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Ultrastructure of *Galaxea fascicularis* from El-Ain El-Sukhna, Gulf of Suez Red Sea

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ABSTRACT

Galaxea fascicularis is a hermatypic (reef-building) scleractinain coral that forms massive colonies of calcium carbonate skeleton. The ultrastructural investigations of the skeleton and the polyps of *G. fascicularis* that collected from the reef edge of El Ain El Sukhna (Gulf of Suez, Red Sea) have been observed by using the Scanning Electron Microscope (SEM). In this study, SEM was used to observe and understand the occurrence and distribution of microflora which may cause diseases to coral species before doing any further molecular or genomic studies. The collected colonies of *G. fascicularis* had green or brown colored polyps. Each polyp lives in a skeleton cup, called a corallite that had a circle of septa around the calice opening. The septa occurred in hexamerous cycles (6 primary, 6 secondary, 12 tertiary, and 24 quaternary). The deposition of two crystal types, aragonite (blade-shaped crystals) and calcite (fusiform crystals) at the growth surface of the septa were investigated. Formation of semi-solid masses by fusiform crystals suggests that the crystals might play a structural role in septal extension. Microbial communities were observed at the surface of the polyp. Fungi appeared to be a regular component of all investigated polyps, while the bacteria were observed on some polyps. The muco-polysaccharides surface of *G. fascicularis* might provide a matrix for microbial colonization leading to the formation of biofilm-forming microbial communities. These communities might lead to tissue degradation and holes formation.

Key words: Scleractinian Coral, *Galaxea fascicularis*, Skeleton, Crystals, Microbial Communities, Scanning Electron Microscopy.

INTRODUCTION

Coral reefs are indeed a major marine ecosystem, because those species diversity greatly exceeds that of any other marine environment. They are generally known as the rainforest of the oceans. It is assumed that, while their total area is less than 0.2% of the sea surface [(1)]; coral reefs host almost 30% of all the marine biodiversity [(2)].

Egypt coastline possesses a significant proportion and considerable range of the coral reefs found in the Red Sea with about 3800 Km^2 of reef area and 1,800 km long. Of about 300 hard coral species found in the Red Sea, 2/3 are found in the Egyptian reefs, including some endemic species [(3,4)]. Scleractinian corals are the most important hermatypic (reef-building) organisms in the Red Sea. They are highly mineralised animals that possess a massive $CaCO_3$ skeleton (limestone) in the form of aragonite, covered by two-layered epithelium[(5,6)]. Skeletal growth occurs extracellularly at the interface between the underlying skeleton and a single-cell epithelial layer called the calicoblastic ectoderm. This external skeleton then creates a 3D framework that forms a complex habitat, increasing species abundance and total productivity. Such limestone structures may reach 1.3 km thick and up to 2,000 km long [(2)]. The $CaCO_3$ skeleton shape is characteristic at the species level[(7, 8,9,10)].

Two kinds of crystals; fusiform and blade or needle-shaped crystals, have been observed on the surface of skeletons of hermatypic corals[(11, 12,13,14,15)].

Studies of the crystalline and overall skeletal structure of scleractinian corals required the removal of the surrounding epithelia to visualize the $CaCO_3$ skeleton underneath. Many treatments have been used to dissolve the epithelial tissue, including H_2O_2 (Jell, 1974; Clode and Marshall, 2003b)[16,17] freshwater [18], NaOH [17, 18, 19] and commercial bleach (sodium hypochlorite)[11, 12, 13, 14, 15, 17, 20, 21, 22, 23, 24, 25, 26].

There is a recent increase in information concerning total microbial communities associated with healthy[27,28] and diseased corals[29]. Coral-specific microbial communities are hypothesized to have important physiological and ecological roles on coral reefs [30,31].

Euendolithic microorganisms are known to penetrate actively, by chemical dissolution, into substrates forming a network of tunnels conforming to the shape of their bodies [32], and also are boring phototrophic and organotrophic microorganisms that include cyanobacteria, chlorophytes, rhodophytes, and fungi [33]. They develop in a large variety of carbonate substrates, including crustose coralline algal thalli and coral skeletons [34, 35].

Euendolithic cyanobacteria and chlorophytes penetrate by an unknown chemical dissolution process into calcareous skeletons (i.e. shells and corals), as well as carbonate rocks, play an important role in the destruction of the invaded substrate in marine and freshwater environments[32, 36, 37,38, 39, 40, 41,42].

Fungi are known to associate with shallow-water corals, both as potential symbionts[43,43,44] and pathogens[45, 46, 47,48].

In live corals, Euendoliths are known to have different activities. Boring heterotrophic fungi appear to inflict damages to their live hosts[42, 44, 49, 50], while autotrophic (cyanobacteria and algae) euendoliths may provide benefits, especially in cases of bleaching events, through the release of nutrients and organic compounds [51, 52,53,54].

Galaxea fascicularis is a hermatypic scleractinian coral in the family Oculinidae. It is usually ball or dome shaped in captivity. Shapes that include spires, plates, encrustations, and branches are sometimes found. *G. fascicularis* can be green, grey, pink or brown, but always with contrasting colored tips. This species has polyps which are amongst the most beautiful of all corals. Each polyp has coloured translucent tentacles, which usually have white tips. The tentacles will be out during the day [55]. The polyp lives in a corallite which has a circle of septa. The septa that extend above the top of the corallite wall are referred to as exsert septa. The exsert septa are one of the primary sites of CaCO3 deposition and skeletal extension in the scleractinian coral *Galaxea fascicularis* [56].

Compared to some other reef corals, *G. fascicularis* is relatively resilient to stress from bleaching [57] and from sedimentation[58,59], however it can affected by microbial communities such as bacteria [31] and fungi [60].

In this study, we describe the ultrastructure of the polyps and skeleton of the scleractinian coral *G. fascicularis* and its associated microbial communities by using scanning electron microscope.

MATERIALS AND METHODS

Collection and maintenance

Colonies of scleractinian coral (*Galaxea fascicularis* Linnaeus, 1767) were collected from the reef edge of El Ain El Sukhna (western coast of Gulf of suez, Red Sea, Egypt) at a depth of 3-5 meters. The corals were transported in buckets of seawater to the laboratory, where they were maintained in sunlit, well-aerated, flow-through aquaria in natural seawater at 24–25 °C. Different samples of *G. fascicularis* were photographed by a digital camera.

Polyp preparation

Ten individual polyps were separated from different live colonies and fixed for ultrastructural investigations by immersing them immediately in $_4F_1G$, phosphate buffer solution (PH 7.2) at 4°C overnight. The polyps were then post fixed in 2% OsO_4 in the same buffer at 4°C for 2 hours, then washed in the buffer and dehydrated at 4°C through a graded series of ethanol, and dried.

Skeleton preparation

Galaxea fascicularis 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_2O to remove the overlying soft tissues. The skeletons were then dried at 60 °C for 24 h. Ten corallites of different sizes were easily separated from the skeletons with forceps; five of them were divided crosswise to investigate the internal structure. Pieces of peritheca (skeletal area between the corallites) and endothecal dissepiments (skeletal area beneath the corallites) were separated.

Ultrastructural investigations

The prepared polyps, corallites, peritheca and endothecal dissepiments were mounted by using carbon paste on an Al-stub and coated with gold up to a thickness of 400Å in a sputter- coating unit (JFC-1100E). Investigations of the samples were performed in a JEOL JSM-5300 scanning electron microscope operated between 15 and 20 KV.

RESULTS

G. fascicularis collected colonies were solid, ball or dome shaped and green or brown in color. Each colony consisted of many polyps with different sizes. The polyps had long coloured finger-like tentacles arranged in cycles around the mouth opening (Fig. 1a, b, c). The polyp embedded in a skeleton cup made of calcium carbonate, called a corallite. The corallites were irregular in shape and rounded or slightly elliptical in cross section with vertical walls. They were separated, distinct and had mixed sizes. The corallite diameter varied from 1.5 mm to about 15 mm. The skeletal area between the corallites was called the peritheca while the area beneath them known as endothecal dissepiments. The corallites were generally at least 2 to 3 mm apart, often more and rised at least 2 mm and sometimes over 15 mm above the peritheca (Fig. 1d, e, f).

Ultrastructural investigations of *G. fascicularis* skeleton

The investigated corallites had the same structure. The upper, open face of the corallite from which the living polyp protrudes is called the calice. Each corallite had a circle of septa surrounded the calice opening. The septa protruded above the level of the corallite wall (theca) and were clearly visible as thin, sharp blades to form exserted septa (Fig. 2a, b). The septa occurred in hexamerous cycles (6 primary, 6 secondary, 12 tertiary, and 24 quaternary) (Fig. 2a, b, c). Septal margins were smooth, granular or dentate with septal teeth or spines, while the septal sides were smooth or dentate (Fig. 2c, d, e, f). The inner ends of the primary septa were fused in the center of the corallite forming upright column called the columella (the central axis of the corallite found below the calice). The columella had a styliform (rod-like) (Fig. 2g, h).

Costae continued a short way down the outside of the theca but were absent from perithecal areas. Theca and costae had many bores (Fig 2i). The peritheca was slightly rough due to the presence of irregular low vesicles and had bores (Fig. 2j). The endothecal dissepiments had overlapping lamellae and fusiform crystals between them (Fig. 2k).

Upper view of crosswise divided corallite showed that the structure of the septal edge was differentiated between the upper and inner margins. Inner margins have only 3 cycles of septa (Fig. 2 l, m). There were large elongate fusiform crystals precipitated at the growing lateral edges of the septa (Fig. 2 m, n, o).

Microbial communities associated to *G. fascicularis* polyps

Fungi appeared to be a regular component of all investigated polyps. The fungal hyphae were observed attached to the tentacles and septal margins (Fig. 3a, b). These hyphae associated to the mucous which secreted by the polyp and had fungal conidia (Fig. 3c). The fungal hyphae were found attached to fusiform and blade-shaped crystals (Fig. 3b, c, d). These crystals were observed around the tentacles and septa (Fig. 3b, e), sometimes were seen attached to each other (Fig. 3f).

Bacterial communities were observed above the oral surface of some polyps attached together and form a web-like structure (Fig. 3g, h, i, j).

Skeletal spines or cones and bores were appeared at the surface of all investigated polyps (Fig. 3k, l). The bores caused tissue degradation and mineralization by micro-granular calcite (Fig. 3m, n, o).

DISCUSSION

In *G. fascicularis* collected samples, corallites were separated and had mixed sizes. corallite diameter varies from 1.5 mm to about 15 mm and was irregular in shape at the surface. Corallites were irregular in shape, that was depending on how closely they were packed and on their position on the corallum as revealed by Veron and Pichon [61].

The septa of *G. fascicularis* had fasciculate surfaces as reported for other corals [20]. The fasciculi were composed of blade-shaped crystals ran parallel to each other. In addition to the blade-shaped crystals, fusiform crystals of various sizes were observed at the growing lateral edges of the septa. Fusiform crystals have been suggested to be calcite, in contrast to the bulk of the skeleton, which is formed from aragonite as reported by Gladfelter [12].

In our study, blade-shaped and fusiform crystals were seen attached together around the septa of *G. fascicularis*. Hidaka ([15] suggested that blade-shaped crystals are deposited on the surface of the fusiform crystals and that further growth and addition of blade-shaped crystals result in parallel bundles of crystals. Tips of these bundles protrude above the surface of the skeleton to form irregular-shaped, fish scale-like fasciculi. However, it is not clear whether the fasciculi are formed only in this manner and are always derived from fusiform crystals. Also, Gladfelter [12] reported that, clusters of needlelike crystals extended from fusiform crystals to ultimately form fasciculi.

Fusiform crystals on investigated *G. fascicularis* septa were typically observed at the lateral edges where centers of calcification do not persist as reported by Cuif and Dauphin, [62] and Clode and Marshall [17]. This is consistent with the suggestion of Constantz [26] that centers of calcification were not required for nucleation and growth of fusiform crystals.

Variability in the reported distribution of fusiform crystals on septa[12,63] made interpretation and understanding of crystal deposition and skeletal extension in corals difficult. Reasons for these reported differences are unknown, but preparatory techniques and environmental conditions may have significant effects upon skeletal microstructure[64,65].

The investigated crystals at the growth edges of the septa were one of the primary sites of CaCO3 deposition and skeletal extension in the scleractinian coral *Galaxea fascicularis*. Gladfelter [12] reported that fusiform crystals form loose scaffolding on the surface of exsert septa at night and acicular crystals nucleate on the fusiform crystals during the day, ultimately giving rise to fasciculi. This cycle of deposition of fusiform crystals was proposed to account for skeletal extension in zooxanthellate corals. Hidaka[13] also proposed

that, no fusiform crystals or needle-shaped crystals were found on the septa of polyps kept in darkness lead to low rate of calcification in the coral.

Bacterial and fungal communities were observed in this study on *G. fascicularis* polyp. Pascal and Vacelet[30] revealed that, the coral surface was covered by muco-polysaccharides, which provided a matrix for bacterial colonization leading to the formation of biofilm-forming microbial communities. Kim [66] postulated that, mucus-covered coral surfaces are often colonized by bacteria and other microorganisms. However, a few studies suggested that corals might be associated with specific bacteria. Differences in the composition of the surface mucus produced by specific corals resulted in different populations of associated microbes[28]. Furthermore, mucus-associated bacteria had specific carbon source utilization patterns that were consistently associated with certain coral species and varied among different species of coral as reported by Ritchie and Smith [67].

The present study established the abundant presence of fungi in *G. fascicularis* polyp. Fungal hyphae were found to be common in corals and assumed the following ecological roles as recorded by Le Campion-Alsumard *et al*.([68]: as euendoliths they penetrate coral skeleton; as cryptoendoliths they resided within pore spaces; and as endophytes they grew inside filaments of endolithic algae. In addition, fungi were found inside the soft coral tissue, where they produced conidia in situ.

This study disclosed that endolithic fungi elicit defensive behavior on the part of the coral, indicating a parasitic rather than saprophytic relationship. Formation of conical carbonate structures has been reported earlier for species of the corals *Colpophyllia* and *Monastrea* by Scherer [69], who observed cones rising from pore walls by algae. Those structures reported in this paper as skeletal spins or cones. Scherer speculated on the mechanisms that might have caused precipitation by algae, but did not consider that the deposition might be a response of the coral to endolith action.

Bores were detected in this study on the skeleton and tissues of *Galaxea fascicularis* lead to tissue degradation, so the tissues became mineralized by micro-granular calcite. Prokaryotic penetration into coral tissue has been described previously, resulting in the bleaching (loss of zooxanthellae) of the tissue [70]. It has previously been proposed that 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 [71,72].

Glynn [73] revealed that, the bioeroding potential of bacteria and the various taxa involved is very limited, preliminary observations suggested that these organisms might be important under certain conditions. Fungi were capable of deep penetration into coral skeletons by chemical dissolution. The hyphae produce narrow borings and penetrate the deepest recesses of coral skeletons, probably because of their ability to utilize the organic matrix of coral skeletons.

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Fig. 1. Photograph pictures of *Galaxea fascicularis*: (a) Part of green colony, (b) Part of brown colony, (c) Individual polyps from brown colony, (d) Part of skeleton of green colony, (e) Part of skeleton of brown colony, (f) Part of skeleton of brown colony shows endothecal dissepiment (asterisk). Abbr.: c, corallite; mo, mouth opening; p, polyp; pe, peritheca; t, tentacle. Scale bar= 5 mm.

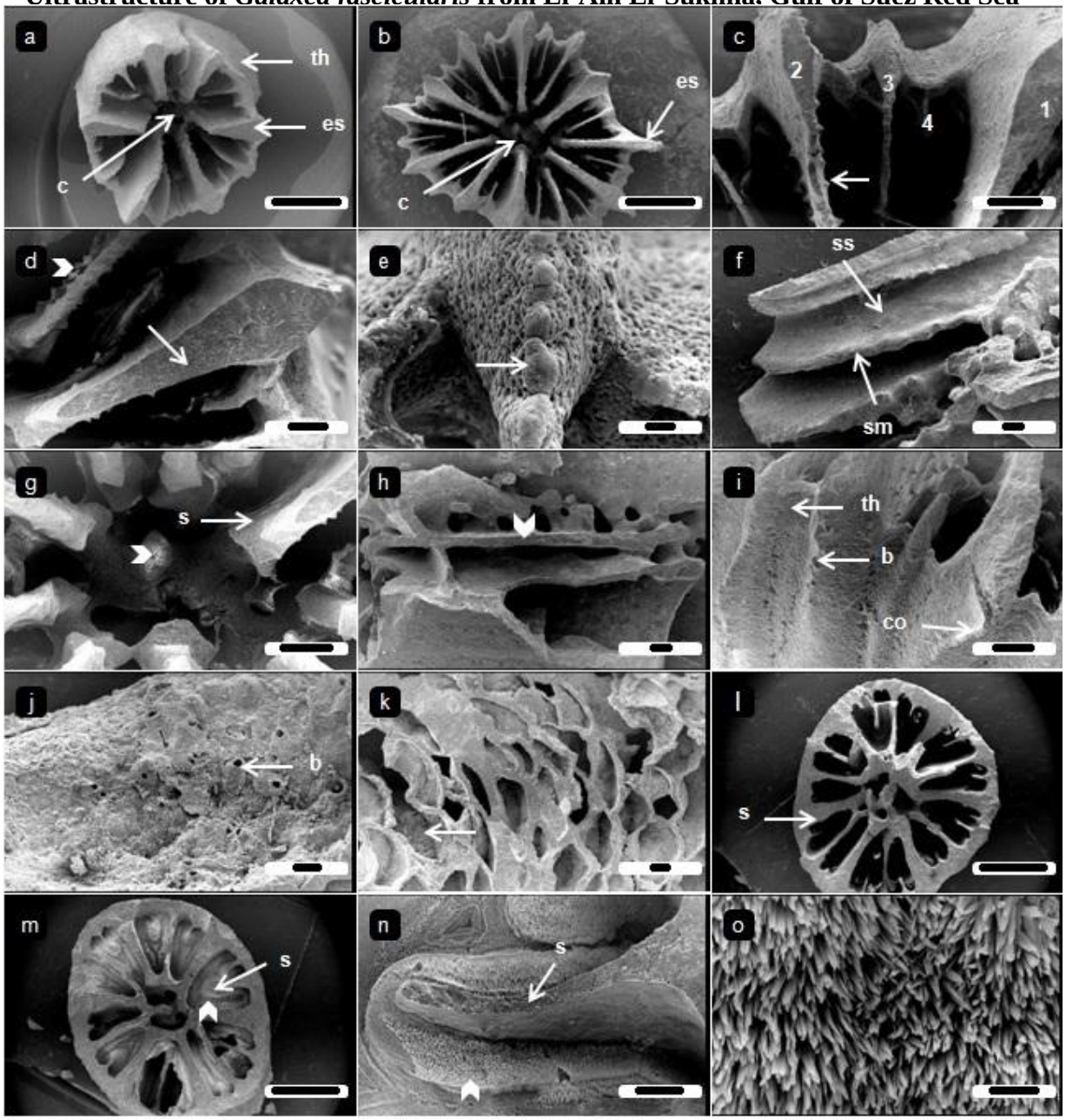

Fig. 2. Scanning electron micrographs of *G. fascicularis* skeleton: (a, b) Upper view of the corallite, (c) 1-4 are septa arranged in four cycles and the arrow refers to the dentate septal margin, (d) Smooth septal margin (arrow) and dentate septal side (head arrow), (e) Granular septal margin (arrow), (f) Septal margin and smooth septal side, (g) Upper view of the corallite shows the columella (head arrow) below the calice, (h) Lateral view of columella (head arrow), (i) Outer surface of corallite wall, (j) Peritheca, (k) Endothecal dissepiments shows the fusiform crystals (arrow) between the lamellae, (l) Upper view of crosswise divided corallite shows septal cycles, (m) Upper view of crosswise divided corallite shows the precipitation of crystals (head arrow) at the growth surface of the septa, (n) Fusiform crystals (head arrow) at the growth surface of the septa, (o) High magnification of the fusiform crystals. Abbr.: b, bore; c, calice; co, costa;

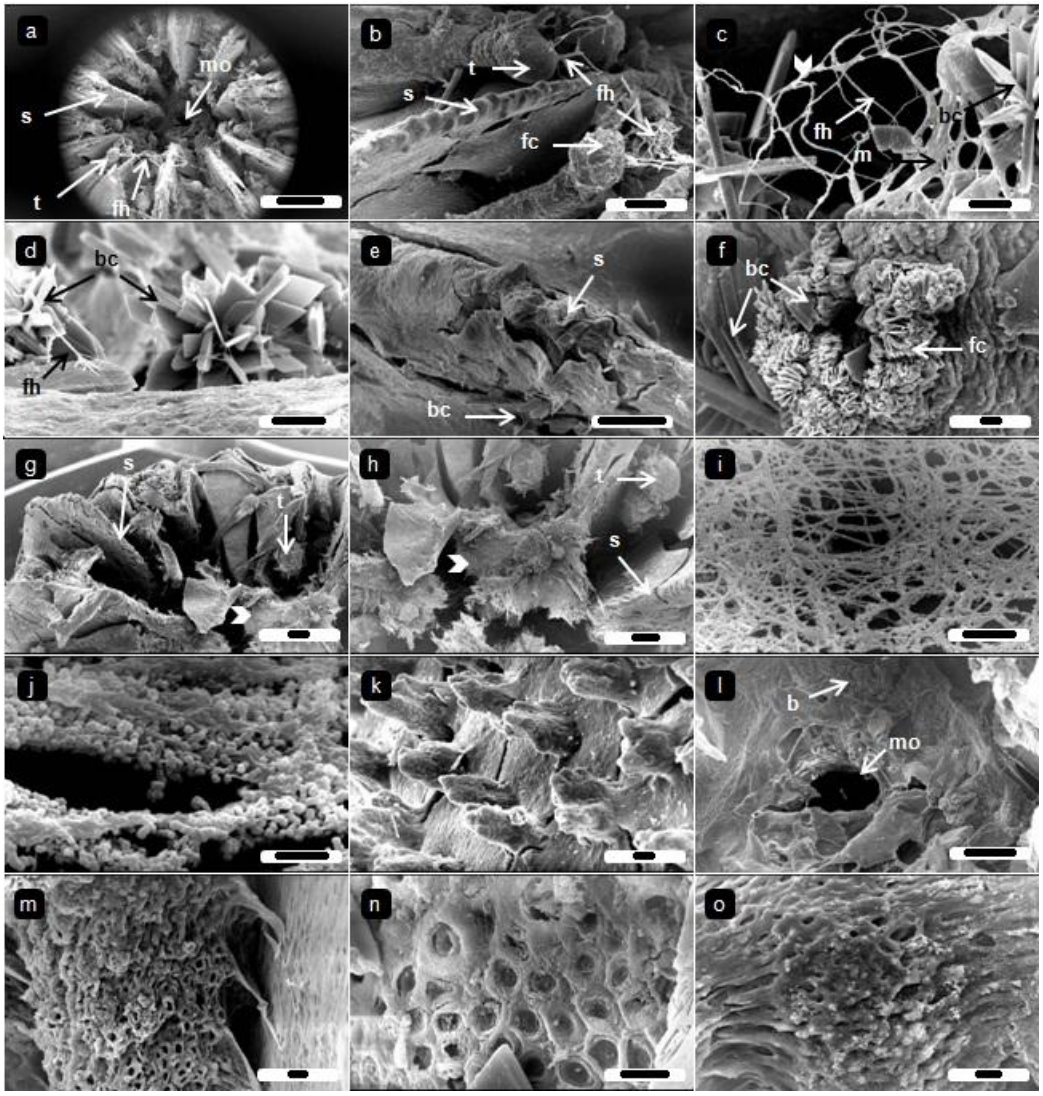

Fig. 3. Scanning electron micrographs of microbial communities associated with *G. fascicularis* polyps: (a) Individual polyp infected with fungal communities, (b) Fungal communities attached to tentacles, septa and fusiform crystals, (c) Fungal hyphae associated with the mucous which secreted by the polyp, head arrow refers to fungal conidia, (d) Fungal hyphae attached to blade-shaped crystals, (e) Blade-shaped crystals around the septa, (f) Fusiform and blade-shaped crystals attached to each other, (g) Individual polyp infected with bacterial communities(head arrow), (h) Web-like structure of bacteria (head arrow) above the oral surface, (i, j) Bacterial communities attached together in the form of web-like structure, (k) Skeletal spines, (l, m, n) Bores formed by microbial communities at the surface of the polyp, (o) Bores and micro-granular calcite on the tissue of the polyp. Abbr.: b, bores; bc, blade-shaped crystals; fc, fusiform crystals; fh, fungal hyphae; m, mucous; mo, mouth opening; s, septa; t, tentacle. Scale bar: a= 500 µm; b, g, h, l= 100 µm; e= 50 µm; c, d, f, i, k,

Ultrastructure of *Galaxea fascicularis* from El-Ain El-Sukhna, Gulf of Suez Red Sea

التركيب الدقيق لاحدى المرجانيات المتكلسة *Galaxea fascicularis* من منطقة العين السخنة في خليج السويس

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تمثل *Galaxea fasicularis* احدى المرجانيات التي تبني مستعمرات ضخمة من الشعاب و يتكون هيكلها من كربونات الكالسيوم . فى هذه الدراسة تم دراسة التركيب المجهري للهيكل و الكائن الذي تم تجميعه من منطقة الشعاب في العين السخنة (خليج السويس- البحر الاحمر). وقد استخدمت تقنية المجهر الالكتروني لدراسة وفهم حدوث وتوزيع الميكروفلورا التي من الممكن ان تسبب امراض لانواع المراجين . وقد وجد ان لدى المستعمرات المجمعه الوان اما خضراء او بنيه للكائن. و ان كل كائن يعيش داخل كاس هيكلي يسمى corallite لديه مجموعه من الحواجز تحيط بفتحة calice. هذه الحواجز تتكون في دورات من العدد 6 ومضاعفاته (6 ابتدائية، 6 ثانوية،12 فوق ثانوية،24 رباعية). وقد وجد ان المسئول عن نمو سطح الحواجزهو ترسيب نوعين من البلورات ا لاراجونيت (بلورات حاده تكون على شكل شفرات) و الكالسيت (بلورات مغزلية). تلعب البلورات المغزلية دورا هيكليا وهاما في زيادة وتمديد الحواجز حيث انها تكون كتل نصف صلبة. كما لوحظ ان المجتمعات الميكروبية متواجدة على سطح الكائن. ظهور الفطريات على سطح الكائن في جميع العينات هو عنصر مألوف ،ولكن ظهرت البكتيريا في بعض العينات فقط. وجود السطح المخاطي على *G. fascicularis* يشكل بيئة مناسبة للجراثيم والميكروبات تؤدي الى تشكيل سطح رقيق من المجتمعات الميكروبية على الكائن ، ووجود هذه الكائنات يساعد في تكوين الثقوب والذى يمكن ان يؤدى الى تدهور الانسجة.