



Mineralogical and biological study on the bryozoan *Calyptotheca alexandriensis* collected from Alexandria, Egypt

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ABSTRACT

Calyptotheca alexandriensis, has been recently recorded at the Eastern Harbour of Alexandria, Egypt, and described as a new bryozoan species. This study focuses on the mineralogical structure of the calcareous shell of *C. alexandriensis* and the morphological features of its polypides using XRD, SEM and EDS. The results revealed that the shell of *C. alexandriensis* contains aragonite in dendritic crystal habit and calcite in rhombohedral structure. These findings were confirmed by the presence of some aragonite-related cations such as Ba and Pb and other calcite-related cations as Mg. The autozooid polypides have an elongated cylindrical body with a lophophore of 14-16 tentacles.

INTRODUCTION

The adverse impacts of climate change; especially ocean acidification, encourage the mineralogical studies of the calcareous skeletons of marine invertebrates. It was believed that animals with variable CaCO_3 mineralogy and chemistry, such as the Bryozoa, might be able to act as a “bellwether” for climate change and ocean acidification (Fabry *et al.*, 2009).

Some invertebrates incorporate two or more minerals in an organized multilayered skeleton. Analysis of such biomineral combined skeletons is necessary for understanding how such complex calcifying invertebrates might behave in the lower-pH marine bodies of a high- CO_2 world (Orr *et al.*, 2005; Raven *et al.*, 2005; McNeil and Matear, 2008). These organisms can act as repositories for information about seawater conditions (Loxton *et al.*, 2018).

Bryozoans represent a major component of most benthic ecosystems from the intertidal to abyssal depths (Schwaha *et al.*, 2020), significantly contribute to the carbonate budget in the sea (Taylor and Allison, 1998; Taylor *et al.*, 2008) through their capability of building calcareous skeletons (Kuklinski and Taylor, 2009; Smith, 2009). Nearly all the calcareous bryozoan skeletons comprise mineral polymorphs with crystal

structures mainly of calcite, aragonite, and rarely vaterite (Taylor *et al.*, 2008) which are secreted in distinct skeletal layers (Smith *et al.*, 2006; Lombardi *et al.*, 2008; Taylor *et al.*, 2008; Gobac *et al.*, 2009).

Two-thirds of the analyzed bryozoan colonies had calcitic skeletons against aragonitic or biminerale skeleton in the others (Smith *et al.*, 2006). The biminerale form is taxonomically widespread among the cheilostomes. The magnesium (Mg) content in bryozoan calcite varied widely among the different species, but it was mostly at low to intermediate level (Smith *et al.*, 2006; Lombardi *et al.*, 2008; Kuklinski and Taylor, 2009; Taylor *et al.*, 2009). This variation can be ascribed to environmental, physiological or phylogenetic influences (Swezey *et al.*, 2017).

A bryozoan colony consists of a number of zooids, each comprises a polypide (soft tissue comprising a lophophore associated with a U-shaped gut and retractor muscles) and a cystid (body wall) (Winston, 1978; Schwaha *et al.*, 2020). Regardless of high morphological diversity of cystids and polypides, phylogenetic and taxonomic studies of Bryozoa were based mainly on the external skeleton (Schwaha *et al.*, 2020), while a few studies were done on polypides morphological characteristics (Winston, 1978; Schäfer, 1985; Boardman *et al.*, 1992; d'Hondt, 2005, 2015).

The cheilostome bryozoan, *Calypotheca alexandriensis*, has been recorded for the first time from the Eastern Harbour of Alexandria, Egypt, and described by Abdel-Salam, Taylor and Dorgham (2017). Since that, no further studies were made on this species except that of Hamdy and Dorgham (2019) on the fauna associated with it.

The present study is an attempt to increase the knowledge about the bryozoan *C. alexandriensis*, particularly the mineralogical structure of its shell and the morphological characteristics of the polypides.

MATERIALS AND METHODS

1. Study area

The Eastern Harbour (EH) is a small shallow semi-closed bay on the central part of Alexandria coast on the Egyptian Mediterranean Coast with depth less than 10 m (Said and Maiyza, 1987). It is isolated from the open sea by a high breakwater, except two openings in the middle and at the eastern side. The harbor has a cultural significance as it contains large numbers of artefacts from ancient Egypt (Schwartz, 1980), but it is considered as eutrophic bay due to long term sewage discharge and other anthropogenic impacts which caused fundamental changes in its environmental and biological characteristics (Dorgham, 2011).

2. Sampling and Lab work

During November 2018, three masses of the *C. alexandriensis* colonies were collected from a rocky bottom at 1.5 m depth and from metallic stand of a marina (Figure 1) and placed in plastic jars containing sea water. Subsamples of the collected colony were first narcotized using MgCl₂ solution in order to facilitate the extraction of the polypides. Under a stereomicroscope, small pieces of the bryozoan colony were carefully and gently crushed to release the polypides. The biked polypides were then fixed and preserved in 70% ethanol and were examined as uncoated specimens by scanning

electron microscopy (SEM) using a low-vacuum scanning electron microscope (LEO 1455-VP) at the NHMUK.

The rest of the collected bryozoan colonies were left to dry at room temperature and then were used for the skeletal mineralogy analysis.



Figure 1. The Eastern Harbour of Alexandria, including the position of the sampling area.

3. Skeletal mineralogy analysis

3.1. X-ray diffractometry (XRD)

XRD was performed at the Central Lab, Faculty of Science, Alexandria University. Samples were powdered in an agate mortar and placed on single-quartz-crystal substrates. Mineral phase determinations; qualitative analysis, of the bulk samples were done using a MeasSrv (D2-208219) powder diffractometer with $\text{CuK}\alpha$ radiation filtered with a graphite monochromator running at 40 kV and 40 mA. The XRD had a fixed source-sample-detector geometry, and samples were measured in reflection mode. An X-ray diffraction data set was collected from 20 to $60^\circ 2\theta$. The tilt angle between the source and the sample was 5.97 , and the horizontal slit system was set to 0.14 mm to confine the x-ray beam to pure cobalt $\text{K}\alpha_1$. Samples were rotated during the measurements to randomize the grain orientations in the x-ray beam.

3.2. Scanning electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS)

Morphological observations on the samples were carried out using a low-vacuum scanning electron microscope (SEM) (JEOL JSM- 6360 LA). Samples of bryozoans were

first cleaned from their organic parts by bleaching in diluted commercial sodium hypochlorite, fractured, finely ground, polished, mounted on the SEM stubs, and then sputtered with carbon. For examination of the samples, the SEM was operating in back scattered (BSE) mode at an accelerating voltage of 25 kV and current of 10 mA. The same SEM equipped with the ISIS Energy Dispersive Spectrometer (EDS), coupled with INCA 250 system, was used for elemental distribution analysis in the samples. EDS qualitative analysis and elemental mapping was performed on the carbon coated samples at an accelerating voltage of 20 kV.

RESULTS

1. Polypides

Systematics

Phylum: Bryozoa

Class: Gymnolaemata Allman, 1856

Order: Cheilostomata Busk, 1852

Suborder: Flustrina Smitt, 1868

Superfamily: Smittinoidea Levinsen, 1909

Family: Lanceoporidae Harmer, 1957

Genus: *Calyptotheca* Harmer, 1957

Calyptotheca alexandriensis Abdel-Salam, Taylor and Dorgham, 2017

(Figure 2A, B and C)

Material examined. Mediterranean, Alexandria, Eastern Harbour, 0.5-1 meter depth on metallic marina supports, collected by Prof. Dr. Mohamed. M. Dorgham, Oceanography Department, Faculty of Science, Alexandria University, November 2018. Detailed description of the zooids was provided by **Abdel-Salam et al. (2017)**.

Description. Polypides highly pigmented, colour orange in fresh specimens and faint to pale orange in preserved specimens. Polypide size about 790-843 μm length. Lophophore simple, cup-shape, forming circular funnels with tentacles of equal length (equi-tentacled type) (Figure 2B). Lophophore diameter varies from 472-500 μm . Tentacle number from 15 to 16 and length of tentacular filaments ranged between 443 and 456 μm .

The lophophore is connected to the cystid via the tentacular sheath (Schwaha et al., 2020). When the lophophore retracted it enclosed in the tentacular sheath (Figure 2A), while when expanded (Figure 2B) it is withdrawn forming what is sometimes referred to as the 'introvert'. Figure 2 AandB shows the tentacular sheath (introvert) which seems to be composed of 2 lobes appeared at the distal end of the retracted lophophore, while when the lophophore expanded the 2 lobes appeared withdrawn on the lateral sides of the body. However, it is not assured whether it is the ordinary structure of the tentacular sheath in this species or these lobes were caused during polypides stripping from their cystids. Sensory structures such as Lateral cilia present on tentacles. Intertentacular pits (pits between the tentacles) are also obvious (Figure 2C).

The gut is U-shaped, the foregut (pharynx and oesophagus) followed by stomach consisting of cardia and a sac-like caecum and then pyloric region that is divided by a constriction from the rectal region that ends to the anus (Figure 2A). The foregut and cardia are elongated, which is a common characteristic in Gymnolaemata.

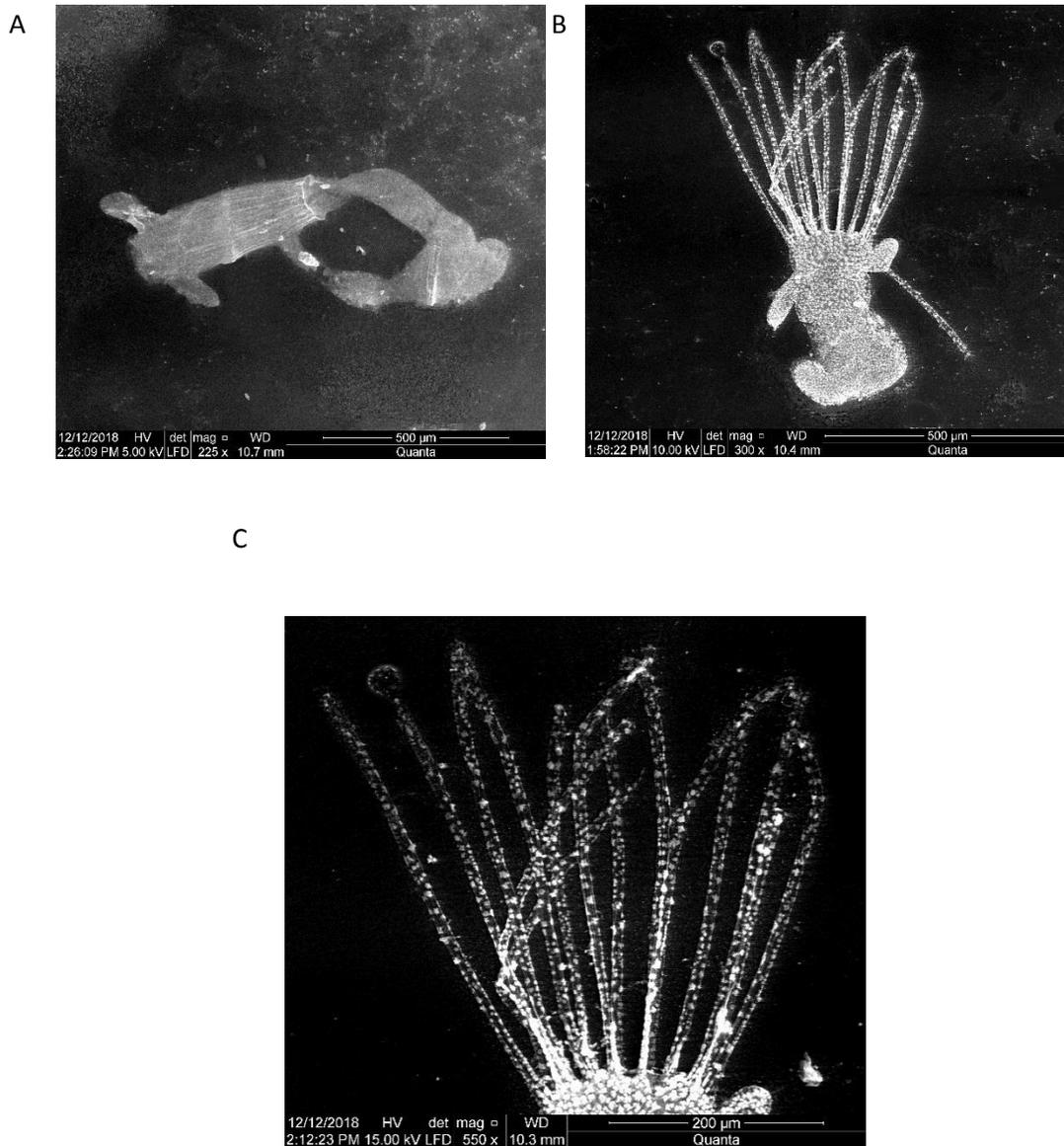


Figure 2. *Calyptotheca alexandriensis*, scanning electron micrographs of the polypides. **A**, whole mount with retracted lophophore: tentacular sheath enclosed the lophophore, two lobes of tentacular sheath appeared on the distal end of the lophophore one on each side; digestive tract appeared (foregut, stomach and rectum). **B**, polypide with lophophore expanded: tentacular sheath with the two lobes withdrawn on the lateral sides. **C**, lophophore: with lateral cilia and intertentacular pits (sensory structures) (lateral cilia obvious on the first tentacular filament to the left).

2. Skeletal mineralogy

The XRD qualitative examination of the bulk samples revealed that the carbonate shell of the bryozoan *C. alexandriensis* is composed of a mixture of aragonite and calcite (Figure 3).

The SEM equipped with back scattered electron detector provides crystal structure of both skeletal components, whereas Calcite has common crystal habit;

rhombohedral crystals (Figure 4) and aragonite has dendritic crystal structure; not in the common sharp and needle-like crystal habit (Figure 5).

Using of EDS qualitative analysis and elemental mapping showed the presence of some aragonite-related cations such as Ba and Pb and other calcite-related cations as Mg (Figure 6).

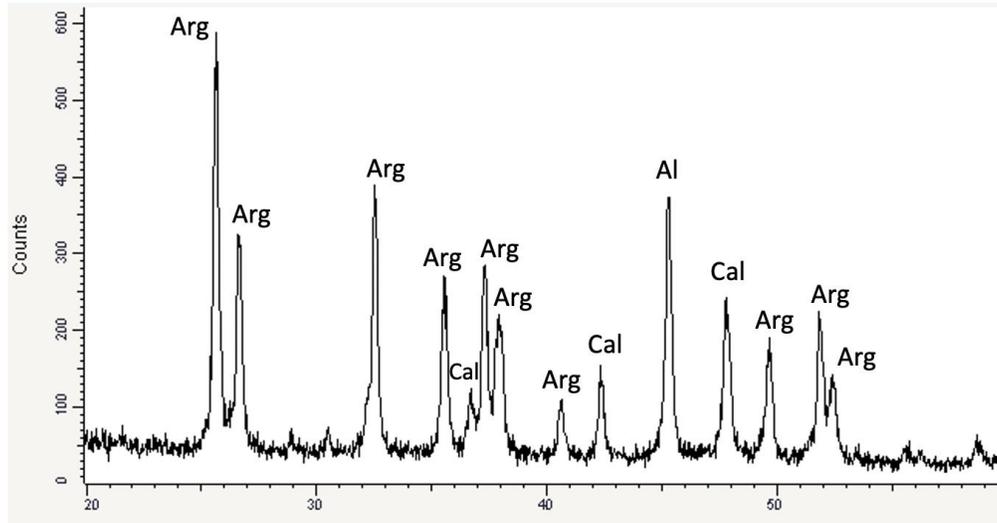


Figure 3. XRD pattern of the analyzed sample of *C. alexandriensis* showing a mixture of aragonite and Calcite (Arg – Aragonite, Cal – Calcite, Al – Aluminum from the sample holder).

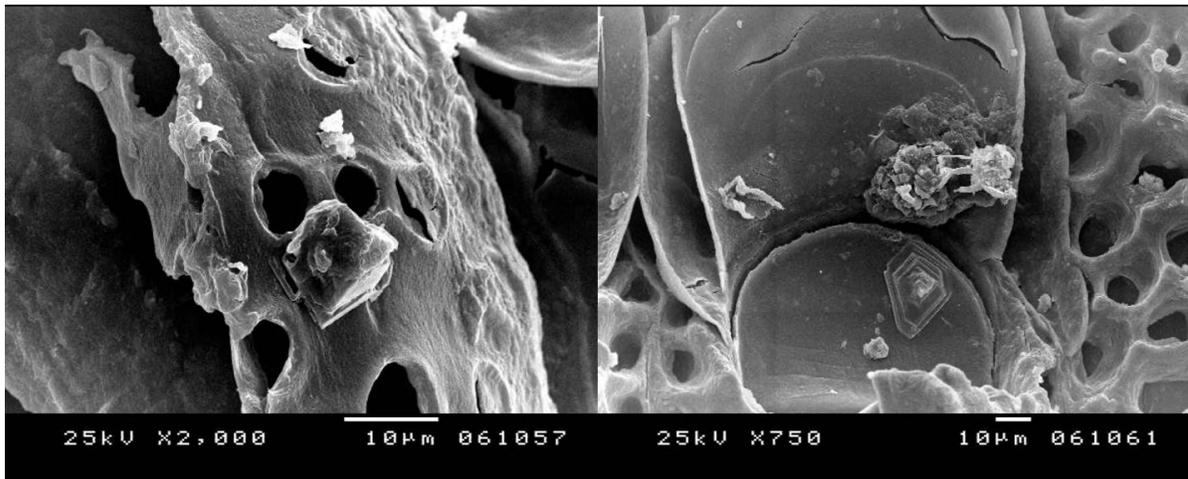


Figure 4. SEM image of rhombohedral calcite crystals of *C. alexandriensis* shell.

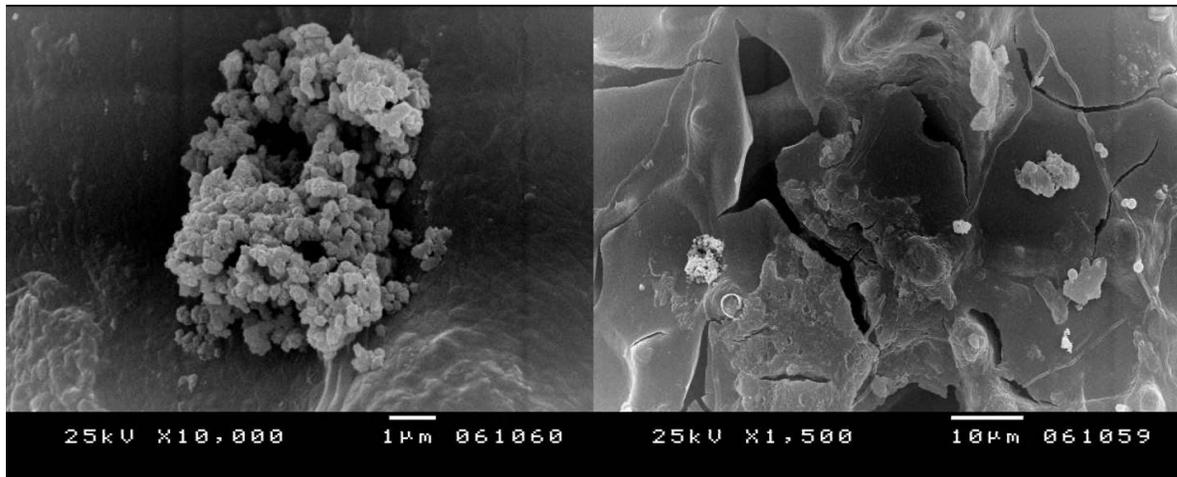


Figure 5. SEM image of dendritic aragonite crystals of *C. alexandriensis* shell.

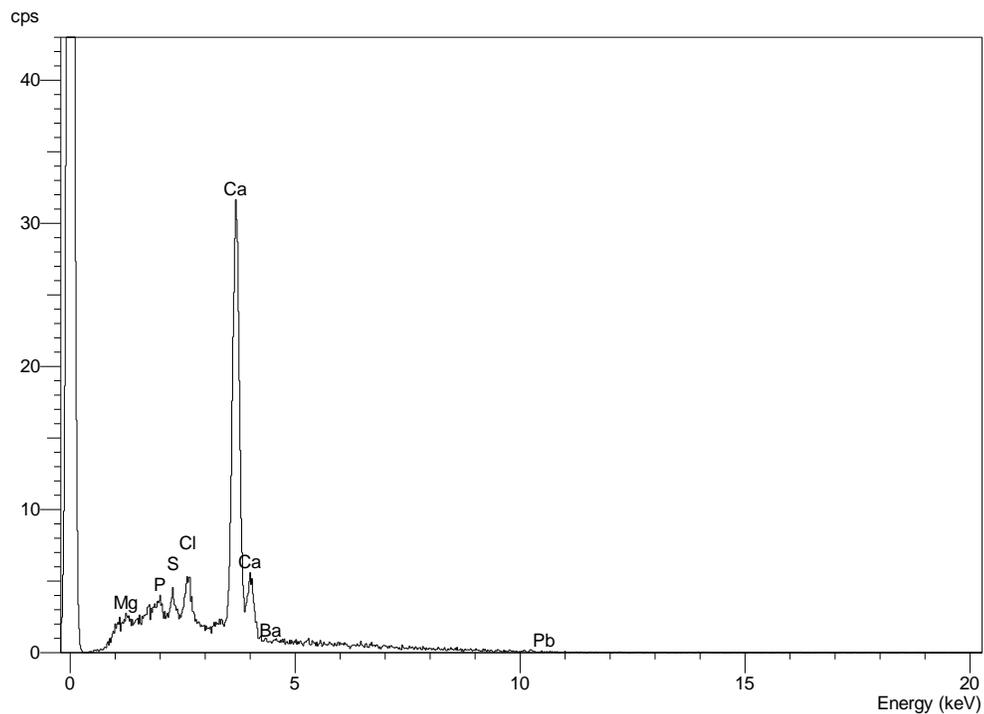


Figure 6. EDS spectrum of the biomineralized carbonate shell of *C. alexandriensis* with minor contents of Mg, Ba, and Pb.

DISCUSSION

Despite the high bryozoan diversity in the Mediterranean when compared with other regions of the globe, bryozoans are still scarcely known in the Eastern Mediterranean (Harmelin *et al.*, 2016; Sokolover *et al.*, 2016). In the Egyptian Coast, a

few studies were conducted about the Bryozoa (**Abdel-Salam and Ramadan, 2008a, b; Abdel-Salam, 2014; Abdel-Salam et al., 2017**).

Although **Abdel-Salam et al. (2017)** stated that *C. alexandriensis* represents a new species of unknown origin, **Hamdy and Dorgham (2019)** reported it as important benthic bioconstructor, providing suitable substrate and refuge for numerous invertebrate taxa.

Abdel-Salam et al. (2017), showed that the zooids of *C. alexandriensis* has unique disposition of the adventitious avicularia, being oriented transversely while in other related species, the disposition was parallel to the length of the autozoooid (**Cumming and Tilbrook, 2014**). Polypide morphology gives information on polypide structure and function (**Winston, 1978**). For example, feeding is greatly affected by the size and shape of the lophophore and the length of the tentacles in addition to the size of the mouth that may set physical limits on the type and size of collected food particles (**Strathmann, 1973**). Tentacle number is related both to taxonomic position and to polypide size. Factors such as zooid size and polypide ages in addition to external factors such as nutrition can influence the tentacle number (**Jebram, 1973; Dudley, 1973**). Variation in tentacle number may occur between species and within polypides of the same colony of a species (**Winston, 1978**). The polypide morphology help in understanding forms and functions of bryozoan polymorphisms and Modularity as well as inferring the evolution and phylogeny of phylum Bryozoa (**Schwaha et al., 2020**).

The polypide morphology can affect ecology and behaviour of bryozoan species, particularly in encrusting cheilostomes with moderate polypide size, as the formation of temporary clusters of lophophores produces strong water current which increases the direct feeding of polypides (**Winston, 1978**). This type of behaviour could be also found in *C. alexandriensis* as one of the encrusting cheilostomes with moderate polypide size. The number of tentacles in polypides of *C. alexandriensis* (15-16) are comparable to those (8 and 31) recorded in other cheilostomes (**Winston, 1978**).

The present study revealed the bimineralic composition of the *C. alexandriensis* shell—including both calcite and aragonite. This pattern is widespread among the cheilostomes (**Smith et al., 2006; Loxton et al., 2018**).

Calcite and aragonite are the most common polymorphs of calcium carbonate in the calcareous shells of numerous organisms with the same chemical composition. However, they have different crystal structure; calcite is a hexagonal mineral with a variable crystal habits (prismatic, rhombohedral, tabular, ...etc.), while aragonite is orthorhombic mineral with a common needle-like crystal habit and in very few cases a dendritic crystal (**Milliman, 1974; Wang et al., 2012**).

In our species, the X-ray diffraction analysis showed the alternation of aragonite and calcite peaks, but it was difficult to describe the textural distribution of these minerals. On the other hand, SEM analysis provided the common rhombohedral crystal habit for calcite and a dendritic crystal structure for aragonite, but not in the common

sharp and needle-like. This may be attributed to the fact that organisms produce skeletons of carbonate minerals mostly with close packing crystals (**Taylor et al., 2008; Gobac et al., 2009**), and /or crystal habit may be modified because of the chemical and physical conditions during the crystal growth (**Taylor et al., 2008**). This agrees with **Speer (1983)**, who stated that, during biomineralization orthorhombic carbonates; including aragonite, may be used by different organisms to support and protect themselves.

The EDS qualitative analysis and elemental mapping confirmed the occurrence of some aragonite-related cations (Ba and Pb) and calcite-related cations (Mg). This is in consistence with the fact that calcite crystals include many divalent cations (like Mn, Fe, Mg, Zn, Co, or Ni), while aragonite crystals contain large cations (like Sr, Pb and Ba) (**Gobac et al., 2009**). This is supported by **Gobac et al. (2009)**, who supposed that the difference between aragonite and calcite can be detected by EDS from the large and small cation content of aragonite and calcite respectively.

However, the present study suggests the importance of using Raman spectroscopy in the future work for better identification of the carbonate mineral phases at submicron spatial resolution, allowing the distributions of calcite and aragonite within bryozoan skeletons to be determined with unprecedented precision.

Aragonite is a metastable form of CaCO_3 and is more soluble than calcite, hence, it is rarely preserved in fossil bryozoan (**Milliman, 1974**). Such conditions may dramatically alter the appearance of bryozoans skeletons (**Greeley, 1969**), and consequently cause more dissolution of the aragonite in the bryozoans' shells with future predicted increasing ocean acidification.

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