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Discussions

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. I am a little concerned about the reliance on molecular methods to characterize the associations. I understand such methods are necessary given the nature of the problem, but they sometimes overlook obvious features. In this particular case, the authors describe two differently pigmented layers in the cryptoendolithic habitat. The

cause of this difference is not addressed. However, in Wierzchos et al. (2015), gypsum samples collected from a spot only a few miles away showed a similar pattern in the cryptoendolithic habitat. In this case the upper, orange-pigmented layer was dominated by eukaryotic algae. These do not appear in the present analysis. Are they absent? Or are they not picked up by the molecular methods used? There is also no discussion of whether the orange and green layers mentioned in the present paper represent different morphologies of the same association or different associations in the same microhabitat.

Authors: We appreciate referee comment on the orange pigmented layer and its possible relation with the presence of eukaryotic algae. In this case algae were absent in all endolithic microhabitats of gypcrete in contrast with Wierzchos et al. (2015) samples and Meslier et al. (2018), where algae were not considered for analysis due to the OTU relative abundance filtering. We performed PCR of 18S rRNA gene in order to obtain eukaryotic sequences and obtained no amplification, also we did not find any OTU sequence belonging to algae chloroplast, which can occur when amplifying 16S rRNA gene from field samples in case algae are present. Also, microscopy observation of all three endolithic microhabitats in gypcrete did not reveal the presence of the algae. The following sentence has been added in the discussion to clarify the absence of algae in these gypcrete endolithic samples (lines 348-350): In contrast with results of Wierzchos et al. (2015) in gypcrete endolithic communities, no eukaryotic algae were found in neither microscopy nor molecular analyses, being Cyanobacteria the phototrophic phylum observed in all gypcrete endolithic microhabitats.

2. In the discussion on page 11 it is suggested that the water relations in the three microhabitats differ as a result of the architecture. A little elaboration here might help with the argument that architecture determines the association.

Authors: The discussion regarding the water relation with the specific features of each microhabitat is developed from line 298 to line 323. However, we agree with the referee that the text should be clarify. To indicate that this suggestion is related to what

is deeply discussed previously, that sentence now reads: Lines 330-333: While the three types of gypcrete microhabitats are exposed to the same climatic conditions, we suggest that differences in micro-architectures resulted in drastically different sets of characteristics for water retention discussed previously: CR counts on water capillary porous condensation and sepiolite water absorption properties, CH has an easier access to liquid water, and HE suffers less water loss.

3. Have the authors considered the proposal by Friedmann and Sun concerning the relative proportions of mycobionts and phycobionts in lichens in response to temperature (Microbial Ecology 49:523-535) in the in relation to the authors hypothesis concerning the relative proportions of phototrophs and heterotrophs in extreme environments?

Authors: We know the work of Friedmann and Sun (2005) in cryptoendolithic lichens. We understand their proposal about the ratio of photobiont and mycobiont in lichens. However, lichenic assemblages between algae and fungi are specific and no other organisms are involved in the symbiotic relationship. In our work we focused in the phototrophic members of the endolithic microbial community and that is why we only discussed about their influence with the ratio of all other members

Technical comments:

1. Does the infrared camera used to measure surface temperature need to be calibrated to gypcrete in order to get an accurate temperature? Most systems need to take the emissivity of the surface material into account first

Authors: Almost all infrared cameras need to be calibrated to measure surface temperature of any material. Gypcrete is almost composed by gypsum and for this material the emissivity values range from 0.8 to 0.95. However, we have introduced the value of 0.92. This value was obtained for gypcrete from sampling place equilibrated to temperature of 25 °C during 5 hours and the value of rock surface temperature detected by FLIR camera was adjusted to 25 °C by introducing adequate value (0.92) of emissivity. Following phrase was added to the text in M&M section (lines 93-94): Calibration of

the FLIR camera for measurements of gypcrete surface temperature was performed introducing the emissivity value of 0.92.

2. I am not clear concerning the numbers of samples and replicates. It looks like three rocks were used. Two of these contained cryptoendoliths, three contained hypoendoliths, and all contained chasmoendoliths, and more than one chasmoendolithic association was sampled for each rock. Does this give enough statistical power for the analysis?

Authors: Due to the problems associated with finding gypsum samples that had at least two of the three endolithic microhabitats under study, it was not possible to count on a large number of samples. However, the samples obtained, although few, were sufficient for the analyses carried out to have necessary statistical power.

3. The CT scans by themselves are difficult to interpret (Figure 2).

Authors: The Figure 2 information has been rewritten to clarify the interpretation. Now it reads: Figure 2: CT-Scan images of a colonized piece of gypcrete. 3D reconstruction of gypcrete sample with spatial distribution of pores (orange colour) and complete reconstructions of the scanned volume (grey colour) on lateral, front and top views of gypcrete. Porous micromorphology is capillary-shaped in vertical position due to gravity movement direction of water. Arrows in top view images point to the deepest cracks. Scale bar = 1cm. Also, 2D images of lateral and front view have been included in Supplementary Material to enable the correct interpretation of CT-Scan images.

4. I did not see any discussion of UAM811, which seems to hold a somewhat anomalous position in maximum likelihood tree (Figure 5)

Authors: Since the aim of this study is a multidisciplinary approach to the impact of microhabitat architecture in the diversity and composition of gypcrete endolithic microbial communities, we used several techniques and approaches obtaining diverse pieces of information. On the one hand, it allows us to combine all that information and helps us

to interpret it, giving a more complete picture of the endolithic communities of gypcrete. However, it makes an in-depth discussion of all the data obtained quite difficult, as is the case of isolated cyanobacteria, which would require a specific study on their own. Nevertheless, we agree with the referee that the phylogenetic position of UAM811 should be at least mentioned and taken into account in the discussion. Thus, that paragraph now reads: Lines 354-363: Further supporting the different micro-environmental conditions and community composition between the top CR and CH habitats and the bottom HE habitat, was the discovery of an unclassified cyanobacterial OTU (UC-OTU, New Reference OTU2), which was almost exclusive to the HE microhabitat and the phylogenetic distance of the hypoendolithic Chroococcidiopsis UAM811 strain with the different Chroococcidiopsis clusters. Regarding the so called UC-OTU, although the low percentage of sequence similarity did not allow for an accurate taxonomical assignment, its closest relatives ($\sim$94% sequence identity for 450 nt of the 16S rRNA gene) were from habitats where light is the limiting factor for photosynthesis such as a pinnacle mat at 10 m depth from a sinkhole (Hamilton et al. 2017) and groundwater sample from a tectonically-formed cavern (Table S2). Both observations, the inability to identify the UC-OTU and the phylogenetic position of the UAM811 strain, highlight the importance of greater efforts in terms of isolation and characterization of cyanobacteria, especially from these environments.

Minor issues:

1. Azuá-Bustos et al. 2015 is missing from the references. Replaced by Azuá-Bustos & Gonzálex-Silva 2014? Authors: Corrected (lines 404-405) 2. Wierzchos et al 2012a and Wierzchos 2012b need to be differentiated in the references. Authors: Corrected (lines 555, 563) 3. Cockell is misspelled in line 61 Authors: Corrected (line 64) 4. I prefer to put genus and phyla ahead of the names: “genus Chroococcidiopsis” instead of “Chroococcidiopsis genus”. Authors: Modified genus (lines 63, 227, 374), genera (238, 240) and phyla (66, 68, 207) 5. Change “the limit established by Nienow (2009)” to “the established limit (Nienow 2009)” (line 264)âËŸAËĞ TNienow cited the

limits but they were established previously Authors: Changed (line 277) 6. Camara et al 2015 should be Camara et al 2014 (line 270) Authors: Corrected (line 284) 7. Changes “consolidates” to “supports” (line 280) Authors: Changed (line 293) 8. instead of “unidentification” might be better to say “inability to identify.” (line342) Authors: Changed (lines 355-356) 9. Pointing references are run together. (line 485) Authors: Corrected (line 513)

Please also note the supplement to this comment:
https://bg.copernicus.org/preprints/bg-2020-245/bg-2020-245-AC2-supplement.pdf

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