

# ***Interactive comment on “The composition of endolithic communities in gypcrete is determined by the specific microhabitat architecture” by María Cristina Casero et al.***

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We like to thank the reviewer for his/her comments, which helped us to improve our manuscript.

Specific comments:

1. The last sentence on the Abstract (line 27) includes “. . . might be an essential factor in. . .”. This rather ambiguous expression on the importance of microscopic features of endolithic habitats does not agree with the stronger terms found in the text on this major point (see Conclusions). I suggest that authors reconsider how to express their

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findings.

Authors: We agree with the reviewer comment and changed the sentence in the abstract, where now reads "plays an essential role in shaping the diversity and composition of endolithic microbial communities" (line 28).

2. In reference to the UV radiation in Atacama by Cordero et al. (2018) at the Introduction (lines 49-50), authors should clarify that the highest measurements came from high altitude coastal and Andean sites and it does not apply to the whole territory since, as we know it today, UV radiation increases with the altitude.

Authors: We agree with the reviewer and rephrase this section: "the highest surface ultraviolet radiation (UV), photosynthetic active radiation (PAR) and annual mean surface solar radiation (Cordero et al. 2018) in the Coastal Cordillera and Andean sites." (lines 51-52)

3. I do not agree completely with the authors when they state (line 51) that life has found refuge in very specific endolithic (inside rocks) microhabitats. Microbial life has found not only endolithic habitats to cope with similar environmental conditions and several examples have reported life in other lithic locations at the coastal and hyperarid core of Atacama. Then, that sentence should be revisited.

Authors: We agree with the reviewer that microbial life has not only been found in endolithic microhabitats but also in epilithic and hypoenolithic locations in the substrate. The sentence has been rephrased and now reads "In this inhospitable polyextreme desert, microbial life has been found in different lithic habitats, as epilithic (on rocks), hypolithic (under rocks) (Azua-Bustos et al., 2012) and endolithic (inside rocks) microhabitats (rev. by Wierzchos et al. 2018; Wierzchos et al. 2012b)." (lines 53-55).

4. On lines 60-61, authors emphasize the importance of the dominant genus *Chroococcidiopsis* leaving behind another cyanobacterial genus (*Halothece*), as part of more diverse lithic microbial communities than previously reported in earlier Atacama studies

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that include an accompanied microflora made of fungi and viruses and, supported by recent publications not properly credited in the manuscripts.

Authors: Chroococciopsis genus is emphasized in the manuscript as the main member of most endolithic communities, especially in the Atacama Desert, and due to its demonstrated tolerance to diverse extreme environmental conditions. However, we agree with the reviewer that Chroococciopsis is not the only genus previously found in endolithic communities from this desert. Thus we rephrased that section as follows: “Molecular and microscopy characterization of these endolithic microbial communities shows that, overall, these communities are dominated by Cyanobacteria, mostly from the extremely resistant to ionizing radiation and desiccation Chroococciopsis genus (Meslier et al. 2018, Crits-Christoph et al. 2016, Billi et al. 2000, Cockel et al. 2005) as well as members from Gloeocapsa (Crits-Christoph et al. 2016) and Halothece (de los Ríos et al. 2010; Robinson et al. 2015; Uritskiy et al. 2019) genera” (lines 62-66).

5. Authors indicate that they have coined “Microbiogeography” (line 73) as a new term. An important conceptual contribution whose scientific value will be validated by further studies in other lithic habitats, showing that gypcrete is not only a peculiar case. If the authors have information on this, they should stress it here to support the introduction of this new term and the international scientific community will have the opportunity to adopt it. Gypcrete samples came from a 3,000 m pre-Andean site, a quite different habitat when compared with others along Atacama but also, other endolithic substrates have other microscopic architectures depending upon their composition and crystal formation. I would like to have that author comments in their responses but also on the next revised manuscript.

Authors: We are grateful to Referee #1 for him/her comments regarding the importance of the conceptual contribution of the term “microbiogeography”. However, our results show, for the first time, that the differences in the architecture of a microhabitat, even within the same piece of lithic substrate, plays an essential role in shaping the diversity and composition of endolithic microbial communities. In this context, we are aware that

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more “microbiogeographic” studies should be done with other endolithic habitats from the Atacama Desert and elsewhere. Thanks to Referee comment following sentence was added to the end of the Discussion section: . . .“However, we are aware that more “microbiogeographic” studies should be done with other endolithic microhabitats from the Atacama Desert and elsewhere showing that gypcrete is not only a peculiar case where differences in the architecture of a microhabitat play an essential role in shaping the diversity and composition of endolithic microbial communities”. (lines 364-366)

6. Some parts of sections of Experimental procedures are brief, lack information and must be expanded or appropriate references should be added. Samples were taken during 2015; then, how storage time may have influenced the samples biodiversity? This a recurrent question and is important to know the authors position on this.

Authors: Sampling was performed in December 2015 and DNA extraction was done in March 2016. During that period, samples were kept in sterile bags and stored at room temperature, dry and dark conditions as explained in lines 80-81. We believe that those conditions do not facilitate the growth of microorganisms (lack of humidity and light – for phototrophs) and therefore minimize the effect that storage can have on the diversity observed after DNA extraction compared to that in the original sample.

RC- 6 (cont.) Considering the microscale of the work, authors should clarify how they obtained samples from the three microhabitats involved in the study without “contamination”. To learn about this strategy is of major importance if someone would replicate or apply the protocols involved. This is finally the objective of the having a Material and Methods section in a paper.

Authors: We agree with the reviewer and explained in detail the followed procedure to avoid contamination between samples in 2.6 section (lines 121-126) that now reads: “Colonization zone was scrapped and ground for DNA extraction. To avoid contamination between samples from different microhabitats, the scraping of material was carried out in the following way: due to the possible proximity of both chasmoendolithic and

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cryptoendolithic microhabitats, on the top of the rock, chasmoendolithic colonization zones more distant from cryptoendolithic colonization zones were selected. In addition, material from each of them was scraped avoiding the edges, so that material from different microhabitats could not be mixed. In the case of the samples coming from hypoendolithic samples, the distance from the other two microhabitats allowed their full scraping.”

RC- 6 (cont.) Cyanobacterial isolation was carried out from a bulk endolithic sample. Did the isolation strategy was independent of the inner location within the sampled rock? Did I understand correctly? Please, explain.

Authors: Cyanobacterial isolation was performed from independent samples of each microhabitat, in the same manner as in the case of DNA extraction. Thus, scrapped material from each of the three different microhabitats in the study (cryptoendolithic, chasmoendolithic and hypoendolithic) was transferred to different BG11-agar plates. This procedure allowed us to classify the cyanobacterial isolates taking into account their original microhabitat as described in section 3.4 (lines 186-191) and Table S1. To clarify this procedure, the text has been rephrased: “Scrapped material from each endolithic colonization zone of gypcrete was transferred to different BG11 1.5%-agar plates (Purified agar, Condalab, Spain)” (lines 110-111).

RC- 6 (cont.) DNA extraction was done with minor modifications. Well, modifications must be indicated.

Authors: The description of the DNA isolation protocol has been updated including detailed modification. Now it reads: “This DNA extraction was performed using 0.3 g of samples and the UltraClean DNA isolation kit (MoBio Laboratories, Solana Beach, CA, USA) including a three-cycle step of freezing 0.3mL aliquots of sample suspended in buffer, breaking them down by using an adapted drill and melting in 60°C water bath, as described in Loza et al. (2013) and Becerra-Absalón et al. (2019)”.

7. Line 183. Alpha diversity differences were not found among the microhabitats. Then,

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do microhabitats affect colonization? Please, explain.

Authors: In this work, we did not focus on how microhabitat structure affects colonization but in how they affect the distribution of microorganisms related to their inner architecture. What we try to show is that, although the three gypcrete endolithic communities are not significantly different in terms of alpha-diversity, they are significantly different in terms of their composition and the distribution (relative abundances) of OTUs in each of these communities (Figure 4).

Technical corrections.

Line 22: “investigations”: did you mean investigation? Authors: We agree with the reviewer; the word should be “investigation”. Corrected in the manuscript.

Line 107: add period after (Philips). Authors: corrected

Line 123: check for spaces at “score Authors: checked and corrected

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