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Aluminosilicate haloes preserve complex life ~800 million years ago

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ABSTRACT

Mudstone-hosted microfossils are a major component of the Proterozoic fossil record, particularly dominating the record of early eukaryotic life. Early organisms possessed no biomineralised parts to resist decay and controls on their fossilisation in mudstones are poorly understood. Consequently, the Proterozoic fossil record is compromised—we do not know whether changing temporal/spatial patterns of microfossil occurrences reflect evolution or the distribution of favourable fossilisation conditions. We investigated fossilisation within the ~1,000 Ma Lakhanda Group (Russia), and the ~800 Ma Svanbergfjellet and Wynniatt formations (Svalbard and Arctic Canada). Vertical sections of microfossils and surrounding matrices were extracted from thin sections by focused ion beam milling. Elemental mapping and synchrotron-based infrared microspectroscopy revealed that microfossils are surrounded by haloes rich in aluminium, likely hosted in kaolinite. Kaolinite has been implicated in Cambrian Burgess Shale-type (BST) fossilisation and is known to slow the growth of degraders. The Neoproterozoic mudstone microfossil record may be biased to tropical settings conducive to kaolinite formation. These deposits lack metazoan fossils even though they share fossilisation conditions with younger BST deposits that are capable of preserving non-mineralising metazoans. Thus metazoans, at least those typically preserved in BST deposits, were likely absent from sedimentary environments before ~800 Ma.

KEYWORDS

Proterozoic Eon, early eukaryotes, clay minerals, complex life, metazoan antiquity, taphonomy

INTRODUCTION

Due to a lack of organisms with readily fossilised hard parts, it has long been observed that the pre-Cambrian fossil record is impoverished compared to its Phanerozoic counterpart [1]. However, over the last ~70 years palaeontologists have discovered a number of exceptionally preserved assemblages of diverse microfossils in Proterozoic rocks [2, 3]. Three principal lithologies record microfossils: early diagenetic cherts and phosphates, and mudstones [4]. Evidence from the record of eukaryotic microfossils suggests that the mudstone record dominates—47 of 59 fossiliferous assemblages in a recent compilation are mudstone-hosted [4]. We know little of the taphonomic processes that operated in mudstones [5]. What factors promoted fossilisation? What determines which mudstones preserve non-biomineralised microfossils? Understanding taphonomy in Proterozoic mudstones is vital to our efforts to chart Proterozoic evolution. Furthermore, we need to understand whether there are temporal or spatial biases to the Proterozoic microfossil record. Patterns of microfossil diversity may represent real ecological patterns, but they could also represent spatial/temporal changes in the availability of environmental conditions conducive to fossilisation. Our inability to unravel these scenarios fundamentally compromises the use of mudstone-hosted microfossils to reveal Proterozoic evolutionary history.

Examples of soft-tissue fossilisation [6] in Phanerozoic mudstones provide a basis for taphonomic hypotheses that can be tested on Proterozoic deposits. Cambrian Burgess Shale-type (BST) deposits are the most studied, preserving early metazoans including diverse organisms that lacked mineralised skeletons [7]. A variety of factors have been proposed to influence BST fossilisation, including oxidant supply, sediment composition, the propensity of soft tissues for replication in authigenic minerals (e.g., pyrite, phosphate, and clay minerals), and cementation of

the overlying sediment [see 8 for review]. A role for clay minerals has been posited more recently: experiments have shown that the aluminium- and iron-rich clays kaolinite and berthierine, which are major constituents of a large proportion of Cambrian strata hosting BST fossils [9, 10], slow the growth of decay bacteria [11] and increase decay resistance through clay-organic matter interactions [12-18]. Evidence is also emerging that kaolinite, in particular, may attach to or precipitate on some tissues early in diagenesis, helping to conserve them by providing a protective coating and/or facilitating polymerisation [14, 19, 20]. Intriguingly, elements indicative of these clays (e.g., aluminium and iron) have also been reported adjacent to some Proterozoic fossils, but their host phases are difficult to constrain [21-23]. Do aluminium- and/or iron-rich clay minerals promote fossilisation in Proterozoic strata? Do they promote fossilisation not only of metazoans but of other phylogenetic groups such as eukaryotic algae, or even cyanobacteria, which have diverse biopolymer compositions?

We investigated the taphonomy of microfossils (both probable eukaryotes and cyanobacteria—table 1) from three exceptional Tonian (1,000–717 Ma) deposits: the ~1,000 Ma Lakhanda Group (Russia) [24, 25], and the ~800 Ma Svanbergfjellet (Svalbard) [26] and Wynniatt (Canada) formations [27]. These assemblages include some of the best preserved and most diverse pre-Ediacaran fossils, and often include microfossils that are more fragile than the decay-resistant spheroidal acritarchs commonly recovered from Proterozoic strata [2, 4]. More fragile forms include relatively large (up to mm-scale) multicellular morphologies and those that possess intricate spines or processes [7, 24-27]. For example, microfossils from the Svanbergfjellet Formation may represent some of the oldest examples of multicellular eukaryotic green algae (chlorophytes) [26, 28]. We used a novel combination of microanalytical techniques to probe sediment mineralogy immediately adjacent to microfossil cell walls. These data

illuminate the role of clay minerals in Proterozoic taphonomy, allowing comparisons to BST fossilisation.

MATERIALS AND METHODS

Microfossils were identified in thin sections cut sub-parallel to sedimentary laminae that were obtained for previous studies (as in BST fossilisation [7], fossils are compressed into two dimensions parallel to laminae). Thin section number, university collection, and England Finder coordinates are given in table 1 for each microfossil/population studied. Thin-sections from Harvard University are deposited in the Paleobotanical Collections of the Harvard University Herbaria, while the sections from the University of Cambridge are in the collections of N. Butterfield. A filamentous microfossil (?sheathed cyanobacterium), *Siphonophycus*, was examined from the Lakhanda Group, whereas three microfossils were analysed from the Svanbergfjellet Formation: the chlorophyte *Proterocladus major*, the acanthomorphic acritarch (?eukaryote) *Germinosphaera fibrilla*, and an unidentified fragment of a larger microfossil (?eukaryote). A monostromatic population of spheroids, possibly *Ostiana* (?cyanobacterium) or the ?chlorophyte *Palaeastrum* (we refer to this population as ?*Ostiana*) and another *Siphonophycus* specimen were analysed from the Wynniatt Formation. For details on phylogenetic assignments, see table 1. The microfossils from each deposit were derived from single rock samples, one per deposit (Lakhanda, LK62, Cambridge; Svanbergfjellet, 86-G-62, Harvard; Wynniatt, 88-KL-131, Cambridge). Vertical sections (~30 µm x 10 µm x 1 µm) of microfossils and adjoining matrix were extracted perpendicular (or sub-perpendicular) to sedimentary laminae from the thin sections using focussed ion beam (FIB) milling at the Harvard Center for Nanoscale Systems (CNS) and attached to copper transmission electron microscopy

(TEM) grids. Milling was performed on a FEI Helios 660 Dual-Beam FIB/SEM (scanning electron microscope), equipped with an Autoprobe 400 micromanipulator.

Vertical sections were imaged using the SEM on the FEI Helios 660 at CNS and also on a Carl Zeiss Merlin SEM equipped with an Oxford Instruments X-Max^N 150mm^2 X-ray detector at the University of Oxford Department of Materials (ODM). Energy dispersive X-ray spectroscopy (EDS) at ODM was used to map elemental distributions across vertical sections for all samples except *Germinosphaera* (which was destroyed in sample manipulation before maps could be generated). In order to reduce sampling volume (thereby increasing spatial resolution) and reduce charging effects, EDS was carried out at voltages below 10 kV, with higher energy analysis to confirm the identify of elements when required (see Supplementary Material table S1 for configurations). Maps were processed using AZtec v3.3 and the TruMap function.

Synchrotron-based Fourier Transform Infrared (FTIR) microspectroscopy at the MIRIAM beamline of Diamond Light Source was used to identify the mineral hosts of elemental variations in all vertical sections. The beamline is coupled to a Bruker Vertex 80V FTIR spectrometer and Hyperion 3000 microscope equipped with a 50 µm, LN_2 cooled, midband MCT detector. 256 scans were coadded at 4 cm^{-1} spectral resolution per point. The infrared focal spot was confined to a diffraction limited area using slits of effective aperture 3 x 3 µm at the sample, via x36 optics in transmission geometry with numerical aperture (NA) = 0.5. The sample was mapped across this aperture with step size 1 µm, oversampling with respect to the aperture size. In these conditions, the resulting spatial resolution is expected to be diffraction limited and wavelength (ʎ) dependent, approximately at the Abbe resolution limit = ʎ /2NA = ʎ (at NA 0.5). The spatial resolution is about 2.8 µm for the M-OH spectral region of interest.

Finally, powder X-ray diffraction (XRD) was performed on each rock sample using a PANalytical Empyrean diffractometer at the Oxford Department of Earth Sciences, employing a Co Kα source and a PIXcel-1D detector. A substitute for the Svanbergfjellet sample, from the same locality/stratigraphic horizon, was obtained from Cambridge collections (sample 99-L-15) as no 86-G-62 material remains. Mineral identifications were confirmed using the ICDD (International Centre for Diffraction Data) Powder Diffraction File-4+ database (http://www.icdd.com/products/pdf4.htm) and the reference intensity ratio method [29]. Clay mineral species were distinguished by weak but diagnostic peaks commonly manifested as a composite reflection from 060 and/or 33-1 [30].

RESULTS

SEM imaging of the vertical sections revealed the cross-sectional structure of the microfossils (Fig. 1), which vary in thickness. The Svanbergfjellet microfossil *Proterocladus* is only tens of nm thick and hardly perceptible, while the Lakhanda *Siphonophycus* specimen and both the *Germinosphaera* specimen and the unidentified microfossil fragment from Svanbergfjellet are up to ~0.5 μm thick. All specimens from the Wynniatt Formation are intermediate in thickness (~0.2–0.4 μm). In some cases, microfossil walls have split and infilled with matrix, as in some of the Wynniatt ?*Ostiana* individuals, but the majority are intact. The Wynniatt ?*Ostiana* cluster is evident on two sedimentary laminae in the sample and thus may represent multiple colonies or one colony that has been infiltrated by sediment during deposition (Fig. 1). The *Siphonophycus* specimen from Lakhanda may be split into two or represents two superimposed filaments (Fig. 1). SEM images confirmed that microfossils are compressed sub-parallel to sedimentary laminae. The lithologies of the samples are similar, with most sediment

grains <<1 μm and only sparse grains ~1 μm, around which microfossils may be deflected: e.g., the vertical section of the Lakhanda *Siphonophycus* (Fig. 1). Larger grains are normally rounded, although some oblong sub-angular grains, which can reach >5 μm in maximum dimension (Fig. 1) with long axes parallel to sedimentary laminae, are associated with the Svanbergfjellet microfossils.

EDS analysis revealed that the microfossils are composed of carbon (Fig. 1), although the result was ambiguous for the Svanbergfjellet *Proterocladus* specimen, probably reflecting its very thin cross-section and the relatively large volume producing X-rays which, even at 5–10 kV, can have a masking effect on local elemental enrichments. The Lakhanda *Siphonophycus* specimen and the Wynniatt ?*Ostiana* specimens are enriched in sulphur. The sediment surrounding all the microfossils is largely similar (see Supplementary Material, Fig. S2), dominated by silicon and oxygen with minor aluminium, carbon, chlorine, iron, magnesium, nitrogen, phosphorous, potassium, sodium, and sulphur, reflecting a siliciclastic matrix with contributions from both organic matter and diagenetic minerals. Calcium is also present but sparsely distributed, likely reflecting a minor admixture of carbonate minerals. Larger rounded quartz grains are present (identified by a dominantly silicon and oxygen composition). Larger sub-angular grains in Svanbergfjellet vertical sections are enriched in magnesium and iron, indicative of chlorite.

Aluminium is generally enriched adjacent to microfossil carbon compared to the matrix, often forming a halo (Fig. 1). Aluminium haloes with a thickness <3 μm are particularly apparent surrounding the Svanbergfjellet unidentified microfossil fragment and the Lakhanda *Siphonophycus* specimen. None of the Wynniatt vertical sections shows a contiguous halo, but there are discontinuous aluminium enrichments adjacent to microfossils. The only vertical

section for which aluminium enrichments adjacent to microfossils were not identified unambiguously is that of the Svanbergfjellet *Proterocladus* specimen; however, as noted regarding its carbon composition, this may be due to its thin cross-section and EDS masking effects.

SEM imaging provides evidence that the aluminium enrichments occur in platy materials, presumably clays. SEM of the Wynniatt *Siphonophycus* vertical section shows a halo of platy material, parallel to sedimentary laminae, extending <0.5 μm around the microfossil (Fig. 2). A layer of matrix characterised by a lack of the larger grains or pseudo-hexagonal crystals that are common elsewhere (possibly muscovite) extends <1 μm beyond this layer. Coarser crystals often display planar interlocking boundaries, suggesting a phase of overgrowth after physical deposition. These observations indicate differential chemistry and mineralogy adjacent to the microfossil compared to that of the matrix. The finely crystalline and platy nature of the material hosting the aluminium enrichments identified by EDS suggests that aluminium may be bound largely in a clay mineral structure.

Synchrotron-based FTIR microspectroscopy confirmed that the most likely clay mineral host of these aluminium enrichments is kaolinite and its metamorphic products. Kaolinite can be identified using FTIR by a series of three M-OH bands at ~3620, ~3652, and ~3694 cm^{-1} [31]. For randomly oriented powders, the ~3620 and ~3694 cm^{-1} bands are more intense than the ~3672 cm^{-1} band; their frequencies are also sufficiently distinct from illite-group and chlorite-group minerals to allow unambiguous identification of kaolinite. FTIR microspectra from all Lakhanda and Svanbergfjellet vertical sections clearly differentiated these bands (Fig. 3). Vertical sections of the Wynniatt Formation fossils showed a broad M-OH band between ~3500 and ~3700 cm^{-1}, with no observable kaolinite bands (Fig. 3). This likely reflects a dominance of

illite/muscovite in Wynniatt clay mineralogy [31]. Any kaolinite, if present, is diluted beyond the detection limit in FTIR microspectra. Mild contact metamorphism of Wynniatt strata [32] may have transformed kaolinite to illite/muscovite [33]. Microfossil carbon is not recorded in our FTIR microspectra. Other Proterozoic microfossils are known to produce FTIR spectra indicative of their carbon composition [34]. Its absence in our analysis likely reflects a drowning of any organic signature given the limited amount of microfossil material compared to the mineral matrix.

The total area under each band provides a proxy for the distribution of minerals. When mapped across the vertical sections the areas under the ~3694 cm^{-1} band and the entire M-OH region show some variation between specimens (Fig. 4) but cannot be fully matched to the EDS maps (Fig. 1, S2). In the Lakhanda *Siphonophycus* and Svanbergfjellet vertical sections both FTIR bands are weaker on and around the microfossils, a contrast that is especially evident near hotspots (red) where the bands are locally strong. This is indicative of a lack of OH within microfossils at the ~2.8 μm resolution of the FTIR map, rather than a lack of OH groups around microfossils. In the Lakhanda *Siphonophycus* vertical section the region where OH bands are weaker extends down vertically, perhaps corresponding to the larger quartz grain below the fossil (Fig. S2). The source of the FTIR band hotspots is not evident in light microscopy or EDS images (Fig. 1, S2), but overall we can conclude that kaolinite is present across the bulk of the mineral phase of the four FTIR maps of the vertical sections in Fig. 4. The large black region on the left side of the Lakhanda *Siphonophycus* FTIR maps is where the band strength is high and saturated with respect to the colour scale in the image (>4.6 or >13 for the 3694 cm^{-1} and full M-OH band respectively). This apparent increase in band strength may include optical effects of the interaction between the infrared beam and the metallic weld close (<5 μm) to the attachment of

the sample to the TEM grid. The ratio of the ~3694 cm^{-1} band to the entire M-OH region reflects the distribution of kaolinite with respect to total illite [31]. At the ~2.8 μm spatial resolution available, a fairly constant abundance of kaolinite, with respect to total illite, across each vertical section is implied by the similar ratios, with no clear correspondence to the microfossils. The black zig-zag patterns along the edges of the vertical section are artefacts resulting from optical scattering from the vertical section. These biomineralogical data from synchrotron-based FTIR microspectroscopy confirm that kaolinite and/or its metamorphic products are present in all vertical sections. Although the FTIR maps do not fully resolve the distribution of minerals with the necessary spatial resolution, in concert with EDS maps they provide a compelling case for kaolinite enrichment adjacent to microfossils.

Further confirmation that kaolinite represents a component of the fossiliferous rocks is evident in bulk powder XRD analyses of hand samples from each locality (table 2), although these do not sample individual laminae. All samples are dominated by muscovite (34–74%, mean = 57.7, std. dev. = 21, n=3) and quartz (26–58%, mean = 37, std. dev. = 18.2, n=3); the Svanbergfjellet sample contains minor chlorite (7%) in addition. The reference intensity ratio method detected kaolinite (8%) only in the Lakhanda sample. However, weak but diagnostic peaks manifested as a composite reflection from 060 and/or 33-1 showed that kaolinite is also present in the Svanbergfjellet sample. Relative abundance of clay species can be obtained as a linear function of the area underlying these peaks [30]. This method shows that kaolinite represents 30.7% of total clay (glauconite + illite type 1 + illite type 2 + kaolinite) in the Svanbergfjellet sample, and 66.2% in the Lakhanda sample. The high kaolinite content of the Lakhanda sample is consistent with mineralogical studies of these strata [35]. The absence of kaolinite from powder XRD and the high illite content (90%) of the Wynniatt sample is

consistent with the broad M-OH band in the FTIR data, perhaps reflecting complete transformation of any precursor kaolinite to illite/muscovite during contact metamorphism [32]. 060 and/or 33-1 composite reflections suggest that glauconite (10%) is also present in the Wynniatt sample.

DISCUSSION

Kaolinite and Proterozoic fossilisation

The identification of aluminosilicate haloes surrounding microfossils, hosted in kaolinite and its metamorphic products illite/muscovite, prompts comparisons to Cambrian BST fossilisation. Strata with BST fossilisation globally are enriched in berthierine (a diagenetic product of kaolinite when iron is present) [9, 10]. Recently it has also been observed that metazoan fossils from the Burgess Shale at the Walcott Quarry (Canada) are themselves enriched in kaolinite compared to their surrounding mudstone matrix [19, 20]. The kaolinite enrichment on Burgess Shale fossils is attributed to bonding between the fossil organic matter and the mineral during organism decay, which is inferred to have slowed or arrested subsequent transformation of fossil-associated kaolinite to other minerals during greenschist facies metamorphism [20, 36]. This interpretation implies that local decreases in pH induced by decay [37] result in positively charged carcass organic matter, facilitating bonding with negatively charged edge sites on kaolinite [e.g., 38, 39, 40]. These edge sites, which account for 10–20% of the surface area of the mineral [39], are known to be relatively acidic, increasing the likelihood that they would persist in a negatively charged state at relatively low pH, maximising the probability of binding to organic matter [41, 42]. It is unclear whether kaolinite attached to fossil organic matter was from the sediment, or whether it precipitated *in situ*. It has been argued that

the chemical properties of kaolinite were a significant factor in the fossilisation of Burgess Shale metazoans [19, 20]. Kaolinite is known to promote polymerisation and adsorption of a variety of organic molecules [38, 39, 43], and to trigger kerogen maturation upon pyrolysis [44]. It can stabilise pre-existing organic cross-links via the donation of electrons, reducing double bonds [45], as well as inhibit the growth of bacteria that promote decay [11]. Experimental studies have shown that kaolinite-organic interactions often promote the conservation of morphology in decaying metazoan tissues [12-18].

The aluminosilicate haloes surrounding the microfossils studied here suggest that kaolinite attachment and/or *in situ* precipitation onto microbial cell-walls or enclosing sheaths, via a similar process to BST fossilisation, was a significant factor in the preservation of microfossils in Proterozoic mudstones. These data add to recent studies that report concentrations of elements indicative of clays (e.g., aluminium and iron) adjacent to other Proterozoic fossils [21-23]. The organisms studied here likely represent a variety of evolutionary clades, including eukaryotic chlorophyte algae and cyanobacteria [24-28]. The cell walls and extracellular sheaths of these organisms were composed of different biomolecules (table 1) than those of the metazoans preserved in BST deposits, with variable resistance to decay [46, 47]. Cyanobacteria cells are commonly enclosed by sheaths composed of chemically distinct carbohydrate fibrils which are relatively resistant to decay [48]—in the case of *Siphonophycus*, for example. ?*Ostiana*, on the other hand, may not possess a sheath; the cell walls likely comprised lipids and proteins [49]. Chlorophytes are dominated by carbohydrates, including cellulose, which make up the cell walls. And acanthomorphic acritarchs, such as *Germinosphaera*, are commonly characterised by highly resistant walls of aliphatic composition similar to sporopollenin or algaenan [50]. In contrast, metazoans, like those represented in BST

deposits, are composed of a range of biomolecules including proteins, carbohydrates and lipids, although sclerotised and cuticularised tissues are preferentially preserved [51]. The similarity of kaolinite enrichments in fossils representing these different clades suggests that this type of fossilisation is not biopolymer specific. Nor is it morphology dependent—the fossils studied here range in morphology from filaments and spheroids, to multicellular remains, and in the case of Burgess Shale fossils, macroscopic forms. Indeed, numerous studies have already shown that clay minerals commonly attach to the organics of microbial organisms such as cyanobacteria in natural and experimental systems [52-57]. The enrichments of kaolinite adjacent to microfossil cell walls argues not only for a role for kaolinite in fossilisation but, through organism-mineral interactions, a role for incipient microfossils in facilitating kaolinite enrichment in the first place. A similar process has also been used as an explanation for silica enrichments surrounding microfossils [58].

Biases of the Proterozoic shale-hosted microfossil record

Identifying a taphonomic role for kaolinite in Proterozoic mudstones, the dominant lithology for early microfossils [4], indicates that the Proterozoic mudstone-hosted fossil record may be biased to environmental settings that were rich in kaolinite or hosted conditions conducive to its formation, giving us pause for thought when considering temporal/spatial patterns in the Proterozoic microfossil record. Kaolinite today is primarily sourced from tropical weathering regimes where drainage is high and soil pH low [33]. Palaeogeographic reconstructions suggest that each of the studied deposits (Lakhanda Group – Siberia, Svanbergfjellet Formation – East Svalbard, and Wynniatt Formation – Laurentia) formed in tropical to mid palaeolatitudes [59], consistent with this interpretation. Moreover, reconstruction

of seawater pH through geologic time suggests that Neoproterozoic oceans were characterised by lower pH than their modern counterparts [60]. Such a palaeoenvironmental bias to fossilisation is likely to be more acute for organisms where preservation of delicate morphology is required. The three assemblages studied include rarely preserved morphologies that were presumably more fragile (e.g., multi-celled, slender spines/processes) than those from other Proterozoic localities with microfossils [24-27]. It is not clear from our work whether the presence of kaolinite in the local environment is sufficient or kaolinite is required at a specific concentration.

Implications for our understanding of the antiquity of metazoans

Our results have specific implications for understanding the emergence of metazoan life. There is a disconnect between molecular clock estimates for the antiquity of metazoans and their earliest body fossils. A recent molecular analysis placed the last common ancestor of extant metazoans at 833–650 Ma [61], yet unambiguous body fossil evidence extends only as far as ~580 Ma [e.g., 62]. Where are the missing pre-580 Ma metazoan fossils? Can the gap be explained by taphonomic bias [63]? BST fossilisation provides an unusually comprehensive picture of Cambrian metazoan diversity [61, 64-66]; even microscopic metazoans are represented [67, 68]. The demonstration that some Neoproterozoic mudstones share taphonomic pathways with BST deposits identifies at least three Tonian targets for early metazoan fossils. However, the microfossils in these assemblages represent microbial eukaryotes and bacteria [2, 24-28, 69-71]. Some large morphologically complex microfossils in Ediacaran mudstones have been interpreted as egg and/or diapause cysts of metazoans [72] and a similar interpretation has been advanced for a population of Tonian fossils [73], but other interpretations have been proposed [74]. Macroscopic fossils, e.g., *Chuaria* and *Tawuia*, occur in two of the deposits

(Svanbergfjellet and Wynniatt) [24-27] and, although recent evidence suggests that some of these may be multicellular, there is no evidence that they represent metazoans [75]. The lack of metazoan fossils in these deposits despite BST conditions conducive to their fossilisation, may indicate that metazoans had not evolved by ~800 Ma. If so, this provides a soft maximum age (a maximum age based on the probability that fossils of this age are not metazoans) for their appearance, as suggested previously [67] and employed in some molecular clocks [64] but without consideration of taphonomy. It could be argued that, even though metazoans are not a component of the three assemblages that we investigated, they could be present in others of similar age, or that small early metazoans are not preserved even by BST fossilisation. Nonetheless this soft maximum age can now be applied with greater confidence.

Clay mineral-organic interactions and the search for fossils on Mars

Our results may also be relevant to the search for fossilised life on Mars [76]. A variety of clay minerals have been identified on the basis of orbital infrared spectroscopy, including illite, kaolinite, smectites, chlorites, and serpentine minerals [77-79]. Clay minerals have been identified in crustal rocks that may encompass a wide range of palaeoenvironmental conditions. These deposits have been variably interpreted as *in situ* weathering profiles, fine-grained clastic sediments, and hydrothermally altered crust [77-79]. Our data suggest that specific interactions between kaolinite mineral surfaces and solution may have facilitated the polymerisation of organic molecules – a key step in promoting fossilisation. These reactions, though, are dependent on the pH of the solution relative to the acidity of clay edge sites. It is reasonable to assume that these same principles apply to the early Martian surface; in suitable chemical conditions, kaolinite may have served a similar taphonomic role to that envisaged for samples described

here. However, available data indicate that clay-rich deposits on early Mars experienced a much broader range in pore/bottom water pH, variable contact times with liquid water, and large fluctuations in ionic strength [79, 80]. Thus, taphonomically favourable clay mineral assemblages on Mars are best diagnosed in light of depositional and diagenetic constraints on fluid chemistry and a mechanistic consideration of clay mineral-organic interactions.

CONCLUSIONS

An understanding of taphonomy is critical to the interpretation of morphology and the environmental/temporal ranges of fossil taxa, and is particularly important for Proterozoic palaeobiology where biomineralised tissues are absent. Kaolinite or its metamorphic equivalents are associated with all microfossils in the three Tonian deposits we studied, despite their phylogenetic, compositional, and stratigraphic diversity. Our search for new microfossils, particularly fragile metazoans, in Proterozoic strata should focus on sites where kaolinite is likely to be present. These search criteria may also be valid when exploring other planets, such as Mars [76], for evidence of past life. Further studies should expand this investigation to a wider variety of Proterozoic fossiliferous mudstones, such as those of the Ediacaran Doushantuo Formation which do not contain complex metazoans [81], in order to determine the prevalence of appropriate taphonomic conditions across time and palaeoenvironments.

AUTHOR CONTRIBUTIONS

RPA, NJT, KDB, and DEGB designed the research. AHK provided fossils. AA prepared vertical sections. RPA and GMH analysed vertical sections using SEM and EDS. RPA, GC, MDF, and IL analysed vertical sections using synchrotron-based FTIR microspectroscopy. RPA and NJT

analysed fossiliferous rocks using XRD. RPA wrote the manuscript with discussion and input from all authors.

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DATA ACCESSIBILITY

Synchrotron-based Fourier Transform Infrared microspectroscopy data collected at the Diamond Light Source and used to build figure 4 are available in the Supplementary Material.

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FIGURE CAPTIONS

Figure 1: Studied microfossils with elemental distributions. Light photomicrographs of fossils in thin section. Locations of extracted vertical sections shown by pink bars. SEM micrographs of extracted vertical sections and EDS maps showing carbon and aluminium distributions. Carbon comprises the microfossils which are surrounded by a concentration of aluminium. Secondary electron (SE) or backscatter electron (BSE) SEM images were utilised to minimise charging effects. Specimen details are listed in table 1. SEM and EDS operating conditions for each map/image are listed in Supplementary Material (table S1). Light and SEM images of the Svanbergfjellet *Germinosphaera* specimen/vertical section are given in Supplementary Material (Fig. S1).

Figure 2: SEM secondary electron (SE) micrograph of a focussed ion beam-milled vertical section of *Siphonophycus* from the Wynniatt Formation. Microfossil cross-section is surrounded by a halo of a platy mineral likely to be kaolinite. Courser crystals with planar interlocking boundaries (likely muscovite) and large quartz grains are also highlighted. SEM operating conditions are listed in Supplementary Material (table S1).

Figure 3: Representative synchrotron-based FTIR microspectra. Top: Representative total spectra showing an average of spectra numbers 188, 189, 190, 203, 204, 205, 218, 219, 220 from Lakhanda *Siphonophycus*. Both M-OH bands are present plus a broad silicate peak at ~1000 cm^{-1}

which is not resolved in sufficient detail to interpret its mineralogical components. Below are representative spectra from the M-OH region for each vertical section plotted on the same vertical scale. Characteristic bands at ~3620, ~3652, and ~3694 cm^{-1} are recorded for the vertical sections from Lakhanda and Svanbergfjellet, while a single broad band between ~3500 and ~3700 cm^{-1} is recorded for the vertical sections from Wynniatt. Representative spectra numbers: Lakhanda *Siphonophycus* 237, Svanbergfjellet *Proterocladus* 150, *Germinosphaera* 150, and microfossil fragment 263, and Wynniatt ?*Ostiana* 240 and *Siphonophycus* 204. See Supplementary Material (table S2) for FTIR data spectra numbers.

Figure 4: Synchrotron-based FTIR microspectroscopy maps of compositional variation indicated by the areas below the ~3694 cm^{-1} band, the entire M-OH region (integrated band intensities in arbitrary units), and the ratio of these two areas, for Lakhanda and Svanbergfjellet vertical sections which show clearly defined M-OH bands. The light microscopy images of vertical sections (left) indicate the position of the microfossils (red boxes). See text for further details.

Table 1: Interpreted phylogenetic affinities and associated biopolymers of the studied microfossils: Lakhanda [24, 25], Svanbergfjellet [2, 26, 28, 70], Wynniatt [27]. Biopolymers taken from [46-50].

Table 2: Bulk powder XRD Results. Percentage of each rock sample composed of different minerals is calculated by the Reference Intensity Ratio Method [29]. Clay speciation is also presented as a % of total clay (glauconite + illite 1 + illite 2 + kaolinite) using the relative areas of weak but diagnostic peaks from 060/33-1 [30]. Qtz = quartz, Musc = muscovite, Chl =

chlorite, Kaol = kaolinite, Gl = glauconite, and Il1 and Il2 = two varieties of illite. Lakhanda sample LK62, Svanbergfjellet sample 99-L-15, Wynniatt sample 88-KL-131.

Figure S1: Left, light photomicrograph of Svanbergfjellet *Germinosphaera* specimen in thin section; pink bars show location of extracted vertical sections. Right, SEM backscatter electron (BSE) micrograph of extracted vertical section. SEM operating conditions are listed in Supplementary Material (table S1).

Figure S2: Selected EDS maps of vertical sections. Distributions of calcium, chlorine, iron, potassium, magnesium, oxygen, sulphur, and silicon are plotted. Pink bars on light photomicrographs of fossils in thin section show locations of extracted vertical sections. Scanning electron micrographs of extracted vertical sections are shown for reference. Secondary electron (SE) or backscatter electron (BSE) SEM images were utilised to minimise charging effects. SEM and EDS operating conditions for each map/image are listed in Supplementary Material (table S1).

Table S1: SEM and EDS operating conditions: accelerating voltages and detector types.

Table S2: Raw synchrotron-based FTIR microspectroscopy data.

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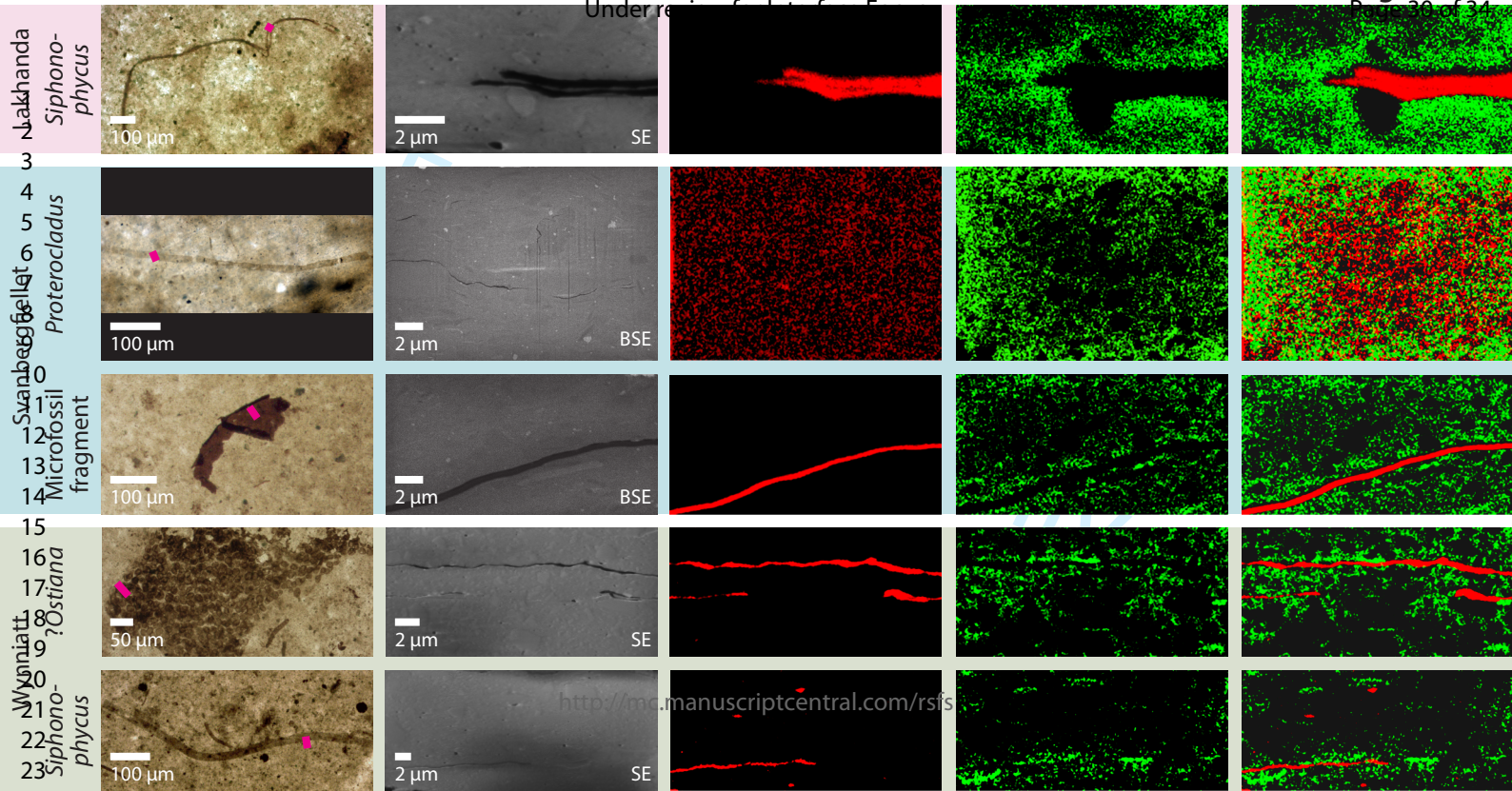
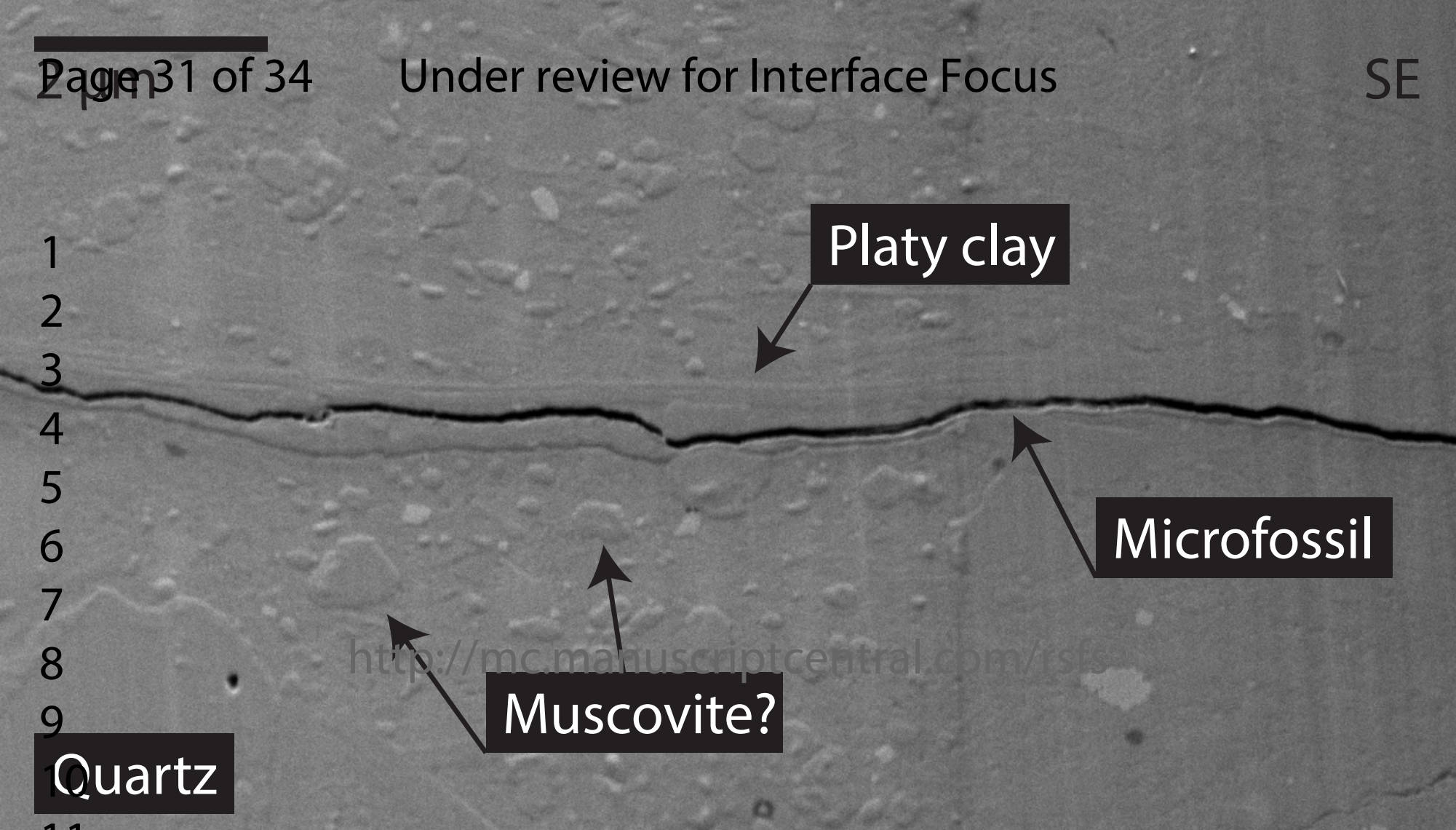
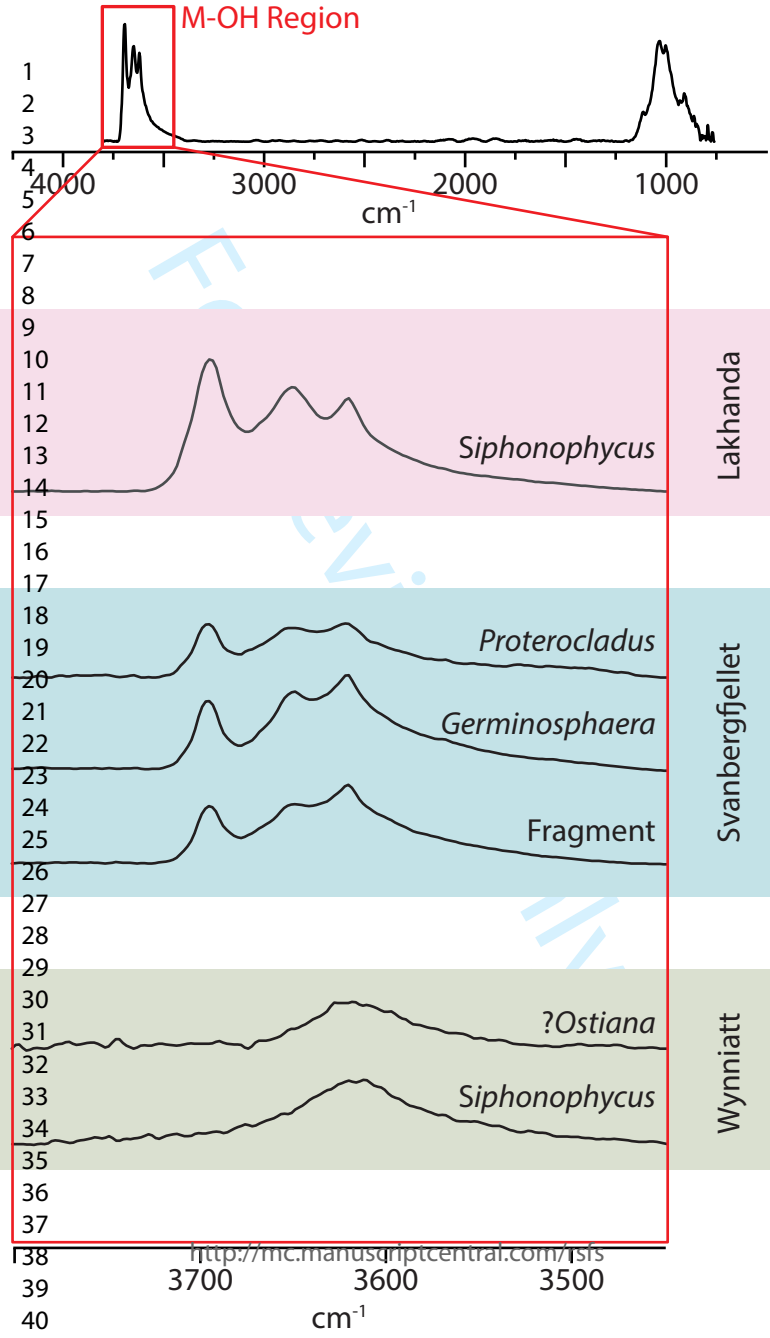
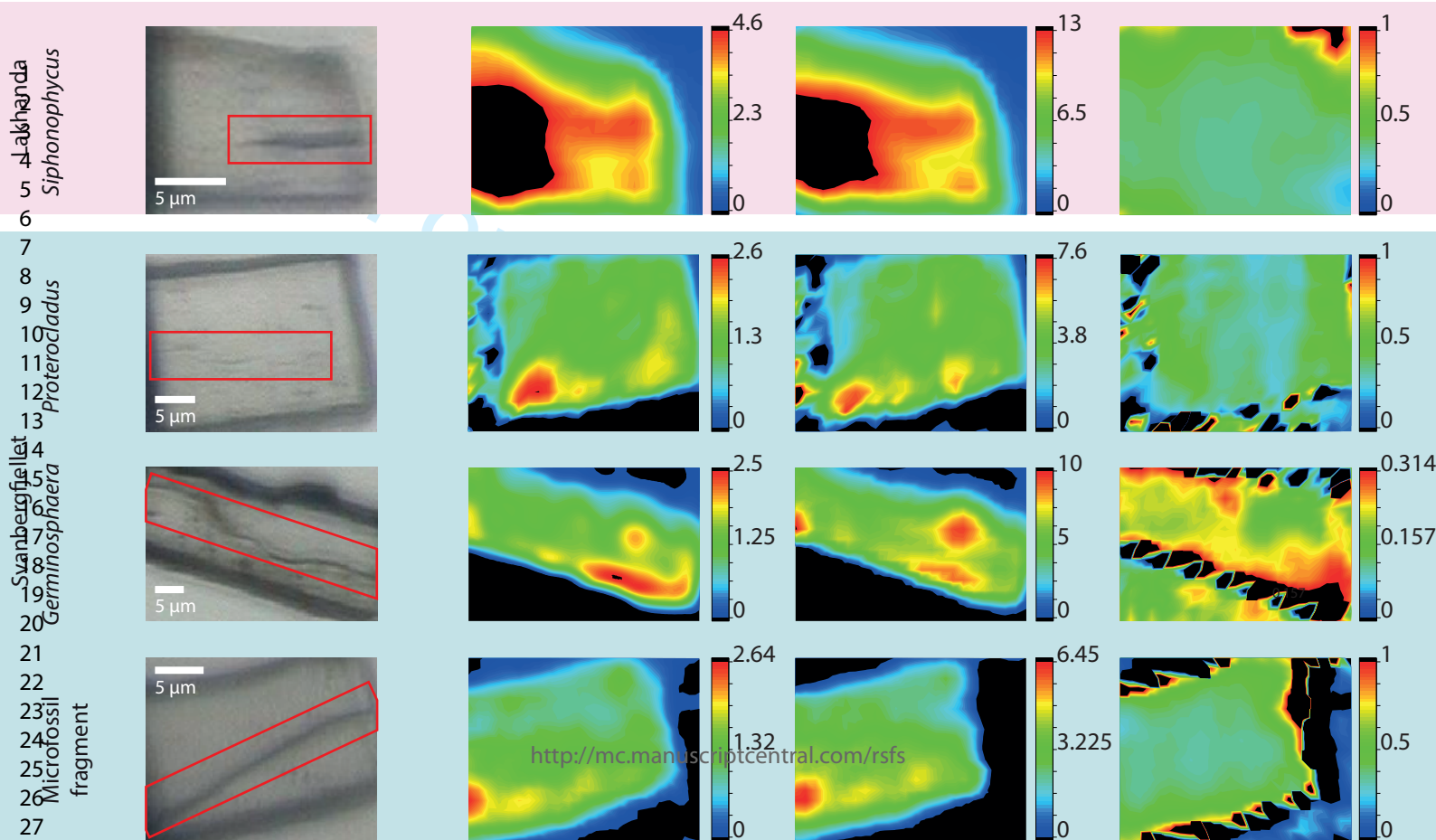





M-OH Region
4000
3000
2000
1000
cm-1
Siphonophycus
Lakhanda
Proterocladus
Germinosphaera
Fragment
Svanbergfjellet
?Ostiana
Siphonophycus
Wynniatt
3700
3600
3500
cm-1



	Phylogenetic affinity	Principal biopolymer	Thin-section, University collection, England finder coordinates
Lakhanda			
Siphonophycus	?cyanobacterium	Sheaths of carbohydrate fibrils	LK62, Cambridge, N32/2
Svanbergfjellet			
Proterocladus major	chlorophyte	Carbohydrates including cellulose	86-G-62-52, Harvard, N47/2
Germinosphaera fibrilla	?eukaryote (possible vaucheriacean alga or fungus)	Likely aliphatic composition similar to sporopollenin or algaenan	86-G-62-68, Harvard, Q48/4
Microfossil fragment	?eukaryote	Undetermined	86-G-62-54, Harvard, L26/2
Wynniatt			
?*Ostiana* (or possibly *Palaeastrum*)	?cyanobacterium (or ?chlorophyte)	Lipids and proteins with possible sheaths of carbohydrate fibrils (or, in the case of ?chlorophyte, carbohydrates including cellulose)	88-KL-131-2, Cambridge, D35/2
Siphonophycus	?cyanobacteria	Sheaths of carbohydrate fibrils	88-KL-131-2, Cambridge, Q46/2

	Reference Intensity Ratio %				060/33-1 % Total Clay			
	Qtz	Musc	Chl	Kaol	Gl	Il1	Il2	Kaol
Lakhanda	58	34	0	8	0	0	33.8	66.2
Svanbergfjellet	27	65	7	0	0	6.6	62.6	30.7
Wynniatt	26	74	0	0	10	90	0	0