1 **Remarkably preserved tephra from the 3430 Ma Strelley Pool Formation,**
2 **Western Australia: Implications for the interpretation of Precambrian**
3 **microfossils**

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14 **Running title: 3430 Ma microfossil-like tephra**

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26 **ABSTRACT**

27 The ~3430 Ma Strelley Pool Formation (SPF), Pilbara, Western Australia contains
28 some of the most diverse microfossil evidence for early life on Earth. Here we report
29 an assemblage of tephra (scoria, tubular pumice, plus vesicular and non-vesicular
30 volcanic glass shards) from two stratigraphic levels in the SPF, including
31 morphotypes that closely resemble previously described microfossils from this unit
32 and elsewhere.

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34 Clasts of scoria are characterised by numerous spheroidal vesicles, with subordinate
35 eye- and lens-shaped morphotypes, commonly lined with anatase (TiO₂) and small
36 amounts of organic material. Their diameters range from 5-180 µm with 80% in the
37 10-50 µm range. Fragments of tubular pumice are also lined with anatase +/- carbon
38 and have tube diameters of 5-15 µm. Other volcanic ejecta particles include a
39 multitude of sub-angular shard particles with or without vesicles, plus more rounded
40 vase-shaped, eye-shaped, and hair-like morphologies; once again, most of these are
41 coated by anatase +/- carbon and are several tens of micrometres in size. Many of the
42 tephra fragments are now entirely silicified with no compositional difference between
43 the former volcanic glass, the vesicle infill and the clast matrix. However, some
44 examples retain a partial aluminosilicate composition, either as a vesicle infilling
45 phase or as isolated lath-like grains within the formerly glassy groundmass.

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47 Isolated occurrences of some of these tephra morphotypes strongly resemble simple
48 microbial morphologies including pairs and clusters of cells (cf. scoria), filamentous
49 microbes (cf. tubular pumice) and larger sheaths/cysts (cf. sub-rounded glass shards).
50 Furthermore, some tephra-containing clasts occur in a SPF sandstone unit that hosts

previously described microfossils, while others are interbedded with chert layers from which microfossils have also been described. In light of our new volcanogenic data, we evaluate the robustness of previous microfossil evidence from the SPF in the East Strelley greenstone belt. We find that the majority of previously illustrated microfossils from this greenstone belt possess multiple features that are consistent with a biological interpretation and are unlikely to be volcanogenic, but at least one previously illustrated specimen is here reinterpreted as volcanic in origin.

The importance of this work is that it serves to highlight the common occurrence of volcanogenic microstructures resembling biological fossils (i.e. pseudo-fossils) in Archean environments that are habitable for life. Such structures have until now been largely overlooked in the assessment of putative Precambrian microfossils. Our data show that tephra-derived microstructures should be considered as a null hypothesis in future evaluations of potential signs of life on the early Earth, or on other planets.

Keywords: Strelley Pool Formation; pseudo-fossils; microfossils; tephra; Archean life

1. Introduction

The ~3430 Ma Strelley Pool Formation (SPF) has emerged as one of the most important rock units in the study of early life on Earth. It occurs over about 30,000 km² in 11 greenstone belts across the East Pilbara granite greenstone terrane, marking a regional hiatus in volcanic activity of up to 75 Ma (Hickman, 2008). Several biosignatures have been reported from the SPF, including carbonaceous microfossils (Sugitani et al., 2010, 2013, 2015a, 2015b; Wacey et al., 2011a, 2012; Lepot et al.,

2013; Brasier et al., 2015), stromatolites (Hofmann, et al., 1999; Allwood et al., 2006, 2007, 2009; Wacey, 2010; Van Kranendonk, 2011), and other potential biofilms and biominerals (Wacey et al., 2010a, 2011b), some of which arguably represent the most robust indicators of early Archean life.

In a recent paper (Wacey et al., 2018) we reported spheroidal to lenticular volcanogenic microstructures from the ~3480 Ma Dresser Formation, Pilbara, Western Australia, some of which closely resemble previously reported putative Archean microfossils from the SPF and other stratigraphic units (e.g., Walsh, 1992; Sugitani et al., 2007). Here we extend this work to report a much more diverse suite of volcanogenic microstructures from the SPF, several of which have microfossil-like morphologies.

Our study samples come from the East Strelley greenstone belt (ESGB) of the SPF close to the type locality of Strelley Pool (Lowe, 1983; Wacey et al., 2010b). Sample 1 (SPZ 1) comes from the eastern portion of Strelley Ridge, approximately 80 m east of Strelley Pool (Fig. 1a and c; GR 0722362E 7664053N). It was obtained from the basal sandstone unit of the SPF (Fig. 1e), just below the transition to laminated grey chert (that hosts stromatolites in places), and some 3-4 m stratigraphically above the level from which microfossils and trace fossils were reported in Wacey et al. (2011a, b). Sample 2 (SPC 2) comes from the central portion of Strelley Ridge, approximately 1.2 km west of Strelley Pool (Fig. 1b and d; GR 0721110E 7663764N). It was obtained from a conglomeratic unit near the top of the SPF (note that the contact between the SPF and the overlying Euro Basalt is not exposed here but is no more than ~10 m above SPC 2), some 18 m stratigraphically above Sample 1 (Fig. 1e). The

conglomerate clasts are diverse and include both angular and sub-rounded grey chert, green chert and banded black and white chert, all heavily silicified and cemented by clear silica (Fig. 1d). The clasts are interpreted to be mostly locally reworked fragments of units lower of the SPF stratigraphy, but older pre-SPF lithologies are also represented.

Fig. 1. Locality and nature of study samples. (a) Photograph looking south towards the central section of Strelley Ridge with position of study sample SPZ 1 marked by pink arrow. (b) Photograph looking south towards the eastern section of Strelley Ridge with position of study sample SPC 2 marked by red arrow. (c) Thin section of sample SPZ 1 with the two clasts of interest indicated by pink arrows. (d) Thin section of sample SPC 2 with the four vesicular clasts of interest indicated by arrows (see text for reference to different colours). Boxed area is enlarged in Fig. S1. (e) Stratigraphic log of the Strelley Pool Formation (SPF) close to Strelley Pool showing the relative positions of samples SPZ 1 (pink star) and SPC 2 (red star).

2. Methods

2.1. Optical microscopy

Petrographic analysis was carried out on uncovered polished geological thin sections (30 μm and 100 μm thick) using a *Leica DM2500M* microscope, with 5x, 10x, 20x, and 50x objective lenses (plus 10x eyepieces), 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. Clasts of interest were mapped in transmitted and reflected light in order to gain an understanding of the distribution of volcanic shards and scoria-like vesicles, measure their dimensions, and select the most appropriate targets for higher resolution study.

2.2. Laser Raman microspectroscopy

Confocal laser Raman microspectroscopy was performed using a *WITec alpha 300RA+* instrument with a *Toptica Photonics Xtra II* 785 nm laser source at CMCA, UWA. The laser was focused through either a 20x/0.4, or 100x/0.9 objective, the latter obtaining a spot size of smaller than 1 μm , and the laser excitation intensity at the sample surface was in the 1-5 mW range. Spectral acquisitions were obtained with a 600 l/mm grating and a peltier-cooled (-60 °C) 1024 x 128 pixel CCD detector. Laser centering and spectral calibration were performed daily on a silicon chip with characteristic Si Raman band of 520.4 cm^{-1} . Count rates were optimised prior to point spectra acquisition or hyperspectral mapping using the dominant quartz Raman band of 465 cm^{-1} . Spectra were collected in the 100-1800 rel. cm^{-1} region in order that both 1st order mineral vibration modes and 1st order carbonaceous vibration modes could be examined simultaneously. Raman maps were acquired with the spectral centre of

the detector adjusted to 944 cm^{-1} , with a motorised stage allowing XYZ displacement with precision of better than $1\text{ }\mu\text{m}$. Spectral decomposition and subsequent image processing were performed using WITec Project FOUR software, with baseline subtraction using a 3rd or 4th order polynomial. Carbon maps were created by integrating over the $\sim 1600\text{ cm}^{-1}$ ‘carbon G’ Raman band, quartz maps using the $\sim 465\text{ cm}^{-1}$ Raman quartz band and anatase maps using the $\sim 145\text{ cm}^{-1}$ Raman anatase band. Anatase can be distinguished from other common TiO phases such as rutile and brookite by characteristic strong Raman bands (in order of intensity) at $\sim 145\text{ cm}^{-1}$, $\sim 400\text{ cm}^{-1}$ and $\sim 515\text{ cm}^{-1}$, plus a minor band at $\sim 640\text{ cm}^{-1}$. All analyses were conducted on material embedded below the surface of the thin section to avoid artefacts in the Raman spectra resulting from polishing and/or surface contamination. Spectra were monitored for any potential contamination from epoxy resin/glue; these results were negative.

2.3. Focussed ion beam (FIB) preparation of TEM samples

A dual-beam FIB system (*FEI Nova NanoLab*) at the Electron Microscopy Unit, University of New South Wales was used to prepare TEM wafers from the thin sections described above, coated with $\sim 30\text{ nm}$ of gold. Electron beam imaging within the dual beam FIB was used to identify microstructures of interest in the thin sections allowing site-specific TEM samples to be prepared. The TEM sections were prepared by a series of steps involving different ion beam energies and currents (see Wacey et al., 2012 for details), resulting in ultrathin wafers of c. $100\text{--}150\text{ nm}$ thickness. These TEM wafers were extracted using an *ex-situ* micromanipulator and deposited on continuous-carbon copper TEM grids. FIB preparation of TEM sections allows

features below the surface of the thin sections to be targeted, thus eliminating the risk of surface contamination producing artefacts.

2.4. TEM analysis of FIB-milled wafers

TEM data were obtained using a *FEI Titan G2 80-200* TEM/STEM with *ChemiSTEM Technology* operating at 200 kV, located in CMCA at UWA. Data obtained included bright-field TEM images, HAADF (high angle annular dark-field) STEM images, and EDS (*ChemiSTEM*) maps.

2.5. SEM-EDS

Semi-quantitative elemental analysis of thin sections was performed on a *FEI Helios Nanolab G3 CX* instrument equipped with an *Oxford Instruments X-Max 80* EDS system and *Oxford Instruments AZtec 3.0* nano-analysis software, located in CMCA, UWA. Analyses were performed on FIB-milled faces below the surface of the thin section to avoid potential surface contamination.

2.6. NanoSIMS ion mapping

High spatial resolution and high sensitivity ion mapping was performed using a *CAMECA NanoSIMS 50*, located in CMCA, UWA. A disc of c. 10 mm diameter containing the region of interest was extracted from the thin section, mounted on a NanoSIMS stub, and coated with a thin (c. 10 nm) layer of gold to provide conductivity at high voltage. Details of qualitative elemental mapping using NanoSIMS in multi-collector mode are given in Kilburn and Wacey (2011a, 2015). Briefly, a focused primary Cs^+ ion beam, with a beam current of 2–4 pA, was rastered over the sample surface, and the sputtered ions were extracted to a double focusing

mass spectrometer. Images with sub-100 nm spatial resolution mapping relative ion intensity were acquired over fields of view ranging from 20 μm to 45 μm . Prior to each analysis, the sample area was pre-sputtered to remove surface contamination, implant Cs^+ ions into the sample matrix and attain an approximate steady state of secondary ion emission. Ion maps of oxygen ($^{16}\text{O}^-$), carbon ($^{12}\text{C}^-$), nitrogen ($^{12}\text{C}^{14}\text{N}^-$), sulfur ($^{32}\text{S}^-$) and titanium oxide ($^{48}\text{Ti}^{16}\text{O}^-$) were then produced simultaneously from the same sputtered volumes of sample. Only relative concentrations of elements can be obtained using this NanoSIMS methodology. Without multiple standards, no inferences can be made from these data concerning either the absolute concentration of elements, or the percentage concentration of one element compared to another.

3. Results

3.1. Distribution and morphology of SPZ 1 microstructures

Diverse morphotypes of microstructures, commonly outlined by very dark brown to black granular material, occur in two rounded clasts within a dark grey sandstone from the lower portion of the SPF, 80 m east of Strelley Pool (Fig. 1c, 2a). Four main microstructure morphotypes are present (here described in order of abundance):

- 1) Fragments of tubular microstructures that may consist of a single tube, or several closely spaced and aligned tubes (Fig. 2b-h). The diameters of single tubes range between approximately 5 and 20 μm , while a ‘multi-tube microstructure’ can attain several hundred micrometres in both diameter and length (e.g., Fig. 2h).

Transverse sections through ‘multi-tube microstructures’ reveal closely spaced pseudo-spheroidal objects (Fig. 2b) while oblique sections reveal eye-like and lens-like morphologies (Fig. 2d and e).

2) Sub-angular to sub-rounded non-vesicular objects. These have highly diverse morphologies, ranging from cuneiform and tear-shaped (Fig. 3a, arrow), to lenticular (Fig. 3b, arrow), to more complex jigsaw piece-like (Fig. 3c). They also have a wide variation in size, from $< 10 \mu\text{m}$ up to approximately $150 \mu\text{m}$ in diameter, and with lengths of up to several hundred micrometres (e.g., Fig. 3c). Their long axes are also commonly aligned (Fig. 3a and c).

3) Sub-angular to sub-rounded vesicular objects. These also have diverse morphologies, ranging from lenticular (Fig. 3d), to spear-head and tear-shaped (Fig. 3e), and complex jigsaw piece-like (Fig. 3f). They are less abundant than the corresponding non-vesicular objects and also span a smaller size range, commonly being $20\text{-}50 \mu\text{m}$ in diameter.

4) Large coalesced hemispherical to spheroidal vesicles. Three clusters of large (hemi)spheroids (that are clearly not merely transverse sections through tubes) occur in one clast within SPZ 1. Individual examples may have long axes up to approximately $100 \mu\text{m}$, and these long axes are commonly aligned in a given cluster (Fig. 3g). The clusters as a whole may be several hundred micrometres in diameter. These objects strongly resemble spheroidal vesicles in sample SPC 2 (described below in section 3.2).

In addition to the four morphotypes described above there are some rare objects that do not fit into any of these categories. These include two vase-shaped morphotypes around $50 \mu\text{m}$ in diameter (Fig. 3h, yellow arrow) and a handful of narrow ($<10 \mu\text{m}$ diameter) hair-like objects (Fig. 3h, red arrow).

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247 **Fig. 2.** Overview of microstructures in sample SPZ 1. (a) Clast within medium
248 grained sandstone containing microstructures interpreted as reworked shards of
249 volcanic glass (yellow arrows) and tubular pumice (red arrow). (b) Longitudinal (left)
250 and transverse (right) sections through fragments of tubular pumice. (c-h)
251 Longitudinal and slightly oblique sections through fragments of tubular pumice
252 showing the range of typical tube diameters and how oblique sections of the tubes
253 may result in eye-like and lens-like microstructures (for example in d and e).

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262 **Fig. 3.** Diverse microstructures from sample SPZ 1. (a-b) Objects interpreted as non-
263 vesicular glass shards, some with rather angular morphologies, others more rounded,
264 all outlined by a thin layer of black granular material. Note areas where
265 microstructures become indistinct due to absence of black granular material around
266 their edges (arrow in a). (c) A selection of microstructures here interpreted as volcanic
267 glass shards showing slightly rounded edges and black granular coatings. Note also
268 the significant amount of pale to medium brown material within the clast matrix. (d)
269 Putative fragment of vesicular volcanic glass showing rounded vesicles within a
270 fragment of a possible tube. (e) Rounded glass shard with 'spear-tip' morphology and
271 small internal vesicles. (f) Highly vesicular jigsaw-piece-like object with both its
272 edges and internal vesicles outlined by black granular material. (g) Cluster of
273 hemispherical to lenticular microstructures comprising dark brown to black granular
274 'walls'. (h) More complex morphotypes including vase-shaped microstructures
275 (yellow arrow) and hair-like microstructures (red arrow).
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3.2. Distribution and morphology of SPC 2 microstructures

Spherical and elliptical microstructures (from here on referred to as spheroids), plus rare lens-shaped morphologies, occur in one large clast and three subordinate clasts within a conglomerate in the upper portion of the SPF, 1.2 km west of Strelley Pool (Fig. 1d). The large clast is grey-green-brown in colour, has sub-angular margins, is rather irregular in shape, and has a maximum diameter of 4.5 mm (Figs. 1d, S1a, red arrow). Three sides of the clast have distinct margins, but one side of the clast is partially eroded and flooded with silica cement (Fig. S1a). Where distinct margins are present these are in places darker in colour than the interior of the clast (Fig. S1a). A second smaller clast occurs adjacent to the eroded side of the main clast (Figs. 1d, S1a, green arrow) and this is here interpreted to be an eroded portion of the main clast. Two further small clasts containing equivalent spheroids occur elsewhere in the thin section (Fig. 1d, blue arrows). These four clasts are different in colour and texture from all other clasts found in the thin section.

Spheroidal microstructures are numerous within the clasts and show no organised geometrical pattern of distribution (Figs. 4 and S1). There are zones where the microstructures are particularly densely clustered and they do not occur in later cross-cutting quartz veins or outside the boundary of the clasts (Fig. 4a, S1a). Morphology varies from almost perfectly spherical (Fig. 4f, yellow arrow), to elliptical (Fig. 4b, yellow arrow), to occasionally highly lenticular (Fig. 4a, arrow), though the vast majority (74 %) show only small amounts of ellipticity (having sphericity in the range of 0.8–1). No correlation is seen between size and sphericity, except for examples with diameters $> 50 \mu\text{m}$ which tend to show greater degrees of ellipticity. The long and short axes of 154 spheroids were measured. Maximum diameters ranged from 4

μm to 181 μm, with 80% of all spheroids having diameters between 10 μm and 50 μm. The cumulative frequency distribution of the spheroids is shown in Figure S2. Overlain on this for comparison are cumulative frequency distributions for populations of coccoidal unicellular microfossils from the 1878 Ma Gunflint chert, 850 Ma Bitter Springs chert (Schopf, 1976), and SPF (Wacey et al., 2011a), plus a cumulative frequency distribution of volcanic vesicles from the ~3480 Ma Dresser Formation (Wacey et al., 2018). The differences between these distributions are discussed below (see section 4.1).

Most spheroids in sample SPC 2 have distinct narrow black walls. In petrographic thin section these appear to have variable thickness, with some spheroids having distinctly darker, thicker walls (e.g., the arrowed example in Fig. 4f). A small proportion of the spheroids have walls that possess a brown tinge when examined at high magnification (Fig. 4c and e). All walls have a distinctly granular appearance, best observed when looking at the top surface of a spheroid (Fig. 4e). Occasionally, this granular texture is disrupted by pale lath-like crystals (e.g., Fig. 4e, arrow). When a spheroid falls entirely within the plane of the thin section, its wall appears superficially continuous (e.g., small spheroids in Fig. 4b; but also see higher spatial resolution data in Fig. 6 below where small gaps in the wall are common). There are also some examples where pairs of spheroids appear to share a wall, or be connected by a perforation in the wall (Fig. 4c, arrow).

Fig. 4. Details of vesicular textures from sample SPC 2. (a) Low magnification transmitted light micrograph giving an overview of a vesicular clast showing the general distribution of spheroidal to lenticular vesicles. (b) Higher magnification view showing wide range of spheroid diameters plus rarer eye-shaped morphotype (arrow). (c) Pair of spheroids showing coalescence via a perforation in a shared wall (arrow), a feature typical of volcanic vesicles. (d) Lenticular vesicle with two smaller spheroids (arrows) attached or contained within. (e) Cluster of spheroids showing granular walls that have a brown tinge in places. Lath-like minerals occasionally interrupt this granular texture (arrow). (f) Pair of spheroids, the upper example showing a particularly thick black granular wall.

3.3. Chemistry of the SPZ 1 and SPC 2 microstructures

Multiple correlative techniques (Raman spectroscopy, EDS in the TEM, and NanoSIMS) show that the walls of nearly all analysed microstructures are composed of anatase, one of the common mineral forms of titanium dioxide (TiO_2 ; Figs. 5-7). The granular nature of the walls observed in light microscopy is reinforced by higher resolution data, with TEM imaging showing SPC 2 spheroid walls to comprise hundreds of sub-micron crystals of anatase (Fig. 6c). Even smaller anatase crystals, often < 100 nm, are found concentrated in zones a few hundred nanometres either side of the main wall (Fig. 6d and e). Anatase crystals are also occasionally found elsewhere within the analysed SPC 2 clasts, showing that they are not merely confined to the spheroid walls. A similar pattern emerges with the SPZ 1 microstructures although the anatase crystals tend to be less densely packed along the edges of the microstructures (Fig. 7b and c). Most SPC 2 and SPZ 1 microstructures have been heavily silicified and their interiors generally comprise 100 % quartz (e.g., Fig. S1b, red arrow). However, a small proportion of SPC 2 spheroids can be observed to have a somewhat dusty (often slightly brown) mineral infill (e.g., Fig. S1c) or partial pale green mineral infill (Fig. S1b, yellow arrows). SEM-EDS elemental mapping of the 'dusty brown' mineral reveals a potassium-rich aluminosilicate phase (Fig. S1d) consistent with altered volcanic protoliths reported from elsewhere in the Pilbara and other Archean terranes (Oberger et al., 2006; Lepot et al., 2011; Westall et al., 2011). The green tinge that some of these vesicles exhibit under the light microscope suggests that some of these aluminosilicates have been chloritized during the lower greenschist facies metamorphism that these rocks experienced in the mid- to late-Archean (Van Kranendonk et al., 2007). Some SPZ 1 microstructures also

preserve lath-like aluminosilicate minerals and these can occur both in the interior of the microstructure and in the nearby matrix (Fig. 7b, d-f).

Laser Raman micro-spectroscopy shows that kerogenous organic material is quite commonly associated with both the SPC 2 and SPZ 1 microstructures (Fig. 5). In contrast, organic material (indicated by CN⁻ mapping) was detected in only 1 out of 10 microstructures analysed using NanoSIMS (Fig. 6g), and was not detected in the TEM. The NanoSIMS analyses suggest that, when present, organic material is co-located with the anatase (i.e. occurs within the same narrow wall region of an SPC 2 vesicle). The Raman data do not possess sufficient spatial resolution to determine if this is the case more generally, but in the case of at least one SPZ 1 microstructure the organic material appears to be coating a grain of anatase (Fig. 5d), while in the case of at least two of the SPC 2 spheroids, organic material may preferentially occur within the anatase walls and in the interior of the vesicles (Fig. 5b and c). Both Raman and NanoSIMS are very sensitive to extremely small amounts of organic matter, much more so than TEM. The data here are therefore consistent with the kerogen occurring as disseminated nanoparticles or nano-coatings on TiO₂, a situation that is very different to the kerogen distribution in microfossils (e.g., Wacey et al., 2012).

Fig. 5. Laser Raman analysis of Strelley Pool microstructures. (a-c) Spheroidal vesicles from sample SPC 2. (d) Glass shard-like microstructure from sample SPZ 1. In each case the upper panel is a reflected light image of the microstructure, the second panel is a Raman map of anatase (TiO_2) created using the $\sim 145 \text{ cm}^{-1}$ Raman band, the third panel is a Raman map of kerogen created using the $\sim 1600 \text{ cm}^{-1}$ carbon G Raman band, and the lower panel is a Raman map of quartz created using the $\sim 465 \text{ cm}^{-1}$ Raman band.

Fig. 6. Nanoscale chemistry of SPC 2 vesicle walls. (a-b) Reflected light photomicrographs showing vesicles analysed in (c-h). Red line in (a) marks position of extracted TEM wafer analysed in (c-e); yellow box in (b) marks area analysed using NanoSIMS in (f-h). (c) ChemiSTEM elemental map showing that the vesicle wall region is enriched in Ti. (d) Dark-field STEM image showing detail of the vesicle wall region. (e) ChemiSTEM elemental maps of Ti and Si showing that all nanoparticles in the wall region are enriched in Ti and these sit in a Si-rich matrix. (f-h) NanoSIMS ion maps showing TiO^- rich vesicle walls associated with low levels of organic material (CN^-) and possible minor sulfur (S^-).

Fig. 7. Nanoscale analysis of SPZ 1 microstructure walls. (a) Reflected light photomicrograph of the spheroidal to lenticular microstructures illustrated in Fig. 3g. Red line indicates position where TEM wafer was extracted. (b) Dark-field STEM image of the extracted TEM wafer showing distinct high mass globular mineral grains (white/light grey), lower mass acicular mineral grains (medium grey) and an even lower mass mineral matrix (dark grey). (c-f) ChemiSTEM elemental maps of Ti, Al, Si, and O respectively. Ti-rich particles (with some O) comprise the ‘walls’ of the microstructures, while aluminosilicates occur both within and exterior to the microstructures. The remainder of the matrix is quartz.

4. Discussion

4.1. Are the SPC 2 and SPZ 1 microstructures microfossils or volcanogenic objects?

Many of the SPC 2 spheroids exhibit morphological features that may be considered consistent with an interpretation as microfossils. These features include: clustering, that may be interpreted as colony of coccoidal cells (Fig. 4b and e); paired spheroids

sharing a common wall, that may be interpreted as dividing or newly divided cells (Fig. 4e and f); small spheroids enclosed within, or closely associated with a larger spheroid or lense, that may be interpreted as cells within a protective vesicle/sheath or cells just released from a vesicle (Figs. 4b and d); pieces missing from the spheroid wall, that may be interpreted as taphonomic decay features (Fig. 4c and e). Likewise, some of the SPZ 1 microstructures superficially resemble filamentous sheaths (Fig. 2b) or more complex vase-shaped microfossils (Fig. 3h). Some chemical signals could also be interpreted as consistent with a microfossil origin for a number of the microstructures, notably the association of carbon with the microstructure walls, as observed by laser Raman micro-spectroscopy, and in rare examples, by NanoSIMS (Figs. 5 and 6).

Evidence that counters against these structures being microfossils comes from the morphology of the population as a whole. For example, clear differences are seen between the cumulative frequency distributions of the SPC 2 spheroids and *bona fide* coccoidal microfossils from the Gunflint Formation (*Huroniospora*) and Bitter Springs Formation (*Glenobotrydion*), plus putative coccoidal microfossils from the SPF (Fig. S2). Populations of true microfossils are almost entirely composed of specimens < 15 µm in diameter, and have positively skewed frequency distributions. They also have much smaller standard deviations (1.9 µm for Bitter Springs with n=519; 2.5 µm for Gunflint with n=805; 2.8 µm for Strelley Pool with n=218) compared to 25 µm (n=154) for the SPC2 spheroids. Although large spheroidal and lenticular microfossils have been described from the SPF (e.g., Sugitani et al., 2010; Wacey et al., 2011) and other Archean deposits (e.g., Sugitani et al., 2007) the frequency distributions of the SPC 2 spheroids are not compatible with those of

known biological populations. The cumulative frequency distribution of SPC 2 spheroids shares many similarities with that of recently described volcanic vesicles from the nearby ~3480 Ma Dresser Formation (Fig. S2; Wacey et al., 2018).

Furthermore, the morphological traits cited in favour of a biological origin soon fall down on closer examination. Clustering of spheroids of unequal diameters is more logically explained as concentrations of vesicles in zones of increased gas content in a magma. Likewise, paired spheroids may be explained as coalescence of vesicles within a magma. Patches of missing wall may be explained as precipitation of crystals of different mineralogy to the anatase; given that most of the gaps in the walls appear lath-like (reinforced by TEM analysis revealing aluminosilicates; Fig. 7) this appears to be a more likely explanation than a taphonomic one. Small spheroids appearing to be enclosed by larger spheroids or lenses may simply be an artefact of a 2D projection of a 3D phenomenon, where the smaller spheroids lie above or below the plane of the larger object. Most tubular microstructures (Fig. 2c-h) contain too many tubes for a trichome-in-sheath explanation to be sustained, while unusual vase-like and hair-like morphologies (Fig. 3c) can easily be replicated by extruding volcanic glass (cf. Pele's hair; Heiken, 1974). Furthermore, there are multiple morphotypes (especially in SPZ 1) that find no morphological analogue in the microbial world (e.g., Fig. 3a-f).

Morphological comparisons with modern volcanic textures are striking (Fig. 8). For example, fragments of vesicular volcanic rock (e.g., scoria shown in Fig. 8a) provide a compelling analogy for the whole population of microstructures observed in SPC 2 (Fig. 4). Likewise, fragments of 'tubular pumice' (Fig. 8b) provide a near perfect analogue for the 'multi-tube microstructures' found in SPZ 1 (Fig. 2b-h). The highly

diverse vesicular and non-vesicular objects from SPZ 1 (Fig. 3a-g) closely compare to small shards of volcanic glass expelled from modern erupting volcanoes (Fig. 8c and d). Finally, some of the more unusual morphotypes in SPZ 1 (Fig. 3h) may find analogues with variously shaped extruded volcanic glass such as Pele's hair (Fig. 8e).

Fig. 8. Volcanogenic textures comparable to those observed in our Strelley Pool material. (a) 3D volume rendering of a block of scoria from the Ambrym volcano, Vanuatu. Note the range of vesicle morphotypes and compare with Fig. 4. Image reproduced with permission from Zandomeneghi et al. (2010). (b) Tubular vesicular ash from the 600 Kyr Rockland Ash Fall, California (compare with Fig. 2). Image credit, Wikipedia. (c) Modern basaltic vesicular tephra (compare to Fig. 3d-f). Image credit, Mark Shapley, University of Minnesota. (d) Non-vesicular ash from the 75 Kyr Mt. Toba eruption (compare to Fig. 3a-c). Image credit, Christine Lane, University of Oxford. (e) Modern basaltic Pele's hair fragments from Hawaii (compare with Fig. 3h). Image reproduced with permission from Clague et al. (2000).

Geochemical data also point towards a volcanogenic origin. While the presence of organic (kerogenous) material may be consistent with a microbial origin, all microstructure walls are composed of anatase, and kerogen is far from ubiquitous across all microstructures analysed. Two of the elements that are common in volcanic glass and are relatively immobile are Ti and Al. Hence, the ubiquitous presence of a Ti-rich mineral (anatase), plus the presence of Al-rich silicates within some of the microstructures and in the nearby matrix (Figs. 7d, S1) strongly favours a volcanogenic origin over a microfossil origin (cf. Lepot et al., 2011).

Further support for a volcanogenic origin for the microstructures in clasts within SPC 2 and SPZ 1 comes from previous work in the Pilbara and Barberton. These Archean cratons are dominated by volcanic rock sequences, including volcanoclastic components (e.g., Westall et al., 2006; Van Kranendonk et al., 2007) and massive scoriaceous flows with up to 70% vesicularity (Buick and Dunlop, 1990). Hence, it would come as no surprise to see fragments of reworked scoria or volcanoclastic debris in the SPF. In addition, similar (but much less diverse) microstructures have been found elsewhere in the Pilbara, within the ~3446 Ma Kitty's Gap chert (Westall et al., 2006, 2011), ~3460 Ma 'Apex chert' (Brasier et al., 2013), and the ~3480 Ma Dresser Formation (Buick and Dunlop, 1990; Wacey et al., 2018). In the former, Westall et al. (2011) noted shards of volcanic glass replaced by silica and hydromuscovite, plus clasts of pumice; many of these microstructures were closely associated with kerogen and anatase (plus minor rutile). In the 'Apex chert', Brasier et al. (2013) noted that clasts of pumice or scoria contained vesicles commonly lined by anatase, with kerogen found throughout the pumice matrix and as geopetal infills to some vesicles. In the Dresser Formation, Buick and Dunlop (1990) described blocky

and cuneiform shards of volcanic glass, plus matrix Ti, which they took to imply derivation from basaltic magmas. These authors also described massive beds of coarse pumice sand from the Dresser Formation that they interpreted as stranded pumice rafts. Wacey et al. (2018) also described volcanic vesicular microstructures from the Dresser Formation, these being associated with kerogen and pyrite, rather than with anatase. Lastly, Kazmierczak et al. (2013) reported silicified volcanic glass shards with envelopes of organic material from the ~3400 Ma Kromberg Formation, Barberton Greenstone Belt, South Africa.

4.2. Re-evaluating microfossil evidence for life from the Strelley Pool Formation (SPF) in the East Strelley greenstone belt (ESGB)

In light of the new data presented above it is timely to re-examine previous reports of microfossils from the SPF, in particular from the ESGB in the vicinity of these newly described volcanogenic samples. Wacey et al. (2011a) reported three microfossil morphotypes from black sandstones in the ESGB. These were: 1) clustered small coccoids (average 10.6 µm in diameter) found as biofilms coating quartz sand grains and in early pore-filling silica cements (Fig. 9a and d); 2) tubular sheath-like microfossils, mostly of around 10 µm in diameter, also occurring in early pore-filling cements in between sand grains (Fig. 9b and c); 3) rare, larger microfossils (up to 80 µm diameter) described as ‘cellular envelopes’ (Fig. 9e and f). The small coccoids and sheath-like tubes co-occurred in samples found to the east of Strelley Pool, with the best examples from sample SPV 3 found 4 km east of Strelley Pool on Unconformity Ridge (Wacey et al., 2010b, 2011a). In contrast, the rare large microfossils occurred in two samples (SPE 1, SP9) found 1-2 km west of Strelley Pool on Six Mile Ridge (Wacey et al., 2010b, 2011a).

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565 **Fig. 9.** Evaluating previously described microfossils from the Strelley Pool Formation
566 in the East Strelley greenstone belt. (a-d) Small coccoidal and tubular microstructures
567 from sample SPV 3, ~4 km east of Strelley Pool (from Wacey et al., 2011a). These
568 are not comparable to the volcanic microstructures described herein and we concur
569 with the original interpretation as microfossils. (e-f) Larger vesicular microstructures
570 from sample SPE 1, ~2 km west of Strelley Pool (from Wacey et al., 2011a). These
571 show close comparison to the volcanic microstructures described herein (g-h) and we
572 suggest that a volcanogenic origin provides a more plausible explanation to the
573 previous microfossil interpretation.

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576 The vast majority of the geochemical and high-resolution morphological data
577 presented by Wacey et al. (2011a, 2012) came from Unconformity Ridge, where type
578 1 and type 2 microfossils are abundant. These data, together with the biofilm-like
579 distribution of the microstructures, provided strong evidence for an interpretation as
580 *bona fide* microfossils and these arguments are outlined in detail in Wacey et al.
581 (2011a) and subsequent papers (Wacey et al., 2012; Brasier et al., 2015). In summary:
582 Raman micro-spectroscopy confirmed that microfossil walls comprise indigenous

organic (kerogenous) material; NanoSIMS confirmed cell walls contain both carbon and nitrogen; TEM demonstrated cell wall ultrastructure (comprising almost continuous organic matter up to a few hundred nm in thickness with impinging quartz nano-crystals) that is almost identical to younger *bona fide* microfossils (e.g, Gunflint biota; Wacey et al., 2012). All three of these techniques show that cell walls are curved and (semi) continuous, are composed only of organic material permineralised in three dimensions by silica, and contain no additional mineral phases. In addition, $\delta^{13}\text{C}$ values of -33 to -46 per mil are consistent with biological carbon fixation (Wacey et al., 2011a) and with other $\delta^{13}\text{C}$ data from this unit (Lepot et al., 2013).

The only high spatial resolution data presented from the type 3 large microfossils shows a much more patchy distribution of carbon within a zone interpreted to be the cell wall, plus the presence of small pyrite grains within and adjacent to the wall (Wacey et al., 2011a, fig 4b). Thin section images also appear to show these microfossils having a more granular wall texture compared to the type 1 and type 2 specimens (Wacey et al., 2011a, fig 4a). The presence of pyrite plus kerogen in the wall zone is reminiscent of volcanic vesicles recently described from the nearby ~3480 Ma Dresser Formation (Wacey et al., 2018) but it could also be consistent with organisms metabolising sulfur (Wacey et al., 2011a) or fossilised by a later sulfur-iron-rich fluid (cf. some Gunflint organisms; Wacey et al., 2013).

To provide a clearer picture regarding the type 3 microfossils, we have performed new laser Raman analysis of a cluster of type 3 large spheroidal microfossils from sample SPE 1. These were first illustrated in figure 1b of Wacey et al (2011a) and are shown again here in Figures 9e and 10. These new data clearly show that anatase has

an almost perfect 1:1 correlation with the walls of the microstructures (Fig. 10 b and c), and this mirrors the distribution of anatase seen in the SPC 2 and SPZ 1 volcanogenic microstructures (e.g., Fig. 5). Kerogen is much less abundant and where present occurs both inside and outside of the vesicles but only extremely rarely in the walls (Fig. 10 b and c); again, this resembles kerogen distribution in the SPC 2 and SPZ 1 microstructures but not that seen in the type 1 and type 2 microfossils of Wacey et al. (2011a).

Fig. 10. Laser Raman analysis of a zone of potential microfossils from sample SPE1. (a) Thin section photomicrograph of a cluster of potential large spheroidal microfossils (previously shown in Wacey et al., 2011a, fig. 1b) with dashed boxes representing the areas analysed using laser Raman. (b and c) Three colour overlay Raman maps of quartz (blue), kerogen (carbon 'G'; red) and anatase (green). It is clear that while kerogen is associated with some of the microstructures it does not correlate with the wall zones, which are instead composed of anatase.

It is possible that *bona fide* microfossils could be mineralised by anatase (cf. Lekele Baghekema et al., 2017) that replaces much of the original organic content, just as younger Precambrian microfossil examples have been mineralised by pyrite or iron oxides (e.g., Gunflint Formation; Planavsky et al., 2009; Wacey et al., 2013). However, the combined morphological and geochemical evidence, when combined with the new observations from SPC 2 and SPZ 1, strongly suggest a volcanogenic origin for this particular cluster of spheroids. We suggest that other large type 3 ‘microfossils’ (for example, Wacey et al. (2011a), figs 1a, g, 4a) may similarly be reinterpreted as volcanogenic in origin, either being volcanic vesicles lined with anatase +/- kerogen +/- pyrite, or volcanic glass shards coated with a similar suite of minerals. In contrast, the evidence remains strongly in favour of the type 1 coccoids and type 2 tubular sheaths of Wacey et al. (2011a) being microfossils.

By extension, these new data also raise questions about the authenticity of some other putative Archean microfossils. In a recent paper (Wacey et al., 2018) we have already suggested that some large spindle-shaped putative microfossils reported from Archean rocks in South Africa (Walsh, 1992) and Australia (e.g., Sugitani et al., 2007) may have volcanogenic origins. In addition, some of the microfossil-like volcanogenic morphologies presented in this contribution are reminiscent of recently reported 3770 Ma putative microfossils mineralised by iron oxides from Canada (Dodd et al., 2017; compare their fig. 2e with Fig. 2c and g herein) and of comparatively large spheroidal objects from multiple Pilbara and Barberton units (e.g., Engel et al., 1968; Walsh, 1992; Ueno et al., 2006; Sugitani et al., 2007). However, further analysis of each of these occurrences would be necessary before any firm conclusions could be drawn.

5. Conclusions

This contribution describes a diverse suite of volcanic tephra particles preserved within a small number of sandstone and conglomerate clasts within the 3430 Ma Strelley Pool Formation in the East Strelley greenstone belt of the Pilbara Craton. The tephra includes fragments of scoria with spheroidal to lenticular vesicles, fragments of tubular pumice containing single and multiple tubes, plus vesicular and non-vesicular shards of volcanic glass. Vesicles, tubes and shards are all heavily silicified but are commonly lined or coated with anatase (TiO_2) and are sometimes associated with kerogenous organic material and/or aluminosilicate minerals.

The morphology and geochemistry of the tephra populations as a whole leaves no doubt that they are volcanogenic in origin. However, several morphotypes strongly resemble simple microbial fossils (e.g., coccoids, filamentous sheaths) and when these occur in isolation, for example when pieces of clasts are eroded and reworked in a carbon-rich environment, they may be difficult to distinguish from true microfossils.

We use the new data gathered here to re-evaluate previous reports of microfossils from the East Strelley greenstone belt. We find that most previously illustrated specimens have morphologies, community structure, wall chemistry and ultrastructure that do not match the volcanogenic objects described herein (i.e. an origin as microfossils is sustained). However, new chemical analyses of one previously figured cluster of large spheroidal ‘microfossils’ shows that their walls are composed of anatase, not kerogen. This leads us to reinterpret these ‘microfossils’ as volcanogenic pseudo-fossils. Given the huge amount of volcanic activity in the Archean, we urge that fragments of vesicular or glassy volcanic rocks be added to the list of potential

abiogetic artefacts that must be considered in any evaluation of putative signs of early
or extraterrestrial life.

Acknowledgements

The authors acknowledge the facilities, scientific and technical assistance of the
AMMRF at the Centre for Microscopy Characterisation and Analysis, The University
of Western Australia, plus the Electron Microscopy Unit, University of New South
Wales. These facilities are funded by the Universities, State and Commonwealth
Governments. We thank the late Prof. Martin Brasier for his assistance during
fieldwork and Paul Guagliardo for help with acquisition of NanoSIMS data. DW is
funded by the Australian Research Council via a Future Fellowship grant (FT
140100321). Eugene Grosch and another anonymous reviewer are thanked for
improving the quality of this manuscript, and Tamsin Mather is thanked for her
editorial handling of the manuscript.

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