungi are common in skeletons of live corals, where they attack microboring algae as well as coral polyps, and may represent a major hazard to coral health<sup>(30)</sup>. Following the death of corals and encrusting coralline algae, colonization by different microborers starts at the surface of the substrates. Colonization occurs within a few days<sup>(31)</sup> followed by a succession of microborer communities.

The aim of the present work was to study the coral tissue and the associated skeleton in *S. pistillata*, in order to better understand the organization and the structure of the skeletal forming tissue associated with different skeletal ultra-structural components on healthy and diseased scleractinian coral *Stylophora pistillata* from the Red Sea characteristics before doing any further molecular or genomic studies.

### **MATERIALS AND METHODS**

#### **Sampling:**

All observations were made on the *Stylophora pistillata*, collected from El-Ain El-Sukhna (western coast of Gulf of Suez, Red Sea, Egypt) at a depth of 3-5 meters with an average water temperature 19°C. Following collection, the corals were kept in shaded plastic tanks with sea water until transferred to the laboratory aquarium. On the arrival, sea water was renewed. Pieces of coral were broken from healthy and unhealthy colonies of *S. Pistillata*.

#### **Sample preparation:**

Samples of live healthy and unhealthy colonies of *S. pistillata* were fixed in 4% formaldehyde 1% glutaraldehyde, phosphate buffer solution (PH 7.2) at 4°C overnight.

#### **Skeleton preparation**

*Stylophora pistillata* colonies were immersed in commercial bleach (12% NaOCl) at 60 °C for 30 min. The resultant colonies were rinsed well in running water and then several times in dH<sub>2</sub>O to remove the overlying soft tissues. The skeletons were then dried at 60 °C for 24 h.

#### **Morphological analyses:**

*S. pistillata* samples were photographed by a digital camera. Pieces of samples (0.5 Cm) were fixed for SEM for micro morphological characters by immersing them immediately in 4F1G, phosphate buffer solution (PH 7.2) at 4°C for 3 hours. Samples were washed in the buffer and dehydrated at 4°C through a graded series of ethanol. Samples of *S. pistillata* were dried by means of the critical point method, mounted using carbon paste on an Al-stub and coated with gold up to a thickness of 400Å in a sputter-coating unit (JFC-1100E). Observations of the samples in the coded *S. pistillata* were performed in a Jeol JSM-5300 scanning electron microscope operated between 15 and 20 KeV.

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## RESULTS

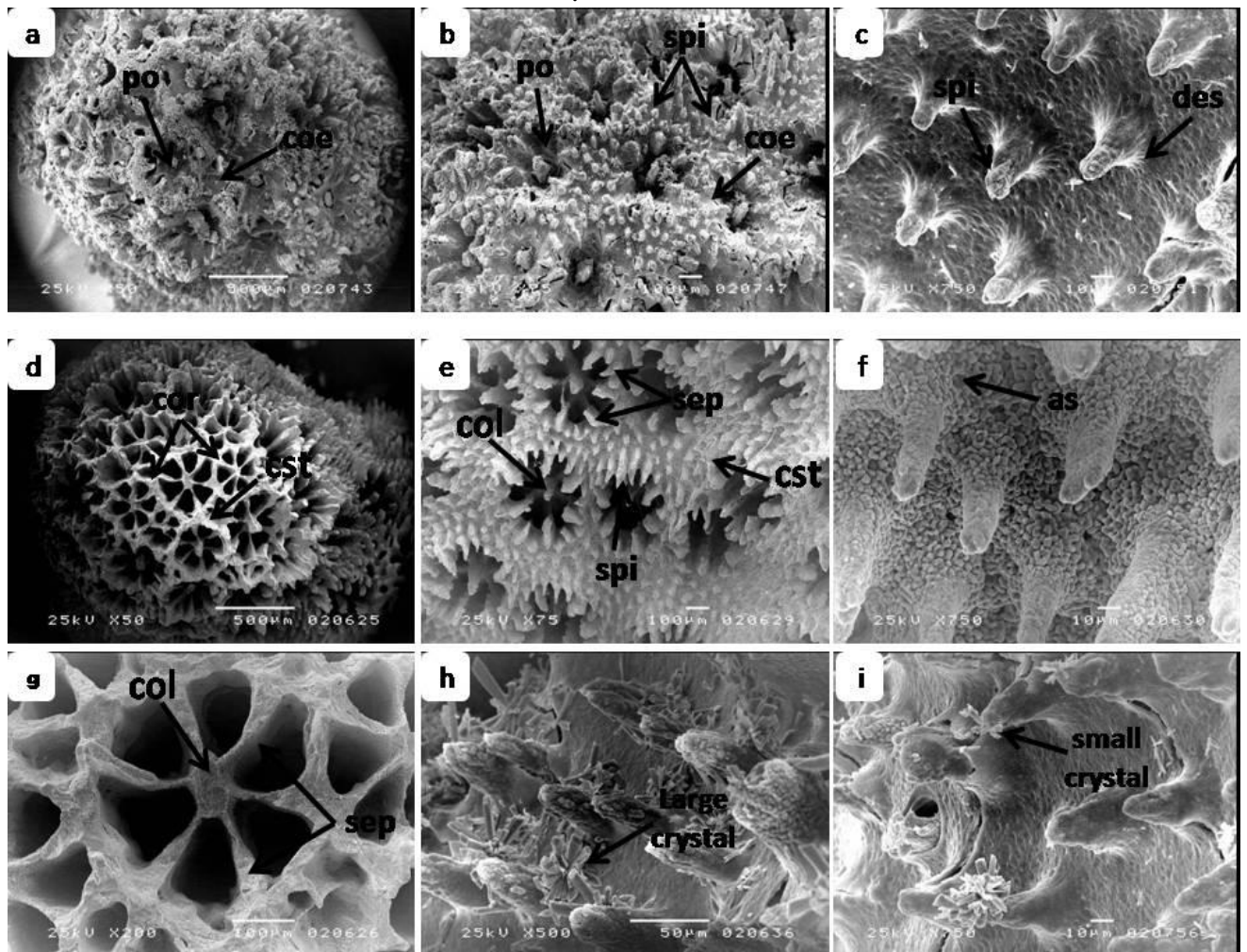
### Micro structural investigations of healthy *Stylophora pistillata*:

Stylophora corals are most commonly cream and pink as shown in (Photo 1). *S. pistillata* is a colonial coral characterized by the presence of numerous polyps, linked together by a common tissue usually referred to as the coenosarc (Fig.1 a,b), The tissue is anchored to the skeleton by desmocytes which appear as small bumps on the polyp and coenosarcs (Fig. 1c), the coral polyps lie in a cup-like skeleton of calcium carbonate called the corallite, Corallites are linked together by the coenosteum ornamented with fine spinules(Fig.1 d,e), they have a solid style-like columella , six primary septa fused with the columella(Fig. 1g), and sometimes six short secondary septa(Fig.1e). pits or depressions in the skeleton are readily visible (Fig.1f). They are interpreted as attachment scar patterns, i.e., points of attachment of desmocytes. crystals were detected in the tissue, on the surfaces of both adherent and non-adherent proto-polyps .Crystals on proto-polyps formed distinct flower-shaped bundles(Fig.1 h,i).



**Photo (1). Colony of *Stylophora pistillata***

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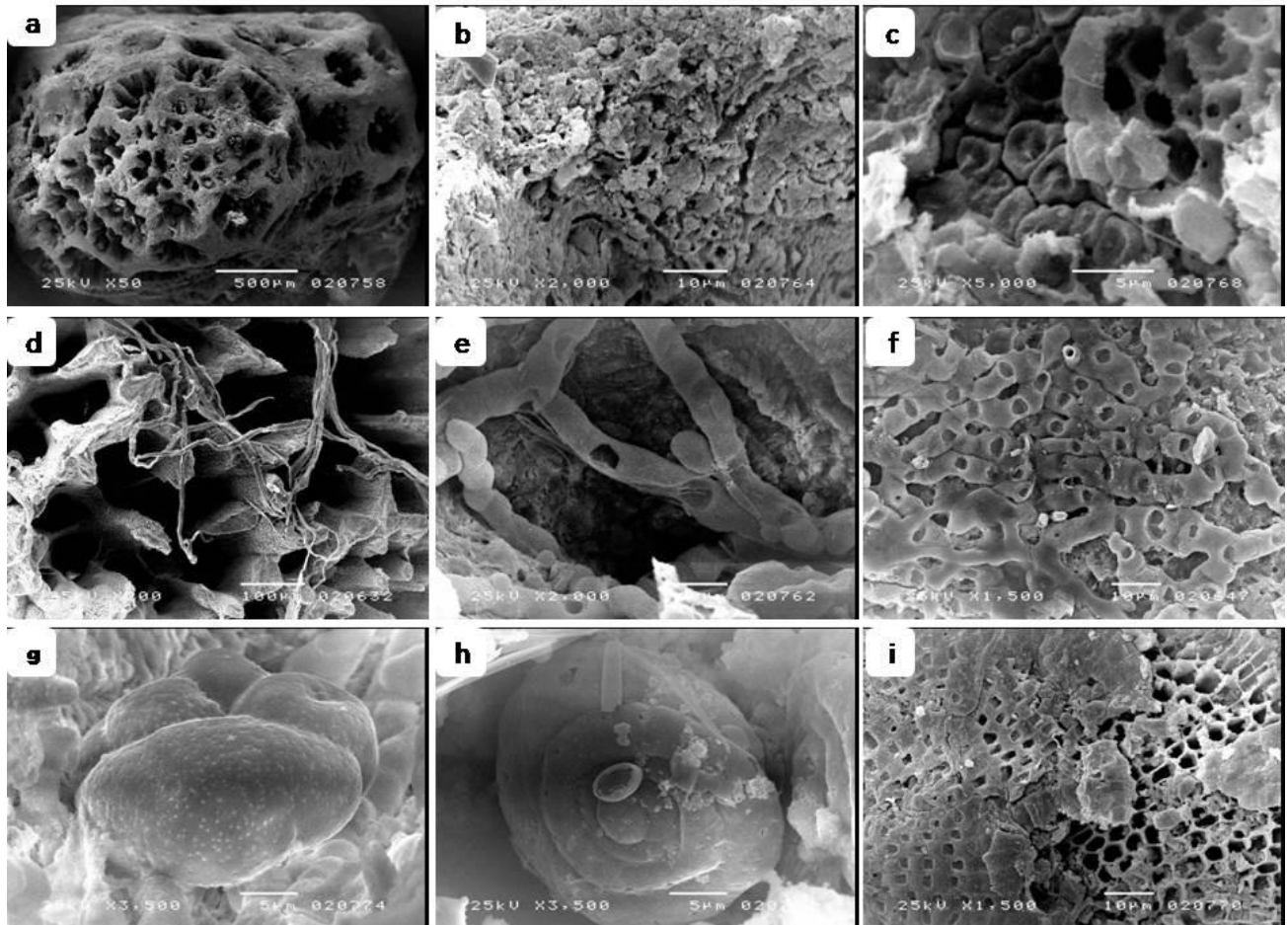


**Fig. 1: Scanning electron micrographs of *Stylophora pistillata*: (a, b) Tissue surface of *S. pistillata* showing polyps, spines and coenosarcs, (c) Coenosarc showing several desmocytes extending beyond the calicoblastic epithelial surface, (d,e) surface of *S. pistillata* skeleton showing many corallites linked by coenosteum which decorated with spines, six long septa and six short septa are observed not connected with columella, (f) Skeletal surface revealing pits interpreted as desmocyte attachment scars, (g) corallite has six primary septa fused with the columella, (h,i) Crystal growth between intact pore wall areas (large crystals) and areas of repair carbonate (small crystals). Abbr.: po, polyp; coe, coenosarc; Spi, spine; des, desmocyte; cor, corallite; Cst, coenosteum; Sep, septa; Col, columella; as, attachment scar. Scale bar: a, d= 500  $\mu$ m; b, e, g=100  $\mu$ m; c, f, i=10  $\mu$ m; h= 50  $\mu$ m.**

**Microbial communities associated to *S. pistillata*:**

Coral disease and coral mortality have increased, characteristics such as the extent of tissue loss and exposure of coral skeleton (Fig. 2a). Bioerosion is considered as the main force of reef degradation, Traces of microbioerosion in the tissue were detected as bioeroders make microscopic holes into the surface of the substrate (Fig. 2b), they also erode coral

skeleton (Fig. 2c) mechanically by removing calcium carbonate or chemically by excreting acidic compounds and convert massive reef structures to rubble, sand and silt. The bioeroders included the microborers endolithic algae, fungi (Fig. 2d, *Ostreobium quekettii* which are shown in (Fig. 2e,f) and foraminifera (Fig. 2g,h). coralline algae skeleton without the algae was observed in samples due to Coralline Lethal Disease in which bacterial pathogen causes death of the reef-building coralline algae (Fig. 2i).



**Fig. 2:** Scanning electron micrographs of unhealthy *S. pistillata* and microbial communities associated with it: (a) unhealthy *S. pistillata* showing tissue loss on both polyps and coenosarcs and exposure of skeleton, (b,c) microbioerosion on the tissue and skeleton, (d) Fungal Septate hyphae and algal filaments from the polyp zone, visible after decalcification of the surface layer, (e,f) *Ostreobium quekettii* (Siphonales, Chlorophyta), a ubiquitous microborer in skeletons of live corals. Shown are resin casts of *O. quekettii* borings in the skeleton of live *S. pistillata* (g,h) foraminifera (g, *Morozovella* sp.), (h, *Miogypsina* sp.), (i) structure of crustose coralline algae reveals an internal structure that looks like a honeycomb, coralline algae skeleton without the algae observed on the right side.

Scale bar: a= 500 $\mu$ m; b, e, f, i = 10 $\mu$ m; c, g, h= 5 $\mu$ m ;d=100  $\mu$ m.



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### DISCUSSION

*Stylophora pistillata* is a colonial coral characterized by the presence of numerous polyps, linked together by a common tissue usually referred to as the coenosarc or coenenchyme<sup>(32)</sup>, it is completely agree with the present study as shown in (Fig.1 a,b). The morphology of the skeletal parts is specific to each species and used as a taxonomic criterion<sup>(33)</sup>. The present study shows that there is a strong morphological correspondence between the soft tissues and the calcareous skeleton from microscopic level of observation. At a microscopic level, the skeleton of *S. pistillata* consists of Coralla which are branching; Calices in this species have rudimentary septa consisting of six primary septa fused with the columella and sometimes six short secondary septa. The calicoblastic ectoderm is firmly attached to the skeleton by anchoring cells, the desmocytes, the morphology of which, and also the mechanism of anchorage to the skeleton have been described in detail<sup>(34)</sup>. In this study, the distribution of desmocyte cells in *S. pistillata* is variable depending on the zones and the stage of tissue growth. On the growing front of the tissues, desmocytes are present and distributed in the grooves delimited by the coenosteal spines. Desmocyte cells are ubiquitous at the interface with the skeleton. In earlier studies, Muscatine et al. (1997)<sup>(34)</sup> found high densities of desmocytes in the calicoblastic ectoderm of *S. pistillata*, whereas Clode and Marshall (2002)<sup>(35)</sup> rarely observed desmocytes in frozen-hydrated polyps of *G. fascicularis* and therefore suggested that the density of desmocytes depends on the region of the skeleton: a high density of desmocytes in low-calcifying regions and a low density in high-calcifying regions. Contrary to these observations of Clode and Marshall (2002)<sup>(35)</sup>. The distribution of desmocytes may, therefore, not only vary among species, but also within the growth zones of an individual colony. It seems that desmocytes are most abundant once skeletal morphological development is complete.

Skeletal elements will be referred to as 'crystal fiber bundles'. Crystal fiber bundles consist of individual crystals arranged into bundles of about 1–10  $\mu$ m in diameter<sup>(36)</sup>. Goldberg (2001a)<sup>(37)</sup> noted that the "compartment-like structure" of the calicoblastic ectoderm corresponds roughly to bundles of crystals in *Mycetophyllia reesi*. In *P. damicornis*, Brown et al. (1983)<sup>(38)</sup> observed that skeletal spines display either "fasciculate" or "smooth" surfaces, and that these features corresponded to different sections of the colony, fasciculate at the apex and smooth at the base, and were dependent on growth rates. In previous study, fasciculate and smooth surfaces described as 'cup-like' or 'flat' surfaces, respectively, effectively correspond to two different stages of growth, e.g., of the spines. When the spine is actively growing, its surface is comprised of curved crystal fiber bundles oriented perpendicular to the surface of the skeleton, whereas when the spine growth is nearly complete, the crystal fiber bundles become oriented parallel to the surface, i.e., the surface appears flat<sup>(39)</sup>. In our study as crystal fiber bundles appear parallel to the surface of the skeleton when the spine is actively growing and perpendicular when the spine growth is nearly complete. Coral disease is considered an important factor in the recent decline of coral reefs worldwide<sup>(40)</sup>. Reports of disease and disease-like syndromes in reef-building corals have increased substantially since first being reported in 1973<sup>(41)</sup>. This increase in the incidence of disease is due in part to a better awareness of coral health but is also linked to the increased environmental stresses affecting coral reefs<sup>(42)</sup>. Coral tissue loss due to a variety of diseases can be substantial. For example, "black line disease" or "black band disease", the result of a cyanobacterial infection<sup>(43)</sup>, may consume one-half of the living tissues of a coral during a single warm season infestation. bioerosion has probably had some effect on reef carbonate budgets. Only four taxa of microborers and their traces were regularly observed in diseased corals. These assemblages are represented by two algae

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(*Ostreobium quekettii* and unidentified algae), foraminifera (*Morozovella* sp. and *Miogypsina* sp.) and unidentified fungi. Low diversity of microboring organism, restricted mainly to *Ostreobium quekettii*, *Plectonema terebrans* and fungi, is known for live corals<sup>(44)</sup>. It is also known that *Ostreobium* growth intensifies in coral skeletons when the coral accretion slows down, but other microbial euendoliths can join only when the skeleton is exposed following coral death<sup>(45)</sup>. Fungal microborers are not constrained by water depth because they are heterotrophic<sup>(46)</sup> and can thus occur over wide bathymetric ranges including abyssal depths<sup>(47)</sup>. A fungal trace were present as septate hyphae. A few species of boring foraminifera and sponges, which possess phototrophic symbiont zooxanthellae, similar to those of corals, are, however, only distributed within the photic zone<sup>(48)</sup>. In this study two foraminifera were detected the first one is *Morozovella* sp. and the second is *Miogypsina* sp.

In this study coralline algae appear as a honeycomb. Corallines were thought to be animals. However, in 1837 Philippi<sup>(49)</sup> recognized that coralline algae were plants and proposed the two generic names Lithophyllum (for flat forms) and Lithothamnium (for erect branched forms). Instances of coralline algal pathogens were unknown until 1993, when CLOD (Coralline Lethal Orange Disease) was first discovered by Littler<sup>(50)</sup>. Coralline Lethal Disease (CLD) was observed on our study as CLD leaving the coralline algae skeleton and destroys the algae this may be due to bacterial pathogen.

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## Morphological structure of healthy and unhealthy *Stylophora pistillata* from Gulf of Suez, Red Sea

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### التركيب المورفولوجي لأحد أنواع الشعاب المرجانية المتحجرة *Stylophora Pistillata* السليمة والمريضة في البحر الأحمر

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تم دراسة الخصائص المورفولوجية للأنسجة والهيكل لأحد أنواع الشعاب المرجانية (*Stylophora pistillata*) (الغطاء المرجاني أو القرنبيط المرجاني الناعم) والتي تعيش على حافة المرجان على عمق من 3 إلى 5 متر في منطقة العين السخنة الموجودة على خليج السويس بالبحر الأحمر بمصر. أجريت عملية التصوير للأنسجة الحية والهيكل لـ *S. pistillata* باستخدام الميكروسكوب الإلكتروني للتعرف على الخصائص التركيبية وأظهرت المستعمرات الخلوية العديد من polyps (الوحدة البنائية للشعاب المرجانية) التي ترتبط ببعضها عبر نسيج مشترك والتي عادة ما يشار إليه باسم coenosarcs وقد وجد بالهيكل Corallites (الوحدة التركيبية لهيكل المرجان) مستديرة في الشكل وموزعة بانتظام على طول المستعمرات الخلوية والتي ترتبط معا بـ coenosteum ومزخرفة بأشواك مشتركة coenosteal spines وقد لوحظ أيضا وجود مجموعة من بلورات الأراجونيت المجاورة لـ coenosteal spines على الأنسجة. يقع الهيكل تحت الأديمية لطبقة البشرة calicoblastic للـ polyps أو coenosarcs. يوجد على سطح الهيكل تجايف سطحية التي قد تمثل ندوب مرافقة لخلية رباطية desmocyte. وأظهرت بعض الـ polyps وجود ثواقب دقيقة microborers مثل البكتيريا الخضراء المزرقة (السيانوبكتيريا) وطحالب داخلية النمو endolithic وقواقع أولية foraminifera التي تسبب تآكل حيوي bioerosion في شكل ثقوب ميكروسكوبية في سطح الخلايا المرجانية polyps التي تؤدي إلى تلف الأنسجة الحية والهيكل. التآكل الحيوي Bioerosion يزيل كربونات الكالسيوم من الهيكل والذي بدوره يضعف إطار الهيكل المرجاني ويمكن أن يقلل من الأستقرار الميكانيكي للشعاب المرجانية.