Comparative multi-scale analysis of filamentous microfossils from the c. 850 Ma Bitter Springs Group and filaments from the c. 3460 Ma Apex chert

David Wacey1*, Kate Eiloart1 & Martin Saunders1,2

1 Centre for Microscopy, Characterisation and Analysis, The University of Western Australia, 35 Stirling Highway, Perth, WA 6009, Australia
2 School of Molecular Sciences, The University of Western Australia, 35 Stirling Highway, Perth, WA 6009, Australia

Abstract: Filamentous microfossils belonging to Cephalophytarion from the 850 Ma Bitter Springs Group have previously been used as key analogues in support of a biological interpretation for filamentous objects from the 3460 Ma Apex chert. Here we provide a new perspective on this interpretation by combining Raman data with correlative electron microscopy data from both Cephalophytarion and Apex specimens. We show that, when analysed at high spatial resolution, the Apex filaments bear no morphological resemblance to the younger Bitter Springs microfossils. Cephalophytarion filaments are shown to be cylindrical, comprising chains of box-like cells of approximately constant dimensions with lateral kerogenous walls and transverse kerogenous septa. They exhibit taphonomic shrinkage and folding, possess fine cylindrical sheaths and are permineralized by sub-micrometric quartz grains. They fulfil all established biogenicity criteria for trichomic microfossils. In contrast, Apex filaments do not possess lateral cell walls, are not cylindrical in nature, and vary considerably in diameter along their length. Their kerogenous carbon does not have a cell-like distribution and their chemistry is consistent with an origin as exfoliated phyllosilicate grains. This work demonstrates the importance of high-resolution data when interpreting the microstructure, and origins, of putative Precambrian microfossils.

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Filamentous microstructures found within hydrothermal black chert veins intruding the c. 3460 Ma Apex Basalt of Western Australia have been at the centre of a long-running and high-profile controversy surrounding Earth’s oldest cellular life. First described and interpreted as an assemblage of at least 11 species of filamentous prokaryotes (Schopf & Packer 1987; Schopf 1993), doubts were subsequently raised over the authenticity of these objects (Brasier et al. 2002, 2004, 2005, 2006, 2011) but a biological interpretation was in turn vigorously defended (Schopf et al. 2002, 2007; Schopf & Kudryavtsev 2009, 2012, 2013). More recently, evidence for an abiotic origin was provided by two studies, by Brasier et al. (2015) and Wacey et al. (2016a), that used electron microscopy to show that many Apex filaments comprise elongated stacks of angular aluminosilicate grains onto which carbon could have migrated. These researchers suggested that the morphology and chemistry of the Apex filaments could be explained by the hydration, heating and exfoliation of mica minerals, plus the redistribution and adsorption of barium, iron and carbon within an active hydrothermal system, a scenario consistent with the hydrothermal feeder vein geological setting (Brasier et al. 2005, 2011) of the host rock. One putative Apex species (Eoleptonema apex) has also convincingly been shown to be a carbon-filled intra-granular crack (Bower et al. 2016). Most recently, contrary to these data, proponents of a cellular origin have inferred that multiple biological metabolisms are represented within the assemblage of filamentous objects based on in situ carbon isotope data (Schopf et al. 2018).

Here, we focus on the most fundamental line of evidence that must be provided to assign a microfossil origin to an object, the possession of plausible biological morphology. In so doing, we revisit previous claims that the morphology of Apex filaments is comparable with that of younger definitive Precambrian permineralized filamentous organisms (e.g. Schopf & Kudryavtsev 2005, 2009; Schopf et al. 2007), filamentous organisms of the genus Cephalophytarion from the 850 Ma Bitter Springs Group were used as morphological analogues for Apex filaments, especially for Apex filaments assigned to the genus Primaevifilum, which are reported to be the most abundant in the assemblage (Schopf 1993) and from which most data have been presented (e.g. Schopf & Kudryavtsev 2012). This contribution provides correlative light microscopy, Raman microspectroscopy and higher spatial resolution electron microscopy data from Cephalophytarion filaments from the Ross River locality (as first described by Schopf 1968) of the Bitter Springs Formation. We then compare and contrast these data with data obtained in precisely the same manner from Primaevifilum filaments from the ‘Chinaman Creek microfossil locality’ of the Apex chert (Schopf 1993). We go on to show that as one increases the spatial resolution of data obtained the Apex Primaevifilum filaments are shown to be in no way morphologically comparable with Cephalophytarion microfossils, possessing no distinctive cellular morphology, and hence it is very difficult to envisage an origin as microfossils of filamentous prokaryotes. This work provides an important correlative link between different spatial scales of data collection, showing that higher resolution electron microscopy data can be deconstructed to construct a landscape of lower resolution Raman-scale mapping. In turn, this permits more refined abiotic models to be advanced for complex microstructures that have previously been explained solely using biological reasoning.

Material and methods

Sample localities

The Bitter Springs material studied here (sample RH5; GR [−23.58367, 134.49767]) was collected in 2017 from the Ross
River area c. 65 km ENE of Alice Springs. It was collected from a ridge 1.1 km NNE of the Ross River Resort (previously known as the Ross River Tourist Camp or Love’s Creek Homestead), and comprises part of a carbonaceous black chert lens hosted within a sedimentary carbonate succession, the same unit (to the best of our knowledge as global positioning system coordinates are not present in the original report) from which the original Ross River locality microfossils were described by Schopf (1968). Details of the geology of the Ross River area have been given by Wells et al. (1970).

The Apex material studied here (sample CHIN-3) comes from the original Chinaman Creek locality (Schopf & Packer 1987; Schopf 1993) and was collected in 2001. Data come from a series of thin sections all prepared from the same CHIN-3 hand sample as used by Brasier et al. (2015) and Wacey et al. (2016a, b). As detailed by Wacey et al. (2016a), it is prohibited to perform any destructive or intrusive analyses on the type specimens held by the Natural History Museum (NHM), London. Hence, standard (c. 30 µm thick) polished geological thin sections of CHIN-3 are as close to the type specimens as we can feasibly analyse by modern techniques (see Schopf et al. 2018). Crucially, filamentous microstructures found in CHIN-3 are morphologically comparable with those found in the type material held at NHM (which are thicker sections, hence the filaments often appear darker; see also extensive details given by Brasier et al. 2015 and Wacey et al. 2016a, plus discussion herein) and in the non-type material recently investigated by Schopf et al. (2018). Details of the geology of the Chinaman Creek area of the Apex Basalt, including evidence for a hydrothermal setting for the host black chert, have been given by Brasier et al. (2005, 2011). This hydrothermal geological setting for the host chert is now widely agreed upon, including by proponents of a cellular microfossil origin for these structures (e.g. Schopf & Kudryavtsev 2012).

Optical microscopy

The c. 30 µm thick, polished, uncovered thin sections were examined by optical microscopy (transmitted and reflected light) to gain an understanding of the filament distributions and morphologies, and to select the most appropriate samples for detailed study. This was carried out using Leica DM2500M and Zeiss Axioskop microscopes, with 5×, 10×, 20×, 50× and 100× objective lenses, located within the Centre for Microscopy, Characterisation and Analysis (CMCA) at The University of Western Australia (UWA). Images were captured using a digital camera and Toupview imaging software.

Confocal laser Raman microspectroscopy

Raman microspectroscopy was performed on a WITec alpha 300RA+ instrument with a Toptica Photonics Xtra II 785 nm laser source at the CMCA, UWA. Laser excitation intensity at the CMCA, UWA. Laser beam currents (0.79 nA then 0.23 nA). Final thinning was performed at lower voltage (16 kV and 1.3 nA current) followed by milling and imaging parameters optimized to suit the specific type of sample (i.e. carbonaceous and phyllosilicate-rich objects within a silica matrix). Briefly, regions of interest (ROI) were covered with a protective (c. 2 µm thick) platinum layer. Initial large trenches were milled either side of the ROI with a 21 nA Ga+ ion beam, and the trench faces were cleaned up using a 9.3 nA beam. Element mapping within the FIB-SEM using energy-dispersive X-ray spectroscopy (EDX) was performed on some cleaned trench faces to gain a preliminary understanding of the chemistry of the filaments and their surrounding matrix. SEM-BSE imaging was also performed on cleaned trench faces during the thinning process. On reaching a thickness of c. 1.5–2 µm the ROI was extracted using an in situ micromanipulator and attached to a Pelco copper TEM grid by welded platinum strips. This ‘welding’ protocol means that there is no carbon film underneath the wafer, simplifying subsequent carbon elemental mapping in the TEM. Subsequent thinning of the ROI was performed with decreasing ion beam currents (0.79 nA then 0.23 nA). Final thinning was performed at lower voltage (16 kV and 1.3 nA current) followed by face cleaning at 5 kV and 15 pA. Average final wafer thicknesses were in the range of 150–200 nm.

TEM analysis of FIB-milled wafers

TEM and STEM (scanning transmission electron microscopy) data were obtained using an FEI Titan G2 80-200 TEM/STEM with ChemiSTEM technology operating at 200 kV equipped with a Gatan SC1000 camera located in the CMCA at UWA. Crystal orientation and mass/density difference data were gained from high-
angle annular dark-field (HAADF) and bright-field (BF) STEM imaging. Energy-dispersive X-ray spectroscopy (EDX) via the ChemiSTEM system provided elemental maps. Lattice spacings of crystals were obtained via high-resolution TEM (HRTEM).

Reconciling EM and Raman data

We applied a Gaussian blurring filter using ImageJ software to our electron microscopy carbon elemental map data to mimic the effect that the lower resolution Raman technique would have on our ability to interpret the microstructure. The width of the Gaussian filter was progressively increased, starting with a 20 pixel full width half maximum (FWHM) followed by increases in increments of 10 pixels until a 150 pixel FWHM was reached. This was done to test how decreases in spatial resolution affect the imaged morphology of the structures of interest. Depending on the image magnification, the application of a Gaussian blur filter with an FWHM of between 100 and 150 pixels resulted in our electron microscopy data having a similar appearance to our Raman data and that of previously reported Raman data (e.g. Schopf et al. 2018). This is consistent with the ratio of the pixel size of our EDX maps and the resolution of the Raman technique.

Results

Nanoscale characterization of 850 Ma Bitter Springs microfossils

Optical and Raman data show that Cephalophytarion filaments comprise distinct, almost cubic compartments outlined by carbonaceous walls with kerogen-like composition (Fig. 1a–d), and these compartments are each infilled by quartz (Fig. 1d). Higher spatial resolution electron microscopy data from longitudinal sections through Cephalophytarion filaments reinforce the Raman data, showing both distinct carbonaceous lateral walls and carbonaceous transverse walls (septa) outlining cellular compartments (Fig. 1f).

Individual cells are c. 4 µm in maximum diameter and 3–5 µm in length, joined to form a cylindrical filament that is demonstrably circular to elliptical in latitudinal cross-section (Fig. 1e). Although there is some minor variation in cell morphology owing to taphonomic effects such as cell wall shrinkage, the carbonaceous septa in individual Cephalophytarion filaments are more or less regularly spaced and each are of very similar thickness (Fig. 1f). For example, septa maximum thicknesses for the filament shown in Figure 1 are between 70 and 100 nm, and the spacings between septa in this organism show a relatively narrow range of between 3.1 and 4.2 µm (n = 11).

Individual cellular compartments had evidently lost much of their cell contents prior to silicification, although preservation in places is of such high quality that potential remnants of organic cell contents remain, mostly found close to the inner margins of the cell walls (Figs 1e, f and 2a, b). This organic material frequently outlines subvertical features; on rare occasions such a carbon distribution is entirely consistent with previous Raman analyses of Apex filaments (Schopf et al. 2002, 2007, 2018; Schopf & Kudryavtsev 2009, 2012; see for example figs 1 and 2 of Schopf et al. 2018). For the most part, structures that could be interpreted as lateral cell walls are absent, with the interiors of filaments characterized by clumps of carbon, which are significantly thicker than the septa observed in Cephalophytarion, interspersed with non-carbonaceous regions of variable morphology and chemical composition (Figs 4b–d and 5e, d). Frequently, the pattern of carbon distribution expected for cells (i.e. narrow carbonaceous cell walls interspersed with thicker compartments filled by quartz or other minerals) is reversed, with apparent thick solid masses of carbon (but see electron microscopy data below for true distribution of carbon) being separated by very narrow mineralic ‘walls’ (Fig. 5b and c, arrows).

Carbonaceous particles are also dispersed throughout the matrix in the vicinity of the filaments (Figs 4b, c and 5b–d). Anatase is relatively common in the matrix (see Bower et al. 2016) and can also occur within the filaments where the morphology of the grains may give a false impression of cellular compartmentalization (i.e. an anatase grain surrounded by carbon mimics the morphology of a cell; Fig. 4d).

Correlative electron microscopy performed on the same Primaevifilum filaments provides a clearer picture of their morphology. Lateral carbonaceous ‘walls’ outlining the filaments are mostly absent (Figs 4g, 5e, f and 6a, b). Instead, the carbon dominantly forms narrow subvertical features; on rare occasions these may superficially resemble the septa of filamentous organisms but throughout the majority of the filaments they define angular structures, with variable orientations, variable lengths and thicknesses, and irregular spacings between one another (Figs 4g, 5e, f and 6a, b). Compared with the septa of Cephalophytarion, these subvertical features have significantly more variable thicknesses (<10 to 300 nm; n = 40), and much more variable spacings between one another (<30 nm to c. 2 µm; n = 38) within a single filament. More than 80% of measured spacings are <250 nm meaning that there are an order of magnitude more vertical carbonaceous components in a given length of Primaevifilum filament compared with the same length of Cephalophytarion filament, and the majority of ‘septa’ spacings in Primaevifilum are too small to feasibly equate to cellular compartments (Figs 4g, 5e, f and 6a, b).
In longitudinal cross-section, many *Primaevifilum* filaments change width dramatically along their length, varying between <1 and >4 µm in width perpendicular to the plane of the geological thin section (Figs 4g and 6a). The filaments are not cylindrical, having transverse cross-sectional profiles of diverse, mostly angular morphologies (Fig. 4e and f). Branching is also common (Wacey et al. 2016a).

Electron microscopy also shows that the *Primaevifilum* filaments possess a much more complex chemistry than is observed using Raman microspectroscopy alone. This chemistry was first described by Brasier et al. (2015) and Wacey et al. (2016a) from specimens in a separate CHIN thin section, and the new specimens examined herein provide some additional data. The filaments comprise chains of angular sheet-like aluminosilicate mineral grains. As well as ubiquitous Al, Si and O, these contain minor K (c. 2–4%), a patchy distribution of Ba (<1%) (Figs 6 and 7) and unevenly distributed trace amounts of Fe and Mg. Al and Si occur in a roughly equal amount. HRTEM data herein (Fig. 8b) reinforce the electron diffraction data of Wacey et al. (2016a) showing the dominant 1 nm spacing of the Al–Si–O sheets characteristic of 2:1 phyllosilicates; for example, micas and some clay minerals such as illite. It is not possible to constrain their exact mineralogy such is the heterogeneity of the distribution of minor elements (but see ‘Plausibility of an abiogenic formation mechanism’ in the Discussion below for the possible origin of this phase).

At least three other phases, in addition to kerogen, are present within the filaments. An iron–oxygen-rich phase occurs as small needles between sheets of the phyllosilicate and towards the outer tips of many of the silicate sheets (Fig. 6b). This phase also contains minor As (c. 1%) (Figs 6d and 7), and sometimes trace amounts of Ni and/or Cr. HRTEM reveals a crystal structure consistent with the iron oxyhydroxide mineral goethite (Fig. 8c), potentially with minor distortions owing to the small amounts of As, Ni and/or Cr in the lattice. Titanium oxide (anatase) occurs infrequently as nano- to micro-scale grains within and just exterior to the filaments (Fig. 4d). Finally, quartz, which forms the vast majority of the matrix of the host rock, is often found intergrown with these other phases within the filaments (Figs 4d and 6a, f).
The plate-like aluminosilicates also frequently occur near the *Primaevifilum* filaments (Fig. 8d–f) and elsewhere in the CHIN thin sections, and here they may not be organized into chains and thereby lack a filamentous appearance; some of these occurrences are also associated with carbon and goethite. Hence, there is a morphological continuum of aluminosilicate objects, only some of which resemble filamentous microfossils. Within *Primaevifilum* filaments carbon does not appear to preferentially occur associated with any specific mineral phase (Fig. 6b). Carbon is also found elsewhere within the quartz matrix, where it can occur at quartz grain boundaries or, most notably, in partially carbon-filled fractures in the quartz, which occur in close proximity to some *Primaevifilum* filamentous objects (Fig. 8e–i).

**Discussion**

### Microfossil criteria

In any test of a microfossil origin for an ancient microstructure, the primary trait that must be demonstrated is that of biological morphology (Schopf & Walter 1983; Buick 1990; Schopf et al. 2010). If an object does not demonstrate a plausible biological morphology it cannot be considered a microfossil even if it possesses secondary characteristics that may be consistent with biology (e.g. kerogenous chemistry with a δ¹³C of biological magnitude). This point cannot be overstated and it is this point that separates a true cellular microfossil from other objects that may be remnants of life but are composed of remobilized organic material. Indeed, proponents of a microfossil origin for the Apex filaments have...
stated that resolution of the Apex microfossil controversy hinges on ‘whether the Apex fossils are cellular and composed of kerogenous carbon’ [present author’s italics] (Schopf & Kudryavtsev 2012).

Three of the critical morphological features of fossilized filamentous trichomes highlighted in previous appraisals of putative microfossils are (after Schopf et al. 2010): (1) they should be cylindrical (or, if distorted during preservation, initially cylindrical) with their shape defined by distinct carbonaceous lateral walls; (2) they should have essentially uniform diameter throughout their lengths (again, with allowances for distortion during preservation, and for tapering towards their apices); (3) they should be partitioned by septa into discrete cells (box-like or spheroidal) of more or less uniform size and shape that are predominantly hollow (later mineral infilled). These morphological features are obviously specific to a discussion of filamentous trichomes (the interpretation put forward by Schopf (1993) for these Primacavifillum objects) and different, less stringent, criteria may apply for potential non-cellular cylindrical microfossils such as those derived from sheaths.

It should be noted at this point that we are not using Cephalophytarion as an exact potential analogue for the Primacavifillum filaments as the geological settings, and hence types of biota probably inhabiting the two localities, are different. We are using Cephalophytarion for two reasons: (1) Cephalophytarion was one of the main organisms used by Schopf and colleagues in their initial comparative work with Apex filaments (e.g. Schopf & Kudryavtsev 2009) so it must be investigated whether these comparisons hold up under higher resolution scrutiny; (2) Cephalophytarion is a well-preserved example of a bona fide filamentous Precambrian organism, fossilized by silica, that should contain the fundamental morphological features of a fossilized trichome outlined above.

Our electron microscopy analysis of Cephalophytarion filaments shows that they fully satisfy the criteria for a filamentous microfossil listed above, being of essentially uniform diameter, with cylindrical cross-sections outlined by clear carbonaceous lateral cell walls, and angular, plate-like mineralic morphology. (g) SEM-BSE image of a longitudinal section through the filament (from dashed blue line in (a)) showing absence of lateral carbonaceous walls and significant changes in diameter along its length. The filament mostly comprises angular stacks of plate-like aluminosilicate mineral crystals (light grey) interspersed with quartz grains (mid grey; blue arrows), carbon and void space (black), plus iron oxyhydroxides (white). Significant differences between SEM-BSE images in (e)–(g) and those shown in Figure 1e and f should be noted.
aluminosilicate minerals; transverse carbonaceous features have morphologies inconsistent with biological septa; and the spaces between these features are too small to be compatible with an origin as cellular compartments. Nor is the morphology of the \textit{Primaevifilum} filaments comparable with that of other potential filamentous microfossils such as those derived from sheaths. The lack of fundamental cellular features cannot be explained by simple physical distortion during preservation as there is no evidence for folding, tearing or flattening of original cell walls.

\textbf{Could Apex filaments be heavily degraded microfossils?}

The above data and discussion show that the Apex \textit{Primaevifilum} specimens cannot be well-preserved filamentous bacteria as previously claimed (e.g. Schopf 1993), as they possess none of the morphological features of such organisms, particularly when viewed at high resolution. However, we next need to consider whether \textit{Primaevifilum} filaments could be heavily degraded versions of ancient organisms. That is, we must address whether it is plausible that biodegradation, thermal decay, and diageneric and metamorphic mineral growth or replacement could be responsible for transforming an original trichome of box-like cells (or indeed any other biological morphology) into the acellular morphology now observed.

It has previously been claimed that the Apex filaments may represent heavily degraded sheathed colonies of coccolid cyanobacteria (Kazmierczak & Kremer 2002, 2009). This was based on superficial morphological similarities (examined using only low-resolution light microscopy) between some Apex filaments and thermally altered remnants of colonial coccolid cyanobacteria from Silurian cherts of Poland. However, 3D analysis of these Silurian colonies show that although they may have flattened, roughly filamentous cross-sections perpendicular to bedding, they are disc-shaped parallel to bedding (Kazmierczak & Kremer 2009). Three-dimensional analysis of Apex specimens clearly shows that they are not disc-shaped in any orientation (Schopf et al. 2007; Schopf & Kudryavtsev 2009; Wacey et al. 2016b; and herein) so they are not analogous to the younger Silurian objects. It should be noted also...
that the Apex filaments do not occur in bedded cherts, as erroneously assumed by Kazmierczak & Kremer (2002, 2009); they occur in a black chert vein intruded at a high angle into basalt, a very different environment from that of their Silurian specimens. Furthermore, based on recent molecular clock estimates, such an interpretation seems implausible because sheathed colonial coccoid cyanobacteria probably evolved some 600 myr or more after the formation of the Apex chert (Sanchez-Baracaldo et al. 2017).

Where heavily degraded carbonaceous objects co-occur in assemblages with much better preserved individuals that possess clear biological morphology (e.g. Knoll et al. 1988) a case can be made for their interpretation as microfossils. In such cases this can be a valid argument so long as one can show a logical progression from well-preserved to poorly preserved morphotypes. Such an argument cannot be made for the Apex chert because there are no specimens that possess biological morphology. Furthermore, the presence of additional identical aluminosilicate grains in the host chert in the vicinity of the Primacovifilum filaments, often occurring in pairs or as short stubby chains but without the distinct microfossil-like filamentous organization, emphasizes that the Primacovifilum filaments are merely one end-member of a morphological continuum of mineralic objects.

The mineralogy of the Apex filaments does little to support or refute a biogenic origin. Micro-organisms can occasionally be fossilized by diagenetic aluminosilicates, and K-rich clays such as illite have been reported within c. 1 billion-year-old cells from NW Scotland (Wacey et al. 2014). However, the small crystal size of clay minerals usually results in very high quality preservation of the organisms, with cell walls (and even some cell contents) remaining intact and only nano-scale modification of the overall morphology of a given cell (Wacey et al. 2014). There is also usually some spatial relationship between the chemistry of the clays and cellular components: for example, Wacey et al. (2014) found that Fe- and Mg-rich clays preferentially occurred in the vicinity of cell walls, and K-rich clays preferentially occurred in cell interiors, suggesting some biological influence on the nano-scale distribution of clay chemistry. Although it is possible that increased thermal degradation and chemical modification could have significantly overprinted original biochemical patterns in the silicates, some zonation of silicate phases relative to biological components may still be expected to be preserved even in highly metamorphosed rocks (Bernard et al. 2007, 2008). It must also be remembered that the oldest example of this style of microfossil preservation is c. 2.5 billion years younger than the Apex chert and occurred in a freshwater lacustrine setting (Wacey et al. 2014) not in a complex hydrothermal system.

There is some evidence that nano-domains between sheets of clays may be more conducive to preservation of organic matter (e.g. Curry et al. 2007) than, for example, interfaces between clay minerals and quartz, which may partially account for the greater amount of organic material in the interior of the filaments than at the outer border. However, if the Apex filaments are highly degraded filamentous organisms it would require the lateral cell walls of a trichome (or sheath walls) to have been almost completely destroyed by mineral growth, and a large portion of this carbon to be redistributed along crystal faces perpendicular to the original walls in the interior of the filament, a scenario that to the best of our knowledge is unproven in the geological record. Alternatively, it would require the lateral cell walls to have been destroyed but much of the cell contents to have been preserved along crystal boundaries, which is contrary to the known degradation pathways of organisms (e.g. Knoll & Barghoorn 1975).

**Plausibility of an abiogenic formation mechanism**

If the Apex filaments are not heavily degraded microfossils then what alternative mechanism may explain their formation? Here, additional data from the newly examined specimens permit us to propose a more comprehensive abiotic formation mechanism, building upon the work of Brasier et al. (2015) and Wacey et al. (2016a, b).

Micas (e.g. biotite, muscovite) are common accessory minerals in several rock types that originally formed the Pilbara Craton, in particular the massive granitoids that form >50% of the East Pilbara Terrane (Van Kranendonk et al. 2007). Micas are 2:1 layered silicates where each layer comprises two (Si,Al)O₄ tetrahedra and...
one MO₆ octahedron, where M is commonly Al, Mg or Fe. The strong negative charge of these layers is counterbalanced by interlayer cations such as K⁺, with these electrostatic forces holding the layers together (Deer et al. 2013). The chemistry of the Apex 2:1 phyllosilicates is complex but is closest to muscovite in composition, with an approximate 1:1 ratio of Al:Si, and K present as the next most abundant element. Other micas such as the biotite subgroup, or K-rich 2:1 clay minerals such as illite, would be expected to have much lower Al:Si ratios (Deer et al. 2013). However, the Apex phyllosilicate is severely depleted in K compared with natural muscovite and has elevated levels of Ba, plus trace amounts of Fe and Mg, which are all rather heterogeneously distributed. HRTEM shows that the 1 nm sheet spacing has been retained throughout much of the phyllosilicate structure but there are hundreds of occasions where the layers have separated and are now filled with carbon, goethite or void space.

Muscovite layers are not as easily separated (exfoliated) as some other micas such as biotite, but exfoliated muscovite has been observed in natural geological settings (e.g. Rutherford 1987) and created in the laboratory (e.g. Jia et al. 2017). Muscovite exfoliation typically involves heating in the presence of water and organic solvents to replace some of the interlayer K ions and weaken the interlayer electrostatic attraction (Nicolosi et al. 2013; Tominaga et al. 2017). We propose that the Apex hydrothermal dykes provided suitable conditions for the partial exfoliation of muscovite creating a range of mineralic objects, some of which resemble filamentous microfossils. This is consistent with partial loss of K and presence within the filaments of Ba, As, Ni and Cr, elements typically found within the Apex hydrothermal system (see Brasier et al. 2005). The Pilbara black chert dykes are carbon-rich (Lindsay et al. 2005) and this organic material may have helped facilitate exfoliation of the muscovite, and would also have readily migrated onto muscovite crystal faces and/or been trapped in the narrow gaps that opened up between the muscovite sheets (see Medeiros et al. 2009). This indigenous carbon was supplemented by non-syngenetic carbon ingress as potentially evidenced by multiple partially carbon-filled...
fractures that occur close to several *Primaevifilum* filaments (Fig. 8e–i). This is consistent with previous reports of multiple generations of carbon within these samples, including a demonstrably later, less thermally mature phase (Olcott Marshall et al. 2012). Whether some or all of this carbon ultimately had a biological source is still open to debate but abiotic synthesis via Fischer–Tropsch-type reactions (Fu et al. 2007; McCollom 2013) remains, in our opinion, a logical scenario for carbon found in these hydrothermal systems.

The presence of goethite in the filaments indicates that there has been modern weathering of these samples, consistent with the study material being obtained from surface outcrop. Based on the presence of sporadic, partially weathered sulphide grains elsewhere in the CHIN thin sections, and abundant sulphides elsewhere in Apex chert dyke samples (e.g. Brasier et al. 2005), we infer that the goethite is a weathering product of small grains of iron (plus As, Ni) sulphides initially formed within the hydrothermal system. Goethite could also have resulted from the modern weathering of Fe-rich micas such as biotite (see Banfield 1985) but we see no firm evidence of the residual silicate component of that reaction, kaolinite.

In summary, the observed mineral phases, their distribution relative to one another, their minor elemental composition and the distribution of kerogen-like carbon in the Apex filaments can be most parsimoniously explained by alteration of a micaceous mineral within a carbonaceous hydrothermal environment, followed by a modern weathering event. The variety of colours of Apex filaments observed in optical microscopy, from almost colourless, to orange, to very dark brown (e.g. Schopf 1993; Brasier et al. 2005, 2015; Wacey et al. 2016a; and herein) can simply be attributed to differences in the initial Fe content of the micas or abundance of Fe-sulphides, differences in the amount of carbon accreted onto the phyllosilicates and variations in the thickness of the thin sections used for analysis.

**Reconciling Raman and electron microscopy data**

Previous data concerning the Apex filaments have mostly been obtained using Raman spectroscopy; this is especially true for the proponents of a microfossil origin who have used Raman mapping of kerogen-like carbon to demonstrate the apparent cellularity of the Apex filaments (e.g. Schopf & Kudryavtsev 2009, 2012; Schopf et al. 2007, 2018). In contrast, higher spatial resolution electron microscopy data herein (plus examples given by Brasier et al. 2015 and Wacey et al. 2016a, b) show no such evidence of cellularity. Hence, it is necessary to reconcile the differences in carbon patterns observed using Raman microspectroscopy with those observed using electron microscopy. To investigate whether the spatial resolution of the respective imaging techniques can explain the differences in these data we applied a Gaussian blurring filter (see the methods section above) to our electron microscopy data to mimic the lower spatial resolution of the Raman method (Figs 9 and 10).

This approach is imperfect as it cannot take into account the differences in z-depth imaged by the different techniques. For example, our TEM samples are 150–200 nm thick but the confocal Raman technique is collecting signal across a depth of more than 500 nm in a single image, which means that a greater volume of carbon has potentially been sampled by Raman microspectroscopy. Application of the Gaussian filter to the electron microscopy carbon data can realistically account for only the lower x and y spatial resolution of the Raman carbon data, and will probably lead to an underestimation of the carbon feature size in the resultant blurred

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**Fig. 8. Insights into the mineralogy and carbon syngeneity of Apex filaments from electron microscopy.** (a) Overview of part of a *Primaevifilum* filament showing the location of the HRTEM images in (b) and (c). (b) HRTEM image of the phyllosilicate phase showing the 1 nm spacing characteristic of 2:1 phyllosilicates such as muscovite. (c) HRTEM image of the Fe–O–rich phase consistent with the <102> zone axis of goethite. White lines denote the [211] planes. (d) Aluminosilicate grains (yellow arrows), in close proximity to *Primaevifilum* filaments, which are not arranged into chains and therefore lack a filamentous microfossil-like morphology; carbon can sometimes be associated with such grains. (e) A partially carbon-filled crack (white arrows) that occurs in close proximity to a branched *Primaevifilum* filament. The aluminosilicate grains associated with carbon but not part of the main filament (yellow arrow) should also be noted. (f, g) A network of partially carbon-filled cracks (white arrows) occurring close to one end of a *Primaevifilum* filament. Again, the small aluminosilicate grain associated with carbon and iron oxyhydroxide some way away from the main filament (yellow arrow) should be noted. (h, i) A further carbon-filled fracture adjacent to a *Primaevifilum* filament.
features can give the impression of cellular compartments in (Fig. 9f) interspersed with rather random zones of carbon-poor material 2018) that show predominantly solid carbonaceous filaments microscopy data emulating the Raman data (Fig. 9i) of previous Apex Raman data (e.g. Schopf Cephalophytarion filter to the Raman maps (Figs 9a–e and 10a–l). Moreover, this simple experiment provides a consistent and logical explanation of previous Apex Raman data (e.g. Schopf et al. 2002, 2007, 2018) that show predominantly solid carbonaceous filaments interspersed with rather random zones of carbon-poor material (Fig. 9f–h). On rare occasions, fortuitous spacing of carbonaceous features can give the impression of cellular compartments in Raman maps (e.g. Fig. 4b and c, arrows; see also Schopf et al. 2018, fig. 11–z) but here we demonstrate that these can be explained as coincidental artefacts of the limited spatial resolution of the Raman data.

A recent report, based on Raman data plus a small δ13C dataset from 11 carbonaceous Apex microstructures (averages of δ13C = −31‰ to −39‰), inferred that multiple metabolic pathways were present amongst Apex microfossils (Schopf et al. 2018).

None of the new objects analysed in that study have cellular organization, with Raman maps showing mostly solid carbonaceous filamentous objects, some with angular facets, plus large quantities of carbon and potential iron staining in the surrounding matrix (see Schopf et al. 2018, figs 1 and 2); hence, none of those newly described objects pass accepted criteria for classification as microfossils (Schopf et al. 2010). Doubts also exist within the carbon isotopic data presented by Schopf et al. (2018): attempts were made to claim that five different taxa are represented within their δ13C analyses (averages of −31‰ to −39‰), including both Archaea and Proteobacteria, exhibiting up to three different metabolisms, yet only 11 filaments were analysed in total and two supposed taxa yielded only a single δ13C data point. Of the supposed taxa that yielded more than one data point, the variability
Regardless of the quality of the Schopf et al. (2018) isotopic data, our demonstration here that Apex filaments lack a plausible biological morphology, can simply be explained by alteration of flakes of mica and probably contain a later generation of carbon renders the inference of multiple metabolic pathways in these objects untenable. The $\delta^{13}C$ patterns observed by Schopf et al. (2018) are more probably a result of the multiple sources and generations of carbon present within these hydrothermally influenced Apex samples (see Olcott Marshall et al. 2012; Sforna et al. 2014). As mentioned previously, it is of course possible that one or more of these carbon sources could be remobilized biological material, as life was very probably established on Earth by this time (e.g. Noffke et al. 2013). However, abiotic sources of carbon within the filaments, for example resulting from Fischer–Tropsch-type synthesis of organic carbon (Fu et al. 2007; McCollom 2013), remain consistent with the geological setting (see Holm & Charlou 2001), associated Ba-rich and As-rich hydrothermal minerals, structure and bonding (see De Gregorio & Sharp 2006), and isotopic signature (see McCollom & Seewald 2006) of the Apex carbon.

**Conclusion**

We have here examined filamentous microfossils belonging to Cephalophytarium from the 850 Ma Bitter Springs Group plus filamentous objects (Primaevifilum) from the 3460 Ma Apex chert at a range of spatial scales. We have shown that the previously held view that these two types of filaments are morphologically comparable (e.g. Schopf & Kudryavtsev 2005, 2009; Schopf et al. 2007) is unsupported, particularly when analysed at high spatial resolution. Raman analyses show superficial morphological similarities between Cephalophytarium and Primaevifilum but there are subtle differences in the distribution of carbon in each case. In the former, carbon clearly corresponds to cell walls outlining hollow, almost cubic cellular compartments. In the latter, carbon occurs as more random clumps without cellular distribution.

Higher spatial resolution electron microscopy data highlight the complete lack of morphological comparison between Cephalophytarium and Primaevifilum filaments. The former satisfy established biogenicity criteria for fossilized filamentous micro-organisms, having cylindrical cross-sections defined by distinct carbonaceous lateral walls, uniform diameter (excepting their apices), and being partitioned by septa into discrete box-like cells of more or less uniform size and shape. The latter fulfil none of these criteria: they lack lateral carbonaceous walls; are angular in transverse cross-section; have rapidly changing diameters along their length; and are not partitioned into box-like cells.

Most previous work on the Apex filaments (e.g. Schopf et al. 2018, and references therein) has tended to resort to biologial explanations for the preserved microstructures, perhaps owing to a lack of abiological models to explain complex mineral–organic morphology. By providing high-resolution electron microscopy data, and deconstructing these data to inform upon previous Raman-scale mapping, we provide additional morphological and compositional details that were not available using traditional light microscopy and Raman analyses. In so doing, this contribution builds upon previous work (Brasier et al. 2015; Wacey et al. 2016a, 6) to continue to refine abiological models that can act as alternative explanations in the assessment of Archean microfossil-like objects.

In summary, when examined at an appropriate spatial scale, Apex Primaevifilum filaments do not possess any of the cellular features found in bona fide Precambrian fossilized filamentous organisms. Hence, they fail the most essential criterion established for the evaluation of putative microfossils and can no longer be interpreted as some of Earth’s oldest cellular life. Rather, these new data provide further support to a hypothesis that the Apex filaments are...
