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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.

Reply to reviewer n°3

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 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.

Authors' response:
For detailed answers to this point, please refer to questions 3, 5 and 12 (see detailed comments).

Detailed comments

1) 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.

   Authors' response:
   We thank the reviewer for this remark. As requested, in the revised version of the manuscript we will refer to our previous publication where the term micropearl was first coined. This article, however, was published four years after the first description of ACC in cyanobacteria and, thus, we do not rule out the possibility that the ACC inclusions produced by the

cyanobacteria could be also considered as micropearls. We will cite the publications about cyanobacteria in this part of the ms as suggested by the reviewer.

2) Line 20. "two freshwater organisms", could mean either two individuals or two species: Only one is cited (cordiformis), what is the other one?

Authors' response:
As answered to question 5 of Reviewer n°1:
*We will replace the present sentence by the following: "*Until now, micropearls had been observed only in two freshwater species: the unicellular green alga *Tetraselmis cordiformis* (Chlorodendrophyceae, Chlorophyta) producing micropearls enriched in Sr and a second freshwater microorganism producing micropearls enriched in Ba, yet to be identified (Martignier et al., 2017)."
For the moment, the only thing we know about this second organism is its approximate size (20 microns) and that it has at least two flagella. We are currently trying different methods to investigate the question (isolation by cytometry, pipette subsampling).

3) 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?

Authors' response:
As explained in the Samples and Methods part (2.1): Samples for microscopic observation were prepared directly after the organisms' arrival in our laboratory".
At the time of this study, we did not have the necessary infrastructure or experience to maintain these algal cultures in satisfactory conditions on the long term. Therefore, as stated in paragraph 2.1, the present manuscript does not study the evolution of the cells with time, but describes an "instantaneous picture" of the micropearls in the cultures at the moment of their reception from the different algal culture collections. Giving a precise time scale for the different cultures is impossible, but these were all cultures with "mature cells" at the time of observation. So, the precision which we can add to the manuscript, is that "most cells in these cultures were mature at the time of observation".
Regarding the variability of the spatial localization (or patterns) of micropearl distribution in the cells, they don't seem to change during time (meaning that one species will not change pattern in the course of its life cycle). If it were the case, several different patterns should be observed simultaneously in the cultures (representing younger or older cells), which never occured. See also answer to question 1 of Reviewer n°1.

We can just add that our group recently obtained funds to finance a bi-disciplinary PhD student (microbiology / earth sciences) who is currently starting cultures and will work, amongst other aspects, on the study of the complete process of cell growth (and micropearl formation) from the start to the total maturation of the cells.
Preliminary analyses seem to confirm that the general pattern of micropearls localization seems to stay the same in one given species. Nevertheless, this study just started, the culture, analyses and sampling techniques still need to be improved and many more observations and analyses will be needed before these points can really be clarified.

4) Is the rate growth of the micropearls known?

Authors' response:
Regarding the growth rate of the micropearls, the only indication we have for the moment is theoretical: Thien et al. (2017) made an evaluation based on thermodynamic modelling of the formation of the micropearls: "If the size of the micropearls ranges between 0.3 and 2.5 µm, their potential growth time would then be between 0.6 and 72 days."
Reference:
Thien, B., Martignier, A., Jaquet, J. M. and Filella, M.: Linking environmental observations and solid solution thermodynamic modeling: The case of Ba- and Sr-rich micropearls in Lake Geneva, Pure Appl. Chem., 89, 645–652, doi:10.1515/pac-2017-0205, 2017.

5) 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?

Authors' response:
Compositional variations (e.g. oscillatory zoning) can occur generally in two different ways. Firstly, the composition of the growth medium is varying with time and this variation is documented in the zoning pattern. In this case, the growth zonation is temporally coupled to the variation of the external medium (marker of time) but often modified by variations in the growth rate. Such external control has to be ruled out here, since the culture cells grew from a medium of constant composition (no cyclic variations). Secondly, compositional variations in a solid can be induced by nonlinear dynamics involving a coupling between solid and fluid composition, and the oscillations are an example of chemical self-organisation in diffusion-reaction system (e.g. Liesegang rings). However, a biological control could influence the growth patterns, but is not yet identified.

6) 2.3.The coating was gold, therefore the authors should state why carbon, nitrogen and oxygen where not taken into account in the semi-quantitative analyses.

Authors' response:
Indeed, the Au peaks do not overlap with the peaks of these light elements. Nervertheless, we did not include C, N and O into the analyses because they are main components of organic matter and we have no means to distinguish how much of these elements comes from the micropearls and how much comes from the surrounding organic matter.
*We wrote, in paragraph 3.4, that "*It should be noted that the size of micropearls is close to or even below the resolution limit of the SEM-EDXS analysis technique. This means that the interaction volume of the electron beam with the sample is often larger than the micropearls themselves and that therefore the technique yields compositions that include the micropearl and the surrounding organic matter (…)".

7) 2.5. Some EDXS have been done so the title should be changed accordingly

Authors' response:
We thank the reviewer for spotting this error. We will change the title as suggested.

8) 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).

Authors' response:
We understand the remark of the reviewer. We agree to change the subtitles for a more homogenous naming, as suggested by the reviewer. We would nevertheless prefer to keep paragraphs 3.2 and 3.3 separated. We consider TEM-EDXS mapping to produce sufficiently different results to TEM-EDXS analyses to justify separate paragraphs.

9) 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.

Authors' response:
We totally agree with this remark. We will complete the legend of this figure. The strain used for image (d) is cord-M_cc and the strain used for (j) is tetrath_ac. We will similarly indicate that the strain used for image (a) is chui_cc.

10) In addition in Table 1 there is a mistake in the sample names for cui_sa and chui_cc.

Authors' response:
For chui, the first line/last column should be chui_cc, and the second line/last column should be chui_sa. Regarding chui_cc, we also realised that the name of the strain is SAG 8-6 instead of SAG 8.6 as indicated. We will correct these mistakes.

11) Line 11. "strains"or "species"?

Authors' response:
At page 6, line 11, both terms would be correct. We agree to change to "species", which is more in accordance with the rest of the paragraph.

12) 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?

Authors' response:
Please refer to our answer to question n° 3 above and to question n°1 of Reviewer n°1.
As explained in our answer to question n°3, the present research project does not observe the evolution of the micropearls through the lifetime of a cell. This manuscript simply states the presence of micropearls in 10 species of Tetraselmis (mature cells), with a given arrangement at the time of observation (time of arrival of the cultures in our lab). It is an "instantaneous picture".
We will add a sentence in the introduction or in the Results part (or both), stating this more clearly, in order to avoid misunderstanding.
Although, as detailed in the answer to question 1 of Reviewer 1, it appears clearly that the general pattern of micropearl distribution in the cell does not drastically change during time. As stated in the ms, the pattern of micropearls seems to be characteristic of a species.

Finally, to answer precisely the question: at p7, Line 1, the exact sentence we wrote is : "T. striata shows a similar central micropearl distribution, but the lateral points of the "trident" are absent (Fig. 1g), possibly due to poorly developed micropearls at the time of

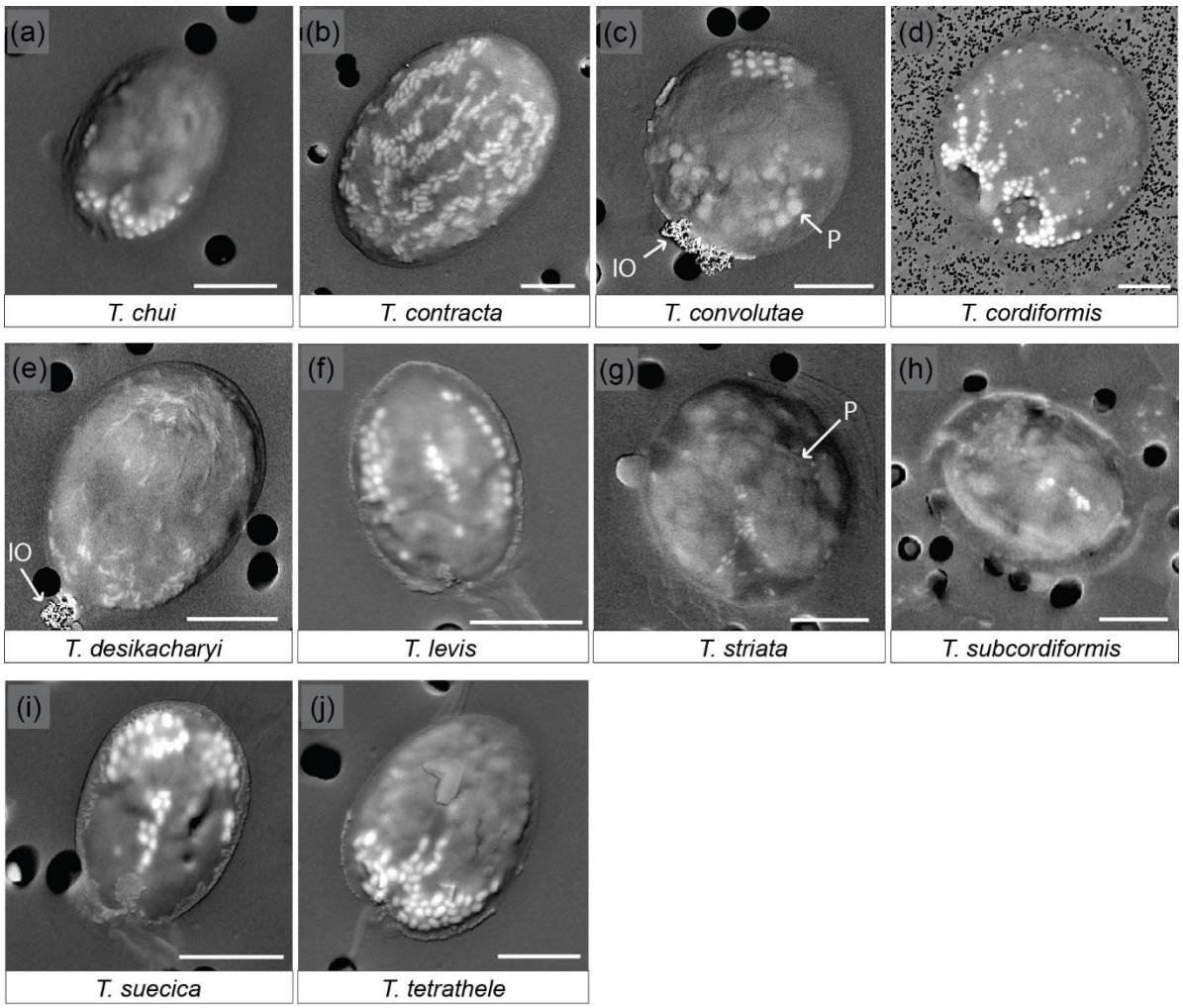
observation". *When we speek of "poorly developed micropearls", it does not refer to a "time problem", but states an apparent poor state of the micropearl development, which might be due to numerous factors. The new observations we provided for question 1 of Reviewer n°1 made us realise that this might be linked to the fact that this species was maintained on Agar, which seems to hinder the micropearl development. We will modify the text to include this new observation.*

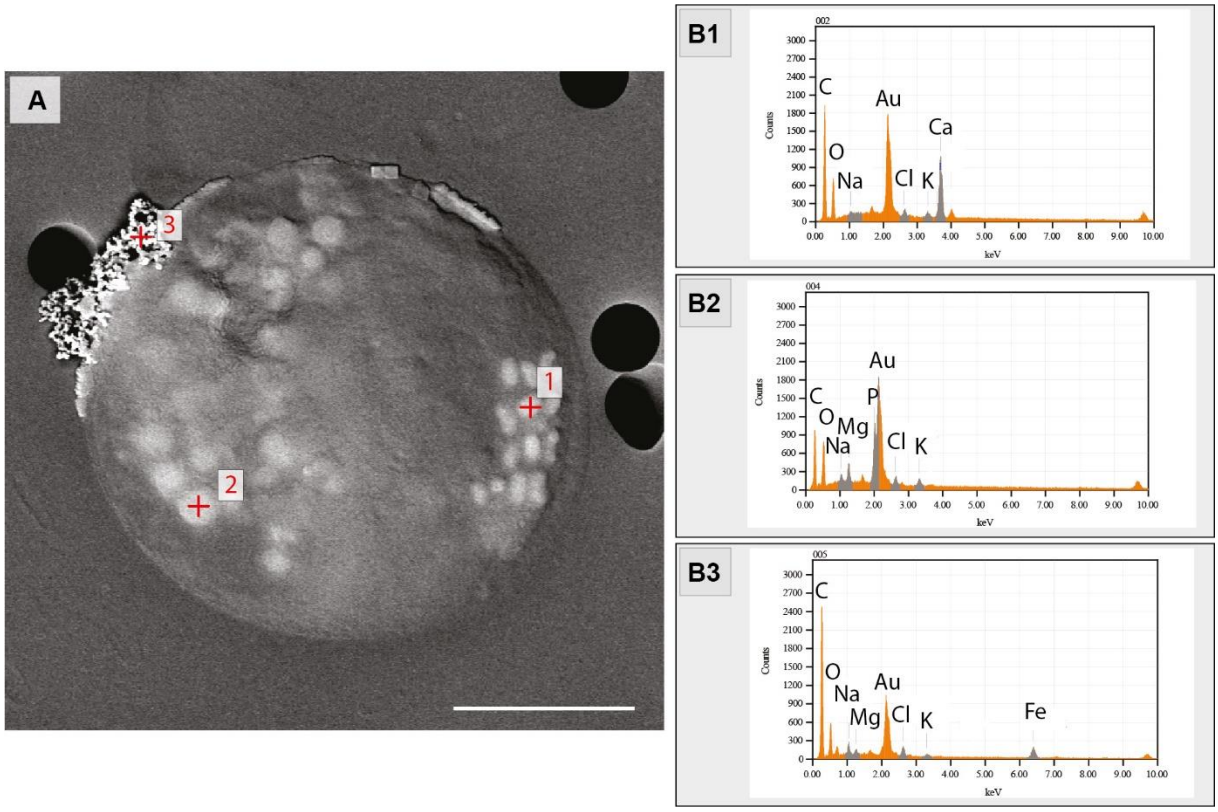
13) Polyphosphate inclusions are not easily seen in Figure 1. Higher magnifications would be useful. Or relate to the TEM- EDXS observations? Did the authors observed EDXS signal from the P in these SEM images? Same for Iron-oxide minerals.

Authors' response:

We agree that the reviewer's suggestions might indeed improve this figure, although we feel that the polyphosphate inclusions are large enough to be seen in Figure 1 without higher magnification, as they are larger than the micropearls. Therefore, we modified the figure by adding arrows pointing to the polyphosphate inclusions (P), as well as to the iron oxides (IO), in order to clearly identify them. See attached modified figure.

Regarding the composition of these items, we indeed performed EDXS analyses. We have attached a new figure, which could be inserted into the ms as a supplementary figure. It shows the EDXS analyses done in T. convolutae (Fig. 1c). This example shows raw EDXS analyses of polyphosphates, micropearls and iron oxides. Please note that the scale bar is 5 micrometers. Au, O and C were not taken into account for the semi-quantitative quantification (and these peaks stayed therefore in orange).





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

Authors’ response:
Indeed, the word “species” might be more appropriate here than the word “organisms”. Will modify the text accordingly.

15) 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”.

Authors’ response:
We understand the reviewer’s concern. We agree to apply the suggested changes.

16) 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.

Authors’ response:
The choice of species for FIB-sections was indeed made to have the best chances of detecting possible compositional zonations in the micropearls of the marine species, similar to the ones already observed in the freshwater species. We agree that it would also be very interesting to have FIB-sections made in the cultured strains of T. cordiformis, as well as in other marine

species such as T. desikacharyi (where micropearls also contain small amounts of Ba). Nevertheless, FIB preparation is very time-consuming and costly. Therefore, for this publication, we reduced our choice to the three species that appeared to be the most promising ones.

17) 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.

Authors' response:
We agree to apply the suggested changes. Regarding the higher magnification images of individual micropearls, they are already presented in Fig. S4. We prefer to leave these higher magnification pictures in a separate supplementary figure as they are now, to avoid adding anymore element to Figure 2, which is already composed of many different elements.

18) 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).

Authors' response:
Images in Fig S4 are not taken at higher magnifications, but are details extracted from Fig 2 and 3. Due to the instability of the samples under the electron beam, repeated imaging (e.g. at higher magnification) is not possible. The HAADF signal is sensitive to changes in the mass-thickness (combination of mean atomic number, density and thickness of the sample). However, if compositions vary continuously or if sharp interfaces between zones are inclined to the electron beam, then the zoning will not be visible in HAADF images or EDXS maps.
T. contracta has a very low concentration of Sr (close to or below the detection limit) in TEM-EDXS. No zoning was detected here (this is stated in page 9 (lines 4-6)). While T. chui is clearly zoned, T. suecica appears almost unzoned, but with quite elevated Sr concentration (Sr/Ca close to 1). However, two additional thin zones close to the rim are visible, but they are too small to do meaningful TEM-EDXS analyses. We have changed the figure caption of Fig S4 to clarify this.

Figure S4: TEM-EDXS analyses of T. contracta, T. chui and T. suecica micropearls.
Cut-out of single micropearls (left) from STEM – HAADF images of the FIB section shown in Fig 2 and 3. The location of the EDXS analyses (right hand-side table) is indicated by the corresponding numbers. Results are normalized to 100 at%. O is calculated stoichiometrically based on the cation concentrations (with absorption correction for sample thickness). (a) The micropearl of T. chui is showing a clear zonation which is well documented in the TEM-EDXS results. (b) The micropearl of T. suesica appears almost unzoned, but with elevated Sr concentration (Sr/Ca close to 1). However, 2 additional thin zones close to the rim are visible, but they are too small to do meaningful TEM-EDXS analyses.(c) T. contracta has a very low concentration of Sr (close to or under the detection limit) in TEM-EDXS. No zoning was detected here. In contrast, a low but significant presence of K was detected. However its analysis n°1 does not fulfil carbonate stoichiometry, which may be due to the excess C from organic matter. Note that the calculation mode for the analyses presented in this figure differ from those presented in the rest of the manuscript, as C and O are included in the composition in order to perform a meaningful absorption correction.

19) 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 ?

Authors’ response:

T*he presence of organics has not been formally established inside the micropearls because of the lack of suitable analytical techniques. The application of NanoSIMS to micropearls, reported in our previous paper (Martignier et al., 2017) revealed the presence of organic signatures (CN and S) at the periphery of the micropearls, but the relatively low resolution (8-15 um) prevented the mapping of OM inside the micropearls. Hence, at this stage, we can neither exclude nor assert the presence of OM inside the micropearls. What is obvious is the high reactivity of the micropearls under the EDS beam (see Fig. 3 in the reference above). This could be ascribed to the presence of OM associated with water or, alternatively, to hydrated ACC, or both. We have not tested the “exploded” micropearls after EDXS analysis for crystallinity, but we observed that their reactivity persists even after months of storage. This might be an indication that ACC occurs in micropearls as a stable, hydrated form. Beam damage was also observed in ACC microspheres precipitated in vitro and was explained by dehydration (Rodriguez-Blanco et al., 2008). Granted this, we propose to modify our phrasing thus: “*As already pointed out previously (Martignier et al., 2016), the micropearls are extremely sensitive to the action of the electron beam, indicating a vaporization of some of its components: either organic matter associated with water, or water contained in the amorphous calcium carbonate (Rodriguez-Blanco et al., 2008), or both. This ACC seems to be rather stable, for the beam sensitivity persists after more than five months of storage of dry samples at room temperature.”

20) 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.

Authors’ response:

Proposed modification for lines 21-25, p. 14:

*“*Non-biogenic ACC is unstable and will rapidly crystallize into calcite or aragonite (Addadi et al., 2003; Bots et al., 2012; Weiner and Addadi, 2011), often through the intermediate form of vaterite (Rodriguez-Blanco et al., 2011). In contrast, long-term stabilization of ACC implies the presence of mineral or organic additives (Aizenberg et al., 2002). Magnesium is known to play a key role in the stabilization of ACC by introducing a distortion in the host mineral structure (Politi et al., 2010). This might well be the case for the *Tetraselmis*-hosted micropearls, in which Mg content is around 2 mol%. Although the phosphate ion has also been reported to inhibit ACC crystallization (Albéric et al., 2018), it does not seem to be the case here, the phosphorus concentration of the micropearls being below the detection level of EDXS. Stabilization of ACC is also enhanced by certain proteins, polyphosphonates, citrates, amino acids (Levi-Kalisman et al., 2002; Addadi et al., 2003; Cam et al., 2015; Cartwright et al., 2012). The presence of these macromolecules inside the micropearls could be postulated by their observed sensitivity to beam damage. As for the possible role of Sr in the ACC long-term stability, we did not find in literature any reference thereof. However, in an *in vitro* experiment, Littlewood et al. (2017) found, in the presence of Mg, a correlation between added Sr and the reaction time to transform ACC into calcite (2 h to a maximum of 24 h).”

21) 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?

Authors' response:
We agree to add a more detailed description of these variations, as observed in Fig. 2. Regarding the reason for these variations, these questions are almost impossible to answer given the current stage of knowledge. We know next to nothing about the dynamics of the micropearls, their formation and whether they can be dissolved or are only diluted to daughter cells during division. Intracellular calcium is required during the early stages of scale formation in the Golgi apparatus during cell division (which occurs in the dark period of the daily cycle). What controls the number and distribution of micropearls within a cell is also unknown.
Please also refer to our answer to question n°5.

22) 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.

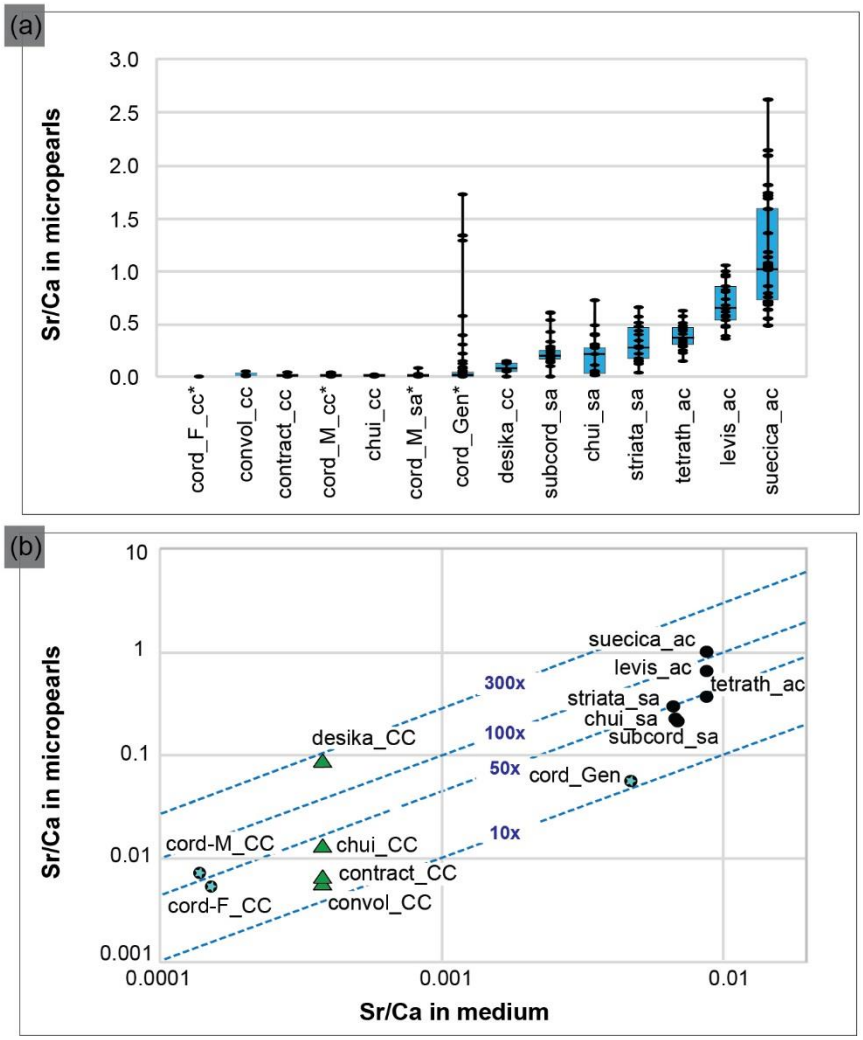
Authors' response:
We can indeed provide zoom-in images of the chloroplasts, starch grains and mitochondrial profiles. We suggest to add them as a supplementary figure.
Organelles were tentatively identified based on both structural as well as positional information derived from previous transmission electron microscopy studies of T. cordiformis (Melkonian, 1979). There is one cup-shaped, reticulate chloroplast per cell. Except for its anterior open end, it is closely associated with the cell surface. It has been identified based on the presence of a smooth stroma and parallel arranged thylakoids (the latter usually in negative contrast). Starch grains are always located inside the chloroplast, otherwise they resemble micropearls (which are located outside the chloroplast) in overall shape and size, being however more irregular in their size distribution and revealing no structured core. Finally, there is only a single highly reticulated mitochondrion, consisting of an anastomosing network of tubules. When cross-sectioned these tubules give the impression of separate organelles. The mitochondrial profiles are predominantly located near the inner surface of the chloroplast.

Reference:
Melkonian, M.: An ultrastructural study of the flagellate *Tetraselmis cordiformis* stein (Chlorophyceae) with emphasis on the flagellar apparatus, Protoplasma, 98, 139–151, doi:10.1007/BF01676667, 1979

23) 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?

Authors' response:

We can indeed indicate which species are from a freshwater environment. We attach an accordingly modified version of Fig. 4: In Fig. 4a, the freshwater strains are distinguished by an asterisk following their name. In Fig. 4b, the freshwater strains are identified by a blue star in the center of the black circle in the graph.
All these EDXS analyses were carried out on SEM. We will add this information in the legend.



24) 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?

Authors' response:
*Fig. S5 is linked to section 3.3, not 3.4: (P11, line 11) "*TEM-EDXS mapping provides compositional information improving the identification of the cellular constituents and organelles visible in the section (Fig. 3c and S5)."
It is true that we do mention again Fig. S5 in paragraph 3.4, but it is just to illustrate why some elements were not taken into account when calculating the composition of the micropearls, using the SEM-EDXS results. As Fig. S5 is not part of the results presented in paragraph 3.4, we do not think there is a need to change the text.

25) 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?

Authors' response:
As illustrated in Fig. S6, although the overall composition of the culture media are rather similar, there are nevertheless clear differences. We will delete this first sentence of the paragraph, in order to avoid any misunderstanding.

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

Authors' response:
We thank the reviewer for this suggestion. We will modify the text p. 12, as from line 3:
"The Sr/Ca ratio seems to be influenced by several parameters, amongst which we identified the composition of the culture medium and the Sr concentrating capacity of each *Tetraselmis* species (dotted isolines in Fig.4b). The broad trend seen in this diagram could indicate a kind of adaptation of the ACC precipitation to the medium composition. However, a more relevant information is given by the enrichment factor (*E factor*), which can be ranked amongst species (Table S4), from low values (12-16) to more than 200. The reality of this ranking could be tested by cultivating the species in different media (eg. the *convolutae* group in ES and the *tetrathele* group in ASP-H) and comparing the new enrichment factor with the present values. The very high *E factor* for *desikacharyi* could be linked to distinctive morphological features (a six-layered theca, a novel flagellar hair subtype) not found in other strains of Tetraselmis (Marin et al., 1996)."
Table S4 : Ranking of the Enrichment factor amongst species

E factor	Strain	Medium	*E* factor	Environment
	cord_L	Lake Geneva	12	Freshwater
Low	*convol_cc*	ASP-H	14	Marine symbiotic
	contract_cc	ASP-H	16	Brackish
	subcord_sa	Porph Ag	30	Marine
Mdium	*cord-F_cc*	SFM	33	Freshwater
	chui_cc	ASP-H	33	Marine
	chui_sa	1/2 SWEg Ag	33	Marine
	tetrath	ES	42	Brackish
High	*striata_sa*	SWES Ag	43	Marine
	cord-M_cc	Waris-H	51	Freshwater
Very High	*desika_cc*	ASP-H	219	Marine sand

Reference:
Marin, B., Hoef-Emden, K. and Melkonian, M.: Light and electron microscope observations on *Tetraselmis desikacharyi* sp. nov.(Chlorodendrales, Chlorophyta), Nov. Hedwigia, 112, 5 461–475, 1996.

27) 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.

Authors' response:
Please refer to our answer to question 19.

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

Authors' response:
We will modfy the text according to the reviewer's suggestion.

29) 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.

Authors' response:
We would greatly appreciate to get the reference related to these statements. Our samples are not stored in a desiccator, but at normal "ambient conditions".

30) 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?

Authors' response:
Unfortunately, it is impossible to answer these questions at the present stage of the research. For the moment, we have only been looking at dried cells with TEM or SEM and we still lack 3D views and sections of unaltered Tetraselmis cells (with preserved micropearls) to accurately locate the micropearls with respect to the various organelles and cell constituents. Work is planned to clarify these points using cryo TEM. But for the moment, as mentioned in the text (p. 15, lines 1-4), the possible role of the micropearls in Tetraselmis remains hypothetical.

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

Authors' response:
We suggest to modify this paragraph as follows:
"Micropearls represent a new intracellular feature. This study shows that they can be clearly distinguished from other cellular constituents and are not randomly distributed in the cell. On the contrary, micropearls seem to be essentially located just under the cell wall and they draw a pattern which suggest to be characteristic for each species. Strong correlations hint that this might have a link with the species habitat.
It appears that, for most of the observed Tetraselmis species, the biomineralization process leading to the formation of micropearls enables a selective concentration of Sr."