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Interactive comment on "Diversity and mineral substrate preference in endolithic microbial communities from marine intertidal outcrops (Isla de Mona, Puerto Rico)" *by* Estelle Couradeau et al.

Anonymous Referee #1

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Diversity and mineral substrate preference in endolithic microbial communities from marine intertidal outcrops (Isla de Mona, Puerto Rico).

Garcia-Pichel et al.

This study investigated substrate specificity of endolithic communities in phosphate rock, limestone and dolostone outcrops from Isla de Mona, Puerto Rico. Authors implemented a high-throughput 16SrDNA genetic diversity approach that reveled the dominance of euendolithic cyanobacteria associated to a high community diversity. Results did not support the hypothesis that community composition would relate to mineral substrate but particular euendolithic cyanobacteria seemed to be specialized at the mineral substrate level. Also, the question regarding the existence of a specialized community

associated to dolostone vs limestone could not be resolved. Since authors used a very short region of the 16S rDNA and then used a culture-collection of euendoliths to extract the euendolith sequences, there seems to be a lot diversity that was not included, thus they conclude that only a small fraction of the community (3.5%) is influenced by substrate. The data analysis need to be redone. The method used provides short fragments and does not allow for thorough phylogenetic analysis. This study would greatly benefit from longer reads and maybe a metagenomic approach.

The main question addressed was if there is a highly adapted endolithic flora to specific mineral substrates, yet in lines 299-301, authors state "At this level of taxonomic resolution, we did not detect any significant association of substrate mineralogy and community composition". To answer the main question authors used high-throughput sequencing to describe the microbial diversity and test the effect of different substrates on community composition.

Specific comments: Abstract- The last claim "The cationic mineral component was. . .. existence in nature of alternatives to the boring mechanism. . .. based. . .on transcellular calcium transport" is not sustained from the results presented.

Methods- Authors justify the need to re-assess the diversity of euendolithic cyanobacteria yet only include a high-throughput sequencing approach that produced very short reads, which are not informative for phylogenetic analysis. Itag sequencing is not the best platform to analyze deep phylogenetic affiliations and to resolve the mentioned issues on euendolithic cyanobacteria for this study model.

Lines 196-201, repeated phrase. Line2 209-212, I don't understand why mention another site, and sequences that are afterwards not discussed in this analysis. Lines 237-238, by using a dataset with proven boring cultured strains and using that to assess which of the cyanobacteria OTUs could be euendolyths, this study is losing the power to identify other euendoliths. Why compare only to the known euendolith dataset?

Results and Discussion Lines 274-275, please give information on coverage.

Lines 307-314, in this paragrapha, authors mention that the sequences obtained in this study clustered together and discuss that 1) this could happen since euendolithic assemblages are distinct in a microbiological and adaptive way, or that alternatively 2) the clustering pattern reflects a biogeographical island effect, since all samples come from a small area. Authors discuss the second is unlikely given the cosmopolitan nature of marine cyanobacteria. Nonetheless the references cited are for a cosmopolitan, nonmarine cyanobacterium, M. vaginatus and for M. chthonoplastes. This discussion should be revised; there are different methods to proof for biogeography in communities, and to analyze diversity patterns related to biogeography. Also, it is possible to do analysis to disentangle which environmental variables, in this case including mineral composition, are relevant and explain community composition.

Line 323, are there cyanobacterial communities? Or populations that interact with others to form communities? Line 327, The cyanobacterial community (diversity?) appeared quire diverse (elevated?) with a specific. . ..

Lines 348-349, What percentage of the community to euendolithis represent?

Lines 353-355, Authors could do microscopic observations to make sure the issue regarding the lack of P. terebrans.

Lines 451-458, This discussion s very interesting bot out of place since this study did not focus on M. testarum but on the overall boarer diversity.

Conclusion- Lines 462-466, please revise use of English. “These complex communities likely host. . . This phrase is stating the obvious. Lines 468-471, the claim regarding different boring mechanisms than those known for M. testarum is not sustained from these results.

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