

## ***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.***

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I read with great interest the manuscript entitled “Marine and freshwater micropearls: Biomineralization producing strontium-rich amorphous calcium carbonate inclusions is widespread in the genus *Tetraselmis* (Chlorophyta)” by Martignier et al.

This paper reports the observation of micropearls, which are intracellular amorphous carbonate formed by unicellular eukaryotes in 14 samples (out of the 16 samples examined) encompassing 11 strains of the genus *Tetraselmis*. The samples were ob-

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tained from culture collections and cells were dehydrated upon arrival on a membrane filter to be further observed using SEM coupled to elementary X-EDS analyses. Some FIB sections were also prepared and analyzed by TEM. This piece of work deepens our view of micropearl formation showing that it is not limited to the freshwater *T. cordiformis* previously found in lake Geneva but also occurs in a large set of marine species. It also shows that the micropearls form in standard culture conditions and that they can express the Sr-Ca zonation pattern in constant culture condition. Interestingly the authors looked at the nucleus of the micropearls and showed that it is a rod shaped organic nucleus suggesting the importance of organic template to initiate the nucleation of the micropearl and to maintain it in an amorphous stage. The authors suggest that the Sr bioremediation properties attributed to the genus *Tetraselmis* could be linked to their ability to concentrate Sr in mineral.

I found this paper very interesting and well written. It is easy to follow and I don't have any concern that that could preclude its publication in BG.

I have a couple of general comments/suggestions that hopefully will help make this paper an even stronger contribution:

It is unclear to me from reading the manuscript if all the cells from a species had the micropearls. I would like to see some kind of measurement of how many cells had them and if the pattern of biomineralization was more homogenous within a strain as compared to between strains. If it is the case (especially for strains grown in the same media) it would suggest a high level of control of the number/size/organization of the micropearls.

Regarding the <sup>90</sup>Sr remediation potential of *Tetraselmis*, I was wondering if the author could calculate from their estimates of the composition of the micropearls the contribution of the mineral phase to the “Sr absorption capacities of several *Tetraselmis*” P15 L19. In other words can we quantitatively link the potential of micropearl forming to the Sr absorption?

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I was also wondering how these species compared in term of Sr concentration to the one forming Sr and Ba sulfates, that could make an additional interesting point to discuss. See for instance: Krejci, M. R., Wasserman, B., Finney, L., McNulty, I., Legnini, D., Vogt, S., et al. (2011). Selectivity in biomineralization of barium and strontium. *J. Struct. Biol.* In Press,. Available at: <http://www.sciencedirect.com/science/article/pii/S1047847711002346>.

Detailed comments:

P2 L4 why are the micropearls “non-skeletal”, your data suggest that the micropearls are organized probably along the cytoskeleton; they could be an organizing component of the cell serving as skeleton/internal spatial organization principle.

P2 L20 “two freshwater organisms” > what is the second one?

P3 L29 resuspended instead of “diluted”

P4 L4 Barium and strontium (instead of Sr)

P4 L12 what is RSD?

P4 L20 what is ZAF?

P7 L20 iron oxide are extracellular? are they always in contact with the cell?

P7 L25 to be exclusive TO a limited number

P15 L6 “require a certain concentration of Ca”, which is ?

Figure 4 (a) tetrah is not in the Table 1, is it Tetrah\_ac or \_sa? (b) is the ES medium Enriched Seawater ? If so you could use the S and Ca concentration of seawater as proxy for this medium as it is composed of filtered seawater amended with metal and vitamins mostly (no addition of Sr & Ca). That would allow you to plot the marine species on the part

(b) of the graph which would be interesting because they have the highest Sr/Ca ratio

C3

in the micropearls.

I will now answer the 15 BG questions:

1. Does the paper address relevant scientific questions within the scope of BG? Yes, the topic of biomineralization is within the scope of BG

2. Does the paper present novel concepts, ideas, tools, or data? Yes the fact that micropearls are widespread beyond the one species already described and also occur in marine environment is new. Although the techniques are not novel, the FIB prep shown in figure 3 is outstanding knowing that the sample was not fixed or preserved in any way.

3. Are substantial conclusions reached? Yes

4. Are the scientific methods and assumptions valid and clearly outlined? Yes

5. Are the results sufficient to support the interpretations and conclusions? Yes

6. Is the description of experiments and calculations sufficiently complete and precise to allow their reproduction by fellow scientists (traceability of results)? Yes

7. Do the authors give proper credit to related work and clearly indicate their own new/original contribution? Yes

8. Does the title clearly reflect the contents of the paper? I found the title too long but indeed it reflects the content of the paper

9. Does the abstract provide a concise and complete summary? Yes

10. Is the overall presentation well structured and clear? Yes

11. Is the language fluent and precise? Yes.

12. Are mathematical formulae, symbols, abbreviations, and units correctly defined and used? Yes

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13. Should any parts of the paper (text, formulae, figures, tables) be clarified, reduced, combined, or eliminated? No

14. Are the number and quality of references appropriate? Yes

15. Is the amount and quality of supplementary material appropriate? Yes

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