# An anaerobic ~3400 Ma shallow-water microbial consortium: Presumptive evidence of Earth's Paleoarchean anoxic atmosphere

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## ABSTRACT

A microbial consortium preserved in shallow water carbonaceous chert of the Paleoarchean (~3400 Ma) Strelley Pool Formation (Pilbara Craton, northwestern Western Australia) is composed of copiously abundant globular "cobweb-like" swirls of randomly oriented filaments intermingled with laterally extensive flat-laminated masses of subparallel filaments. The shallow-water (possibly mudflat-like) habitat of the biocoenose is established by its geological setting and intimately intermixed association with millimetric quartz radiating laths and rosettes interpreted to be pseudomorphs after evaporitic gypsum/anhydrite. The indigenousness and syngenicity of the permineralized fossils to the encompassing chert; their uncompacted minimally altered "life-like" spatial distribution; and their biogenicity and bacterial affinity are shown by optical microscopic, Raman spectroscopic and confocal laser scanning microscopic analyses *in situ* that document the mineralogy of their embedding matrix and their cylindrical filamentous morphology, cellularity, and kerogenous composition.

By comparison with mid-Precambrian and modern sulfur-cycling ecosystems, and consistent with sulfur isotopic data and their intimate co-existence with copious microgranular pyrite and pseudomorphs evidently after sulfate, the swirled aggregates are interpreted to be anaerobic sulfuretums. In contrast, the intermixed flat-laminated components, like microbes of fossil and modern stromatolitic mat communities, are interpreted to be co-occurring light-requiring phototrophs. Whether such phototrophs were anoxygenic photosynthesizers, like extant photosynthetic bacteria (e.g., chloroflexaleans) or facultatively anaerobic cyanobacteria (cf. *Oscillatoria limnetica*), their anoxygenic metabolism seems plausibly established. Given its great age, shallow water habitat, and anaerobic/microaerophilic physiology, this evidently first reported occurrence from the fossil record of anoxygenic phototrophs and of a microbial sulfuretum-phototroph consortium is consistent with the supposition that Earth's Paleoarchean environment was essentially anoxic and suggests that $O_2$-producing photosynthesis may have been a later evolutionary innovation.

## 1. Introduction

Possible microscopic fossils have previously been reported from greenstone belts of the Paleoarchean (~3400 Ma) Pilbara Craton Strelley Pool Formation at five principal localities:

(1) "East Strelley" (from the East Strelley greenstone belt, ~21°5′S, 119°8′E, ~104 km SSE of Port Hedland, Western Australia; Wacey et al., 2006);

(2) "Anchor Ridge" (Panorama greenstone belt, ~21°12′S, 119°18′E, ~123 km SSE of Port Hedland; Sugitani et al., 2010);

(3) "Marble Bar Road" (Warralong greenstone belt, ~20°52′S, 119°48′E, ~144 km SE of Port Hedland; Sugitani et al., 2010);

(4) "Waterfall Ridge" (Goldsworthy greenstone belt, ~20°21′S,

119°25′E, ~88 km E of Port Hedland; Sugitani et al., 2010, 2015a, 2015b; Lepot et al., 2013); and

(5) "Unnamed ridge south of Trendall Locality" (Panorama greenstone belt, south of ~21°13′S, 119°18′E, ~126 km SSE of Port Hedland; Sugitani et al., 2013, 2015a).

Interpretations of these microscopic objects as *bona fide* microfossils have been presented judiciously, the objects having been variously described as "putative biologic structures," "putative evidence of biogenicity," "microstructures we identify [as exhibiting] indicators of biological affinity" (Wacey et al., 2006, 2011a), and as "morphologically diverse structures that *may* constitute organic microfossils" [italics added] – followed by the caveat that "further investigations are required to confirm the biogenicity of carbonaceous structures from the Strelley Pool Formation" (Sugitani et al., 2010) – an initial interpretation later altered to an affirmative assessment (Lepot et al., 2013; Sugitani et al., 2015a, 2015b).

Coupled with reluctance to affirm the veracity of "claims for life in the [Archean] earliest 2.0–2.5 billion years of Earth's history" (Moorbath, 2005), the uncertainty expressed in the initial reports appears to stem from the lack of similarity in habitat and organismal and cellular morphology of various such Paleoarchean fossil-like objects to microbes of younger Precambrian, Phanerozoic, and modern assemblages. For example, "putative biologic structures" from the East Strelley locality occur in a "coarse-grained, moderately sorted, quartz-rich sandstone" (Wacey et al., 2006), a notably atypical rock-type for cellular preservation of minute fragile carbonaceous fossils; and neither these cigar-shaped "putative microtubular biosignatures" nor the lenticular microscopic structures reported from other Strelley Pool localities (e.g., Sugitani et al., 2010) exhibit phylogenetically definitive morphological similarity to Proterozoic, Phanerozoic or modern microorganisms. If biogenic, as their abundance, carbonaceous composition and "life-like" form suggest, they could be members of now extinct lineages (perhaps like *Eosphaera* and *Kakabekia* of the classic ~1900 Ma Gunflint microbiota; Barghoorn and Tyler, 1965) or members of the Archaea, an early-evolved "extremeophile" methanogen-including lineage inferred from carbon isotopic data to have been present during the Archean (e.g., Hayes, 1983) of which morphological fossils are as yet undocumented.

In contrast with the initial reports, evidence presented here from studies of Strelley Pool carbonaceous chert collected at the "Anchor Ridge" locality establishes that the microbe-like objects we report – and by plausible extension, many of those earlier described as well – are, in fact, *bona fide* fossils. We interpret the 3.4 Ga "Anchor Ridge" biocoenose to be a near-surface shallow water symbiotic anaerobic microbial consortium composed of $H_2S$-producing sulfuretums and $H_2S$-consuming anoxygenic phototrophs. If this interpretation is correct, this community provides the first evidence of microbial consortia and of anoxygenic phototrophs identified in the geological record; indicates that the availability of biologically useable sulfur was not biotically limiting and that the oxygen content of Earth's Paleoarchean atmosphere was evidently quite low; and suggests that stromatolites of this age may have been produced by anoxygenic phototrophs rather than by oxygen-producing cyanobacteria, constraining speculations about the time of origin of $O_2$-producing photosynthesis, arguably among the most significant evolutionary innovations in the history of life.

## 2. Geologic setting

The ~3400 Ma Strelley Pool Formation crops out across numerous greenstone belts of the Pilbara Craton, having an age bracketed between those of the ~3315–3450 Ma Kelly Group and the ~3427–3515 Ma Warrawoona Group in the East Pilbara region, a depositional area of $\geq 30,000 \text{ km}^2$ (Van Kranendonk, 2006; Hickman, 2008).

Studies reported here were carried out on specimens in petrographic

thin sections TRD-2a-1, TRD 2a-6 and TRD 2a-7 prepared from samples of carbonaceous cherts collected from outcrop by K.S. in 2005 at the "Anchor Ridge" locality (Panorama greenstone belt) situated on the south bank of an unnamed creek that cuts through the formation along the Shaw River at 21°11′ 46″S, 119°18′23″E where they, like associated Strelley Pool Formation strata have been interpreted to have been deposited in a shallow water setting (Sugitani et al., 2010). For relevant maps and detailed discussion of the Pilbara Craton Strelley Pool Formation see Allwood et al. (2006, 2007), Hickman and Van Kranendonk (2008) and Wacey et al. (2010b).

## 3. Analytical methods

### 3.1. Optical microscopy

Optical images of the thin section-embedded specimens studied here were acquired at the University of California, Los Angeles (UCLA) using fluorescence-free microscopy immersion oil and a Leitz Orthoplan 2 microscope equipped with a Nikon DS Microscope Digital Camera.

### 3.2. Raman spectroscopy

Molecular-structural compositional analyses of the fossils and associated minerals were carried out at UCLA using a T64000 triple-stage confocal laser-Raman system that permits acquisition both of point spectra and of Raman images that display the two-dimensional spatial distribution of the molecular-structural components of the specimens and their associated minerals. A Coherent Innova argon ion laser provided excitation at 457.9 nm permitting data to be obtained over a range from ~300 to ~3000 $cm^{-1}$ using a single spectral window centered at 1800 $cm^{-1}$. The laser power used was ~6–8 mW over a ~1 μm spot, a configuration well below the threshold resulting in radiation damage to kerogenous fossils, and the thin sections were covered by a veneer of fluorescence-free microscopy immersion oil, the presence of which has been shown to have no discernable effect on the Raman spectra acquired (Schopf et al., 2005). Varying pixel intensities in the acquired two-dimensional Raman images correspond to the relative concentrations of the material analyzed.

### 3.3. Confocal laser scanning microscopy

Confocal laser scanning micrographs were obtained at UCLA using an Olympus Fluoview 300 confocal laser scanning biological microscope system equipped with two Melles Griot lasers, a 488 nm 20 mW-output argon ion laser and a 633 nm 10 mW-output helium-neon laser. Images were acquired using a 100× oil-immersion objective, fluorescence-free microscopy immersion oil, and filters in the light-path to remove wavelengths < 510 nm (for 488 nm laser excitation) and < 660 nm (for 633 nm laser excitation) from the laser-induced fluorescence emitted by the specimens. Image-sets were subsequently processed by use of the VolView v3.4 3D-rendering computer program that permits image manipulation and interpretation in three dimensions.

### 3.4. Scanning electron microscopy (SEM)

To facilitate identification and targeting of pyrite grains prior to their sulfur-isotopic analyses by secondary ion mass spectrometry (SIMS), and to confirm the location and quality of SIMS analytical pits, scanning electron microscopy was performed in the JPL Astrobiogeochemistry Laboratory (abcLab). A Hitachi SU3500 variable pressure scanning electron microscope (VP-SEM) was used to acquire images in secondary and backscattered electron modes. After applying a 2-nm-thick Pt/Pd veneer to the surface of polished samples, SEM images were acquired using an accelerating voltage of 15 keV at a working distance of 10 mm.

### 3.5. Secondary ion mass spectrometry (SIMS)

At the University of Wisconsin-Madison WiscSIMS Laboratory, isotopic analyses of sulfur were carried out on micron-sized pyrite grains in polished petrographic thin section TRD-2a-6. WiscSIMS pyrite standard UWPy-1 ($\delta^{34}S_{VCDT}$ = 16.04 ± 0.18‰; Ushikubo et al., 2014; Table S1) was emplaced into the thin section near the area containing the pyrite grains identified and selected for study at UCLA by use of Raman spectroscopy. For isotopic analyses using a SIMS CAMECA IMS 1280, the polished thin section was cut and the part to be analyzed was epoxy-mounted at the center of a 25-mm-diameter disc and gold-coated. Analyses of the isotopic composition of sulfur in the minute pyrite grains were acquired using a $^{133}Cs^+$ primary ion beam-spot size of 5 μm (the majority of which was concentrated in an area < 3 μm broad) at an intensity of 35–37 pA and a secondary ion accelerating voltage of 10 kV. Measurements of sulfur standards were performed using the same corresponding analytical conditions. Multiple consecutive measurements of the pyrite standard were performed before each set of 15–23 sample analyses (Table S1).

For use of this 5 μm beam, external precision was 0.39‰ (two standard deviations of spot-to-spot variability)), a relatively poor analytical reproducibility in comparison with pyrite grains analyzed with a larger (10 μm) spot at the WiscSIMS Laboratory (e.g., Ushikubo et al., 2014). The "Anchor Ridge" specimens yielded low ion count rates due to the small size of the grains analyzed that required use of a 5 rather than a 10 μm spot size (Ushikubo et al., 2014). To evaluate the reliability of the data obtained, particularly from small-size grains having low count rates, secondary ion yields, calculated as the relative count rate [RCR, % = (cps of sample ÷ cps of standard UWPy-1) × 100], were compared to measured $\delta^{34}S$ values. Sample analyses having less than 50% of the count rate for pure pyrite were on average a few permil higher than for grains having higher count rates, and because no standard exists for a mixed pyrite-quartz matrix it was not possible to rule out instrumental artifacts for the S-poor pits that yielded lower count rates. No correlation of $\delta^{34}S$ with count rates was observed for ion yields above 50% (Fig. S1).

### 4. Results

Figs. 1–5 document the microscopic carbonaceous filamentous fossils reported here, showing that they are preserved in a pyrite- and originally evidently gypsum/anhydrite-rich evaporite (Fig. 1); that the sulfur isotopic composition of the associated pyrite is consistent with the presence of sulfate-reducing bacteria (Fig. 2, Table S1); that many of the fossils are organically permineralized in aggregated swirls (Fig. 3); that flat-laminated microbial fabric is intermixed with such swirls (Fig. 4); and that the fossils are cellular and not uncommonly exceptionally long (Figs. 3 and 5). These data – acquired *in situ*, in petrographic thin sections, documenting the syngenicity and indigenousness of the assemblage to the chert studied – establish the biogenicity of the fossils, support interpretation of the swirls as sulfate-reducing microbial sulfuretums, and are consistent with interpretation of the flat-laminated fabric as evidencing phototrophs.

Fig. 1 shows the Strelley Pool "Anchor Ridge" microbial swirls, interpreted to be sulfur-cycling sulfuretums, intermixed with closely associated quartz pseudomorphs evidently after evaporitic gypsum/anhydrite, siliceous pseudomorphs notably similar to those occurring in the Mississippian (~345 Ma) Keokuk Limestone of Kansas, USA (Franseen, 2006). In comparison with the grain size of the microcrystalline quartz of the encompassing chert matrix, that of the evaporite-replacing pseudomorphs is appreciably larger (Fig. 1B, D, H) and the globular intermixed close-packed originally gelatinous microbial swirls are unflattened (Figs. 1I, K; 3A, C–F), both evidencing early diagenetic silicification, prior to sediment-compaction. Similarly, because evaporitic gypsum/anhydrite is readily compacted during diagenesis (Maiklem et al., 1969; Shearman and Fuller, 1969), the preservation of

intact, unbroken and uncompacted radiating bladed siliceous pseudomorphs of evaporite crystals (Fig. 1A–D), rosettes (Fig. 1A, B, G, H) and nodules (Figs. 1A, I, K; 3A, C–F) indicates that evaporite emplacement and silica replacement occurred during early stages of diagenesis.

Although firm identification of the original mineralogy of pseudomorphs after evaporitic crystals is fraught with difficulty, siliceous evaporitic pseudomorphs interpreted as evidencing the early silicification of gypsum/anhydrite are well documented in the geological record (e.g., Chowns and Elkins, 1974; Geeslin and Chafetz, 1982; Franseen, 2006); evaporitic sulfate crystal laths and rosettes morphologically comparable to those of the "Anchor Ridge" specimens occur in Holocene coastal sediments of the Persian Gulf (Alsharhan and Kendall, 2003; Kendall and Alsharhan, 2011; Court et al., 2017), a near-surface shallow-marine setting like that inferred for the deposits studied here; and similar radiating-upward evaporitic sulfate crystals have been described from seven Recent, Miocene and Permian deposits where, like the "Anchor Ridge" evaporites, they are demonstrably microfossiliferous (Schopf et al., 2012).

Of particular note, in the "Anchor Ridge" chert both the evaporitic pseudomorphs (Fig. 1A–D, G, H) and the associated globular swirls (Fig. 1A, B, I, K) are enshrouded by particulate kerogen (Fig. 1E, J, L) – microbe-derived degraded remnants of Strelley Pool organics – as well as micron-sized pyrite granules (Fig. 1F, M, N), a firm indicator of the anoxia of the early diagenetic environment consistent with the anaerobic to microaerophilic metabolism of sulfuretum bacteria, both fossil (Schopf et al., 2015; Czaja et al., 2016) and modern (Baumgartner et al., 2006; Gallardo and Espinoza, 2007a, 2007b).

SIMS analyses of the sulfur isotopic compositions of the ~3400 Ma "Anchor Ridge" fossil-associated pyrite are consonant with interpretation of the swirls as evidencing sulfate-reducing microbes. Analyses of 16 specimens of this granular pyrite (Fig. 2, Table S1) yielded $\delta^{34}S_{VCDT}$ values ranging from −2.6 to +5.4‰, within the range of those obtained on micron-sized pyrite grains ($\delta^{34}S_{VCDT}$ = −12 to +6‰) from Strelley Pool Formation sandstone at the East Strelley locality (Wacey et al., 2010a). Such ranges of $\delta^{34}S$ values have been interpreted as indicating the occurrence of microbial sulfate-reduction (Lyons et al., 2015), a process inferred to have been extant at ~3490 Ma (Shen et al., 2001, 2009; Philippot et al., 2007; Ueno et al., 2008) and thus predating preservation of the "Anchor Ridge" fossils.

In laboratory cultures, extant sulfate-reducing microbes exhibit decreasing amounts of sulfur isotopic fractionation as specific rates of sulfate reduction increase, and some taxa exhibit higher specific rates, and therefore lower fractionations, with increasing temperature (Kaplan and Rittenberg, 1964; Chambers et al., 1975). Thus, sulfur isotopic values of the "Anchor Ridge" pyrite grains may indicate that they formed as a result of rapid microbial sulfate reduction (Canfield et al., 2000) in a locally sulfate-rich (Canfield and Raiswell, 1999) relatively high-temperature (i.e., ~65 °C) Paleoarchean environment (Knauth and Lowe, 1978, 2003; Garcia et al., 2016), likely using $H_2$ as an electron donor (Canfield, 2001) – an interpretation that, interestingly, suggests that their associated sulfate evaporites may have originally been anhydrite rather than lower temperature-precipitating gypsum.

Fig. 3 shows representative examples of Anchor Ridge permineralized carbonaceous sulfuretum swirls, the interlinked polycyclic aromatic hydrocarbon-rich kerogenous composition of their component filaments consistent with results of fluorescence-based confocal laser scanning microscopy (Fig. 3D, F) and established by Raman spectroscopy (Fig. 3B). Like morphologically similar filaments that enclose ellipsoidal nodules interpreted as quartz pseudomorphs after anhydrite in mid-Precambrian sulfuretums (Van Kranendonk et al., 2012; Schopf et al., 2015), those of the Archean "Anchor Ridge" chert exhibit a random "cobweb-like" fabric (Fig. 3A, C, E) that encloses but does not penetrate such nodules, with relatively large-diameter long-celled arcuate filaments commonly occurring on nodule surfaces (Fig. 3C, D).

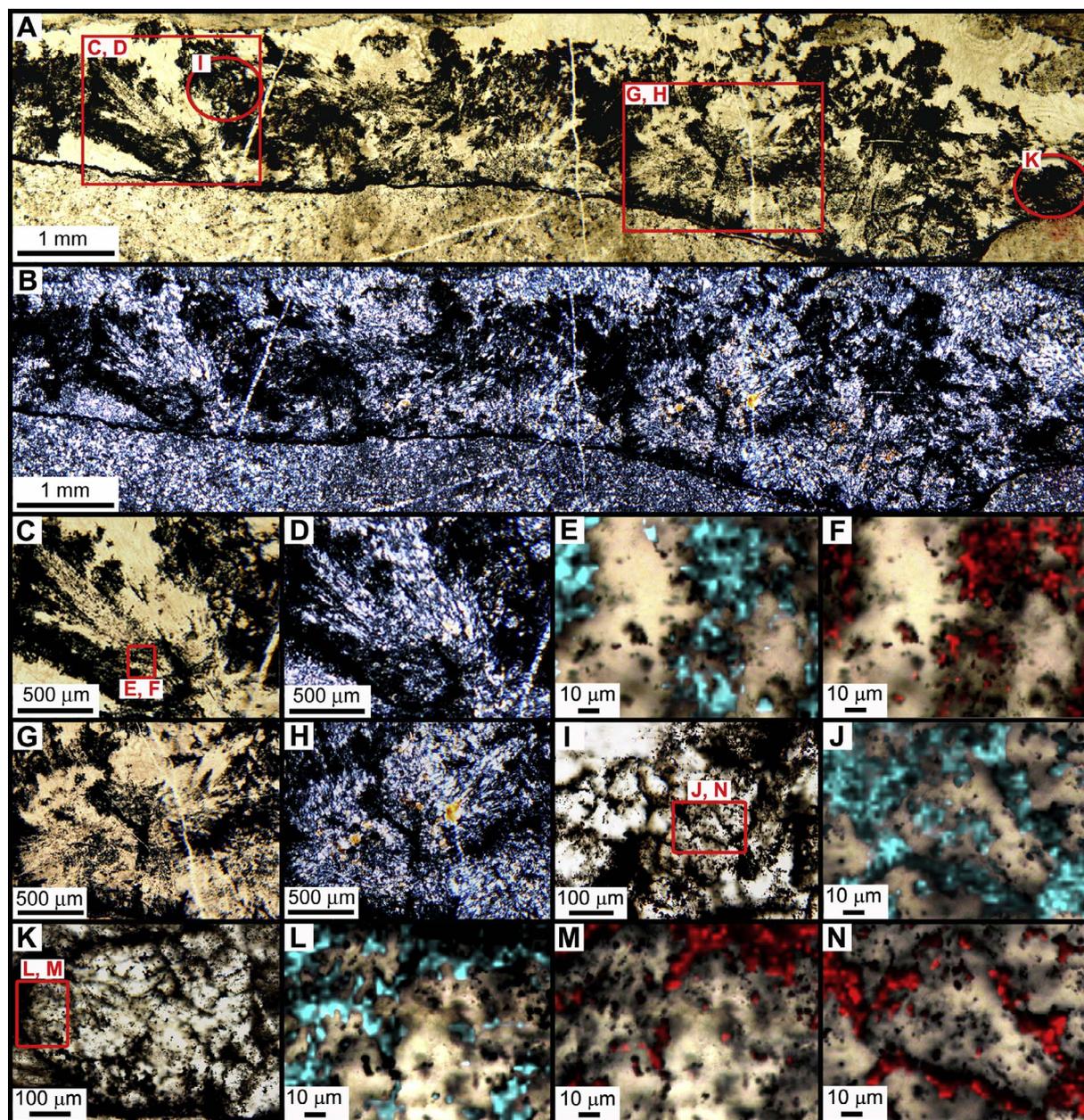In Fig. 4 are shown examples of microbially produced laterally

**Fig. 1.** Evaporitic bed, illustrated in petrographic thin section TRD-2a-6 of carbonaceous chert from the ~3400 Ma Strelley Pool Formation at the "Anchor Ridge" locality, shown in optical photomicrographs, acquired in transmitted white light (A, C, G, I, K) and crossed nichols plane polarized light (B, D, H), and Raman images that document the distribution of kerogen (E, J, L; blue, acquired in a spectral window centered on the kerogen "G" band at ~1605 cm$^{-1}$) and pyrite (F, M, N; red, acquired at its major ~384 cm$^{-1}$ band). (**A, B**) A laterally continuous sediment-surface layer in which the red rectangles in (A) denote quartz pseudomorphs after evaporitic gypsum/anhydrite shown at higher magnification in (C, D, G, H) and the red ellipses, intermixed (I) and basal (K) sulfuretum swirls. (**C–H**) Upward-radiating evaporitic (originally sulfate) laths (C, D) and intermixed rosettes (G, H) in which the vertically oriented rectangle in (C) denotes the area of the horizontally oriented Raman images in (E), showing particulate kerogen (blue), and (F), showing micron-sized granular pyrite (red). (**I–N**) Originally globular sulfuretum swirls outlined by kerogen and pyrite at their margins, the red rectangle in (I) denoting the area shown at higher magnification in kerogen (J) and pyrite (N) Raman images, and that in (K), the area in kerogen (L) and pyrite (M) Raman images. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

extensive flat laminae, a fabric that differs decidedly from that of the globular sulfuretum swirls. Such highly ordered flat-laminated organization is well documented in Proterozoic stromatolitic cherts, particularly those deposited in quiescent lagoonal or shallow-water mudflat environments (e.g., Schopf, 1968; Schopf and Blacic, 1971), and is exhibited by many fossil and modern microbial mat communities (e.g., Walter, 1976; Cohen et al., 1984). In such instances, the laminar texture typically reflects the phototactic response of microbial phototrophs as they spread across surfaces in their need for light.

Representative "Anchor Ridge" filaments are illustrated in Fig. 5 showing examples that document their incompletely preserved original

cellularity (Fig. 5B–D) and notable length (Fig. 5A, B), in some instances in excess of 500 μm (Fig. 5A), that like the well-ordered flat-laminated organization of the mat assemblages are consistent with their preservation in a quiescent low-energy setting devoid of filament-fragmenting and unlithified microbial mat layer-disrupting vigorous wave action.

## 5. Discussion

As is shown in Fig. 4, the "Anchor Ridge" flat-layered microbial mats are intimately intermixed with (Fig. 4A, B) and are surrounded by
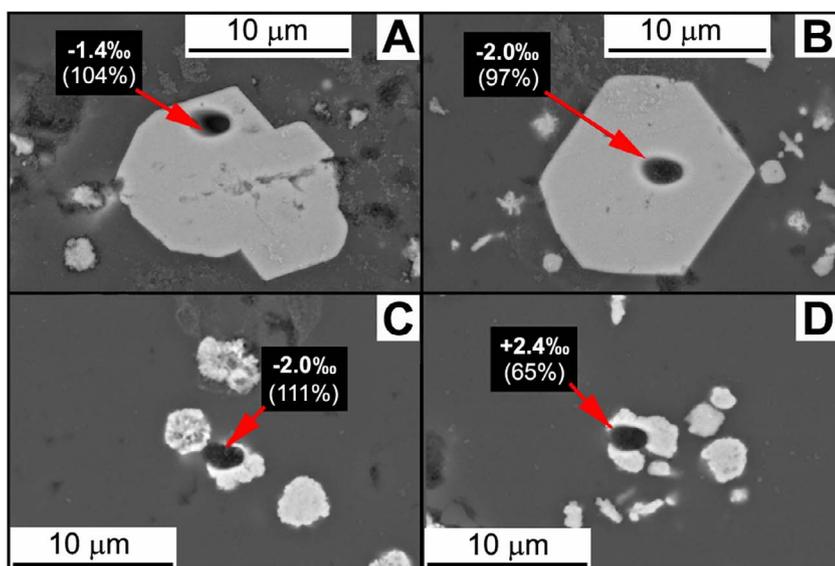
**Fig. 2.** Backscattered electron images of pyrite grains from the area of thin section TRD-2a-6 illustrated in Fig. 1A, showing the SIMS pits analyzed (denoted by the red arrows) and the measured $\delta^{34}S_{VCDT}$ values and ion count rates (%) relative to the average for UWPy-1 pyrite standard (Table S1). (**A, B**) Relatively large euhedral grains (A, specimen TRD-2a-6_033; B, _019). (**C, D**) Smaller pyrite granules (C, specimen TRD-2a-6_011; D, specimen TRD-2a-6_026). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

globular sulfuretum swirls. This intimate co-existence – not previously reported from the geological record – seems ecologically implausible. Mat-forming phototrophs require light. Sulfuretum microbes do not. Phototrophs inhabit the near-surface photic zone whereas modern sulfur-cycling assemblages typically occupy habitats devoid of visible light such as the sub-surface regions of microbial mat communities (Jørgensen et al., 1992) and sub-seafloor mud (Gallardo and Espinoza, 2007a, 2007b) as do the deep-sea mid-Precambrian fossil sulfuretums previously recorded (Schopf et al., 2015; Czaja et al., 2016). Moreover, and although studies of hundreds of Proterozoic microbial communities establish that the laminar fabric of stromatolites evidences the presence of $O_2$-producing cyanobacteria (e.g., Schopf, 2009), in the Paleoarchean "Anchor Ridge" chert the flat laminae-forming phototrophs are intimately close-packed with enormous numbers of sulfuretum swirls

(Fig. 4C) composed of anaerobic (Gallardo and Espinoza, 2007a, 2007b) to minimally oxygen-tolerant sulfate-reducing bacteria (Baumgartner et al., 2006).

How could such metabolically differing microbes co-exist in the "Anchor Ridge" sulfuretum-phototroph biocoenose?

Understanding of the ecology and metabolism of modern microbes may provide an answer. In extant bacterial consortia, it is common for effluents of one microbial group to be consumed by another (e.g., Clark et al., 2009), in this instance $H_2S$ produced by sulfate-reducing bacteria providing a product inferred to have been consumed and used as a metabolic reactant by physically closely associated anoxygenic phototrophs. And because concentrated $H_2S$ is toxic to sulfate reducing bacteria (Reis et al., 1992), this metabolically interdependent relationship would have been beneficial to both components, the $H_2S$
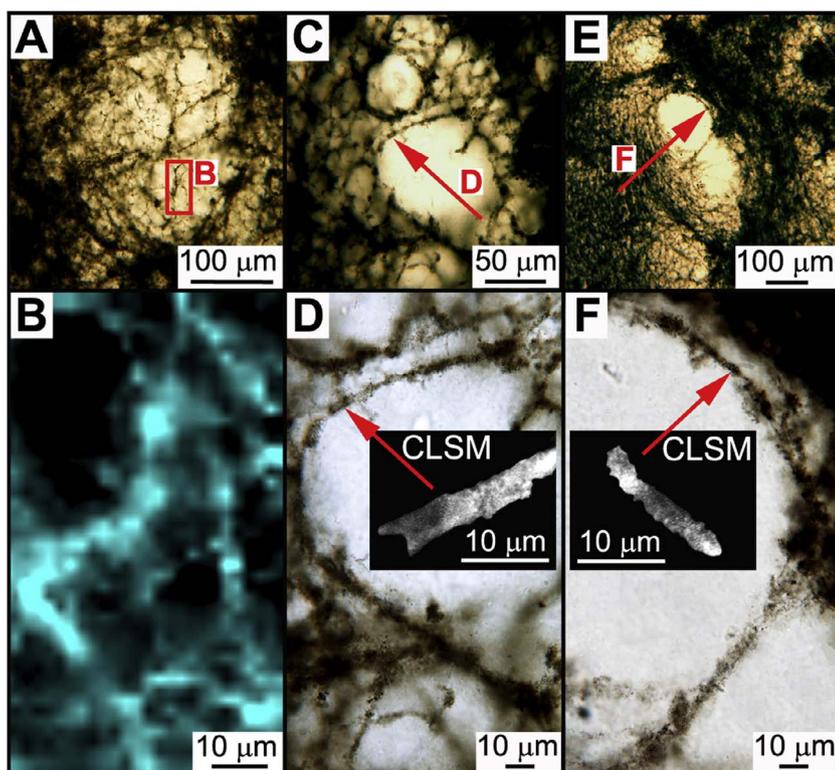


**Fig. 3.** Organically preserved sulfuretum swirls, illustrated in petrographic thin section TRD-2a-7 of carbonaceous chert from the ~3400 Ma Strelley Pool Formation at the "Anchor Ridge" Locality, shown in optical photomicrographs (A, C–F), confocal laser scanning micrographs (insets in D and F), and a Raman kerogen image (B, blue, acquired in a spectral window centered on the kerogen "G" band at ~1605 cm$^{-1}$). (**A, B**) A representative swirl illustrating the randomly oriented "cobweb-like" fabric characteristic of such microbial aggregates, the red rectangle in (A) denoting the area shown at higher magnification in the Raman kerogen image (B) that establishes the carbonaceous, kerogenous composition of the interlaced filaments. (**C–F**) Similar sulfuretum swirls, the arrows in (C, E) denoting the arcuate filaments shown at higher magnification in (D) and (F), respectively, in which the CLSM images confirm the cylindrical cellularity and kerogenous composition of the filaments that, as is typical of Mid-Precambrian sulfuretums (Van Kranendonk et al., 2012; Schopf et al., 2015), encompass quartz nodules interpreted to be pseudomorphs after anhydrite. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
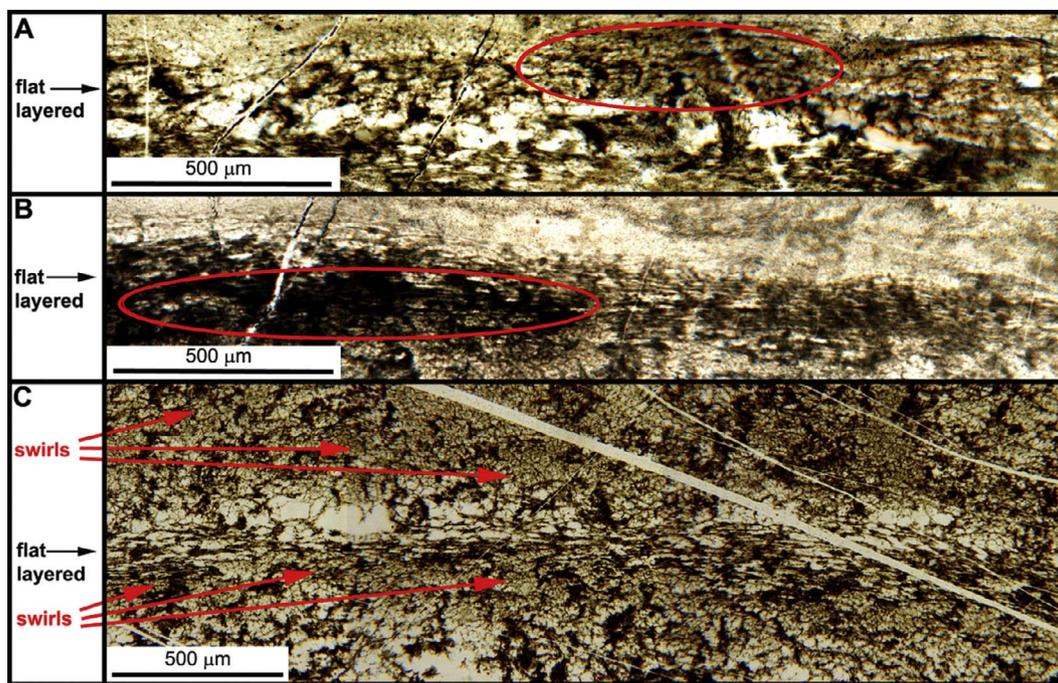
**Fig. 4.** Carbonaceous intermixed flat-layered and sulfuretum-swirl microbial fabrics, illustrated in petrographic thin section TRD-2a-7 of carbonaceous chert from the ~3400 Ma Strelley Pool Formation at the "Anchor Ridge" Locality, shown in optical photomicrographs. (**A, B**) Predominantly flat-layered fabrics intermingled with (encircled by ellipses in B and C) and enclosed above and below by less distinct sulfuretum swirls. (**C**) A laterally continuous flat-layered region (center) enclosed both above and below by thousands of sulfuretum swirls.

produced by the sulfuretum swirls being scavenged from the immediate environment by the H$_2$S-consuming phototrophs. By analogy, we thus interpret the intermingled co-occurrence of these two metabolically interdependent but phylogenetically disparate microbial groups in the "Anchor Ridge" chert to constitute a microbial consortium, an example of ecological symbiotic mutualism known well in modern microbial biocoenoses including such familiar examples as the microbial assemblages of sewage treatment facilities (Manz et al., 1994) and bovine rumens (Brulc et al., 2009).

Among the more notable findings of the study reported here of the Strelley Pool Fm. "Anchor Ridge" biocoenose, evidently the first such microbial consortium to be reported from the geological record, are data documenting its preservation in a quiescent, near-surface shallow water environment evidenced by its geological setting (Sugitani et al.,

2010); its intimate co-occurrence with millimetric quartz pseudomorphs evidently after evaporitic gypsum/anhydrite; and the laterally extensive flat layers and notably long unbroken filaments of its evidently phototrophic components. In this habitat, nodules interpreted to be pseudomorphs after anhydrite are surrounded by globular sulfuretum swirls of anaerobic or minimally oxygen tolerant sulfate-reducing microbes (Baumgartner et al., 2006) and abundant diagenetic micron-sized pyrite grains (Fig. 1F, M, N), indicators of an anoxic or virtually anoxic setting.

In concert with reports of possible sulfur bacteria from a near-shore shallow water sandstone of the Strelley Pool Formation (Wacey et al., 2006, 2010a, 2011a,b), evidence presented here strongly suggests that the oxygen content of Earth's Paleoarchean atmosphere was quite low; that recently expressed concerns (Homann et al., 2016) that Archean
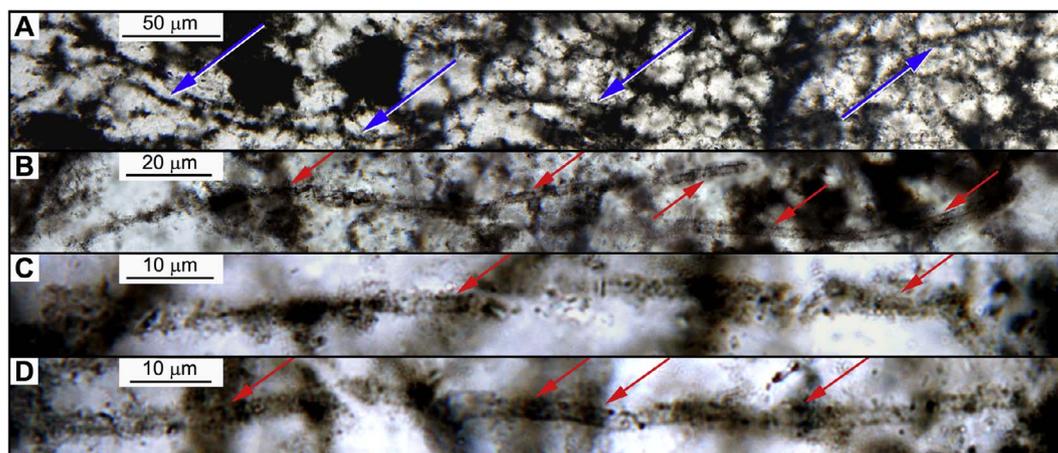


**Fig. 5.** Chert-permineralized organically preserved microbial filaments, illustrated in petrographic thin sections of carbonaceous chert from the ~3400 Ma Strelley Pool Formation at the "Anchor Ridge" Locality, shown in optical photomicrographs. (**A**) A gently sinuous > 500-μm-long filament (blue arrows) intermixed with other filaments and organic detritus (thin section TRD-2a-1), such exceptionally lengthy filaments being typical of the quiescent environment inhabited by sulfuretums (Gallardo and Espinoza, 2007a,b; Schopf et al., 2015). (**B–D**) Similar filaments illustrating the incomplete preservation of the exceptionally ancient Strelley Pool fossils in which the red arrows point to regions of optically discernable lateral and partially preserved transverse cell walls (B, thin section TRD-2a-1; C, TRD 2a-6; D, TRD-2a-6). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

microbes may have lacked "adequate protection against the intense ultraviolet radiation that characterized the Archean ozone-free atmosphere" are misplaced; and that, at least locally, the availability of biologically useable sulfur was not biotically limiting – a rather surprising finding given the widely accepted assumption that environmental $SO_4^=$ increased globally only after the billion-year-later, mid-Precambrian (2400–2200 Ma) "Great Oxidation Event" (e.g., Catling and Claire, 2005; Sessions et al., 2009).

In addition, given the widespread occurrence of microbially produced stromatolites in the Strelley Pool Formation – initially described by Hoffman et al. (1999) from the Panorama greenstone belt at the "Trendall locality" and subsequently reported from numerous other sites (e.g., Allwood et al., 2006, 2007, 2009) – and their presence also in the older ~3490 Ma Dresser Formation of the Pilbara Craton (Walter et al., 1980; Van Kranendonk et al., 2008), the current report presents the first direct microfossil-based evidence suggesting that these Paleoarchean stromatolites, variable in scale and laminar characteristics, may have been produced by anoxygenic phototrophs rather than by oxygen-producing cyanobacteria. This interpretation is consistent with the copious occurrence in such units of spheroidal micron-sized pyrite grains – possibly diagenetically altered sulfur granules like those produced extracellularly by early-evolved *non*-$O_2$-producing bacterial photosynthesizers (e.g., chloroflexaleans; Madigan and Brock, 1975) – an inference that if substantiated will constrain speculations about the time of origin of $O_2$-producing photosynthesis, arguably among the most significant evolutionary innovations in the history of life.

The known fossil record is consistent with the inferred anaerobic metabolism of the Strelley Pool "Anchor Ridge" sulfuretum swirls and their associated flat-laminated phototrophs. The deep sea mud-inhabiting sulfuretums of mid-Precambrian (2300 and 1800 Ma) units are similarly anaerobic (Schopf et al., 2015) as are those of modern sub-sediment sulfur-cycling assemblages (Gallardo and Espinoza, 2007a, 2007b). Moreover, neither the narrow-filament-composed "Anchor Ridge" sulfuretums nor the two comparable mid-Precambrian biocoenoses includes such broad-diameter (and readily identifiable) microaerophilic sulfate-reducers as *Thioploca* and *Beggiatoa* (Jørgensen and Gallardo, 1999), taxa inferred to have evolved later in geological history (Schopf et al., 2015).

Nevertheless, can it be accepted that the microorganisms of the "Anchor Ridge" sulfuretum swirls were necessarily obligate anaerobes, unable to survive in the presence of oxygen? No, probably not, given the presence of oxygen-tolerant sulfate-reducing microbes in the extant biota (e.g., Cypionka et al., 1985; Teske et al., 1998; Baumgartner et al., 2006). Similarly, were the "Anchor Ridge" sulfuretum swirl-associated flat-laminated filamentous phototrophs necessarily strict anaerobes? Again, probably not, given the fact that some modern anoxygenic photosynthetic bacteria are tolerant to low oxygen concentrations (e.g., Visscher et al., 1992; Teske et al., 1998).

The precise physiology of the "Anchor Ridge" fossils – whether strictly anaerobic or both anaerobic and microaerophilic – cannot be determined on the bases of the available fossil evidence and analogy with modern microbes. Moreover, it seems certain that at least small concentrations of ambient oxygen would have been present in the Paleoarchean environment produced by UV-induced photodissociation of $H_2O$ and $CO_2$, perhaps similar in quantity to that contributed by such processes to the current atmosphere of Mars (~1200 ppm $O_2$) but orders of magnitude less than that of oxygenic photosynthesis-derived oxygen in Earth's present-day atmosphere (~210,000 ppm). To remain viable in the presence of such ambient oxygen, which would have yielded biologically deleterious peroxides (e.g., $H_2O_2$), superoxide ($O_2^-$), hydroxyl radicals ($OH^\cdot$), and singlet oxygen, ($O_2 [^1\Delta_g]$), near-surface Paleoarchean microbes would have required intracellular enzymes such as peroxidases, superoxide dismutase, and catalase, the times of evolutionary origin of which are unknown.

Despite these uncertainties, it is well established that anoxygenic bacterial photosynthesis was the evolutionary precursor of oxygen-producing cyanobacterial photosynthesis (Blankenship, 2010) and, given the great age of the ~3400 Ma "Anchor Ridge" microbial consortium and current uncertainties about the time of origin of $O_2$-generating photosynthesis (Schopf, 2011), it is plausible and seems likely that the flat laminated fabric-producing filaments of the "Anchor Ridge" cherts were anoxygenic photosynthetic bacteria (e.g., members of the early originating Chloroflexales, green non-sulfur bacteria), a supposition consistent with the presence of abundant micron-sized pyrite grains – both in this unit (Fig. 1F, M, N) and in other shallow-water Strelley Pool deposits, whether clastic (sandstone of the East Strelley locality; Wacey et al., 2010a) or stromatolitic (carbonate units of the Trendall locality; Hoffman et al., 1999; Allwood et al., 2006, 2007) – minute granules that may represent diagenetically altered micron-sized extracellular sulfur granules produced by modern anoxygenic phototrophs such as *Chloroflexus aurantiacus* (Madigan and Brock, 1975).

The occurrence of such copious micron-sized pyrite, shown by the analyses presented here (Fig. 2) to be consistent with the presence of sulfur-metabolizing microbes and atypical of post-Archean shallow water microbially laminated units, suggests that microbial anaerobic sulfur-cyclers were a major component of the Paleoarchean biota. Consonant with this interpretation – including the $H_2S$-production of such cyclers and its consumption by co-occurring anoxygenic consortial phototrophs as suggested here – are recent studies based on analyses of ~2.7 Ga rain drop imprints (Som et al., 2012) and ~3.0–3.5 Ga fluid inclusions in volcanics (Som et al., 2016) suggesting the Archean origin of oxygen-poisoned nitrogen fixation, a biochemical reaction encoded by the early-evolved *nif* gene complex. The primary enzyme of this complex is nitrogenase that mediates the conversion of ambient $N_2$ to biologically useable products and that is decoupled, rendered inoperable, by its interaction with $O_2$ – but, notably, is virtually universal in primitive anoxygenic phototrophs (Schopf, 1978; Madigan, 1995; Ludden and Roberts, 2002; Shridhar, 2012) as well as in early-evolved (Hayes, 1983) archaeal methanogens (Leigh, 2000).

Given the close-packed association of the "Anchor Ridge" phototrophs and the $H_2S$-producing sulfuretum bacteria (Fig. 4), it is evident that both of these principal components of the consortium were adapted to the same anoxic or oxygen-deficient ecologic setting. Nevertheless, and although the photosynthesis of these phototrophs was presumably anoxygenic rather than being oxygen-producing, it does not necessarily follow that they were photosynthetic bacteria rather than being primitive cyanobacteria. In this regard, the energy relations of the hydrogen (i.e., electron) source used in the earlier-evolved anoxygenic and later-evolved oxygenic (viz., cyanobacterial) forms of photosynthesis are of particular relevance, bond dissociation of the $H_2S$ used in anoxygenic photosynthesis requiring 91 kcal/mol (377 kJ/mol) and that for the generation of $O_2$ from $H_2O$, 119 kcal/mol (498 kJ/mol; Blanksby and Ellison, 2003). Because of these differing bond-breaking requirements and the evolutionary derivation of oxygenic photosynthesis from its anoxygenic precursor (Blankenship, 2010), it is not surprising that some extant typically $O_2$-producing cyanobacteria (e.g., *Oscillatoria limnetica*) are capable of oxygenic/anoxygenic facultative photosynthesis, using as their electron donor the less energy-requiring $H_2S$, if available, rather than more energy-needing $H_2O$, and thus carrying out anoxygenic rather than oxygenic photosynthesis (Cohen et al., 1975; Padan, 1979).

Finally, it is conceivable that $H_2S$-production by the "Anchor Ridge" sulfuretums and its inferred consumption by intimately co-existing phototrophs were sequentially partially diurnal, local build-up of $H_2S$ occurring largely during the night, in the absence of its light-driven photosynthetic use, the accumulated $H_2S$ being subsequently consumed by phototrophs during daylight hours, a strategy somewhat similar to that well established in modern cyanobacterial mat-forming communities for the diurnal temporal separation of oxygen-inhibited nitrogenase-dependent $N_2$-fixation from oxygenic photosynthesis (Griffiths et al., 1987; Khamees et al., 1987; Villbrandt et al., 1991).

Although atmospheric oxygen has been "shown to be necessary for the photodestruction of $H_2S$" (Canela et al., 1998, p. 73), in the virtually anoxic Paleoachean environment postulated here and the concomitant absence of an atmospheric ozone layer, UV-induced photolysis of $H_2S$ would have occurred (e.g., Cleaves and Miller, 1998). Thus, such diel alternation of $H_2S$ production and use in a sulfuretum-phototroph biocoenose would necessitate that diffusion of accumulated $H_2S$ into the environment occurred slowly – a possibility not implausible, given that $H_2S$ is highly soluble (Fogg and Young, 1988); that microbial mats are typically anoxic at night, which creates a microenvironment particularly suitable for sulfate-reducing microbes during that period (Visscher et al., 2002; Baumgartner et al., 2006); and that the sulfuretum and phototroph components of the "Anchor Ridge" consortium are close packed in the same gas diffusion-inhibiting mucilage-embedded microbial layers (e.g., Fig. 4A, B).

In sum, the Paleoarchean (~3.4 Ga) evidently anaerobic, shallow water (possibly mudflat-like) evaporitic sulfate-associated "Anchor Ridge" sulfuretum-phototroph microbial consortium described here is notably similar to that envisioned by Canfield and Raiswell (1999, p. 697) who postulated the existence of "a primitive early Earth terrestrial ecosystem … with anoxygenic photosynthesis producing organic matter and oxidized sulfur species," and suggested that "by 3.5 Ga anoxygenic photosynthesis was established and provided a weak source of sulfate to the global ocean [that] in some instances [could produce] locally high concentrations of sulfate [which] could accumulate and precipitate as evaporitic sulfate minerals."

## 6. Conclusions

Carbonaceous chert from the "Anchor Ridge" locality of the Paleoarchean ~3400 Ma Strelley Pool Formation contains a diverse assemblage of permineralized filamentous microbial fossils, among the oldest evidence of life now known.

(1) The reported fossils are demonstrably syngenetic with and indigenous to the fossil-hosting chert, and are assuredly *bona fide* and bacterial as documented by optical microscopy, Raman spectroscopy, confocal laser scanning microscopy, and SIMS analyses of "Anchor Ridge" organics (Lepot et al., 2013), the occurrence of sulfur-cyclers in the assemblage being supported here by SIMS analyses of co-occurring pyrite.

(2) The intermingled occurrence of these microfossils with millimetric quartz-replaced gypsum/anhydrite evaporites and the uncompacted globular and laterally continuous flat-laminated form of the exceptionally long filament-containing assemblages they comprise, coupled with geological evidence of their environmental setting and the mineralogy of their embedding chert matrix, document their early diagenetic permineralization in a quiescent, shallow water, possibly mudflat-like habitat.

(3) Globular aggregates of swirls of "cobweb-like" microbial filaments are closely associated with flat-laminated microbes, establishing their shared ecologic requirements.

(4) As evidenced by their intermingled association with pyrite-rich originally sulfate evaporites and by comparison with younger biocoenoses, both fossil and modern, the globular swirls are interpreted to be sulfuretums composed of oxygen-sensitive sulfate-reducing microbes. In contrast, the fossil microbes of the flat-laminated assemblages are regarded most probably to be anoxygenic phototrophs, the first microfossils to be so interpreted from the geological record.

(5) The intimate physical intermixing and inferred metabolic interdependence of the two anaerobic to minimally oxygen-tolerant components of this sulfuretum-phototroph microbial consortium, evidently the first such mutualistic symbiotic community to be identified in the geological record, strongly suggest that their near-surface shallow water habitat was essentially devoid of ambient

oxygen.

Given the foregoing, we infer that sulfur-cycling assemblages constituted a major component of the Paleoarchean near-surface biosphere; that phototrophs in the ~3400 Ma environment, whether photosynthetic bacteria or their cyanobacterial evolutionary derivatives, carried out anoxygenic photosynthesis; and, therefore, that stromatolites of this age are perhaps most likely to have been produced by anoxygenic phototrophs rather than by $O_2$-producing cyanobacteria as has been widely assumed. These suppositions, if confirmed by future studies, may help to constrain the time of origin of oxygenic photosynthesis.

## 7. Statement of significance

An anaerobic ~3400 Ma shallow-water microbial consortium: presumptive evidence of Earth's Paleoarchean anoxic atmosphere.

Carbonaceous chert from the "Anchor Ridge" locality of the Paleoarchean ~3400 Ma Strelley Pool Formation of Western Australia contains intermingled globular "cobweb-like" assemblages and laterally continuous flat-laminated masses of permineralized, uncompacted, filamentous microbial fossils. The local geological setting, exceptionally long unbroken filaments exhibited by these microfossils, and their intermixed occurrence with pyrite-rich millimetric originally sulfate evaporites document their preservation in a quiescent, shallow water, possibly mudflat-like habitat. By comparison with younger biocoenoses, both fossil and modern, the globular swirls are interpreted to be composed of obligately anaerobic sulfate-cycling microbes whereas the fossil microbes of the flat-laminated assemblages are regarded to be anaerobic anoxygenic phototrophs. The intimate physical association of these two biotic components evidences their shared ecologic requirements and identifies this biocoenose as a sulfuretum-phototroph microbial consortium, $H_2S$ produced by the sulfuretum bacteria providing a product consumed and used as a metabolic reactant by closely associated anoxygenic phototrophs. And because concentrated $H_2S$ is toxic to sulfate reducing bacteria, this metabolically interdependent relationship was beneficial to both components, the $H_2S$ produced by the sulfuretum swirls being scavenged from the immediate environment by the $H_2S$-consuming phototrophs.

We thus interpret the 3.4 Ga "Anchor Ridge" biocoenose to be a near-surface shallow water symbiotic anaerobic microbial consortium composed of $H_2S$-producing sulfuretums and $H_2S$-consuming anoxygenic phototrophs. If this interpretation is correct, this community provides the first evidence of microbial consortia and of anoxygenic phototrophs identified in the geological record; indicates that the availability of biologically useable sulfur was not biotically limiting and that the oxygen content of Earth's Paleoarchean atmosphere was evidently quite low; and suggests that stromatolites of this age may have been produced by anoxygenic phototrophs rather than by oxygen-producing cyanobacteria, constraining speculations about the time of origin of $O_2$-producing photosynthesis, arguably among the most significant evolutionary innovations in the history of life.

## Appendix A. Supplementary data

Analytical data for *in situ* secondary ion mass spectrometry (SIMS) measurements at WiscSIMS of the sulfur isotopic compositions of pyrite grains in petrographic thin section TRD-2a-6 of carbonaceous chert from the "Anchor Ridge" locality of the ~3400 Ma Strelley Pool Formation of northwestern Western Australia. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.precamres.2017.07.021.

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