What is eating my rocks? A possible novel biological niche in limestone

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Abstract. Metamorphic limestones in Namibia and Oman were found to be consumed inside the rock mass by microbiological activity of a thus far unknown nature that created bands of parallel tubules. Tubule of up to 0.5 mm wide and 30 mm long collectively form bands of tens of meters long. These bands formed along fractures in the rock and only surfaced after erosion. In fresh outcrops the tubules are filled with white calcium carbonate with internal structures. This filling is depleted for several metal elements that can be incorporated into biomatter and was deposited by the microorganisms as they grew inside the rock. The rim of the tubules contain1 µm wide growth rings enriched for P and S. Fluorescence microscopy confirmed presence of biological material. The rocks are too old to conserve DNA or protein. We consider this a new niche for life that has so far not been described

1 Introduction

The global carbon cycle describes how carbon atoms are exchanged between the Earth's atmosphere (gaseous CO_2) and the main global carbon sinks: the oceans (dissolved CO_2 giving carbonate ions), terrestrial carbonate deposits, and living and fossilized biomatter (Prentice et al., 2001). Most of the global carbon is present as terrestrial carbonate rock (calcite, limestone, marble). Storage of atmospheric carbon into carbon sinks is mainly via dissolution of CO_2 into the oceans and by incorporation into biomatter via photosynthesis by terrestrial and marine plants and microorganisms. Release of CO_2 is by biological activity (respiration and decomposition), anthropogenic activity (burning of fossil fuels) and geological processes (volcanism) and erosion and weathering of carbonate rocks. The so-called slow carbon cycle describes the process of chemical reactions and tectonic activity by which carbon atoms cycle between rocks, soils, oceans and the atmosphere. This slow cycle has been estimated to involve 61,000 million metric tons of carbon per year (Falkowski et al., 2000).

In addition to calcite formation by spontaneous dissolution, occurring when concentrations of Mg and Ca are high enough, microorganisms can also immobilize carbon into carbon sinks by the formation of calcite. This microbial deposition is mainly driven by marine processes (Tanhua et al., 2013), but occurs on land on a smaller scale as well. The term endostromatolites is used to describe fissure calcrete of biological origin that is formed in extreme terrestrial environments

such as the Arctic and other periglacial regions (Lacelle et al., 2009; Clark et al., 2004). However, microorganisms may also assist in local removal of calcite. In 1981, endolithic microorganisms were described from three habitats with terms that are still in use: cryptoendoliths inhabit crypts in the rock, chasmoendoliths colonize fissures, and hypoendoliths occupy cavities that are positioned deeper inside the rock (Golubic et al., 1981). Endolithic microorganisms may create their own niche by removal of rock material (most often calcite) and typically survive extreme environments. For instance, as recently reviewed by Wierzchos et al. (2018), cyanobacteria and other endoliths colonize rocks in the hyperarid Atacama Desert (Chile). The contribution of carbon release by such biological activity towards the global carbon cycle is most likely negligible. However, where biological activity assists in weathering of terrestrial carbonate rocks on a larger scale, that contribution may be significant. Although many large-scale biologically assisted weathering processes have been described, our observations a suggest the presence of additional organisms.

Here, we describe metamorphic limestones collected in Namibia and (in limited numbers) in Oman that have been 'eroded and replaced' by microorganisms, forming bands of tubules, inferred as a result of biological activity. The product of this activity is observed on a relatively large scale in outcrops of these limestones which are at present located in a hyperarid climate.

2 Materials and methods

2.1 Field observations

Field observations presented here are mainly from Namibia. Local geology is characterized by the presence of two older Proterozoic cratons, the Angola and Kalahari cratons, separated by a belt of strongly deformed marine sediments of Neoproterozoic age, which were deformed and metamorphosed in the Cambrian (Frimmel, and Miller, 2009; Porada, 1979; Miller, 1983; Prave and Hoffmann, 1995). The marbles consist of banded coarse-crystalline $CaCO_3$ with traces of Si, Mg, Fe, Al, Sr, Mn, and other elements. The composition of the marbles is still comparable to marine limestone. The marble layers are folded, causing variable *in situ* orientation of the layering. After the Cambrian deformation and metamorphism, the rocks were long buried and covered by Mesozoic sediments but surfaced in the Tertiary and weathered to their recent outcrop geometry. Calcrete of probably Neogene - Pleistocene age (Pickford, 2000) formed on top of the marbles and in fractures.

The coastal strip in Namibia is extremely arid (Heine, 1998), but wetter periods associated with calcrete formation have occurred in the past, the last 16,000 - 13,000 years before present (BP) (Shaw and Thomas, 1996). Significant weathering of the structures and associated calcrete described here, however, suggest that they must be older, probably between 1-3 My (Pickford, 2000; Heine, 1998). Observations and samples collected in Oman were from the southern flank of the Jebel Akhdar anticlines.

2.1 Microscopy

Petrographic thin sections were produced at 30 µm and 100 µm thickness. Microscopy was performed with a LEICA
petrographic microscope (Leitz DMRD). Thin sections of 30 µm were investigated under plain and crossed polarized light, and 100 µm thin sections were photographed in reflected and in transmitted light and used for analyses.

2.2. Laser Ablation-Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS)

Trace element analyses were performed by LA-ICP-MS using an ArF Excimer laser system (ESI NWR193, TwoVol2 ablation cell) with an output wavelength of 193 nm coupled to an Agilent 7500ce ICP-MS. Reference material is described elsewhere (Jochum et al, 2005, 2011). Analyses were carried out on polished 100 µm thin sections, creating lines of spots with a spot size of 100 or 50 µm and a spacing of 100 µm using a repetition rate of 10 Hz and an energy density of approximately 3.0 J/cm2. For further details see Supplementary file.

2.3. Electron Microprobe Analysis (EMPA)

Element compositions of thin-section surfaces were determined by Wavelength-Dispersive Spectroscopy (WDS) with a JEOL JXA-8200 electron microprobe (JEOL, Eching, Germany). The conditions were 15 kV accelerating voltage, 8 nA beam current with a beam diameter set to 5 µm. Peak counting times were 20 s for major elements, 30-40 s for minor elements and 60 s for S and P. Sets of reference materials with well characterized natural and synthetic oxides and minerals (P&H Development Ltd., Whitby, England and Astimex Scientific Ltd., Toronto, Canada) were used for calibration. The routine ZAF procedure in the JEOL software was used for data processing. The applied conditions for X-ray element maps of Mg, Al, Si, P, and S (including backscattered images) were 15 kV, 30 nA with a resolution of 0.5-2 µm/pixel and a dwell time of 280 ms/pixel.

2.4. Fluorescence microscopy

Epifluorescence microscopy was carried out using an Olympus IX70 inverted microscope with an UPLANF 4x (NA 0.13) and CPLANFL 10x (NA 0.3) objective. Thin section fluorescence was excited with an UV-mercury lamp (100 W, Olympus U-RFL-T) and observed with a U-MNU2 excitation filter (Olympus, BP 360–370 excitation, DM 400 dichromatic mirror, 420LP emission filter). Images were taken with a Canon EOS 550D camera and processed using ImageJ.

2.5. Terminology used

A graphical description of the terminology used is given in figure S1. The main features described here are named 'tubules', with 'tubular bands' specifying a collection of parallel narrow tubes that are mostly evenly spaced in the rock. The tubules can be void or filled with white material. Their edges are called straight if all tubules are aligned at one edge, or ragged if their length differs. Other terminology is summarized in figure S1.

3 Results

3.1 Field and macroscopic observations

The first examples are from the desert of western Namibia. Bands of tubules were observed in marbles at various locations situated between 10-150 km from the coast between 20-29°S. A landscape picture (Fig. 1A) shows three bands composed of vertical tubules in the foreground. The tubules are present as small, parallel cavities in the rock. A single band (Fig. 1B) illustrates how this differs from the typical irregular karst erosion. Multiple tubular bands in the outcrop of figure 1C have undergone various degrees of erosion. Alternating blue, grey and white layers in the marble, caused by minor differences in composition, are still visible in the remaining tubule walls, suggesting that they formed by removal of original rock material. Fractures or joints commonly develop parallel to layering, or in other orientations where marbles are folded. The tubules are mostly growing perpendicular to such fractures. Both fractures and tubules have become surface exposed due to erosional removal of rock mass.

In fresh outcrop with rock on both edges of a tubular band (see figure S1 for descriptive terminology), the tubules are loosely filled with white material. The upper edge of a tubular band to the original rock is quite sharp and straight, while the lower edge is more often irregular, since tubules can penetrate with variable lengths, to up to 30 mm depth into the rock for 0.5 mm wide tubules. All tubules are strictly parallel, they never cross or diverge, and when they are slightly bent, all bend in the same direction. A thin white band is often visible along the straight edge, as in figure 2A. Commonly, calcrete is present at the straight edge of a tubular band (Fig. 2B,C) again often separated by a thin white layer. The tubules in figure 2C are nearly parallel to the rock layering.

Figure 3A shows a branched tubular band, where a thin band is branching off at an angle from a broader band, with all tubules positioned in the same orientation. The shorter tubules along the lower branch fade into a fracture. This and similar observations (Fig. 3B-C) suggest that the bands originated along fractures inside the rock mass. Although in most examples the tubules are tightly aligned, their density does vary from outcrop to outcrop. In figure 3B, a surface-exposed upper band is now weathered, but a second band with less densely distributed tubules is branching off it, with remnants of a thin white layer at its upper edge. A third thin white layer with even lesser developed tubules is also present. In the loose specimen of figure 3C calcrete separates original rock fragments containing tubules. Figure 3D shows an outcrop *in situ*, demonstrating that the calcrete lies on top of the tubules. More examples of tubular bands combined with calcrete are shown in figure S2

The tubular bands are obviously the result of removal of rock material. No natural erosion mechanism is known to produce this kind of pattern, and we observe it inside rocks at fresh cuts as well as on exposed surfaces, so whatever is responsible for the formation of the tubules, that process must take place inside the rock. The tubular bands are different to deposits caused by endostromatolites, which are also present in the investigated area (Fig. 4A). Endostromatolites build rock material that no longer resembles the original rock, so the rock layering is not conserved, and the built-up material does not contain regular tubules that span the complete width of a layer. Instead, their deposits contain columnar structures with

macroscopically visible growth layers (Fig. 4A, inset). The specimen shown in figure 4B contains tubules on one side and a layer of endostromatolites on the other side.

In situ, the orientation of the tubules is mostly vertical with - when present - a thin white layer at the straight (upper) edge. Figure 4C shows a rare example of an incipient tubular band above a white layer from which well-developed tubules run downwards. When tubules grow upwards, they are much shorter and less well developed. In combination, these observations suggest that the tubules started from a rock fracture which now forms the straight edge of a tubular band, which we call the basal end of the tubule, while the apical end penetrates the rock, most often downwards, to reach various end-points at the ragged edge.

Where the rock layering is folded, the tubular bands do not follow those folds but cut through the inclined layering (Fig. 4D). Tubules are never observed to penetrate calcrete, so we conclude the latter is formed later. Calcrete deposits have relatively coarse structures (Fig. 3C) or lack structural features but contain large clastic grains (Fig. 3D). We consider the calcrete that is deposited on top of tubular bands to be of an abiotic origin.

When one edge of a tubular band is surface-exposed, their third dimension becomes visible. Figure 5A shows a loose specimen with a narrow tubular band that on the surface appear as pores (Fig. 5B), more clearly visible in the zooms. Similar pores are observed *in situ* (Fig. 5C), and when weathered, this produces a strongly pockmarked karst surface (Fig. 5D). The *in situ* block in figure 5E contains a heavily weathered tubular band, partly covered by calcrete. Below it a second tubular band is also covered with calcrete. Once an edge of a tubular band is surface-exposed, it increases weathering of the rock, as the cavities vastly increase the exposed surface. Large-scale karst-weathering of marble outcrops as a result of presence of tubules is illustrated in figure S3.

Similar structures were also observed in Oman, where they are present in layered, impure metamorphic limestones of Cretaceous age (Grobe et al., 2019) exposed on the southern flank of Jebel Akhdar anticline. The tubules usually developed conformal to the layering but are also found along vertical fractures. Figure 6A shows an example of a tubular band resembling the examples from Namibia. The tubules observed in Oman are often less well developed and more widely and irregularly spaced, up to 50 mm long (Fig. 6A). They commonly start from a white layer (Fig. 6B, C). Endostromatolites are common and in figure 6D they are attached to a rock containing tubular bands.

3.2 Microscopic observations

In polished thin sections investigated under plain and crossed polarized light, the marble from Namibia mainly consists of coarse calcite crystals of up to 0.2 mm in diameter, with minor traces of mica and opaque minerals (results not shown). The tubules cut off calcite crystals without clear changes in microstructure. Freshly cut rocks frequently display a discoloured zone around the tubules, as shown for two examples from Namibia (Fig. 7). These discoloured areas are also devoid of distinct changes in the crystal structure of the rock.

Tubules in standard thin sections of 30 μm thickness were mostly void, partly caused by the polishing procedure (Fig. 7B-D). Thin sections of 100 μm thickness were more informative, especially when investigated with alternating transmitted and

reflected light settings (Fig. 7F-M). Under transmitted light, the filling inside tubules appears as a grey mass, and at higher magnification internal structures are visible, especially when a thin section was cut normal to the direction of the tubules (Fig. 7L). Commonly, this material is loosened from the tubule wall.

3.3 LA-ICP-MS and Electron Microprobe Analysis (EMPA)

Line measurements by LA-ICP-MS were used to determine whether the composition of the white tubule fillings and the white thin layer differed from the marble from Namibia. Metal concentrations were locally increased in the rock close to tubules, but the signals were quite variable, as the composition of the marbles varied from outcrop to outcrop, and even within a sample. More consistently, both the white filling within the tubules and the thin white layer consists of calcite with lower amounts of a number of metal elements than the host marble (Fig. S4). In particular, Sr, Mn, Fe and a number of Rare Earth elements (Nd, Th, Ce, Pr, La) are present at lower abundance in the white material (Fig. S5).

EMPA was applied to produce maps of normal cut, filled tubular holes. Elemental maps were produced for Ca, Mg, Si, P, S, Al, K, Fe, Mn, and Cl (Fig. S6). Of these, Ca, K, Fe, Mn and Cl did not reveal variation, but the other elements produced highly interesting patterns (Fig. 8). The electron micrographs (last panel of each series) shows that the white filling is very fine crystalline (micritic) calcite and Mg-calcite, with delicate internal structures, that are also visible in the distribution of Mg and, in one case (Fig. 8B), by Al. The rim of each filling is enriched in P and S, and at some locations in Mg and Si. P and S create contours of the tubule fillings, as the white carbonate inside the tubules is mostly depleted of these elements. Some of the tubule rims contain spectacular ring structures that resemble growth rings (Fig. 8D). An electron micrograph zoom is shown in figure 8E. The phases of these rings are approximately 1 µm wide. P and S are abundant elements of nucleic acids and proteins, respectively. The other main elements of biomolecules, C and O, would not result in useful EPMA signals due to their abundance in calcite, while N could not be determined. Likewise, Ca signals were not informative as this was used as an internal standard. The scale of local concentration differences seen in figure 8 explains why LA-ICP-MS, though more sensitive than EMPA, did not reveal these, as the measured LA-ICP-MS sampling spots were 50 to 100 µm wide, averaging highly localized concentration peaks.

As the observed growth rings strongly suggests the white fillings of tubules were deposited by biological growth, microscopic fluorescence was applied. This identified fluorescence in the tubules that was strongest at the border of the white fillings, which itself was poorly fluorescing (Fig. 9). A conical cut through one of the tubules revealed its three-dimensional features (Fig. 9E, F).

4 Discussion

The observations presented here suggest that in the past, carbonate rock was locally consumed to create parallel macroscopic tubules, whose length, diameter and morphology varies. Some tubular bands now lie at the surface, resulting in visible tubules, pores and holes, but even these examples have probably originated inside a rock, and the covering layer has eroded away since.

As no known weathering mechanism can explain this phenomenon, and the tubules form inside rocks, we propose that they are of biological origin. We infer that microorganisms, which we collectively name 'rock borers', have consumed the rock

195   material, leaving behind the tubules that are filled with white material, in a process that takes place inside the rock. The tubules were clearly created after the metamorphic limestones were uplifted to the surface, as these fragile porous structures would not have survived the metamorphic conditions deep down. The observed tubules are currently located close to the surface; whether they occur in deeper rocks as well was not determined. The observations suggest that radial biological growth occurred towards the walls of the tubules, of which fluorescent remnants are still present. This interpretation is supported by the local

200   enrichment of P and S at the edge of a tubule: if microbial growth had formed these tubules and filled them with calcite, the microbes would multiply and grow in a radial direction, feeding on dead material (with recycling of biomaterial) while depositing the white calcite at the centre where growth was no longer sustained. The most recent growth phase would occur at the outside of a tubular disk, which is where P and S were highly enriched and fluorescence was the strongest. Although intact biological macromolecules will have disintegrated over time because of the considerable age of the tubules (estimated at more

205   than 1 Ma) and the strong weathering in desert conditions, the elements from which they were made up are still locally detectable and degraded biological material still resulted in fluorescence.

A typical weathering pattern of limestone in which biological activity is involved is surface pitting, which is very different from what we present here. Carbonate weathering in an arid region of Tunisia that may have been formed by biological activity (Smith et al., 2000) also does not resemble our observations. Investigations of endolithic systems in East

210   Antarctica included field photographs (Mergelov et al., 2018), but those presented features were also different from our findings. We have observed endostromatolite deposits, both in Namibia and, far more common, in Oman, but those were clearly different from tubular bands that were formed by removal, not deposition of material. To the best of our knowledge, the rock-boring patterns we report here have so far not been described, although they are quite common in Namibia and were also observed in Oman.

215   A biotic origin of these structures supposes the presence of liquid water, without which biological growth would be impossible. The investigated areas are currently arid, but wet periods occurred in the past, at 1-3 My in Namibia (Pickford, 2000; Heine, 1998) and in Oman (Nicholson et al., 2020). Fractures in rock commonly carry water and from such moist fractures the tubules extend downwards, probably in response to gravitational water movement, into the rock. The importance of downwards growth is supported by rare observations where tubules grow upwards from a fracture, in which case they only

220   reach a few mm in length. The parallelism of tubules suggests some competing mechanism. The tubules are nearly always straight, but their diameter can be somewhat variable along their length, and without weathering, the tubules become narrower towards the apical tip. Based on this shape, macro-organisms are unlikely candidates to have formed the tubules. Animals such as worms, or the pedestals that molluscs or other boring animals use for attachment, would produce burrows with a constant diameter, which is not what we observe here. Moreover, in most (and possibly all) cases the tubules formed along closed

225   fractures inside the rock, places no macro-organism could have reached. Instead, we propose that microorganisms have built

these structures (bacteria, archaea or monocellular eukaryotes). Note that the tubules are much wider than the width of typical single cells of microorganisms, which would be more in the order of micrometres than the observed millimetres.

Bacteria have been isolated from deep rocks, where they feed on inorganic and organic material dissolved in water circulating in small fissures and fractures (Pedersen, 1997). Carbonate deposits, which are often the product of previous
230 biological activity, can support bacterial growth. Certain photosynthetic cyanobacteria can digest carbonates and assist in the destruction of biogenic carbonates as well as coastal limestones (Schneider and Campion-Alsumard, 1999). The microscopic evidence of these carbonate-borers is well documented, however, the cavities they produce are typically no deeper than 1 mm (Garcia-Pichel, 2006). To the best of our knowledge, tubules tens of mm long have not been described as the result of cyanobacterial carbonate destruction.

235 The terrestrial calcium carbonate deposits in which the tubules are observed have all been formed as marine sediments. A biotic origin and the contribution of 'marine snow' would have introduced biomatter in these deposits (Macquaker et al., 2010), which in Namibia have since been exposed to high pressures and temperatures due to tectonic activity, before they were uplifted to the surface. This would have disintegrated the biomatter to short-chain unsaturated carbohydrates or methane that might serve as a carbon and electron source. The amount of biologically available carbon couldn't be assessed, as its signal is
240 overshadowed by C present in the form of carbonate. SO_2 may have formed from proteins, and N may be present in small amines or amides, all of which are molecules that can serve as nutrients. In the Oman limestone small amounts of hydrocarbons (bitumen) are present (Grobe et al., 2019) which may provide nutrients and energy there.

All life forms depend on nutrients that must provide a combination of elements, an energy source, and a suitable electron donor (reducing power). Apart from Ca, C, H and O present in carbonate, life also depends on presence of P, N, S, and, in
245 lower amounts, Cl, K, Na, Mg, Se, Zn, Fe, Mn, Cu, Co, Ni, and Mo (Wackett et al., 2004). During anabolism, microorganisms must reduce oxygenated carbon to a less oxygenated state, to produce the C-C, C-N or C-P bonds that are present in biomolecules. Cyanobacteria and other phototrophs do this by means of the electrons generated by the photosynthetic reaction, together with the energy they harvest from sunlight, but they can only grow in light-exposed niches, not inside a rock. Lithotrophic bacteria use reduced metal ions (Jones et al., 1984), as an electron donor for instance ferric iron (Fe^{3+}) present in
250 minerals such as pyrite, biotite or hornblende, but these are notoriously absent in the marbles of Namibia, excluding canonical lithotrophs as culprits for tubule formation.

Based on the observations we hypothesize a mechanism for the formation of parallel tubules that lead away from the fracture from which they formed (Fig. 10). The proposed process starts with microorganisms entering the rock via water that penetrates a fracture (Fig. 10A). These multiply while they consume short-chain hydrocarbons and other nutrients that are
255 captured in the carbonate and dissolved in fracture water, at the same time dissolving $CaCO_3$, possibly by secretion of acid. As they multiply, the microorganisms form monocultures or possibly even complex communities. Other elements required for growth are taken up from the water or diffuse from the surrounding rock. As the fracture environment becomes depleted of nutrients and essential elements, the community expands by growing downwards, away from the fissure. A zone of depleted nutrients and minerals eventually forms around each growing community, which not only hampers growth in any direction

260   other than away from the fissure where competing communities are absent (further downwards), but also defines the minimal width *a* of the remaining tubule wall (Fig. 10A). Growth occurs at the apical tip of the tubule, where competition for nutrients and elements ensures all colonies grow in the same direction (Fig. 10B).

       Material that is removed from the apical side of a beginning cavity must either be completely incorporated into biomatter, or (actively or passively) transported towards the opening at the top. This waste product, mostly calcite, forms the

265   white filling. Redundant calcium ions would have to be moved towards the basal end of the growing tubule in order to create the space required for growth. From there it would 'overflow' into the fracture to form the thin white layer. The movement of calcium ions can be enabled by passive diffusion, provided there is a concentration gradient along the tubule, or by water, but an upwards direction of movement makes this unlikely. Alternatively, calcium ions may have been actively transported from cell to cell along the tubule, starting from cells accumulating it at the apical side that pass it on towards cells located more

270   basally. Living organisms contain specific calcium pumps in their membranes, and for cyanobacteria that form micro-cavities in shells, it has been suggested these calcium pumps can be used to create low calcium concentrations at the end of their cavity, to allow diffusion (Garcia-Pichel, 2006). If this also occurred in the hypothesized microbes forming the tubules described here, a higher order of organization would be necessary to ensure Ca transport happens in the correct (longitudinal) direction towards the base. Such transport may pose a maximum length of any cavity that can be formed, which according to our observations

275   is about 30 mm. Ultimately, the calcium is secreted behind the growth phase where it forms a deposit. Such calcium mineral deposits have been described for *Dictyostelium*, which are multicellular eukaryotic microorganisms, when grown on calcite (Eder et al., 2016).

       In addition to growth in the longitudinal direction, radial growth would widen the tubule (Fig. 10C) and this requires Ca transport towards the centre of the cavity, where over time a column is formed that fills the tubule. Recycling of bio-

280   material inside the tubule would move essential elements from the middle of a tubule (representing earlier growth stages) towards the outer ring that represents the latest zone of growth. As a result, the white filling is waste material of nearly pure calcite. Once a community is present, it can maintain growth by consumption of diffusing nutrients and of dead cells, until nutrients (and energy) become limited. When eventually the biological material decays, diffusion of degraded material into the surrounding rock may be responsible for the observed discolouring.

285   As water would have been sparse it is quite possible that the organisms produced a protective hygroscopic extracellular matrix, of which the local enrichment of Si and Mg that was sometimes observed may be remnants. Extracellular polymeric substances containing Mg and Si have contributed to the fossilization of benthic marine cyanobacteria living in the Proterozoic (Moore et al., 2021), and extracellular polymeric substances rich in Ca, Mg, Si, Al and S have been detected in a microbial mat from peritidal Qatar (Perri et al., 2018). That the white waste material inside the tubules had a low content of certain metals

290   suggest that these had been incorporated in biomatter at the growing border of the tubules. This included a number of Rare Earth elements that are not typically found in microbial metalloproteins. Nevertheless, certain dehydrogenases can incorporate La, Ce, Pr or Nd ions (Lv and Tani, 2018), all of which we observed at low abundance in the white matter (Fig. S5). Interestingly, this trend was not seen for U and V. This selectivity supports our interpretation that a microbiological process is

behind the described observations. It is not known if these rock-eating processes currently still take place, and it would be interesting to investigate carbonate rocks in other desert areas around the world for their presence.

The cavities formed by the rock-eating microbes proposed to be responsible for the observations presented here are now contributing to weathering. If the rock-eating organisms were active on a global scale in the past, this would have strong implications on the global carbon cycle in those periods, and they still affect present rates of erosion and weathering. Their contribution towards global carbon cycles deserves further studies. If this limestone-consuming and erosion-enhancing biological activity has taken place on a large enough scale, its contribution to the global carbon cycles would need to be incorporated in models to accurately describe past processes. Whether this would affect predictions about future carbon cycling remains to be seen. The intriguing observations described here are a first description of what may become a whole novel field of litho-biology.

Author contributions. CWP first observed the rock borer tubules in Namibia and subsequently in Oman, initiated the project and collected most field data. TMW assisted in the field, wrote the manuscript draft and assisted in interpretation of the data. NS performed microprobe analyses. AJ performed fluorescence microscopy. RM performed LA-ICP-MS. JU collected samples in Oman and provided support. All authors contributed to writing the final manuscript.

Competing Interests. None of the authors have a conflict of interest to declare.

Availability of data. The raw data from EMPA and ICPMS analyses are available upon request.

Acknowledgements. Field studies in Namibia were funded by the Schürmann Foundation, the Netherlands (CWP). We thank Corinna R. Schott for preliminary investigations performed during her undergraduate study.

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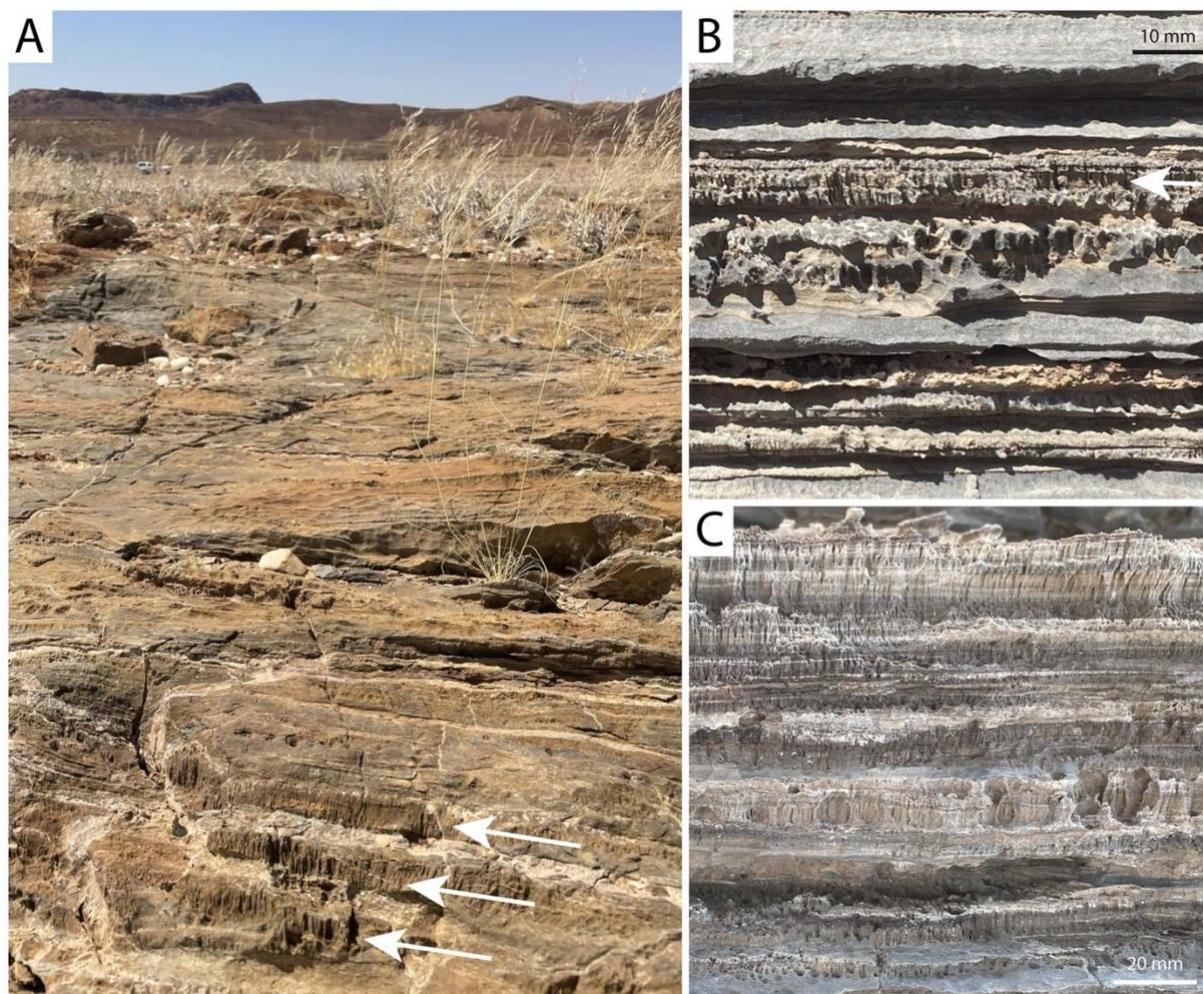

Figures

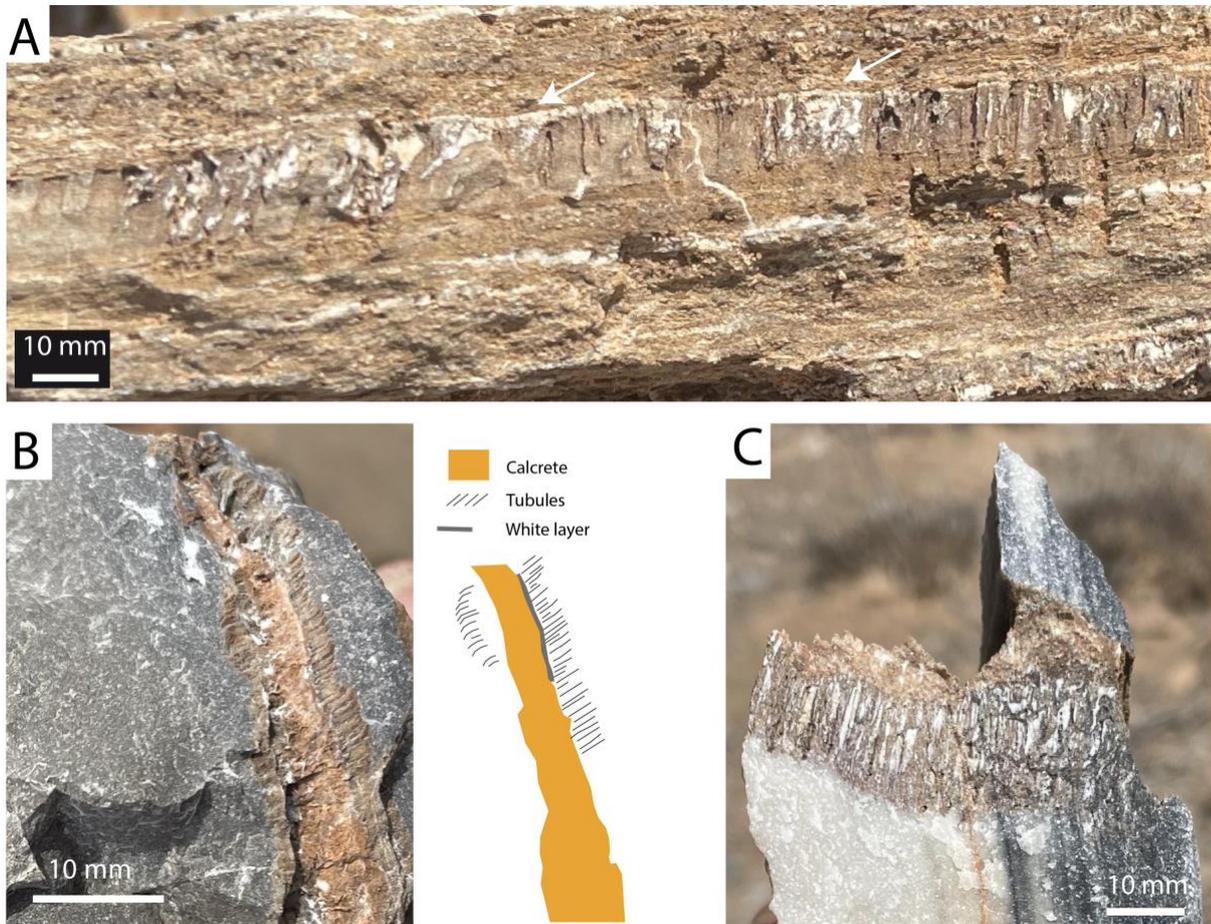
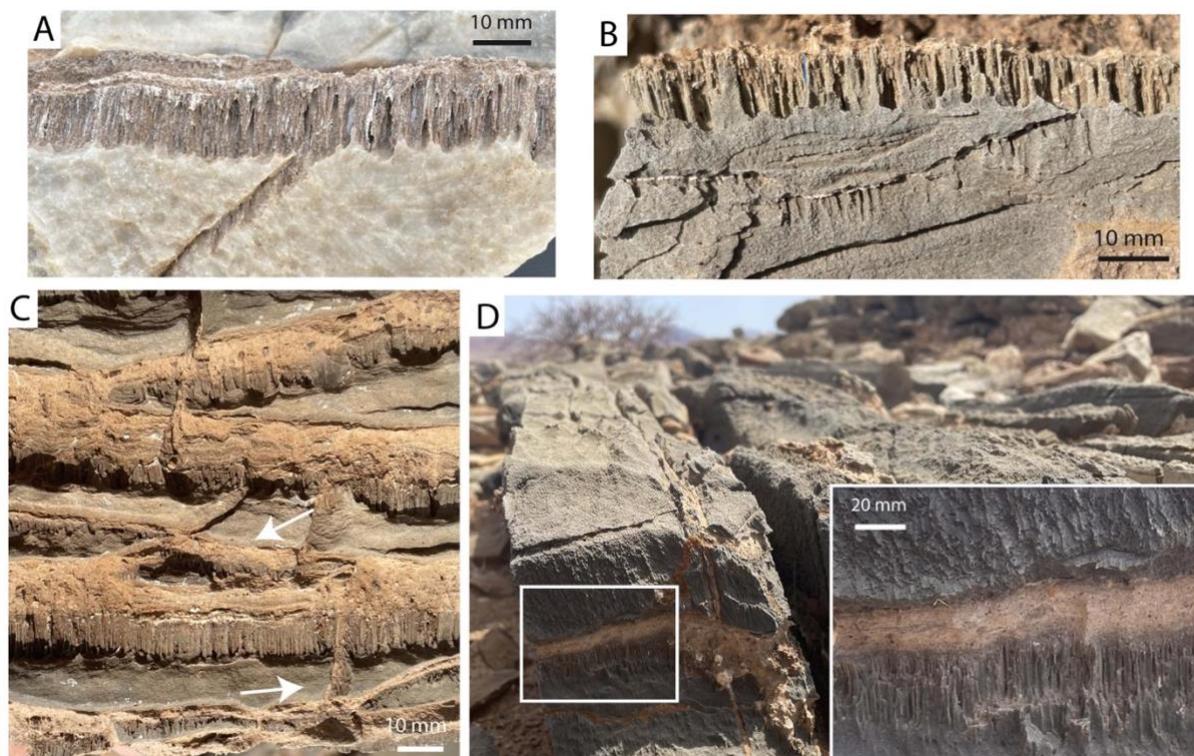
Figure 1: Field observations from Namibia. A: Namibian desert landscape. In the foreground three layers of marble in
395 **which tubular bands are visible (white arrows). B: Example of a single tubular band (white arrow) that is clearly**
distinguishable from irregular karst weathering directly below it. C: Multiple tubular bands in an outcrop with various
degrees of weathering. The layering in the rock is still visible in the tubular bands.

400 **Figure 2: Tubular bands vary in appearance. A: Tubules are commonly filled with white material and are separated from the original rock by a sharp, straight border (white arrows). B: The tubules are frequently bordered by calcrete, often with a thin white layer in between. C: Where layering is inclined, the tubules can be nearly parallel to the rock layering; remnants of calcrete is present above tubules. B and C are loose specimens.**

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Figure 3: The tubular bands appear to form in fractures. A: Branched tubular band with tubules having the same orientation in both branches. The lower branch ends in a fracture. B: Three bands, the main one (top) is heavily weathered as it is surface-exposed. A thin white layer is visible above the second and third band, whose density of tubules is lower than that of the main band. C: In this loose specimen, calcrete separates original rock fragments as it was formed in fractures without tubules (as indicated by the white arrows) and in fractures where tubules were present. In the upper part of the photo calcrete separated what originally seemed to have been a Y-shaped tubular band. Remnants of a white band separating the calcrete from tubules are visible in the lowest tubular band. D: *In situ*, the calcrete lies on top of the tubules. A close-up of the white square is shown in the zoom inset.

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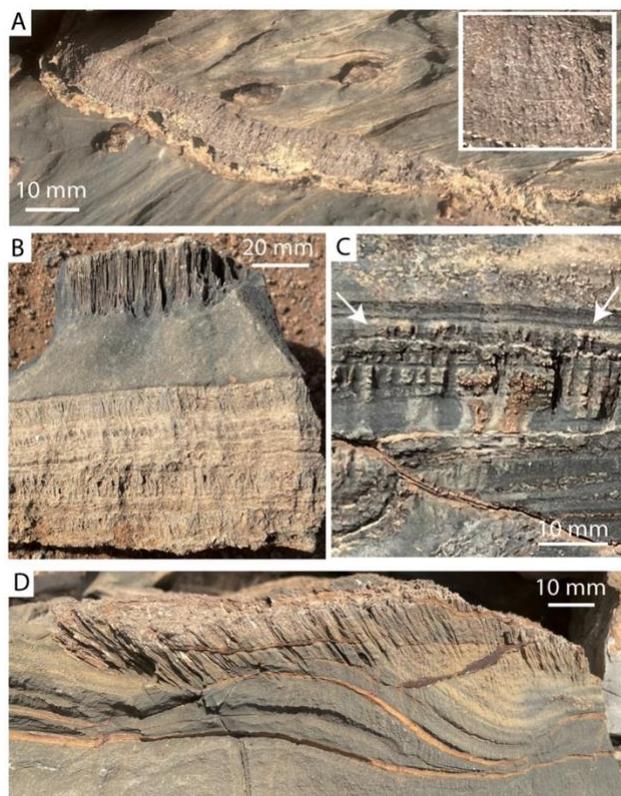
Figure 4: Tubules bands differ from endostromatolites. A: Endostromatolites that fill fractures result in deposits that no longer resemble the original rock and do not contain tubules that extend to the complete width of a band. Inset: zoom showing the columnar structure with macroscopic growth layers that are typical for endostromatolites. B: Loose specimen containing tubules on one side and an endostromatolite deposit on its other side. C: In this heavily weathered outcrop, a minor band (indicated by the arrows) formed above the thin white layer from which the main tubular band developed downwards. D: Tubules formed in a folded outcrop where they maintain their orientation despite the fold. Part of the tubules are intersected by a fracture.
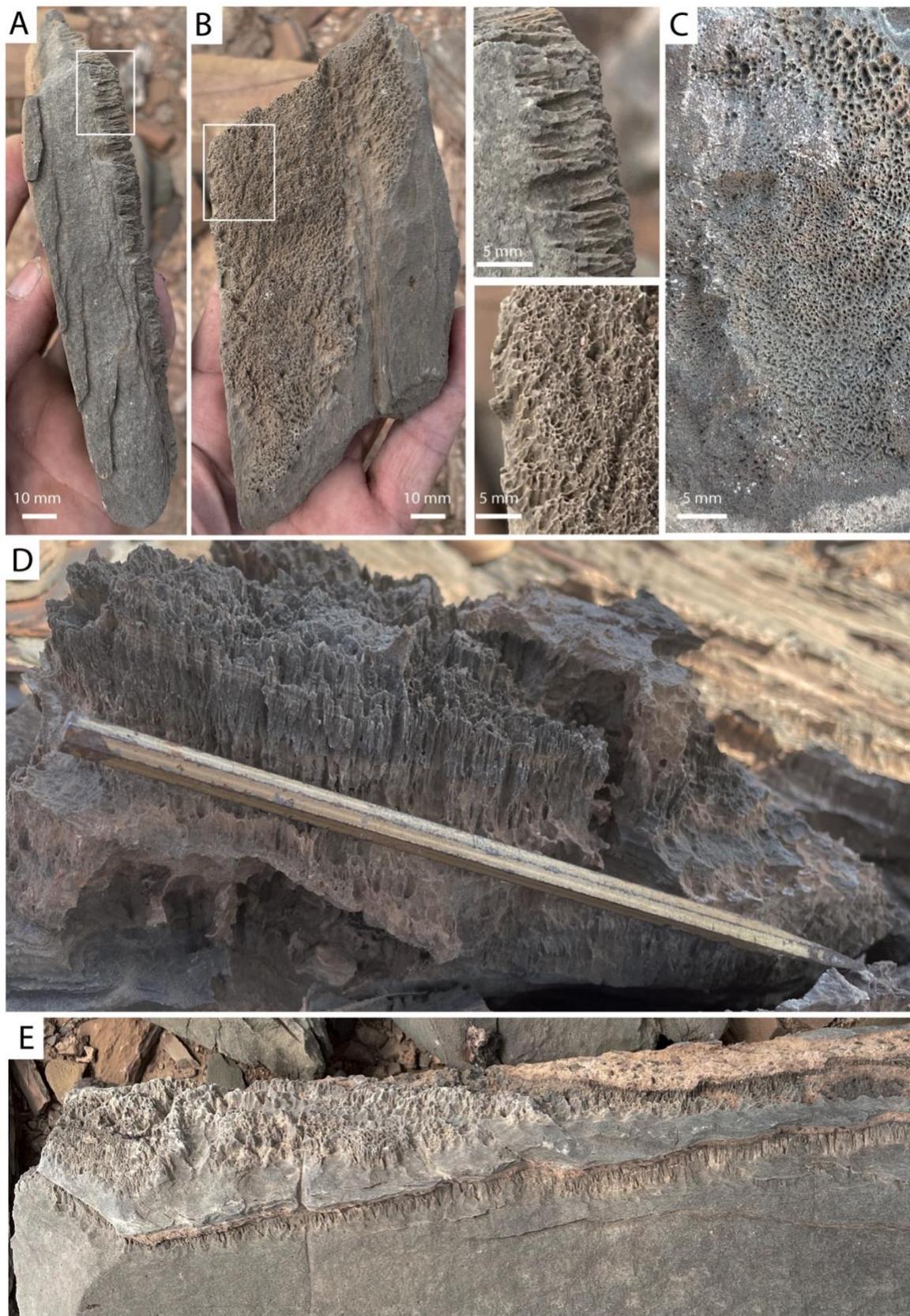
Figure 5: Tubules contribute to carbonate weathering. A: Loose specimen with a narrow tubular band shown from the
440 **side and from the surface where pores are visible (B). The two white squares are shown as zooms to the right of B. C:**
***In situ* surface showing a similar porous structure. D: When weathered, the tubules result a strongly pockmarked**
surface. Chisel length: 20 cm. E: *In situ* outcrop with a weathered tubular band, still partly covered with calcrete on its
top, with below it a second tubular band that is still inside the rock and is also covered by calcrete.

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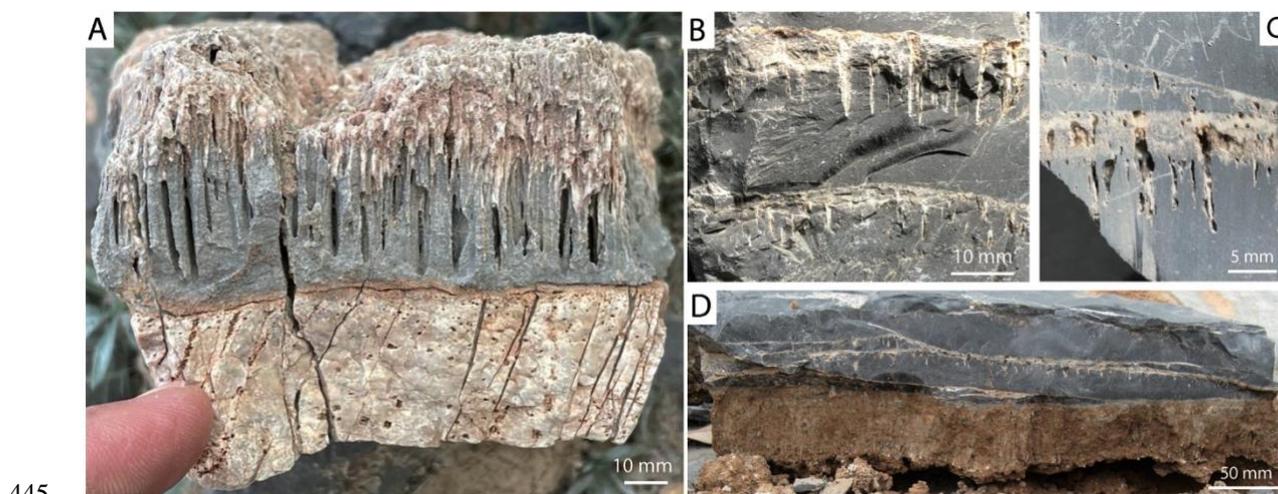
Figure 6: Examples of tubular bands from Oman. A: Loose specimen with high-density tubules (top half) that are
partly filled with white deposit. B: Two *in situ* bands of low-density tubules filled with white material that start from a
white layer. C: Freshly cut surface showing one well-developed and two less developed, low-density tubular bands
starting from a white layer. D: Loose fragment with branched, low-density tubular bands. Its bottom side is covered
450 **with a layer of endostromatolites.**
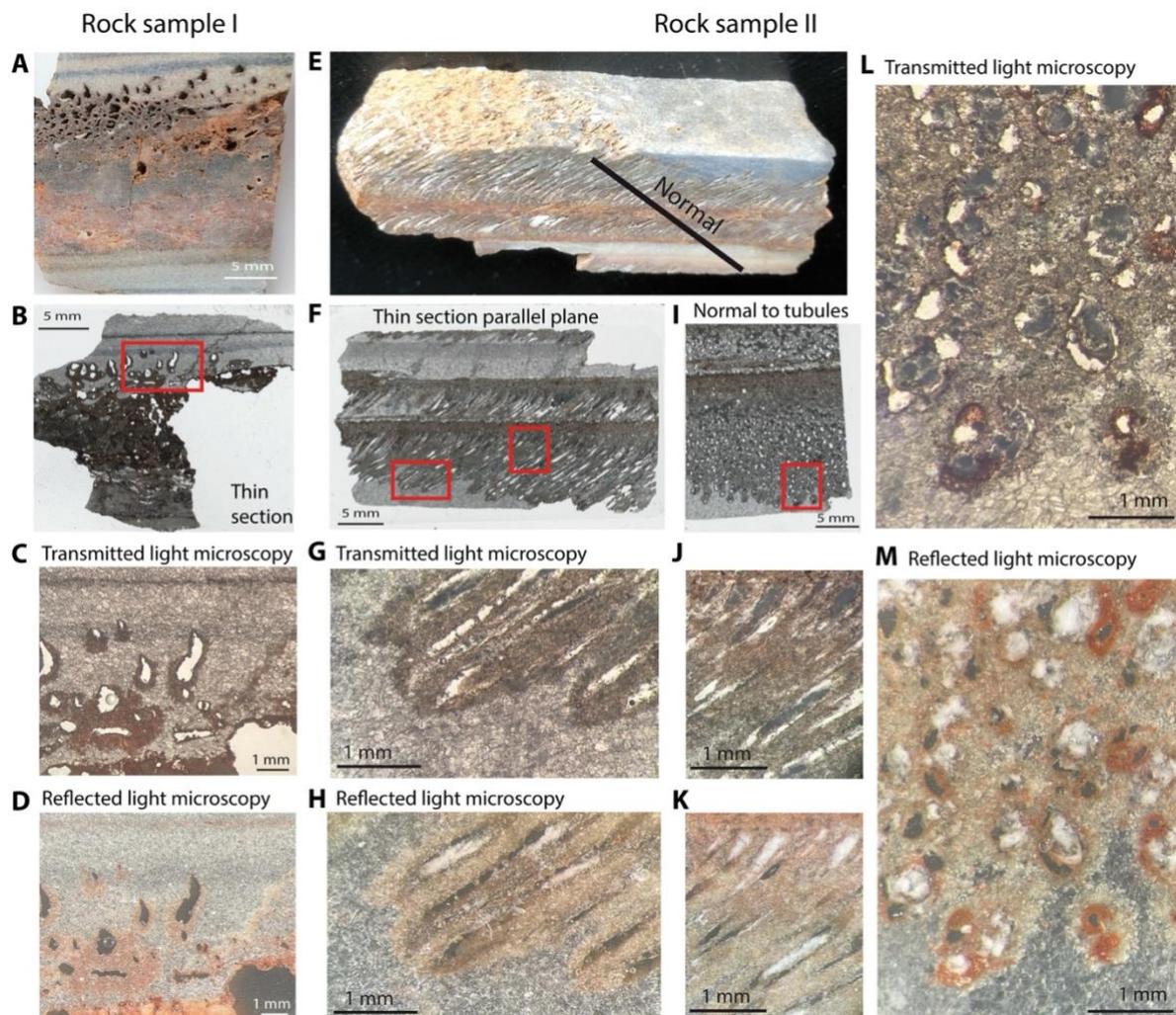
Figure 7: Microscopic observations for two typical samples from Namibia. Freshly cut surfaces often show discolouring around the tubules. A: Rock sample I was cut normal to the slanting tubules, giving thin section B. The tubule holes were empty in this specimen, but the discolouring around the tubules was clearly visible under transmitted light (C) and reflected light (D). E: Rock sample II was cut to give thin sections parallel (F) and normal (I) to the tubules. Areas enlarged in the micrographs are shown in red. The white filling appears dark grey under transmitted light (G, J, L), and white under reflected light (H, K, M). Various degrees of discolouring can be seen in M, and internal structures inside the tubules are visible in L.
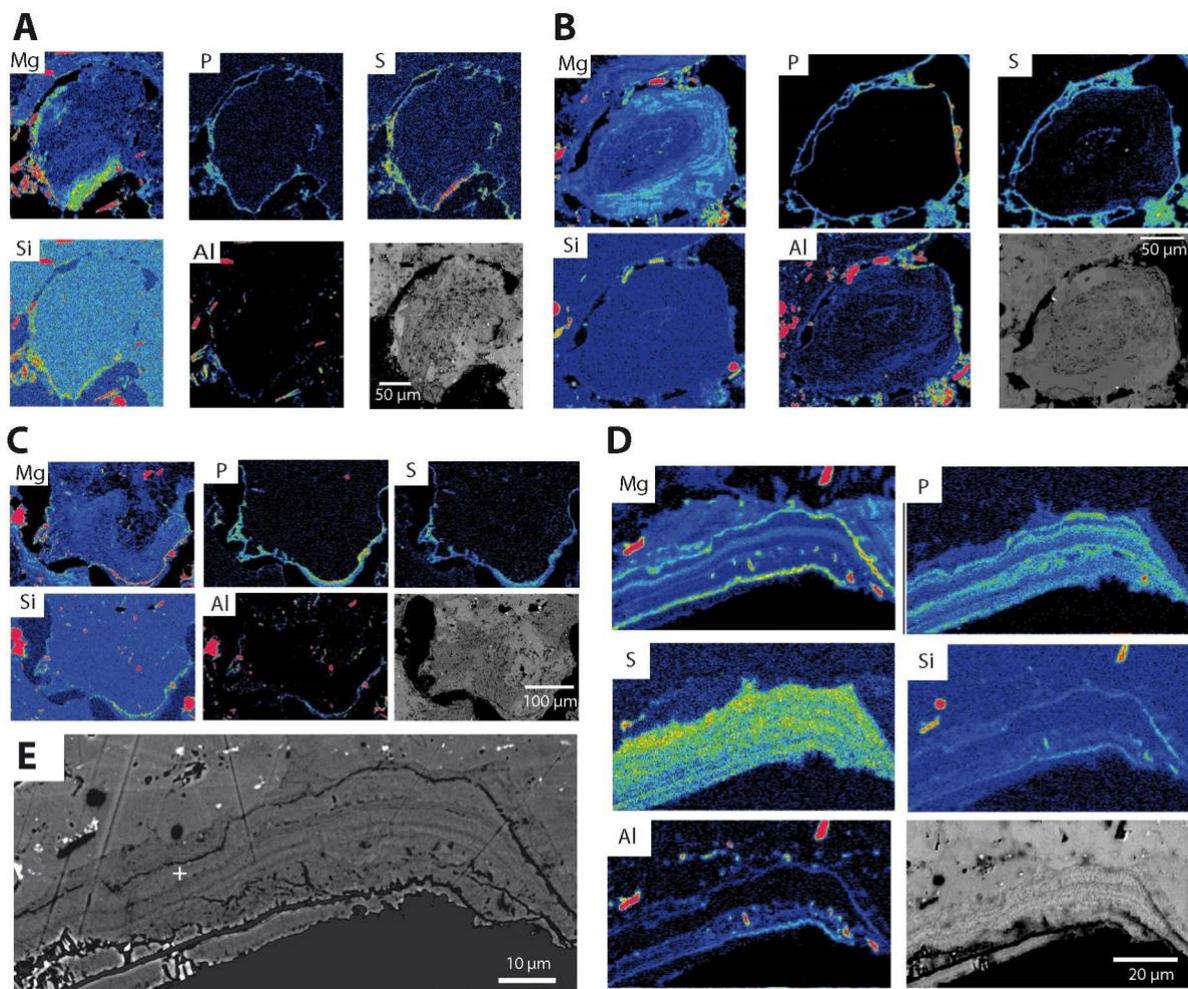
Figure 8: Electron-Microprobe Analysis. Four elemental maps (A to D) are shown for cross-sections of tubules, for the five elements indicated, complemented with the electron micrograph. Blue to red show low to high concentrations. E: enlargement of the electron micrograph of D, clearly showing growth rings of approximately 1 μm wide. The complete maps with colour scales are presented in figure S5.

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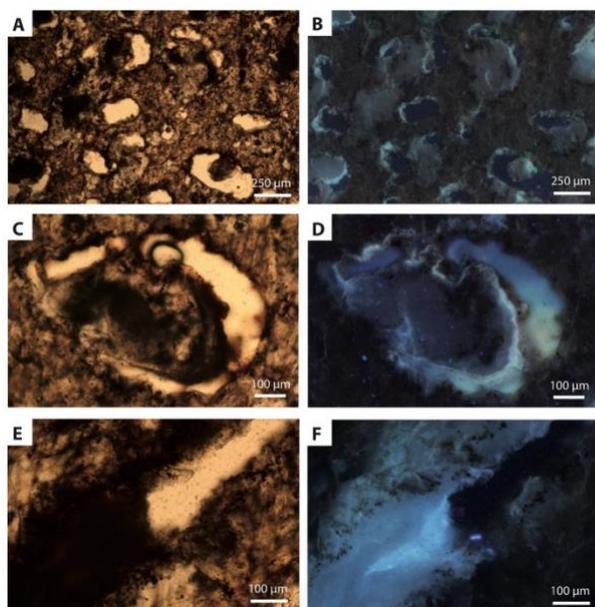
Figure 9: Fluorescence microscopy. Three examples are shown, under visible light (left) and UV (right). A-D are normal sections, E and F show a conical cut through a tubule. The white filling of the tubules is dark brown in visible light and
480 **dark blue under UV, except for their strongly fluorescent rims. Voids appear cream coloured under visible light.**
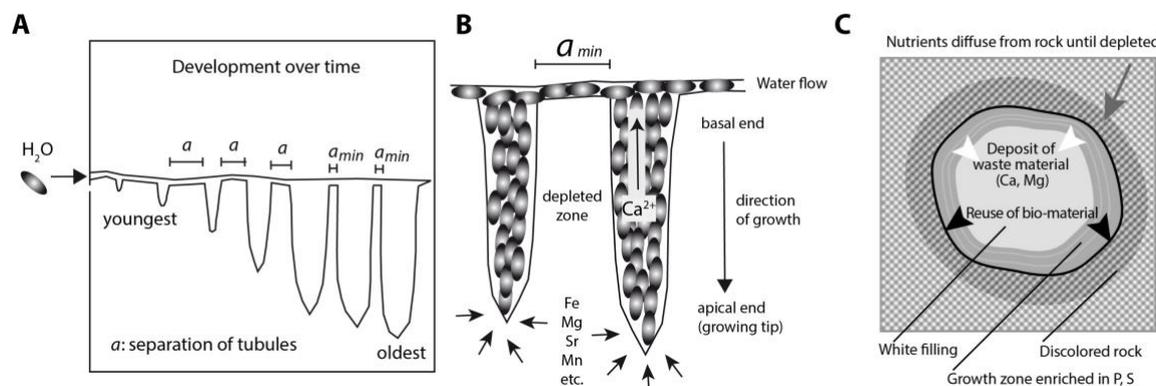
Figure 10: Schematic drawing of the proposed formation of the tubules by microbes. A: Various developmental stages towards a minimal separation a_{min} of parallel tubules. B: Zoom showing microorganism cells (not drawn to scale), the direction of growth and
485 **movement of nutrients from the rock. C: Cross section showing how growth takes place at the rim of a tubule in a radial direction, while a deposit of waste (mostly calcium minerals) is formed at the centre of the tubule. The discolouring of the surrounding rock may be caused by material diffusing out of the tubule during decay.**