

Comment on bg-2021-140

Anonymous Referee #1

Referee comment on "A stable ultrastructural pattern despite variable cell size in *Lithothamnion corallioides*" by Valentina Alice Bracchi et al., Biogeosciences Discuss., <https://doi.org/10.5194/bg-2021-140-RC2>, 2021

General Comments

In this paper, the microstructure of the chosen coralline algae was carefully observed at each depth using electron microscopy. The study can make a significant contribution to the community that is studying the diversity of coralline alga. It is important to note that the patterned microstructure in this species is also helpful for identifying these algae. The suggestion that there may be a correlation between water depth and cell size could also enhance the value of this study.

However, the fact that the number of individuals used for observation and measurement has not been clarified is an essential deficiency when considering the reproducibility of this study. The number of individuals in the samples used should be clearly stated. In addition, to refer to the "very early diagenesis," it is essential to describe the more detailed processing and storage conditions from sample collection to observation. We believe that the current description does not go as far as to say that diagenesis effects were found in living individuals. The authors should also concern about the lack of environmental data other than the water depth. Currently, they are discussing the correlation with water depth, but various parameters such as light intensity, water pressure, water temperature, and others co-vary with water depth. In order to examine which of these parameters has more influence, environmental data other than water depth needs to be presented. In addition, even though the report is about microstructure, it is not easy to make comparisons at different water depths. Moreover, it would be better to observe the micromorphology from more angles to compose diagrams for comparison. That diagrams can be helpful for general comparison among other groups of organisms and others. The crystal photographs from various angles would make the crystal's morphology easier to understand for a general audience.

Individual comments

P2L34 Why does examining GCA reveal the primary production of macroalgae? Is it correlated with the local/regional overall macroalgae?

P3L77 This reviewer is not sure of the motivation that led to this objective. The authors' working hypothesis is that the elements depend on environmental factors, or are they stable within the region?

If the motivations of this study are not clear, it is not possible to judge whether this study is appropriate for BG audiences. This reviewer is not confident that the authors have drawn appropriate conclusions (P8L237) that will be of interest to the BG audience. Perhaps it would be better to publish the paper as a classification study in a more paleontological journal to reach the right audience.

Further, the authors should clarify what elements are included in the "ultrastructural mineralogical features" and "ultrastructure pattern."

P3L86 Is the identification of samples done by molecular biology? Since authors are discussing variation in microstructural morphology, would not species identification by morphology be a circular argument?

P3L87 The authors discuss the very early diagenesis of crystals. It is crucial to describe the details of the method from collection to sample preparation to ensure reproducibility. Please describe it. It is acceptable to make supplemental material.

Readers need to know the environmental information of the sample collection point. For example, in Fig.4, we can consider whether the depth or environmental information such as temperature is correlated with the morphological features.

P3L95 Could the authors list the number of samples for each branch at each depth in the text or Table 1.

P4L116 The number of cells measured and other information for each sample should be indicated. The authors can also write the number of samples (n=) in Figure 4 or Table 2.

P4L127 To enable the reader to easily compare differences between samples, a picture of the long and short cells at all water depths should be shown in a single figure for comparison.

P4L131 If the combination of short and long cells is the seasonal variation, please show the reason for the classification into two types: short and long. If it is a seasonal variation, some may have a transitional feature. For example, if authors make a scatter plot, it may divide into two. Another possibility is that the cells appear to be short due to the difference in cutting direction. In order to prove this, MXCT measurement is recommended to show that the cells are smaller in three dimensions.

If short cells and long cells seem to have different cytological roles, to begin with, that explanations should be introduced beforehand in the introduction.

P4L144 Are "flat rectangular tiles" the form of crystal defined in previous studies? If there is already terminology for similar forms in previous studies, please cite it to avoid future confusion. Also, this reviewer is not convinced that the name "tiles" and the schematic diagram are appropriate for the crystal form seen in this picture. It would be good if SEM pictures from various angles could be available.

P5L160 It needs to be shown how the growth rate can be calculated; according to the description in P1L55, is not the reason for the band formation still under argument? If so, wouldn't the growth rate be based on the "assumption" that the band is one year old?

P5L169 If secondary crystals were there, how the samples were stored. Describe the conditions from the time of collection to the time of observation.

P7L224 The reviewer thinks it would be easier to convey the idea if authors make a comprehensive schematic diagram of the microstructure patterns that are not affected by the environment.

Fig.8 c): The black arrows are hard to see. Please border the arrows with white.

Fig.8 h) The black arrows are hard to find. Please border the arrows with white.