

Morphological characteristics of ultra structure of *Lobophyllia hemprichii* from El-Ain El-Sukhna, Gulf of Suez, Red Sea

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ABSTRACT

This study discusses the morphological characteristics by using the Scanning Electron Microscope (SEM) of tissue and skeleton of *Lobophyllia hemprichii* coral species that collected from the reef edge at El- Ain El-Sukhna (Gulf of Suez, Red Sea). *L. hemprichii* has a large polyp stony coral often referred to as a lobed, colored, carpet, flat, or open brain coral, meat coral, modern coral, or large flower coral. It has fleshy polyps that hide in calcareous skeleton. It is found in a variety of textures and color forms. Some are smooth, while others are pimply, and look like carpet. Colors vary from bright red, green, orange, gray, tan, or brown. Colonies are flat to hemispherical, phaceloid to flabello-meandroid, the latter with valleys dividing irregularly as growing space permits. Septa taper in thickness from the wall to the columella and have tall sharp teeth. Retracted polyps are thick and fleshy, with either smooth or rough surfaces. The symbiotic algae zooxanthellae hosted within its body provide the majority of its nutritional requirements through photosynthesis. It will also benefit from additional food in the form of micro-plankton or brine shrimp fed in the evening when its tentacles are visible.

Key words: Morphology, ultra-structure, *Lobophyllia hemprichii*, scleractinian coral, *Lobophyllia tissue*, *Ostreobium quekettii*, microborers, scanning electron microscopy (SEM).

INTRODUCTION

The Red Sea contains some of the world's most unique and diverse marine and coastal habitats. Also, it is one of the most important repositories of marine biodiversity in the world⁽¹⁾. Its relative isolation has given rise to an extraordinary range of ecosystems, biological diversity and endemism, particularly among reef fishes and reef-associated organisms. The coral reefs of the Red Sea comprised more than 200 species of scleractinian corals, representing the highest diversity in the Indian Ocean and Red Sea^(1, 2). The coast of the northern Red Sea is fringed by an almost continuous band of coral reef, which physically protects the shoreline. Further south the shelf becomes much broader and shallower and the fringing reefs gradually disappear and are replaced with shallow, muddy shorelines. Coral reefs become more numerous in the offshore parts of this coast^(3, 5).

Although many reef areas in the Red Sea are still in a pristine state, threats are increasing rapidly and reefs are being damaged by coastal development and other human activities. Major threats include: landfilling and dredging for coastal expansion; destructive fishing methods; damage by the recreational SCUBA diving industry, shipping and maritime activities, sewage and other pollution discharges, lack of public awareness, and insufficient implementation of legal instruments that affect reef conservation^(2,5,6).

Coral reefs are distributed throughout the tropics, and large proportions are located in developing countries^(3, 4). Reefs are constructed by a host of reef-building (hermatypic) coral species, but also are home to ahermatypic, or non-calcium carbonate depositing corals such as soft corals, black corals and gorgonians. Coral reefs are made up of thousands of minute coral polyps each of which secretes a calcium carbonate skeleton. The deposition rate for individual coral species varies, but is generally considered to range between 0.1 mm and 10.0 cm per year^(6,7). The accumulation of these skeletons over an enormously long period of time results in massive, three dimensional geological structures. The actual living tissue however, is only a very thin layer lining the surface. Coral polyps feed by filtering plankton, aided by tentacles tipped with stinging-cells (nematocysts); they also receive organic matter through their symbiotic relationship with minute algae (dinoflagellates) called zooxanthellae^(7,8).

Coral reefs support complex food and energy webs that are inter-linked with nutrient inputs from outside sources (such as those brought with ocean currents and run-off from nearby rivers) and from the reef itself (where natural predation and die-off recirculate organic matter)^(9,10). These complex webs mean that any effect on one group of individuals will ultimately impact another, and single disturbances can have multiple effects on reef inhabitants. For example, the complete eradication of the giant Triton *Charonia trinis* through overfishing usually results in outbreaks of Crown-of-Thorns starfish *Acanthaster planci*⁽¹¹⁾. This in turn leads to massive coral mortalities as the starfish reproduces and feeds on the coral polyps. Habitats and food sources for reef fishes are then reduced leading to declines in the population of larger predatory fishes. Human disturbances and their general effects on coral reefs were studied⁽¹²⁾.

The popular and durable lobed brain coral *Lobophyllia hemprichii* is a favorite beginner's large polyp stony (LPS) coral. It is distinct from other *Lobophyllia* species because of its colorful variations and textures. Colors vary from bright red, orange, blue, green, gray, white, tan, or brown and can be a single color or have a contrasting centre⁽¹³⁾. Their large polyps are thick and fleshy with either a smooth or rough surface. Each polyp forms curves, almost like an hour glass shape, but often with a few more curves and an open center.

The *Lobophyllia* species form massive colonies in the wild, often more than 15 feet (4.5 m) across. A giant colony of *L. hemprichii* is very impressive^(13,14). Although this coral is semi-aggressive towards other corals, its colonies are often groupings of multiple individual colonies of this species. Each individual group will have its own distinct coloration and texture, making a tapestry of color and design. *Lobophyllia* colonies are either flat or domed. Typical of many brain corals, the *Lobophyllia* grows in two basic shapes^(15,16). They can develop as flabello-meandroid forms, which means that there are elongated meandering valleys between separate corallite walls; or they can be placeloid, which means that tall separate corallites rising up from the skeleton which are very predominant. They can have polyps on the ends of branches, and these can be very long branches up to 12' (30 cm") in length. Their large prominent polyp tissue usually cloaks the underlying skeleton from view⁽¹⁷⁾.

L. hemprichii has been propagated in captivity with great success. Some of the common names they known for are Lobed Brain Coral, Flat Brain Coral, Wrinkle Coral, Open Brain Coral, and Meat Coral⁽¹⁸⁾. This genus of corals can be confused with *Symphyllia* species, however they can be distinguished by the skeletal structure. *Symphyllia* corals have corallite walls that are joined or fused together rather than separate. They can also be confused with the *Scolymia* genus when young or just a single polyp, this is just until they develop their curved (hourglass) shaped polyps⁽¹⁹⁾.

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The *Lobophyllia* genus is found in many types of environments, but are most common on upper reef slopes and fore reef slopes. Though colonies are usually in fairly protected areas, they are found in both shaded and brightly lit waters and in both vertical and horizontal orientations^(19,20). They are found at depths from 10 - 115 feet (3 - 35 m) in various types of water movement. *Lobophyllia* form massive colonies that can reach over 15 feet (4.5 m) in diameter. They feed at night, extending whitish tipped tentacles. The *Lobophyllia* genus can grow in different colony formations. The colonies can be a branched formation where the corallite (main polyp) walls are tall and separate from the others, coming up from the skeleton of the colony like a single branch. The other formation is when the colony has corallites with long winding valleys, but each corallite or polyp has its own separate walls^(21, 22).

L. hemprichii or the Flat Lobed Brain Coral forms one of the largest colonies. The polyps can reach 2" (5 cm) and are at the ends of 12" (30 cm) long branches. You cannot usually see the branches since they are hidden by the collection of polyps of the colony⁽²³⁾.

In this study, the morphological ultrastructure of tissue of *lobophyllia hemprichii* polyps coral and its associated microbial communities were studied by using scanning electron microscope (SEM), which help in explaining reasons of bleaching, white bands disease, and presence of microbial communities such as bacteria and fungi.

MATERIALS AND METHODS

Sampling:

Specimens of the coral reef *Lobophyllia hemprichii* were 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. Corals were kept in strong plastic tanks with sea water until transferred to the laboratory at Faculty of Science, Benha University. In laboratory corals were allowed to be in aquaria in natural sea water at 24-25°C, sea water was renewed.

Morphological analyses:

Pieces of samples were taken, examined and photographed by light microscope for macro morphological characters.

For micro morphological characters studies by SEM, sample pieces (0.5 cm) of live colonies of *L. hemprichii* were fixed in 4% formaldehyde 1% glutaraldehyde, phosphate buffer solution (PH 7.2) at 4°C overnight. This followed by immersing them immediately in Parniczky solution (6 parts, 2% osmium tetroxide in 0.2 m. filtered seawater: 1 part, saturated aqueous mercuric chloride) for 60 min. After fixation, the specimens were washed 3 times for 15 min in 0.2 m. filtered seawater, dehydrated with 70% ethanol and stored for up to 4 days at 4°C. The final dehydration was performed in graded ethanol solutions up to 100%, then some specimens were cryofractured in a frozen glass of liquid-N₂-cooled ethanol. All coral fragments were then critical-point-dried with liquid CO₂, mounted on aluminum stubs (while most were still attached to the original splints), and coated with gold up to a thickness of 400Å in a sputter-coating unit (JFC- 1100E). Examination of *L. hemprichii* were performed with a Jeol JSM-5300 scanning electron microscope operated between 15 and 20 KeV. Scanning electron microscope images were obtained to examine selected samples of interest in further detail.

RESULTS

The popular and durable lobed brain coral *Lobophyllia hemprichii* is a favorite beginner's large polyp stony (LPS) coral. It is distinct from other *Lobophyllia* species because of its colorful variations and textures. Colors vary from bright red, orange, blue, green, gray, white, tan, or brown and can be a single color or have a contrasting centre. Their large polyps are thick and fleshy with either a smooth or rough surface. Examination of samples of *L. hemprichii* shows an unhealthy affected colony with white band disease that separates between live and dead corals (Fig.1a, b). Morphological structure of healthy colony of *Lobophyllia hemprichii* appears in Figure (1c, d).

Ultra-structural investigations of *L. hemprichii* tissues:

Fusion of neighboring large-sized calcification centres and a better marked incremental zonation of fibers immediately were surrounding the centres *Lobophyllia hemprichii* (Fig.2 a, c). Incremental zonation results from gradual thickening of individual biocrystals within each growth increment (blade-shaped & Fusiform crystal) (Fig. 2b). A structure of crustose coralline algae was observed in tissue of *L. hemprichii* that looks like a honeycomb. Coralline algae skeleton without the algae was observed in tissue of *L. hemprichii* as shown in Figure (2 d).

Scanning electron micrographs of unhealthy corals and microbial communities associated with them:

Examination of SEM micrographs of **unhealthy corals of *L. hemprichii*** shows bores formed by microbial communities at the surface of the polyps and micro-erosion on its tissue and skeleton as shown in Figure (3 a, b). In the interior of a skeletal pore, bundles of genetically grown skeletal aragonite with smooth areas of repair aragonite (darker region) are deposited in response to fungal attack bleached polyp (Fig.3c). *Ostreobium quekettii* (Siphonales, Chlorophyta) were observed in tissue. Also, a ubiquitous microborer in skeletons and tissue of live coral were observed (Fig. 3d) .

SEM micrographs of coral tissues also show bacterial communities attached together in the form of web-like structure and traces of microbioerosion due to filaments of *Ostreobium quekettii* (Fig.4a). On the other hand, normal *lobophyllia* sp. fractured surface of mesentery and zooxanthellae in abundance in separate vacuoles in gastrodermal cells, and flagellated coelenteric were seen by SEM (Fig. 5 b). Also, diagenetic syntaxial growth of skeletal carbonate smooth-planed skeletal wall, formed in contact with the calycolytic polyp epithelium, is comprised of 'truncated' (across the c-axis) aragonite crystals and is located deeper within the coral skeleton than the structure shown in Fungal protuberances (Fig. 5 c, d).

DISCUSSION

lobophyllia hemprichii Colonies are hemispherical to flat, often attaining several meters in diameter. Corallites are phaceloid, being monocentric to highly meandroid, or a mixture of both, with the length of the valley being determined by the competition for space between branches of the same or neighboring colonies^(25, 26). Septa may be arranged in two alternating orders, four distinct orders, or with the orders indistinguishable. Half of the septa belong to the first order, which are very exsert, with 2-10 large lobate to echinulate dentations. Dentations become finer and more numerous in higher order septa^(27, 28). All septal margins are finely serrated, and septal sides are finely granulated. Columella is usually trabecular⁽²⁹⁾. Costae usually appear as parallel ridges with rows of sharp dentations. Living colonies may be almost any

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colors, each polyp are usually distinguished into three different colored, concentric zones: the central oral disc, corallites wall or the inner valley, and the outer wall⁽³⁰⁾.

Lobophyllia hemprichii exhibited high bleaching and mortality⁽³¹⁾. In longitudinal sections, calcification centers are arranged along axes parallel to the overall growth direction. Since the section is likely to be oriented slightly oblique to the axis of centers, the axial areas are often viewed like a discontinuous file of dome-shaped areas occupied by the minute crystals of calcification centers.⁽³²⁾

SEM reveals that bores were detected in this study on the tissues of *lobophyllia hemprichii* lead to tissue degradation, so the tissues became mineralized by microgranular calcite. Prokaryotic penetration into coral tissue has been described previously, resulting in the bleaching (loss of zooxanthellae) of the tissue. It has previously been proposed the physical penetration of bacteria into the coral tissue is aided by chemical degradation that results in a mat containing decaying coral tissue as it migrates across the coral colony^(33,34).

Ostreobium filaments have been found in *lobophyllia* tissue which are straight or somewhat knotted and tangled, mostly 2–5 µm in diameter and morphologically closely resemble *O. quekettii*^(35,36,37).

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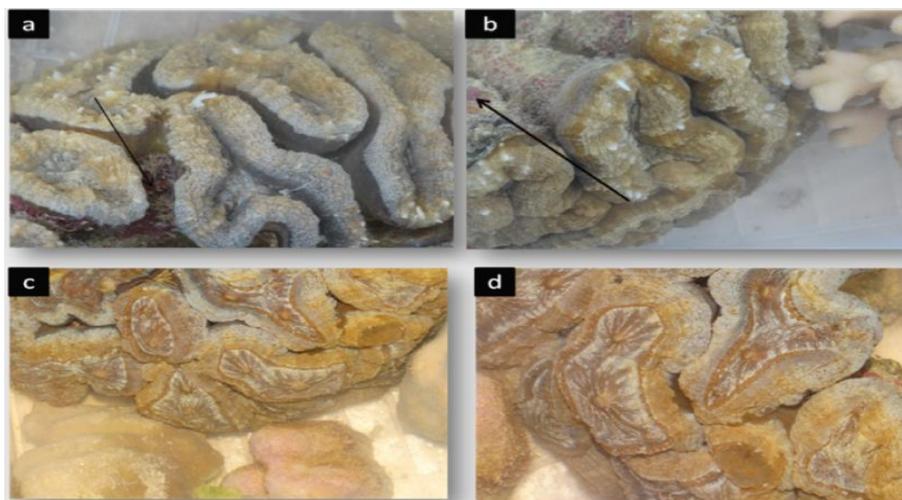


Fig.1: Photomicrograph of healthy and unhealthy *Lobophyllia hemprichii* samples ; (a, b) show unhealthy affected colonies with white band disease that separates between live and dead corals (arrows). (c, d) show morphological structure of healthy colony.

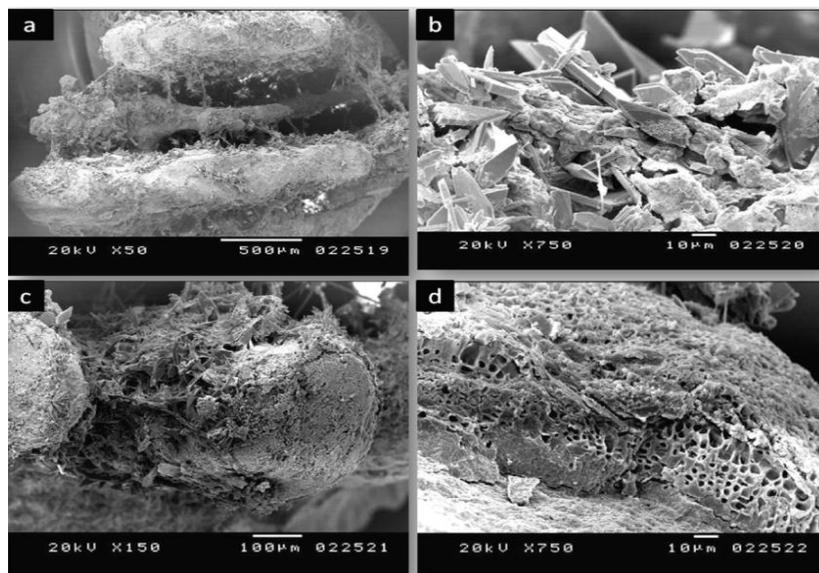


Fig. 2: (a,c) showing fusion of neighboring large-sized calcification centres and a better marked incremental zonation of fibers immediately surrounding the centres of *L. hemprichii*. (b) high magnification view showing incremental zonation of *L. hemprichii* that results from gradual thickening of individual biocrystals within each growth increment (blade-shaped & Fusiform crystal). (d) 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.

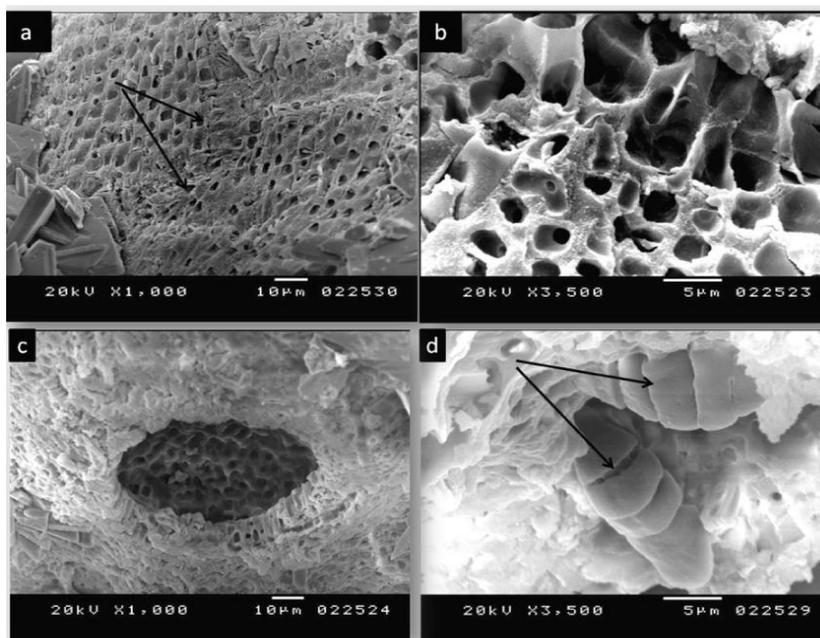


Fig. 3: Scanning electron micrographs of unhealthy and microbial communities associated with it: (a,b): show pores formed by microbial communities at the surface of the polyp & microbial bioerosion on the tissue and skeleton. (c): View of the interior of a skeletal pore showing bundles of didgenetically grown skeletal aragonite, with smooth areas of repair aragonite (darker region) deposited in response to fungal attack bleached polyp. (d): *Ostreobium quekettii* (*Siphonales*, *Chlorophyta*), a ubiquitous microborer in skeletons & tissue of live corals.

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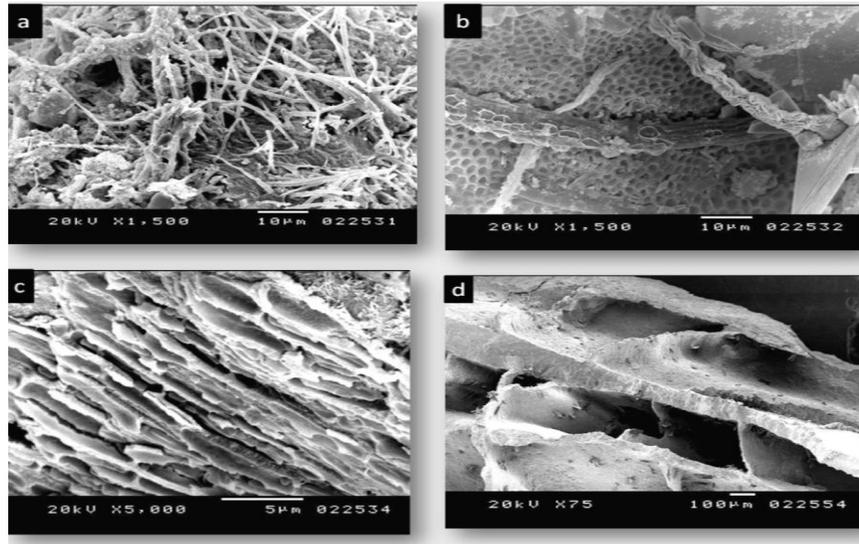


Fig. 4: SEM micrographs of coral tissues. (a): Bacterial communities attached together in the form of web-like structure & traces of microbioerosion due to filaments of *Ostreobium quekettii*. (b): Normal *Lobophyllia hemprichii* fractured surface of mesentery. Note zooxanthellae in abundance in separate vacuoles in gastrodermal cells, and flagellated coelenteric. (c,d): Diagenetic syntaxial growth of skeletal carbonate located deeper within the coral skeleton.

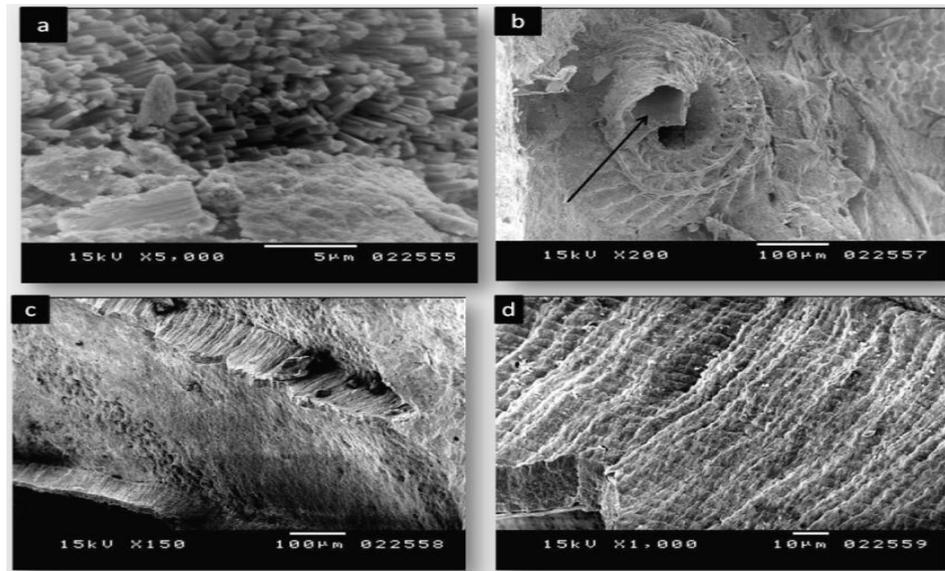


Fig.5: (a) normal calcite crystals which helps in coral growth, indicates zoomed area of calcite crystals. (b) structure of crustose coralline algae reveals an internal structure that looks like a honeycomb. (c,d): cracks in tissue caused by environmental stressors

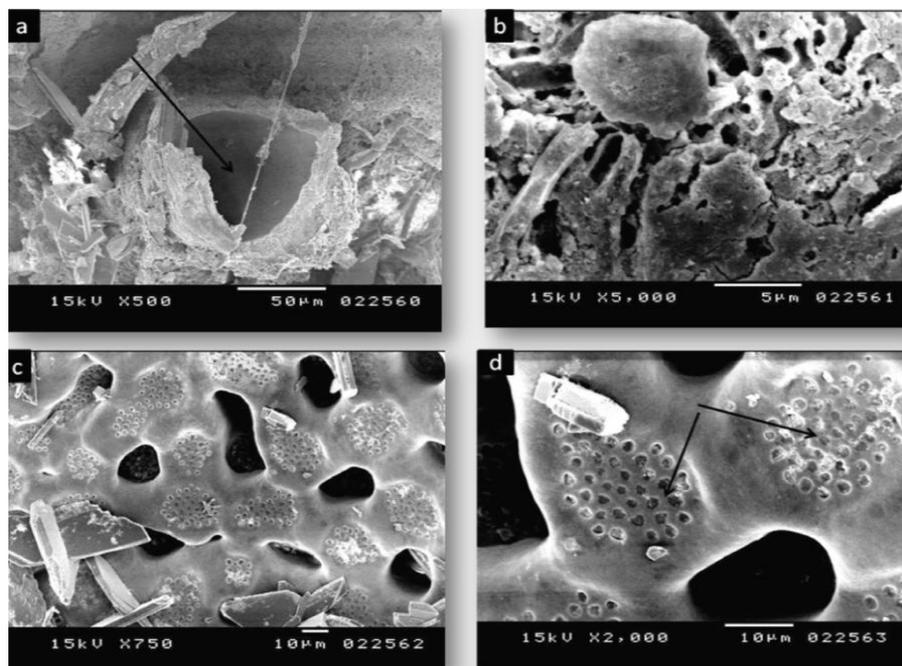


Fig.6: (a) Growth of fungal hyphae in empty pore spaces (crypto-endoliths). (b) Fungal septate hyphae and algal filaments from the polyp zone. (c,d) structure of crustose coralline algae reveals an internal structure that looks like a honeycomb & fusiform crystals.

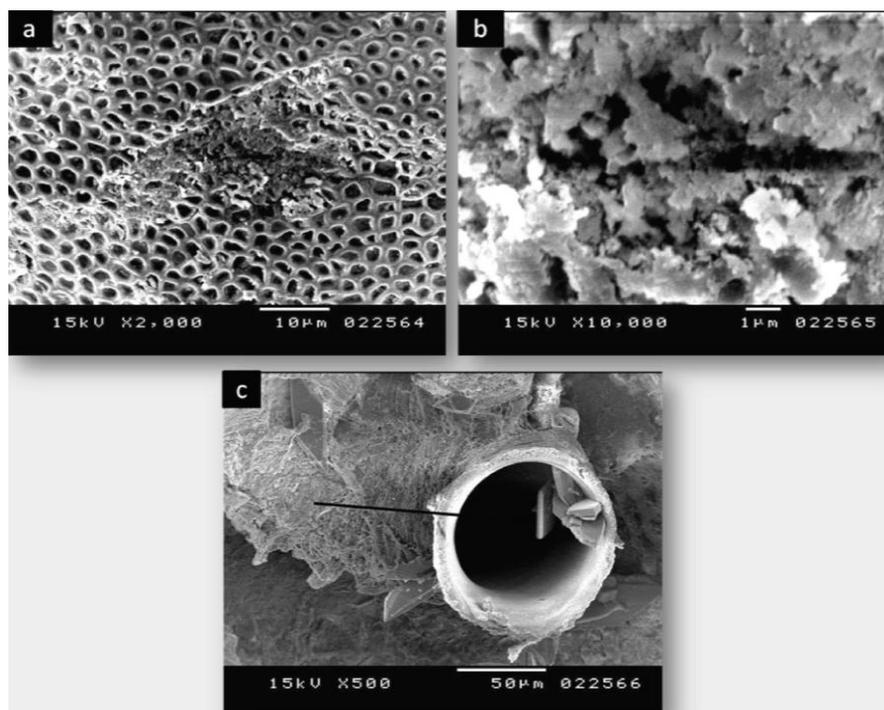


Fig. 7: (a) The coenosarcs at the growing front showing the desmocytes between fingerprints of the coenosarcs spines. (b) Protruding fungal hypha surrounded by fields of fine aragonitic needles syntaxially growing from an area of repair carbonate. (c) Top view of a fungal cone surrounded by a 'halo' of dense repair carbonate, where syntaxial crystal growth is delayed. Syntaxial growth of normal skeletal carbonate is seen peripheral to the smooth repair carbonate.

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الخصائص المورفولوجية الدقيقة لانسجة وهيكـل المرجان *Lobophyllia hemprichii* من العين السخنة - خليج السويس - البحر الأحمر

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1-قسم علم الحيوان - كلية العلوم - جامعة بنها - مصر

2-المعهد القومى لعلوم البحار والمصايد - السويس - مصر

تتناول هذه الدراسة الخصائص المورفولوجية باستخدام المجهر الإلكتروني (SEM) لأنسجة وهيكـل المرجان *L. hemprichii* التي تم جمعها من على حافة الشعاب المرجانية في العين السخنة (خليج السويس - البحر الأحمر).

انها تتواجد في مجموعة متنوعة من القوام. ولديها بوليبيات لحمية تختفي في الهيكل العظمي الجيري والأشكال بعضها على نحو سلس، والبعض الآخر كثير البثور. والحاجز (سبته) يتفتق في سمك الجدار إلى (الكوليوملا) ولها أسنان حادة طويلة القامة.

الطحالب التكافلية يتم استضافتها داخل جسم الحيوان وهي توفر لها الاحتياجات الغذائية من خلال عملية التمثيل الضوئي. و تستفيد أيضا من أغذية إضافية في شكل عوالق صغيرة مثل الأرتيميا وذلك في المساء عندما تخرج زوائدها وتصبح مرئية.