

Answer to Anonymous Referee #2

The study presented by Couradeau and colleagues nicely demonstrates that the submicrometer-scale research on modern microbialite might provide tools to recognize ancient microfossils. This study is well constructed and the authors use a set of microscopic technique that nicely complement each other. As a result the authors present convincing data and propose a model of biomineralization in Pleurocapsales (Fig. 5) that is supported by their observations. At this point, it must be noted that in contrary to the promising title, the conclusion of the authors only apply to the pleurocapsales. Still, some comment and question remains about this study:

We used "cyanobacterial" because Pleurocapsales are indeed cyanobacteria, and this represents one well-described modern example of calcified cyanobacteria. It may be extensive to other lineages/species (but we did not specifically looked for that here) or not, but this does not take the cyanobacterial qualification to Pleurocapsales.

1) It seems from the authors discussion that precipitation of aragonite and hydromagnesite is directly linked to the oxygenic photosynthetic activity of pleurocapsales. However, during night time, when photosynthesis is not possible, many microbial matforming cyanobacteria are know to be able to turn to respiration or even fermentation, thus releasing organic acid (Stal, 2012). If only Pleurocapsales are considered, then during night time dissolution of carbonate minerals is likely to occur as well (Dupraz et al., 2009).

We agree that during night time respiration or even fermentation may lead to dissolution of carbonate minerals and that additional processes may be playing in Alchichica microbialites. We now specify this in the manuscript and cite (Dupraz et al., 2009) and(Stal, 2012).

2) It is clear that the focus of this paper is on Pleurocapsales. This group is indeed of interest. However, when looking at the microbial community present in such microbialite, it seems that pleurocapsales are rather not dominant at the depth indicated in this paper (i.e., 4m). At this depth, Oscillatoriales seem more numerous. The only sample where this group dominated the community was found at 14m depth (Couradeau et al., 2011). Microscopy does not allow to investigate large samples and the set of techniques deployed in this study cannot be applied on a very large number of samples. Although the model for the mineralization of the Pleurocapsales presented by the authors is well supported by their observations, it would be great to consider the contribution of other groups (Dupraz and Visscher, 2005) (cyanobacteria, but also other bacteria such as sulfate reducers) in the formation of the microbialite found in the lake Alchichica. As it has been nicely demonstarted for stromatolite, the formation of microbialites is usually the result of interaction between different key player in the microbial community(Reid et al., 2000).

Even if the Pleurocapsales are not dominant they are present at all depth. 14 microbialites sections treated in the same way and collected from 10cm to 14m depth were studied. The association between Pleurocapsales and aragonite has been observed systematically. These data are shown in Gerard et al., ISME J. in press. We agree with the reviewer that the accretion is probably the net result of a delicate biogeochemical balance, including several process (such as induced biomineralization, particle trapping, diagenesis) and metabolic groups (including sulfate reducers and maybe anoxygenic phototroph also, see FigS14 (Couradeau et al., 2011)). The understanding of how

quantitatively aragonite biomineralized by Pleurocapsales contributes to it is another question that will required more investigation. In this study we chose one sample which exhibits a particularly well developed gradient of encrustation of Pleurocapsales by aragonite to describe further the encrustation process, establishing a well described modern case of cyanobacterial calcification. We will add a sentence in the manuscript to clarify that point.

3) Looking at the paper it seem that the claims of the authors would be even better supported if such process could be reproduced in the laboratory. Especially what would happen if a pure culture of those Pleurocapsales (or eventually strains from culture collection ATCC 29393 or ATCC 29394) would be inoculated to sterile filtered lake water. There is no doubt that the technique deployed by the authors would more than appropriate to study the mineral formation under such controlled conditions.

We agree with the reviewer that an experimental study of this process would be interesting. A model strain of calcifying Pleurocapsales would indeed be very helpful to study the ongoing biomineralization process (in order to test the model we propose in Fig 5) as well as the role of the cells in the orientation of precipitation towards aragonite or hydromagnesite. However, it is possible that not all Pleurocapsales can do that and therefore we would need to isolate the strain from Lake Alchichica; moreover, we have no clue of how long it takes. Therefore, such a study might take a long time and we now specify in the revised manuscript that such a work would open interesting perspectives for a better understanding of the mechanisms at stake.

In addition some minor points would benefit from clarifications:

4) In the discussion (section 4.2) the authors claim that Pleurocapsales exhibit “cell wall integrity”. Usually cell wall integrity can be tested by ethidium bromide staining or propidium iodide staining (the latter being found in many live/dead staining kit) which are normally excluded of healthy cells. Autofluorescence can persist some time after the membrane has been compromised. Thus I would not rely on this only to assess cell wall integrity.

We agree with the reviewer that this is not a proper use of “cell wall integrity” we then rephrase the sentence as “cell wall appeared preserved.”

5) The authors should put the composition and condition used for modeling with Visual minteq available as supplementary material (the water composition is usually not equivalent to the matrix used in such software since pH is often “adjusted” with H⁺ or OH⁻ and charge balance is often achieved with addition or subtraction of Cl⁻. This would make the comparison with other models or other site easier (Gallagher et al., 2013)

The water lake composition used as described in (Kaźmierczak et al., 2011). It has now been added to the material and methods section. The temperature was set at 15°C. Ks values were retrieved from Minteq thermodynamic database. We used the Multi problem/Sweep section to vary the pH in the 8 to 12 range, using the default parameters of Visual Minteq 3.0. We also added in the discussion part (4.2) a reference to (Gallagher et al., 2013) : “Previous studies have shown that subtle modifications of the solution chemical composition can impact significantly the nature of precipitated mineral phases and that this can be predicted by chemical equilibrium modeling (Gallagher et al., 2013) “

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