

Response to reviewer 1,

In summary, reviewer 1 identifies some issues of concern relating particularly to our use of the term biomineralization and concludes that '*extrapolation to the geological history of dolomite formation is unwarranted*'. We start with a general reply to the reviewer's final comment and then address individual comments.

Reviewer 1 contends that *until all the various aspects of this proposal are convincingly demonstrated, extrapolation to the geological history of dolomite formation is unwarranted*. The reviewer does not specify what aspects need to be demonstrated and saying 'all' is quite broad. Therefore we will attempt to define what aspects need to be demonstrated, before being able to extrapolate to the geological history.

Our central claim is that dolomite and magnesite were discovered in living crustose coralline algae.

The proofs required to support this proposition are

1. Confirmation of the presence of the mineral forms of dolomite and magnesite
2. The algae were living at the time of collection

Lines of evidence were provided for both of these in the ms and the reviewer did not question the validity of these results, i.e. XRD, SEM-EDS, ICP-AES for the mineralogy and composition and Pulse Amplitude Modulated fluorometer to confirm photosynthetic activity of the surface layer. By establishing both elements needed to demonstrate our central claim we provide solid ground for extrapolating to the geological history. This extrapolation, while new, is really no more extraordinary than discovering that corals form aragonite and extrapolating this to the geologic record.

Although the extrapolation does not rely on definitions of biomineralization for support we will outline why we applied that term. The reviewers' main issue seems to be that we did not prove the in-filled cells were living, and thus should not claim intracellular biomineralization, as the filling of cell lumens by mineral could be a "*result of post-mortem precipitation*". There are two main points relating to this issue.

1. Definition of biomineralization- concepts of 'biologically induced' biomineralization from the deVrind-de Jong and de Vrind reference (line 25, p5893) do seem appropriate for this situation. Biologically induced concepts include secondary precipitation of minerals that occurs as a result of interactions between biological activity and the environment and this includes minerals forming in enclosed spaces, not necessarily even cell spaces, within the organism and biological surfaces acting as nucleation sites. The reviewer, by stating that we need to prove the cells were living seems to be invoking the 'biologically controlled' concept of biomineralization. In our manuscript we use the terms 'influenced by', 'caused by', and 'precipitation contemporaneous with organism growth' to describe the mineralization. On page 5893 twice we refer to 'biologically induced', we note the

manner of cell in-fill ‘suggests a biologically induced rather than controlled reaction’ thus we are clearly relying on the broader concept of biologically induced biomineralization.

2. At line 23, p5888, we noted the presence of magnesite cell in-fill in the top photosynthetically active layer. We demonstrate photosynthetic activity (line 13, p 5886) , therefore living cells and biological activity in association with the mineralization. We consider this reasonable evidence for cellular in-fill in association with biological activity

In summary, it seemed appropriate to apply the biomineralization term to our manuscript. Regardless of biomineralization being used to describe the situation, given that we have demonstrated both magnesite and dolomite present and biological activity, this supports extrapolation to the geological history as this extrapolation relies on the close association of the mineralogy to the biological activity of the coralline by virtue of the fact that these minerals are contained within the attached crust. This situation is very different from the existing paradigm requiring some process, unrelated to biological activity, to take place when the entire organism, if not the entire reef, is dead.

The reviewer comments that our *proposal for the multiple methods of biomineralization, one that deposits minerals in the cell walls and another, never before seen, that deposits a different mineral within the cell, is not supported by convincing evidence.*

Some clarification is required here. We do not propose multiple methods of biomineralization. We measure and identify multiple minerals throughout the ms and *speculate* as to the processes (line 16, p5893). We are not sure if the reviewer is referring to the normal cell wall calcification and a separate one for dolomite and magnesite, or two different methods for each dolomite and magnesite, however the manuscript focus is on identification of minerals in a biological organism, not method of biomineralization.

The reviewer comments that ‘*the protodolomite rims are stated to be part of the cell wall surrounding living cells*’. The reviewer does not provide a line reference for this comment and as we do not make this statement in the ms we are not sure what the reviewer is referring to. We use the term protodolomite ‘rims’ to describe the shape of the dolomite mineralization.

We do not claim cell in-fill is ‘*never before seen*’. Cell in-fill by different calcite minerals has been well documented in previous published work and on page 5890, in our paragraph on previous work on coralline algae we reference a paper that had measured magnesite composition in cell in-fill and we build the case that this study had in fact seen, just not recognized, both dolomite and magnesite.

Added to p5890 – (reference Moberly)’ also noted the presence of cell in-fill by calcite and Mg-calcite in tropical corallines’.

## Response to specific comments

1. *The authors attempt to provide a solution to the ‘dolomite problem’ by proposing that dolomite formation is the product biomineralization by coralline red algae.*

**Suggestion adopted.** As both reviewers interpret our claim as being that coralline algae are responsible for all dolomite formation, which was not our intention, we have changed the ms to clarify that the discovery of dolomite (and magnesite) forming in crusts of living algae demonstrates that the formation of dolomite can be biological (not all by coralline algae), in contrast to the existing paradigm that it is abiotic,

### Note of edits made in response to reviewer 2.

2. *After giving a brief account of early red algae, the authors state: “They have a high magnesium calcite skeleton.” A reader might conclude that ‘They’ refers to all the preceding red algae listed, but, in fact, it is known to be true of only members of the Corallinales, whose fossil record before the Cretaceous is sparse. Whether Paleozoic solenopores deposited magnesium rich calcites is unknown*

**Suggestion adopted.** We thank the reviewer for noting this. We did not intend to suggest that all the preceding red algae had Mg-calcite skeletons, rather only the modern corallines - as reflected at line 13 referring to capacity to form low Mg skeletons. Paragraph edited to clarify.

3. *and there is no basis for extending this characterization to the cited Proterozoic red algae, because they are not believed to have been calcified.*

**Suggestion adopted.** The sentence referring to the proterozoic red algae has been edited clarify these are not the calcifying red algae.

4. *Even if one assumes coralline red algae have always deposited high magnesium carbonate, the comparison to the geological history of dolomite formation can only begin in the Cretaceous. Therefore, the ability of this study to solve the “dolomite problem” based only on a single species of extant algae is severely constrained.*

**Suggestion adopted.** We refer to our previous comment that we do not claim coralline algae formed all the dolomite. We note that although coralline algae is not confirmed, dolomite presence in shallow facies in association with calcifying red algae eg Triassic dolomites, is consistent with confirmed presence in comparable environments in Cenozoic reefs. Noting the concerns also of reviewer 2, the second part of the paragraph ‘applying our results to interpret fossil dolomite formation’ has been rewritten to separate the pre- and post -Cenozoic comparison Note of edits in response to reviewer 2

Regarding the *single species* –

*H.onkodes* may only be one species however it dominates shallow reef front / crest coralline assemblages and has been recorded as being the only component in some samples . To highlight the substantial role of *H.onkodes* the following addition has been made to the ms, in 1.2 ‘Background on coralline algae’

Added in - *H. onkodes* can dominate shallow reef front / crest coralline assemblages (Rasser and Piller, 1997; Littler and Doty, 1975; Matsuda, 1989) and has been recorded as being 100% of species coverage in parts of the reef crest (Littler and Doty, 1975). *H. onkodes* can grow prolifically in the right environmental conditions and out-compete other calcifying algae. Its role in building and maintaining the reef edge places *H. onkodes* among the most ecologically important of the tropical crustose coralline algae (Littler and Doty 1975).

While we focused our discussion on the *H.onkodes*, there was supporting evidence in the ms that dolomite could be in other corallines. We draw attention to the XRD results (line 15, p5889) for the sample having an asymmetrical curve and SEM-EDS confirming dolomite composition. The caption to these results in SupplI ... “This sample appeared to contain multiple genera, and identification to the species level was not possible. However, it seemed that both *Hydrolithon* sp and *Lithophyllum* sp were likely to be present”. Moreover, studies since the 1950’s using XRD on tropical corallines have all noted the asymmetry of these XRD curves. Having demonstrated in our paper that such peak asymmetry can indicate the presence of protodolomite it is possible that many other tropical corallines exhibiting similar XRD asymmetry could also contain protodolomite.

Added in- Comments have been moved from Supplementary Information into the main text XRD results and this paragraph edited to better link our results to earlier studies. (This is further addressed in other comments).

5. *The Methods section provides no information about the methods by which sections were prepared. Use of a resin is mentioned in a legend, but not in the Methods.*

**Suggestion adopted.** Added to SEM-EDS methods- Samples were cut with a rock saw and embedded in resin, polished and carbon coated and held in with carbon tape, one sample was coated with Platinum for one session.

6. *The extent to which resin infiltrates the fabric of the thallus, especially the cell lumens and conceptacles, should be stated, because it could profoundly affect interpretations*

**Suggestion adopted.** Added to SEM-EDS methods- When conducting the SEM-EDS a measurement was taken of the resin surrounding the sample and this enabled us to identify when a measurement included resin. These measurements were discarded. The extent to which the resin infiltrated the thallus was impossible to quantify, however measurements returning a resin signature were infrequent and resin infill was not considered problematic.

7. *Given the authors' assertion of a novel type of intracellular biomineralization, it is important to demonstrate that the cells were, in fact, living, and that the filling of cell lumens by mineral was not the result of post-mortem precipitation.*

We commented on this on the first page. If the editor prefers we can add a small paragraph to the ms discussing the concepts of biologically induced biomineralization.

8. *The possibility that the specimens overgrew other coralline thalli or other calcareous organisms also needs to be ruled out. (In discussing aragonite findings, the authors do mention the possibility of growth on a coral that could have affected their analytical results).*

**Suggestion adopted.** We do not rule out the possibility of specimens overgrowing other coralline thalli, rather the opposite, as discussed in our earlier response noting the dolomite seen in a crust with multiple genera in the XRD results section. While the majority of the *H.onkodes* imaged by SEM show cell structures consistent with the *H.onkodes* thallus, there were small patches that had a few layers of cells that were not consistent with the *H.onkodes* thallus structure and may represent a second unidentified species. These very interesting images also showing patches of atypical mineralization have been added to the manuscript Supplementary information (Figures 1 and 2) and referred to in SEM-EDS results.

The presence of aragonite whether it is from a remnant coral piece or within the coralline thalli as per Fig 3. does not affect our results for identification of dolomite and magnesite mineralogy by XRD or SEM-EDS, however it does affect the ICP-AES results by reducing the mol.% Mg, and these results were excluded from our mass balance calculations as noted at table 3, p8 of the supplement.

9. *Parallel histological study of the same specimens to show well preserved cell contents is the only way to establish that cells deep in the thallus were living; SEM of dried specimens alone is not sufficient.*

As we are relying on the broader definition of ‘biologically induced’ biomineralization as discussed above, there is no reason to demonstrate that each individual cell containing dolomite was living at the time of mineralization. However consideration was given to other analyses that could enlighten as to the biological features involved. TEM was investigated as a possible method to obtain more information on the biological structures, however the expert advice we were given suggested that this would be problematic as it either had to be treated as biological, i.e. decalcified, or mineralogical, i.e. similar to the SEM-EDS. While it will be fascinating to see more on the associated biological features it is beyond the scope of this paper.

10. *An effective use of conventional secondary electron imaging and backscattered electron imaging would have provided correlated and complementary interpretations. None of the images shown demonstrate identifiable cell structures, such as starch grains or organelles.*

**Suggestion adopted.** As our interest was in confirming the presence and composition of the minerals dolomite and magnesite and not the biological structures associated with this, SEM-EDS carried out on polished samples was regarded as sufficient. However we saw what appeared to be starch grains in the top layer below cells containing magnetic in-fill but since these were not necessary to demonstrate the presence of the minerals, they were not investigated further or included in the paper.

Added to supplementary information (Figures 3 and 4) and referred to in SEM-EDS results - Figures showing starch grains along with cell in-fill.

11. *Fig. 6 attempts to use the difference in the amount of specimen charging as a basis for comparing different cells, concluding that the upper cells in the thallus have less content. Normally, cells near the upper surface of the thallus would be much richer in cellular organelles.*

**Suggestion adopted.** We thank the reviewer for this comment as it reminded us we are writing for a multidisciplinary journal and not only for geologists. The manuscript has been edited to change void to ‘void of mineral infill’. As we were only interested in the mineral infill, if the cell didn’t have mineral infill we considered it ‘void’. This is our error in the use of language and reveals our geological outlook.

*12. High magnification SEM images should reveal the crystal structure of each of the different mineral phases.*

SEM is not able to reveal crystal structure, only crystal shape, and we are not sure what the reviewer means. We used XRD to reveal the structure of the minerals. For the SEM-EDS we were using, the magnification was at its highest and sufficient to allow us to confirm the presence, distribution and mineral composition of dolomite and magnesite. Although it will be interesting to see the morphology of the different crystals, it is beyond the scope of the study.

*13. The results from compositional analysis are intriguing. However, elemental localization is complicated by the presence of organic material and the porous texture and locally complex contours of the cell lumens. These features will affect beam penetration and X-ray emission*

**Suggestion adopted.** Yes, the results are intriguing and once we became familiar with what we were seeing, we found the consistency was quite surprising. The influence of the cell organics on the SEM-EDS measurements were recognised by us in our first SEM session. This is referred to on page 3 of the supplementary notes, that some of the weight totals of the SEM analysis of Mg-calcite, protodolomite and especially magnesite are slightly lower than would be expected for ideal minerals and we attributed this to the presence of organic material.

Added to page 3 Supplementary information Using the secondary electron imaging we were able to see porous textures in some cells, and cells that were not completely mineralized, i.e., there was beam charging. When analysing such features, the analysis total (weight %) was noticeably lower. Such analyses were not considered reliable and were not included in our final datasets.

*14. The “protodolomite rims” are stated to be part of the cell wall surrounding living cells. Intercellular connections, the pit plugs, form organic bridges between living cells. The plugs may be sealed off after cell death, but otherwise there should be no wall or mineral between cells that share pit connections. The presumed extracellular “protodolomite rims” shown in Fig. 4 are present in places that would cover the ends of the pit plugs, and therefore would have to be present in an intercellular location. The homogeneous texture of this layer of apparently intercellular material and its radial cracking do not fit with the features of any native cell structures.*

The reviewer claims that the ‘ “protodolomite rims” are stated to be part of the cell wall surrounding living cells’, without providing a line reference. As mentioned earlier at no

place in our manuscript do we state that the rims are part of the cell wall. We therefore conclude that this comment must be based on a misunderstanding of the usage of the word ‘cell’, which the reviewer regards as the biological cell with nucleus and membrane etc., whereas we are using it as a descriptive term to describe the cell spaces (this term was used in the abstract) inside the original honeycomb wall structure before magnesite or dolomite were precipitated. As we do not know the actual process by which the deposition takes place we are unable to state whether the crystallization happens inside out outside the living cells. Alternatively the misunderstanding could come from our captions to figures 1 and 2 where we refer to details of cell infill and rims within Mg-calcite wall structure, this however refers to all of these features being located within the thallus. Without a line reference we are unable to specifically address this comment.

*15. In the Discussion, the authors state, “we could find no previous record of magnesite in coralline algae. We propose that the standard method of bleaching prior to analysis... may ... remove ... the magnesite within the cell space.” This speculation, which explains away a major point of contradiction with existing literature, should have been tested.*

The reference provided to support this comment measured a decrease of Mg % by bulk measurement after the sample of *Porolithon* sp. (the equivalent of *Hydrolithon*) was treated in H<sub>2</sub>O<sub>2</sub> to remove organic matter. The decrease was nearly 1% by weight, equating to a shift from 29 to 25 mol.% MgCO<sub>3</sub>, a significant decrease which was presumed to be organically complexed however could be ~4% magnesite or ~8% dolomite. This speculation was not attempting to ‘explain away’ a point of contradiction as is suggested by the reviewer as we did note 2 sentences after this at line 1, p 5891 that there was no literature on *H.onkodes* mineralogy and this seems the most reasonable explanation as to why the magnesite feature had not previously been reported.

**Suggestion adopted.** The speculation on the bleaching has since been tested by comparing XRD before and after bleaching and has been found to make a noticeable difference in some samples

Added in to 4.1 Results compared to previous work on coralline algae - 2 paragraphs and a figure on bleaching

*16. The authors’ proposal that this alga has multiple methods of biomineralization, one that deposits minerals in the cell walls and another, never before seen, that deposits a different mineral within the cell, is not supported by convincing evidence. Until all the various aspects of this proposal are convincingly demonstrated, extrapolation to the geological history of dolomite formation is unwarranted.*



We addressed most this comment on the first page. We do not propose that this alga has multiple methods of biomineralization. We identify and measure multiple minerals. Clearly the reviewer would like more details on the biological structures of the mineralization, however while this is interesting this is not required to prove the presence of dolomite and magnesite as this has already been confirmed by XRD and SEM-EDS. Given we have demonstrated the presence of these minerals and that the organism was living, and then applying the broad definition of biologically induced biomineralization, we disagree with the reviewer that this proposal of biomineralization has not been convincingly demonstrated.

Littler, M. M., and Doty, M. S.: Ecological components structuring seaward edges of Tropical Pacific reefs: Distribution, communities and productivity of Porolithon, *Journal of Ecology*, 63, 117-129, 1975.

Matsuda, S.: Succession and growth rates of encrusting crustose coralline algae (Rhodophyta, Cryptonemiales) in the upper fore-reef environment off Ishigaki Island, Ryukyu Islands, *Coral Reefs*, 7, 185-195, 1989.

Rasser, M., and Piller, W. E.: Depth distribution of calcareous encrusting associations in the northern Red Sea (Safaga, Egypt) and their geological implications, 8th International Coral Reef Symposium, 1997, 743-748,