RESPONSES TO REVIEWERS AND CHANGES IN MANUSCRIPT

ANONYMOUS REFEREE #1

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 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.*

We agree with the reviewer that microbial life has not only been found in endolithic microhabitats but also in epilithic and hypoendolithic 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 that include an accompanied microflora made of fungi and viruses and, supported by recent publications not properly credited in the manuscripts.

Authors: *Chroococcidiopsis* genus is emphasize 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 *Chroococcidiopsis* 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 *Chroococcidiopsis* 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.*

We are grateful to the 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 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.

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 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."

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).

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 60ºC 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, do microhabitats affect colonization? Please, explain.*

Authors: In this work we did not focus on how microhabitat structure affects to 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

ANONYMOUS REFEREE #2

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 ºC during 5 hours and the value of rock surface temperature detected by FLIR camera was adjusted to 25 ºC 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 (~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)âˇA˘ 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)

ANONYMOUS REFEREE #3

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

1. *Abstract: suggesting that the lithic substrate "might" be an essential factor does not instill*
   confidence in the results and conclusions, which contrasts with the term "confirms"
   in the Conclusions. In addition, the abstract should be more concise in describing the
   results of this work, not general observations of the results. For example, it currently
   points out that the hypoendolithic community was the least diverse and hosted unique
   taxa; explaining "why" here is important for the reader.

Authors: We agree with Reviewer 3 regarding of the use of the term "might" in the abstract. We changed the text and now reads: "These 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". Also, to better describe the results of the work, as suggested by the reviewer, we added "as a result of a lower access to sun radiation" after that sentence.

2. *What is the significance of "Preandean Atacama Desert" within the context of this study?*

Authors: Due to the huge extension of the Atacama Desert, the term Preandean is used in this manuscript to better localize the region of the sampling site, which also determines the climatic conditions of the area of study. The Preandean region of the Atacama Desert has been well defined in several Chapters of the book: "Microbial Ecosystems in Central Andes Extreme Environments.Biofilms, Microbial Mats, Microbialites and Endoevaporites" edited by M.E. Farías, Springer Nature Switzerland AG, 2020.

In this book the Part II is referred to Preandean and Andean Atacama Desert: Life at Limits with Chapter 3: The Desert Polyextreme Environment and Endolithic Habitats by Jacek Wierzchos, Carmen Ascaso, Octavio Artieda, and María Cristina Casero and Chapter 4: Preandean Atacama Desert Endolithic Microbiology by María Cristina Casero, Victoria Meslier, Jacek Wierzchos, and Jocelyne DiRuggiero.

We are grateful to Referee #3 for this comment and bibliographic reference of elsewhere mentioned book (chapter 4) was introduced to the manuscript were Preandean region of the Atacama Desert was mentioned.

3. *Section 2.2: It is unclear how this climate data is directly relevant to the results of this manuscript. Other than thermal measurements, it does not appear to have been collected specifically for this work and so only needs to be mentioned in the Discussion*

Authors: Our paper is focused on microbial ecology of very singular endolithic microbial communities within gypcrete rocks in an extreme environment. Indeed, microclimate parameters such as air temperature and RH over a period of 22 months in the sampling place was only described once by Wierzchos et al. (2015). We consider these data of interest to the readers, such as microbial ecologists of extreme environments. Moreover, we have measured colonized gypcrete surface temperature revealing maximum of 68°C, which is very high temperature, even for desiccated endolithic microbial communities. Also this data was considered by us as of interest to the readers. We preferred to introduce detailed values of climatic and thermal measurements data in description of Sampling Site section (Results) as also these data were discussed in Discussion section.

4. *Section 2.5: Title should include "DNA extraction procedures" to be consistent with*
   Section 2.6.

Authors: Included in the title.

5. *Section 3: Results – Lines 139-141 are note necessary, nor is Section 3.1 with exception of gypcrete surface temperatures if they were measured for this study.*

Authors: Please, consider as correct our response as in point 3.

6. *Section 3.3: Use present tense to describe observations, such as "… colonization zone is close… "*

Authors: We agree with Reviewer 3 and so we changed observations to present tense.

7. *Section 4: What is the distance between the cryptoendolithic/chasmoendolithic habitats in the upper part of the substrate and the hypoendolithic habitat in the lower part of the substrate? Are they separated by millimetres? Centimetres?*

Authors: Cryptoendolithic/chasmoendolithic microhabitats and hypoendolithic microhabitat are separated by centimetres (~ 4 cm).

Technical corrections:

Line 21 – "…a combination of microscopic investigations and…" Line 22 – "…the endolithic communities and their habitats at the microscale…" Line 23 – replace "lithic substrate" with "gypcrete"
Authors: It now reads: "A combination of microscopy investigation and high-throughput sequencing approaches were used to characterize the endolithic communities and their habitats at the microscale within the same piece of gypcrete"

Line 39 – replace "noticeable" with "plausible" Line 39 – "…only by microorganisms that can survive and/or thrive under physical or geochemical extremes such as temperature…"

Authors: It now reads: "Regarding the second half of the statement (but, the environment selects) extreme environments present some of the most plausible scenarios since they are inhabited only by microorganisms that can survive and/or thrive in their respective physical or geochemical extremes such as temperature, solar radiation, pressure, desiccation, pH"

Line 43 – replace "stress" with "limitations":

Authors: Replaced

Line 45 – "…able to survive under such conditions"

Authors: accepted and changed

Line 46 – "The Atacama Desert…on Earth, with scarce precipitation events…and extremely low mean annual relative humidity"

Authors: accepted and changed

Line 54 – replace "living inside the rock but close to the bottom" with "living on the underside of the rock"

Authors: We agree with Reviewer 3 that the expression used is not clear. However, we cannot change it by the expression "underside of the rock" since it could be understood as the hypolithic colonization. Thus, we changed it and now it reads "hypoendolithic (living inside pores in the bottom part of the rock)".

Line 56 – "…PAR radiation levels… "

Authors: accepted and changed

Line 67 – ": : :architecture on the diversity: : :"

Authors: accepted and changed

Line 71 – ": : :the microscale dimensions: : :"

Authors: accepted and changed

Line 71 – How do you define "peculiar"?

Authors: The term "peculiar" in this context is ambiguous so that we decided to change it by a term that better describes what we want to say. Now it reads: "The microscale dimensions and differential diversity distribution in this unique environment has led us to coin the new term "microbiogeography"

Line 72 – Can you provide a definition for the term "microbiogeography"?

Authors: Biogeography is a known term. However, our investigation describes for the first time the composition of endolithic communities at microscale, namely within few cm^3 of lithic substrate. For this reason, we introduced in our manuscript the term "microbiogeography", since the composition of microbial communities is changing even at microscale, what was demonstrated in our work.

Line 75 – "The area experiences…"

Authors: accepted and changed

Line 78 – "…we sampled gypcrete…"

Authors: accepted and changed

Line 80 – dry and dark environment – a lab drawer?

Authors: Thank to Reviewer. #3 for this indication. Exactly, samples were stored in a lab drawer. However, to clarify the description we changed the sentence, that now reads: "…dry and dark conditions…".

Line 92 – "Light microscopy (LM) was used to examine cell aggregates…" Line 93 – "…on cyanobacterial isolates cultured from…"

Authors: accepted and changed

Line 97 – "…were run on pieces…"

Authors: accepted and changed

Line 101 – "…to reduce beam hardening."

Authors: accepted and changed

Line 102 – "..performed using VG Studio Max Version 2.2 software."

Authors: accepted and changed

Line 105 – "Biological material removed from gypcrete: : :"

Authors: accepted and changed

Line 105 – Was it BG11+N or BG11-N?

Authors: The culture medium used was BG11 with nitrogen ($NaNO_3$). When BG11 culture medium contains no $NaNO_3$, it would be called $BG11_0$. (sensu Rippka et al. 1979)

Line 107 – Include a period after "Philips"

Authors: accepted and changed

Line 107 – "After incubation for 15 days, visible…"

Authors: Accepted and changed. It now reads: "After incubation for 15 days, visible cyanobacterial growth appeared. Colonies were isolated by repeated plating on 0.8%-agar with BG11 medium (Rippka et al. 1979), and successfully isolated colonies were transferred to liquid BG11 medium"

Line 116 – "Colonization zones were scraped…" (not scrapped)

Authors: accepted and changed

Line 130 – "Sequences of 16S rRNA genes…"

Authors: accepted and changed

Line 151 – Replace "visualization" with "representation"

Authors: accepted and changed

Line 152 – Replace "following" with "exhibit"

Authors: accepted and changed

Line 155 – Can you better describe "undulated furrows"?

Authors: We agree with Reviewer 3 that the description should be clarify. After consulting the literature, a better term for the observed dissolution features over gypcrete was found. Thus, we changed it by "microrills weathering features (DiRuggiero et al. 2013)"

Line 160 – How did you differentiate pigmentation in layers? Light microscopy?

Authors: The observation of different pigmentation layer was performed by stereoscopic microscopy

Line 161 – "…with high carotenoid content closest to…"

Authors: accepted and changed

Line 219 – "…included only one…"

Authors: accepted and changed

Line 220 – Which Chinese desert are you referring to?

Authors: After revising the literature corresponding to the cited sequence and GenBank database, it is not possible to assign that *Chroococcidiopsis* sequence to a specific location of those studied by Pointing et al. (2007): Qaidam Basin, Turpan Depression and Taklimakan Desert. However, to clarify the climatic conditions of the studied deserts by those authors, the sentence now reads: "Chroococcidiopsis sp. strains isolated from quartz hypolithic communities from hyperarid Chinese deserts (Pointing et al. 2007)"

Line 221 – Please provide a reference for University Valley

Authors: Included. Cumbers, J. and Rothschild, L. J.: Salt tolerance and polyphyly in the cyanobacterium *Chroococcidiopsis* (Pleurocapsales)., J. Phycol., 50(3), 472–482, doi:10.1111/jpy.12169, 2014.

Line 222 – "…no sequences from isolates…"

Authors: accepted and changed

Line 230 – "…with our isolate sequences…"

Authors: accepted and changed

Line 234 – Can you think of another way to say "differentially abundant"?

Authors: "Differentially abundant taxa" is a common term in this type of studies to define those taxa/features whose different abundance across samples is statistically significant. Examples:
Taye et al. (2020) https://doi.org/10.3389/fmicb.2019.03007
Shatzkes et al. (2017) https://doi.org/10.1038/srep43483
Jiang et al. (2017) https://doi.org/10.1128/mSystems.00092-17

Thus, we consider that the term is correctly used in this study.

Line 235 – "Both OTU11…"

Authors: accepted and changed

Line 255 – The last sentence of this paragraph is not necessary.

Authors: We removed that sentence

Line 259 – "Both substrates show…"

Authors: accepted and changed

Line 263 – "…based on the ratio of mean…"

Authors: accepted and changed

Lines 265-267 – I do not know if you can compare temperatures on terrestrial rock surfaces with those in hot springs as an approximation for the upper temperature limit for photosynthesis. Can you estimate the temperatures within the endolithic habitats?

Authors: We agree with the reviewer that the comparison of rock surface temperatures with those in hot springs is inaccurate and that its relation to the temperature in the endolithic microhabitats should be mentioned. Thus, we revised that paragraph and now it reads:

Specific measurements of surface temperature on gypcrete revealed values of close to 70°C. This value was detected at zenith, when microbial communities are desiccated and metabolically inactive (Cockell et al. 2008). The temperature within the endolithic habitats is expected to be close to that in the rock surface as shown by Wierzchos et al. (2012) for halite endoliths.

Line 273 – ": : :water to metabolise and grow." Line 273 – ": : :gypcrete restricts water loss"

Authors: accepted and changed

Line 280 – I don't think "consolidates" is the correct word in this sentence

Authors: It now reads "supports the proposal of Wierzchos et al. (2015)"

Line 282 – "following water gravity flow" is unclear

Authors: We agree with Reviewer 3 and this phrase was corrected as follow:

…" This structure reveals different dissolving and crystallization processes of the gypsum following the water displacement from the surface to the bottom of the rock (gravity flow). This water gravity flow giving rise to the cave-shaped pores, thus providing this HE microhabitat with a hard permeable bottom gypsum layer" …

Line 295 – end the sentence with a period

Authors: accepted.

Line 297 – "EPSs" should be "EPS" Line 300 – see line 297

Authors: accepted and changed

Line 302 – "The aggregate-like structure…"

Authors: accepted and changed

Line 303 – "…and heterotrophic bacteria also helps…"

Authors: accepted and changed

Line 308 – "…in such an oligotrophic environment."

Authors: accepted and changed

Line 330 – can you provide a reference that would support the statement that light intensity should be considered a crucial factor in understanding differences in community composition between top and bottom habitats?

Authors: Recently the light intensity, as driving factor of spatial heterogeneity within halite endolithic microbial communities was reported by Uritskiy et al. (2020). This phrase and ref. was introduced to the manuscript text.

Line 342 – replace "unidentification" with "lack of positive identification"

Authors: Changed. It now reads "the inability to identify the UC-OTU"

Line 353 – Replace "confirmed" with "hypothesized"; I would not say that this work confirms that liquid water availability is a driver of community composition, as no experimental evidence was provided in the manuscript to substantiate this claim. A more convincing argument for how microenvironmental conditions determines microbial distribution would strengthen the manuscript.

Authors: We agree with Reviewer 3 that confirmed is not an appropriate word to define the findings included in this work. Thus, the term it was changed and now it reads "In

this study, liquid water availability was proposed to be a driver of community composition…”

Line 369 – “…draw conclusions…”

Authors: accepted and changed.

Figure 1 – Latitude and longitude markers should be included in the study are map

Authors: GPS coordinates are already included in site description and sampling section

Figure 3 – It would be helpful to point out what samples are polished blocks/thin sections vs whole mounts for SEM work.

Authors: no thin sections were included in Figure 3. All samples are polished blocks

RESPONSE TO EDITOR AND ANONYMOUS REFEREE #2

We are grateful for Ref. # 2 and your comments focused on microhabitat's architecture and its relations with environmental factors in that very microhabitat. It was several years ago when we discovered the presence of endolithic microorganisms within several substrates in the hyperarid Atacama Desert as the last refugees of life in these harsh environmental conditions (review in Wierzchos et al. 2018). The attached Table summarizes the presence of dominant microorganisms within three different endolithic habitats (crypto-, chasmo- and hypoendolithic) in only one Ca-sulfate lithic substrates.

Table 1. Dominant microorganisms within three different endolithic habitats. *Endolithic algae-fungi association: **The works where endolithic microhabitats were well defined.

Endolithic habitats in Ca-sulfate-bearing substrates	Nature of Ca-sulfate substrates / Locality	Algae	Fungal hyphae	Proto-lichens*	Cyanobacteria	Heterotrophic bacteria	References**
Cryptoendolithic	Gypsum/anhydrite crusts on soil surface / Tarapacá						Wierzchos et al. (2011), Vítek et al. (2013)
Cryptoendolithic	Gypsum+anhydrite crusts on soil surface / Salar Navidad						Culka et al. (2017)
Cryptoendolithic	Gypcrete / Cordon de Lila						Wierzchos et al. (2015)
Chasmoendolithic	Gypsum crust on the surface of rhyolite / Tilocalar						DiRuggiero et al. (2013)
Hypoendolithic	Gypsum/anhydrite crusts on soil surface / Tarapacá						Wierzchos et al. (2011)
Hypoendolithic	Gypcrete / Cordon de Lila						Wierzchos et al. (2015)
Crypto-, Chasmo- and Hypoendolithic	Gypcrete / Cordon de Lila						**This work**

Hence the endolithic habitat could be the same, their dwelling microbial communities' composition could be very different. It means that the denomination and nature of endolithic habitat is not a driver of microbial structure. If we compare the Ca-sulfate substrates from different climatic regimes of the Atacama Desert it is obvious that indeed these characteristics of climate regimes (T, RH, rainfall, dewfall, etc.) must have the main influence on the endolithic microbial communities' composition. Definitely, for this reason, our study was performed within the same external climate regime and more: within the same piece of gypcrete with three well defined endolithic microhabitats. This was a challenging question: how is the structure and composition of endolithic colonization within the same climatic regimen and within the same piece of the rock? Our work answers that there are certain differences in endolithic microbial structure among crypto-, chasmo- and hypoendolithic habitats. We would like to again underline that the external climatic regime was absolutely the same for studied rock pieces and one could expect the same or very similar microbial structure colonization within all three endolithic habitats. However, our results have shown that indeed the structure of these microbial colonization's is different among endolithic habitats. How is a driver of these differences? Of course different microclimatic regimes at the micro-scale within different endolithic microhabitats. Obviously, it is impossible to measure microclimate parameters such as: T, RH, dewfall, gravity water flow, water nanopore condensation, evaporation rates, solar irradiance, heat irradiance, etc. within endolithic microhabitats. However, it is and it was possible to describe and characterize the "architecture" of these microhabitats (this work and references in a review in Wierzchos et al., 2018). The term rock architecture was for the first time introduced by Wierzchos et al. (2015) as follows:

…"4.6. Architecture of a lithic substrate

As observed with microscopy techniques, internal structural elements such as porosity, pore-size distribution, presence of large pores and cavities, light transparency and light scattering properties, dissolution and crystallization features, and sepiolite nodules distribution varied significantly within various location of the gypsum substrate. These all together structural, physical, chemical and mineral elements give rise to a new understanding of the features and functions relevant to the rock bioreceptive characteristics. We suggest using the term of "rock architecture" instead of "rock structure" to emphasize the functional role of the rock interior. As such, this new concept of the architecture of a lithic substrate encompasses the internal structures of a rock with all mentioned elements that are essential as a habitat for microbial life. It is about perceiving the rock interior from the existence of porous spaces of different sizes and shapes, interconnected or not; the solid structures that divide and support these spaces, and the minerals and salts that can be transformed. All these components and elements are interrelated and influence one another, thus fulfilling a requisite: they might shape a suitable architecture to hold microbial life. The architecture of habitable rocks provides resources (water, light and nutrients, above all) and guarantees effective protection from excessive evapotranspiration, thus assuring efficient gas exchange and provides long-time stable fabric. Considering the architecture of a rock can provide an integrated view of its potential habitability for endolithic microbial communities. All porous rocks have a structure, yet very few show such a suitable architecture for endolithic microbial colonization, even under extreme environmental conditions, as the Atacama's gypsum do."…

Following this definition, we can distinguish different architecture of the substrate within different endolithic microhabitats, and indeed these differences will induce to different microenvironmental characteristics on the microscale. And these microenvironmental characteristics shaping the different microbial structures within different endolithic microhabitats what was shown in our paper. As so, we do not pretend to separate the influence of the architecture of the microhabitat from the myriad of other environmental variables. Quite opposite. We consider that indeed distinct differences in microarchitecture of the microhabitats have an influence on environmental variables at the microscale and shape microbial colonization structure. We consider that the endolithic communities are determined by endolithic microhabitat architecture and not by the endolithic microhabitat type (crypto-, chasmo- and hypo endo (see Table 1). However, we agree that a much more precise conceptual definition of the above-mentioned relationships is needed and appropriate corrections were introduced in the text of the manuscript as follows:

Introduction section:

The concept of rock architecture was introduced by Wierzchos et al. (2015) for colonized gypcrete substrate and encompasses the internal structures of rock with all elements that are essential for microbial life. Microhabitat architecture allows perceiving the rock interior from the existence of porous spaces of different sizes and also the solid structures that divide and support these spaces. All these components and elements are interrelated and influence one another, thus fulfilling a requisite: they might shape a suitable architecture to hold microbial life.

Discussion section:

Our work answers that there are certain differences in endolithic microbial communities' structure among crypto-, chasmo- and hypoendolithic habitats. Considering that the external climatic regime was the same for studied pieces of rock, our results have shown that the structure of these microbial communities was different among endolithic habitats. Following the definition of microhabitat architecture by Wierzchos et al. (2015) we can distinguish different architecture of the substrate within different endolithic microhabitats. In this context, our work suggests that distinct features of microhabitat architecture that have an influence on microenvironmental variables at the microscale would shape microbial communities' structure.

The composition of endolithic communities in gypcrete is determined by the specific microhabitat architecture

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Abstract. Endolithic microhabitats have been described as the last refuge for life in arid and hyper-arid deserts where life has to deal with harsh environmental conditions. A number of rock substrates from the hyper-arid Atacama Desert, colonized by endolithic microbial communities, such as halite, gypsum crusts, gypcrete, calcite, granite and ignimbrite, have been characterized and compared using different approaches. In this work, three different endolithic microhabitats are described, each one with a particular origin and architecture, found within a lithic substrate known as gypcrete. Gypcrete, an evaporitic rock mainly composed of gypsum ($CaSO_4 \cdot 2H_2O$) and collected in the Cordón de Lila area of the desert (Preandean Atacama Desert), was found to harbour cryptoendolithic (within pore spaces in the rock), chasmoendolithic (within cracks and fissures) and hypoendolithic (within microcave-like pores in the rock-bottom layer) microhabitats. A combination of microscopy investigation~~s strategies~~ and high-throughput sequencing approaches were used to characterize the endolithic communities and their habitats at the microscale ~~in these microhabitats~~ within the same piece of ~~lithic substrate~~gypcrete. Microscopy techniques revealed differences in the architecture of the endolithic microhabitats and ~~in~~ the distribution of the microorganisms within those microhabitats. Cyanobacteria and Proteobacteria were dominant in the endolithic communities, of which the hypoendolithic community was the least diverse and hosted unique taxa, as a result of lower access to sun radiation. These results show, for the first time, that the differences in the architecture of a microhabitat, even within the same piece of a lithic substrate, plays ~~might be~~ an essential ~~factor~~ role in shaping the diversity and composition of endolithic microbial communities.

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1. Introduction

The statement developed by Professor Lourens Gerhard Marinus Baas-Becking (1934) "everything is everywhere but the environment selects" which established the most referred principle for microbial biogeography remains in discussion regarding the first half of the statement (everything is everywhere) (de Wit and Bouvier 2006, O'Malley 2008, Bass and Boenigk 2011, Fontaneto and Hortal 2012, van der Gast 2015). Regarding the second half of the statement (but, the environment selects) extreme environments present some of the most plausible ~~noticeable~~ scenarios since they are inhabited only by microorganisms that can~~, which are able to~~ survive and/or thrive in their respective physical or geochemical ~~extreme conditions~~extremes~~,~~ such as~~:~~ temperature, solar radiation, pressure, desiccation, pH (Rothschild and Mancinelli 2001).

Hyper-arid deserts, where aridity index is lower than 0.05 (Nienow, 2009) constitute the most extreme deserts on Earth, and usually combine a series of simultaneous stress conditions such as water ~~stress~~limitation, extreme high and low temperatures, scarcity of organic carbon, high solar radiation and osmotic stress (Pointing and Belnap 2012). While these environments are considered polyextreme, they are inhabited by microbiota able to survive ~~in~~ under such ~~extreme~~ conditions. Hence, polyextreme environments are excellent microbial ecosystem models to study adaptive mechanisms to environmental stress. ~~Among others deserts,~~ Tthe Atacama Desert (North Chile) is perhaps the most challenging polyextreme environment on Earth and the most barren region imaginable, with scarce precipitation events~~s~~ (McKay et al. 2003; Wierzchos et al. 2012a) and ~~an~~ extremely low mean annual relative humidity (RH) ~~(~~(Azua-Bustos et al.~~,~~ 2015)~~Azúa-Bustos et al. 2015)~~. Further, this desert holds another world record: 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 Andes sites.

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 ~~refuge in very specific~~ endolithic (inside rocks) microhabitats (rev. by Wierzchos et al. 2018; Wierzchos et al. 2012b). Three different locations of these endolithic habitats have been described within rocks of the Atacama Desert: cryptoendolithic (occupying pore spaces in the rock), chasmoendolithic (living within cracks and fissures in the rock), and hypoendolithic (living inside pores in the bottom part of the rock ~~but close to the bottom~~). Endolithic colonization can be viewed as a stress avoidance strategy whereby the overlying mineral substrate provides protection from incident lethal UV and PAR radiation levels~~,~~ and also offers enhanced moisture availability (Walker and Pace 2007; Wierzchos et al. 2012b). These microbial communities, regardless of the position they occupy in the rock, or the type of rock, are supported by oxygenic phototrophic primary producers supporting a diversity of heterotrophic microorganisms (rev. in Wierzchos et al. 2018). 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 genus *Chroococcidiopsis* ~~genus~~ (Meslier et al. 2018, Crits-Christoph et al. 2016, Billi et al. 2000, Cockell et al. 2005) as well as members from genera *Gloeocapsa* (Crits-Christoph et al. 2016) and *Halothece* (de los Ríos et al. 2010; Robinson et al. 2015 and Uristkyi et al 2019)~~,~~ and phyla ~~,~~Actinobacteria, Proteobacteria, Chloroflexi, Bacterioidetes and Euryarchaeota ~~phyla~~ (Meslier et al. 2018). In gypcrete and gypsum crust from ~~P~~preandean Atacama Desert (Casero et al.,

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2020), previous studies reported endolithic communities dominated by the phyla Cyanobacteria (36-83%), Actinobacteria (10-25%) and Proteobacteria (13-30%) ~~phyla~~ (Wierzchos et al. 2015; Dong et al. 2007; and Meslier et al. 2018; rev. in Casero et al. 2020), however, these studies did not differentiate between microhabitats, even though the occurrence of different endolithic microhabitats in gypcrete had already been described (Wierzchos et al. 2015).

This work addresses the impact of microhabitat architecture ~~in~~ on the diversity and composition of gypcrete endolithic microbial communities (EMCs). The concept of rock architecture was introduced by Wierzchos et al. (2015) for colonized gypcrete substrate and encompasses the internal structures of rock with all elements that are essential for microbial life. Microhabitat architecture allows perceiving the rock interior from the existence of porous spaces of different sizes and also the solid structures that divide and support these spaces. All these components and elements are interrelated and influence one another, thus fulfilling a requisite: they might shape a suitable architecture to hold microbial life.

The study is based on the hypothesis that the different~~ial~~ architecture of endolithic microhabitats involves small-scale differences in the micro-environmental conditions, which in turn determine the distribution of organisms in each community. The hypothesis is tested here for the first time by using a multidisciplinary approach combining microscopy and molecular tools for their characterization. The microscale dimensions and ~~peculiar~~ differential diversity distribution in this unique environment ~~has~~ have led us to coin the new term “microbiogeography”.

2. Experimental procedures

2.1 Site description and sampling

Colonized rocks were collected in the Atacama Desert in December 2015 from the Monturaqui area (MTQ) (GPS coordinates 23°57’S; 068°10’W; 2868 m.a.s.l.) located in an N-S trending depression of the Cordón de Lila Range. ~~This area exhibits~~The area experiences a pronounced rain shadow effect by the western slope of the central Andes from 15° to 23°S (DiRuggiero et al. 2013; Wierzchos et al. 2015). In order to study endolithic communities that inhabit the same piece of a lithic substrate, we ~~looked for~~sampled gypcrete pieces that harboured at least two of the three endolithic microhabitats of interest, that were collected within a 50 m^2 area. All samples were packed in sterile bags and stored at room temperature in ~~,~~ dry and dark conditions~~environment~~ before further processing.

2.2 Microclimate data

Microclimate data (Meslier et al. 2018) were recorded using an Onset HOBO® Microweather Station Data Logger (H21-USB), as previously described by Wierzchos et al. (2015). Air temperature (T), air relative humidity (RH in %) and Photosynthetic Active Radiation (PAR in µmol photons m^{-2} s^{-1}) were recorded from January 2011 to February 2013 (22 months) (Wierzchos et al. 2015). Rainfall data were obtained from DiRuggiero et al. (2013). Thermal measurements of the

gypcrete surface were acquired at zenith time at 20 cm distance from the substrate. Thermal images were taken using a thermal infrared camera (FLIR® E6, FLIR Systems, Oregon, USA) whose technical specifications are: ±2°C or ±2% of reading; < 0.06°C pixel sensitivity with a resolution of 160 × 120 pixels. Calibration of the FLIR camera for measurements of gypcrete surface temperature was performed introducing the emissivity value of 0.92.

2.3 Microscopy analyses

Colonized gypcrete samples were processed for scanning electron microscopy in backscattered detection mode (SEM-BSE) according to methods described by Wierzchos and Ascaso (1994) and Wierzchos et al. (2011). Light microscopy (LM) in differential interference contrast mode (DIC) was ~~performed on~~used to examine cell aggregates gently isolated from the cryptoendolithic, chasmoendolithic and hypoendolithic microhabitats and on cyanobacterial isolate~~sd cultures~~ cultured from those microhabitats. The samples were examined using a microscope AxioImager M2, Carl Zeiss, Germany in DIC mode equipped with Apochrome 63x n=1.4 oil immersion objective.

2.4 CT-Scan analysis

Micro-CT scans were run on ~~a~~ pieces of gypcrete with an X-Ray Computed Tomography system (CT-scan) — HMXST 225 micro-CT system (Nikon Metrology, Tring, UK) to observe volume, bulk density, and variations in internal density. For volume and bulk density measurements a Nikon X-Tek CT-Scan device was used, with an X-ray peak voltage of 146 kV and current of 65 mA, collecting 1583 sections at 1000 micro-seconds on average from four frames. The system operates with an X-ray tube and added filtration (0.875 mm Cu) to reduce ~~the~~ beam hardening. ~~Three~~ Three-dimensional viewing and analyses of the obtained X-ray sections were performed by software VG Studio Max Version 2.2. software. The auto-threshold feature determined the grey-scale intensity for 3-D surface segmentation and subsequent analysis.

2.5 Cyanobacteria isolation and characterization and DNA extraction procedures from isolates.

~~Scrapped~~ Biological material removed from each endolithic colonization zones of gypcrete was transferred to different BG11 1.5%-agar plates (Purified agar, Condalab, Spain). All samples were incubated in a growth chamber at 28±2°C with illumination of 20 µmol photons m^{-2} s^{-1} by cool white 40W fluorescent tubes (Philips). After incubation for 15 days, ~~of incubation, when~~ visible cyanobacterial growth appeared., C~~c~~olonies were isolated by repeated plating on 0.8%-agar with BG11 medium (Rippka et al. 1979), and successfully isolated colonies were transferred to liquid BG11 medium. Culture material from each strain (2 mL) was harvested during exponential growth and centrifuged (10,000 *g*, 5 min). Genomic DNA was extracted from the cell pellet using the UltraClean DNA isolation kit (MoBio Laboratories, Solana Beach, CA, USA). 16S rRNA was amplified using primers PA (Edwards et al. 1989) and B23S (Lepère et al. 2000), PCR reaction and sequencing were performed as described in Casero et al. (2014).

2.6 DNA extraction procedures from natural samples, 16S rRNA gene libraries preparation and sequencing

Three individual rocks harbouring at least two of the three endolithic microhabitats were processed, which resulted in 11 samples, including technical replicates: cryptoendolithic (n=2), chasmoendolithic (n=6) and hypoendolithic (n=3). Colonization zones ~~was~~ were 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 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.

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 60ºC water bath, as described in Loza et al. (2013) and Becerra-Absalón et al. (2019) ~~with minor modifications~~. A two-step PCR strategy was used to prepare the sequencing libraries of endolithic microbial communities, as previously described (Robinson et al. 2015). DNA was amplified using primers 338F and 806R (V3-V4 hypervariable region) barcoded for multiplexing; amplicons from 2 PCR reactions were pooled after the first step. Illumina paired-end sequencing (2 x 250bp) was performed using the MiSeq platform at the Johns Hopkins Genetic Resources Core Facility (GRCF).

2.7 Computational analysis

After demultiplexing and barcode removal, sequence reads with ~~phred~~ Phred score <20 and length <100bp were discarded using sickle (Joshi and Fass, 2011), representing only 2% of the initial reads count. The Qiime package (v1.6.0) was used to further process the sequences (Caporaso et al. 2010) and diversity metrics were calculated based on Operational Taxonomic Units (OTUs) at the 0.03% cutoff against the Ribosomal Database Project (RDP) database release 11 (Cole et al. 2014). The resulting OTUs table was filtered of the rare OTUs (total abundance across all samples below 1%), representing 40% of the initial count (1511 OTUs).

2.8 Phylogenetic analysis

Sequences of 16S rRNA genes from Cyanobacterial OTUs that showed significant differences in their relative abundance between endolithic microhabitats and 16S rRNA gene sequences from cyanobacterial isolates~~,~~ were aligned with sequences obtained from the NCBI GenBank using the Clustal W 1.4 software (Thompson et al. 1994). 16S rRNA gene sequences from GenBank were selected using the NCBI MegaBlast tool (http://blast.ncbi.nlm.nih.gov/ Blast.cgi, accessed 28.08.18). The final alignment length was 400 bp. Phylogenetic trees of each of the genes were constructed in MEGA 7.0 using the Maximum Likelihood (ML) method (Kumar et al. 2016). The best-fitting evolutionary model, chosen following the BIC (Bayesian

Inference Criterion) in MEGA 7.0, was the Kimura 2-parameter model (Kimura 1980) for 16S rRNA genes. 1000 bootstrap replicates were performed for all trees.

3. Results

We combined microclimate measurements, microscopy analyses and high throughput culture-independent molecular data to identify the effect of micro-biogeography and the factors underlying the structure and composition of microbial assemblages of gypcrete endoliths from the hyper-arid Atacama Desert.

3.1 Sampling site

Gypcrete samples were collected from the Monturaqui area (MTQ), located in the Preandean Depression of the Atacama Desert (Casero et al., 2020) (Fig. 1) ~~on~~ in December 2015. Climate data recorded over a period of 22 months described a mean air temperature about 15°C, with strong amplitude between minima and maxima (from -4.7°C to 49.3°C), average diurnal PAR ~ 1000 µmol photons m^{-2} s^{-1} with a maximum of 2553.7 µmol photons m^{-2} s^{-1}, providing evidence for the extremely intense solar irradiance found in this region (Cordero et al. 2014). This area experiences extremely dry conditions, with an average air RH of about 20% with frequent lows of 1% and precipitations extremely scarce with mean annual values of 24.5 mm (Wierzchos et al. 2015). Gypcrete surface temperature examined with a thermal infrared camera revealed a maximum temperature of 68°C.

3.2 Micromorphology of gypcrete

CT-Scan images provided a 3D spatial ~~visualization~~ representation of pore shapes and their distribution inside the gypcrete rock (Figs. 2). The pores revealed capillary-like micromorphology ~~following~~ exhibit a vertical orientation as is shown in both top and lateral views. Detailed 3D images pointed to the apparent absence of connectivity with the surface of most of the pores (Figs. 2). However, the presence of this connectivity cannot be discarded due to the limited resolution of the CT-Scan technique and the conditions of acquisition. Moreover, CT-scan images of the gypcrete surface reveal microrills weathering features (DiRuggiero et al. 2013)~~undulated furrows~~ due to the dissolution of gypsum after scarce rains (Video S1).

3.3 Endolithic microhabitats

~~Cross~~ Cross-sections of the gypcrete rocks revealsed the presence of three clearly differentiated microhabitats where a significant heterogeneity in micromorphology and structure was found (Figs. 3). The cryptoendolithic colonization zone ~~was~~ is close to the compact gypcrete surface layer (up to 5mm depth). Within cryptoendolithic microbial communities, two characteristic pigmented layers ~~were~~ are distinguished. The observed orange colour belongs to microorganisms with high carotenoids content ~~laid~~ closest to the gypcrete surface. The green colour layer beneath the orange layer belongs to microorganisms with chlorophyll and phycobiliproteins content. The presence of these pigments was previously reported by Wierzchos et al. (2015) and Vítek et al. (2016) (Fig. 3, A1). The chasmoendolithic colonization zone reachesd a deeper (up to

8mm depth) position in the substrate and ~~was~~ is directly connected to the surface (Fig. 3, B1). Finally, the hypoendolithic colonization zone~~, was~~ is located close to the compact bottom gypcrete crust, shaped like micro-caves (Fig. 3, C1).

Cyanobacteria were found in the cryptoendolithic habitat among lenticular gypcrete crystals, filling up vertically elongated pores, and aggregated around sepiolite nodules (Figs. 3, A2-A3), a clay mineral with high water retention capacity, previously identified in gypcrete by Wierzchos et al. (2015). SEM-BSE also revealed dense arrangements of cyanobacterial cells embedded in concentric sheets of EPSs (Figs. 3, A3). By contrast, the microbial assemblages inhabiting the chasmoendolithic and hypoendolithic microhabitats were coating the walls of the cracks and caves previously described (Figs. 3, B2, B3, C2, C3). Detailed SEM-BSE (Figs. 3, A3-C3) and LM images (Figs. 3, A4-C4) of each microhabitat showed mainly Cyanobacteria with different size and morphology accompanied by heterotrophic bacteria.

3.4 Cyanobacterial isolates from endolithic microhabitats

A total of 12 cyanobacterial strains were isolated from the three different endolithic microhabitats (Table S1): 3 from cryptoendolithic, 3 from chasmoendolithic and 6 from hypoendolithic. The cyanobacterial strains were identified, following Komárek et al. (2014), as *Chroococcidiopsis* sp. (UAM800, UAM801, UAM802, UAM805, UAM808, UAM809, UAM810, UAM811), *Gloeocapsa* sp. (UAM803, UAM804) and *Gloeocapsopsis* sp. (UAM806, UAM807).

3.5 Structure and composition of endolithic communities

High throughput sequencing of 16S rRNA gene amplicons across 11 samples and 3 microhabitats resulted in a total of 385,440 V3-V4 SSU rDNA reads, with an average number of paired-end reads per sample of 35,040 ± 6,288 and an average length of 456 ± 11 bp. Diversity metrics, calculated from OTUs clustered at 97%, revealed no significant differences between microhabitats in terms of alpha-diversity (Table 1).

A total of 11 bacterial phyla with a relative abundance > 0.1% were found across all microhabitats. Of these only 7 had a relative abundance over 1% of sequences across the different microhabitats (Figs. 4). Cyanobacteria, Proteobacteria, Actinobacteria and Gemmatimonadetes were the most abundant phyla, representing 82%–83% of the total community (Fig. 4, A). Cyanobacteria dominated the communities inhabiting all endolithic microhabitats; in the cryptoendolithic and chasmoendolithic communities, Cyanobacteria did not exceed 40% of the sequences, while in the hypoendolithic community they reached a relative abundance of 60% (Fig. 4, A). Proteobacteria were the second most abundant phylum, contributing ~30% of the sequences in the cryptoendolithic and chasmoendolithic communities, and less than a half in the hypoendolithic community (13%). The relative abundance of Actinobacteria was even across all microhabitats, never exceeding 10% of the sequence reads. Gemmatimonadetes relative abundance showed differences across microhabitats representing 7%, 4.4% and 2.3% of sequences in the cryptoendolithic, chasmoendolithic and hypoendolithic communities, respectively (Fig. 4, A). The phyla Bacteroidetes and Thermi ~~phyla~~ also exhibited variation between the different endolithic communities, showing the higher relative abundance in the hypoendolithic (8.2%) and cryptoendolithic (4.9%) microhabitats. Firmicutes and

Planctomycetes were also found in all three microhabitats at very low relative abundance (0.003% and 0.002%). No archaeal OTUs were detected before or after the quality filtering of sequences during the ~~samples processing~~processing of the samples. The four main phyla constituted ~ 80% of OTUs, clustered at 97% identity, across all microhabitats, which was quite different from the distribution of sequence reads (Fig. 4, B). The three major phyla, Cyanobacteria, Proteobacteria and Actinobacteria, has similar OTUs relative abundances across all three microhabitats (25%, 32% and 21% respectively). The greatest difference between the distribution of the relative abundance of sequences and that of OTUs is observed for Cyanobacteria in the hypoendolithic community.

Compared to other microhabitats this phylum showed the highest relative abundance in terms of sequences (60.4%) but the lowest in terms of OTUs (21.9%), thus revealing the high abundance of a very low number of cyanobacterial OTUs. Average Bray Curtis distance confirmed that dissimilarity between microhabitats (CR-CH= 0.36, CR-HE= 0.44, CH-HE= 0.44) was higher than between replicates of the same microhabitat (CR=0.36, CH= 0.29, HE=0.32). ~~—~~Adonis and ANOSIM tests, performed with the 3 microhabitats categories (cryptoendolithic, chasmoendolithic and hypoendolithic), confirmed the statistical significance of the grouping (R^2 =0.38, *p*- value=0.014 and R^2=0.48, *p*-value=0.003 for adonis and ANOSIM, respectively).

3.6 Cyanobacterial composition

As the major component of the endolithic communities from the 3 described microhabitats, Cyanobacteria OTUs and isolates were studied in detail. A phylogenetic analysis of the 15 major cyanobacterial OTUs (relative abundance > 1%)~~,~~ and 12 isolates revealed 6 main clusters supported by high bootstrap values (Fig. 5).

Most of the OTUs (9 out of 15) and isolates (8 out of 12) were assigned to the genus *Chroococcidiopsis* ~~genus~~ and were distributed in three clusters (I, III and V), each with representatives of *Chroococcidiopsis* isolates and clones' sequences from various deserts. Cluster I had the highest number of sequences from this study: six of the *Chroococcidiopsis* strains (UAM801, UAM810, UAM802, UAM809, UAM800, UAM808) and four of the cyanobacterial OTUs (OTU1, OTU497, OTU8, OTU112). This cluster also included two reference *Chroococcidiopsis* sp. sequences of soils from the Atacama Desert (Patzelt et al. 2014). Cluster III included only one *Chroococcidiopsis* isolate (UAM805), three OTUs sequences (OTU1772, OTU420 and OTU4), and reference sequences of *Chroococcidiopsis* sp. strains isolated from quartz hypolithic communities from hyperarid Chinese deserts (Pointing et al. 2007) and from University Valley (Antarctica)(Cumbers and Rothschild, 2014). The last *Chroococcidiopsis* sp. cluster, number V, had no sequences from isolates, two OTUs sequences (OTU7 and OTU98), sequences from cloning libraries from two deserts, Atacama and Jordan (Dong et al. 2007), and one *Chroococcidiopsis* sp. sequence from a Mediterranean biocrust (Muñoz-Martín et al. 2019).

Cluster II comprised cyanobacterial sequences belonging to the Nostocales order from the genera *Fischerella* and *Calothrix* ~~genera~~ to which OTU18 and OTU11 were respectively assigned. A total of 6 Cyanobacteria of this study were clustered with members of the genera *Gloeocapsa* and *Gloeocapsopsis* ~~genera~~ (order Chroococcales), four isolates (UAM806, UAM807,

UAM804, UAM803) and two OTUs (OTU9, OTU854), forming cluster IV. Two reference sequences of *Synechococcus* together with OTU5 constituted Cluster VI.

Because of the low % identity of OTU2 with its closest relatives in the database (< 95%) (Table S2) and with our isolates sequences, it was not possible to provide an accurate taxonomical assignment for this OTU (Fig. 5). Hits were found between two of the isolates (*Chroococcidiopsis* UAM801 and *Gloeocapsopsis* UAM806) and two of the most abundant OTUs (OTU1 and OTU9, respectively).

Differential abundance analysis using DESeq2 test revealed that 9 out of 15 of the cyanobacterial OTUs were differentially abundant in the three microhabitats (Fig. 6). Both OTUs 11 (*Calothrix* sp.) and OTU18 (*Fischerella* sp), phylogenetically assigned to the Nostocales order, were significantly more abundant in the chasmoendolithic community (3.8% and 1.5%, respectively) than in cryptoendolithic and hypoendolithic communities (< 0.4% for both OTUs) (p-value < 0.01). OTUs clustered with *Gloeocapsa* and *Gloeocapsopsis* (cluster IV), with *Synechococcus* (cluster VI), and with *Chroococcidiopsis* sp. from three clusters (I, III and V), showed significantly different abundances (p-value < 0.001) between the hypoendolithic community and that of the two communities from the upper side of the substrate (cryptoendolithic and chasmoendolithic). OTU8 (*Chroococcidiopsis* sp.) was the only one displaying a higher abundance in the hypoendolithic community, while OTU9 (*Gloeocapsopsis* sp.), OTU5 (*Synechococcus* sp.), OTU854 (*Gloeocapsa* sp.) and OTUs 1772 and 7 (*Chroococcidiopsis* sp.) had a higher abundance in the cryptoendolithic and chasmoendolithic communities. The unassigned Cyanobacterial OTU2 was mostly found in the hypoendolithic community (p-value < 0.0001) with an average relative abundance of more than 39%, of the total community while its relative abundance in the other two communities was ~ 0.4%.

4. Discussion

In this study, we characterized the microbial communities inhabiting gypcrete collected from the Monturaqui area (Preandean Depression), which is of particular interest due to its location in the hyper-arid zone of the Atacama Desert. While endolithic colonization of the gypsum crust and gypcrete in this area has previously been studied (Dong et al. 2007, DiRuggiero et al. 2013, Wierzchos et al. 2015, Meslier et al. 2018), this is the first work in which cryptoendolithic, chasmoendolithic and hypoendolithic communities have been characterized separately. The novelty of this study lies in the consideration of two different EMCs inhabiting two endolithic microhabitats located in the upper part of the substrate, and in the description of the structure and composition of the hypoendolithic microhabitat and its endolithic community, located at the bottom part of the substrate. This work was based on a multidisciplinary approach to elucidate for the first time the relationship between microhabitat architecture and community composition of EMCs hosted in these different endolithic microhabitats coexisting within the same piece of rock.

The Monturaqui region, located in the Preandean Depression of the Atacama Desert has been found to harbour two different substrates colonized by microbial communities, namely gypcrete (Wierzchos et al. 2015) and ignimbrite, a volcanic rock (Wierzchos et al. 2013). Both substrates showed endolithic colonization and a lack of epilithic colonization (rock surface

colonization). The absence of this second type of colonization in any substrates from the Monturaqui region may be explained by the extremely arid microclimate of this area, including low relative humidity, high fluctuation of air and surface temperature, extreme high solar irradiation and scarce precipitation (Wierzchos et al. 2015). Monturaqui has been described as a hyper-arid area, showing an aridity index of 0.0075 (Wierzchos et al. 2013), based on the ratio of mean annual precipitation (P) and potential evapotranspiration rate (PET) (P/PET), up to one order of magnitude lower than the ~~limit~~ established limit ~~by~~ (Nienow ~~(~~2009) for the classification of hyper-arid regions (0.05). Specific measurements of surface temperature for gypcrete revealed values ~~of almost~~close to 70°C. This value was detected at the zenith, when microbial communities are desiccated and metabolically inactive (Cockell et al., 2008). The temperature within the endolithic habitats is expected to be close to that in the rock surface as shown by Wierzchos et al. (2012a) for halite endoliths. ~~on its surface at zenith, thus approximating the upper temperature limit for photosynthesis of 74°C, under which thermophilic cyanobacteria in hot springs have been found to live (Castenholz et al., 2001)~~. The combination of these environmental conditions has led to the avoidance of epilithic colonization in favour of endolithic colonization.

Potential endolithic habitability is tightly linked to the porosity of a lithic substrate because the distribution and size of pores are often directly related to the substrate's water retention capacity (Cámara et al. 2014~~5~~; Herrera et al. 2009; Matthes et al. 2001; Omelon 2008; Pointing et al. 2009; Meslier et al. 2018). Porosity in gypcrete allows microbial communities to survive in different microhabitats, providing sufficient space for the communities, while receiving enough light and having enough water to metabolise and grow. The porous network of gypcrete ~~slows down~~restricts water loss by rapid evaporation and helps its retention by capillary forces acting in small capillary-like shape pores. The inner architecture of gypcrete allows the habitability of three different locations inside the substrate. The CT-Scan and SEM-BSE images from this work showed that all three types of microhabitats shared a vertical axis of morphology with vertical cracks constituting the chasmoendolithic (CH) microhabitat and capillary-like pores constitute the cryptoendolithic (CR) and hypoendolithic (HE) microhabitats. This ~~capillary~~ capillary-like pore architecture found in the CR microhabitat could be explained by the progressive substrate dissolution due to scarce rains and by the water retained and condensed within the micropores, as it occurs in halite endolithic microhabitats (Wierzchos et al. 2012a). The observed HE microhabitat architecture ~~consolidates~~ supports the proposal of Wierzchos et al. (2015), in which the authors described the presence of a dense crust delimiting the bottom part of the HE microhabitat. This structure reveals different dissolving and crystallization processes of the gypsum following the water ~~gravity flow~~displacement from the surface to the bottom of the rock (gravity flow). This water gravity flow ~~and~~ giving rise to the cave-shaped pores, thus providing this HE microhabitat with a hard permeable bottom gypsum layer.

The larger distance between the HE microhabitat and the top surface microhabitats CR and CH~~,~~ might be thought as a limiting factor for the development of HE communities, especially in terms of water availability. However, the location of the HE microhabitat at the bottom of the rock could reduce water losses due to evaporation processes. Thus, the micro-cave structures we observed in the HE microhabitat might retain liquid water for longer times, leading to cyanobacterial growth.

The structural characteristics of the crypto- and chasmoendolithic microhabitats, located at the top of the substrate, also allow access to water for the EMCs. Within the CR microhabitat, the labyrinth of pores directly or indirectly connected to the surface

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may act as cavities where water might be retained, condensed, and also be present in form of saturated water vapour (high RH) through the substrate, and be available to the microbial communities. Additionally, the presence of sepiolite inclusions improves water retention in those pores, as previously described (Wierzchos et al. 2015, Meslier et al. 2018), leading to lower rates of water loses by evaporation and gravitational forces. In contrast, the CH microhabitat provides direct access to rainfall liquid water for its community, via its fissure and cracks, while at the same time lowering water retention capacity by higher evaporation rates and losing liquid water by percolation through the rock.

Microbial communities inhabiting all three microhabitats were found in the form of large aggregates and were often embedded in an EPS~~s~~ matrix. These characteristics are closely linked to survival strategies under harsh environmental conditions related to low water and nutrient availability (Billi 2009, Wright et al. 2005)~~ .~~. Since water is the most limiting factor for the development of microbial communities inhabiting endolithic microhabitats of gypcrete, it is the component on which adaptive strategies are primarily focused. EPS~~s~~, because of their role in hydration and dehydration processes in lithobiontic communities from Antarctic deserts (de los Ríos et al 2007) and ~~from~~ the Atacama Desert (Dong et al. 2007; Wierzchos et al. 2011; Wierzchos et al. 2015; Crits-Christoph et al. 2016) are an essential adaptation strategy against hyper-aridity. The aggregate~~s~~-like structure of these communities composed by cyanobacteria and ~~other~~ heterotrophic bacteria ~~with a different physiological status~~ also helps their survival against drought, since dead cells could provide physical protection against desiccation processes (Postgate 1967; Roszak and Colwell 1987; Billi 2009; de los Ríos et al. 2004). In the case of the CR community, a special strategy against dryness was observed in this work, since microorganisms were located close to the sepiolite, as previously reported ~~with respect to~~concerning gypcrete endolithic communities (Wierzchos et al. 2015, Meslier et al. 2018). EPS and dead cells taking part in the aggregates can also act as a nutrient reservoir in such an oligotrophic environment ~~as the endolithic microhabitats~~; since low amounts of ~~water~~ water-soluble ions were previously detected in the MTQ gypcrete (Meslier et al. 2018).

The absence of significant differences in diversity metrics between the three EMCs of gypcrete is in accordance with the diversity values of previously reported EMCs in the Atacama Desert (rev. in Casero et al. 2020). At a phylum level, the community was composited of three main dominant phyla, Cyanobacteria, Proteobacteria and Actinobacteria (Fig. 4) as in other EMCs of the Atacama Desert (Wierzchos et al. 2015, Meslier et al. 2018, Dong et al. 2007). However, a switch in the Proteobacteria and Actinobacteria relative abundances was found compared to gypcrete cryptoendolithic communities previously described (Meslier et al. 2018). That difference is presumably associated with different DNA extraction methods and the inherent associated biases. 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.

While the communities from the 3 microhabitats had similar alpha diversity metrics, we found the composition of these communities was statistically different, which is supported by the relative abundance of the main phyla, Cyanobacteria, Proteobacteria and Actinobacteria, across the microhabitats distributed differentially, exhibiting differences between the CR

and CH communities as compared to the HE community, especially regarding cyanobacterial OTUs. This notable difference in the relative abundance of cyanobacteria could be related to the particular resources of the phototrophic community. The differential access to solar irradiance could explain the contrast between cyanobacterial proportions on both sides, at the top (CR and CH) and bottom (HE) of the substrate. Thus, an update to the proposal by Wierzchos et al. (2018) is here suggested, in which a causal link is evoked to explain the higher abundances of phototrophs as opposed to heterotrophs in EMCs, which has been observed previously (Robinson et al. 2015, DiRuggiero et al. 2013, Wierzchos et al. 2015, Meslier et al. 2018). According to that work, the scarcity of water was suggested to cause a lower metabolic activity in phototrophs, thus leading to ~~a~~ lower support of the heterotrophic community. However, in this scenario, light intensity should also be considered a crucial factor in understanding the differences between the composition of top and bottom EMCs, since the HE community has ~~a~~ notably lower access to sun radiation. Recently the light intensity, as a driving factor of spatial heterogeneity within halite endolithic microbial communities was reported by Uritskiy et al. (2020). Thus, for EMCs communities based on phototrophic microorganisms, a limitation to one of those resources essential for photosynthesis would further lead to low rates of CO_2 fixation and, consequently, to a smaller heterotrophic community.

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. While we found multiple phylotypes of cyanobacteria among the gypcrete microhabitats, most of them belonged to the genus *Chroococcidiopsis*. Several strains of this genus have previously been described in EMCs from both hot and cold deserts (Friedmann 1980) as a result of their capacity to cope with extreme environmental conditions (Billi et al. 2011; Verseux et al. 2017). 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, a~~A~~lthough the low percentage of sequence similarity did not allow for an accurate taxonomical assignment, its closest relatives (~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, ~~T~~the ~~unidentification~~ inability to identify ~~of~~ the UC-OTU and the phylogenetic position of the UAM811 strain, highlight~~s~~ the importance of greater efforts in terms of isolation and characterization of cyanobacteria, especially from these environments.

The differential distribution of key members of these EMCs among microhabitats in the same lithic substrate and the same piece of rock, as their primary producers, reveals an "environmental filtering" process (Kraft et al. 2015). This concept focuses on the relationship between an organism and the environment, recognizing that not all organisms will be able to establish themselves successfully and persist in all abiotic conditions. Thus, in this scenario, the abiotic conditions linked to the architecture and location of the endolithic microhabitat would force the development of community assemblages highly specialized to small scale differences, thereby exhibiting a microbiogeographical behaviour.

Our work answers that there are certain differences in endolithic microbial communities' structure among crypto-, chasmo- and hypoendolithic habitats. Considering that the external climatic regime was the same for studied pieces of rock, our results have shown that the structure of these microbial communities was different among endolithic habitats. Following the definition of microhabitat architecture by Wierzchos et al. (2015) we can distinguish different architecture of the substrate within different endolithic microhabitats. In this context, our work suggests that distinct features of microhabitat architecture that have an influence on microenvironmental variables at the microscale would shape microbial communities' structure.

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.

5. Conclusions

This study is the first to address differences between microbial communities inhabiting three differentiated endolithic microhabitats within the same lithic substrate. In this study, liquid water availability was ~~confirmed~~ proposed to be a driver of community composition because the specific architectural features of each microhabitat facilitated water input and retention in different ways. Water, light, and CO_2, are indispensable resources for photosynthetic activity. Thus, we support the cause and effect relationship where the restriction of these factors may affect the proportion of phototrophic and heterotrophic components in the EMC communities as proposed by previous works (Robinson et al. 2015, Wierzchos et al. 2018 and Meslier et al. 2018).

The genus *Chroococcidiopsis* ~~genus~~ displayed a variety of strains distributed among all microhabitats, proving its high capacity to colonize effectively endolithic microhabitats under polyextreme conditions. Nevertheless, the presence of a singular cyanobacterial OTU stresses the need for additional efforts in cyanobacterial characterization from these extreme environments.

Findings from this work reveal the importance of using an appropriate scale for the study of microbial communities. Indeed, we found that the microstructural and microarchitectural features of the endolithic habitats were key factors in determining the composition of endolithic microbial communities. Thus, this study suggests a cautious use of "macroenvironmental" parameters in characterizing differences between endolithic communities from different deserts or substrates. Our results point to the need for a more thorough description of the micro-environmental conditions that directly exert an effect on microbial assemblages: light, water and CO_2. Therefore, once the relationship between factors affecting the absence and/or presence of certain taxa, the actual environmental filtering in these microhabitats could be described in more detail, it will be possible to draw ~~on~~ conclusions on the interactions and specific roles of the different members in the community and their microbiogeography.

Data availability

All the sequencing data sets generated in this study have been submitted to the National Center for Biotechnology Information (NCBI) SRA database~~,~~ and can be found under the BioProject ID PRJNA637482.~~Author contributions~~

Author contributions

MCC and JW designed and performed the research. JW conceived the original project. MCC, JW and OA carried out the sampling. MCC, JW and AQ wrote the manuscript; MCC, JW and CA performed the microscopy; TK contributed to CT-SCAN analysis; MCC, VMA and JDR contributed to the molecular data, analysis, and performed the sequencing. All authors contributed to editing and revising the manuscript and approved this version for submission.

Competing interest

The authors declare that they have no conflict of interest.

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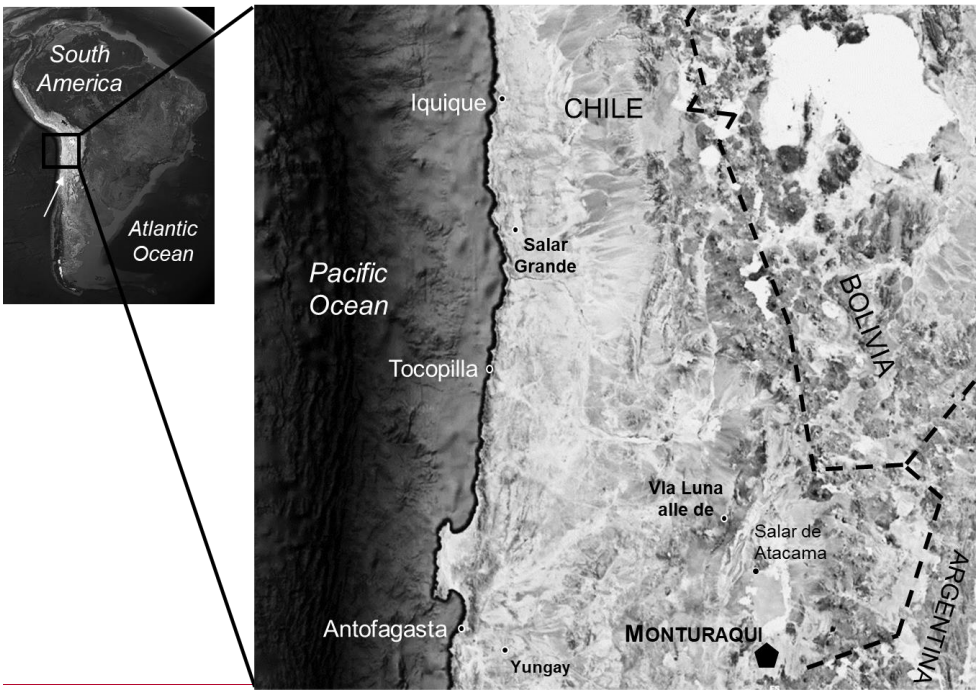
Table 1: Diversity estimates of microbial communities in the endolithic microhabitats of gypcrete.

Microhabitats		Chao	OTU Richness	Shannon
Cryptoendolithic	Avg	583.8	430	6.3
	SD	43.2	38	0.2
Chasmoendolithic	Avg	574.9	419	6.1
	SD	46.0	29	0.1
Hypoendolithic	Avg	564.9	409	4.6
	SD	31.7	32	1.0


South America
Atlantic Ocean
Iquique
CHILE
Salar Grande
Pacific Ocean
Tocopilla
BOLIVIA
Vla Luna alle de
Salar de Atacama
ARGENTINA
Antofagasta
MONTURAQUI
Yungay

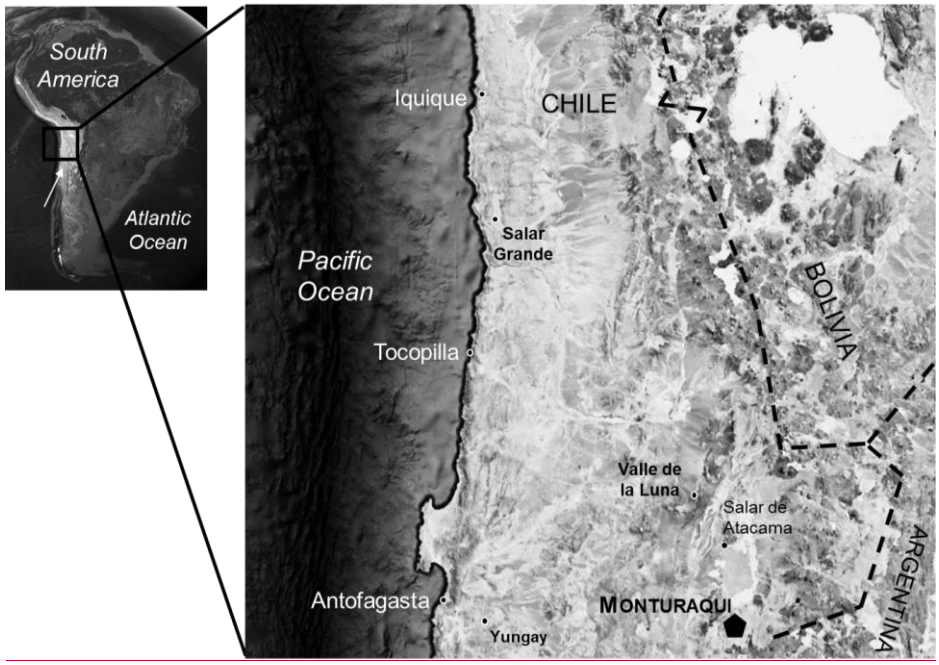
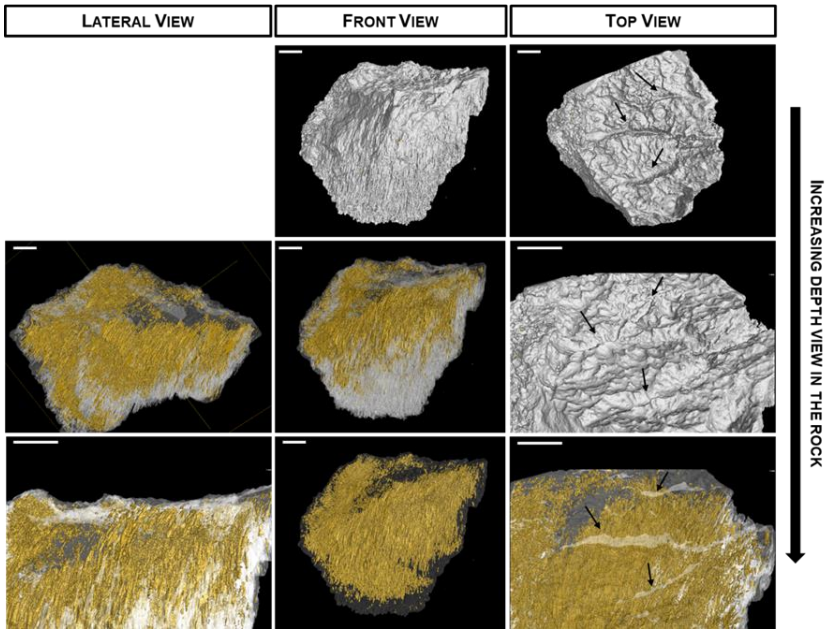
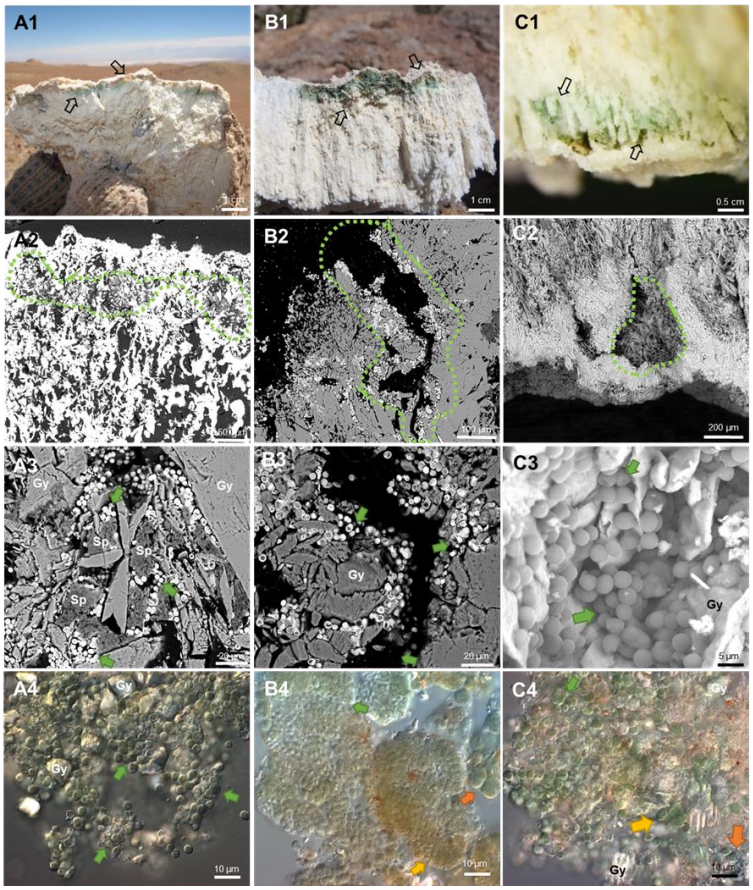


Figure 1: Sampling location in the Atacama Desert. Monturaqui area: MTQ (black diamond). (Google Earth, image providers: Landsat /Copernicus)



635 **Figure 2: CT-Scan images of a colonized piece of gypcrete.** 3D reconstruction of a gypcrete sample with the spatial distribution of pores (orange colour) and ~~external view of the rock~~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 the water. Arrows in top view images point to the deepest cracks. Scale bar = 1cm.



640 **Figure 3: Endolithic colonization zones characterization.** Series A: Cryptoendolithic; Series B: Chasmoendolithic; Series C: Hypoendolithic. Series 1: Macro images of gypcrete cross-section of colonized zones; Series 2 and 3: SEM-BSE images of gypcrete cross-section of colonized zones; Series 4: LM-DIC images of scrapped cyanobacteria from gypcrete. **Series 1**: Black arrows indicate green and orange coloured endolithic colonization zones of 5 mm thick on A1 (CR), 8 mm thick on B1 beneath the surface (CH) and 5-9 mm thick on C1 above bottom gypsum crust (HE). **Series 2**. CR, CH and HE microhabitats with aggregates of endolithic microbial communities
645 surrounded by the green dotted lines, inside the pores of gypcrete: A2, under a white dense surface crust; B2, inside the cracks of gypcrete and C2, inside the micro caves of gypcrete at bottom of the rock. **Series 3** Green arrows point to aggregates of cyanobacteria among gypcrete (Gy) crystals (A3, B3), surrounding by sepiolite (Sp) nodules (A3) and on the gypcrete (Gy) walls (C3). **Series 4** aggregates of different morphotypes of cyanobacteria, shown by green, yellow and orange arrows and gypcrete crystals (Gy).

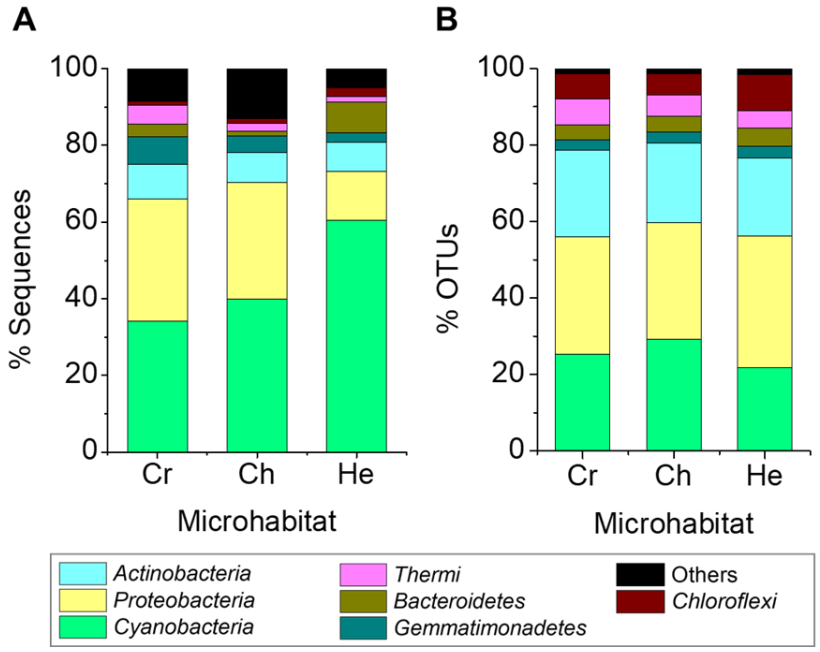


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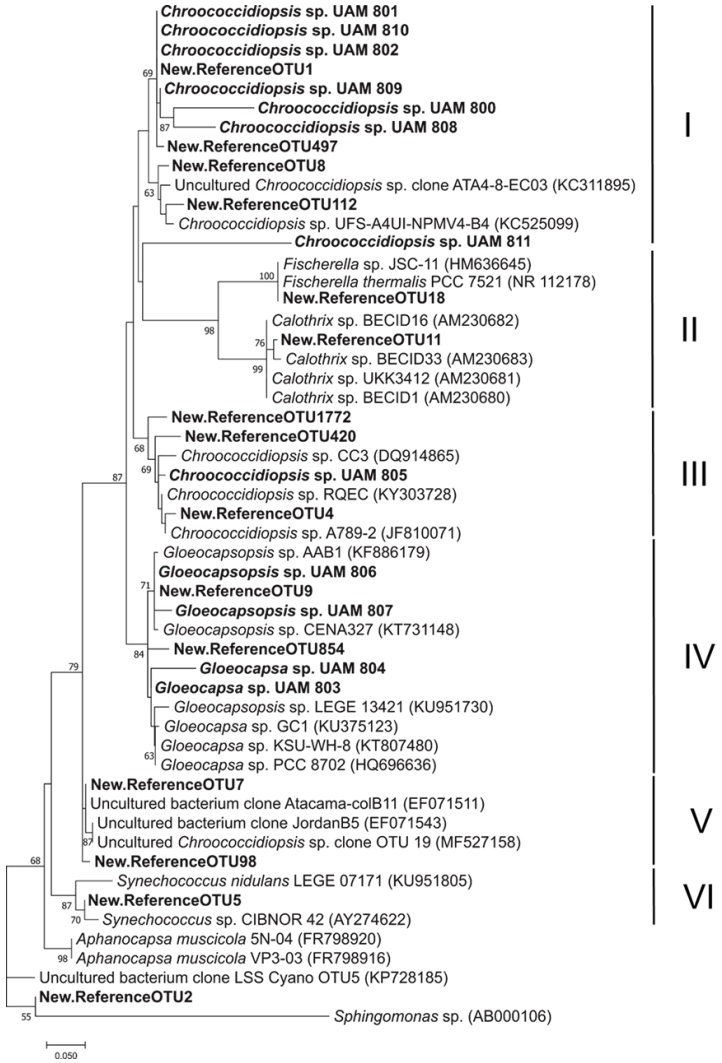
Figure 4: Average relative abundance of sequence reads (A) and OTUs at the 97% clustering cut-off (B) of major bacterial phyla (at least 1% across all samples) of microbial assemblages in the cryptoendolithic (Cr) chasmoendolithic (Ch) and hypoendolithic (He) microhabitats of gypcrete.



655 **Figure 5: Maximum likelihood tree based on partial 16S rRNA sequences of Cyanobacteria OTUs above 1% relative abundance and cyanobacterial strains isolated from the three gypcrete microhabitats**. Bold indicates sequences from this study. Scale bar~~s~~ indicates 5% sequence divergence.

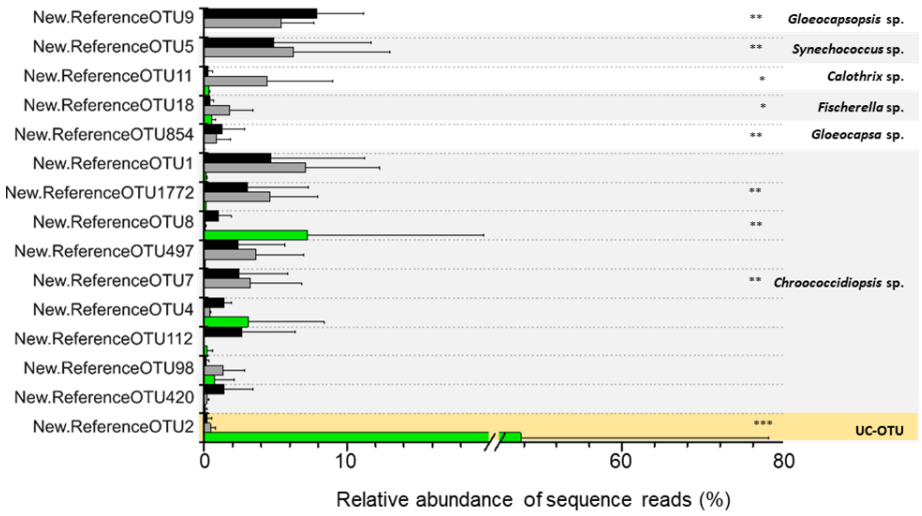


Figure 6: Average relative abundance of cyanobacterial OTUs. Differentially abundant cyanobacterial OTUs across the three microhabitats are represented by *(Diff-OTUs p-value <0.01 Ch / Cr-He), ** (Diff-OTUs *p*-value <0.001 He / Cr-Ch), ***(Diff-OTUs *p*-value <0.0001 He / Cr-Ch). UC-OTU (Unclassified Cyanobacterial OTU). Only sequences > 1% relative abundances were used.