

Interactive comment on “Novel water source for endolithic life in the hyperarid core of the Atacama Desert” by J. Wierzchos et al.

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We thank Prof. Dr. Emmanuelle J. Javaux for handling our manuscript entitled: Novel water source for endolithic life in the hyperarid core of the Atacama Desert and thank the Anonymous Referee #1 for her(his) insightful and helpful comments.

It was our request to have our paper reviewed by an expert in rock salt microstructure and LT-SEM investigation strategy and as we were able to figure out Referee #1 is indeed such an expert. We would like to express our gratitude to Referee #1 for her(his) insightful comments, which have helped us improve our paper. Below we have addressed each specific comment. We have also described changes to the previous version of the manuscript that we feel have enhanced the quality of our work, making it suitable for consideration for publication in BG. Each original Referee's comments are

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provided, with our response right below each point (Referee's suggestions are highlighted in blue text).

Response to Referee #1 general argumentation:

In our paper we have attempted to explain the occurrence of liquid water inside the halite pinnacles below halite DRH and without rainfall, fog or dew during the time span of one year. The key hypothesis in our paper is that water vapour could condense in nano-porous spaces. Our revised work shows the presence of significant nono-porosity with pores smaller than 100 nm, both by means of mercury intrusion porosimetry (MIP) (Table 1) and diverse electron microscopy techniques. MIP data shows that the average pore radius in non-colonized and colonized halite regions is 42.65 nm and 153.10 nm respectively. We have correlated this porosimetry data with SEM observations of a nano-porous phase, which covers the large halite crystals and occurred in grain boundaries, and is intimately associated with the endolithic communities. We proposed that this nano-porous phase is responsible for water condensation at RH below DRH.

Ad. (I) As mentioned above we appreciate all comments by Referee #1 regarding our original LTSEM work. Thanks to these comments we questioned our previous results and made further observations of the halite samples with new techniques. First of all, and according to the Referee's suggestion, we considered that the use of the LTSEM with etching, and thus sublimation of ice crystals, might result in the formation of hydrohalite structures not present in the natural sample. We see it likely that this was the case of our misinterpretation of nano-porous structures in some of the LTSEM images in the first version of the MS. All these images were removed from the MS and new LTSEM images were taken for the new version of the manuscript. The presence of this brine remnant is however a clear demonstration that deliquescence results in sufficient liquid water to drive the endolithic microbial population, confirming earlier suggestions by our group (Davila et al. 2008). Hence we used the LTSEM with etching as a means to reveal the brine remnants due to deliquescence (Fig. 5d-5e). As such, we agree that LTSEM cannot be used to study the original structure of halite within

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the grain boundaries but provide indication of earlier presence of brine in the grain boundaries.

Since the etching process could mask the true structure of the halite we decided to work with samples equilibrated at 0%, 20%, 37%, 65% RH, and those samples were not etched in the LTSEM (Fig. 5a-5c). All these new images show the presence of a phase with pitted surface of halite occurring among massive halite crystals. We studied further this granular phase with a high-resolution SEM technique using "Auriga" field emission SEM (Zeiss) with innovative in-lens detection system of secondary electrons operating in only 3 kV accelerating potential. According to our knowledge this apparatus is the most powerful high resolution SEM in the market nowadays. The obtained images again reveal the nano-porous phase within grain boundaries as well as forming skin cover on the surface of larger halite crystals (Fig. 6). This phase is shown to be composed of small (<100nm) porous. Since the samples analyzed in the HRSEM were completely dry and were visualized in high vacuum, we also tried to observe the same phase at lower resolution using the ESEM at low RH (Fig. 7 and Fig 8). Again, we show the presence of the nano-porous phase in samples

Ad (II) ... "I am not telling that this nano porous phase seen by ESEM does not exist but a robust discussion is needed since it is the key point of the paper.

We thank the reviewer for this suggestion and to address this we have strengthened ESEM technique description and we have added further corresponding discussion of newly obtained results. Additionally we have added new bibliographic data regarding to that point.

... "Authors never discussed how such a porous phase network can develop in grain boundaries and the possible artifacts due to the preparation of sample are also never discussed". ... "How the resulting foam/sponge structure (called by us micro- and nano-phases) can develop?"

We understand the valuable suggestion of Referee #1 to discuss how such a porous

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phase network can develop in grain boundaries. However, this is not a trivial study, and we have found several important issues related to sample preparation and three SEM techniques gives us three different kinds of images. This is not surprising to us as different preparation methods and different electron signals formed in different ways should produce slightly different images. However, all the observations seem to agree. In addition we should not forget that we are studying the halite pinnacles from the Atacama Desert formed during millions of years in natural and unique environmental conditions, and not pure NaCl crystals. Moreover, the interior of these rocks is also composed of small quantities of many other salts and minerals (gypsum, glauberite, sylvite, etc) and microbial communities containing life and dead cells, as well as large amounts of organics molecules (e.g. scytonemin, EPS-es, etc.). We consider that any attempt to explain how such a porous phase network can develop should be very complex and taken with care. Nevertheless, while unravelling this mechanism might indeed be very interesting, we feel that such an analysis would fall outside of the scope of this study.

Response to Referee #1 some remarks:

Ad. a. ... "Did you observe macropores with smooth walls under ESEM?"

Usually in ESEM we have observed smooth surfaces with continuous (not porous) walls covering large halite crystals on samples equilibrated at low (20%-65%) RH. During the ESEM visualization, we used a gaseous secondary electron detector (GSED), which collects secondary electrons very efficiently. Design of this detector allows us the study the external micromorphology of fine halite structures. The GSED detector applied a positive bias to collect secondary electrons released by the sample surface after the primary electron beam penetrated the sample. During the travel of those secondary electrons to the detector, they collide with water vapor molecules that generate secondary electrons themselves producing an amplification of the signal. However, this amplification is affected significantly by backscattered electrons, which gives us information from up to 2-3 microns in deep beneath smooth (viewed by SE) surface.

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Nevertheless, the SE signal affected by backscattered electron signal is rather viewed as an advantage for image interpretation, because simultaneously we have the possibility to obtain information of what lies beneath the surface (akin to observing aquatic plants in a pond with a quiet water table). Here we observed a foam/sponge-like phase (Fig. 8a-a"). Please find below the images which also illustrate this situation:

There is one aspect of these two images interpretation that follow the Referee #1 question about the smooth walls under ESEM. It should be noted that the granular phase with macropores seems to reveal some 3D relieve, when observed in narrow (sharp) incident electrons path angle, as indicated by the white arrowhead in Fig. R1a. Additionally some irregularities following the macropores rims on brine surface were also observed in Fig. 1Rb, as indicated by the white arrowhead. However, we do not discuss this issue in the new manuscript, because we feel that such image analysis would fall outside of the scope of this study.

Ad. b. Therefore, which is the effect of drying of brine in pore in the development of precipitate/recrystallization/efflorescence artifacts?

We consider that the brine drying process within pores could lead to small crystal precipitates, their recrystallization and/or formation of efflorescence. This results in the formation of micro- and nono-porous spaces. We do not consider this phase as an artifact. This phase should be formed in natural systems during many drying – wetting events in natural environment of the Atacama Desert. Keeping this in mind, we studied the samples also in their natural conditions of RH by equilibrating the sample at RH =37% before LTSEM analysis, and through in situ equilibration in the ESEM chamber to low RH during ESEM analysis. We chose this investigation strategy indeed in an attempt to avoid any artifacts.

Which could be the effect of change of temperature and pressure on the state of residual brine? The temperature of the halite samples was always constant and of 5 °C. Changes of the water vapour pressure, in fact changes of RH, might lead to brine

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evaporation/condensation processes observed and described in the original MS and in the new version (Fig. 7 and 8).

Ad. c. Why is it necessary to oven-dry the sample? Can they just break the natural sample directly inside the ESEM without pre-drying? We thank the reviewer for this insight. We used also natural samples directly introduced to the ESEM chamber without pre-drying process. These new results are included in the new MS.

Ad. d. Are these nano porous phases frequent under ESEM? Yes, they are frequent and relatively easy to identify after long enough time of sample equilibration, and when adequate operating condition of ESEM and purges are carefully selected.

Ad. e. ...“what happens if the RH is decreasing?” Will the nano-porous phase re-condensate? If not: how could you explain the presence of such a preserved nanoporous phase after several natural RH cycles during the lifetime of a halite pinnacle?

We have attempted to perform this experiment inside the ESEM chamber, which was already reported in first version of the MS (Fig. 7) and the same result are reported in the new version. During this dehydration process (as observed at relatively low resolution) the brine is disappearing but still present at 30% of RH. The formation of nano-porous phase might be a trace left by evaporating brine. Our results show that halite deliquescence can leave these traces in the form of structures with micro-and nano-pores. These structures were found within the grain boundaries, as well as thin coating covering surfaces of large halite crystals. It is conceivable that this nano-phase is formed during drying process and is dissolved during deliquescence process. Their total or partial dissolution might depend on deliquescence time and the maximum RH.

Ad (IVa) I have maybe a naïve question about the state of water needed for endolithic brine: are we speaking about pure water or brine (or salty water)? If the bacteria need really pure water, I do not understand the relevance of the process suggested by the authors since the deliquescence of halite-made nano-porous phase results in brine.

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The reviewer poses one of the key questions regarding the halite endolithic system. Microorganisms, including some cyanobacteria, are known to thrive in saturated brines (i.e. NaCl). These are the so-called halophilic or halotolerant organisms. In the first case (halophilic) microorganisms only grow in high salt concentrations, and in the second case (halotolerant) microorganisms can grow at high or low salt concentrations. Because none of the species inside the Atacama halite have been isolated in the laboratory, we still do not know whether the microorganisms are halophilic, or halotolerant, however common sense dictates that they must be adapted to high salt concentrations because briny water appears to be the only kind of water they have access to.

Ad. (IVb) In the case that brine can be sufficient for endolithic life, I am wondering if the story about the water source should be less complicated just by considering the LTSEM experiment which conclude (see I)) that brine is present everywhere in large amount at grain boundaries (indicated by foam pattern) independently of RH and the presence of a nano-porous phase. Gas inclusions (interpreted as macro-pore by the authors) embedded in brine indicate that the grain boundary reservoir is very tight (e.g. because of grain the boundary healing process, Desbois et al., 2012; Van Noort et al., 2008; Ghossoub and Leroy, 2001).

Indeed, our data shows that brine is present in relatively large amounts inside the halite, and while we find this to be a key result we also think that the true relevant science to be gained here is to understand the mechanisms by which water is incorporated in the halite and retained for long periods of time. This is the only way we can go from a purely descriptive study to a well-defined and understood system.

Figure 1R legend Figures: R1a, ESEM image of halite crystals and microbial cell aggregates (open white arrow) equilibrated with RH=65% note the observed foam/sponge-like phase covering the surface of halite crystals (arrowheads). R1b, ESEM image after deliquescence process (RH=76%); white open arrows point to the microbial cell aggregates and arrowheads show the foam/sponge-like phase covering the surface of halite crystals.

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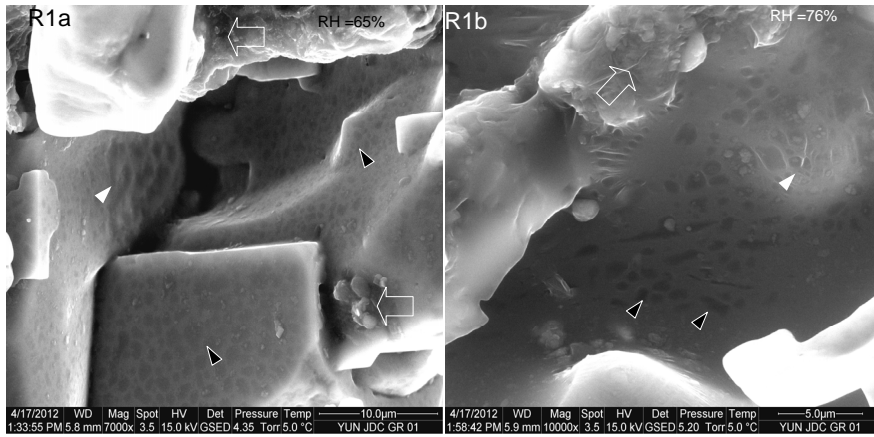


Fig. 1.

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