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Fungus-like mycelial fossils in 2.4-billion-year-old vesicular basalt

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Geological setting

The Ongeluk Formation is up to 900 m thick and comprises mainly massive basalt, hyaloclastites and pillow lavas that extruded under seawater^{1,2}. Individual flows are typically 2–3 m thick and contain amygdales towards the top. Flows may be capped by flow breccias and separated from overlying flows by thin layers of sediment and jasper². The lavas have undergone a remarkably low degree of deformation and only very low-grade (sub-greenschist) metamorphism³. Geochemical and petrographic data indicate that the basalts have undergone low-temperature alteration by seawater after extrusion².

The basalts are grey to green and preserve igneous textures ranging from subophitic and intergranular to intersertal and glomeroporphyritic⁴. They contain mainly augite and plagioclase laths with minor ilmenite, zircon and titanomagnetite. The primary minerals have undergone alteration, with the growth of chlorite, pumpellyite, quartz, albite, calcite and sulphide minerals. Vesicles and fractures are commonly filled with chlorite, calcite, quartz and sulphides.

An earlier generated Pb–Pb isochron has indicated a ca. 2.2 Ga eruption age of the basalt². However, whole-rock U–Pb and Pb–Pb isochron ages from dolomite of the overlying Mooidraai Formation suggest that the Ongeluk Formation is older than 2.39 Ga^{5,6}, and U–Pb dating of baddeleyite in sub-volcanic sills in the lower part of the Ongeluk Formation gives an age of ca. 2426±3 Ma⁷.

The timing of metamorphism of the Ongeluk Formation is uncertain, but significant seafloor alteration apparently occurred shortly after extrusion². The mineral assemblages of the basalts may also have been affected by later episodes of metamorphism, although direct dates for these are lacking. In situ U–Pb geochronology of monazite, xenotime and titanite in the Transvaal Supergroup sediments indicates that the Kaapvaal craton has been affected by regional events at ~2.15 Ga and ~2.06 Ga^{8,9}. Paleomagnetic data from the Kalahari mineral field suggests a complex thermal history with possible events at ~1.9 Ga, 1.8–1.75 Ga and

1.25–1.1 Ga¹⁰. A paleosol developed on the Ongeluk Formation yielded a Rb/Sr date of 1257±11 Ma¹¹, which may represent localized metasomatic alteration along faults and lithological contacts.

Vesicles vs. varioles

A number of different spherical or globular structures are found in volcanic and subvolcanic rocks (see Phillips, 1973¹², for nomenclature and brief descriptions). The most common are vesicles, formed due to the exsolution of a vapour phase and formation of gas bubbles from the magma as it rises to the Earth's surface. Vesicles are found in all types of magma, in subaerial and submarine volcanic rocks, in pillowed and non-pillowed basaltic rocks. They form cavities, called amygdales (or amygdules), in many cases filled by secondary, low-temperature, minerals. The minerals forming the amygdales nucleate on the walls of the vesicles and grow inwards to fill the empty space. The most common minerals forming amygdales are zeolites, clays, chlorite, chalcedony, quartz, barite and calcite. In Precambrian rocks, which have experienced at least low-temperature metamorphism, zeolites and clay minerals are generally replaced by chlorite, other phyllosilicates and in some cases, low-temperature feldspars, while quartz and calcite are recrystallized.

Varioles are centimeter-scale, leucocratic spherical or globular structures found in basaltic rocks. They are comparatively common in, although not restricted to, Archaean basalts. They are mostly composed of feldspars that show radial growth from the centre of the variole outwards. They generally result from spherulitic growth of plagioclase, which may be nucleated on an amygdale or crystal nucleus^{13,14}. They grow from a point outwards, in contrast to amygdales. The feldspars within variolitic spherulites may have a dendritic habit. Dendrites are characterized by branching from a central 'stem' and are crystallographically controlled. The stem and branches become progressively finer away from the point of origin, and there is a regular arrangement of side branches. Less commonly, varioles result

from the crystallization of droplets of felsic magma in mafic magma, due to mixing of magmas with different composition. These varioles have the compositions of magmas.

The spherical structures that we describe from the Ongeluk lavas do not have the composition of magmas and do not have a spherulitic, i.e. radial, structure. They are <2 mm in diameter and filled with minerals, e.g., calcite, quartz and chlorite, that are characteristic of amygdalites. Amygdalites containing these minerals are common in the Ongeluk lavas², whereas varioles have not been described from there.

Chlorite thermometry

Chlorite defining the filament structures was analysed from two amygdalites containing microfossil filaments in Thin Section IOS004 (Supplementary Table 1). The chlorite comprises aggregates of very fine grains (<1 µm), so analyses are derived from composites rather than single grains. Minor amounts of CaO are recorded in all analyses, suggesting that the aggregates include some fine calcite as well as chlorite. Analyses with >1 wt% CaO have been excluded from the temperature calculations. The chlorites are Fe-rich members of the chamosite–clinocllore series (Mg numbers 0.35–0.37) with ~0.9 apfu (atoms per formula unit based on 14 oxygen atoms) of tetrahedral Al and 1.1 apfu octahedral Al (Supplementary Table 1). Temperatures were estimated following the method of Bourdelle et al.¹⁵, which is independent of the oxidation state of Fe and applicable to chlorites formed at temperatures <300°C. Temperature estimates for chlorite in the two amygdalites are indistinguishable, and seven analyses give temperatures in the range 198–234°C with an average of 209±14°C (2σ).

Chlorites from both amygdalites and groundmass were analysed in Thin Section ZBF061 (Supplementary Table 2). The amygdalites contain very fine-grained chamosite (Chlorite 1) associated with poorly defined microfossil filaments as in Section IOS004, but also some pale-green, coarser-grained flakes (Chlorite 2). In some places, Chlorite 2 forms overgrowths on Chlorite 1. The Chlorite 1 is chamosite with only minor Ca in some analyses and has Mg numbers of 0.31–0.36 and ~0.8–0.9 apfu tetrahedral Al and 1.0–1.3 apfu octahedral Al, similar to Section IOS004. Temperature estimates from the Bourdelle thermometer range from 179°C to 260°C with an average of 202±26°C (n=16) from EDS analyses and 211±29°C (n=25) from WDS analyses.

Chlorite 2 is more Fe-rich and aluminous, with no Ca, and Mg numbers 0.24–0.26. It has 1.2–1.3 apfu tetrahedral Al and 1.3–1.4 apfu octahedral Al. Temperatures estimated using the Bourdelle et al. calibration¹⁵ are >300°C and therefore out of the range employed in the calibration of this thermometer. The thermometer of Lanari et al.¹⁶ is applicable for chlorites with <3 apfu Si and a crystallization temperature up to 500°C. The formulation where all Fe is treated as Fe²⁺ and vacancies are >0.03 (thermometer 2 of Lanari et al.¹⁶) is appropriate for the analyses of Chlorite 2 and gives temperatures in the range 319–411°C with an average of 368±33°C (n=10) for EDS analyses and 335±67°C (n=22) for WDS analyses.

The groundmass of the basalt in thin section ZBF061 has been totally altered, with plagioclase laths replaced by K-feldspar and more mafic minerals and interstitial groundmass or glass by fine-grained chlorite, quartz, K-feldspar and calcite. The age relationship between the chlorite in the groundmass and chlorite in the amygdalites is not clearly defined. The composition of the chlorite in the groundmass is intermediate between Chlorite 1 and Chlorite 2. It has Mg numbers 0.25–0.29, and 1.2–1.4 apfu octahedral Al and 1.0–1.2 apfu tetrahedral Al. Both the Bourdelle and Lanari thermometers are applicable to these analyses. Temperature estimates for seven analyses have a range of 214–304°C with an average of 262±34°C (thermometer of Bourdelle et al.¹⁵) and 121–278°C with an average of 232±54°C (thermometer of Lanari et al.¹⁶). These estimates overlap within the accuracy of the methods, which is estimated at ±30°C.

Raman carbonaceous-material geothermometry

The ordering of the structure of carbonaceous material (CM) reflects the maximum metamorphic temperature condition that has affected the carbon compound in the rock, and the transformation of the structure from its original carbonaceous precursor is irreversible during retrogression. The temperature–CM structure relationship can therefore be used as a geothermometer^{17,18}. In order to characterize the CM, Raman spectrometry was performed on thin sections of the volcanic matrix in the lower part of the Ongeluk Formation, a basal sandstone in the Ongeluk Formation, and from a diamictite of the underlying Makganyene Formation. Inspections for the presence of CM were also made of a dolerite dyke immediately above the Ongeluk sample and of calcite-filled cavities from the lower part of the Ongeluk Formation. All spectra were recorded on CM below the sample surface and with low laser power to avoid laser-induced modifications of the CM. The Raman spectra of CM^{17,18} have a first-order region with two main bands at ~1350 (D1) and ~1580 cm⁻¹ (G) together with minor bands at ~1250 cm⁻¹ (D4) as a shoulder on D1, a wide band at ~1510 cm⁻¹ (D3) and at ~1620 cm⁻¹ (D2) as a shoulder on G. Second-order overtone bands occur in the region 2600–3200 cm⁻¹. The G band is the only band in well-ordered graphite and with increasing disorder structure, the D1, D2, D3 and D4 bands appear. The D1 band becomes more wide and intense with decreasing order of the CM, and the width of D2, D3 and D4 increases. In poorly ordered CM it is impossible to separate the G and D2 bands, and thus only one broad band is observed at ~1600 cm⁻¹. The obtained Raman spectra are shown in Supplementary Fig. 7 where the difference of the CM structures between the Ongeluk and the Makganyene samples is clearly demonstrated. There was no CM detected neither in the dolerite (Supplementary Fig. 7) nor in the calcite-filled cavities including the hyphae fossils (Supplementary Fig. 2). For the Raman CM geothermometer at temperatures above 330°C, Beyssac et al.¹⁷ proposed that there is a linear correlation of the band area ratio $R2 = D1 / (D1 + G + D2)$ and the

temperature T ($^{\circ}\text{C}$), according to the equation $T = -445 \times R2 + 641$. The ratios of the band areas measured on 10 isolated CM from the Makganyene diamictite and the Ongeluk sandstone are between 0.59 and 0.61 suggesting an average maximum temperature of around 370°C . The shape of the Raman spectra for the CM in the matrix of the Ongeluk volcanic sample (Supplementary Fig. 7b) with a wide D1 band, visible D4 shoulder, a wide G and D4 that cannot be separated, high intensity of the D3 band and significantly suppressed second-order bands points to a poorly ordered structure and low ($<300^{\circ}\text{C}$) metamorphic temperature^{17,18}. For temperatures below 300°C , the band height ratio $R1 = D1/G$ changes regularly with metamorphic grade, and based on this Rahl et al.¹⁸ presented a modified Raman CM geothermometer using the equation $T = 737.3 + 320.9(R1) - 1067(R2) - 80.638(R1)^2$. The $R1$ ratios of five spot measurements on the Ongeluk CM range from 0.65 to 0.75 and $R2$ values around 0.65–0.70. To determine the $R1$ and $R2$ values, the spectra were decomposed into D1/G bands with Lorentzian shape using the LabSpec 5 software. With this method an approximate temperature of $200 \pm 30^{\circ}\text{C}$ was obtained. However, the shape of the bands together with the rare occurrence of CM in the Ongeluk volcanic sample gives a high uncertainty in estimates of temperatures from the spectra.

Assessment of antiquity and biogenicity

In the following we investigate how the Ongeluk filaments stand up against criteria of antiquity and biogenicity established in the recent literature^{19,20} for ancient microfossil-like structures. We have chosen this approach, rather than setting up our own list of criteria, in order to avoid the possibility of ad-hoc rules potentially adding bias to the comparisons. Lines in boldface denote the fulfillment (or non-fulfillment) of the criteria by the Ongeluk structures.

The following list of criteria distinguishing crypto- and chasmoendolithic filaments from biogenic and abiogenic tunnels in rock was set up by McLoughlin et al.¹⁹.

Cryptoendolithic filaments

Timing of formation: Pre-metamorphic growth into a fluid-filled cavity.

Criterion fulfilled.

Morphology: Filaments with circular cross-section, of a non-uniform diameter are curvilinear, or branched, and may show swellings along their lengths.

Criterion fulfilled.

Infilling mineralogy: Clays with or without organics.

Criterion fulfilled.

Host matrix: Carbonate-filled vesicles.

Criterion fulfilled.

Distribution: Grow into cavities.

Criterion fulfilled.

Mechanical abrasion: No.

Criterion fulfilled.

Dissolution: Possibility of secondary chemical dissolution.

Criterion fulfilled.

Chasmoendolithic filaments

Timing of formation: Pre-metamorphic growth into a fluid-filled vein or fracture.

Criterion fulfilled.

Morphology: Filaments with circular cross-section, of non-uniform diameter, may be curvilinear, branched, show internal septae or terminal swellings.

Criterion fulfilled.

Infilling mineralogy: Iron-oxides or clays.

Criterion fulfilled.

Host matrix: Carbonate or zeolite-filled veins.

Criterion fulfilled.

Distribution: Grow into fluid-filled vein or fracture, nucleate on walls.

Criterion fulfilled.

Mechanical abrasion: No.

Criterion fulfilled.

Dissolution: Possibility of secondary chemical dissolution.

Criterion fulfilled.

The following list of antiquity and biogenicity criteria for ancient microfossils is adopted from Wacey²⁰.

General antiquity criteria

(1) Structures must occur in rocks of known provenance; i.e., detailed location information must be presented so that independent re-sampling is possible.

Criterion fulfilled; sample is from specified drill core.

(2) Structures must occur in rocks of demonstrable or established age; i.e., the host rock must be dated directly by radiometric techniques, or the age of the rocks can be accurately inferred by correlation to nearby rocks that have been dated.

Criterion fulfilled; see “Supplementary Information: Geological Setting”.

(3) Structures must be indigenous to the primary fabric of the host rock; i.e., they must be physically embedded within the rock, not products of sample collection or preparation. They should, therefore, be present in petrographic thin sec-

tions of the rock. Other identification techniques such as acid maceration and acid etching are valuable accessory techniques but may accidentally incorporate post-depositional contaminants.

Criterion fulfilled; specimens are identified in petrographic sections as well as in closed vesicles examined by X-ray techniques.

(4) Structures must be syngenetic with the primary fabric of the host rock; i.e., they must not have been introduced by ancient or modern post-depositional fluids.

Criterion not relevant to cryptoendolithic biota, which by definition occupies cavities in already existing rock.

(5) Following from 4; any structures found within metastable mineral phases, void filling cements, veins, or cross-cutting fabrics must be viewed with extreme caution.

See comments to point 4 above.

(6) Structures should not occur in high-grade metamorphic rocks, because delicate organic structures will not survive these extremes of pressure and/or temperature; the likelihood of non-biological artefacts in such rocks is substantially increased.

Criterion fulfilled; chlorite thermometry shows that the low-grade metamorphism represented by Chlorite 2 has not affected crystals of Chlorite 1 replicating the fossil filaments.

(7) The geological context of the host rock must be fully understood at a range of scales; i.e., the host unit must show geographical extent and fit logically within the regional geological history.

Criterion fulfilled; see “Supplementary Information: Geological setting”.

Additional antiquity criteria specific to microfossils

(8) Potential microfossils should not be significantly different in colour from that of particulate carbonaceous material in the remainder of the rock matrix. For example, brown ‘microfossils’ in a largely black carbonaceous chert would immediately be suspicious.

Criterion not applicable to non-organic preservation, as in the Ongeluk structures.

(9) There should be evidence for organo-sedimentary interaction, e.g., sediment grains trapped or supported by fossils, coatings of distinctive composition or texture precipitated around the fossils, or perhaps alternating layers of prostrate and erect filaments in stromatolite-like sediments.

Criterion not applicable to non-sedimentary environments.

General biogenicity criteria

(10) Structures should exhibit biological morphology that can be related to extant cells, sheaths, traces of activity or waste products. Ideally life cycle variants should be identifiable (reproductive stages), comparable to that found in morphologically similar modern or fossil microorganisms.

Criterion fulfilled; filaments closely comparable to hyphae of Phanerozoic and modern fungi.

(11) More than a single step of biology-like processing should be evident. These steps may take the form of biominerals (e.g., pyrite), geochemical fractionations of isotopes (e.g., carbon and sulphur), specific organic compounds (e.g., hopanoid biomarkers) or distinctive elemental ratios.

Criterion not fulfilled; neither the calcite nor the chlorites in the amygdalae appear to be biological in origin, and there are no preserved organics.

(12) Structures should occur within a geological context that is plausible for life; i.e., at temperatures and pressures that extant organisms are known to survive.

Criterion fulfilled; vesicles in lavas are known to harbour cryptoendoliths.

(13) Structures should fit within a plausible evolutionary context.

Criterion fulfilled with question; current understanding of eukaryote evolution has fungi evolving considerably later.

(14) Structures should be abundant and ideally occur in a multi-component assemblage.

Criterion fulfilled in so far as the filaments are abundant within the basalt vesicles, but no direct evidence for the presence of other taxa has been found.

(15) Following from 14, ideally they should show colonial/community behaviour.

Criterion fulfilled; the filaments form mycelium-like structures.

(16) Following from 15, a preferred orientation indicating a role in the formation of biofabrics would be an additional bonus criterion.

Criterion fulfilled; the filaments are consistently organized within the vesicles.

Additional biogenicity criteria specific to microfossils

(17) Microfossils should ideally be composed of kerogenous carbon. However, if mineralised this should be a result of microbially mediated precipitation. Later mineral replacement of carbonaceous material may also be permissible but then doubts upon antiquity will be raised.

Criterion fulfilled in the “permissible” sense that filaments have been replaced by chlorite. The antiquity is-

sue, however, is dealt with using the known sequence of metamorphism of the rock (see below).

(18) Microfossils should be largely hollow. Cell walls and sheaths are by far the most likely parts of the microbe to be preserved; cellular constituents are rarely preserved in more modern examples. Mineral artefacts are unlikely to be hollow.

Criterion not fulfilled; filaments are preserved as solid chlorite without internal cavities.

(19) Ideally the microfossils should show some sort of cellular elaboration; e.g., not just smooth cell walls.

Criterion not applicable to fungal mycelia.

(20) Microfossils should show taphonomic degradation; i.e., collapse of cells, folding of films, fracturing. This may not occur in exceptional preservational circumstances, for example, in situ rapid silicification of living communities.

Criterion not fulfilled; no obvious taphonomic degradation, but fossilization processes inside basalt vesicles are poorly understood.

(21) The object must exceed the minimum size for independently viable cells ($\sim 0.25 \mu\text{m}$ diameter). Note: The recent discovery of nano-bacteria may modify this criterion to even smaller sizes.

Criterion fulfilled; filaments concur in size with fungal hyphae.

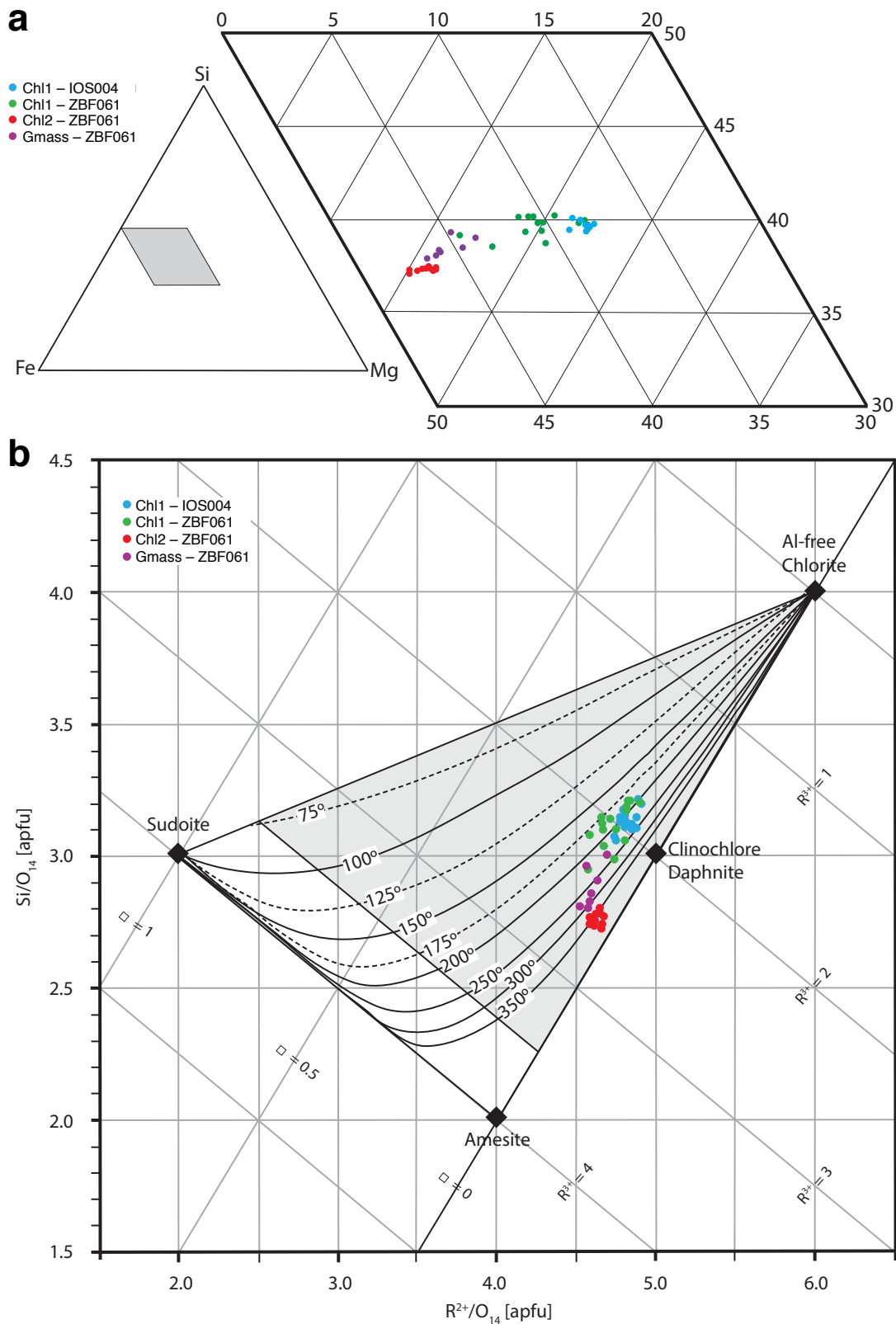
(22) Microfossils should be demonstrably dissimilar from potentially co-existing non-biological organic bodies (e.g., self organising spherulitic structures), and should occupy a restricted biological morphospace.

Criterion fulfilled; structures well defined in shape and distinctly different from known non-biological features in similar environments.

(23) Evidence of extra-cellular polymeric substances surrounding the putative microfossils would be an added bonus criterion.

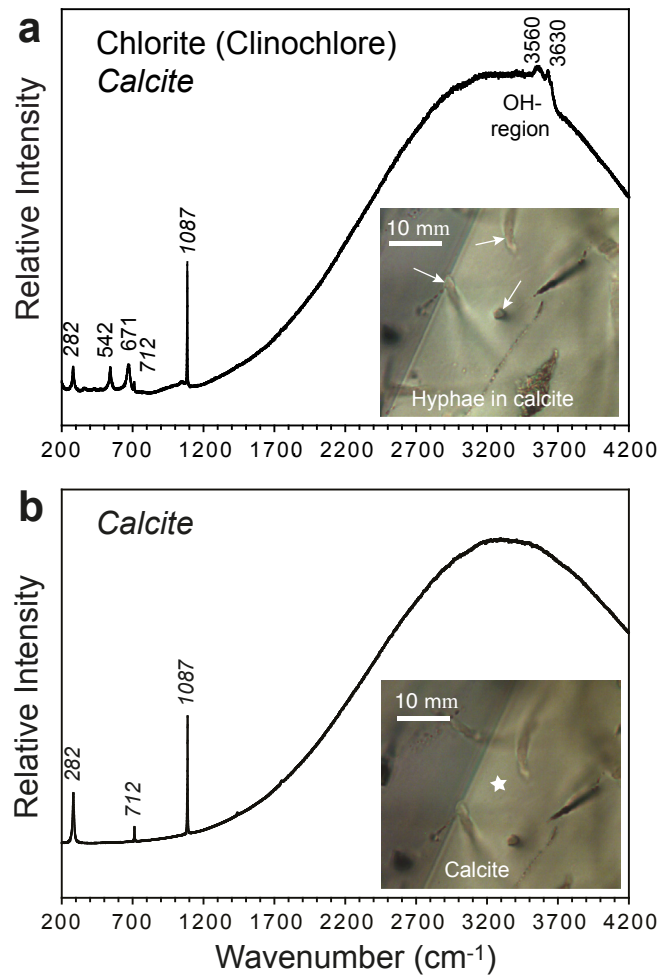
Criterion not applicable to fungal hyphae.

The Ongeluk filaments thus fulfill the criteria of McLoughlin et al. 2010¹⁹ for crypto- and chasmoendoliths. They also meet Wacey's²⁰ relevant criteria for antiquity and biogenicity, with the exception of three (11, 18 and 20) that relate to taphonomic breakdown and hollowness of fossil structures. None of the three can be considered compelling in the case of fossils being preserved by diagenetic/metamorphic mineralization in basaltic vesicles. The fossil filaments are composed of chlorite that probably formed from smectite during metamorphism and are encased in calcite cement. This sequence of mineral growth is consistent with observations from vesicles that preserve microfossils in younger basalts^{21–23} and with the main stages of mineral growth during the low-temperature alteration of oceanic crust²⁴. Isotopic dating of secondary minerals in submarine basalts suggests that smectite synthesis occurs within three million years of eruption whereas calcite cementation is complete within 10 million years²⁴, suggesting that cavities in the Ongeluk basalt were largely occluded within 10 million years of eruption. Some time after burial, likely during metamorphism at ~ 2.15 Ga and/or ~ 2.06 Ga⁸, the clays that mineralized the filaments, as well as clays within the vesicles and the matrix of the basalts, were converted to Chlorite 1 at crystallization temperatures of 179–260°C, followed by a partial recrystallization to Chlorite 2 at 319–411°C (see “Chlorite Thermometry” above).

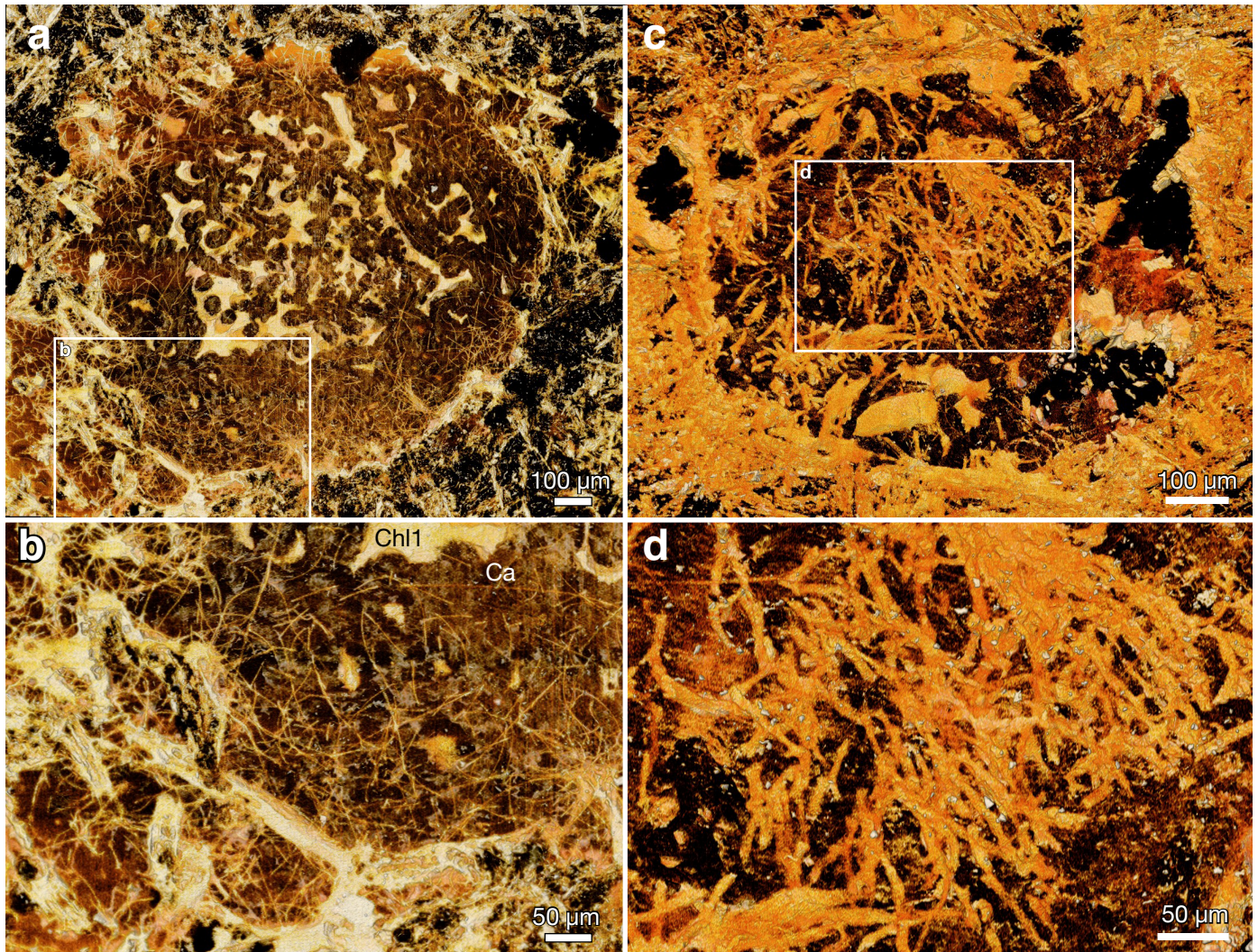


Supplementary Figure 1 | Plots showing chlorite composition. a, Compositions of Chlorite 1 from amygdals in IOS004 and ZBF061, Chlorite 2 from an amygdale in ZBF061, and Chlorite from the groundmass of ZBF061 in a triangular Si-Fe-Mg composition diagram. Chlorite 1 and Chlorite 2 are compositionally distinct, while groundmass chlorite falls between Chlorite 1 and Chlorite 2. **b,** Chlorite compositions plotted on the graphical chlorite thermometer of Bourdelle and Cathelineau²⁵. R²⁺ represents divalent cations

(Fe, Mg), R³⁺ represents trivalent cations (Al, Fe) and □ represents vacancies in atoms per formula unit (apfu) in chlorite based on 14 oxygen atoms. The shaded area is the compositional space for which the Bourdelle et al.¹⁵ thermometer is calibrated. The compositions of Chlorite 1 have >3.0 Si apfu and indicate crystallization temperatures of 175–250°C; Chlorite 2 compositions have <3.0 apfu Si and cluster around temperatures ≥350°C; Compositions and temperatures for groundmass chlorite plot between Chlorite 1 and Chlorite 2.

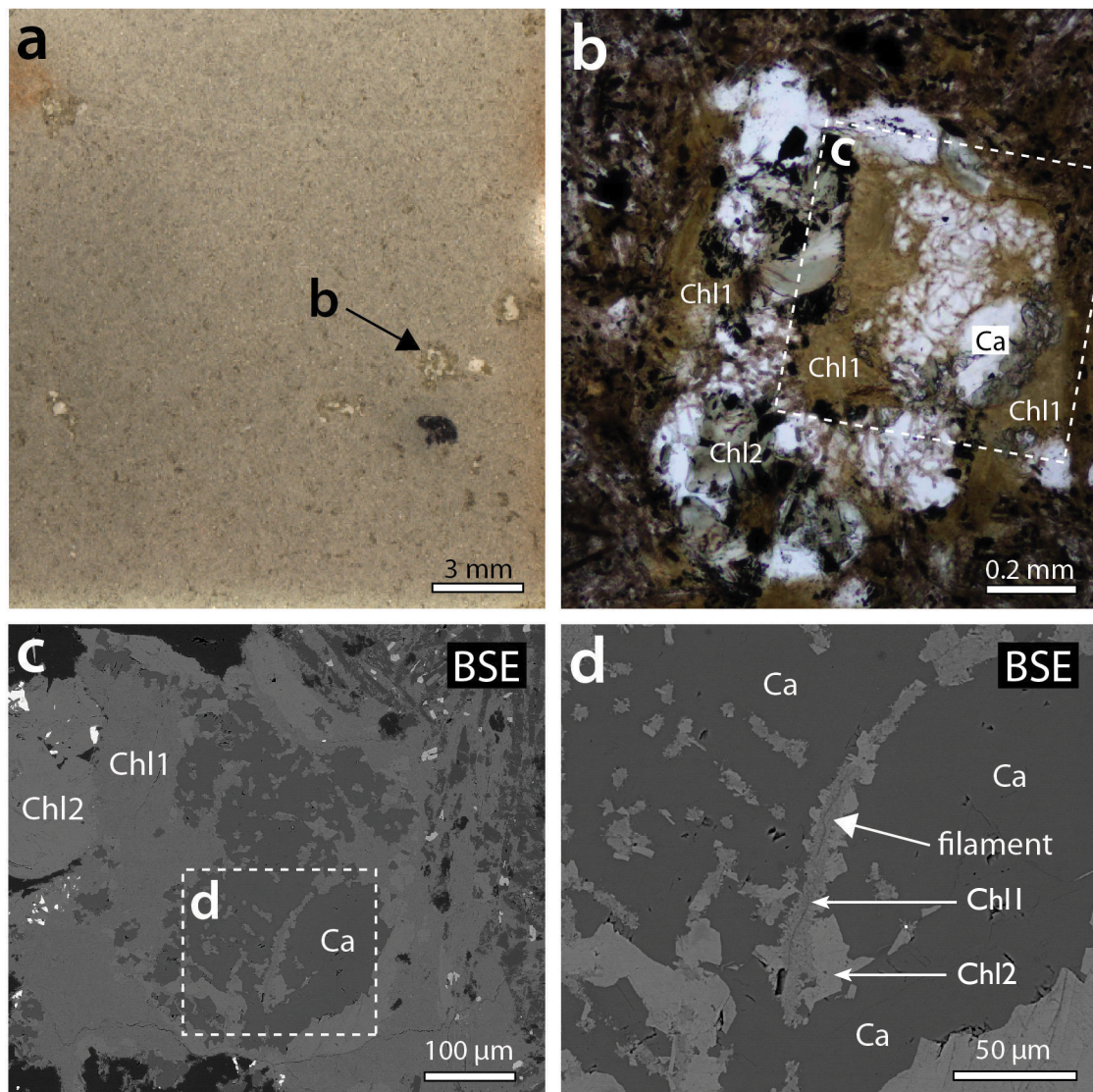


Supplementary Figure 2 | Diagrams showing unprocessed Raman spectra from a calcite-filled cavity from the lower part of the Ongeluk Formation. Spectrum (a) from the hyphae fossils demonstrates that they consist of chlorite, but no carbonaceous matter could be detected. A comparison can be made with spectrum (b) from the surrounding calcite. Chlorite bands (in Roman) are identified after data in Kleppe and Jephcoat²⁶ and calcite bands (in italics) after data in Downs²⁷.

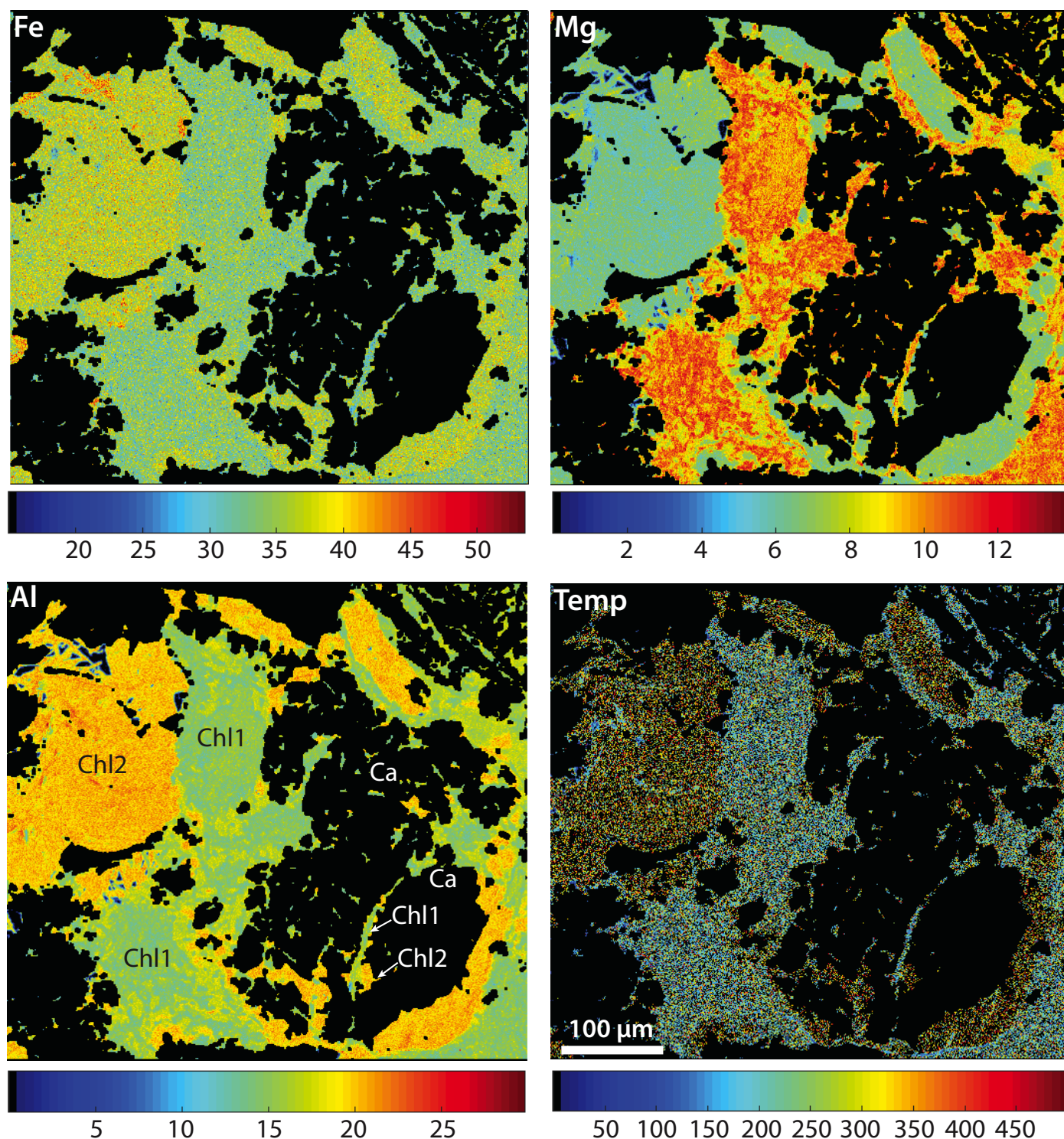


Supplementary Figure 3 | Ongeluk vesicles with filamentous fossils, SRXTM surface/volume renderings of thin section. a, b, Vesicle showing density difference between marginal and central filament network and succes-

sive deposition of calcite (dark) and chlorite (light) cement; Swedish Museum of Natural History X6138. c, d, Vesicle with conspicuous brooms; X6139. Legend: Ca, Calcite; Chl1, Chlorite 1.

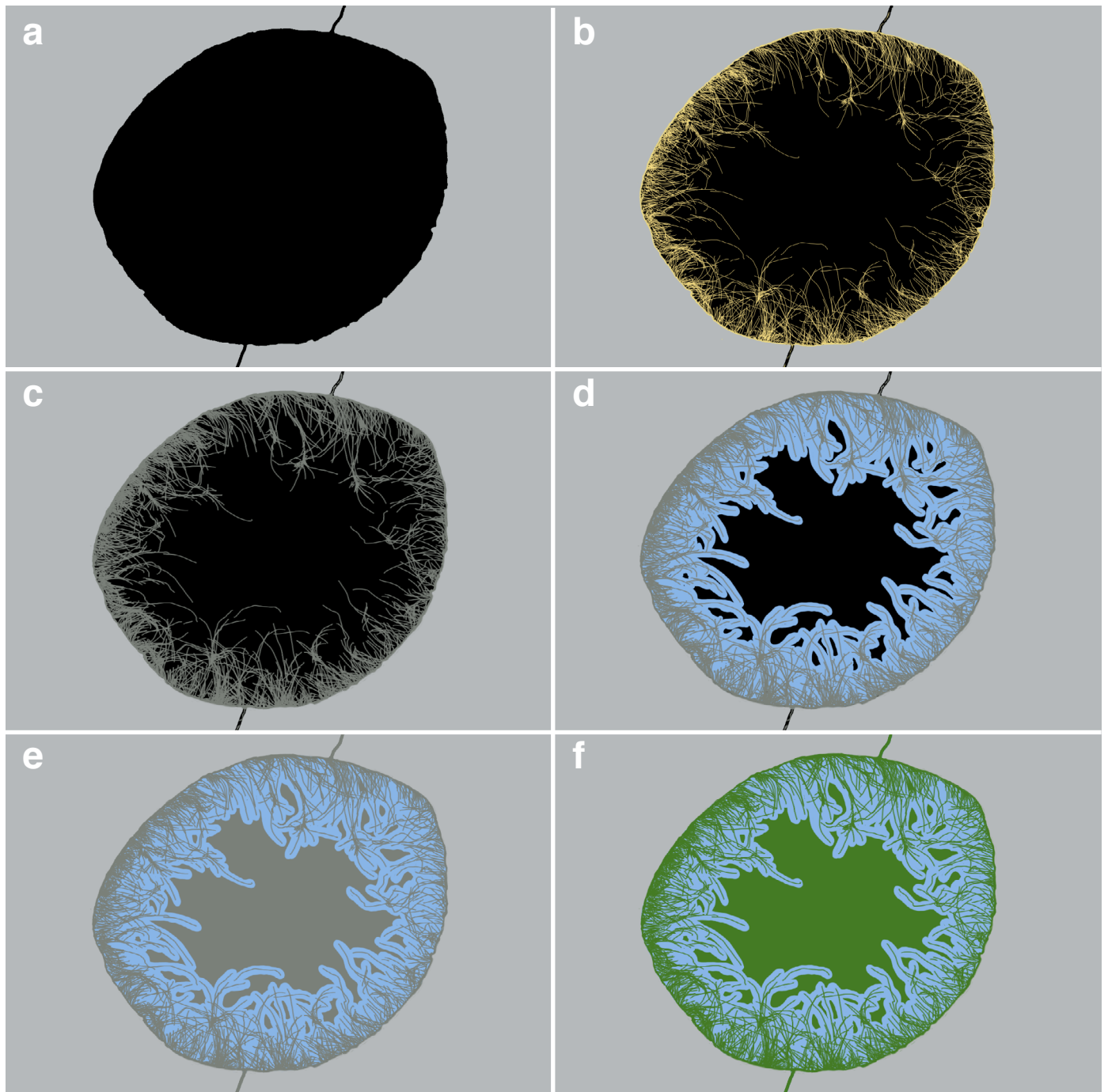


Supplementary Figure 4 | Ongeluk vesicular basalt, petrographic thin section, ZBF061, Swedish Museum of Natural History X6409. **a–b**, Plane-polarized transmitted light; **c–d**, Back-scattered electron images. Image region the same as for WDS maps in Supplementary Fig. 5. Legend: Ca, calcite; Chl1, Chlorite 1; Chl2, Chlorite 2.



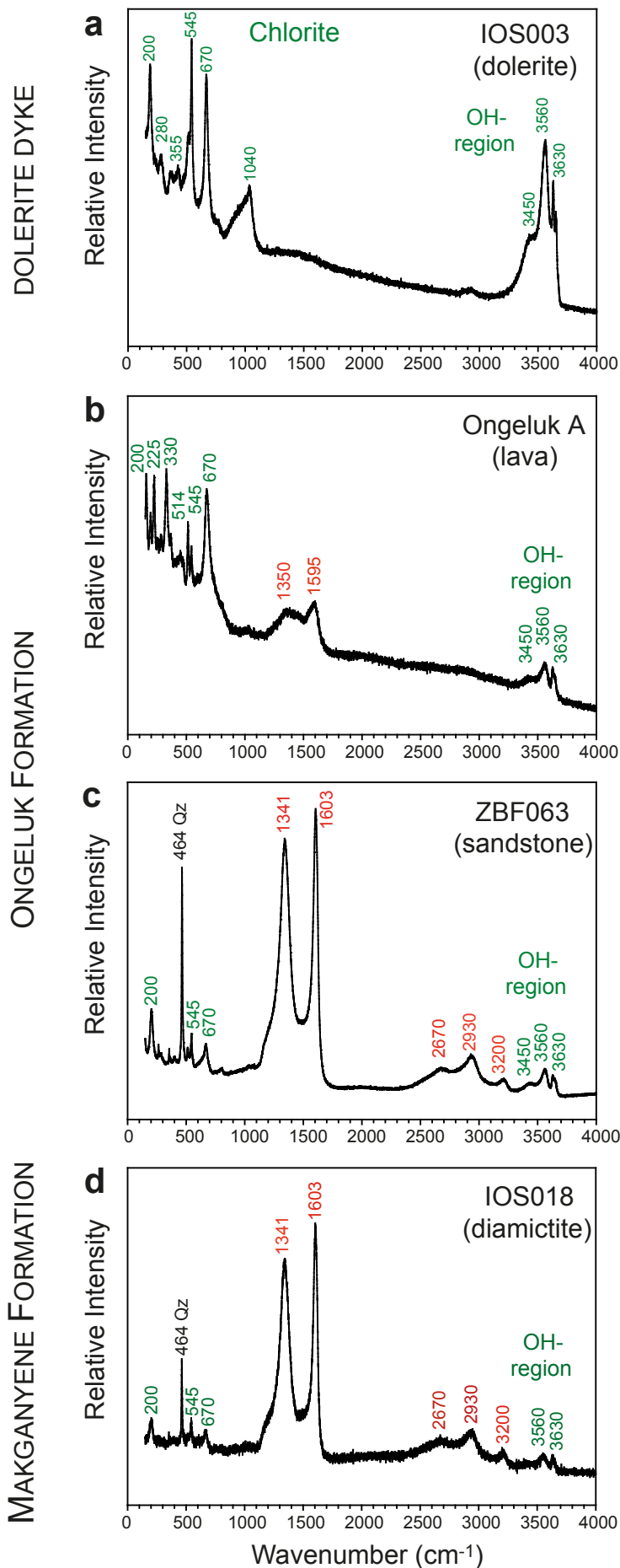
Supplementary Figure 5 | Quantitative element distribution maps for FeO, MgO and Al₂O₃ for chlorite from part of an amygdale in ZBF061, Swedish Museum of Natural History X6409. The maps were generated in XMapTools²⁸ from raw X-ray counts collected with wavelength dispersive spectrometers (WDS). A line of 25 point analyses (WDS) across Chlorite 1, and a separate line of 25 analyses across Chlorite 2, were used to calibrate the element maps (analysis points not shown). The temperature map (Temp)

was generated in XMapTools from the element distribution maps, as well as those of SiO₂, TiO₂, CaO, K₂O and Na₂O, using a pixel by pixel calculation based on the Bourdelle et al.¹⁵ geothermometer. The element maps show the distinct compositions of Chlorite 1 and Chlorite 2, which are reflected in the temperature map. A filamentous structure within calcite is composed of a central layer of Chlorite 1, but shows some growth of Chlorite 2 on its margins (see arrows). Legend: Ca, calcite; Chl1, Chlorite 1; Chl2, Chlorite 2.

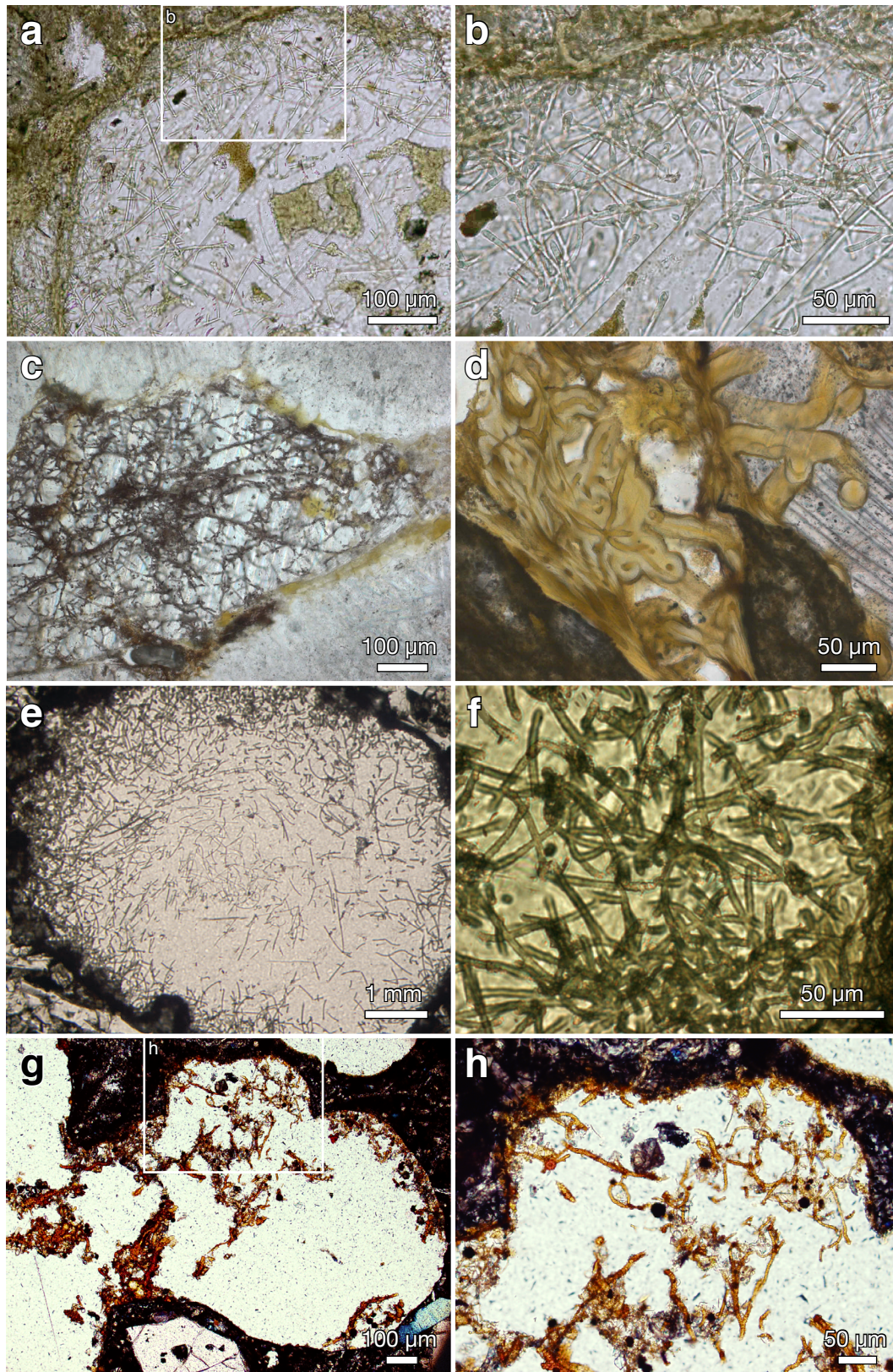


Supplementary Figure 6 | Proposed fossilization sequence of filaments in Ongeluk vesicle. **a**, Empty vesicle, connected to outside via a crack to allow inflow of water; **b**, Colonization of vesicle by mycelial organism; **c**, Mineraliza-

tion of filaments by smectite clay; **d**, Encrustation of filaments by calcite rims; **e**, Infilling of empty space with diagenetic clays; **f**, Metamorphic chloritization (Chlorite 1) of clay minerals in filaments and cavities.



Supplementary Figure 7 | Diagrams showing unprocessed Raman spectra of samples from a stratigraphic sequence comprising **(a)** a dolerite immediately above the Ongeluk sample, **(b)** volcanic matrix in the lower part of the Ongeluk Formation, **(c)** a sandstone at the base of the Ongeluk Formation, and **(d)** a diamictite in the underlying Makganyene Formation. Chlorite bands (green) are identified after data in Kleppe and Jephcoat²⁶, quartz (Qz) after data in Downs²⁷ and bands from carbonaceous material, CM (red), are identified after data in Rahl et al.¹⁸. The ordering of the CM structure is reflected by its Raman spectra and there is a correlation between the ~1350 and ~1600 cm⁻¹ bands (intensity, area, width) and maximum metamorphic temperature. The Raman spectra of CM in **b–d** can therefore be used as a geothermometer^{17,18} and indicate metamorphic temperatures for the sedimentary base of the Ongeluk Formation **(c)** and the Makganyene Formation **(d)** of around 370°C and for the disordered CM from the Ongeluk Formation **(b)** of approximately 200±30°C.



Supplementary Figure 8 | Comparative images of fossilized mycelia in vesicular volcanics of different ages. a, b, Palaeoproterozoic Ongeluk Formation; Swedish Museum of Natural History X6130, same specimen as in Fig. 2b. **c, d,** Middle Devonian, Arnstein, Germany; images courtesy of J. Peckmann²². **e, f,** Eocene, Ocean Drilling Programme Site 1224, North Pacific; from Schumann et al. 2004²¹, fig. 1A, B, reprinted by permission of the publisher (Taylor & Francis Ltd, <http://www.tandfonline.com>). **g, h,** Quaternary, Vesteris Seamount, North Atlantic, Geoscience Museum of the University of Göttingen, Germany (GZG-PB.4041)²⁹.

Supplementary Table 1. EDS spectra of Section IOS004.

IOS004	Am 1 Chl 1	Am 1 Chl 1	Am 1 Chl 1	Am 1 Chl 1	Am 2 Chl 1	Am 2 Chl 1	Am 2 Chl 1
Label	194	205	206	215	222	223	225
SiO ₂	27.11	27.61	27.79	27.30	27.80	28.12	27.79
TiO ₂	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Al ₂ O ₃	15.76	14.84	14.55	14.57	14.75	14.86	14.70
Cr ₂ O ₃	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FeO	32.18	31.67	31.93	31.46	31.66	31.71	32.32
MnO	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MgO	9.79	10.01	10.23	10.23	10.34	10.37	10.50
CaO	0.37	0.68	0.50	0.49	0.55	0.77	0.68
Na ₂ O	0.12	0.00	0.00	0.00	0.00	0.00	0.00
K ₂ O	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TOTAL	85.33	84.81	85.00	84.05	85.10	85.82	85.99
Cations/14 O×							
Si	3.06	3.13	3.15	3.12	3.14	3.14	3.12
Ti	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Al	2.10	1.98	1.94	1.97	1.96	1.96	1.94
Cr	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fe ²⁺	3.04	3.00	3.02	3.01	2.99	2.97	3.03
Mn	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mg	1.65	1.69	1.73	1.75	1.74	1.73	1.76
Ca	0.05	0.08	0.06	0.06	0.07	0.09	0.08
Na	0.03	0.00	0.00	0.00	0.00	0.00	0.00
K	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TOTAL	9.91	9.88	9.89	9.90	9.89	9.88	9.92
ToC	218	198	199	214	201	199	234

References

- Grobler, N. J. & Botha, B. J. V. Pillow-lavas and hyaloclastite in the Ongeluk Andesite Formation in a road-cutting west of Griquatown, South Africa. *Trans Geol Soc S Afr* **79**, 53–57 (1976).
- Cornell, D. H., Schütte, S. S. & Eglinton, B. L. The Ongeluk basaltic andesite formation in Griqualand West, South Africa: submarine alteration in a 2222 Ma Proterozoic sea. *Precambrian Res.* **79**, 101–123 (1996).
- Miyano, T. & Beukes, N. J. Physicochemical environments for the formation of quartz-free manganese oxide ores from the early Proterozoic Hotazel Formation, Kalahari manganese field, South Africa. *Economic Geology* **82**, 706–718 (1987).
- Gutzmer, J. et al. Ancient sub-seafloor alteration of basaltic andesites of the Ongeluk Formation, South Africa: implications for the chemistry of Paleoproterozoic seawater. *Chemical Geology* **201**, 37–53 (2003).
- Bau, M., Romer, R. L., Lüders, V. & Beukes, N. J. Pb, O, and C isotopes in silicified Mooidraai dolomite (Transvaal Supergroup, South Africa): implications for the composition of Paleoproterozoic seawater and “dating” the increase of oxygen in the Precambrian atmosphere. *Earth Planet Sci Lett* **174**, 43–57 (1999).
- Fairey, B., Tsikos, H., Corfu, F. & Polteau, S. U–Pb systematics in carbonates of the Postmasburg Group, Transvaal Supergroup, South Africa: Primary versus metasomatic controls. *Precambrian Res.* **231**, 194–205 (2013).
- Gumsley, A. et al. The timing and tempo of the Great Oxidation Event. *Proc. Natl. Acad. Sci. USA* **online**, 6 pp., doi:10.1073/pnas.1608824114 (2017).
- Rasmussen, B., Fletcher, I. R., Muhling, J. R., Mueller, A. G. & Hall, G. C. Bushveld-aged fluid flow, peak metamorphism, and gold mobilization in the Witwatersrand basin, South Africa: Constraints from in situ SHRIMP U–Pb dating of monazite and xenotime. *Geology* **35**, 931–934 (2007).
- Rasmussen, B., Bekker, A. & Fletcher, I. R. Correlation of Paleoproterozoic glaciations based on U–Pb zircon ages for tuff beds in the Transvaal and Huronian Supergroups. *Earth Planet Sci Lett* **382**, 173–180 (2013).
- Evans, D. A. D., Gutzmer, J., N. J. Beukes & Kirschvink, J. L. Paleomagnetic constraints on ages of mineralization in the Kalahari Manganese Field, South Africa. *Economic Geology* **96**, 621–631 (2001).
- Macfarlane, A. W. & Holland, H. D. The timing of alkali metasomatism in paleosols. *Canadian Mineralogist* **29**, 1043–1050 (1991).
- Phillips, W. J. Interpretation of crystalline spheroidal structures in igneous rocks. *Lithos* **6**, 235–244 (1973).
- Fowler, A. D., Berger, B., Shore, M., Jones, M. I. & Ropchan, J. Supercooled rocks: development and significance of varioles, spherulites, dendrites and spinifex in Archaean volcanic rocks, Abitibi Greenstone belt, Canada. *Precambrian Res.* **115**, 311–328 (2002).
- Sandstå, N. R., Robins, B., Furnes, H. & de Wit, M. The origin of large varioles in flow-banded pillow lava from the Hooggenoeg Complex, Barberton Greenstone Belt, South Africa. *Contributions to Mineralogy and Petrology* **162**, 365–377 (2011).
- Bourdelle, F., Parra, T., Chopin, C. & Beyssac, O. A new chlorite geothermometer for diagenetic to low-grade metamorphic conditions. *Contributions to Mineralogy and Petrology* **165**, 723–735 (2013).
- Lanari, P., Wagner, T. & Vidal, O. A thermodynamic model for di-trioctahedral chlorite from experimental and natural data in the system MgO–FeO–Al₂O₃–SiO₂–H₂O: applications to P–T sections and geothermometry. *Contributions to Mineralogy and Petrology* **167**, 19 pp., doi:10.1007/s00410-014-0968-8 (2014).
- Beyssac, O., Goffé, B., Chopin, C. & Rouzaud, J. N. Raman spectra of carbonaceous material in metasediments: a new geothermometer. *Journal of Metamorphic Geology* **20**, 859–871 (2002).
- Rahl, J. M., Anderson, K. M., Brandon, M. T. & Fassoulas, C. Raman spectroscopic carbonaceous material thermometry of low-grade metamorphic rocks: Calibration and application to tectonic exhumation in Crete, Greece. *Earth Planet Sci Lett* **240**, 339–354 (2005).
- McLoughlin, N., Staudigel, H., Furnes, H., Eickmann, B. & Ivarsson, M. Mechanisms of microtunneling in rock substrates: distinguishing endolithic biosignatures from abiotic microtunnels. *Geobiology* **8**, 245–255 (2010).
- Wacey, D. in *Early Life on Earth. A Practical Guide. Topics in Geobiology* **31** (ed David Wacey) 47–53 (Springer, 2009).
- Schumann, G., Manz, W., Reitner, J. & Lustrino, M. Ancient fungal life in North Pacific Eocene oceanic crust. *Geomicrobiol. J.* **21**, 241–246 (2004).
- Peckmann, J., Bach, W., Behrens, K. & Reitner, J. Putative cryptoendolithic life in Devonian pillow basalt, Rheinisches Schiefergebirge, Germany. *Geobiology* **6**, 125–135 (2008).
- Ivarsson, M., Bengtson, S., Skogby, H., Belivanova, V. & Marone, F. Fungal colonies in open fractures of subseafloor basalt. *Geo-Mar. Lett.* **33**, 233–243 (2013).
- Staudigel, H., Hart, S. R. & Richardson, S. H. Alteration of the oceanic crust: processes and timing. *Earth Planet Sci Lett* **52**, 311–327 (1981).
- Bourdelle, F. & Cathelineau, M. Low-temperature chlorite geothermometry: a graphical representation based on a T–Si–R²⁺ diagram. *European Journal of Mineralogy* **27**, 617–626 (2015).
- Kleppe, A. K. & Jephcoat, A. P. in *Earth's Deep Water Cycle. Geophysical Monograph Series* **168** (eds Steven D. Jacobsen & Suzan Van Der Lee) 69–93 (2006).
- Downs, R. T. The RRUFF Project: an integrated study of the chemistry, crystallography, Raman and infrared spectroscopy of minerals. *Program and Abstracts of the 19th General Meeting of the International Mineralogical Association in Kobe, Japan*, O03–13 (2006).
- Lanari, P. et al. XMapTools: A MATLAB®-based program for electron microprobe X-ray image processing and geothermobarometry. *Comput Geosci* **62**, 227–240 (2014).
- Ivarsson, M. et al. Zygomycetes in vesicular basanites from Vesteris Seamount, Greenland Basin – a new type of cryptoendolithic fungi. *PLoS ONE* **10**, 16 pp., doi:10.1371/journal.pone.0133368 (2015).