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Interactive comment on "Marine and freshwater micropearls: Biomineralization producing strontium-rich amorphous calcium carbonate inclusions is widespread in the genus *Tetraselmis* (Chlorophyta)" *by* Agathe Martignier et al.

M. Alberic (Referee)

marie.alberic@mpikg.mpg.de

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The presence of intracellular amorphous calcium carbonate inclusions (called micropearls) has been identified for the first time in marine unicellular micro-algae (genus Tetraselmis). A wide range of marine species has been studied and compared to a fresh water species from the same genus. Careful and high quality structural and chemical investigations were performed, which allow characterizing the main structural features (shape, size, spatial localization of the micropearls and other cellular components) as well as chemical composition (Sr/Ca ratio) of the micropearls for each

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species. The results are discussed in terms of biomineralization processes, possible functions of the micropearls are proposed and bioremediation application highlighted. I believe this study is very interesting for the readership of Biogeosciences and the manuscript is very well written. The authors may address the following comments that mainly concern the organization of the results and a more advanced discussion part that could impact more fields as for example the “ACC stabilization” research community. My only small concern is about the timescale of the biomineralization processes. It will strengthen the paper if some scale can be provided.

Detailed comments

Introduction. The first sentence giving the definition of “micropearls” should be precised because this term was first proposed in the last study of the authors (Martignier 2017) for one genus (Tetraselmis). “Intracellular mineral inclusions of ACC” have been identified before in others species (in particular cyanobacteria) and were not called micropearls. Therefore, the genus should be stated and the reference (Martignier et al. 2017) added. In addition, previous studies (Couradeau et al 2012 and later ones Benzerara et al. 2014), should be cited even if they concern prokaryote organisms. Line 20. “two freshwater organisms”, could mean either two individuals or two species... Only one is cited (cordiformis), what is the other one?

2.1. The presence of micropearls might depend on the time of observation of the cells. A time scale should be therefore indicated approximately, in order to make sure that it is the same for all culture cells. Would it be possible that micropearls belonging to different species could have different sizes, shapes, spatial localizations, Sr/Ca ratios just because of different time scales and not because they are from different species? Is the rate growth of the micropearls known? Does the compositional zonation (number of lines or spacing) could be a marker of time? or a marker of the different steps in the biomineralization process?

2.3.The coating was gold, therefore the authors should state why carbon, nitrogen and

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oxygen where not taken into account in the semi-quantitative analyses.

2.5. Some EDXS have been done so the title should be changed accordingly

3. Results. In general the subtitles are not homogenous. If the authors choose to name the subtitles according to the techniques they use they should be more consistent, and therefore called 3.1 “SEM observation. . .” , and possibly put 3.2 and 3.3 together and call it TEM-EDXS, (because EDXS analysis are also reported in 3.2).

3.1. In Figure 1, the full name of the samples are not reported for d) and j) that have different strains. The different strains might be very similar but this should be specified. In addition in Table 1 there is a mistake in the sample names for cui_sa and chui_cc. Line 11. “strains”or “species”?

p.7 line 1. The “problem” of the time of observation appears here, it is reported just for T. sriata, but the different organization in the different species could not be also related to different time scale? For T. Levis “the aggregate is missing” again, is this related to time?

Polyphosphate inclusions are not easily seen in Figure 1. Higher magnifications would be useful. Or relate to the TEM- EDXS observations? Did the authors observed EDXS signal from the P in these SEM images? Same for Iron-oxide minerals.

Line 22 and 25 “organisms” or “species”?

The authors cannot really state that “most flagellates do not produce micropearls” if they studied only two other species of one genus. They should be more careful, and maybe write instead of “most, . . . do not produce” “not all, . . . produce”.

3.2. The choice of the samples for the FIB-sections is not clear. Why T. cordiformis from the culture was not considered? it would have been useful to compare with the natural environment one. It looks like the choice of the species was made in order to observe the compositional zonation. However, the compositional zonation of the different species in barely describe in the paper.

Figure 2. The red line is not visible in a black and white printed version, a dashed white line will be more useful. Higher magnification of individual micropearls like in Martignier et al. 2017 will be useful to better see the compositional zonation.

Fig. S4 shows higher magnifications, but it is difficult to see the zoning pattern. Is it because of the image quality? It looks like T. contracta does not show zoning pattern at all? (in Fig S4,"c)"is missing).

p.9 line 8. "the highly hydrated" state of the ACC should be speculate more carefully. Could it be that the water associated with organic molecules around or within(?) the ACC micropearls could lead to the "strong response under the electron beam"? When dehydration occurs, does ACC eventually crystallize into calcite or is it still stable? Because of the presence of the Sr ?

The unexpected stability of ACC in living systems is still highly debated. And to my knowledge not much studies so far reported the role of Sr on ACC stability, therefore, it will be worth discussing it in the discussion part.

line 14. Describe in which sense (number of lines, spacing. . .) the zoning pattern varies within one cell. Could it be related to different stage of biomineralization within one cell? The same for the different composition within one cell?

3.3. line 22-27. The low magnification of Figure 3a and 3b does not allow properly visualizing the different cellular components, namely starch grains and chloroplasts. On which criteria the authors based the identification of these elements? Structural features? If so, higher magnifications of the areas presenting the chloroplasts and starch grains are needed. Do starch grains and chloroplasts can be characterized by specific chemical elements like PolyP and the scales? What about mitochondrial profiles? Are they also identified according to their shape? Higher magnification is then needed.

3.4. In Fig. 4, it would help the reader to indicate which species are from marine and

fresh environment. Are this data coming from SEM-EDXS or TEM-EDXS or both?

Fig. S5 reports TEM-EDXS, and the section is about SEM-EDXS. No SEM-EDXS figures seem to be reported neither in the text nor in the supplementary info? Or Fig. S5 is a SEM-EDXS figure. Could the authors please clarify this point?

3.5. p13. Line 2. If the “overall composition of all culture media is rather similar” how could one study the influence of the composition of the media on the micropearls composition?

An interesting result is the one line 18, which would I think deserve more discussion in 4.1.

4.Discussion. 4.2. p.14 line 18 to 25. Stabilization of ACC might also be achieved by inorganic ions (phosphates, magnesium, strontium?). The concentration of these ions might still be controlled by the organism. More recent papers about the stabilization of synthetic ACCs in presence of inorganic ions should be cited.The role of Strontium in the stabilization ACC should be further discussed by reporting the literature.

To what refers “ACC in its “pure form”? this is rather vague, the authors could use the term “synthetic ACC with no additives” instead?

Moreover, synthetic ACC even if without inorganic or organic additives can be stable if it is stored in a desiccator for example. ACC in solution crystallizes indeed rapidly but not in air.

4.3. The spatial localization of the micropearls in a cell as well as the differences between cells of different species should be discussed. Does the specific localization close to the flagella have a role in swimming capacities? Center of gravity?

5. Conclusion. p.16 line 6to 9 belongs to discussion not really to the conclusion

BG questions: 1. Yes 2. Yes 3. Yes 4. Yes 5. Yes 6. Yes in general, only one detail might be needed to precise p. 4. L 12. What is RSD what is the “home made

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standard solution”? 7. Yes in general, but it would be more precise if they include in the introduction the presence of such intracellular ACC inclusions also in other organisms as cyanobacteria (Couradeau 2012, Benzerara 2014) 8. Yes 9. Yes 10. Yes 11. Yes 12. Yes 13. Some clarification need to be provided (see detailed comments) 14. More recent reference on the stabilization of ACC would be needed 15. Yes

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